In Vitro Fermentation of Various Food Fiber Fractions

James L. Casterline, Jr., Carolyn J. Oles, and Yuoh Ku*

Division of Science and Applied Technology, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 200 C Street S.W., Washington, D.C. 20204

Dietary fiber fractions (DFF) were fermented *in vitro* with human fecal inocula to study fiber fermentability and production of short-chain fatty acids (SCFA). Amylolytic and proteolytic enzyme treatment using the Association of Official Analytical Chemists enzymatic-gravimetric procedure reduced processed food fiber products to DFF by removing gastric enzyme-digestible (nonresistant) components. Total 24-h SCFA production from DFF decreased in the following order: fig > oat = soy > pea > apple > corn = wheat > pear. SCFA production was proportional to the fermentability of these fibers. Fermentation of DFF from fruits such as pear, apple, and fig produced low amounts of butyrate. In contrast, less acetate and more propionate and butyrate were produced by fermentation of oat and soy DFF. Major fiber constituents, resistant starch (RS), β-glucan, and pectin were also fermented. RS, isolated from starch, produced acetate more slowly than the starch. β-Glucan produced propionate and butyrate in higher amounts than did pectin, starch, and RS. This study demonstrates that the fermentability of DFF and the production of SCFA differ among food products. *In vitro* fermentation of DFF is useful in estimating SCFA production in the human colon.

Keywords: *Dietary fiber fractions; fermentation; short-chain fatty acids*

INTRODUCTION

On the basis of a correlation of high-fiber intake with improved health in the general populace, government agencies, particularly the U.S. Department of Health and Human Services, have recommended that dietary fiber intake be increased. Additionally, researchers have investigated the physiological effects of fiber products, and their results demonstrated that dietary fiber has several physiological benefits (Demigne and Remesy, 1991; Wolever, 1991). The United States Food and Drug Administration recently authorized the use on foods of a health claim on the relationship between diets low in saturated fat and cholesterol and high in fruits, vegetables, and grain products that contain fiber, particularly soluble fiber, and risk of coronary heart disease (Code of Federal Regulations, 1996). In addition, in 1997, the agency also authorized the use on food labels of a more specific health claim relating intake of soluble fiber from whole oats (e.g., oat bran, rolled oats, and whole oat flour) and reduced risk of coronary heart disease (Federal Register, 1997).

Fiber products in foods vary in composition or structure, depending on plant origin, age, and method of food processing used (Van Soest, 1978; Prosky, 1986; Chang, 1990). These compositional or structural variations lead to different physiological effects in humans (Englyst et al., 1987). Significant amounts of polysaccharide and lignin residues, known as dietary fibers, remain after gastric digestion of processed food products. The residues reach the colon and, except for lignin (Garleb et al., 1988), are metabolized or fermented by anaerobic saccharolytic microflora to carbon dioxide, methane, hydrogen, and short-chain fatty acids (SCFA). Sometimes the residues contain a small but significant amount of gastric resistant starch (RS) formed during food processing (Eerlingen et al., 1993). Several studies have demonstrated that structurally different polysaccharides and fiber constituents produce C2−C4 SCFA in different proportions (McBurney and Thompson, 1987, 1990; Mortensen et al., 1988; Titgemeyer et al., 1991). Production of SCFA has been implicated in colon carcinogenesis (Kruh, 1982), cholesterol metabolism (Chen and Anderson, 1986), and glucose metabolism (Wolever, 1991).

Two important techniques available for this study are the AOAC (1990) enzymatic method 985.29, referred to as the Prosky method, and the *in vitro* dietary fiber fermentation method using human bacterial microflora. The Prosky method is used to determine total dietary fiber (TDF) in accordance with the physiological definition of TDF in the United States (Hall, 1989; Lee and Prosky, 1992). With a minor modification, this method can be used to obtain fiber fractions from foods for research purposes. Previous researchers showed that *in vitro* fermentation of fibers can be used to estimate SCFA production in the human colon (McBurney and Thompson, 1988; Bourquin et al., 1993). Because humans absorb SCFA quickly (McNeil et al., 1978), determining the rates of *in vivo* production of SCFA is difficult. *In vitro* batch fermentation of dietary fibers, however, allows collection, extraction, and measurement of SCFA. Differences in the production of individual SCFA by different types of fibers can also be determined. The work of McBurney et al. (1988) suggests that the fiber fractions obtained from foods by an enzymatic procedure similar to that of Prosky et al. (1984) can be used as representative of human dietary fibers for *in vitro* fermentation experiments. McBurney et al. (1988) demonstrated that SCFA production from ileal effluent samples collected from an ileostomate was significantly correlated with fiber fractions isolated from the same whole foods that were fed to the ileostomate. The objective of this project was to assess several different dietary fiber fractions (DFF) and starches in terms of SCFA production using the *in vitro* fermentation system. The fermentation of major fiber constitu-

* Author to whom correspondence should be addressed [telephone (202) 205-4603; fax (202) 205-4594; e-mail yxk@vm.cfsan.fda.gov].

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Table 1. Production of Short-Chain Fatty Acids (SCFA)[a] after 24 h of Fermentation of Dietary Fiber Fractions (DFF)

DFF sources	SCFA			
	acetate	propionate	butyrate	total[b]
fig	2.55 ± 0.06	0.33 ± 0.01	0.04 ± 0.002	2.92[c]
oat	1.32 ± 0.05	0.96 ± 0.01	0.38 ± 0.01	2.66[d]
soy fibrium 2000	1.19 ± 0.08	1.14 ± 0.05	0.22 ± 0.01	2.55[d]
vegetable pea	1.46 ± 0.11	0.42 ± 0.01	0.11 ± 0.01	1.99[e]
apple	1.06 ± 0.03	0.41 ± 0.01	0.09 ± 0.002	1.56[f]
corn	0.76 ± 0.02	0.21 ± 0.01	0.16 ± 0.01	1.13[g]
wheat	0.62 ± 0.01	0.36 ± 0.01	0.11 ± 0.003	1.09[g]
pear	0.66 ± 0.02	0.24 ± 0.01	0.05 ± 0.002	0.95[h]

[a] mmol/g of DFF ± standard error of the mean; $n = 6$. [b] Means in this column with different letters differ by one-way analysis of variance followed by LSD test ($P < 0.01$).

ents, RS, β-glucan, and pectin found in processed foods that have been suggested to influence the production of SCFA was also studied.

EXPERIMENTAL PROCEDURES

In this study, dietary fiber from processed foods and starches was incubated with human fecal inocula to determine the amounts of SCFA formed.

Materials. Commercially processed food products (PF) used in this study were wheat bran from the American Association of Cereal Chemists (St. Paul, MN) and Fibrium 2000 (soy) from Ralston Purina Co. (St. Louis, MO). Oat bran, Centra III vegetable fiber (pea), fig fiber, apple fiber, and Sunlite corn bran from MidAmerica Food Sales Ltd. (Northbrook, IL) were also used. Pear fiber was obtained from Tree Top, Inc. (Selah, WA). Starch, pectin, and β-glucan were obtained from Sigma Chemical Co. (St. Louis, MO).

Preparation of Dietary Fiber Fractions. DFF for *in vitro* fermentation were extracted from PF according to the Association of Official Analytical Chemists (1990) enzymatic–gravimetric method using a Total Dietary Fiber Kit purchased from Sigma Chemical Co. (St. Louis, MO). RS was extracted from autoclaved starch samples using the same method. After hydrolysis, four volumes of 95% ethanol were added to precipitate soluble fibers. The resulting residues were filtered through No. 541 ashless paper and stored in a desiccator. This extraction procedure was repeated as needed to obtain sufficient amounts of DFF for this study. For TDF, portions of DFF were treated, including ashing, according to the Prosky method. Solutions without PF were carried through the same procedure for use as controls.

Before extracting RS, the content of RS in starch was increased by autoclaving (Berry, 1986; Eerlingen et al., 1993). Starch in each of two flasks was autoclaved for 1 h at 121 °C and cooled. The second flask was autoclaved again for an additional hour.

***In Vitro* Fermentation Technique.** The conditions and reagents for the *in vitro* fermentation technique used to degrade PF and DFF were those of Goering and Van Soest (1970). Sodium sulfite was not used (Jeraci, J. L., Cornell University, Ithaca, NY, personal communication, 1989). Six experiments were conducted, each with the eight fibers listed in Table 1. Triplicate 0.5-g test portions of fiber were hydrated for 17 h in a medium under carbon dioxide before addition of human fecal inoculum. Four healthy male donors, 35–61 years old, were selected for this study. The donors had not been treated with any medication, including antibiotics, for 3 months prior to the study. Fresh feces were collected in plastic bags from two donors for each experiment. Donors 1–4 were scheduled in the following order for collection: 1,2; 3,4; 2,3; 1,4; 1,3; and 2,4. The inoculum was prepared immediately by pooling and mixing feces of equal weights with CO_2-saturated incubation medium. The final mixture containing 0.2 g of feces/mL was filtered through several layers of cheesecloth and through a 30-cm^2 20-μm nylon membrane (Spectrum Scientific, Los Angeles, CA). To begin fermentation, 10 mL of this inoculum was added to the incubation flasks containing medium with and without 0.5 g of PF or DFF. Flasks without PF or DFF (blanks) were used to enable corrections. Aliquots of the fermented solutions for gas chromatographic determination of SCFA were removed at 0, 6, 24, and 48 h in one group of experiments using only wheat, oat, soy, and apple DFF and PF for comparison purposes and at 0 and 24 h in another group to assess different DFF and starches. Individual and total amounts of SCFA (mmol/g of fiber) were calculated. For bacterial production of SCFA without fiber, total SCFA were expressed as mmol/g of feces. The procedure for starch, RS, β-glucan, and pectin samples was as described above with the exception that smaller amounts were fermented (0.2–0.3 g).

The remaining solutions of the 0- and 24-h trials, except those of starch, RS, β-glucan, and pectin, were used to determine the fermentabilities of the fiber fractions. Four volumes of 95% ethanol were added to the solutions. After standing overnight, the contents of each flask were filtered through Celite using a Fibertec filtration module (Tecator, Hoganas, Sweden) and treated, including ashing, according to the Prosky method.

Gas-Chromatographic Determination of Short-Chain Fatty Acids. SCFA were isolated according to the method of Lombard and Dowell (1982). Duplicate 10-mL aliquots of fermented solution were acidified with 0.4 mL of 50% H_2SO_4 and allowed to stand for 30 min. Each mixture was extracted four times with 2 mL of ethyl ether, the extracts were combined in a 10-mL volumetric flask, and the solution was diluted to volume with ethyl ether. Prefired (~650 °C) and ether-washed sodium sulfate was added to remove water. SCFA were determined by gas chromatography on a Nukol fused silica capillary column, 30 m × 0.25 mm i.d., 0.25-μm film (Supelco, Bellefonte, PA). The column temperature was held at 90 °C for 5 min and then increased 4 °C/min to 200 °C. The flow rate was 18 mL of He/min, the flame ionization detector was set at 220 °C, and the injection port was set at 210 °C. Ether extracts were stored in autosampler vials and kept in a freezer for no more than 3 days before analysis. The extracts were analyzed using an autosampler.

Standard SCFA mix (Matreya, Inc., Pleasant Gap, PA) and individual pure acetate, propionate, and butyrate (Sigma Chemical Co.) were used for quantitation and identification of the SCFA.

Statistical Analysis. For statistical comparison of the results, analysis of variance followed by a least significant difference (LSD) test (*SAS/STAT user's guide*, 1988) was used wherever applicable. Regression and correlation (Youmans, 1973) were used to measure the extent of the relationship between fermentability and SCFA production. All figures were computer-generated using Harvard Graphics, v.3.0 (Software Publishing Corp., Santa Clara, CA).

RESULTS AND DISCUSSION

Figure 1 shows low production of SCFA in fecal solutions incubated without fiber. The production of SCFA, particularly acetate and propionate, was generally maximal at 24 h. Previous studies show that a 24-h incubation time is considered an appropriate period for comparing the fermentation variables of fibers (McBurney and Thompson, 1989; Titgemeyer et al., 1991; Bourquin et al., 1993).

Figures 2 and 3 show that, with PF and DFF, the fermentation is prolonged and the production is much higher. SCFA production was greatly reduced by removing enzyme-digestible (nonresistant) components such as starch from PF. The 24-h fermentation data for each DFF decreased significantly ($P < 0.05$) in the following order: wheat (72%) > apple (67%) > soy (61%) > oat (28%). Generally, fermentation of DFF began to slow somewhat after 24 h of incubation. Fermentability (disappearance) and SCFA production after a 24-h fermentation of various DFF are shown in Figure 4 and

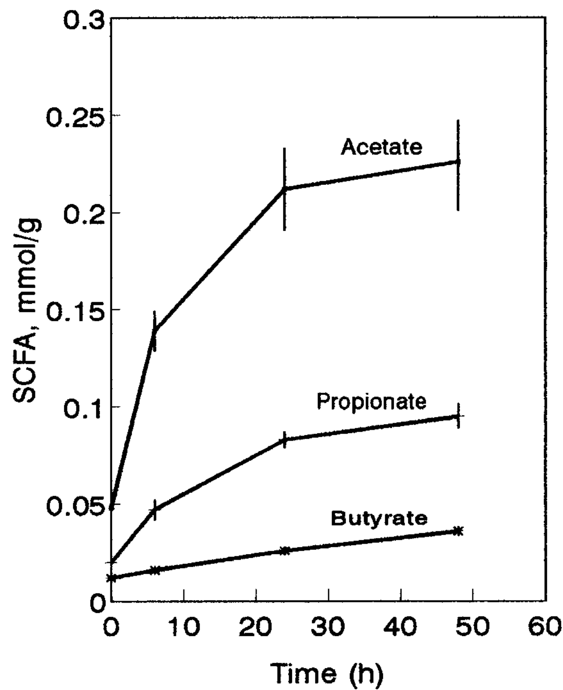

Figure 1. *In vitro* production of short-chain fatty acids (SCFA) in test solutions incubated without PF and DFF. Mean SCFA produced (mmol/g of feces) and standard errors of the mean are plotted against incubation time.

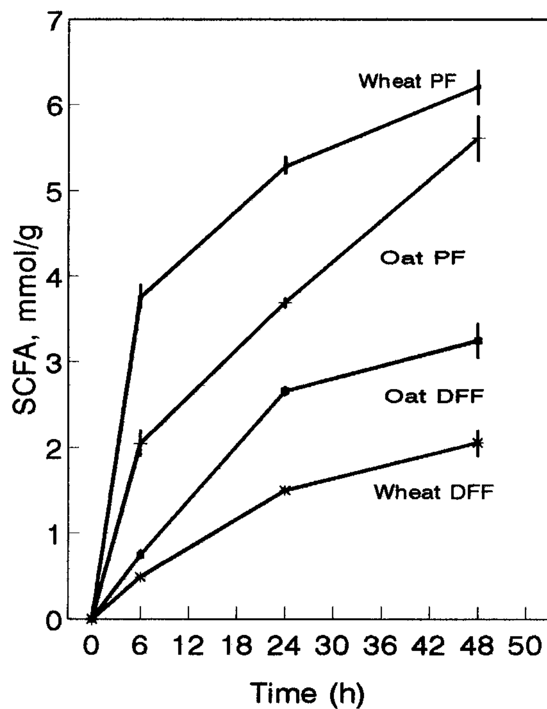

Figure 2. Production of short-chain fatty acids (SCFA) from *in vitro* fermentation of wheat and oat samples. Mean total SCFA produced (mmol/g of sample) and standard errors of the mean are plotted against fermentation time.

Table 1, respectively. Figure 4 shows that although minor short-chain fatty acids such as isobutyrate and valerate were not taken into account, the total amounts of SCFA formed by fermentation were proportional to DFF fermentability ($r = 0.953$). Table 1 data indicate that the total SCFA production decreased in the following order: fig > oat = soy > vegetable pea > apple > corn = wheat > pear. The data also show that different DFF yield SCFA in different proportions. The total amount of SCFA produced by fig fermentation is great-

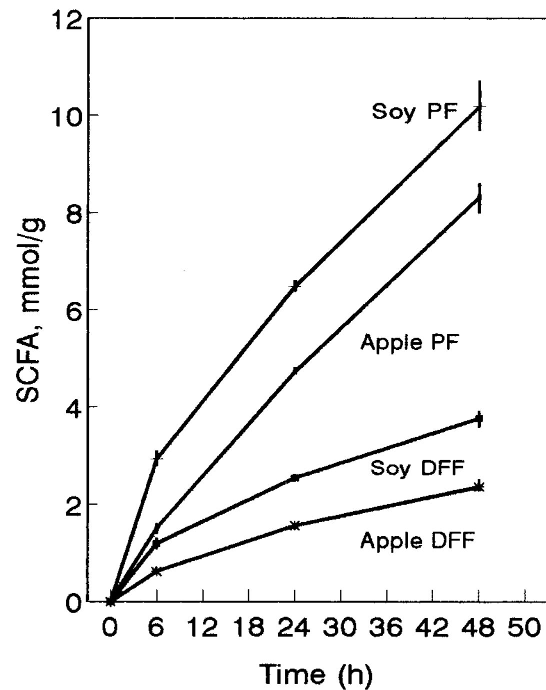

Figure 3. Production of short-chain fatty acids (SCFA) from *in vitro* fermentation of apple and soy samples. Mean total SCFA produced (mmol/g of sample) and standard errors of the mean are plotted against fermentation time.

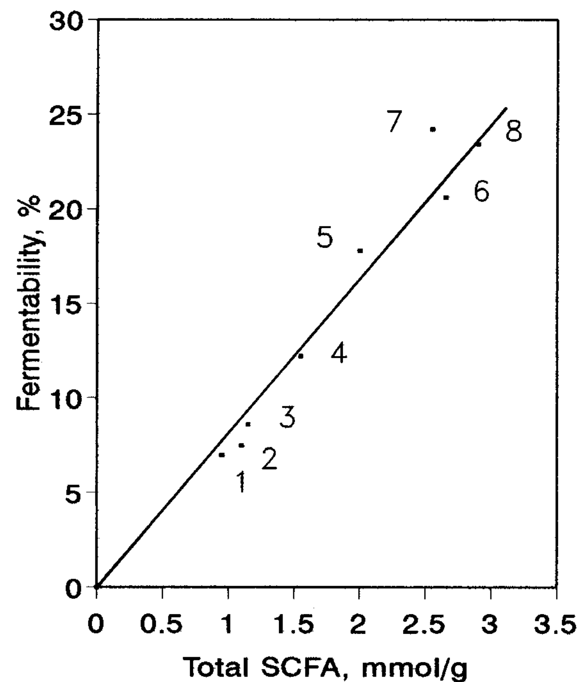

Figure 4. *In vitro* production of total short-chain fatty acids (SCFA) versus fiber fermentability: 1 = pear; 2 = wheat; 3 = corn; 4 = apple; 5 = vegetable pea; 6 = soy; 7 = oat; 8 = fig. Regression analysis indicates a significant correlation ($r = 0.953$) between SCFA produced and fiber fermented after 24 h of fermentation.

est primarily because of the very high production of acetate. The fermentation of fruit DFF (pear, apple, and fig) produced the smallest amounts of butyrate. In contrast, less acetate and more propionate and butyrate were produced during the fermentation of oat and soy DFF. Of the eight DFF, soy produced the greatest amount of propionate.

Since the major constituents of fiber, RS, β-glucans, and pectins, in certain foods such as bran cereals and

Table 2. Production of SCFA[a] after 24 h of Fermentation of Polysaccharide

polysaccharide	RS yield, %	SCFA acetate	propionate	butyrate	total
starch (whole)	2.3	2.39 ± 0.07	0.96 ± 0.01	1.00 ± 0.02	4.35
rs (1c)[b]	6.6	1.16 ± 0.04	0.72 ± 0.02	0.94 ± 0.04	2.82[c]
rs (2c)[b]	8.1	1.30 ± 0.04	0.89 ± 0.02	1.23 ± 0.05	3.42[c]
pectin		5.18 ± 0.20	0.76 ± 0.05	0.57 ± 0.02	6.51[c]
β-glucan		2.41 ± 0.04	1.69 ± 0.06	1.44 ± 0.01	5.54[c]

[a] mmol/g of polysaccharide ± standard error of the mean; $n = 6$. [b] RS(1C), RS after 1 autoclaving cycle; RS(2C), after 2 autoclaving−cooling cycles. [c] Total SCFA differ from that of starch by analysis of variance followed by LSD test ($P < 0.01$).

fruits have been suggested to influence the production of SCFA (Kritchevsky, 1985; Englyst et al., 1987; Demigne and Remesy, 1991), they were also tested in this study (Table 2). Whole starch was included in this test for comparison purposes. The starch and pectin data agree with the finding of Englyst et al. (1987). The production of propionate and butyrate from pectin was lower than that from starch during the fermentation. The yield of acetate by pectin was higher. According to product literature provided by the suppliers, vegetable pea, apple, and pear used in this study contain 16%, 13%, and 5% (w/w) pectin, respectively. Although other types of fiber in these DFF may be involved in the production of SCFA, the production of acetate is related to the amounts of pectin. On the basis of the total amounts of SCFA formed, RS fermented more slowly than the whole starch. This was largely the result of decreased acetate production. The increase in RS yield was due to autoclaving-cooling processes (Garleb et al., 1988). Our data also show that fermentation of β-glucan produced propionate and butyrate in higher amounts than did pectin, starch, and RS. Oat bran is known to contain an appreciable amount of soluble fiber, β-glucan (Shinnick et al., 1988), which is composed entirely of glucose units and, unlike other starches, is not susceptible to human digestion. It was reported to be beneficial to humans (e.g., it reduces the risk of coronary heart disease). This study shows that wheat and corn DFF produced SCFA in lower amounts than did oat and soy DFF. Both wheat and corn brans were reported to have little effect on blood cholesterol levels, whereas oat bran and soy fiber reduce cholesterol levels in patients with hypercholesterolemia (Kirby et al., 1981; Anderson et al., 1984; Lo et al., 1986; Shinnick et al., 1988).

The present results confirm the importance of using dietary fiber fractions or isolates in research studies. They demonstrate that the fermentability of such fibers and the production of SCFA differ among food products. There are numerous studies pertaining to the complex physiological effects of dietary fibers, and it is becoming clear that some of the effects are mediated by SCFA. Studies on the nutritive role of SCFA in epithelial cells of the colonic mucosa or colonocytes are still being conducted. In addition, there is limited information available on the metabolic impact of specific SCFA in tissues or organs other than the colon. Demigne and Remesy (1991) reported that, in rats fed various fiber diets, approximately 60% of the absorbed SCFA is metabolized by the liver. More studies on the involvement of SCFA in human metabolism are needed. The fermentation data together with the data on the utilization of SCFA as metabolic fuels should provide important information that may be relevant to the role of the fibers and SCFA in human nutrition and health. This is especially important because consumer demand for high fiber foods such as cereals is increasing. Also, dietary fiber products derived from a variety of foods such as vegetables and cereals are available for use in food formulations.

ABBREVIATIONS USED

DFF, dietary fiber fractions; LSD, least significant difference; PF, processed food products; RS, resistant starch; SCFA, short-chain fatty acids; TDF, total dietary fiber.

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