BIOCHEMICAL ASSESSMENT OF COWPEA PROTEINS AND OTHER CONSTITUENTS +

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ABSTRACT

Chemical analysis of ten Philippine recommended and promising varieties/lines of cowpea showed protein and starch contents ranging from 25 to 29% and 47 to 55% (dry weight basis), respectively. As with other legumes, cysteine and methionine were found limiting as shown by the amino acid composition of UPL Cp2 (VCS 18). The trypsin inhibitor activity ranged from 30 to 72 units/mg protein for the ten cultivars. No phytohemagglutinins were detected. Stachyose and raffinose were found to be 3.8 and 1.8%, respectively while only traces of verbascose were detected in UPL Cp2.

Albumins, globulins and glutelins were isolated and found to be heterogeneous by polyacrylamide gel electrophoresis. Their subunit molecular weights ranged from 12,000 to 90,000 daltons as determined by SDS gel electrophoresis. Prolamin had a single wide band with a molecular weight of 12,000 on SDS gel electrophoresis. Amino acid analysis revealed that albumin had the best chemical score. The other fractions had inferior chemical scores. VCS 18 cowpea proteins consisted of: 9%albumins, 83% globulins, 8% glutelins and 0.01% prolamins.

Lipoxygenase specific activity was found highest in cowpea (205-316 units/mg protein) as compared to that of soybean (11-48 units/mg protein) and winged bean (14-30 units/mg protein). This enzyme was isolated, purified and characterized. The role of lipoxygenase in beany flavor formation, nutritional quality and cowpea resistance to pests was discussed.

INTRODUCTION

In recent years, cowpea *(Vigna unguiculata* (L) Walp) has increasingly

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+ Paper presented at the Ninth Annual Convention of the Kapisanan ng mga Kimiko sa Pilipinas, Los Baños Chapter with the Integrated Chemists of the Philippines and its affiliate organizations, October 20-21, 1980, Los Baños, Laguna.

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become an important source of high protein food due to three major factors: (1) high protein content of about 24%, (2) high yield even in tropical regions not suitable for the growth of other legumes and (3) relatively lower anti-nutritional factors. Table 1 shows the relatively higher potential protein productivity of cowpea as compared to other legumes and other tropical crops.

Cowpea traces its origin to West Africa where it has been a regular constituent of the West African diet. In the Philippines and in Asia, cowpea or "paayap" is cultivated for green pods or dry beans. Local vegetable production statistics do not show the actual production and consumption of cowpea, either as snap or dry bean, although its use may be more widespread than is commonly thought of as shown by its availability in public markets. Asian production of cowpea is, however, only 2% of the world production, amounting to 27 metric tons out of 1,146 metric tons (PAG Bulletin, 1973).

Research and breeding work on cowpea in the Philippines, have been geared towards developing and selecting high yielding and disease and pest resistant varieties. As a result of these studies, several varieties have been recommended, namely: UPL Cp1 (VCS 6-1), UPL Cp2 (VCS 18), UPL Cp3 (VCS 6-12), BPI Cp1 (BPI-Imp Gr #1) and BPI Cp2 (Mecan Pea).

Presently, the use of cowpea is rather limited to that of vegetable food. However, the use of cowpea can be extended to that for animal feed or as substitute for regularly used beans in processed foods like navy beans in the manufacture of pork and beans and in meat extenders and other processed foods. Del Rosario et. al (1980) has reported the successful substitution of cowpea beans for navy beans for this purpose.

This paper reports on the various factors which affect the biochemical and nutritional qualities, as well as acceptability of local cowpea varieties.

NUTRITIONAL QUALITY

A good amino acid composition of a protein is not an assurance of its high nutritional quality. The latter is influenced by protein digestibility which in turn depends on several factors among which are the so-called anti-nutritional factors like trypsin inhibitors, hemagglutinins and flatulence factors, and even regular cell wall substances like tannins. The enzyme lipoxygenase has also been implicated in lowering the nutritive values of foods due to its ability to catalyze the production of lipid hydroperoxides. The hydroperoxides as well as their products of decomposition are potentially reactive substances which can damage protein and amino acids (Gardner, 1979).

Proximate Analyses and Amino Acid Composition

The total protein content of cowpea varies from 25 to 29% (d.w.b.) in ten recommended and promising varieties or lines (Table 2). Table 2 also

Table 1. Potential protein productivity of certain tropical food crops

Crop	Programmable yield, kg/ha*	Protein Content %	Amino Acid Score %	Crop duration days	Protein productivity kg/ha/day
Legumes					
1. Soybean	2800	38	81	95	8.9
2. Lima bean	3200	25	64	115	4.4
3. Cowpea	1800	25	65	80	3.6
4. Pigeon Pea	2000	22	60	80	3.3
5. Winged bean	1400	31	78	112	3.0
6. Chick pea	2500	20	68	125	2.7
7. Mung bean	900	24	65	75	1.9
Root Crops					
1. Yam	25000	2.0	74	185	2.0
2. Sweet Potato	20000	1.3	63	120	1.4
3. Potato	15000	2.0	54	125	1.3
4. Cassava	20000	1.2	57	220	0.6
Cereals					
1. Rice	5000	7.5	72	140	1.9
2. Maize	4000	9.5	49	120	1.6
3. Sorghum	3500	10.1	47	110	1.4

*From Luse et al (1975), based on estimates made in early 1975.

shows the fat, starch and total sugars composition of these cowpea cultivars.

Like other legume proteins, cowpea proteins are rich in lysine but limiting in the sulfur amino acids–methionine and cysteine. Table 3 summarizes the amino acid content of cowpea and mungbean as well as rice and whole egg. The chemical score of cowpea is 68% based on an FAO/WHO amino acid pattern. Since legume proteins are high in lysine and cereal proteins are usually low in lysine but high in the other essential amino acids, legumes complement cereals. It can be noted that the protein efficiency ratio (PER) (gram weight gain per gram protein eaten) of a rice diet increases from 2.25 to 2.62 if rice is mixed with red *P. vulbaris* in 80-20 ratio (distribution of protein), compared to PER of 2.71 and 1.24 for casein and cooked beans, respectively (Bressani, 1973). It has also been confirmed that a rice mungo beans diet greatly improved the protein value of a diet of rice alone (Bunce et al., 1970).

In a comparative study on the nutritional quality of locally available beans by Gonzales et. al. (1972), white cowpea (variety not known), was

Table 2. Chemical Composition of Several Cowpea Cultivars.

Cultivar	% Composition (dry weight)				
	Moisture	Fats	Protein (N x 6.25)	Total Sugar	Starch
VCS 6-1 (RV)	9.8	1.9	29.4	6.04	49.9
VCS 18 (RV)	12.1	2.4	28.3	4.1	50.7
V 59-41 (PL)	12.5	2.3	28.3	7.3	49.4
BPI IMP Gr No.1 (RV)	11.8	2.2	28.1	8.24	46.9
VCS 6-12	11.8	2.4	28.0	4.1	49.0
Mecan Pea (RV)	11.7	2.0	27.6	6.17	55.0
All Season (RV)	12.0	2.5	27.2	4.1	49.1
TVX 289-4G	11.7	2.4	26.2	4.6	51.0
CES 26-12 (PL)	10.0	2.3	25.9	4.0	52.0
CES 42-2 (PL)	11.7	2.5	24.9	5.7	53.0

RV — Recommended Variety

PL — Promising Line

found to have digestibility, biological value (BV) and net protein utilization (NPU) of 76, 60 and 46 compared to control value (casein) of 91, 78 and 70 respectively. Black cowpea, on the other hand, had digestibility, BV and NPU values of 75, 72 and 54 compared to control values of 96, 79 and 76, respectively. Only the biological value of black cowpea is not significantly different from the corresponding control value. Among the legumes tested, chickpea was rated best followed by black cowpea and red mungbean as second in nutritional quality.

Antinutritional factors. The low nutritive values of legume proteins have been ascribed to and/or affected by the following factors: (a) Inhibitors of trypsin, a proteolytic enzyme in the intestine, have been found and studied in various beans. Nonspecific protease inhibitors have also been detected (Liener, 1976). (b) Protein components which agglutinate red blood cells are present in legumes. Although these factors are usually heat labile, boiled beans might still contain undestroyed toxic hemagglutinins which could result in diarrhea and other signs of toxicity. (c) Consumption of legume foods results in increased flatulence or gas production. (d) Some of the salt-soluble globulins which are the major class of proteins in legumes are resistant to hydrolytic enzymes (Seidl et. al., 1969).

Table 3. Amino acid composition of dried defatted cowpea and its various protein fractions[a], and mungbean, rice, and whole egg.

Amino Acid	Amino acid content, g aa/100 g protein							
	Cowpea Fractions				Defatted Meal			
	Albumin	Globulin	Glutelin	Prolamin	Cowpea[a]	Mungbean[b]	Rice[c]	Whole Egg[b]
Arginine	6.06	7.43	7.11	2.05	8.16	9.76	8.06	6.60
Cystine	1.84	0.57	0.50	1.39	0.86	0.52	4.32	4.80
Histidine	2.81	3.63	2.98	1.02	3.44	3.09	2.20	2.40
Isoleucine	5.05	5.70	5.60	2.25	6.35	5.15	4.28	7.70
Leucine	6.37	9.16	8.59	3.68	9.06	7.49	8.68	9.20
Lysine	8.25	7.64	7.63	1.67	7.54	14.26	3.15	7.00
Methionine	1.87	1.35	1.31	0.60	1.51	2.35	2.55	4.00
Phenylalanine	4.30	6.60	6.17	2.26	5.91	5.70	5.24	6.30
Threonine	6.27	3.34	4.47	2.65	4.16	3.26	3.42	4.30
Tryptophan	1.52	0.91	1.21	0	1.51	0.74	1.36	1.50
Tyrosine	2.70	2.80	3.71	0.82	2.64	2.55	5.28	5.00
Valine	6.46	6.43	5.54	2.62	6.32	5.89	5.88	7.20
Chemical Score, %[d]	96	55	52	30	68	82	57	100%

[a]UPL Cp 2 variety was used.

[b]From Gonzales et. al. (1972)

[c]From Juliano (1978)

[d]Defined as the mg amino acid in 1 gm test protein compared to the amount of that amino acid in 1 gm of reference protein. The chemical score of a protein is the lowest score for any of the essential amino acids. The amino acid reference pattern used here was the FAO/WHO 1973 recommended pattern.

1. *Trypsin inhibitors.* Following the method of Kakade et. al., (1972), the trypsin inhibitor activity (TIA) of the recommended and promising varieties/lines was found to range from 30 to 72 trypsin inhibitor units/mg protein. The TIA values for cowpea are generally lower than those reported for other beans (Table 4).

The trypsin inhibitors from cowpea have recently been isolated and purified (Gatehouse et. al., 1980). The total trypsin inhibitors with a molecular weight of about 17,000 daltons could be further divided into inhibitors active against trypsin only and active against trypsin and chymotrypsin.

It is well established that trypsin inhibitors are inactivated by heat treatment and that their destruction is accompanied by a marked increase in the nutritive quality of the protein as measured in experimental animals like rat or chick. Recent studies showed that trypsin inhibitor accounts for about 40% of the growth inhibition observed with raw soybeans (Kakade et. al., 1972). Furthermore, crude extract from which the trypsin inhibitor has been removed by affinity chromatography still caused growth inhibition and pancreatic hypertrophy. With cowpea, the PER of raw beans is 1.2 while cooked beans had a PER of 1.5 (Rockland & Nishi, 1979). This low increase

Table 4. Trypsin Inhibitor Activity of Cowpea Varieties

Variety/Line	Trypsin Inhibitor Activity units/mg protein
Mecan Pea	29.6
V59-41	38.4
VCS-18	40.3
TVX 289-4G	43.4
BPI Imp Gr #1	43.5
VCS 6-12	44.1
All Season	51.7
VCS 6-1	53.7
CES 42-2	53.8
CES 26-12	72.1
Soybean (108)[a]	66-233

[a] From Kakade et al. (1972), 108 varieties used.

in PER after heat treatment could be due to the presence of heat stable trypsin inhibitors like tannins as discussed later and the low methionine-cysteine levels in cowpea.

Interestingly, Gatehouse (1979) recently reported that trypsin inhibitors could partially account for the resistance of cowpea to the brushed beetle *Callosobruchus maculatus.*

2. *Phytohemagglutinins* or *lectins.* The hemagglutinating activities of UPL Cp2 and nine other recommended and promising varieties/lines of cowpea were found to be nondetectable or zero. Similar observation has been reported for chickpeas, pigeon peas and mungbean (Honavar and Liener, 1962). The absence of phytohemagglutinins from cowpea is beneficial to its nutritive quality since these substances are known to repress or inhibit growth of experimental rats.

3. *Flatulence factor.* Raffinose, stachyose and verbascose are ∞-D-galactooligosaccharides which cause flatulence in man and animals. These oligosaccharides escape digestion and are not absorbed into the blood. Consequently, the bacteria in the lower intestinal tract metabolize them to produce large amounts of carbon dioxide and hydrogen. As an example, a diet of defatted soy flour and navy bean meal will result in gas production of 71 and 179 cc/hour average, respectively, compared to 13 cc/hour for a basal diet containing no legume product (Rackis, 1978).

The values of oligosaccharide contents in cowpea are within the range of those reported for soybean (Table 5). No studies on the effects of cowpea or cowpea products on gas production in man have been reported. Various methods of eliminating the oligosaccharides from mature soybean involve leaching, soaking and boiling, aqueous alcohol extraction, and by patented enzyme processes. Hymowitz and Collins (1974) have reported that although the raffinose and stachyose contents of soybean varieties differ, elimination of these oligosaccharides by genetic means does not look promising.

4. *Tannin content.* Besides the presence of toxic heat-labile factors like the trypsin inhibitors and hemagglutinins, beans also contain a heat resistant factor (tannins) which also inhibits trypsin activity and lowers nutritional value of food. Studies on *Phaseolus vulgaris* showed that white cultivars containing the lowest tannin acid content had the highest digestibility. Furthermore, red-colored beans showed the lowest protein digestibility and the highest tannic acid content (de España, 1977 and Fukuda Suzuki, 1978).

In a survey of the seeds of ten varieties each of four legume species, namely cowpea, chickpeas, pigeon peas and mungbeans, cowpea was the only species found to contain amounts of tannin that may be nutritionally harmful (Price et. al., 1980). The tannin content (as % vanillin) was estimated to range from 0-0.59 in the cowpea varieties. None of the ten varieties of chickpea and mungbean had tannin, while only two of 10 varieties of pigeon pea had tannin (0.03 and 0.08).

The relatively high content of tannin in cowpea could be a major factor in the rather low nutritional quality of cowpea and could account for the slight increase in PER of cowpea even after cooking. More studies should be undertaken to clarify the role of tannins in the nutritional quality of cowpea and to determine the level of tannin which would be harmful to cowpea's nutritional value.

Table 5. Oligosacchride Content of Cowpea and Soybean Meal

Constituent	% Oligosaccharide		
	Cowpea (UPL Cp2)	Cowpea[a]	Soybean[b]
Sucrose	3.1	1.8-3.1	6-8
Raffinose	1.8	1.1-1.3	1-2
Stachyose	3.8	2.9-3.8	4-5
Verbascose	Trace	0.6-1.0	Trace

[a] From Akpapunam and Markakis (1979), using 13 American cultivars of cowpeas.

[b] From Kawamura (1967).

COWPEA PROTEIN FRACTIONS

Based on their solubility in different solvents, seed proteins are divided into four fractions (Osborne, 1895): the water-soluble albumins, salt-soluble globulins, alcohol-soluble prolamins and acid or alkali-soluble glutelins.

As in other legumes, globulins constitute the bulk of cowpea proteins, about 83% with albumin at 9%, glutelin, 8% and promalin, 0.01% (Table 6). The heterogeneity and wide range of proteins comprising these fractions are shown in the polyacrylamide gel patterns of Figure 1. On SDS gel electrophoresis, the albumin fraction had 10 polypeptides with molecular weight ranging from 11,500 to 66.000 daltons. The globulin fraction had 10 polypeptides, 20,400 to 83,000 daltons, and the glutelin fraction had 9 polypeptides, 23,000 to 90,000 daltons. The prolamin fraction had only one wide band of protein with an average molecular weight of 12,000 daltons.

Amino acid analysis of the different protein fractions revealed that albumin had the best chemical score, exhibiting more than 100% for all

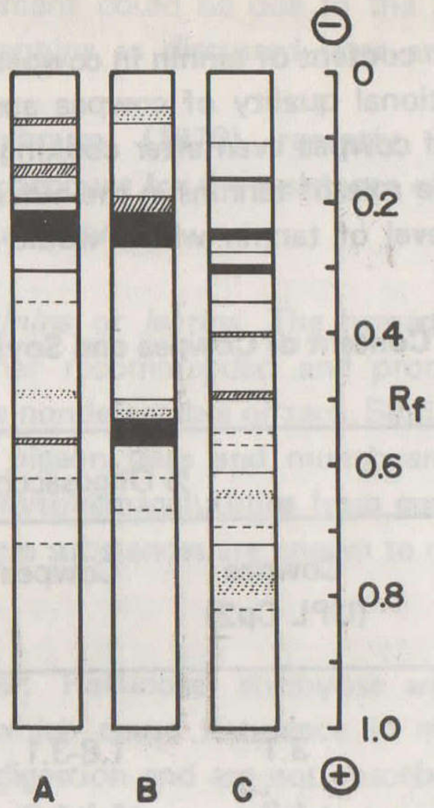

Figure 1. Polyacrylamide disc gel patterns of cowpea crude extract (A), albumins (B), and globulins (C).

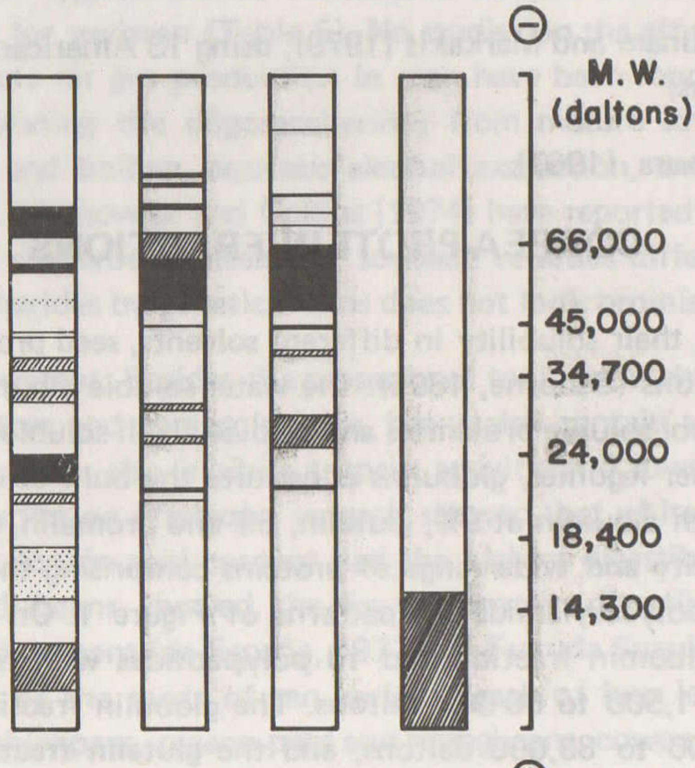

Figure 2. SDS gel electrophoresis patterns of cowpea albumins, globulins, glutelins and prolamin (from left to right).

Table 6. Cowpea Protein Composition

Fraction	Solubility	%	No. of Polypeptides	Molecular Weight Range
Albumins	Water-soluble	9	10	15,000-66,000
Globulins	Salt-soluble	83	10	20,400-83,000
Prolamins	Alcohol-soluble	0.01	1	12,000
Glutelins	Alkali-soluble	8	9	23,000-90,000

essential amino acids except for leucine where it still scored a high 96% (Table 3). Globulin had a low chemical score for methionine and cysteine (55%) and threonine (83%). Glutelin had high scores except for methionine and cysteine (52%) while prolamin was low (66) in all the essential amino acids.

The trypsin inhibitors were found to be 87% localized in the albumin fraction while 90% of the lipoxygenase activity was in this fraction and the rest in the globulin fraction.

Fractionation and characterization studies were done only on one variety of cowpea, UPL Cp2. These studies will have to be extended to other cowpea varieties to establish if the amino acid patterns of the different fractions will be similar. It is interesting to note that the albumin fraction, although only 6% of total nitrogen, had an excellent rating as a protein. One goal of legume researchers is to find a way of improving the quality of legume proteins. This may be possible through changing the relative proportions of protein fractions in the grain, e. g. lowering globulin and increasing the higher quality albumin. Indeed the superior protein quality of opaque-2 maize (Mertz et. al., 1964), floury-2 maize (Nelson et. al., 1965) resulted from changes in the relative amounts of the protein fractions and specifically, from a decrease in prolamin synthesis.

LIPOXYGENASE ACTIVITY

The enzyme lipoxygenase (linoleate: oxygen oxidoreductase E.C. 1.13.11.12) is primarily involved in the conversion of polyunsaturated fatty acids to aldehydes and alcohols which contribute to the off-and beany flavor of legume seeds and products. Its role in the beany flabor formation in soybean and peanuts is well established (Rackis, et. al., 1979). More recently, lipoxygenase was shown to be involved in the beany flavor formation in winged bean (Truong et. al., 1980).

Among several legume species tested, cowpea was found to contain the highest level of lipoxygenase specific activity (205-316 units/mg protein) as compared to that of winged bean (14-30 units/mg protein) and soybean (11-48 units/mg protein) (Table 7) (Truong et. al., 1979). Disc gel electrophoresis of crude cowpea extracts and partially purified enzyme followed by specific activity staining revealed at least four isoenzymes of cowpea lipoxygenase with a major isoenzyme of Rf = 0.21-0.27. The average molecular weight of this enzyme is 68,000 daltons. Pure cowpea lipoxygenase has optimum activity at about pH 6.2 and 30°C. The apparent Km and substrate concentration at Vmax of this enzyme which follows Michaelis-Menten kinetics are 0.25×10^{-3}M and 1.6×10^{-3}M, respectively, for the enzyme from Sephadex G-150 pooled fraction.

Recently Truong and Mendoza (1981) have purified the major cowpea lipoxygenase isoenzyme to about 90-95% purity. Figure 3 shows the polyacrylamide gel electrophoretic pattern and gel scan of this enzyme. This

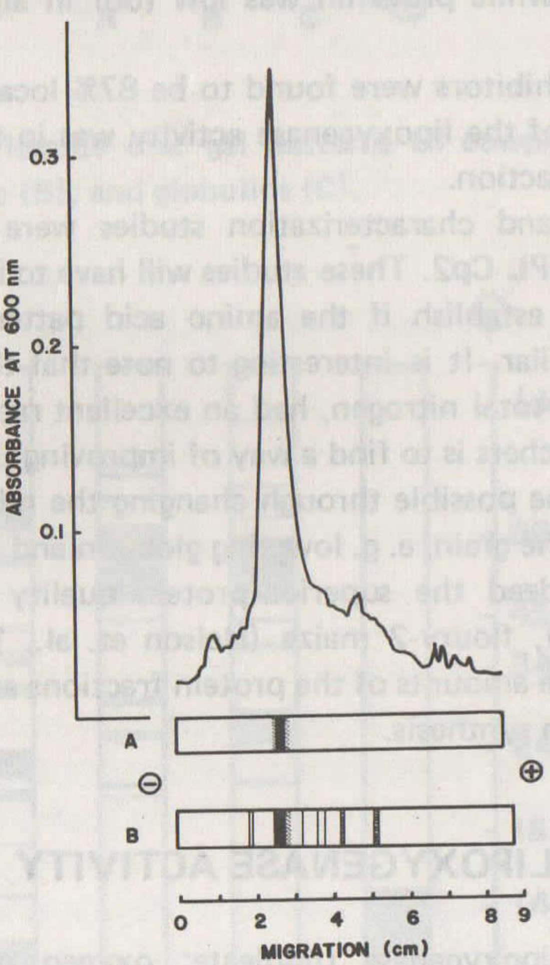

Figure 3. Polyacrylamide gel electrophoresis patterns of purified cowpea lipoxygenase (A) and active pooled fractions from Sephadex G-200 column chromatography (B). Densitometric sean is shown above.

Table 7. Lipoxygenase Activity in Cowpea, Soybean, and Winged Bean Extracts[a]

Legume Variety or Line	Lipoxygenase Specific Activity (Unit/mg protein)
COWPEA	
CES 26-12	316
VCS-61	296
BPI Imp Gr #1	279
CES 42-2	272
Mecan Pea	260
VCS 18	258
VCS 6-12	242
V 59-41	211
All Season	209
TVX-289-4G	205
SOYBEAN	
UPSSY2	48
Cobb	37
CES-12	33
Commercial	14
WINGED BEAN	
Batangas medium	30
PI 7041	30
TPT-2	28
TPT-1	23
PI-7256	14

[a] From Truong et al. (1980a).

highly purified preparation of lipoxygenase will be used in a series of experiments to establish its role and mechanism of action in beany flavor formation in cowpea.

The beany flavor detected in cowpea products consequently limits the use of cowpeas. Okaka and Potter (1979) were able to reduce the beany flavor in cowpea powder by using acid and heat treatment. Del Rosario et. al., (1980) have also reported the substitution of cowpea of relatively lower lipoxygenase activity for navy beans in preparing pork and beans. The resulting formulation was found acceptable and comparable to pork and beans with navy beans. These reports indicate that the prospects of wide utilization and acceptance of cowpea processed foods are promising and would greatly depend on selection/hybridization work to produce varieties of appropriate characters, as well as on processing technology.

The physiological and biochemical role of lipoxygenase in cowpea and other legumes has become complicated because of the reported damage on proteins by lipid hydroperoxides as well as their secondary products which could lower the nutritive quality of these legumes. Some chemical changes caused by interaction of lipid hydroperoxide and protein are: protein-protein crosslinks, protein scission, protein-lipid adducts and amino acid damage. Among the secondary products, aldehydes form Schiff base adducts with amino groups (Gardner, 1979). The extent to which lipoxygenase primary and secondary products can damage proteins and lower nutritional quality has not been reported. With the high lipoxygenase activity in cowpea, this could be another factor that affects and eventually lowers the protein quality of cowpea.

Another aspect of lipoxygenase study which merits attention is its possible role in pest resistance of legume grain. The presence of substances in plants which are toxic to pests and/or diseases but not to the plants themselves is well documented. The primary or secondary products of lipoxygenase reaction which produce the off-and beany flavor could also provide a mechanism for plant resistance to pests or diseases. This aspect of the lipoxygenase problem is now being looked into in this laboratory. A simple correlation between pest or disease resistance with lipoxygenase level may not be possible because of the many factors involved in plant resistance mechanisms.

RESEARCH NEEDS

The following points were raised or implied in this paper and should be worked on:

1. Role of tannin in low nutritive value of cowpea
2. Process of eliminating or reducing flatulence factors
3. Improving protein quality by changing proportion of protein fractions
4. Role of lipoxygenase in nutritional quality and resistance mechanism

Because of the increasing use of cowpea as source of high protein food, improvement of cowpea should include besides high yield and disease- and pest-resistance characters, nutritional quality and acceptability. However, work should not stop here. Developing the appropriate technologies to process cowpea into acceptable and good quality processed foods ought to come alongside the breeding work. Moreover, promotion of the improved and recommended varieties for use in the farmer's fields, as well as that of the cowpea products, is highly imperative for the majority to benefit from this legume grain.

ACKNOWLEDGEMENT

The authors wish to thank the Food and Nutrition Research Institute and the staff members of its Amino Acid Analysis Laboratory –Ms. E. U. Castillo, Ms. T. R. Portugal and Ms. N. C. Pangilinan for the amino acid analysis of the cowpea samples. Some of the data presented in this paper are from the M.S. thesis of Ms. V. S. Hernandez.

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