Effect of Resistant Starch on Intestinal Responses in Rats\textsuperscript{1}

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**ABSTRACT**

Native starch (NS) extracted from wheat and subjected to five autoclaving and cooling cycles contained 11.5% resistant starch (RS), which was measured as insoluble fiber; NS contained a mere 0.5% RS. Both starches were fed to groups of rats for four weeks (RS was fed with or without antibiotics). Compared with rats fed NS, those fed treated starch showed a sixfold (RS diet) or nearly 18-fold (RS with antibiotics) increase in fecal wet weight; increases in fecal volumes paralleled increases in fecal weight. Rats fed treated starch (no antibiotics) digested 37.1% RS; those fed antibiotics digested only 14.3% RS. RS thus appears to be highly resistant to mammalian enzyme and may be classified as a component of fiber.

Heat processing of starchy foods increases the digestibility of starch (Ring et al 1988). However, a fraction of the starch may also be rendered resistant to mammalian enzymes and thus escape digestion. Retrogradation of amylose is believed to lead to the formation of resistant starch (RS) (Berry 1986, Ring et al 1988).

Many bakery products contain up to 3% RS; some breakfast cereals contain even more (Englyst and Cummings 1985, Englyst et al 1987). By the enzymatic-gravimetric method now widely used in the United States to measure fiber (Prosky et al 1988), RS is measured as insoluble fiber.

The occurrence of RS in foods may have a significant health implication, especially if the diet is modified as recommended (Cronin and Shaw 1988) to include more complex carbohydrates (starches and fiber) or if RS is added to foods as a fiber component. Even when not added as a fiber component, the formation of RS in traditional foods can be substantially enhanced, if desired, by modifying selected processing parameters.

When present in significant amounts, RS lowers the caloric density of foods. RS is also reported to elicit a low glycemic response (Jenkins et al 1982, Ring et al 1988). In addition, RS can modify the intracolonic environment to favorably alter toxicological functions (Mallet et al 1988). It may also provide protection against colorectal cancer (Burkitt et al 1972, Cummings 1985) by shortening intestinal transit time and by providing fecal bulk. This study was undertaken to assess processing parameters that favor RS formation in isolated wheat starch and to examine the effect of RS on fecal bulking characteristics. Rats were used as the test model.

**MATERIALS AND METHODS**

**Preparing Resistant Starch**

Wheat flour, kneaded into a dough using distilled water, was washed with water several times to recover native starch. This starch was centrifuged (medium speed, IEC EXD centrifuge, Needham, MA) several times to remove starch tailings (scraped from the top), air-dried, and then stored at 4°C. At a later date, the starch was mixed with distilled water (20% starch suspension, w/w), autoclaved (126°C, 15 psi) for 1 hr for five successive days, freeze-dried, and then finely ground (to pass through a 0.5-mm diameter screen). After each 1-hr autoclaving step, samples were stored at 4°C for 24 hr, because prolonged cooling after autoclaving is a prerequisite for RS formation. This method, which evolved from a series of preliminary studies (Table 1), was used to prepare bulk quantities of RS for use in test diets.

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Test Diets
The native starch used to formulate diet A (Table II) contained only 0.5\% RS, a value similar to that reported by Berry (1986). Treated starch contained 11.47\% RS, almost all (97-100\%) measured as insoluble fiber (Prosky et al 1988). Native starch and treated starch constituted 74.9\% of the diets; other diet components added to meet the rats' nutrient requirements (NRC 1987) accounted for the remaining 25.1\% (Table II). Antibiotics, where used, were added to the drinking water and included streptomycin sulfate (5 mg/ml), neomycin sulfate (4 mg/ml), bacitracin (4 mg/ml), and amphotericin B (0.1 mg/ml); the levels used were as prescribed by Srivastava et al (1976). Water containing antibiotics was freshly prepared on a regular basis.

Animals
Three groups of male, weanling rats (10 rats per diet) of the Sprague-Dawley strain (Harlan Sprague-Dawley, Indianapolis, IN) were housed individually in mesh-bottom, stainless steel cages in a controlled environment. Each rat was allowed to consume an adequate, and identical, amount of total diet during the four-week test period. Deionized water was offered ad libitum. Body weight records were maintained.

Fecal Collection
For each rat, feces were collected quantitatively throughout the four-week test period, pooled, air-dried, weighed, and stored under refrigeration. A few freshly voided fecal pellets were also collected at frequent intervals, analyzed for moisture content, and then added to the pool collection.

Analytical
RS in starch samples was determined by the enzymatic-gravimetric method of Prosky et al (1988); this method measures insoluble fiber and soluble fiber separately. Moisture in freshly voided fecal samples was determined by air-drying the feces at room temperature. Calculated factors (Table III) were used to convert dry fecal weight (air-dried feces) to wet fecal weight. Fecal volume was measured in a long-stem graduated cylinder using fine sand as the embedding medium (Table III). Feces recovered from the sand were finely ground and analyzed for RS again using the method of Prosky et al (1988).

Statistical
Mean comparisons were made with Duncan’s multiple-range test using the Statistical Analysis System (SAS 1982).

RESULTS AND DISCUSSION
Formation of Resistant Starch
A positive correlation is reported to exist (Berry 1986, Sievert and Pomaranz 1989) between amylase content of the starch and RS formation. Changes in processing parameters such as autoclaving and cooling cycles and freeze-drying steps also affect RS formation. Autoclaving and cooling cycles appear to favor RS formation more than autoclave temperature (above 100°C) or the freeze-drying step (Bjorck et al 1987).

<table>
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<th>Sample</th>
<th>Autoclaved</th>
<th>Cooled</th>
<th>Freeze-dried</th>
<th>RS (%)</th>
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</table>

*In a 24-hr period. 
Resistant starch.

Growth Response
Because all diets contained the same level (12\%) and source of protein (casein) and were fed to the rats in identical amounts (Table II), the growth response of groups of rats fed these diets differed minimally (Table III).
Fecal Bulking Effect

Human epidemiological studies show a lower incidence of colorectal cancer in population groups that consume diets high in fiber (Trowell et al. 1985). Various hypotheses are put forward to explain this, including one postulating that a higher fecal bulk would result in the dilution of potential carcinogens in the intestinal lumen.

Fecal bulking capacity of fiber sources varies. Sources that are high in insoluble fiber such as wheat bran provide more fecal bulk than those that are high in soluble fiber, a fraction highly fermentable in the colon (Nyman and Asp 1982, Nyman 1985).

Including treated starch in the diet increased the wet fecal weight over sixfold in comparison with native starch (diet B vs. A); the increase in dry fecal weight was of a similar magnitude (Table III). When the hind-gut bacterial population was effectively, if not completely, suppressed by antibiotics, the increase in wet fecal weight was even greater, nearly 18-fold (diet C vs. A). Increases in fecal volume paralleled increases in fecal weight; volume increases were fivefold for diet B and over eightfold for diet C compared with diet A (Table III). Thus, fecal weight and volume measurements suggested that the fecal bulking capacity of RS (with or without antibiotics) was substantial.

Digestibility of Resistant Starch

Measurement of RS digestibility was based on intake and fecal output data (Table III). RS is viewed by some (Berry 1986, Bjorck et al. 1987) as a component like soluble fiber that is easily fermented by bacteria. It is, however, measured as essentially all insoluble fiber and expected to ferment less readily than soluble fiber. In the group of rats fed treated starch but not antibiotics, the increase in wet fecal weight was even greater, nearly 18-fold (diet C vs. A). Increases in fecal volume paralleled increases in fecal weight; volume increases were fivefold for diet B and over eightfold for diet C compared with diet A (Table III). This suggests that the fecal bulking capacity of RS (with or without antibiotics) was substantial.

LITERATURE CITED


