EFFECT OF HEAT TREATMENT ON NUTRITIONAL VALUE OF WINGED BEAN (*Psophocarpus tetragonolobus*) AS COMPARED TO SOYBEAN
I. CHEMICAL CHARACTERISTICS OF TREATED WINGED BEAN

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Summary

The effect of heat treatment (autoclave) on nutritional value of winged bean as compared to soybean has been investigated. The winged bean and soybean were obtained from local cultivar grown in Indonesia. The beans were autoclaved at 120℃ for 15, 30, 45, 60 or 90 minutes, respectively before being ground for chemical analysis. Trypsin inhibitors of winged bean and soybean decreased ($p < 0.05$) along with decreasing of urease activity as heating time increased from 0 to 90 minutes. Heat treatment significantly ($p < 0.05$) reduced protein solubility in 0.2% potassium hydroxide of winged bean as well as soybean. *In vitro* protein digestibility was significantly ($p < 0.05$) improved by heating treatment (15 to 60 min of autoclaving), however, excessive heating (90 min of autoclaving) decreased the digestibility of winged beans. Excessive heating had adverse effect on lysine, cystine and methionine contents of winged beans. The results of this study suggested that autoclaving at 120℃ within 45 minutes should be adequate to remove protease inhibitors and could improve protein digestibility of winged beans.

(**Key Words**: Winged Bean, Soybean, Heat Treatment, Trypsin Inhibitor, *In vitro* Protein Digestibility, Amino Acids Composition)

Introduction

The winged bean (*Psophocarpus tetragonolobus* L. Dc) is tropical crop has a high protein content comparable to the soybean. Winged bean seeds have protein and oil content ranging from 32-37% and 15-18%, respectively (Okezie and Martin, 1980). The winged bean proteins have an amino acid composition similar to that of soybean proteins with methionine and cystine being limiting amino acids (Cerny et al., 1971; Okezie and Martin, 1980; Wyckoff et al., 1983). Many researchers have focused mainly on utilization of the winged bean as human food rather than as animal feed. While its potentiality as a direct source of protein for human use is well recognized, its potential use in animal feed is just as valuable. Under sufficient supply, winged bean can be valuable in some countries where soybean meal has to be imported for animal feeds (de Lumen et al., 1982).

The nutritive value of winged bean has been studied using rats, chicks and Japanese quails. Cerney et al. (1971) fed winged bean to rats and found the net protein utilization (NPU) and protein efficiency ratio (PER) were significantly lower than that of a skim milk control. de Lumen et al. (1982) reported that replacement of soybean meal with autoclaved winged bean at level 75% and 100% decreased metabolizable energy and led to poorer broiler performance. In a preliminary study by Wyckoff et al. (1983) found that raw winged bean was lethal to Japanese quail. However, autoclaved winged bean with an additional 0.5% methionine were able to improve the performed of Japanese quails as well as the soy bean control.

In general, legume proteins have been reported to have low levels of sulphur containing amino acids and protein digestibility along with the presence of anti-nutritional factors (Liener, 1976). Protease (trypsin and chymotrypsin) inhibitors have been suggested as factors that responsible for the low digestibility of winged bean seeds.

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Received May 9, 1992
Accepted September 21, 1992

The presence of trypsin inhibitors in this bean is well documented (de Lumen and Salamat, 1980; de Lumen and Belo, 1981; de Lumen and Chan, 1982; Khor et al., 1982). Many studies have shown that trypsin inhibitors in this bean can be destroyed by moist heat treatment (Kadam, et al., 1987; Ekpeyong and Borchers, 1980; Wyckoff et al., 1983). Tan et al. (1984) reported an improvement in *in vitro* protein digestibility with a concomitant decrease in trypsin inhibitors through heat treatment.

The purpose of the present study was to evaluate the effect of heat treatment (autoclave) on chemical properties of winged bean as compared to soybean. In the next study, the experiment will be continue with animals.

Materials and Methods

Materials

The winged bean and soybean seeds of local cultivars grown in Indonesia were obtained from Indonesian Agricultural Department. The beans were cracked to pass a 0.5 cm screen and then were autoclaved at 120℃ for 15, 30, 45, 60, or 90 minutes, respectively. The beans were allowed to cool at room temperature before being grounded to pass a 1 mm screen.

Trypsin, chymotrypsin and peptidase were purchased from Sigma Chemicals, USA. All other chemicals used in this experiment were of analytical grade.

Analysis

Proximate analysis

Moisture, total mineral (ash), crude fat, crude fiber and total nitrogen (Kjeldahl's N) were determined according to the standard procedure of AOAC (1980).

Trypsin inhibitors assay

The winged bean and soybean extracts for trypsin inhibitors assay were prepared by extracting 1 g of sample with 50 ml of 0.01 N NaOH (pH 8.4-10.0) for 3 hrs. The trypsin inhibitors was determined according to the method described by Hamerstrand et al. (1981).

Urease index

The difference between pH of the test sample and pH of the blank was used as an index of urease activity following the method of Caskey and Knapp (1944).

Protein solubility

Protein solubility in 0.2% potassium hydroxide was determined by the method of Araba and Dale (1990).

In vitro digestibility

The assay of *in vitro* digestibility was carried out according to the method described by Hsu et al. (1977).

Amino acids analysis

The amino acid analysis were determined by a JEOL Model JLC-6 AH amino acid analyser, after acid hydrolysis of samples in 6 N HCl in evacuated sealed tubes at 110℃ for 22 hours. At the same time, free amino acids were determined in an aqueous extract of sample with 75 % (v/v) boiling ethanol.

Statistical analysis

The data were analyzed by analysis of variance using SAS (1987). Significant differences between means were calculated according to Duncan's multiple range tests (1955).

Results and Discussion

Chemical composition

Chemical composition of raw winged bean and soybean seeds are presented in table 1. The protein and ash content of soybean (42.66 and 5.00%) were higher than that of winged bean (36.79 and 4.11%). However, fat and nitrogen free extract contents of the winged bean were slightly higher than those of soybean. Okezie and Martin (1980) reported the high protein and fat content of winged bean were comparable to soybean. Autoclaving increased the dry matter and protein content of winged bean (table 2).

Urease activity

The urease test is one of the most common assays used to evaluate soybean meal from over-processing. The level of urease activity also can

TABLE 1. PROXIMATE COMPOSITION OF RAW WINGED BEAN AS COMPARED TO SOYBEAN

Content	Winged bean	Soybean
Moisture (%)	10.57	6.23
Crude protein (% DM)	36.79	42.66
Crude fat (% DM)	18.73	17.35
Crude fiber (% DM)	4.95	4.95
Ash (% DM)	4.11	5.00
NFE (% DM)	24.85	23.81

be used as an indicator of the presence of toxic factors such as trypsin inhibitors (Araba and Dale, 1990). The urease activity of raw winged bean was higher than that of soybean. After heating in 15, 30, 45, 60 or 90 minutes, the urease activity levels decreased significantly ($p < 0.05$) in both winged bean and soybean (table 2). These findings were agree with McNaughton and Reece (1980) who reported that urease activity value were decreased quickly by autoclaving. Furthermore, they stated that urease enzyme found in soybean was not considered to be detrimental to non-ruminant, but its destruction by heat treatment closely parallels the destruction of anti-nutritional factors, especially the protease inhibitors.

Trypsin inhibitors

As shown in table 2, trypsin inhibitors of raw winged bean were lower than that of soybean. The initial levels of anti-nutritional factors in beans could be important and would determine the type and length of heat treatment required (de Lumen and Salamat, 1980; de Lumen et al., 1982). Trypsin inhibitors content of winged bean decreased significantly ($p < 0.05$) from 16.92 to 2.08 mg/g as heating time increased from 0 to 90 minutes. Reduction of trypsin inhibitors was parallels to destruction of urease activity (table 2). Numerous studies on trypsin inhibitors have showed that heat treatment would reduced trypsin inhibitors content of winged bean as showed by Kadam et al. (1987) and de Lumen et al. (1982).

TABLE 2. EFFECT OF HEAT TREATMENT ON DRY MATTER CONTENT, CRUDE PROTEIN, CRUDE FAT, UREASE INDEX, TRYPSIN INHIBITORS, *IN VITRO* DIGESTIBILITY AND PROTEIN SOLUBILITY

Item	Winged bean					
	Heating time (min)					
	0	15	30	45	60	90
DM (%)	89.43^{a}	91.46^{b}	91.61^{b}	90.89^{c}	89.96^{d}	90.07^{d}
Protein (% DM)	36.79^{a}	37.37^{ab}	37.49^{b}	37.53^{b}	37.01^{ab}	36.97^{ab}
Crude fat (% DM)	18.73^{a}	19.11^{b}	18.72^{a}	18.56^{bc}	18.22^{d}	18.30^{cd}
Urease index (∠pH)	0.34^{a}	0.13^{b}	0.06^{c}	0.02^{d}	0.01^{d}	0.02^{d}
Trypsin inhibitors (mg/g)	16.92^{a}	3.20^{b}	2.61^{bc}	2.22^{c}	2.29^{c}	2.08^{c}
In vitro digestibility (%)	75.24^{a}	82.84^{c}	82.48^{bc}	82.84^{c}	82.57^{bc}	80.94^{b}
Protein solubility (%)	98.20^{a}	90.97^{b}	84.88^{c}	74.23^{d}	52.13^{e}	43.44^{f}

Item	Soybean					
	Heating time (min)					
	0	15	30	45	60	90
DM (%)	93.77^{a}	90.74^{b}	90.91^{b}	91.11^{b}	90.59^{b}	90.74^{b}
Protein (% DM)	42.66	43.58	42.56	42.92	43.25	42.96
Crude fat (% DM)	17.35^{a}	18.71^{b}	18.75^{b}	18.39^{b}	18.63^{b}	17.96^{c}
Urease index (∠pH)	0.96^{a}	0.01^{b}	0.01^{b}	0.03^{b}	0.02^{b}	0.01^{b}
Trypsin inhibitors (mg/g)	19.40^{a}	2.20^{b}	2.50^{b}	1.61^{c}	1.43^{c}	1.68^{c}
In vitro digestibility (%)	77.95^{a}	85.55^{b}	86.10^{b}	83.65^{c}	83.83^{c}	83.93^{c}
Protein solubility (%)	96.79^{a}	87.79^{b}	78.51^{c}	66.60^{d}	64.59^{d}	48.24^{e}

[a-f] Means in the same row with different superscripts were significantly different ($p < 0.05$).

Although the tannin content of winged bean in this study was not determined, the residual trypsin inhibitors activity could be due to tannin as suggested by de Lumen and Salamat (1980). The activity of trypsin inhibitors in this bean has been shown to be influenced by a heat-unstable factor (true trypsin inhibitors) and a heat-stable factor (tannin). Tannin may responsible for trypsin inhibitors activity and heat resistance of trypsin inhibitors activity was accountable by the tannin content of winged bean (de Lumen and Salamat, 1980).

In vitro protein digestibility

Table 2 shows that *in vitro* protein digestibility values of unheated winged bean was slightly lower than that of soybean. The low digestibility of raw beans is due to the presence of protease inhibitors which reduced the activity of the digestive enzymes (Ekpeyong and Borchers, 1980). Heat treatment had significant effect to improved protein digestibility of both beans. There results agree with the finding of Tan et al. (1984) who reported an improvement in *in vitro* digestibility with a concomitant decrease in trypsin inhibitors activity as a result of heat treatment. The improvement of protein digestibility is probably due to protein denaturation which destroys the cellular structure that proteolytic enzyme attacked easily. Furthermore, heat treatment caused inactivation of the antiphysiological factors, particularly the trypsin inhibitors (Finot, 1981). In this experiment, protein digestibility of winged bean increased from 75.24 to 82.84% as heat treatment increased from 0 to 45 minutes. However, further heating would decreased protein digestibility, it is probably due to overheating of the bean.

Protein solubility in potassium hydroxide

The protein solubility of winged bean in a solution of 0.2% potassium hydroxide decreased significantly ($p < 0.05$) following increasing of heating time from 0 to 90 minutes. Protein solubility was reduced from 98.20% to 43.44% for winged bean and from 96.79% to 48.24% for soybean. This marked decrease of protein solubility in an alkaline solution supports the finding of Araba and Dale (1990), who reported a decrease in solubility of soybean protein in aqueous potassium hydroxide after autoclaving the soybean meal. The meals with protein solubility below 70% probably would impaired the nutritive value

TABLE 3. THE AMINO ACIDS COMPOSITION OF UNHEATED WINGED BEAN AS COMPARED TO SOYBEAN

Component	Total amino acids		Free amino acids	
	Winged bean	Soybean	Winged bean	Soybean
	(g/16 g N)		(mg/16 g N)	
Lysine	8.29	5.48	6.35	3.63
Histidine	3.19	2.13	9.47	4.27
Arginine	6.96	6.05	5.63	4.33
Aspartic acid	13.13	10.85	25.63	3.31
Threonine	4.41	3.47	3.29	2.53
Serine	5.98	4.89	7.95	2.55
Glutamic acid	17.69	17.32	45.09	3.57
Proline	7.33	4.93	ND*	ND
Glycine	5.05	4.09	7.57	1.84
Alanine	4.85	4.19	28.28	2.17
Cystine	0.85	1.26	1.53	ND
Valine	5.61	4.50	9.71	5.21
Methionine	1.02	0.77	1.69	4.65
Isoleucine	6.16	4.65	2.28	3.34
Leucine	11.10	7.31	2.36	3.17
Tyrosine	4.63	2.86	14.20	4.36
Phenylalanine	5.71	5.02	3.95	3.88

*ND = Not determined.

as reflected by decrease in protein digestibility. Araba and Dale (1990) reported that protein solubility value of less than 65% almost certainly indicated overprocessing of soybean meal. Perhaps, autoclaving denatured the proteins of winged bean and reduced their solubility in potassium hydroxide.

Amino acids composition

The nutritional quality of a protein depends upon the total amounts of amino acids present in the protein, the relative proportion of the constituent amino acids (pattern of amino acid), and the degree to which the animal can liberate and utilize the amino acids from the protein (Knipfel et al., 1975). The amino acids composition from acid hydrolysis and ethanol extraction samples of winged bean as compared to soybean are presented in table 3. In this study, the value of all amino acids, except cystine of unheated winged bean were higher than those of soybean. A similar pattern is shown in free amino aicds composition of both raw winged bean and soybean that all amino acids content of winged bean, except methionine, isoleucine and leucine were higher than those of soybean. The amino acids composition of winged bean seeds have been determined by a number of workers. Like soybean and other legumes, the sulfur amino acids in the winged bean seem to be the most limiting amino acids (Cerney et al., 1971; Okezie and Martin, 1980). Heat treatment had adverse effect on lysine, cystine and methionine of winged bean (table 4). These amino acids tend to decrease as autoclaving time was increased from 60 to 90 minutes. The same tendency is also shown in other amino acids of winged bean. Sibbald (1980) reported that the most severe heat treatment reduced the amounts of arginine, methionine and lysine. Furthermore, excessive heating may either destroy or render certain available heat sensitive amino acids (McNaughton and Reece, 1980). Several investigations have shown that lysine to be more subject to reductions in availability caused by heating than methionine. Protein with lysine as first limiting amino acid might be subject to more severe reductions in nutritive value than would those deficient in methionine (Knipfel et al., 1975).

The free amino acids of winged bean which heated for 45 minutes were the lowest values (table 6). However, hydrolysis sample of winged bean heated for 45 minutes had the highest values

TABLE 4. EFFECT OF HEAT TREATMENT ON AMINO ACIDS COMPOSITION OF PROTEIN IN WINGED BEAN

Amino acid	Heat Treatment (min)					
	0	15	30	45	60	90
	(AA g/16 g N)					
Lysine	8.29[ab]	8.49[a]	7.27[ab]	7.52[ab]	7.67[ab]	6.43[b]
Histidine	3.19	3.32	2.56	3.12	2.71	2.48
Arginine	6.96	7.41	5.97	7.76	5.90	5.49
Aspartic acid	13.13	13.21	12.09	13.65	11.87	11.06
Threonine	4.41	4.43	4.06	4.41	4.08	3.82
Serine	5.98	6.16	5.61	6.27	5.85	5.18
Glutamic acid	17.69	17.65	16.32	20.18	16.52	14.90
Proline	7.33	7.39	6.25	6.93	4.79	5.91
Glycine	5.06	4.90	4.52	5.15	4.56	4.16
Alanine	4.85	4.80	4.43	5.07	4.36	4.13
Cystine	0.85[ab]	1.03[a]	0.72[b]	0.81[ab]	0.79[ab]	0.46[c]
Valine	5.61	5.29	5.56	5.25	5.14	4.92
Methionine	1.02[ab]	1.03[ab]	0.78[b]	1.11[a]	0.95[ab]	0.84[ab]
Isoleucine	6.16	5.78	5.34	6.10	5.41	4.75
Leucine	11.10	10.18	9.35	9.79	9.64	8.69
Tyrosine	4.63	4.64	4.22	3.80	4.19	3.71
Phenylalanine	5.71	5.52	5.20	5.88	5.26	4.82

[a-c] Means in the same row with different superscripts were significantly different ($p < 0.05$).

TABLE 5. EFFECT OF HEAT TREATMENT ON AMINO ACIDS COMPOSITION OF PROTEIN IN SOYBEAN

Amino acid	Heat Treatment (min)					
	0	15	30	45	60	90
	(AA g/16 g N)					
Lysine	5.48[ab]	6.05[a]	6.05[a]	5.92[a]	5.46[ab]	4.82[b]
Histidine	2.13	2.25	2.28	2.34	2.19	2.05
Arginine	6.05[ab]	6.38[a]	6.29[a]	6.33[a]	5.93[ab]	5.31[b]
Aspartic acid	10.85	11.30	11.85	11.64	11.41	11.43
Threonine	3.43	3.48	3.59	3.62	3.41	3.46
Serine	4.81	4.89	5.14	5.15	4.87	5.02
Glutamic acid	17.07	17.38	18.27	18.52	17.57	18.13
Proline	4.73[a]	4.95[ab]	5.26[b]	4.86[ab]	4.84[ab]	4.98[ab]
Glycine	4.04	4.11	4.33	4.31	4.12	4.26
Alanine	4.08	4.13	4.28	4.31	4.06	4.18
Cystine	1.16[a]	1.09[a]	0.91[b]	0.77[bc]	0.74[bc]	0.65[c]
Valine	4.44	4.67	5.01	4.67	4.60	4.73
Methionine	0.67	0.77	0.94	0.76	0.95	0.89
Isoleucine	4.51[a]	4.76[ab]	5.09[b]	4.84[ab]	5.02[b]	5.13[b]
Leucine	7.27	7.45	7.85	7.77	7.27	7.48
Tyrosine	2.84[ab]	2.95[ab]	3.04[a]	2.81[ab]	2.72[b]	2.74[b]
Phenylalanine	5.03[ab]	5.06[ab]	5.23[a]	5.06[ab]	4.83[b]	4.92[ab]

[a-c] Means in the same row with different superscripts were significantly different ($p < 0.05$).

TABLE 6. EFFECT OF HEAT TREATMENT ON FREE AMINO ACIDS COMPOSITION OF PROTEIN IN WINGED BEAN

Amino acid	Heat Treatment (min)					
	0	15	30	45	60	90
	(AA mg/16 g N)					
Lysine	6.35[a]	6.01[a]	5.29[a]	3.33[b]	5.16[a]	4.87[ab]
Histidine	9.47[a]	10.55[a]	8.05[abc]	5.81[c]	7.71[bc]	7.78[bc]
Arginine	110.63[a]	106.99[a]	88.35[a]	48.80[b]	88.82[a]	83.97[ab]
Aspartic acid	25.63[a]	27.57[a]	22.58[ab]	12.49[b]	24.59[a]	27.34[a]
Threonine	3.29[a]	3.88[a]	3.28[a]	1.66[b]	3.06[ab]	3.00[ab]
Serine	7.95[a]	8.01[a]	5.98[ab]	3.42[b]	5.74[ab]	6.06[ab]
Glutamic acid	45.09[a]	41.06[ab]	28.77[bc]	15.06[c]	24.46[c]	19.44[c]
Glycine	7.57[a]	7.72[a]	5.86[ab]	3.37[b]	5.49[ab]	3.51[b]
Alanine	28.28[ab]	30.36[a]	24.12[ab]	13.80[b]	23.56[ab]	26.28[ab]
Cystine	1.53	1.68	ND*	ND	ND	ND
Valine	9.71[ab]	10.64[a]	7.72[ab]	3.64[b]	6.78[ab]	6.11[ab]
Methionine	1.69	ND	ND	ND	ND	ND
Isoleucine	2.28[a]	2.46[a]	1.48[ab]	0.99[b]	1.40[ab]	1.55[ab]
Leucine	2.36[ab]	2.79[a]	1.82[ab]	1.29[b]	1.67[ab]	1.74[ab]
Tyrosine	14.20	17.76	14.62	10.74	16.44	20.79
Phenylalanine	3.95	4.83	3.49	2.54	3.37	3.99

*ND = Not determined.

[a-c] Means in the same row with different superscripts were significantly different ($p < 0.05$).

of some amino acids (table 4). In other word, autoclaving for 45 minutes would reduced free amino acid content and increased some amino acids from hydrolysis sample of winged bean. These results well correlated with protein digestibility value. Autoclaving of winged bean for 45 minutes increased protein digestibility to the highest value compared with the other treatments.

In conclusion, autoclaving reduced the trypsin inhibitors, urease activity, protein solubility and improved protein digestibility of winged bean as well as soybean. However, excessive heating would damage lysine and sulphur-amino acid content of winged bean. Based on these results, now the experiment with rats, chicks and Japanese quails are in progress.

TABLE 7. EFFECT OF HEAT TREATMENT ON FREE AMINO ACIDS COMPOSITION OF PROTEIN IN SOYBEAN

Amino acid	Heat Treatment (min)					
	0	15	30	45	60	90
	(AA mg/16 g N)					
Lysine	3.63[a]	4.59[c]	3.99[b]	3.94[b]	3.93[b]	2.89[d]
Histidine	4.27	5.44	4.91	4.66	5.08	4.82
Arginine	4.33[a]	4.26[a]	4.49[a]	4.45[a]	4.39[a]	35.60[b]
Aspartic acid	3.31[a]	3.33[a]	3.42[a]	3.44[a]	3.37[a]	34.91[b]
Threonine	2.53[a]	ND*	2.73	3.73	3.62	3.80
Serine	2.55[a]	2.59[a]	2.61[a]	2.57[a]	2.57[a]	3.77[b]
Glutamic acid	3.57[a]	3.58[a]	3.67[a]	3.59[a]	3.59[a]	21.74[b]
Glycine	1.84[a]	1.89[a]	1.89[a]	1.87[a]	1.86[a]	3.98[b]
Alanine	2.17[a]	1.98[a]	2.61[a]	2.58[a]	2.81[a]	28.60[b]
Cystine	ND	ND	ND	ND	ND	ND
Valine	5.21[a]	4.22[c]	5.01[ac]	4.83[cd]	4.59[d]	7.78[b]
Methionine	4.65	ND	ND	ND	ND	ND
Isoleucine	3.34	3.46	3.30	3.25	3.22	3.19
Leucine	3.17[a]	3.38[a]	3.32[a]	3.24[a]	3.25[a]	3.76[b]
Tyrosine	4.36[a]	4.24[a]	4.49[a]	4.48[a]	4.47[a]	18.30[b]
Phenylalanine	3.88[a]	4.09[a]	4.05[a]	4.01[a]	4.01[a]	11.59[b]

*ND = Not determined.
[a-d] Means in the same row with different superscripts were significantly different ($p < 0.05$)

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