Hypocholesterolaemic effect of banana (*Musa sapientum* L. var. Cavendishii) pulp in the rat fed on a cholesterol-containing diet

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The pulp of banana fruit (*Musa sapientum* L. var. Cavendishii) was examined for its cholesterol-lowering effect with male rats fed on a diet containing lard (50 g/kg) and cholesterol (5 g/kg). Freeze-dried banana pulp showed a marked cholesterol-lowering effect when incorporated into a diet at the level of 300 or 500 g/kg, while the banana pulp dried in a hot-air current (65°) did not. Starch and tannin prepared from banana pulp were not responsible for the cholesterol-lowering effect. The results also suggest that banana lipids did not affect the concentration of serum cholesterol. Feeding of dopamine, *n*-epinephrine and serotonin tended to raise the concentration of serum cholesterol. Thus, all the substances tested which were thought to be susceptible to influence by hot-air drying were unlikely to be responsible for the hypocholesterolaemic effect. However, both soluble and insoluble fibres fractionated from banana pulp had a cholesterol-lowering effect, with the exception of cellulose. It was assumed that a browning reaction undergone during hot-air drying might be related to the disappearance of the hypocholesterolaemic effect of banana pulp dried in a hot-air current. The results obtained support the conclusion that soluble and insoluble components of dietary fibre participate in the hypocholesterolaemic effect of banana pulp.

Banana: Hypocholesterolaemic effect: Dietary fibre: Rat

The banana (*Musa sapientum*) is popular as an appetizing and nutritious fruit not only in many tropical countries but also in many other countries. The banana is consumed throughout the year even in Japan and accounts for about 50% of fruit imports to Japan (773700 tons in 1989). Agot (1968) and Sharaf *et al.* (1979) reported that the amount of protein and fat in banana pulp was nutritionally insignificant but that banana pulp had useful quantities of vitamins, except for vitamin B_{12}. It is also noted from the report of the Resources Council of Japan (1982) that banana pulp is a good source of potassium and energy. On the other hand, banana-starch granules are largely indigestible in rats (Sugimoto *et al.* 1980) and in humans (Englyst & Cummings, 1986). Englyst & Cummings (1986) suggest that banana-starch granules have a compact structure like potato starch and this does not, or does only to a limited degree, allow penetration and hydrolysis by mammalian amylase (*EC* 3.2.1.1). However, Carroll *et al.* (1978) reported that raw potato starch, which is also indigestible, prevents hypercholesterolaemia in rats. In addition, banana pulp contains pectic substances (Kondoh *et al.* 1928; Meyer, 1960; Sharaf *et al.* 1979) which are well known to have a hypocholesterolaemic effect in animals (Wells & Ershoff, 1961; Fisher *et al.* 1965, 1967; Vigne *et al.* 1987) and in humans (Keys *et al.* 1961; Palmer & Dixon, 1966; Kay & Truswell, 1977; Miettinen & Tarpila, 1977; Jenkins *et al.* 1979). These facts suggest that banana pulp may possibly show a cholesterol-lowering

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effect. Usha *et al.* (1984) reported that rats given neutral-detergent fibre (NDF) prepared from unripe banana showed significantly lower levels of serum cholesterol and triacylglycerols in both cholesterol-containing and cholesterol-free diets compared with control rats fed on a fibre-free diet, while NDF from ripe banana had no such effect. If the whole banana has a hypocholesterolaemic effect it may be a suitable food for both the middle-aged and the elderly to prevent atherosclerotic disease.

The present investigation was undertaken to estimate the hypocholesterolaemic activity of banana pulp on rats with a view to providing fundamental information.

METHODS

Banana fruit

The banana fruit of *Musa sapientum* L. var. Cavendishii which is grown in the Philippines was used for this work. The green banana was imported from the Philippines to Japan and then further ripened artificially. The mature but green banana (unripe banana) was purchased directly from a local wholesaler in Okayama before the application of any ripening stimulus. The ripe banana (yellow with some green) was purchased from local food markets. All the bananas used for the experiments were Chiquita brand. The slices of banana pulp were dried either by freeze-drying ($-30°$, 50 h) or in a hot-air current (65°, 10 h). The moisture of the dried pulps ranged from 55 to 70 g/kg. The dried pulp was ground using a porcelain pestle and mortar.

Preparation of banana-starch granules

Banana-starch granules were prepared from unripe banana by the method of Sugimoto *et al.* (1980), using the banana pulp without the skin. The starch granules obtained were washed repeatedly with acetone and air-dried. The yield of white starch was 440 g from 16 kg banana pulp. Moisture and starch contents of the starch sample were 87 and 895 g/kg respectively.

Preparation of banana tannin

Matsuo & Itoo (1981) reported that young banana fruit contained tannin which was designated as proanthocyanidin. Crude tannin mixture was prepared from unripe banana fruit (pulp and skin) by the procedure described in a previous paper (Horigome *et al.* 1988).

Extracted banana pulp

The acetone powder of banana pulp was also prepared by extracting freeze-dried ripe pulp with acetone–water (70:30, v/v) to remove tannin (banana pulp A). Half the acetone powder was further extracted with diethyl ether to remove lipids (banana pulp E).

Preparation of banana dietary fibre

As a general rule, in the determination and preparation of dietary fibre starch should be removed by amylase digestion after its gelatinization (Southgate, 1969). In the case of banana pulp, however, heat treatment for gelatinization of starch causes a browning reaction which might impair the components of banana pulp (Meyer, 1960; Horigome & Kandatsu, 1968; Pierpoint, 1969*a*, *b*; Hurrell & Finot, 1982). Therefore, the procedure using amylase was not appropriate to remove starch from dietary fibre in this case and the procedure for the preparation of banana dietary fibre was carried out as follows, by reference to the analytical methods of Schweizer & Wursch (1979) and Ayano *et al.* (1983). Ripe banana pulp (500 g) without the skin was cut into small slices and homogenized in a mixer with 1·5 l acetone–water (70:30, v/v) containing ascorbic acid (2 g/l) as an

antioxidant. After centrifugation of the mixture at 10000 *g*, the sediment was re-extracted with the same solvent. This procedure was repeated twice more to remove tannin and phenolic components from the sediment. The final sediment was mixed with 1 litre 0·033 M-phosphate buffer (pH 8·0) containing actinase E (200 mg, protease produced by *Streptomyces griseus*, 1000 tyrosine units/mg; Kaken Pharmaceutical Co., Tokyo) and the reaction mixture was continually stirred under nitrogen at 30° for 16 h, and adjusted frequently to pH 8·0 with 6 M-sodium hydroxide to allow for the hydrolysis of protein in the mixture and extraction of soluble dietary fibre. The reaction mixture was then centrifuged at 15000 *g* for 30 min and the sediment thoroughly mixed with 3–4 vol. water. The mixture was centrifuged again at 10000 *g* for 10 min. The resulting sediment was composed of a stiff lower layer containing mainly starch granules, and the soft, water-holding upper layer containing insoluble fibre. The upper layer was collected and washed repeatedly with water followed by acetone. The fibrous substance obtained in this way was insoluble fibre. The yield of the insoluble fibre was 5 g from 500 g banana pulp. The insoluble fibre contained (g/kg): moisture 82, crude protein (N × 6·25) 70, crude ash 21, starch 53, NDF 284, acid-detergent fibre (ADF) 192. The difference between NDF and ADF is hemicellulose (92 g/kg), and the difference between 1 kg and the sum of moisture, crude protein, crude ash, starch and NDF may be pectic substance (490 g/kg). Accordingly, the insoluble fibre prepared from banana pulp could be mainly composed of a pectic substance which was not extractable under the conditions of this procedure (pH 8·0).

The supernatant fraction obtained from the reaction mixture by high-speed centrifugation was adjusted to pH 6·0 by the addition of 6 M-hydrochloric acid and mixed with 4 vol. ethanol to cause coagulation. The resulting coagulum was collected by filtration and suspended in a small quantity of water. The suspension of the coagulum was dialysed in a cellophane tube against running distilled water for 3 d. The dialysed suspension was mixed with 4 vol. ethanol and the resulting coagulum, i.e. soluble fibre, was washed repeatedly with acetone followed by diethyl ether. The yield of soluble fibre was 3 g from 500 g banana pulp. The soluble fibre contained (g/kg): moisture 94, N 15, crude ash 167.

Animals

Five feeding experiments, i.e. Expts 1–5, were conducted with male Wistar rats weighing about 80 g (Ishibe Breeding Institution, CLEA Japan Inc.). The rats were fed on a powdered commercial diet (CE-2; CLEA Japan Inc.) for 3 d before feeding experiments and then were randomly divided into groups of five rats in each experiment. The rats were individually housed in stainless-steel cages with wire-mesh floors in a temperature-controlled room (23–25°) which was kept on a 12 h artificial lighting cycle. In each experiment, control and experimental diets containing cholesterol at 5 g/kg diet were given for 2 weeks. Rats in control and experimental groups were offered the same amount of their respective diets daily in each experiment. Food was placed in a stainless-steel food vessel to prevent food spillage (Natsume Seisakusho Co. Ltd, Tokyo). Food refusals were weighed daily and body-weight of rats was measured twice weekly. At the end of the feeding period rats were fasted for 12–13 h and then blood was drawn by puncture of the carotid artery under diethyl ether anaesthesia. Serum was prepared by centrifugation. Stomach, small intestine, caecum and large intestine were removed and weighed with their contents in Expt 1. In Expts 3 and 5, the weight of caecum was measured.

Estimation of digestibility

Digestion experiments were conducted by collecting all faeces during the last 3 d of an experimental period. A sheet of filter paper was placed under the wire-mesh floor of the rat cage to minimize faecal contamination by urine. The apparent digestibility of protein was

Table 1. *Expt 1. Composition (g/kg) of diets*

Diet...	Control	Unripe banana		Ripe banana	
Drying method...		Hot-air drying	Freeze-drying	Hot-air drying	Freeze-drying
Banana pulp†	—	500	500	500	500
Maize starch	393	—	—	—	—
Cellulose powder	30	—	—	—	—
Sucrose	270	218	218	218	218
Casein	200	175	175	175	175
Lard	50	50	50	50	50
Cholesterol	5	5	5	5	5
Mineral mixture*	40	40	40	40	40
Vitamin mixture*	10	10	10	10	10
Choline chloride	2	2	2	2	2

* Mineral and vitamin mixtures according to Harper (1959) were obtained from Oriental Yeast Co., Tokyo.
† Starch (393 g), cellulose (30 g), sucrose (52 g) and casein (25 g) were replaced by 500 g banana pulp in the experimental diets.

determined by measuring N intake and faecal N. The apparent digestibility of dry matter (DM) was also evaluated by estimating DM intake and faecal DM.

Expt 1. Feeding experiment using banana pulp dried by freeze-drying or in a hot-air current

A comparison was made of the hypocholesterolaemic properties of banana pulp dried by either freeze-drying or in a hot-air current. A comparison of unripe banana and ripe banana was also carried out at the same time. As shown in Table 1, therefore, a control diet and four experimental diets were used. Ground banana pulp was incorporated into the experimental diets at 500 g/kg. The control diet contained maize starch instead of banana pulp. The control diet contained 200 g casein/kg but the experimental diet contained 175 g casein/kg since dried banana pulp had an average crude protein content of 53 g/kg. The detailed compositions of the diets are shown in Table 1.

Expt 2. Feeding experiment using freeze-dried banana pulp at two dietary levels

Rats were fed on experimental diets containing two levels (150 and 300 g/kg) of freeze-dried pulp from ripe banana. The composition of the control diet (g/kg) was: maize starch 43, cellulose powder 20, sucrose 630, casein 200, mineral mixture 40, vitamin mixture 10, lard 50, cholesterol 5, choline chloride 2. The amounts of casein, mineral mixture, vitamin mixture, lard, cholesterol and choline chloride in the two experimental diets were the same as those of the control diet; the remaining ingredient of the experimental diets was sucrose at 543 and 393 g/kg respectively.

Expt 3. Feeding experiment using banana starch, banana tannin and extracted banana pulp

In Expt 3 banana starch, banana tannin, banana pulp A and banana pulp E were compared. Banana pulps A and E contained 94 and 112 g crude protein/kg respectively, but casein was incorporated into all diets at 200 g/kg. The diet containing freeze-dried banana pulp was also included in this experiment as a reference diet. The detailed compositions of the diets are presented in Table 2.

Table 2. *Expt 3. Composition (g/kg) of diets*

Diet ...	Control	Banana pulp†	Banana starch	Banana tannin	Banana pulp A‡	Banana pulp E‡
Material on test	—	500	250	5	300	280
Maize starch	250	—	—	245	—	—
Cellulose powder	20	—	20	20	—	—
Sucrose	423	193	423	423	393	413
Casein	200	200	200	200	200	200
Lard	50	50	50	50	50	50
Cholesterol	5	5	5	5	5	5
Mineral mixture*	40	40	40	40	40	40
Vitamin mixture*	10	10	10	10	10	10
Choline chloride	2	2	2	2	2	2

* For details, see Table 1.
† Ripe-banana pulp dried by freeze-drying.
‡ For details, see p. 232.

Expt 4. Feeding experiment using diets containing amines

The amines used for this experiment were dopamine hydrochloride (H 8502; Sigma), *n*-epinephrine hydrochloride (E 7386; Sigma) and serotonin hydrochloride (H 5755; Sigma). The composition of the control diet was the same as that used for Expt 2. Experimental diets were prepared by mixing each amine with the control diet since the amounts of amines added were very small. The amount of amine added into each experimental diet (mg/kg) was: dopamine hydrochloride 185, *n*-epinephrine hydrochloride 18, serotonin hydrochloride 181. In addition, a feeding experiment was conducted with a diet containing a combination of the three amines.

Expt 5. Feeding experiment using soluble and insoluble banana fibre

The experimental diets contained 25 g soluble or 50 g insoluble fibre/kg diet. The composition of the control diet (g/kg) was: cellulose powder 50, sucrose 643, casein 200, mineral mixture 40, vitamin mixture 10, lard 50, cholesterol 5, choline chloride 2. Cellulose powder (20 g) and sucrose (5 g) were replaced by 25 g soluble banana fibre in the experimental diet containing the soluble fibre. Ceullulose powder (50 g) was replaced by 50 g insoluble banana fibre in the experimental diet containing the insoluble fibre.

Chemical analyses

Serum total cholesterol was measured enzymically using a commercially available kit (Fuji Lebio Co., Tokyo). High-density-lipoprotein (HDL) was separated by the heparin–manganese precipitation method followed by determination of HDL-cholesterol. Serum triacylglycerol and HDL-cholesterol were measured enzymically with commercial kits (Wako Pure Chemical Ind. Co., Osaka). Low-density-lipoprotein (LDL)-cholesterol was calculated by subtracting HDL-cholesterol from total cholesterol. Moisture, N and crude ash of samples and faeces were determined by conventional methods. Banana pulp amino acids were determined using banana pulp E according to the procedure reported in a previous paper (Horigome & Uchida, 1980). NDF and ADF in the insoluble banana fibre were determined by the methods of Van Soest & Wine (1967) and Van Soest (1963) respectively. Starch in the samples of banana starch and insoluble fibre was measured according to the Association of Official Analytical Chemists (1975) procedure using glucoamylase (*EC* 3.2.1.3) from *Rhizopus niveus* (Seikagaku Kogyo Co., Ltd, Tokyo).

Table 3. *Expt 1. Food intake, body-weight gain, wet weight of alimentary tract, apparent digestibilities of dry matter and protein and serum total cholesterol of rats fed on diets containing 500 g/kg of various forms of banana pulp**

(Mean values with their standard errors)

Dietary group...	Control		Unripe banana pulp				Ripe banana pulp			
			Freeze-drying		Hot-air drying		Freeze-drying		Hot-air drying	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Food intake (g/14 d)	182·4	0·6	183·3	0·2	183·9	0·1	177·7	2·2	183·3	0·6
Weight gain (g/14 d)	39·9	1·9	36·7	4·4	36·8	3·3	33·4	2·3	37·9	2·9
Alimentary tract† (g)	13·1[a]	0·4	20·0[b]	0·8	21·7[b]	0·6	14·7[a]	0·6	14·6[a]	0·4
Digestibility (%):										
Dry matter	96·1[c]	0·4	83·9[a]	1·4	87·3[a]	0·8	93·6[b]	0·8	94·4[ab]	0·9
Protein	95·3[c]	0·4	71·6[a]	1·6	71·8[a]	1·7	85·9[b]	2·4	88·5[b]	0·8
Cholesterol (mmol/l)	4·98[b]	0·48	2·64[a]	0·21	3·82[b]	0·30	2·57[a]	0·09	4·77[b]	0·55

[a, b, c] Values with different superscript letters in the same horizontal line were significantly different: $P < 0·05$.

* For details of procedures, see pp. 232–234, and for composition of diets, see Table 1.

† Stomach, small intestine, caecum and large intestine with their contents.

Statistical analyses

The results obtained in the feeding experiments are presented as means with their standard errors. The statistical significance of differences between the control and experimental groups was assessed by Student's *t* test. In Expt 1 the statistical significance of the difference between the means was evaluated by analysis of variance (Snedecor & Cochran, 1967).

RESULTS

Expts 1 and 2. Hypocholesterolaemic effect of banana pulp

The results of feeding experiments with the pulp from unripe and ripe bananas are shown in Table 3. Although food intake and body-weight gain were similar in all dietary groups, the highest weight of alimentary tract and the lowest digestibilities of DM and protein were observed in both groups fed on unripe banana pulp dried by freeze-drying and in a hot-air current. These facts suggest that the unripe banana pulp has an abundance of indigestible components. On the other hand, the groups fed on the freeze-dried pulp obtained from unripe and ripe bananas had significantly lower serum total cholesterol than the control group, while the pulp dried in a hot-air current had no such effect with either unripe or ripe bananas. Incidentally, these findings suggest that the low digestibility of unripe banana pulp was not responsible for the cholesterol-lowering effect of banana pulp.

Table 4 shows the concentrations of serum cholesterol and triacylglycerols in rats fed on the freeze-dried pulp of ripe banana at 150 and 300 g/kg. There were no differences in body-weight gain among the three groups. The group fed on banana pulp at 300 g/kg showed a marked reduction in total cholesterol, as did the group fed on 500 g banana pulp/kg (Table 3). The total cholesterol concentration of the group fed on 150 g banana pulp/kg was only slightly lower than that of the control group but the difference was still significant. The HDL-cholesterol concentrations of the control group and the group fed on 300 g banana pulp/kg were similar and consequently a large difference in LDL-cholesterol concentration between the groups was observed. Although triacylglycerol concentrations

Table 4. *Expt 2. Body-weight gain and concentration of serum lipids in rats fed on diets containing 150 or 300 g banana pulp/kg*†

(Mean values with their standard errors)

Dietary group...	Control		Banana pulp			
			150 g/kg		300 g/kg	
	Mean	SE	Mean	SE	Mean	SE
Body-wt gain (g/14 d)	65·5	1·7	66·3	3·0	65·5	2·0
Serum lipids (mmol/l):						
Total cholesterol	4·54	0·19	3·55*	0·35	2·48**	0·23
HDL-cholesterol	0·42	0·04	—	—	0·44	0·03
LDL-cholesterol	4·12	0·19	—	—	2·03**	0·23
Triacylglycerol	2·00	0·35	2·19	0·07	2·69	0·44

HDL, high-density-lipoprotein; LDL, low-density-lipoprotein.
Mean values were significantly different from those for the control diet: * $P < 0·05$, ** $P < 0·01$.
† Ripe banana pulp dried by freeze-drying.

tended to be somewhat higher in the groups fed on the banana pulp, the difference was not statistically significant.

Expt. 3. Serum lipid concentrations of rats fed on banana starch, banana tannin and banana pulps A and E

The results of Expt 3 are given in Table 5. Although food intake was similar in all the groups (values not shown), the body-weight gain tended to be higher with diets containing banana starch and three types of banana pulp. In particular, the weight gain of the group fed on banana starch was significantly higher than that of the control group fed on maize starch. This may be attributable to an enlargement of the caecum in rats fed on banana starch. The enlarged caecum in rats fed on banana starch was packed with white undigested starch, which further resulted in lower digestibilities for DM and protein. There were no differences between the control and experimental groups in the corrected gain which was calculated by subtracting caecum weight from body-weight gain (Table 5). As would be expected, the protein digestibility was reduced by feeding banana tannin. The reduction, however, was not large enough to explain the low digestibility of protein in the group fed on banana pulp.

Serum total cholesterol was not reduced in the groups fed on banana starch and banana tannin when compared with the control group. In contrast, the groups fed on banana pulps A and E showed a marked reduction in total cholesterol, as did the group fed on the original type of banana pulp. The diets containing banana starch, banana tannin and banana pulps A and E did not produce any significant change in serum triacylglycerol concentration, while the diet containing the original type of banana pulp led to an increase in triacylglycerol concentration.

Expt 4. Serum lipid concentrations of rats given dopamine, n-epinephrine and serotonin

It can be seen from Table 6 that ingestion of dopamine, *n*-epinephrine, serotonin and their combination did not affect food consumption and body-weight gain and that serum total cholesterol tended to be higher with diets containing amines, in particular, *n*-epinephrine when compared with the control diet, while triacylglycerol concentration was similar in all

Table 5. *Expt 3. Body-weight gain, caecum weight, apparent digestibilities of dry matter and protein, and concentrations of serum lipids in rats fed on diets containing the original form of banana pulp§, banana starch, banana tannin, banana pulp A or banana pulp E‡*

(Mean values with their standard errors)

Dietary group...	Control		Banana pulp		Banana starch		Banana tannin		Banana pulp A		Banana pulp E	
Dietary level (g/kg)...			500		250		5		300		280	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Weight gain (g/14 d)	37·8	1·0	39·4	2·2	43·2*	1·3	38·0	1·6	40·0	1·5	38·2	0·4
Caecum weight (g)	1·6	0·1	4·7*	0·1	7·7*†	0·2	1·3†	0·2	3·4*†	0·1	3·3*†	0·1
Corrected gain (g)‖	36·2	1·0	34·7	2·1	35·5	1·5	36·7	1·6	36·6	1·3	34·9	0·3
Digestibility (%):												
Dry matter	97·1	0·2	94·0*	0·3	94·5*	0·4	96·8†	0·4	92·1*†	0·2	93·0*	0·3
Protein	96·8	0·3	88·8*	0·6	92·6*†	0·4	93·0†	0·2	86·4*	0·4	86·5*	1·8
Serum lipids (mmol/l):												
Total cholesterol	5·12	0·26	2·90*	0·29	4·89†	0·37	5·59†	0·37	2·71*	0·23	2·97*	0·25
Triacylglycerol	1·21	0·10	2·08*	0·29	1·46	0·32	1·28	0·16	1·38	0·14	1·26†	0·10

Mean values were significantly different from those for the control diet: * $P < 0·05$.
Mean values were significantly different from those for the reference group fed on banana pulp dried by freeze-drying: † $P < 0·05$.
‡ For details of composition, see Table 2.
§ Ripe banana pulp dried by freeze-drying.
‖ Calculated by subtracting caecum weight from body-weight gain.

Table 6. *Expt 4. Food intake, body-weight gain and concentration of serum lipids in rats fed on diets containing various amines*†

(Mean values with their standard errors)

Dietary group...	Control		Dopamine		Epinephrine		Serotonin		Combination	
Dietary level (mg/kg)‡			185		18		181		§	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Food intake (g/14 d)	187·8	0·8	188·1	1·1	189·2	0·8	190·5	1·2	188·7	1·4
Weight gain (g/14 d)	64·8	1·4	66·1	1·3	63·8	1·5	64·9	0·9	64·5	1·6
Serum lipids (mmol/l):										
Total cholesterol	4·75	0·18	5·71	0·39	6·61*	0·38	6·04	0·55	5·68	0·53
Triacylglycerol	1·88	0·18	1·92	0·22	1·60	0·14	1·74	0·08	1·62	0·21

Mean value was significantly different from that for the control diet * $P < 0·05$.
† For details of composition, see p. 235.
‡ As the hydrochloride.
§ The diet contained (mg hydrochloride/kg): dopamine 185, *n*-epinephrine 18, serotonin 181.

Table 7. *Expt 5. Food intake, body-weight gain, caecum weight, apparent digestibility of protein and concentration of serum lipids in rats fed on diets containing banana fibre*†

(Mean values with their standard errors)

Dietary group...	Control		Soluble fibre‡ (25 g/kg)		Insoluble fibre‡ (50 g/kg)	
	Mean	SE	Mean	SE	Mean	SE
Food intake (g/14 d)	138·9	0·1	138·8	0·1	138·9	0·1
Body-wt gain (g/14 d)	46·3	1·5	50·0	0·9	52·8*	1·7
Caecum wt§ (g)	2·56	0·15	2·14	0·12	2·28	0·18
Faeces voided (g/3 d)	1·53	0·04	1·17*	0·10	0·65**	0·05
Protein digestibility (%)	97·7	0·1	97·6	0·2	97·4	0·2
Serum lipids (mmol/l):						
Total cholesterol	5·57	0·14	4·54*	0·31	4·08*	0·12
Triacylglycerol	1·13	0·08	1·09	0·10	1·39	0·17

Mean values were significantly different from those of the control: * $P < 0·05$, ** $P < 0·01$.
† For details of composition, see p. 235.
‡ For details of preparation, see pp. 232–233.
§ Including contents

dietary groups. Dopamine, *n*-epinephrine and serotonin are contained in the banana pulp (Riggin *et al.* 1976; Feldman & Lee, 1985; Feldman *et al.* 1987) and, consequently, it is clear that none of the three amines are responsible for the hypocholesterolaemic effect of banana pulp.

Expt 5. Hypocholesterolaemic effect of soluble and insoluble dietary fibre prepared from ripe banana pulp

Table 7 shows the results obtained in Expt 5. Although food intake and protein digestibility were similar in all groups, body-weight gain tended to be higher in the groups given dietary fibre from banana pulp. The difference in body-weight gain between the control group and the group given insoluble fibre was statistically significant. On the other hand, the weight

of faeces voided increased with increasing levels of cellulose powder in the diet. Incidentally, ingestion of banana fibre did not cause an enlargement of caecum nor any change in the apparent digestibility of protein. These findings are compatible with the low contents of ADF and starch in insoluble fibre and suggest that the banana fibre was more fermentable and available in the hind-gut when compared with cellulose in the control diet. Serum total cholesterol was depressed in the groups fed on soluble and insoluble fibre when compared with the control groups fed on cellulose powder. There were no differences in serum triacylglycerol concentrations between the control and experimental groups. The results suggest that dietary fibre, with the exception of cellulose, may be responsible for the hypocholesterolaemic effect of banana pulp.

DISCUSSION

The present study confirms the effect of banana pulp in lowering serum cholesterol of rats receiving a diet containing 50 g lard and 5 g cholesterol/kg. This effect, however, was observed only in the freeze-dried banana pulp but not in the banana pulp dried in a hot-air current for both unripe and ripe bananas (Tables 3 and 4). Furthermore, it was suggested that the dietary fibre component in banana pulp was responsible for its cholesterol-lowering effect (Table 7). Usha *et al.* (1984) reported that NDF prepared from unripe banana showed a hypocholesterolaemic effect while NDF from ripe banana did not have this effect. NDF used for their experiment was prepared according to the Goering–Van Soest method (including boiling treatment) and, hence, their NDF was free from pectin and other soluble dietary fibre. In addition, they used banana fruits with the skin for the preparation of NDF. Thus, their NDF would differ in chemical and physiological properties from the dietary fibre used for the present experiment. Therefore, a direct comparison of their results and the present findings is limited.

On the other hand, it was demonstrated that starch, tannin, lipids and amines, which are labile or readily modified during hot-air drying of banana pulp, were not involved in the hypocholesterolaemic effect of banana. Raw potato starch is poorly utilized by rats (Jelinek *et al.* 1952) and prevents the hypercholesterolaemic response in rats fed on a semi-purified diet (Carroll *et al.* 1978). Raw banana starch is also poorly digested in rats (Sugimoto *et al.* 1980) and this might be reflected in the presence of large alimentary tracts and low digestibility of DM in the rats fed on various types of banana pulp (Tables 3 and 5). However, the addition of banana starch to a diet containing cholesterol failed to prevent the hypercholesterolaemic response. This discrepancy may be related to whether cholesterol is in the diet.

Sklan (1980) and Roy & Schneeman (1981) suggested that undigested protein binds bile acids and impairs the absorption of bile acids in the small intestine of chicks and mice. An increase in the excretion of bile acids in faeces was observed in antibiotic-treated rats fed on a diet containing condensed tannin in which the protein digestibility was depressed (Horigome *et al.* 1988). Furthermore, several researchers (Huff & Carroll, 1980; Kuyvenhoven *et al.* 1989) have assumed that an increased excretion of bile acids in faeces would depress body cholesterol and consequently result in lower serum cholesterol. From these findings the inclusion of tannin in the diet would be expected to have a cholesterol-lowering effect. Contrary to expectations, however, the diet containing banana tannin did not cause any change in serum cholesterol (Table 5). Furthermore, feeding banana pulp A, which was freed of tannin, produced as marked a hypocholesterolaemic response as the original form of banana pulp. This finding also suggests that banana tannin is not concerned with the hypocholesterolaemic effect of banana pulp.

The diet containing *n*-epinephrine raised serum total cholesterol (Table 6). Nor-

epinephrine administration results in an increased cholesterol synthesis by rat liver slices (Bortz, 1968) and an increased serum total cholesterol in cholesterol-fed rabbits (O'Donnell *et al.* 1988). These findings support our present finding. It was also observed that dopamine and serotonin were not responsible for the hypocholesterolaemic effect of banana pulp. Feldman *et al.* (1987) reported that the concentrations (mean with SE) of dopamine and serotonin in the pulp of yellow banana were 42·0 (SE 4·1) and 22·1 (SE 5·0) mg/kg wet weight. Accordingly, the respective concentrations of dopamine and serotonin in the experimental diets used for Expt 1 are 70 and 37 mg/kg diet (moisture content of wet banana pulp was approximately 700 g/kg). The maximum concentration of *n*-epinephrine in the banana fruit is 5·8 mg/kg wet weight according to Riggin *et al.* (1976), thus, the approximate concentration of *n*-epinephrine is 10 mg/kg for the experimental diets of Expt 1. Accordingly, the concentrations of amines in the diets used for Expt 4 were 1·5–4·0 times greater than those in the diets for Expt 1 and might be sufficient to determine the effect of the amines present in the banana pulp.

The diets containing banana pulps A and E used for Expt 3 had 28 and 31 g banana protein/kg diet respectively and 200 g casein. The addition of glycine to a diet containing casein and cholesterol prevents the rise in serum cholesterol levels in rats and rabbits (Katan *et al.* 1982). However, the amount of banana protein in the diets and the glycine content of banana protein (256 mg/g N, Table 8) were not high enough to mediate hypocholesterolaemia. Additionally, the rats fed on various forms of banana pulp showed a low protein digestibility when compared with the control diet (Table 3). Many factors, e.g. browning reaction, tannin, starch and protein itself in banana pulp, probably had an influence on protein digestibility. However, there was no significant difference in protein digestibility between the rats fed on banana pulp dried in a hot-air current and that dried by freeze-drying within the ripe- and unripe-banana-pulp diets, although the drying method of banana pulp had a marked effect on the cholesterol-lowering activity. The effect of banana pulp on cholesterol metabolism, therefore, was independent of the low digestibility of protein in the diets. These findings demonstrated that banana protein was not related to the cholesterol-lowering effect of banana pulp. It also appears that lipids were not responsible for the cholesterol-lowering effect of banana pulp (extracted banana pulp E, Table 5).

On the basis of the arguments presented previously, it seems reasonable to conclude that the active principle of banana pulp responsible for the hypocholesterolaemic action may be soluble and insoluble dietary fibre. In addition, Englyst & Cummings (1986) showed that the amount of dietary fibre in banana pulp, which was measured as non-starch polysaccharide by the Englyst procedure (Englyst *et al.* 1982; Englyst & Hudson, 1987; Englyst *et al.* 1987), was relatively constant during banana ripening while the amount of starch decreased as ripeness increased. It is also reasonable, therefore, that both the unripe and ripe bananas exerted a cholesterol-lowering effect.

What then is the reason for the disappearance of the cholesterol-lowering effect of banana pulp associated with hot-air drying? In the present study remarkable browning of slices of banana pulp was observed during hot-air drying, and browning of soluble fibre was also noticed when the extraction of fibre from banana pulp was achieved without ascorbic acid or at higher pH (around 10·0). The browning reaction in banana pulp is due to the oxidation and polymerization of dopamine and other phenolic compounds (Griffiths, 1959; Palmer, 1963; Galeazzi & Sgarbieri, 1981). Also, the amino group of serotonin may react with dopamine quinone as well as with the amino groups of amino acids and proteins (Pierpoint, 1969*a*, *b*; Hurrell & Finot, 1982). The soluble fibre component, if it is polygalacturonide, will be negatively charged and, thus, may interact with the oxidizing dopamine or may be absorbed onto a polymerizing product. The resulting brown fibre may

Table 8. *Amino-acid content of ripe banana pulp* (mmol/g nitrogen)*

Lysine	2·79	Glycine	3·41
Histidine	1·58	Alanine	3·57
Arginine	1·44	Valine	3·77
Aspartic acid	4·72	Methionine	0·92
Threonine	2·25	Isoleucine	2·75
Serine	2·71	Leucine	4·58
Glutamic acid	9·46	Tyrosine	1·70
Proline	2·96	Phenylalanine	2·36

* Washed with acetone–water (70:30, v/v) followed by diethyl ether.

undergo a modification in its chemical and physiological functions. Thus, the modification of fibre caused by the browning reaction was presumed to be the principal reason for the disappearance of the hypocholesterolaemic effect when the banana pulp was dried in a hot-air current. In this connection it would be interesting to study the interaction of pectin with dopamine undergoing oxidation, using a model system.

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