

Short Communication

Dietary fibre and fermentability characteristics of root crops and legumes

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The dietary fibre and fermentability characteristics of local root crops and legumes were determined. Total, soluble and insoluble fibre were determined in six root crops (kamote, gabi, potato, tugi, ube, cassava) and ten legumes (mungbean, soyabean, peanut, pole sitao, cowpea, chickpea, green pea, lima bean, kidney bean and pigeon pea) using Association of Official Analytical Chemists methods. The dietary fibre from test foods was isolated and fermented *in vitro* using human faecal inoculum simulating conditions in the human colon. The SCFA, e.g. acetate, propionate, butyrate, produced after fibre fermentation was measured using HPLC. The dietary fibre content of root crops ranged from 4.6 to 13.5 g/100 g while legumes ranged from 20.9 to 46.9 g/100 g, suggesting that root crops and legumes are good sources of dietary fibre. Significant amounts of SCFA were produced after *in vitro* fermentation of the fibre isolate of both root crops and legumes. The best sources (as mmol/g fibre isolate) of acetate among the legumes were pole sitao (5.6 (SEM 0.5)) and mungbean (5.3 (SEM 0.1)) and among the root crops, tugi (2.5 (SEM 0.4)) and cassava (2.4 (SEM 0.1)); of propionate, kidney bean (7.2 (SEM 1.5)) and pigeon pea (3.3 (SEM 0.2)) for legumes, and tugi (1.8 (SEM 0.2)) for root crops; and of butyrate, peanut (6.0 (SEM 0.2)) and cowpea (5.4 (SEM 0.2)) for legumes, and tugi (0.8 (SEM 0.0)) and cassava (0.8 (SEM 0.0)) for root crops. In conclusion, root crops and legumes are good sources of dietary fibre and produced SCFA after fibre fermentation, such as acetate, propionate and butyrate. SCFA production after *in vitro* fermentation can be estimated using human faecal inoculum and can be used to model the human colon.

Dietary fibre: Legumes: Root crops: Fermentation

Increased intake of dietary fibre is recommended in the proper control of chronic diseases, e.g. diabetes mellitus, CVD and cancer^(1,2). It is now well established that dietary fibre is not metabolized in the small intestine and reaches the colon where it is fermented to produce SCFA such as acetate, propionate and butyrate, and gases such as carbon dioxide, methane and hydrogen^(3,4). SCFA contributes 6.3–8.4 kJ/g dietary fibre (1.5–2.0 kcal/g dietary fibre)⁽⁵⁾. Propionate has been shown to inhibit the activity of the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase, the limiting enzyme for cholesterol synthesis⁽⁶⁾. Butyrate enhances cell differentiation thus preventing tumour formation in the colon⁽⁷⁾. Dietary fibre can be soluble or insoluble depending upon solubility in water. Water-soluble fibre can form viscous solutions. Increased viscosity in the intestine slows intestinal transit time, delays gastric emptying^(8,9), and slows glucose and sterol absorption by the intestine^(10,11). Viscous soluble fibre can control the release of glucose with time thus lowering postprandial blood glucose and insulin levels, and serum cholesterol⁽⁹⁾. Insoluble fibre consists of lignin, cellulose and hemicellulose. It has usually high water-holding capacity and contributes to increased faecal bulk and frequency of defecation^(12,13).

Moreover, all fibres, both soluble and insoluble, can entrap bile acid and prevents its re-absorption in the liver thus inhibiting cholesterol synthesis⁽¹⁴⁾. The consumption of root crops and tubers in the Philippines is 19 g/d while consumption of beans, nuts and seeds is 10 g/d⁽¹⁵⁾. The present study will determine the dietary fibre and fermentability characteristics of root crops and legumes.

Material and methods

Test foods

In the present study, six root crops (ube (*Dioscorea alata*), gabi (*Colocasia esculenta*), tugi (*Dioscorea esculenta*), potato (*Solanum tuberosum*), camote (*Ipomoea batatas*) and cassava (*Manihot esculanta*)) and ten legumes (cowpea (*Vigna uruguiculata* (L.) Walp.), mungbean (*Vigna radiate* (L.) Wilczek), pole sitao (*Vigna uruguiculata* subsp. *Sesuipealosa* L. Verde), chickpeas (*Cicer arietinum*), green peas (*Pisum sativum* L.), peanut (*Arachis hypogaea* L.), pigeon pea (*Cajanus cajan*), kidney beans (*Phaseolus vulgaris* L.), lima beans (*Phaseolus lunatus*) and soyabeans (*Glycine soja*)) were used as test foods. Food

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samples were bought in local markets. Root crops were cooked in water while legumes were soaked in water overnight, and boiled to cook the next day. Both root crops and legumes were freeze-dried before analysis.

Analytical methods

The proximate analysis of all test food was determined using Association of Official Analytical Chemists methods, and the test foods were also analysed for total, soluble and insoluble fibre using Association of Official Analytical Chemists methods^(16,17) against a standard wheat flour (NBS Standard Reference Material 1567 A; Gaithersburg, MD, USA).

In vitro fermentation

Dietary fibre isolates from each test food were fermented *in vitro* using human faecal inoculum⁽¹⁸⁾. Dietary fibre was isolated from test samples using Association of Official Analytical Chemists methods. Fibre isolate (0.5 g) was weighed in a serum bottle. Fermentation media (40 ml of a mixture of 2 litres deionized water, 1 litre 0.5 M-sodium bicarbonate buffer solution, 1 litre macromineral solution, 5 ml 0.1 % resazurin) and reducing solution (2 ml of a mixture of 1.25 g cysteine-HCl + fifty pellets of potassium hydroxide in 100 ml deionized water and 1.25 g sodium sulphide in 100 ml deionized water) were added to the serum bottle and flushed with carbon dioxide until colourless. The bottles were sealed with rubber stoppers and an aluminium seal and stored at 4°C. The next day the bottles were placed in a water-bath for 1–2 h at 37°C. A 10 ml faecal inoculum (1:15 dilution of fresh faeces from a healthy female human volunteer eating an unspecified diet with no intake of antibiotics for a year) was added into each bottle and the mixture was incubated for 24 h at 37°C without mixing. The fermented digest was filtered and read in a high-pressure liquid chromatograph (LC10 Shimadzu, Shimadzu, Tokyo, Japan) to measure SCFA against a volatile acid standard mixture of acetate, propionate and butyrate (Supelco, Philadelphia, PA, USA). No internal standards were used.

Statistical analysis

Differences between test foods and food analyses were determined by ANOVA and Duncan's multiple range test using the Statistical Analysis System program (SAS Institute Inc., Cary, NC, USA).

Results

Table 1 shows the nutritional composition of root crops and legumes. Legumes are the best sources of protein and are significantly greater than root crops (Table 1; $P < 0.05$). Root crops have significantly lower fat content than legumes (Table 1; $P < 0.05$). The dietary fibre content of root crops ranged from 4.6 to 13.5 g/100 g while legumes ranged from 20.9 to 46.9 g/100 g, suggesting that both root crops and legumes are good sources of dietary fibre (Table 1). The dietary fibre content of legumes was significantly greater than that of root crops. Soyabean has significantly greater dietary fibre content (46.9 (SEM 3.4)g/100 g) among the legumes

while gabi has significantly greater dietary fibre content (13.5 (SEM 0.1)g/100 g) among the root crops (Table 1; $P < 0.05$). The ratio of insoluble to soluble fibre in root crops ranged from 1:1 to 2:1 while for legumes 5:1 to 5:19 excluding kidney beans, which was approximately 99% insoluble fibre. However, the ratio of insoluble to soluble fibre in all test foods was not significantly associated with production of SCFA (Table 1; $P < 0.05$). Significant amounts of SCFA were produced after fermentation of the fibre isolate of both root crops and legumes, with cowpea, kidney bean and peanut having the greatest production of SCFA for legumes, and tugi and cassava for root crops (Table 1). The best sources (as mmol/g fibre isolate) of acetate among the legumes were pole sitao (5.6 (SEM 0.5)) and mungbean (5.3 (SEM 0.1)) while among the root crops, tugi (2.5 (SEM 0.4)) and cassava (2.4 (SEM 0.1)); for propionate, kidney bean (7.2 (SEM 1.5)) and pigeon pea (3.3 (SEM 0.2); Table 1); and for butyrate, peanut (6.0 (SEM 0.2)) and cowpea (5.4 (SEM 0.2)) among the legumes, and tugi (0.8 (SEM 0.0)) and cassava (0.8 (SEM 0.0); Table 1) among the root crops.

Discussion

Colonic fermentation studies ideally should be done *in vivo*. However, the difficulty of experimental variables often makes *in vivo* studies difficult to conduct. Moreover, the rate and extent of fermentation determined from SCFA production cannot be estimated with much accuracy *in vivo*⁽¹⁸⁾. The *in vitro* fermentation method using human faecal inoculum has been used to study rate and extent of fermentation⁽¹⁸⁾. The method provided SCFA molar ratios which are similar to those seen *in vivo* and therefore can be used to model the human colon. It demonstrated that faecal materials collected from man at different times of the year provide a sufficiently uniform source of micro-organisms so that fermentation values such as fibre digestibility and gas or SCFA production can be compared between studies⁽¹⁸⁾. It also showed the differences in fermentability of different fibre sources. For example, pectin was found to be most rapidly fermented followed by psyllium gum, tragacanth gum and cellulose⁽¹⁸⁾. However, in the present study we did not determine the rate and extent of fermentation of the different test foods since it was not one of the objectives of the study. We only determined the SCFA produced after 24 h of fermentation which mimics human fermentation in the colon.

The study showed that root crops and legumes are good sources of dietary fibre. Although both root crops and legumes have significant amounts of dietary fibre, there must be some differences in their physiological effects. Root crops contained low fat and high fibre and can be a good substitute for bread and rice. Flaxseed (28.0 g/100 g dietary fibre) may be considered a legume and has been shown to have some protective and preventive effects on CVD⁽¹⁹⁾ and colon and breast cancer^(20–22). The physiologic effect of legumes may be similar to flaxseed. Legumes maybe a better laxative than root crops because of the high percentage of insoluble fibre. Intakes of legumes may increase faecal bulk and decrease transit time due to excellent water-holding capacity. Legumes may also serve to dilute secondary bile acids in the colon, thereby reducing colonic exposure to these substances. The higher percentage of soluble fibre in root crops compared to legumes may have phys-

Table 1. Nutritional composition of root crops and legumes (g/100 g sample)
(Mean values with their standard errors)

Test foods	Fat		Protein		CHO*		Dietary fibre		Soluble fibre		Insoluble fibre		Acetate†		Propionate†		Butyrate†	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Root crops																		
Kamote	0.6 ^k	0.2	3.4 ^h	0.0	89.6 ^c	0.3	8.1 ^j	0.1	4.8 ^c	0.4	3.2 ⁱ	0.2	1.1 ^{gx}	0.2	0.4 ^{hz}	0.0	0.5 ^{ly}	0.0
Gabi	0.4 ^k	0.0	1.1 ^j	0.0	90.7 ^b	0.2	13.5 ^g	0.1	3.6 ^e	0.1	9.5 ^f	0.2	1.1 ^{gx}	0.1	0.2 ^{ly}	0.0	0.2 ^{ky}	0.0
Potato	1.0 ^j	0.1	10.0 ^e	0.1	77.1 ^e	0.4	7.6 ^k	0.2	3.6 ^e	0.1	4.0 ^h	0.2	0.2 ^{hy}	0.0	0.1 ^{iy}	0.1	0.6 ^{hijx}	0.1
Tugi	0.2 ^l	0.1	4.8 ^g	0.1	75.6 ^f	0.8	10.3 ^l	0.1	3.6 ^e	0.1	7.0 ^g	0.2	2.5 ^{dex}	0.4	1.8 ^{dey}	0.2	0.8 ^{gz}	0.0
Ube	0.4 ^k	0.0	5.2 ^f	0.1	87.0 ^d	0.3	11.8 ^h	0.2	4.4 ^d	0.1	7.4 ^g	0.2	0.2 ^{hx}	0.0	0.1 ^{iy}	0.0	0.1 ^{kx}	0.1
Cassava	0.3 ^l	0.0	2.4 ⁱ	0.0	91.1 ^a	0.1	4.6 ^l	0.2	1.4 ^g	0.4	3.2 ⁱ	0.1	2.4 ^{ex}	0.1	0.7 ^{fz}	0.1	0.8 ^{gx}	0.0
Legumes																		
Mungbean	5.8 ^a	0.4	14.6 ^{de}	4.9	64.1 ^{gh}	5.4	31.7 ^c	0.1	4.8 ^c	0.3	26.9 ^c	0.2	5.3 ^{ax}	0.1	1.7 ^{dz}	0.0	2.5 ^{dy}	0.2
Soyabean	2.8 ^d	0.1	33.3 ^a	1.8	55.8 ⁱ	1.9	46.9 ^a	3.4	8.0 ^a	0.2	38.9 ^a	0.2	3.8 ^{cx}	0.5	1.4 ^{ey}	0.2	0.0	0.0
Peanut	1.9 ^h	0.9	22.1 ^{bc}	8.5	62.0 ^{gh}	9.5	24.1 ^e	1.7	4.2 ^{cde}	0.7	20.0 ^d	0.7	2.9 ^{dy}	0.1	2.2 ^{cz}	0.1	6.0 ^{ax}	0.2
Pole sitao	4.2 ^b	0.1	11.5 ^e	4.4	75.8 ^{efg}	4.6	35.0 ^b	0.2	5.5 ^b	0.3	29.5 ^b	0.6	5.6 ^{ax}	0.5	1.9 ^{dx}	0.1	0.7 ^{hz}	0.0
Cowpea	3.2 ^c	0.1	22.3 ^c	3.9	67.0 ^g	4.1	34.0 ^b	0.6	4.0 ^{de}	0.2	29.8 ^b	0.6	4.8 ^{by}	0.1	2.1 ^{cdz}	0.2	5.4 ^{bx}	0.2
Chickpea	2.3 ^f	0.1	20.7 ^c	0.1	69.5 ^g	0.3	26.2 ^e	0.7	1.3 ^g	0.1	24.9 ^d	0.6	2.8 ^{dx}	0.2	0.6 ^{ghz}	0.2	0.9 ^{fy}	0.0
Green pea	1.0 ^j	0.1	21.5 ^{cd}	0.1	69.4 ^g	0.3	29.7 ^d	0.9	2.1 ^f	0.1	27.6 ^c	0.6	1.9 ^{fx}	0.1	0.7 ^{fy}	0.1	0.6 ^{ly}	0.0
Lima bean	2.0 ^g	0.1	23.3 ^c	1.9	60.1 ^h	2.5	20.9 ^f	0.1	3.7 ^e	0.1	17.7 ^e	0.2	2.0 ^{fx}	0.1	0.6 ^{gy}	0.0	0.1 ^{kz}	0.1
Kidney bean	2.5 ^e	0.3	28.3 ^b	0.0	60.4 ^h	0.3	29.8 ^d	0.3	0.4 ^h	0.1	29.4 ^b	1.8	0.4 ^{hz}	0.2	7.2 ^{ax}	1.5	3.6 ^{cy}	0.7
Pigeon pea	1.3 ⁱ	0.2	24.5 ^c	0.1	63.2 ^h	0.4	21.8 ^f	1.1	2.4 ^f	0.2	19.4 ^d	0.6	0.0		3.3 ^{bx}	0.2	1.5 ^{ey}	0.1

Dietary fibre from root crops and legumes

CHO, carbohydrates.

^{a-l} Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).

^{xyz} For SCFA mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

* Calculated by difference (100 – (ash, moisture, fat, protein)).

† SCFA (mmol/g fibre isolate).

iological effects related to glucose and cholesterol metabolism^(7,8). Generally, soluble fibres have a tendency to have long-term effects associated with better glycaemic control and lower insulin requirements due to increased insulin sensitivity or changes in insulin receptors as well as cholesterol-lowering effects. In terms of SCFA production, a good source of butyrate, e.g. tugi and cassava (root crops) and peanut and cowpea (legumes), may have an important role in the prevention for risk of colon cancer, while a good source of propionate, e.g. kidney beans and pigeon pea (legumes), may have an important role in cholesterol-lowering effects.

In conclusion, both root crops and legumes are good sources of dietary fibre and produced SCFA after fibre fermentation, such as acetate, propionate and butyrate. SCFA production after *in vitro* fermentation can be estimated using human faecal inoculum and can be used to model the human colon.

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