BIOCHEMICAL COMPOSITION OF MATURE WINGED BEANS

Psophocarpus tetragonolobus (L.) DC

by

Virgilio Villegas Garcia

Dissertation submitted to the Graduate Faculty
of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Food Science and Technology

Approved:

James K. Palmer, Chairman

George J. Flick, Jr.

John R. Vercellotti

Charles B. Wood

Roderick W. Young

March, 1979
Blacksburg, Virginia

ACKNOWLEDGMENTS

I wish to express my appreciation to Dr. James K. Palmer for his advice, interest and guidance in this research study. I am grateful to and the staff of the Department of Food Science and Technology for making available the equipment and facilities to carry out this research. I would also like to express my gratitude to each member of my advisory committee: Dr. James K. Palmer, Professor Charles B. Wood, Dr. George J. Flick, Jr., Dr. John R. Vercellotti and Professor Roderick W. Young for their suggestions and constructive criticisms.

, and , Department of Human Nutrition and Foods, receive special thanks for their valuable help in the amino acid analysis and the use of their equipment. I would also like to thank Professor Roderick W. Young and his technical staff (Department of Biochemistry and Nutrition) for assistance in the fatty acid analysis.

Gratitude is also expressed to the International Institute of Tropical Agriculture, the Asia Foundation and , USDA, for supplying the seeds.

I am very grateful to the University of the Philippines at Los Baños and to the Education Development Projects Implementing Task Force, Republic of the Philippines for the award of a faculty development fellowship.

I am most grateful to my wife, and my daughters, and , for their patience, companionship, understanding and love during the tenure of my studies. I am also most grateful to my parents, , for their letters and encouragement and love, and to my brother, and his family for helping us in all the way they did. I am grateful to Professor and Mrs. Charles B. Wood for being our parents as well as grandparents to my family.

To the endless string of friends that I have made, thank you for your friendship, wit and candor.

TABLE OF CONTENTS

INTRODUCTION

There is an urgent need for modern man to seek additional food resources. A large portion of the world's population, particularly in the developing countries, lacks adequate food supplies resulting in protein-calorie malnutrition. Malnutrition is not limited to the less developed countries. Even in highly technological societies, malnutrition can and does exist (Lachance, 1972). In the developing countries, there is a strong demand for more calories and good quality protein as well as other nutrients such as Vitamin A, riboflavin and iron. A more vigorous program is needed on the scientific, economic and social fronts to meet this demand.

One approach has been to seek new potential sources of food proteins. In the past decade, single-cell proteins, leaf proteins and other plant proteins have been introduced as a partial solution to the protein problem. Many plant proteins in the form of isolates and concentrates have also been tested and developed as food additives (Circle and Johnson, 1958; Smith and Wolf, 1961; Anson, 1962; Circle, 1971; Wolf, 1970) and are expected to play a major role in the new dairy type products such as whipped toppings, coffee whiteners, and frozen desserts (Berba, 1955; Meyer, 1966). Protein gels have also been studied for use in the manufacture of textured products or as

functional components of semi-solid foods (Frank and Circle, 1959; Smith and Wolf, 1961; Anson, 1962; Kelley and Pressley, 1966; Wolf, 1970).

Edible legumes, because of their high protein content (20-40%) are likely sources for development of protein rich mixtures. Legumes provide the same number of calories (300-350 per 100 grams edible portion) as that of cereals, but legumes contain considerably more protein and a higher content of lysine. Lysine is often deficient in cereals, thus, legumes complement cereals in cereal-legume formulated foods.

Among the legumes, soybean is the most utilized in the preparation of protein-rich foods, including soy milk, soy curd (fermented and non-fermented), tempeh and extruded food products. The production of soybeans has increased from 25.3×10^6 metric tons (MT) in 1967 to 36.6×10^6 MT in 1976 (Smith and Circle, 1978).

In 1975, the U.S. National Academy of Science brought to the attention of the scientific community a little-known tropical legume, the winged bean (Psophocarpus tetragonolobus (L.), DC) grown almost exclusively for food in Papua New Guinea and Southeast Asia. According to Newell and Hymowitz (1979), the winged bean is one of nine species of the genus Psophocarpus in existence. Eight species of Psophocarpus are indigenous to Africa while the winged bean (Psophocarpus tetragonolobus (L.), DC) appears to be native to Southeast Asia and

Papua New Guinea with Papua New Guinea as the most likely center of geographical origin (Hymowitz and Boyd, 1977).

The winged bean plant is a climbing perennial requiring trellises and produces large four-sided pods. It is grown in Asia mostly in backyard gardens for immediate consumption and for the local market. The winged bean grows well and yields abundantly in tropical and sub-tropical regions, where protein deficiency is prevalent and where soybeans do not grow well.

The winged bean* is unique among legumes in that all parts of the plant are edible. The young green pods, the leaves and the flowers are rich in protein and vitamins and are consumed as a vegetable. The underground tubers are quite unique among tubers in that they contain a high level of protein (up to 20%). The mature seeds contain about 34% protein and 17% oil which is very similar to soybeans. If extraction of the edible oil proves commercially feasible, the residue should be an excellent source of protein, analogous to soybean meal.

Limited chemical data show that immature pods, the beans and the tubers are also rich in minerals and vitamins. The beans are also rich in tocopherol, an antioxidant that improves utilization of Vitamin A in

---

*Winged beans" or "beans" refers to mature winged bean seeds in all subsequent discussions; "winged bean" refers to the plant as a whole.

the human body. This is significant since Vitamin A deficiency is common in many tropical countries.

The raw, mature beans, like the soybean and other legume seeds, contain various antinutritional factors such as trypsin inhibitor (Sohonie and Bhandarkar, 1954; Cerny et al., 1971), hemeagglutinin (Renkonen, 1948) and amylase inhibitors (Jaffe and Korte, 1976). These factors can be destroyed by moist-heat or boiling water (Cerny et al., 1971).

The fatty acid profile of the bean oil appears to be quite similar to that of soybeans. Cerny et al. (1971) reported that winged bean oil contains parinaric acid (C 18:4), a potential antinutritional factor. However, Kleiman (as cited in Newell and Hymowitz, 1979) found no parinaric acid using UV and GLC analytical methods.

Full utilization of winged beans as a food will require a concerted international effort in developing its full potential. The Winged Bean Steering Committee (WBSC) was formed in January 1978 at the conclusion of the International Winged Bean Workshop/Seminar held in the Philippines. The workshop/seminar identified research priorities at the international and national level. Food technology and nutrition research was one of the research priorities identified in this meeting.

The suitability of winged beans as a source of protein deserves further study. The information to date indicates they contain approximately three to four times the protein found in cereal grains and

sufficient quantities of all the amino acids except methionine, cystine and tryptophan. The deficiency in sulfur-containing amino acids could be partly corrected by combining winged beans with appropriate cereal grains containing complementary amino acids. For example, a 19% increase in net protein utilization (NPU) was observed when winged beans and corn were combined (Cerny et al., 1971). The above mentioned information on winged beans is based on only a few studies, with a limited number of varieties. Varieties used in many of the earlier research studies were not identified.

The present state of knowledge may be compared with that available for soybean 60 years ago. There is a pressing need for vigorous study of the biochemical and nutritional and sensory properties of winged beans including the inherent variability of the available varieties. Favorable results could lead to the development of a tropical crop with all the impact of the soybean in temperate climates.

The overall objective of the present study was to compare the biochemical and nutritive properties of the mature winged beans to other legumes, especially to soybeans. Included in this study are the following:

1. Proximate analysis of five promising varieties of winged beans.
2. Extraction, isolation and partial characterization of the proteins from variety TPT-2.

3. Determination of the amino acid composition of the five varieties of winged beans and of the protein extracts and/or isolates obtained from variety TPT-2.
4. Quantitative determination of the soluble sugars including the oligosaccharides, in the five varieties.
5. Quantitative determination of the fatty acids in the five varieties.

The results of the studies will hopefully provide a sound basis for further research on food uses of winged beans. They will also complement and facilitate the agronomic and genetic studies aimed at further improving the nutritive, functional, and sensory properties.

REVIEW OF LITERATURE

The winged bean, Psophocarpus tetragonolobus (L.), DC has received considerable attention and recognition from the scientific community (Levy, 1977a, 1977b, 1978) since 1975. The winged bean shows much potential as a food crop for the humid tropics because of the high protein content of the foilage, immature pods, dried seeds and tubers. In addition, this legume survives well in the humid tropics, where soybean as a crop has failed.

The literature on the winged bean is meager and largely inaccessible, scattered through old journals, mimeographed papers and annual reports from experiment stations (NAS, 1975).

The winged bean was grown and consumed as early as the 17th century in Southeast Asia and Melanesia (Burkhill, 1966 as quoted by Claydon, 1975; Purseglove, 1968), where the earliest record of the bean was in the Moluccas as mentioned by Rumpf (Masefield, 1973). The winged bean which is grown in Asia and on various Pacific islands, is the cultivated species of the genus Psophocarpus Necker. It is not known in the wild state in comparison to the other eight species of Psophocarpus which are native in Africa (Newell and Hymowitz, 1979). The winged bean has been found to grow throughout Asia with Papua New

Guinea and Southeast Asia (Indonesia, Philippines, Thailand, Malaysia) considered as the center of dispersion or origin.

Proximate Composition

The first analysis of the composition of the winged bean was probably that of Greshoff et al. in the early 1950's (as quoted by Masefield, 1973) who found that the immature seeds contained 29.8% protein and 15.0% oil, indicating exceptional promise as a food resource. Since then, additional analyses have confirmed the promising properties of the plant (NAS, 1975). Proximate analysis have been reported on the flowers (Claydon, 1975), immature green pods (Cerny, 1978), leaves (Cerny, 1978; Ekpenyong and Borchers, 1978), the tubers (Claydon, 1975) and the "beans" (mature seeds) (NAS, 1975; Jaffe and Korte, 1976; Claydon, 1975; Ekpenyong and Borchers, 1978) all of which have been reported to be edible.

Mature winged beans have never been produced as a marketable commodity, except for the small quantities required for planting the small acreage grown. Pospisil et al. (1971) were the first to record seed yields. They obtained 1254 lbs/acre (1.4 tons/ha) in their cultivation of winged beans in Ghana. This result created attention because it was higher than the seed yields that could be obtained for soybeans in the humid tropics. At Ibadan, multiple harvests have yielded 2.2 tons and 1.8 tons per ha of dry, mature beans. These

figures are still below the seed yield potential of 2.7 tons/ha attainable for soybeans (NAS, 1975).

Table 1 shows the proximate composition of winged beans. Although the varieties analyzed were specified in only one case (Ekpenyong and Borchers, 1978) all the results confirm the high amount of protein and lipid present in winged beans. The protein and fat content compares well with that of soybean but is higher than most other legumes (cowpea, mung bean, chickpea, etc.).

Winged beans are also a good source of minerals and vitamins (Cerny, 1978). They also contain adequate amounts of tocopherol, an antioxidant, that improves utilization of Vitamin A as well as increasing its absorption and storage.

The edible roots or tubers of winged bean appear to hold the most interest after the seeds. This is especially true for the population whose staple food are root crops and/or plantains. The yield for the edible tubers from winged bean have not been reliably measured. In Burma, in which the winged bean is grown for its tubers, fresh tuber yields exceeding 4 tons/ha have been reported (NAS, 1975). The tubers are also consumed in Papua New Guinea (Claydon, 1975). The tubers are eaten raw or boiled. Purseglove (1968) stated that the roots are best liked for eating when they are a little thicker than the human thumb. They are usually harvested when they reach 2-4 cm in diameter and 8-12 cm in length. Claydon (1976) observed that conditions of low

Table 1. Proximate composition of winged beans

Analysis	1[a/]	2[b/]	3[c/]	4[d/]	5[e/]	6[f/]	7[g/]	8[h/]	9[i/]
Moisture	8.54	9.7	14	6.7-24.6	8.7	11.6	10.4	9.5-10.4	9.7
Crude protein	41.86	32.8	33	29.8-37.4	36.6	30.6	35.9	34.4	37.3
Fat	13.11	17.0	16	15.0-20.4	15.3	18.3	15.8	16.9	18.1
Crude fiber	5.27	5.2	5	5.0-12.5	3.7	9.4	9.2	10.7	5.4
Ash	-	4.0	-	3.6-4.0	3.8	3.7	4.9	4.2	4.3
Carbohydrates	31.22	36.5	-	31.6-28.0	35.6	26.4	23.9	34.1	25.2

[a/] Padilla and Soliven, 1933.
[b/] Institute of Nutrition (Philippines), 1957.
[c/] Tindall, 1968 (as cited by Pospisil, 1978).
[d/] National Academy of Science, U. S., 1975.
[e/] Claydon, 1975.
[f/] Jaffe and Korte, 1976.
[g/] Ekpenyong and Borchers, 1978.
[h/] Gandjar, 1978.
[i/] Kordylas, 1978.

temperature (13.5°C) and high humidity (82%) permit storage of winged bean roots for at least 2 months, if mold growth can be arrested.

Table 2 shows the composition of winged bean roots as determined by Claydon (1975) in comparison with other tropical root crops. The winged bean roots contain a high protein content, 5 to 10 times higher than other legume tubers and other staple root crops of the tropics. Thus, it provides a good source of protein as well as carbohydrates where it is available (Burma and Papua New Guinea). Choo, 1975 (as cited by Cerny, 1978) reported that the essential amino acid pattern is poor compared to other root crops (sweet potato, cassava and yam). Ekpenyong and Borchers (1979) also determined the amino acid content of the roots. Their analysis show that the essential amino acids are adequate. The limiting amino acids were methionine and tryptophan. More amino acid analyses of the root will be needed to determine its nutritional value.

In the Philippines, as well as in other parts of Asia where winged bean is consumed as a vegetable, the young green pods are the most relished part of the plant. These pods make a tender crunchy vegetable that can be eaten as such, included in salads or cooked in other recipes. They are highly palatable and taste like green beans (Cerny, et al. 1971; NAS, 1975). The composition of the green pods (Table 3) corresponds well with the green pods of other legumes. However, they tend to have a higher calcium content, 236 mg % (Brown, 1954) and iron content, 12 mg % (Institute of Nutrition, Philippines, 1957).

Table 2. Constituents of some tropical root crops (composition per 100 grams edible portion)

Analysis	Winged bean[a/] (*Psophocarpus tetragonolobus*)	Yambean[b/] (*Padhyrhizus erosus*)	Yam[b/] (*Dioscorea alata*)	Cassava[c/] (*Manihot esculenta*)	Sweet potato[b/] (*Ipomea batatas*)
Calories (g)	150[d/]	46	87	135	115
Moisture	51.3-67.8	87.4	76.4	65.5	70.7
Crude protein	4.7-29	1.6	1.9	1.0	1.2
Fat	0.1-0.4	0.2	0.2	0.2	0.3
Carbohydrates	27.2-30.5	10.3	19.9	32.4	27.1
Fiber	1.5-1.6	1.3	1.6	1.0	0.8
Ash	0.9-1.7	0.5	1.6	0.9	0.7
Calcium (mg)	25[d/]	18	38	26	36
Iron (mg)	0.5[d/]	0.8	1.1	0.9	0.9
Ascorbic acid	26.2[d/]	15	6	41	35

[a/] Newell and Hymowitz, 1979.
[b/] FAO, 1972.
[c/] Food and Nutrition Research Center (Philippines), 1968.
[d/] Claydon, 1975.

Table 3. Composition of the young green pods of winged bean as compared to other legume green pods (in 100 g) of edible portion.

Analysis		Winged bean[a/]	Cowpea[b/] (*Vigna sinensis*)	Green beans[b/] (*Phaseolus vulgaris*)	Hyacinth bean[b/] (*Dolichos lablab*)
Moisture	%	76-93	88.3	90.6	87.5
Fat	g	0.1-3.4	0.2	0.2	0.3
Crude protein	g	1.9-3.0	3.0	2.1	3.1
Crude fiber	g	0.9-2.6	1.6	1.3	1.9
Ash	g	0.4-1.9	0.6	0.7	0.9
Carbohydrates	g	1.1-7.9	7.9	6.4	8.2
Calcium	mg	53-236	44	50	75
Phosphorus	mg	26-60	45	48	50
Sodium	mg	3	6	8	5
Potassium	mg	205	233	250	279
Iron	mg	0.2-12.0	0.7	0.7	1.2
Vitamin A	I.U.	340-595	225	110	160
B_1	mg	0.06-0.24	0.12	0.07	0.08
B_2	mg	0.08-0.12	0.11	0.08	0.13
Niacin	mg	0.5-1.2	1.0	1.8	0.6
Vitamin C	mg	21-37	22	16	16

[a/] Cerny, 1978.
[b/] FAO, 1972.

The winged bean flowers are white to purple in color. They may be eaten raw, added to salads, and when fried in oil taste like mushrooms (NAS, 1975). The flowers of winged bean are also used as food coloring (Claydon, 1975). Table 4 shows the proximate composition of the winged bean flavor in comparison to other edible flowers. The protein content of the winged bean flower is higher than the other edible flowers, except for the dried banana flower which is used mainly for flavoring.

Many kinds of leaves are used as food by people in the tropics. Some are good sources of protein and the dark green ones are rich in Vitamin A precursors and other vitamins and minerals. Table 5 shows the composition of winged bean leaves in comparison to other leaves consumed in the tropics. The crude protein content of winged bean leaves compare favorably with the dark green leaves. The protein content is twice as high when compared to the leaves of a root crop such as sweet potato. Senanayake and Sumanasinghe (1976) found the leaf protein content of several varieties of winged bean to vary from 24.48% to 31.46% with an average of 27.85% dry weight basis. Pospisil et al. (1971) found the haulm (silage) palatable to stock.

It is evident from the results presented (Tables 1-5) that considerable variation exists in the analyses. Differences can be attributed to the year of analysis, locations, analytical and sampling methods and germ plasm sources (varieties). However, the values reported do provide an approximation of the chemical compositions of the winged bean seeds, leaves, flowers, immature pods and tubers.

Table 4. Composition of winged bean flowers as compared to other edible flowers (in 100 g edible portion).

	Winged bean[a/]	Banana[b/] (*Musa sapientum*)		Squash[b/] (*Cucurbita maxima*)	Sesban flower[b/] (*Sesbania grandiflora*)
		flower	flowering stalk (aerial stem)		
Moisture	84	14.9	87.8	89.8	86.3
Fat	0.9	1.3	0.4	0.2	0.6
Crude protein	5.6	9.6	1.6	0.6	1.6
Carbohydrates	3.0	69.6	8.0	4.3	10.7
Fiber	-	5.3	1.1	0.7	0.8
Ash	-	4.6	1.1	2.1	0.8

[a/] Claydon, 1975.

[b/] Food and Nutrition Research Center (Philippines), 1968.

Table 5. Proximate composition of winged bean leaves as compared to other edible leaves.

Analysis	Winged bean		Sweet potato[c] (Ipomea batatas)	Jute[c] (Corchorus oliterius)	Spineless amaranth[c] (Amaranthus gracilis)
	1[a]	2[b]			
Moisture	75.0-85.0	75.8	84.8	81.6	84.4
Crude protein	5.0-7.6	6.8	2.8	5.9	4.6
Fat	0.5-1.1	2.47	0.5	0.3	1.1
Crude fiber	-	2.95	2.2	1.8	1.4
Ash	1.0-2.9	2.52	1.6	2.5	2.5
Carbohydrates	3.0-8.5	9.46	10.3	9.7	7.4

[a] Cerny, 1978.

[b] Ekpenyong and Borchers, 1978 (calculated from moisture-free data).

[c] Food and Nutrition Research Center (Philippines), 1968.

The winged beans (seeds) are expected to play a major role in the development of food products because of their high protein and oil content. Like soybeans, it is possible to exploit the oil content for industrial and food uses. After defatting, the beans can be milled into flour for making composite flours and a starting material for other food products. Since the flour is a good source of proteins, protein concentrates and isolates offer other uses such as in the fortification of cereal foods, texturization and other uses similar to that of soy protein concentrates and isolates. The whole beans can also be processed into tempeh (Gandjar, 1978), winged bean milk and curd (Cerny and Addy, 1973; Shurtleff, 1978; Ruberte and Martin, 1978), winged bean yogurt (Smoot et al., 1979) and weaning foods (Kordylas, 1978).

Protein and Amino Acid Composition

The results presented in Tables 1-5 show that the different parts of the winged bean plant are indeed rich in protein. The dried beans, because of their potential use in aleviating human malnutrition, have received the most attention with regard to protein content and amino acid composition.

Kordylas (1978) studied the distribution of nutrients by separating winged beans into grits and seed coat. He found the grits to contain the major portion of the protein, fat and ash. The seed coat was found to be rich in iron (11 mg %).

Kulkarni and Sohonie (1956) were the first to determine the amino acid content of winged beans. It is unclear whether the whole pod or the immature seed inside the pod was used for the analysis. They found that 44.2% of the total nitrogen was soluble in 2.5% trichloroacetic acid and of this, 36.4% was amino nitrogen.

More detailed amino acid composition data were reported by Pospisil et al. (1971), Cerny et al. (1971), Ekpenyong and Borchers (1978), and Gillespie and Blagrove (1978). Except for Ekpenyong and Borchers who utilized TPT-2, the varieties used for analyses were not mentioned. The results show similar amino acid patterns, although there is considerable variability in results for individual amino acids (Table 6). The amino acid profile of winged beans compares favorably with that of soybeans. The winged bean is richer in lysine than soybean. Like soybean, the winged bean is deficient in cystine and methionine.

Gillespie and Blagrove (1978) have studied the storage globulins of winged beans and their amino composition. They found one globulin fraction rich in sulfur-containing amino acids.

The amino acid composition of the roots, leaves and immature pods have been reported by Ekpenyong and Borchers (1978). Choo, 1975 (as cited by Cerny, 1978) has also reported the amino content of the roots. These are shown in Table 7. The results on the roots show wide variation. More analyses are needed to determine the nutritional value of the above mentioned parts of the winged bean.

Table 6. Amino acid composition of protein in mature winged beans (mg/g N)

Amino acid	Cerny 1978	Cerny 1971	Jaffe & Korte 1976	Ekpenyong & Borchers 1978	Gillespie & Blagrove 1978	Gandjar 1978
Isoleucine	306-350	306	-	288	244	263
Leucine	462-564	562	-	520	550	506
Lysine	413-600	500	600	494	425	488
Methionine	75-87	75	88	54	56	58
Cystine	73-162	100	73	88*	106*	-
Phenylalanine	214-362	362	-	290	256	321
Tyrosine	195-200	200	-	296	262	281
Threonine	269-287	269	-	244	294	294
Tryptophan	47-63	N.D.	47	59	-	104
Valine	242-306	306	-	307	281	265
Arginine	400-440	406	-	471	331	283
Histidine	169-183	169	-	187	150	176
Alanine	230-269	269	-	257	381	296
Aspartic acid	719-781	919	-	433	694	751
Glutamic acid	956-1154	956	-	937	844	1080
Glycine	259-269	269	-	254	456	268
Proline	425-431	431	-	373	475	449
Serine	306-327	306	-	285	456	360

*determined as cysteic acid

Table 7. Amino acid composition of the winged bean tuber, immature pod and young leaves (mg/g N)

Amino Acid	Tuber		Immature pods[b]	Leaves[b]
	1[a]	2[b]		
Isoleucine	171	270	266	344
Leucine	229	460	430	595
Lysine	N.D.	351	410	162
Methionine	48	122	152	102
Cystine	14	164*	120*	84*
Phenylalanine	106	216	284	414
Tyrosine	72	267	243	296
Threonine	195	269	231	300
Tryptophan	N.D.	70	59	130
Valine	150	421	319	402
Arginine	N.D.	268	388	205
Histidine	N.D.	158	198	78
Alanine	113	320	276	403
Aspartic Acid	594	709	761	651
Glutamic Acid	406	616	705	720
Glycine	137	295	250	370
Proline	106	384	329	331
Serine	171	397	262	264

[a] Choo (1975) as cited by Cerny (1978); recalculated to mg/g N.
[b] Ekpenyong and Borchers (1978) recalculated to mg/g N.
*determined as cysteic acid.

Protein Extraction and Characterization

There were no reports concerning the extraction, isolation and characterization of winged bean proteins prior to 1977. Gillespie and Blagrove (1977) reported the presence of major components with sedimentation coefficients of 2S and 7S in winged bean proteins extracted with 0.15 M phosphate buffer at pH 7. They also found three globulin fractions (which they named psophocarpin A, B, and C in order of increasing electrophoretic mobility) via cellulose acetate electrophoresis. The major 2S component was found to be psophocarpin B; the major 7S component, psophocarpin C; and psophocarpin A was a minor component.

Gillespie and Blagrove (1978) found that winged bean proteins are soluble to the extent of 60% at pH 6.6, 80% at pH 11 but only 12% at pH 5. They extracted the soluble winged bean proteins at pH 4.5 using acetate-chloride buffer followed by ammonium sulfate precipitation. They also found that psophocarpin A, a minor component, is rich in sulfur containing amino acids. Blagrove and Gillespie (1978) extracted the proteins from winged beans representing 80 pure lines. They utilized sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to separate the proteins and found little diversity in the electrophoretic patterns of the storage proteins.

Ekpenyong and Borchers (1978) extracted and precipitated the winged bean proteins using combinations of water, hydrochloric acid, sodium chloride and sodium hydroxide. Their results showed maximum protein

extraction with sodium hydroxide (pH 12). The minimum point of nitrogen solubility was found to be pH 4.0, the apparent isoelectric point.

Numerous publications report information on legume proteins (in particular soybean protein) and suggest approaches applicable to winged beans. One such early paper by Smith and Circle (1938) investigated the effect of several precipitating agents on soy protein including HCl, trichloroacetic acid, H_2SO_4, and oxalic acids with Na^+ and Ca^{++} salts at various concentrations. They concluded that acid extraction was not preferable to water extraction. They also correlated the effects of pH and salt indicating that an increased salt concentration would decrease pH effects. Smith and Circle (1939) later compared the methods of electrodialysis and acid precipitation from water and alkaline media. They found 0.05 N NaOH extraction followed by H_2SO_4 precipitation gave 85% recovery of protein and showed that electrodialysis offered no advantage over acid precipitation.

Smith et al. (1966) extracted 90-95% of the total nitrogen from defatted soybean meal using water (pH 6.5-6.8) or dilute alkali (pH 7.2). In an earlier study, Smith and his coworkers (Smith and Circle, 1938; Smith et al., 1938) found water, dilute alkali (pH 7-9) and 0.5-2 M sodium chloride solutions were the most efficient solvents for extracting proteins in an undenatured state.

The preparation of protein isolates from soybean extract is made possible by acidification to pH 4.2, where protein solubility is minimal

(Smith and Circle, 1938). The soluble soybean "whey" proteins are separated from the precipitate by centrifugation or filtration. Solubility-pH relationships have also been reported for a large number of other legumes (Ayres et al., 1974; Bhatty, 1974, Fan and Sosulski, 1974; Hang et al., 1970a; Puztai, 1965).

Flink and Christiansen (1973) extracted broad bean (Vicia faba) flour at pH 8-10, centrifuged to remove the starch and other insoluble materials, and then recovered the protein by precipitation at pH 3.5. Rhee et al. (1972) dispersed ground peanuts (Arachis hypogea) in dilute alkali to give a final pH of 8.0. Screening removed the insoluble residues from the aqueous extract which was then acidified to precipitate the peanut globulins.

Hang et al. (1970a) studied the dispersing effects of pH and a variety of salt solutions on the nitrogenous constituents of mung bean (Phaseolus aureus), pea beans (Phaseolus vulgaris), and red kidney beans (Phaseolus vulgaris). Like earlier workers they obtained the best extraction yield at high pH. Alkaline salts were judged to be fairly effective dispersing agents but results depended strongly on salt concentration. Some dilute salts were suggested to have an inhibitory effect on dispersion.

Djang et al. (1953) expressed the opinion that only salt extraction should be considered in extraction of mung bean proteins as this yields protein in the least altered state. Thus, 0.1 M Na_2SO_4 or K_2SO_4 or

0.4 M NaCl were recommended for extraction of proteins. Pant and Tulsiani (1969) agreed NaCl was best for extraction, with subsequent removal of salt by dialysis of the extract. They cited strong alkaline or acid treatment as detrimental to the nutritive value of the protein.

Hang et al. (1970b) found enhanced extractability of the nitrogenous constituents of mung bean after enzyme hydrolysis with Cellulase 36 (Rohm and Haas Co., Philadelphia, Pennsylvania) from Aspergillus niger.

Chromatographic separation of soybean proteins has been reported using hydroxyl apatite (Wolf and Sly, 1964, 1965, 1967), modified polysaccharides (Rackis et al., 1959; Catsimpoolas et al., 1967, Wolf et al., 1962; Birk et al., 1963; Mitsuda et al., 1967 and gel filtration (Hasegawa et al., 1963; Obara and Kimura, 1967, Eldridge and Wolf, 1967, Koshiyama, 1965, 1968, 1969; Catsimpoolas and Leuthner, 1969).

The water extractable soybean proteins were separated into 11 fractions by Hasegawa et al. (1963) employing a Sephadex G-200 gel column. The eluted proteins were further characterized by ultracentrifugation which showed that soybean protein contained four major components (2S, 7S, 11S and 15S). Their results supported previous sedimentation studies of Wolf and Briggs, 1958; Watanabe and Nakayama, 1962; Wolf and Briggs, 1969. Obara and Kimura (1967) were able to identify the trypsin inhibitor fraction in a water-extracted soy protein using potassium phosphate-sodium chloride buffer containing 0.01M 2-mercaptoethanol to elute the soy proteins from a Sephadex G-200 column.

Smith et al. (1955) characterized the soybean proteins by moving boundary electrophoresis, separating the acid precipitated proteins into 3-5 components. Shibasaki and Okubo (1966) and Puski and Melnychyn (1968) employed starch gel electrophoresis in buffers containing dissociating solvents to separate the soybean proteins into 14 bands.

Catsimpoolas et al. (1967) employed polyacrylamide gel disc electrophoresis for characterizing the proteins of soybean. They purified the 11S protein until a single major band was obtained. The 11S protein separated into more than 12 bands when treated with guanidine hydrochloride. This methodology was later applied to the separation of soybean proteins during germination (Catsimpoolas et al., 1968), of soybean whey proteins (Catsimpoolas et al., 1969b) and hemeagglutinins (Catsimpoolas and Meyer, 1969). Vaintraub (1967) reported 18 bands on polyacrylamide gel electrophoresis of 11S protein after treatment with 6 M urea at pH 6.76. Several of the bands migrated slightly at pH 8.6.

In spite of the voluminous literature, there are still many gaps in the knowledge of proteins in soybean. However, the past studies on soybean proteins can serve as a guide to the extraction and characterization of winged bean proteins.

Lipids of Winged Bean

Winged bean seeds are rich in lipid (Table 1). Padilla and Soliven (1933) recognized that the winged beans could be a good source of oil

for commerce. Pospisil et al. (1971) and Cerny et al. (1971) reported the fatty acid analysis of oil from winged beans (Table 8). Their results show that 71% of the fatty acids are unsaturated, with oleic (39%) and linoleic (27.2%) acids predominating.

The fatty acid profile of winged beans is similar in quality to that of soybean. Cerny et al. (1971) reported that winged bean oil contains C 18:4 (parinaric acid), a potential antinutritional factor. Kleiman, however (in a personal communication to Newell and Hymowitz, 1979) found no parinaric acid, employing UV and GLC analytical procedures. Further analysis will be required to confirm the presence or absence of parinaric acid in winged beans.

Carbohydrates

There are at present no research data on the carbohydrates of winged beans other than crude fiber and total carbohydrates obtained by difference (Table 1). Since winged beans resemble soybeans in so many other respects, it is possible that the carbohydrates of winged beans are similar to those of soybeans. Dehulled, defatted soy flour was found to contain 15-18% high molecular weight polysaccharides by Aspinall et al. (1967) and about 15% soluble oligosaccharides (Kawamura et al., 1963). The oligosaccharides (verbascose, stachyose and raffinose) are important in human nutrition in that they have been reported to cause flatulence in man and animals. These three sugars escape digestion and

Table 8. Fatty acid composition of the oil of winged beans (percent composition by weight).

Fatty acid	Cerny et al. (1971)	Kleiman[a/]
14:0 Myristic acid	0.1	0.1
16:0 Palmitic acid	9.7	7.4
16:1 Palmitoleic acid	0.8	0.1
18:0 Stearic acid	5.7	2.8
18:1 Oleic acid	39.0	33.9
18:2 Linoleic acid	27.2	28.8
18:3 Linolenic acid	2.0	1.4
18:4 Parinaric acid	2.5	none
20:0 Arachidic acid	2.0	1.3
20:1 Gadoleic acid	-	4.0
20:2	-	0.1
22:0 Behenic acid	13.4	15.9
22:1 Erucic acid	-	0.7
24:0 Lignoceric acid	-	3.4

[a/] as cited by Newell and Hymowitz, 1979.

absorption in the upper gastrointestinal tract, but are microbially degraded and fermented to yield H_2 and CO_2 when they reach the colon.

Hardinge et al. (1965) and Cristofaro et al. (1974) have reported the oligosaccharide content of various legumes. Soybeans contain the highest levels of raffinose and stachyose whereas other legumes have the highest levels of verbascose. Hymowitz et al. (1972) determined the oligosaccharide content of varieties and strains of soybean to determine whether these carbohydrates can be eliminated genetically. They found that elimination of flatulence by breeding holds little promise since there is a high degree of stability of oligosaccharides in the varieties of soybeans tested.

Kawamura et al. (1977) determined the free monosaccharides and sugar alcohols present in mature soybeans using paper partition and gas liquid chromatography. The cotyledon contained glucose and fructose while the hulls contained galactose, glucose, fructose, arabinose and xylose. Sorbitol, arabinitol, xylitol and manitol were also present. The free monosaccharides and sugar alcohols were present in minute amounts (about 0.001% - 0.4% of the whole seeds). Schweizer et al. (1978) found a new disaccharide, galactopinitol, present in soybeans, chickpeas, lentils and green beans.

There is an urgent need to determine the soluble carbohydrates present in winged beans if they are to become a major food source, since winged beans could also contain flatulence-producing oligosaccharides.

Biological Value (Nutritive Value) of Winged Bean

The biological value of winged beans has been assessed in Ghana by Pospisil et al. (1971) and Cerny et al. (1971) using rats and human subjects. In rat experiments, Cerny et al. (1971) found that at a 10% protein level winged beans were superior to peanuts in terms of protein efficiency ratio (PER) and net protein utilization (NPU). The PER and NPU values obtained with winged beans were quite similar to that of soybean. An experimental diet of 3 parts corn to 2 parts winged bean resulted in PER and NPU values similar to that of skim milk.

Jaffe and Korte (1976) observed that supplementation of autoclaved winged beans with 0.3% methionine greatly increased the PER value bringing it close to that of casein. Digestibility was considerably improved by autoclaving the raw, mature winged beans. Cerny and Addy (1973) showed that winged bean milk is suitable in the treatment of children suffering from kwashiorkor.

Ekpenyong and Borchers (1978) studied the effect of processing method on fat content, protein and in-vitro digestibility. Autoclaving was found to increase the content of cysteic acid, tyrosine and tryptophan. However, lysine, leucine, histidine and arginine were found to decrease. They concluded that heat processing must be maximized to prevent protein damage. In-vitro digestibility was also found to increase by autoclaving.

Toxic Factors in Winged Beans

Winged beans were found to contain trypsin inhibitors by Sohonie and Bhandarkar (1954) who stated that the activity of acid extracted inhibitor was not affected by one hour boiling in water. Cerny et al. (1971) found that by soaking the seeds in water for 10 hours, followed by boiling in water for 30 minutes, the trypsin inhibitor activity is eliminated. Autoclaving the presoaked seeds at 130°C for 10 minutes gave the same results. When a lower temperature is used (i.e. 120°C), the antitryptic activity is reduced only by a third. Dry heat application was found to be ineffective in reducing trypsin inhibitor activity. Ekpenyong and Borchers (1978) found that autoclaving inactivates the antinutritional factors present in winged beans.

Claydon (1975) observed a 50% death rate in rat feeding experiments using uncooked raw winged beans in balanced diets. The rats showed symptoms of renal failure, possible liver cirrhosis and general malnutrition. Winged beans also contain a non-specific hemeagglutinating activity (Renkonen, 1948; Schertz et al., 1960; Bhatia and Allen, 1962; all cited by Claydon, 1975). Claydon (1975) also found cyanide (5 mg %) present in winged beans which is a non-toxic level.

Jaffe and Korte (1976) found trypsin and amylase inhibitor activity present in winged beans, as well as hemeaglutinins.

MATERIALS AND METHODS

Sample Preparation

Dried seeds of winged bean (Psophocarpus tetragonolobus (L.) DC) varieties* TPT-2, CHIMBU, WB-19, Selections 10 and 12 were used. These varieties were used because they were the most promising varieties grown in various experiment stations. TPT-2 was a gift from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Dr. W. Steele); CHIMBU was a gift from the Asia Foundation, San Francisco, California (Mr. Louis Lazaroff); WB-19 was a gift from the University of Puerto Rico, Mayaguez, Puerto Rico (Dr. F. W. Martin); Selections 10 and 12 were procured from 27 Farms, Homestead, Florida.

The dried winged bean seeds were ground in a Wiley Laboratory Mill (Arthur H. Thomas, Philadelphia, Pennsylvania) to pass through a 40 mesh sieve. For the purpose of analyzing the seed coat and endosperm separately, winged beans (TPT-2) were soaked in water for 16 hours, cracked, and dried in an oven at 100°C for 30 minutes. The seed coat

---

*WB-19 is the accession number assigned for TPT-12 (IITA); Selections 10 and 12 are the numbers assigned to UPS 76 and UPS 105, respectively and originally came from Papua New Guinea.

was separated from the cotyledon with the aid of a stainless steel scalpel. The seed coat and the cotyledon were then ground separately in the Wiley Laboratory Mill to pass through a 40 mesh sieve.

Proximate Analysis of Winged Bean Flour and Seed Fractions

Crude protein, crude fiber, ash, moisture, and crude fat analyses were performed on the whole flour, dehulled flour and seed coat fractions of winged beans. Methods used were those given in the Official Methods of Analysis of the AOAC (1975) for analysis of soy flour (pp. 234). Total nitrogen was determined by micro-Kjeldahl (pp. 937) as described also in AOAC (1975) except that 100 ml digestion flasks were used. Both full fat and fat-free samples were analyzed for crude protein. Protein was calculated as N x 6.25 for winged beans.

Acid detergent fiber and neutral detergent fiber were determined according to the methods of Van Soest (1963a, 1963b) as described in the AOAC (1975) and by Southgate (1976).

Nitrogen Solubility

The nitrogen solubility of winged bean flour (TPT-2) was determined at room temperature (20°C) using the combined methods of Hang et al. (1970a) and Mattil (1971). Two and a half grams of sample were dispersed in 85 ml distilled water. The dispersions were stirred

continuously with a magnetic stirrer for 30 minutes and the pH frequently checked (every 5 min.) and adjusted with 0.5 N HCl or 0.5 N NaOH. A Corning pH meter Model No. 110 (Corning, Medfield, Massachusetts) was used for all pH determinations and adjustments. After final pH adjustment, the volume was adjusted to 100 ml to give a solvent:flour ratio of 40:1 (v/w). The solution was centrifuged at 1465 xg for 20 min. at 4°C. The supernatant fluid was filtered on a Whatman No. 1 V-folded filter paper. Ten ml aliquots were used for total nitrogen determination by the micro-Kjeldahl method (AOAC, 1975). Nitrogen solubility tests were made at pH 1, 2, 3, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, and 12. Calculations were based on the supernatant in relation to the total nitrogen present in the winged bean flour. The results were expressed as per cent soluble nitrogen, i.e., per cent soluble nitrogen at a given pH.

Extraction and Isolation of Proteins

Extraction Procedure

The schematic outline for preparing the protein extract is shown in Figure 1. Five grams of hexane-defatted, ground winged beans and 200 ml of extracting solution were placed in a 300 ml Erlenmeyer flask and shaken in a Lab-Line Orbit Environ-Shaker (Lab-Line Industries, Inc., Melrose, Park, Illinois) at 220 rpm for 1 hour at 25°C. The dispersions were transferred to 250 ml polyethylene centrifuge bottles

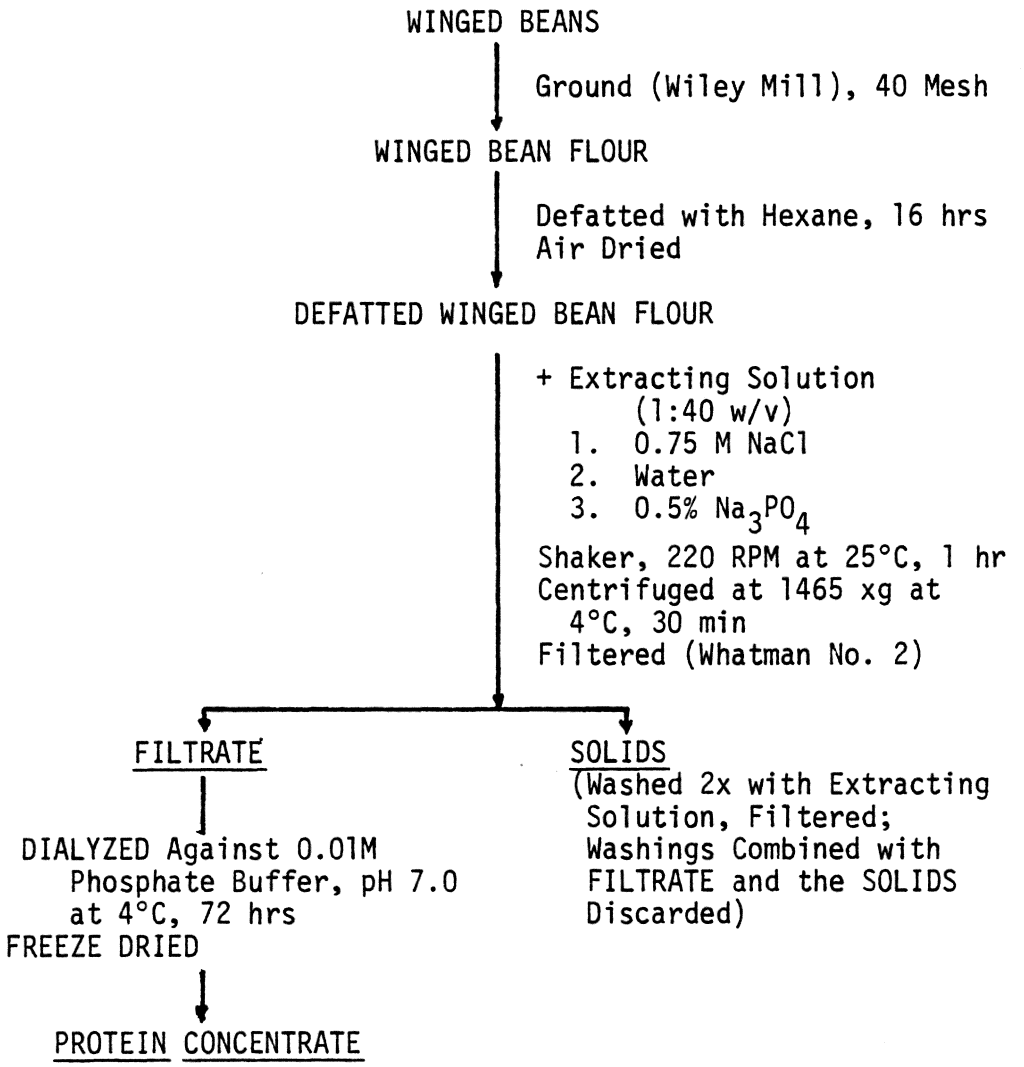

FIGURE 1. Schematic Outline for the Preparation of Protein Extract From Winged Bean Flour.

and centrifuged in a Sorvall RC 2B (Sorvall Inc., Newtown, Connecticut) employing a GSA head at 1465 xg at 4°C for 30 minutes. The supernatant was filtered through a Whatman No. 2 fluted filter paper. The solids were washed twice with 50 ml of extracting solution and the mixture filtered. The washings were combined with the original filtrate and the solids were discarded. The protein extract was dialyzed against 0.01M phosphate buffer, pH 7.0 for 3 days with 3 changes of buffer every day at 4°C. The dialyzed protein extract was freeze dried in a Virtis mobile freeze dryer, Model 10-145 MR-BA (Virtis, Inc., Gardiner, New York) to obtain the protein concentrate.

In the protein extractability (solubility) studies, the washing operation was omitted and ten ml aliquots of the filtrate were analyzed for protein (% N x 6.25) by the micro-Kjeldahl method (AOAC, 1975).

The extracting solutions used were NaCl (pH 6.95), Na_2SO_4 (pH 7.20), Na_2HPO_4 (pH 9.25), 0.5% Na_3PO_4 (pH 11.0) and deionized distilled water (pH 6.70). NaCl and Na_2SO_4 were used at concentrations of 0.01 M to 1.0 M. Na_2HPO_4 solutions were used at concentrations of 0.01 M to 0.5 M.

Preparations of Acid Soluble and Acid Insoluble Proteins

To the filtrate plus washings obtained from protein extraction (Fig. 1), 0.5 N HCl was added to effect precipitation. Precipitation

was considered complete when the solution pH reached pH 4.0. The precipitated protein (acid insoluble) was separated by centrifugation at 1465 xg using a Sorvall RC 2B refrigerated centrifuge for 30 min. at 4°C. The supernatant fluid was filtered using a Whatman 40 V-folded filter paper. The precipitate was dissolved by dispersing in water and adding 0.5 N NaOH until the pH of the protein solution was 7.0. The protein was re-precipitated by adding 0.5 N HCl to pH 4.0. This operation (dissolving and precipitation of the proteins) was repeated twice. Finally, the precipitated protein was again dissolved by dispersion in water and addition of 0.5 N NaOH to pH 7.0. The solubilized protein was dialyzed against 0.1 M phosphate buffer, pH 7.0 at 4°C for 3 days with 3 changes of buffer every day and freeze dried.

The filtered supernatant solution (containing the "whey" or acid soluble proteins) was also dialyzed against 0.01 M phosphate buffer, pH 7.0 at 4°C for 3 days with 3 changes of buffer every day, and freeze dried. The method is shown schematically in Figure 2.

Amino Acid Analyses

Fat-free samples of the five varieties of winged bean flour and winged bean protein extracts (from winged bean variety TPT-2) extracted with water, 0.75 M NaCl and 0.5% Na_3PO_4, respectively and their respective isoelectric precipitated protein and "wheys" were analyzed for amino acid content as outlined in the Technicon Operation Manual (1973).

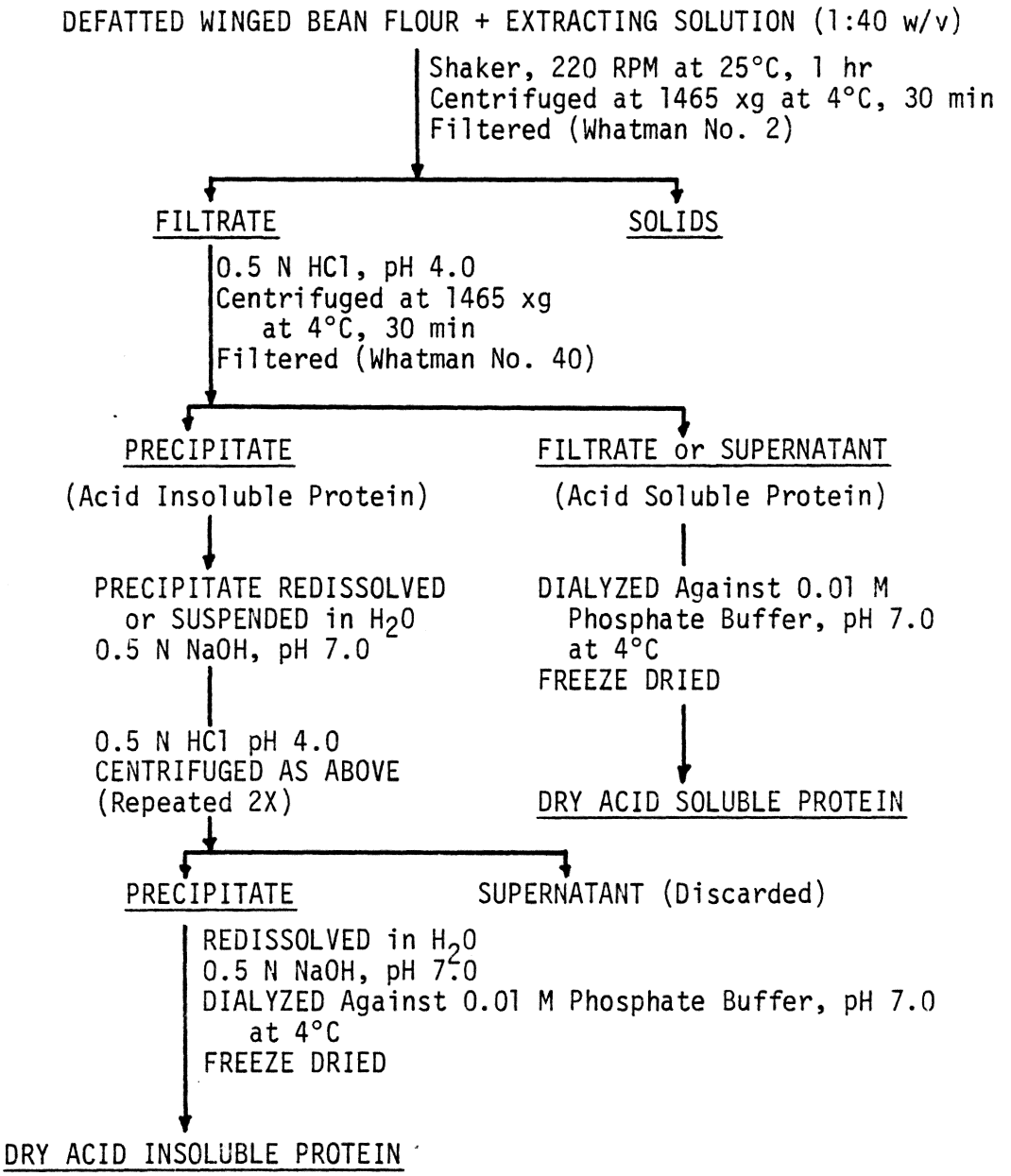

FIGURE 2. Schematic Outline for the Preparation of the Acid Insoluble (Precipitate) and Acid Soluble Protein ("Whey") Fractions From Winged Bean Flour.

Samples containing about 35-40 mg protein were hydrolyzed with 10 ml of 6 N HCl for 24 hours at 110°C in sealed ampoules. The hydrolysate was filtered through glass wool. Two ml of the amino acid solution was freeze dried and dissolved in 5 ml of cartridge buffer solution (pH 2.00). A 50 µl aliquot was analyzed on the Technicon Amino Acid Analyzer Model-TSM (Technicon Instruments Corp., Tarrytown, New York). Separation of amino acids was by ion-exchange chromatography. A short (8 x 0.6 cm ID) column containing Type 3C Chromobeads resin was used for the separation of the basic amino acids. The acidic amino acids were separated on a longer (25 x 0.6 cm ID) column using the same resin. Quantitation was based on the ninhydrin reaction. Calculations were accomplished manually using the Height-Width (triangulation) method of determining the area under the curve. No analysis for tryptophan was done, because of destruction by the hydrolysis procedure employed.

Polyacrylamide Disc-Gel Electrophoresis (PAGE)

Anodic disc-electrophoresis was carried out essentially as described by Davis (1964). This involved an electrophoresis system consisting of a 7.5% acrylamide separating gel (pH 8.9), 2.5% acrylamide stacking gel (pH 6.7), a tris-glycine electrode buffer, pH 8.3, and 10.0 x 0.6 cm ID tubes. An ISCO Model 1270 electrophoretic apparatus (ISCO, Lincoln, Nebraska) was used along with a Gelman power supply Model No. 38520 (Gelman Instrument Co., Ann Arbor, Michigan) at a constant current of

2 ma per gel for the first 30 min. and a constant current of 4 ma per gel through the end of the run. The voltage did not exceed 300 V. The samples were applied to the gels on an equal protein basis (50 µg protein). After the electrophoresis (3 to 4 hours) the gels were stained with 1% Amido black in 7% acetic acid for about one hour, and were subsequently destained with 7% acetic acid in BioRad diffusion destainer (BioRad, Richmond, California). The destained gels were appraised visually for the number and intensity of components and their relative mobilities were computed relative to the tracking dye (bromphenol blue). All reagents used in the electrophoresis were obtained from BioRad.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The molecular weights of the protein subunits present in the total extracts, precipitates and wheys obtained from the three extracting solutions (water, 0.75 M NaCl and 0.5% Na_3PO_4) were determined using the method of Weber and Osborne (1969). Briefly, this involved an electrophoresis system in 5% acrylamide containing 0.1% SDS and 0.1 M sodium phosphate buffer, pH 7.0 using 10.0 x 0.6 cm ID tubes. The electrode buffer consisted of 0.1% SDS-0.1 M sodium phosphate, pH 7.0. An ISCO Model No. 1270 electrophoretic apparatus (ISCO, Lincoln, Nebraska) was used along with a Gelman power supply Model No. 38520 (Gelman Instrument, Co., Ann Arbor, Michigan) at a constant current of

5 ma per gel. The samples were applied at a level of 20 μg protein/gel. After electrophoresis (9 to 10 hours), the gels were stained in 0.25% Coomasie brilliant blue (1.25 g Coomasie brilliant blue in 454 ml 50% methanol and 46 ml glacial acetic acid) for 12 hours. The gels were then destained in 14% acetic acid- 7% methanol-water in a BioRad diffusion destainer (BioRad Laboratories, Richmond, California). The mobility of the proteins in the destained gels were calculated as

$$\text{mobility (M)} = \frac{\text{distance migrated by protein}}{\text{distance migrated by tracking dye}} \times \frac{\text{length of gel before staining}}{\text{length of gel after staining}}$$

Prior to loading of the samples in the gel, the proteins were incubated at 37°C for 2 hours in 1% SDS, 1% mercaptoethanol, 5M urea and 0.01M sodium phosphate, pH 7.0. The molecular weight standards (mixtures of myosin, β-galactosidase, phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozme- BioRad Laboratories, Richmond, California) were incubated in the same dissociating solution, and separated in the same electrophoretic system.

Gel Chromatography on Biogel A- 1.5 m

Materials

Biogel A- 1.5m (BioRad Laboratories, Richmond, California), 100-200 mesh, is an agarose gel in the form of spherical beads. Biogel A- 1.5m was used because higher flow rates can be achieved as compared to Sephadex gels (especially Sephadex G-200) which tend to compress, thus limiting the flow rates. The agarose gel was used for the chromatographic separation of the total protein extracts (obtained from aqueous, 0.75 M NaCl and 0.5% Na_3PO_4 extractions) and of the acid-insoluble precipitate and acid-soluble proteins from the aqueous extract.

Sample preparation

The freeze dried proteins were prepared for gel chromatography by dissolving them in 0.01 M phosphate buffer, pH 7.0 at a protein concentration of 10 mg/ml, followed by dialysis at 4°C against 0.01 M phosphate buffer, pH 7.0 for 2 days. The protein content of the dialyzed samples was determined using the micro-Kjeldahl method (AOAC, 1975).

Packing and operation of column

Biogel A- 1.5m was packed in a 25 cm ID Sephadex column SR 25 (Pharmacia Fine Chemicals, Piscataway, New Jersey) to a bed height of 35 cm using the procedure given in the BioRad catalog (1978). The void

volume of the column was determined to be about 55 ml by eluting 1 ml of 0.2% blue dextran solution. The actual buffer in which the proteins had been dialyzed was used to elute the proteins from the gel column, in order to ensure a uniform ionic strength. For each run, protein samples were applied at equal protein load (44 mg). Elution of proteins in the column was at 25°C. The column was equilibrated with 300 ml of the dialysate prior to addition of the protein samples.

A Fractomette Alpha 400 Model 3-4700 (Buchler Instruments, Fort Lee, New Jersey) was used to collect the fractions eluted from the gel column. The fractions (5 ml) were delivered to the collector with an ISCO Volumeter Model 400 (ISCO, Lincoln, Nebraska). The eluate was continuously monitored at 280 nm using a Fracto-Scan (Buchler Instruments, Fort Lee, New Jersey) and the elution pattern recorded on a dual channel Fisher Recordall Series 5000 (Fisher Scientific Company, Raleigh, North Carolina). Selected fractions were pooled and the protein recovered by freeze drying.

Carbohydrate Analysis (Soluble Carbohydrates)

Sample Preparation

Five grams of ground, defatted winged beans were refluxed in 50 ml 85% ethanol (AOAC, 1975) for 30 min. in a boiling water bath. The ethanol extract was filtered through Whatman 541 V-folded filter paper. The residue was washed 5X with 10 ml volumes of 85% ethanol, the

mixture filtered after each wash and the washings combined with the filtrate. The ethanol was allowed to evaporate in the hood until the sample was dry. The dry sample, which contains the soluble carbohydrate, was made up to 10 ml with water, mixed and filtered through a 0.4 μm pore diameter membrane filter to remove any insoluble material. The filtered sample was deionized on a 10 x 0.7 cm ID column containing a mixed bed ion exchange resin (AG 501 X-8(D), 20-50 mesh, BioRad Laboratories, Richmond, California), with a bed height of 5 cm. The top was layered with a 1 cm bed height of 1:1 mixture of AG 50W -X8, H^+ form, 200-400 mesh and AG 3-X4A, OH^- form, 200-400 mesh (BioRad Laboratories) to give a slow flow rate. The sample was deionized in 1 ml increments, the first 3 ml discarded and the rest collected in a scintillation vial and frozen when not in use.

HPLC standards

Verbascose, stachyose, raffinose and sucrose were dried at 60°C in 26 inches (99 mm) Hg of vacuum for 16 hours. Standard solutions of these carbohydrates (except verbascose) in water were prepared at concentrations ranging from 20 mg/ml to 50 mg/ml. Dr. D. K. Salunkhe (Utah State University) kindly supplied 20 mg of verbascose (68% purity) while the other sugars were procured from Sigma Chemical Co., St. Louis, Missouri. A mixed standard solution was prepared, containing verbascose at a concentration of 5 mg/ml and the other sugars at 10 mg/ml. Standard

solutions of individual sugars (except verbascose) were also prepared at a concentration of 10 mg/ml. The standard solutions were kept frozen when not in use.

Equipment

The oligosaccharides and sucrose were examined by high performance liquid chromatography (HPLC). A Model ALC 201 chromatograph was equipped with a Model 6000 A solvent delivery system, a model R- 401 Refractive Index Detector, a Model 440 UV absorbance detector and a Model U6K Injection System (Waters Associates, Milford, Massachusetts). All data were recorded with a dual channel Omni-Scribe recorder (Houston Instruments, Austin, Texas).

Packing of HPLC column

Aminex 50 W X-4, H^+ (BioRad Laboratories, Richmond, California) was converted into the Ca^{++} form as described by Scobell et al. (1977) and the BioRad Catalog (1978). The Ca^{++} form resin was packed in 30.0 cm x 0.5 cm ID stainless steel precision bore column with a 10 μm pore-size fritted disc end fitting. An aqueous slurry of the resin was continuously stirred in a Micromeritics column packer Model 705 (Micromeritics Instrument Corp., Norcross, Georgia) and pumped into the column at 1.5- 2.0 ml/min. with a Milton Roy pump, at a maximum pressure of about 2000 psi.

Separation and quantitation

The samples and the standards were injected in 10 μl volume. The samples and standards were eluted with deionized water which had been filtered in a 0.45 μm membrane filter. The column length used was 60 cm (two 30 cm columns) and was operated at a flow rate of 0.50 ml per min. The column was equipped with an aluminum water jacket and operated at 85°C, employing a Haake FS circulating water bath (Haake, Saddlebrook, New Jersey).

The soluble sugars in winged beans were tentatively identified from the elution times relative to the individual standard sugars. Confirmation of identities was obtained by thin layer chromatography on phosphate treated plates (Supelco, 1977) of individual sugars collected during HPLC. A periodate-p-anisidine hydrochloride solution was used for detection of the sugars (Hough and Jones, 1962). Quantification of the sample components were achieved from HPLC peak area measurements (Height (H) x Width at half height ($W_{1/2\ H}$) relative to the peak areas of the standard.

Carbohydrates (Starch Determination)

Staining in I_2-KI solution was used as a qualitative test for the presence of starch. The qualitative tests were confirmed by employing the method of Palmer (1978) which was used for starch determinations in plantains. To 100 mg of the air dried residue of winged beans from the

soluble carbohydrates determination, was added 3 ml of 0.05 M NaAc buffer, pH 5 in a 15 ml centrifuge tube. The mixture was stirred with a magnetic bar to suspend the sample uniformly. The mixture was then heated in a boiling water bath for 5 min. and cooled. Two ml of glucoamylase solution (prepared by stirring Sigma Type II glucoamylase in 50 ml cold NaAc buffer for 10 minutes, centrifuging at 15,000 xg for 15 min. at 4°C and recovering the supernatant enzyme solution) was added to the mixture. The solution was mixed and incubated at 25°C for 1 hour. The solution was then made to volume with distilled water, filtered in a 0.45 μm pore size membrane filter and deionized as described in the oligosaccharide analysis. Analysis of glucose was performed as described in oligosaccharide analysis except that a glucose standard (10 mg/ml) was used. Mg glucose x 0.90= mg starch.

Fatty Acid Analysis

Sample preparation

The fatty acids of the five varieties of winged bean were analyzed by the Pesticide Residues Laboratory, VPI&SU, (Prof. R. W. Young). Approximately 150 mg of the lipids (prepared by ether extraction of vacuum dried winged bean flour) were methylated in a 50 ml volumetric flask with 10 ml methanolic NaOH, heated in a steam bath for 2 to 5 min., after which 5 ml of BF_3-methanol was added. The mixture was boiled for 2 min. Five ml of distilled hexane was added to the flask and the

mixture was boiled for 1 min. Saturated NaCl was added to bring the liquid level into the neck of the flask. The upper layer (hexane) was pipetted into a glass stoppered centrifuge tube. The volume was adjusted to 1 ml by the addition of hexane or by evaporation of hexane under a stream of nitrogen gas. Anhydrous sodium sulfite (0.10 g) was added to bind traces of water. One μl of this solution was injected into a gas chromatograph.

Gas chromatography

A Bendix gas chromatograph (Bendix Process Instruments Division, Ronseverte, West Virginia) Model 2600 was used with a flame ionization detector (FID). A 4 mm OD x 122 cm glass column was packed with 15% diethylene glycol succinate (DEGS) in 80-100 mesh Chromosorb W AW.

The detector was set at 255°C and the injector port at 190°C. Air pressure to the hydrogen flame detector was held at 600 ml/min., hydrogen at 30 ml/min., and helium at 90 psi to maintain a constant flow rate of 9 ml/min.

The fatty acids found in the fat sample were identified and quantified with reference to known concentrations of known fatty acids. Fatty acid standards were obtained from Supelco, Inc. (Bellefonte, Pennsylvania). Relative percentages of the fatty acids were calculated by using integrator counts (Spectra-Physics Minigrator, Spectra-Physics, Santa Clara, California) adjusted to a standard attenuation.

RESULTS AND DISCUSSIONS

Proximate Analysis of the Winged Bean

The results of the proximate analyses (Table 9) of the five varieties of winged beans showed that the protein content ranged from 38.06% for WB-19 to 41.25% for TPT-2 while the fat content ranged from 15.44% for TPT-2 to 18.46% for CHIMBU all on a moisture-free basis. These results compare favorably with the previous work shown in Table 1. The protein and fat content are similar to commercial varieties of soybeans in the U.S. which contain an average of 40.5% protein and 20% fat.

In more recent studies not included in Table 1, Harding et al. (1978) analyzed the protein and oil content of 32 winged bean accessions that were grown in Puerto Rico. Based on one planting, they found the protein content of the winged bean to vary from 29.2% to 40.9% with a mean protein content of 34.25%. The oil content was found to vary from 14% to 19%. The varieties TPT-2, CHIMBU, and WB-19 (originally TPT-12) were included in their studies. The results in the present study cannot be compared directly with those of Harding et al. (1978) because of the absence of moisture data for the Harding et al. samples. Ekpenyong and Borchers (1978) found that TPT-2 beans contained 40.07% protein and 17.67% fat (dry weight basis), which is similar to the values obtained in this study.

Table 9. Proximate chemical composition of the five varieties of winged beans (dry weight basis)[a/]

Analysis[b/]	Variety				
	TPT-2	WB-19	Sel. # 10	Sel. # 12	CHIMBU
Protein	41.25	38.06	41.16	38.95	41.20
Fat	15.44	18.00	15.92	16.87	18.46
Ash	4.16	4.66	4.76	4.69	4.05
Carbohydrates[c/]	31.44	32.04	31.10	32.21	29.80
Crude fiber	7.71	7.24	7.06	7.28	6.49
(Acid detergent fiber)	(13.85)	(13.21)	(13.06)	(12.84)	(12.27)
(Neutral detergent fiber)	(17.18)	(17.00)	(16.05)	(15.77)	(14.29)

Source of winged beans: TPT-2 (IITA, Ibadan, Nigeria), WB-19 (Puerto Rico, Selections 10 and 12 (Florida), and Chimbu (Asia Foundation).

[a/] Average of 3 analyses.

[b/] Percent moisture of seed samples: TPT-2, 9.42%; WB-19 11.62%; Selection 10, 11.22%; Selection 12, 11.38%; and Chimbu, 8.19%.

[c/] Calculated by difference.

The earlier data plus the results in Table 9 shows that the protein content of winged beans is higher than other food legumes (dry beans, pigeon peas, cowpeas, chickpeas, broad beans and peas) recommended by the Protein Advisory Group of the United Nations (PAG) in 1973 for intensified research in relation to human nutrition and food technology. These legumes contain 22-25% protein (Esh and Som, 1952; Lal et al., 1963; Singh et al., 1968; Oyenuga, 1966). Three of these legumes (cowpeas, chickpeas and pigeon peas) and mung beans were also identified by Araullo (1974) as possessing the potential for increased food utilization in Asia because they are well accepted in the diet.

The distribution of nutrients in the hulls (seed coat) and the cotyledon (endosperm) was studied in variety TPT-2. The cotyledon was found to constitute 84.1% and the hulls 15.9% of the dry weight of the seed. Table 10 shows the proximate composition of the isolated seed coat and the cotyledon. The cotyledon contained the greater portion of the protein and fat of winged beans. The hulls contained most of the fiber. Kordylas (1978) separated winged beans into seed coat and grits (cotyledon). Neither the variety nor the relative contribution of the seed coat and hulls were reported. He found the grits to contain 44.3% protein and 8.8% fat (calculated on a dry weight basis) while the seed coat contained 13.0% protein and 1.3% fat. These results are similar to the results in Table 10 for TPT-2.

Table 10. Proximate analysis of winged beans (TPT-2) and seed fractions (dry weight basis)[a/].

Analysis[b/]	Whole beans	Dehulled beans*	Hulls*
Protein	41.25	50.13	10.18
Fat	15.44	17.65	0.99
Ash	4.16	3.98	2.13
Carbohydrates[c/]	31.44	25.53	44.17
Crude fiber	7.74	2.71	42.53
(Acid detergent fiber)	(13.85)	(10.77)	(57.48)
(Neutral detergent fiber)	(17.18)	(12.86)	(71.52)

* Dehulled beans constitute 84.1% of the bean and the hulls constitute 15.9% of the bean.

[a/] Average of three analyses.

[b/] Percent moisture of samples: whole beans, 9.42%; dehulled beans, 5.51%; and hulls, 4.80%.

[c/] Calculated by difference.

The crude fiber content of the five varieties of winged beans varied from 6.49% (CHIMBU) to 7.28% (Selection 12). The average value of the crude fiber (7.16%) is higher when compared to previous data (5.42% on a dry weight basis) obtained through 1975 but were significantly lower when compared to the three values reported from 1976 (Table 1). Ekpenyong and Borchers (1978) also analyzed winged beans (TPT-2) for crude fiber. Their results (11.89%) do not agree with the crude fiber (7.71%) obtained in this analyses. The wide difference may be attributed to difference in experimental techniques and strength of alkali and acid used since AOAC methods were employed in both of the analyses.

The ash content of winged beans is similar to the previous values reported (Table 1). The carbohydrate content (obtained by difference) of the winged beans was within the ranges of values previously reported (Table 1).

The neutral and acid detergent fibers were analyzed in the five varieties of winged beans because they provide a more reliable estimate of the digestibility than crude fiber (Goering and Van Soest, 1970). Acid detergent fiber (ADF) estimates cellulose and lignin. Neutral detergent fiber (NDF) estimates the total cell wall material. The difference between NDF and ADF estimates hemicelluloses. The NDF values of winged beans ranged from 14.29% to 17.18% (mean, 16.06%) while the ADF varies from 12.27% to 13.85% (mean, 13.05%). The NDF is thought

to give a reliable estimate of the total fiber in a vegetable foodstuff which are available for microbial fermentation in the gut. There are no detergent fiber data on soybeans or other legumes.

Some variation in the chemical composition of winged bean seeds can undoubtedly be attributed to agronomic factors (soil, climate, moisture, etc.) as well as to varietal differences. Cartter and Hopper (1942) studied the influence of variety, environment, and fertility level on the chemical composition of soybeans. They concluded from their results that the chemical composition of soybeans (in particular the protein content) resulted primarily from two factors: agronomic (locality and environment) and variety. Varietal differences exert a greater effect on protein content. Additional data will be required to establish the extent and source of the variability in winged beans.

Nitrogen Solubility

Effect of pH

The water-soluble nitrogen in defatted winged bean flour (TPT-2) was determined at pH values 1 to 12 (Figure 3). The solubility was lowest at pH 4.0 and it increased dramatically as the pH was adjusted to either more acid or more alkaline values. Thus, test runs at pH 1.0 and pH 12.0 yielded the highest per cent soluble nitrogen (about 76% and 92% respectively). In general, pH values less than 2 and greater than 6 yielded 50% or more soluble nitrogen. The soluble nitrogen at

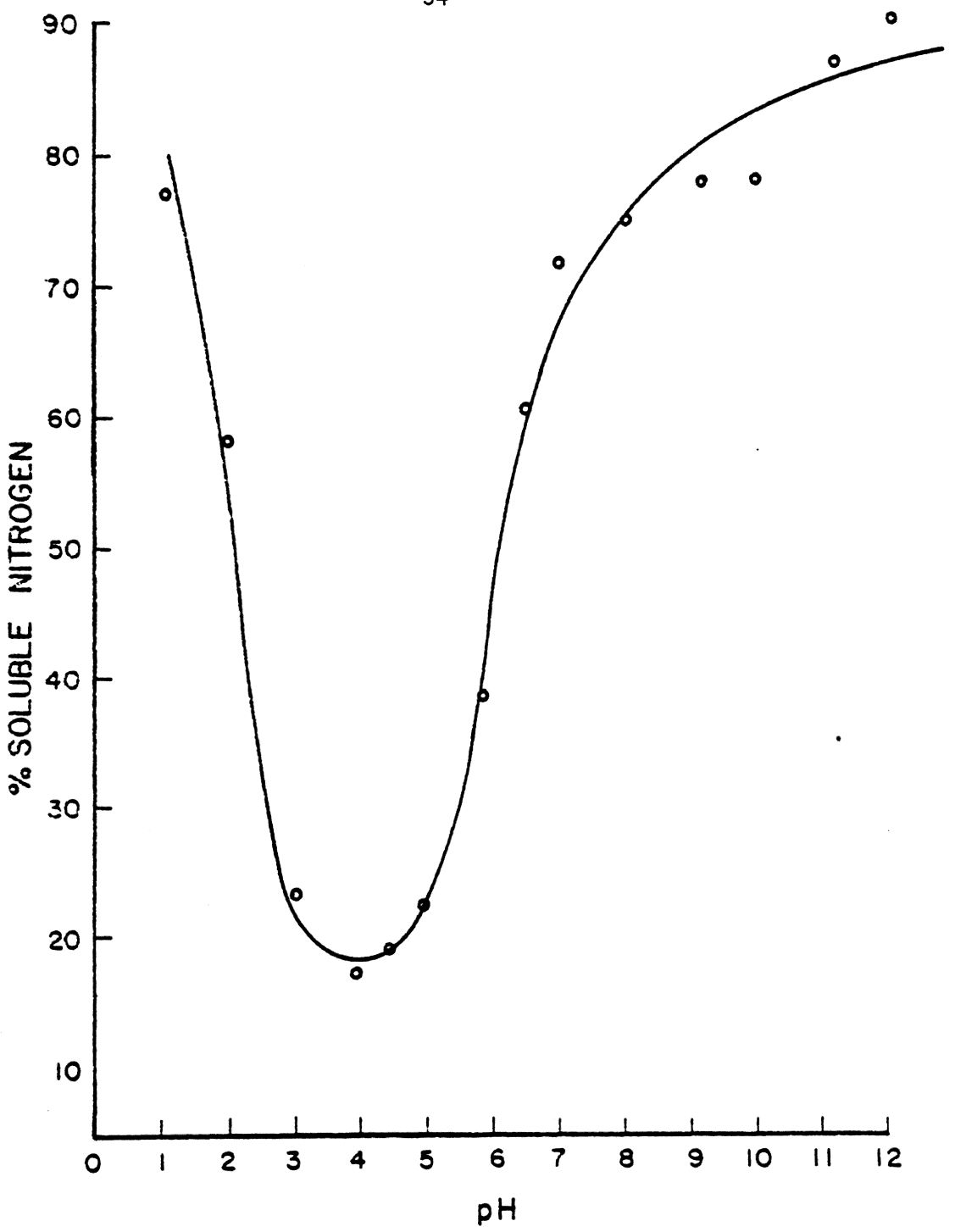

Figure 3. Nitrogen solubility of winged bean proteins as a function of pH at 25°C ($\mu \leq 0.0012$)

pH 4.0 (the apparent isoelectric pH of the major winged bean proteins) was about 17.6%. Extraction with water at neutral pH (pH 6.70) yielded 60.4% soluble nitrogen. Ionic strength during this study did not exceed 0.0012. At somewhat higher ionic strengths, Gillespie and Blagrove (1978) found 80% of the winged bean proteins soluble from pH 5 to 9.

The results in Figure 3 indicate that nearly 90% of the nitrogeneous constituents can be extracted by dilute NaOH at pH 11-12 and that a major portion of this soluble nitrogen should be precipitated by reducing the pH to 4.0.

These findings compared favorably with the results of Ekpenyong and Borchers (1978). They observed the lowest solubility (19.5%) to occur at pH 4.0 and the highest solubility (90.0%) at pH 12.0. The ionic strengths of the solutions used were not mentioned. The results of the present study do differ somewhat with the findings of Gillespie and Blagrove (1978), who reported that the minimum solubility of winged bean proteins (about 8%) was found at pH 4.5 at low ionic strengths. The difference in results could probably be attributed to differences in the ionic strengths of solutions used in each study.

The pH-solubility curve obtained from winged beans is also similar to that obtained by Hang et al. (1970a) for mung beans, pea beans, and red kidney beans. The protein of these beans all had minimum solubility at pH 4.0. It is also similar to the pattern for soybeans

as described by Smith and Circle (1938), except that soy protein had a minimum solubility at pH 4.2. Puztai (1965) and Evans and Kerr (1963) have shown similar patterns of pH nitrogen solubility with kidney beans and navy beans.

It should be noted that the soluble nitrogen extracted from winged beans is essentially protein nitrogen. The total nitrogen content of the defatted TPT-2 winged bean flour was found to be 7.57%. Trichloroacetic acid (TCA) insoluble nitrogen was found to be 6.96%. Thus, 92% of the total nitrogen was protein nitrogen (TCA insoluble nitrogen) and these solubility curves reflect closely the solubility of winged bean proteins.

Effects of salts

Aqueous NaCl, Na_2SO_4 and Na_2HPO_4 solutions in the range of concentrations from 0.025 to 1.0 M were used for extracting proteins from winged beans. The results (Table 11 and Figure 4) show that when NaCl solutions (pH 6.95) were used, minimum solubility in winged bean protein nitrogen occurred at 0.075 M. As the concentration was increased, increased solubility was observed until a maximum solubility was reached at 0.75-1.0 M. With Na_2SO_4 (pH 7.20), minimum solubility occurred at 0.025 M, after which the solubility increased to a maximum at 0.25 M. Increasing the salt concentration above 0.25 M resulted in a sharp decrease in its solubilizing power. Na_2HPO_4 (pH 9.25) showed

Table 11. Comparative extraction of soluble nitrogen from winged beans (TPT-2) with NaCl, Na_2SO_4 and Na_2HPO_4.[a/]

Concentration	Percent Soluble Nitrogen[b/]		
(M)	NaCl	Na_2SO_4	Na_2HPO_4
0.010	57.7	58.1	60.0
0.025	52.0	55.0	60.9
0.050	48.2	56.3	62.1
0.075	47.8	60.1	63.7
0.100	49.0	62.4	64.5
0.200	-	-	66.0
0.250	60.4	68.8	-
0.300	-	-	66.0
0.400	-	-	64.9
0.500	68.6	67.1	64.7
0.750	70.3	62.4	-
1.00	70.9	55.9	-

[a/] Average of three analyses.

[b/] Total nitrogen of defatted winged beans, 7.57%. TCA soluble nitrogen in defatted winged beans, 0.61%.

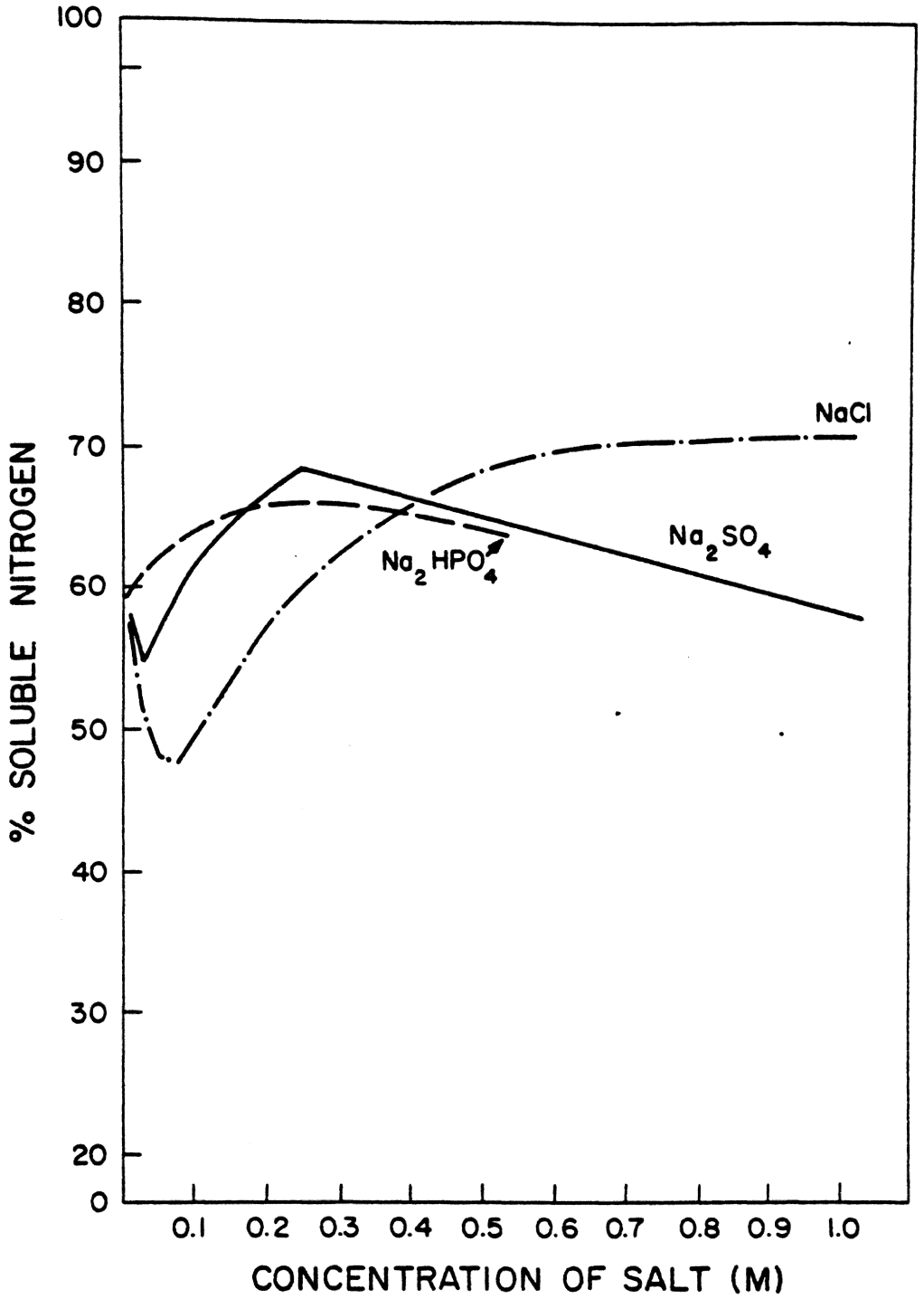

Figure 4. Effect of salt concentration on the nitrogen solubility of winged bean proteins at 25°C.

maximum solubility at 0.20-0.30 M. Decreased solubility was observed at concentrations above or below 0.20-0.30 M. NaCl solutions (0.75-1.0 M) extracted more protein nitrogen from winged beans than Na_2SO_4 and Na_2HPO_4 solutions.

Na_3PO_4 (0.5%) at pH 11.0 was observed to give the highest extraction (86.0% soluble nitrogen) of the four salt solutions used in this study. This was expected because of the alkaline pH of 0.5% Na_3PO_4. The previous pH-solubility study (Figure 3) showed that more protein nitrogen is solubilized at alkaline pH. Ekpenyong and Borchers (1978) have shown that the most effective solvents for extracting winged bean proteins are 0.10 M NaOH (pH 12.4) and water adjusted to pH 10.0. However, extraction at high alkaline conditions may present protein peptization problems. It is also likely that the proteins will dissociate and reassociate to form large protein polymers, which are different from the native proteins present in winged beans.

The results of this study are in agreement with Gillespie and Blagrove (1978), who also found that winged bean proteins are soluble to the extent of 60% at pH 6.6 at low ionic strengths. The data above on nitrogen solubility on winged beans are similar to those reported on other legume seeds (Puztai, 1956; Smith et al., 1959; Shemer et al., 1973; Thompson et al., 1976; Padhye and Salunkhe, 1977). Soybean proteins were found to be efficiently extracted with water, water plus dilute alkali (pH 7-9), and NaCl solutions (0.5-2.0 M) by Smith

and Circle (1938) and Smith et al. (1938, 1966). They found these extracting solutions to be mild and yield the soy proteins in the undenatured form. Hang et al. (1970a) found that protein extraction for mung beans, pea beans and red kidney beans was favored at alkaline pH extraction.

Isolation of Proteins

From the nitrogen solubility studies, 0.75 M NaCl, water and 0.5% Na_3PO_4 were chosen for further studies on the preparation of the acid insoluble proteins (precipitate) and the acid soluble proteins ("wheys"). NaCl and water were chosen because they are mild extractions as well as efficient protein dispersing solvents compared to Na_2SO_4 and Na_2HPO_4. Na_3PO_4 (0.5%) was also chosen to study the effect of alkaline extraction on the proteins.

The isolation of the precipitate and the "whey" were carried out as outlined in Figure 2. The apparent isoelectric point of pH 4.0 was chosen based on the pH-nitrogen solubility profile of winged beans (Figure 3). Table 12 shows the yields of the precipitates and "wheys" obtained with the three extracting solvents employed.

Of the three extracting solvents, 0.5% Na_3PO_4 was the most efficient yielding 34.9 g of crude protein from 100 g defatted winged bean flour. Water and 0.75 M NaCl gave approximately the same yields of protein from the total extracts (29.9% and 30.3% respectively). The yield of

Table 12. Comparative yields of protein concentrates and isolates (acid precipitates) and "wheys" employing three extracting solvents, g/100g defatted bean flour (dry weight basis)[a/]

Extracting solvent	weight of freeze dried protein[b/] (g/100g)		
	Precipitate	"whey"	total extract
Water	13.8	8.3	29.9
0.75 M NaCl	1.8	20.4	30.3
0.5% Na_3PO_4	21.8	9.5	34.9

Precent total protein of defatted flour, 47.3 (total N X 6.25). Percent TCA insoluble protein in defatted flour, 43.5.

[a/] Average of three trials.

[b/] Percent protein of concentrates isolates and "wheys".
Water: total extract, 76.25%; acid precipitate, 86.46%; and "whey", 40.39%.

NaCl: total extract, 79.57%; precipitate, 73.46%; and "whey", 73.63%.

Na_3PO_4: total extract, 76.24%; precipitate, 83.20%; and "whey", 55.42%.

acid precipitate was highest for 0.5% Na_3PO_4 (21.8%) followed by water (13.8%). The NaCl extract yielded the least amount of precipitate (1.8%). The solubility of the bean proteins must be enhanced by 0.75 M NaCl, even at low pH (pH 4.0). Gillespie and Blagrove (1978) observed this during acetate-chloride extraction of winged bean proteins at pH 4.5. Hence, most of the proteins (20.4%) in the NaCl extract were found to be present in the "whey". The amount of "whey" protein recovered from water and 0.5% Na_3PO_4 extraction were 8.3% and 9.5% respectively.

The total protein recoveries for the combined precipitate and "whey", based on the TCA insoluble protein of the defatted flour, are 35.2% for water, 37.5% for NaCl, and 53.7% for Na_3PO_4. Smith et al. (1966) were able to obtain protein isolate (precipitate) yields of 39 to 56 g/100 dehulled defatted meal (moisture free basis) on 26 soybean strains. The protein content of the isolates were 83.8% to 95%. The large loss in recovery in this study can be attributed mostly to procedural losses especially in the purification of the proteins, since the total non-protein nitrogen in defatted winged beans was found to be only 0.61% (8% of the total nitrogen).

Amino Acid Composition

The amino acid composition of the five winged bean varieties (Table 13) is similar to that previously reported by other workers (Table 6).

Table 13. Amino acid composition of the five varieties of winged beans (mg/gN).

Amino Acid	Winged beans					Range	Soybeans[a/]
	TPT-2	CHIMBU	WB-19	Sel. #10	Sel. #12		
Aspartic acid	693	628	619	570	652	570-693	728
Threonine	238	232	237	208	240	208-240	247
Serine	269	255	265	239	266	239-269	309
Glutamic acid	864	756	795	744	818	744-864	1185
Proline	382	340	368	320	374	320-382	332
Glycine	253	242	261	227	246	227-261	259
Alanine	252	265	248	232	252	232-265	428
Cystine	38	35	42	43	46	35-46	81
Valine	326	328	342	286	332	286-342	291
Methionine	56	55	53	47	58	47-58	84
Isoleucine	282	276	274	251	280	251-282	290
Leucine	474	471	459	426	480	426-480	494
Tyrosine	290	271	297	242	272	242-272	165
Phenylalanine	278	262	274	250	280	250-280	341
Lysine	446	453	458	436	424	424-458	391
Histidine	244	228	229	232	264	228-264	168
Arginine	405	426	400	418	407	400-426	279
Tryptophan	-	-	-	-	-	-	76

[a/] FAO, 1972.

The amino acid composition of winged beans is also similar to soybeans. However, winged beans contain more lysine than soybeans. The lowest value obtained for lysine was 424 (Selection 12) and this was 33 mg/g N higher than soybeans. Winged beans will be a good supplement for lysine deficient cereals. Histidine which is an essential amino acid for infants is also high in winged beans. This is an important consideration in the formulation of weaning foods with winged beans in developing countries where malnutrition is prevalent.

Among the essential amino acids other than lysine and histidine, arginine and tyrosine are higher in winged beans than that found in soybeans (Table 13), while phenylalanine and methionine are lower. The sulfur containing amino acids, cystine and methionine, are the limiting amino acids. Pospisil et al. (1971) and Cerny (1971) reported that the total sulfur-containing amino acids are slightly higher in winged beans than in soybeans. Ekpenyong and Borchers (1978) do not support this view. The differences in the sulfur-containing amino acids is probably due to varietal differences. All other essential amino acids are similar to soybens and are present in adequate amounts.

The deficiency in cystine and methionine was clearly demonstrated by the experiments of Jaffe and Korte (1976) when supplementation with methionine resulted in increased PER value of winged beans. The complementary nature of winged beans with corn was also demonstrated with an increased PER value in rats.

The amino acid composition of the winged bean extracts, of the acid-precipitates and "whey" obtained from the three extracting solvents was also determined (Table 14). The results of the analyses did not differ markedly from that for the winged bean flour except for the acid precipitate obtained after NaCl extraction. Methionine was found to be at least twice the concentration found in the other isolates and extracts. Gillespie and Blagrove (1978) reported a minor globulin (psophocarpin A) protein present in winged beans which is rich in S-containing amino acids. In contrast to the present studies, they reported that the amino acid composition of their acetate-chloride extracts differed considerably from the amino acid composition of winged beans they used.

Many workers (Circle and Johnson, 1958; Meyer, 1966; Wolf, 1970) have studied the amino acid content of various soybean isolates and soybean meal. They observed no significant changes in amino acid composition of the meal from the isolates. The variations that did exist were explained as resulting from varietal differences (Meyer, 1966), fractionation of protein (Wolf, 1970) or partial protein hydrolysis by alkali (Circle and Johnson, 1958).

Gel Chromatographic Separation on Biogel A-1.5m

Preliminary studies showed that a sample size in excess of 44 mg yielded poor separations on the 35 x 2.5 cm Biogel A-1.5m column. Therefore, a 44 mg sample of each protein preparation was separated by

Table 14. Amino acid composition of the winged bean protein extracts and isolates obtained from water, 0.75 M NaCl, and 0.5% Na_3PO_4 extraction (mg/gN).

	Water			0.75 M NaCl			0.5% Na_3PO_4		
	TE*	PPT*	"whey"	TE	PPT	"whey"	TE	PPT	"whey"
Aspartic acid	786	726	879	780	688	797	674	690	921
Threonine	254	217	340	264	280	276	263	239	366
Serine	328	338	351	343	313	330	341	310	384
Glutamic acid	914	955	707	907	800	911	874	903	727
Proline	422	439	441	441	330	431	417	390	474
Glycine	244	232	342	276	293	270	302	258	343
Alanine	258	239	286	257	352	240	276	252	289
Cystine	39	37	42	46	40	44	41	42	48
Valine	343	304	427	350	404	344	370	304	426
Methionine	44	28	70	54	112	46	68	60	56
Isoleucine	296	262	312	308	335	299	325	270	308
Leucine	471	470	427	516	553	523	576	520	515
Tyrosine	373	333	350	346	319	353	352	320	302
Phenylalanine	295	276	302	301	335	303	311	297	287
Lysine	484	516	464	482	526	511	472	422	529
Histidine	268	225	220	245	298	237	214	216	283
Arginine	400	412	379	420	420	407	404	393	436

* TE, total extract; PPT, acid precipitate.

elution with 0.01 M phosphate buffer (pH 7.0) at 25°C and a flow rate of 24 ml/hr.

Figures 5 and 6 show the elution patterns obtained when the various protein extracts and the acid precipitate and "whey" from the water extract were separated by gel filtration, utilizing the general procedures described under Methods. Operating conditions for particular separations are shown in the figure captions.

The total aqueous extract (Figure 5a) yielded four peaks, labelled A, B, C, and D in order of elution. Peak B was a shoulder on the largest peak, A. Peaks C and D were not well resolved from each other. The acid precipitate yielded three peaks (Figure 5b). Two of these were labelled A and B because they corresponded closely in retention time and relative heights to peaks A and B of the total extract. The third acid precipitate peak was labelled E, since its retention time did not correspond to any of the peaks from the total extract. The acid "whey" pattern (Figure 5c) indicated that this sample was free of high molecular weight protein components responsible for peak A and B in the previous samples. The acid "whey" yielded only two poorly-resolved peaks with retention time corresponding approximately to peaks C and D of the total extract. However, since the relative heights of the two peaks were different from C and D, the two peaks from the acid whey were presumed different and were labelled F and G.

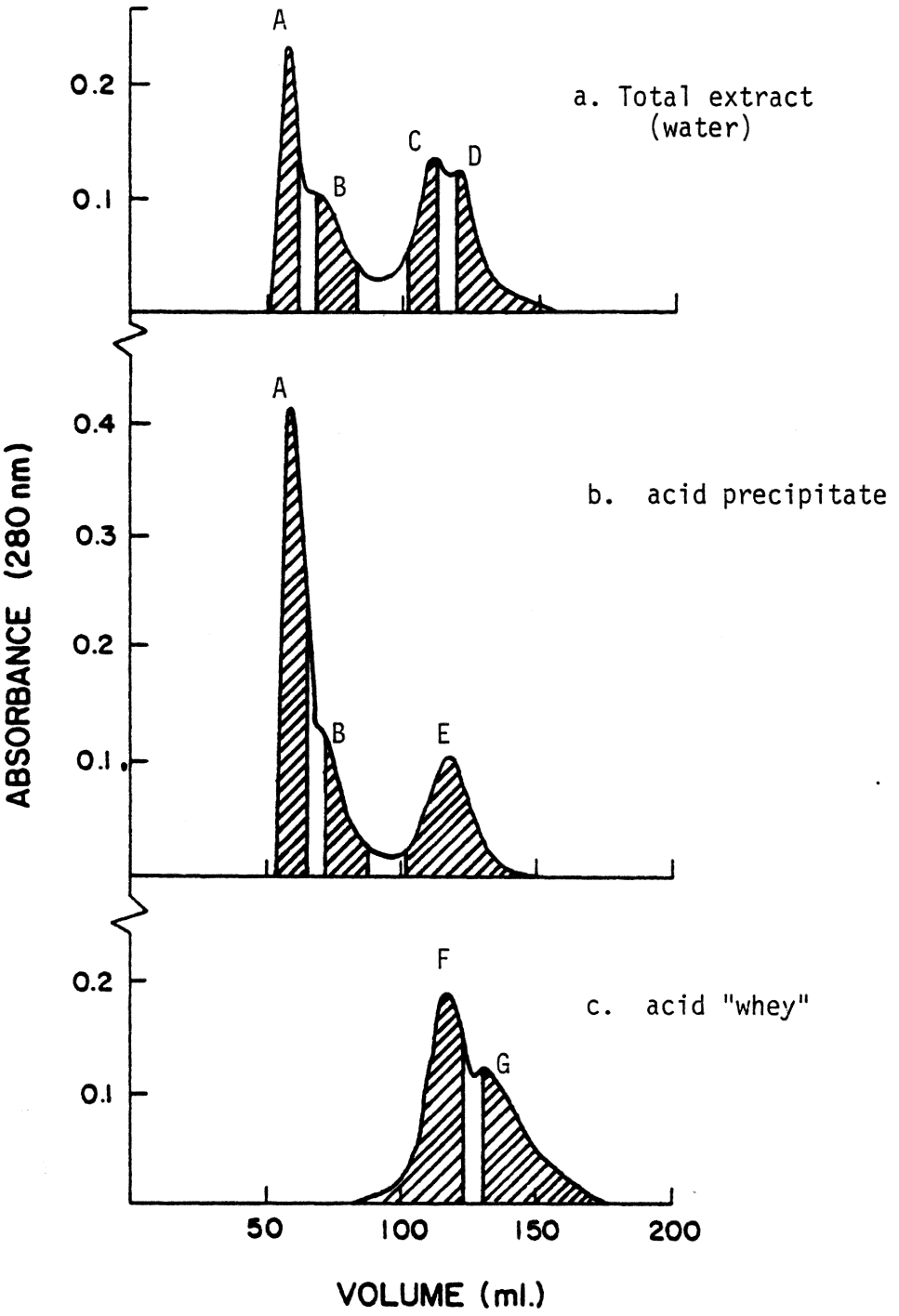

Figure 5. Gel chromatography of water-extractable winged bean proteins on a 35 x 2.5 cm Biogel A-1.5m column eluted with sodium phosphate buffer (0.01 M, pH 7.0) at 25°C. Shaded area represent pooled fractions.

The collected fractions were pooled as indicated on the figure caption, freeze dried and subsequently analyzed employing PAGE and SDS-PAGE procedures.

The elution pattern obtained from 0.75 M NaCl extract (Figure 6a) was similar to the elution pattern of the aqueous extract and the peaks were labelled A, B, C, and D as before. The elution pattern for the 0.5 % Na_3PO_4 extract (Figure 6b) differed significantly from the other two total extracts, having two peaks (A and C) and a poorly-resolved shoulder B.

Water extracted soybean proteins were successfully fractionated on Sephadex G-200 by Obara and Kimura (1967) into five fractions using phosphate-chloride buffer (pH 7.6). The first four fractions were found to be protein fractions while the fifth fraction was a non protein fraction. The trypsin inhibitor activity was found in the fourth fraction.

Polyacrylamide Gel Electrophoresis of Winged Bean Protein Concentrates and Isolates

Polyacrylamide gel electrophoresis (PAGE) was employed to further characterize the winged bean protein concentrates (total extracts) and isolates (acid precipitates and "wheys"). Figure 7 shows the gel electrophoretograms of the protein concentrates and isolates. For ease

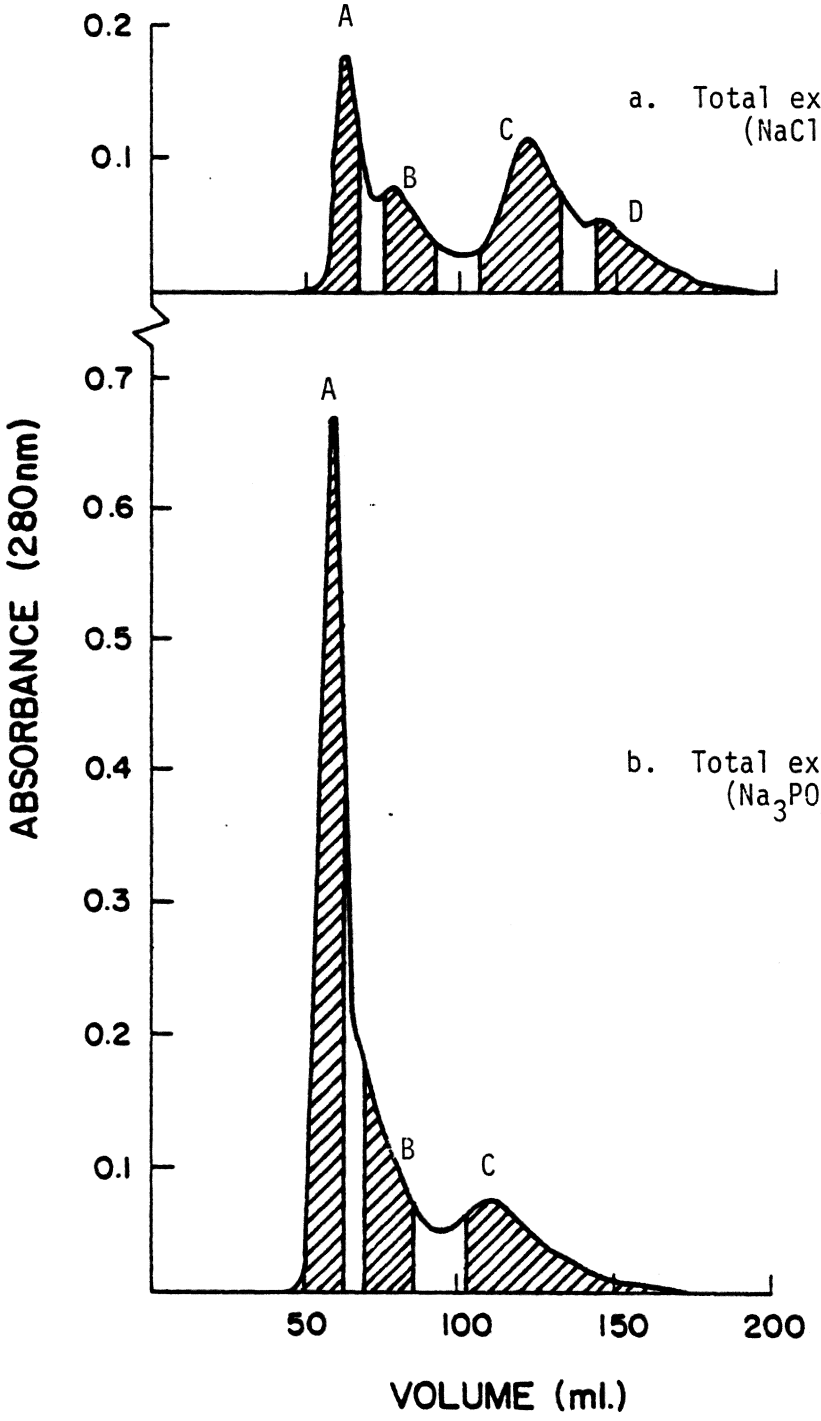

Figure 6. Gel chromatography of winged bean proteins on a 35 x 2.5 cm Biogel A-1.5m column eluted with 0.01 M sodium phosphate buffer (pH 7.0) at 25°C. Shaded area represent pooled fractions.

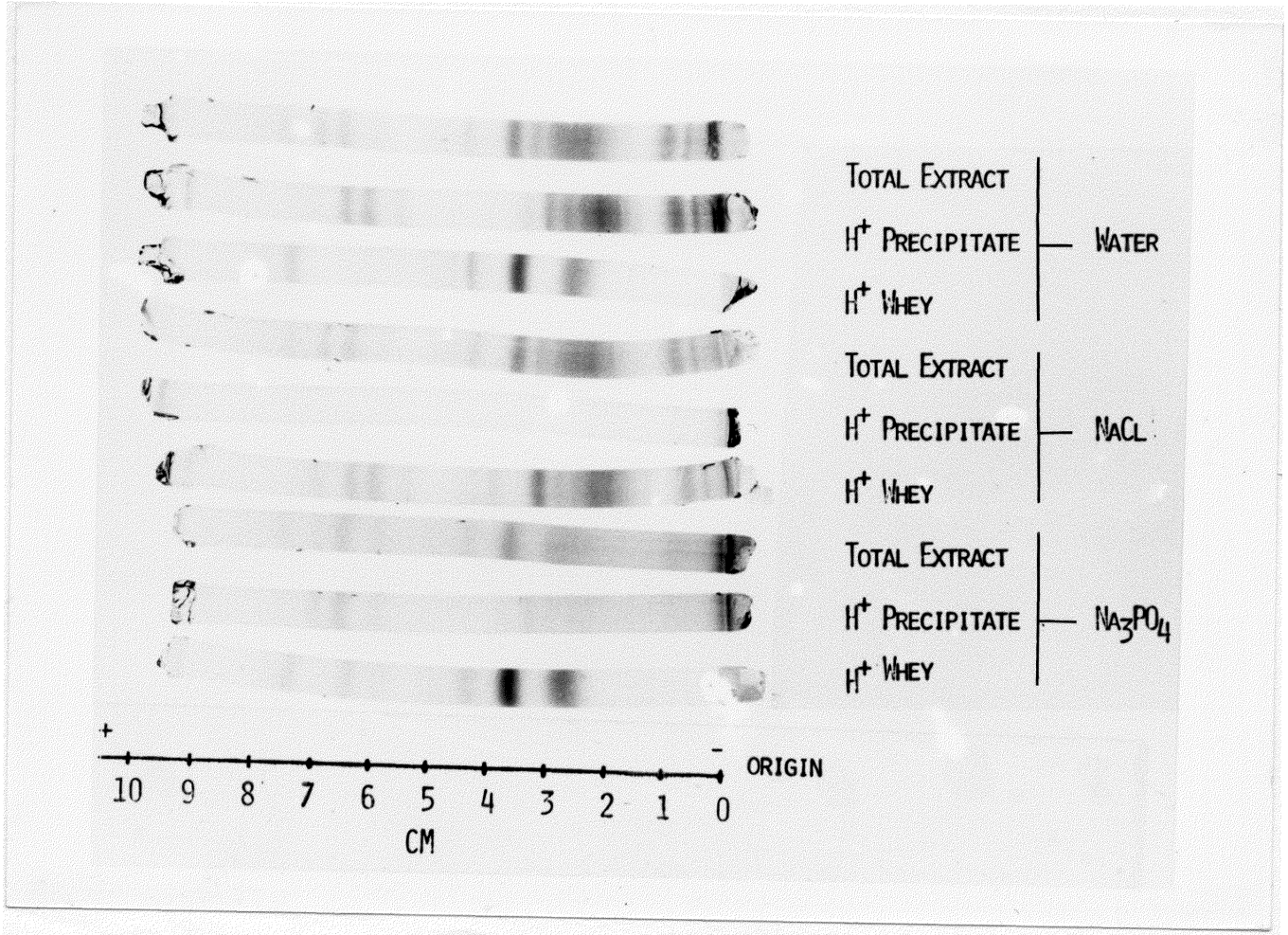

Figure 7. PAGE disc patterns of the total extracts, acid precipitates and wheys of water, 0.75M NaCl and 0.5% Na_3PO_4 extracted winged bean proteins.

of comparison, the electrophoretogram data are summarized in Tables 15, 16 and 17.

As shown in Figure 7 and Table 15, there was a difference in the number of protein bands obtained from the three extracting solutions used in this study. Water extraction of winged beans gave 14 protein bands compared to the 12 protein bands from the NaCl extract and 9 protein bands from the Na_3PO_4 extract. The two major* bands in the total extract for all 3 extracts occurred at R_m values of 0.01 and at 0.36 ± 0.03. The relatively strong band at 0.01 suggests that considerable protein did not migrate. Either the isoelectric point of the protein in this fraction must be very close to pH 8.3 (the buffer used in the system) or the proteins are highly aggregated.

The acid precipitate from the water extract yielded a pattern (Figure 7 and Table 16) quite similar to the total water extract, except that the band at 0.36 was reduced from a major band to a trace band. This was distinctly different from the situation with the NaCl and Na_3PO_4 extracts. With these two extracts, the acid precipitates gave only faint bands except for a strong band at the origin.

The whey proteins (Figure 7 and Table 17) from the water extract contained the major protein band (R_m = 0.36) missing from the acid

---

*All bands are classified as "major", "minor" or "trace bands"; "major" being the darkest, most obvious bands and "minor" the bands of intermediate color density. All other bands were "trace" bands, clearly visible, but light to faint in color.

Table 15. Relative mobilities (R_m) of the bands in the polyacrylamide gel electrophoresis of the total extracts.

Water			0.75 M NaCl			0.5% Na_3PO_4		
Band no.[a/]	D(cm)	R_m	Band no.[a/]	D(cm)	R_m	Band no.[a/]	D(cm)	R_m
SZ	-	-	SZ	-	-	SZ	-	-
1	0.10*	0.01	1	0.10*	0.01	1	0.10*	0.01
2	0.55	0.06	2	0.55	0.06	2	0.60	0.06
3	0.80	0.08	3	2.00	0.20	3	2.85**	0.30
4	1.55	0.16	4	2.30**	0.23	4	3.70*	0.39
5	2.00	0.20	5	2.60	0.27	5	4.50	0.47
6	2.30**	0.23	6	3.20	0.33	6	6.10	0.64
7	2.60	0.26	7	3.60	0.37	7	6.70	0.70
8	3.20	0.33	8	4.30	0.44	8	7.10	0.75
9	3.50*	0.35	9	5.90	0.60	9	7.70	0.81
10	4.35	0.44	10	6.50	0.66	TD	9.50	1.00
11	5.90	0.60	11	6.90	0.70			
12	6.40	0.65	12	7.50	0.77			
13	6.90	0.70	TD	9.80	1.00			
14	7.50	0.76						
TD	9.90	1.00						

[a/] SZ - sample zone; TD - tracking dye.

* major band
** minor band

Table 16. Relative mobilities (R_m) of the bands in the polyacrylamide gel electrophoresis of the acid insoluble proteins (precipitates).

Water			0.75 M NaCl			0.5% Na_3PO_4		
Band no.[a/]	D(cm)	R_m	Band no.[a/]	D(cm)	R_m	Band no.[a/]	D(cm)	R_m
SZ	-	-	SZ	-	-	SZ	-	-
1	0.10*	0.01	NO BANDS OBSERVED			1	0.10*	0.01
2	0.55**	0.06	TD	9.80	1.00	2	1.35	0.14
3	0.80*	0.08				3	3.10	0.33
4	1.55	0.19				4	3.50	0.37
5	1.80	0.19				5	3.90	0.41
6	1.95	0.20				6	6.10	0.64
7	2.30	0.24				7	6.70	0.71
8	2.60*	0.27				8	7.20	0.76
9	2.80	0.29				TD	9.50	1.00
10	3.10**	0.31						
11	3.50	0.36						
12	5.80	0.60						
13	6.30	0.65						
14	6.60	0.70						
TD	9.70	1.00						

[a/] SZ - same zone; TD - tracking dye.

* major band
** minor band

Table 17. Relative mobilities (R_m) of the bands in the polyacrylamide gel electrophoresis of the acid soluble proteins ("wheys").

Water			0.75 M NaCl			0.5% Na_3PO_4		
Band no.[a/]	D(cm)	R_m	Band no.[a/]	D(cm)	R_m	Band no.[a/]	D(cm)	R_m
SZ	-	-	SZ	-	-	SZ	-	-
1	2.65**	0.27	1	0.10**	0.01	1	2.80**	0.29
2	3.55*	0.36	2	0.55	0.06	2	3.78*	0.40
3	4.30	0.44	3	0.80	0.08	3	4.50	0.47
4	4.60	0.47	4	1.55	0.16	4	6.60	0.69
TD	9.80	1.00	5	2.00**	0.20	5	7.70	0.81
			6	2.30**	0.23	TD	9.50	1.00
			7	2.60	0.27			
			8	3.20*	0.33			
			9	3.50	0.36			
			10	4.25	0.43			
			11	5.80	0.59			
			12	6.30	0.64			
			13	6.80	0.69			
			14	7.40	0.76			
			TD	9.80	1.00			

[a/] SZ - sample zone; TD - tracking dye.

* major band
** minor band

precipitated proteins. It also contained a substantial quantity of protein with R_m = 0.27. The whey from the NaCl extract yielded a pattern quite similar to the NaCl extract. The whey from the Na_3PO_4 extract contained 2 major protein bands at R_m = 0.01 (non-migrating) and 0.39, plus a few trace bands.

Minor bands of the water extract and NaCl extract were observed to be at R_m = 0.23 while a minor band of the Na_3PO_4 extract was at R_m = 0.30. Minor bands of the acid precipitate of the water extract were observed to be at R_m = 0.06 and R_m = 0.31. No minor band was observed for the acid precipitate of the Na_3PO_4 extract. The "wheys" from the water extract and Na_3PO_4 extract were observed to contain a minor band at R_m = 0.27 and R_m = 0.29 respectively. The "whey" from the NaCl extract contained 3 minor bands measured at R_m = 0.01, 0.20, and 0.23.

The results of the PAGE analyses show that the proteins of winged bean have a complexity similar to soybean proteins. Briggs and Mann (1950) were the first to use moving boundary electrophoresis for the analyses of soybean proteins. They reported partial separation of the water soluble proteins into "seven or more" components. Kondo et al. (1953) reported that at least five components were present in a 1.5 M NaCl extract of soybean meal. Smith et al. (1955) reported a minimum of eight peaks in the acid precipitate and whey proteins of soybeans. They obtained good resolution only with the whey proteins. Catsimpoolas et al. (1968) were able to separate soybean protein bodies soluble in

10% NaCl by PAGE. They found seven protein bands. They categorized these seven bands by disc immunoelectrophoresis and followed their metabolism during germination.

Polyacrylamide Gel Electrophoresis of the Protein Fractions Obtained from Gel Chromatography

The protein fractions obtained from gel chromatography of the water extract, the acid precipitate and the "whey" of the water extract, the NaCl extract and the Na_3PO_4 extract were also analyzed using PAGE to study the relative distribution of the protein fractions.

Figure 8 and Table 18 show the disc gel electrophoretic patterns and the relative mobilities respectively of the 4 fractions obtained from the gel chromatography of the water extract. Four protein bands were observed for fractions A and B, 6 protein bands for fraction C and 8 protein bands for fraction D. The major protein band of fraction A was at R_m = 0.02, suggesting that the protein did not migrate. This protein band was present in fraction B as a minor band. The major band(s) of fraction B was observed to be contained within a smeared band measured at R_m = 0.24 to 0.36. This smeared band was also present in fraction A as a minor component. The disc gel electrophoretic patterns for fractions C and D are well resolved. The major protein band of fraction C was at R_m = 0.45. Fraction D yielded two major bands (R_m = 0.31 and 0.40).

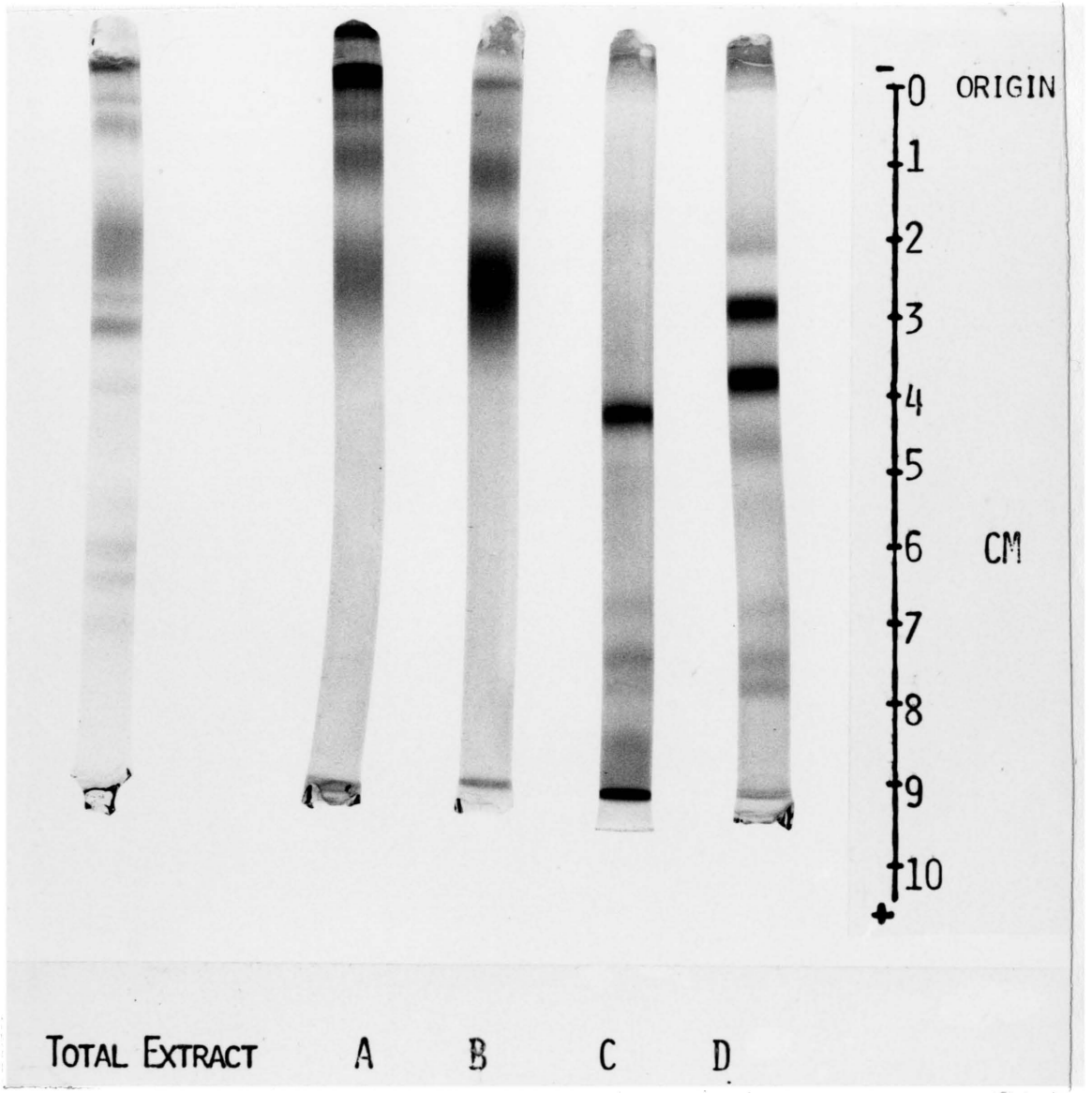

Figure 8. PAGE disc patterns of the total extract fractions (water) obtained from gel chromatography in Biogel A-1.5m.

Table 18. Relative mobilities (R_m) of the bands obtained by polyacrylamide gel electrophoresis of the protein fractions from gel chromatography of the water extracted winged bean protein.

R_m[a/] of fractions from gel chromatography			
A	B	C	D
0.02*	0.02**	0.45*	0.20
0.07**	0.07	0.73	0.31*
0.12**	0.12**	0.81**	0.40*
0.24-0.36[b/]**	0.24-0.36[b/]*	0.85	0.50**
		0.93**	0.59
		0.97	0.72
			0.80**
			0.85**

[a/] Tracking dye moved 9.4 cm from sample application zone for protein fractions A and B; 9.2 cm for fraction C; and 9.25 cm for fraction D.

[b/] smeared band

* major band

**minor band

The disc gel electrophoretic patterns of the fractions obtained from the acid precipitate of the water extract are shown in Figure 9 and the relative mobilities are shown in Table 19. Four protein bands were observed for fractions A and B. This was similar to that previously observed for A and B fractions of the water extract. This indicated that fractions A and B are the same for both the acid precipitate and the water extract. The major protein band of fraction A was observed to be at R_m = 0.02, indicating the same non-migrating or very slow migrating protein band observed in the water extract. The major protein band of fraction B was found to be within a smeared band measured at a R_m = 0.24 to R_m = 0.36. This was also present in the water extract. Six protein bands were observed for fraction E, three of which were major bands at a R_m = 0.33, 0.72 and 0.80.

The disc gel electrophoretic patterns of fractions F and G from the "whey" of the water extract are shown in Figure 10. The relative mobilities are also shown in Table 19. The disc gel patterns were well resolved compared to the patterns of the water extract and the acid precipitate. Three protein bands were observed for fraction F and 6 protein bands for fraction G. The major protein bands of fraction F was measured at a R_m = 0.87. Fraction G gave two major protein bands with their R_m = 0.27 and 0.36.

The contribution of the precipitate and the "whey" to the water extract are complex. This is evident from the disc gel electrophoretic

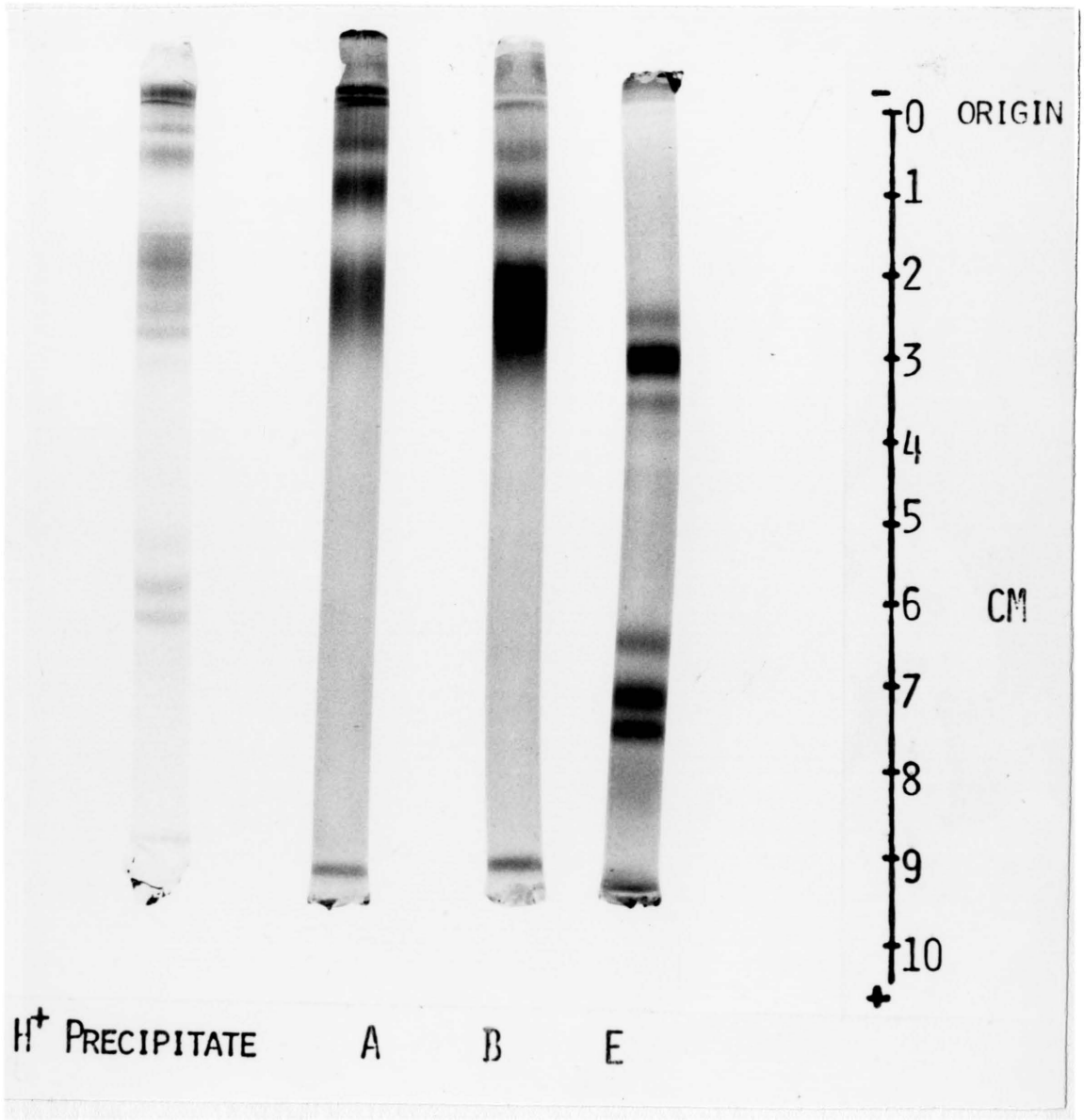

Figure 9. PAGE disc patterns of the acid precipitate (water) fractions obtained from gel chromatography in Biogel A-1.5m.

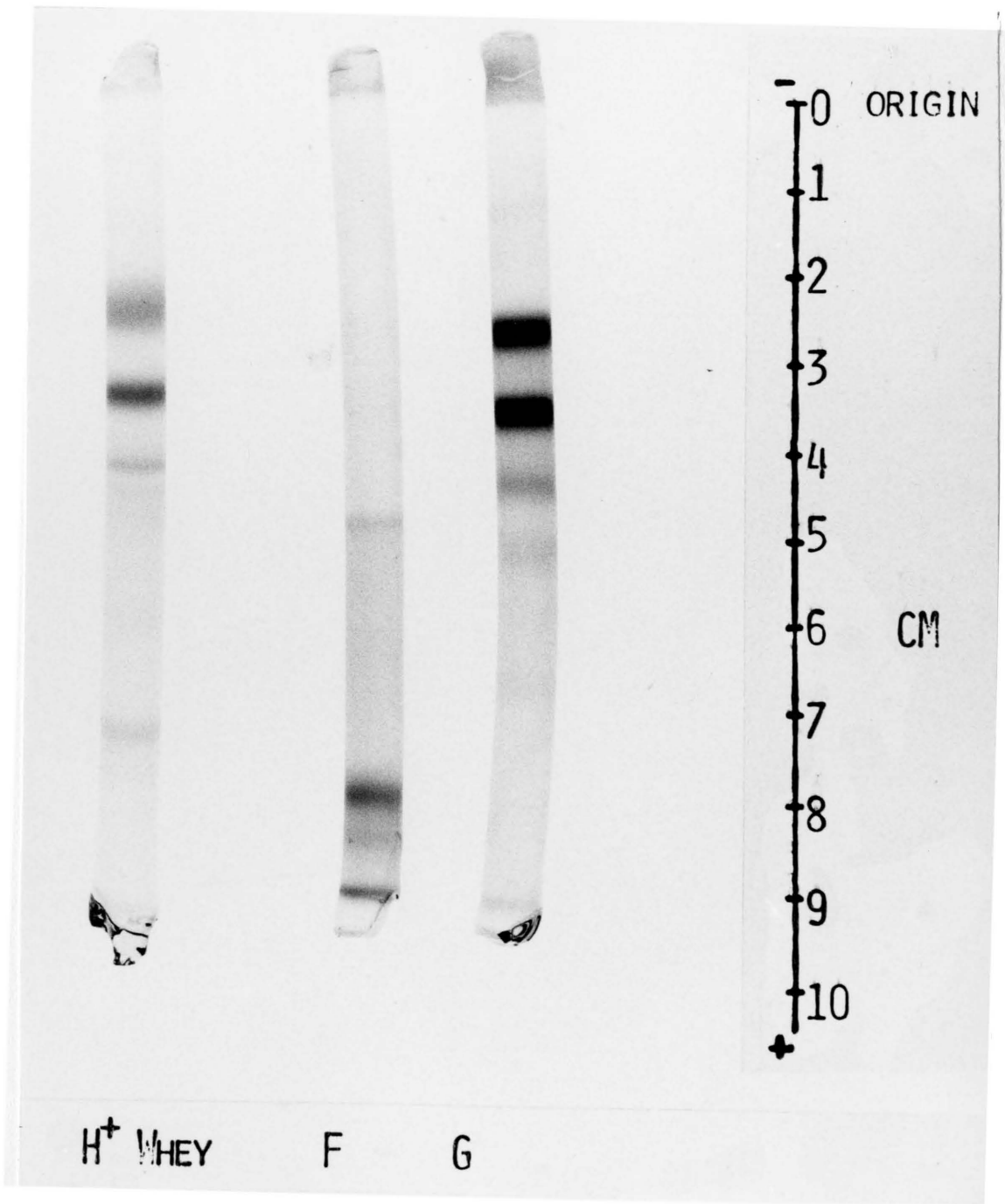

Figure 10. PAGE disc patterns of the "whey" (water) fractions obtained from gel chromatography in Biogel A-1.5m.

Table 19. Relative mobilities (R_m) of the bands obtained by polyacrylamide gel electrophoresis of the protein fractions from gel chromatography of the acid precipitate and "whey" of the water extracted winged bean protein.

R_m [a] of fractions from gel chromatography				
precipitate			"whey"	
A	B	E	F	G
0.02*	0.02	0.27	0.51	0.12
0.07**	0.07	0.33*	0.87*	0.27*
0.12**	0.13**	0.37	0.93	0.36*
0.22-0.32[b]*	0.23-0.34[b]*	0.69**		0.45**
		0.72*		0.54
		0.80*		0.69

[a] Tracking dye moved 9.4 cm from sample zone for protein fractions A and B; 9.2 cm for fraction E and F; and 9.3 cm for fraction G.

[b] smeared band

* major band

**minor band

patterns obtained from the fractions of the gel chromatography of the water extract, the acid precipitate and the "whey".

The disc gel electrophoretic patterns of the fractions from the gel chromatography of the NaCl extract are shown in Figure 11 and the relative mobilities are shown in Table 20. Three bands were observed for fraction A. These bands were either trace or faint, thus no major bands could be determined. Fraction B had three bands plus a smeared band measured at a R_m = 0.22 to 0.40. The major band of fraction B appeared to be between R_m = 0.24 to 0.32. Fraction C yielded 7 protein bands with the major band measured at a R_m = 0.39. Fraction D contained 4 protein bands with 2 major bands measured at a R_m = 0.30 and 0.40. The protein bands exhibited by the NaCl extract fractions were similar with the water extract fractions especially in fraction D (R_m = 0.30 and 0.40).

Figure 12 and Table 21 show the disc gel electrophoretic patterns and the relative mobilities of the 3 fractions obtained from the gel chromatography of the Na_3PO_4 extract. The patterns were not similar to any of the other extracts. Fraction A yielded 4 protein bands and a smeared band measured at R_m = 0.32 to 0.38. Fraction B yielded 9 protein bands and fraction C yielded 8 protein bands. The major bands of fraction A and B were measured to be at R_m = 0.01 (non-migrating protein band) and at R_m = 0.40 (very slow migrating). Fraction B yielded one other major band measured at a R_m = 0.28. Fraction C was observed to

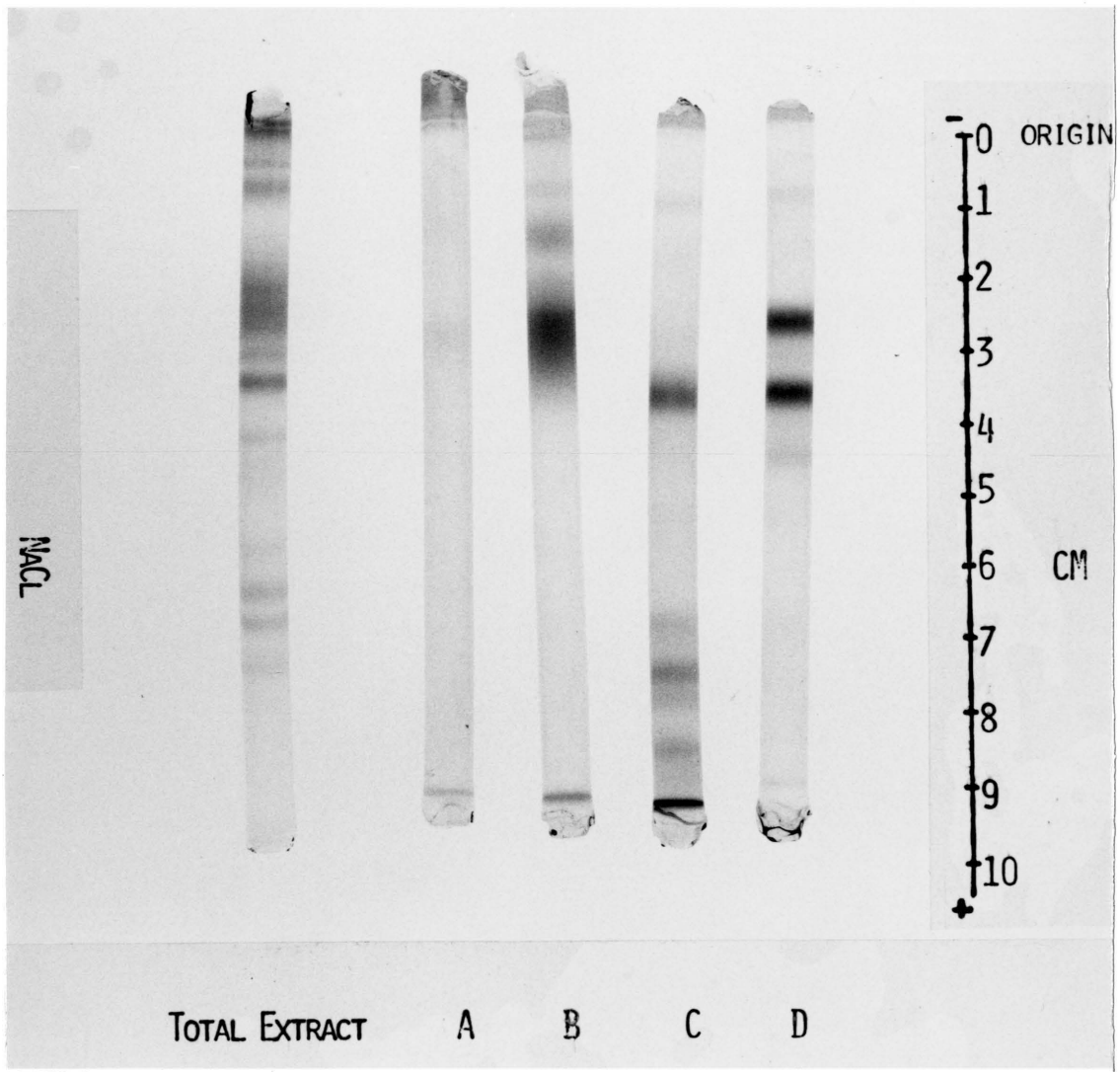

Figure 11. PAGE disc patterns of the total extract (0.75M NaCl) fractions obtained from gel chromatography in Biogel A-1.5m.

Table 20. Relative mobilities (R_m) of the bands obtained by polyacrylamide gel electrophoresis of the protein fractions from gel chromatography of 0.75 M NaCl extracted winged bean protein.

R_m[a] of fractions from gel chromatography			
A	B	C	D
0.02	0.02	0.11	0.11
0.08	0.08	0.30	0.30*
0.18[b]	0.16**	0.39*	0.40*
	0.22-0.40[b]	0.57	0.51
	0.26-0.32*	0.73**	
		0.81	
		0.85	

[a] Tracking dye moved 9.3 cm from sample zone for A and D fractions; 9.4 cm for fraction B; and 9.5 cm for fraction C.

[b] smeared band

* major band

**minor band

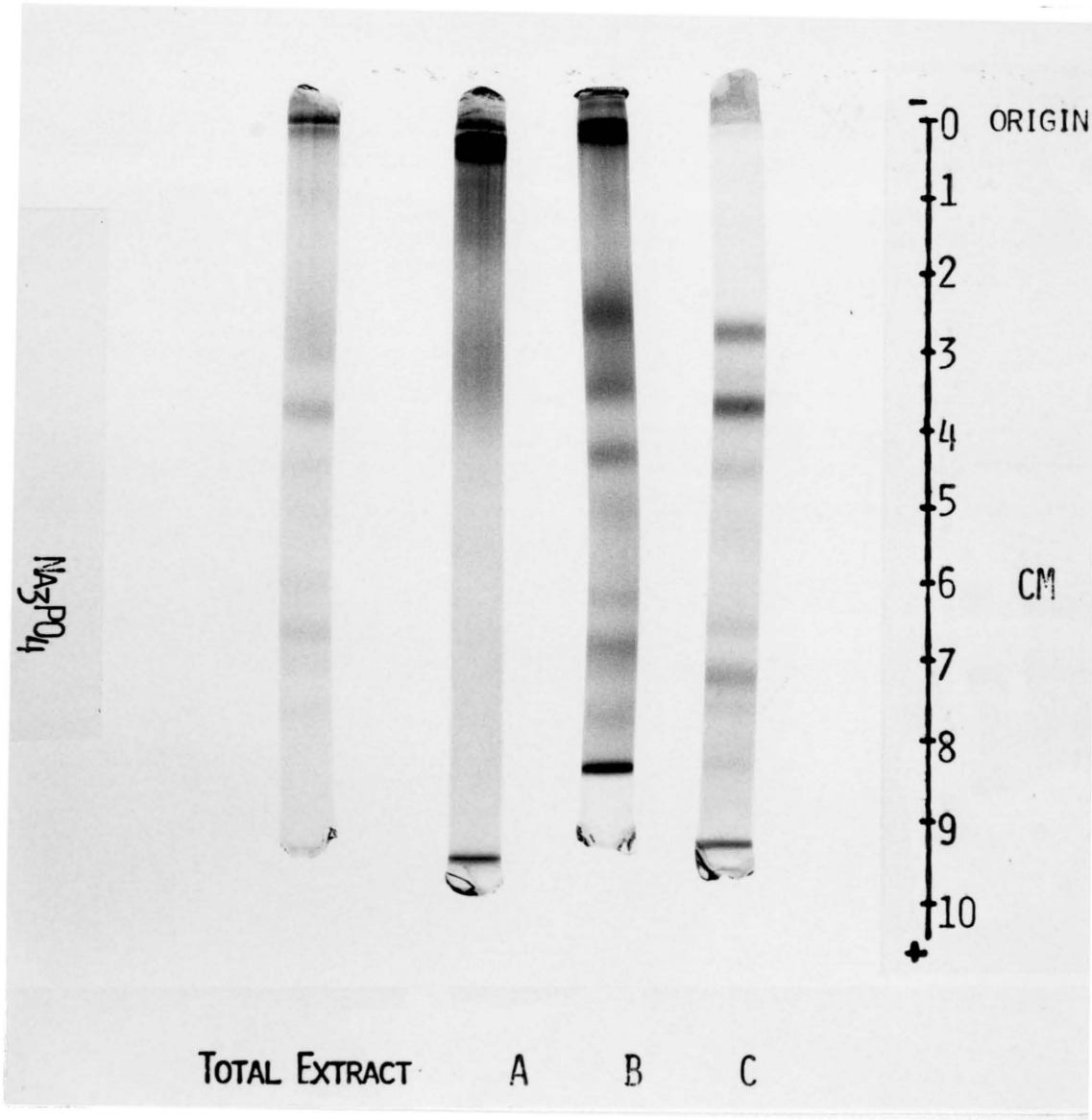

Figure 12. PAGE disc patterns of the total extract (0.5% NA_3PO_4) fractions obtained from gel chromatography in Biogel A-1.5m.

Table 21. Relative mobilities (R_m) of the bands obtained by polyacrylamide gel electrophoresis of the protein fractions from gel chromatography of 0.5% Na_3PO_4 extracted winged bean protein.

R_m [a] of fractions from gel chromatography		
A	B	C
0.01*	0.01*	0.29*
0.04*	0.04*	0.40*
0.09	0.28*	0.49
0.18	0.38**	0.58
0.32-0.38[b]	0.48**	0.70
	0.58	0.78**
	0.70	0.81
	0.77**	0.90
	0.86	

[a] Tracking dye moved 9.0 cm from sample zone for fraction A; 8.9 cm for fraction B; and 9.3 cm for fraction C.

[b] smeared band

* major protein band

**minor protein band

have one major band measured at a R_m = 0.40. The protein bands of Fraction C was well resolved when compared to fractions A and B.

The separation of the protein fractions in polyacrylamide gel electrophoresis showed that water and NaCl are better protein extractants than Na_3PO_4 because they are mild. Alkaline extraction appeared to have a deleterious effect on the winged bean proteins, thus less protein bands were obtained during electrophoresis.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of Winged Bean Protein Concentrates and Isolates

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was employed to characterize and determine the molecular weights of the proteins and/or subunits of the proteins present in winged beans. The results of the SDS-PAGE disc gel analyses for the protein concentrates (total extracts) and isolates (acid precipitates and "wheys") are shown in Figure 13. The data obtained from these disc gel electrophoretic patterns are summarized in Tables 22, 23 and 24. The molecular weight (MW) of the winged bean proteins were obtained from a standard plot of 8 protein standards against their respective mobilities (M) in SDS-PAGE (Figure 14).

As shown in Figure 13 and Table 22, there was a difference in the number of protein bands obtained from the three extracting solutions

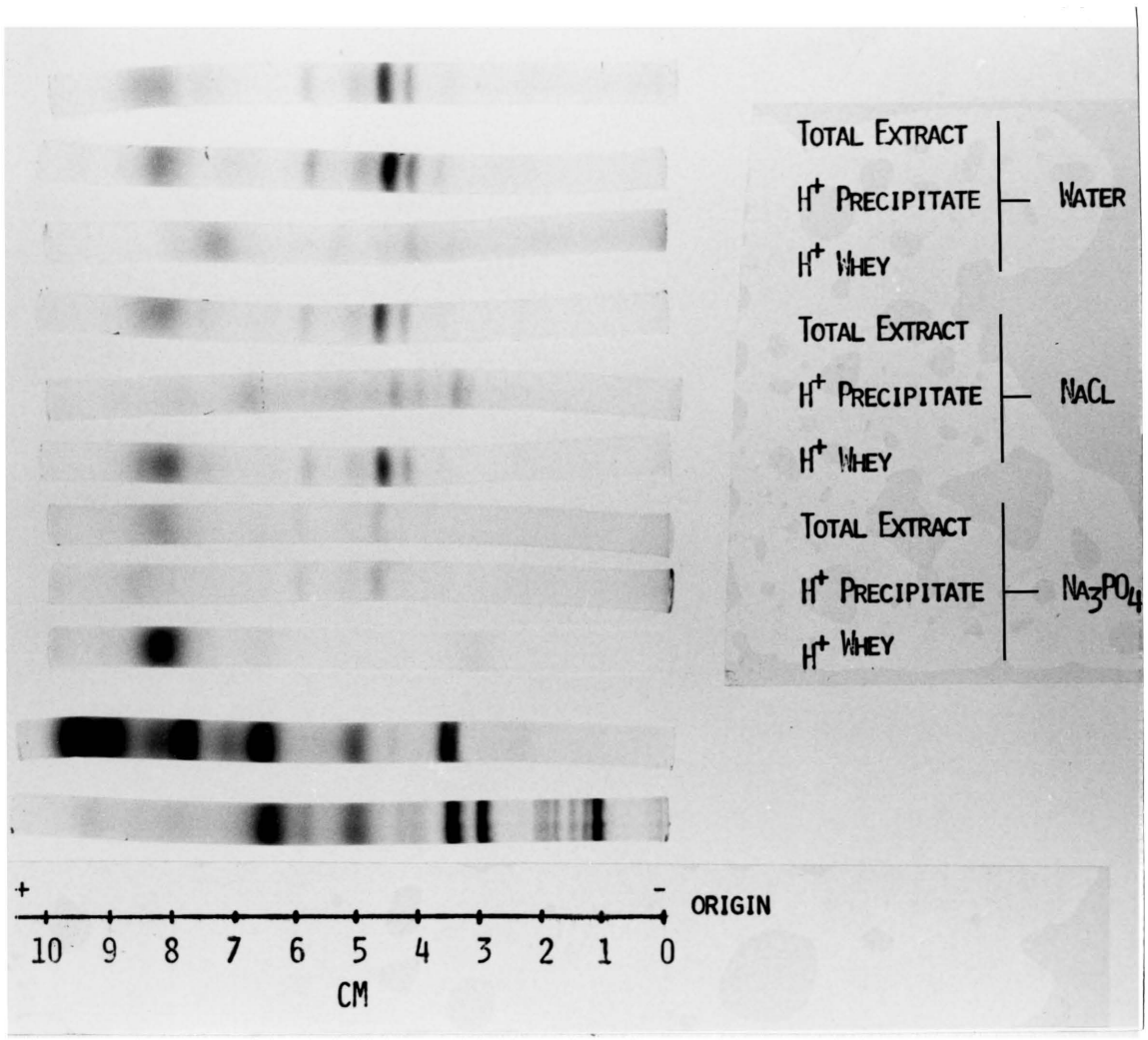

Figure 13. SDS-PAGE disc patterns of the total extracts, acid precipitates and "wheys" of water, NaCl and Na_3PO_4 extracted winged bean proteins.

Table 22. Comparative analysis of the mobilities (M) and molecular weights (MW) of the total protein of winged beans extracted with water, 0.75 M NaCl and 0.5% Na_3PO_4 using sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Mobility (M)			
Water	0.75 M NaCl	0.5% Na_3PO_4	MW
0.39			97,000
		0.43	86,000
	0.45		82,000
0.46			80,000
0.49*	0.49*		74,000
		0.51	69,000
0.55			61,000
		0.57	59,000
	0.62		51,000
0.64			48,000
		0.66	45,000
	0.84		27,000
0.87			24,500
		0.89*	23,300

* major band.

Table 23. Comparative analysis of the mobilities (M) and molecular weights (MW) of the acid precipitate of water-, 0.75 M NaCl- and 0.5% Na_3PO_4 extracted winged bean proteins using sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Mobility (M)			
Water	0.75 M NaCl	0.5% Na_3PO_4	MW
0.39	0.39		97,000
0.45			82,000
		0.47	78,000
0.48*			76,000
	0.49		74,000
		0.50	72,000
	0.53		66,000
0.54			64,000
		0.56	60,000
	0.58		57,000
0.62		0.62	51,000
0.74	0.74		35,500
0.90		0.90	22,500

* major band.

Table 24. Comparative analysis of the mobilities (M) and molecular weights (MW) of the acid "wheys" of water-, 0.75 M NaCl- and 0.5% Na_3PO_4 extracted winged bean proteins using sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Mobility (M)			
Water	0.75 M NaCl	0.5% Na_3PO_4	MW
		0.34	112,500
0.43			86,000
	0.44		85,000
	0.49*		74,000
	0.53		66,000
	0.62		51,000
		0.70	40,000
	0.80		30,000
0.83			27,500
	0.84*		27,000
		0.85*	26,000
0.88			25,000

* major band.

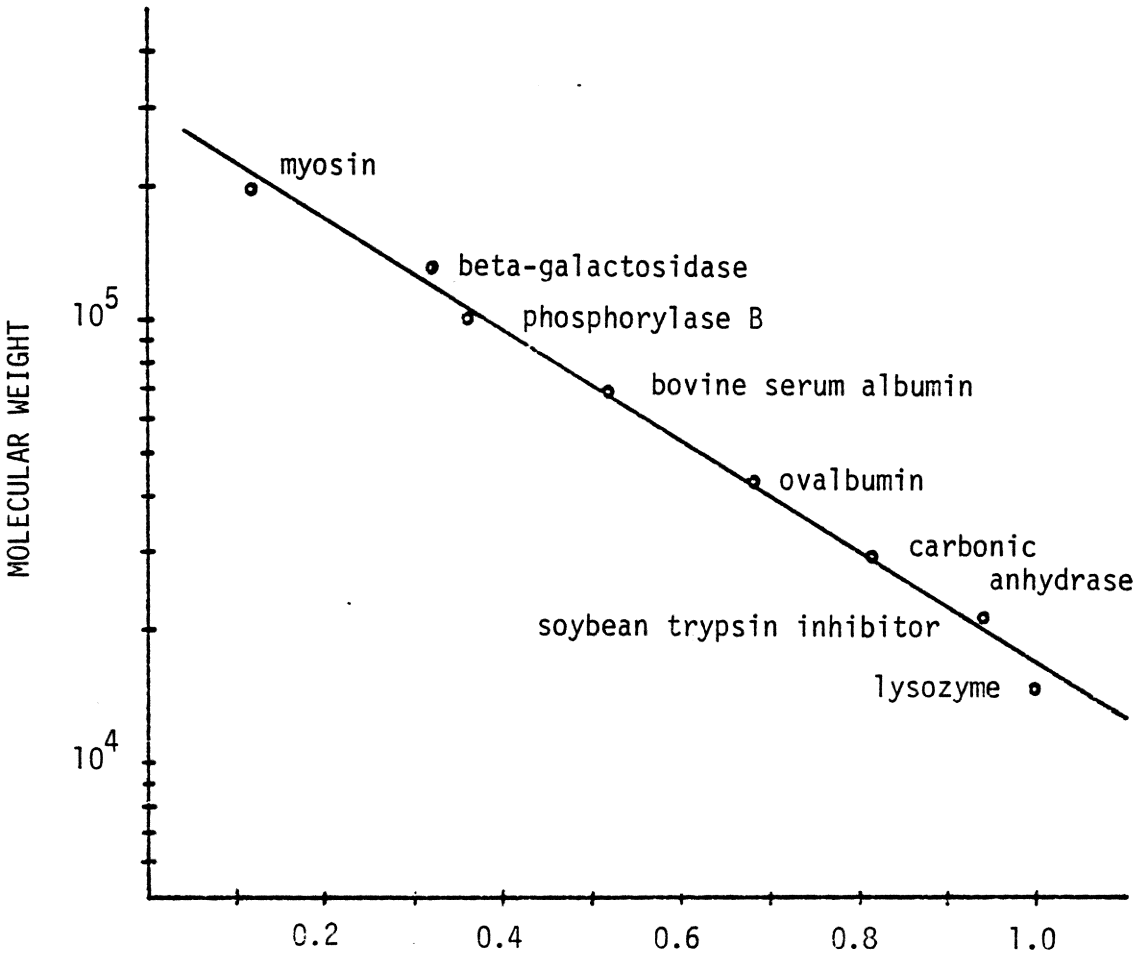

Figure 14. Standard curve of molecular weights against monilities of protein standards in SDS-PAGE.

used in this study. Water extraction of winged beans gave 6 protein bands compared to the 4 protein bands from the NaCl extract and 5 protein bands from the Na_3PO_4 extract. The major bands observed for the water extract and NaCl extract were similar with a measured mobility, M = 0.49 corresponding to a molecular weight of 74,000. This strong band appeared as a trace band for the Na_3PO_4 extract. The Na_3PO_4 extract yielded no major protein bands although one protein band (M = 0.89) approaches that of a major band corresponding to MW = 23,300. The patterns of the extracts were quite similar except for M = 0.39 of the water extract which was absent in both the NaCl and Na_3PO_4 extracts and M = 0.56 ± 0.01 which was absent in the NaCl extract.

The acid precipitates (Figure 13) were observed to have similar mobilities (Table 23) to that of the total extracts. The acid precipitate from the water extract was observed to have 7 protein bands compared to the 5 bands from the NaCl and Na_3PO_4 extracts. The major band in the water extract occurred at M = 0.48 with a corresponding molecular weight of 72,000. Major bands were not distinct for the acid precipitates from the NaCl and Na_3PO_4 extracts.

The "wheys" from the water and Na_3PO_4 extracts contained 3 protein bands compared to the 6 protein bands from the NaCl extract (Figure 13 and Table 24). The "whey" from the water extract appeared not to contain a major protein band although a distinct band was observed at M = 0.88 (MW = 25,000). The "whey" from the NaCl extract yielded

2 major protein bands with a M = 0.49 and 0.84 corresponding to molecular weights of 74,000 and 27,000 respectively. The major band for the "whey" from the Na_3PO_4 extract was observed at M = 0.85 corresponding to a molecular weight of 26,000.

The molecular weights of the protein bands obtained from the isolates and concentrates ranged from 22,500 to 97,000. The total extracts and the precipitates appear to contain the high molecular weight proteins while the "wheys" appear to contain the low molecular weight proteins of winged beans.

The molecular weights obtained from the mobilities (Tables 22, 23 and 24) can be subdivided into 5 groups: 97,000; 80,000 - 86,000; 59,000 - 74,000; 45,000 - 51,000; and 23,300 - 26,700. These molecular weight groups agree in part with the findings of Gillespie and Blagrove (1978). They obtained protein subunits with molecular weights of 17,000, 26,000, 43,000 and 68,000 in acetate-chloride winged bean protein. It is probable that extraction was incomplete since they were able to isolate only the globulin fractions at pH 4.5 (extraction pH). They observed that they obtained traces of minor proteins in cellulose acetate electrophoresis which were not present in the SDS-PAGE gels.

Gillespie and Blagrove also observed that psophocarpin A is disaggregated by SDS to a simple monomeric specie with an apparent molecular weight of 40,000 which after reduction in β-mercaptoethanol yields 2 polypeptides with molecular weights of 24,000 and 16,000.

Psophocarpin B, a 2S protein fraction was observed to give a major band with a molecular weight of 20,000. Psophocarpin C, the major 6S component, was found to be dissociated by SDS to give subunits with molecular weights ranging from 15,000 to 80,000.

Eldrige et al. (1966) were able to fractionate soybean whey proteins into at least 24 bands using PAGE with glycine buffer (pH 9.2) containing 8 M urea. The acid precipitated proteins of soybeans as well as the water extracted proteins proved to be as complex as the soy whey proteins. They observed at least 8 protein bands in both the water and the acid precipitate using PAGE with an electrolysis buffer containing 0.1 M 2-mercaptoethanol.

The results of this study show that the winged bean proteins are as complex as the soybean proteins. Molecular weight of soybean proteins have been studied employing an analytical ultracentrifuge.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of Protein Fractions from Gel Chromatography

The fractions obtained from gel chromatography were also analyzed by SDS-PAGE to determine the molecular weight of the proteins and/or protein subunits present in each fraction.

Figure 15 shows the SDS-PAGE disc gel patterns of the protein fractions from the gel chromatography of the water extract. Table 25 shows the mobilities of the protein bands present in each fraction

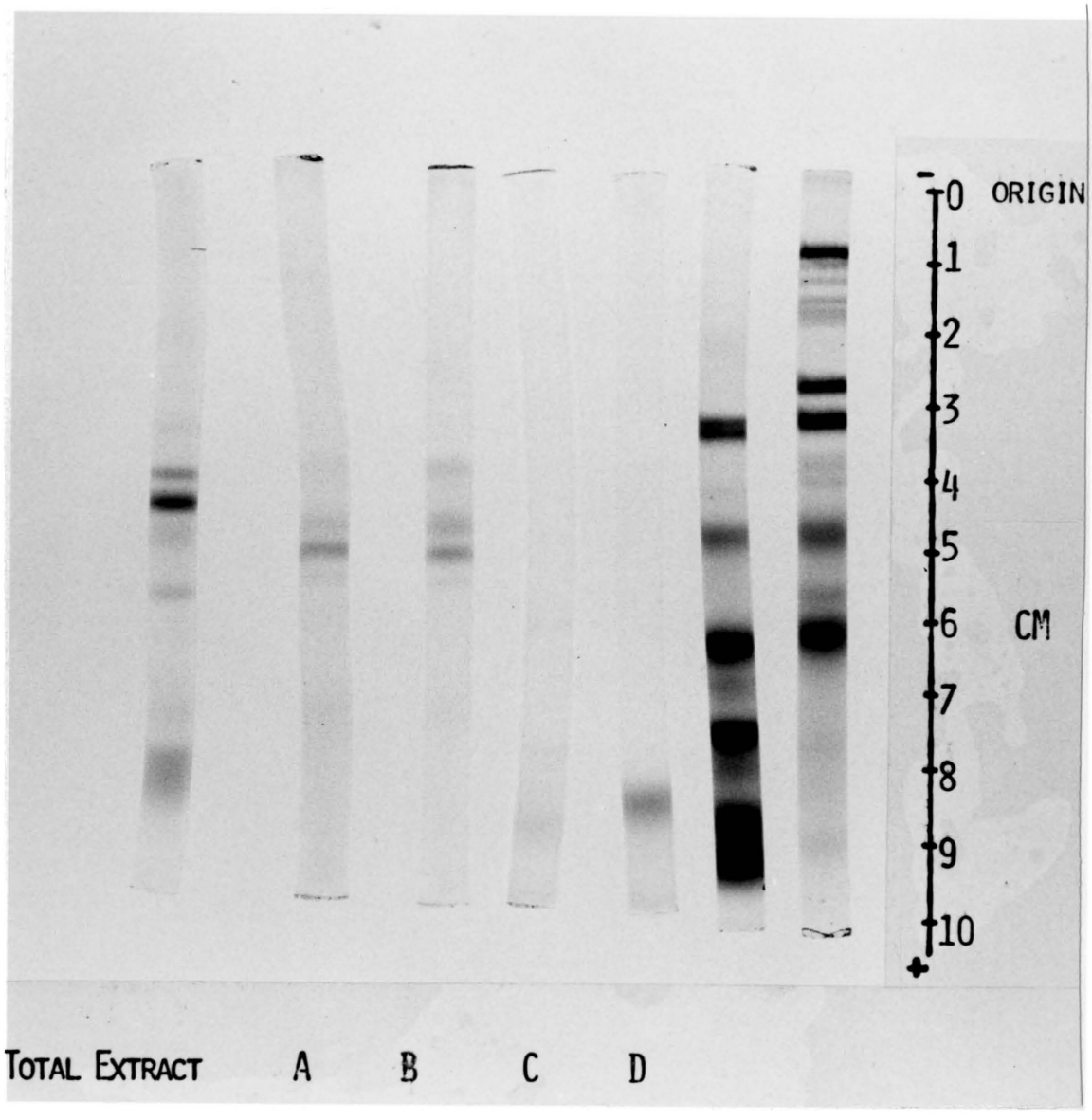

Figure 15. SDS-PAGE disc patterns of the total extract (water) fractions obtained from gel chromatography in Biogel A-1.5m.

Table 25. Mobilities (M) and molecular weights (MW) of the protein fractions from the gel chromatography of the water extracted protein using SDS-PAGE.

Mobilities (M) of bands in protein fractions				
A	B	C	D	MW
0.44	0.44			85,000
0.52				68,000
	0.53**			67,000
0.56*				60,000
	0.57*			59,000
0.60				54,000
	0.62			52,000
		0.65		46,000
		0.83		27,500
			0.89*	23,000
		0.93		20,500

* major band.
**minor band.

with their corresponding molecular weights. Fraction A and B had 4 similar protein bands compared to the 3 bands from fraction C and a single band from fraction D. The major protein band of fraction A and B were observed to be at M = 0.56 and 0.57, corresponding to molecular weights of 60,000 and 59,000 respectively. The major band in fraction C was not distinct. However, it appeared to be measured at M = 0.93 with a corresponding molecular weight of 20,500. Fraction D consisted essentially of one protein band with an observed M = 0.89 corresponding to a molecular weight of 23,000.

The results obtained from the SDS-PAGE of the acid precipitate fractions from gel chromatography are shown in Figure 16 and Table 26. Fractions A and B contained 4 similar protein bands. These were also similar to fractions A and B observed with the water extracted proteins. The major bands in fractions A and B had a measured M = 0.51 and 0.52 (MW = 69,000 and 68,000 respectively). Fraction E yielded only one protein band with a measured M = 0.92 corresponding to a molecular weight of 21,000.

Figure 17 shows the disc gel patterns of the "whey" fractions from gel chromatography in SDS-PAGE. The mobilities are also shown in Table 26. Fraction F and G were observed to have a single protein band with measured M = 0.82 and 0.90 corresponding to molecular weights of 28,000 and 22,500 respectively.

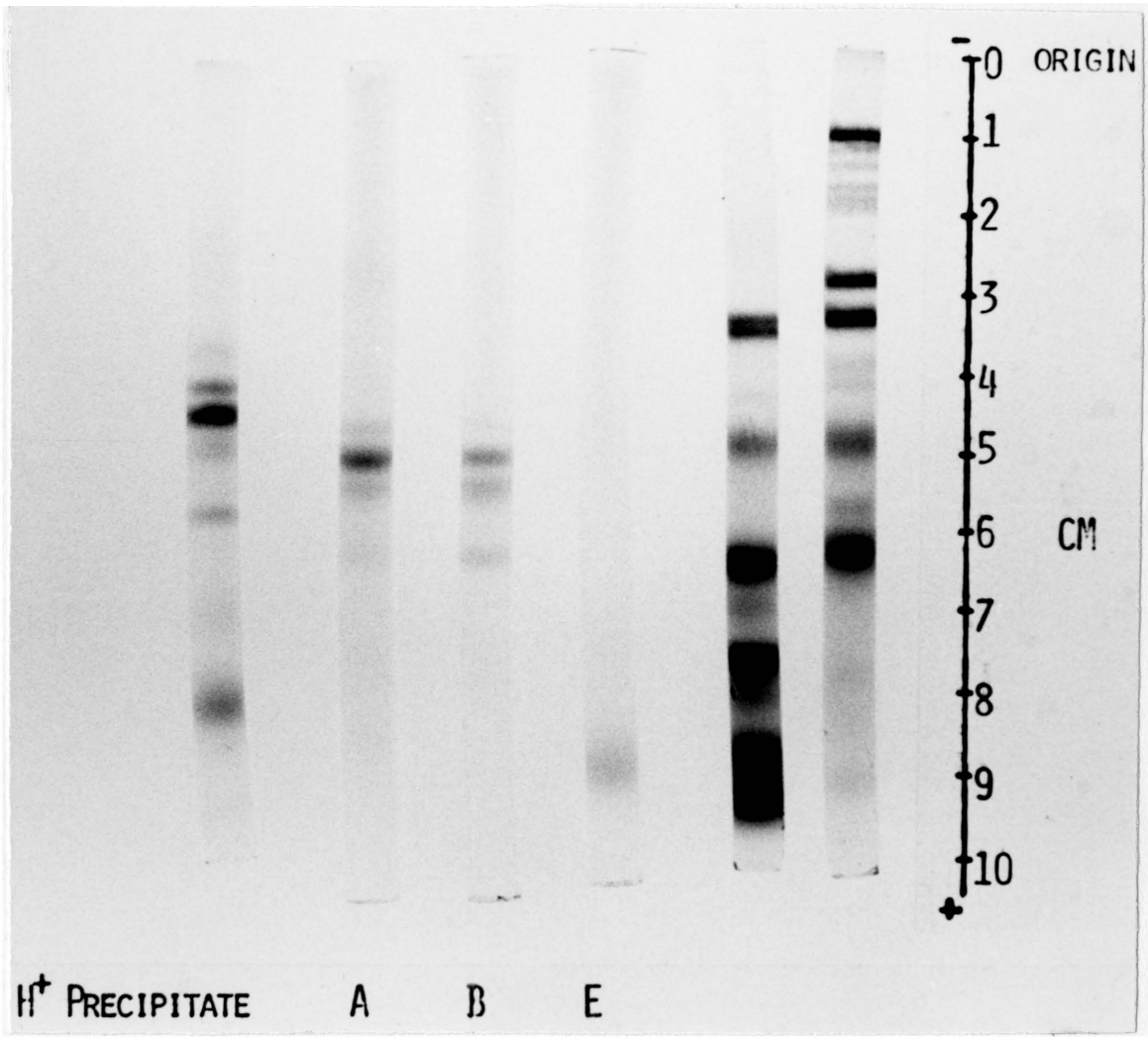

Figure 16. SDS-PAGE disc patterns of the acid precipitate (water) fractions obtained from gel chromatography in Biogel A-1.5m.

Table 26. Mobilities (M) and molecular weights (MW) of the protein fractions from gel chromatography of the acid precipitate and "whey" of the water extracted protein using SDS-PAGE.

Mobilities (M) of bands in protein fraction					
precipitate			"whey"		MW
A	B	E	F	G	
0.47	0.47				78,000
0.51*					69,000
	0.52*				68,000
0.55					62,000
	0.60				60,000
0.64	0.64				48,000
			0.82*		28,000
				0.90*	22,500
		0.92*			21,000

* major band.

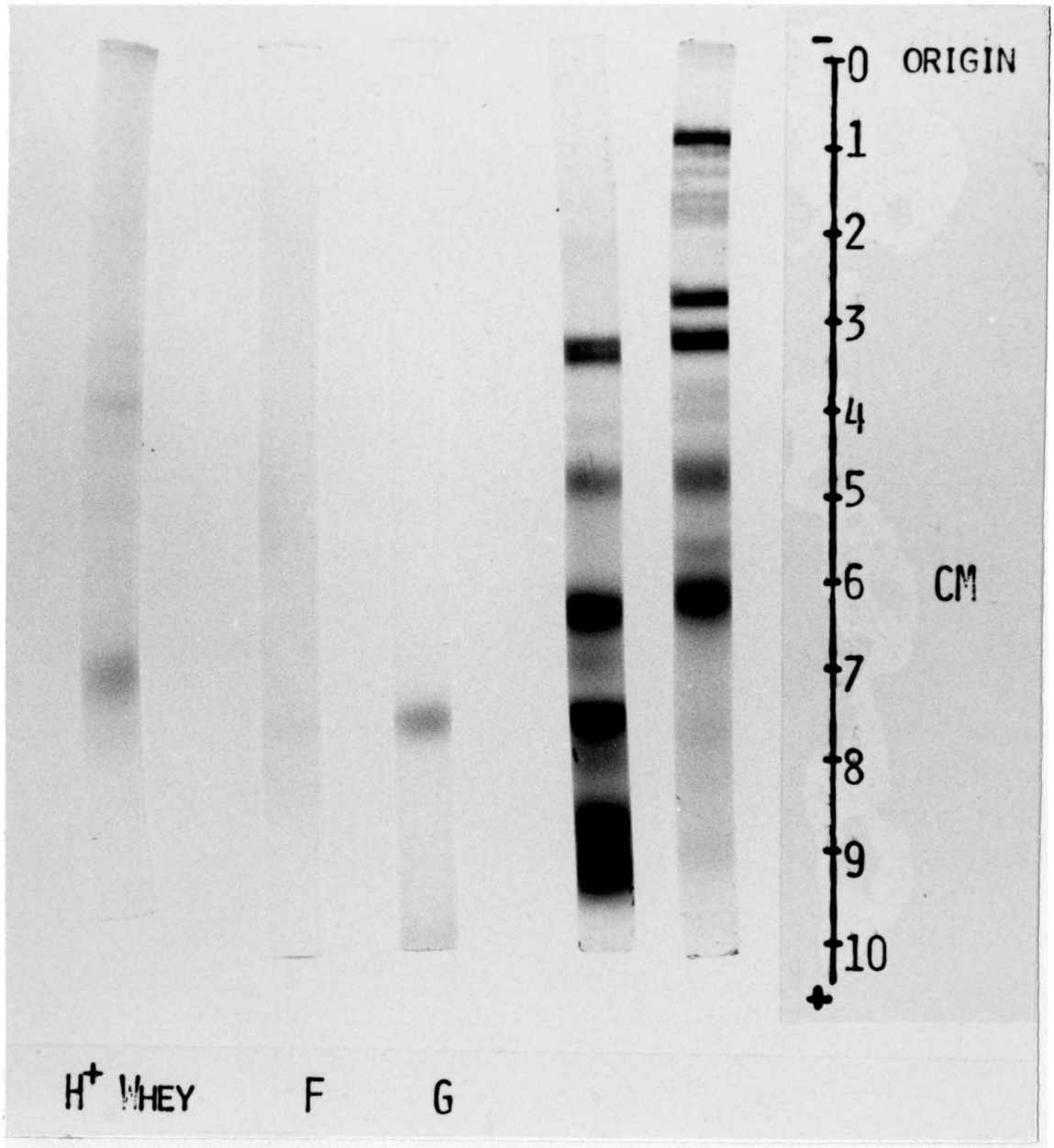

Figure 17. SDS-PAGE disc patterns of the "whey" (water) fractions obtained from gel chromatography in Biogel A-1.5m.

The SDS-PAGE disc gel patterns of the fractions from the chromatography of the 0.75 M NaCl are shown in Figure 18. The mobilities obtained from these disc gels are shown in Table 27. Fraction A contained 6 protein bands compared to the 3 bands from fraction B and a band each from fractions C and D. Fraction A yielded 2 major protein bands with a measured M = 0.51 and 0.55 corresponding to molecular weights of 69,000 and 62,000. Fraction B contained 1 major band with a measured M = 0.56 corresponding to a molecular weight of 60,000. This is equal to M = 0.55 of fraction A. The major protein band of fractions C and D was observed to have measured M = 0.93 and 0.90. These corresponds to molecular weights of 20,500 and 22,000 respectively.

Figure 19 shows the SDS-PAGE disc gel patterns of the 0.5% Na_3PO_4 fractions from gel chromatography. Table 28 shows the mobilities and the corresponding molecular weights of the protein bands. Fraction A and B yielded 3 similar protein bands compared to the single band obtained from fraction C. The major bands of fractions A and B were also similar with measured M = 0.61 (MW = 52,000) and 0.62 (MW = 50,500). The single band obtained from fraction C had a measured M = 0.87, corresponding to a molecular weight of 24,500.

The results of the SDS-PAGE of the protein fractions from gel chromatography show that the winged bean proteins consists of a large number of proteins with a molecular weight range of 20,500 to 85,000. The high molecular weight proteins and/or protein subunits were with

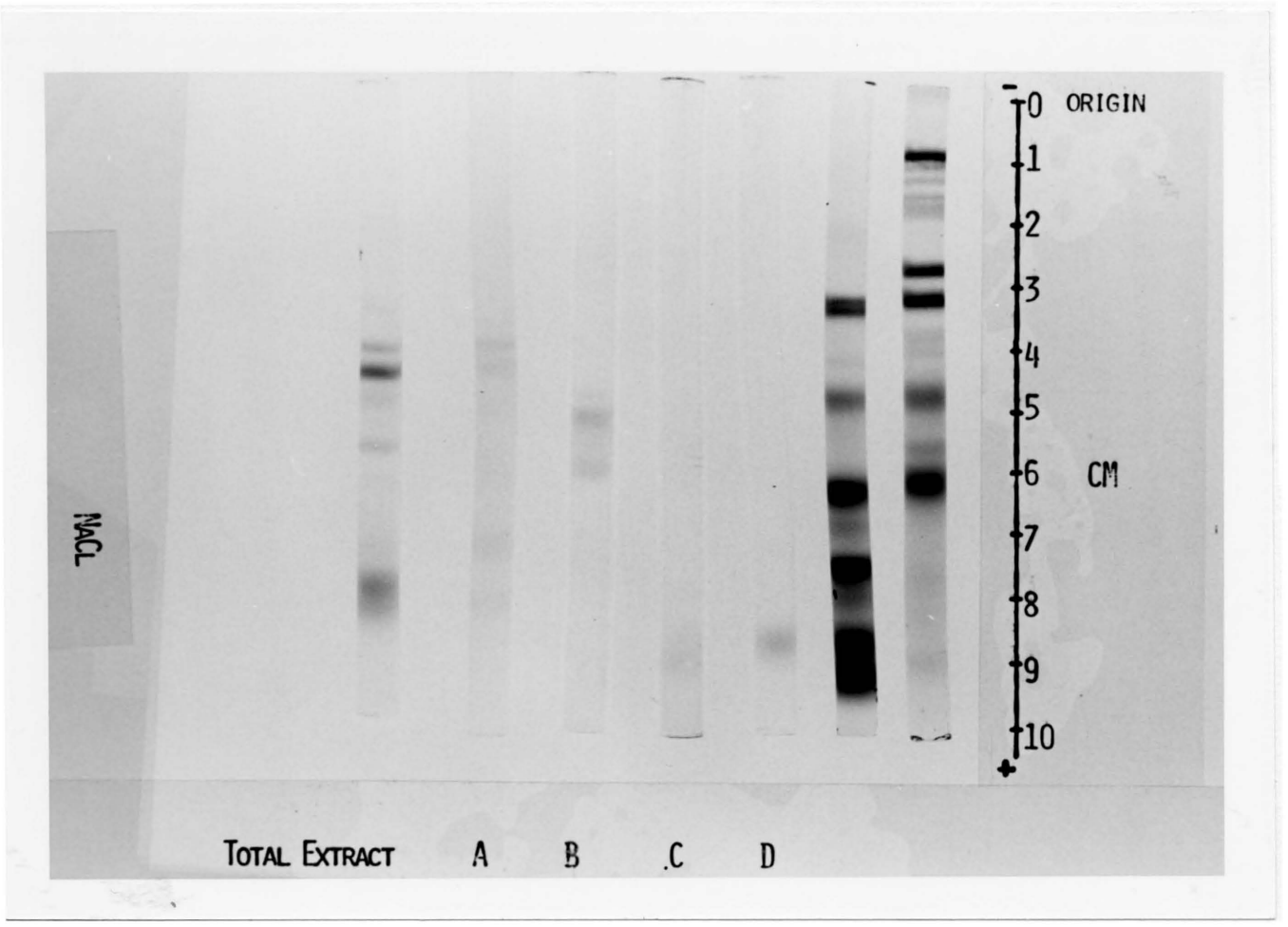

Figure 18. SDS-PAGE disc patterns of the total extracts (0.75M NaCl) fractions obtained from gel chromatography in Biogel A-1.5m.

Table 27. Mobilities (M) and molecular weights of the protein fractions from the gel chromatography of the 0.75 M NaCl extracted protein using SDS-PAGE.

Mobilities (M) of bands in protein fractions				
A	B	C	D	MW
0.51*				69,000
	0.53			66,000
0.55*				62,000
	0.56*			60,000
0.65				46,000
	0.66			45,000
0.70				40,000
0.82				28,000
0.90			0.90*	22,500
		0.93*		20,500

* major band.

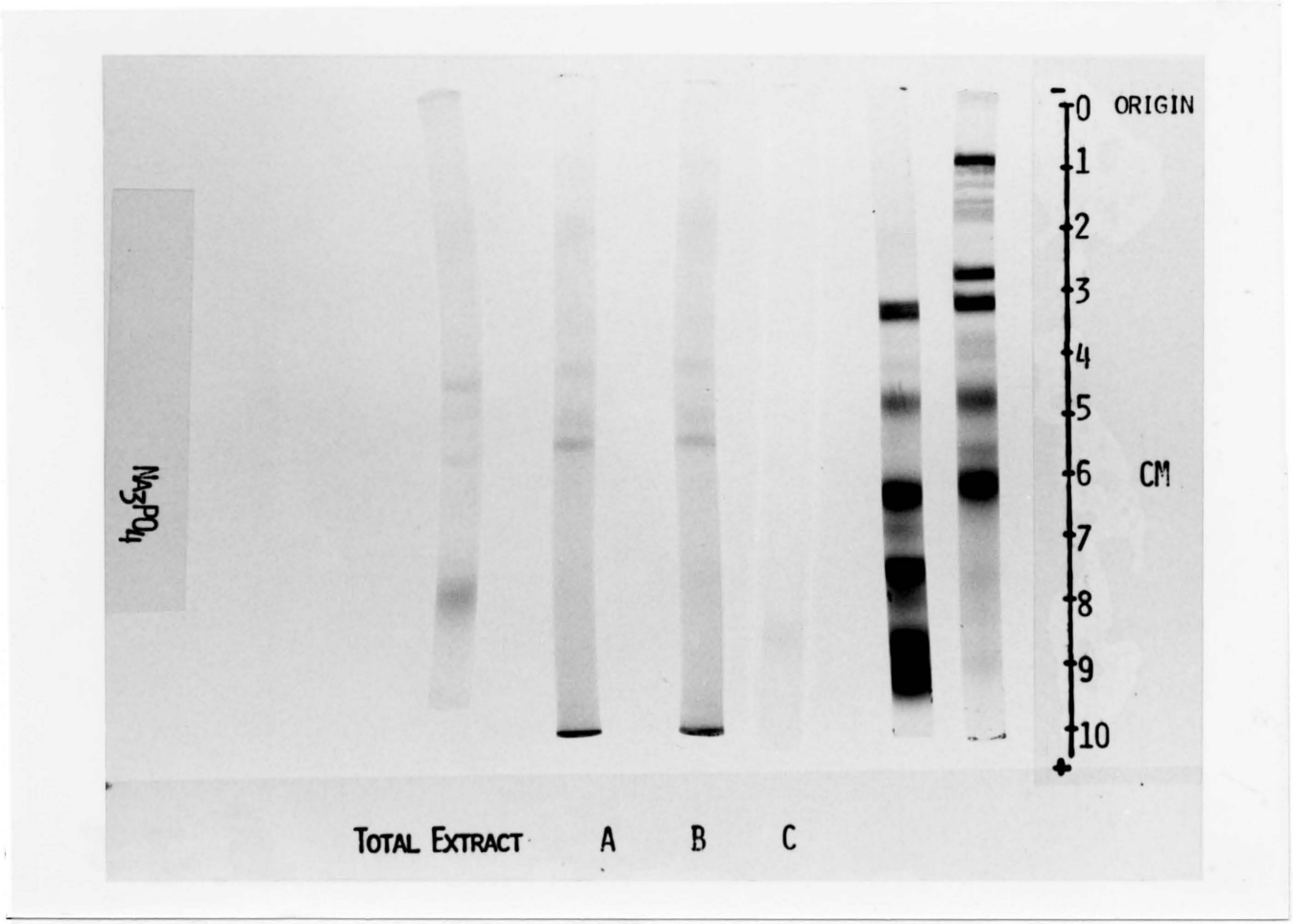

Figure 19. SDS-PAGE disc patterns of total extract (0.5% Na_3PO_4) fractions obtained from gel chromatography in Biogel A-1.5m.

Table 28. Mobilities (M) and molecular weights (MW) of the protein fractions from gel chromatography of the 0.5% Na_3PO_4 extracted protein using SDS-PAGE.

Mobilities (M) of bands in protein fractions			
A	B	C	MW
0.49	0.49		73,000
	0.56		60,000
0.57			59,000
0.61*			52,000
	0.62*		50,500
		0.87	24,500

* major band

fractions A and B. Fractions C and D of the water extract and NaCl extract contain the low molecular weight fractions. These were also observed for fractions E from the acid precipitate of the water extract and F and G from the "whey" of the water extract. Fraction C of 0.5% Na_3PO_4 extract was also observed to contain a low molecular weight component.

Carbohydrate Analyses

Starch content determination

Based on iodine staining and on enzymatic determination, there was no starch in the five varieties of mature winged beans. Thus, winged beans are similar to mature soybeans which do not contain starch. In other food legumes, starch is the predominant carbohydrate. It is possible that during the early stage of the development of the winged beans (i.e., green immature pods), the seed contains starch. This is the case with soybeans. An amylase inhibitor was found to be present in winged beans by Jaffe and Korte (1976). The activity, however, was found to be low.

Oligosaccharide content

An analysis of the oligosaccharides in winged bean was performed because legumes commonly contain relatively high amounts. The oligosaccharides are important in nutrition because they cause

flatulence in man and animals. Claydon (1975) observed that raw mature winged beans cause abdominal pains.

The typical legume oligosaccharides (verbascose, stachyose and raffinose), as well as sucrose, were found to be present in all five winged bean varieties. These analyses are shown in Table 29 and a representative elution profile is shown in Figure 20.

Verbascose was present at levels of 0.19% in the CHIMBU to 0.91% in the WB-19. Stachyose was present at 2.18% in the TPT-2 to 3.56% in the WB-19. Raffinose was present from 1.34% in Selection 10 to 1.98% in the CHIMBU. The total oligosaccharides were present in the range of 4.10- 6.18%. Sucrose was found to be present in large amounts in Selection 12 (8.18%). Sucrose was lowest with TPT-2 (5.64%).

The total oligosaccharide content was about the same level as found in soybeans. Raffinose in winged beans is present at about the same concentration as that present in soybeans (1.3%). Stachyose is present in winged beans (3.2%) at a lower level with that found in soybeans (5.3%) by Kawamura, 1967 (as cited by Rackis, 1977). Verbascose was found to be present in the range of 0.19-0.91% in winged beans whereas in soybeans it was found to be present in trace amounts. The oligosaccharide content of winged bean is low when compared to other food legumes (chickpea, cowpea, field beans, lentils, etc.), which usually contain 5.7 to 7.9% oligosaccharides (Hardinge et al., 1965; Cristofaro et al., 1974).

Table 29. Oligosaccharide content of winged beans (g/100g sample).

Variety	Verbascose	Stachyose	Raffinose	Total oligo-saccharides	Sucrose	Total soluble sugars
CHIMBU	0.19	3.40	1.98	5.57	6.36	11.93
Selection 10	0.82	3.32	1.34	5.48	6.03	11.51
Selection 12	0.68	3.53	1.40	5.61	8.18	13.79
TPT-2	0.77	2.18	1.15	4.10	5.64	9.74
WB-19	0.91	3.56	1.71	6.18	6.79	12.97

Average of four analyses

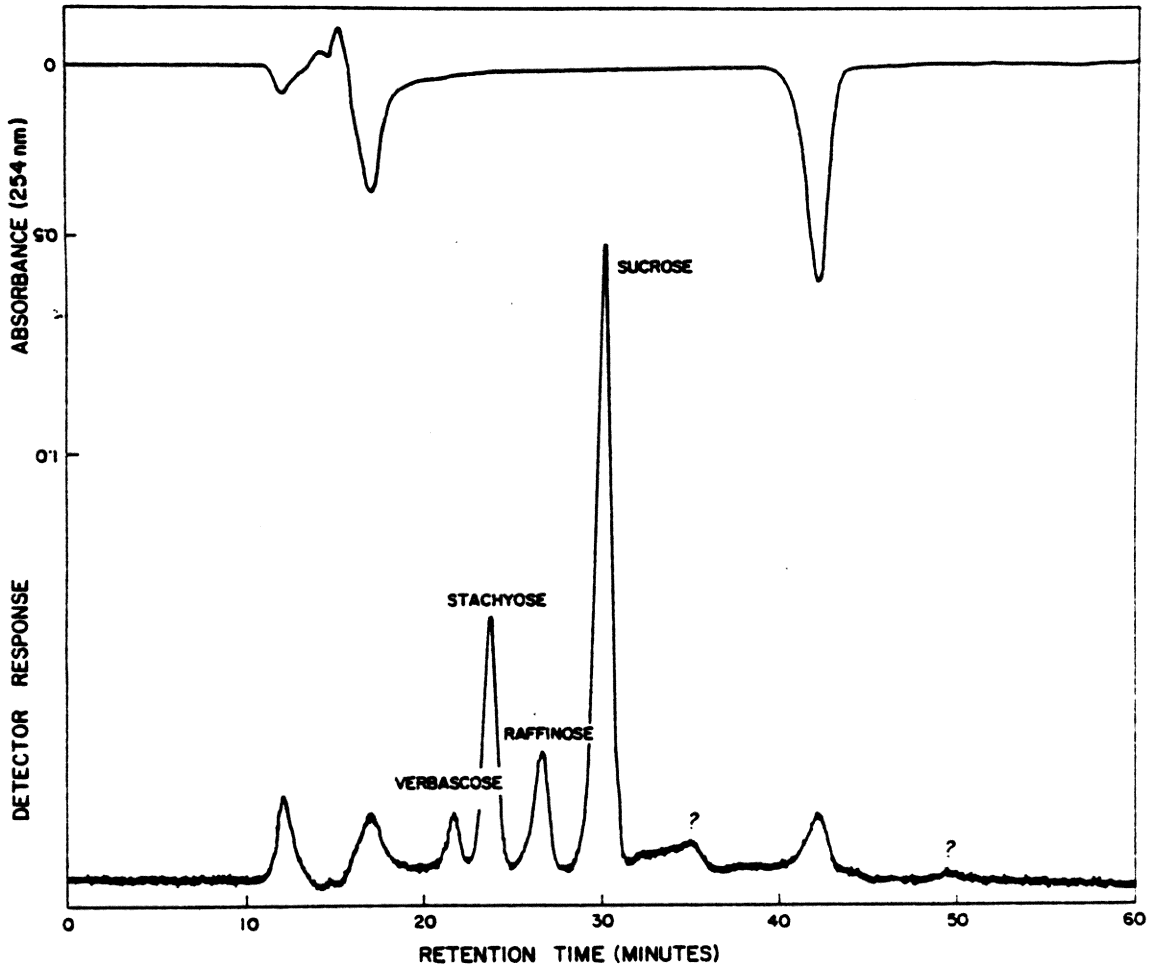

Figure 20. Chromatogram of the ethanol extract from defatted winged bean flour on a 60 cm column packed with Aminex 50 W X-4, Ca^{++}, eluted with water at 85°C. Bottom trace: RI, detector. Top trace: UV absorption shows non-carbohydrate components during elution. (0 min. - sample injection)

Sucrose levels are about the same as found in soybeans. However, one variety (Selection 12) contained 8.18% sucrose, a level which is 30% higher than the average of the other four varieties of winged beans.

The elimination of flatulence factors by breeding in winged beans holds little promise if winged beans are like soybeans. Hymowitz et al. (1972) have shown that there exists a high degree of stability in the oligosaccharide content of soybeans which cannot be eliminated by breeding. The only recourse would be to find varieties which contain low concentrations of the flatulence factors.

The total soluble sugar content (oligosaccharide and sucrose) found in each variety when added to their corresponding neutral detergent fiber values (Table 9) amounted to a total carbohydrate content of 26.9% in TPT-2, 26.2% in CHIMBU, 29.6% in Selection 12, 27.5% in Selection 10 and 30.0% in WB-19. These values account for 85.7% in TPT-2, 87.9% in CHIMBU, 91.9% in Selection 12, 88.4% in Selection 10 and 93.8% in WB-19 (mean, 90% ± 4) of the total carbohydrates obtained by difference in the proximate analyses of the winged beans shown in Table 9. The neutral detergent fiber which is an estimate of the total cell wall materials (hemicellulose, lignin and cellulose) should correlate very well with the indigestible materials (for man and animals) in winged beans due to the absence of starch. The other carbohydrates unaccounted for possibly consist of small quantities of monosaccharides and sugar

alcohols which have been found to be present in soybeans and other legumes.

Fatty Acid Composition of Winged Beans

The fatty acid composition of the oil of the five varieties of winged beans (Table 30) are similar to the results of Kleiman (as cited by Newell and Hymowitz, 1979) previously shown in Table 8. The fatty acid profile is similar to soybean oil. The total unsaturated fatty acids of winged beans ranged from 53.80 to 68.55% assuming the contribution of linolenic acid is one half of the combined values of arachidic and linolenic acids in Table 30. These values are considerably lower than those of Cerny et al. (1971), who reported 71% total unsaturated acids (Table 8). Kleiman (as cited by Newell and Hymowitz, 1979) reported 69% total unsaturated fatty acids (Table 8). This is quite similar to the values obtained in this study for Selection 12. Neither Kleiman nor Cerny reported the variety used in their study.

Oleic acid and linoleic acids were the major unsaturated fatty acids present with oleic acid present in the largest amount. This is similar to peanut oil. Oleic acid content of winged bean oil ranged from 35.04% (Selection 10) to 41.04% (CHIMBU). Linoleic acid content ranged from 15.28% (CHIMBU) to 31.77% (Selection 10). Linolenic acid and arachidic acid were eluted as a peak under the conditions employed (described in methods section), hence they are reported as the combined

Table 30. Fatty acid composition of the oil of winged beans, soybeans and peanuts (percent).

	Winged beans						
	CHIMBU	Selection 10	Selection 12	TPT-2	WB-19	Soybeans*	Peanuts*
Myristic acid	0.11	0.06	0.06	0.10	0.10	0.1	-
Palmitic acid	10.35	8.53	8.58	11.30	11.99	10.5	11.0
Stearic acid	7.36	5.00	4.19	4.90	7.51	3.2	2.3
Oleic acid	41.01	35.04	35.57	38.38	36.15	22.3	51.0
Linoleic acid	18.14	31.77	31.55	17.61	15.28	54.5	30.9
Linolenic acid	4.00	2.64	2.43	3.67	4.51	8.3	-
Arachidic acid						0.2	0.7
Eicosenoic acid	-	-	-	-	-	0.9	-
Behenic acid	15.71	13.35	13.97	19.29	19.74	-	2.3
Erucic acid	0.06	0.27	0.22	0.27	0.12	-	-
Lignoceric acid	3.32	3.34	3.43	4.46	4.60	-	0.8
Total unsaturated	61.21	68.40	68.55	58.09	53.80	86.0	81.9
Total saturated	38.85	31.60	31.45	41.89	45.20	14.0	17.1

*Weiss (1970)

value for all five varieties. These values ranged from 2.43% (Selection 12) to 4.51% (WB-19). Arachidic acid and linolenic acid (Table 8) appears to be present in equal amounts as reported by Kleiman (as cited by Newell and Hymowitz, 1979) and Cerny et al. (1971).

Behenic acid was the major saturated fatty acid found in winged beans, followed by palmitic, stearic and lignoceric acids. Behenic acid content ranged from 13.35% (Selection 10) to 19.74% (WB-19). Stearic acid content ranged from 4.19% (Selection 12) to 7.51% (WB-19); lignoceric acid content from 3.32% (CHIMBU) to 4.60% (WB-19); and palmitic acid content from 8.53% (Selection 10) to 11.99% (WB-19). Behenic acid is not present in soybeans and is present only in a small amount (2.3%) in peanuts (Table 30).

Erucic acid and myristic acid were present in trace amounts in all five varieties of winged beans. Parinaric acid (9, 11, 13, 15 octadecatetraenoic acid) a toxic fatty acid first isolated from an oil bearing tree (Parinarium laurinum) by Farmer and Sunderland (1935), was not present in the winged bean oils analyzed. This finding is not in agreement with Cerny et al. (1971) who found parinaric acid in winged beans. The results of Kleiman (as cited by Newell and Hymowitz, 1979) showed the absence of parinaric acid in the winged beans he analyzed. More winged bean varieties will have to be analyzed to prove the presence or absence of parinaric acid.

SUMMARY AND CONCLUSIONS

The proximate biochemical composition has been determined and information on the carbohydrates and fatty acid composition has been obtained for the mature seeds of five varieties of the winged bean, Psophocarpus tetragonolobus (L.) DC. The proteins of one of the varieties (TPT-2) have been fractionated and partially characterized.

The results confirm that winged beans have a high protein and fat content (Table 9) similar to soybeans. The protein content of the varieties analyzed ranged from 38.1% for WB-19 to 41.2% for TPT-2, while the fat content ranged from 15.4% for TPT-2 to 18.5% for CHIMBU, all on a moisture free basis. The protein content of winged beans is higher than most food legumes (mung bean, cowpea, chickpea, broad beans and peas). The non-protein nitrogen in winged beans is about 8%.

The cotyledon was found to constitute 84.1% and the hulls 15.9% of the dry weight of the seeds in TPT-2 beans (Table 10). The cotyledon contained the greater portion of the protein and fat of winged beans. The hulls contained most of the fiber. These findings are in agreement with Kordylas (1978).

The crude fiber content varied from 6.5% (CHIMBU) to 7.3% (Selection 10). The ash contents (4.0% - 4.7%) are similar to those previously

reported (Table 1). The neutral detergent fiber values of winged bean ranged from 14.29% for the CHIMBU to 17.2% for the TPT-2, while the acid detergent fiber ranged from 12.3% for the CHIMBU to 13.8% for TPT-2.

Oligosaccharides (verbascose, stachyose and raffinose) were found to be present in the five varieties of winged beans (Table 29). Raffinose is present at about the same concentration found in soybeans (1.3%). Stachyose is present at a lower level (3.2%) than that found in soybeans (5.3%). Verbascose is present in the range 0.19-0.91% in winged beans whereas in soybeans it is present in trace amounts. The oligosaccharide content of winged beans is low when compared to chickpea, cowpea, field beans, mung beans and lentils, which usually contain 5.7% to 6.9% total oligosaccharides (Harding et al., 1965; Cristofaro et al., 1974). Sucrose levels are about the same as found in soybeans. One variety, Selection 12, contained 8.18% sucrose, a level which was 30% higher than the average of the other four varieties.

The total soluble sugars found in each variety plus their corresponding neutral detergent fiber values account for about 90% ± 4 of the total carbohydrates obtained by difference in the proximate analyses of winged beans (Table 9). The neutral detergent fiber, which is an estimate of the total cell wall materials (hemicellulose, lignin and cellulose) should correlate highly with the indigestible materials present in winged beans, due to the absence of starch in mature beans.

The fatty acid composition of winged bean oil (Table 30) is similar to soybean oil. The total unsaturated fatty acids ranged from 53.80% to 68.55% which is not in agreement with the findings of Cerny et al. (1971) who reported a value of 71% (Table 8). The 69% value reported by Kleiman (as cited by Newell and Hymowitz, 1979) is quite similar to the 68.55% obtained for Selection 12. Oleic and linoleic acids were the major unsaturated fatty acids present. The major saturated fatty acids present were behenic and stearic acids. Parinaric acid, a toxic fatty acid reported earlier (Cerny et al. 1971), was not present to occur in winged beans in the oil of the five winged bean varieties.

The solubility of the winged bean proteins was found to be a function of pH and salt concentration. The nitrogen solubility of winged beans was lowest at pH 4.0 (17.6% soluble nitrogen). This increased dramatically as the pH was adjusted to either acid or more alkaline. Test runs at pH 1 and pH 12 yielded the highest percent soluble nitrogen (about 80% and 90% respectively). The nitrogen solubility of winged beans in water at neutrality (pH 6.70) was 60.4%.

The solubility of winged bean protein nitrogen in salt solutions was found to be a function of the concentration and nature of the salt (Table 11 and Figure 4). The maximum solubility of winged bean proteins was observed to be at 0.75-1.0 M for NaCl solutions (pH 6.95), 0.25 M for Na_2SO_4 (pH 7.20) and 0.20-0.30 M for Na_2HPO_4 (pH 9.25). NaCl (0.75-1.0 M) extracted more protein nitrogen than Na_2SO_4 and Na_2HPO_4

solutions. Na_3PO_4 (0.5%) at pH 11.0 extracted the most protein nitrogen (about 86%).

The proteins extracted by water, NaCl and Na_3PO_4 solutions from defatted winged bean flour were further studied by subjecting them to acid precipitation. The crude protein of the freeze-dried water and Na_3PO_4 extracts contained about 77% protein and the acid precipitates about 85%. Little protein was precipitated on acidification of the NaCl extract. The freeze-dried acid precipitate from the water extract represented 32% of the protein originally present in the defatted flour; 50% for the Na_3PO_4 and only 4% for the NaCl.

Amino acid analyses of the defatted flour, the total extracts, the acid-precipitates and the "wheys" remaining after precipitation, showed only slight differences in the amino acid patterns. The only exception was the protein precipitated from the NaCl solution; it had a high content of methionine (Table 14). It was predicted from the amino acid analyses that the winged bean protein will be of high nutritive quality with cystine and methionine as the limiting amino acids. Lysine was considerably higher than in soybeans.

Gel filtration of the proteins extracted with water, NaCl and Na_3PO_4 solutions revealed the presence of three to four fractions. The acid-precipitated protein and the "whey" protein from the water extract were also separated by gel filtration. The "whey" protein yielded two fractions, both of relatively low molecular weight. The acid precipitate

yielded two fractions of high molecular weight, plus one fraction of lower molecular weight.

The extracts, the acid precipitate, the "whey" and the fractions from gel filtration were all analyzed by polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

The overall conclusions derived from the protein extraction, gel filtration and PAGE analyses were as follows: Na_3PO_4 extracted the most protein and yielded the highest recovery of acid precipitable proteins (Table 12). However, much of the protein was apparently aggregated during extraction (as indicated by emergence near the void volume in gel filtration and failure to migrate in PAGE). Water and NaCl solutions extracted about the same proportion of the total protein and the extracted proteins appear to be similar with regard to complexity and molecular size. However, it was found impossible to obtain adequate amounts of purified protein from NaCl extracts by acid precipitation. Therefore, most subsequent studies emphasized the proteins and fractions from the water extract.

The proteins in the water extract are also partially aggregated, as indicated by the relatively large peak at the void volume on gel filtration (Figure 5a) and by the major protein band remaining at the origin during PAGE (Figure 7 and Table 15). However, a substantial proportion of the proteins extracted with water is of lower molecular

weight. Acidification precipitates some of the low molecular weight proteins and all the higher molecular weight and/or aggregated protein, leaving only lower molecular weight proteins in the "whey". In comparison, the PAGE patterns of the gel filtration fractions from the NaCl extract appear nearly identical to those from the water extract, while those from the Na_3PO_4 fractions were significantly different and indicated a preponderance of aggregated proteins in all but the lower molecular weight fractions.

Analysis of the water extract by SDS-PAGE (Figure 17 and Table 22) indicated the presence of only one major protein band with an estimated molecular weight of 74,000. There were five minor bands corresponding to molecular weights of 24,500 to 97,000. There was little or no protein at the origin, indicating that the SDS had broken down any aggregation. The acid precipitated proteins of the water extract contained the same proteins in the same relative amounts, except for the presence of a minor amount of a protein with molecular weight about 35,500. The whey contained only three proteins with molecular weights 86,000, 27,500, and 24,000.

By comparison, the single major protein of the NaCl extract (Figure 13 and Table 22) had a molecular weight identical to the major protein in the water extract (74,000), and there were three minor bands detectable at molecular weights 82,000, 51,000, and 27,000. In this case, since little protein precipitated on acidification, the major proportion

of the protein of the NaCl extract remained in the "whey". The SDS-PAGE pattern of the "whey" was similar to the pattern of the total extract. The pattern for the total proteins in the Na_3PO_4 extract was quite different from the other two, with a single major protein band corresponding to a molecular weight of 23,000 and minor bands corresponding to molecular weights of 86,000, 69,000, 59,000, and 45,000. The pattern for the acid precipitate from the Na_3PO_4 indicated 5 minor or trace bands at molecular weights ranging from 22,500 to 78,000. The major protein in the "whey" (26,000) apparently corresponded to the major protein of the total extract. The only other bands from the "whey" were faint and corresponded to molecular weights of 112,500 and 40,000.

SDS-PAGE analysis of the gel filtration fractions of the water extract showed that the high molecular weight fractions (labelled A and B, Figure 5a) contained a mixture of at least four proteins ranging in molecular weights from 52,000 to 85,000. The third fraction (C) contained a mixture of at least three proteins with molecular weights from 20,500 to 46,000. The fourth fraction (D) was predominantly the major protein of the water-extracted protein with a molecular weight of 23,000. The acid precipitate tended to contain the high molecular weight proteins (Figure 16), although it also contained 2 protein (or group of proteins) with a molecular weight of 21,000. The "whey" yielded only one faint band for fraction F (Figure 17) corresponding to a

molecular weight of 28,000 and one fairly strong band for fraction G corresponding to a molecular weight of 22,500.

The SDS-PAGE patterns of the fractions from gel filtration of the NaCl extract (Figure 18) showed the high molecular weight fractions containing six bands for fraction A (molecular weight range of 22,500 to 69,000) and three bands for fraction B (molecular weight range of 45,000 to 69,000), which are similar to the first three bands of fraction A. The third fraction (C) contained only one band corresponding to a molecular weight of 20,500. The fourth fraction (D) also contained one band with a molecular weight of 22,500.

SDS-PAGE analysis of the gel filtration fractions of the Na_3PO_4 extract (Figure 19) showed that the high molecular weight fractions (labelled A and B, Figure 6b) contained a mixture of three proteins ranging in molecular weights from 50,500 to 73,000. The third fraction (C) contained only one band corresponding to a molecular weight of 24,500. The number of bands obtained for fractions A and B were less than the bands obtained from the water- and NaCl extracts.

Overall, the results of the SDS-PAGE studies showed that the high molecular weight proteins and/or protein subunits elute with the A and B fractions of the total extracts as well as with the acid precipitate from water extraction. The low molecular weight components elute with the fractions C and D of the total extracts and fraction E of the precipitate of the water extract and fractions F and G of the "whey" of

the water extract. The molecular weights of the proteins and/or protein subunits obtained in this study agree in part with the results of Gillespie and Blagrove (1978). However, components with molecular weights higher than 70,000 were not observed by Gillespie and Blagrove, probably because they used acetate-chloride buffer at pH 4.5, in comparison with water, NaCl and Na_3PO_4 used in the present study.

More analyses are needed on the proximate composition of winged beans in the available varieties. These will help explain the variability in the proximate composition, especially the protein and fat content. Varieties with a low oligosaccharide content need to be identified. The influence of environment and variety in the growing of the seeds should also be studied in order to assess the possiblility of improving the nutritional value of winged beans.

A serious effort must be expended in identifying high yielding varieties of winged bean as well as in the development of dwarf varieties of the plant if winged beans are to compete with soybeans. The studies presented here should provide a basis for further research on the proteins and carbohydrates in winged beans and for further research on food utilization of winged beans.

REFERENCES

Anson, M. L. 1962. Oilseed protein in foods. Arch. Biochem. Biophys. Supp. 1:68-77.

AOAC. 1975. "Official Methods of Analysis", 12th ed., Association of Official Agricultural Chemists. Washington, D.C.

Aspinall, G. O., R. Begbie and J. E. McKay. 1967. Polysaccharide components of soybeans. Cereal Sci. Today 12 (6):223-228,260.

Araullo, E. V. 1974. Processing and utilization of cowpea, chickpea, and pigeon pea, and mung bean. In "Interaction of Agriculture with Food Science" ed. R. MacIntyre. IDRC. Ottawa, Canada.

Ayres, J. L., L. L. Branscomb and G. M. Rogers. 1974. Processing of edible peanut flour and grits. J. Am. Oil Chem. Soc. 51:133-136.

Baker, E. C. and G. C. Mustakas. 1973. Heat inactivation of trypsin inhibitor, lipoxygenase and urease in soybeans: effect of acid and base additives. J. Am. Oil Chem. Soc. 50:137-141.

Berba, B. M. 1955. Hydrolysis of dehulled mongo beans with HCl (Phaseolus aureus Roxb.). Unpublished B.S. thesis, U.P. College of Liberal Arts.

Bhatia, H. M. and F. H. Allen, Jr. 1962. 'Nonspecific' seed agglutinins and blood group specificity. Study of fifteen lectins. Vox Sanguinis 7:83-85. (as cited by Claydon, 1975).

Bhatty, R. S. 1974. Chemical composition of some faba bean cultivars. Can J. Plant Sci. 54:413-421.

BioRad. 1978. Materials, Equipment and Systems for Chromatography, Electrophoresis, Membrane Filtration and Immunochemistry. Richmond, California.

Birk, Y., A. Gertler and S. Khalef. 1963. A pure trypsin inhibitor from soya beans. Biochem. J. 87:281-284.

Black, L. T. and E. B. Bagley. 1978. Determination of oligosaccharides in soybeans by high pressure liquid chromatography using an internal standard. J. Am. Oil Chem. Soc. 55:228-232.

Blagrove, R. J. and J. M. Gillespie. 1978. Variability of the seed globulins of winged beans, Psophocarpus tetragonolobus (L.) DC. Aust. J. Plant Physil. 5:371-375.

Brown, W. H. 1954. Useful plants of the Philippines, Vol. 2. Technical Bulletin No. 10. Bureau of Prints, Manila. 160-162.

Burkhill, I. H. 1966. A Dictionary of the Economic Products of the Malay Peninsula. Vol 2. Ministry of Agriculture and Cooperatives, Kuala Lumpur, Malaysia. Repr. 1st ed. London, 1936. 1850-1851. (as cited by Claydon, 1975).

Cartter, J. L. and T. H. Hopper. 1942. Influence of variety, environment and fertility levels on the chemical composition of soybean seed. USDA Tech. Bull. 787.

Catsimpoolas, N. and C. Ekenstam. 1969. Isolation of alpha, beta, and gamma conglycinins. Arch. Biochem. Biophys. 129:490-497.

Catsimpoolas, N. and E. Leuthner. 1969. The major pH 4.5 soluble proteins of soybean cotyledons 1. Separation by gel filtration, disc electrofocusing and immunoelectrophoresis. Biochem. Biophys. Acta 181:404-409.

Catsimpoolas, N. and E. W. Meyer. 1968. Immunochemical properties of the 11S component of soybean proteins. Arch. Biochem. Biophys. 125:742-750.

Catsimpoolas, N. and E. W. Meyer. 1969. Isolation of soybean hemagglutinin and demonstration of multiple forms by isoelectric focusing. Arch. Biochem. Biophys. 132:279-285.

Catsimpoolas, N., T. G. Campbell and E. W. Meyer. 1968. Immunochemical study of changes in reserve proteins of germinating soybean seeds. Plant Physiol. 43:799-805.

Catsimpoolas, N., T. G. Campbell and E. W. Meyer. 1969. Association-dissociation phenomena in glycinin. Arch. Biochem. Biophys. 131: 577-586.

Catsimpoolas, N., C. Ekenstan, D. A. Rogers and E. W. Meyer. 1968. Protein subunits in dormant and germinating soybean seeds. Biochem. Biophys. Acta 168:122-131.

Catsimpoolas, N., C. Ekenstam and E. W. Meyer. 1969a. Separation of soybean whey proteins by isoelectric focusing. Cereal Chem. 46: 357-369.

Catsimpoolas, N., C. Ekenstam and E. W. Meyer. 1969b. Isolation of the pH 4.5 soybean trypsin inhibitor by isoelectric focusing. Biochem. Biophys. Acta 175:76-81.

Catsimpoolas, N., D. A. Rogers, S. J. Circle and E. W. Meyer. 1967. Purification and structural studies of the 11S component of soybean proteins. Cereal Chem. 44:631-637.

Cerny, K. 1978. Comparative nutritional and clinical aspects of the winged beans. Workshop/Seminar on the Development of the Potential of the Winged Bean. Los Banos, Philippines.

Cerny, K. and H. A. Addy. 1973. The winged bean (Psophocarpus palustris Desv.) in the treatment of kwashiorkor. Br. J. Nutr. 29:105-112.

Cerny, K., M. Kordylas, F. Pospisil, O. Svabensky and B. Zajic. 1971. Nutritional value of the winged bean (Psophocarpus palustris Desv.) Br. J. Nutr. 26:293-299.

Circle, S. J. 1971. Functional properties of commercial edible soybean protein products. Am. Chem. Soc. 161: AGFD 36.

Circle, S. J. and D. W. Johnson. 1958. Edible isolated soybean protein. In: Processed Plant Protein Foodstuffs. A. M. Altschul, ed. (1958). Academic Press, New York. 339-418.

Claydon, A. 1975. A review of the nutritional value of the winged bean Psophocarpus tetragonolobus (L.) DC. with special reference to Papua New Guinea. Science in New Guinea 3:103-114.

Claydon, A. 1976. An investigation into storage of winged bean roots, Psophocarpus tetragonolobus (L.) DC. 10th Waigani Seminar, Lae, Papua New Guines. 13 pp.

Cristofaro, E., F. Mottu and J. J. Wuhrmann. 1974. Involvement of the raffinose family of oligosaccharides in flatulence. In Sugars in Nutrition. Eds. H. L. Sipple and K. W. McNutt. Academic Press, N.Y., N.Y. 10003.

Davis, B. J. 1964. Disc electrophoresis II. Method and application to human serum proteins. Ann., N.Y. Acad. Sci. 121:404-427.

Djang, S. S. T., C. D. Ball and H. A. Lillevik. 1953. Factors affecting solubilization of the nitrogeneous constituents of the mung bean (Phaseolus aureus). Cereal Chem. 30:230-235.

Ekpenyong, T. E. and R. L. Borchers. 1978. Nutritional aspects of the winged bean. Workshop/Seminar on the Development of the Potential of the Winged Bean. Los Banos, Philippines.

Eldridge, A. C., R. L. Anderson and W. J. Wolf. 1966. Polyacrylamide-gel electrophoresis of soybean whey proteins and trypsin inhibitors. Arch. of Biochem. Biophys. 115:495-504.

Eldridge, A. C. and W. J. Wolf. 1967. Purification of the 11S component of soybean protein. Cereal Chem. 44:645-652.

Eldridge, A. C. and W. J. Wolf. 1969. Polyacrylamide-gel electrophoresis of reduced and alkylated soybean trypsin inhibitors. Cereal Chem. 46:470-478.

Esh, C. S. and J. M. Som. 1952. Nutritional survey on available food materials. Part III. Nutritive value of pulses. Indian J. Physiol. Allied Sci. 6:61-70.

Evans, R. J. and M. H. Kerr. 1963. Extraction and precipitation of nitrogeneous constituents of dry navy beans (Phaseolus vulgaris). J. Agr. Food Chem. 11:26.

F.A.O. 1972. Food Composition Table for Use in East Asia. Food and Agricultural Organization of the United Nations. Food Policy and Nutrition Division. Rome (1972).

Fan, T. Y., and F. W. Sosulski. 1974. Dispersibility and isolation of proteins from legume flours. Can. Inst. Food Sci. Technol. J. 7:256-259.

Farmer, E. H. and E. Sunderland. 1935. Unsaturated acids of natural oils Part II. the highly unsaturated fatty acid of the kernels of Parinarium laurinum. J. Chem. Soc. (London): 759-761.

Flink, J. and I. Christiansen. 1973. The production of a protein isolate from Vicia faba. Lebensm.-Wiss. Technol. 6:102-106.

Food and Nutrition Research Center. 1968. Food Composition Table Recommended for Use in the Philippines. NSDB. Manila, Philippines.

Frank, S. S. and S. J. Circle. 1959. The use of isolated soybean protein for non-meat, simulated sausage products- frankfurter and bologna types. Food Technol. 13:307-313.

Gandjar, I. 1978. Fermentation of the winged bean seeds. Workshop/ Seminar on the Development of the Potential of the Winged Bean. Los Banos, Philippines.

Gillespie, J. M. and R. J. Blagrove. 1977. The proteins of the winged bean seed. Aust. Biochem. Soc. Proc. 10:23.

Gillespie, J. M. and R. J. Blagrove. 1978. Isolation and composition of the seed globulins of winged bean, Psophocarpus tetragonolobus (L.) DC. Aust. J. Plant Physiol. 5:357-369.

Goering, H. K. and P. J. Van Soest. 1970. Forage Fiber Analysis (apparatus, reagents, procedures and some applications). Ag. Handbook No. 379, ARS/USDA. Washington, D.C.

Hang, Y. D., K. H. Steinkraus and L. R. Hackler. 1970a. Comparative studies on the nitrogen solubility of mung beans, pea beans, and red kidney beans. J. Food Sci. 35:318-320.

Hang, Y. D., W. F. Wilkens, A. S. Hill, K. H. Steinkraus and L. R. Hackler. 1970b. Enzymatic solubilization of nitrogeneous constituents of mung beans. J. Agr. Food Chem. 18:9-12.

Harding, J., F. W. Martin and R. Kleiman. 1968. Seed protein and oil yields of the winged bean Psophocarpus tetragonolobus in Puerto Rico. Trop. Agric. (Trinidad) 55:307-314.

Hardinge, M. G., J. B. Swarner and H. Crooks. 1965. Carbohydrates in foods. J. Am. Dietetic Assoc. 46:197-204.

Hasegawa, K., T. Kusano and H. Mitsuda. 1963. Fractionation of soybean proteins by gel filtration. Agr. Biol. Chem. 27(2):878-880.

Hough, L. and J. K. N. Jones. 1962. Chromatography on paper. In Methods in Carbohydrate Chemistry. Vol. I. Preparation and Analysis of Sugars. Ed. Whistler, R. L. and M. L. Wolfrom. Academic Press, New York, NY.

Hymowitz, T. and J. Boyd. 1977. Origin, ethnobotany and agricultural potential of the winged bean, Psophocarpus tetragonolobus. Economic Botany 31:180-188.

Hymowitz, T., F. I. Collins, J. Panczner and W. M. Walker. 1972. Relationship between the content of oil, protein, and sugar in soybean seed. Agron. J. 64:613-616.

Institute of Nutrition, Philippines. 1957. Handbook No. 1. pp 28-29. Manila.

Jaffe, W. G. and R. Korte. 1976. Nutritional characteristics of the winged bean in rats. Nutr. Rep. Intl. 14:449-455.

Kawamura, S., K. Nagao and T. Kasai. 1977. Determination of free monosaccharides and detection of sugar alcohols in mature soybean seeds. J. Nutr. Sci. Vitaminol. 23:249-255.

Kelley, J. J. and R. Pressley. 1966. Studies with soybean protein and fiber formation. Cereal Chem. 43:195-206

Kondo, K., S. Mori and M. Kajima. 1953. Studies on proteins (56). On the components of soybean protein. I. Kyoto Univ. Res. Inst. Food Sci. Bill. 11:1-23.

Kordylas, J. M., Y. D. Osei and E. A. Berko. 1978. The processing and formulation of weaning foods based on the winged bean (mns.).

Koshiyama, I. 1968. Chromatographic and sedimentation behavior of a purified 7S proteins in soybean globulins. Cereal Chem. 45:405-412.

Koshiyama, I. 1969. Distribution of the 7S proteins in soybean globulins by gel filtration with Sephadex G-200. Agr. Biol. Chem. (Tokyo) 33:281-284.

Koshiyama, I. and N. Iguchi. 1965. Studies on soybean protein. 1. A ribonucleoprotein and ribonucleic acids in soybean casein fraction. Agr. Biol. Chem. (Tokyo) 29:144-150.

Kulkarni, L. and K. Sohonie. 1956. Non-protein nitrogen in vegetables. Ind. Jour. Med. Res. 44(3):511-518.

Lachance, P. A. 1972. Nutrification: a concept for assuring nutritional quality by primary intervention in feeding systems. J. Agr. Food Chem. 20:522-525.

Lal, B. M., V. Prakash and S. C. Verma. 1963. The distribution of nutrients in the seed parts of Bengal gram. Experimentia 19:154-155.

Levy, J. W. 1977a. The Winged Bean Flyer (Department of Agronomy, University of Illinois in cooperation with the Steering Committee of the Winged Bean, 1977), Vol. 1(1).

Levy, J. W. 1977b. The Winged Bean Flyer (Department of Agronomy, University of Illinois in cooperation with the Steering Committee of the Winged Bean, 1977), Vol. 1(2).

Levy, J. W. 1978. The Winged Bean Flyer (Department of Agronomy, University of Illinois in cooperation with the Steering Committee of the Winged Bean, 1978), Vol 2(1).

Mattil, K. F. 1971. Functional requirements of proteins for foods. J. Am. Oil Chem. Soc. 48:477-480.

Masefield, G. B. 1973. Psophocarpus tetragonolobus- a crop with a future? Field Crops Abs. 26:157-160.

Meyer, E. W. 1966. Soy protein concentrates and isolates. In Proceedings of International Conference on Soybean Protein Foods. Peoria, Illinois. USDA-ARS 71-35:142-154.

Meyer, E. W. and L. D. Williams. 1976. Soy protein concentrates and isolates. Ed. L. O. Hill, World Soybean Research. The Interstate Printers and Publishers, Inc., Danville, Illinois.

Mitsuda, H., K. Yasumoto, A. Yamamoto and T. Kusano. 1967. Study on soybean lipoxygenase. 1. Preparation of crystalline enzyme and assay by polarographic method. Agr. Biol. Chem. (Tokyo) 31:115-118.

National Academy of Sciences. 1975. The Winged Bean: A High Protein Crop for the Tropics. Washington, D.C.

Newell, C. A. and T. Hymowitz. 1979. The potential of the winged bean- Psophocarpus tetragonolobus (L.) DC - as an agricultural crop. In

New Agricultural Crops. Ed. G. A. Ritchie. Westview Press, Boulder, Colorado (in press).

Obara, T. and M. Kimura. 1967. Gel filtration fractionation of the whole water-extractable soybean proteins. J. Food Sci. 32:531-534.

Okubo, K., M. Asano, Y. Kimura, and K. Shibasaki. 1969. On basic subunits dissociated from C (11S) component of soybean proteins with urea. Agr. Biol. Chem. (Tokyo) 33:463-465.

Oyenuga, V. A. 1966. Improvement of nutritional status in developing countries by improved production method. In Proceedings of the Seventh International Congress of Nutrition. Hamburg.

Padilla, S. P. and F. A. Soliven. 1933. Chemical analysis for possible sources of oils for forty-five species of oil bearing seeds. Phil. Agriculturist 22:408-415.

Padhye, V. W. and D. K. Salunkhe. 1977. Biochemical studies on black gram (Phaseolus mungo): 1. Solubilization and electrophoretic characterization of the proteins. J. Food Biochem. 1:111-129.

Palmer, J. K. 1978. Personal communication.

Pant, R. and D. R. P. Tulsiani. 1969. Solubility, amino acid comparison, and biological evaluation of protein isolation from legume seeds. J. Agr. Food Chem. 17:361-366.

Pospisil, F., B. Hlava and M. Buresova. 1978. The winged bean (Psophocarpus tetragonolobus (L.) DC. Workshop/Seminar on the Development of the Potential of the Winged Bean. Los Banos, Philippines.

Pospisil, F., S. K. Karikari and E. B. Mensah. 1971. Investigations of winged bean in Ghana. World Crops 23:260-264.

Protein Advisory Group. 1973. Upgrading human nutrition through the improvement of food legumes. P.A.G. Statement No. 22 (Protein Advisory Group of the United Nations System, New York).

Purseglove, J. W. 1968. Tropical Crops. Dicotyledons I pp. 315-318. John Wiley and Sons, Inc., New York, New York.

Puski, G. and P. Melnychyn. 1968. Starch-gel electrophoresis of soybean globulins. Cereal Chem. 45:192-201.

Puztai, A. 1965. Studies on the extraction of nitrogeneous and phosphorus-containing materials from the seeds of kidney beans (Phaseolus vulgaris). Biochem. J. 94:611-616.

Rackis, J. J., D. H. Honig, D. J. Sessa and F. R. Steggerda. 1970. Flavor and flatulence factors in soybean protein products. J. Agr. Food Chem. 18(6):977-982.

Rackis, J. J., H. A. Sasame, R. L. Anderson and A. K. Smith. 1959. Chromatography of soybean proteins. 1. Fractionation of whey proteins on diethylaminoethylcellulose. J. Am. Chem. Soc. 81: 6265-6270.

Renkonen, K. O. 1948. Studies on haemagglutinins present in seeds of some representative of the family of Leguminoseae. Annales medicinae experimentalis biologiae fenniae 26:66-72. (as cited by Claydon, 1975).

Rhee, K. C., C. M. Cater and K. F. Mattil. 1972. Effect of processing pH on the properties of peanut protein isolates and oil. Cereal Chem. 50:395-404.

Ruberte, R. M. and F. W. Martin. 1978. Cooking of winged bean seed. J. Agr. Univ. Puerto Rico 62:321-329.

Schertz, K. F., W. C. Boyd, W. Jugelsky, Jr. and E. Cabanillas. 1960. Seed extracts with agglutinating activity for human blood. Economic Botany 14(3):232-240.

Schweizer, T. F., I. Horman and P. Wursch. 1978. Low molecular weight carbohydrates from leguminous seeds; a new disaccharide: galactopinitol. J. Sci. Fd. Agric. 29:148-154.

Scobell, H. D., K. M. Brobst and E. M. Steele. 1977. Automated liquid chromatographic system for analysis of carbohydrate mixtures. Cereal Chem. 54(4):905-917.

Senanayake, Y. D. A. and V. A. D. Sumanainghe. 1976. Leaf protein content of Psophocarpus tetragonolobus (L.) DC. J. Natl. Agric. Soc. of Ceylon 13:119-121.

Shemer, M., S. Mizrahi, Z. Berk and S. Mokady. 1973. Effect of processing conditions on isolation of cotton seed proteins by sodium hexametaphosphate extraction method. J. Agr. Food Chem. 21:460-462.

Shibasaki, K. and K. Okubo. 1966. Starch gel electrophoresis of soybean proteins in high concentration of urea. Tohoku J. Agr. Res. 16:317-329.

Shurtleff, W. R. 1978. Household preparation of winged bean milk, tofu, and miso. Workshop/Seminar on the Development of the Potential of the Winged Bean. Los Banos, Philippines.

Singh, S., H. D. Singh and N. C. Sikka. 1968. Distribution of nutrients in the anatomical parts of common Indian pulses. Indian J. Agric. Sci. 45:13-18.

Smith, A. K. and S. J. Circle. 1938. Peptization of soybean protein. Extraction of nitrogeneous consituents from oil-free meal by acids and bases with and without added salts. Ind. Eng. Chem. 30:1414-1418.

Smith, A. K. and S. J. Circle. 1939. Soybean protein precipitation from water and alkaline dispersions by acids and by electrophoresis. Ind. Eng. Chem. 31:1284-1288.

Smith, A. K. and S. J. Circle. 1978. In Soybeans, Chemistry and Technology Vol. I. Proteins. AVI Publishing Co., Westport, Connecticut.

Smith, A. K. and W. J. Wolf. 1961. Food uses and properties of soybean protein. I. Food Technol. 15(5):4-6,8,10.

Smith, A. K., S. J. Circle and G. H. Brother. 1938. Peptization of soybean proteins. The effect of neutral salts on the quantity of nitrogeneous constituents extracted from oil-free meal. J. Am. Chem. Soc. 60:1316-1320.

Smith, A. K., E. N. Schubert and P. A. Belter. 1955. Soybean protein fractions and their electrophoretic patterns. J. Am. Oil Chem. Soc. 32:274-278.

Smith, A. K., J. J. Rackis, P. Isnardi, J. L. Cartter and O. A. Krober. 1966. Nitrogen solubility index isolated protein yield and whey nitrogen content of several soybean strains. General Chem. 43:261-270.

Smith, C. R., Jr., F. R. Earle, I. A. Wolf and Q. Jones. 1959. Comparison of solubility characteristics of selected seed proteins. J. Agr. Food Chem. 7:133-136.

Smoot, L. A., V. V. Garcia and M. D. Pierson. 1979. Fermentation of soy and winged bean milks by lactic acid bacteria - paper accepted for presentation to the Annual Meeting of Int'l Assn. of Milk, Food and Environmental Sanitarians, Inc. Aug. 12-16, 1979. Orlando, Florida.

Sohonie, K. and A. P. Bhandarkar. 1954. Trypsin inhibitors in Indian foodstuffs. Part I. Inhibitiors in vegetable. J. Sci. Ind. Res. 13B:500-503.

Southgate, D. A. T. 1976. Determination of Food Carbohydrate. Applied Science Publishers. Essex, England.

Technicon Instrument Corporation. 1978. Technicon Operations Manual, Tarrytown, New York.

Thompson, L. U., P. A. Allum -Poon, and C. Procope. 1976. Isolation of rapeseed protein using sodiumm hexamataphosphate. J. Inst. Can. Sci. Technol. Aliment. 9:15-19.

Vaintraub, I. A. 1967. The heterogeneity of the subunits of the 11S protein of soybean seeds. Mol. Biol. 1:807-814; Mol. Biol. (USSR) (English Transl.) 1:671-676.

Van Soest, P. J. 1963a. Use of detergents in the analysis of fibrous feeds. Preparation of fiber residues of low nitrogen content. J. Assoc. off. Agric. Chem. 46:825.

Van Soest, P. J. 1963b. Use of detergents in the analysis of fibrous feeds. A rapid method for the determination of fiber and lignin. J. Assoc. off. Agric. Chem. 46:829.

Watanabe, T. and O. Nakayama. 1962. Study of water-extracted protein of soybean. J. Agr. Chem. Soc. Japan 36:890-895.

Weber, K. and M. Osborne. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406-4412.

Weiss, T. J. Food Oils and Their Uses. AVI Publishing Company, Inc. Westport, Connecticut. 1970. pp. 26-31.

Wolf, W. J. 1970. Soybean proteins: their functional, chemicals, and physical properties. J. Agr. Food Chem. 18(6):969-976.

Wolf, W. J. and D. R. Briggs. 1958. Studies on the cold insoluble fraction of the water-extractable soybean proteins. II. Factors influencing conformational changes in the 11S component. Arch. Biochem. Biophys. 76:377-393.

Wolf, W. J. and D. A. Sly. 1964. Effects of buffer cations on chromatography of proteins on hydroxyl apatite. J. Chromatog. 15:247-250.

Wolf, W. J. and D. A. Sly. 1965. Chromatography of soybean proteins on hydroxylapatie. Arch. Biochem. Biophys. 110:47-56.

Wolf, W. J. and D. A. Sly. 1967. Cryoprecipitation of soybean 11S protein. Cereal Chem. 44:653-668.

Wolf, W. J., G. E. Babcock and A. K. Smith. 1962. Purification and stability studies of the 11S component of soybean proteins. Arch. Biochem. Biophys. 99:265-274.

The two page vita has been removed from the scanned document. Page 1 of 2

The two page vita has been removed from the scanned document. Page 2 of 2

BIOCHEMICAL COMPOSITION OF MATURE WINGED BEANS

Psophocarpus tetragonolobus (L.) DC

by

Virgilio Villegas Garcia

(ABSTRACT)

The proximate composition has been determined and information on the carbohydrates and fatty acid composition has been obtained for the mature seeds of five varieties of winged bean, *Psophocarpus tetragonolobus* (L.) DC. The proteins of one of these varieties have been fractionated and partially characterized.

The results confirm that winged beans have a high protein and fat content, which is similar to soybeans. The mean protein content of the five varieties analyzed was 40.12%; the mean fat content was 16.94%. The endosperm in TPT-2 bean was found to constitute about 84% of the dry weight of the seeds, the hulls about 16%. Whole beans contain about 17% "dietary fiber", mostly (72%) in the hulls.

Similar to soybeans, starch could not be detected in the mature beans and the soluble sugars (9.7 to 13.8%) consisted almost entirely of verbascose (0.2 to 0.9%), stachyose (2.2 to 3.6%), raffinose (1.1 to 2.0%) and sucrose (5.6 to 8.2%).

The fatty acid composition of winged beans was also similar to soybeans, with a high degree of unsaturation (mean, 62.0%). The major unsaturated fatty acids were oleic and linoleic. Behenic and stearic acids were the major saturated fatty acids. Parinaric acid was not present in the five varieties of winged beans analyzed, although the presence of this toxic acid in winged beans had been reported earlier. The solubility of protein nitrogen was found to be a function of pH and salt concentration. The nitrogen solubility in water was lowest (17.6%) at pH 4.0 (the apparent isoelectric point). The maximum solubility in water at neutrality (pH 6.70) was about 60.4%. Solutions of Na_3PO_4 (0.5%, pH 11.0) extracted the most protein (86.0%), compared to 70% in 0.75-1.0 M NaCl (pH 6.95), 69% in 0.25 M Na_2SO_4 (pH 7.20) and 66% in 0.20-0.30 M Na_2HPO_4 (pH 9.20).

This study also describes the preparation of protein concentrates (extracts), isolates (acid precipitates) and "wheys" from defatted bean flour. The amino acid composition of the concentrates, isolates, and "wheys" obtained from water, NaCl and Na_3PO_4 extraction were similar to the defatted bean flours, except that the precipitate from NaCl extraction contained a high amount of methionine. Lysine was higher than that found in soybeans with cystine and methionine as the limiting amino acids.

Fractionation of the extracts on Biogel A-1.5M gave three to four fractions, two high molecular weight fraction components and one or two low molecular weight fractions. The acid precipitated protein from the water extract yielded two fractions of higher molecular weight

proteins and a fraction of lower molecular weight. The "whey" from the water extract yielded two fractions, both of relatively low molecular weight proteins.

The fractions from gel filtration, the original extracts, the acid precipitate and "whey" were all analyzed via polyacrylamide gel electrophoresis. The Na_3PO_4 extract was apparently highly aggregated as indicated by non-migration of the proteins. The NaCl and water extracts appeared to be similar with regard to complexity and molecular size. These extracts also showed evidence of aggregation. The acid precipitates were also highly aggregated especially that of the NaCl extract which did not migrate at all. The wheys were all well resolved.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the winged bean proteins indicated the presence of well resolved proteins and/or subunits. SDS-PAGE patterns did not show any aggregation. The high molecular weight fractions appeared to consist of proteins with a molecular weight of about 74,000 while the low molecular weight fractions consisted of proteins with molecular weights of about 20,000.