

In Vitro Digestion of RS4-Type Resistant Wheat and Potato Starches, and Fermentation of Indigestible Fractions

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ABSTRACT

Cereal Chem. 88(1):72–79

RS4-type resistant wheat starch (RWS) and resistant potato starch (RPS) were subjected successively to in vitro digestion with pepsin and pancreatin-bile, and the indigestible residues (82.1% db and 74.1% db, respectively) were recovered and subsequently fermented by in vitro techniques using fresh human fecal microbiota as inoculum. Scanning electron microscopy of the indigestible residues showed surface erosion on the residual granules. Total gas production during the in vitro fermentation increased almost linearly over time with the two resistant starches exhibiting similar gas production rates, as well as a similar rate of pro-

duction of total short-chain fatty acids (SCFA). The indigestible fractions from both starches produced acetate as the major SCFA and relatively higher levels of butyrate than propionate, but wheat starch tended to produce more butyrate over time than potato starch. Fractional molar ratios of acetate, propionate, and butyrate from the RWS and RPS were 0.586:0.186:0.228 and 0.577:0.200:0.223, respectively. The calculated caloric contributions of the RWS and RPS are ≈33% lower than for unmodified starch and are comparable to those reported in the literature for RS2 and RS3 high-amylose maize starches.

Classic studies, both in vitro and in vivo, have demonstrated that raw starch as well as starch in baked products can be partially or completely digested by amylases (Langworthy and Deuel 1920). While developing an in vitro dietary fiber assay for nonstarch polysaccharides, Englyst et al (1982) showed that some starch even in cooked foods resisted amylase digestion and remained in the fiber residue. The presence of “resistant” starch in the human digesta was confirmed by examining the ileal effluents of ileostomy patients (Englyst and Cummings 1985). Resistant starch is currently defined as “the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals” (Asp 1992). Five classes or types of resistant starch are reported where the nature of enzyme resistance depends on the physical accessibility of α -amylase or on chemical structural features of the substrate (Nugent 2005; Brown et al 2006). RS1 is physically shielded from digestion and occurs in pasta, legumes, and whole or coarsely milled grains. Ungelatinized starch granules with B-type crystals such as those from raw potatoes, green bananas, high-amylose corn, and some legumes are classified as RS2. Retrograded starch polymers belong to RS3 and are found in cooked and cooled potatoes, bread, and cornflakes. Starches chemically modified by cross-linking, substitution, oxidation, acid treatment, or combinations thereof belong to RS4. Food and beverage products formulated with chemically modified starches may contain varying amounts of RS4. RS5 is an amylose-lipid complex that exists as the V-type polymorph (Brown et al 2006).

Isolated and purified forms of RS1, RS2, RS3, and RS4 classes of resistant starch derived from various botanical sources are available commercially (Nugent 2005; Witwer 2008; Woo et al 2009). On the other hand, a commercial source of RS5 resistant starch is not currently available. Because resistant starches demonstrate similar physiological benefits as dietary fiber when consumed by humans, resistant starch is included in the definitions of dietary fiber by several organizations such as the American Association of Cereal Chemists International and Codex Alimentarius Commission (Anonymous 2001; Codex Alimentarius Commission 2009). Commercially manufactured resistant starches are used as vehicles to increase the total dietary fiber content of food

products, which allows for “good source” or “excellent source” of fiber claims.

RS4-type resistant wheat starch (RWS) has potential applications in high-fiber (low carbohydrate) food products (Maningat et al 2005, 2008). This particular RWS was commercially produced by reacting starch with sodium trimetaphosphate and sodium tripolyphosphate to yield a heavily cross-linked and lightly substituted product belonging to the chemical classification of phosphorylated distarch phosphate (Seib and Woo 1999). Compared to RS2 and RS3 high-amylose corn resistant starches, the RWS delivers a higher level of total dietary fiber when analyzed by the enzymic-gravimetric methods such as AOAC Method 991.43 (Maningat et al 2005; Woo et al 2009). In general, resistant starches, like traditional fiber sources, exhibit physiological benefits such as attenuation of blood glucose and insulin responses, and improved bowel health through decreased transit time, increased fecal bulking, and production of short chain fatty acids (SCFA) (Nugent 2005). In particular, RWS attenuates glycemic and insulin response (Al-Tamimi 2010; Haub et al 2010) and selectively increases the cell counts of *Bifidobacterium adolescentis* in the large bowel of human subjects (Walter 2009).

The gastrointestinal tract is considered one of the human body's metabolically active organs and is home to some 100 trillion microorganisms of at least 400 species (McKenna et al 2008). This complex community of anaerobic microbes with their exposure to the environment and their metabolic activities influences human health, physiology, and immune system (Venema et al 2007; Tannock 2008). Dietary carbohydrates, specifically resistant starch and dietary fiber, are substrates for large bowel fermentation that evolve gases and produce beneficial SCFA primarily acetic (C2), propionic (C3), and butyric (C4) as end products (Topping and Clifton 2001; Nugent 2005). C2, which is the principal SCFA in the colon, enters the peripheral circulation to be metabolized by peripheral tissues. After absorption, C2 increases cholesterol synthesis. C3 is largely taken up by the liver and has been shown in animal studies to inhibit cholesterol synthesis (Chen et al 1984). A decrease in the ratio of C2:C3 production may potentially reduce hepatic synthesis of cholesterol from C2 (Wolever et al 1991) and, consequently, may reduce the risk of cardiovascular disease. C4 is the major energy source of the cells lining the colon (colonocytes) and has been implicated in the prevention of colon cancer through its role in cell differentiation. In vitro studies demonstrated that C4 stimulates cell proliferation of normal colonic cells, whereas it stimulates apoptosis of cancer cells (Hague and Praskeva 1995). It has also healed damaged mucosa in inflammatory bowel diseases, such as ulcerative colitis (Scheppach et al 1992).

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Human fecal material is considered the most accessible and noninvasive source of microbiota that is similar to that found in the human large bowel. In vitro fermentation techniques using human fecal inoculum are useful in determining SCFA production potential because SCFAs produced in vivo are difficult to directly quantify as they are absorbed by the host, utilized as an energy source by large bowel microbiota; the remainder are found in the gut contents. The first aim of this study was to determine and compare the extent of digestion by pepsin and then pancreatin-bile of RS4-type resistant starches from wheat and potato, and second, to determine the rate of fermentation of indigestible residues using an in vitro method using fresh human fecal microbiota as inoculum. This study will show, for the first time, the in vitro fermentability characteristics of RS4-type resistant starches using human fecal inoculum, which may provide clues to a possible role in promoting health, particularly of the large bowel.

MATERIALS AND METHODS

The chemicals and enzymes used in this study are all reagent-grade. The RWS (Fibersym RW, 10.2% moisture) and RS4-type resistant potato starch (RPS; Fibersym 80 ST, 13.9% moisture) were obtained from MGP Ingredients, Atchison, KS. RS4-type resistant starches from tapioca and high-amylose maize were prepared in the laboratory according to the procedure of Woo and Seib (2002). An aqueous slurry of starch (40%, w/w) was reacted with 12% (starch basis) of a 99:1 mixture of sodium trimetaphosphate and sodium tripolyphosphate at pH 11.5 and 45°C in the presence of 10% sodium sulfate (starch basis). The reaction was terminated after 3 hr when the phosphorus level of the modified starch reached $\approx 0.4\%$. ^{31}P -NMR showed that phosphorylated wheat starch (0.38% P) prepared under the same conditions contained 63% of phosphorus as distarch monophosphate ester (cross-link, D.S. = 0.026) and 37% of phosphorus as monostarch monophosphate ester (not cross-link, D.S. = 0.008) (Sang et al 2007).

In Vitro Digestion with Pepsin and Pancreatin-Bile

Starch digestion similar to small intestinal conditions was conducted in vitro according to established methods (Trinidad et al 1996). Duplicate 20-g RWS and RPS were dispersed in 80 mL of distilled water and the mixtures placed in a boiling water bath for 30 min. After cooling, the dispersions were adjusted to pH 2.0, pepsin-HCl solution was added, and the digests then incubated for 3 hr in a water bath at 37°C. Aliquots of the pepsin digests were placed in duplicate dialysis bags containing pancreatin-bile solution plus bile extract in 0.1M NaHCO₃, and merthiolate solution. They were then digested and dialyzed against sodium bicarbonate solution (pH 7.5) for 12 hr at 37°C, with changes in dialysate every 3 hr. A blank control was tested at the same time as the starches.

To determine the amount of 0.5M NaHCO₃ needed during dialysis, a 20-g aliquot of each pepsin digest, to which the pepsin/pancreatin-bile mixture had been added, was titrated to pH 7.5 with the NaHCO₃ solution. This calculated volume of NaHCO₃ was then made up to 100 mL with deionized water and used for dialysis.

After digestion, the dialysis retentates were freeze-dried and weighed. The duplicate freeze-dried retentates were pooled and portions were used for in vitro fermentation and examination by scanning electron microscopy. Weights were corrected for the weight obtained with the blank control.

In Vitro Fermentation with Fresh Human Fecal Inoculum

In vitro fermentation was conducted using methods previously described by McBurney and Thompson (1987). Duplicate freeze-dried starch dialysis retentates (indigestible residues) were weighed into 100-mL serum bottles and the fermentation medium (40 mL)

was added. Blank control samples contain only the medium. The serum bottle contents, which were first reduced using the conditions and reducing agents described by Goering and van Soest (1970), were sealed with a butyl rubber stopper crimped with a metal seal and stored overnight at 4°C. One to two hours (1–2 hr) before inoculation with fecal inoculum, the bottles were put into a water bath at 37°C.

Feces was collected from a healthy individual into a tared blender bowl that was continuously flushed with CO₂, diluted with deoxygenated distilled water to a concentration of 66.6 g of wet feces/L, blended for 30 sec, filtered through glass wool to remove fibrous particles, and squeezed through a 41- μm Nitex membrane. This inoculum (10 mL) was then injected through the septum of a serum bottle. Fecal contents and containers were kept under a continuous flow of CO₂ at all times. Serum bottles were incubated in a water bath at 37°C with swirling at regular intervals. Duplicate samples were removed for analysis at 0, 4, 8, 12, and 24 hr. Immediately after removal of the serum bottles from the water bath, gas production was measured. A 60-mL syringe with a three-way stopcock and needle was used to sample gas contents in the serum bottle to determine total gas production as previously described by McBurney and Thompson (1987). The serum bottle was then opened and 1 mL of 1% copper sulfate solution was added to inactivate the microorganisms. An aliquot was taken from each flask, centrifuged, filtered through 0.22 Millipore filter, and analyzed for SCFA using the HPLC method with diode array detector as previously described (McBurney and Thompson 1987). The SCFA values were corrected for the values obtained with the blank control.

Calculated Food Energy Values of RS4 Resistant Starches

The food energy value of the resistant fraction of the RS4 resistant starches was assumed to be the sum of the energy in the SCFA released during fermentation after 24 hr. The energy content for each specific SCFA in kcal/mol is formic (C1), 61; C2, 209; C3, 365; C4, 522; isobutyric (iC4), 522; and isovaleric (iC5), 678 (McBurney and Thompson 1988). A correction is applied assuming 90% absorption of SCFA in the gut.

Indigestible Residues

RS4-type resistant starches from wheat, potato, tapioca, and high-amylose corn were subjected to total dietary fiber determination by Official Method 991.43 (AOAC International 2005), and the indigestible residues after each step in the method were collected, washed, and freeze-dried. In addition, RWS was subjected to the Englyst assay method (Englyst et al 1992), and the indigestible residues after hydrolysis for 20 min and after 120 min were collected, washed, and freeze-dried.

Scanning Electron Microscopy (SEM)

The freeze-dried indigestible starch residues from the in vitro digestion used in this work and from the AOAC and Englyst methods were sprinkled onto double-sided adhesive tapes on top of specimen stubs and then coated with gold. Microscopic examination was performed using an Etec-Autoscanning electron microscope (Hitachi S-3000N Variable Pressure SEM, Pleasanton, CA) at an accelerating potential of 20 Kv.

Statistical Analyses

In vitro digestibility was analyzed by *t*-test. The gas and SCFA production were analyzed using two-way analysis of variance (ANOVA) for the effect of time, starch, and starch \times time interactions. One-way ANOVA followed by Tukey's multiple range test was used to determine the effect of time on individual SCFA. All analyses used SigmaStat (Aspire Software International, Ashburn, VA).

RESULTS AND DISCUSSION

In Vitro Digestion and Mode of Enzyme Attack

Under the conditions of the in vitro starch digestion, RWS had more indigestible residue ($73.7 \pm 1.0\%$) than RPS ($63.8 \pm 0.6\%$) ($P < 0.001$). Corrected for moisture content of the samples, the indigestible residues amounted to 82.1% and 74.1%, respectively. Despite the heat treatment in a boiling water bath and subsequent pepsin-pancreatin enzyme treatments at the corresponding optimum pH and temperature, the swollen granules of the resistant starches remained intact due to the stabilizing effect of the high degree of cross-linking as similarly observed by several workers (Seib and Woo 1999; Woo et al 2009).

The general mechanism of amylase attack on starch granules has been reported to be either exo-corrosion, endo-corrosion, or a combination of exo- and endo-corrosion (Manelius et al 1997; Planchot et al 1997; Apinan et al 2007). In the SEM images, the indigestible residue from RWS displayed surface erosion as evidenced by a roughened surface of the granules and by pieces peeling off from the surface (Fig. 1). There were no visible signs of random pitting (holes) or the formation of furrows, canals, or tunnels that lead to the granule interior. Furthermore, internal layered structures (growth rings) were not evident. The same surface erosion was observed for RPS, but mostly pieces peeling from the surface were observed (Fig. 2). Some granules have a

smooth surface, and the dimpled appearance in some granules is probably an artifact due to cooking of the modified starch followed by the loss of moisture in the freeze-drying procedure. Cooked RWS without undergoing enzyme treatment showed the same dimpled appearance in the dry starch powder (data not shown). By comparison, indigestible residues isolated after each digestion step of AOAC Method 991.43 displayed surface erosion and pieces peeling off both RWS (Fig. 3) and RPS (Fig. 4). Other RS4-type resistant starches prepared from tapioca and high-amylose corn yielded indigestible residues that demonstrated a similar mode of enzyme attack as described above (data not shown). The cocktail of amyloglucosidase, pancreatin, and invertase enzymes used in the Englyst method at 37°C with agitation appears to roughen the surface of RWS as evidenced clearly by pieces peeling off after 20 min of digestion (Fig. 5). After a total of 120 min of Englyst digestion conditions, a more extensively roughened surface is visible with the formation of deep etches that probably could lead to bore holes (Fig. 6).

RS2-type native high-amylose maize starch granules that were recovered from human ileostomy effluents and from the large bowel of a pig with a cannula inserted into the cecum and proximal large bowel, exhibited substantial etching and pitting (Topping et al 1997; Topping and Clifton 2001). To explain our SEM observations, we considered the surface features of the granules and the effects of cross-linking modification. We speculate that the

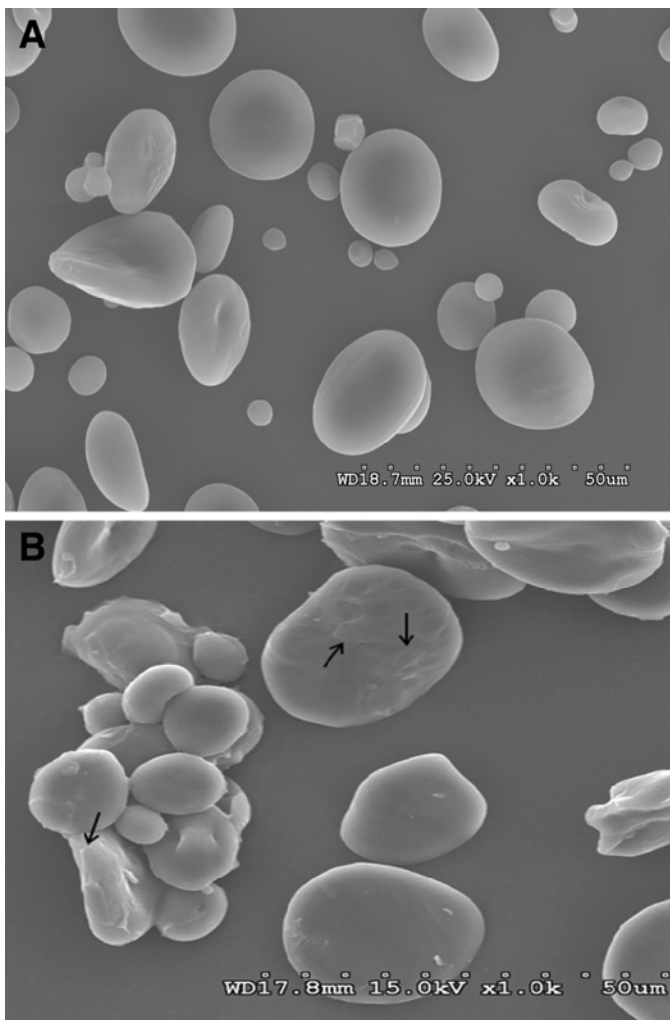


Fig. 1. Scanning electron micrographs of RS4 resistant wheat starch (A) and indigestible residues after cooking in water (boiling water bath) followed by in vitro digestion with pepsin and pancreatin-bile (B). Arrows indicate areas with surface erosion or pieces peeling off.

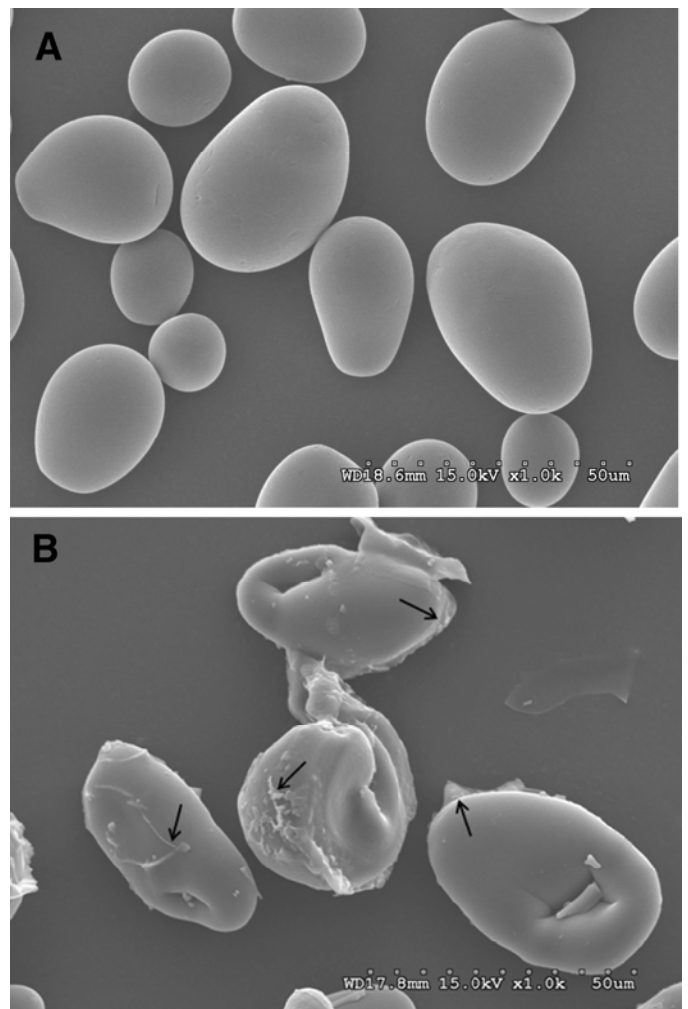


Fig. 2. Scanning electron micrographs of RS4 resistant potato starch (A) and indigestible residues after cooking in water (boiling water bath) followed by in vitro digestion with pepsin and pancreatin-bile (B). Arrows indicate areas with surface erosion or pieces peeling off.

protruding nodules or blocklets observed by atomic force microscopy on a starch granule surface (Baldwin et al 1997) are readily accessible and therefore most susceptible to amylase attack. Phosphate cross-linking bonds restrict granular swelling (Woo and Seib 2002) during heat treatment and help stabilize granular structure.

Furthermore, phosphate groups could sterically hinder formation of amylase-starch complex, resulting in incomplete hydrolysis. Surface pores and channels common in wheat starch (Kim and Huber 2008) are probably blocked or obstructed by phosphate groups, thereby inhibiting diffusion of amylase molecules.

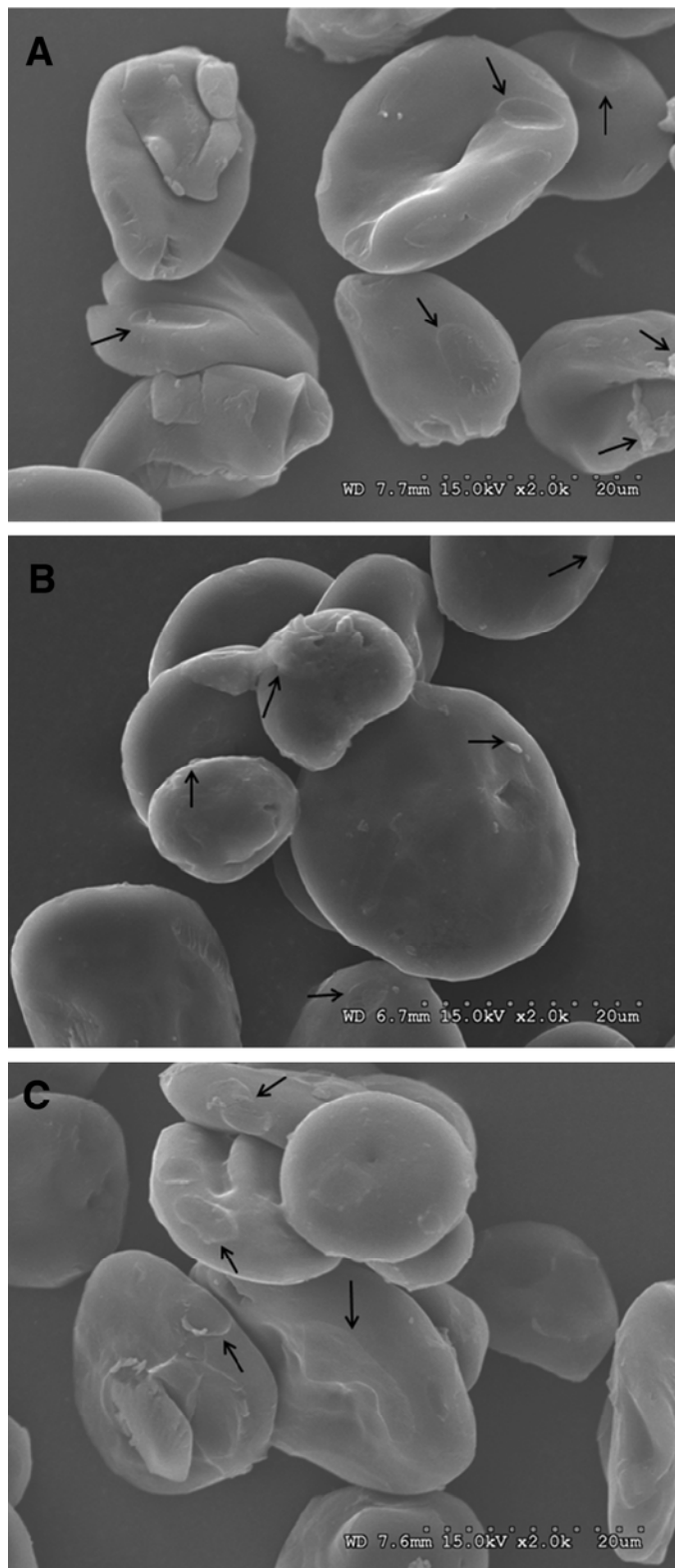


Fig. 3. Appearance of RS4 resistant wheat starch after hydrolysis with α -amylase (A); after hydrolysis with α -amylase and protease (B); and after hydrolysis with α -amylase, protease and glucoamylase (C) according to total dietary fiber determination by AOAC Method 991.43. Arrows indicate areas with surface erosion or pieces peeling off.

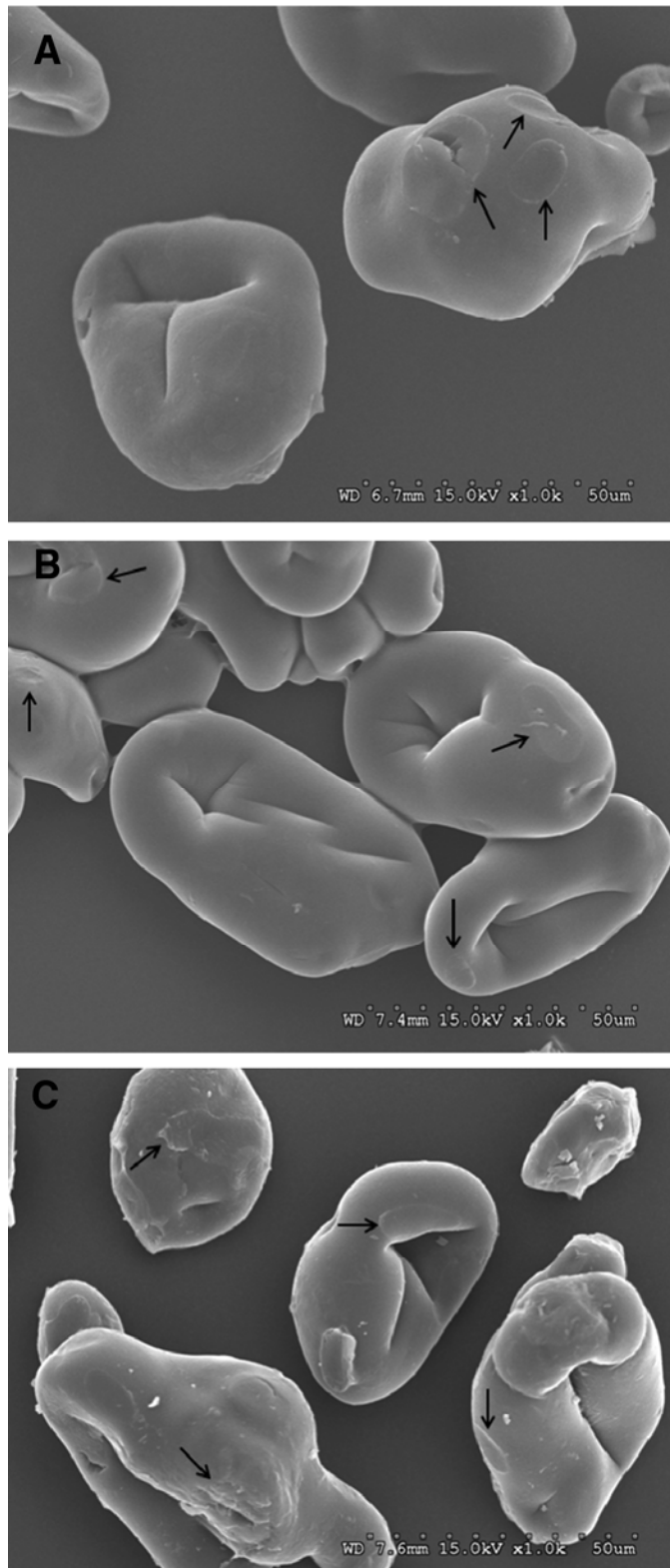


Fig. 4. Appearance of RS4 resistant potato starch after hydrolysis with α -amylase (A); after hydrolysis with α -amylase and protease (B); and after hydrolysis with α -amylase, protease and glucoamylase (C) according to total dietary fiber determination by AOAC Method 991.43. Arrows indicate areas with surface erosion or pieces peeling off.

In Vitro Fermentation

During in vitro fermentation of the indigestible residues from RWS and RPS after pepsin and pancreatin-bile treatments, total gas production increased almost linearly over time, indicating that fermentation occurred during the 24-hr period (Table I). McBurney and Thompson (1989b) indicated that gas production was significantly affected by donor fecal inoculum and that a 24-hr incubation time seemed to be the most appropriate or preferred period for comparing fermentation variables such as gas and SCFA production. There was a significant time effect ($P < 0.001$) on gas production; that is, it increased with time of fermentation but the two starches did not differ significantly.

There was a significant time effect ($P < 0.001$) as well on total SCFA production per gram of resistant starch fermented, but again the two starches overall did not differ (Table II). The higher amounts of total SCFA from RWS than RPS (6.58 vs. 6.10 mmol/g) did not reach statistical significance. In both starches, the main SCFA were C2, C3, and C4, which increased during the 24-hr period. However, the SCFA produced at 8 hr did not differ significantly from those produced at 12 and 24 hr, indicating a leveling off after 8 hr. An exception is C4, which further increased significantly after 12 hr ($P < 0.05$), indicating a shift of fermentation toward C4 production. Previous studies have shown that most of the fermentable starch is consumed after 12–24 hr (McBurney

et al 1990). There tends to be a difference in C4 production ($P < 0.08$) for the two different sources of starch, and the amounts produced from the indigestible RWS residue were greater than those from the indigestible RPS residue.

The fractional molar ratios of C2, C3, and C4 at the end of fermentation were 0.586:0.186:0.228 for RWS and 0.577:0.200:0.223 for RPS in relative agreement with the stoichiometry of the fermentative reaction in the human colon as described by Cummings (1997) as well as in the SCFA ratio of $\approx 57:22:21$ reported for the cecum and rectum contents of sudden death victims (Cummings et al 1989). The starches produced C2 as the major SCFA and relatively higher amounts of C4 than C3. By comparison, in an in vitro fermentation test by Brouns et al (2007), pyrodextrinized maltodextrins produced slightly higher total amounts of SCFA than RS3-type retrograded resistant maltodextrins from tapioca and potato; however, C4 production showed the opposite trend. RS3-type retrograded resistant maltodextrins exhibited a significantly faster substrate disappearance and higher total SCFA at 8 hr of incubation compared to retrograded debranched high-amylose corn starch and thermally modified granular high-amylose corn starch (Arrigoni et al 2002). In another study, Fassler et al (2006) reported roughly similar SCFA ratios of $\approx 69:8:23$ and $\approx 69:9:22$ for resistant fractions of RS2 high-amylose maize starch and RS3 tapioca retrograded maltodextrins, respectively, after 8 hr

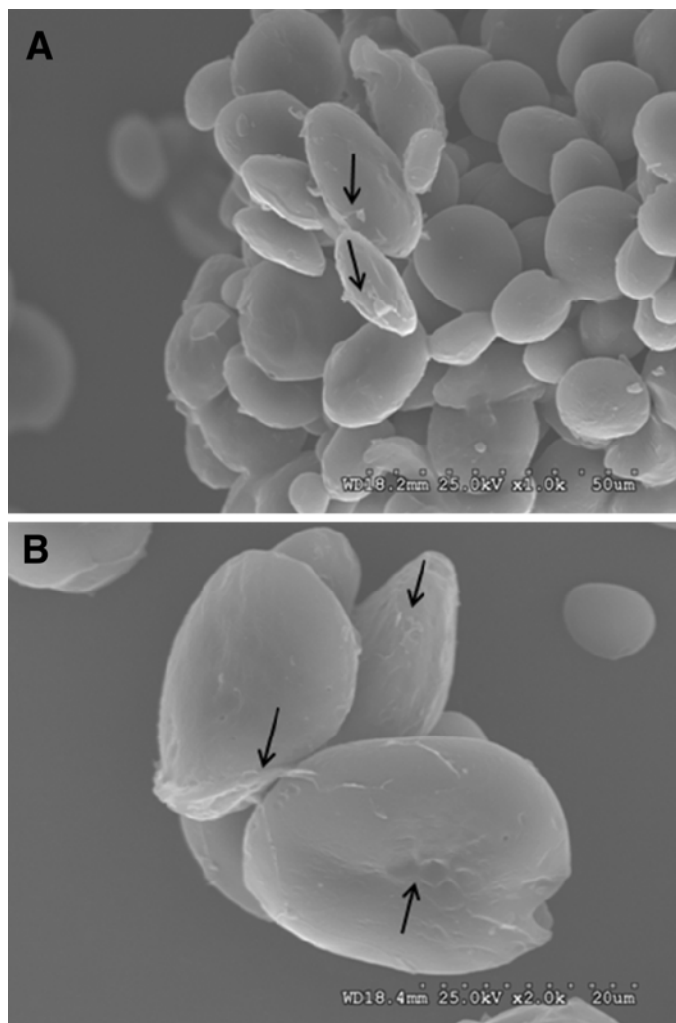


Fig. 5. Appearance of RS4 resistant wheat starch after hydrolysis for 20 min at 37°C using an enzyme mixture of pancreatin, amyloglucosidase, and invertase according to the Englyst method. **A** and **B** are 1000× and 2000× magnification, respectively. Arrows indicate areas with surface erosion or pieces peeling off.

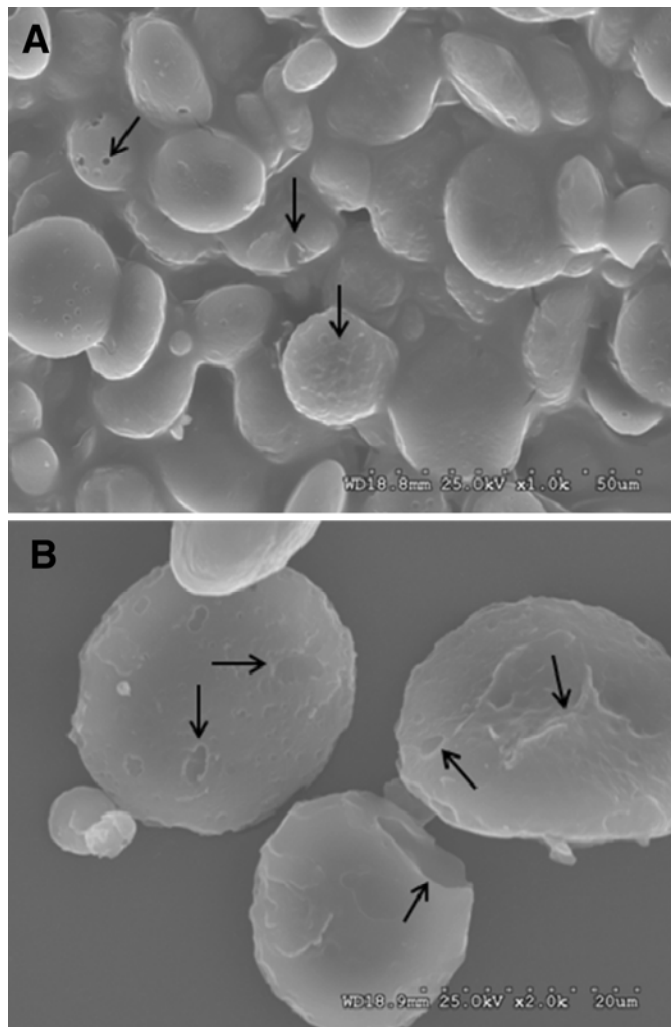


Fig. 6. Appearance of RS4 resistant wheat starch after hydrolysis for 120 min at 37°C using an enzyme mixture of pancreatin, amyloglucosidase, and invertase according to the Englyst method. **A** and **B** are 1000× and 2000× magnification, respectively. Arrows indicate areas with surface erosion, deep etches, or pieces peeling off.

of in vitro fermentation, in which the resistant starch fractions are nearly completely fermented. Furthermore, their work showed that metabolite productions and starch degradation rates were similar for both RS2 and RS3 samples. Crystalline polymorphism, granular structure, and retrograded structure affect the fermentability of resistant starch (Arrigoni et al 2002; Lesmes et al 2008).

In our study, C1, iC4, C5, and iC5 were initially produced during fermentation but underwent deterioration by 24 hr (Table II), which is in agreement with previous observations on raw starch fermentation (McBurney et al 1990). Fermentation of indigestible residues of the two resistant starches into SCFAs occurred mostly in the first 12 hr.

The total SCFA production of the two RS4 resistant starches (6–6.6 mmol/g of starch) in the present study was less than the previously reported SCFA production after fermentation of several raw starches (8–10 mmol/g of starch) (McBurney et al 1990) and dietary fiber (8–12mmol/g of fiber) (McBurney and Thompson 1989a). This seems to indicate that the resistant starch samples may be less fermentable than the other raw starches or fiber. However, the reduced fermentability could also be due to inoculum differences during fermentation between our study and that of McBurney et al (1989a, 1990). Based on our calculations using the 24-hr data in Table II, a 90% absorption factor, and the energy content of each separate SCFA (McBurney and Thompson 1988), predicted energy value absorbed as SCFA is 1.82 kcal/g for undigested residue (resistant portion) of RWS and 1.70 kcal/g for undigested residue of RPS. Taking into account in vitro digestibility of 17.9% for RWS and 25.9% for RPS the total caloric values are 2.54 and 2.74 kcal/g, respectively. Hence, replacing nonresistant starches (4 kcal/g) with RWS or RPS reduces the caloric density of the starch by 36.5% and 31.5%, respectively.

The caloric values for the RWS and RPS showed discordant, but generally comparable results when matched with published information for fiber or resistant starch. For example, the U.S. Food and Drug Administration explicitly assigned a value of 4 kcal (16.7 kJ/g) metabolizable energy/g of soluble fiber and a zero value for insoluble fiber (FDA 1993; Livesey 2008). In Australia, carbohydrates are assigned a value of 4 kcal/g (Goldring 2004); unavailable carbohydrate, which includes dietary fiber, is assigned 1.8 kcal/g (7.5 kJ/g). For carbohydrates that reach the colon, the energy value is set at 2 kcal/g (8.4 kJ/g) for nutritional and labeling purposes according to FAO/WHO (1998). In Japan, fermentable fiber is assigned a value of 2 kcal/g (Goldring 2004).

Birkett and Brown (2008) discussed the work in human subjects by Behall and Howe (1996) where a RS2 high-amylose maize starch contributed digestible energy (dietary energy minus fecal energy) of 2.8 kcal/g (11.7 kJ/g), which was 67% of that for corn starch, whereas a RS3 high-amylose maize starch was 62% of that for wheat starch when measured in rats by Aust et al (2001). By comparison, Kinoyama et al (2007) calculated the effective energy value of a RS3 high amylose corn starch to be 2.5 kcal/g (10.5 kJ/g) when determined in vivo by ¹³CO₂, H₂, and CH₄ breath tests. Baer and Rumpler (2008) reported the metabolizable energy of resistant maltodextrin was lower at 1.0 kcal/g (4.2 kJ/g) in a human study and 1.05 kcal/g (4.4 kJ/g) in a rat study.

TABLE I
Cumulative Total Gas Production (mL/g of starch) at Various Times Upon Fermentation of Indigestible Residues Obtained from RS4 Resistant Starches^a

Sample	Fermentation Time (hr)			
	4	8	12	24
Wheat starch	78.0 ± 0a	118.5 ± 5.8b	153.3 ± 5.8c	214.2 ± 2.8d
Potato starch	77.9 ± 0a	112.8 ± 11.6b	150.4 ± 8.7c	214.2 ± 14.5d

^a Mean values (± SEM) in the same row followed by different letters are significantly different ($P < 0.001$).

Our study showed, for the first time, the pattern or mechanism of amylase digestion of RS4-type resistant starch granules when resistant starches were subjected to three different in vitro digestion procedures. Resistant starch fractions were then tested for fermentability to SCFA. Stoichiometry of carbohydrate fermentation as summarized for a hexose is represented by the equation from Cummings (1997): $59 C_6H_{12}O_6 + 38 H_2O \rightarrow 60 CH_3COOH + 22 CH_3CH_2COOH + 18 CH_3CH_2CH_2COOH + 96 CO_2 + 268 H^+ + \text{heat} + \text{additional bacteria}$. The rate and amount of SCFA production depends on the species and the amount of microflora present in the colon, substrate source, and gut transit time (Wong et al 2006). As stated earlier, both C2 and C3 have physiological roles in hepatic cholesterol synthesis and, consequently, in modulating the risk of cardiovascular disease (Chen et al 1984; Wolever et al 1991). C4, the primary energy source of colonocytes, not only stimulates the proliferation of normal colonic cells, but also stimulates programmed cell death of cancerous cells (Hague and Praskeva 1995).

A limitation of this study is the use of only one fecal inoculum to conduct in vitro fermentation. However, previous studies have shown that feces collected from the same donor on multiple occasions were sufficiently uniform to yield similar in vitro findings (McBurney and Thompson 1987). Also, while different fecal donors may differ in fermentation abilities, the variability is low after 24 hr (McBurney and Thompson 1989b), the endpoint used in this study. Furthermore, different fecal donors ranked the fermentability of substrates similarly. Therefore, although the accuracy of fermentation data may be increased by using more fecal donors, the results of this study may be considered acceptable in demonstrating fermentability of the two resistant starches relative to each other or to other starches.

CONCLUSIONS

The RWS and RPS contain ≈75% of indigestible residue, which can be fermented by human colonic bacteria to yield SCFA accompanied by evolution of gases. In fermentation of both indi-

TABLE II
Cumulative Short Chain Fatty Acid (SCFA) (mmol/g of starch) Production at Various Times After Fermentation of Indigestible Residues Obtained from RS4 Resistant Starches^a

SCFA ^{b,c}	Fermentation Times (hr)			
	4	8	12	24
RWS				
C1	0.50 ± 0.08ab	0.69 ± 0.10ab	0.76 ± 0.15a	0.00 ± 0.00b
C2	1.85 ± 0.35a	2.87 ± 0.27ab	3.44 ± 0.03ab	3.85 ± 0.21b
C3	0.39 ± 0.02a	1.05 ± 0.03b	1.10 ± 0.04b	1.22 ± 0.04b
iC4	0.01 ± 0.01a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
C4	0.40 ± 0.10a	0.93 ± 0.08b	1.32 ± 0.00bc	1.50 ± 0.05c
iC5	0.04 ± 0.03a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
C5	0.05 ± 0.03a	0.04 ± 0.00a	0.04 ± 0.01a	0.01 ± 0.01a
Total	3.25 ± 0.60a	5.58 ± 0.48ab	6.66 ± 0.21b	6.58 ± 0.30b
RPS				
C1	0.65 ± 0.02a	0.63 ± 0.07a	0.35 ± 0.02b	0.00 ± 0.00c
C2	2.12 ± 0.04a	2.87 ± 0.17ab	2.93 ± 0.18ab	3.52 ± 0.07b
C3	0.41 ± 0.01a	0.93 ± 0.04b	1.21 ± 0.06b	1.22 ± 0.06b
iC4	0.02 ± 0.01a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
C4	0.47 ± 0.00a	0.83 ± 0.07b	1.06 ± 0.01b	1.36 ± 0.01c
iC5	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
C5	0.08 ± 0.00a	0.03 ± 0.00b	0.02 ± 0.00b	0.00 ± 0.00c
Total	3.75 ± 0.07a	5.28 ± 0.35ab	5.58 ± 0.21b	6.10 ± 0.14b

^a Mean ± SEM; Mean values (± SEM) in the same row followed by different letters are significantly different ($P < 0.05$).

^b SCFA, short chain fatty acids; C1, formic acid; C2, acetic acid; C3, propionic acid; iC4, isobutyric acid; C4, butyric acid; iC5, isovaleric acid; C5, valeric acid. RWS, resistant wheat starch. RPS, resistant potato starch.

^c Fractional molar ratio of C2:C3:C4 at 24 hr for RWS = 0.586:0.186:0.228 and RPS = 0.577:0.200:0.223.

gestible residues, C2 was the major SCFA that was produced, but higher amounts of C4 were produced than C3. The amylase-resistant fraction of the two RS4 starches is predicted to have a food energy value of 1.70–1.82 kcal/g (7.11–7.61 kJ/g). Taking into account the digestible portion, the total caloric contribution of the two RS4 starches is 2.54–2.74 kcal/g (10.62–11.45 kJ/g), which is ≈33% lower than those of unmodified starches, but comparable to literature reports for RS2 and RS3 high-amylose maize starches. Surface erosion is the primary mode of amylase attack on the granules of the RS4 resistant starches during *in vitro* digestion with pepsin followed by pancreatin-bile. Indigestible residues collected after digestion by either AOAC Method 991.43 or the Englyst method exhibited the same pattern of surface erosion.

ACKNOWLEDGMENTS

We thank Jian-min Chen and Zhen Liu for technical assistance.

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[Received July 8, 2010. Accepted November 23, 2010.]