

To the Graduate Council:

I am submitting herewith a thesis written by Sevim Caglar Burklin entitled "Enzymatic Modification of Proteinaceous Vegetable Materials for Infant Food in Turkey." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology.

J. L. Callin's
Major Professor

We have read this thesis and
recommend its acceptance:

M. R. Huelo

John T. Smith

Accepted for the Council:

Stanton A. Smith
Vice Chancellor for
Graduate Studies and Research

156

ENZYMATIC MODIFICATION OF PROTEINACEOUS VEGETABLE
MATERIALS FOR INFANT FOOD IN TURKEY

A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Sevim Caglar Burklin
August 1973

ACKNOWLEDGMENTS

The author wishes to express her sincere appreciation to Dr. M. R. Johnston for his valuable and enthusiastic advice and guidance in this study.

The author is indebted to Dr. J. L. Collins for his valuable and patient assistance throughout this study and for improving both style and content of this thesis. Sincere appreciation is extended to Dr. J. T. Smith for his valuable assistance in this study.

Special thanks are due to Dr. J. A. Corrick for his valuable advice and assistance, to Mr. M. Newman for his assistance with the statistical analysis and to Miss R. Hill for her assistance with laboratory work.

Deep appreciation is expressed to her husband and her family for their encouragement and support during the period of this study.

ABSTRACT

This experiment was designed to study the use of a commercial proteolytic enzyme preparation for the partial hydrolysis of proteinaceous vegetable materials so that they may be utilized for the purpose of child feeding practices. The effect of pH, temperature, time, denaturation period, concentrations of enzyme and substrate, and sodium chloride content were factors of the investigation from which optimum conditions for proteolytic activity could be ascertained. Another objective was the evaluation of protein quality by estimating the amounts of limiting essential amino acids of the individual raw materials after enzyme treatment. The raw materials were chick peas, whole wheat flour, sunflower seed press cake and a mixture consisting of 44 percent sunflower seed press cake, 28.5 percent chick peas, and 27.5 percent whole wheat flour.

Effect of pH, temperature, time, denaturation period, concentration of enzyme and substrate, and sodium chloride content on enzymatic solubilization of proteins and production of sugars from the individual sources were determined.

The macro-Kjeldahl method ($N \times 6.25$) was used to express the proteolytic activity of Rhozyme-41. The estimated amount of free amino groups in terms of total ninhydrin positive material (NPM) was determined by the ninhydrin-colorimetric method. The amount of glucose was determined by the Fermco-test S.F.G. The amount of maltose was determined by the A.O.A.C. maltose method. The quantities of limiting amino acids were

estimated by one dimensional thin-layer chromatography. The quality of protein for each protein source was evaluated by the chemical score.

Maximum protein solubilization occurred in chick peas between pH 7.5 and 8.5, in whole wheat flour at pH 8.0 and in sunflower seed press cake and the mixture at 8.5. The greatest release of free amino groups in terms of ninhydrin positive material was found between pH 8.0 and 8.5 for individual sources. There was a trend for a reduction in the amount of reducing sugars above pH 6.0 for each protein source.

High levels of soluble protein, ninhydrin positive material, glucose and maltose were observed at 50°C for the individual proteinaceous materials.

The maximum enzymatic hydrolysis was completed within 6 hours for each protein source. However, there was a trend for an increase in NPM content until reaction time was extended to 8 hours or beyond. High maltose contents were observed at 6 hours while no change was observed in glucose level after 2 hours digestion for each plant protein source.

All four protein sources required denaturation of proteins prior to enzyme treatment. The cooking time was found to be 20 minutes for chick peas and 15 minutes for whole wheat flour, sunflower press cake and the mixture for optimum solubilization. Higher amounts of NPM were observed at 20 minutes denaturation period followed by a decrease at a 25 minute cooking period. Glucose and maltose were not affected by the length of denaturation.

For maximum solubility of proteins, whole wheat flour, chick peas, sunflower press cake and the mixture required 1.5, 2.0, 2.5 and 2.5

enzyme preparation, respectively. Except for the mixture, high NPM concentrations occurred at the 2.5 percent enzyme level for the individual protein sources.

There were no significant changes in soluble protein and free amino acid content as the amount of enzyme added reached the 3.0 percent level of each protein source. The amount of sugar increased directly with the amount of enzyme added.

The amount of soluble protein increased only slightly as the substrate was increased from 5 grams to 9 grams.

The maximum protein solubilization of chick pea, whole wheat flour and the mixture occurred between the 0.2 and 0.3 M concentrations of sodium chloride, while the maximum protein solubilization occurred at 0.1 M sodium chloride in sunflower seed press cake. There were no significant changes in free amino acid and sugar content for each protein source.

The soluble protein content of the enzyme hydrolyzates from chick pea, whole wheat flour, sunflower seed press cake and the mixture were 187.7, 111.2, 412.0, and 257.7 mg/g, respectively. These corresponded to 134, 94.8, 387 and 235.6 micromoles of NPM per gram of protein source.

The protein scores based on the quantity of limiting amino acid, determined by thin-layer chromatography, were found to be: 46 percent in relation to reference protein for chick pea, which was deficient in methionine; 73 percent in relation to reference protein for whole wheat flour, which was deficient in lysine; and 84 percent in relation to reference protein for sunflower seed press cake, which is deficient in lysine. The protein score for the mixture was 75 percent.

TABLE OF CONTENTS

CHAPTER		PAGE
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	6
	The Use of Proteolytic Enzymes	9
	Source of Proteolytic Enzymes and Mode of Action . . .	10
	Choice of Proteolytic Enzymes	11
	Present Activities on the Development of Food Mixtures	
	in Turkey	12
	Possible composition of a baby food developed in	
	Turkey	13
	Evaluation of Nutritive Value of Proteins	14
	Protein value of foods	14
	Protein and amino acid requirements	14
	Effect of heat on nutritive value of proteins	15
	Measurement and expression of protein quality	16
	Amino acid analysis	18
III.	MATERIAL AND METHODS	21
	Source of Material Utilized	21
	Source of Proteolytic Enzyme	22
	Experimental Methods	22
	Standardization of conditions for optimum enzyme	
	activity	22
	Effect of pH on enzymatic protein extraction	22

Effect of temperature on enzymatic protein	
extraction	24
Effect of reaction time on enzymatic protein	
extraction	24
Effect of denaturation period on enzymatic protein	
extraction	25
Effect of enzyme concentration on enzymatic protein	
extraction	26
Effect of substrate concentration on enzymatic	
protein extraction	26
Effect of sodium chloride content on enzymatic	
protein extraction	27
Ninhydrin-colorimetric determination of total free	
amino acids	28
Reducing sugars	29
Determination of glucose	29
Determination of maltose	30
Preparation of proteinaceous material for thin-layer	
chromatography	32
Oxidation	32
Acid hydrolysis	33
Alkaline hydrolysis	33
Standard amino acid solutions	34
Thin-layer chromatography of samples	34

CHAPTER

PAGE

Calculation of the protein score of the predigested samples	35
Statistical analysis	36
IV. EXPERIMENTAL RESULTS	37
Standardization of Conditions for Optimum Enzyme Activity	37
Effect of pH on enzymatic hydrolysis of proteinaceous vegetable material	37
Effect of temperature on enzymatic hydrolysis of vegetable proteinaceous material	45
Effect of reaction time on enzymatic hydrolysis of proteinaceous vegetable material	55
Effect of denaturation time on enzymatic hydrolysis of proteinaceous vegetable material	62
Effect of enzyme concentration on enzymatic hydrolysis of proteinaceous vegetable material	72
Effect of substrate concentration on enzymatic solubilization of proteinaceous vegetable material	79
The effect of sodium chloride content on enzymatic solubilization of proteinaceous vegetable material	89
Evaluation of Protein Quality	98
V. DISCUSSION	105

CHAPTER	PAGE
VI. SUMMARY	118
LITERATURE CITED	122
VITA	130

LIST OF TABLES

TABLE		PAGE
1.	Summaries of Analysis of Variance for the Effect of pH on Enzymatic Solubilization of Proteins and Production of Sugars from Vegetable Proteinaceous Materials	38
2.	Means for the Effect of Protein Source on Enzymatic Solubilization of Proteins and Production of Sugars when pH of Hydrolysis was from 6.0 to 9.5	40
3.	Means for the Effect of pH on Enzymatic Solubilization of Proteins and Production of Sugars from Vegetable Proteinaceous Materials	42
4.	Effect of Protein Source x pH Interaction on Enzymatic Solubilization of Proteins from Vegetable Proteinaceous Materials	43
5.	Effect of Protein Source x pH Interaction on Sugar Content from Enzymatic Solubilization of Vegetable Proteinaceous Materials	46
6.	Summaries of Analysis of Variance for the Effect of Temperature on Enzymatic Solubilization of Proteins and Production of Sugars from Vegetable Proteinaceous Materials	48
7.	Means for the Effect of Protein Source on Enzymatic Solubilization of Proteins and Production of Sugars when Temperature of Hydrolysis was from 40° to 60°C	49

TABLE

PAGE

8.	Means for the Effect of Temperature on Enzymatic Solubilization of Proteins and Production of Sugars from Vegetable Proteinaceous Materials	51
9.	Effect of Protein Source x Temperature Interaction on Enzymatic Solubilization of Proteins from Vegetable Proteinaceous Materials	52
10.	Effect of Protein Source x Temperature Interaction on Production of Sugars from Enzymatic Solubilization of Vegetable Proteinaceous Materials	54
11.	Summaries of Analysis of Variance for the Effect of Reaction Time on Enzymatic Solubilization of Proteins and Production of Sugars from Vegetable Proteinaceous Materials	56
12.	Means for the Effect of Protein Source on Enzymatic Solubilization of Proteins and Production of Sugars when Reaction Time of Hydrolysis was from 2 to 10 Hours . . .	57
13.	Means for the Effect of Reaction Time on Enzymatic Solubilization of Proteins and Production of Sugars from Vegetable Proteinaceous Materials	59
14.	Effect of Protein Source x Reaction Time Interaction on Enzymatic Solubilization of Proteins from Vegetable Proteinaceous Materials	61
15.	Effect of Protein Source x Reaction Time Interaction on Production of Sugars from Enzymatic Solubilization of Vegetable Proteinaceous Materials	63

TABLE

PAGE

16.	Summaries of Analysis of Variance for the Effect of Denaturation Time on Enzymatic Solubilization of Proteins and Production of Sugars from Proteinaceous Vegetable Materials	65
17.	Means for the Effect of Protein Source on Enzymatic Solubilization of Proteins and Production of Sugars when Hydrolysis of Denaturation Period was from 5 to 25 Minutes	66
18.	Means for the Effect of Denaturation Time on Enzymatic Solubilization of Proteins and Production of Sugars from Vegetable Proteinaceous Materials	68
19.	Effect of Protein Source x Denaturation Time Interaction on Enzymatic Solubilization of Proteins from Vegetable Proteinaceous Materials	69
20.	Effect of Protein Source x Denaturation Time Interaction on Production of Sugars from Enzymatic Solubilization of Proteinaceous Vegetable Materials	71
21.	Summaries of Analysis of Variance for the Effect of Enzyme Concentration on Enzymatic Solubilization of Proteins and Production of Sugars from Proteinaceous Vegetable Materials	73
22.	Means for the Effect of Protein Source on Enzymatic Solubilization of Proteins and Production of Sugars when Enzyme Concentration of Hydrolysis was from 1.0 to 3.0 Percent	74

TABLE

PAGE

23.	Means for the Effect of Enzyme Concentration on Enzymatic Solubilization of Proteins and Production of Sugars from Proteinaceous Vegetable Materials	76
24.	Effect of Protein Source x Enzyme Concentration Interaction on Enzymatic Solubilization of Proteins from Vegetable Proteinaceous Materials	78
25.	Effect of Protein Source x Enzyme Concentration Interaction on Production of Sugars from Enzymatic Solubilization of Proteinaceous Vegetable Materials . . .	80
26.	Summaries of Analysis of Variance for the Effect of Substrate Concentration on Enzymatic Solubilization of Proteins and Production of Sugars from Vegetable Proteinaceous Materials	81
27.	Means for the Effect of Protein Source on Enzymatic Solubilization of Proteins and Production of Sugars when Substrate Concentration of the Hydrolysis was from 5 to 9 Grams	82
28.	Means for the Effect of Substrate Concentration on Enzymatic Solubilization of Proteins and Production of Sugars from Proteinaceous Vegetable Material	84
29.	Effect of Protein Source x Substrate Concentration Interaction on Enzymatic Solubilization of Proteins from Vegetable Proteinaceous Materials	86
30.	Effect of Protein Source x Substrate Concentration Interaction on Production of Sugars from Enzymatic Solubilization of Proteinaceous Vegetable Materials . . .	88



TABLE

PAGE

31. Summaries of Analysis of Variance for the Effect of Sodium Chloride Concentration on Enzymatic Solubilization of Proteins and Production of Sugars from Proteinaceous Vegetable Materials	90
32. Means for the Effect of Protein Source on Enzymatic Solubilization of Proteins and Production of Sugars when Sodium Chloride Content of Hydrolysis was from 0.1 to 0.5 M	91
33. Means for the Effect of Sodium Chloride Concentration on Enzymatic Solubilization of Proteins and Production of Sugars from Proteinaceous Vegetable Materials	93
34. Effect of Protein Source x Sodium Chloride Concentration Interaction on Enzymatic Solubilization of Proteins from Proteinaceous Vegetable Materials	95
35. Effect of Protein Source x Sodium Chloride Concentration Interaction on Production of Sugars from Enzymatic Solubilization of Proteinaceous Vegetable Materials . . .	97
36. Limiting Essential Amino Acid Content of Proteinaceous Vegetable Materials as Determined by Thin-Layer Chromatography	99
37. Literature Values for the Essential Amino Acid Content of Proteinaceous Vegetable Materials as Compared with Those of Whole Hen's Egg Proteins	102
38. Chemical Scores of Proteinaceous Vegetable Material Based on Limiting Essential Amino Acid Content as Compared with Corresponding Amino Acids of Whole Egg Proteins	103

CHAPTER I

INTRODUCTION

In developing countries the major nutritional problem among weaned infants and preschool children is malnutrition. While protein deficiency may predominate, calorie deficiency is often a contributing factor. Protein deficiency is most serious in children between the ages of weaning and school entrance. Children are fed mostly local carbohydrate staples such as cereals.

These foods are deficient in proteins both quantitatively and qualitatively. About 50 percent of children of the world do not receive adequate protein. Fish, meat, eggs, and milk, which are classified as protective foods, occupy a very small place in their diet. Under these circumstances it is not surprising that in many populations up to 40 percent of the infants die before reaching school age. Children who survive protein deprived childhood may have their mental capacity permanently impaired because of retarded brain growth.

Protein deficiency also renders infants and children susceptible to respiratory and gastrointestinal infections. In the developing countries the mortality rate of children between the ages of one and four is 20 to 50 times greater than that in the developed countries.

Protein deficiency arises either from a lack of adequate food supplies within the country or from social, economic, and cultural factors which limit the consumption of protein by infants and children.

In Turkey the nutritional status of infants and children is not too different from that of any other developing country. At present there is no overall nutritional survey in Turkey. Although a few individual surveys of certain groups have been carried out in certain areas of the country, there exists very little information to indicate the morbidity and mortality rates of the children in relation to malnutrition. Baysal (10) reported that one nutritional survey was conducted with 346,263 children in 1968. It was found that for each thousand live births, 205 died by the end of 12 months and 100 more died between one and four years of age. According to the United States Interdepartmental Committee on Nutrition for National Defense (ICNND) nutritional survey in Turkey, 50 percent of the children and infants coming to hospitals were malnourished. Authorities found an extremely high incidence of malnutrition of marasmus type, which indicated a severe degree of both protein and calorie symptoms (83).

Recent surveys of Neyzi et al (56) indicated that average birth weight was comparable to that of children born in Europe and the U. S. Infants showed normal growth rates up to six months of age since they are breast fed during this period. The growth rate decreased as the flow of milk became insufficient. Supplementary foods did not provide sufficient quantities of protein, total calorie requirements, vitamins and minerals. Prolonged breast feeding up to 18 to 24 months is universal among lower socioeconomic groups in Turkey. Semisolids are usually introduced in the diet at about 9 to 12 months. The most suitable food

for infants is probably "muhallebi" which is a pudding made from a mixture of rice, flour, sugar and water. The other starch-sugar mixtures "Paro" and "Ari" are advertised highly as the best food for growth and development. About 50 percent of infants and children do not get milk and milk products; meat and eggs are seldom fed to the weaned infants of low income groups.

Factors that affect the feeding patterns of infants are: ignorance of food nutrition, the unavailability of high quality, inexpensive baby foods, poor economic conditions, and social and cultural limitations. A large portion of the population which lives in rural areas is uneducated. The great majority of the mothers are illiterate; they are unaware of food values and food requirements for infants and young children even though this information is often common knowledge over most of the world. Religious, traditional and superstitious sanctions usually interfere in the intelligent use of available food sources. For instance, when meat and legumes are available, they are eaten by adults, but are not given to infants since the parents believe that they are too difficult to be digested by the infants. Furthermore, the socioeconomic conditions play important roles in the feeding of young children. In Turkey the annual income per capita is around 200 U. S. dollars. Thus, the buying capacity of many families is quite low. This makes most families dependent on cereals and legumes. Another factor is the unavailability of high quality, food at a low price. Therefore, unavailability of high quality, inexpensive baby foods becomes an important factor in the proper feeding of infants and young children.

In an attempt to raise the level of nutrition and standard of living among the populations of developing countries, the United Nations provides worldwide coordinated programs dealing with the most urgent nutritional problems. The agencies of The Food and Agriculture Organization (FAO), World Health Organization (WHO), the United Nations Educational Scientific and Cultural Organization (UNESCO), and the United Nations Children's Fund (UNICEF) are supplying food, agricultural machinery and many other essential items to the developing areas of the world. However, the improvement in nutritional levels can be brought about only through the economic and scientific development within the country on a national basis. In Turkey food supplies are more or less adequate at the national level. The existing malnutrition and undernutrition are not related solely to the food supply shortage. Although, foods of animal origin are limited, foods consisting of cereals, legumes and oil seeds are being consumed in greater quantities. There is an urgent need for instruction of the kinds of food that should be produced in the country for improving the diets of infants and young children as well as adults.

In view of acute shortage of animal protein, the need for providing low cost nutritious food supplements has been stressed by several workers in different countries and by international organizations. A considerable amount of work has been conducted on the utilization of protein-rich foods of plant origin for infant feeding. The most promising protein-rich materials are legumes and oil seed meals from soybean, sunflower seed, peanut, sesame and cottonseed. However, foods of vegetable origin which

are used for infant feeding are poorly digested and possess unacceptable textural characteristics and flavor. In general, very little attention has been given to the plant protein mixtures with low digestibility that are used for infants who cannot even easily masticate at one year of age.

The purpose of the present investigation was to study the use of commercial proteolytic enzyme preparation for the partial hydrolysis of plant proteins for baby food mixtures. The effect of pH, temperature, time, denaturation period, concentrations of enzyme preparation and substrate and sodium chloride content were factors of the investigation so that optimal conditions for proteolytic activity could be ascertained. Other objectives were to determine the amounts of total free amino acids and reducing sugars and also to evaluate protein quality by estimating the amounts of limiting essential amino acids of the individual raw materials and the mixture after enzyme treatment.



CHAPTER II

REVIEW OF LITERATURE

Protein-calorie deficiency is the most common nutritional disorder of early childhood particularly in Latin America, Africa, the Far East and the Near East. Protein-calorie malnutrition in infants and children from kwashiorkor (mainly a protein deficiency disease) to marasmus is caused by inadequate proteins and calories in diet. Deficiencies may be due either to the lack of total protein or to the absence of the essential amino acids.

The consequences of protein deficient diets are found among the weaned infants and young children of the lower income groups. Milk, eggs, meat and fish are not available in adequate quantities.

Animal sources of protein are generally too scarce and expensive, and long term efforts are needed to bring about a substantial increase in supplies of such foods. Therefore, much attention has been given to other sources of proteins. These are available locally for consumption at the present time or could be made available readily. Most of these food sources are of plant origin, particularly, processed oil seed meals and legumes of various kinds.

Plant proteins are usually deficient in one or more essential amino acids, but Bressani (18) and Howe (40) reported that these deficiencies could be corrected by one of the following methods: (a) amino acid supplementation, (b) supplementation with other protein sources which

have high biological value, and (c) the appropriate combination of two or more proteins which are deficient by themselves. The latter method has wider applicability for infant and child feeding. Studies indicated that supplementation through such food combinations is practical and acceptable (41, 42, 61). Autret and Van Veen (5) gave short accounts of the possible infant formula combinations based on food mixtures of plant origin. Using locally available protein sources, inexpensive and nutritious infant food formulae have been developed in different countries sponsored by local governments and international agencies.

Saridele was one of the first vegetable infant foods developed and manufactured under a joint UNICEF and FAO project in Indonesia. Teply (81) and Guthkar et al. (35) reported that a blend of soybean and sesame promoted favorable growth in premature babies.

The Institute of Nutrition for Central America and Panama developed a high quality vegetable protein mixture known as Incaparina, consisting of corn and cottonseed. Bressani (19) and Scrimshaw (69) described the high nutritional value for the Incap Vegetable Mixture. A blend of peanut and chick pea flour was used to formulate an Indian Multi-purpose Food. Feeding trials with children produced highly significant improvements in their nutritional status (60, 77). A high quality protein-rich mixture known as Laubina has been developed from local sources in Lebanon. It is a mixture of parboiled wheat and chick peas (80).

Selection of ingredients for infant food mixture depends not only on the protein content but also on acceptability, lack of toxicity,

local availability, stability under poor storage conditions, calorie intake and digestibility.

Early experiments by Osborne and Mendel (58) showed that soybeans and other legumes will not support growth unless cooked for several hours. Consequently, it was found that raw legumes contained a substance which inhibits the action of trypsin. Liener (45) reported that so-called trypsin inhibitor did not appear to interfere with the ultimate digestion of protein, but it did retard the liberation of methionine so that methionine can not be used for protein synthesis. A list of the most known toxins in plant foods was given by Coon (26); these are cyanogenetic glucosides (linseed), saponin (soybean), trypsin inhibitors (soybeans and other legumes), hemagglutinins (soybean and other legumes) and gossypol (cottonseed). However, these toxic substances can be reduced or eliminated to safe limits by appropriate preparations and by cooking.

The calorie yield of any food and the nutritive value of protein and other nutrients are influenced by digestibility of the substance. Digestibility of plant food is lower than that of animal protein. FAO reports that plant proteins which give constantly low digestibility values are cereals, legumes and oil seeds (43). There is little knowledge of the causes for the lower digestibility. It has been suggested that crude fiber concentration is the responsible factor (20). Another opinion is that low digestibility is due to lack of complete hydrolysis of the proteins in the gastrointestinal track.

I. THE USE OF PROTEOLYTIC ENZYMES

Utilization of vegetable proteins for human consumption has presented difficulties because of poor digestibility, unacceptable flavor and textural properties. Thus, mothers with various food prejudices are reluctant to give such plant protein mixture to their babies. To improve digestibility as well as flavor and textural characteristics of plant protein foods, attention has been focused on the use of enzymes in the preparation of infant foods. Sreekantiah, et al. (74) reported that enzymatic treatment enhanced the extractibility of proteins in defatted sesame and peanut meals and in chick peas, green and black grams and field beans. Criswell et al. (27) and Connelley et al. (25) investigated acid and enzyme hydrolysis for recovery of proteins from raw materials. These authors found that the use of fungal proteolytic enzymes gave satisfactory results in separating proteins from tissues of plant and animals. Abdo and King (1) and Hang et al. (38) proposed the use of enzymes to improve the nutritional quality of plant proteins.

Through many centuries, soybean and fish have been modified by fermentation using molds, yeasts, and a mixture of organisms to produce more digestible and palatable foods in the Far East (71, 72). One of the oldest enzyme-modified plant proteins is Natto (67) of Japan. It is produced by inoculating cooked soybeans with Bacillus natto. Other enzyme-modified soybean products are miso, soysauce and tempeh. Soysauce and miso have been made from soybeans inoculated with Aspergillus oryzae. Tempeh, an Indonesian food, has received much attention in recent

years (36, 54, 76). It is an inexpensive and a readily digested product. The soybean proteins are broken down into amino acids and other water soluble products thereby making tempeh a rich source of readily assimilable amino acids.

A number of investigations have been made on the nutritive value of these enzyme-modified soybean products. Arimoto (4), Gyorgy (36) and Omans et al. (57) reported that water soluble nitrogen, digestibility, and biological values are higher than those of cooked beans.

Source of Proteolytic Enzymes and Mode of Action

Proteases, along with amylases are probably the most important hydrolytic enzymes. Proteolytic enzymes are distributed widely in plants, animals, molds and bacteria. The well-known digestive enzymes are papain, ficin, bromelain, trypsin, chymotrypsin, and pepsin. Fungal proteases are produced mostly from Aspergillus oryzae (14), A. niger and A. satoi (65). These enzymes attack all proteins. They hydrolyze denaturated proteins more easily than native ones (13). There are two practical methods for measuring proteolytic activity: the increase in the free carboxyl content of amino groups may be followed titrametrically at a constant pH and the increase in ninhydrin color development reflects the proteolytic activity through the increase in new amino terminal groups. In the latter case, the increase in amino groups in the extract can be measured by the Kjeldahl method (16,30,66).

Choice of Proteolytic Enzymes

Production of enzyme-modified foods and protein hydrolyzates for flavor purposes requires enzymes of broad specificity which leads to extensive hydrolysis of proteins into low molecular weight substances. Rao et al. (64) and Cheftel et al. (23) reported that fungal proteases are most suitable for the general cleavage of peptide bonds. On the other hand, in the formation of milk curd in cheese making, the enzyme rennin which splits specific bonds, is more satisfactory.

Heat stable enzymes are preferred in the food industry. Their extensive use is attributed to preventing microbial spoilage during the enzymatic hydrolysis and to increasing the rate of enzymatic reactions (11). Moreau (53) found that papain was more heat stable than fungal proteases. Heat stability of papain is very important in meat tenderizing. A major part of the hydrolysis occurs while the meat is cooking. However, sometimes high heat stability can be a disadvantage. Usually canned hams are tenderized with papain before canning. Schleich and Arnold (78) observed that the enzyme survived during the heat processing and caused the decomposition of the hams during storage.

Enzymes are affected greatly by pH changes. There is considerable variation in pH values for optimum activity of proteases (23). For most enzymes the optimal pH is in the neutral or slightly acidic regions. Some proteases, however, exhibit activity at extremely low pH range such as pepsin at pH 1.5-2.5, and some exhibit activity at a fairly high alkaline pH range such as trypsin at pH 8-11 (64, 73).

The presence of inhibitors and activators influence the choice of enzyme for a given application. Fungal proteases are used widely in baking bread while trypsin and plant proteases have not been used due to the presence of natural inhibitors in wheat flour and the effect of oxidizing agents on plant proteases (44).

The effect of salt on proteolytic activity has been studied by Miller (48) and by Hang et al. (38). They found that the addition of 0.3 M sodium chloride had a significant effect on the extractibility of proteins by proteases.

II. PRESENT ACTIVITIES ON THE DEVELOPMENT OF FOOD MIXTURES IN TURKEY

In Turkey the consumption of animal protein has not reached a satisfactory level. The people consume a high level of cereals. The major portion of calories and proteins is furnished by wheat. Only 16 grams of protein per day are furnished by animal sources (61).

While many countries have been using the most advanced techniques in food science and technology, Turkey as late as 1956 did not offer a single course in nutrition nor feeding programs at any educational level (82). To improve nutritional conditions among school children, UNICEF sent a nutrition instructor and a dietician to Turkey at the request of the government. For seven years the nutrition expert collected information on food sources, food habits and nutritional status of the people. In 1966, another FAO/UNICEF team (22) visited Turkey to determine the possibilities for the development and production of protein

rich mixtures for the infants and young children. They concluded that raw materials were available for the development of such mixtures.

Possible Composition of a Baby Food Developed in Turkey

Wheat, Triticum vulgare, is the most widely cultivated food plant in Turkey. It is eaten in the form of bread and burgul and it contributes most to calorie and protein intake. However, the nutritive value of wheat is lower than legumes and oil seed meals due to a lysine deficiency. Foods rich in lysine can be used for the supplementation of wheat. Thus, using wheat flour or burgul, the development of plant food mixtures, namely Laubina (80), superamine (42) and bal-ahar (29) has been accomplished in different countries. Experimental studies with these mixtures showed satisfactory results in the growth rates.

Chick pea, Cicer arietinum, is the most popular legume in the Mediterranean countries including Turkey. Chick pea is consumed by children as a snack after lime-roasting. It is also used in some candied sweets and in some vegetable dishes. It contains about 18 to 25 percent protein and is rich in lysine and threonine, but limited in methionine (6, 62).

In search for a suitable food mixture, the use of a combination of cereals and legumes is a logical choice. This is not only because of the known mutual supplemental effects, but also because of the wide acceptance of these products in local dietary patterns. Hanafy et al. (37) and Parpia et al. (61) reported on the successful use of food mixtures supplemented with chick pea in the treatment and prevention of malnutrition.

Optimal heat treatment has been suggested to bring about an increase in nutritive value of chick pea by inactivating the trypsin and growth inhibitors present (26, 45).

Sunflower seed (Helianthus annus), press cake is obtained by extracting oil from the kernels. Clandinin (24) reported that sunflower seed meal contains 45 to 50 percent protein. It has been used in the preparation of milk substitutes for infant feeding (7, 21). It is a good source of methionine but is deficient in lysine. Feeding trials with a mixture of defatted fish flour and sunflower seed press cake have been conducted in Chile (8). Results indicate that the mixtures were promising for infant feedings.

III. EVALUATION OF NUTRITIVE VALUE OF PROTEINS

Protein Value of Foods

Proteins are essential to life. The primary functions of food proteins are to provide amino acids in appropriate proportions for the synthesis of tissue proteins during growth, for the maintenance of cellular nitrogenous constituents and for other metabolic needs. The nutritive value of food in the terms of protein is dependent on both the quantity and the quality of the protein.

Protein and Amino Acid Requirements

In order to calculate protein needs, it is necessary to assume that the intake of other nutrients, particularly calories, is adequate. According to FAO/WHO (33) experts, protein requirements of an individual

are expressed either as a requirement of a given amount of protein per day or a percentage of a total diet. Another method of expression and perhaps the most useful, is to express the requirement in the terms of grams of protein per kg body weight per day.

The expert group stated that the daily protein requirements of infants should not be less than 2.2 g/kg, assuming that the protein is completely digested. According to Fomon (32), if a child is fed very little milk and a mixture of plant protein, the desirable figure is 2.5 to 3.5 g/kg/day at the age of four months.

When protein foods are fed at the level needed to meet the total protein requirements, the overall proportion of amino acids available is more important in determining the quality than simply the absolute amount of the individual essential amino acids. To establish a balanced amino acid reference, a number of amino acid patterns have been proposed (55).

A reference amino acid pattern for infants and children should be similar to the pattern of their amino acid requirements. An estimate of average requirements for essential amino acids can be obtained by utilizing data on volumes of intake by normal infants.

Effect of Heat on Nutritive Value of Proteins

The nutritive value of most legumes is improved by heating. This is due partly to the destruction of trypsin inhibitors and toxic substances and to improved digestibility of sulfur-containing amino acids (12,45).

Similar beneficial effects of heat have been reported with cereals, but it appears that the effect was due to the greater palatability of the food. Millner et al. (59) showed that cooked wheat was more palatable to rats than raw wheat.

Severe heat treatment impairs the nutritive value of proteins. It has been established that temperature, time of heating and the presence of reducing substances affect the values of protein. One of the major causes of the reduction in nutritive value is the Maillard reaction between lysine and reducing sugars. The impairment is frequently accompanied by an increased tendency of the protein to resist enzymatic digestion (2, 45).

Measurement and Expression of Protein Quality

The evaluation of protein in mixed diets can be accomplished most accurately in the species for which the proteins are intended as food. Since this can be seldomly accomplished, the quality of proteins in food is generally estimated by either analyzing the amino acid composition of the protein or by utilizing biological assay techniques (55). The latter is carried out on experimental animals having analogous nutritional requirements with human subjects. The most widely used biological methods are protein efficiency ratio, net protein utilization, biological value, gross protein value in the estimation of nutritive quality of proteins.

The first reasonably successful method for the chemical evaluation of proteins based on amino acid content was developed by Mitchell and

Block (50). The nutritive value of a protein was based on the ratio of the quantity of an essential amino acid in a test protein to the quantity of the respective amino acid in whole egg which was computed and called the egg ratio. Originally, the nutritive quality of a protein, expressed as a chemical score, was equal to the greatest percentage deficit in an essential amino acid in a protein being evaluated. A score of 100 meant the protein completely lacked one of the essential amino acids. Later, the definition of chemical score was changed to 100 minus the greatest percentage deficit of an essential amino acid relative to that present in egg protein. The whole egg protein, which was used as a reference, has been shown to be completely utilized in the growing rats and children (70).

In 1963 the FAO/WHO joint expert group (33) proposed an alternative method for scoring proteins on the basis of their limiting essential amino acids. The provisional pattern of amino acid requirements was used to determine a protein score. The expert group noted that the proportion of methionine, cystine, tryptophan and lysine in the FAO provisional pattern was too high. Correction of the FAO pattern for these high values made the pattern resemble that of whole egg and human milk. Therefore, the FAO/WHO expert group recommended adoption of the essential amino acid pattern of either egg or human milk for reference purposes.

In order to calculate protein scores by the FAO/WHO procedure it is necessary to know the amino acid composition with some degree of

accuracy. Then one can sum up the contributions of all essential amino acids together with those of cystine and tyrosine, calculate the percentage contributions of the potentially limiting essential amino acids to this total and compare these percentages with the corresponding one of the reference protein.

The greatest obstacle to the use of a scoring system is the question of availability of amino acids in a protein. During processing, especially under heat treatment, food proteins undergo certain changes which impair their nutritional value without disturbing the amino acid composition as ordinarily determined (12). Furthermore, alterations in the protein structure may improve or lessen the digestibility. Browning reactions between amino acids and reducing sugars lead to the formation of enzyme resistant compounds, thereby influencing the availability of amino acids.

In spite of these obstacles, chemical scores remain valid in the evaluation of proteins (55). These disadvantages are probably negligible when the scoring procedure is applied to ordinary diets.

Amino Acid Analysis

In the estimation of amino acid composition of food proteins, considerable progress has been made after the introduction of microbiological and chromatographic methods during the last twenty years. In order to determine the kinds and amounts of amino acids present in a protein, it is necessary first to hydrolyze the protein either by heating it with

an acid or alkali. Sometimes proteolytic enzymes are used for this purpose (15, 52).

Hydrolysis with acids remains the best method for the total splitting of proteins into single amino acids (15). The best method recognized is the treatment with 6 N HCl at 110° C for 24 to 70 hours where conditions exclude oxygen. However, during acid hydrolysis tryptophan was completely destroyed. For the determination of tryptophan, barium hydroxide is commonly used as the hydrolyzing agent.

Also cystine and methionine are affected adversely to some extent. In order to determine these amino acids they must be oxidized with performic acid to cystic acid and methionine sulfone (39). During chromatography, cystic acid and methionine sulfone can be determined more accurately than cystine and methionine.

After hydrolyzation of proteins, the determination of amino acids may be carried out by chromatographic or microbiological procedures. Dean (28) pointed out that the quickest and the most reliable procedure was column chromatography with ion exchange resins. Other useful examples of partition chromatography are paper, thin-layer and gas chromatography. These methods are used primarily for identification of amino acids. Because of convenience, simplicity, sharpness of separations, sensitivity and the speed of separation thin-layer chromatography has been used widely (46). It is simple and an inexpensive method that permits very rapid and efficient qualitative and semi-quantitative analysis (63).

Brenner and Niederwiser (17) reported that quantitative evaluation of amino acids was satisfactory when one dimensional separations were performed. Direct quantitative determination on thin-layer method is desirable because of its simplicity. This is possible by visual comparison of spot size and color intensity between an unknown amino acid and a standard amino acid. An aliquot of the unknown and the standard are applied on the chromatogram side by side. The intensity of the unknown is matched with standards and the approximate concentrations are noted.

CHAPTER III

MATERIAL AND METHODS

I. SOURCE OF MATERIAL UTILIZED

The raw material preparations used were chick peas, sunflower seed press cake, and whole wheat flour. The mature, dry chick peas of current production were imported from Egypt. They were grounded in a micro-mill to a fine flour. Sunflower seed press cake was obtained from the vegetable oil division of Cargill Inc., Minneapolis, Minnesota. The press cake had a high husk content and a large amount of this material was removed by passing the press cake through No. 60 and 100 mesh sieves. Whole wheat flour was purchased from a local source.

The protein content of chick peas, whole wheat flour, sunflower seed press cake, and the mixture was determined by the macro-Kjeldahl method ($N \times 6.25$) and reported on the dry weight basis.

The mixture was prepared by combining 28.5 percent chick peas, 27.5 percent whole wheat flour, and 44.0 percent sunflower seed press cake. In formulating the mixture the following items from the child's standpoint were considered: food intake, age, daily amino acid requirements of normal healthy infants, and the amino acid composition of the raw material preparations.

Source of Proteolytic Enzyme

In this study Rhozyme-41, a food-grade enzyme prepared from Aspergillus oryzae, was used to extract the proteinaceous materials from the plant origin. Rhozyme-41 was kindly provided by Rohm and Haas Co., Philadelphia, Pennsylvania. The enzyme had a proteolytic activity of about 64,000 hemoglobin units and a diastatic activity of about 30 starch dextrinizing units. Rhozyme-41 has a wide pH activity ranging from 6.0 to 9.0. The optimum temperature for the enzyme activity is between 40° and 50°C.

II. EXPERIMENTAL METHODS

The enzyme digestion with Rhozyme-41 was conducted in different experiments for standardizing the optimum conditions. Proteolytic activity was determined by measuring the increase in amounts of soluble nitrogen available after separating from the insoluble part with 1 percent picric acid (79). The nitrogen content of the extracted proteinaceous material was measured by the Kjeldahl method; percentage N x 6.25 was equal to percentage of protein.

Standardization of Conditions for Optimum Enzyme Activity

The following experiments were conducted for the individual proteinaceous materials.

Effect of pH on enzymatic protein extraction. In establishing the effect of pH on enzymatic hydrolysis of proteins in the raw materials, five grams of each of the four proteinaceous protein sources were suspended in fifty milliliters of sodium phosphate (0.1 N NaOH and 0.1 M KH_2PO_4) or sodium borate (0.1 N NaOH and 0.1 M H_3BO_3) buffer

solutions with eight pH levels ranging from 6.0 to 9.5 with intervals of 0.5 pH units. The suspensions were heated at 100°C for five minutes and cooled to 40°C. To the slurries, 1 percent enzyme preparation, (w/w on a substrate basis), in fifty milliliters of sodium phosphate or sodium borate buffer solutions were added and allowed to digest the substances for two hours at 40°C in a water bath. At the end of digestion period, the enzyme reaction was terminated by raising the temperature to 80°C and holding for ten minutes. To the slurries fifty milliliters of 1 percent picric acid were added and allowed to precipitate the insoluble protein for five minutes. The picric acid precipitates were centrifuged at 894 X G for ten minutes. The supernatants were filtered with Whatman No. 1 filter paper. Controls (without enzyme) were prepared in the same manner. Two observations for each of two replications were made for each of eight treatments. The protein-free extracts of samples and controls were transferred to shallow aluminum dishes and dried in a vacuum oven at 50°C for 24 hours.

Kjeldahl analyses for nitrogen were made on dried, powdered samples and controls. The nitrogen content of controls and enzyme preparation were subtracted from that of the appropriate samples. The findings showed that the soluble protein content of sunflower seed press cake and the mixture was highest at pH 8.5 under the enzymatic conditions of 40°C, two hours digestion period, five minutes denaturation time, 1 percent enzyme preparation (w/w on a substrated basis) and five grams substrate. Degree of protein hydrolysis in chick peas was quite similar

at pH 7.5 to 8.5. On the other hand, maximum protein solubilization of whole wheat flour occurred at pH 8.0 under the same enzymatic conditions. In the view of these findings, pH of 8.5 was selected for the remainder of the studies.

Effect of temperature on enzymatic protein extraction. In studying the influence of temperature on enzymatic solubilization of proteinaceous constituents of the raw material preparations, five grams of each of the samples were blended with fifty milliliters of sodium borate (0.1 N NaOH and 0.1 M H_3BO_3) buffer solution at pH 8.5. The samples were heated at 100°C for five minutes to denature the proteinaceous constituents of the preparations. The remainder of the experiment was carried out in the same manner as before except that the digestion temperature, which was the objective of the experiment, was varied from 40° to 60°C with increments of 5°C. Two observations were made for each of two replications of five treatments. Controls without enzyme were prepared in the same manner.

The highest amount of soluble protein determined for each of four proteinaceous vegetable materials was produced at 50°C under the defined conditions which were pH 8.5, two hours reaction time, five minutes denaturation period, 1 percent enzyme preparation and five grams substrate.

Effect of reaction time on enzymatic protein extraction. This experiment was designed to determine the time required for maximum hydrolysis of proteins in the samples under optimum conditions based

on the data of the previous experiments. Five grams of the proteinaceous material preparations were dispersed in sodium borate buffer solution with pH 8.5. After denaturation at 100°C for five minutes, 1 percent enzyme preparation (w/w on a substrate basis) was added in fifty milliliters of buffer solution at pH 8.5 and the samples were allowed to digest for ten hours. Aliquots were drawn at two hour intervals. Two observations for each of two replications were made for five treatments. Controls without enzyme were prepared in the same manner as stated above.

Maximum protein hydrolysis of each of proteinaceous preparations was observed in six hours digestion time under the study conditions of pH 8.5, 50°C, denaturation time five minutes, 1 percent enzyme preparation and 5 grams of substrate.

Effect of denaturation period on enzymatic protein extraction.

Denaturation of proteinaceous foods is a prerequisite for enzyme hydrolysis. In order to investigate optimum denaturation time by heat, five grams of the individual raw materials were suspended in fifty milliliters sodium borate buffer solution at pH 8.5. The suspension was heated to 100°C for different lengths of time and cooled to 50°C. To the slurries, 1 percent enzyme preparation in fifty milliliters of sodium borate solution with the pH 8.5 was added and allowed to digest for six hours at 50°C. The remainder of the experiment was carried out in the same manner as presented in the previous section. Denaturation period was varied from 5 minutes to 25 minutes with 5 minutes intervals. Two observations were made for each of two replications of five treatments. Controls without enzyme were prepared.

Optimum denaturation time appeared to be 20 minutes for all four proteinaceous materials under specified conditions which were pH 8.5, 50°C, six hours reaction time, 1 percent enzyme preparation (w/w) on a substrate basis and five grams of substrate.

Effect of enzyme concentration on enzymatic protein extraction. In order to determine the enzyme concentration required for the maximum proteolytic activity on the raw materials, various amounts of enzyme preparation ranging from 1 percent to 3 percent with increments of 0.5 percent (w/w on a substrate basis) were added to five grams of each of the proteinaceous samples. Other conditions of the experiment were based on data from previous experiments. Two observations were made for each of the two replications of five treatments. Controls without enzyme were prepared.

Amounts of enzyme preparation required for maximum protein solubilization was found to be 2.5 percent for all samples under the enzymatic conditions of pH 8.5, 50°C, reaction time six hours, denaturation period 20 minutes, and 5 grams of substrate.

Effect of substrate concentration on enzymatic protein extraction.

The investigation of the effect on substrate content was conducted by utilizing various concentrations of the proteinaceous materials. The amounts of samples was varied from five to nine grams with increments of one gram. The digestion was carried out at the previously standardized conditions. There were two observations for each of two replications for five treatments. Controls without enzyme were prepared.

The results showed that there was a little change in the degree of hydrolysis as the substrate concentration was increased from five to nine grams at the conditions of pH 8.5, 50°C, a six hours digestion period, 20 minutes denaturation, and 2.5 percent enzyme preparation. Therefore, five grams of substrate concentration were selected for the experiments.

Effect of sodium chloride content on enzymatic protein extraction.

In establishing the effect of NaCl on the enzymatic solubilization of proteinaceous materials, five grams of each of the samples were blended with fifty milliliters of sodium borate buffer solution at pH 8.5. The samples were heated at 100°C for 20 minutes and cooled to 50°C. To the slurries, different concentrations of NaCl in fifty milliliters of sodium borate buffer solution were added and allowed to digest the substances for six hours. Sodium chloride concentrations ranged from 0.1 to 0.5 M with increments of 0.1 M. The remainder of the experiment was conducted in the same manner as previously stated. Two observations were made for each two replications of five treatments. Controls without enzyme were prepared.

Use of 0.3 M NaCl was sufficient for maximum solubilization of each of four proteinaceous materials under the study conditions of pH 8.5, 50°C, reaction time six hours, denaturation period 20 minutes, 2.5 percent enzyme preparation (w/w on a substrate basis) and five grams of substrate.

Ninhydrin-Colorimetric Determination of Total Free Amino Acids

Total ninhydrin positive material (NPM) determination was used to estimate the total free amino groups in the enzyme digested samples. The method is based on the formation of blue color by the reaction of ninhydrin and the compounds having free amino groups. In this study, the methods of Moore and Stein (51) for colorimetric determination of the total amino acids were used to estimate the amount of free amino acids in enzyme modified samples which were previously digested under defined conditions.

The enzyme modified samples of proteinaceous vegetable materials were dried in a vacuum oven after deproteinizing with 1 percent picric acid followed by centrifuging and filtering with Whatman No. 1 filter paper. The protein-free, oven-dried samples were transferred to airtight bottles and stored at -18°C until analyzed.

Upon removing from the freezer, one gram of each of four enzyme modified samples was redissolved in 250 milliliters of water. From the solution a one milliliter aliquot was transferred to a test tube and two milliliters of ninhydrin reagent were added. The tube was capped with aluminum foil and placed in boiling water for twenty minutes. Five milliliters of a mixture of water and n-butanol (1:1, v/v) diluent were pipetted to the tube. Absorbance was measured with the Bausch and Lomb spectronic 20 colorimeter at 570 nm. The concentration of amino acids and peptides containing free alpha-amino groups was calculated from the standard monosodium glutamate curve.

Three observations were made for each three replications of each treatment. The amino acid content of controls were estimated in the same manner as stated above and subtracted from that of the appropriate samples.

A standard curve was prepared with seven concentrations of monosodium glutamate solution ranging from 0.5 to 2.0 micromoles with 0.1 M citrate buffer solution at pH 5.0. One milliliter of each of seven standard solutions were transferred to a test tube and to this were added two milliliters of ninhydrin reagent. The tube was capped and placed in boiling water for twenty minutes. At the end of the heating period, the contents of the tube were diluted with five milliliters of the water-n-butanol diluent and absorbance was measured. The values were plotted against corresponding concentrations of monosodium glutamate on arithmetic graph paper.

Reducing Sugars

Determination of glucose. The Fermco-Test (31) is designed for measuring the glucose content in the presence of maltose, sucrose, mannose, galactose, dextrans and starch. To determine the amount of glucose in predigested proteinaceous vegetable materials the Fermco-Test SFG was used.

After removing enzyme hydrolyzed and oven-dried samples from the freezer at -18°C , five hundred milligrams of predigested samples from the individual preparations were placed into 500 milliliter Erlenmeyer flasks and two hundred fifty milliliters of distilled water were added. The contents in the flasks were shaken vigorously and two milliliters of each solution were pipetted into each test tube and temperature

of the contents of the test tubes was brought to 30°C. After equilibrium, one milliliter of Fermco-Test SFG reagent was added to each tube. The reaction mixtures were incubated at 30°C for thirty minutes. At the end of the incubation period, twelve milliliters of sulfuric acid reagent were added to stop the enzyme reaction. Absorbance of each solution was measured with the Bausch and Lomb spectronic 20 colorimeter at 540 nm. The milligrams of glucose were calculated from the glucose standard curve. Three observations for each of three replications were made for the individual treatments. The Fermco-Test SFG was carried out in the same manner as stated previously for the controls which were prepared without enzyme addition and the amount of glucose found in controls were subtracted from the corresponding samples.

The glucose standard curve was prepared with five concentrations of glucose solution ranging from 0.04 to 0.20 milligrams of glucose. Two milliliters of each of five standards were transferred to test tubes. The temperature of the glucose standard solutions was adjusted to 30°C. To each tube one milliliter of Fermco-Test SFG was pipetted and the reaction mixture was allowed to stand for thirty minutes. Absorbance of the glucose solutions was obtained by using the spectronic 20 colorimeter at 540 nm. The values were plotted against corresponding milligrams of glucose for each aliquot on arithmetic graph paper.

Determination of maltose. Enzyme modified and vacuum oven dried samples of proteinaceous vegetable materials were removed from the

freezer at -18°C for the determination of maltose. Two grams of dried samples were placed into 500 milliliter Erlenmeyer flasks and fifty milliliters of acetate buffer solution at pH 4.6 were added. The flasks were stoppered and shaken to bring the sample into suspension. Immediately, two milliliters of sodium tungstate were pipetted into the suspensions and each sample was mixed thoroughly. The suspensions were filtered with Whatman No. 4 filter paper and the first ten drops on each filtrate were discarded.

To determine the maltose content of each sample, five milliliters of each filtrate were pipetted into a test tube and ten milliliters of 0.05 N potassium ferricyanide solution were added. After mixing, the tubes were immersed in a boiling water bath. They were cooled under running tap water after the twenty minute heating period and the contents were transferred to 250 milliliter Erlenmeyer flasks. The test tubes were rinsed with twenty-five milliliters of acetic acid reagent and this wash was added to corresponding flasks. One milliliter of potassium iodide solution was added to each flask followed by two milliliters starch indicator solution and mixed thoroughly. The contents of each flask were titrated with 0.05 N sodium thiosulfate solution until the blue color disappeared. The milliliters of $0.05\text{ N Na}_2\text{S}_2\text{O}_3$ solution used in the titration were subtracted from that of the reagent blank to obtain the volume of potassium ferricyanide reduced to potassium ferro cyanide by the reducing sugar in the sample filtrate. The quantity of reducing sugar which corresponds to the amount of potassium ferricyanide

reduced was calculated as maltose from the A.O.A.C. maltose conversion table (3). Three observations for each of three replications were made for the individual treatments. Maltose content of the controls was determined in the same manner as above and the values were subtracted from the appropriate samples.

Preparation of Proteinaceous Material for Thin-layer Chromatography.

Vegetable proteinaceous preparations were hydrolyzed enzymatically under previously standardized conditions, which were pH 8.5, 50°C, digestion time six hours, denaturation period 20 minutes, 2.5 percent enzyme preparation, five grams of substrate and 0.3 M sodium chloride content. In order to estimate the limiting amino acids of enzyme digested materials quantitatively, the samples were oxidized and completely hydrolyzed prior to chromatographic assay.

Oxidation. During acid hydrolysis of proteins, particularly when carbohydrates are present, sulfur-containing amino acids and tryptophan are destroyed or undergo alteration that can lead to inaccuracies in quantity estimations. To overcome these difficulties Hirs (39) suggested the use of performic acid to oxidize cystine and cysteine residues to cysteic acid and methionine to methionine sulfone, both being stable to acid hydrolysis.

Seventy-five milligrams of enzyme hydrolyzed, dry samples were placed in a small round bottomed flask and four milliliters of performic reagent were added. The reaction mixture was allowed to stand for four hours at 0°C in an ice bath. After standing, 0.3 milliliter of 38 percent

hydrogen bromide was added and the oxidized mixture was concentrated to dryness with a rotary evaporator in a 40°C water for twenty minutes.

Acid hydrolysis. To the previously oxidized samples, fifteen milliliters of 6 N HCl were added and the contents were transferred to heavy-walled pyrex test tubes. The samples in the test tubes were frozen in an acetone-solid CO₂ bath. A section of the tubes about three centimeters from the top was constricted to about one milliliter bore by using an oxygen flame. The tubes were connected to a vacuum line and the system was evacuated with an oil pump to 0.1 inch Hg and the tubes were sealed under vacuum. The hydrolysis was conducted at 110°C for twenty-four hours in a thermostatically controlled oven and the hydrolyzates were stored at -18°C until analyzed (52).

The hydrolyzates were transferred to a 50 milliliter round-bottomed flask prior to concentration in the rotary evaporator operated with a condenser axis at a downward tilt (about 30°). Concentration was conducted at 40°C and the hydrochloric acid was removed in about thirty minutes. The residues were dissolved in ten milliliters of water. Aliquots of these solutions were used for the chromatographic analysis.

Alkaline hydrolysis. Since tryptophan is destroyed with acid hydrolysis, the alkaline hydrolysis was carried out for the estimation of tryptophan (15). Seventy-five milligrams of enzyme modified and vacuum oven dried samples were suspended in fifteen milliliters of 5 N barium hydroxide solution and the remainder of the experiment was carried out in the same manner as acid hydrolysis. Prior to chromatographic

assay, the alkaline hydrolyzates were adjusted to pH 6.0 with 2N sulfuric acid, heated to boiling and centrifuged to remove the BaSO_4 from the mixture. The latter was washed with a small amount of water and the washings were added to the supernatant liquid. The supernatant was evaporated to dryness with a rotary evaporator. The residue was dissolved in ten milliliters of 0.1 N hydrochloric acid and aliquots of this solution were used for chromatography.

Standard amino acid solutions. Twenty-five milligrams of pure lysine, methionine and tryptophan were dissolved into two hundred fifty milliliters of 0.1 N HCl solution containing 0.1 milligram per milliliter of each standard amino acid. Amino acids occurring in acid hydrolyzates of protein and peptides are mostly present as hydrochlorides. Therefore, the aqueous solutions correspond approximately to a solution of the standard amino acid in 0.1 N hydrochloric acid.

Thin-Layer Chromatography of Samples

One-dimensional thin-layer chromatography was used in the separation, identification, and estimation of limiting essential amino acids (17).

Ten lambda of the standards and test solutions were applied eight millimeters apart on silica Gel G thin-layer plates and at a distance of fifteen millimeters from the lower edge of the plates. After air drying the samples on the plates, ten millimeters of the plates below where the samples were applied were submerged in a solvent containing n-butanol/acetic acid/water (8:2:2 v/v/v). This procedure was conducted in a

developing tank containing one hundred twenty milliliters of the solvent. The developing tank was allowed to become saturated with solvent vapors before the plates were submerged. The plates were removed when the solvent front had moved ten centimeters from the point of sample application. The plates were air dried, sprayed with ninhydrin reagent (Ninspray Reagent, N.B.C. Research Biochemicals, Cleveland, Ohio) for the detection of amino acids and dried at 110°C for ten minutes. The size and intensity of the color of the spots were compared visually with those of the standards. The determined amounts of lysine, methionine and tryptophan were used for the protein score in the estimation of protein quality of predigested proteinaceous materials.

Calculation of the Protein Score of the Predigested Samples

The reference protein, which was whole hens egg, contained 3.215 milligrams of total essential amino acids per gram of total nitrogen. Methionine plus cystine furnished 346 milligrams (10.8 percent) of the total amino acids in the egg protein. Lysine furnished 403 milligrams (12.4 percent) and tryptophan furnished 100 milligrams (3.1 percent) of the total essential amino acids (33).

To calculate the protein scores of the samples, the amounts of total essential amino acids per gram of total nitrogen were calculated together with those of cystine and tyrosine. The percentage of potential limiting amino acids were compared with the corresponding values for the reference protein.

Statistical Analysis

The Statistical Analysis System (9) computer program at the University of Tennessee Computing Center was used for the analysis of variance calculations. The data, reflecting the effects of pH, temperature, reaction time, denaturation period, enzyme concentration, substrate concentration and sodium chloride content on enzymatic solubilization of crude protein, ninhydrin positive material, glucose and maltose contents were analyzed as factorial arrangements of a completely randomized design. Significant difference between means were determined by Duncan's New Multiple Range Test (75).



CHAPTER IV

EXPERIMENTAL RESULTS

Proteinaceous vegetable materials were analyzed as raw materials for extractable proteins after enzymatic hydrolysis. An objective was to determine the conditions for maximum enzymatic solubilization of proteins from vegetable material. Experiments were designed to study the effect of some factors on the amount of soluble protein and sugar produced by enzymatic hydrolysis. The factors tested were studied in the following order: pH, temperature, reaction time, denaturation period, enzyme concentration, substrate concentration, and salt concentrations. Also, to evaluate the quality of proteins, thin-layer chromatographic analysis was conducted to determine the quantity of limiting amino acids in the protein extracts.

I. STANDARDIZATION OF CONDITIONS FOR OPTIMUM ENZYME ACTIVITY

Effect of pH on Enzymatic Hydrolysis of Proteinaceous Vegetable Material

The analysis of variance summaries for the effect of pH on the enzymatic solubilization of the protein and production of sugars from the vegetable proteinaceous sources are presented in Table 1. The effect of protein source, pH and interactions was significant at the 0.01 level of probability. Replications were not significant at the 0.05 level of probability.

Under specified enzymatic conditions which were 40°C, reaction time 2 hours, denaturation period 5 minutes, 1 percent enzyme, the means for

TABLE 1. SUMMARIES OF ANALYSIS OF VARIANCE FOR THE EFFECT OF pH ON ENZYMIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM VEGETABLE PROTEINACEOUS MATERIALS

Source of Variance	D. F.	Mean Squares Soluble Protein	D. F.	NPM ¹	Mean Squares	
					Glucose	Maltose
Total	63	365.5	95	428.9	110.5	3.3
A. Protein Source	3	4171.0**	3	8167.7**	518.3**	34.0**
B. pH	7	1015.3**	7	1519.5**	1165.2**	19.8**
A x B	21	144.5**	21	258.9**	29.8**	2.9**
Replication	1	7.7	2	6.4	0.4	0.2
Residual Error	31	11.7	62	2.5	2.6	0.2

** Significant at the 0.01 level of probability.

¹Ninhydrin positive material.

the effect of preparation on soluble protein, ninhydrin positive material (NPM), glucose and maltose are shown in Table 2. Among the protein-containing vegetable materials the greatest amount of soluble protein was observed for the sunflower seed press cake and for the mixture. The amount of soluble protein between the chick peas and whole wheat flour was not different prior to hydrolysis, the protein content of chick peas and whole wheat flour was 21.6 and 12.8 percent respectively. However, there was no significant difference in soluble protein between these two preparations at the 0.05 level of probability.

Free amino acids, peptides and proteins having free amino groups were estimated as total ninhydrin positive material (NPM). The quantity of ninhydrin positive material found for the sunflower seed press cake was larger than that found for the mixture which was larger than that for chick peas and whole wheat flour.

The source of vegetable proteinaceous materials influenced the amount of glucose produced. The greatest amount of glucose was observed for the chick peas and whole wheat flour; these were not significantly different. The mixture exhibited a lower quantity of glucose than chick peas or whole wheat flour, but sunflower seed press cake exhibited the lowest amount of glucose for all samples.

The highest maltose content was found in whole wheat flour while the lowest maltose content was observed in sunflower seed press cake. Maltose values of chick peas and the mixture were at an intermediate level between whole wheat flour and sunflower seed press cake.

TABLE 2. MEANS FOR THE EFFECT OF PROTEIN SOURCE ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS WHEN pH OF HYDROLYSIS WAS FROM 6.0 TO 9.5

Protein Source	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
CP	40.0 ^c	29.8 ⁿ	25.6 ^r	3.9 ^x
WWF	40.1 ^c	35.5 ^m	25.4 ^r	5.1 ^w
SSPC	71.9 ^a	68.7 ^k	15.8 ^t	2.3 ^z
Mixture	62.6 ^b	58.4 ^l	23.9 ^s	3.1 ^y

¹Mg per gram of proteinaceous vegetable material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of proteinaceous vegetable material, dry weight basis, expressed as monosodium glutamate.

³Means of 16 observations.

⁴Means of 24 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44% SSPC.

Mean values for the effect of pH on soluble protein, ninhydrin positive material (NPM), glucose and maltose as influenced by the enzymatic hydrolysis for each proteinaceous vegetable material are shown in Table 3.

There was an increase in soluble protein content at each increment of pH increase from 6.5 to 8.5; the values at pH 6.0 and 6.5 were not different. The highest soluble protein mean value was found at pH 8.5. With further increases in pH, the enzymatic solubilization of proteinaceous preparations decreased significantly.

Ninhydrin positive material increased continuously as the pH was raised from 6.0 to 8.5. The amount of ninhydrin positive material was reduced markedly as the pH was raised to 9.5.

Conversely, there was a trend for a reduction of the glucose content when the pH was increased from 6.0 to 9.5. The highest glucose production occurred at pH 6.0. There was no apparent change in glucose content between pH 6.5 and 7.0.

The highest maltose mean value was observed at pH 6.0. The amount of maltose decreased gradually when the pH was raised from 6.0 to 9.5. The maltose levels between pH 7.5 and 8.0 and between pH 9.0 and 9.5 were not different.

Means for the effect of the interaction between protein source and pH on enzymatic solubilization of proteins are given in Table 4. The pH at which each vegetable proteinaceous material was held influenced significantly the amount of soluble protein production and the level of ninhydrin positive material.

TABLE 3. MEANS FOR THE EFFECT OF pH ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM VEGETABLE PROTEINACEOUS MATERIALS

pH	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
6.0	40.4 ^e	30.9 ^l	36.9 ^m	5.6 ^t
6.5	42.2 ^e	35.1 ^k	31.3 ⁿ	4.9 ^u
7.0	47.1 ^d	42.7 ^j	30.2 ⁿ	4.5 ^v
7.5	57.1 ^c	50.4 ⁱ	24.3 ^o	3.5 ^w
8.0	66.1 ^b	57.9 ^g	21.9 ^p	3.2 ^w
8.5	71.1 ^a	64.0 ^f	15.3 ^q	2.6 ^x
9.0	59.4 ^c	53.8 ^h	11.4 ^r	2.3 ^z
9.5	46.8 ^d	50.1 ⁱ	9.9 ^s	2.1 ^z

¹Mg per gram of proteinaceous vegetable material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of proteinaceous vegetable material, dry weight basis, expressed as monosodium glutamate.

³Means of 8 observations.

⁴Means of 12 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

TABLE 4. EFFECT OF PROTEIN SOURCE x pH INTERACTION ON ENZYMATIC SOLUBILIZATION OF PROTEINS FROM VEGETABLE PROTEINACEOUS MATERIALS

pH	Soluble Protein of Protein Source ^{1,3}				NPM of Protein Source ^{2,4}			
	CP	WWF	SSPC	Mixture	CP	WWF	SSPC	Mixture
6.0	31.7 ^{o-q}	33.4 ^{n-p}	51.4 ^{h-k}	45.1 ^{j-m}	17.3 ^s	23.7 ^r	45.8 ^{ij}	36.7 ^{n-p}
6.5	26.8 ^{pq}	39.9 ^{l-n}	53.6 ^{g-i}	48.3 ^{i-k}	24.2 ^r	27.3 ^o	47.2 ⁱ	41.0 ^{lm}
7.0	32.0 ^{o-q}	43.5 ^{k-m}	60.6 ^{fg}	52.5 ^{h-g}	34.8 ^{op}	35.5 ^{op}	53.7 ^h	46.9 ⁱ
7.5	49.0 ^{h-k}	46.4 ^{i-l}	70.4 ^e	62.7 ^f	42.9 ^{kl}	38.6 ^{mn}	63.2 ^f	56.9 ^g
8.0	48.9 ^{h-k}	56.4 ^{f-h}	79.8 ^{cd}	79.1 ^d	40.5 ^{lm}	43.0 ^{kl}	75.7 ^c	72.2 ^d
8.5	50.3 ^{h-k}	44.0 ^{k-m}	98.5 ^a	91.6 ^a	36.5 ^{n-p}	44.1 ^{jk}	90.3 ^a	85.3 ^b
9.0	43.6 ^{k-m}	32.1 ^{o-q}	86.7 ^{bc}	71.2 ^e	23.3 ^r	37.5 ^{no}	88.2 ^a	66.1 ^e
9.5	37.8 ^{m-o}	25.1 ^q	73.8 ^{de}	50.6 ^{h-k}	19.2 ^s	33.9 ^p	85.5 ^b	61.9 ^f

¹Mg soluble protein per gram of vegetable proteinaceous material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of vegetable proteinaceous material, dry weight basis, expressed as monosodium glutamate.

³Means of 2 observations.

⁴Means of 3 observations.

Means followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44.0% SSPC.

The largest amount of soluble protein in chick peas was found between pH 7.5 and 9.0. The soluble protein content was maintained at a constant level from 7.5 through pH 8.5. Further increase in pH showed a reduction of soluble protein in chick peas. The highest amount of soluble protein for whole wheat flour was found at pH 8.0. Raising the pH from 6.0 to 8.0 caused a steady increase in soluble protein content. Beyond the pH 8.0, the amount of soluble protein was reduced markedly. The soluble protein content of sunflower seed press cake was highest at pH 8.5. The amount of soluble protein of sunflower seed press cake was significantly larger than that of chick peas and whole wheat flour at pH 6.0. There was a steady increase in soluble protein content until pH reached to 8.5. However, subsequent increase in pH showed a trend for a reduction in soluble protein of sunflower seed press cake. In the case of the mixture, the largest amount of soluble protein was produced at pH 8.5. There was a continuous increase in soluble protein when pH was raised from 6.0 to 8.5. But, the additional increase in pH caused a marked reduction of soluble protein content in the mixture.

The maximum release of ninhydrin positive material in chick peas occurred between pH 7.5 and 8.0. But subsequent increase in pH showed a trend for a reduction in ninhydrin positive material level of chick peas. The highest level of ninhydrin positive material in whole wheat flour was found between pH 8.0 and 8.5. However, there was a significant decline in the amount of ninhydrin positive material when the pH was

raised from the pH 8.5 to 9.0. The largest amount of ninhydrin positive material from sunflower seed press cake was produced between pH 8.5 and 9.0. Ninhydrin positive material concentration of sunflower seed press cake showed a continuous increase as the pH was raised from 6.0 to 8.5. Beyond the pH 9.0, the level of ninhydrin positive material in sunflower seed press cake was reduced significantly. The highest amount of ninhydrin positive material for the mixture was found at pH 8.5. However, with the additional increase in pH showed a marked decline in ninhydrin positive material levels.

Means for the effect of the interaction between protein source and pH on sugar content resulting from enzymatic solubilization of vegetable proteinaceous materials are shown in Table 5.

The highest level of glucose for the individual protein sources was found at pH 6.0. With the mixture there was no difference in the glucose concentration between pH 6.0 and 7.0. The highest values for maltose in whole wheat flour was observed at pH 6.0; for the other proteinaceous materials the highest amount of maltose was produced over the pH range of 6.0 to 6.5. There was a trend for a reduced amount of glucose and maltose for all samples as the pH was raised.

Effect of Temperature on Enzymatic Hydrolysis of Vegetable Proteinaceous Material

The summaries of the analysis of variance for the effect of temperature on enzymatic solubilization of proteins and production of sugars from

TABLE 5. EFFECT OF PROTEIN SOURCE x pH INTERACTION ON SUGAR CONTENT FROM ENZYMATIC SOLUBILIZATION OF VEGETABLE PROTEINACEOUS MATERIALS^{1,2}

pH	Glucose of Protein Source			Maltose of Protein Source				
	CP	WWF	SSPC	Mixture	CP	WWF	SSPC	Mixture
6.0	42.1 ^a	39.8 ^a	29.4 ^{cd}	36.1 ^b	5.8 ^{DE}	8.2 ^A	3.0 ^{K-N}	5.2 ^{EF}
6.5	36.1 ^b	34.4 ^b	18.9 ^{i-k}	35.7 ^b	5.2 ^{EF}	7.3 ^B	2.3 ^{N-S}	4.8 ^{FG}
7.0	35.4 ^b	31.1 ^c	18.1 ^{j-l}	36.3 ^b	4.3 ^{GH}	7.0 ^{BC}	2.9 ^{L-P}	3.8 ^{H-K}
7.5	26.3 ^{ef}	29.7 ^c	17.2 ^{k-m}	23.8 ^{fg}	3.3 ^{I-L}	6.3 ^{CD}	1.6 ^S	2.9 ^{L-O}
8.0	22.0 ^{gh}	26.9 ^{de}	17.6 ^{j-m}	21.3 ^{g-i}	4.1 ^{G-I}	3.9 ^{H-J}	2.1 ^{N-S}	2.5 ^{L-O}
8.5	20.3 ^{h-j}	15.9 ^{l-n}	10.2 ^{qr}	14.7 ^{m-o}	3.0 ^{L-N}	3.2 ^{J-M}	2.5 ^{L-Q}	2.0 ^{P-S}
9.0	13.7 ^{n-p}	13.0 ^{o-q}	7.4 ^s	11.7 ^{pq}	3.3 ^{I-M}	2.1 ^{O-S}	2.2 ^{N-S}	1.6 ^S
9.5	8.8 ^{rs}	11.9 ^{pq}	7.2 ^s	11.5 ^{p-r}	2.6 ^{L-R}	2.4 ^{M-S}	1.7 ^{Q-S}	1.7 ^{Q-S}

¹Mg sugar per gram of vegetable proteinaceous material, dry weight basis.

²Means of 3 observations.

Means followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44.0% SSPC.

the vegetable proteinaceous materials are given in Table 6.. The factors and interactions were significant at the 0.01 level of probability except the interaction was not significant at the 0.05 level of probability for maltose.

The means for soluble protein, ninhydrin positive material, glucose and maltose content as influenced by the individual protein sources are presented in Table 7. The conditions of study were pH 8.5, reaction time 2 hours, denaturation period 5 minutes and 1 percent enzyme preparation. Enzymatic solubilization of proteins was affected significantly by each of the vegetable sources. The highest soluble protein concentration was observed for sunflower seed press cake followed by the mixture. The lowest soluble protein concentration was found in whole wheat flour. The amount of soluble protein from the chick peas and the whole wheat flour was roughly one half of that obtained from the sunflower seed press cake and the mixture.

The largest amount of ninhydrin positive material was observed for the sunflower seed press cake followed by the mixture, the chick peas and whole wheat flour in order of decreasing levels and each amount being significantly different from the others.

The quantity of glucose produced from the chick peas and whole wheat flour was higher than that produced from the sunflower seed press cake and the mixture. There was no difference in glucose content between chick peas and whole wheat flour. The lowest quantity of glucose was observed for the sunflower seed press cake.

TABLE 6. SUMMARIES OF ANALYSIS OF VARIANCE FOR THE EFFECT OF TEMPERATURE ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM VEGETABLE PROTEINACEOUS MATERIALS

Source of Variance	D. F.	Mean Squares Soluble Protein	D. F.	NPM ¹	Mean Squares	
					Glucose	Maltose
Total	39	907.8	59	803.0	55.5	1.34
A. Protein Source	3	7857.3**	3	11535.1**	552.8**	8.1**
B. Temperature	4	2371.2**	4	2296.9**	299.2**	9.3**
A x B	12	189.8**	12	288.6**	24.5**	0.8
Replication	1	2.0	2	4.5	3.2	0.1
Residual Error	19	3.5	38	3.0	3.2	0.2

** Significant at the 0.01 level of probability.

¹ Ninhydrin positive material.

TABLE 7. MEANS FOR THE EFFECT OF PROTEIN SOURCE ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS WHEN TEMPERATURE OF HYDROLYSIS WAS FROM 40° TO 60°C

Protein Source	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
CP	51.3 ^c	46.8 ^g	24.1 ⁱ	3.5 ^m
WWF	45.8 ^d	44.6 ^h	23.1 ⁱ	4.1 ^l
SSPC	101.1 ^a	100.6 ^e	10.9 ^k	2.3 ⁿ
Mixture	91.9 ^b	83.9 ^f	17.4 ^j	3.3 ^m

¹Mg per gram of vegetable proteinaceous material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of vegetable proteinaceous material, dry weight basis, expressed as monosodium glutamate.

³Means of 10 observations.

⁴Means of 15 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44.0% SSPC.

The highest maltose concentration was found in the whole wheat flour sample while the lowest maltose concentration was observed in sunflower seed press cake. Maltose content of the chick peas and the mixture, not significantly different from each other, showed an intermediate level between that observed in sunflower seed press cake and whole wheat flour.

The means for the influence of temperature on soluble protein, ninhydrin positive material, glucose and maltose contents resulting from enzymatic hydrolysis of proteinaceous vegetable material are reported in Table 8.

As the temperature was raised from 40° to 50°C, there was an increase in the amount of soluble protein, ninhydrin positive material, glucose and maltose; the amounts of these substances decreased progressively as the temperature was raised from 50°C to 60°C. Soluble protein, ninhydrin positive material, glucose and maltose levels were higher at 50°C than at 45°C or 55°C with one exception. There was no significant difference in the amounts of glucose between 45° and 50°C.

The means for the effect of protein source X temperature interaction on enzymatic solubilization of proteins in the vegetable proteinaceous materials are presented in Table 9. The results showed that whole wheat flour, sunflower seed press cake and the mixture had the highest values for soluble protein at 50°C. For the chick pea sample, the soluble protein content at 45°C was not different from the amount at 50°C.

TABLE 8. MEANS FOR THE EFFECT OF TEMPERATURE ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM VEGETABLE PROTEIN-ACEOUS MATERIALS

Temp. °C	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
40	72.2 ^c	65.3 ^h	19.5 ^k	2.9 ^p
45	84.2 ^b	78.4 ^f	23.0 ^j	4.0 ^o
50	91.4 ^a	83.8 ^e	23.4 ^j	4.3 ⁿ
55	69.5 ^c	69.4 ^g	17.2 ^l	3.0 ^p
60	46.4 ^d	47.9 ⁱ	11.2 ^m	2.3 ^q

¹Mg per gram of vegetable proteinaceous material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of vegetable proteinaceous material, dry weight basis, expressed as monosodium glutamate.

³Means of 8 observations.

⁴Means of 12 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

The highest ninhydrin positive material concentrations were observed for the sunflower seed press cake and the mixture at 50°C. There was no significant difference in ninhydrin positive material concentration between 45° and 50°C for chick peas and between 45°C and 55°C for the whole wheat flour sample. The amounts decreased markedly beyond the temperature 50°C for the chick peas, sunflower seed press cake and the mixture and beyond the temperature 55°C for the whole wheat flour sample.

Means for the influence of the protein source X temperature interaction on the sugar content resulting from enzymatic hydrolysis of proteinaceous vegetable material are found in Table 10. The amount of glucose produced from the chick pea and the whole wheat flour was not affected significantly as the temperature was raised from 40°C to 50°C. There was a progressive decrease in the glucose content when the temperature was increased from 50° to 60°C. Glucose values for the sunflower seed press cake showed no increases as the temperature was raised from 40° to 60°C. In the case of the mixture, there was a significant change in the glucose content when the temperature was increased from 40° to 45°C. The amount of glucose for the mixture remained constant between the temperature 45° and 50°C. Further temperature increases caused a lower yield of glucose content for the mixture.

The interaction between the protein source and temperature had no significant effect on maltose content.

TABLE 10. EFFECT OF PROTEIN SOURCE x TEMPERATURE INTERACTION ON PRODUCTION OF SUGARS FROM ENZYMATIC SOLUBILIZATION OF VEGETABLE PROTEINACEOUS MATERIALS^{1,2}

Temp. °C	Glucose of Protein Source			Maltose of Protein Source		
	CP	WWF	SSPC	Mixture	CP	WWF
40	26.3 ^{ab}	26.7 ^a	10.2 ^{h-j}	14.7 ^{fg}	3.3 ^A	3.9 ^A
45	28.4 ^a	29.3 ^a	12.5 ^{g-i}	21.6 ^{cd}	4.4 ^A	5.1 ^A
50	28.8 ^a	28.8 ^a	14.3 ^{g-i}	21.9 ^{cd}	3.8 ^A	5.6 ^A
55	23.5 ^{bc}	19.2 ^{de}	9.3 ^{ij}	17.0 ^{ef}	3.2 ^A	3.2 ^A
60	13.3 ^{gh}	11.5 ^{g-i}	8.1 ^j	11.7 ^{g-i}	2.5 ^A	2.4 ^A
					2.2 ^A	2.1 ^A
					2.5 ^A	3.8 ^A
					3.3 ^A	4.7 ^A
					1.9 ^A	3.5 ^A
					1.5 ^A	2.3 ^A

¹Mg sugar per gram of vegetable proteinaceous material, dry weight basis.

²Means of 3 observations.

Means followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour, SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44.0% SSPC.

Effect of Reaction Time on Enzymatic Hydrolysis of Proteinaceous Vegetable Material

Summaries of the analysis of variance for the effect of reaction time on enzymatic solubilization of protein and production of sugars from the proteinaceous vegetable materials are given in Table 11. The factors and the interactions were significant at the 0.01 level of probability except that the interaction was not significant on glucose content. Replications for soluble protein was significant at the 0.05 level of probability.

The means for the effect of the individual proteinaceous materials on soluble protein, ninhydrin positive material, glucose and maltose are shown in Table 12. The enzymatic conditions were pH 8.5, 50°C, denaturation time 5 minutes, 1 percent enzyme preparation and 5 grams of substrate. The results showed that the greatest amount of soluble protein was observed for the sunflower seed press cake followed by the mixture, chick peas and whole wheat flour in order of decreasing amount.

The concentration of ninhydrin positive material for sunflower seed press cake and the mixture was considerably higher than that for the chick peas and whole wheat flour samples. The highest concentration of ninhydrin positive material was observed for the sunflower seed press cake while the concentration found for the whole wheat flour sample was the lowest.

The highest amount of glucose was found in whole wheat flour followed by chick peas, the means being significantly different. The sunflower seed press cake had the lowest glucose level.

TABLE 11. SUMMARIES OF ANALYSIS OF VARIANCE FOR THE EFFECT OF REACTION TIME ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM VEGETABLE PROTEINACEOUS MATERIALS

Source of Variance	D. F.	Mean Squares Soluble Protein	D. F.	NPM ¹	Mean Squares	
					Glucose	Maltose
Total	39	1898.8	59	1968.8	37.4	2.1
A. Protein Source	3	22537.4**	3	34842.8**	494.1**	18.1**
B. Reaction Time	4	1379.1**	4	2468.1**	117.7**	10.8**
A x B	12	72.4**	12	139.5**	9.7	1.6**
Replication	1	17.1*	2	5.1	2.1	0.1
Residual Error	19	2.0	38	1.9	3.5	0.2

** Significant at the 0.01 level of probability.

* Significant at the 0.05 level of probability.

¹ Ninhydrin positive material.

TABLE 12. MEANS FOR THE EFFECT OF PROTEIN SOURCE ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS WHEN REACTION TIME OF HYDROLYSIS WAS FROM 2 TO 10 HOURS

Protein Source	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
CP	76.8 ^c	70.3 ^g	31.9 ^j	4.7 ^o
WWF	67.2 ^d	61.4 ^h	33.6 ⁱ	7.3 ^m
SSPC	166.9 ^a	162.3 ^e	21.7 ^l	5.5 ⁿ
Mixture	134.4 ^b	129.2 ^f	24.4 ^k	5.7 ⁿ

¹Mg per gram of vegetable proteinaceous material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of vegetable proteinaceous material, dry weight basis, expressed as monosodium glutamate.

³Means of 10 observations.

⁴Means of 15 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44.0% SSPC.

The amount of maltose produced in whole wheat flour was considerably higher than that produced in chick peas which had the lowest level of maltose. The mixture and sunflower seed press cake yielded on intermediate maltose content between whole wheat flour and chick peas.

The extent of enzymatic solubilization of proteinaceous vegetable materials was studied as a function of time. The means are presented in Table 13. The maximum hydrolysis of proteins occurred within six hours. There was no significant difference in the amount of soluble protein when digestion time was extended from six to ten hours.

Mean values for the ninhydrin positive material concentration, however, showed a significant increase at each two-hour interval of the reaction time from two to ten hours. The rate of increase between two and six hours digestion was considerably larger than that in the later stages of incubation time.

There was a trend for an increase in the amount of glucose when the reaction time was extended from two to ten hours. There was no significant difference in the glucose content between six and eight hours digestion period. The highest amount of glucose was found at ten hours reaction time.

Maltose content increased with an increase of incubation time from two to six hours. The quantity of maltose showed a reduction at eight hours digestion followed by an increase when the reaction time reached to ten hours. There was no difference in the maltose level between six and ten hours digestion.

TABLE 13. MEANS FOR THE EFFECT OF REACTION TIME ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM VEGETABLE PROTEINACEOUS MATERIALS

Reaction Time Hrs.	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
2	91.2 ^c	83.7 ^h	23.4 ^l	4.5 ^p
4	105.0 ^b	98.9 ^g	26.3 ^k	5.3 ^o
6	119.4 ^a	113.1 ^f	28.8 ^j	6.5 ^m
8	120.4 ^a	115.1 ^e	29.5 ^j	6.0 ⁿ
10	121.8 ^a	118.0 ^d	31.5 ⁱ	6.8 ^m

¹Mg per gram of vegetable proteinaceous material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of vegetable proteinaceous material, dry weight basis, expressed as monosodium glutamate.

³Means of 8 observations.

⁴Means of 12 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

Effect of protein source X digestion time interaction on enzymatic solubilization of proteins are shown in Table 14. There was a progressive increase in the amount of soluble protein from the chick peas as the incubation period was extended from two to six hours. The amount was maintained at a constant level from six through ten hours digestion period. The greatest amount of soluble protein for the whole wheat flour and the sunflower seed press cake samples was observed at six hours digestion period. Both proteinaceous materials showed, however, no significant change in the amount of soluble protein between six and eight hours and between eight and ten hours reaction time. For the mixture soluble protein content showed an increase until the reaction time reached to eight hours. With further increase in reaction time, there was no change in soluble protein level.

The highest ninhydrin positive material concentration for the chick peas was observed between six and ten hours time period. The lowest level of ninhydrin positive material was produced when the incubation was two hours. Whole wheat flour exhibited the maximum amount of ninhydrin positive material after eight hours reaction time. The amount of ninhydrin positive material after ten hours was not different from the amount after the eight hour digestion period. For the sunflower seed press cake sample there was an increase in ninhydrin positive material concentration when the duration of reaction time was increased from two to eight hours. There was no significant difference in the concentration between eight and ten hours digestion period. The mixture showed an

TABLE 14. EFFECT OF PROTEIN SOURCE x REACTION TIME INTERACTION ON ENZYMATIC SOLUBILIZATION OF PROTEINS FROM VEGETABLE PROTEINACEOUS MATERIALS

Reaction Time Hrs.	Soluble Protein of Protein Source ^{1,3}				NPM of Protein Source ^{2,4}			
	CP	WWF	SSPC	Mixture	CP	WWF	SSPC	Mixture
2	60.9 ^l	56.4 ^m	133.1 ^f	114.2 ^h	56.0 ^o	46.8 ^p	125.6 ^g	106.5 ⁱ
4	70.1 ^k	62.6 ^l	158.6 ^c	128.6 ^g	64.6 ^m	58.9 ⁿ	154.9 ^c	117.3 ^h
6	84.0 ⁱ	69.6 ^k	179.1 ^b	140.9 ^e	76.5 ^j	65.2 ^{lm}	174.2 ^b	136.5 ^f
8	83.6 ⁱ	72.3 ^{jk}	181.4 ^{ab}	144.3 ^d	76.7 ^j	67.1 ^{kl}	177.2 ^a	139.4 ^e
10	85.6 ⁱ	75.1 ^j	182.4 ^a	144.2 ^d	77.5 ^j	69.0 ^k	179.4 ^a	146.3 ^d

¹Mg soluble protein per gram of vegetable proteinaceous material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of vegetable proteinaceous material, dry weight basis, expressed as monosodium glutamate.

³Means of 2 observations.

⁴Means of 3 observations.

Means followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44.0% SSPC.

increase in the amount of ninhydrin positive material when the reaction time was extended from two to ten hours.

The effect of protein source X reaction time interaction on sugar content resulting from enzymatic hydrolysis of proteinaceous vegetable material is given in Table 15. There was no significant effect of interaction on glucose content at the 0.05 level of probability.

The maltose level of chick peas remained constant as the incubation time was increased from two to four hours. The largest amount of maltose for the chick peas was found at six and ten hour periods. A progressive decrease in the amount of maltose occurred when the time was extended from six to eight hours. For whole wheat flour, the maltose level showed an increase as the reaction time extended from two to six hours. No increase was found between six and eight hours. The highest maltose value occurred after ten hours. The quantity of maltose for the sunflower seed press cake sample showed an increase as the reaction time was extended from two to six hours. With further increase in reaction time, the maltose value remained unchanged. The mixture exhibited an increase in maltose level when the digestion period was raised from two to four hours. There was no significant difference in the maltose level between four and ten hours digestion period.

Effect of Denaturation Time on Enzymatic Hydrolysis of Proteinaceous Vegetable Material

The summaries of analysis of variance for the effect of denaturation time on enzymatic solubilization of proteins and production of sugars

from vegetable proteinaceous materials are presented in Table 16. All factors and interactions are significant at the 0.01 level of probability with the exception that denaturation time and the interaction for glucose are not significant at the 0.05 level of probability.

The means for soluble protein, ninhydrin positive material, glucose and maltose content as affected by proteinaceous vegetable materials are shown in Table 17. The mean for each substance was determined under the following conditions: pH 8.5, 50°C, reaction time 6 hours, five grams of substrate and 1 percent enzyme preparation. The greatest amount of soluble protein and ninhydrin positive material was found in sunflower seed press cake followed by the mixture, chick peas and whole wheat flour in order of decreasing amounts. Each amount of substance was significantly different from the others.

The highest level of glucose was produced from the chick peas and whole wheat flour among which there was no significant difference. Sunflower seed press cake had the lowest amount of glucose while the mixture yielded an intermediate level.

Whole wheat flour yielded the highest maltose mean value followed by the mixture. The lowest maltose mean values were observed in chick peas and sunflower seed press cake; these means were not significantly different.

Mean values for the effect of denaturation time on soluble protein, ninhydrin positive material, glucose and maltose content as influenced by the enzymatic hydrolysis of proteinaceous vegetable material are

TABLE 16. SUMMARIES OF ANALYSIS OF VARIANCE FOR THE EFFECT OF DENATURATION TIME ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM PROTEINACEOUS VEGETABLE MATERIALS

Source of Variance	D. F.	Mean Squares Soluble Protein	D. F.	NPM ¹	Mean Squares	
					Glucose	Maltose
Total	39	2710.6	59	2254.9	29.4	2.9
A. Protein Source	3	33873.1**	3	43304.7**	479.5**	40.8**
B. Denaturation Time	4	864.1**	4	516.4**	10.1	2.7**
A x B	12	49.7**	12	78.1**	7.3	2.3**
Replication	1	6.2	2	1.5	0.7	0.2
Residual Error	19	1.9	38	3.1	4.4	0.3

** Significant at the 0.01 level of probability.

¹Ninhydrin positive material.

TABLE 17. MEANS FOR THE EFFECT OF PROTEIN SOURCE ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS WHEN HYDROLYSIS OF DENATURATION PERIOD WAS FROM 5 TO 25 MINUTES

Protein Source	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
CP	98.3 ^c	88.5 ^g	34.7 ⁱ	5.7 ⁿ
WWF	76.1 ^d	71.4 ^h	34.3 ⁱ	9.3 ^l
SSPC	203.0 ^a	187.9 ^e	22.6 ^k	5.9 ⁿ
Mixture	161.9 ^b	147.5 ^f	28.8 ^j	6.8 ^m

¹Mg per gram of proteinaceous vegetable material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of proteinaceous vegetable material, dry weight basis, expressed as monosodium glutamate.

³Means of 10 observations.

⁴Means of 15 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44.0% SSPC.

reported in Table 18. There was a steady increase in soluble protein content when duration of denaturation was increased from 5 to 20 minutes. With a further increase in the denaturation period the enzymatic solubilization of proteinaceous material was not changed. The highest soluble protein mean values occurred at the 20 and 25 minute denaturations.

Means for ninhydrin positive material showed a progressive increase until duration of the denaturation was allowed to reach 15 minutes. As the duration of cooking was extended to 25 minutes, there was a reduction in ninhydrin positive material from that found at 15 minutes.

The duration of denaturation period did not influence the mean values of glucose content.

The maltose mean values showed an increase in the amount of sugar when the period of denaturation was extended from 5 to 10 minutes. As the time of denaturation was increased to 25 minutes the amount of maltose produced was progressively reduced. The value at 25 minutes was not different from that at 5 minutes denaturation.

Means for the effect of the protein source X denaturation time interaction on enzymatic solubilization of proteins are given in Table 19. Soluble protein concentration of chick peas showed a continuous increase until denaturation time reached to 20 minutes. The highest amounts of soluble protein were observed at the 20 and 25 minutes denaturation periods. The maximum soluble protein concentration for whole

TABLE 18. MEANS FOR THE EFFECT OF DENATURATION TIME ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM VEGETABLE PROTEINACEOUS MATERIALS

Denaturation Time Min.	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
5	118.4 ^d	113.1 ^h	28.8 ⁱ	6.5 ^m
10	130.8 ^c	121.9 ^g	30.1 ⁱ	7.5 ^k
15	139.3 ^b	129.1 ^e	30.7 ⁱ	7.2 ^{kl}
20	142.6 ^a	127.8 ^{ef}	29.8 ⁱ	7.0 ^l
25	143.0 ^a	127.1 ^f	31.1 ⁱ	6.4 ^m

¹Mg per gram of proteinaceous vegetable material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of proteinaceous vegetable material, dry weight basis, expressed as monosodium glutamate.

³Means of 8 observations.

⁴Means of 12 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

TABLE 19. EFFECT OF PROTEIN SOURCE x DENATURATION TIME INTERACTION ON ENZYMATIC SOLUBILIZATION OF PROTEINS FROM VEGETABLE PROTEINACEOUS MATERIALS

Denaturation Time, Min.	Soluble Protein of Protein Source ^{1,3}				NPM of Protein Source ^{2,4}			
	CP	WWF	SSPC	Mixture	CP	WWF	SSPC	Mixture
5	84.0 ^l	69.6 ^o	179.1 ^d	140.9 ^h	76.5 ^M	65.2 ^o	174.4 ^D	136.5 ^H
10	90.5 ^k	74.1 ⁿ	201.5 ^c	157.2 ^g	85.6 ^K	72.7 ^N	179.0 ^C	150.5 ^E
15	100.2 ^j	78.4 ^m	209.9 ^b	168.8 ^f	94.4 ^I	79.5 ^L	192.4 ^B	150.0 ^E
20	109.7 ⁱ	78.9 ^m	211.3 ^{ab}	170.7 ^{ef}	95.4 ^I	73.3 ^N	196.2 ^A	146.4 ^F
25	107.1 ⁱ	79.6 ^m	213.1 ^a	172.1 ^e	90.6 ^J	66.3 ^o	197.5 ^A	144.1 ^G

¹Mg soluble protein per gram of vegetable proteinaceous material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of vegetable proteinaceous material, dry weight basis, expressed as monosodium glutamate.

³Means of 2 observations.

⁴Means of 3 observations.

Means followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44.0% SSPC.

wheat flour was found at 15, 20, and 25 minutes denaturation. The means at these periods of denaturations were not significantly different. The amount of soluble protein in the sunflower seed press cake and the mixture was increased as the duration of time was extended from 5 to 15 minutes. The amount of soluble protein produced at 20 minutes was not significantly higher than that at 15 minutes, but the level at 25 minutes was greater than that at 15 minutes.

The highest amount of ninhydrin positive material in chick peas was found at 15 and 20 minutes denaturation. The largest amount of ninhydrin positive material for the whole wheat flour sample was observed at 15 minutes denaturation. The concentration of NPM for sunflower seed press cake increased until the denaturation time was allowed to reach 20 minutes. The amount of NPM remained unchanged when the duration of denaturation was extended to 25 minutes. The maximum amount of ninhydrin positive material for the mixture was produced at 10 and 15 minutes denaturation and these levels were not different.

Means for the effect of the protein source X denaturation time interaction on production of sugars resulting from enzymatic solubilization of proteinaceous vegetable material are found in Table 20. The amount of glucose was not significantly influenced by the interaction.

The amount of maltose for the chick peas and sunflower seed press cake were unchanged as the denaturation time was increased from 5 to 20 minutes. The level of sugar at 25 minutes denaturation was significantly lower. The quantity of maltose for whole wheat flour remained

unchanged when denaturation time was extended from 10 to 25 minutes; the maltose level at the 5 minute denaturation period was lower. The highest maltose level was found in the mixture after 15 minutes denaturation. However, the level showed no significant difference between 5 and 10 minutes and between 15 and 25 minutes.

Effect of Enzyme Concentration on Enzymatic Hydrolysis of Proteinaceous Vegetable Material

The summaries of analysis of variance for the effect of enzyme concentration on enzymatic solubilization of proteins and production of sugars from proteinaceous vegetable materials are presented in Table 21. All the factors and interactions were significant at the 0.01 level of probability. The replication for glucose was significant at the 0.05 level of probability.

The means for soluble protein, ninhydrin positive material, glucose and maltose values as influenced by the protein source are shown in Table 22. The mean levels of substances were produced under defined conditions of pH 8.5, 50°C, reaction time 6 hours, denaturation period 20 minutes and 5 grams of substrate. The greatest amount of soluble protein was found in sunflower seed press cake. The mixture exhibited the next highest level followed by chick peas. The amount of soluble protein for whole wheat flour was the lowest of all the proteinaceous materials.

The highest quantity of ninhydrin positive material was observed in sunflower seed press cake followed by the mixture, chick peas and

TABLE 21. SUMMARIES OF ANALYSIS OF VARIANCE FOR THE EFFECT OF ENZYME CONCENTRATION ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM PROTEINACEOUS VEGETABLE MATERIALS

Source of Variance	D. F.	Mean Squares Soluble Protein	D. F.	NPM ¹	Mean Squares	
					Glucose	Maltose
Total	39	10685.5	59	10346.7	130.4	25.0
A. Protein Source	3	114023.5**	3	172730.8**	1110.6**	210.2**
B. Enzyme Conc.	4	11654.5**	4	13350.5**	969.6**	152.2**
A x B	12	2333.3**	12	3228.0**	30.9**	17.5**
Replication	1	1.2	2	0.3	12.0*	0.8
Residual Error	19	2.4	38	3.3	2.3	0.6

** Significant at the 0.01 level of probability.

* Significant at the 0.05 level of probability.

¹Ninhydrin positive material.

TABLE 22. MEANS FOR THE EFFECT OF PROTEIN SOURCE ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS WHEN ENZYME CONCENTRATION OF HYDROLYSIS WAS FROM 1.0 TO 3.0 PERCENT

Protein Source	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
CP	149.4 ^c	122.8 ^g	48.5 ^j	12.3 ⁿ
WWF	96.4 ^d	90.2 ^h	50.4 ⁱ	16.6 ^m
SSPC	344.3 ^a	332.0 ^e	31.7 ^l	8.0 ^p
Mixture	209.9 ^b	192.8 ^f	39.5 ^k	9.7 ^o

¹Mg per gram of proteinaceous vegetable material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of proteinaceous vegetable material, dry weight basis, expressed as monosodium glutamate.

³Means of 10 observations.

⁴Means of 15 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower press seed cake; mixture = 28.5% CP, 27.5% WWF, 44.0% SSPC.

whole wheat flour, each mean level being significantly different from the others.

The amount of glucose produced was influenced significantly by the proteinaceous vegetable source. The maximum amount of glucose was found in whole wheat flour followed closely by chick peas; however, the means were significantly different. The sunflower seed press cake exhibited the lowest amount of glucose.

The highest maltose content was observed for whole wheat flour while the lowest was found for sunflower seed press cake. Maltose values for the individual protein sources were significantly different from each other.

Means for the effect of enzyme concentration on enzymatic solubilization of proteins and production of sugars from protein-containing vegetable materials are reported in Table 23. The amount of protein solubilized showed a progressive increase as the level of enzyme concentration was raised from 1.0 to 2.5 percent. A further increase in enzyme concentration did not produce a greater amount of soluble protein.

There was an increase in the quantity of ninhydrin positive material with each increase of the enzyme level from 1.0 to 3.0 percent. The highest amount of ninhydrin positive material was observed when 3.0 percent of the enzyme was used.

Mean values for glucose and maltose content exhibited a steady and significant increase at each increment of enzyme concentration as the amount of enzyme was increased from 1.0 to 3.0 percent.

TABLE 23. MEANS FOR THE EFFECT OF ENZYME CONCENTRATION ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM PROTEINACEOUS VEGETABLE MATERIALS

Enzyme Conc. %	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
1.0	139.6 ^d	130.2 ⁱ	30.3 ⁿ	7.0 ^s
1.5	187.2 ^c	175.7 ^h	37.5 ^m	9.5 ^r
2.0	212.3 ^b	197.6 ^g	43.0 ^l	11.6 ^q
2.5	229.9 ^a	207.3 ^f	48.7 ^k	13.9 ^p
3.0	231.1 ^a	211.5 ^e	53.0 ^j	16.1 ^o

¹Mg per gram of proteinaceous vegetable material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of proteinaceous vegetable material, dry weight basis, expressed as monosodium glutamate.

³Means of 8 observations.

⁴Means of 12 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

Effect of protein source X enzyme concentration interaction on enzymatic solubilization of protein from protein-containing vegetable material is given in Table 24. The quantity of soluble protein for chick peas was increased as the enzyme level was raised from 1.0 to 2.0 percent. The soluble protein content remained unchanged with additional increases in the enzyme level. The amount of soluble protein produced for whole wheat was significantly higher at 1.5 percent. When the amount of enzyme was raised to 3.0 percent, there was a trend for higher amounts of soluble protein. There were substantial and significant increases in the amount of soluble protein content for sunflower seed press cake and for the mixture for each increment increase of enzyme as it was raised to 2.5 percent. Raising the amount of enzyme to 3.0 percent did not alter the amount of soluble protein from that produced by 2.5 percent enzyme.

There was a steady increase in the quantity of ninhydrin positive material for the chick peas as the concentration of the enzyme was raised from 1.0 to 2.0 percent, but no change occurred when the amount of enzyme was raised to 3.0 percent. A significant increase in ninhydrin positive material for whole wheat flour was produced with each increment increase of enzyme to 2.5 percent. For sunflower seed press cake and the mixture, the rate of increase in ninhydrin positive material was significant with each increment increase of the enzyme to 3.0 percent.

The effect of protein source X enzyme concentration interaction on production of sugars resulting from enzymatic solubilization of

TABLE 24. EFFECT OF PROTEIN SOURCE x ENZYME CONCENTRATION INTERACTION ON ENZYMATIC SOLUBILIZATION OF PROTEINS FROM VEGETABLE PROTEINACEOUS MATERIALS

Enzyme Conc. %	Soluble Protein of Protein Source ^{1,3}				NPM of Protein Source ^{2,4}			
	CP	WWF	SSPC	Mixture	CP	WWF	SSPC	Mixture
1.0	109.7 ^j	78.4 ^m	201.5 ^f	168.8 ^h	94.4 ^{LM}	69.5 ^O	196.7 ^G	149.9 ^I
1.5	128.7 ⁱ	98.6 ^l	329.0 ^c	192.4 ^g	114.7 ^K	78.0 ^N	310.4 ^D	175.8 ^H
2.0	168.9 ^h	99.9 ^{kl}	373.0 ^b	207.9 ^e	134.2 ^J	91.5 ^M	349.6 ^C	195.2 ^G
2.5	169.1 ^h	102.5 ^k	390.6 ^a	229.3 ^d	135.5 ^J	96.1 ^L	379.3 ^B	218.2 ^F
3.0	170.8 ^h	102.8 ^k	387.3 ^a	231.4 ^d	134.9 ^J	95.6 ^L	390.2 ^A	225.1 ^E

¹Mg soluble protein per gram of proteinaceous vegetable material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of proteinaceous vegetable material, dry weight basis, expressed as monosodium glutamate.

³Means of 2 observations.

⁴Means of 3 observations.

Means followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44.0% SSPC.

protein-containing vegetable material is shown in Table 25. There was a trend for an increase in the amount of glucose and maltose for all the proteinaceous materials as the level of enzyme concentration was raised from 1.0 to 3.0 percent. The highest absolute amount of glucose and maltose for the individual protein sources was found at 3.0 percent enzyme concentration; however, the quantity was not always significantly higher than that found at 2.5 percent of enzyme used. The amount of sugars for 2.5 percent enzyme was significantly greater than the amount found for 2.0 percent enzyme except that maltose concentration in the mixture showed no significant difference between 2.0 and 2.5 percent enzyme concentration.

Effect of Substrate Concentration on Enzymatic Solubilization of Proteinaceous Vegetable Material

The summaries of analysis of variance for the effect of substrate concentration on enzymatic solubilization of proteins and production of sugars from proteinaceous materials are reported in Table 26. Protein source had a significant effect on soluble protein, ninhydrin positive material, glucose and maltose values at the 0.01 level of probability. Substrate concentration and the interaction influenced significantly the contents of ninhydrin positive material and glucose at the 0.01 level of probability. Substrate concentration and the replication for soluble protein was significant at the 0.05 level of probability.

Means for the effect of protein-containing vegetable material on enzymatic solubilization of proteins and production of sugars are presented in Table 27. Conditions of the study were pH 8.5, 50°C,

TABLE 25. EFFECT OF PROTEIN SOURCE x ENZYME CONCENTRATION INTERACTION ON PRODUCTION OF SUGARS FROM ENZYMATIC SOLUBILIZATION OF PROTEINACEOUS VEGETABLE MATERIALS^{1,2}

Enzyme Conc. %	Soluble Protein of Protein Source ^{1,3}			NPM of Protein Source ^{2,4}				
	CP	WWF	SSPC	Mixture	CP	WWF	SSPC	Mixture
1.0	34.2 ^k	34.5 ^k	22.8 ⁿ	29.7 ^l	5.7 ^L	9.6 ^{GH}	5.9 ^L	7.0 ^{KL}
1.5	38.8 ^{ij}	49.0 ^e	26.5 ^m	35.9 ^k	9.2 ^{HI}	12.3 ^{EF}	8.0 ^{I-K}	8.7 ^{H-J}
2.0	49.1 ^e	52.4 ^d	29.9 ^l	40.8 ^{hi}	13.3 ^E	15.6 ^D	7.6 ^{JK}	10.0 ^{GH}
2.5	57.2 ^c	56.1 ^c	36.4 ^{jk}	45.3 ^{fg}	15.0 ^D	20.7 ^B	8.8 ^{H-J}	10.9 ^{FG}
3.0	63.1 ^a	59.7 ^b	43.2 ^{gh}	46.0 ^f	18.1 ^C	24.9 ^A	9.6 ^{GH}	11.9 ^F

¹Mg sugar per gram of vegetable proteinaceous material, dry weight basis.

²Means of 3 observations.

Means followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF; 44.0% SSPC.

TABLE 26. SUMMARIES OF ANALYSIS OF VARIANCE FOR THE EFFECT OF SUBSTRATE CONCENTRATION ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM VEGETABLE PROTEINACEOUS MATERIALS

Source of Variance	D. F.	Mean Squares Soluble Protein	D. F.	NPM ¹	Mean Squares	
					Glucose	Maltose
Total	39	13694.3	59	13230.7	44.7	2.8
A. Protein Source	3	177993.5**	3	259812.3**	661.5**	38.5**
B. Substrate Conc.	4	14.4*	4	156.8**	39.3**	2.8
A x B	12	0.6	12	33.9**	29.5**	0.9
Replication	1	5.8*	2	5.7	1.5	0.6
Residual Error	19	1.3	38	3.3	3.7	0.7

** Significant at the 0.01 level of probability.

* Significant at the 0.05 level of probability.

¹Ninhydrin positive material.

TABLE 27. MEANS FOR THE EFFECT OF PROTEIN SOURCE ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS WHEN SUBSTRATE CONCENTRATION OF THE HYDROLYSIS WAS FROM 5 TO 9 GRAMS

Protein Source	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
CP	169.3 ^c	133.6 ^g	55.3 ⁱ	13.9 ^m
WWF	99.9 ^d	93.6 ^h	50.7 ^j	12.0 ⁿ
SSPC	410.7 ^a	389.7 ^e	40.3 ^l	10.0 ^o
Mixture	241.9 ^b	227.5 ^f	44.4 ^k	12.3 ⁿ

¹Mg per gram of proteinaceous vegetable material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of proteinaceous vegetable material, dry weight basis, expressed as monosodium glutamate.

³Means of 10 observations.

⁴Means of 15 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44.0% SSPC.

reaction time 6 hours, denaturation period 20 minutes and 2.5 percent enzyme concentration (w/w) on a substrate basis. The largest yield of soluble protein was observed for sunflower seed press cake followed by the mixture; the lowest yield was found for the whole wheat flour sample.

The largest quantity of ninhydrin positive material was found in sunflower seed press cake followed by the mixture, chick peas and whole wheat flour in decreasing quantities of NPM. The amount of ninhydrin positive material in the whole wheat flour sample was very much lower than that found in sunflower seed press cake and in the mixture.

The concentration of glucose observed for the chick pea sample was higher than that observed for the whole wheat flour which was higher than that observed for the mixture. The mean amount of glucose for sunflower seed press cake was significantly lower than that of the mixture.

The highest content of maltose was found in chick peas while the lowest content of maltose was observed for sunflower seed press cake. There was no significant difference in the amount of maltose produced from whole wheat flour and from the mixture.

Means for the effect of substrate concentration on soluble protein, ninhydrin positive material, glucose and maltose content as influenced by the enzymatic activity are shown in Table 28. An increase in the quantity of substrate from 5 to 6 grams did not cause any significant change in the level of soluble protein formed after enzymatic treatment. This was also true when substrate level was raised from 7 to 8 grams.

TABLE 28. MEANS FOR THE EFFECT OF SUBSTRATE CONCENTRATION ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM PROTEINACEOUS VEGETABLE MATERIALS

Substrate Conc. gm/100 ml Buffer	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
5	228.8 ^c	205.0 ^f	45.0 ⁱ	11.3 ^k
6	229.5 ^c	210.6 ^e	46.8 ^h	11.8 ^k
7	230.8 ^b	213.8 ^d	48.4 ^g	12.3 ^k
8	231.0 ^b	212.8 ^d	48.8 ^g	12.4 ^k
9	232.2 ^a	213.2 ^d	49.5 ^g	12.5 ^k

¹Mg per gram of proteinaceous vegetable material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of vegetable proteinaceous material, dry weight basis, expressed as monosodium glutamate.

³Means of 8 observations.

⁴Means of 12 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

However, there was a change in the amount of soluble protein as the substrate concentration was increased from 6 to 7 grams and from 8 to 9 grams. The highest increase in soluble protein content occurred when 9 grams of substrate was used.

There was an increase in the quantity of ninhydrin positive material when the level of substrate was raised from 5 to 7 grams. The quantity of NPM remained constant with further increases in the substrate concentration to 9 grams.

The glucose content showed a significant increase with each increment of substrate increase from 5 to 7 grams. Additional increases in substrate level caused no change in glucose content.

The quantity of maltose was not affected by the increase of substrate level from 5 to 9 grams.

The means for the effect of protein source X substrate concentration interaction on enzymatic solubilization of proteins are presented in Table 29. Increasing the amount of substrate from 5 to 9 grams did not cause significant differences in soluble protein mean values.

The quantity of ninhydrin positive material for the chick pea sample remained constant as the level of substrate was raised from 5 to 9 grams. With the whole wheat flour, there was an increase in the amount of ninhydrin positive material between the level of substrate at 5 and 6 grams. No change occurred in the quantity of ninhydrin positive material when the level of substrate was raised to 8 grams. The mean value at 9 grams was significantly higher than that at 6

grams. Mean values of ninhydrin positive material for the sunflower seed press cake showed an increase as the substrate concentration was raised from 5 to 7 grams. The highest NPM value was observed at 7 grams of substrate. Further increases in the level of substrate caused a reduction of NPM mean values. In the case of the mixture, the amount of ninhydrin positive material was increased significantly when the level of substrate was raised from 5 to 6 grams. Raising the substrate level from 6 to 7 grams did not alter the amount of NPM. An increase in NPM value was observed at 8 grams of substrate. The amount of ninhydrin positive material at 9 grams of substrate was not significantly different from 8 grams of substrate.

Means for the effect of the protein source X substrate concentration interaction on production of sugars resulting from enzymatic solubilization of proteinaceous vegetable material are shown in Table 30. There was a trend for an increase in the amount of glucose for chick peas as the level of substrate was raised from 5 to 9 grams. The highest amount of glucose for the chick pea sample was observed at 9 grams of substrate level. The quantity of glucose in the whole wheat flour sample was maintained at the same level at 5 and 6 grams of substrate. Whole wheat flour yielded the highest amount of glucose at 7 grams of substrate. At 8 grams of substrate, there was a significant reduction in the quantity of glucose. No significant change was found in the yield of glucose between the substrate concentration at 8 and 9 grams. The content of glucose for sunflower seed press cake remained at a constant

TABLE 30. EFFECT OF PROTEIN SOURCE x SUBSTRATE CONCENTRATION INTERACTION ON PRODUCTION OF SUGARS FROM ENZYMATIC SOLUBILIZATION OF PROTEINACEOUS VEGETABLE MATERIALS^{1,2}

Substrate Conc. gm/100 ml Buffer	Glucose of Protein Source			Maltose of Protein Source		
	CP	WWF	SSPC	Mixture	CP	WWF
5	49.1 ^{ef}	49.0 ^{ef}	36.4 ^k	45.5 ^{gh}	13.3 ^A	12.3 ^A
6	53.7 ^{cd}	49.7 ^{ef}	38.7 ^{jk}	44.9 ^{gh}	14.0 ^A	11.7 ^A
7	54.7 ^{bc}	53.9 ^{bc}	38.6 ^{jk}	46.5 ^{fg}	13.6 ^A	11.9 ^A
8	57.5 ^b	51.1 ^{de}	42.4 ^{hi}	44.1 ^{g-i}	14.0 ^A	11.9 ^A
9	61.5 ^a	50.0 ^e	45.6 ^{gh}	40.9 ^{ij}	14.7 ^A	12.0 ^A
					10.8 ^A	10.5 ^A
					10.3 ^A	9.6 ^A
					8.8 ^A	12.3 ^A
					13.1 ^A	13.3 ^A
					13.5 ^A	10.9 ^A

¹Mg sugar per gram of proteinaceous vegetable material, dry weight basis.

²Means of 3 observations.

Means followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44.0% SSPC.

level as the substrate concentration was raised from 5 to 7 grams. The content of glucose showed an increase at 8 grams of substrate. The amount of glucose remained unchanged when the level of substrate was increased to 9 grams. For the mixture, the amount of glucose showed no significant changes as the amount of substrate was raised from 5 to 9 grams.

The yield of maltose was not influenced by the interaction of the factors.

The Effect of Sodium Chloride Content on Enzymatic Solubilization of Proteinaceous Vegetable Material

The analysis of variance summaries for the effect of sodium chloride content on enzymatic solubilization of proteins and production of sugars are given in Table 31. The factors and the interactions were significant at the 0.01 level of probability. The replication for ninhydrin positive material was significant at the 0.05 level of probability.

The effect of proteinaceous vegetable source on the mean values of soluble protein, ninhydrin positive material, glucose and maltose contents is found in Table 32. The conditions of the enzymatic activity were pH 8.5, 50°C, reaction time 6 hours, denaturation period 20 minutes and 2.5 percent enzyme preparation. The highest amount of soluble protein and the largest release of ninhydrin positive material were found in sunflower seed press cake followed by the mixture, chick peas and whole wheat flour in decreasing concentrations.

TABLE 31. SUMMARIES OF ANALYSIS OF VARIANCE FOR THE EFFECT OF SODIUM CHLORIDE CONCENTRATION ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM PROTEINACEOUS VEGETABLE MATERIALS

Source of Variance	D. F.	Mean Squares Soluble Protein	D. F.	NPM ¹	Mean Squares	
					Glucose	Maltose
Total	39	13050.3	59	13114.4	97.1	7.1
A. Protein Source	3	169076.3**	3	257455.5**	1453.2**	54.5**
B. NaCl Conc.	4	324.5**	4	123.9**	217.7**	38.1**
A x B	12	34.6**	12	61.8**	29.7**	7.1**
Replication	1	0.2	2	11.6*	4.9	0.3
Residual Error	19	3.1	38	3.2	3.4	0.5

** Significant at the 0.01 level of probability.

* Significant at the 0.05 level of probability.

¹ Ninhydrin positive material.

TABLE 32. MEANS FOR THE EFFECT OF PROTEIN SOURCE ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS WHEN SODIUM CHLORIDE CONTENT OF HYDROLYSIS WAS FROM 0.1 to 0.5 M

Protein Source	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
CP	177.6 ^c	133.6 ^g	52.4 ⁱ	14.1 ^m
WWF	102.1 ^d	92.2 ^h	49.0 ^j	12.1 ⁿ
SSPC	407.2 ^a	387.6 ^e	30.1 ^l	9.5 ^o
Mixture	246.4 ^b	224.7 ^f	42.3 ^k	12.5 ⁿ

¹Mg per gram of proteinaceous vegetable material, dry weight basis.

²Micromoles ninhydrin positive material (NPM) per gram of proteinaceous vegetable material, dry weight basis, expressed as monosodium glutamate.

³Means of 10 observations.

⁴Means of 15 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44.0% SSPC.

Chick peas showed the highest yield of glucose and maltose while sunflower seed press cake had the lowest levels of glucose and maltose. The amount of maltose between the whole wheat flour sample and the mixture was not different. However, there was a significant difference in the amount of glucose between the two protein sources.

The means for the influence of sodium chloride content on soluble protein, ninhydrin positive material, glucose and maltose are presented in Table 33. There was a progressive increase in the amount of soluble protein as the level of sodium chloride was raised from 0.1 to 0.3 M. The highest amount of soluble protein was found at 0.3 M NaCl content. Beyond that point, the quantity of soluble protein decreased significantly.

The concentration of ninhydrin positive material showed an increase with the rise of NaCl level from 0.1 to 0.3 M. The largest NPM mean value was observed at 0.3 M NaCl. At 0.4 M NaCl level, there was a significant reduction in the quantity of NPM value which was not different from that of 0.5 M NaCl level.

The glucose content remained unchanged as the addition of salt was increased from 0.1 to 0.2 M. When the level of salt was raised to 0.3 M, the glucose content increased significantly. Further additions of salt caused a significant decrease in the glucose content.

The highest amount of maltose was observed at 0.2 M NaCl level. There was a decrease in the amount of maltose as the concentration of NaCl was increased from 0.2 to 0.5 M.

TABLE 33. MEANS FOR THE EFFECT OF SODIUM CHLORIDE CONCENTRATION ON ENZYMATIC SOLUBILIZATION OF PROTEINS AND PRODUCTION OF SUGARS FROM PROTEINACEOUS VEGETABLE MATERIALS

NaCl Conc. moles/l	Soluble Protein ^{1,3}	NPM ^{2,4}	Glucose ^{1,4}	Maltose ^{1,4}
0.1	228.7 ^c	204.9 ^g	44.9 ⁱ	11.9 ⁿ
0.2	235.5 ^b	210.0 ^f	45.4 ⁱ	14.2 ^l
0.3	242.2 ^a	213.8 ^e	48.0 ^h	13.5 ^m
0.4	234.4 ^b	210.1 ^f	42.2 ^j	10.8 ^o
0.5	225.8 ^d	208.7 ^f	36.2 ^k	9.9 ^p

¹Mg per gram of proteinaceous vegetable material, dry weight basis.

²Moles ninhydrin positive material (NPM) per gram of proteinaceous vegetable material, dry weight basis, expressed as monosodium glutamate.

³Means of 8 observations.

⁴Means of 12 observations.

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

Means for the effect of protein source X sodium chloride concentration interaction on enzymatic solubilization of proteins from vegetable proteinaceous material are reported in Table 34. The amount of soluble protein for chick peas, whole wheat flour and for the mixture exhibited a progressive increase as the level of sodium chloride was raised from 0.1 to 0.3 M. Levels of salt above 0.3 M caused a significant reduction in the amount of soluble protein for all the three proteinaceous preparations. The quantity of soluble protein for sunflower seed press cake remained constant as the level of salt was raised to 0.3 M. Further increases in NaCl content produced a marked decrease in soluble protein.

The yield of ninhydrin positive material in chick peas was not altered as the level of salt was increased to 0.5 M. The mean value of NPM for the whole wheat flour sample was not different between 0.1 and 0.2 M NaCl but an increase in the NaCl level to 0.3 M caused a significant increase in NPM value which was maintained at a constant level when NaCl content was raised to 0.5 M. Sunflower seed press cake exhibited an increase in the amount of NPM between salt concentration 0.1 and 0.2 M. No further changes were observed in NPM mean values as the amount of salt was increased to 0.4 M. However, an additional increase in the concentration to 0.5 M caused a significant increase in the quantity of NPM. There was a continuous increase in the level of NPM for the mixture until NaCl concentration was raised to 0.3 M but subsequent increases of salt produced a marked decrease in NPM.

The means for the effect of protein source X sodium chloride content interaction on production of sugars resulting from enzymatic solubilization of proteinaceous vegetable material are given in Table 35. The amount of glucose in chick peas showed an increase when NaCl content was raised from 0.1 to 0.3 M. There was no change in the level of glucose between 0.3 and 0.4 M salt but the amount of glucose was decreased at 0.5 M. Whole wheat flour showed an increase between salt content 0.1 and 0.2 M. The level of glucose was not different at 0.2 and 0.3 M. A significant decrease in the amount of glucose was observed with the additional increases of salt content. The maximum amount of glucose for sunflower seed press cake was produced at 0.1 M NaCl. The amount was reduced gradually when the salt addition was increased. The quantity of glucose in the mixture was constant as the level of salt was varied from 0.1 to 0.3 M. Further increases in NaCl concentration caused a reduction of glucose values.

The highest values for maltose was found at 0.2 M salt in chick peas; at 0.3 M salt in whole wheat flour and at 0.2 to 0.3 M salt in the mixture. The highest level of maltose in sunflower seed press cake occurred between 0.2 and 0.3 M salt content. The amount of maltose for chick pea was decreased when the level of salt was raised from 0.2 to 0.5 M. Between 0.4 and 0.5 there was no difference in the amount of maltose. Whole wheat flour showed a reduction in the maltose content at 0.4 M NaCl which was not different from the maltose content at 0.5 M salt. Sunflower seed press cake did not exhibit any change between 0.3 and 0.4 and also

between 0.4 and 0.5 M NaCl content. In the case of the mixture, there was a reduction in maltose content as the level of NaCl was increased from 0.3 to 0.5 M.

II. EVALUATION OF PROTEIN QUALITY

Prior to separation, identification and estimation of lysine, methionine and tryptophan by chromatography the individual protein sources were hydrolyzed enzymatically under the conditions of pH 8.5, 50°C, reaction time 6 hours, denaturation period 20 minutes, 2.5 percent enzyme preparation on a substrate basis and 0.3 M sodium chloride. The enzyme digested samples for each protein source were hydrolyzed completely by hydrochloric acid or 5 N Barium hydroxide.

The limiting essential amino acid contents as determined by thin-layer chromatography from enzyme, enzyme + acid and enzyme + alkali hydrolyzates of proteinaceous vegetable materials are shown in Table 36. The mean values of lysine content were highest in enzyme + acid hydrolyzates of sunflower seed press cake followed by chick peas and the mixture. The lysine content of whole wheat flour was comparatively low. The quantity of lysine in the mixture was slightly lower than that of sunflower seed press cake and chick peas.

A large amount of methionine was observed for enzyme + acid hydrolyzates of sunflower seed press cake, whereas the amount of methionine was low in enzyme + acid hydrolyzates of the chick pea sample. The mixture yielded the second highest level of methionine while whole wheat

TABLE 36. LIMITING ESSENTIAL AMINO ACID CONTENT OF PROTEINACEOUS VEGETABLE MATERIALS AS DETERMINED BY THIN-LAYER CHROMATOGRAPHY^{1,2}

Protein Source	LYSINE		METHIONINE		TRYPTOPHAN	
	Enzyme ³ + Acid	Enzyme ⁴	Enzyme ³ + Acid	Enzyme ⁴	Enzyme ⁵ + Alkali	Enzyme ⁴
CP	1279	183	197	--	142	--
WWF	320	100	223	86	94	--
SSPC	1454	860	995	146	408	115
Mixture	1071	133	550	194	279	90

¹Mg per grams of proteinaceous vegetable material, dry weight basis.

²Means of 18 observations.

³Means based on thin-layer chromatography of 6 N HCl hydrolysis of enzyme hydrolyzates.

⁴Means based on thin-layer chromatography of enzyme hydrolyzates.

⁵Means based on thin-layer chromatography of 5 N Ba(OH)₂ hydrolysis of enzyme hydrolyzates.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44% SSPC.

flour had an intermediate level of methionine between the mixture and chick peas.

The quantity of tryptophan was low in enzyme + alkaline hydrolyzates of whole wheat flour and chick peas. However, the yield of tryptophan was comparatively higher in sunflower seed press cake and the mixture.

The enzymatic hydrolysis of each of proteinaceous material, without acid or alkaline hydrolysis prior to chromatography, revealed comparatively smaller amounts of detectable limiting essential amino acids. Lysine was the first amino acid separated on the chromatogram. The highest lysine content was observed for sunflower seed press cake followed by the chick pea sample and the mixture. The amount of lysine for whole wheat flour was the lowest. The value of methionine for the enzyme hydrolyzates of the mixture was higher than that of the mixture. Whole wheat flour yielded a lower amount of methionine whereas, chick peas did not have any detectable quantity of methionine. The concentration of tryptophan in enzyme hydrolyzates of sunflower seed press cake was higher than that of the mixture. The chick pea and whole wheat flour samples did not produce detectable concentration of tryptophan. Therefore, in the chemical score calculations, mean values of limiting amino acids observed from the only enzyme + acid and enzyme + alkaline hydrolyzates of the individual proteinaceous materials were used.

The total essential amino acid values which were based on the individual essential amino acid contents of protein-containing vegetable materials and whole hen's egg were obtained from literature (34) and they are

presented in Table 37. The values of each essential amino acid content for the mixture were calculated from the published values of essential amino acids of chick peas, whole wheat flour and sunflower seed press cake. In recent recommendations on protein and amino acid requirements (33), cystine and tyrosine were added to the total essential amino acid content of each protein source even though these are not considered as essential amino acids.

The essential amino acid pattern of whole hen's egg was used for reference purposes. The highest content of total essential amino acids per gram of nitrogen was observed for whole egg followed by the mixture. Although sunflower seed press cake had the largest amount of total nitrogen content, its total essential amino acid level per gram of nitrogen was lower than those of chick peas, mixture and whole egg. Whole wheat flour exhibited the lowest quantity of total essential amino acids per gram of nitrogen as well as the total amount of nitrogen. The amounts of limiting amino acids for the individual proteinaceous materials as determined by thin-layer chromatographic method were considerably lower than those obtained from the published values except methionine which was higher than that of corresponding literature value in whole wheat flour.

The chemical scores of the limiting essential amino acids of vegetable protein sources, as percentage of the reference egg pattern by modified (A/E) method of calculation (33), are reported in Table 38. The data indicates that chick peas is a good source of lysine, but deficient

TABLE 37. LITERATURE VALUES FOR THE ESSENTIAL AMINO ACID CONTENT OF PROTEINACEOUS VEGETABLE MATERIALS AS COMPARED WITH THOSE OF WHOLE HEN'S EGG PROTEINS^{1,2}

Amino Acid	CP	WWF	SSPC	Mixture ³	Whole Egg
Isoleucine	277	204	267	271	415
Leucine	468	417	399	430	553
Lysine	428(380)*	179(160)*	231(196)*	272(230)*	403
Methionine	65(59)*	94(110)*	154(134)*	135(118)*	197
Cystine	74	159	110	113	149
Phenylalanine	358	282	304	323	365
Tyrosine	183	187	162	176	262
Threonine	234	183	228	232	317
Tryptophan	54(42)*	68(47)*	76(55)*	73(60)*	100
Valine	284	286	322	536	454
Total	2425	2049	2253	2561	3215

¹Mg per gram of total nitrogen from proteinaceous material.

²Literature values based on column chromatography except tryptophan.

³Calculated amino acid content of the mixture based on literature values of proteinaceous materials.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44% SSPC.

*Values based on thin-layer chromatography.

TABLE 38. CHEMICAL SCORES OF PROTEINACEOUS VEGETABLE MATERIAL BASED ON LIMITING ESSENTIAL AMINO ACID CONTENT AS COMPARED WITH CORRESPONDING AMINO ACIDS OF WHOLE EGG PROTEINS^{1,2}

Amino Acid	CP	WWF	SSPC	Mixture
Lysine	149	73	84	86
Methionine + Cystine	46	107	98	75
Tryptophan	58	75	90	78
Total Essential Amino Acids	2359	2025	2177	2489
Protein Score	46	73	84	75

¹Values expressed as percentage of the ratio in egg proteins.

²Calculations based on thin layer chromatographic values of limiting essential amino acids.

CP = chick peas; WWF = whole wheat flour; SSPC = sunflower seed press cake; mixture = 28.5% CP, 27.5% WWF, 44% SSPC.

in respect to sulphur-containing amino acids and tryptophan. On the other hand, whole wheat flour and sunflower seed press cake were reasonably rich in sulphur amino acids with the chemical score of 107 and 98, respectively. Whole wheat was low in the tryptophan while sunflower seed press cake yielded the highest amount of tryptophan. The mixture showed the lowest chemical score for the sulphur amino acids. The second lowest score in the mixture was for tryptophan. But as far as sulphur amino acids and tryptophan are concerned, the quality of protein in the mixture was superior to those of chick peas and was slightly inferior to the quality of protein in sunflower seed press cake. The chemical score in the terms of lysine for the mixture showed that the quality was higher than that for whole wheat flour.

in respect to sulphur-containing amino acids and tryptophan. On the other hand, whole wheat flour and sunflower seed press cake were reasonably rich in sulphur amino acids with the chemical score of 107 and 98, respectively. Whole wheat was low in the tryptophan while sunflower seed press cake yielded the highest amount of tryptophan. The mixture showed the lowest chemical score for the sulphur amino acids. The second lowest score in the mixture was for tryptophan. But as far as sulphur amino acids and tryptophan are concerned, the quality of protein in the mixture was superior to those of chick peas and was slightly inferior to the quality of protein in sunflower seed press cake. The chemical score in the terms of lysine for the mixture showed that the quality was higher than that for whole wheat flour.



CHAPTER V

DISCUSSION

A solution to the problem of animal protein shortage in areas, where protein-calorie malnutrition is prevalent, should be based on the use of local, inexpensive sources of high quality protein which could supplement diets of infants and preschool children of the poor in a community. High quality protein food can be prepared by proper processing oil seed meals, pulses and cereals. But it is a well-known fact that the utilization of plant protein in food for child feeding has limitations such as low digestibility and poor flavor and texture. To improve the digestibility and organoleptic acceptability of plant protein foods, the use of a proteolytic enzyme system has been suggested (1, 38, 74). Nevertheless, a search of literature revealed that a systematic and comprehensive study of conditions affecting enzymatic digestion of proteinaceous vegetable material was lacking. Such studies, therefore, seem to be warranted.

This experiment was designed to study the effects of conditions on enzymatic modification of plant proteins that can be utilized for the purpose of child feeding practices. Also an attempt has been made to evaluate the protein quality of enzyme hydrolyzed proteinaceous vegetable materials by means of a chemical score.

From the analytical data presented, the enzymatic solubility of proteins from sunflower seed press cake and the mixture was increased

significantly as the pH was raised from 6.0 to 8.5. The maximum solubility of proteins for these materials was observed at pH 8.5. At pH levels above 8.5, solubility of the proteins was decreased sharply. The pH values for optimum protein solubilization were found at 7.5 through 8.5 for the chick pea sample and at 8.0 for the whole wheat flour at 40°C, reaction time 2 hours, denaturation period 5 minutes, 5 grams of substrate and 1 percent enzyme preparation. Rhozyme-41 is reported to exhibit an optimum pH between 6.0 to 9.0. Present investigations revealed that the proteolytic activity of the enzyme was most effective between pH 7.5 and 8.5. Furthermore, the results showed that the extent and the rate of protein solubilization with Rhozyme-41 were higher for sunflower seed press cake and the mixture than for proteins of chick peas and whole wheat flour. These optimum pH findings of the enzyme for the individual proteinaceous materials, of course, depends not only on the nature of the enzyme, but also on the type of substrate, substrate concentration, stability of the enzyme, temperature of the reaction, and length of the reaction time. Since the enzymes are protein in nature, pH changes will affect primarily the ionic character of the free amino and carboxylic groups of the protein molecule. Consequently, the catalytic nature of the enzyme will be altered by the changes in ionization of either the enzyme, the substrate or the enzyme substrate complex with varying pH levels.

On the other hand, maximum release of the total free amino acids, peptides and proteins having free amino groups, expressed as ninhydrin positive material, was found for chick peas to be between pH 7.5 and 8.0;

for whole wheat flour, pH 8.0 and 8.5; for sunflower seed press cake, pH 8.5 and 9.0; and for the mixture, pH 8.5.

Both the macro-kjeldahl ($N \times 6.25$) and ninhydrin-colorimetric methods were used to determine the protein content. The former is a measure of the soluble crude protein which was used to express the proteolytic activity of Rhozyme-41; the latter method provides an estimate of the amount of free amino groups as total ninhydrin positive material (NPM).

The concentration of glucose and maltose resulting from enzymatic hydrolysis was reduced when pH was increased from 6.0 to 9.5. This indicated that amylolytic power of the enzyme exhibited an increased activity at lower pH levels while proteolytic capacity showed a maximum activity at higher pH ranges. The highest yields of glucose were observed for the chick pea and whole wheat flour at pH 6.0, followed by the mixture and sunflower seed press cake. Whole wheat flour yielded the largest amount of maltose while sunflower seed press cake produced the lowest quantity of maltose.

The temperature of enzymatic hydrolysis had a significant effect on the solubility of proteins as well as the release of the amino acids. There was a substantial increase in the amounts of soluble protein and NPM for sunflower seed press cake when the temperature was raised from 40 to 50°C with pH at 8.5 and reaction time of 2 hours. The maximum quantity of soluble protein and NPM was observed for chick pea at 45° and 50°C. Whole wheat flour exhibited the highest yield of soluble protein at 50°C while the NPM level remained unchanged at the temperature 45° through 55°C. The higher rate of protein solubility for sunflower

seed press cake and the mixture indicated that under the same study conditions, Rhozyme-41 exhibited a high proteolytic activity in sunflower press cake and the mixture when compared to chick peas and whole wheat flour. This could be due to the presence of enzyme inhibitor or the moderate degree of enzyme inhibition by the products of hydrolysis in chick peas and whole wheat flour.

A considerable decrease in the amounts of enzyme extractable protein and NPM for the individual sources was observed at temperatures above 50°C. The decrease was possibly due to the rapid degradation of the enzyme at the higher temperatures.

Generally, the contents of glucose and maltose for each proteinaceous material remained constant as the temperature was raised from 40°C to 50°C. The glucose level was higher in chick pea and whole wheat flour held at 45° and 50°C than in sunflower seed press cake held at the same temperature range. The amount of glucose for the mixture was at an intermediate level. Although temperature and source of protein had a significant effect on maltose content, the amounts of maltose for the individual sources were extremely low when compared to the corresponding values of glucose at each increment of temperature increase. There was a slight but not always significant decrease in both glucose and maltose content when the temperature was elevated beyond 50°C. The higher pH levels and temperatures probably contributed to inactivation of the amylase in Rhozyme-41 that led to the hydrolysis of starches.

The time factor had a greater effect on the enzymatic extraction of proteins and liberation of amino acids when the length of reaction time

was extended from 2 to 6 hours. The level of these two substances in most cases remained unchanged at 6 and 8 hours digestion except that the mixture showed a significant increase in both substances and sunflower seed press cake in NPM values. However, the increase in the soluble protein and NPM were significant between 6 and 10 hour digestion except for chick peas which exhibited no increase beyond 6 hours. Since the enzymatic hydrolysis was very slight after 6 hours digestion, 6 hours reaction time was adopted for the remainder of the experiment. Reaction time for maximum protein-hydrolysis is dependent on many factors such as type and nature of the substrate, pH, temperature and the enzyme concentration. At pH 8.5, 50°C, 5 minute denaturation, 5 grams of substrate and 1 percent enzyme the largest quantity of soluble protein and NPM was found for sunflower seed press cake followed by the mixture, chick peas and whole wheat flour in an order of decreasing concentrations. In the earliest experiment, whole wheat flour exhibited the highest yield of soluble protein at pH 8.0 while the other three protein sources yielded larger amounts of soluble protein at pH 8.5. For the practical purposes, the pH 8.5 was selected for the rest of the studies. This could be the reason for the low enzymatic solubility of proteins in whole wheat flour.

In general, there were significant overall changes in the glucose and maltose content as the reaction time was extended from 2 to 10 hours. Most of the glucose and maltose production for proteinaceous sources occurred within 6 hour reaction time. The rate of glucose and maltose conversion was reduced after 6 hour digestion for individual sources except that the level of both sugars in the mixture remained constant

between 2 and 10 hours reaction time. It appeared that conversion of starch into glucose and maltose for sunflower seed press cake and the mixture was lower than that for whole wheat flour and chick peas which produced similar amounts of glucose at 6 hours digestion.

Studies on denaturation time requirement for enzymatic hydrolysis indicated that the extent of precooking of protein sources prior to enzyme treatment exhibited significant effects on the soluble protein and NPM contents. The amount of soluble protein was increased as the duration of denaturation period was extended from 5 to 20 minutes. These results agree with the findings of Sreekantiah, et al. (74). However, the level of NPM remained unchanged between 15 and 20 minutes then showed a slight reduction at 25 minutes. The highest amounts of soluble protein were observed for chick peas, sunflower press cake and the mixture between 20 and 25 minutes. Sunflower seed press cake and the mixture did not show any change in the amounts of soluble protein between 15 and 20 minutes. No difference was observed for whole wheat flour at 15 through 25 minutes denaturation.

The values of NPM were highest for chick peas at 15 and 20 minutes; for whole wheat flour at 15; for sunflower press cake at 20 and 25 minutes; and for the mixture at 10 and 15 minutes. The level of ninhydrin positive material showed a decrease at 25 minutes, for whole wheat flour and the mixture between 15 and 25 minutes cooking. One explanation for the reduction could be the destruction of certain amino acids due to excessive heat. Another explanation for the reduction of amino acids could be the formation of substances resulting from an interaction

between proteins and reducing sugars. These substances are resistant to enzymatic digestion. Therefore, the length of denaturation period is a very critical factor as far as nutritional value and enzymatic digestion of proteinaceous material are concerned.

Denaturation period had no significant effect on glucose content. On the other hand, an increase was observed for the maltose values when cooking time was extended from 5 to 10 minutes. No changes occurred between 10 and 20 minutes and also between 15 and 20 minutes. A reduction occurred at 25 minutes. Chick peas and sunflower press cake showed no difference in maltose level at 5 through 20 minutes. The level was reduced at 25 minutes. The amount of maltose for whole wheat flour remained constant beyond 10 minutes denaturation. Only between 10 and 15 minutes the mixture showed an increase in maltose value.

The enzymatic solubilization of proteinaceous materials was affected significantly by the enzyme concentration factor. The amounts of soluble protein and NPM continued to increase as the addition of the enzyme was increased from 1 percent to 2.5 percent. The rate of increase in both substances was substantial for sunflower seed press cake and the mixture until enzyme level reached to 2.5 percent. The greatest soluble protein yield occurred for chick peas at 2 percent and for whole wheat flour at 1.5 percent. The amount of soluble protein at 2 percent was different than the amount 1.5 percent enzyme in whole wheat flour. No change in the amounts of soluble protein was observed for chick peas and whole wheat flour beyond 2 percent enzyme level.

Similarly, the quantity of ninhydrin positive material for chick peas remained constant between 2 percent and 3 percent enzyme, while the

quantity of NPM for whole wheat flour continued to increase until the enzyme concentration was raised to 2.5 percent. It appeared that for maximum protein hydrolysis, the amount of enzyme required was 1.5 percent for whole wheat flour, 2 percent for chick peas and 2.5 percent for sunflower seed press cake and the mixture when other conditions were pH 8.5, 50°C, 6 hours reaction time, 20 minutes denaturation, and 5 grams of substrate.

The amount of sugars was also influenced by the level of enzyme. Amylolytic activity of Rhozyme-41 continued until enzyme concentration was raised to 3 percent. The continuous enzyme activity indicated that high enzyme concentrations did not have any detrimental effect on amylolytic nature of the enzyme considering the inhibitory effect on the proteolytic activity at 3 percent enzyme level. However, reduced proteolytic enzyme activity at 3 percent enzyme level could be due to the insufficient amount of substrate among other causes. The highest glucose level was observed for chick pea followed by whole wheat flour, the mixture and sunflower seed press cake in a decreasing order at 3 percent. The highest maltose content occurred in whole wheat flour followed by chick peas, the mixture, and sunflower seed press cake.

The results of the study indicated that there was a significant change in soluble protein content as the level of substrate was increased from 5 to 9 grams while the changes in the quantity of ninhydrin positive material occurred between 5 and 7 grams of substrate. However, increasing the amount of substrate did not cause significant differences in soluble protein mean values for each of the individual sources under the

conditions of pH 8.5, 50°C, reaction time 6 hours, denaturation period 20 minutes and 2.5 percent enzyme. NPM levels of the protein sources increased slightly due to the rise in the amount of substrate except for chick peas in which there was no change. It appeared that high substrate concentrations inhibited further proteolytic activity of the enzyme. Therefore, 5 grams of substrate were chosen for the entire enzymatic hydrolysis.

The level of glucose produced was affected by the source of protein and by the amount of substrate while the level of maltose was influenced only by the protein source. Under the same conditions chick pea had the highest level of both sugars while the lowest values were observed for the press cake. Even though whole wheat flour had a higher carbohydrate content when compared to chick pea, upon their enzymatic hydrolysis, larger values for sugars were found in chick peas. The increased amounts of glucose were observed for chick peas at 9 grams, for whole wheat flour at 7 grams, for sunflower seed press cake between 8 and 9 grams and for the mixture between 5 and 7 grams of substrate.

The enzymatic solubilization of proteinaceous vegetable materials occurred without the addition of sodium chloride. However, the amount of substances resulting from enzymatic solubilization indicated that when salt was added, the catalytic power of the enzyme was considerably increased. It appeared that the solubility of the proteins from chick pea, whole wheat flour and the mixture was influenced significantly by the salt concentration while no change in the soluble protein level was observed for sunflower seed press cake. The results showed that the

highest protein solubility occurred at the 0.3 M NaCl level for the individual materials. A progressive decrease in the amounts of soluble protein was found for the all the samples above 0.3 M NaCl.

The values of ninhydrin positive material for chick pea remained constant with NaCl increases. The level of NPM for whole wheat flour was the same at 0.1 and 0.2 M followed by an increase at 0.3 M. The level was maintained until NaCl concentration was raised to 0.5 M. For sunflower seed press cake the only increase in NPM was observed between 0.1 and 0.2 M NaCl. For the mixture, the highest amount of ninhydrin positive material was found at 0.3 M salt followed by a progressive decrease beyond 0.3 M salt.

Addition of sodium chloride had a significant effect on the production of sugars. The highest levels of glucose and maltose were produced for individual protein sources at 0.2 and 0.3 M salt concentrations, except that the amount of glucose was reduced for press cake beyond 0.1 M NaCl.

The Rhozyme-41 is capable of extensively hydrolyzing the proteins of proteinaceous vegetable material under the study conditions of pH 8.5, 50°C, reaction time 6 hours, denaturation period 20 minutes, 2.5 percent enzyme concentrate, 5 grams of substrate and 0.3 M sodium chloride content. The increase in the amount of soluble nitrogen and formation of reducing sugars are the results of enzymatic hydrolysis of both proteins and carbohydrates. The increases in soluble proteins for chick peas, whole wheat flour, sunflower press cake and the mixture were 187.7, 111.2, 412.0 and 257.7 mg/g, respectively; while corresponding figures for the

total free amino groups in the terms of ninhydrin positive material, expressed as monosodium glutamate were 134, 95, 387.5, and 236.6 micro-moles/g. As a result of enzymatic hydrolysis, the amount (mg/g) of glucose and maltose produced were 57.2 and 14.0 for chick peas; 55.7 and 15.1 for whole wheat flour; 33.0 and 11.0 for sunflower seed press cake; and 46.0 and 15.1 for the mixture, respectively.

The thin-layer chromatographic analysis of enzyme hydrolyzates for the individual protein sources showed lower values of lysine, methione and tryptophan compared with the published values of the amino acids (34) except that methionine content for whole wheat flour was higher than the value published. Protein scores, based on the three limiting essential acids mentioned above, revealed that enzyme treated chick pea proteins were deficient both in methionine (46 percent) and tryptophan (58 percent) but superior in lysine (149 percent) to that of the reference egg pattern. Proteins of whole wheat flour were lacking in lysine (73 percent) and in tryptophan (75 percent) while they were relatively rich in methionine (107 percent). The chemical score for sunflower seed press cake showed that lysine was deficient (84 percent). On the other hand, the mixture, which consisted of 44.0 percent sunflower seed press cake, 28.5 percent chick peas and 27.5 percent whole wheat flour, was relatively low in methionine (75 percent) and tryptophan (78 percent). The chemical score of lysine (86 percent) of the mixture indicated that the amount of lysine was fairly high when compared to that of whole wheat flour and sunflower seed press cake alone. Thus, these mentioned deficiencies were improved by proper mixing of chick pea, whole wheat flour and sunflower

seed press cake so that the amino acid pattern of one protein source complemented the amino acid patterns of the other two protein sources. The protein quality of the mixture was higher than the quality of the individual protein sources. The limiting amino acids in one component were balanced by the excesses in the second and the third component and vice versa.

The report on protein requirements by the joint FAO/WHO expert group (33) stated that 60 and 70 mg of tryptophan per gram N seems to be preferable to the level of 100 mg in the reference egg pattern. It further concluded that the proportion of sulfur containing amino acids in the egg protein was also too high and instead of 346 mg/g N, in the range of 190 to 220 mg/g N would be sufficient. Since the contents of methionine and tryptophan estimated by thin-layer chromatography for the individual protein sources fell between the lower and higher limits of the range proposed by the joint expert group, it can be concluded that the mixture would adequately supplement the diets of the infants and preschool children. However, further investigation of the mixture is warranted in animal and human studies to determine its utility as a protein source.

Apart from protein quality, the enzymatic hydrolysis of proteinaceous vegetable material did not improve the organoleptic characteristics. A bitter taste, a strawy flavor and a brown coloration develop for all the protein sources after enzyme treatment. These were more pronounced for sunflower seed press cake and the mixture. An increase in the intensity of color for sunflower seed press cake and the mixture was caused by

chlorogenic acid which is a compound found naturally in sunflower seed press cake (84). Therefore, further study is required to improve organoleptic characteristics and to determine the physical and chemical properties as well as bacterial count of the enzyme hydrolyzed mixture for human consumption.



CHAPTER VI

SUMMARY

In this study experiments were conducted to determine the effect of pH, temperature, reaction time, duration of denaturation period, enzyme concentration, substrate concentration and salt concentration on soluble protein and sugar levels as affected by enzymatic hydrolysis of chick pea, whole wheat flour, sunflower seed press cake, and the mixture consisting of 28.5 percent chick pea, 27.5 percent whole wheat flour, and 44 percent sunflower seed press cake. Also in order to evaluate the quality of proteins, thin-layer chromatographic analysis was performed to determine the quantity of limiting amino acids in vacuum oven-dried enzyme hydrolyzates.

Based on the results of this study, the following conclusions were made:

1. Maximum protein solubilization occurred in chick pea between pH 7.5 and 8.5; in whole wheat flour at pH 8.0; in sunflower seed press cake and the mixture at pH 8.5. Highest release of total free amino acids, in terms of ninhydrin positive material, expressed as monosodium glutamate were found between pH 8.0 and 8.5 in all the protein sources except chick pea. The greatest increase in free amino acid in chick pea was observed between pH 7.5 and 8.0.

Glucose and maltose content of each protein source, as affected by enzymatic hydrolysis, was reduced significantly as pH values increased

from pH 6.0 to 9.5. The rate of reduction was very sharp between pH 9.0 and 9.5.

2. Mean soluble protein and free amino acid values indicated that the rate of enzymatic hydrolysis increased as the temperature of the reaction raised from 40° to 50°C. Beyond that temperature the rate decreased. Therefore, maximum temperature was between 45° and 50°C. Also at this range of temperatures, glucose and maltose values were high in all four sources.

3. Six hours reaction time was found to be sufficient for the complete enzymatic hydrolysis of proteins in each formulation. There was a very small increase in soluble proteins and total free amino acids and sugar content when digestion time lasted for 8 and 10 hours.

4. Five percent buffer suspension was most suitable for denaturing the protein-containing vegetable material, which has been found to be a prerequisite for enzyme attack. Chick pea required a denaturation time of 20 minutes; however, sunflower seed press cake, whole wheat flour and the mixture required 15 minutes. There was a slight reduction in soluble protein content as well as amino acid values when protein-containing vegetable material denaturated for 25 minutes. However, there was no loss in sugar content when cooking was extended to 25 minutes.

5. For economical solubilization of protein-containing vegetable materials, high enzyme concentrations were used to achieve a rapid rate and high conversion efficiency. The results revealed that chick pea, whole wheat flour, sunflower seed press cake and the mixture required 2.0, 1.5, 2.5, and 2.5 percent enzyme, on a substrate basis, respectively.

There were no significant changes in soluble protein and free amino acid content as amounts of enzyme addition increased to the 3.0 percent level in each protein source. On the other hand, the amount of sugar increase appeared to be proportional to the amount of enzyme added.

6. The results indicated that the amount of protein solubilized is only slightly affected by increasing substrate concentration from 5 grams to 9 grams. Therefore, a 5 grams substrate concentration has been selected for each protein-containing vegetable material.

7. Maximum protein solubilization of chick pea and whole wheat flour and the mixture occurred between 0.2 and 0.3 M concentrations of sodium chloride, while the corresponding figure for sunflower seed press cake was 0.1 M sodium chloride. There were no significant changes in free amino acid and sugar content of each protein source.

8. Having established the conditions for maximum enzymatic hydrolysis of each protein source, soluble protein content of enzyme hydrolyzates from chick pea, whole wheat flour, sunflower seed press cake and the mixture were 187.7, 111.2, 412.0, and 257.8 mg/g, respectively. While corresponding figures were 134, 94.8, 387 and 235.6 micromoles of NPM per gram of protein source. The enzyme hydrolyzates also showed appreciable increases in glucose and maltose content even though Rhozyme-41, a food grade protease, had a low amylolytic power.

9. Protein scores of enzyme hydrolyzates showed that chick pea was deficient in both sulphur-containing amino acids and rich in lysine; whole wheat flour was lacking in lysine and tryptophan while rich in methionine; sunflower seed press cake was found to be deficient in only lysine.

Suitable combination of these products showed that protein scores of the mixture were significantly higher than those of chick pea, sunflower seed press cake or whole wheat flour alone.





LITERATURE CITED

LITERATURE CITED

1. Abdo, K. M., and K. M. King. 1967. Enzymatic modification of the extractibility of proteins from soybeans. J. Agr. Food Chem. 15, 83-85.
2. Alexander, J. C., and D. C. Hill. 1952. The effect of heat on lysine and methionine in sunflower seed oil meal. J. Nutr. 48, 149.
3. A.O.A.C. 1965. Official Methods of Analysis. 10th ed., pp. 195-197. Association of Official Chemist, Washington, D. C.
4. Arimoto, K. 1961. Nutritional research on fermented soybean products. In "Meeting Protein Needs of Infants and Children," Proceedings of an International Conference, National Research Council, Publication 843, pp. 269-273, Washington, D. C.
5. Autret, M., and A. G. Van Veen. 1955. Possible sources of proteins for child feeding in underdeveloped countries. Am. J. Clin. Nutr. 3, 234-266.
6. Aykroyd, W. R., and J. Doughty. 1964. Legumes in human nutrition. No. 19, pp. 54-66. Food and Agriculture Organization, Rome.
7. Ballester, D. 1968. Sunflower presscake: chemical composition, biological quality of the protein and test of toxicity in rats. Nutr. Abstr. and Rev. 38, 425.
8. Ballester, D., I. Barja, E. Yanez, and G. Donoso. 1968. Protein-rich mixtures for human consumption based on fish flour, sunflower dry skim milk and wheat flour. Brit. J. Nutr. 22, 225-228.
9. Barr, A. J., and J. H. Goodnight. 1971. Statistical Analysis System. North Carolina State University. Raleigh, North Carolina.
10. Baysal, A. 1968. Present situation in Turkey regarding malnutrition in infants and preschool children--types of problems, magnitude of problem, location. In "Combating Malnutrition in Preschool Children," pp. 45-59. Central Treaty Organization, Islamabad, Pakistan.
11. Beckhorn, E. J., M. D. Labbee, and L. A. Underkofler. 1965. Production and use of microbiological enzyme in food processing. J. Agr. Chem. 13, 30-34.

12. Bender, A. E. 1970. Factor affecting nutritive value of protein foods. In "Evaluation of Novel Protein Products," eds. Bender, A., B. Lofqvist, R. Kihlberg, and L. Munk, vol. 14, pp. 319-327. Proceedings International Biological Program and Wenner-Green Center Symposium. Pergamon Press, New York.
13. Bennett, J. C. 1967. Denaturation of polypeptides. In "Methods in Enzymology," eds. Colowick, S. P., and N. O. Kaplan, vol. 11, pp. 211-214. Academic Press Inc., New York.
14. Bergvist, R. 1963. The proteolytic enzymes of Aspergillus oryzae II. Properties of proteolytic enzymes. Acta. Chem. Scand. 17, 1541-1551.
15. Blackburn, S. 1968. "Amino Acid Determination Methods and Techniques," pp. 11-29. Marcel Dekker, Inc., New York.
16. Bowlby, C., H. Tacker, B. S. Miller, and J. A. Johnson. 1953. A comparison method for determining proteolytic activity. Cereal Chem. 30, 480-491.
17. Brenner, M., and A. Niederwiesser. 1967. Thin-layer chromatography (TLC) of amino acids. In "Methods in Enzymology," eds. Colowick, S. P., and N. O. Kaplan, vol. 11, pp. 39-59. Academic Press, New York.
18. Bressani, R. 1969. Formulation and testing of weaning and supplementary foods containing oilseed proteins. In "Protein-enriched Cereal Food for World Needs," ed. Milner, M., pp. 49-66. The American Association of Cereal Chemists, St. Paul, Minn.
19. Bressani, R., L. G. Elias, and N. S. Scrimshaw. 1962. All-vegetable mixtures for human feeding. VIII. Biological testing of INCAP Vegetable Mixture 9 in rats. J. Food Sci. 27, 203-209.
20. Bressani, R., and L. G. Elias. 1968. Processed vegetable mixtures for human consumption in developing countries. In "Advances in Food Research," eds. Chichester, C. O., E. M. Mrak, and G. F. Stewart, vol. 16, pp. 1-104. Academic Press, New York.
21. Bricker, M. L., and J. M. Smith. 1951. Biological value of sunflower seed meal. J. Nutr. 44, 553-573.
22. Buffa, A., and G. D. Kapsiotis. 1966. Possibilities of manufacturing protein-rich food mixtures for infants and children. Report of a Joint FAO/UNICEF, No. 1231, Rome.
23. Cheftel, C., M. Ahern, D. I. C. Wang, and S. R. Tannenbaum. 1971. Enzymatic solubilization of fish protein concentrate: batch studies applicable to continuous enzyme recycling process. J. Agr. Food Chem. 19, 155-161.

24. Clandinin, D. R. 1958. Sunflower seed oil meal. In "Processed-Plant Protein Foodstuffs," ed. A. M. Altschul, pp. 557-591. Academic Press, Inc., New York.
25. Connelly, J. J., V. G. Vely, W. H. Mink, G. F. Sachsel, and J. H. Litchfield. 1966. Studies on improved recovery of proteins from rendering plant raw materials and products. III. Pilot plant studies on an enzyme hydrolysis process. Food Technol. 20, 125-128.
26. Coon, J. M. 1969. Naturally occurring toxins in foods. Food Technol. 23, 1041-1045.
27. Criswell, L. G., J. H. Litchfield, V. G. Velly, and G. F. Sachsel. 1964. Studies on improved recovery of protein from rendering-plant raw materials and products. II. Acid and enzyme hydrolysis. Food Technol. 18, 247-248.
28. Dean, J. A. 1969. "Chemical Separation Methods," pp. 182-185. Van Nostrand Reinhold Co., New York.
29. Desai, B. L. M., V. A. Danial, S. Venkatrao, M. Swaminathan, and H. A. B. Parpia. 1970. Supplementary value of low cost balanced foods based on cereals, cottonseed, peanut flour and chick pea flour to poor Indian diets. J. Nutr. Dietet. 7, 21-26.
30. Dixon, M., and E. C. Webb. 1964. "Enzymes," pp. 19-26. Academic Press, Inc., New York.
31. Fermco Laboratories. 1966. Fermco-Test S. F. G., Tech. Bul. 2. Fermco Laboratories, Inc., Chicago.
32. Fomon, S. J. 1967. "Infant nutrition," pp. 57-79. W. B. Saunders Co., Philadelphia.
33. FAO. 1965. Protein requirements, Report of a Joint FAO/WHO Expert Group. FAO Nutrition Meeting Report Series 37; World Health Organization Technical Report Series 301, Rome.
34. FAO. 1970. Amino acid content of foods and biological data on proteins, FAO Nutritional Series 24, Rome.
35. Guttikar, M. N., M. N. Rao, and R. Rajagopalan. 1965. Studies on processed protein foods based on ground nut, Bengal gram, soybean and sesame flours and fortified with minerals and vitamins. J. Nutr. Dietet. 2, 21-23.
36. Gyorgy, P. 1961. Nutritive value of Tempeh. In "Progress in Meeting Protein Needs of Infants and Children," Proceedings of an International Conference, National Research Council Publication 843, pp. 281-289, Washington, D. C.

37. Hanafy, M. M., Y. Seddik, and M. K. Aref. 1970. Formulation of a protein-rich vegetable mixture for prevention of protein-calorie malnutrition. J. Sci. Fd. Agric. 21, 9-12.
38. Hang, Y. D., W. F. Wilkens, A. S. Hill, K. H. Steinkraus, and L. R. Hackler. 1970. Enzymatic solubilization of nitrogenous constituents of mung beans. J. Agr. Food Chem. 18, 2-12.
39. Hirs, C. H. W. 1967. Performic acid oxidation. In "Methods in Enzymology," eds. Colwick, S. P., and N. O. Kaplan, vol. 11, pp. 197-199. Academic Press, New York.
40. Howe, E. E., E. W. Gilfillan, and M. Milner. 1965. Amino acid supplements of protein cereal grains as related to the world food supply. Am. J. Clin. Nutr. 16, 1315.
41. Jansen, G. R., and E. E. Howe. 1964. World problems in protein malnutrition. Am. J. Clin. Nutr. 15, 262-265.
42. Kapsiotis, G. D. 1969. History and status of specific protein foods. FAO/WHO/UNICEF Food Program and Products. In "Protein Enriched Cereal Foods for World Needs," ed. M. Milner, pp. 255-265. The American Association of Cereal Chemists, St. Paul, Minn.
43. King, K. W. 1963. Modification of plant utilization by the poorly metabolized components of plants. Document, WHO/FAO/UNICEF Protein Advisory Group Meeting, Geneva.
44. Learmonth, E. E., and J. C. Wood. 1963. A trypsin inhibitor in wheat flour. Cereal Chem. 40, 61-65.
45. Liener, I. E. 1958. Effect of heat on plant proteins. In "Processed Plant Protein Foodstuffs," ed. Altschul, A. M., pp. 107-122. Academic Press, New York.
46. Maier, R., and H. K. Mangold. 1964. Thin-layer chromatography. In "Advances in Analytic Chemistry and Instrumentation," ed. Reilley, C. N., vol. 3, pp. 369-468. Interscience Publishers, New York.
47. Milic, B., S. Stojanovic, N. Vucurevic, and T. Turcic. 1968. Chlorogenic and quinic acids in sunflower meal. J. Sci. Food Agr. 19, 108.
48. Miller, W. C. 1964. Degradation of synthetic polypeptides by proteolytic enzymes in 0.2M sodium chloride. J. Am. Chem. Soc. 86, 3913-3918.
49. Milner, C. K., and K. J. Carpenter. 1969. Effect of wet heat processing on nutritive value of whole wheat protein. Cereal Chem. 46, 425-435.

50. Mitchell, H. H., and R. J. Block. 1946. A method for determination of nutritive value of proteins. J. Biol. Chem. 163, 599-603.
51. Moore, S., and W. H. Stein. 1948. Photometric ninhydrin method for use in the chromatography of amino acids. J. Biol. Chem. 176, 367.
52. Moore, S., and W. H. Stein. 1963. Hydrolysis of Protein substrates. In "Methods in Enzymology," eds. Colowick, S. P., and N. O. Kaplan, vol. 6, pp. 819-822. Academic Press, New York.
53. Moreau, J. R., and E. E. Jankus. 1963. An assay for measuring papain in meat tissue. Food Technol. 17, 1047-1049.
54. Muto, S., E. Takahashi, M. Hara, and Y. Konuma. 1963. Soybean products as proteins sources for weaning infants. J. Am. Dietet. Assoc. 43, 451-456.
55. NRS Council. 1963. Evaluation of Protein Quality, Publication 1100. National Academy of Sciences, National Research Council, Washington, D. C.
56. Neyzi, O., F. Tanman, B. Oztekin, C. Ozalp, and C. T. Gürson. 1966. Physical measurements of children of different social background. Proceedings VII International Congress of Nutrition, vol. 4, p. 224, Hamburg.
57. Omans, W. B., N. Leuterer, and P. Gorgy. 1963. Feeding value of soymilk for premature infants. J. Pediat. 62, 98-106.
58. Osborne, T. B., and L. B. Mendel. 1917. The use of soybean as food. J. Biol. Chem. 32, 369-387.
59. Parpia, H. A. B. 1966. Development of food mixes for preschool children in India. In "Preschool Malnutrition, Primary Deterrent to Human Progress," An International Conference, National Research Council, Publication 1283, pp. 181-212, Washington, D. C.
60. Parpia, H. A. B. 1969. Protein foods of India based on cereals, legumes, and oilseed meal. In "Protein-rich Cereal Foods for World Needs," ed. Milner, M., pp. 129-139. The American Association of Cereal Chemists, St. Paul, Minn.
61. Parpia, H. A. B., M. N. Roa, R. Rajagopalan, and M. Swaminathan. 1964. Recent developments in the production and use of foods in treatment and prevention of protein malnutrition in children. J. Nutr. Dietet., 1, 114-130.
62. Patwardhan, V. N. 1962. Pulses and beans in human nutrition. Am. J. Clin. Nutr. 11, 12-30.

63. Randerath, K. 1964. "Thin-layer Chromatography," pp. 37-70. Academic Press, New York.
64. Rao, K. S. N., K. R. Sreekantial, and T. N. N. Rao. 1970. Studies of fungal proteolytic enzymes. Part 1. Selection and evaluation of strains to hydrolyze proteins of animal and plant origin. J. Food Sci. and Technol. 7, 26-29.
65. Reed, G., and L. A. Underkofler. 1966. "Enzymes in Food Processing," pp. 150-153. Academic Press, New York.
66. Rupley, J. A. 1967. Susceptibility to attack by proteolytic enzymes. In "Methods in Enzymology," eds. Colowick, S. P., and N. O. Kaplan, vol. 11, pp. 905-917. Academic Press, New York.
67. Sakurai, Y., and M. Nokano. 1961. Production of high protein food from fermented soybean products. In "Progress in Meeting Protein Needs of Infants and Preschool Children." Proceedings of an International Conference, National Research Council, Publication No. 843, 251-255, Washington, D. C.
68. Schleich, H., and R. S. Arnold. 1962. Composition and methods for processing meat products. U. S. Patent 3,037,870.
69. Scrimshaw, N. S. 1959. Progress in solving world nutrition problems. J. Am. Diet. Assoc. 35, 441-445.
70. Sheffner, A. L. 1967. In vitro protein evaluation. In "Newer Methods of Nutritional Biochemistry," ed. Albanese, A. A., vol. 3, pp. 125-192. Academic Press, New York.
71. Smith, A. K. 1963. Foreign uses of soybean protein foods. Cereal Sci. Today 8, 196-199.
72. Smith, A. K., J. J. Rackis, C. W. Hesseltine, M. Smith, D. J. Robbins, and A. N. Booth. 1964. Tempeh nutritive value in relation to processing. Cereal Chem. 41, 173-181.
83. Smyth, D. G. 1967. Techniques in enzymatic hydrolysis. In "Methods in Enzymology," eds. Colowick, S. P., and N. O. Kaplan, vol. 11, pp. 214-231. Academic Press, New York.
84. Sreekantiah, K. R., H. Ebine, T. Ohta, and M. Nakano. 1969. Enzymatic processing of vegetable protein foods. Food Technol. 23, 1055-1061.
75. Steel, R. G., and J. H. Torrie. 1960. "Principles and Procedures of Statistics," pp. 107-109. McGraw-Hill Book Company, New York.

76. Stilling, B. R., and L. R. Hackler. 1965. Amino acid studies on the effect of fermentation time and heat processing of Tempeh. J. Food Sci. 30, 1043-1048.
77. Subrahmanyam, V., K. Joseph, T. R. Doraiswamy, M. N. Rao, A. N. Sankaran, and M. Swaminathan. 1957. The effect of supplementary multipurpose food on growth and nutritional status of school children. Brit. J. Nutr. 11, 382-388.
78. Swaminathan, M., V. A. Daniel, and H. A. B. Parpia. 1971. Protein-enriched cereal foods for overcoming malnutrition among preschool children in developing countries. Ind. J. Nutr. Dietet. 9, 22.
79. Tallon, H. M., S. Moore, and W. H. Stein. 1954. Studies in the free amino acids and related compounds in the tissues of the cat. J. Biol. Chem. 211, 927-931.
80. Tannous, R. I., J. W. Cowan, F. Rinnu, R. Y. Asfour, and Z. I. Sabry. 1965. Protein-rich food mixtures for feeding infants and preschool children in the Middle East. I. Development and evaluation of Laubina mixtures. Am. J. Clin. Nutr. 17, 143-148.
81. Teply, L. J., and P. Gyorgy. 1962. Vegetable proteins in infant feedings. J. Pediat. 61, 925-933.
82. Van Dam, B. E. 1964. School feeding and nutrition education. Food and Agriculture Organization. No. 1801. Rome.
83. Wray, J. D. 1961. Kwashiori and maramus in Turkey. In "Meeting Protein Needs of Infants and Children," Proceedings of an International Conference, pp. 189-197. National Research Council, Publication 843, Washington, D. C.
84. Yasuddin, S. G., C. M. Caten, and K. F. Mallil. 1970. Effect of several variables on the extractibility of sunflower seed proteins. J. Food Sci. 35, 454-461.

VITA

Sevim Caglar Burklin was born on February 4, 1940, in Antalya, Turkey. She attended high school at Bursa, Turkey. In June, 1964, she graduated from the University of Ankara with a degree in veterinary medicine. For two years she worked for the Turkish Ministry of Defense. In 1966, she came to the United States under a scholarship provided by the Turkish government. She attended the American Language Institute at New York University. In 1968, she enrolled in the Department of Food Technology of The University of Tennessee. Since that time, she has been working to complete the requirements for the degree of Master of Science.

She is married to Richard Burklin, a graduate physics student attending The University of Tennessee.