Prior Exposure of Cecal Microflora to Grape Pomaces Does Not Inhibit in Vitro Fermentation of Pectin

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Grape pomace is mainly composed of dietary fiber (DF) and polyphenols (PP), components of the undigestible residue and substrates for colonic fermentation. Male Wistar rats were fed fiber-free diets supplemented with 5% cellulose or 10% red grape peels (RGP), white grape peels (WGP), or white grape seeds (WGS) as source of DF for 6 weeks, and rat cecal contents from fasted rats were used as inoculum to in vitro ferment apple pectin. Short-chain fatty acid (SCFA) production, pH, and pressure were measured as indications of fermentative capacity at different times. SCFA production at 24 h, expressed as micromoles per milligram of dry substrate, was significantly higher in RGP (9.7) and WGS (9.6) groups than in the control group (8.8). Fermentative capacity of cecal contents from WGP-fed rats did not differ from that of the control group (8.5 μmol/mg of dry substrate). The presence of the three test materials in the diets did not modify the fermentation rate. SCFA production was significantly correlated with pH values ($r = -0.969$) and gas production ($r = 0.960$). In conclusion, DF rich in PP did not inhibit colonic fermentation; furthermore, some materials enhanced the process.

Keywords: *Dietary fiber; polyphenols; in vitro fermentation; rats*

INTRODUCTION

Dietary nondigestible components reach the colon and are fermented by the endogenous microflora, producing short-chain fatty acids (SCFA), gas, and water (Cummings and MacFarlane, 1991). SCFA have some beneficial effects for the host: butyrate is an important fuel source for the colon (Roediger, 1991) and it has been shown to be a potent trophic agent in vivo (Sakata, 1987); therefore, it may be implicated in the prevention of colorectal cancer and in the maintenance of epithelial mucosa (McDougall et al., 1996). Propionate is cleared by the liver and may modulate hepatic carbohydrate and lipid metabolism (Chen et al., 1984). Acetate largely escapes colonic and hepatic metabolism and serves primarily as a fuel for peripheral tissues (Scheppach et al., 1991). Acetate and propionate have been related to hypocholesterolemic effects (Gallaher et al., 1993).

The principal substrates for colonic fermentation are resistant starch, dietary fiber (DF), sugar alcohols, oligosaccharides, and polyphenolic compounds (Gibson et al., 1996). Besides the beneficial effects of SCFA, undigestible compounds seem to play an important role in modifying the microflora (Monsma and Marlett, 1996; Bouhnik et al., 1997).

Polyphenols (PP) are absorbed in part from the digestive tract, but an important fraction is not absorbed and reaches the colon, where it is susceptible to bacterial metabolism (Bravo et al., 1994a; Martín-Carrón et al., 1997). PP are compounds extensively distributed in vegetable foods (legumes, cereal, fruits) (Hertog et al., 1992) and beverages (tea, wine, cider) (Hertog et al., 1993); among fruits, grapes contain a high percentage of polyphenolic compounds.

The main constituents of grape pomaces are DF and PP (Saura-Calixto et al., 1991; Valiente et al., 1995). DF has been demonstrated to exert beneficial effects on human health (Kritchevsky and Bonfield, 1995). PP have been traditionally considered as antinutritional factors, but at present they have interest as bioactive compounds with positive effects on health. PP have been demonstrated to exert a hypocholesterolemic effect on high-cholesterol-fed rats (Tebib et al., 1994). Ahn et al. (1993) found that tea PP modify positively the microflora composition in vitro by enhancing the growth of some *Bifidobateria* and inhibiting the growth of some *Clostridia*. Despite these effects, little is known about the physiological and nutritional implications of the consumption of PP-rich DF in man.

The objective of this work was to study the effect of three grape fractions, red grape peels (RGP), white grape peels (WGP), and white grape seeds (WGS), with a high content of DF and associated polyphenolic compounds on the fermentative capacity of cecal flora from Wistar rats. In vitro fermentations with cecal inocula from rats fed with the materials were conducted to determine the production of total and individual SCFA of a highly fermentable substrate, as indications of fermentative capacity.

MATERIALS AND METHODS

Materials. Red grape pomaces (*Vitis vinifera* var. Cencibel) and white grape pomaces (*Vitis vinifera* var. Airén) were obtained from Bodega Los Llanos (Valdepeñas, Spain). Stems were manually removed, and pomaces were dried in an air-circulating oven at 60 °C overnight. Dry samples were sieved (5 and 2 mm mesh size) to separate seeds and peels. RGP, WGP, and WGS were selected. Samples were milled to a

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Table 1. Composition of the Experimental Diets, Expressed as Grams per Kilogram of Diet

ingredient	cellulose	RGP	WGP	WGS
casein	140	140	140	140
cornstarch	465.5	415.5	415.5	415.5
dyetrose[a]	155.2	155.2	155.2	155.2
sucrose	100	100	100	100
cellulose	50	0	0	0
salt mix AIN-93M	35	35	35	35
vitamin mix AIN-93VX	10	10	10	10
L-cystine	1.8	1.8	1.8	1.8
TBHQ	0.008	0.008	0.008	0.008
soybean oil	40	40	40	40
choline bitartrate	2.5	2.5	2.5	2.5
RGP		100		
WGP			100	
WGS				100

[a] Dextrinized cornstarch (Dyetrose, Dyets, Bethlehem, PA).

particle size of <0.5 mm in a Cyclone sample mill (Tecator, Höganäs, Sweden), to obtain test products. DF contents (expressed as percentage of dry matter) of RGP, WGP, and WGS were 54.2, 59.0, and 56.2%, respectively; the detailed composition of the grape fractions has been reported by Bravo and Saura-Calixto (1998).

Animals and Diets. Forty male Wistar rats of the same age and with an average body weight of 195 ± 7.3 g were supplied by the breeding center at the Facultad de Farmacia (Universidad Complutense, Madrid, Spain). The study was approved by the Department of Nutrition of the Universidad Complutense. Animals were housed in individual metabolic cages in a room maintained at 22 (±1) °C, with 12 h light/dark cycles. Food and water were available ad libitum. Animals were fed with a standard diet with cellulose as source of DF (fiber-free AIN-93M purified rodent diet, DYETS Inc., Bethlehem, PA) for a 4 day adaptation period.

After the adaptation period, the animals were randomly divided into four groups of 10 animals. The control group was fed with the standard diet with cellulose as source of DF. The three experimental diets were prepared from the standard diet by addition of the experimental product, RGP, WGP, or WGS (10% w/w). Since the products contained ~50% fiber (Bravo and Saura-Calixto, 1998), the test diets gave 5% DF. Control diet was prepared with 5% DF as cellulose microcrystalline (no. 401855, DYETS Inc.) and made up to 10% with cornstarch (no. 401200, DYETS Inc.). The four groups of rats were fed control and test diets for 6 weeks. The experimental diets follow the American Institute of Nutrition guidelines (Reeves et al., 1993). The composition of the diets is shown in Table 1.

In Vitro Fermentation. Samples were fermented in vitro in a batch culture system under strict anaerobic conditions for 24 h. Fresh rat cecal contents were used as inoculum. Anaerobic conditions were maintained using oxygen-free carbon dioxide. The in vitro fermentation method was adapted from that of Abia et al. (1993) and modified following the fermentation procedure from a European interlaboratory study (Barry et al., 1995).

Inoculum. After 12 h of food deprivation, the rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg of body weight) and ceca were removed through abdominal midline incisions. Cecal contents from the 10 rats of each group (control, RGP, WGP, and WGS) were used to prepare four inocula.

Rat cecal contents from each experimental group were scraped, weighed, and added to a flask containing sterile and anaerobic medium to give a 10% (w/v) inoculum. The inoculum was mixed for 10 min in a Stomacher 80 Lab Blender (Seward Medical, London, U.K.) and filtered (1 mm mesh) before use. These steps were carried out in an oxygen-free CO_2-saturated atmosphere in an Atmosbag (Aldrich no. 836-2, Milwaukee, WI).

Substrate. Apple pectin (P-2157) and lactulose (L-7877) were obtained from Sigma (Sigma-Aldrich Química S.A., Madrid, Spain). According to the certificate from Sigma, apple pectin does not contain protein and the contents of galacturonic acid, methoxy groups, and ash were 76.0, 7.0, and 6.3%, respectively. The moisture content determined at 105 °C overnight was 8.1%.

Medium. The fermentation medium has been adapted from that of Goering and Van Soest (1979). It contained the following (per liter of distilled water): 2.5 g of trypticase (no. 2340, Biolife, Milano, Italy), 125 μL of micromineral solution, 250 mL of buffer solution, 250 mL of macromineral solution, and 1.25 mL of resazurine solution 0.1% (w/v) (R-2127, Sigma-Aldrich Química S.A). The micromineral solution contained (per liter of distilled water) the following: 132 g of $CaCl_2 \cdot 2H_2O$, 100 g of $MnCl_2 \cdot 4H_2O$, 10 g of $CoCl_2 \cdot 6H_2O$, and 80 g of $FeCl_3 \cdot 6H_2O$. The buffer solution contained (per liter of distilled water) the following: 4 g of $(NH_4)HCO_3$ and 35 g of $NaHCO_3$. The macromineral solution was prepared with 5.7 g of Na_2HPO_4, 6.2 g of KH_2PO_4, and 0.6 g of $MgSO_4 \cdot 7H_2O$ per liter of distilled water. Reducing solution (33.5 mL), containing 6.25 g of cysteine hydrochloride (C-1276, Sigma-Aldrich Química S.A.) 6.25 g of $Na_2S \cdot 9H_2O$, and 40 mL of 1 M NaOH per liter of distilled water, was added to 1 L of medium, and they were sterilized at 100 °C for 15 min. The reactives were supplied by Panreac Química S.A., Barcelona, Spain.

Procedure. One hundred milligrams of substrate was weighed in 50 mL serum vials (Supelco), and 8 mL of medium was added. Vials were sealed with rubber caps (no. 407-0-13, Ormacisa, Madrid, Spain). Substrates were hydrated at 4 °C for 16 h. Two milliliters of inoculum was then added to each vial. The concentrations of substrate and inoculum were 1 and 2% (w/v), respectively. Vials were placed in a shaking water bath (80 strokes/min) at 37 °C for 24 h. Fifteen fermentation flasks were prepared from each inoculum source to provide triplicate fermentations with durations of 0, 2, 5, 8, and 24 h. Four controls for each time point, two containing no substrate and two containing lactulose (L-7877, Sigma-Aldrich Química S.A.), were included in the experiments as zero and completely fermentable substrate, respectively. The amount of lactulose in the medium after 24 h of fermentation was determined gravimetrically, and it was negligible.

At 0, 2, 5, 8, and 24 h, gas pressure and pH were measured in the vials. Fermentation was stopped by adding an excess of 1 M NaOH (2.5 mL), samples were centrifuged at 2500*g* for 10 min, and 3 mL of supernatant in duplicate was taken for SCFA determinations.

Calculations. SCFA results obtained for controls without substrate and time 0 h were subtracted from the samples to correct the SCFA production from the inoculum.

Since the experiments were carried out on different days and lactulose was used as a fermentative reference in each incubation, experimental values for SCFA production, pH, and pressure were calibrated using the formula (Michel et al., 1996)

$$V_{\text{pect cal}} = V_{\text{pect expt}}(M_{\text{lact}}/V_{\text{lact expt}})$$

where $V_{\text{pect cal}}$ corresponds to the calibrated value for a given variable, $V_{\text{pect exp}}$ and $V_{\text{lact exp}}$ are the values for the given variables obtained from the same experiment for the pectin sample and the fermentation reference (lactulose), and M_{lact} is the mean value calculated for each variable for the reference from all of the experiments.

The percentage of fermentability of apple pectin was calculated by considering total SCFA produced from lactulose as 100% fermentability.

SCFA produced were expressed as micromoles per milligram of dry substrate and as molar proportions (percent) acetate/propionate/butyrate.

Chemical Analysis. *SCFA Analysis by Gas Chromatography.* The method of Spiller et al. (1980), slightly modified, was followed. A 400 μL aliquot of supernatant from the fermentation samples with 100 μL of internal standard, 50 μmol mL^{-1} 4-methylvaleric acid (no. 27,782-7, Sigma-Aldrich Química S.A.), and 50 μL of 850 g L^{-1} phosphoric acid 85% (no. 21,510-4, Sigma-Aldrich Química S.A.) was made up to 1

Table 2. Polyphenolic Content of RGP, WGP, and WGS[a,b]

	RGP	WGP	WGS
soluble polyphenols	3.8 ± 0.1	5.0 ± 0.2	5.4 ± 0.4
condensed tannins	26.2 ± 0.3	21.0 ± 1.3	18.8 ± 1.4

[a] Mean value ± standard deviation; $n = 3$. [b] Data expressed as percentage on a dry matter basis.

mL with Milli-Q water and centrifuged (4 °C, 7300*g*, 15 min). Two microliters of supernatant was injected into a 5890 Hewlett-Packard gas chromatograph equipped with a flame ionization detector and a fused silica column (Carbowax 20M, 10 m × 0.53 mm i.d.). Nitrogen was the carrier gas at a pressure of 17 kPa. Injector and detector temperature were 200 °C, and column temperature was 120 °C (isothermal). SCFA were identified and quantified by comparison with known fatty acid standards.

Polyphenols. Total soluble PP were extracted from RGP, WGP, and WGS by shaking at room temperature with methanol/water (50:50 v/v, 50 mL/g of sample, 60 min) and acetone/water (70:30 v/v, 50 mL/g of sample, 60 min). PP were spectrophotometrically determined in the combined extracts according to the Folin−Ciocalteu method using tannic acid (T-0125, Sigma-Aldrich Química S.A.) as standard (Montreau, 1972).

Residues obtained from the previous extractions were treated with 5% HCl−butanol (100 °C, 3 h), and condensed tannins were analyzed by reading the absorbance at 555 nm of the anthocyanidin solutions formed (Reed et al., 1982). Carob pod condensed tannins (Nestec, Ltd., Vers-chez-les-Blancs, Switzerland) were used as a standard according to a previous spectral study (Saura-Calixto et al., 1991).

Analysis were conducted in triplicate.

Statistical Analysis. Results are reported as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to determine the significance of mean differences between groups, by using the Stat Graphics computer program (SAS/STAT version 6, SAS Institute, Cary, NC). Significance level was $P < 0.05$. Homogeneity of variance was assumed (Cochran's test).

RESULTS AND DISCUSSION

Grape pomaces and grape seeds are the byproducts obtained after the wine-making process. Analysis of these products shows that their main component is DF, at >50% of the dry matter (Bravo and Saura-Calixto, 1998), and they also contain important amounts of polyphenolic compounds, extractable or soluble PPO, and nonextractable or condensed tannins (Table 2). The content of condensed tannins was high in the three materials, the highest value corresponding to RGP.

The physicochemical characteristics of the three materials are different. Peels from red and white grapes have similar physical characteristics but different chemical composition; WGP presents a higher percentage of soluble PP and a lower content of condensed tannins (Table 2). WGS have different physical characteristics due to their fat content (12.4%) and their cell wall structure, and polyphenolic content is also different. These different characteristics may influence their physiological effects and fermentative pattern.

Condensed tannins have been demonstrated to exert an inhibitory effect on growth by decreasing protein digestibility (Longstaff and McNab, 1991). PP content in the diets did not cause negative effects on animal growth in RGP- and WGP-fed animals with respect to the control group (Table 3). The food efficiency was lower in WGS-fed rats than in the other groups, although there was not a great difference. In a previous work, no differences in growth were reported in rats fed

Table 3. Effect of RGP, WGP, and WGS on Food Intake, Weight Gain, and Food Efficiency for 6 Weeks in Rats[a]

	food intake (g of dm)	wt gain (g)	food efficiency[b]
control	758.7 ± 53.1a	186.5 ± 18.4a	0.25 ± 0.03b
RGP	857.2 ± 64.0b	207.0 ± 27.8ab	0.24 ± 0.02ab
WGP	878.4 ± 90.9b	216.3 ± 29.7b	0.25 ± 0.02b
WGS	836.5 ± 88.3b	184.7 ± 29.7a	0.22 ± 0.02a

[a] Mean value ± standard deviation; $n = 10$. Values in a column not sharing a common letter are significantly different when tested by ANOVA ($p < 0.05$). [b] Food efficiency = weight gain × food intake^{-1}.

10% grape pomaces for 8 weeks with respect to the same control diet (Martín-Carrón et al., 1997).

Fermentable components of the diet could affect the metabolic activity and composition of the colonic microflora (Monsma and Marlett, 1996; Bouhnik et al., 1997); in consequence, they could indirectly modify bacterial fermentative activity. Feeding rats with a purified diet with DF from canned peas, psyllium seed husk, and a mixture of fiber from soybeans, corn, and wheat during 2 weeks has been shown to increase the in vitro fermentation rate due to adaptation of the colonic bacteria to the substrate (Monsma and Marlet, 1995). Tea PP have been shown to selectively modify colonic microflora (Ahn et al., 1993). The test products used in the present work (RGP, WGP, and WGS) were chosen because of their high content in DF and polyphenolic compounds and, therefore, their possible influence on colonic flora and their potential application as food ingredients.

Estimation of fermentative capacity and colonic microflora composition in vivo is difficult due to the inaccesibility of proximal colon. Several in vitro methods using rat or human inocula have been developed to study this process (Titgemeyer et al., 1991; Barry et al., 1995). Despite the inconveniences of in vitro methods to extrapolate results to humans, different studies confirm that in vitro fermentations with cecal inocula mimic what takes place in vivo in the proximal large intestine (Edwards and Eastwood, 1992; Monsma and Marlett, 1996). No differences between human fecal and rat cecal inocula were found by Barry et al. (1995) using conditions similar to those used in the present work.

SCFA production and fermentability of apple pectin by rat cecal inocula are shown in Table 4. The addition of RGP and WGS to the standard diet increased the fermentative capacity of the colonic flora: SCFA production (acetic, propionic, and butyric acids) after 24 h of fermentation in these groups was higher than in the control one. The stimulation of fermentative activities could be due to the influence of DF (Maciorowski et al., 1997; Morishita and Konishi, 1994) or polymeric grape tannins (Tebib et al., 1996) on bacterial enzymatic activities.

The addition of polyphenolic compounds to the diet seems to increase fermentation by cecal bacteria. Tebib et al. (1996) observed a significant increment of SCFA pool and a pH decrease in the cecal contents of rats fed grape polymeric tannins for 12 weeks compared to controls. On the contrary, feeding prefermented condensed tannins from Quebracho to rats for 3 weeks depressed the cecal SCFA concentrations compared to control group (Levrat et al., 1993). This difference could be due to the different degrees of polymerization of the products in the experiments; analysis of Quebracho established that it is rich in monomers and trimers with

Table 4. In Vitro Fermentation at 24 h of Apple Pectin Using Cecal Inocula from Rats Fed Cellulose, RGP, WGP, and WGS for 6 Weeks[a]

	(μmol/mg of dry substrate)				
	acetate	propionate	butyrate	total SCFA	fermentability[b] (%)
cellulose	7.1 ± 0.2ab	1.3 ± 0.03a	0.4 ± 0.03a	8.8 ± 0.2a	94.3 ± 2.5a
molar proportion[c] (%)	80.7	14.8	4.5		
RGP	7.7 ± 0.2b	1.5 ± 0.05b	0.5 ± 0.04b	9.7 ± 0.3b	103.9 ± 3.5b
molar proportion[c] (%)	79.4	15.4	5.2		
WGP	6.8 ± 0.4a	1.3 ± 0.04a	0.4 ± 0.01a	8.5 ± 0.4a	91.4 ± 4.6a
molar proportion[c] (%)	80.0	15.3	4.7		
WGS	7.7 ± 0.3b	1.4 ± 0.1ab	0.5 ± 0.01b	9.6 ± 0.4b	102.1 ± 4.5b
molar proportion[c] (%)	80.2	14.6	5.2		

[a] Mean value ± standard deviation; $n = 3$. Values in a column not sharing a common letter are significantly different when tested by ANOVA ($p < 0.05$). [b] Percentage of fermentability with respect to lactulose = ($SCFA_{pectin}/SCFA_{lactulose}$) × 100. [c] Molar proportions (%) of the SCFA acetate, propionate, and butyrate.

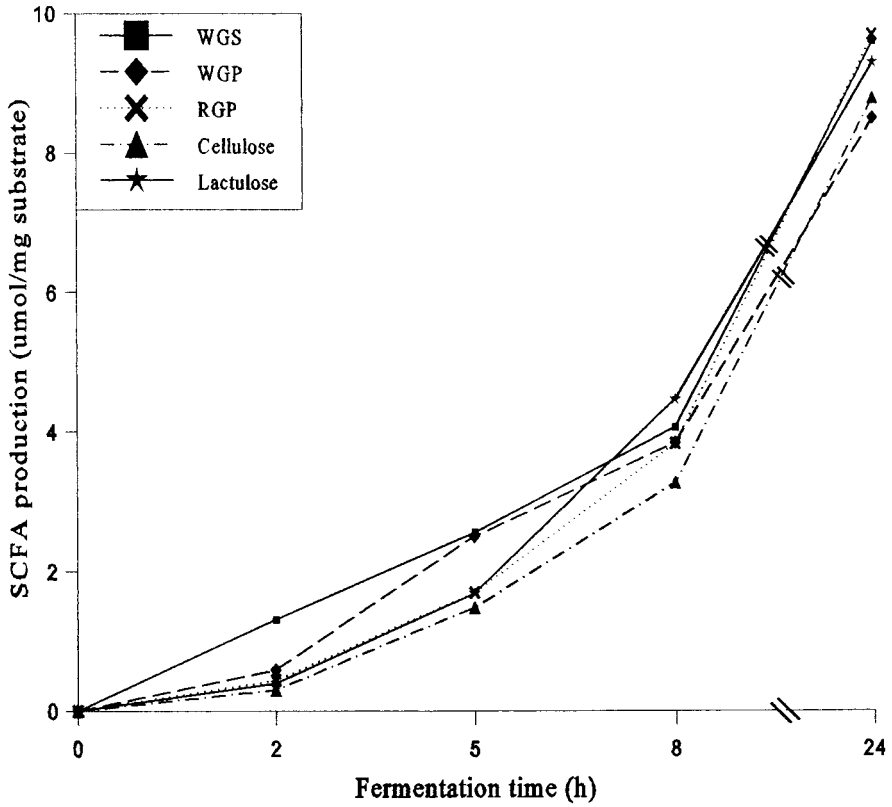

Figure 1. Production of SCFA (μmol/mg of dry substrate) during in vitro fermentation of apple pectin using cecal inocula from rats fed cellulose, RGP, WGP, and WGS as source of dietary fiber for 6 weeks.

little highly condensed polymers, while the grape products used by Tebib et al. (1996) and in the present experiment are rich in polymeric tannins or procyanidins.

Another explanation for the different fermentative capacities of the colonic flora could be the effect of these polymeric substances or condensed tannins on the composition of the microflora. Ahn et al. (1993) attributed to procyanidins from tea a selective change in the microflora. The three byproducts tested in this study are rich in condensed tannins, especially RGP, which produced the highest amount of SCFA (Table 4). The lower fermentative capacity observed in the WGP group, despite its high condensed tannin content, may be due to the presence of a higher PP proportion than in RGP, as a result of the different wine-making process (Shahidi and Naczk, 1995).

Although the DF and PP content of WGP was similar to that of RGP and WGS, SCFA production from the group fed with the WGP diet did not differ from that of the control group. This suggests that the influence of RGP, WGP, and WGS on the microflora could be different as a result of the different physicochemical characteristics of the materials or the possible presence of PP with acid structure, which have been shown to exert an inhibitory effect on the colonic microflora and may therefore influence the fermentative capacities of the inocula (Chesson et al., 1982; Bravo et al., 1994b).

Figure 1 shows SCFA production at different incubation times. In all groups there was a rapid increase in the SCFA production during the first 8 h, after which time the SCFA production increased but at a slower rate. There were no differences among fermentation rates from the four inocula.

pH and gas pressure evolution during fermentation are shown in Figures 2 and 3, respectively. pH variations can be used as an index of fiber fermentation (Guillon et al., 1992). SCFA production was significantly correlated with pH values ($r = -0.969$) and gas production ($r = 0.960$).

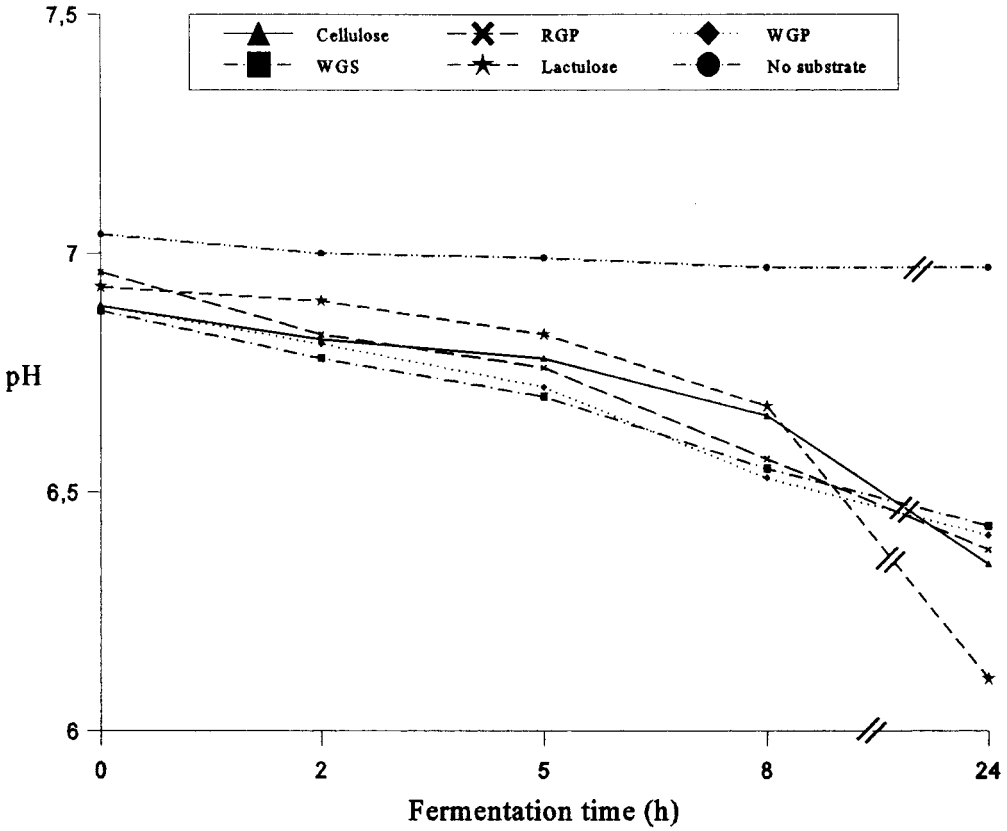

Figure 2. Changes in pH during in vitro fermentation of apple pectin using cecal inocula from rats fed cellulose, RGP, WGP, and WGS as source of dietary fiber for 6 weeks.

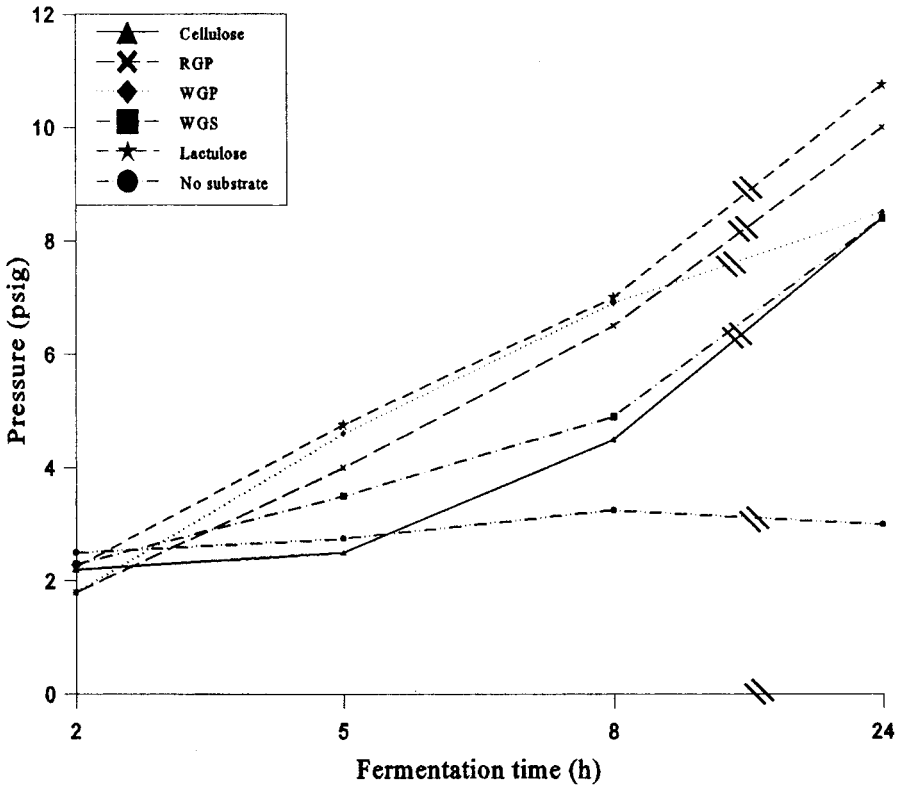

Figure 3. Changes in pressure during in vitro fermentation of apple pectin using cecal inocula from rats fed cellulose, RGP, WGP, and WGS as source of dietary fiber for 6 weeks.

The products originated in the fermentative process are related to the bacterial strains involved as well as to the type of substrate available. The main SCFA produced in all of the experiments was acetate, as was predictable since apple pectin is constituted of uronic acids, which seem to be involved principally in the production of acetic acid (Salvador et al., 1993). The molar proportions of acetate, propionate, and butyrate

were 80:15:5 (Table 4) and were similar for all groups. Similar results have been obtained in in vitro studies with human fecal bacteria (81:11:7) (Titgemeyer et al., 1991) or rat cecal inocula (75:12:13) (Barry et al., 1995) and in in vivo studies with rats (79:13:7) (Berggren et al., 1993).

Apple pectin is degraded extensively in the colon. The percentage of fermentability with respect to lactulose, considered as a totally fermentable substrate, ranged from 91 to 103% in WGP and RGP groups, respectively (Table 4). These data are similar to those from a European interlaboratory study (Barry et al., 1995) in which a 97.4% apple pectin degradability with human inoculum was reported.

In summary, our results show that DF rich in polyphenolic compounds, compared to purified cellulose, does not inhibit fermentation or diminish fermentation rate in in vitro experiments with rat cecal inoculum; on the contrary, RGP and WGS seem to enhance the fermentative process. Although PP have been considered as antinutritional factors, they could have positive effects on health.

ABBREVIATIONS USED

SCFA, short-chain fatty acids; DF, dietary fiber; PP, polyphenols; RGP, red grape peels; TBHQ, *tert*-butylhydroquinone; WGP, white grape peels; WGS, white grape seeds.

LITERATURE CITED

Abia, R.; Buchanan, C. J.; Saura-Calixto, F.; Eastwood, M. A. Structural changes during the retrogradation of legume starches modify the *in vitro* fermentation. *J. Agric. Food Chem*. **1993**, *41*, 1856−1863.

Ahn, Y. J.; Sakanata, S.; Kim, M.; Dawamura, T.; Fujisawa, T.; Mitsuoka, T. Effect of green tea extract on growth of intestinal bacteria. *Microb. Ecol. Health Dis*. **1993**, *6*, 3−9.

Barry, J.-L.; Hoebler, C.; Macfarlane, G. T.; Macfarlane, S.; Mathers, J. C.; Reed, K. A.; Mortensen, P. B.; Nordgaard, I.; Rowland, I. R.; Rumney, C. J. Estimation of the fermentability of dietary fibre in vitro: a European interlaboratory study. *Br. J. Nutr*. **1995**, *74*, 303−322.

Berggren, A. M.; Björck, I. M. E.; Nyman, M. Short-chain fatty acid content and pH in caecum of rats given various sources of carbohydrates. *J. Sci. Food Agric*. **1993**, *63*, 397−406.

Bouhnik, Y.; Flourié, B.; D'Agay-Abensour, L.; Pochart, P.; Gramet, G.; Durand, M.; Rambaud, J.-C. Administration of transgalacto-oligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. *J. Nutr*. **1997**, *127*, 444−448.

Bravo, L.; Saura-Calixto, F. Characterization of dietary fiber and the *in vitro* indigestible fraction of grape pomace. *Am. J. Enol. Vitic*. **1998**, in press.

Bravo, L.; Abia, R.; Saura-Calixto, F. Polyphenols as dietary fiber associated compounds. Comparative study on *in vivo* and *in vitro* properties. *J. Agric. Food Chem*. **1994a**, *42*, 1481−1487.

Bravo, L.; Abia, R.; Eastwood, M. A.; Saura-Calixto, F. Degradation of polyphenols (catechin and tannin acid) in the rat intestinal tract. Effect on colonic fermentation and faecal output. *Br. J. Nutr*. **1994b**, *71*, 933−946.

Chen, W. J. L.; Anderson, J. W.; Jennings, D. Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol fed rats. *Proc. Soc. Exp. Biol. Med*. **1984**, *175*, 215−222.

Chesson, A.; Stewart, C. S.; Wallace, R. I. Influence of plant phenolic acids on growth and cellulolytic activity of rumen bacteria. *Appl. Environ. Microbiol*. **1982**, *44*, 597−603.

Cummings, J. H.; MacFarlane, G. T. A review: the control and consequences of bacterial fermentation in the human colon. *J. Appl. Bacteriol*. **1991**, *70*, 443−459.

Edwards, C. A.; Eastwood, M. A. Comparison of the effects of ispaghula and wheat bran on rat caecal and colonic fermentation. *Gut* **1992**, *33*, 1229−1233.

Gallaher, D. D.; Hassel, C. A.; Lee, K. J.; Gallaher, C. M. Viscosiy and fermentability as attributes of dietary fiber responsible for the hypocholesterolemic effect in hamsters. *J. Nutr*. **1993**, *123*, 244−252.

Gibson, G. R.; Williams, A.; Reading, S.; Collins, M. D. Fermentation of nondigestible oligosaccharides by human colonic bacteria. *Proc. Nutr. Soc*. **1996**, *55*, 899−912.

Goering, H. K.; Van Soest, P. J. Forage fiber analysis (apparatus, reagents, procedures and some applications). *Agricultural Handbook 379*; U.S. Department of Agriculture; U.S. Government Printing Office: Washington, DC, 1979.

Guillon, F.; Barry, J-L.; Thibault, J. F. Effect of autoclaving sugarbeet fiber on its physicochemical properties and in vitro degradation by human faecal flora. *J. Sci. Food Agric*. **1992**, *60*, 69−79.

Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumend in The Netherlands. *J. Agric. Food Chem*. **1992**, *40*, 2379−2383.

Hertog, M. G. L.; Hollman, P. C. H.; Van de Putte, B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J. Agric. Food Chem*. **1993**, *41*, 1242−1246.

Kritchevsky, D.; Bonfield, C. In *Dietary Fiber in Health and Disease*; Kritchevsky, D., Bonfield, C., Eds.; Eagan Press: St. Paul, MN, 1995.

Levrat, M. A.; Texier, D. O.; Régerat, F.; Demigné, C.; Rémésy, C. Comparison of the effects of condensed tannin and pectin on cecal fermentations and lipid metabolism in the rat. *Nutr. Res*. **1993**, *13*, 427−433.

Longstaff, M.; McNab, J. M. The inhibitory effects of hull polysaccharides and tannins of field beans (*Vicia faba* L.) on the digestion of amino acids, starch and lipid and on digestive enzyme activities in young chicks. *Br. J. Nutr*. **1991**, *65*, 199−216.

Maciorowski, K. G.; Turner, N. D.; Lupton, J. R.; Chapkin, R. S.; Shermer, C. L.; Ha, S. D.; Ricke, S. C. Diet and carcinogen alter the fecal microbial populations of rats. *J. Nutr*. **1997**, *127*, 449−457.

Martín-Carrón, N.; García-Alonso, I.; Goñi, I.; Saura-Calixto, F. Nutritional and physiological properties of grape pomace as a potential food ingredient. *Am. J. Enol. Vitic*. **1997**, *48*, 328−332.

McDougall, G. J.; Morrison, I. M.; Stewart, D.; Hillman, J. R. Plant cell walls as dietary fibre: range, structure, processing and function. *J. Sci. Food Agric*. **1996**, *70*, 133−150.

Michel, C.; Lahaye, M.; Bonnet, C.; Mabeau, S.; Barry, J-L. In vitro fermentation by human faecal bacteria of total and purified dietary fibres from brown seaweeds. *Br. J. Nutr*. **1996**, *75*, 263−280.

Monsma, D. J.; Marlett, J. A. Rat cecal inocula produce different patterns of short-chain fatty acids than fecal inocula in in vitro fermentations. *J. Nutr*. **1995**, *125*, 2463−2470.

Monsma, D. J.; Marlett, J. A. Fermentation of carbohydrate in rat ileal excreta is enhanced with cecal inocula compared with fecal inocula. *J. Nutr*. **1996**, *126*, 554−563.

Montreau, F. R. Sur le dosage des composés phénoliques totaux dans les vins par le méthode Folin-Ciocalteu. *Connaiss. Vigne Vin* **1972**, *24*, 397−404.

Morishita, Y.; Konishi, Y. Effects of high dietary cellulose on the large intestinal microflora and short-chain fatty acids in rats. *Lett. Appl. Microbiol*. **1994**, *19*, 433−435.

Reed, J. D.; McDowell, R. E.; Van Soest, P. J.; Horvath, P. J. Condensed tannins: a factor limiting the use of cassava forage. *J. Sci. Food Agric*. **1982**, *33*, 213−220.

Reeves, P. G.; Nielsen, F. H.; Fahey, G. C. AIN-93 purified diets for laboratory rodents: finalñ report of the American Institute of Nutrition ad hoc writing committee on the

reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123*, 1939−1951.

Roediger, W. E. W. Cellular metabolism of short-chain fatty acids in colonic epithelial cells. In *Short-Chain Fatty Acids: Metabolism and Clinical Importance*; Roch, A. F., Ed.; Ross Laboratories: Columbus, OH, 1991.

Sakata, T. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal factors. *Br. J. Nutr.* **1987**, *58*, 95−103.

Salvador, V.; Cherbut, C.; Barry, J.-L.; Bertrand, D.; Bonnet, C.; Delort-Laval, J. Sugar composition of dietary fibre and short-chain fatty acid production during in vitro fermentation by human bacteria. *Br. J. Nutr.* **1993**, *70*, 189−197.

Saura-Calixto, F.; Goñi, I.; Mañas, E.; Abia, R. Klason lignin, condensed tannins and resistant protein as dietary fibre constituents: determination in grape pomaces. *Food Chem.* **1991**, *39*, 299−309.

Scheppach, W.; Pomare, E. W.; Elia, M.; Cummings, J. H. The contribution of the large intestine to blood acetate in man. *Clin. Sci.* **1991**, *50*, 177−182.

Shahidi, F.; Naczk, M. Phenolic compounds of beverages. In *Food Phenolics: Sources, Chemistry, Effects and Applications*; Technomic Publishing: Lancaster, PA, 1995; pp 136−148.

Spiller, G. A.; Chernoff, M. C.; Hill, R. A.; Gates, J. E.; Nassar, J. J.; Shipley, E. A. Effect of purified cellulose, pectin and low-residue diet on fecal volatile fatty acids, transit time and fecal weight in humans. *Am. J. Clin. Nutr.* **1980**, *33*, 754−759.

Tebib, K.; Bitri, L.; Besançon, P.; Rouanet, J-M. Polymeric grape seed tannins prevent plasma cholesterol changes in high-cholesterol-fed rats. *Food Chem.* **1994**, *49*, 403−406.

Tebib, K.; Besançon, P.; Rouanet, J.-M. Effects of dietary grape seed tannins on rat cecal fermentation and colonic bacterial enzymes. *Nutr. Res.* **1996**, *16*, 105−110.

Titgemeyer, E. C.; Bourquin, L. D.; Fahey, G. C.; Garleb, K.,A. Fermentability of various fiber sources by human fecal bacteria in vitro. *Am. J. Clin. Nutr.* **1991**, *53*, 1418−1424.

Valiente, C.; Arrigoni, E.; Esteban, R. M.; Amado, R. Grape pomace as a potential food fiber. *J. Food Sci.* **1995**, *60*, 818−820.

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