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Analysis of Sweet Potato (*Ipomoea batatas*) from the Highlands of Papua New Guinea: Relevance to the Incidence of *Enteritis necroticans*

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Analyses have been made for crude protein, amino acids, and trypsin inhibitor content of 21 cultivars of sweet potato collected from two regions of the highlands of Papua New Guinea, one (Upper Mendi region) of high incidence of *Enteritis necroticans* (EN) and the other (Erave region) of low incidence of EN. The incidence of EN occurs in populations that are reported to be low in protein; hence, the analysis of the staple food (sweet potato) *may* give a clue to the difference between the two regions. No significant differences were found in the crude protein content, amino acid scores, or trypsin inhibitor contents between the sweet potatoes from the two regions. The range of crude protein content is 0.5–2 g of protein/100 g of fresh sweet potato; the S-containing amino acids (cystine plus methionine) are limiting in 65% of cases, followed by lysine (23%), leucine (6%), and other amino acids. The average chemical score is 0.6. The trypsin inhibitor content varies greatly over a 67-fold range. No significant correlation (r = 0.057) is found between trypsin inhibitor and crude protein.

Enteritis necroticans (EN) is endemic to the Highlands of Papua New Guinea (P.N.G.), where until recently it was the main cause of death of children over 1 year of age (Lawrence et al., 1979a). The disease is caused by *Clostridium perfringens* type C present in the gut, which produces protein toxins (particulary the β-toxin) that damage the intestinal wall. The diet in the Highlands is very low in protein with sweet potato as the staple food; very occasionally there occurs a high-protein meal or feast. The occurrence of EN normally follows the consumption of high-protein food, usually pig meat, hence the local name "pig-bel" for EN. It is postulated that this causes rapid growth of *C. perfringens* type C, which is either contaminating the meat or already present in the gut (Millar, 1983), with the production of β-toxin, that causes necrosis of the intestinal wall (Lawrence, 1979; Lawrence and Walker, 1976). An effective vaccine has been developed that is in use in the Highlands (Lawrence et al., 1979b), but its ultimate efficacy is limited by its restricted delivery to the population at risk (Lawrence et al., 1979c). It is estimated that at best only about 50% of children will receive full vaccination. Severely protein deficient diets in monkeys (Gyr et al., 1975) are known to cause a diminution of exocrine pancreatic function, hence a lowered concentration of proteases in the gut and a consequent reduction in the attack of β-toxin by trypsin and chymotrypsin. Furthermore, the presence of appreciable levels of trypsin inhibitor in sweet potatoes (Sugiura et al., 1973; Sumathi and Pattabiraman, 1975; Lin and Chen, 1980) that constitute the major component of the diet of the Highland people may retard the tryptic breakdown of the necrotising β-toxin produced by *C. perfringens* type C (Lawrence, 1979).

In this paper we report the analysis for crude protein, amino acids, and trypsin inhibitor of 21 representative varieties of sweet potato from two regions of the Highlands of P.N.G.; in one region there is a high incidence of EN and in the other region there is a low incidence of the disease. The objective is to establish if the difference in

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incidence of EN between the two regions is related to dietary differences in the staple food, sweet potato.

MATERIALS AND METHODS

Sweet Potato. Roots were obtained from 11 different varieties of sweet potato from the Erave region where there is low incidence of EN and from 10 different varieties from the Upper Mendi region, where there is a high incidence of EN. The fresh weight of the roots was measured and the weight loss during transport to Australia amounted to 4–19%. The skin was removed either by scraping (mean amount removed 2.5%) or by peeling (mean amount removed 8.9%) and was dried at 40 °C for 3 days. The root, was cut into small pieces with a blender and dried to constant weight at 40 °C for 4–5 days. The dry chips were powdered, and the moisture content of each sample was determined by weighing.

Nitrogen and Crude Protein. Analyses for total nitrogen were made using a Hewlett-Packard HP18513 CHN analyzer by the Microanalytical Unit at the Australian National University. Results for % N are the mean of four analyses (average standard deivation = 0.10). The crude protein content was calculated by multiplying the % N by 6.25 (Paul and Southgate, 1978).

Amino Acid Analysis. Samples containing about 1 mg of nitrogen were hydrolyzed under nitrogen gas in autoclavable vials in 6 M hydrochloric acid at 110 °C for 22 h, the HCl was removed by rotary evaporation, and the amino acid analysis was determined (Bradbury et al., 1980). The amount of each amino acid in mg of amino acid/g of N of sample was calculated from a knowledge of the N content of the sample and the weight of the hydrolyzed sample loaded on the column. The reproducibility of duplicate amino acid analyses was about ±5%. Tryptophan was not determined. Cystine was determined as cysteic acid (Moore, 1963). No corrections were made for losses of other amino acids (serine, threonine, methionine, lysine) by decomposition during hydrolysis.

The amino acid score of each essential amino acid was calculated by using the value for a reference protein as follows: Thr 250, Val 310, Cys plus Met 220, Ile 250, Leu 440, Tyr plus Phe 380, Lys 340, and Trp 60 mg of amino acid/g of N (Food and Agriculture Organization/World Health Organization ad hoc Expert Committee, 1973). The chemical score is the amino acid score of the first limiting amino acid. The amount of nitrogen present in each of the amino acids recovered was calculated and summed and compared with the total amount of nitrogen loaded on the column. The percent recovery of nitrogen thus calculated amounts to 50–79%, in reasonable agreement with Oomen et al. (1961). The value was appreciably less than 100%, due largely to the presence of non–protein nitrogen in the sweet potato samples. If non-protein nitrogen is removed from the system by precipitation of the water-soluble protein from sweet potato, prior to analysis of the protein, then recoveries of almost 90% are obtained (Purcell et al., 1972).

Trypsin Inhibitor Assay. The activity of trypsin (Worthington Biochemicals 165 units mg) was measured by using the specific substrate TAME (Sigma Chemical Co.) in preference to the unspecific substrate casein, with which we did not obtain reproducible results. Using a Cary 215 spectrophotometer, there was a linear increase with time over a period of 4 min at 30.0 °C in the absorbance at 247 nm (Hummel, 1959). The gradient of this line gave the initial rate of hydrolysis of TAME.

A weight of dry sweet potato powder equivalent to 40 g fresh weight (calculated by using the moisture content, Table I) was homogenized in a blender and stirred for 30 min with 120 mL of Tris–HCl buffer (0.04 M Tris, 0.01 M $CaCl_2$, adjusted to pH 8.1 with HCl (Hummel, 1959). The homogenate was centrifuged at 9000 rpm for 30 min. Aliquots of the supernatant were diluted to 5.0 mL with Tris–HCl buffer to give a wide range of about five different inhibitor activities.

Trypsin (0.050 mL of a 1 mg/mL solution in 0.001 M HCl) was added to 5.0 mL of the supernatant solution of the inhibitor and incubated at 30.0 °C for 10 min. Exactly 0.10 mL was stirred into a 1-cm cuvette containing 3.0 mL of TAME solution (1.00×10^{-3} M) at 30.0 °C and the control cuvette also contained TAME solution of the same concentration. There was a linear change in absorbance A with time t followed over 4 min, from which $\Delta A/\Delta t$ was determined. The residual activity of trypsin, units (μmol of substrate reacted/min) per milligrams of trypsin, for each mixture in a concentration series was calculated by the equation (Bergmeyer, 1974)

$$\text{units/mg of trypsin} = V\Delta A/(\epsilon d v c \Delta t)$$

where V is the volume (mL) of solution in the cell during the assay, ϵ is the extinction coefficient ($cm^2/\mu mol$), d is the light path in cuvette (1 cm), v is volume of sample mixture (mL) added to cuvette, and c is the trypsin concentration (mg/mL) in the incubation mixture. The residual trypsin activity (units/mg) was plotted against concentration of trypsin inhibitor for each sweet potato sample, and straight lines were obtained from 0 to 30–70% inhibition with correlation coefficients of 0.97–1.00. We define a trypsin inhibitor unit (TIU) as the amount of sweet potato trypsin inhibitor required to cause 50% inhibition of trypsin (50 μg) under the conditions of our experiment. In those cases where curvature occurred before 50% inhibition was reached, the straight line was extrapolated to 50%. It is then possible to express our final result in terms of the number of trypsin inhibitor units per gram of fresh sweet potato (TIU/g).

RESULTS

The results for nitrogen, crude protein, and moisture content of 21 sweet potato tubers from regions of low and high incidence of EN and of samples of skin are given in Table I. Amino acid analyses and amino acid scores for these samples are given in Tables II and III, and the amino acid analyses of other workers (recalculated in comparable units) are summarized in Table IV. Trypsin inhibitor activities are given in Table V.

DISCUSSION

Nitrogen and Crude Protein Content. In general, the nitrogen determinations were made on dry samples, but results on fresh sweet potato samples gave similar results. The crude protein content of skin is 50–90% higher than that of the bulk of the root. In agreement with these results, Purcell et al. (1976) found a higher crude protein content in the outside 0.1 radius thick layer, but apart from this, they found no evidence of radial gradients in cultivars. In Table I the average percent crude protein is significantly higher ($P < 0.01$) in the samples from Upper Mendi where only 2.5% of the skin is removed by scraping than where 8.9% of the skin is peeled off. This shows that there is a higher crude protein content close to the skin surface. The moisture content of sweet potato samples varies from 61 to 74%, in reasonable agreement with Purcell et al. (1972). The amount of crude protein present in fresh sweet potato varied from 0.5 to 2.0% and showed no significant differences between the Erave and Upper Mendi samples (Student's t test). This variability in crude protein content agrees reasonably well with that of other workers, 0.8–1.4%

Table I. Nitrogen, Moisture, and Crude Protein Content of Sweet Potato (SP) Roots and Skin Samples from Erave and Upper Mendi Regions

region	name of cultivar	% N in dry SP	% moisture in SP	% crude protein in fresh SP	% crude protein in fresh skin
Erave	Kiumnake	0.43	63.5	1.00	1.88[c]
	Sekamonde	0.87	63.7	2.00	2.38
	Momondo	0.53	65.5	1.19	1.50
	Poronogo	0.58	67.6	1.19	2.25
	Waris[a]	0.46	72.8	0.81	1.25[c]
	Koe	0.46	66.8	0.94	1.50
	Kaume	0.59	67.2	1.19	2.31
	Undawe[a]	0.68	65.1	1.50	1.81[c]
	Kalua	0.68	68.8	1.31	1.56
	Wanmun[b]	0.74	66.2	1.56	1.94
	Omibo	0.48	69.7	0.94	1.63[c]
	mean	0.59	67.0	1.24	1.82
Upper Mendi	Takion	1.06	69.1	2.06	2.94[c]
	Wanmun[b]	0.35	60.8	0.88	1.81[c]
	Pulupori	0.47	66.8	1.00	2.38[c]
	Hopomehene	0.31	74.0	0.50	1.94[c]
	Sapel	0.35	66.3	0.75	2.25[c]
	Simbul Sowar	0.72	62.4	1.69	2.56[c]
	Kariko[a]	0.67	61.9	1.63	2.38[c]
	Soii	0.70	69.0	1.38	3.00[c]
	Kariap[a]	1.01	67.9	2.00	3.00[c]
	Tomun	0.67	62.1	1.56	2.81[c]
	mean	0.63	66.0	1.34	2.51

[a] Most commonly grown varieties. [b] Wanmun from Erave and Upper Mendi may not be identical cultivars. [c] Denotes skin was scraped from tuber; otherwise tuber was peeled.

Table II. Amino Acid Analyses (mg of Amino Acid/g of N of Sweet Potato) and Amino Acid Scores for Sweet Potatoes from Erave

	name of cultivar										
	Kuminake	Sekamonde	Momondo[a]	Poronogo	Waris[a]	Koe	Kaume	Undawe[a]	Kalua	Wanmun	Omibo
nonessential amino acids											
alanine	249	236	312	349	275	234	243	251	271	257	333
arginine	185	133	202	232	154	132	209	190	218	196	300
aspartic acid	657	672	674	862	493	428	605	634	551	631	802
glutamic acid	715	385	494	686	467	518	528	518	486	543	588
glycine	219	219	194	364	204	137	250	219	221	250	356
histidine	73	91	122	117	78	86	93	102	88	97	184
proline	271	176	314	262	168	180	182	229	193	217	323
serine	229	180	165	294	157	154	224	173	263	255	335
amino acid scores											
S containing (Cys + Met)	0.51[b]	0.63[b]	0.81	0.79[b]	0.42[b]	0.57[b]	0.69	0.61[b]	0.76	0.83	0.53[b]
isoleucine	1.11	0.91	0.86	1.64	0.97	0.86	1.18	1.03	1.15	1.28	1.19
leucine	0.76	0.78	0.84	0.88	0.62	0.59	0.64[b]	0.76	0.63	0.66[b]	1.09
lysine	0.98	0.64	0.80[b]	0.87	0.54	0.68	0.64[b]	0.75	0.57[b]	0.79	1.06
aromatic (Phe + Tyr)	0.70	1.22	1.20	1.36	0.64	0.83	1.00	1.14	1.17	1.07	1.23
threonine	0.83	1.07	0.90	1.67	0.72	0.94	0.97	1.17	0.85	1.11	1.34
valine	1.02	1.43	1.09	1.62	0.81	0.80	1.27	1.24	1.02	1.03	2.53
% recovery of N	59	54	60	77	46	46	57	58	56	59	83

[a] Most commonly grown varieties. [b] Refers to the first limiting amino acid.

(Oomen et al., 1961) and 0.5–2.2% (Purcell et al., 1972), although an extremely wide range, 0.5–23% protein on a dry weight basis, has been reported by Selleck (1982).

Amino Acid Analysis and Protein Quality. The results of 21 analyses are given from our study in Tables II and III and those of 12 analyses from 4 other sources in Table IV. There are considerable variations between the amino acid analyses of the protein from different cultivars, e.g., aspartic acid gives a 2.96-fold range (428–1269) and alanine a 1.76-fold range (229–404), but these are all less than the 4-fold range of protein contents.

Comparison of amino acid analyses and amino acid scores shows no significant differences between the sweet potato protein from Erave (low incidence of EN) and Upper Mendi (high incidence of EN) (see Tables II and III). Furthermore, the chemical score, which is directly related to the quality of the protein, averages 0.61 from Erave and 0.59 from Upper Mendi, a negligible difference.

Considering the 33 results in Tables II–IV, it is found that the S-containing amino acids are limiting in 65% of the cases, lysine in 23%, leucine in 6%, and threonine and tryptophan each in 3% of cases. Since we have not determined tryptophan, tryptophan may have been limiting in more than 1 case in 33. Thus the S-containing amino acids are most often limiting, but recent results show that a range of different amino acids may be limiting (Bradbury et al., 1983). The average chemical score of 0.39 obtained by Oomen et al. (1961) is lower than our result (0.60), which is less than that of 0.89 due to Purcell et al. (1972). Amino acid analyses were made on the precipitated proteins by Purcell et al. (1972), whereas Oomen et al. (1961) and ourselves worked on the sweet potato as such, hence

Table III. Amino Acid Analyses (mg of Amino Acid/g of N of Sweet Potato) and Amino Acid Scores for Sweet Potatoes from Upper Mendi

	name of cultivar									
	Takion	Wanmun	Pulu-pori	Hopome-hene	Sapel	Simbul Sowar	Kariko[a]	Soii	Kariap[a]	Tomun
nonessential amino acids										
alanine	252	399	304	378	313	306	287	229	241	271
arginine	220	215	205	208	180	222	134	134	207	215
aspartic acid	649	578	563	751	674	868	1012	575	627	835
glutamic acid	688	678	690	1336	633	543	505	468	589	632
glycine	134	265	307	405	278	225	227	209	212	253
histidine	56	97	103	114	106	104	92	81	99	116
proline	176	182	193	185	234	190	168	144	144	185
serine	204	269	263	288	240	234	188	191	184	228
amino acid scores										
S containing (Cys + Met)	0.43[b]	0.80	0.68[b]	0.51[b]	0.73[b]	0.65[b]	0.66	0.65	0.49[b]	0.49[b]
isoleucine	0.83	1.28	1.32	1.25	1.35	1.20	0.86	0.63	0.95	1.29
leucine	0.58	0.79[b]	0.73	1.23	0.77	0.70	0.85	0.82	0.64	0.67
lysine	0.75	0.79[b]	0.86	1.33	0.77	0.80	0.64[b]	0.52[b]	0.70	0.82
aromatic (Phe + Tyr)	0.92	0.96	1.20	1.01	1.07	1.06	1.08	1.19	1.08	1.19
threonine	0.77	1.34	1.23	1.10	1.08	1.22	0.99	0.83	0.85	1.06
valine	0.88	1.25	1.33	1.38	1.22	1.20	1.11	0.93	0.96	1.19
% N recovery	52	65	63	81	63	63	58	49	56	67

[a] Most commonly grown varieties. [b] Refers to the first limiting amino acid.

Table IV. Amino Acid Analyses (mg of Amino Acid/g of N of Sweet Potato) and Amino Acid Scores of Sweet Potato Samples Recalculated from the Work of Others

	Oomen et al. (1961)				Purcell et al. (1972)							
	Gen-jem-1	Gen-jem-2	Kada-koga	Buto-katoga	Porto Rico	Jewel Mutant	Cen-tennial	Jewel	Julian	171 × 196-3	Nagase (1957)	FAO (1970)
nonessential amino acids												
alanine	294	294	343	303	343	345	346	326	347	404	381	298
arginine	318	356	250	255	255	346	403	378	288	406	401	307
aspartic acid	498	879	583	597	1269	1018	914	898	1038	1118	819	825
glutamic acid	501	428	624	553	566	599	625	541	597	696	737	541
glycine	184	210	279	230	347	316	309	269	317	350	163	234
histidine	45	84	96	83	77	108	142	153	109	111	375	84
proline	193	176	276	239	307	311	312	338	270	324	269	219
serine	246	224	274	241	335	379	383	321	360	398	344	255
amino acid scores												
S containing (Cys + Met)	0.38[a]	0.34[a]	0.42[a]	0.40[a]	0.79	0.96[a]	0.87[a]	0.86[a]	0.97[a]	1.08	1.16	0.80
isoleucine	0.96	0.73	1.09	0.85	1.40	1.05	1.40	1.33	2.54	1.49	1.33	0.92
leucine	0.69	0.57	1.00	0.82	1.18	1.18	1.20	1.11	1.18	1.31	1.24	0.77
lysine	0.48	0.50	0.71	0.68	0.78[a]	0.98	1.33	1.26	0.97[a]	1.07	1.20	0.63[a]
aromatic (Phe + Tyr)	0.96	0.87	0.98	0.97	2.24	2.09	2.02	1.96	2.08	2.11	1.58	1.02
threonine	0.78	0.76	1.02	0.82	1.42	1.42	1.53	1.37	1.46	1.58	1.15[a]	0.94
tryptophan	1.74	1.80	3.13	2.14	1.12	1.05	0.94	1.12	1.21	0.88[a]	1.87	
valine	0.87	0.78	0.99	0.86	1.58	1.57	1.42	1.36	1.57	1.67	1.59	0.91

[a] Refers to the first limiting amino acid.

Table V. Trypsin Inhibitor Activity (TIU[a]/g of Fresh Sweet Potato) of Sweet Potato Cultivars from Erave and Upper Mendi

Erave (low incidence of EN)		Upper Mendi (high incidence of EN)	
cultivar	TIU/g	cultivar	TIU/g
Kuminake	16.6	Takion	12.7
Sekamonde	2.85	Wanmun	0.64
Momondo	0.66	Pulupori	0.43
Poronogo	22.1	Hopomehene	0.33
Waris[b]	5.89	Sapel	9.06
Koe	9.28	Simbul Sowar	8.16
Kaume	18.3	Kariko[b]	6.43
Undawe[b]	20.9	Soii	0.80
Kalua	1.69	Kariap[b]	5.24
Wanmun	19.6	Tomun	10.8
Omibo	19.0	mean	5.5
mean	12.4		

[a] TIU = trypsin inhibitor unit = amount of trypsin inhibitor to give 50% inhibition of 50 μg of trypsin (165 units) under the conditions of the experiment. [b] Most commonly grown varieties.

the inclusion of non-protein nitrogen in our calculation, which has the effect of lowering the chemical score. The presence of non-protein nitrogen in sweet potato, identified largely as amino acids (Purcell et al., 1978; Purcell and Walter, 1980), also accounts for the low recovery of nitrogen from the ion-exchange column (see Tables II and III).

Trypsin Inhibitor. Results in Table V were obtained from samples dried to constant weight at 40 °C. Measurements to be reported elsewhere made on fresh sweet potato and on material dried at 40 °C from the same sample showed that no loss of trypsin inhibitor occurred as a result of heating. There is a very large (67-fold) range of sweet potato trypsin inhibitor concentrations (Table V). If the incidence of EN were simply related to high trypsin inhibitor content, we would have expected the Upper Mendi results to be higher than those from Erave. In fact, the results are in the opposite direction, although not significantly ($P \sim 0.07$). Studies are in progress on the stability of trypsin inhibitor to cooking of sweet potato (Sumathi and Pattabiraman, 1975; Lin and Chen, 1980),

which may throw light on the problem.

A rough estimate of the percent of trypsin inhibitor present in the total protein of sweet potato may be made by comparing the trypsin inhibitor activity of the sweet potato with that of a pure sample of soybean trypsin inhibitor (molecular weight 21 500). Sweet potato trypsin inhibitors have a molecular weight of 23 000–24 000 (Sugiura et al., 1973), and if we assume the same 1:1 reaction stoichiometry as that of soybean trypsin inhibitor, then it is found experimentally that 11.9 μg of soybean trypsin inhibitor has the inhibiting activity of 1 TIU. When an average crude protein content of 1.3% for fresh sweet potato (Table I) is used, it is calculated that the percent of inhibitor in total crude protein is 0.092%/TIU. Thus the range of values of percent of inhibitor in total crude protein in the samples in Table V (0.33–22.1 TIU/g) would be 0.03–2%. Because of the assumptions in the calculation, this result is only of the correct order of magnitude, but it shows that trypsin inhibitor is only a small fraction of the total crude protein.

For 53 sweet potato cultivars, Lin and Chen (1980) found a significant positive correlation between the crude protein content and percent trypsin inhibitor and between water-soluble protein and percent inhibitor, using casein as the substrate. Analysis of the inhibitor results in Table V gives no such correlation, $r = 0.057$. The difference between our results and those of Lin and Chen (1980) may be due to differences between the trypsin inhibitor assay procedures, since we were unable to achieve reproducible results with the nonspecific substrate casein. Indeed, in some cases using casein, we obtained a negative value for the amount of trypsin inhibitor present, as they also reported. This apparently results from proteolysis of casein by another protease present in the sweet potato extract, which more than balances the reduced rate of attack by the trypsin due to the trypsin inhibitor present. If there were a positive correlation, this could be a problem, because of the likely decreased digestibility of sweet potato protein in the presence of appreciable amounts of trypsin inhibitor. Also, if the latter were a significant factor in the incidence of EN, then the apparently desirable genetic trait of a sweet potato of higher protein content could be counterbalanced by a higher inhibitor content.

Finally, it is clear that the low incidence of EN in Erave and the high incidence of EN in Upper Mendi is *not* due to the differences in protein content, protein quality, or content of trypsin inhibitor between the sweet potato cultivars grown in these two regions, because such differences are not significant. This does not eliminate the possibility that the incidence of EN in Upper Mendi is dietary based, since the diet in the Erave region (at an altitude of 1076 m as compared with 1950 m for Upper Mendi) is more varied. In order to test this postulate it would be necessary to carry through a dietary survey and to analyze the other components of the diet. Unfortunately, for reasons of logistics, such a dietary survey would be very difficult.

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