Effects of Condensed Tannins on the in Vitro Protein Digestibility of Cowpea [*Vigna unguiculata* (L.) Walp.]

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Condensed tannins of eight cowpea [*Vigna unguiculata* (L.) Walp.] cultivars determined by the protein precipitation method were localized in the seed coat and concentrated in the cooking broth. They were positively correlated with seed coat color ($r = +0.64^{**}$), which varied from white to light brown, dark red, and black. Cooking increased in vitro digestibility by 6–8% significantly. The digestibility of the cooked seeds with cooking broth was significantly different from that without the cooking broth. Replacing the cooking broth of the cooked white seeds with the broth of the cooked light brown and black seeds resulted in a decrease of 1.4% in digestibility. Replacing the cooking broth of the cooked light brown and black seeds with that of the white resulted in an increase in digestibility of 3 and 4%, respectively. The change in the relative nutritive value determined by *Tetrahymena pyriformis* W upon interchange of broth was greater (5–31%). Significant negative correlations were observed between condensed tannins and protein digestibility ($r = -0.87^{**}$) and relative nutritive value ($r = -0.96^{**}$). Polyvinylpolypyrrolidone when added to homogenized cooked seeds at 4 and 6 mg/mL increased protein digestibility by 3.7%. Isolated cowpea condensed tannins and commercial tannic acid decreased the digestibility of raw white cowpea and casein by 3.6 and 5.1% and 2.6 and 4.2%, respectively.

Grain legumes are becoming important alternative sources of indigenous plant proteins in a world where there is an increasing demand for protein. According to Hoshiai (1980), of the world's 94.7 million tons of total protein supply (average figures from 1972 to 1974), 61.9 million tons come from vegetable proteins of which 6.4 million tons come from grain legumes. The protein content of grain legumes is relatively high but protein quality is low. The latter has been attributed not only to its deficiency in the sulfur-containing amino acids (especially methionine) but also to its poor digestibility. In addition to trypsin inhibitors, hemagglutinins, cyanogenic glucosides, and other antiphysiological factors such as phytates (Liener, 1972), several investigators have also studied the presence of condensed tannins (polyphenols), which lower the digestibility of grain legumes such as the common beans *Phaseolus vulgaris* (Elias et al., 1979), winged beans *Psophocarpus tetragonolobus* (Lumen and Salamat, 1980), broad and tick beans *Vicia faba* and maple peas *Pisum sativum* (Griffiths, 1981; Griffiths and Moseley, 1980), chick peas *Cicer arietum*, green bean *Vigna radiata*, soybean *Glycine max*, hyacinth bean *Dolichos lablab*, pigeon pea *Cajanus cajan* (Narasinga Rao and Prabhavathi, 1982), horse gram *Dolichos biflorus*, and moth bean *Phaseolus aconitifolius* (Satwadhar et al., 1981).

Among these grain legumes, the cowpea (*Vigna unguiculata*) is a major species of the torrid semiarid to lowland humid regions. It has been increasingly utilized for food due to its high protein content of about 24% and high yielding ability in regions not suitable for the growth of other legumes. In 10 cowpea cultivars evaluated, trypsin inhibitor values were shown to be generally lower than those reported for other beans and their hemagglutinating activities were nondetectable (Mendoza et al., 1980). However, in a survey of seeds of 10 varieties each of cowpea, chickpeas, pigeon peas, and mung bean, cowpea was the only species found with an amount of condensed tannins that may be nutritionally harmful (Price et al., 1980).

The present work was therefore undertaken to investigate the role of condensed tannins in the nutritional quality of cowpea.

EXPERIMENTAL SECTION

Materials. Eight cultivars of cowpea with seed coat color ranging from white to red and black were obtained from the National Plant Genetic Resources Laboratory of the Institute of Plant Breeding, University of the Philippines at Los Baños.

Condensed tannins were isolated from UPL Cp 3, a cowpea cultivar with a red seed coat color, and purified according to the method of Hagerman and Butler (1978). Commercial tannic acid was obtained from Sigma Chemical Co.

The enzymes used for in vitro digestibility were purchased from Sigma Chemical Co. These were pancreatic trypsin (Type IX) with 16 950 BAEE units/mg of protein, bovine pancreatic chymotrypsin (Type II), 48 units/mg of protein, and porcine intestinal peptidase (Type III), 50 units/mg of protein.

The test organism, *Tetrahymena pyriformis* W (10542), was obtained from the American Type Culture Collection, Rockville, MD. The culture was maintained in a *Tetrahymena* medium by transferring the culture every 3–4 weeks in duplicate screw-capped test tubes.

Bovine serum albumin (Sigma fraction V) was used for the protein precipitation test of tannin. Polyvinylpolypyrrolidone, an insoluble, high molecular weight, crosslinked form of polyvinylpyrrolidone was obtained from Sigma.

All other chemicals used were of analytical grade.

Methods. The seeds were hand-cleaned to remove glumes, broken grain, dirt, and other foreign adhering particles. Intact, whole raw seeds were ground to pass a 100-mesh sieve on a cyclone sample mill. Twenty-five grams each of the 10 cultivars were weighed and dehulled by hand. The testa was separated from cotyledon without soaking the seeds in water. The seed coat and the cotyledon were weighed separately and then ground in the same manner as the whole seed. The ground samples were

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Table I. Protein and Condensed Tannin Content of Several Cowpea Cultivars

accession	seed coat color	protein,[a] % N × 6.25		protein-precipitable phenols, % tannin[b]			
		whole seeds	seed coat	whole seed	cotyledon	seed coat	cooking broth
UPL Cp 2	white	25.27	12.78 (8.5)	0.144	0.056	0.126	0.126
acc. 642	white	25.10	12.78 (10.0)	0.176	0.039	5.503	1.009
acc. 603	light brown	23.52	9.14 (12.0)	0.420	0.040	10.882	1.262
acc. 608	light brown	24.40	9.79 (11.0)	0.725	0.043	17.347	1.718
acc. 141	dark red	23.59	8.84 (11.5)	0.474	0.045	14.697	2.155
acc. 1222	dark red	20.34	12.54 (14.0)	0.793	0.027	11.023	2.096
acc. 87	black	22.36	10.62 (13.5)	1.025	0.077	15.088	1.741
acc. 643	black	23.32	16.13 (11.0)	0.447	0.340	7.564	1.799

[a] Protein content is expressed on a dry weight basis; numbers in parentheses refer to percent of whole seed. [b] Calculated by using purified condensed tannins from red UPL Cp 3 cowpea.

Table II. In Vitro Protein Digestibility of Raw and Cooked Cowpea Samples and TIA of Raw Seeds and Cooking Broth[c]

accessions	in vitro protein digestibility[a]				trypsin inhibitor activity[b]	
	raw seeds		cooked seeds			
	whole seed	cotyledon	with broth	without broth	raw seeds	broth
UPL Cp 2	77.64 cde[d]	76.57 ef	80.94 ab	83.07 a	0.97 e	0.13 f
acc. 642	75.20 efg	75.01 efg	80.65 ab	80.71 ab	1.39 cd	0.23 f
acc. 603	76.47 ef	76.57 ef	80.24 b	80.32 b	1.09 de	0.24 f
acc. 608	75.72 ej	78.30 bcd	78.85 bcd	81.44 ab	1.21 cde	0.23 f
acc. 141	74.55 fg	74.90 fg	79.34 bcd	82.87 a	1.43 c	0.41 f
acc. 1222	72.95 gh	74.18 fg	79.06 bcd	80.03 bc	2.28 b	0.30 f
acc. 87	77.16 de	77.38 de	79.27 bcd	80.75 ab	1.09 de	0.19 f
acc. 643	70.80 h	72.81 gh	79.67 bc	79.80 bc	3.00 a	0.20 f

[a] Using the method of Hsu et al. (1977). [b] Determined by the method according to the "Worthington Enzyme Manual" (1977). [c] Two and a half grams of whole seeds was cooked for 30 min with 100 mL of distilled water; the cooking broth was used for TIA determination. Data are expressed in terms of TIU/mg of seeds. [d] Means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test, DMRT).

subjected to the following analyses.

Protein (nitrogen × 6.25) was determined according to the standard AOAC method (1975).

Estimation of condensed tannin content was done by using the protein precipitation method (Hagerman and Butler, 1978). Condensed tannin was purified from a red cowpea cultivar UPL Cp 3 following the method of Hagerman and Butler (1978) and was used to prepare standard curves for this assay.

The method of Hsu et al. (1977) using trypsin, chymotrypsin, and peptidase was used to estimate the digestibility of cowpea proteins. The in vitro protein digestibility was calculated according to the regression equation $Y = 210.464 - 18.103X$ where Y = in vitro digestibility (%) and X = pH of the sample suspension after 10-min digestion with the multienzyme solution (Hsu et al., 1977). Casein was used as the standard.

The relative nutritive value was analyzed according to the method of Stott et al. (1963) as modified by Landers (1975).

Treatments. Whole seeds (2.5 g/100 mL of distilled water) were boiled for 30 min and the broth was set aside. Trypsin inhibitor activity was measured by the decrease in the rate by which trypsin hydrolyzed *p*-toluenesulfonyl-L-arginine methyl ester (TAME) ("Worthington, Enzyme Manual", 1977). One unit of trypsin activity is defined as the enzyme needed to catalyze the hydrolysis of 1 μmol of TAME/min at 25 °C and pH 8.1 in the presence of 0.01 M calcium ions.

In the second set of experiments, cooked seeds (2.5 g/100 mL of H_2O) with and without broth were analyzed for in vitro digestibility (Hsu et al., 1977). Aliquots of the broths were subjected to condensed tannin estimation using the protein precipitation method. The broths of the cooked seeds were interchanged; the in vitro digestibility and the relative nutritive value of the resulting mixture were analyzed.

In the third set of experiments, the in vitro digestibility values of the white-seeded cultivar UPL Cp2 and casein treated with varying levels of purified condensed tannins (from UPL Cp 3) and tannic acid were determined. The effect of adding different levels of polyvinylpolypyrrolidone in the in vitro digestibility was also analyzed.

RESULTS AND DISCUSSION

Condensed Tannins in Cowpea. Seed coats of the different accessions comprised about 7–14% of the seed weight (Table I). Protein content varied from 20 to 25% in the raw whole seeds and 9 to 16% in the seed coats.

The condensed tannins as determined by the protein precipitation method were primarily localized in the seed coats and were highly concentrated in the cooking broth (Table I). Since proteinaceous trypsin inhibitors are inactivated by heating especially at high temperature, the trypsin inhibitor activity (TIA) obtained in the cooking broth is likely to be due to condensed tannins. The values of condensed tannins obtained in the seed coat and cooking broth were 7–10 times greater than the amounts of condensed tannins in the whole seed. These large differences could be due to the interaction of tannins with seed proteins during the analysis of tannins in the whole seed, thereby decreasing their reactivity. Seed coats of the various cultivars have considerable protein content ranging from 9 to 16%, which can also interfere with the analysis of condensed tannins.

The levels of condensed tannins were significantly correlated with the color of the seed ($r = +0.64^{**}$) with the light colored seeds having lower levels while the darker seeds had higher levels.

Effects on in Vitro Protein Digestibility and Relative Nutritive Values. The in vitro protein digestibility values of raw seeds of the light brown sample (75.72–76.47%) did not differ significantly from those of the white accessions (75.20–77.64%), while those of the two

Table III. Effect of Cooking Broth on the in Vitro Protein Digestibility of Cooked Cowpea Samples

samples[a]	% digestibility
UPL Cp 2 plus its CB[b]	80.57 c[c]
UPL Cp 2 plus CB of acc. 608	79.64 d
UPL Cp 2 plus CB of acc. 87	79.43 de
acc. 608 plus CB of UPL Cp 2	81.36 e
acc. 608 plus its CB	78.85 b
acc. 87 plus CB of UPL Cp 2	82.45 de
acc. 87 plus its CB	79.27 ab

[a] Two and a half grams of whole seeds was cooked for 30 min with 100 mL of distilled water. [b] Cooking broth. [c] Means followed by the same letter are not significantly different at the 5% level (DMRT).

dark red and one black accessions were significantly lower (70.80–74.55%) (Table II). The black accession 87 had an in vitro digestibility value similar to that of the white accession. The in vitro digestibility of the cotyledons was not significantly different from that of the raw whole seeds. Cooking increased the in vitro digestibility significantly by 6–8% and resulted in similar values for all accessions. Statistically, the means of the values for cooked seeds without broth were significantly higher than those of cooked seeds with broth.

Replacing the cooking broth of the white cultivar UPL Cp 2 with that of the dark accessions 608 and 87 significantly lowered the digestibility of UPL Cp 2 although by only 1% (Table III). Conversely, replacing the cooking broth of the dark accessions with that of UPL Cp 2 increased the digestibility significantly to a greater degree, up to 3%.

A significant negative correlation ($r = -0.87^{**}$) was observed between in vitro digestibility and condensed tannins in the seed coat, cooking broth, and whole raw seed. A higher significant negative correlation ($r = -0.95^{**}$) was obtained between the condensed tannin content of the added cooking broth and the resulting protein digestibility. This negative correlation was expected since tannins are known to bind dietary proteins (Ariga et al., 1981) and inhibit proteolytic activity in the intestinal tract (Griffiths and Moseley, 1980). Similar negative correlations have been reported for sorghum (Cummings and Axtell, 1973) and *Phaseolus vulgaris* (Bressani and Elias, 1979), although a positive but statistically nonsignificant correlation was reported recently for the same species, *P. vulgaris* (Bressani et al., 1983). The protein digestibility values of cowpea cultivars obtained by the in vitro method are similar to those obtained by the in vivo method (Cheftel, 1979; Gonzales, 1983). Hsu et al. (1977) showed a significantly high correlation ($r = 0.90^{**}$) between the digestibility values obtained by the in vivo animal assay and the in vitro method for 23 samples.

A similar trend was observed when the effect of interchanging broth on relative nutritive values was evaluated (Table IV). The change in relative nutritive values of 5–31% was more marked that that in protein digestibility, which indicates effective precipitation of proteins by the condensed tannins in the broth. A significant correlation ($r = -0.96^{**}$) exists between relative nutritive values of the cowpea accessions with their own cooking broths and the corresponding protein precipitation values. These results indicate the presence of heat-stable factor(s) in the cooking broth, possibly condensed tannins, which lower the protein digestibility and relative nutritive value of cowpea.

Addition of Polyvinylpolypyrrolidone (PVP) and Tannins. When PVP, a tannin complexing agent, was added to the homogenized cooked seeds and broth at 4 and 6 mg/mL, protein digestibility was significantly increased by an average of 3.7% (Table V). The maximum protein digestibility was obtained at 4 mg/mL PVP for the white cultivar UPL Cp 2 and black cultivar 87 and at 6 mg/mL PVP for accessions 608 and 643 (light brown and black, respectively). A preliminary experiment revealed addition of 10, 25, and 50 mg/mL PVP to cooked seeds of accession 643 did not further increase the protein digestibility. The above result is similar to the higher in vitro digestibility observed with tannin-free field beans (*Vicia faba*) as compared with condensed tannin containing white-flowered varieties (Bond, 1976).

Addition of condensed tannins (0.4 mg/mL) isolated and purified from red UPL Cp 3 cowpea to raw whole white cowpea seeds and casein decreased their respective protein digestibility values by 3.6 and 5.1% (Table VI). Tannic acid lowered their digestibility to a lesser degree, by 2.6 and 4.2% for cowpea and casein, respectively.

Our studies show a small but definite lowering of in vitro protein digestibility and a larger decrease in relative nutritive values of cowpea by condensed tannins. Whether this decrease is significant nutritionally has to be further investigated considering the growing importance of cowpea as a food legume.

Table IV. Relative Nutritive Value of Cooked Whole Seeds of Cowpea with Interchanged Broths[a]

sample	relative nutritive value,[b] %
UPL Cp 2 plus its CB[c]	94.30 a[d]
UPL Cp 2 plus CB of acc. 87	69.15 cd
UPL Cp 2 plus CB of acc. 608	85.10 b
UPL Cp 2 plus CB of acc. 643	69.82 cd
acc. 87 plus its CB	60.10 e
acc. 87 plus CB of UPL Cp 2	91.71 a
acc. 608 plus its CB	67.34 d
acc. 608 plus CB of UPL Cp 2	72.80 c
acc. 643 plus its CB	73.57 c
acc. 643 plus CB of UPL Cp 2	83.27 b

[a] Two and a half grams of whole seeds was cooked for 30 min with 100 mL of distilled water; the cooking broths were interchanged as indicated. [b] Determined by the method of Landers (1975). [c] Cooking broth. [d] Means followed by the same letter are not significantly different at the 5% level (DMRT).

Table V. Effect of Polyvinylpolypyrrolidone (PVP) on the in Vitro Protein Digestibility (IVPD) of Cooked Cowpea Samples

	amount of PVP added, mg/mL		
sample	0	4	6
UPL Cp 2	80.46 cd[a]	83.10 ab	83.66 a
acc. 608	78.83 e	81.19 c	82.40 b
acc. 87	79.28 e	82.40 b	82.95 ab
acc. 643	79.51 de	80.36 cd	83.86 a

[a] Means followed by the same letter are not significantly different at the 5% level (DMRT).

Table VI. Effect of Condensed Tannins and Tannic Acid on the in Vitro Protein Digestibility of Raw Whole Seeds of Cowpea var. UPL Cp 2 and Casein

	UPL Cp 2		casein	
tannins, mg/mL	condensed tannins	tannic acid	condensed tannins	tannic acid
0	77.94 i[a]	75.30 jkl	98.08 bc	97.90 bc
0.02	77.91 i	74.53 klmn	97.24 cd	95.83 de
0.04	74.56 klmn	73.23 mop	95.71 de	94.60 efg
0.2	75.28 jkl	72.79 p	93.80 fg	93.70 fg
0.4	74.34 lmno	72.68 p	92.94 g	93.84 fg

[a] Means followed by the same letter are not significantly different at the 5% level (DMRT).

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Measurement of Lysine Damage in Proteins Heated with Gossypol

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Experiments were conducted to investigate which analytical method for lysine determination best predicted the amount of lysine available to the rat from proteins that had been heated with gossypol. Glanded commercial cottonseed flour (CF) was analyzed without further treatment, while samples of glandless CF, egg white, and ovalbumin were studied either as is or after being heated with gossypol acetic acid. Relative to the level of bound gossypol and to the fall in lysine potency estimates determined from a rat growth assay, there were only small reductions in the lysine content as measured by amino acid analysis following sodium borohydride treatment or reaction with fluorodinitrobenzene, but the dye binding procedure with acid orange 12 gave closer agreement. Lysine released after digestion with Pronase underestimated the rat assay response. The reduction of in vivo nitrogen digestibility due to the gossypol treatment served to explain the differences observed between the values for reactive lysine determined from chemical procedures and those from rat growth.

The heating of protein foods during processing can result in a reduction in protein quality through destruction, or a reduction in availability, of susceptible amino acids, particularly lysine. Most attention has been given to the damage to lysine that can occur from reaction with reducing sugars through Maillard reactions as in the drying of milk (Mottu and Mauron, 1967). There have been several studies to determine the analytical method that best predicts the physiologically useful lysine in materials where protein has reacted with reducing sugars (Hurrell and Carpenter, 1974; Hurrell et al., 1983) and also with formaldehyde (Hurrell and Carpenter, 1978) or with a polyphenol, such as caffeic acid (Hurrell et al., 1982). Here we extend the study to complexes of protein with gossypol both in cottonseed flours (CF), where the dialdehyde gossypol occurs naturally and can bind to the protein during processing, and in a purified protein–gossypol mixture. The low value of protein–gossypol complexes as a source of lysine has already been shown in studies of animal growth (Baliga and Lyman, 1957; Smith et al., 1958; Frampton, 1965; Major and Batterham, 1981) and digestibility, both in vitro (Ingram et al., 1950; Horn et al., 1952; Cater and Lyman, 1970) and in vivo (Kuiken, 1952; Craig

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