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IN VITRO DIGESTIBILITY OF CASHEW KERNEL PROTEIN

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PROTEIN content of defatted kernel flour of some cashew varieties has been shown to vary between 32.1 and 43.7%¹. Cashew kernel protein contains all the essential amino acids^{2,3}. Protein efficiency ratio of cashew-nut extraction meal (3.23) compares well with that of casein⁴ (3.12). Lysine content of cashew kernel protein was significantly different among varieties⁵. Detailed studies were initiated to compare the varieties for *in vitro* digestibility of kernel protein by proteolytic enzymes such as trypsin, α -chymotrypsin and pepsin.

Trypsin (from bovine pancreas, 13,000 BAEE units/mg protein), α -chymotrypsin (from bovine pancreas, 47 units/mg protein) and pepsin (from porcine stomach mucosa, 3165 units/mg protein) were obtained from Sigma Chemical Co., USA. All the buffer salts used were of analytical reagent grade. Cashew kernel flour, after defatting with a chloroform-methanol mixture (2:1 v/v), was extracted with double-distilled water (1:20 w/v) at pH 10 and 4 for 60 min at 25 C and the supernatant was precipitated with ammonium sulphate (90% saturation). The precipitate was taken in a suitable buffer (0.1 M potassium phosphate, pH 7.6, for trypsin and α -chymotrypsin; 0.2 M KCl HCl, pH 2.0, for pepsin) and dialysed overnight at 25 C against the same buffer. Protein in the extract was estimated by Bradford's dye method⁶.

In vitro digestibility of cashew kernel protein by

trypsin, α -chymotrypsin and pepsin was studied as described earlier⁷. The ratio of enzyme to protein was 1:100. Protein for the assay varied between 3 and 3.5 mg/ml, and the enzyme concentration was 35 μ g/ml. For pepsin, kernel protein and enzyme were taken separately in 1 ml of 0.2 M KCl-HCl buffer (pH 2.0), while for trypsin and chymotrypsin, enzyme and kernel protein were taken separately in 1 ml of 0.1 M potassium phosphate buffer (pH 7.6). Digestion was carried out at 37°C for 15 min and terminated by addition of 2 ml of 20% trichloroacetic acid (TCA). Absorbance at 280 nm of the TCA supernatant after centrifugation was read against reagent blank.

Cashew kernel protein extracted at pH 10.0 was denatured by heating for 10 min at different temperatures and *in vitro* digestibility by trypsin was studied. Denaturation was also done using SDS by heating the protein extracted at pH 10.0 with SDS and β -mercaptoethanol (SDS 4% and β -mercaptoethanol 10% final concentrations) for 2 min in a boiling water bath. SDS-denatured protein was

Table 1 *In vitro* digestibility of cashew kernel protein by trypsin

Incubation time (min)	Digestibility*	
	Tr. No. 1 kernel protein	BSA
5	0.07	0.036
15	0.084	0.082
30	0.11	0.119
60	0.134	0.201
90	0.144	0.223
120	0.137	0.232

*Expressed as increase in absorbance at 280 nm of TCA supernatant (see text for details).

Table 2 Comparison of *in vitro* digestibility of cashew kernel protein, haemoglobin and BSA

Protein	Digestibility (%)	
	Trypsin	α -Chymotrypsin
Haemoglobin	100 (0.088)*	100 (0.094)*
BSA	100	73
Cashew kernel protein		
i) pH 10.0 extract	133	62
ii) pH 4.0 extract	37	14

*Figures within parentheses are actual increase in absorbance at 280 nm of TCA supernatant. *In vitro* digestibility of BSA and cashew kernel protein is expressed as a percentage, taking digestibility of haemoglobin as 100. Kernel protein from cashew variety H-3-17 was used. Values are mean of three estimations.

Table 3 *In vitro* digestibility of kernel protein from different cashew varieties

Variety	Trypsin		α -Chymotrypsin		Pepsin	
	pH 10.0	pH 4.0	pH 10.0	pH 4.0	pH 10.0	pH 4.0
H-3-17	100 (0.1133)*	100 (0.0367)*	100 (0.0515)*	100 (0.0207)*	100 (0.0373)*	100 (0.0583)*
Ansur-1	52	98	46	110	46	86
Vetore-56	42	136	44	72	131	39
WBDC-V	62	180	30	23	36	127
Morgaon-1	59	147	74	29	56	53
M 10 4	51	169	73	97	131	88
M 44 3	66	121	87	188	117	117
M 76 1	71	118	52	270	134	77
Tr No. 1	69	202	35	184	52	111
Tr No. 40	61	157	83	30	118	120
Tr No. 56	75	128	46	39	88	83
Tr No. 273	74	139	91	35	88	108
Bla-139-1	68	112	35	158	134	118
Bla-256-1	59	124	83	52	177	46
Bla-266-1	63	155	61	55	86	48
Vengurla-1	68	232	97	42	138	60
Vengurla-2	56	141	53	174	103	85
Vengurla-3	42	121	75	135	105	105
Vengurla-4	51	99	85	372	90	62
Vengurla-5	38	95	65	162	242	75

*See footnote to table 2.

used for digestibility studies after dialysis against 0.1 M potassium phosphate buffer (pH 7.6).

In vitro digestion by trypsin of cashew kernel protein from variety Tr. No. 1 extracted at pH 10.0 was carried out for different periods. The results are given in table 1. In the case of BSA the rate of reaction was found to be linear up to 60 min. However, in the case of cashew kernel protein, the rate of reaction was higher in the first 15 min. Hence, further studies were restricted to 15 min incubation.

A comparison of *in vitro* digestibility by trypsin and α -chymotrypsin of cashew kernel protein from variety H-3-17, and haemoglobin and BSA is shown in table 2. The digestibility of kernel protein extracted at pH 10.0 compared well with that of haemoglobin and BSA. However, the digestibility of kernel protein extracted at pH 4.0 was much less compared to that of BSA and haemoglobin.

Different cashew varieties differed in the *in vitro* digestibility of kernel protein (table 3). Protein extracted at pH 4.0 was less susceptible to digestion by trypsin and α -chymotrypsin compared to protein extracted at pH 10.0. Digestibility by pepsin was, however, slightly higher in pH 4.0-extracted protein than in pH 10.0-extracted protein.

In vitro digestibility by trypsin of denatured cashew kernel protein extracted at pH 10.0 increased with increase in denaturation temperature. Digesti-

bility was maximum at 90°C. *In vitro* digestibility of heat- (90°C) and SDS-denatured cashew kernel

Table 4 *In vitro* digestibility of denatured kernel protein from different cashew varieties

Variety	Digestibility denatured (% increase over control)		
	Control*	Heat	SDS
Ansur-1	0.07	76	21
Vetore-56	0.073	103	0
WBDC-V	0.025	150	272
Morgaon-1	0.066	125	19
M 44/3	0.084	66	0
M 76/1	0.046	90	0
M 10/4	0.077	93	0
Tr No. 1	0.051	102	14
Tr No. 40	0.064	77	0
Tr No. 56	0.049	106	62
Tr No. 273	0.047	119	106
Bla-139-1	0.063	70	2
Bla-256-1	0.046	62	134
Bla-266-1	0.066	73	19
H-3-17	0.05	87	86
Vengurla-1	0.053	68	2
Vengurla-2	0.04	25	104
Vengurla-3	0.062	40	0
Vengurla-4	0.059	90	16
Vengurla-5	0.051	83	35

*Figures are actual increase in absorbance at 280 nm of TCA supernatant.

protein is shown in table 4. Both heat denaturation and SDS denaturation enhanced digestibility. However, SDS did not enhance digestibility in all the varieties. The varieties also showed significant differences in digestibility of heat-denatured kernel protein.

The results presented here clearly indicate that cashew varieties differ considerably in *in vitro* digestibility of kernel protein. Lysine content of kernel protein of these varieties has been shown to vary significantly⁵. Cashew kernel meal has been shown to contain proteinase inhibitors⁸. The observed differences in the *in vitro* digestibility of kernel protein among these varieties may possibly be due to variation in the total amino acid composition or variation in levels of proteinase inhibitors.

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