BIOCHEMICAL STUDIES OF PEANUT (*ARACHIS HYPOGAEA* L.) QUALITY

By

Clyde Thomas Young

Bachelor of Science
North Carolina State University
Raleigh, North Carolina
1952

Master of Science
North Carolina State University
Raleigh, North Carolina
1955

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1970

BIOCHEMICAL STUDIES OF PEANUT (~~ARACHIS~~ HYPOGAEA L.) QUALITY

Thesis Approved:

George R. Waller
Thesis Adviser

Leroy V. Odell

Roger E. Koeppe

Ralph S. Matlock

Robert L. Henrickson

A. Dunham
Dean of the Graduate College

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. George Waller (his major adviser), Dr. Ralph S. Matlock and Dr. Michael E. son for their encouragement and counseling during the course of these udies. The suggestions of Dr. George V. Odell, Dr. R. L. Henrickson d Dr. Roger E. Koeppe in the preparation of the manuscript are also knowledged.

The technical assistance of Dr. James Kirby, Yai-Po Tai, Dr. R. O. mmons, Virginia Choate, Eunice Park, Dr. Earl Worthington, Dr. Bob rrison and Delyorce Rebouche is recognized. The author is grateful L. Fowden for the samples of γ-methylene glutamine and γ-methylene utamic acid.

The author is indebted to Departments of Biochemistry and Agron- y, Oklahoma and Georgia Experiment Stations, Corn Products Corpora- on, Best Foods Division, Oklahoma Peanut Commission and Anderson ayton and Company for facilities and/or financial aid during these vestigations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

16:0	palmitic acid
18:0	stearic acid
18:1	oleic acid
18:2	linoleic acid
20:0	arachidic acid (eicosanic)
18:3	linolenic acid
20:1	eicosenoic acid
22:0	behenic acid
24:0	lignoceric acid

gas liquid chromatography	
mass spectrometer	
mature	
high intermediate	
low intermediate	
immature	
irrigated	
nonirrigated	

Asp	aspartic acid
Thr	threonine
Ser	serine
Asn	asparagine
Pro	proline
Glu	glutamic acid
Gly	glycine
Ala	alanine
Val	valine
Cys	cystine
Met	methionine
Ile	isoleucine
Leu	leucine
Phe	phenylalanine
Tyr	tryosine
Pep	peptide
NH_3	ammonia
Lys	lysine
His	histidine
Arg	arginine
Try	tryptophan
Unk	unknown
MG	γ-methylene glutamine
MGA	γ-methylene glutamic acid

CHAPTER I

INTRODUCTION

Dean Bond, Extension peanut Specialist, remarked that "the peanut
stry will experience many changes during the decade of the '70s.
of their biggest advancements will be improved varieties." The
of this study was to develop methods and improve the knowledge of
it chemistry which would aid in the rapid development of improved
eties and to help understand the conditions that affect the "quality
ors" of peanuts. In fact, chiefly because of this study, this
or proposes that the majority of the varieties of the near future
be developed by the Biochemist and then tested by the Agronomist
e being released for commercial production.

Peanuts (Arachis hypogaea L.) fruit are an important segment of
klahoma farm economy valued at 26 million dollars in 1969. A basic
standing of the biochemical and physiological changes that occur
g their growth is essential to improve our knowledge about this
tant food product. Thus, in this study, the relation of biochemi-
agronomic and organoleptic factors to peanut flavor and quality
been emphasized.

To accomplish this, a better knowledge and measure of the contri-
n and effect of variety, maturity, irrigation and planting location
emical constituents such as flavor precursors, fatty acids, and

ino acids were necessary for the improvement of flavor and other
ality factors of roasted peanuts and peanut products.

It was necessary to develop new and to modify existing chemical
ocedures to accomplish the goals of this study. Newer methods such
ion exchange chromatography, gas liquid chromatography and mass
ectrometry were used to give a better understanding of long recognized
oblems, such as changes associated with maturity, flavor, and problems
recent origin associated with harvesting, curing and storing.
thods, and the development of new varieties for the space age.

This dissertation is divided into three broad classifications:

A. Fatty Acids

Major emphasis was on the development of a rapid micro analytical
chnique so that a portion of a raw peanut kernel can be analyzed for
e oleic acid/linoleic acid ratio permitting the remainder of the
rnel to be planted for genetic evaluation. Such a method is now being
ed by the plant breeder as an aid in the scientific selection of
netic material to speed development of new peanut varieties that are
manded by manufacturers of peanut products.

The peanut industry would like to have a Spanish peanut with a
rger oleic/linoleic fatty acid ratio. This would permit a longer
elf-life in peanut butter and oil and would make it feasible to pro-
ce a peanut butter from Spanish peanuts alone instead of mixing in a
rcentage of runner peanuts.

The method employs gas liquid chromatography as the analytical tool
choice. The oil is subjected to trans-esterification and the fatty
ids analyzed as their methyl esters. Parameters investigated include
precision and accuracy study, time required for analysis, significance

contaminants in the oleic and linoleic acid GLC peaks, location of correct portion of the peanut kernel to be sampled to get a represen- ive and reproducible sample and elimination of possible sources of or or interference that may be introduced by having pieces of the nut kernel present in the reaction mixture. The ultimate objective this method was to provide a technique for the rapid and accurate lysis of 50 to 100 samples per day. Peanuts from widely divergent rces were used.

Using this fast GLC technique for analyzing a portion of the seed osite the germ for oleic/linoleic (O/L) ratios, the first F_1 gener- on seed of peanuts were analyzed and will be planted for further luation. Also O/L ratios were determined on approximately 2200 gle peanut seeds in F_2 generation and advanced generations from nish x Runner crosses. Approximately 300 of these were selected by plant breeder on the basis of wider O/L ratios with Spanish type racteristics and were planted in 1969 for further evaluation.

Variations in the total fatty acid composition as influenced by urity, irrigation and planting location were also evaluated on ected varieties. Statistical studies were included.

B. Amino Acids

There is a growing demand for a balanced dietary source of protein free amino acids to supply the needs of the world population. atively little information exists regarding the amino acid composi- n of the many varieties of peanuts. The total amino acids of sev- l varieties were examined to determine differences that exist in the ential amino acids.

Also, free amino acids have been found to be related to flavor of

ısted peanuts. The possible correlation of free amino acid concentra-
ɔn and the concentration of the acidic peptide of raw peanuts with
ɛ roasted flavor as affected by variety, maturity, irrigation and
ınting location is studied.

The Sakaguchi reaction for arginine was evaluated as a method to
ɛermine the degree of maturity in peanuts and the results compared
ɛh those obtained by ion exchange chromatography.

Several of the unknown amino acids found in the raw peanut extract
ve been identified.

C. Isolation and Partial Characterization of an Acidic Peptide

Of particular interest was the characterization of an acidic peptide
the free amino acid extract from Arachis hypogaea fruit. Preliminary
ılyses by Mason et al (1, 2) indicated that the peptide contained
.atively high amounts of aspartic acid, glycine and phenylalanine and
ıller amounts of seven other amino acids. No basic amino acids were
ɛected. The significance of this peptide is not known but its concen-
ıtion appears to increase with maturity of the fruit.

Some progress has been made on purification of the peptide, but
ɛh remains to be done before it is pure enough for complete character-
ıtion.

CHAPTER II

REVIEW OF LITERATURE

The origin of the peanut is still as much a mystery as the "char-
:eristic" peanut component or components and their precursors. Hig-
ıs (3) writing in the book The Peanut, The Unpredictable Legume quoted
:ranslation from the work of August Chevalier (4) which was written
1933 which said "The problem of the origin of the peanut has made
›ods of ink flow". But with the finding of peanuts in ancient graves
:avated at Ancon, Pachacamac (Peru) which are similar in appearance
those peanuts now grown in Peru, South America has been accepted as
: origin of the peanut.

Apparently the South American Indians made a peanut paste which is
ıilar to our peanut butter. Early in the 16th century, both the
:tuguese and Spaniards probably carried peanuts to the East Indies.
:ords of early Spanish explorers show that the peanut was grown in
:ico and Central America and various islands of the West Indies before
: arrival of Europeans. Later the peanut, grown in Africa, was used
food to feed the Negroes on slave ships. Records (5) indicated
ıt the Spanish variety of peanuts was introduced to this country
›m Spain in 1871. In these earlier days the peanuts were roasted and
.d in the shell by street vendors. The first peanut butter was made
about 1890 by a St. Louis, Missouri physician (6) for use by his
:ients. George Washington Carver, in 1921, appeared before the Ways

Means Committee of the United States House of Representatives and sented information on the more than 300 products that he had developec m peanuts (7). Some of these products were milk, cream, buttermilk, ese, coffee, plastics, paper and flour. Even today, "peanut butter erves ample credit for maintaining the health of young Americans ing their years of finicky eating habits" (49). In 1963, Brakman al (8) reported that an extract of peanut flour could be an aid to ophilioid disorders. The extract of raw peanuts was twenty times ter than the extract of roasted peanuts.

Hoffpauir (9) in 1953 published an excellent review of the chemi- composition of the peanut but the values were obtained prior to the mon use of modern chromatographic technique.

The first use of gas liquid chromatography (GLC) in the separation fatty acids was reported by James and Martin (10, 11) in 1952. ce then many improvements in column materials and equipment have e development of the methods reported by Mason (12), Mason and ler (13) and Jellium and Worthington (14) possible. The development the GLC-Mass spectrometer combination instrument as used by Waller) has led to further improvements with identification of minor com- ents now more easily identified.

After the published work of Spackman, Stein and Moore (16) in 1958 which they were separating the amino acids using ion-exchange chroma- raphy, the automation of the procedure has made amino acid determin- ons some what routine.

These analytical tools have made it possible to examine the com- ents thought to be related to peanut quality in more detail than viously. Many of the more important articles dealing with this

sertation have been discussed in their related chapters.

Matlock (17) in 1968 (about one year after the initiation of this earch program) discussed research on peanut quality which sets the ge for the research that is discussed in this dissertation. "Qual-", a poorly understood and widely used term, means different things different individuals. Matlock (17) using the published information m the papers of Sexton, et al. (18) and Sexton (19) listed "19 qual- factors for which objective standardized methods of measurement e been or should be developed," and this table is reproduced: ble I). Quality factors 18 and 19 are related to this research. e again Matlock (17) is quoted. "In order to evaluate the desirable racteristics involved in flavor, odor, appearance and texture, sen- y tests are used. These tests are subjective in nature and diffi- t to use."

He goes on to report on the evidence concerning the hereditary environmental influences on fatty acids and flavor. Matlock (17) ther stated that "evidently, there are many environmental factors t contribute to flavor".

Later in 1968, Thomas (20) published a paper on the effect of igation and maturity on the quality of peanuts and peanut products. report contained only organoleptic results. He reported that igation improved the quality of peanuts and peanut products and re were a higher percent of mature kernels present in the peanut p. Peanut products from mature kernels were superior to those from ature and underdeveloped kernels. It was also observed that the ^{0} F dried peanuts were inferior to both bag cured and field cured nuts.

Figure 1 shows a decrease of arginine and an increase in peptide phenylalanine with increasing maturity as published by Newell (21), on, et al. (1, 2) and Young, Mason and Matlock (22).

Pickett and Holley (23) reported on the changes in free amino ds of peanuts during roasting and found no difference in the number identifiable chromatographic spots. However, the size of all spots reased as the nuts were subjected to progressively heavier roasts. 1962, McOsker (24) published a paper showing the loss by destruction certain essential amino acids (lysine 15 percent, threonine 11 per- t and methionine 10 percent) in roasted peanuts.

An excellent review by Cobb (25) covering the physical and chemi- properties of peanuts will soon be published and will update the mical references found in the book on peanuts by Woodroof (26).

TABLE I

PEANUT QUALITY FACTORS FOR WHICH OBJECTIVE, STANDARDIZED METHODS OF MEASUREMENT SHOULD BE DERIVED (17)

Quality Factor	Type[a]	Available Methods Indicated
Maturity	S	Spectrophotometric evaluation of expressed oil, sugar content, unsaturation of oil.
Resistance to mold	IS or S	None
Color	IS or S	Use of color "chips" similar to those used by the USDA for peanut butter.
Shape	S	Use of slotted screens with relatively small samples.
Density	Raw or Roasted	Beckman air pynometer, count per pound, sand displacement, fluctuation.
Concealed damage	S Raw or Roasted	Federal-State Grading Procedure
Milling quality	IS	Lab sheller
Blanchability	S	Lab blancher, hand blanching
Kernel hardness	S	Penetrometer
Texture of kernel	S	None
Tendency for radicle breakage	S	None
Pod thickness	IS	Micrometer or microscope measurement.
Pod fragility	IS	Impact tester
Mold Count	S	Direct count
Aflatoxin content	S	Chromatographic method
Infestation	IS	Direct Count

TABLE I (continued)

Quality Factor	Type[a]	Available Methods Indicated
Skin Slippage Tendency	S Raw or Roasted	None
Flavor	S Raw or Roasted	Flavor panel evaluation of ground or roasted peanuts.
Chemical constituents	S Raw or Roasted	Moisture - Oven, moisture meter, distillation. Oil - Total, iodine value, fatty acid content, fatty acid composition, rancidity potential, Tocopherol content, Protein - Total. Vitamin.

[a] S = Shelled peanuts; IS = Peanut in the shell.

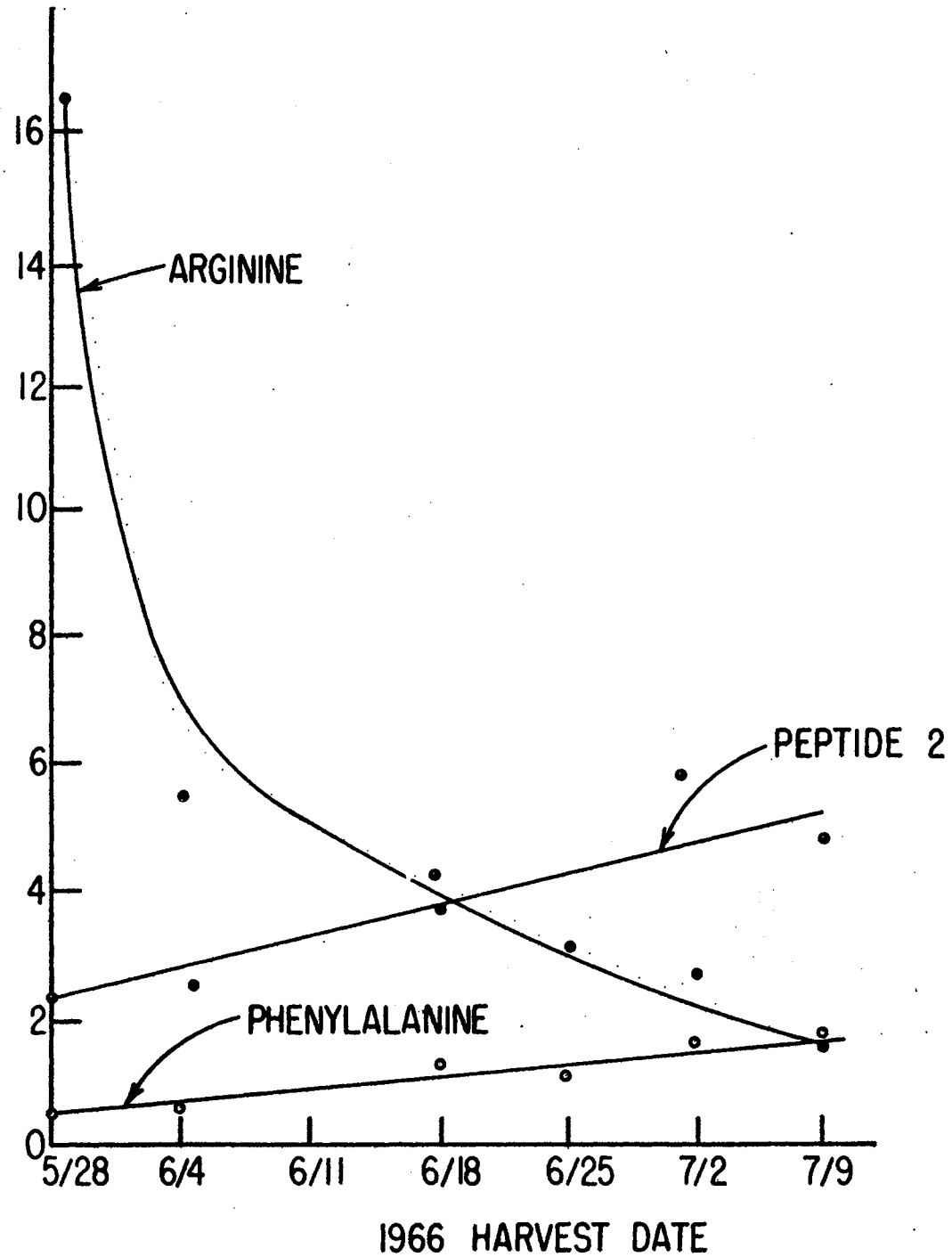


Figure 1. Change in Arginine, Peptide 2, and Phenylalanine Content of Spanish Peanut Fruit as a Function of Maturation (2).

CHAPTER III

A RAPID OLEIC/LINOLEIC MICRO ANALYTICAL PROCEDURE

troduction

The peanut industry desires a Spanish type peanut with a high eic/linoleic (O/L) fatty acid ratio i. e. less than 25 percent lino- ic acid which would give a ratio of two or three. This would allow e production of a longer shelf-life peanut butter, peanut oil and her roasted peanut products. Also, it would be feasible to produce anut butter from Spanish peanuts alone instead of mixing in a percen- ge of runner peanuts so that the correct O/L ratio might be obtained. rmally the breeder will make his crosses and grow several generations seed to obtain proper segregation of seed for chemical analysis. is requires considerable time and expense. By the use of the rapid cro analytical method of oleic and linoleic acids, he will be able to ke his selections sooner. This should help him arrive at his goal fo taining a peanut variety with a lower linoleic acid content in a shorter ne period and perhaps at a lower cost than present selection methods.

In 1958, Rosen (27) proposed that one might be able to produce anut oil with increased unsaturation. Already large changes in tty acid composition of flax (28) have been induced by selective eeding practices. Preliminary work by Mason et al. (29) indicated e genetic variation was present in peanuts that was necessary to

hieve the present goal of decreased unsaturation. Because of possible alth problems, a proper balance of saturation and unsaturation in the tty acids must be considered.

In earlier work by Mason (12), small composite samples of ten to enty peanuts were pressed and analyzed for the O/L ratios. Pressing the oils was the chief limitation of the method. Jellium and Worthgton (14) had developed a similar rapid method of fatty acid analysis oil from individual corn kernels. Seed viability was destroyed in eir method. Only one or two drops of oil were necessary for the alysis and one peanut contains more than enough oil for this analysis. a portion of the kernel could be analyzed and the remaining portion uld be planted it would speed the development of the desired peanut riety. Therefore this procedure was developed for the analysis of a rtion of the individual peanut kernel.

Apparatus and Reagents

paratus

GLC analyses were performed on either a Perkin-Elmer model 800 uipped with a flame ionization detector or a modified Barber-Colman del 5000 gas chromatograph equipped with a flame ionization detector. uminum columns, ¼ inch by six feet (or four feet), packed with 14.5 rcent DEGS or stabilized DEGS on Anakrom 100/110 mesh were used. lium was used as the carrier gas.

agents

DEGS, diethylene glycol succinate (Applied Science Laboratories).

Stabilized DEGS (Analabs, Inc.).

Anakrom, 100 to 110 mesh (Analabs, Inc.).

Benzene, Fisher reagent grade, dried over sodium.

2,2-Dimethoxypropane (Dow Chemical Company). redistilled from 76° to 79° c.

Anhydrous methanolic hydrogen chloride: Methanol, Fisher reagent ade, is dried over Linde molecular sieve #3A (1/16 inch pellets). r HCl was prepared by bubbling the gas (Matheson) through sulfuric id. The dried HCl is then bubbled into the dried methanol which was oled with an ice-water bath. It was standardized with 1/14 N NaOH yield a 2.8 N solution which is then sealed and stored at 4° C.

Procedures

lection of Sound Mature Kernels

Size alone is not sufficient for selection of sound mature kernels MK). Successful and highly reproducible chemical determinations of anuts require a rigid and carefully controlled selection and classi-cation of the kernel (21, 30, 31, 32, 33). A brief outline of the assification (Table II) used in this study follows. Peanuts having rk colored interior pericarp surfaces and very thin faded pink colored sta were classed as mature (M). Those having some white on the in-rior pericarp and with pink were classed as high intermediate (HI). en there was some slight wrinkling of the skin, the testa had not mpletely collapsed and the interior of the pericarp remained white, e kernels were referred to as low intermediate (LI). The remainder the undersized, shriveled, white pericarp and thick testa were ouped in the immature class (I). In most varieties the sound mature rnel was smooth with little or no wrinkling of the testa surface.

nple Preparation

The peanut kernel was held by the germ end. While being careful : to touch the germ, use a sharp scalpel to slice about 1/3rd of the anut from the end opposite the germ. It was important to use at ast ¼ of the seed because of the variability of the oil within the anut (34). With the large-seeded varieties, it was usually best to ove and discard ¼ of the nut before slicing a portion for analysis.

paration of Methyl Esters for O/L Analysis

The chopped portion of the peanut was placed in a 16 by 150 mm test e and the following reagents were added in order: 4 ml sodium dried zene, 0.1 ml of 2,2-dimethoxypropane and 0.5 ml of 0° C methanolic .. This mixture was shaken and the test tubes were covered and left rnight at room temperature (22-25° C) to form the methyl esters. s reaction mixture was analyzed by directly injecting 2-3 µl or by anting, evaporating to near dryness on a hot water bath (80-85° C) l injecting 50 nanoliters of the concentrated mixture on the GLC umn. The latter method gave the best results and also a longer umn life.

Analytical Condition

Operating parameters varied slightly from day to day, thus the ndard oil sample was used to adjust the equipment so as to obtain urate analysis. The injection port should be about 250° C with an n temperature of 235-240° C and a helium flow rate of approximately ml per minute. The temperature of the hydrogen flame ionization ector on the Perkin-Elmer model 800 was the same as the oven temper-

ıre. On instruments with small lines such as the modified Barber-
.man model 5000, it was necessary to operate the detector tempera-
:e at 350° C to avoid clogging. On the Perkin-Elmer model 800, a four
one stream splitter was used since the flame was not suppose to
:eive more than 50 ml per minute of carrier gas. A typical analysis
the Perkin-Elmer model 800 gas chromatograph with a stream splitter
ıuired slightly less than two minutes. When using the Barber-Colman
lel 5000 gas chromatograph, a typical analysis required about three
l one-half minutes.

Results and Discussion

The purpose of this phase of research was to improve upon the-
:hod of Mason and Waller (13) so that an even larger number of oil
ıples could be analyzed so as to aid the peanut breeder in a genetic
ıdy of inherited characteristics to the O/L ratio. This should speed
: development of a Spanish type peanut with a lower linoleic fatty
.d content as sought by the peanut industry. A considerable portion
time was required for checking and rechecking the methyl ester reac-
›n to make sure that the rigid conditions were adhered to as reported
Mason and Waller (13).

The major simplification was the elimination of the time consuming
:p of hydraulically expelling the oil. Also test tubes were used
:tead of expensive and more bulky flasks. Less reagents were used
l lastly it was found not to be necessary to neutralize the methyl
:er preparation before injection on the gas chromatograph. Covering
: samples with a towel gave the same results as stoppered tubes.
.id state injection of samples was attempted but found to be very time

suming because of the time required to load and resume operation. ause there was no solvent present in this latter method, very good aration of oleic and linoleic acids was obtained.

Portions of the peanut were analyzed starting from the tip opposite germ end and on the small seeded type it was necessary to sample rd to ½ of the seed to obtain accurate and reproducible results. tha (34) fractionated peanut cotyledons and then combined similar tions to obtain enough sample to measure the percent oil and iodine ue of the different locations within the cotyledon. The iodine ue is a measure of unsaturation and was reported by Holly and Hammons) to be highly correlated with linoleic acid values. According to Kartha), the internal distribution varies with the tip opposite the germ ng the most unsaturated portion of the peanut kernel. Preliminary dies taking only ¼ of the peanut kernel showed this to be true. The hest percentage of unsaturated fatty acids were found in the more ature peanuts (36, 37, 38) with the saturated fatty acids being the hest in a mature kernel. Since this tip is the furtherest from the nt where nutrients enter the seed, one would also expect the tip to the most unsaturated portion of the kernel. In the same article, tha reported that the interior face in about the middle of the ker- had the lower iodine value. Thus to take a representative sample, must sample sufficiently near the center of the kernel. With the ge-seeded varieties, it was best to remove and discard a portion of seed and then take a cut for the O/L ratio analysis.

A new technique by Yermanos (39) involved the immersion of oil- ds in liquid solvents allowing the extraction of enough oil for lytical purposes without destroying seed viability. This was

empted with peanuts but it was difficult to obtain enough fat in the hours using the technique recommended by Yermanos. Also the ratio somewhat lower due to the extraction of the more unsaturated fatty ds present in the testa (40). At the present time, this method is recommended for use with peanut kernels.

After the reaction was completed, the samples were stable for roximately 30 hours with some changes being observed by 48 hours. s no samples were kept for analysis more than 24 hours when prepared er the above conditions.

To minimize GLC errors, the largest peak should be at least 40 per- ıt full scale deflection. Below this value the peak height error reased at a very fast rate due to changes in base line.

With these fast flow rates and high temperatures, it was felt that heck on the purity of the oleic and linoleic methyl ester peaks was essary. The technique of Sweeley, et al. (41) for the analysis of esolved compounds in gas chromatographic effluents was utilized. s technique using a prototype of the LKB 9000 combination GC-MS (15) loyed an accelerating voltage alternator such that a continuous ording of two values of m/e, separated by not more than one percent the mass range, can be obtained. This technique was used to indi- e the presence of trace amounts of methyl stearate (about one per- ıt) under the methyl oleate peak and methyl linolenate (also about percent) under the methyl linoleate peak and the results on several ınut oils are shown in Table III. These values were within the erimental error that the rapid micro analytical technique gave, so re was no need to apply a correction factor to the O/L ratio.

To test for precision, 100 samples of oils from a wide range of

etic material containing a range of O/L ratios from one to six were
d. These oils had been analyzed earlier for their fatty acid content.
ios were calculated and compared with the values obtained by this
id micro analytical method. Results were plotted (Figure 3) and
ve a 1.8 O/L ratio, it was found necessary to apply a correction fac-
to derive an approximate ratio for the rapid micro analytical tech-
ue. Correction factors can be read directly from this figure as
icated by the dotted line.

Figure 2 is a tracing of a GLC chromatogram showing the separation
t was obtained with this technique. Excellent separation was
ained. Base lines were drawn and peak heights were measured for
nitic, oleic and linoleic acids and the O/L ratio calculated. If
essary, correction factors can be read from Figure 3. The above
ee fatty acids account for approximately 90 percent of the total
ty acids. Oleic and linoleic in most varieties and strains comprised
percent of the total fatty acids.

The standards used in the rapid micro analytical technique to
ermine the O/L ratio on approximately 2,250 samples for the plant
eders in 1969 gave excellent reproducibility (1.125 ± 0.043) of the
ratios. Mason (29) had found that an O/L ratio difference of 0.04
significant at the 95 percent level of probability using his method.

Data (42) recorded in Table IV illustrates the type of data now
ng derived by the peanut breeder using the rapid micro analytical
hnique. Notice that certain crosses give a narrow range of O/L
ios, i.e. P-939 X P-2. Another cross, P-964 X P-2, shows a much
er range of O/L ratios.

Summary

A biochemical rapid micro analytical technique is described whereby rtion of a peanut kernel was analyzed for the oleic/linoleic acid hyl ester) ratio with the remainder being planted for genetic evalun. A detailed description of the preparation of the methyl esters eported. Analysis of the methyl esters by gas liquid chromatography ired two to four minutes per sample depending upon the equipment. analytical techniques were evaluated for precision using 100 peanut eties. This methodology is now in use to aid the breeder in the ction of his genetic material and approximately 2,200 selected pea- have been analyzed. Factors which influence O/L ratios are report- The described procedures should speed the development of new pea- varieties and other oilseed crops that are demanded by the manu- urers of peanut products.

TABLE II

DESCRIPTION OF CLASSIFICATION OF
PEANUTS INTO MATURITY CLASSES

Mature (M) peanuts have: (1) a dark colored interior pericarp surface (2) a very thin faded pink colored testa (skin)
High Intermediate (HI) peanuts have: (1) some white on interior pericarp (2) a thin pink colored testa
Low Intermediate (LI) peanuts have: (1) considerable white on the interior pericarp (2) a testa that isn't completely collapsed (3) slight wrinkling of the skin
Immature (I) peanuts have: (1) a white pericarp (2) a thick fleshly white-pink testa (3) undersized, shriveled kernels

TABLE III

THE USE OF THE MASS SPECTROMETER GAS CHROMATOGRAPH ALTERNATING VOLTAGE ACCELERATOR (AVA) TO ESTIMATE THE CONTAMINATING FATTY ACIDS IN THE OLEIC AND LINOLEIC ACID GLC PEAKS WHEN MEASURED BY THE RAPID O/L PROCEDURE

nut	AVA			AVA		
ple ber	methyl oleate	methyl stearate	methyl stearate	methyl linoleate	methyl linolenate	methyl linolenate
	M+	M+	%	M+	M+	%
dard	296	298	1.10	294	292	1.60
63	296	298	0.66	294	292	0.59
18	296	298	0.65	294	292	0.80
16	296	298	0.99	294	292	1.00
91a	296	298	1.08	294	292	1.20
92	296	298	1.61	294	292	0.89
age			1.01			1.01

TABLE IV

RANGE IN O/L RATIOS FOR SEED OF PLANTS FROM F_3 POPULATIONS, PERKINS, OKLAHOMA, 1968 (42) AS DETERMINED BY THE RAPID MICRO ANALYTICAL TECHNIQUE

Cross	Generation	O/L Ratio Range	Plants Analyzed
P-939 x P-2	F_3	0.66-1.12	80
P-939 x P-6	F_3	0.61-1.23	74
P-190 x P-2	F_3	0.88-1.56	50
P- 25 x P-2	F_3	0.88-2.42	48
P-960 x P-6	F_3	1.06-2.77	19
P-636 x P-6	F_3	0.91-1.32	49
P-962 x P-2	F_3	1.01-2.15	49
P-964 x P-2	F_3	0.98-2.95	50
P- 15 x P-964	F_3	0.98-2.95	100

Figure 2. Gas Liquid Chromatographic Tracing of Five Typical O/L Analyses.

Conditions were as follows:

Column - 6' x 1/4" coiled aluminum tubing

Column Packing - 14.5 percent DEGS on Anakrom 100/110 ABS

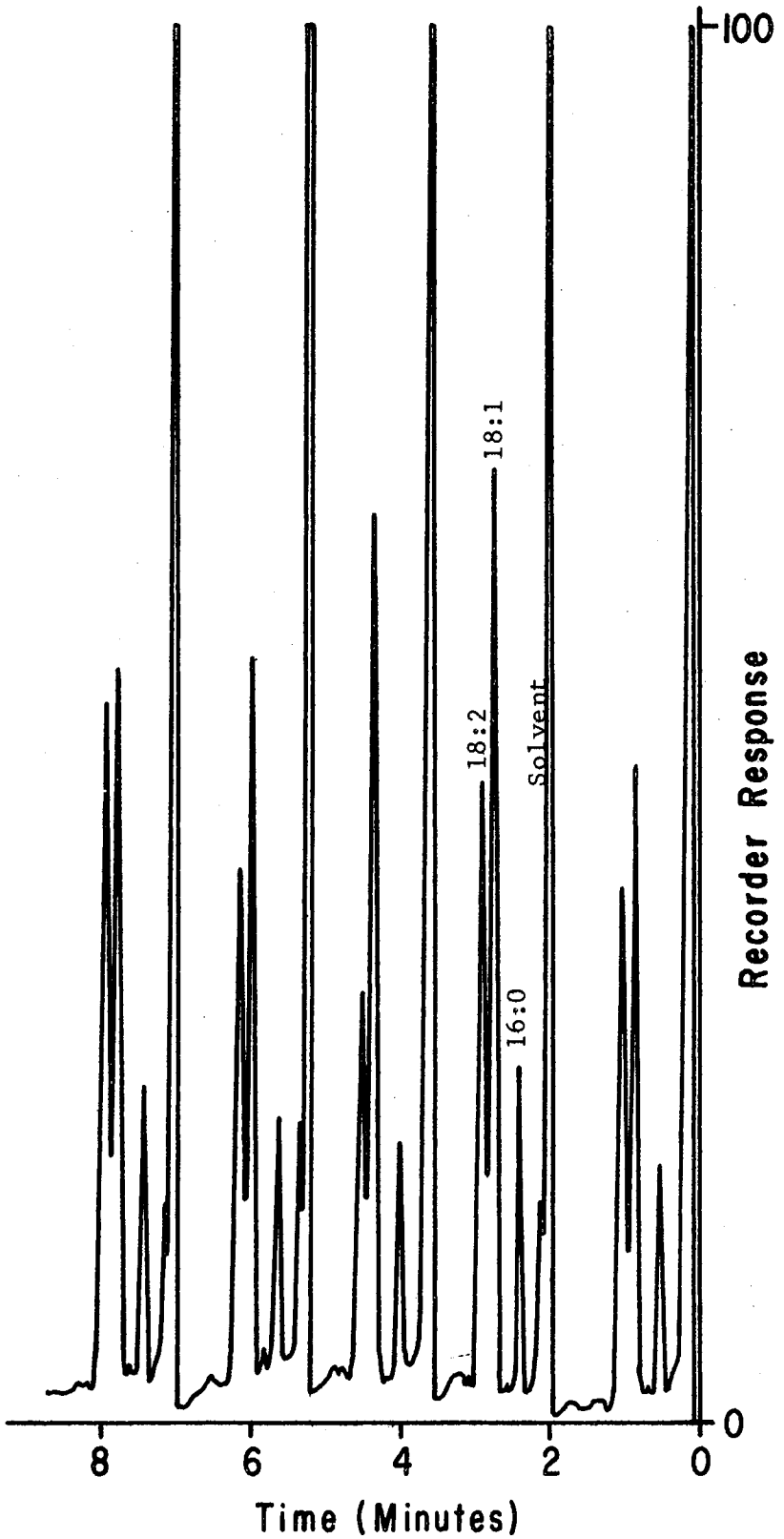
Column Temperature - 240° C

Inlet Temperature - 250° C

Carrier Gas - Helium

Detector - Flame Ionization

Flow Rate - 200 ml/min with 4 to 1 stream splitter


100
0
18:1
18:2
16:0
Solvent
Recorder Response
8
6
4
2
0
Time (Minutes)

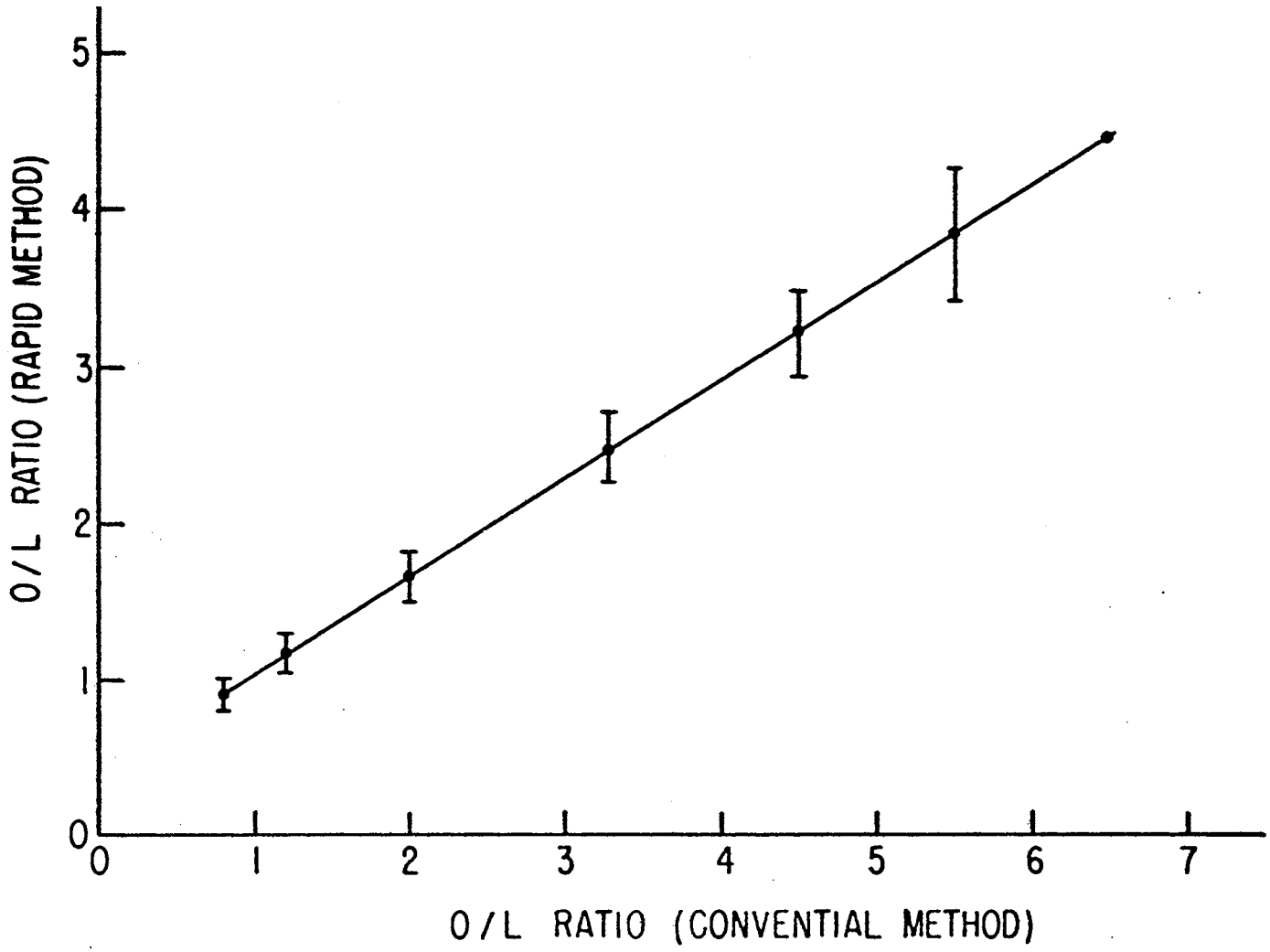


Figure 3. Calibration Curve for O/L Ratios.

CHAPTER IV

FATTY ACID COMPOSITION AND STABILITY OF PEANUT OIL AS INFLUENCED BY VARIETY, MATURITY, IRRIGATION, PLANTING LOCATION AND TIME OF HARVEST

ntroduction

In recent years there has been a determined search for the "quality factor" in peanuts. In earlier work, Stokes and Hull (43) found hat Spanish peanuts had a higher oil content than the runners. Later ohn, et al. (44) indicated that low and deficient rainfall at the ime of maturity reduced the oil content of the kernels. Schenk (38) eported that the percent of oil in developing kernels of Dixie Spanis nd Virginia Bunch 67 peanut fruits increased with maturity. Other orkers (36, 37) have used the iodine number to measure the degree of il unsaturation and have shown that a maximum value was reached early n the development of the peanut fruit. Holley and Hammons (35) found hat the stability of the oil was highly correlated with the linoleic cid concentration and reported that the linoleic acid accounted for bout 85 percent of the variation as measured under their conditions.

With the development of gas liquid chromatography (GLC), more ccurate and complete analysis of the fatty acid in peanut oil were ossible to obtain. Worthington (40) had measured the fatty acid composition of developing peanut fruit in the pericarp, testa, embryonic axis and cotyledon using peanuts grown in the greenhouse. Mason (29) and Tripp (45) have reported the fatty acid composition of some peanut

arieties with the latter work showing considerable variation in the oncentration of fatty acids between the same varieties grown at Per- ins, Oklahoma and those grown at Paradise, Oklahoma. No possible xplanation was proposed.

The primary purpose of this section of the dissertation was to xamine the influence of variety, maturity, irrigation and location on ne fatty acid composition and stability of the peanut oil of peanuts rown under essentially normal but measured field conditions.

Apparatus and Reagents

pparatus

The fatty acids were analyzed as their methyl esters on a Micro ek gas chromatograph equipped with an Infotronics electronic integra- or according to the procedure of Worthington and Holley (46). A DEGS inch six foot glass U-shaped column was used. Fatty acid compositio as determined by normalization of peak areas and the values reported re therefore relative proportions of total fatty acids analyzed by his method.

eagents

Gas chromatographic supports and stationary phases are described n Chapter III, page 13.

All other chemicals were reagent grade.

Procedures

gronomic

The first portion of this study covered eight peanut varieties of

ghly homozygous breeding lines that were grown at the Perkins, Okla-
ma station in 1968. Table V shows the variety, harvest dates and
lentification numbers. These tests were divided into two groups for
nvenience and limitation of drying equipment. After harvest all
anut samples were dried at 90° F in the forced air oven. The total
me in the dryer for each harvest was about 140 hours. Both the tem-
rature and relative humidity were recorded on a Bristol Humidigraph
d Temperature Recorder.

The second portion of this study involved nine varieties or
rains grown in the National variety test in both Oklahoma and Georgia
1968. Mature, sound, machine shelled peanuts were used for analysis
Oklahoma the nonirrigated peanuts were grown at Perkins and the
rigated samples at the Fort Cobb research stations. The samples from
orgia were grown at Tifton.

paration of Peanut Samples into Maturity Groups

See Chapter III, page 14.

orage of Samples Until Analysis

The first group of eight varieties was stored at 4° C until all
e peanuts were harvested and classified into maturity levels and then
ey were stored at -20° C to minimumize chemical changes (particularly
the free amino acids).

The second group of nine varieties from the National variety test
on receipt in the late fall were stored at 34° F and 60 percent rela-
ve humidity until analyzed.

xtraction of the Peanut Oil

Oils for methyl ester determinations were extracted by grinding th ɜanuts in a Serval Omni-mixer, transferring to Whatman #1 filter paper ctraction with diethyl ether, evaporation to dryness at room tempera- ɪre under an explosive-proof-hood (a safety precaution) and storing in ɪe refrigerator in small capped vials until needed for analysis. Thes ɪme oils were used for the quality study of solvent extracted oils.

The hydraulic pressed oils were obtained by pressing; using a ɪrver Hydraulic Press with Silver plated dies.

ɛeparation of Methyl Esters

The method of Jellium and Worthington (14) was used to prepare ɪe methyl esters; a method which used three percent sulfuric acid in ɜthanol, followed by extraction of methyl esters with petroleum ether, ʋaporation to dryness under nitrogen and storage at -20° C.

ɜeping Time in Oven

The method of Olcott and Einset (47) as modified and used by ɔung and Holley (31) was used to evaluate the stability of the peanut ɬls. "An 0.5-ml sample was pipetted into each of three 30-ml beakers ɪich were placed in a forced-draft oven at 60° C. Daily weighings wer ɜde until a weight increase of 1.0 mg was attained. The average num- ɜr of days for each of the three beakers to attain an increase of 1.0 ʒ in weight was recorded as keeping time."

Results and Discussion

The first portion of this study on peanut oil was on eight varie- ɪes grown at Perkins, Oklahoma which are identified in Table V. Parti

ar attention is directed to the P-No. and variety name, for they are d in the following discussion. At the bottom of the Table, the har- t dates are shown for each group. Three of these varieties (P-215, 958 and P-1276) are not well adapted to Oklahoma as they do not ch full maturity under Oklahoma conditions. The other varieties ld normally be harvested about 140 days after planting.

The fatty acid compositions of the varieties are recorded in les VI-XIII and were determined using the method of Jellum and thington (14). A tracing of a typical gas liquid chromatogram is wn in Figure 4. The peaks were identified by numbers and were fol- ed by a numerical designation of the fatty acid. The next two col- s were taken from the printer connected to the GLC-integrator com- ation.

Several figures were drawn to illustrate representative data. ure 5 illustrates the oleic acid composition of peanut oil extracted m three maturity classes of peanuts harvested at different dates for Argentine variety (P-2). The mature classification in this portion the study contains the peanuts of the mature and high intermediate ups. In the arginine study (Chapter VII), they were determined to similar in maturity and thus were combined to provide less samples analysis.

The percent oleic acid acid in the mature group was fairly con- nt (40.48-41.44 percent) throughout the growing season with the imum amount being measured at 141 and 155 days from planting. The rease to 40.67 at 169 days was note-worthy. Some unpublished pre- inary studies on over-mature peanuts indicated that the germination le was essentially a reversal of maturity. Argentine is a non-dor-

ıt type of peanut. The drop in oleic acid found in this variety was ıught to be due to the metabolism of this fatty acid at the initiation the germination cycle.

The oleic acid of the low intermediate group, most of which would in peanut products because they cannot be separated from mature ker-.s by conventional methods, was shown to increase (2.80 percent) with :vesting time reaching a maximum of 41.08 percent at 155 days. Since : quality of oil was highly correlated (-0.988) with linoleic acid ;), the best quality oil would be from peanuts harvested at 155 days : this variety in 1967.

The low oleic acid values of the immature peanuts were associated :h immaturity. At the bottom of Table VI the ratio of oleic and lin-ɜic (O/L) showed that the lower O/L values were also associated with naturity. If the optimum O/L ratio was known for a variety, one ıld predict the degree of immaturity by determining the O/L ratio ɨ comparing with the desired ratio.

Figure 6 shows the oleic acid composition of the OICB1271(P-112) related to harvest dates, recently released under the name Spanhoma. gher oleic acid values occurred earlier in the season with the P-112 ɐn compared with P-2 for the mature group. The low intermediate ɔup had the most oleic acid late in the season. Using the O/L ratio d fatty acid data, it was difficult to decide upon the optimum har-ɜt date. But, based on this author's experience with fatty acid mposition, it would appear that two "crops" of peanuts were obtained this variety in 1967 and the second crop never fully matured. In ture studies, a more careful record of fruit set is needed.

Figure 7 is a plot of the oleic acid content of the mature and low

ermediate kernels from the third harvest date of each variety. Large ferences between varieties are shown. Many companies blend runner nuts (such as P-215 which are grown in the southeast because of the ger growing season) with Spanish types to increase stability (shelf- e) of their product. Such blending information is not released by companies but can be easily calculated from data in a paper by droof et al. (48).

Many other such plots could be made for study, For example, lin- ic acid is very important since it is highly correlated with oleic) and could be plotted as was done in the second part of this study.

A comparison of Dixie Spanish (P-1271) and Argentine (P-2) show t their composition is almost identical. P-1271 was introduced from lia and P-2 was introduced from Argentina but the almost identical ty acid composition and very similar phenotype lead the author to ieve that they probably have a very closely related ancestor.

The second portion of this study on peanut oils was performed on nuts from the National variety test. The nine varieties were grown a randomized split plot design and the data were statistically lyzed. They are very similar in phenotype characteristics with all these of the Spanish types.

These peanuts were machine shelled and graded and represented sentially the type of peanuts that the peanut industry would process.

In Table XIV, the fatty acid composition of the nine varieties as fected by State (Georgia vs Oklahoma) and by treatment (irrigated vs nirrigated) is shown. Two replications in each group were analyzed. eic and linoleic acid concentrations were included to give the O + L lue in the next to the last column. In the last column the O/L

ios were listed. Tables XV and XVI show the stability of either solt extraction or hydraulic pressed oils.

The analysis of variance results are recorded in Table XVII for various fatty acid variables tested. The degree of significance indicated. For simplicity, only three of these variables are examd in detail. These three fatty acids make up more than 90 percent the total fatty acids.

Figure 8 is a graph of palmitic acid (16:0) composition. The rage of the nine varieties are shown on the left side of the graph int A). The variety variation is much less in Oklahoma than in rgia for both the irrigated (IRR) and nonirrigated (NIR). The nificant differences between states are easily seen in this figure. most varieties, the Georgia peanuts contain more palmitic acid redless of treatment. Also this figure shows that more palmitic acid in the nonirrigated peanuts in both states. Only for palmitic d is a state (S) and treatment (L) interaction observed. This sult indicates that the treatment effect was significally different response within each state as noted by the wider spread between IRR NIR in Oklahoma as compared to Georgia. The wider differences ween IRR and NIR in Oklahoma may be due to the fact that the IRR e grown at Ft. Cobb and the NIR at Perkins which are about 150 miles art. There are two exceptions; variety nine (P.I. 268771B) in rgia and variety six (Starr) in Oklahoma. The palmitic acid content variety nine in relation to the other varieties decreased when own in Georgia under irrigation. The opposite was true for variety x grown in Oklahoma. The other significant first order interaction between state and variety. There was little variation on the Okla-

a-NIR peanuts and to a lesser degree the Oklahoma-IRR. The palmitic d content did not vary nearly as much between varieties in Oklahoma it did in Georgia.

At the bottom of Table XVII the coefficient of variation, CV (a) CV (b), values are given. The CV values are low for palmitic acid. se values are a measure of the unaccounted for variation and are due tly to the variation in precision and accuracy. CV (a) is between t variation and CV (b) is within plot variation. Since CV (b) is ger than CV (a), then the variation within plots is greater than the iation between plots.

Figure 9 is a plot of the mean values of oleic acid (18:1). Signi- ant differences in the oleic acid content were found between states, ween irrigated and nonirrigated and between varieties. These dif- ences can be seen by examining the figure in the same way as was ie for palmitic acid. For palmitic acid, there was a significant ond order interaction involving state, treatment and entry (SxLxE).) first order interactions are also present (SxE and LxE). For a re complete interpretation, a further division of the data must be de (50). Often second order interactions are very difficult to in- rpret but some of these significant interactions are discussed ter. It was interesting that the variance for SxL was not signifi- nt. The NIR and IRR tended to respond similarly in each state. The lues for oleic acid in IRR test for Oklahoma was approximately 2.7 rcent lower than the IRR test in Georgia.

A graph is shown for linoleic acid in Figure 10. Differences re noted in the same manner as above but once again the second der interaction is present, however, none of the first order inter-

ons were significant.

The coefficients of variation for these data were small indicating l precision. The range of variations in the other fatty acids of to 30 percent, were probably due to the small peak size.

Arachidic (20:0) and behenic (22:0) saturated acids have been .icated with heart disease (51) but peanut composition was not signi- ıntly affected by any of the parameters utilized in this study. The ficients of variation ranged between 13.0 and 15.1 percent. The ı percentage for arachidic was 1.13 and for behenic was 1.96.

The variance for linolenic and eicosenoic (18:3 and 20:1) acids ı significant between states and locations within states. Those .ances for varieties and interactions were not significant. The :entage of these fatty acids made up a small portion of the total the coefficients of variation were higher than for the other fatty ls.

When the major fatty acids (oleic plus linoleic) were combined, :e were significant differences in the variance between Georgia and ıhoma, the irrigated and nonirrigated and among varieties (Table II). The variances for the four interactions were not significantly ferent.

The O/L ratio is considered to be an important factor in estimat- stability of peanuts, peanut oil and peanut products. This has ı discussed in Chapter III in the development of a rapid micro lytical procedure. The O/L ratio statistical analyses showed the ə type of interactions including the second order interaction that ic and linoleic acids had shown. Thus, the O/L ratio data were sen for examination to see if the pooled data were valid. Figure 11

a plot of mean O/L ratios as shown for palmitic, oleic and linoleic
.ds. The Tifspan (#3) and Spantex (#4) varieties appeared to be the
ples causing the problems in the interpretation of the data. The
ılysis of variance was made on the treatment in state and summarized
Table XIX. The interaction (LxE) variance for the Georgia samples
ı significant while the Oklahoma LxE interaction was not significant.
ı difference appeared to be attributed to the wide variation in Tif-
ın and Spantex in Georgia. Thus it was concluded that the pooled
ılyses testing significance of Georgia vs Oklahoma, NIR vs IRR and
riety were valid for most of the varieties tested. Further studies
Tifspan and Spantex (P-1258 and P-4) should be made to test the
lidity of these results since they do not follow the response for O/L
tio that the other varieties in this study showed.

Oil stability is very important and was reported by Holley and
mmons (35) to be correlated with linoleic acid. A formula was derive
r predicting the shelf-life of the oil by measuring the linoleic acid
eic acid and protein cottent of peanuts. Linoleic acid accounts for
percent of the variation associated with oil stability according to
eir formula. Their formula was computed using a number of varieties.
this was true, then it would be predicted when looking at the plot
ır linoleic acid (Figure 10) that Georgia peanuts would be stable
ınger than the Oklahoma peanuts. This was true on the solvent extract
l oils (Table XV) but not on the hydraulic pressed oils (Table XVI).
.so one would predict a longer stability for the NIR samples. This
ıs true for Oklahoma samples but not Georgia peanuts when the means
: the nine varieties were considered. The statistical data on the
:ability test gave a CV of about 10 percent, thus small differences

tween irrigation and nonirrigation tests would be more difficult to e. Thus the formula of Holley and Hammons (35) would appear to be ther accurate on these solvent extracted oils. But other factors e evidently involved based on the reverse trend when the hydraulic tracted oils were analyzed.

Table XVI shows the results of the stability test on hydraulic essed oils and no significant differences were found. Since iron a catalyst for oxidative rancidity in oils, it was postulated that e silver coating of the dies on the press was probably in need of pair (52). These tests on the stability of hydraulic pressed oils st be repeated.

After the oil samples had been stored at 4° C for eight months, it s decided to recheck their oxidative stability. The stability was creased and it was thought that this was due to storage at an ele- ted temperature. Thus it would be recommended that peanut oil sam- es be stored at -20° C.

Summary

The fatty acid composition of three maturity groups for eight rieties with different harvest dates are reported. Mature peanuts ually contain more stearic (18:0) and oleic (18:1) acids and less noleic acid (18:2) and other fatty acids. Behenic (22:0) and arachi- c (20:0) which were recently implicated in heart disease (51) are wer in the mature nuts.

A second study on nine varieties showed that state (Georgia vs lahoma), treatment (irrigation vs nonirrigation) and variety had gnificant effect on the percentage of fatty acids except behenic

2:0) and arachidic (20:0). Solvent extracted oils from Georgia
owed greater stability to oxidative rancidity than those from Okla-
ma, whereas there was no difference between states for the hydraulic
essed oils.

TABLE V

HARVESTING SCHEDULE FOR EIGHT VARIETIES
GROWN AT PERKINS, OKLAHOMA 1968

Agronomy Entry No.	Okla. P-No.	Variety	Biochem. No.
Group I: Sept. 10, 24, Oct. 8, 22, Nov. 5.			
01	0002	Argentine	1
02	0112	OICB 1271	2
05	0161	Valencia	3
06	1271	Dixie Span.	4
Group II: Sept. 17, Oct. 1, 15, 29, Nov. 12.			
03	0215	Early Runner	5
04	0958	NC 5	6
07	1273	Ga. 61-42	7
08	1276	Va. Bunch 67	8

Total growing days from seeding to harvesting

	Group I	Group II
1st Harvest	113	120
2nd Harvest	127	134
3rd Harvest	141	148
4th Harvest	155	162
5th Harvest	169	176

TABLE VI

FATTY ACID COMPOSITION OF EIGHT VARIETIES GROWN AT PERKINS, 1968

Strain: Argentine - Okla. P-No. 0002, Entry No. 01

ty Acid	Maturity	Harvest Date & No. of Days 9/10/68 : 113	9/24/68 : 127	10/8/68 : 141	10/22/68 : 155	11/5/68 : 169
				% of Total		
16:0	Mature	13.06	13.16	13.07	12.56	11.87
	Low Int.	12.92	12.29	13.14	12.08	11.81
	Immature	13.65	13.29	13.66	13.00	12.60
18:0	Mature	2.74	2.47	2.63	2.86	2.76
	Low Int.	2.48	2.24	2.51	2.88	2.31
	Immature	2.03	2.10	2.40	2.55	1.75
18:1	Mature	40.48	41.19	41.44	41.40	40.67
	Low Int.	38.28	38.44	39.78	41.08	40.30
	Immature	36.03	35.97	37.54	37.14	35.47
18:2	Mature	39.56	38.49	38.31	38.51	39.23
	Low Int.	39.87	41.08	39.65	39.13	40.72
	Immature	40.57	41.32	40.14	40.86	42.74
20:0	Mature	.98	1.15	1.02	1.12	.94
	Low Int.	1.24	1.05	.95	1.05	1.08
	Immature	1.13	1.04	1.10	1.10	.88
18:3 + 20:1	Mature	.72	1.07	.82	.77	.90
	Low Int.	1.09	1.15	.76	.83	1.02
	Immature	1.46	1.44	1.24	1.51	1.87
22:0	Mature	1.84	2.14	2.10	2.27	2.45
	Low Int.	3.30	2.70	2.61	2.14	2.18
	Immature	4.16	3.44	3.18	2.84	3.43
24:0	Mature	.51	.27	.62	.61	.78
	Low Int.	.82	.82	.60	.68	.53
	Immature	.87	1.16	.86	.92	1.25
O + L	Mature	80.04	79.68	79.75	79.91	79.90
	Low Int.	78.15	79.52	79.43	80.21	81.02
	Immature	76.60	77.29	77.68	78.00	78.21
O / L	Mature	1.02	1.07	1.08	1.08	1.04
	Low Int.	.96	.94	1.00	1.05	.99
	Immature	.89	.87	.94	.91	.83

TABLE VII

FATTY ACID COMPOSITION OF EIGHT VARIETIES GROWN AT PERKINS, 1968

Strain: OICB1271 Spanhoma - Okla. P-No. 0112, Entry No. 02

Fatty Acid	Maturity	Harvest Date & No. of Days				
		9/10/68	9/24/68	10/8/68	10/22/68	11/5/68
		113	127	141	155	169
		% of Total				
16:0	Mature	12.88	12.81	12.15	12.06	12.37
	Low Int.	12.70	11.68	11.64	11.40	11.33
	Immature	12.78	13.59	13.38	12.88	12.04
18:0	Mature	2.79	2.90	2.58	2.36	2.34
	Low Int.	2.96	2.86	2.92	2.89	3.28
	Immature	2.66	2.44	2.06	2.21	2.53
18:1	Mature	41.85	42.23	41.46	41.02	41.03
	Low Int.	38.09	38.58	38.49	39.02	39.86
	Immature	36.46	35.14	35.20	35.30	37.09
18:2	Mature	38.08	37.10	38.94	39.66	39.84
	Low Int.	37.52	38.79	38.96	39.20	38.14
	Immature	37.81	38.52	38.93	39.76	39.87
20:0	Mature	1.15	1.18	1.07	1.18	1.09
	Low Int.	1.57	1.50	1.51	1.46	1.61
	Immature	1.60	1.48	1.30	1.36	1.39
18:3 + 20:1	Mature	.71	.73	.89	1.09	.95
	Low Int.	1.24	1.31	1.33	1.25	1.23
	Immature	1.66	1.68	2.18	2.19	1.69
22:0	Mature	1.82	2.28	2.02	2.23	2.27
	Low Int.	4.23	3.80	3.67	3.22	3.23
	Immature	5.32	5.24	4.97	4.59	3.95
24:0	Mature	.51	.60	.62	.55	+
	Low Int.	1.67	1.47	1.47	1.56	1.31
	Immature	1.71	1.90	1.99	1.72	1.43
O + L	Mature	79.93	79.33	80.40	80.68	80.87
	Low Int.	75.61	77.37	77.45	78.22	78.00
	Immature	74.27	73.66	74.13	75.06	76.96
O / L	Mature	1.10	1.14	1.06	1.03	1.03
	Low Int.	1.02	.99	.99	1.00	1.05
	Immature	.96	.91	.90	.89	.93

TABLE VIII

FATTY ACID COMPOSITION OF EIGHT VARIETIES GROWN AT PERKINS, 1968

Strain: Valencia - Okla. P-No. P-0161, Entry No. 05

ty Acid	Maturity	Harvest Date & No. of Days 9/10/68 : 113	9/24/68 : 127	10/8/68 : 141	10/22/68 : 155	11/5/68 : 169
				% of Total		
16:0	Mature	11.75	11.62	11.08	11.05	10.31
	Low Int.	11.04	13.04	10.87	Moldy	Moldy
	Immature	12.21	12.46	12.71	Moldy	Moldy
18:0	Mature	2.78	2.12	2.61	2.12	2.67
	Low Int.	2.55	3.00	2.43		
	Immature	2.16	2.02	1.70		
18:1	Mature	39.96	39.02	38.62	38.11	38.87
	Low Int.	35.82	39.06	36.15		
	Immature	34.41	34.61	33.28		
18:2	Mature	41.22	42.36	42.89	43.73	42.70
	Low Int.	41.36	37.04	42.01		
	Immature	40.36	40.38	41.99		
20:0	Mature	1.05	.96	.98	.94	1.11
	Low Int.	1.40	1.36	1.36		
	Immature	1.36	1.24	1.10		
18:3 + 20:1	Mature	.75	.92	1.06	1.14	1.09
	Low Int.	1.54	1.08	1.57		
	Immature	2.10	2.02	2.32		
22:0	Mature	1.75	2.34	1.96	2.22	2.17
	Low Int.	4.30	3.28	3.77		
	Immature	5.31	5.17	4.70		
24:0	Mature	.62	.58	.72	.61	.87
	Low Int.	1.98	1.72	1.84		
	Immature	2.10	2.10	2.18		
O + L	Mature	81.18	81.38	81.51	81.84	81.57
	Low Int.	77.18	76.10	78.16		
	Immature	74.77	74.99	75.27		
O / L	Mature	.97	.92	.90	.87	.91
	Low Int.	.87	1.05	.86		
	Immature	.85	.86	.79		

TABLE IX

FATTY ACID COMPOSITION OF EIGHT VARIETIES GROWN AT PERKINS, 1968

Strain: Dixie Spanish - Okla. P-No. P-1271, Entry No. 06

ty Acid	Maturity	Harvest Date & No. of Days				
		9/10/68	9/24/68	10/8/68	10/22/68	11/5/68
		113	127	141	155	169
		% of Total				
16:0	Mature	13.77	13.09	13.32	13.37	13.18
	Low Int.	13.04	12.63	12.45	11.76	11.90
	Immature	13.20	13.89	13.54	12.77	12.33
18:0	Mature	2.27	2.59	2.81	2.30	2.41
	Low Int.	2.51	2.40	2.62	2.05	2.73
	Immature	2.35	2.03	2.22	2.22	2.09
18:1	Mature	40.95	41.53	41.39	41.42	40.83
	Low Int.	37.15	36.83	38.41	43.10	39.87
	Immature	34.50	33.47	35.17	35.04	36.32
18:2	Mature	39.72	38.44	37.76	38.12	39.45
	Low Int.	38.32	38.65	39.36	34.55	38.01
	Immature	38.01	39.74	39.55	39.20	39.45
20:0	Mature	.61	.94	.98	1.11	1.00
	Low Int.	1.50	1.55	1.32	1.18	1.44
	Immature	1.58	1.36	1.37	1.38	1.35
18:3 + 20:1	Mature	.53	.77	.85	.96	.97
	Low Int.	1.40	1.52	1.28	1.75	1.25
	Immature	1.96	1.91	1.87	2.14	2.05
22:0	Mature	2.08	1.93	2.07	2.17	2.13
	Low Int.	4.43	4.46	3.29	3.71	3.33
	Immature	6.39	5.15	4.49	5.05	4.52
24:0	Mature	+	.51	.65	.48	.72
	Low Int.	1.63	1.94	1.26	1.67	1.46
	Immature	2.01	1.95	1.78	2.18	1.89
O + L	Mature	80.67	79.97	79.15	79.64	80.28
	Low Int.	75.47	75.48	77.77	77.65	77.88
	Immature	72.51	73.71	74.72	74.24	75.77
O / L	Mature	1.03	1.08	1.10	1.09	1.03
	Low Int.	.97	.95	.98	1.25	1.05
	Immature	.91	.85	.89	.89	.92

TABLE X

FATTY ACID COMPOSITION OF EIGHT VARIETIES GROWN AT PERKINS, 1968

Strain: Early Runner - Okla. P-No. P-0215, Entry No. 03

ty Acid	Maturity	Harvest Date & No. of Days				
		9/17/68 : 120	10/1/68 : 134	10/15/68 : 148	10/29/68 : 162	11/12/(: 176
		% of Total				
16:0	Mature	10.37	10.53	9.58	9.53	9.08
	Low Int.	9.67	9.81	9.39	8.91	9.62
	Immature	10.32	9.85	10.16	9.87	9.11
18:0	Mature	1.71	2.15	1.73	1.83	1.47
	Low Int.	1.83	1.72	1.72	1.74	1.29
	Immature	1.56	1.70	1.50	1.33	1.10
18:1	Mature	45.11	42.63	46.08	44.16	46.20
	Low Int.	43.75	43.78	41.94	44.15	41.81
	Immature	40.66	41.47	38.95	39.72	39.21
18:2	Mature	37.62	36.42	37.34	36.79	37.76
	Low Int.	36.06	36.30	38.04	37.16	39.65
	Immature	36.99	36.82	40.19	39.22	40.08
20:0	Mature	.82	1.23	.92	1.10	.79
	Low Int.	1.18	1.01	1.13	1.11	.83
	Immature	1.01	1.13	.91	.93	.84
18:3 + 20:1	Mature	1.17	1.59	1.33	1.79	1.49
	Low Int.	1.84	1.89	2.02	1.94	2.14
	Immature	2.41	2.42	2.42	2.94	3.34
22:0	Mature	2.24	3.40	1.95	2.96	2.00
	Low Int.	3.82	3.44	3.38	3.02	2.84
	Immature	4.80	4.22	3.76	3.61	3.60
24:0	Mature	.90	2.04	.78	1.82	.96
	Low Int.	1.86	2.05	2.37	1.97	1.81
	Immature	2.25	2.40	2.11	2.38	2.71
O + L	Mature	82.73	79.07	83.42	80.95	83.96
	Low Int.	74.81	80.08	79.98	81.31	81.46
	Immature	77.65	78.29	79.14	78.94	79.29
O / L	Mature	1.20	1.17	1.23	1.20	1.22
	Low Int.	1.21	1.21	1.10	1.19	1.05
	Immature	1.10	1.13	.97	1.01	.98

TABLE XI

FATTY ACID COMPOSITION OF EIGHT VARIETIES GROWN AT PERKINS, 1968

Strain: NC5 - Okla. P-No. P-0958, Entry No. 04

ty Acid	Maturity	Harvest Date & No. of Days 9/17/68 : 120	10/1/68 : 134	10/15/68 : 148	10/29/68 : 162	11/12/ : 176
				% of Total		
16:0	Mature	9.97	10.90	10.72	10.28	9.71
	Low Int.	10.96	11.01	10.20	10.18	9.79
	Immature	11.10	11.79	10.74	10.90	9.60
18:0	Mature	1.74	2.20	1.64	1.91	1.89
	Low Int.	1.63	1.82	1.64	2.06	2.03
	Immature	1.65	1.58	1.51	1.52	1.95
18:1	Mature	48.57	49.98	47.18	47.79	49.80
	Low Int.	45.59	45.54	44.84	48.38	47.63
	Immature	44.08	43.04	42.49	45.23	45.96
18:2	Mature	34.07	30.78	36.01	33.72	33.59
	Low Int.	34.40	33.70	36.04	32.99	33.44
	Immature	34.18	35.52	36.80	34.14	34.10
20:0	Mature	1.20	1.12	.61	1.03	.75
	Low Int.	.99	1.13	.98	1.07	1.09
	Immature	1.08	1.00	1.07	.96	1.09
18:3 + 20:1	Mature	1.77	1.18	1.19	1.47	1.33
	Low Int.	1.70	1.64	1.67	1.37	1.63
	Immature	2.07	1.90	2.03	2.03	1.91
22:0	Mature	1.50	2.57	1.80	2.53	1.84
	Low Int.	3.17	3.24	2.96	2.54	2.86
	Immature	4.01	3.57	3.45	3.45	3.42
24:0	Mature	.75	1.27	.66	1.25	.76
	Low Int.	1.58	1.92	1.66	1.36	1.52
	Immature	1.83	1.61	1.91	1.75	1.96
O + L	Mature	82.64	80.76	83.19	81.51	83.39
	Low Int.	79.99	79.24	80.88	81.37	81.07
	Immature	78.26	78.56	79.29	79.37	80.06
O / L	Mature	1.43	1.62	1.31	1.42	1.48
	Low Int.	1.33	1.35	1.24	1.47	1.42
	Immature	1.29	1.21	1.15	1.32	1.35

TABLE XII

FATTY ACID COMPOSITION OF EIGHT VARIETIES GROWN AT PERKINS, 1968

Strain: Ga. 61-42 - Okla. P-No. P-1273, Entry No. 07

ty Acid	Maturity	Harvest Date & No. of Days				
		9/17/68 : 120	10/1/68 : 134	10/15/68 : 148	10/29/68 : 162	11/12/6 : 176
		% of Total				
16:0	Mature	12.19	11.56	10.95	10.38	10.86
	Low Int.	12.00	11.08	10.89	10.34	10.69
	Immature	11.75	11.08	11.37	10.98	11.19
18:0	Mature	1.56	1.89	1.75	1.85	1.83
	Low Int.	2.52	2.07	1.87	1.88	2.00
	Immature	1.88	1.84	1.56	1.47	1.64
18:1	Mature	43.07	39.14	43.23	42.82	43.13
	Low Int.	35.71	41.00	40.43	41.31	41.45
	Immature	39.54	38.72	38.07	37.22	38.57
18:2	Mature	38.64	36.47	37.57	36.52	37.79
	Low Int.	40.10	36.26	37.41	37.29	36.45
	Immature	34.90	35.09	35.90	37.51	37.27
20:0	Mature	.72	1.20	.88	1.15	1.01
	Low Int.	1.45	1.26	1.18	1.19	1.22
	Immature	1.24	1.29	1.20	1.18	1.07
18:3 + 20:1	Mature	1.04	2.31	1.51	1.56	1.64
	Low Int.	1.33	1.88	1.92	1.83	1.92
	Immature	2.38	1.97	2.18	2.49	2.86
22:0	Mature	1.99	4.86	2.66	3.65	2.38
	Low Int.	4.36	4.24	4.07	4.11	4.07
	Immature	5.99	6.17	6.06	5.37	4.95
24:0	Mature	.65	2.56	1.04	2.07	.97
	Low Int.	2.24	2.20	2.22	2.05	2.20
	Immature	2.30	3.39	3.33	3.36	2.45
O + L	Mature	81.71	75.61	80.80	79.34	80.92
	Low Int.	75.81	77.26	77.84	78.60	77.90
	Immature	74.44	73.81	73.97	74.73	75.84
O / L	Mature	1.11	1.07	1.15	1.17	1.14
	Low Int.	.89	1.13	1.08	1.11	1.14
	Immature	1.13	1.10	1.06	.99	1.03

TABLE XIII

FATTY ACID COMPOSITION OF EIGHT VARIETIES GROWN AT PERKINS, 1968

Strain: Va. Bunch 67 - Okla. P-No. P-1276, Entry No. 08

ty Acid	Maturity	Harvest Date & No. of Days 9/17/68 : 120	10/1/68 : 134	10/15/68 : 148	10/29/68 : 162	11/12/(: 176
				% of Total		
16:0	Mature	10.89	9.94	9.92	9.51	10.80
	Low Int.	10.29	9.82	9.82	9.79	11.03
	Immature	11.33	10.06	10.67		10.92
18:0	Mature	1.61	2.27	1.80	2.23	1.63
	Low Int.	1.84	2.00	2.00	2.08	1.83
	Immature	1.80	1.80	1.77		1.43
18:1	Mature	47.15	49.62	49.94	50.57	47.28
	Low Int.	45.52	44.64	46.81	46.11	46.44
	Immature	42.17	43.79	45.03		42.97
18:2	Mature	36.38	31.37	33.47	30.81	36.20
	Low Int.	34.39	34.38	33.43	34.65	35.66
	Immature	34.48	36.19	35.09		36.75
20:0	Mature	.66	1.25	.83	1.16	.54
	Low Int.	1.16	1.20	1.21	1.11	.93
	Immature	1.17	1.03	.98		.91
18:3 + 20:1	Mature	1.18	1.41	1.14	1.52	1.13
	Low Int.	1.68	1.87	1.77	1.62	1.39
	Immature	2.16	2.32	1.77		2.11
22:0	Mature	1.65	2.74	1.74	2.73	1.79
	Low Int.	3.37	3.90	3.16	2.97	1.87
	Immature	4.78	3.08	3.06		3.09
24:0	Mature	.58	1.39	.75	1.47	.63
	Low Int.	1.74	2.18	1.80	1.66	.85
	Immature	2.10	1.71	1.62		1.81
O + L	Mature	83.53	80.99	83.41	81.38	83.48
	Low Int.	79.91	79.02	80.24	80.76	82.10
	Immature	76.65	79.98	80.12		79.72
O / L	Mature	1.30	1.58	1.49	1.64	1.31
	Low Int.	1.32	1.30	1.40	1.33	1.30
	Immature	1.22	1.21	1.28		1.17

FATTY ACID COMPOSITION OF PEANUT OILS FROM
THE 1968 NATIONAL VARIETY TEST

State	Ident. #	Treat-ment	16:0	18:0	18:1	18:2	20:0	18:3+ 20:1	22:0	24:0	O+L	O/L
					% of total							
					Argentine (P-0002) (#2)							
Ok	1	NIR	12.49	3.01	42.77	37.22	1.11	.65	1.95	.55	79.99	1.15
Ok	2	NIR	13.06	2.82	43.51	37.11	1.05	.51	1.86	+	80.62	1.17
Ga	1	NIR	12.58	3.34	45.15	34.38	1.19	.63	2.01	.60	79.53	1.31
Ga	2	NIR	13.22	2.88	44.96	35.69	1.00	.43	1.77	+	80.65	1.26
Ok	3	IRR	11.72	2.73	41.00	40.00	1.00	.71	2.02	.61	81.00	1.03
Ok	4	IRR	11.78	2.76	42.06	39.26	1.19	.91	2.00	+	81.32	1.07
Ga	3	IRR	12.08	3.30	43.24	36.51	1.26	.68	2.17	.63	79.75	1.18
Ga	4	IRR	12.12	3.21	42.95	37.11	1.24	.63	2.06	.57	80.06	1.16
					Tifspan (Ga C-1-27) (P-1258) (#3)							
Ok	5	NIR	12.95	2.59	43.00	37.98	.94	.54	1.93	+	80.98	1.13
Ok	6	NIR	12.68	2.79	43.19	37.24	1.00	.65	1.68	.50	80.43	1.16
Ga	5	NIR	13.26	2.67	44.34	35.34	1.13	.69	1.91	.56	79.68	1.25
Ga	6	NIR	13.09	2.80	44.64	35.05	1.07	.64	2.05	.58	79.69	1.27
Ok	7	IRR	11.76	2.38	42.15	38.33	1.17	.95	2.14	.58	80.98	1.09
Ok	8	IRR	11.59	2.76	42.29	38.73	1.11	.78	1.94	.57	81.02	1.09
Ga	7	IRR	12.67	2.42	45.95	34.84	1.17	.83	2.07	+	80.79	1.32
Ga	8	IRR	12.81	2.10	45.99	35.39	1.03	.78	1.86	+	81.38	1.30

State	Ident. #	Treat-ment	16:0	18:0	18:1	18:2	20.0	18:3+ 20:1	22:0	24:0	O+L	O/L
					% of total							
					Spantex (P-0004) (#4)							
Ok	9	NIR	12.89	2.34	41.79	39.23	1.05	.77	1.87	+	81.02	1.07
Ok	10	NIR	13.08	2.39	40.74	40.26	.97	.63	1.89	+	81.00	1.01
Ga	9	NIR	13.66	2.98	43.27	36.63	.77	.44	1.80	.40	79.90	1.18
Ga	10	NIR	13.30	2.90	44.49	34.89	1.29	.87	1.77	.37	79.38	1.28
Ok	11	IRR	12.23	2.28	40.73	40.56	1.01	.83	1.96	.26	81.29	1.00
Ok	12	IRR	11.63	2.31	40.72	40.38	1.04	.89	2.12	.67	81.10	1.01
Ga	11	IRR	13.08	2.39	40.74	40.26	.97	.63	1.89	+	81.00	1.01
Ga	12	IRR	12.89	2.34	41.79	39.23	1.05	.77	1.87	+	81.02	1.07
					Starr (P-0006) (#6)							
Ok	13	NIR	12.52	2.65	42.13	38.33	1.05	.78	1.84	.53	80.46	1.10
Ok	14	NIR	13.16	2.45	42.12	38.19	1.17	.90	1.96	+	80.31	1.10
Ga	13	NIR	13.56	2.80	43.58	36.11	1.14	.83	1.93	+	79.69	1.21
Ga	14	NIR	13.44	2.87	43.86	35.67	1.26	.91	1.95	+	79.53	1.23
Ok	15	IRR	12.52	2.01	41.66	39.80	.98	.98	1.98	+	81.46	1.05
Ok	16	IRR	12.44	2.35	42.00	39.94	.93	.53	1.81	+	81.94	1.05
Ga	15	IRR	12.88	3.07	43.24	36.41	1.41	.90	2.05	+	79.65	1.19
Ga	16	IRR	12.61	3.01	44.28	36.46	1.09	.47	2.01	+	80.74	1.21
					Spancross (Ga-C-32-S) (P-1259) (#5)							
Ok	17	NIR	13.00	2.55	43.16	37.76	1.08	.74	1.64	+	80.92	1.14
Ok	18	NIR	12.67	2.46	42.51	38.35	1.14	.85	1.84	+	80.86	1.11

State	Ident. #	Treat-ment	16:0	18:0	18:1	18:2	20.0	18:3+ 20:1	22:0	24:0	O+L	O/L
Ga	17	NIR	13.15	3.34	44.91	35.34	1.05	.38	1.86	+	80.15	1.27
Ga	18	NIR	12.68	3.10	45.91	34.63	1.14	.57	1.88	+	80.54	1.33
Ok	19	IRR	11.81	2.41	41.27	40.37	1.12	.95	2.01	+	81.64	1.02
Ok	20	IRR	11.99	2.25	41.77	39.94	1.12	.86	2.04	+	81.71	1.05
Ga	19	IRR	12.26	3.42	44.88	35.12	1.35	.57	2.12	.21	80.00	1.28
Ga	20	IRR	12.39	2.93	45.71	35.87	.97	.28	1.81	+	81.58	1.27
					PI 268684 (P-0385) (#1)							
Ok	21	NIR	13.26	2.81	42.70	38.02	.89	.45	1.75	+	80.78	1.12
Ok	22	NIR	12.81	2.81	41.76	38.77	1.00	.54	1.78	.42	80.53	1.08
Ga	21	NIR	12.40	3.38	46.42	33.49	1.11	.54	1.91	.54	79.91	1.39
Ga	22	NIR	12.88	3.18	45.84	34.79	1.03	.35	1.87	+	80.63	1.32
Ok	23	IRR	12.10	2.27	41.34	40.09	1.13	.94	1.94	+	81.43	1.03
Ok	24	IRR	12.14	2.44	40.92	40.10	1.26	1.15	1.86	+	81.02	1.02
Ga	23	IRR	12.35	2.90	44.95	34.97	1.17	.57	1.98	+	80.92	1.25
Ga	24	IRR	12.08	2.88	45.19	35.92	1.25	.66	1.86	+	81.11	1.26
					Spanhoma (P-0112) (#7)							
Ok	25	NIR	12.80	2.45	41.21	39.42	1.17	.95	1.87	+	80.63	1.05
Ok	26	NIR	12.93	2.50	41.60	39.45	1.00	.59	1.87	+	81.05	1.05
Ga	25	NIR	13.04	3.04	43.21	37.11	1.15	.60	1.61	+	80.32	1.16
Ga	26	NIR	13.04	2.87	44.06	36.19	1.07	.61	1.90	+	80.25	1.22
Ok	27	IRR	11.67	2.55	40.57	40.40	1.05	.81	2.09	.61	80.97	1.00
Ok	28	IRR	12.07	2.27	39.96	40.97	1.49	1.31	1.63	.23	80.93	1.98
Ga	27	IRR	12.23	3.40	42.99	36.41	1.11	.51	1.94	.81	79.40	1.18
Ga	28	IRR	12.55	2.88	43.48	37.34	1.11	.51	2.01	+	80.82	1.16

State	Ident. #	Treat-ment	16:0	18:0	18:1	18:2	20:0	18:3+ 20:1	22:0	24:0	O+L	O/L
					% of total							
					Dixie Spanish (P-0003) (#8)							
Ok	29	NIR	12.92	2.68	41.06	39.14	1.00	.65	1.88	.53	80.20	1.05
Ok	30	NIR	12.97	2.73	40.40	39.02	1.10	.81	2.13	.68	79.42	1.04
Ga	29	NIR	13.35	3.12	44.02	35.25	1.35	.52	1.80	.45	79.27	1.25
Ga	30	NIR	13.49	3.05	44.21	35.40	1.35	.68	1.81	+	79.61	1.25
Ok	31	IRR	12.39	2.27	40.23	40.58	1.02	.89	2.21	.36	80.81	.99
Ok	32	IRR	11.97	2.72	41.16	39.55	1.13	.84	2.02	.51	80.71	1.04
Ga	31	IRR	12.87	3.15	43.06	36.44	1.12	.57	2.11	.57	79.50	1.18
Ga	32	IRR	12.93	2.98	42.73	37.73	1.03	.47	2.13	+	80.46	1.13
					PI 268771B (P-0931) (#9)							
Ok	33	NIR	13.14	2.39	41.67	38.86	1.46	.74	1.74	+	80.53	1.07
Ok	34	NIR	12.28	3.20	42.52	35.88	1.47	.87	2.71	1.01	78.40	1.19
Ga	33	NIR	13.85	3.30	44.94	35.17	.96	.20	1.58	+	80.11	1.28
Ga	34	NIR	13.38	3.19	44.11	34.81	1.13	.47	2.91	+	78.92	1.27
Ok	35	IRR	12.39	2.52	41.91	39,46	1.05	.70	1.91	+	81.37	1.06
Ok	36	IRR	11.57	2.76	40.17	38.73	1.43	1.04	3.02	1.11	78.90	1.04
Ga	35	IRR	13.04	3.03	44.59	35.61	1.13	.50	2.10	+	80.20	1.25
Ga	36	IRR	12.36	3.20	43.10	35.50	2.08	1.68	1.92	+	78.60	1.21

TABLE XV

STABILITY OF SOLVENT EXTRACTED OILS BASED ON THE OXYGEN UPTAKE METHOD OF OLCOTT AND EINSET (47)

Serial No.	Strain	Georgia		Oklahoma	
		NIR	IRR	NIR	IRR
			days		
1	Argentine	16.5		10.5	
2	Argentine	17.5		11.0	
3	Argentine		17.0		10.0
4	Argentine		16.0		10.0
5	Ga-C-1-27	16.0		12.0	
6	Ga-C-1-27	16.0		11.0	
7	Ga-C-1-27		19.0		9.5
8	Ga-C-1-27		16.5		9.0
9	Spantex	17.5		10.5	
10	Spantex	14.5		13.0	
11	Spantex		17.5		9.0
12	Spantex		19.0		10.0
13	Starr	19.0		10.5	
14	Starr	21.5		11.0	
15	Starr		21.0		10.0
16	Starr		20.0		9.5
17	Ga-C-32S	17.0		13.5	
18	Ga-C-32S	18.0		12.0	
19	Ga-C-32S		15.0		9.0
20	Ga-C-32S		15.0		10.0
21	PI 268684	15.0		13.0	
22	PI 268684	16.5		10.0	
23	PI 268684		13.5		12.5
24	PI 268684		16,5		8.5
25	Okla P-112	14.5		9.0	
26	Okla P-112	16.0		13.0	
27	Okla P-112		15.5		10.5
28	Okla P-112		14.5		9.0
29	Dixie Spanish	15.0		11.0	
30	Dixie Spanish	16.5		14.0	
31	Dixie Spanish		15.5		10.5
32	Dixie Spanish		14.5		10.5
33	PI 268771 B	14.0		11.0	
34	PI 268771 B	16.0		10.5	
35	PI 268771 B		15.0		10.0
36	PI 268771 B		14.0		9.0
	Average	16.5	16.4	11.5	9.8

TABLE XVI

STABILITY OF HYDRAULIC PRESSED OILS BASED ON THE OXYGEN UPTAKE METHOD OF OLCOTT AND EINSET (47)

Serial No.	Strain	Georgia		Oklahoma	
		NIR	IRR	NIR	IRR
			days		
1	Argentine	8.0		7.5	
2	Argentine	9.0		8.5	
3	Argentine		9.5		7.0
4	Argentine		8.5		7.0
5	Ga-C-1-27	9.0		8.5	
6	Ga-C-1-27	8.0		8.0	
7	Ga-C-1-27		9.0		8.5
8	Ga-C-1-27		9.0		8.0
9	Spantex	7.0		9.0	
10	Spantex	9.0		8.5	
11	Spantex		8.5		9.0
12	Spantex		9.0		7.0
13	Starr	9.0		9.0	
14	Starr	9.0		9.0	
15	Starr		9.5		8.0
16	Starr		9.0		8.0
17	Ga-C-32S	8.5		9.0	
18	Ga-C-32S	9.0		7.5	
19	Ga-C-32S		7.5		8.5
20	Ga-C-32S		6.5		8.5
21	PI 268684	7.0		10.0	
22	PI 268684	10.0		9.0	
23	PI 268684		7.5		8.0
24	PI 268684		8.0		8.5
25	Okla P-112	6.5		9.0	
26	Okla P-112	7.5		9.0	
27	Okla P-112		7.5		8.5
28	Okla P-112		7.5		8.0
29	Dixie Spanish	7.0		8.5	
30	Dixie Spanish	7.5		7.5	
31	Dixie Spanish		8.5		8.0
32	Dixie Spanish		8.5		7.5
33	PI 268771 B	8.0		8.0	
34	PI 268661 B	8.0		8.5	
35	PI 268771 B		9.0		7.0
36	PI 268771 B		9.0		8.5
	Average	8.2	8.4	8.6	8.0

SUMMARY OF ANALYSIS OF VARIANCE ON THE POOLED DATA OF FATTY ACID COMPOSITION AND STABILITY OF PEANUT OILS FROM THE 1968 NATIONAL VARIETY TEST

	16:0	18:0	18:1	18:2	20:0	18:3+ 20:1	22:0	O+L	O/L	KTS	KTH
Ga vs Ok (S)	**	**	**	**	NS	**	NS	*	**	**	NS
NIR vs IRR (L)	**	NS	**	**	NS	**	NS	*	**	*	NS
Variety (E)	**	**	**	**	NS	NS	NS	**	**	*	NS
S x L	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
S x E	*	**	**	NS	NS	NS	NS	NS	**	**	**
L x E	NS	NS	*	NS	NS	NS	NS	NS	*	NS	NS
S x L x E	NS	NS	*	*	NS	NS	NS	NS	**	NS	NS
Grand Mean	12.65	2.76	42.92	37.52	1.13	0.71	1.96	80.45	1.15	1.35	.83
C.V. (a)%	1.5	10.1	0.8	1.3	13.4	16.9	13.0	0.8	1.8	9.7	11.3
C.V. (b)%	2.2	6.2	1.3	1.7	15.1	30.2	13.3	0.7	2.6	9.4	7.6

NS Not significant

* 5% level

** 1% level

TABLE XVIII

THE VARIETY MEANS OF OLEIC ACID AND LINOLEIC ACID COMPOSITION OF PEANUT OILS AS AFFECTED BY STATE (GEORGIA AND OKLAHOMA) AND IRRIGATION FROM THE 1968 NATIONAL VARIETY TEST

Variety	Georgia		Oklahoma		NIR		IRR	
	O	L	O	L	O	L	O	L
	% of total							
1	45.6	35.0	41.7	39.2	44.2	36.3	43.1	38.0
2	44.1	35.9	42.3	38.4	44.1	36.1	42.3	38.2
3	45.2	35.2	42.7	38.1	43.8	36.4	44.1	36.8
4	42.6	37.8	41.0	40.1	42.5	37.8	41.0	40.1
5	45.4	35.2	42.2	39.1	44.1	36.5	43.4	37.8
6	43.7	36.1	42.0	39.1	42.9	37.1	42.8	38.2
7	43.4	36.8	40.8	40.1	42.5	38.0	41.8	38.8
8	43.5	36.2	40.7	39.6	42.4	37.2	41.8	38.6
9	44.2	35.3	41.6	38.2	43.3	36.2	42.4	37.3
Mean	44.2	35.9	41.7	39.1	43.3	36.8	42.5	38.2
Grand mean		Oleic 42.9				Linoleic 37.5		

TABLE XIX

RESULTS OF ANALYSIS OF VARIANCE ON THE O/L RATIOS OF IRRIGATED VERSUS NONIRRIGATED IN STATE

	Oklahoma	Georgia
IRR vs NIR (L)	**	*
Variety (E)	**	**
L x E	NS	**

NS Not significant

* 5% level

** 1% level

Figure 4. Gas Liquid Chromatographic Tracing of Fatty Acid
Esters From a Typical Peanut Oil.

ions were as follows:

olumn - 6' x ¼" u-shaped glass

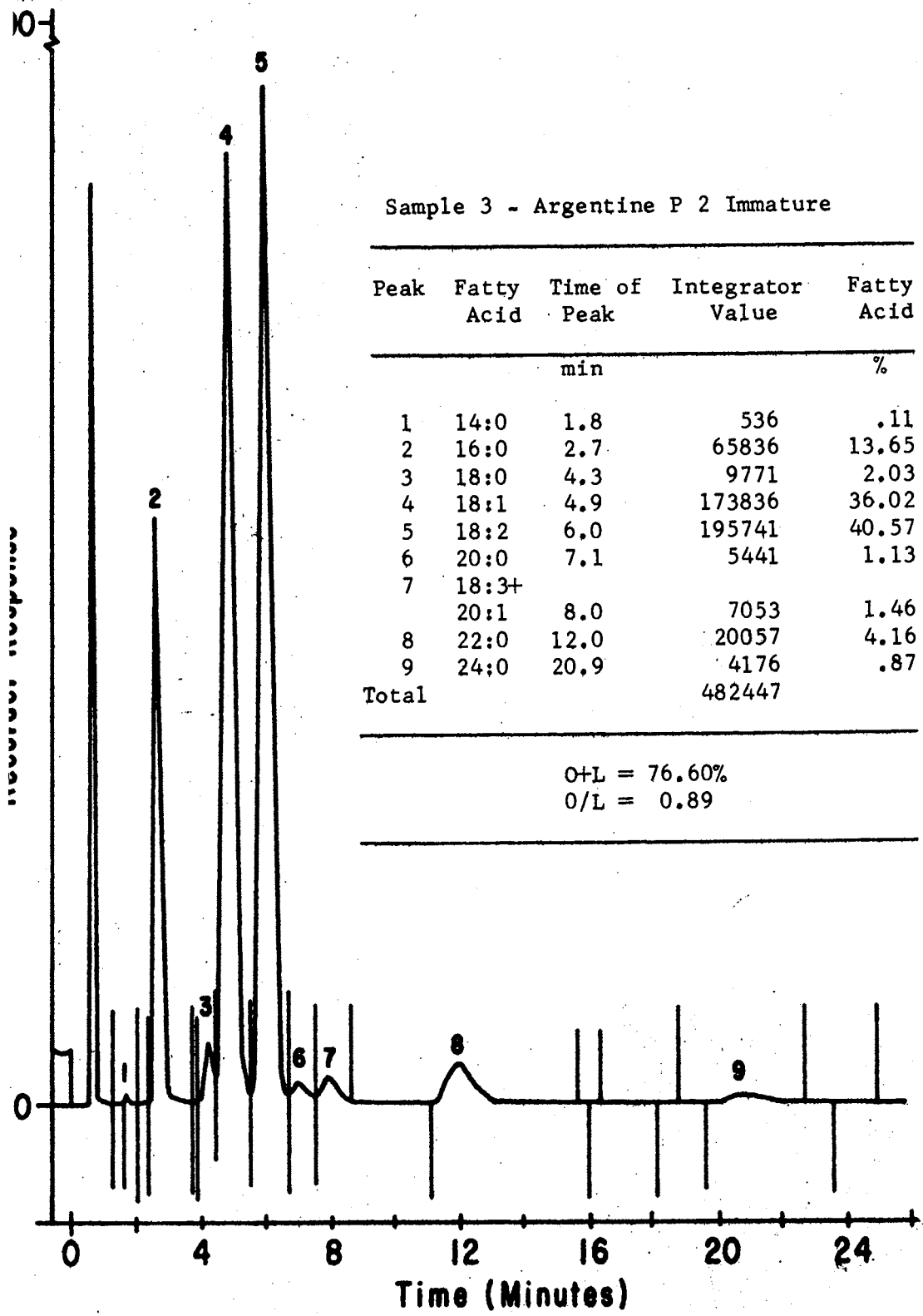
olumn Packing - 14.5 percent DEGS on Chromosorb W

olumn Temperature - 180° C

arrier Gas - Nitrogen

low Rate - 60 ml/min

etector - Flame Ionization



Sample 3 - Argentine P 2 Immature

Peak	Fatty Acid	Time of Peak	Integrator Value	Fatty Acid
		min		%
1	14:0	1.8	536	.11
2	16:0	2.7	65836	13.65
3	18:0	4.3	9771	2.03
4	18:1	4.9	173836	36.02
5	18:2	6.0	195741	40.57
6	20:0	7.1	5441	1.13
7	18:3+ 20:1	8.0	7053	1.46
8	22:0	12.0	20057	4.16
9	24:0	20.9	4176	.87
Total			482447	

O+L = 76.60%
O/L = 0.89

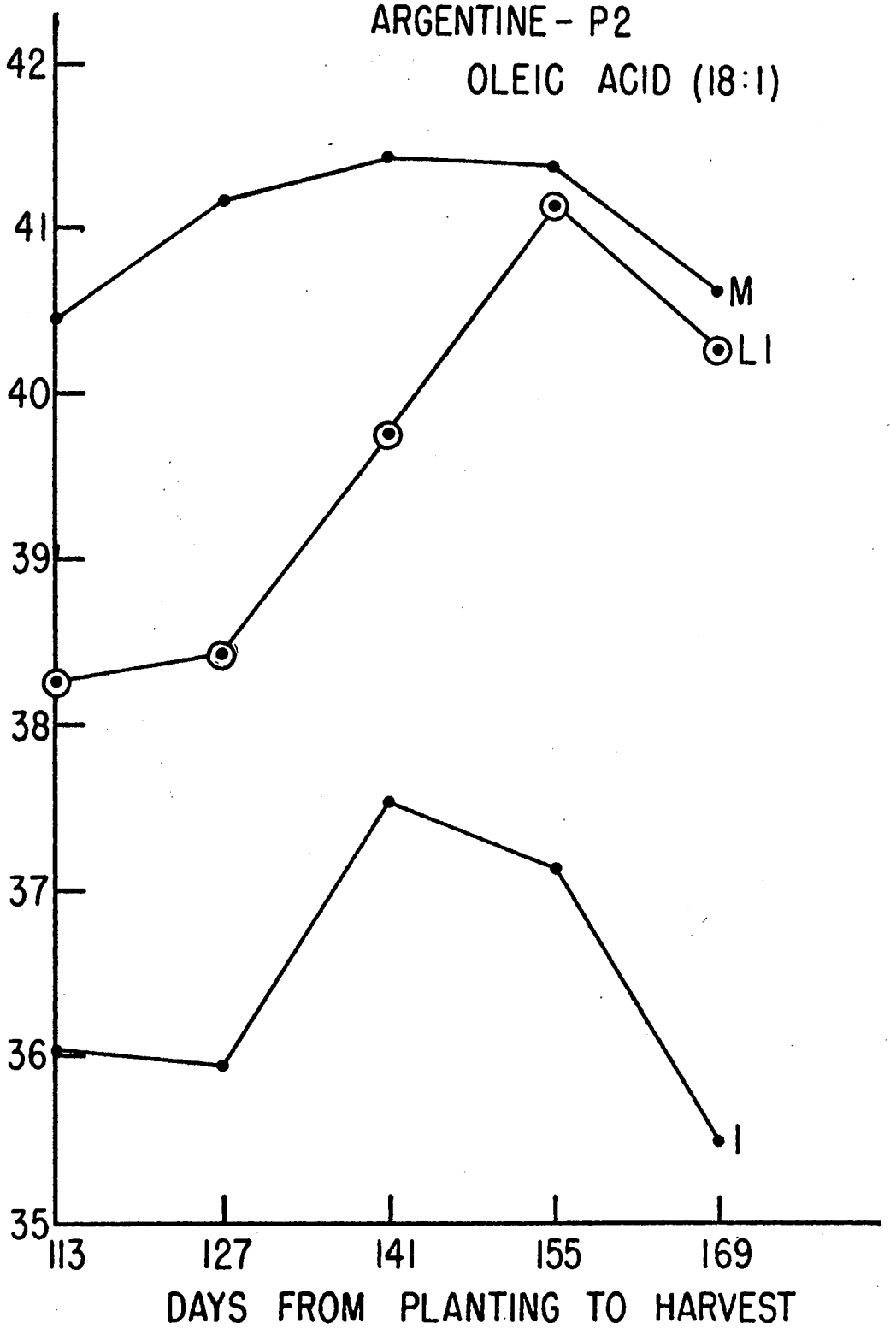


Figure 5. The Effect of Maturity and Harvest Date on Oleic Acid Composition in the Argentine Variety.

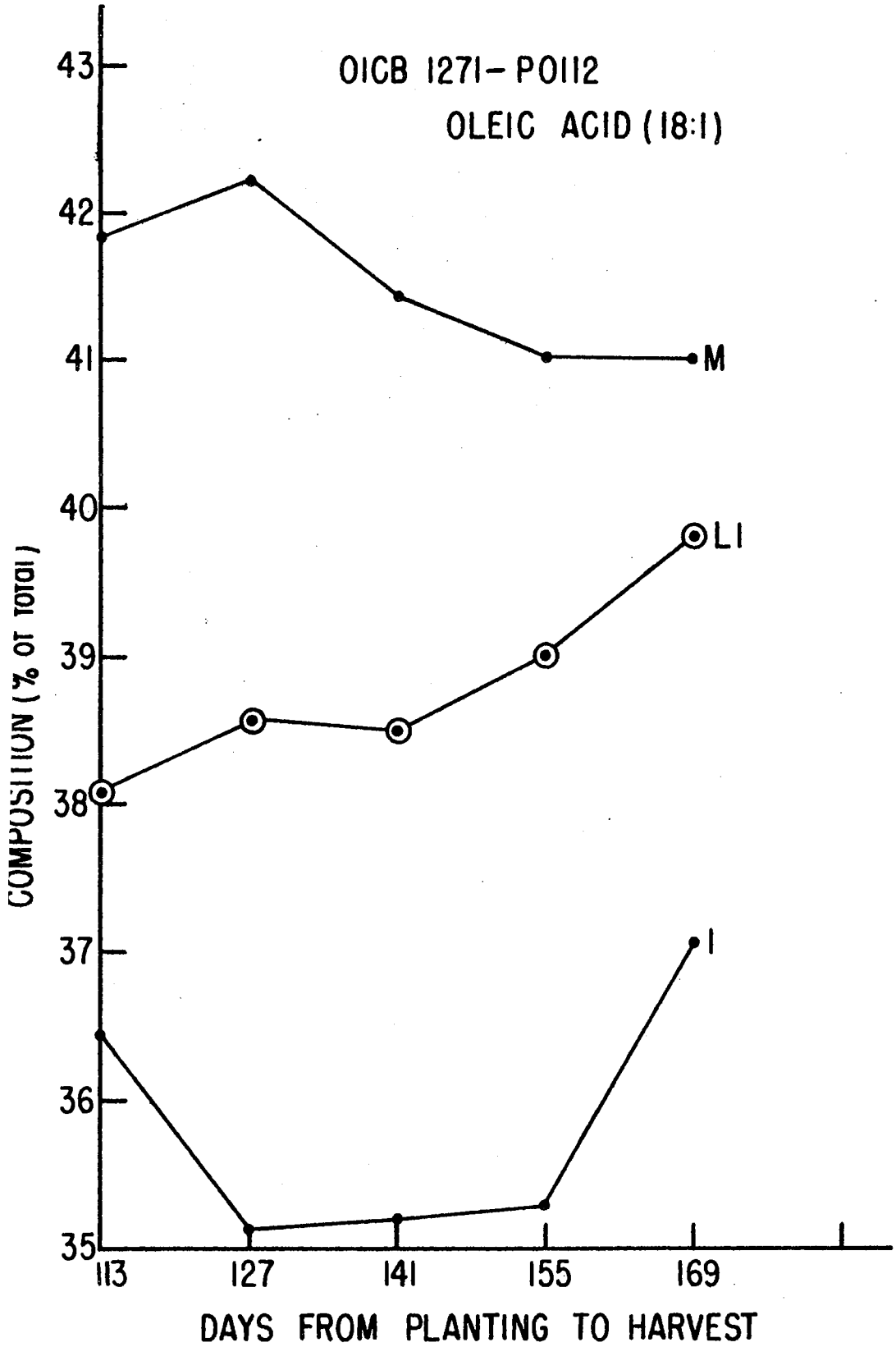


Figure 6. The Effect of Maturity and Harvest Date on Oleic Acid Compostion in the Spanhoma Variety.

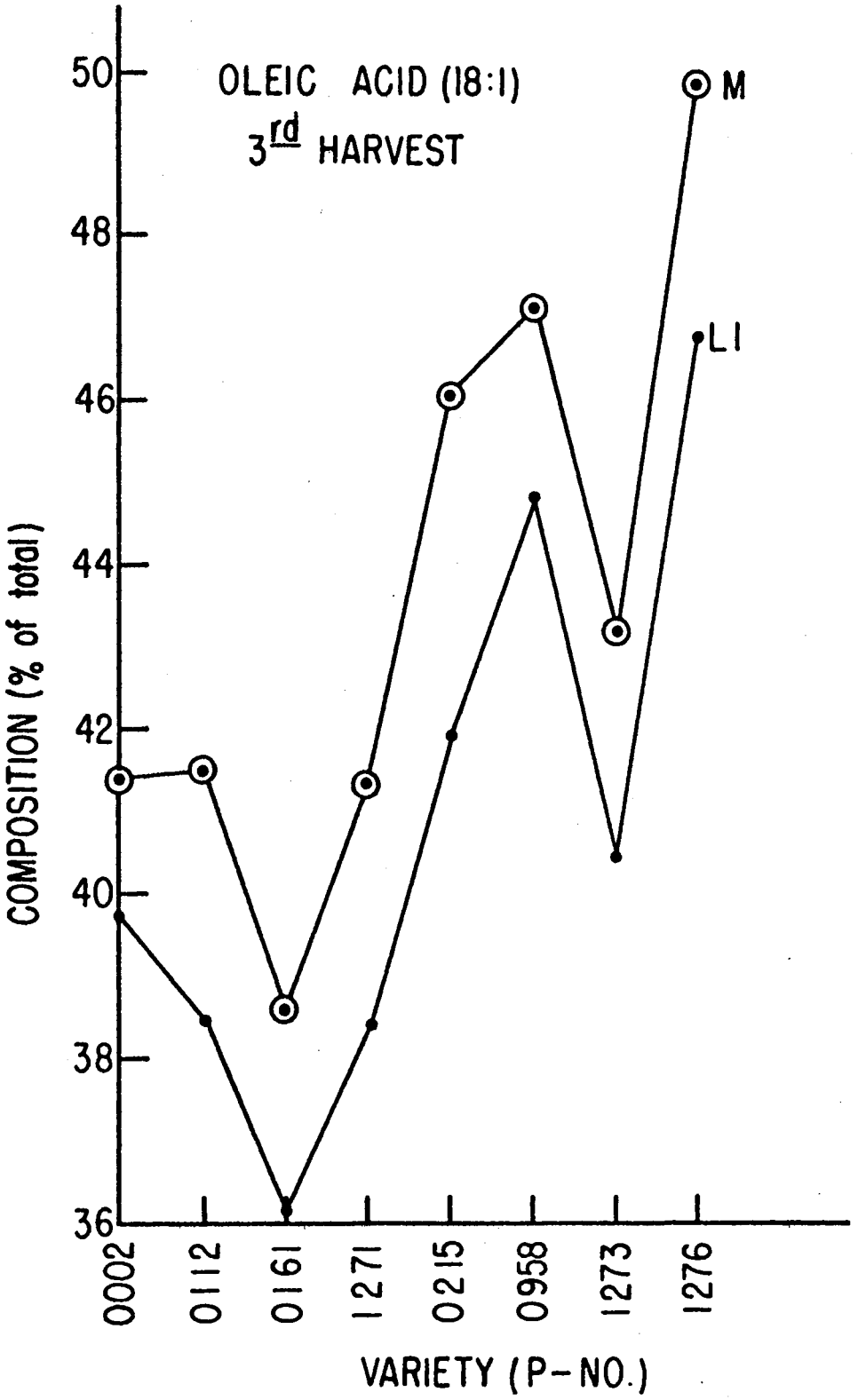


Figure 7. Variety Effect on Oleic Acid Composition.

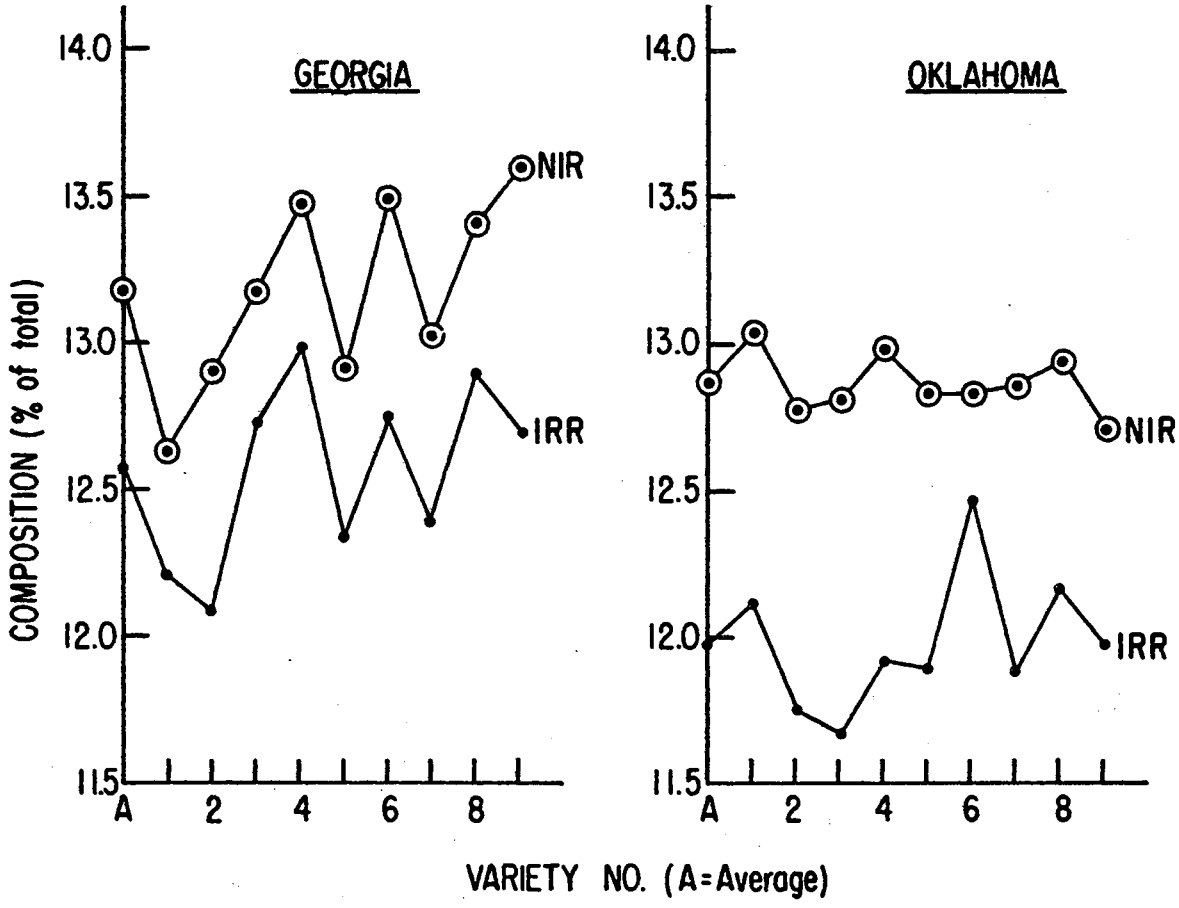


Figure 8. The Effect of State, Irrigation and Variety on Palmitic Acid Composition.

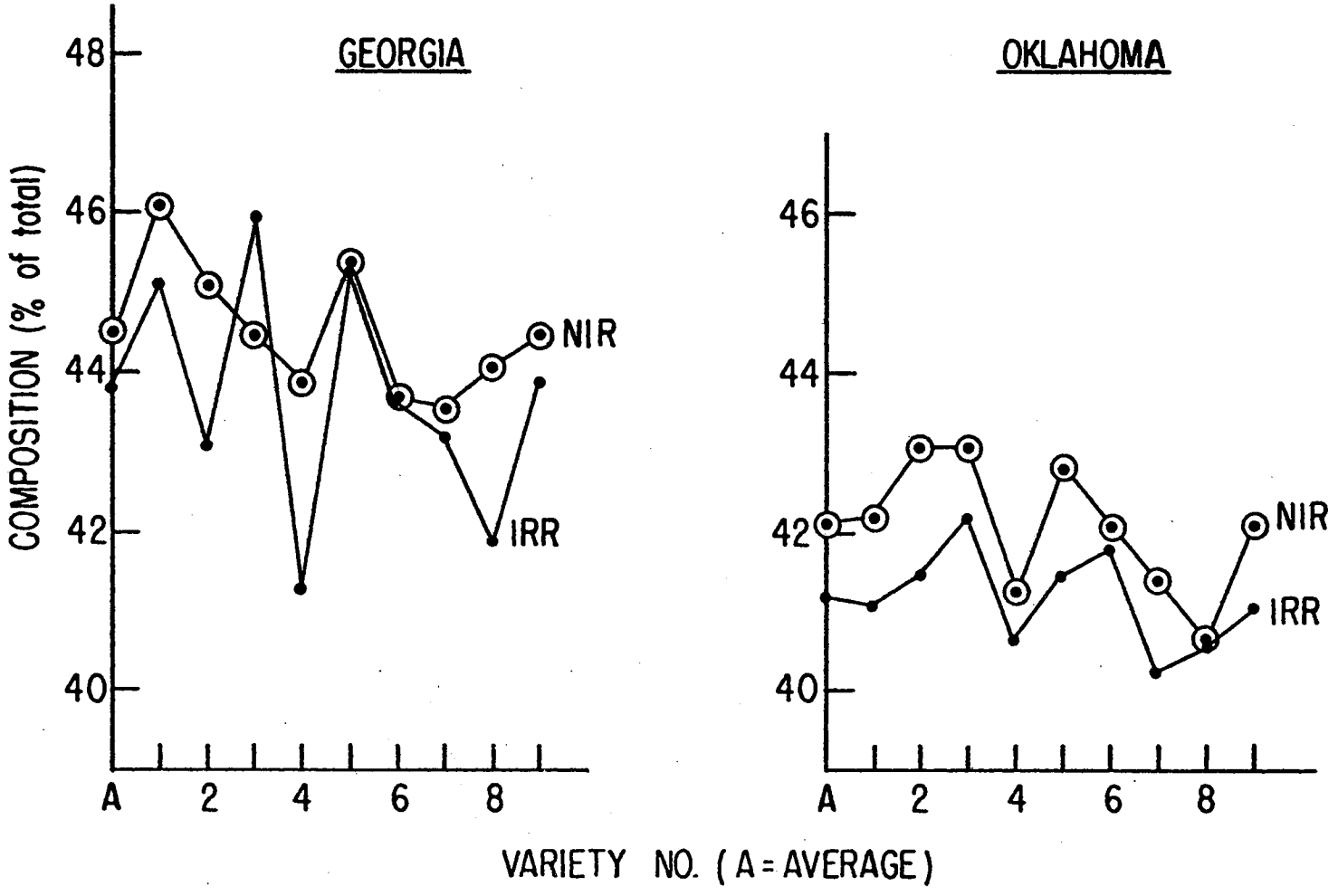


Figure 9. The Effect of State, Irrigation and Variety on Oleic Acid Composition

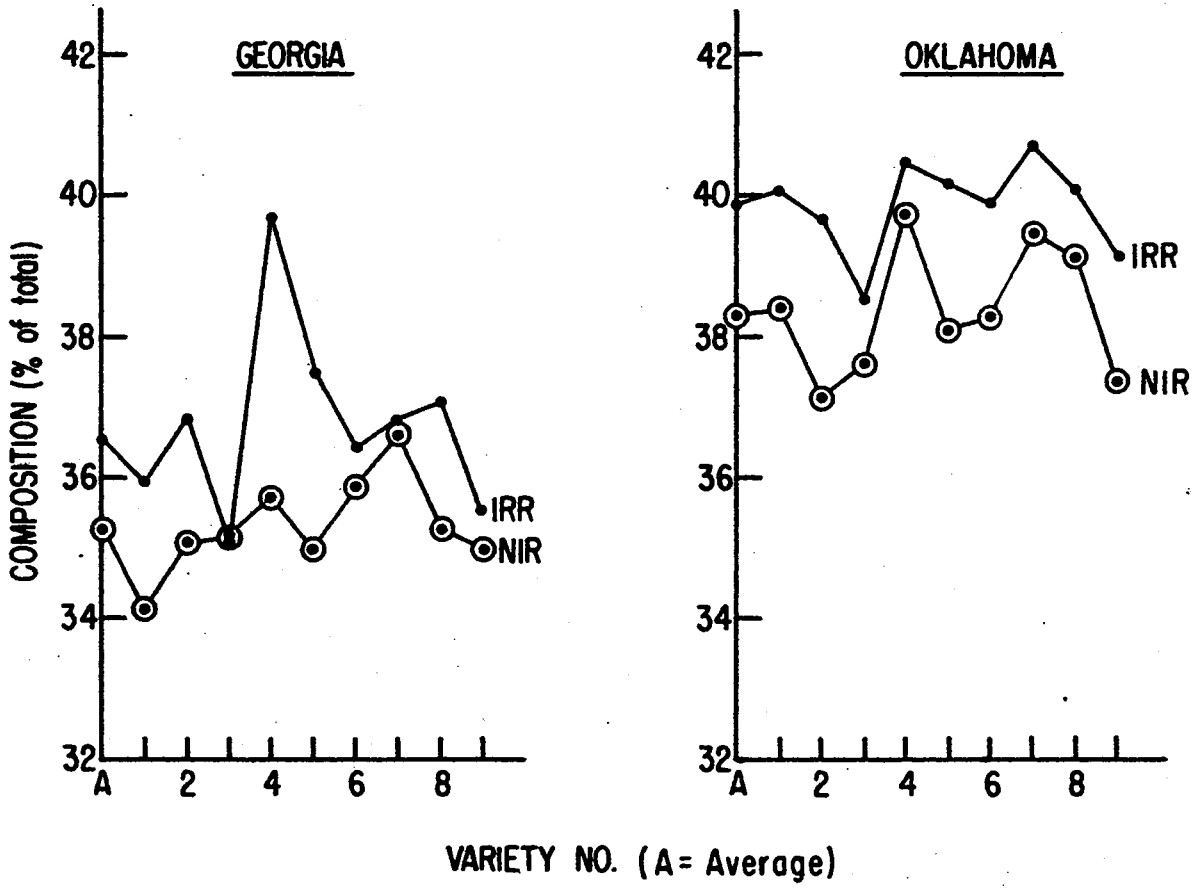


Figure 10. The Effect of State, Irrigation and Variety on Linoleic Acid Composition

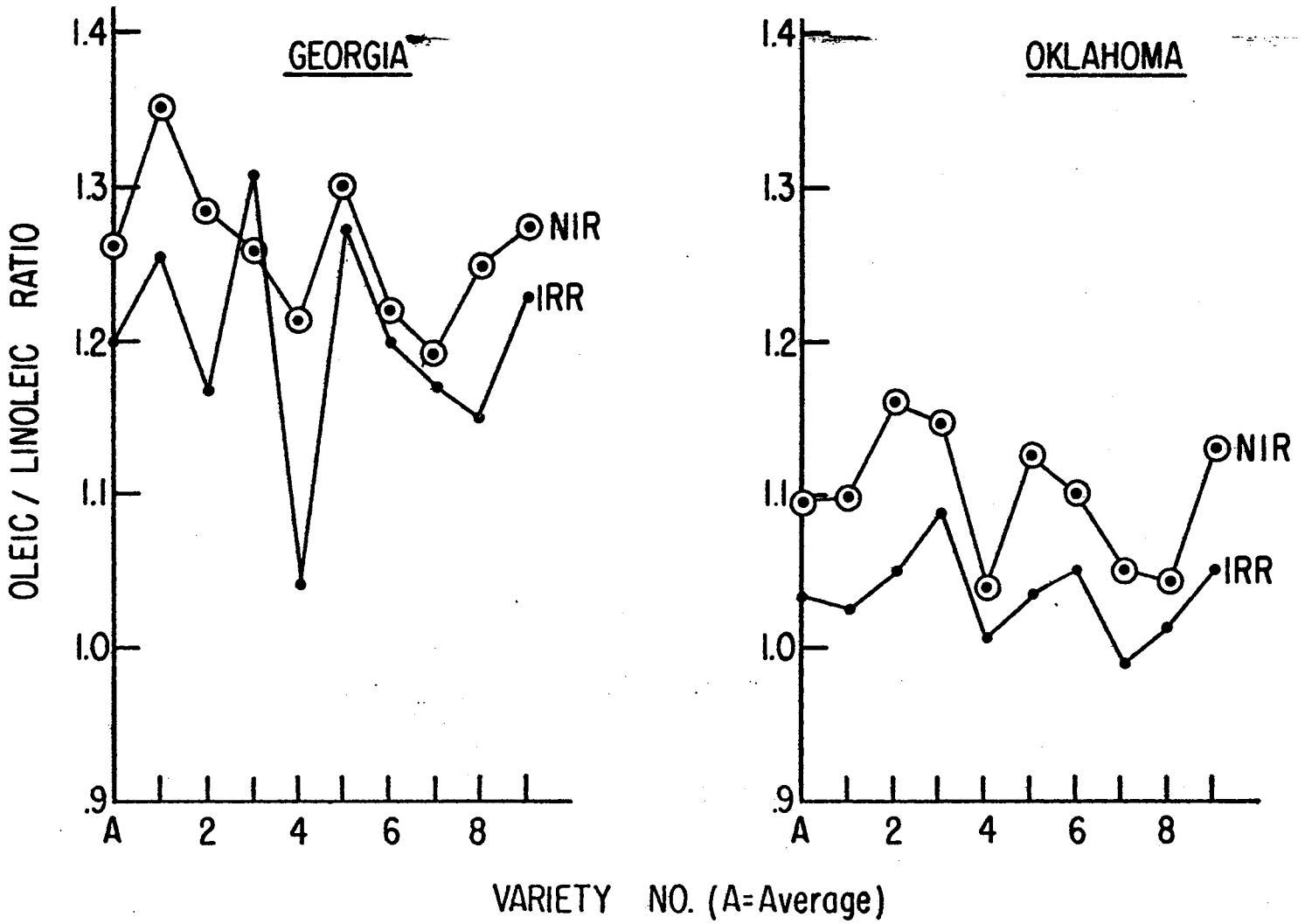


Figure 11. The Effect of State, Irrigation and Variety on the O/L Ratio.

CHAPTER V

ORGANOLEPTIC EVALUATION RELATED TO FATTY ACIDS AS INFLUENCED BY MATURITY, VARIETY AND TIME OF HARVEST

roduction

Most of the biochemical data have been obtained but correlation of data with organoleptic evaluation by a consumer type panel is a atively recent development (32, 45). The major goal of this work to attempt to measure some of the chemical components that may be lated to results from a consumer type panel which had been used very cessfully at Oklahoma State University for several years.

Procedures

Pang (32) has an excellent study that is related to this research d described in detail an organoleptic method in use for evaluating anuts at Oklahoma State University. Kirby, Choate and Collins (53) ve a brief description of the "Organoleptic Test" developed by tlock, et al. (54) and it is quoted:

> Peanut samples are selected for certain experiments to determine the flavor of the roasted peanuts and/or peanut butter.
>
> The raw shelled peanuts are placed in the modified rotisserie oven and roasted to a "golden brown" cotyledon color. After the peanuts are removed from the oven they are cooled with a fan. Twenty kernels for each of four treatments and a coded standard are exposed to each of five

panel members, who rate them for flavor, roast and preference. (CLER SCORE).

To make peanut butter the roasted peanuts are split and degermed with the splitter and the testa and germ (hearts) are separated with a hand sieve and fan. The roasted cotyledons are weighed, 0.5 per cent salt added and ground into peanut butter using the Quaker City Laboratory Mill.

Each of five panel members compare the five fresh peanut butter samples including a coded standard with a known standard with respect to flavor, odor, roast, texture and preference.

The samples used in this study are the ones grown at Perkins, Oklaa in 1967 and were studied in Chapter IV for the fatty acid compoion and in Chapter VIII for free amino acid and peptide composition affected by maturity and harvesting date.

Results and Discussion

A partial record of the more important organoleptic values are ported in Tables XX-XXVII. In many instances the sample was too all to evaluate for both peanut butter and roasted peanuts. The formation obtained on peanut butter was more variable than the sults reported by Pang (32). However, this study had only one plication because of small samples. Pang worked with peanut butter ality while in this study both peanut butter and the CLER scores on asted peanuts were examined.

Figure 12 shows a rather consistent increase in the CLER scores om 74 to 86 on mature roasted peanuts with increasing harvest dates r the Argentine variety. The low intermediate Argentine peanuts were re variable, possibly for two reasons. One, because of the inherent fficulty in visually classifying peanuts intermediate in maturity, it rmally is easier to select peanuts that are definitely mature or mature. Secondly, the major variation in this instance is the low

ER score for the low intermediate for the 141 day harvest. As pointed t elsewhere, these peanuts were dried at an excessively high tempera- re, so the low CLER scores for the 141 day harvest points to the com- tency of the taste panel. CLER scores for the standard showed some riation as indicated by the values of 84 and 90. Though the data re not statistically analyzed, the differences are believed to be significant.

Figure 13 is a plot of the mature peanuts for each variety from e mean of harvest dates. Also plotted is the mean CLER scores of the andard (from irrigated plots of Spanhoma, Ft. Cobb, Oklahoma) used organoleptic tests for each variety. The varieties were evaluated the taste panel over a three-week period and in the order of left right. As the test progressed, there was a reduction in CLER score lues for the standard. The reduction in CLER scores for the standard s probably a result of using a Spanish type peanut as the standard compare with the four non-Spanish types during the last half of the sting period. The non-Spanish types had larger kernels and would troduce some bias against the smaller Spanish standard.

Probably a more meaningful plot is the separation of the peanuts to Spanish type (P-2, P-112, P-161 and P-1271) and non-Spanish type .215, P-958, P-1273 and P-1276). The means of each maturity group each harvest in the Spanish type and non-Spanish type are plotted Figure 14. The mature and low intermediate Spanish type consistently ored higher for all harvest dates except for the 141 day harvest ich was cured at too high temperature as previously discussed. If e were to ignore the 141 day harvest of Spanish type (see dashed ne), the mean CLER score of these four varieties varied only from

) to 80.5 for the harvest season. Immature peanuts when available red lower than either the mature or low intermediate peanuts. The ure and low intermediate non-Spanish type, which are not agronomi- ly adapted to Oklahoma, scored lower than the Spanish type at the inning of the season. On the last harvest date, there was no reciable difference in the mean CLER score on the mature and low ermediate peanuts of the Spanish and non-Spanish types. The non- nish type required a longer growing season which accounted for the e in the CLER score late in the harvest season. These results point the importance of having larger samples and additional replications statistical analyses of the organoleptic data.

The variations observed make it difficult to obtain meaningful relations with fatty acid composition or other chemical components n as free amino acids, peptide and protein. More work needs to be e in the area of relating the chemical components with the consumer el data.

The panel reported low values for the samples of four varieties vested at 141 days. A later examination of the curing temperature ords revealed that the curing temperature reached 110° F which was ficient to cause off-flavor in peanuts (56). It is significant that s difference in curing temperature was observed by the taste panel it is also significant that some differences in the fatty acid com- ition and free amino acid composition were noted for the 141 day vest using chemical methods.

It should be noted that reliable chemical techniques for the luation of maturity, harvest date and variety have been developed this study. From a long-range point of view, the objective analyses

emical) may be more useful than subjective analysis (taste panel); ever, organoleptic values and chemical values must be correlated ore the latter can be used routinely.

Summary

Since this study contained only one replication, it was necessary pool the Spanish type varieties and then the non-Spanish type ieties to utilize the information available. A rather consistent rease in the CLER scores (from 74 to 86) on mature roasted peanuts h increasing harvest dates for the Argentine variety were observed. mean CLER score for the Spanish type mature and low intermediate nuts was high throughout the harvest season except for one harvest ch scored low. An examination of the curing temperatures records ealed that the temperature was sufficiently high to cause off- vor in this sample of peanuts. It is significant that this differ- e in curing temperature was observed by the taste panel and also had effect on the fatty acid composition and free amino acid composition. mean CLER score of mature and low intermediate non-Spanish type nuts increased as the harvesting season progressed. Their CLER res reached the same level as the Spanish type (169 days) by the of the growing season (176 days).

TABLE XX

PEANUT BUTTER AND CLER SCORE ON PEANUTS GROWN AT PERKINS, OKLAHOMA, 1968

Strain: ARGENTINE - Okla. P-No. 0002, Entry No. 01

chem. No.	P.B. No.	Days From Planting	Maturity	wt/100 seeds	Peanut Butter %	Pref. Rank of P.B.	ROASTED PEANUTS CLER Score	Roast	Pref. Rank
1	2353	113	Mature	28.28	84.82	4.0	--	---	---
2	2354	113	Low Int.	17.55	77.58	4.2	74	1.2	3.6
3	2355	113	Immature	13.11	51.66	7.0	68	1.4	4.0
4	2356	127	Mature	28.38	86.28	4.0	74	1.2	2.8
5	2357	127	Low Int.	22.33	81.70	2.0	76	1.3	4.0
6	2358	127	Immature	18.79	79.86	4.2	72	1.3	4.2
7	2359	141	Mature	32.85	86.28	4.2	78	1.2	4.6
8	2360	141	Low Int.	27.22	83.54	6.8	62	1.4	6.6
9	----	141	Immature	-----	-----	---	--	---	---
10	2361	155	Mature	33.62	88.57	4.6	82	1.2	3.4
11	2362	155	Low Int.	27.65	86.14	3.6	82	1.2	3.8
12	----	155	Immature	-----	-----	---	--	---	---
13	2363	169	Mature	32.40	87.58	4.8	86	1.1	2.0
14	2364	169	Low Int.	30.70	84.16	3.4	74	1.3	5.4
15	----	169	Immature	-----	-----	---	--	---	---
12	2352 Spanhoma Std.			36.44	86.82	1.3	84	1.2	2.4
	Ft. Cobb (One standard						90	1.2	2.2
	used 2 times for pea-					Total	174	2.4	4.6
	nut butter.)					Mean	87	1.2	2.3

TABLE XXI

PEANUT BUTTER AND CLER SCORE ON PEANUTS GROWN AT PERKINS, OKLAHOMA, 1968

Strain: OICB1271 - Okla. P-No. 0112, Entry No. 02

Chem. No.	P.B. No.	Days From Planting	Maturity	wt/100 seeds	Peanut Butter %	Pref. Rank of P.B.	ROASTED PEANUTS CLER Score	Roast	Pref. Rank
16	2365	113	Mature	33.12	85.16	4.6	76	1.2	3.8
17	2366	113	Low Int.	21.96	81.98	5.2	86	1.2	2.4
18	2367	113	Immature	16.58	82.43	5.4	61	1.6	6.4
19	2368	127	Mature	34.37	87.60	2.4	73	1.4	4.7
20	2369	127	Low Int.	25.97	84.18	3.0	86	1.2	3.0
21	2370	127	Immature	17.12	78.38	5.4	78	1.4	4.7
22	2372	141	Mature	31.60	87.46	5.6	65	1.4	5.4
23	2373	141	Low Int.	28.24	86.62	6.8	40	1.4	7.0
24	----	141	Immature	-----	-----	---	--	---	---
25	2374	155	Mature	33.62	88.94	3.2	82	1.3	3.4
26	2375	155	Low Int.	28.22	85.05	3.4	86	1.2	2.4
27	----	155	Immature	-----	-----	---	--	---	---
28	2376	169	Mature	34.00	86.62	3.4	72	1.4	4.4
29	2377	169	Low Int.	30.76	86.80	4.0	83	1.2	2.6
30	----	169	Immature	-----	-----	---	--	---	---
--	2371	Spanhoma Std.		35.91	86.80	1.8	81	1.4	3.0
	Ft. Cobb (One standard used 2 times)						88	1.2	2.2
						Total	169	2.6	5.2
						Mean	82	1.3	2.6

TABLE XXII

PEANUT BUTTER AND CLER SCORE ON PEANUTS GROWN AT PERKINS, OKLAHOMA, 1968

Strain: VALENCIA - Okla. P-No. P-0161, Entry No. 05

Chem. No.	P.B. No.	Days From Planting	Maturity	wt/100 seeds	Peanut Butter %	Pref. Rank of P.B.	ROASTED PEANUTS CLER Score	Roast	Pref. Rank
31	----	113	Mature	-----	-----	---	--	---	---
32	----	113	Low Int.	-----	-----	---	--	---	---
33	2378	113	Immature	16.38	86.24	5.0	66	1.4	4.0
34	2379	127	Mature	39.77	88.32	1.8	90	1.2	2.0
35	2380	127	Low Int.	31.56	83.88	3.0	80	1.2	2.9
36	2381	127	Immature	24.44	82.52	4.0	75	1.3	3.6
37	2382	141	Mature	40.90	90.45	4.2	63	1.4	4.0
38	2383	141	Low Int.	32.80	86.81	4.6	55	1.4	5.0
39	2384	141	Mature	-----	-----	---	--	---	---
40	2385	155	Mature	44.36	-----	3.0	84	1.3	2.4
41	----	155	Low Int.	-----	-----	---	--	---	---
42	----	155	Immature	-----	80.03	---	--	---	---
43	2386	169	Mature	48.57	82.90	2.2	81	1.2	2.4
44	----	169	Low Int.	-----	-----	---	--	---	---
45	----	169	Immature	-----	-----	---	--	---	---
112	2384 Spanhoma Std.			34.67	90.00	1.1	83	1.2	2.5
	Ft. Cobb (One standard						90	1.2	1.2
	used 2 times)					Total	173	2.4	3.7
						Mean	86	1.2	1.8

TABLE XXIII

PEANUT BUTTER AND CLER SCORE ON PEANUTS GROWN AT PERKINS, OKLAHOMA, 1968

Strain: DIXIE SPANISH - Okla. P-No. P-1271, Entry No. 06

›chem. ›. No.	P.B. No.	Days From Planting	Maturity	wt/100 seeds	Peanut Butter %	Pref. Rank of P.B.	ROASTED PEANUTS CLER Score	Roast	Pref. Rank
46	----	113	Mature	-----	-----	---	--	---	---
47	2388	113	Low Int.	21.70	90.50	2.2	--	---	---
48	2389	113	Immature	15.29	79.44	5.2	79	1.4	3.0
49	2390	127	Mature	31.77	87.80	3.2	74	1.3	3.1
50	2391	127	Low Int.	25.97	85.58	2.4	74	1.3	3.3
51	2392	127	Immature	19.45	86.42	5.6	71	1.4	3.8
52	2393	141	Mature	33.87	86.54	2.8	55	1.4	5.0
53	2394	141	Low Int.	26.49	82.88	6.6	38	1.6	6.0
54	----	141	Immature	-----	-----	---	--	---	---
55	2395	155	Mature	35.40	86.32	2.8	74	1.3	4.2
56	2396	155	Low Int.	31.96	85.04	2.8	74	1.3	3.4
57	----	155	Immature	-----	-----	---	--	---	---
58	2397	169	Mature	36.03	85.68	3.6	82	1.2	3.6
59	2398	169	Low Int.	33.81	81.86	3.8	74	1.5	4.2
60	----	169	Immature	-----	-----	---	--	---	---
0112	2387 Spanhoma Std.			37.33	91.25	2.4	85	1.2	1.2
	Ft. Gobb (One standard						90	1.2	1.6
	used 2 times)					Total	175	2.4	2.8
						Mean	88	1.2	1.4

TABLE XXIV

PEANUT BUTTER AND CLER SCORE ON PEANUTS GROWN AT PERKINS, OKLAHOMA, 1968

Strain: EARLY RUNNER - Okla. P-No. P-0215, Entry No. 03

›chem. ›. No.	P.B. No.	Days From Planting	Maturity	wt/100 seeds	Peanut Butter %	Pref. Rank of P.B.	ROASTED PEANUTS CLER Score	Roast	Pref. Rank
61	----	120	Mature	-----	-----	---	--	---	---
62	2399	120	Low Int.	30.46	82.90	3.8	--	---	---
63	2400	120	Immature	20.39	90.21	7.0	40	1.6	5.8
64	----	134	Mature	-----	-----	---	--	---	---
65	2401	134	Low Int.	38.04	82.86	4.4	72	1.4	2.9
66	2402	134	Immature	26.90	84.36	6.0	56	1.6	5.0
67	2403	148	Mature	43.23	87.00	3.0	78	1.3	1.9
68	2404	148	Low Int.	24.04	87.92	2.8	64	1.4	3.2
69	----	148	Immature	-----	-----	---	--	---	---
70	2405	162	Mature	48.81	79.63	2.8	64	1.4	3.8
71	2406	162	Low Int.	41.16	86.26	5.0	62	1.5	4.8
72	----	162	Immature	-----	-----	---	--	---	---
73	2407	176	Mature	48.17	87.64	2.6	78	1.3	2.6
74	2408	176	Low Int.	45.94	83.20	3.6	76	1.4	2.8
75	----	176	Immature	-----	-----	---	--	---	---
0112	2416 Spanhoma Std.			33.51	86.66	1.1	76	1.4	2.2
	Ft. Cobb (One standard						88	1.2	1.0
	used 2 times)					Total	164	2.6	3.2
						Mean	82	1.3	1.6

TABLE XXV

PEANUT BUTTER AND CLER SCORE ON PEANUTS GROWN
AT PERKINS, OKLAHOMA, 1968

Strain: NC5 - Okla. P-No. P-0958, Entry No. 04

)chem.). No.	P.B. No.	Days From Planting	Maturity	wt/100 seeds	Peanut Butter %	Pref. Rank of P.B.	ROASTED PEANUTS CLER Score	Roast	Pref. Rank
76	----	120	Mature	-----	-----	---	--	---	---
77	2409	120	Low Int.	40.88	85.16	2.0	peanut butter only		
78	2410	120	Immature	34.06	78.86	5.0	60	1.6	3.6
79	----	134	Mature	-----	-----	---	--	---	---
80	2411	134	Low Int.	51.43	81.78	3.4	72	1.4	2.2
81	2412	134	Immature	44.72	67.30	3.6	64	1.5	3.2
82	2413	148	Mature	64.64	84.16	2.0	60	1.6	3.8
83	2414	148	Low Int.	53.28	89.24	3.2	72	1.6	2.2
84	2415	148	Immature	40.44	84.40	3.8	65	1.7	3.0
85	2418	162	Mature	63.86	87.46	2.4	74	1.5	3.8
86	2419	162	Low Int.	56.94	81.44	5.0	63	1.6	4.8
87	----	162	Immature	-----	-----	---	--	---	---
88	2420	176	Mature	58.79	78.80	4.0	88	1.2	2.0
89	2421	176	Low Int.	56.10	86.24	2.4	84	1.4	2.4
90	----	176	Immature	-----	-----	---	--	---	---
0112	2417 Spanhoma Std. Ft. Cobb			36.66	90.00	1.0	84	1.4	1.0
0112	2417 Spanhoma Std. Ft. Cobb			-----	-----	1.0	86	1.3	1.0
0112	2438 Spanhoma Std. Ft. Cobb			35.02	88.00	1.8	82	1.4	2.0
	Total			71.68	178.00	3.8	192	4.1	4.0
	Mean			23.89	89.00	1.3	84	1.4	1.4

TABLE XXVI

PEANUT BUTTER AND CLER SCORE ON PEANUTS GROWN
AT PERKINS, OKLAHOMA, 1968

Strain: GA. 61-42 - Okla. P-No. P-1273, Entry No. 07

ɔchem. ɔ. No.	P.B. No.	Days From Planting	Maturity	wt/100 seeds	Peanut Butter %	Pref. Rank of P.B.	ROASTED PEANUTS CLER Score	Roast	Pref. Rank
91	----	120	Mature	-----	-----	---	--	---	---
92	2422	120	Low Int.	26.35	88.92	3.0	58	1.8	5.6
93	2423	120	Immature	21.22	88.58	7.8	60	1.4	6.2
94	2424	134	Mature	38.00	96.03	3.6	peanut butter only		
95	2425	134	Low Int.	33.01	81.06	3.8	74	1.4	2.8
96	2426	134	Immature	27.35	63.82	6.0	62	1.6	5.8
97	2427	148	Mature	38.27	81.96	2.6	72	1.4	4.2
98	2428	148	Low Int.	36.12	89.98	4.6	82	1.2	1.6
99	----	148	Immature	-----	-----	---	--	---	---
L00	2429	162	Mature	46.63	83.33	2.8	80	1.2	2.4
L01	2430	162	Low Int.	44.00	86.62	3.2	58	1.5	4.6
L02	----	162	Immature	-----	-----	---	--	---	---
L03	2431	176	Mature	46.14	84.08	2.6	84	1.1	1.8
L04	2432	176	Low Int.	38.28	88.50	3.8	73	1.2	3.0
L05	----	176	Immature	-----	-----	---	--	---	---
)112	2438	Spanhoma Std.	Ft. Cobb	35.02	88.00	2.6	82	1.3	1.8
)112	2446	Spanhoma Std.	Ft. Cobb	34.69	88.06	2.6	76	1.4	3.2
	Total			69.71	176.06	5.2	158	2.7	5.0
	Mean			34.86	88.03	2.6	79	1.4	2.5

TABLE XXVII

PEANUT BUTTER AND CLER SCORE ON PEANUTS GROWN
AT PERKINS, OKLAHOMA, 1968

Strain: VA. BUNCH '67 - Okla. P-No. P-1276, Entry No. 08

ochem. lb. No.	P.B. No.	Days From Planting	Maturity	wt/100 seeds	Peanut Butter %	Pref. Rank of P.B.	ROASTED PEANUTS CLER Score	Roast	Pref. Rank
106	----	120	Mature	-----	-----	---	--	---	---
107	2433	120	Low Int.	42.24	92.10	4.0	84	1.3	1.6
108	2434	120	Immature	32.46	75.94	5.6	73	1.4	3.6
109	2435	134	Mature	60.62	87.56	1.8	peanut	butter	only
110	2436	134	Low Int.	48.53	85.82	3.8	60	1.5	3.4
111	2437	134	Immature	39.15	77.12	4.2	44	1.6	5.0
112	2439	148	Mature	61.42	80.95	2.0	peanut	butter	only
113	2440	148	Low Int.	51.51	88.14	2.6	66	1.5	2.6
114	2441	148	Immature	34.18	66.22	4.0	74	1.4	2.2
115	2442	162	Mature	63.32	84.56	4.8	84	1.3	2.3
116	2443	162	Low Int.	47.21	87.85	3.6	78	1.2	2.6
117	----	162	Immature	-----	-----	---	--	---	---
118	2444	176	Mature	69.46	85.44	3.2	78	1.4	3.0
119	2445	176	Low Int.	57.90	87.26	2.0	83	1.3	2.3
120	----	176	Immature	-----	-----	---	--	---	---
-0112	2446	Spanhoma Std.	Ft. Cobb	34.69	88.06	1.6	82	1.2	1.4
-0112	2447	Spanhoma Std.	Ft. Cobb	33.88	86.66	1.4	84	1.2	1.2
-0112	2447	Spanhoma Std.	Ft. Cobb	33.88	-----	1.4	74	1.4	4.8
	Total			102.45	164.72	4.4	240	3.8	7.4
	Mean			34.15	82.36	1.5	80	1.3	2.5

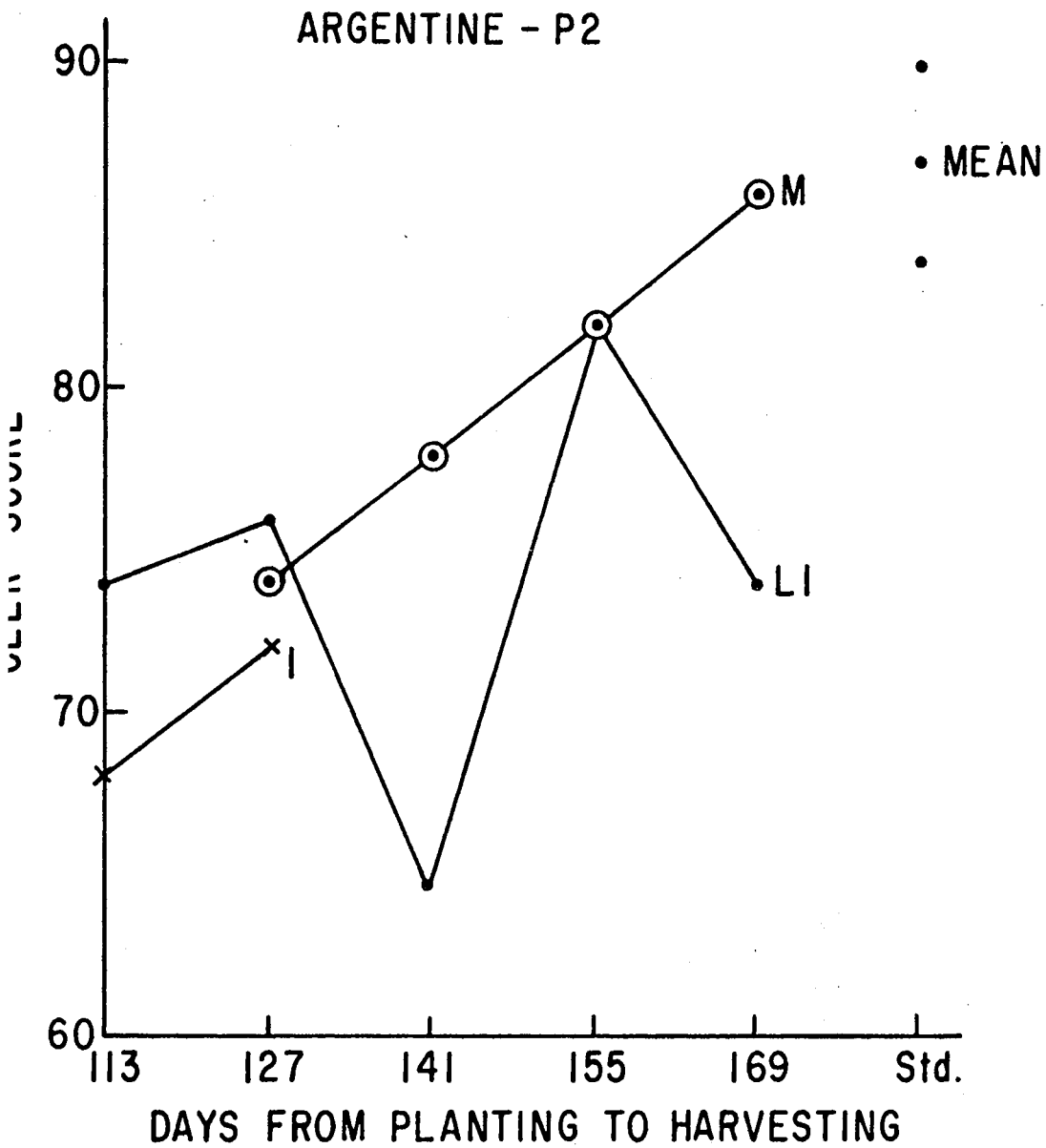


Figure 12. The Effect of Maturity and Harvest Date on CLER Score for the Peanut Variety Argentine P-2.

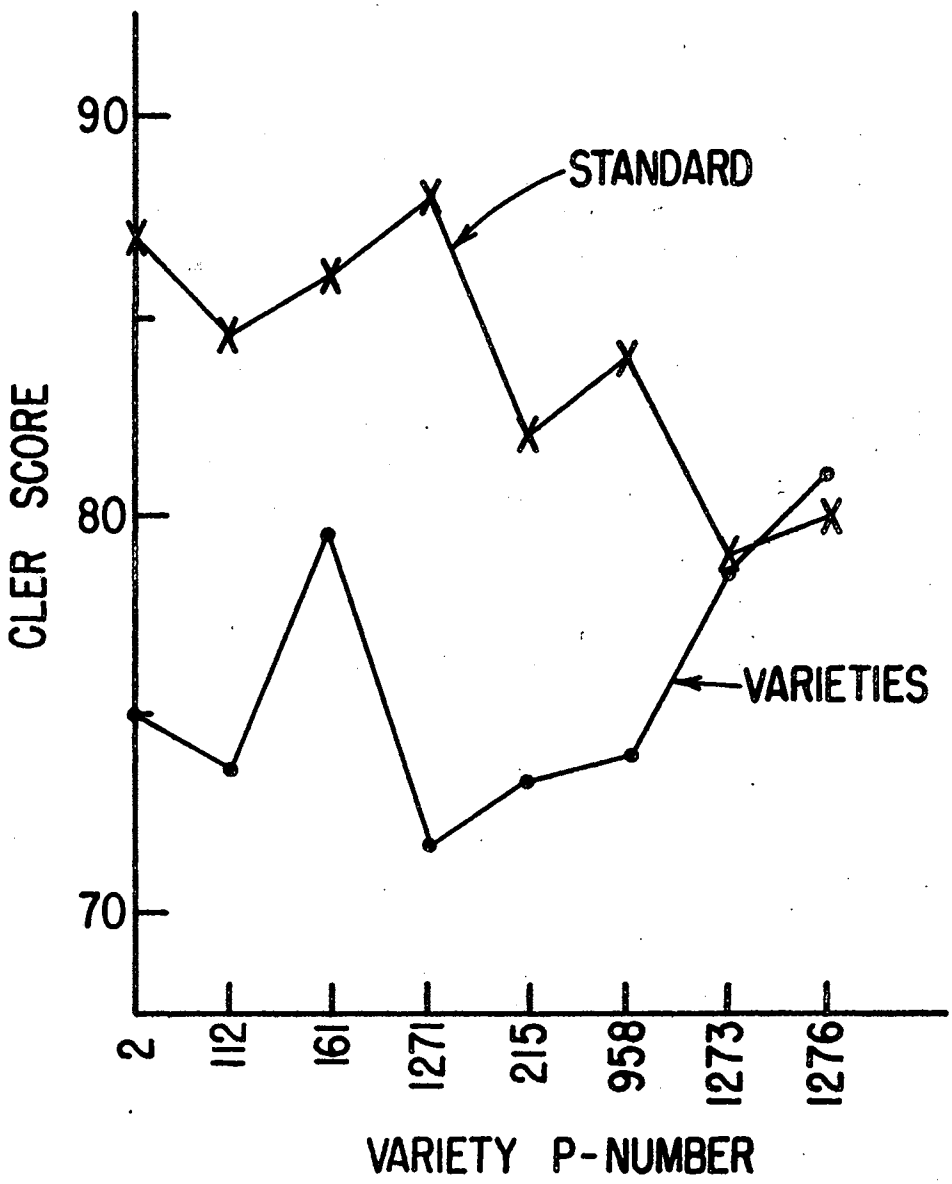


Figure 13. A Comparison of the Mean CLER Score for the Mature Peanuts of each Variety and its Standard.

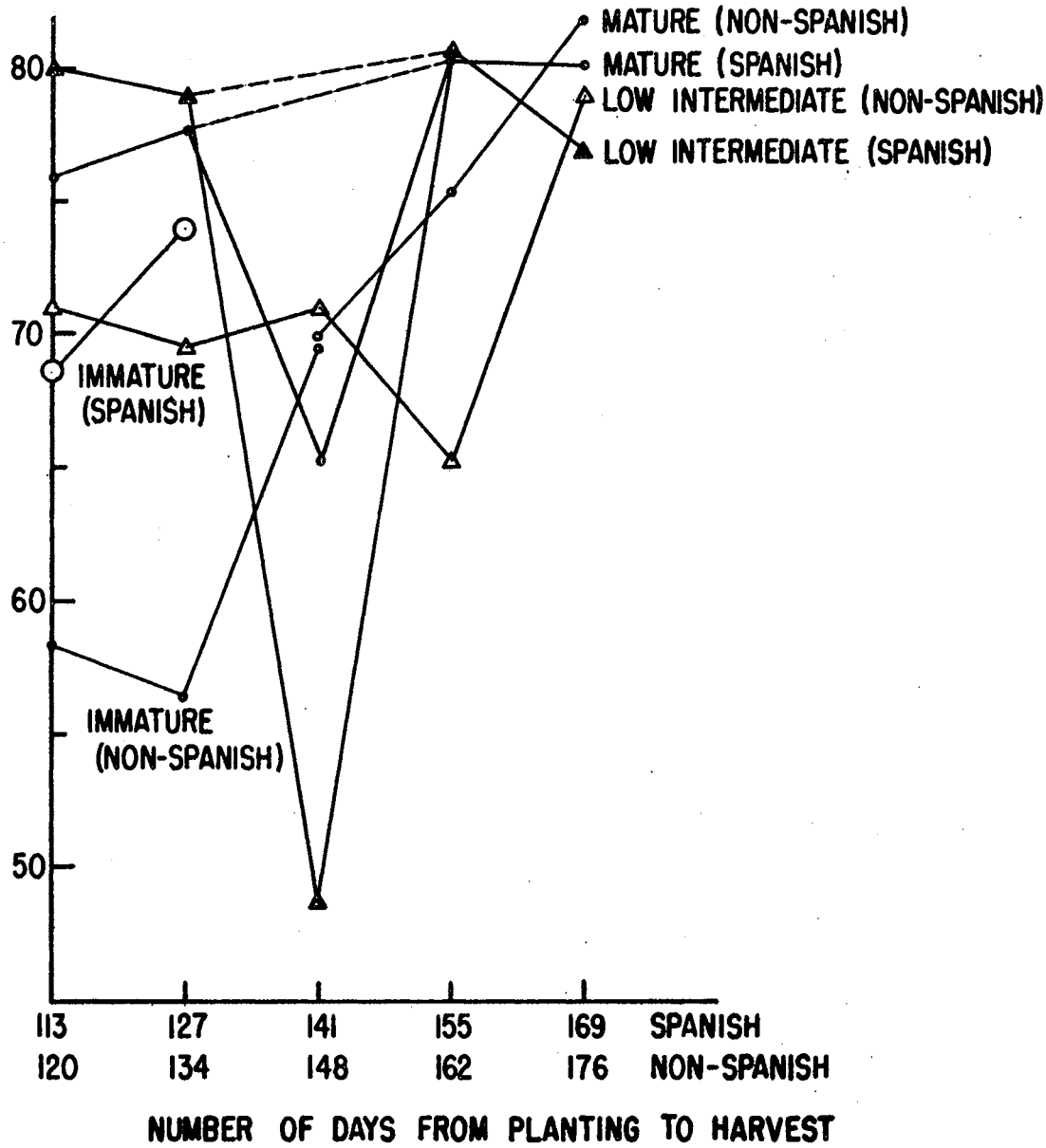


Figure 14. The Effect of Maturity and Harvest Date on the Mean CLER Score for the Spanish type and Non-Spanish type Peanuts.

CHAPTER VI

AMINO ACID COMPOSITION OF PEANUT FLOUR AS RELATED TO VARIETY

ıtroduction

There is a growing demand for a balanced dietary source of protein) supply the needs of the world population. Only recently with the ıtomation of ion-exchange chromatography using the Spackman, Stein ıd Moore (16) technique, has it been possible to obtain accurate ılues for the amino acid composition of food products. In a summary :ticle in 1953, Hoffpauir (9) reported the amino acid composition : peanuts. Since then several other publications (58, 59, 61) have ;ported the total amino acid composition of peanuts which are not in ;reement. Some of these papers (60, 61) report several varieties and) not show large varietal differences, although the latter paper (61) :ated that small but significant differences of nitrogen, serine, lutamic acid, proline, alanine, leucine, phenylalanine, tyrosine, ysine, methionine and cystine content were found. The differences ı nitrogen for nine varieties varied only from 10.69 percent to 10.81 ercent. Results of Young and Holley (31) showed considerable varia- lon in the percentage of nitrogen among peanut varieties. To the nowledge of this author, there has been no study on the effect of ydrolysis time for peanut protein as has been studied by Tkachuk and rvine (62) on wheat.

The first goal of this study was to develop improved methods that d permit uniform and precise determinations of total amino acids to ade in peanuts and peanut products. The second and major goal was xamine various varieties of peanuts for their amino acid patterns eanut flour. Sixteen varieties with a wide variation in protein ent were used for this phase of study.

ratus

Amino acid analyses were made using the ion-exchange column chroma-aphy technique of Spackman, et al. (16) on a Beckman Model 120-C o Acid Analyzer using the P-28 resin for acidic and neutral amino s and the P-35 resin for basic amino acid (as recommended in the man Procedure manual, 63).

Hydrolyzate tubes used in this study were constructed from a mm bore Teflon stopcock (Figure 15).

ents

Reagent grade chemicals were used.

For buffers used in ion-exchange chromatography on the analyzer, Beckman Procedure Manual of instructions was followed (63).

Procedure

aration of peanut meal sample

Peanut samples were selected from samples grown at Tifton, Georgia 965. These were hand shelled and selected for sound mature kernels) (as described in Chapter III, page 14). The peanut meal was pre-d according to the standard method (64).

Nitrogen was determined by the Macro-Kjeldahl method (64) and reported on an oven-dry basis. Nitrogen may be converted to protein sing the conversion factor of 5.5 (64, 66).

olysis of Peanut Meal with HCl

Direct hydrolysis of samples was carried out with 6 N HCl to ob- ı hydrolysates suitable for analysis. Tryptophan was not determined e it was destroyed by acid hydrolysis.

Approximately twenty mg of fat-free peanut meal was accurately hed on a micro analytical balance into the hydrolyzate tubes ribed above. Two ml. of 6 N HCl was added and the tube cooled to C and evacuated with a water aspirator. The stopcock was closed the samples were placed in a 110° C oven for the prescribed length ime. The tube was removed and the hydrolyzed sample transferred ı water moistened filter paper (Whatman #1) in a funnel and filtered emove the insoluble humin (humin must be removed for it binds versibly with the ion-exchange resin). The samples were evaporated ryness on a rotary evaporator. The sample was dissolved in 10 ml H 2.2 citrate buffer (prepared according to the Beckman instruc- ı manual, (63) and stored at -20° C until analyzed. Each column ired 0.5 ml of buffered sample for determination of the amino ds.

no Acid Standards

A series of standards from Spinco Division, Beckman Instruments, . were analyzed to obtain a measure of precision of the instrument methodology (included variation due to application of sample to

umn). The major limiting factors as reported in the Beckman instruc-
n manual are ninhydrin and the technique and skill of the operator.
overies of the amino acids were in the range of 0.25 - 3.0 μm (100 ±
ee percent) under normal operating conditions (63). Each time samp-
were analyzed, standards were run to insure precision and accuracy.

Results and Discussion

Peanut meal samples were analyzed to reveal details of amino acid
coveries as a function of hydrolysis time and to determine how best
analyze for variety variation in amino acid composition.

Preliminary studies using 10 to 15 mg samples did not give satis-
ctory results and thus were increased to 20 mg which were utilized
this study.

Table XXVIII records the data of various hydrolysis times. The
5 hour hydrolysis time was sufficient for methionine only. With
e 12-30 hour period, there were only small differences. It was
served in the preliminary studies that time periods of longer than
hours hydrolysis decreased the amount of many of the amino acids;
finding in agreement with a published study on wheat (62). Thus
e decision was made to hydrolyze the peanut meal for 15 hours.
andards of amino acids during this study gave a recovery of 100 ±
62 percent which is better than the normal expected recovery of
)0 ± three percent (63). In this hydrolyzate study and later the
ıriety study, there was considerable variation in the ammonia
alyses. A recent paper by Thachuk and Irvine (62) pointed out
ıat filter paper must be washed to remove ammonia, thus, now ex-
laining our problem with reproducibility in the ammonia de-

mination. Since ammonia was variable, it was not included in calating the total recovery of the amino acids. Five of the time study nples (12, 18 and 30 hours) were analyzed at the same time and the 15. ır sample at another time. The five samples averaged a total of 423 μm/mg of peanut meal with a ± 2.75 percent variation. The two hour samples had a ± 1.78 percent variation. The average variation r all seven samples was ± 2.47 percent. Since the standard was ± 62 percent the precision of the hydrolyzate method was ± 0.85 percent.

Table XXIX shows nitrogen content of peanuts and of peanut meal the 16 varieties used. These varieties were selected because of eir wide range of protein content which had been noted in earlier udies (31, 35).

The amino acid composition was determined as previously described. sults are recorded in Table XXX. Duplicate analyses were made on ve varieties, with the duplicates being weighed, hydrolyzed, analyzed d calculated at different times using coded sample numbers. This 11 give another estimate of analytical variation which will be used en comparing variety differences. At the time these samples were alyzed on the amino acid analyzer, the aspartic content was subject considerable variation on the column used; however, no explanation n be offered at this time. Thus aspartic acid and ammonia values e not included in the following discussion. The average variation tween duplicate analyses for the five varieties was ± 1.63 percent. is compares very favorably with the ± 1.62 percent value determined for the standards.

Lysine is often considered to be deficient in peanut protein 50, 67) and has been plotted in Figure 16 to illustrate the large

riety differences. The differences between certain varieties were rge enough to be of significance to the plant breeder wanting to crease the lysine content of peanuts. Methionine content another amino id considered to be deficient (60, 67) has been plotted in the same gure for a comparison. Methionine content of meal ranges from .040 /mg for variety 25 to a low of 0.19 μm/mg for variety 61, a 2-fold ıriation.

Figure 17 shows a plot of two other amino acids sometimes referred as being deficient (24, 60) in peanuts. The variability of both isoucine and threonine were clearly demonstrated.

Chopra and Sidhu (61) indicated in their study that the nine rieties they examined would probably not permit development of a riety of superior protein quality. This study for the first time s clearly shown that the variation was present in peanuts for the velopment of superior protein quality. This study was on sixteen rieties. There are more than 3,000 accesions of cultivated varieies in the plant introduction station and more than 20,000 different eeding lines of cultivated peanuts available in this country (86). th these materials available it is possible that there already exists peanut line that is of superior protein quality.

Summary

A hydrolyzate procedure with a precision and accuracy on duplite samples of ± 1.62 percent was described. The procedure was used) examine 16 varieties of peanuts that had a range of protein content :om 24-30 percent in the kernels. Variation of approximately twoɔld for the limiting essential amino acids (lysine, methionine, iso-

ucine and threonine) were found which had not previously been reported ese variations will permit the development of improved quality of pea- t protein.

TABLE XXVIII

AMINO ACID RECOVERIES FROM DIXIE SPANISH PEANUT MEAL

ıino Acid	Hydrolysis Time (hrs)							
	6.5	12	12	15	15	18	18	30
	μm/mg							
ʏsine	.086	.109	.105	.122	.120	.114	.106	.104
ɪstidine	.049	.066	.062	.070	.072	.068	.062	.061
ɯmonia	.660	.672	.635	.596	.642	.658	.592	.594
ːginine	.242	.306	.298	.346	.352	.327	.296	.295
ʒpartic Acid	.428	.462	.440	.454	.484	.477	.448	.432
ɪreonine	.065	.086	.088	.112	.112	.096	.092	.094
ɜrine	.234	.266	.268	.258	.280	.285	.266	.252
ɭutamic Acid	.577	.677	.677	.802	.740	.726	.682	.664
ːoline	.154	.184	.184	.190	.206	.200	.179	.182
ɭycine	.394	.390	.399	.380	.408	.410	.389	.375
ɭanine	.192	.207	.211	.212	.230	.228	.211	.205
ɜlf Cystine	.035	.046	.050	.056	.060	.053	.044	.049
ɜline	.058	.081	.048	.158	.142	.104	.084	.109
ɜthionine	.032	.030	.031	.050	.048	.024	.032	.013
ʒoleucine	.038	.055	.055	.110	.092	.067	.057	.069
ɜucine	.166	.204	.204	.234	.242	.227	.206	.213
ʏrosine	.076	.089	.089	.104	.098	.100	.088	.085
ɪenylalanine	.112	.135	.136	.158	.162	.154	.137	.139

TABLE XXIX

NITROGEN CONTENT OF PEANUTS AND PEANUT MEAL USED IN THE TOTAL AMINO ACID STUDY

Ident. No.	Variety or Strain	Peanut meal	Kernels
		gmN/100 gm	
1	Dixie Spanish	8.95	4.80
23	Tenn. Red	9.07	4.88
25	Ga. 61-42	9.63	4.91
27	Nambyquaras	7.74	4.56
28	Va. B 67	8.85	4.61
33	Argentine	8.99	4.84
41	Jenkins Jumbo	9.80	5.46
45	Early Runner	9.25	4.59
50	Conagina Macrocarpa	8.54	4.69
52	Fla. Jumbo	10.09	5.38
61	McEachem Jumbo	9.54	5.50
70	Bynum Runner	9.32	5.46
75	NC-5	8.45	4.38
84	Tara Pota	8.71	4.40
85	F 334A-B-14	8.67	4.34
86	Ga 186-28	8.75	4.49

AMINO ACID COMPOSITION OF THE KERNELS OF 16 VARIETIES OF PEANUT MEAL

Amino Acid	Sample Number 1	1	23	23	25	25	27	27	28	28
					µm/mg					
Aspartic Acid*	.576	.486	.417	.443	.498	.473	.305	.538	.393	.389
Threonine	.102	.107	.103	.100	.109	.113	.081	.084	.090	.082
Serine	.272	.241	.248	.248	.281	.253	.181	.197	.188	.211
Glutamic Acid	.621	.631	.639	.645	.730	.711	.459	.502	.553	.614
Proline	.192	.187	.177	.188	.218	.196	.149	.154	.158	.170
Glycine	.406	.375	.386	.402	.452	.424	.335	.438	.391	.404
Alanine	.226	.188	.193	.198	.234	.199	.155	.169	.177	.179
Half Cystine	.043	.050	.056	.051	.077	.016	.039	,010	.054	.038
Valine	.111	.126	.121	.118	.139	.143	.102	.093	.112	.078
Methionine	.034	.035	.034	.040	.043	.037	.020	.028	.021	.034
Isoleucine	.073	.084	.077	.078	.072	.102	.073	.062	.078	.051
Leucine	.217	.212	.218	.226	.254	.248	.172	.175	.205	.179
Tyrosine	.093	.091	.092	.102	.100	.105	.075	.080	.085	.082
Phenylalanine	.140	.132	.130	.146	.150	.151	.109	.110	.130	.120
Lysine	.089	.091	----	.147	.098	.101	.096	.072	.086	.081
Histidine	.058	.058	----	.093	.068	.062	.057	.051	.058	.048
Ammonia*	.580	.358	----	.509	.379	.375	.328	.321	.348	.394
Arginine	.277	.269	----	.436	.285	.298	.244	.226	.257	.220
Total*	2.954	2.877	3.150	3.218	3.310	3.159	2.347	2.451	2.643	2.591
					µm/mg Nitrogen					
Average	.330	.321	.347	.354	.344	.328	.303	.317	.299	.293

*Aspartic acid and ammonia have not been included because of analytical variation.

Amino Acid	Sample Number 33	41	45	50	52	61	70	75	84	85	86
						μm/mg					
Aspartic Acid*	.411	.457	.441	.259	.400	.381	.372	.339	.325	.319	.280
Threonine	.088	.096	.093	.066	.085	.077	.079	.070	.067	.062	.059
Serine	.242	.244	.231	.205	.194	.194	.179	.165	.170	.175	.146
Glutamic Acid	.590	.239	.614	.594	.503	.563	.536	.149	.469	.453	.442
Proline	.177	.195	.181	.168	.179	.193	.175	.162	.159	.165	.160
Glycine	.395	.357	.410	.379	.311	.323	.275	.315	.289	.294	.261
Alanine	.207	.192	.183	.177	.171	.164	.154	.139	.136	.130	.111
Half Cystine	.045	.046	.043	.036	.038	.038	.044	.028	.041	.028	.029
Valine	.113	.148	.116	.133	.122	.128	.104	.080	.077	.062	.063
Methionine	.032	.036	.028	.024	.021	.019	.029	.027	.027	.021	.023
Isoleucine	.061	.088	.079	.099	.080	.087	.073	.056	.044	.038	.041
Leucine	.195	.235	.208	.202	.200	.196	.182	.158	.145	.141	.135
Tyrosine	.091	.100	.087	.076	.083	.076	.077	.064	.063	.062	.057
Phenylalanine	.131	.144	.129	.122	.120	.122	.113	.114	.114	.084	.081
Lysine	.115	.092	.082	.091	.131	.088	.099	.087	.085	.066	.070
Histidine	.074	.063	.059	.056	.088	.063	.067	.055	.055	.038	.041
Ammonia*	.676	.434	.317	.473	.501	.378	.429	.297	.405	.241	.240
Arginine	.326	.291	.257	.254	.424	.272	.294	.229	.252	.180	.182
Total*	2.882	2.566	2.800	2.682	2.750	2.603	2.480	1.898	2.193	1.999	1.901
						μm/mg Nitrogen					
Average	.320	.262	.303	.314	.272	.273	.266	.225	.252	.231	.217

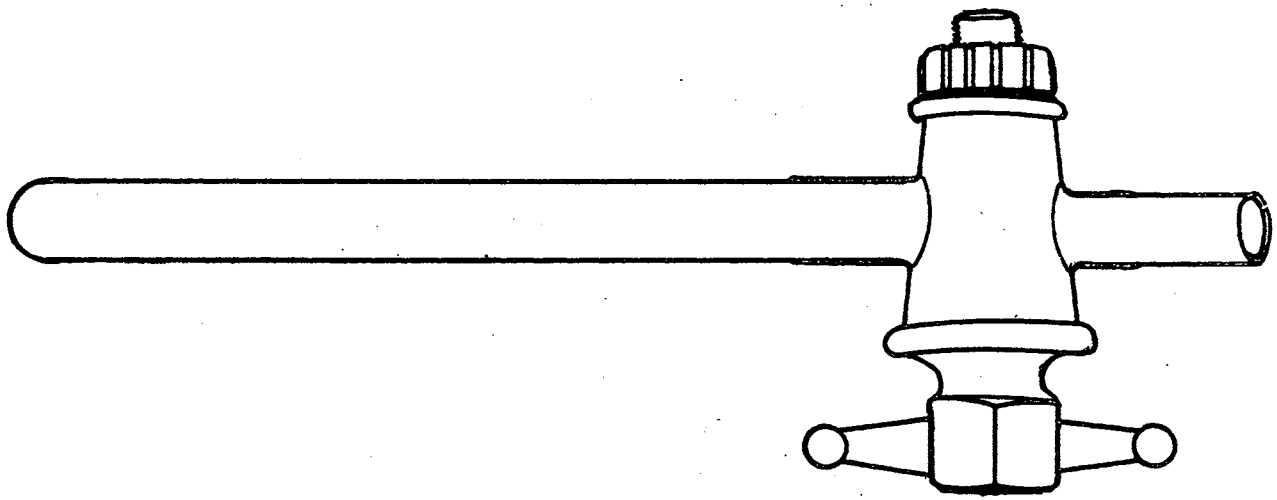


Figure 15. A Drawing of a Protein Hydrolyzate Tube (Drawn to Scale).

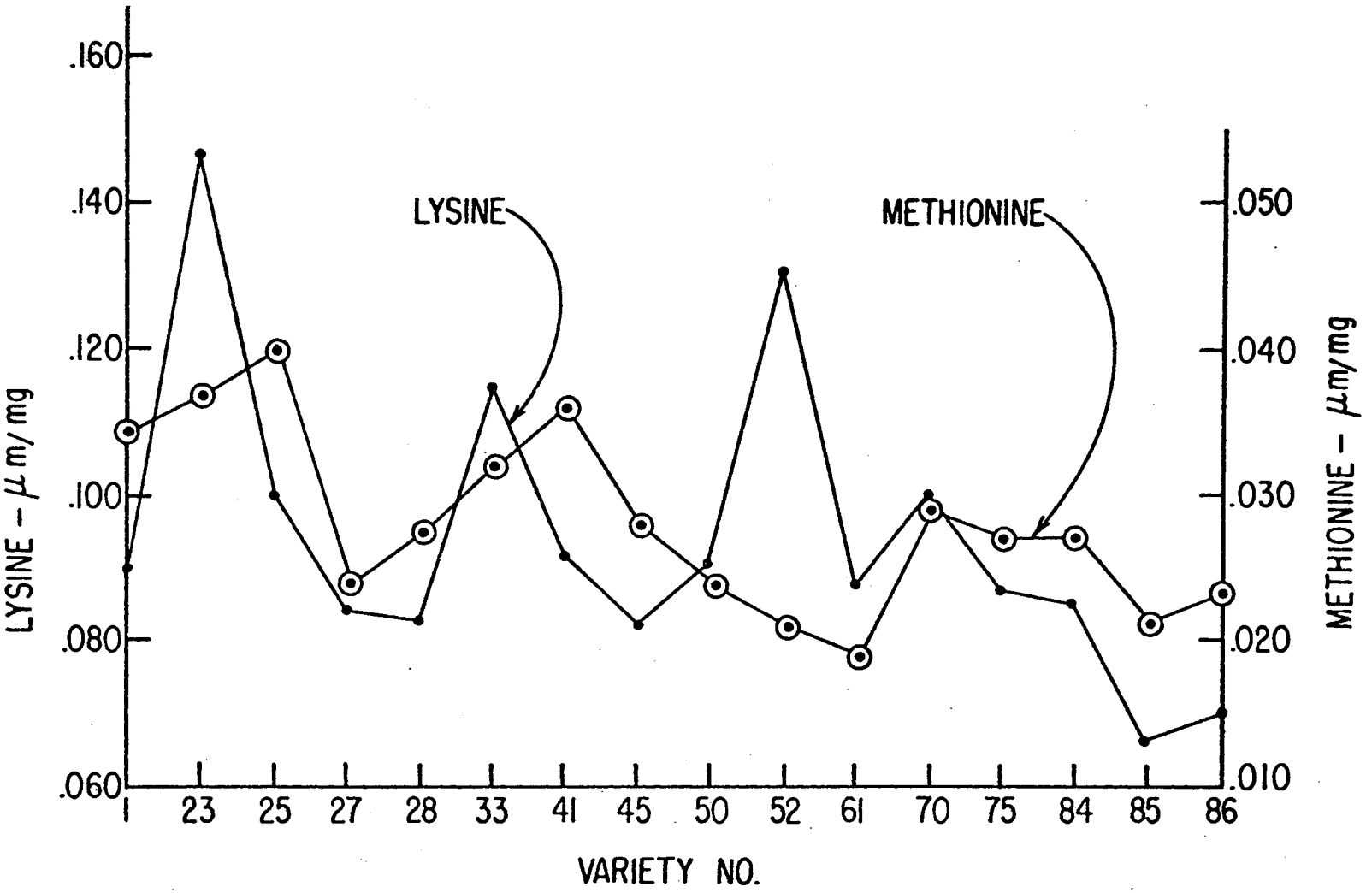


Figure 16. Lysine and Methionine Content of Different Varieties of Peanuts.

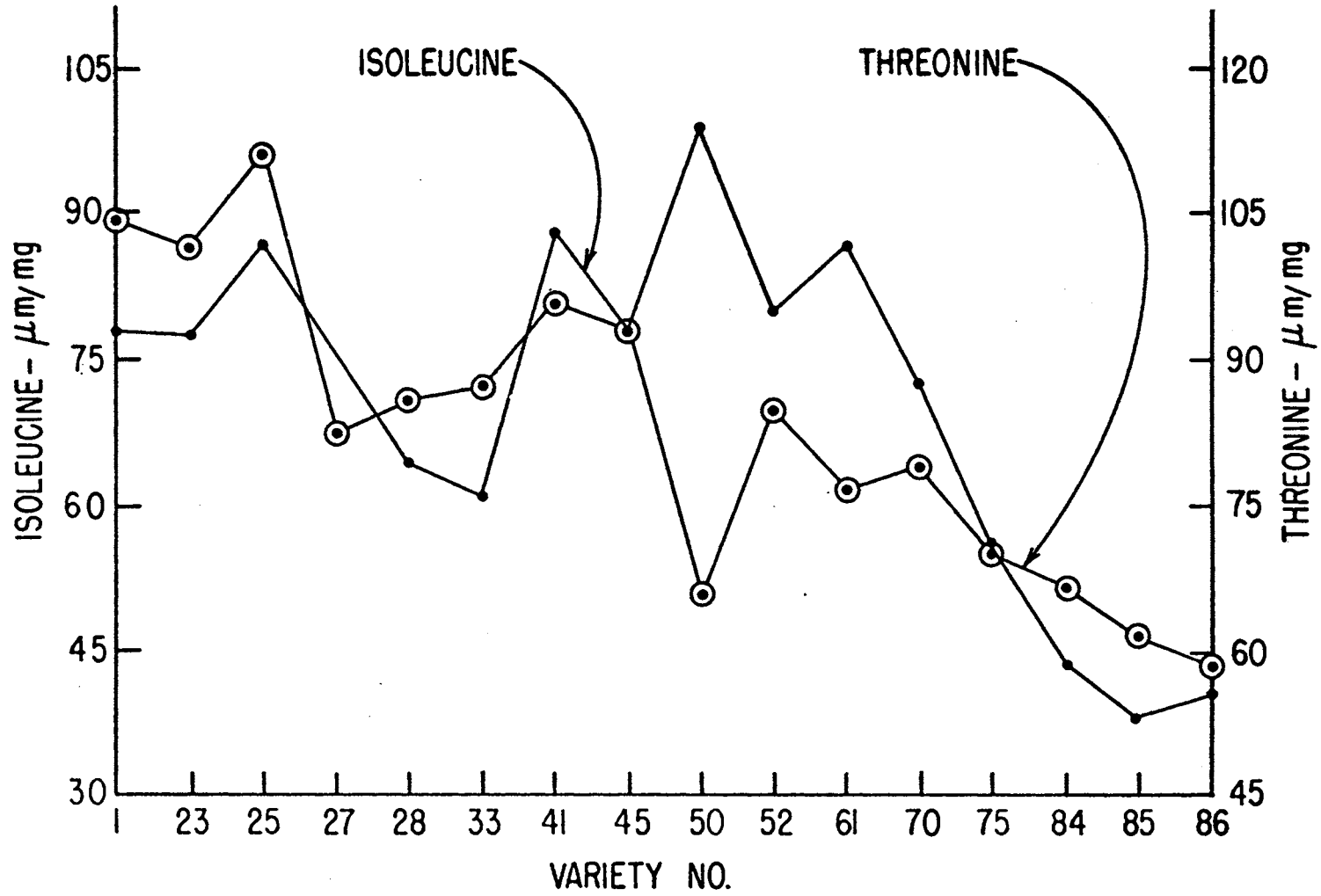


Figure 17. Isoleucine and Threonine Content of Different Varieties of Peanuts.

CHAPTER VII

ARGININE (NON-PROTEIN) ANALYSIS BY SAKAGUCHI REACTION AND ION EXCHANGE CHROMATOGRAPHY AS A MEASURE OF MATURITY

ıtroduction

Because peanuts are indeterminate in their growth habit (69), ıanuts harvested at a given time always possess a certain number of mature fruits. At the present time, the many methods (70, 71, 30, 72), 74) used to determine the degree of immaturity are subjective for ıe most part and are based upon such factors as size, color of the ısta, degree of darkening of the inside of the pod and seed character- ıtics. The need for an objective, quantitative procedure which is ecise and accurate has been apparent to growers, manufacturers and ıientists for some time (30).

A chemical method based on the analysis of carotenoid pigments ıs met with some success in determining the degree of immaturity in ıanuts (30). The data showed that the extract of immature peanuts ɔsorbed light to a greater extent at 435nm than mature peanuts but ɔme overlap occurred and no quantitative interpretations could be made. nery et al. (71) used a pigmentation (absorbance at 455nm) method to ifferentiate maturity of farmers stock peanuts and found it to be the ɔst effective of the methods used. However, the high concentration f pigments were associated with immaturity only when the fruit was ured rapidly and not when they were cured by the traditional stockpil

thod. Thus, the presence of high absorption values was a positive alitative check for immaturity but low absorption was not necessarily nclusive. Use of this procedure as a quantitative measure of degree immaturity appeared to be invalid.

This paper describes a new technique, a reasonably accurate, pre-se, objective measure of immaturity applicable to the major peanut pes based on arginine content. The means by which the procedure was veloped are briefly summarized.

Procedures

eparation of Peanut Samples

Fifty-gram samples of wet or of dry-cured peanuts were homogenized a Waring blender at high speed in 500 ml of 3N $HClO_4$ for 9 minutes. e flask was immerged in an ice-water bath to keep the extraction mix-re cold. The suspension was filtered on a fast flowing fluted fil-r paper and the first 50 ml of filtrate was collected. After adjust-g the pH of the filtrate to 8.0 with 2 N KOH, the precipitate was moved by centrifugation. The supernatant was transferred to a 250 ml lumetric flask and diluted to volume with deionized, distilled water. mples taken from this flask were diluted five-fold before the Saka-ichi determination for arginine was made on 1 ml aliquots.

For recovery studies the procedure was exactly the same as for utine analyses except the filtration was completed and the filtrate shed (washings added to filtrate) before a representative sample was ken. Also, the centrifugate was washed and the washings combined th the supernatant to insure quantitative transfer.

ginine Determination

The procedure for arginine determination was exactly that of Izumi 3, 75). However, a number of precautions need to be enumerated here cause of the nature of samples analyzed in these studies: (1) KOH ould be protected by a soda lime tube since the base was not sufficiently strong if considerable CO_2 was absorbed; (2) protection of the etic anhydride from water vapors was critical because the amount ed in the procedure was critical (150 µliters). If the acetic anhyide were slightly deteriorated, there was insufficient anhydride to mplete the reaction presumably because of the presence of considerle amounts of amino acids other than arginine in the sample. If a ight excess of acetic anhydride was used the amount of KOH was not fficient; (3) Strength and amounts of KOBr added were critical and nsiderable precaution should be taken in storing the KOBr (4° C and the dark). The best policy was to make a new KOBr stock solution ery week.

Standard curves were established with at least ten serial dilu-ons containing from 0 to 30 µg of arginine. Curves were consistently near over the entire range and were similar to those shown in Figure . The developed color for standards and samples were stable for an ur.

Results and Discussion

Since 1965, numerous free amino acid analyses have been performed n Spanish peanuts segregated into mature and immature classes by subctive means. Clearly, immature peanuts contained much higher levels f free arginine than mature peanuts (Table XXXIII). Those which were

clearly mature or immature showed intermediate levels of arginine. apparent decrease of arginine during maturation was confirmed by a dy in plants that were harvested periodically starting 30 days after oming had commenced. Peanuts were dried at 90° F in a forced air n, were shelled and classed into two groups, mature and immature, ng a combination of the subjective criteria eluded to earlier (32). se peanuts which were difficult to classify in one of these two sses were placed in an intermediate maturity class. Arginine con- t of peanuts from each maturity class and harvest date were deter- ed using the Beckman Model 120C amino acid analyzer and the results m the intermediate group are shown in Figure 1.

The data plotted in Figure 1 showed that arginine decreased ymptotically to a very low level with age within the intermediate urity class. The other maturity classes exhibited similar results ept the absolute values were lower for mature peanuts and consider- ly higher and more variable for immature peanuts; mean values of 52 and 21.3 μmoles/gm fat-free meal were found for mature and imma- re peanuts respectively. These results were verified in 1967 grown anuts (Table XXXIII).

Clearly, arginine content would be a sensitive, rapid means of termining the amount of immaturity in any particular sample of peanut a sufficient simple and sensitive quantitative means of determining ginine content would be found and if suitable calibration curves for l types of peanuts could be constructed.

The search for a simple, wet chemical procedure for determining ginine was quickly reduced to a recent modification of the Sakaguchi action (68) which embodies other modifications (75). The procedure

mised to be applicable to product control type laboratories because the simple equipment necessary and the lack of need for highly ined personnel. A review of the literature revealed that it was a hly sensitive and accurate procedure for arginine in which interence from other amino acids present in large amounts was not great. determine whether or not the procedure would measure free arginine the protein free extracts of peanuts was tested by performing recovies on samples to which arginine had been added to the cold perchric acid used in extraction.

Results from a number of recovery studies in which the standard rve was prepared from aqueous solutions of arginine showed that the an recoveries were consistently about 120 percent of the arginine ded. Since the presence of other amino acids such as glutamic acid, partic acid, and phenylalanine result in 10 to 20 percent higher tical density readings (68) and since these amino acids are present considerable amounts in perchlorate extracts of peanuts (21), the gh readings must have been due to the presence of other amino acids. at this was the case was shown by the data plotted in Figure 18. en a standard arginine curve was prepared using the perchlorate exact of the control sample (no added arginine) as diluent rather than ter, the standard curve (curve a) exhibited a slightly lower slope an the curve constructed from standards diluted with water (curve b) d recovery values on two separate recovery studies were near 100 perent (Table XXXI) within experimental sampling error when they were ad from curve A. Thus, free amino acids other than arginine present n perchlorate extracts are measured by this procedure to the extent about 20 percent of the arginine present. This fact would not

:ract from the quantitative usefulness of this procedure as long as
: amount of color measured by the procedure were a function of the
gree of immaturity present in the peanuts.

Calibration curves (Figure 19) for four major peanut types were
nstructed from wet and cured peanuts segregated by subjective means
to four maturity classifications; mature, high intermediate, low
termediate and immature. Peanuts from each group were analyzed by
: procedure described and samples containing from 0 to 100 percent
naturity were formulated from the four groups as follows: The
ginine values for immature peanuts were taken to represent 100 per-
nt immaturity while that of the mature peanuts was arbitrarily
signed zero percent immaturity. Ninety and 80 percent immature
mples were formulated from calculated amounts of each of the immature
d low intermediate group. Seventy, 60 and 50 percent immature sam-
es were formulated by combining calculated amounts of each of the
gh intermediate and mature groups. In order to have sufficient pea-
ts to supply 50 gram samples of all calibration samples this proce-
re was necessary since most of the peanuts fell into the two more
ture segregation categories (high intermediate and mature). Also,
e procedure should have produced calibration samples fairly indica-
ve of the actual immaturity category since the lower immaturity cali-
ation samples were made up of peanuts from the two segregations con-
ining the lower immaturity (mature and high intermediate) while the
gher immaturity calibration samples were made up of peanuts from the
o higher immaturity segregations (low intermediate and immature).

The calibration samples were analyzed according to the outlined
:ocedure and the resulting calibration curves for both raw and cured

anuts of the four major types are plotted in Figure 19. Thus, the gree of immaturity of any sample of raw peanuts may be estimated by rforming the Sakaguchi analyses as described herein and reading the rcentage immaturity from the corresponding calibration curve. Even ough mature peanuts grown in this area have been remarkably consis- nt from year to year in free amino acid content, one should probably tablish calibration curves from peanuts grown in the area of concern the procedure is to be used routinely.

In practice, the important part of the calibration curves was that presenting less than 50 percent immaturity since peanuts harvested der normal conditions with a reasonable growing season would not ssess immaturity higher than 50 percent. Also, the analyst should be minded that this procedure measures mean percentage immaturity since me seeds would be very close to mature while others would be very mature. Even though 100 percent immaturity and zero percent imma- irity have been defined for the purpose of plotting the calibration irves, it was doubtful that the physiologically immature state can be iemically defined. This was because the various states of develop- ent from nearly embryonic to highly differentiated tissue were present i this category. However, chemical definition of the mature state eemed much more certain since all peanuts in this category had reached) very nearly the same physiological state. Thus, the less meaning- il part of the calibration curves (above 50 percent immaturity) would cobably be of little value in actual practice.

The precision and accuracy of the procedure was established with n elaborate experiment (Figure 20) which was designed to allow dif- erentiation of sampling error from inherent error arising from mani-

ılations involved in the Sakaguchi procedure. Figure 20 contains a ow diagram of the sampling procedure starting with replicate 100 gm mples of the same variety of Spanish peanuts. The results of Sakaıchi analyses of the resulting samples are shown in Table XXXII.

A brief inspection of the means and average error values allowed ›nclusions to be drawn concerning sources of error. Average error ›t greater than ± 0.3 between duplicate samples showed that the preısion of manipulations involved in the Sakaguchi procedure was excelent (± two to three percent). This was indicated by submean I since ıbsamples labeled one or two or a or b were all simply duplicates of ıe same subsample (see Figure 20).

Errors that were present due to manipulation during preparation f the samples was apparent from examining values for submean II. Mear alues for subsample A_1 and A_2 were considerably different whereas hose for B_1 and B_2 were very close. Thus considerable errors in ccuracy were apparent in subsampling within the A series even though he precision of the Sakaguchi procedure for arginine was excellent.

Apparently, from the comparison of the values for submean III, onsiderable error in accuracy was incurred between replicate samples and B since the mean value for sample A was 2.32 μgm/gm peanuts ower than that for sample B. The amount of error involved was indiated by the average error of 1.21 for the mean value of 13.67 for the eplicates. After this precision study was completed, replicate samping error was reduced considerably by blending the sample for a longe ›eriod of time. This change was incorporated in the procedure describ ıerein.

Thus, precision and accuracy of the Sakaguchi procedure under

ɜse conditions was well within the sampling error for 100-gram sam-
ɜs. This error amounted to ± nine percent in this study. As a
ɜsequence of these results, differences between routine samples of
ss than 10 percent were not considered significant. Repetition of
is procedure for 50-gram samples revealed sampling error (8.7 percent)
s about the same as that for 100-gram samples.

Repeated analyses of several peanut samples revealed that excel-
nt precision was obtained using the routine procedure even though no
tempt was made for quantitative transfer at the filtration and centri-
gation steps. This protocol was necessary to shorten the time for
eparation of samples. The described technique was equally applicable
both wet and dried peanuts.

The objective of this phase of research was to test its applica-
lity under field conditions.

Peanuts of four varieties were grown at Perkins in 1967 and then
ɪrvested at weekly intervals. Care was taken to harvest all of the
ɜanuts including those that came off the vine while removing the pea-
ɪts from the soil. Freshly dug samples were shelled and segregated into
ɔur stages of maturity using a visible method based on pericarp, seed
ɔat color and thickness, and lastly, seed size. Each group was
ɜighed and analyzed for arginine (Sakaguchi reaction) and moisture.

Table XXXIV gives the amount in percent of peanuts that are withi
ɜch maturity group and is also an excellent guide for maturity and
robably harvest dates. This was possible because the pods in the soil
ere recovered.

Results of the arginine analysis are shown in Table XXXIII and
ignificant differences in each group were observed. Note that the

ıture and high intermediate maturity groups usually gave about the sam ılues for arginine. This was the reason for combining the mature and ıgh intermediate for the other studies in this dissertation in which ıe peanuts were divided into maturity groups.

The low intermediate group contains about 50 percent more arginine ıan the mature and high intermediate groups. Most of these peanut ɜrnels were of the size that they would be processed into peanut butter r used in other peanut products. Pang (32) in his presentation, lmost always rated the peanut butter made with this group of peanuts nferior to those made with mature peanuts. Examining Table XXXIV, rgentine peanuts contained about 10 percent of the low intermediate roup if harvested at about 140-150 days. The immature, usually quite nall, peanuts were extremely high in arginine and Pang (32) scored hese even lower than the low intermediate group. Certainly this upports the possible relation of high arginine and poor flavor of eanut products. Even if a relationship does not exist, the Sakaguchi ethod for arginine could still be used to predict degree of immatur- ty, and therefore, measure the poor flavor that is correlated with mmaturity.

Analyzing the data in both Tables XXXIII and XXXIV, it was con- luded that it would have been best to harvest the Argentine peanuts t 158-172 days to have the highest quality peanuts. Later, organolep ic data on peanuts from the same location and same variety verified his postulate. If the farmer had dug at the time recommended of 140 ays, he would have harvested a less desirable product.

Summary

For establishing the degree of immaturity in freshly harvested or

ed peanuts, arginine was determined using a modified Sakaguchi
hod. Precision and accuracy of the method is reported. Calibra-
n curves to predict the degree of immaturity in peanuts were formu-
ed. Lastly, the modified Sakaguchi method for arginine was tested
er field conditions and found to be an accurate measure of immatur-

TABLE XXXI

ACCURACY OF MODIFIED SAKAGUCHI METHOD USED TO ANALYZE FREE ARGININE

Added	Recovered		Recovered	
	I	II	I	II
μg	μg	μg	%	%
5.0	5.0	5.0	100	100
10.0	10.3	9.3	103	93
15.0	16.2	15.3	108	102
20.0	21.0	19.9	105	99

TABLE XXXII

PRECISION AND ACCURACY STUDY FOR SUBSAMPLES OF REPLICATE SAMPLES (A AND B) OF PEANUTS

Arginine Values (mg/gm nuts) for Duplicate Subsamples		Submeans and Average Error			
		I	II	III	Grand Mean
$a_{1,1}$	12.9	13.0 ±0.1			
$a_{1,2}$	13.1		13.10 ±0.22		
$b_{1,1}$	13.5	13.2 ±0.3			
$b_{1,2}$	12.9			12.57 ±0.53	
$a_{2,1}$	11.6	11.9 ±0.3			
$a_{2,2}$	12.2		12.05 ±0.25		
$b_{2,1}$	12.0	12.2 ±0.2			
$b_{2,2}$	12.4				13.67 ±1.21
$a_{1,1}$	15.2	15.2 ±0.0			
$a_{1,2}$	15.2		14.90 ±0.30		
$b_{1,1}$	14.8	14.6 ±0.2			
$b_{1,2}$	14.4			14.89 ±0.30	
$a_{2,1}$	14.8	15.1 ±0.3			
$a_{2,2}$	15.4		14.88 ±0.27		
$b_{2,1}$	14.8	14.7 ±0.2			
$b_{2,2}$	14.5				

TABLE XXXIII

THE ARGININE IN EACH MATURITY GROUP OF SEVERAL VARIETIES OF UNCURED PEANUTS GROWN AT PERKINS, OKLAHOMA IN 1967 AS MEASURED BY THE MODIFIED SAKAGUCHI REACTION

Variety	Maturity Group	Days from Planting to Harvest							Aver age
		123	130	137	144	151	158	172	
		μg/ml							
ılencia (P-161)	Mature	6.4	5.5	7.9	8.6	11.8	10.2	9.2	8.5
	High Int.	8.6	7.1	11.0	8.6	9.4	9.9	9.8	9.2
	Low Int.	11.4	22.6	22.7	13.5	12.9	----	10.9	15.6
	Immature	23.2	----	----	----	----	----	----	23.2
rgentine	Mature	7.6	7.2	7.6	9.3	10.5	9.3	8.2	8.5
	High Int.	7.7	7.6	7.8	10.6	10.8	11.0	10.1	9.3
	Low Int.	13.9	14.3	15.5	14.0	13.4	----	----	14.2
	Immature	29.3	27.5	23.2	----	----	----	----	26.7
arly Runner	Mature	----	----	7.4	10.2	10.1	10.9	10.4	9.8
	High Int.	12.2	7.4	11.5	9.8	10.7	11.9	10.9	11.0
	Low Int.	19.6	16.4	19.8	14.0	17.1	16.7	14.9	16.9
	Immature	25.3	23.7	25.0	24.6	---	---	---	24.6
C-2 (P-36)	Mature	----	10.5	12.8	10.4	10.6	11.5	10.9	11.1
	High Int.	14.1	8.6	9.9	9.7	10.7	11.0	11.2	10.7
	Low Int.	16.7	15.3	18.1	15.9	16.3	19.8	19.2	17.3
	Immature	23.9	25.0	24.9	----	----	----	----	24.6

TABLE XXXIV

THE AMOUNT OF SHELLED PEANUTS IN EACH MATURITY GROUP OF SEVERAL VARIETIES OF FRESHLY HARVESTED PEANUTS GROWN AT PERKINS, OKLAHOMA IN 1967

Variety	Maturity Group	Days from Planting to Harvest						
		123	130	137	144	151	158	172
					%			
lencia (P-161)	Mature	26	47	48	62	65	74	73
	High Int.	45	33	29	20	14	18	13
	Low Int.	19	16	20	17	18	8	8
	Immature	10	5	3	2	2	1	1
gentine	Mature	42	58	67	77	78	81	82
	High Int.	32	24	19	9	11	13	11
	Low Int.	21	14	9	14	10	5	2
	Immature	4	4	5	1	1	1	0
rly Runner	Mature	--	5	11	8	34	40	49
	High Int.	52	53	53	49	38	34	27
	Low Int.	32	32	28	33	23	21	18
	Immature	16	11	8	9	5	5	4
-2 (P-36)	Mature	2	9	4	16	56	50	62
	High Int.	55	45	65	50	25	26	16
	Low Int.	29	36	22	26	15	20	12
	Immature	14	11	9	8	4	5	2

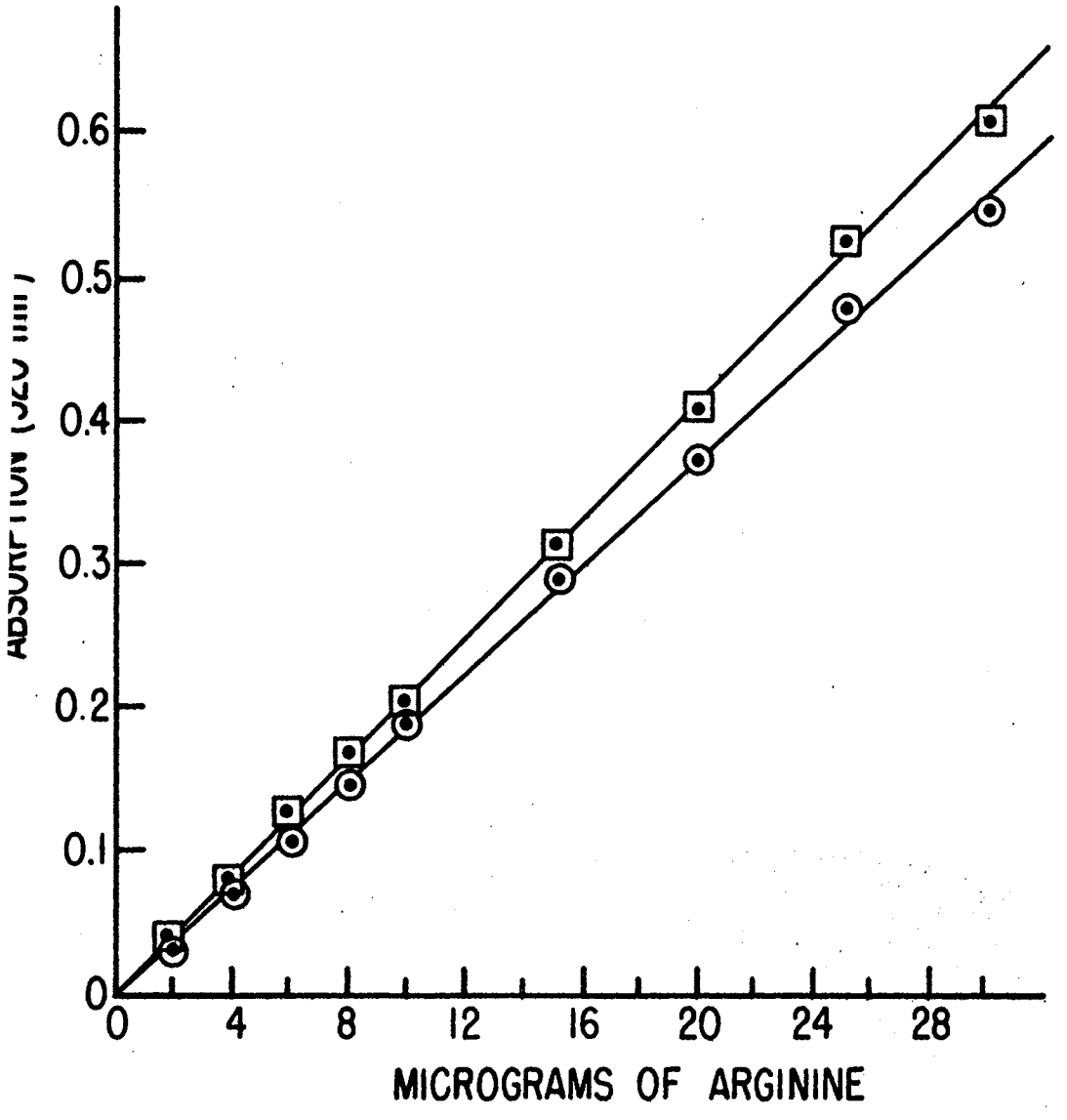


Figure 18. Standard Curve for the Assay of Free Arginine Using Water (⊙) as Diluent and with Control Peanut Extract (⊡) as Diluent.

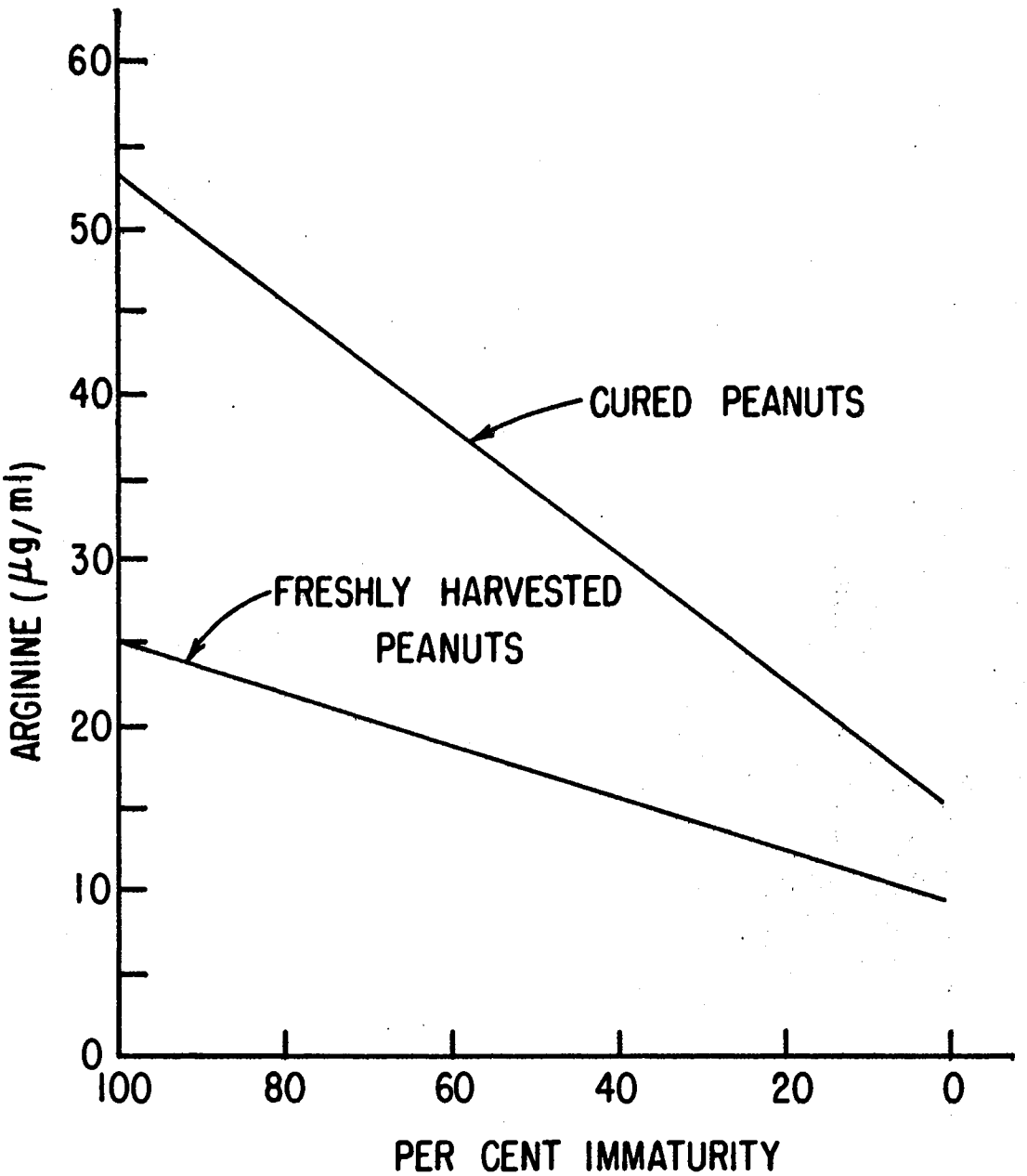


Figure 19. Calibration Curves for Arginine in Relation to the Degree of Immaturity.

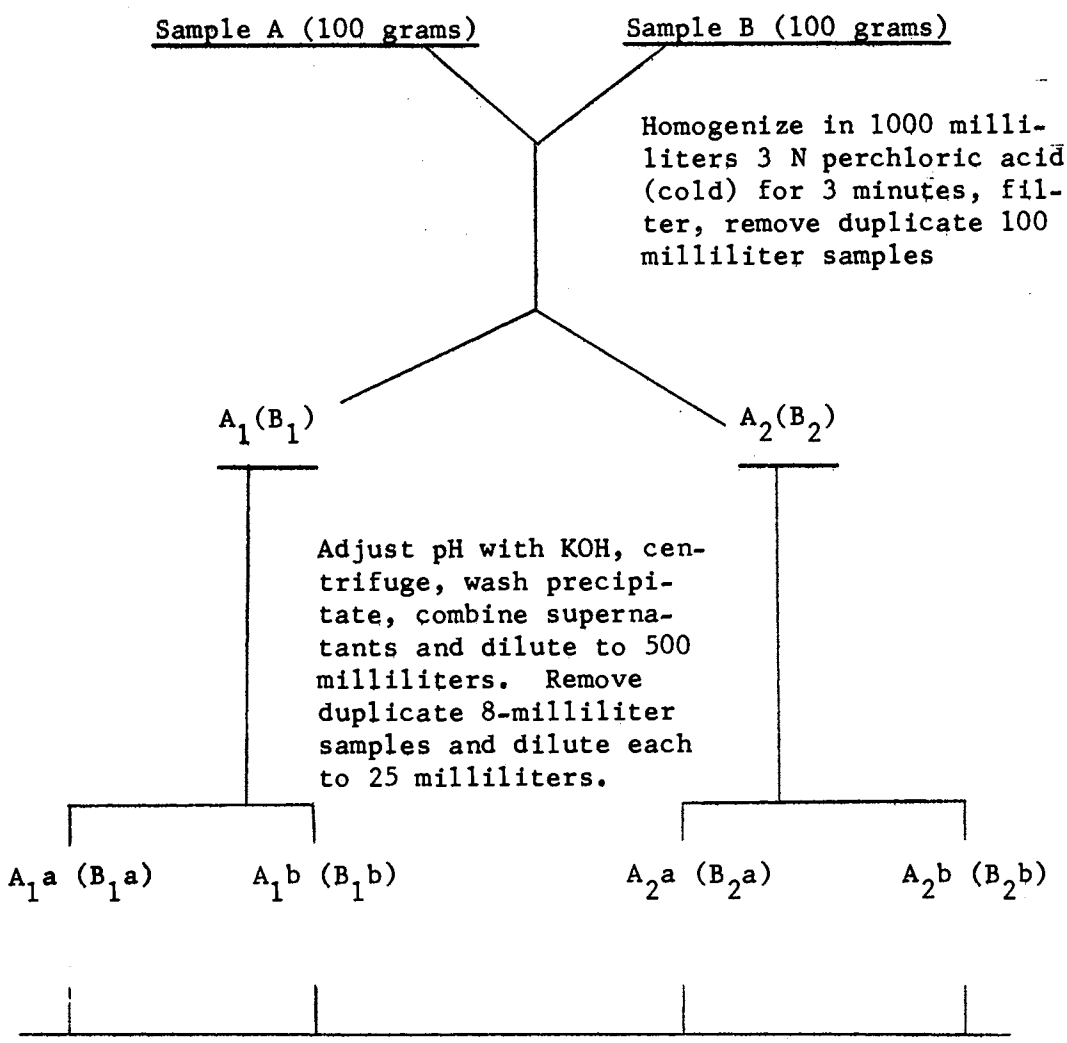


Figure 20. Flow Diagram of Precision and Accuracy Experiment Used in Arginine Assay System

CHAPTER VIII

FREE AMINO ACID, PEPTIDE AND PROTEIN COMPOSITION AS INFLUENCED BY VARIETY, MATURITY IRRIGATION AND PLANTING LOCATION

ntroduction

Recently published work (21, 2, 76) proposes that the roasted-utty flavor of roasted peanuts results largely from the reactions of lucose and fructose, liberated from sucrose, with free amino acids. he "majority" of these amino acids are believed to be released from a arge peptide during roasting.

In this portion of the study, an effort was made to statistically easure some of the genetic and environmental effects on the free amin acid concentration of raw peanuts. Such information might provide a etter understanding of the conditions necessary to produce and maintain a peanut capable of producing a good roasted flavor.

Before undertaking the analysis, several extraction methods were evaluated. These were compared with the method used by Newell (21) and Mason et al. (1, 2). Their methods required considerable techniq and skill to obtain reproducible results. Based upon this author's experience with the extraction of peanut flavor precursors with methy alcohol (76), a method using chloroform, methanol and water (MCW) to extract the free amino acids was developed which was milder than the perchloric acid extraction. The development of an improved method wa

ught to be desirable.

With the MCW method, samples from the National variety test (see pter IV, page 29) were extracted by the detailed method listed under cedures and were analyzed on the amino acid analyzer using a modified ı-exchange procedure of Beckman Instruments.

The peanuts used in this series of studies were the same as those :d for the fatty acid research in Chapter IV.

Apparatus and Reagents

The apparatus and reagents were the same as reported in Chapter VI, ge 84 except that after the development of the methodology Aminex A-5 sin had been added to the long column (containing PA-28) for the sam- es analyzed in Table XL. The data in Tables XXXV-XXXIX were obtained the PA-28 column.

Procedures

Table XXXV gives a very brief description of the various methods ied and Table XXXVI lists the micromoles of each amino acid recovered ı an equivalent amount of a standard peanut sample of Argentine Variet -0002, 1967, Ft. Cobb).

ctraction Procedure for Free Amino Acids and Peptide

A 10 gm $\pm$ 1 mg peanut sample (known moisture content) was thoroughly round (about 20-30 seconds) in a 250 ml stainless steel container sing the Serval Omni-Mixer with the Powerstat setting of 80 V. Di- thyl ether, 100 ml, was added with mixing and filtered with suction sing a coarse sintered glass disk. The residue was washed twice,

ng a wash bottle, with diethyl ether. The ether was evaporated on a ary evaporator and a small bottle of peanut oil was saved for fatty d analysis. The residue was extracted (same container and same 'er-stat settings throughout) by blending three minutes with 150 ml methanol, chloroform and water mixture (60:25:15) (MCW) and filtered, ng the same filter and washed once with a small amount of MCW. The idue was extracted again with 100 ml MCW, and blended one minute, n filtered, washed twice with MCW and then the residue was discarded.

The filtrate was evaporated to near dryness on a rotary evaporator 45° C and diluted to 25 ml volume (for results recorded in Table XL) ıtrifuged and saved a portion of the clear liquid (between the fatty 'er and residue) until ready for the chemical analysis. A portion this sample was diluted with an equal volume of pH 2.2 citrate buffer l analyzed as described below. The samples, in which the data were :orded in Tables XXXVI-XXXIX, were taken after concentration to near 'ness on the rotary evaporator and lyophilized to dryness. Then 20 of pH 2.2 citrate buffer was added, centrifuged, decanted and stored -20° C until analyzed.

ino Acid Analyzer Procedure

The standard Beckman physiological run for acidic amino acids and ıtral amino acids was modified in order to speed up the analyses. e term "Hi'Temp" physiological was used because the analyses was rformed at 56° C throughout without a temperature change. Buffer A s at pH 3.250 and must be very accurately measured and the timer tting to start B buffer (pH 4.26) must be adjusted accordingly. ese steps are necessary to separate the peptide from the other amino ids. The buffer change occurs at about 120-125 minutes with a total

taking approximately 260 minutes (4.5 hours for the standard physio-
ical run). The analysis of the basic amino acids on the short PA-35
umn was found to be adequate. A pH 5.28 buffer was used and required
roximately one hour (one hour vs the recommended physiological run
6.25 hours). This shortening of the time on the analyzer saved about
: hours on each sample with only a slight loss of information on the
e amino acid content of peanuts.

rogen Determination

The standard AOAC macro Kjeldahl method was used (64).

sture Determination

The samples were dried in a static drying oven at 110° C for five
irs.

Results and Discussion

Recent published research (77) shows a methanol, chloroform and
ter mixture (MCW) to be an excellent solvent for extracting the free
ino acids of plant material. Methanol had been found to be
cellent for the extraction of the flavor precursors of peanuts. A
lder and simpler extraction method than used by Mason et al. (1, 2)
d Newell (21) was desired. Thus several methods were evaluated. The
thods are recorded in Table XXXV, with the free amino acid composi-
on recorded in Table XXXVI. The standard consisted of Spanhoma pea-
ts (P-112 Ft. Cobb IRR) and was used for all of these tests. The
rchloric acid extraction method of Newell (21) was used as a base
r comparison (method one), since so much of the flavor precursor

earch was with this method. Methanol and 95 percent ethanol (methods , three, and four) were used as the extraction solvent but did not ract enough of the free amino acids. Repeated extractions probably ld have been better but were not repeated in this study. In method e, 70 percent aqueous methanol was used but the method was discarded ause of poor peptide extraction. The method using MCW (77) (method) extracted about the same amount of free amino acids as the perloric acid extraction and extracted nearly twice as much peptide. ce there existed considerable interest in the peptide as a major ecursor of roasted peanut flavor (21, 1, 2), this was considered the st method thus far. A more complete extraction was obtained by thod seven and supposedly gives a complete extraction of all free ino acids (78), but several time consuming steps employing additional tractions were necessary. Also there was not a significant increase the extraction of the peptide. With the information obtained with thod seven one may calculate an estimate of the total amino acid conent of peanuts when using method six. Several simplifications were ied in methods eight, nine and ten but the poorer extraction of parcularly the peptide made them less desirable. Thus method six was lected for these groups of studies and was described fully under ocedures on page 116. Although not shown clearly in these results of mparing methods one and six, the comparison of results in Table XVII with those published by Newell (21) showed that the MCW extraction method extracted consistently twice as many micromoles of each mino acid except for methdonine.

In Tables XXXVII-XXXIX are recorded the results on peanuts grown t Perkins, Oklahoma in 1968. These are the same samples analyzed for

ıtty acid composition and oil stability described in Chapter IV and ɜsted organoleptically (Chapter V). Only a portion of these peanut ımples were analyzed because both of the limited availability of the ıino acid analyzer and the approximately six hours required for each ımple.

Table XXXVII shows the results of three maturity classes over the .ve harvest dates for the Argentine (P-2) variety. In all cases, ɛcept for aspartic acid and the peptide, the amount of an amino acid ɛcreased with maturity for a given harvest date. Aspartic acid con-ɜnt early (113 days) in the season decreased with maturity (3.34 μm/gm ɔwn to 0.69 μm/gm) but the reversed trend was observed late (169 days) ı the season (an increase from 0.17 to 1.97 μm/gm). The peptide con-ɜnt increased with maturity in each harvest date except in the 155 day ıɾvest in which it was about the same in all three maturity groups.

In the accelerated method, the asparagine and glutamine peak ɛcurred between threonine and serine giving one large peak which will ɜ referred to as asparagine* peak. Mason et al. (1, 2) and Newell (21 ıd listed typical flavor precursors as aspartic acid, asparagine (in-.uded glutamine), glutamic acid, phenylalanine and histidine. If ıe amino acids were ranked in descending order (data from Tables XVII-XXXIX), glutamic acid followed by asparagine* was always the .ghest in mature and low intermediate groups (except P-161 at 141 ıys). The peptide and phenylalanine were usually in the top six. ɜpartic acid occurred in the top six rank fairly often. Of the imma-ıre peanuts studied, there was more arginine present (from 18-38 μm/gm peanuts) than any other amino acid which continues to support the ıeory that the presence of arginine is an indication of immaturity.

The importance of the peptide will be discussed in Chapter IX.

Proline was found in higher concentrations in the immature peanuts, n previously reported. Considerable more variation existed in pro- e content when compared with arginine. Further studies need to be e on the proline variation within mature and immature peanuts and relation to immaturity.

An examination of the alanine values in Tables XXXVII-XXXIX shows alanine content of the 141 day harvest to be two to three fold her than the alanine content on the other harvest days. These pea- samples were cured at a higher temperature (110° F instead of 90°F) to temperature control failure and scored lower on the organolep- test (see Chapter V, page 70). The possibility of a high alanine ıtent because of the increased drying temperature is worthy of fur- ır research. If increased alanine content is related to high drying ıperatures, this might be a valuable handle to help solve the basic)blems of off-flavors in peanuts dried above 95° F (56).

Figures 21, 22 and 23 are ion-exchange column chromatograms of ; extracts of mature, low intermediate and immature peanuts. These ;entine peanut samples were harvested at 141 days after planting. :ticular attention is directed toward the location of the peptide ık following valine and just before methionine essentially riding the buffer change (from pH 3.250 to pH 4.25). The last peak on :h chromatogram was arginine and it showed that the arginine peak :reased in size with immaturity, even though less sample (0.1 ml as npared to 0.3 ml for the mature samples) was used.

Examination of these chromatograms show several unidentified aks. In 1952, Done and Fowden (79) first reported the presence of

thyleneglutamine (MG) and γ-methyleneglutamic acid (MGA) in germina- peanut seedlings. Conkerton and Neucere (80) reported the use of exchange chromatography to identify MG and MGA in ethanol extracts 0-20 germinating peanut seedlings. A reproduction of the area near ine of these three chromatograms is shown in Figure 24. The MG peak ust before proline and the MGA peak follows proline. These two :s were identified by adding known amounts of MG and MGA to both :andard amino acid mixture and to an extract of peanuts. This pre- :ion was taken to ensure that unknowns in the peanut extract would have an effect on elution time of these two non-protein amino acids. ı MG and MGA increase with immaturity.

Fowden (81) reported that MG and MGA have not been isolated from ıre peanut kernels. Although Conkerton and Neucere (80) reported isolation of free amino acids from selected portions of dormant and ninating peanuts, they only reported the presence of MG and MGA in ninating peanut seedlings. Therefore, this appears to be the first ɔrt on the occurrence of MG and MGA from mature kernels. In Figure the chromatogram was produced by the extract from slightly less n 0.2 gm of a mature peanut kernel. To the knowledge of this author s is the first published description of the effect of maturity on se two non-protein amino acids.

Table XL shows the free amino acid content of peanuts from the 8 National variety test. In Table XLI are recorded the dry matter tent and percentage nitrogen. These data were statistically analyzed the results recorded in Table XLII. Also recorded in Table XLII the Coefficients of Variation (CV). These shelled peanuts from the 8 National variety test were stored at 34° F and 60 percent relative

idity. The nonirrigated samples from Oklahoma were grown in the same ld as the 1968 Perkins, Oklahoma samples. They were stored until y, 1969 at which time they were extracted for the free amino acids. s storage system was similar to that used by some of the large mercial storage companies.

The most notable changes (apparently due to storage) were the plete loss of the asparagine and glutamine peak, a loss of most of peptide (approximately a 75 percent reduction) and an increase in ammonia content (approximately a six-fold increase). Earlier work Young and Holley (31) showed increasing amounts of ammonia in the nut volatiles of roasted peanuts after the peanuts were shelled and red at 42° F but did not speculate on the source of the increased onia content. It appears that most of this ammonia, based on these ults, probably came from the breakdown of asparagine and glutamine. on and Matlock (82) had examined the amino acid content of aleurone ins stored at 70° F. With zero-six months of storage, the aspara- ie and glutamine contents did not change significantly. The peanuts this study, at the time of extraction were still viable and probably abolism of the asparagine and glutamine occurred. Prentice, et al.) and Burger, et al. (84) have shown the presence of peptide hydro- es from wheat and barley, respectively. Thus, similarly active zymes were thought to be responsible for the disappearance of aspara- ie, glutamine and the peptide in peanuts.

These peanuts contain some degree of immaturity because they were chine shelled and graded. This probably accounts for about one-half the coefficients of variation being above 10 percent (Table XLII). so Aminex A-5 resin had been added to the PA-28 resin to maintain

ıgth after cleaning. The resulting column did not give as good reso-
:ion on amino acid extracts of the peanuts as seen in the chromato-
ams (Figures 21-23) in which a pure PA-28 resin column was used. This
pports the opinion of this author that two different ion-exchange
sins should not be mixed.

The variance for the following were significantly different among
e samples grown in Georgia and Oklahoma: dry matter, nitrogen,
partic acid, proline, glycine, valine, isoleucine, peptide, ammonia,
d histidine. The variance for the other free amino acids were not
gnificantly different for the irrigated versus the nonirrigated tests
the two states: dry matter, nitrogen, aspartic acid, threonine,
oline, glutamic acid, isoleucine, leucine, tyrosine, phenylalanine,
ptide, ammonia and histidine. The variance for the other free amino
ids did not differ significantly. The variance for the following
re significantly different among the six entries in the four tests:
y matter, glutamic acid, leucine, tyrosine, phenylalanine, ammonia,
stidine, arginine, tryptophan and total amino acids. The variances
r the other free amino acids were not significantly different. The
y matter content, ammonia and histidine were the only items that had
gnificant variance for state, irrigation and entry (variety).

As compared with the fatty acid analysis of variance (Chapter IV,
ge 55). there were no significant variances for LxE and SxLxE and
ere were only five significant variances for SxL and three for SxE
nteractions. For simplification of discussion, assume that glutamic
id was the most important amino acid flavor precursor. Variety was
ost important with the effect of irrigation also being important.
rowth of peanuts in Georgia or Oklahoma had no significant effect on

e glutamic acid content of the varieties of peanuts tested. A similar amination could be made for each of the amino acids. A more meaningl analysis of these data would probably be performed on a combination several of the amino acids, and our knowledge about such a combinaon has yet to be determined. It would appear that the most important ctor was the disappearance of asparagine and glutamine followed by utamic acid as far as good roasted flavor is concerned. The study arginine in a model system as used by Newell (21) and Koehler (76) eds to be made to get a better understanding of the possible role of ginine in off-flavored immature peanuts.

Table XLI shows clearly the effect of irrigation on the protein ntent of peanut kernels (IRR 25.1 percent and NIR 28.2 percent). Proin is metabolized earlier in the season (38) with the fat being storec ward the end of the growing season (38, 66), thus it was not surising that the peanuts grown with less stress (irrigated) contained ss protein.

Summary

An improved method for the extraction of free amino acid and the ptide with a methanol, chloroform and water mixture was described.

The effect of variety, maturity and harvest date on amino acid d peptide content was studied. Glutamic acid and asparagine (inudes glutamine, threonine and serine) were present in highest concenation in the mature and low intermediate peanuts. Arginine was the ghest in immature peanuts. Two non-protein amino acids, γ-methylene utamine (MG) and γ-methylene glutamic acid (MGA) were identified in ture peanut kernels and found to increase with immaturity.

Asparagine, glutamine and most of the peptide disappeared in
elled peanuts stored six months at 34° F and 60 percent relative
midity.

The effect of state, irrigation versus nonirrigation and variety
free amino acid contents under the above storage conditions was
aluated statistically. The analyses of variance were summarized in
bular form to show the responses.

TABLE XXXV

EXTRACTION METHODS FOR FREE AMINO ACIDS AND PEPTIDE

Method	Description
1	Perchloric acid method of Newell (21).
2	10 gm of peanuts extracted with 100 ml of 95% ethanol, filtered, evaporated to dryness, 10 ml of 2.2 citrate buffer added, and filtered for analysis.
3	10 gm of peanuts extracted with 100 ml of 95% ethanol, filter, evaporated to dryness, extracted with ether, and 5 ml of 2.2 citrate buffer added.
4	Same as #2 except methanol is used in the extraction.
5	Same as #2 except using 70% aqueous methanol.
6	10 gm of peanuts extracted with 100 ml of hexane, extracted 3 min. with 100 ml of MCW in Serval Omini-Mixer, filtered, repeated, conc. and lyophilized to dryness, 20 ml of 2.2 buffer added, filtered and analyzed.
7	Same as #6 except after the MCW extraction, two extractions for 3 min. with 100 ml of 80% aq-ethanol were added.
8	Same as #6 except used 250 ml of MCW (one extraction).
9	10 gm ether extracted and extracted once with MCW.
10	Same as #9 except no ether extraction.

TABLE XXXVI

RECOVERY OF AMINO ACIDS AND PEPTIDE FROM PEANUTS BY VARIOUS EXTRACTION PROCEDURES

mino Acid	Procedure 1	2	3	4	5	6	7	8	9	10
						μm/gm				
p	.317	.058	.059	.206	.326	.346	.570		.400	.365
r	.084	.019	.014	.017	.109	.198	.248		trace	nil
r	.228	.044	.053	.073	.169	.198	.278		.158	.165
n	.474	.106	.104	.147	.448	.542	.490		.238	.255
o	.324	.104	.028	.084	.400	.494	.358		.302	.083
u	1.521	.331	.284	.073	1.735	1.336	2.582	1.980	2.335	1.770
y	.101	.015	.009	.063	.090	.146	.288		.115	.135
a	.243	.069	.041	.099	.310	.264	.598		.300	.287
l	.114	.029	.008	.023	.155	.162	.264		.110	.087
s	nil	slight trace	nil	nil	.010	nil	nil		trace	nil
t		slight trace	nil	trace		.022	.052		.025	trace
e	.047	.009	.016	.009	.064	.090	.136	.057	.055	.055
u	.045	.008	.015	.006	.048	.090	.172		.048	.040
e	.436	.031	.016	.039	.344	.494	.620	.394	.435	.367
r	.062	.006	.004	.007	.037	.044	.076		.043	.032
k-1	.024	.049	.779	.028	.011	.146	.176			
k-1a					.025	.024	.054			
k-2	.028	.076	.066	.235	.296	.072	.074			
k-3	.078	.015	trace	trace	.031	.032	.038			
k-2a						.118	.124			
ptide	.238	.086	.056	.151	.111	.424	.432	.307	.365	.350

TABLE XXXVII

THE EFFECT OF MATURITY AND HARVEST DATE ON THE FREE AMINO ACID COMPOSITION OF PEANUTS GROWN AT PERKINS, OKLAHOMA IN 1968

Strain: Argentine-Okla. P-No. 0002, Entry No. 01

ıino Acid	Maturity	Harvest Date + No. of Days				
		9/10 113	9/24 127	10/8 141	10/22 155	11/5 169
				µg/gm		
ɔartic acid	Mature	.69	.90	1.16	1.19	1.97
	Low Int.	2.18	1.63	.83	1.16	1.63
	Immature	3.34	3.97	1.32	.11	.17
ɔaragine*	Mature	3.34	2.62	2.74	2.23	2.81
	Low Int.	6.51	5.54	6.34	3.68	4.41
	Immature	19.72	16.55	19.59	27.07	21.42
ɔline	Mature	1.11	2.15	1.31	.89	.90
	Low Int.	4.32	2.57	1.28	.95	1.23
	Immature	20.83	5.79	4.29	14.56	15.15
ıtamic acid	Mature	4.95	6.45	7.33	6.81	7.02
	Low Int.	7.82	8.14	9.53	6.48	8.44
	Immature	11.52	10.73	13.47	14.56	16.86
ɣcine	Mature	.38	.32	.45	.17	.18
	Low Int.	.67	.56	1.13	.29	.32
	Immature	1.24	.91	2.56	1.02	1.71
ınine	Mature	1.14	1.02	1.43	.54	.68
	Low Int.	2.55	2.22	6.23	.78	.97
	Immature	5.07	3.59	10.92	4.07	7.14
line	Mature	.55	.42	1.43	.28	.39
	Low Int.	.81	.67	1.44	.36	.44
	Immature	1.94	.78	2.33	1.69	1.46
thionine	Mature	.03	.03	.05	.01	.06
	Low Int.	.06	.08	.07	.07	.06
	Immature	.10	.13	.17	.14	.12
ɔleucine	Mature	.17	.18	.36	.23	.17
	Low Int.	.26	.22	.66	.17	.16
	Immature	.70	.24	1.05	.68	.43
ucine	Mature	.15	.13	.24	.12	.13
	Low Int.	.23	.21	.50	.17	.14
	Immature	.51	.25	.72	.53	.39
rosine	Mature	.08	.08	.17	.11	.10
	Low Int.	.10	.09	.48	.11	.10
	Immature	.24	.14	.70	.35	.25

TABLE XXXVII (continued)

ino Acid	Maturity	Harvest Date + No. of Days				
		9/10	9/24	10/8	10/22	11/5
		113	127	141	155	169
				µg/gm		
nylalanine	Mature	.51	1.07	1.43	1.43	1.32
	Low Int.	.35	.40	2.44	1.37	1.55
	Immature	1.30	.41	3.53	3.42	1.37
tide	Mature	.63	1.63	2.40	1.79	1.47
	Low Int.	.30	.68	1.03	1.37	1.03
	Immature	.34	.66	.94	1.64	.34
onia	Mature	.43	.33	.40	.28	.43
	Low Int.	.95	.77	1.23	.49	1.20
	Immature	2.91	2.08	2.42	2.45	3.11
ine	Mature	.08	.09	.10	.08	.09
	Low Int.	.33	.30	.49	.16	.18
	Immature	1.84	1.48	2.14	2.50	3.20
tidine	Mature	.17	.19	.28	.19	.20
	Low Int.	.53	.43	1.05	.35	.36
	Immature	1.99	1.14	2.12	3.54	3.34
inine	Mature	.60	.69	.54	.43	.52
	Low Int.	3.62	2.90	3.00	1.10	1.27
	Immature	25.52	17.93	21.44	33.28	38.31
ptophan	Mature	.05	.06	.14	.09	.07
	Low Int.	.07	.07	.37	.30	.15
	Immature	.35	.08	.54	.61	.30

* A combination of asparagine, glutamine, threonine and serine

TABLE XXXVIII

THE EFFECT OF MATURITY AND HARVEST DATE ON THE FREE AMINO ACID COMPOSITION OF PEANUTS GROWN AT PERKINS, OKLAHOMA IN 1968

Strain: OICB1271 (Spanhoma)-Okla. P-No. 0112, Entry No. 2

Amino Acid	Maturity	Harvest Date & No. of Days				
		9/10	9/24	10/8	10/22	11/5
		113	127	141	155	169
				μm/gm		
spartic Acid	Mature	.71	.80	.93	1.35	2.32
sparagine*	Mature	2.71	2.14	2.97	2.26	2.64
roline	Mature	.88	1.92	1.17	.77	.82
lutamic Acid	Mature	6.25	6.55	6.84	6.35	7.69
lycine	Mature	.37	.29	.76	.22	.18
lanine	Mature	.91	.81	2.23	.76	.61
aline	Mature	.47	.41	.86	.33	.34
ethionine	Mature	.05	.07	.07	.07	.06
soleucine	Mature	.18	.19	.43	.16	.18
eucine	Mature	.14	.13	.38	.12	.13
yrosine	Mature	.09	.08	.28	.07	.12
henylalanine	Mature	.94	1.02	1.14	.89	2.03
eptide	Mature	1.66	1.96	1.72	1.68	2.06
mmonia	Mature	.57	.29	.73	.40	.31
ysine	Mature	.05	.05	.14	.06	.09
istidine	Mature	.13	.14	.03	.19	.22
rginine	Mature	.22	.25	.78	.34	.57
ryptophan	Mature	nil	.05	.10	.06	.10

*This value includes asparagine, glutamine, threonine and serine.

TABLE XXXIX

THE EFFECT OF MATURITY AND HARVEST DATE ON THE FREE AMINO ACID COMPOSITION OF PEANUTS GROWN AT PERKINS, OKLAHOMA IN 1968

Strain: Valencia-Okla. P-No. 0161, Entry No. 5

Amino Acid	Maturity	Harvest Date & No. of Days				
		9/10	1/24	10/8	10/22	11/5
		113	127	141	155	169
		µg/gm				
spartic Acid	Mature	.89	1.27	.52	.88	1.09
sparagine*	Mature	3.65	3.69	4.67	3.30	3.50
roline	Mature	.90	2.41	2.68	.94	1.66
lutamic	Mature	6.32	7.14	6.46	6.53	4.53
lycine	Mature	.43	.41	.86	.39	.63
lanine	Mature	1.27	1.69	5.25	1.23	2.60
aline	Mature	.74	.67	1.62	.54	.43
ethionine	Mature	.10	.10	.17	.06	.01
soleucine	Mature	.36	.27	.89	.28	.23
eucine	Mature	.25	.29	.82	.24	.18
yrosine	Mature	.16	.14	.59	.13	.15
henylalanine	Mature	1.60	.69	1.41	1.25	1.34
eptide	Mature	1.75	1.38	1.23	1.07	1.33
mmonia	Mature	.48	.44	.93	.47	.90
ysine	Mature	.10	.12	.30	.13	.12
istidine	Mature	.24	.22	.41	.20	.19
rginine	Mature	.50	.60	1.36	.65	.69
ryptophan	Mature	.12	.09	.18	.20	.08

* This value includes asparagine, glutamine, threonine and serine.

TABLE XL

THE EFFECT OF STATE, IRRIGATION AND VARIETY ON THE FREE AMINO ACID COMPOSITION OF PEANUTS FROM THE 1968 ..TIONAL VARIETY TEST

:ate	Ident. #	Treat-ment	Asp	Thr	Ser	Pro	Glu	Gly	Ala	Val
						µm/gm				
						Argentine (P-0002) (#2)				
)k	1	NIR	0.40	.33	.40	1.09	5.00	.40	1.35	.65
)k	2	NIR	1.75	.31	.36	0.80	5.70	.39	0.48	.57
a	1	NIR	1.67	.35	.32	0.97	6.70	.54	0.76	.57
a	2	NIR	1.52	.35	.31	0.53	6.35	.51	0.71	.64
)k	3	IRR	2.28	.35	.51	0.56	5.00	.39	0.67	.52
)k	4	IRR	0.45	.36	.58	0.80	5.12	.41	2.08	.54
a	3	IRR	0.36	.35	.31	0.44	4.75	.51	0.80	.65
a	4	IRR	0.66	.30	.65	0.39	6.55	.52	0.86	.98
						Ga C-1-27 (P-1258) (#3)				
k	5	NIR	1.15	.33	.40	0.88	6.20	.42	0.77	.51
k	6	NIR	0.41	.34	.37	0.88	4.23	.39	1.11	.61
a	5	NIR	0.96	.26	.82	0.68	7.15	.46	0.66	.73
a	6	NIR	1.40	.33	.30	0.60	6.15	.54	0.87	.67
k	7	IRR	2.03	.35	.50	0.85	4.90	.38	0.63	.53
k	8	IRR	3.13	.37	.55	0.85	6.70	.36	0.88	.57
a	7	IRR	1.43	.37	.34	0.49	7.70	.59	0.86	.84
a	8	IRR	0.67	.32	.31	0.43	3.50	.43	0.60	.51
						Spantex (P-0004) (#4)				
c	9	NIR	1.60	.32	.36	0.93	6.35	.40	0.55	.52
c	10	NIR	1.55	.33	.38	0.97	5.95	.39	0.64	.48
a	9	NIR	0.86	.35	.70	0.54	7.20	.51	0.88	.88
a	10	NIR	0.68	.31	.32	0.53	6.50	.54	1.27	.52
c	11	IRR	2.00	.33	.48	0.58	5.40	.39	0.74	.54
c	12	IRR	1.89	.34	.50	0.64	5.50	.39	0.64	.54
a	11	IRR	0.92	.35	.35	0.45	5.50	.50	0.74	.60
a	12	IRR	1.32	.37	.38	0.56	5.15	.56	1.14	.63
						Starr (P-0006) (#6)				
:	13	NIR	1.80	.36	.41	1.34	6.20	.41	0.65	.61
:	14	NIR	1.40	.32	.38	0.88	5.83	.39	0.62	.52
i	13	NIR	0.68	.30	.30	0.49	6.25	.50	0.97	.48
i	14	NIR	1.25	.32	.32	0.50	6.63	.55	0.74	.52
:	15	IRR	2.66	.40	.55	0.60	6.05	.41	0.76	.59
;	16	IRR	2.33	.35	.54	0.58	5.83	.39	0.66	.57

Met	Ile	Leu	Tyr	Phe	Pep	NH_3	Lys	His	Arg	Try	Total
						µm/gm					
.24	.32	.38	.46	1.08	.58	1.80	.25	.31	0.45	.40	15.89
.24	.32	.37	.46	1.41	.38	1.31	.26	.31	0.50	.40	16.32
.24	.33	.38	.51	1.74	.18	1.27	.26	.31	0.47	.40	17.97
.24	.33	.38	.47	1.74	.19	1.27	.25	.32	0.48	.40	16.99
.24	.34	.39	.44	1.94	.20	2.32	.29	.34	0.62	.43	17.83
.24	.34	.39	.42	1.81	.39	2.76	.24	.34	0.50	.41	18.18
.23	.29	.39	.46	3.55	.19	1.80	.24	.34	0.38	.40	16.44
.19	.41	.34	.36	3.12	.19	0.48	.21	.35	0.41	.33	17.30
.24	.33	.38	.54	2.12	.28	1.63	.25	.32	0.52	.50	17.77
.24	.32	.38	.43	1.33	.47	2.04	.24	.32	0.48	.41	15.00
.19	.27	.30	.31	0.67	.18	0.54	.22	.30	0.47	.33	15.50
.23	.34	.38	.46	1.41	.18	1.18	.25	.31	0.47	.40	16.46
.23	.34	.39	.44	2.28	.25	2.15	.26	.34	0.52	.43	17.80
.24	.35	.40	.43	2.21	.27	2.17	.30	.37	0.73	.42	21.30
.23	.44	.41	.50	3.75	.18	1.18	.25	.31	0.47	.40	20.74
.23	.35	.37	.46	1.84	.17	1.42	.24	.37	0.50	.44	13.16
.24	.34	.38	.48	3.08	.35	1.52	.26	.32	0.51	.40	18.91
.24	.33	.39	.51	2.32	.26	1.72	.26	.32	0.57	.45	18.06
.19	.36	.36	.36	0.86	.18	0.63	.23	.33	0.50	.33	16.25
.24	.34	.39	.55	2.23	.20	1.55	.32	.43	0.80	.54	18.26
.23	.35	.40	.45	1.98	.19	1.48	.27	.33	0.54	.43	17.11
.23	.35	.40	.46	2.32	.20	1.93	.27	.33	0.54	.43	17.90
.23	.42	.41	.59	5.20	.18	1.44	.35	.47	0.83	.41	19.94
.23	.42	.43	.56	4.50	.17	1.73	.35	.50	0.92	.44	20.36
.23	.34	.39	.48	2.30	.18	1.80	.27	.33	0.60	.41	19.11
.23	.33	.37	.47	2.21	.30	1.55	.26	.31	0.52	.43	17.32
.24	.34	.38	.55	2.36	.19	1.32	.32	.42	0.78	.53	17.40
.24	.34	.39	.55	2.16	.19	1.30	.33	.43	0.78	.42	17.96
.23	.35	.40	.46	2.30	.17	2.96	.28	.34	0.66	.42	20.59
.23	.35	.39	.42	2.07	.23	2.00	.27	.33	0.52	.43	18.49

TABLE XL (continued)

ate	Ident. #	Treat-ment	Asp	Thr	Ser	Pro	Glu	Gly	Ala	Va
						µm/gm				
a	15	IRR	0.72	.35	.34	0.44	7.55	.52	1.20	.6
a	16	IRR	1.00	.33	.34	0.47	4.95	.50	0.83	.7
					Ga C-32-S (P-1259) (#5)					
c	17	NIR	1.28	.33	.43	0.88	10.70	.43	0.94	.5
c	18	NIR	1.75	.34	.44	1.40	8.90	.43	0.88	.5
a	17	NIR	0.38	.37	.39	0.64	8.23	.61	2.16	.6
a	18	NIR	0.83	.33	.35	0.56	7.40	.53	1.40	.4
c	19	IRR	3.00	.36	.60	0.66	7.45	.42	0.83	.6
c	20	IRR	2.00	.39	.71	0.77	9.80	.47	2.63	.6
a	19	IRR	0.66	.33	.35	0.41	4.85	.46	0.78	.5
a	20	IRR	0.95	.35	.37	0.47	7.85	.59	1.43	.6
					PI 268684 (P-0385) (#1)					
:	21	NIR	1.86	.33	.45	1.24	8.90	.43	0.75	.5
:	22	NIR	1.55	.33	.41	0.88	10.40	.43	0.98	.4
i	21	NIR	0.67	.31	.34	0.55	10.20	.63	2.05	.5
i	22	NIR	1.46	.33	.32	0.47	7.35	.52	0.74	.4
:	23	IRR	3.14	.35	.58	0.72	7.45	.40	0.75	.5
:	24	IRR	3.30	.37	.65	0.68	7.70	.42	0.83	.5
:	23	IRR	1.64	.35	.35	0.49	6.10	.50	0.72	.5
:	24	IRR	0.43	.35	.33	0.47	7.15	.55	2.16	.5

Met	Ile	Leu	Tyr	Phe	Pep	NH_3	Lys	His	Arg	Try	Total
						μm/gm					
.23	.43	.40	.57	5.80	.19	1.52	.33	.47	0.81	.45	22.94
.23	.40	.40	.44	2.63	.19	1.50	.24	.35	0.50	.43	16.49
.24	.35	.38	.70	5.05	.27	2.95	.27	.33	0.60	.57	27.22
.24	.35	.39	.60	3.97	.24	2.32	.24	.34	0.59	.50	24.49
.24	.38	.43	.63	3.00	.21	2.35	.27	.33	0.51	.66	22.39
.24	.35	.39	.60	2.86	.21	2.64	.26	.33	0.54	.63	20.94
.24	.38	.43	.48	3.15	.20	4.00	.29	.37	0.66	.46	24.59
.24	.39	.44	.58	5.20	.29	7.20	.27	.39	1.05	.62	34.06
.23	.39	.39	.55	4.18	.19	1.70	.27	.36	0.48	.44	17.57
.24	.44	.43	.62	4.90	.22	1.90	.27	.38	0.52	.51	23.09
.24	.34	.39	.66	3.26	.25	2.80	.27	.33	0.63	.50	24.13
.24	.34	.39	.65	6.20	.28	4.53	.49	.34	0.39	.54	29.85
.24	.35	.40	.69	4.52	.26	2.65	.28	.34	0.44	.77	26.21
.23	.32	.37	.53	2.70	.19	1.68	.26	.32	0.47	.40	19.12
.24	.35	.40	.44	3.00	.21	3.90	.28	.36	0.57	.44	24.15
.24	.36	.41	.47	3.30	.21	4.53	.31	.37	0.89	.45	26.07
.23	.40	.40	.51	3.81	.19	2.27	.27	.39	0.60	.42	20.22
.23	.41	.40	.56	4.83	.18	2.86	.26	.38	0.58	.48	23.20

TABLE XLI

NITROGEN AND DRY MATTER CONTENT OF SPANISH PEANUT VARIETIES OR STRAINS FROM THE NATIONAL VARIETY TEST, 1968

	Georgia				Oklahoma			
			N(DB)				N(DB)	
riety or Strain	No.	DM	NIR	IRR	No.	DM	NIR	IRR
			%				%	
gentine	1	93,69	5.25		1	94.27	5.43	
	2	93.61	5.13		2	94.25	5.16	
	3	94.16		4.72	3	94.56		4.59
	4	93.98		4.55	4	95.27		4.43
C-1-27	5	94.02	5.13		5	94.23	5.29	
ifspan)	6	93.77	5.08		6	94.09	5.40	
	7	94.24		4.39	7	94.43		4.32
	8	94.08		4.38	8	94.78		4.47
antex	9	93.49	5.03		9	94.24	5.01	
	10	93.94	5.00		10	94.01	5.25	
	11	93.79		4.58	11	94.74		4.54
	12	94.05		4.64	12	94.58		4.53
arr	13	94.46	5.07		13	94.53	5.08	
	14	93.70	5.21		14	94.36	5.13	
	15	94.01		4.81	15	94.46		4.68
	16	94.25		4.27	16	94,93		4.59
C-32S	17	93.14	5.13		17	94.17	5.08	
pancross)	18	93.81	5.05		18	94.04	5.06	
	19	94.07		4.59	19	94.03		4.68
	20	95.78		4.41	20	94.50		4.66
268684	21	95.37	4.97		21	93.84	5.50	
	22	95.37	4.99		22	93.74	5.16	
	23	95.47		4.63	23	94.23		4.58
	24	95.55		4.71	24	93.97		4.81
la P-112	25	95.28	5.04		25	94.06	5.17	
panhoma)	26	95.25	5.02		26	93.78	5.20	
	27	95.74		4.47	27	94.12		4.78
	28	95.55		4.48	28	94.26		4.46
xie Spanish	29	95.29	4.66		29	93.87	5.18	
	30	95.29	4.95		30	93.99	4.96	
	31	95.54		4.52	31	94.36		4.62
	32	94.66		4.42	32	94.25		4.46
268771 B.	33	94.21	5.18		33	93.88	5.09	
	34	94.06	5.08		34	93.88	5.07	
	35	94.73		4.71	35	94.40		4.56
	36	94.48		4.74	36	94.21		4.63

TABLE XLII

SUMMARY OF ANALYSIS OF VARIANCE ON THE POOLED DATA OF FREE AMINO ACID COMPOSITION, DRY MATTER AND PROTEIN CONTENT OF PEANUTS FROM THE 1968 NATIONAL VARIETY TEST

	Ga vs Ok(S)	IRR vs NIR(L)	Variety (E)	SxL	SxE	LxE	SxLxE	CV (a)	CV (b)
								%	%
DM%	*	**	**	NS	**	NS	NS	0.25	0.32
Nitrogen	*	**	NS	*	NS	NS	NS	2.4	2.5
Asp	**	*	NS	**	NS	NS	NS	29.5	37.2
Thr	NS	**	NS	NS	NS	NS	NS	4.2	6.6
Ser	NS	NS	NS	NS	NS	NS	NS	35.4	22.5
Pro	**	**	NS	NS	NS	NS	NS	19.8	21.3
Glu	NS	*	**	NS	NS	NS	NS	16.1	18.2
Gly	**	NS	NS	NS	NS	NS	NS	3.9	9.6
Ala	NS	NS	NS	NS	NS	NS	NS	62.3	47.4
Val	*	NS	NS	NS	NS	NS	NS	13.8	16.6
Met	NS	NS	NS	NS	NS	NS	NS	5.3	4.3
Ile	**	**	NS	*	NS	NS	NS	5.5	8.8
Leu	NS	**	*	NS	NS	NS	NS	2.2	5.1
Tyr	NS	*	**	*	NS	NS	NS	8.5	11.8
Phe	NS	*	**	**	NS	NS	NS	24.9	31.1
Pep	**	*	NS	NS	NS	NS	NS	23.0	24.4
NH_3	**	*	**	NS	NS	NS	NS	27.3	31.5
Lys	NS	NS	NS	NS	NS	NS	NS	12.9	14.6
His	**	**	**	NS	**	NS	NS	5.0	7.5
Arg	NS	NS	*	NS	**	NS	NS	20.2	19.2
Try	NS	NS	**	NS	NS	NS	NS	9.5	17.2
Total	NS	NS	**	NS	NS	NS	NS	11.8	14.7

NS Not significant

* 5% level

** 1% level

Figure 21. Chromatogram of Free Amino Acids from Mature anuts, Variety Argentine P-2.

nditions were as follows:

Harvested - 10/8/68 - 141 days

Concentration of Sample - 10 gm peanuts/20 ml of pH 2.2 :rate Buffer

	Acidic & Neutral Amino Acids	Basic Amino Acids
Resin	PA-28	PA-35
Buffer Flow	50 ml/hr	68 ml/hr
Ninhydrin Flow	25 ml/hr	25 ml/hr
Sample Applied	0.3 ml	0.3 ml

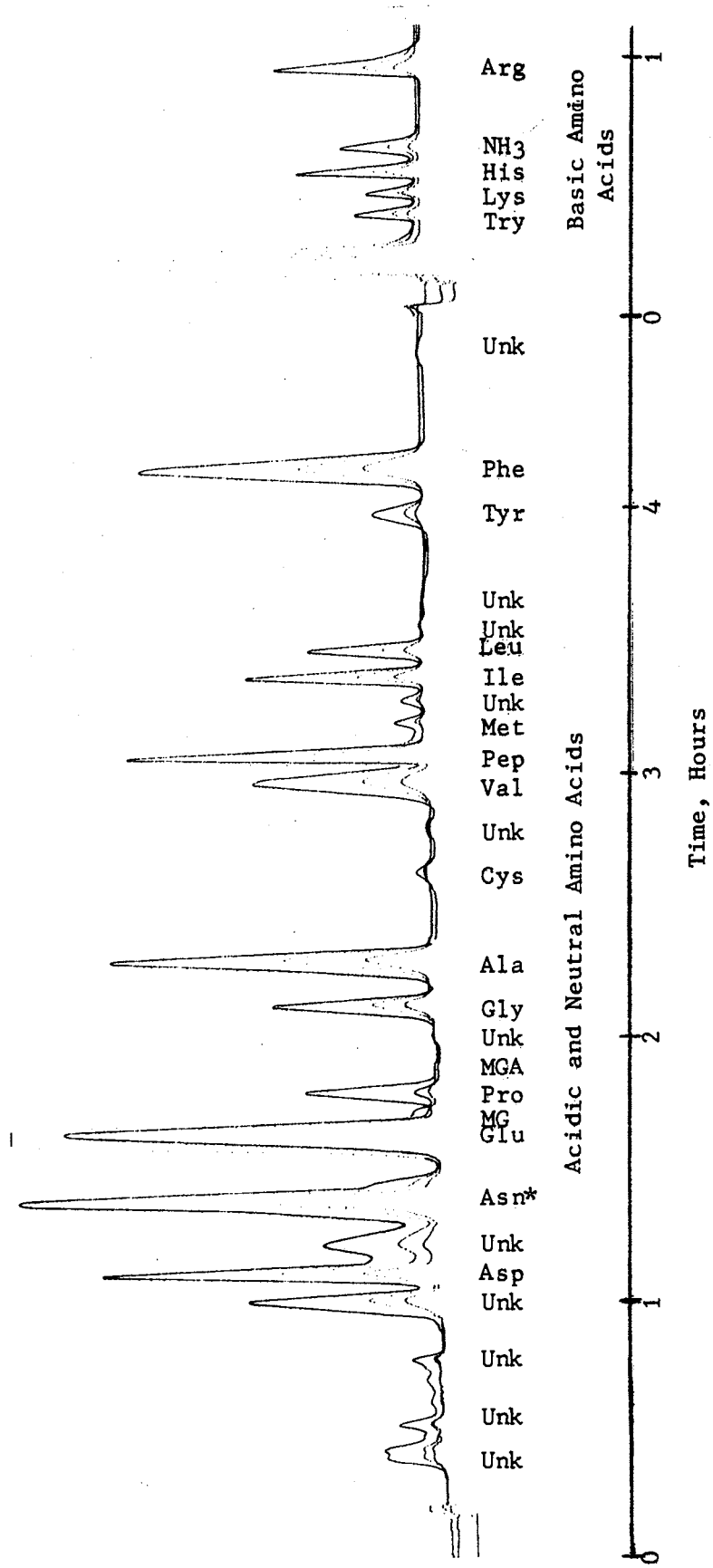

Arg
NH3
His
Lys
Try
Basic Amino Acids
Unk
Phe
Tyr
Unk
Unk
Leu
Ile
Unk
Met
Pep
Val
Unk
Cys
Ala
Gly
Unk
MGA
Pro
MG
Glu
Acidic and Neutral Amino Acids
Asn*
Unk
Asp
Unk
Unk
Unk
Unk
0
1
2
3
4
Time, Hours

Figure 22. Chromatogram of Free Amino Acids from Low
ıtermediate Peanuts, Variety Argentine P-2,
›nditions were as follows:

Harvested - 10/8/68 - 141 days

Concentration of Sample - 10 gm peanuts/20 ml of pH 2.2
.trate Buffer

	Acidic & Neutral Amino Acids	Basic Amino Acids
Resin	PA-28	PA-35
Buffer Flow	50 ml/hr	68 ml/hr
Ninhydrin Flow	25 ml/hr	25 ml/hr
Sample Applied	0.2 ml	0.3 ml

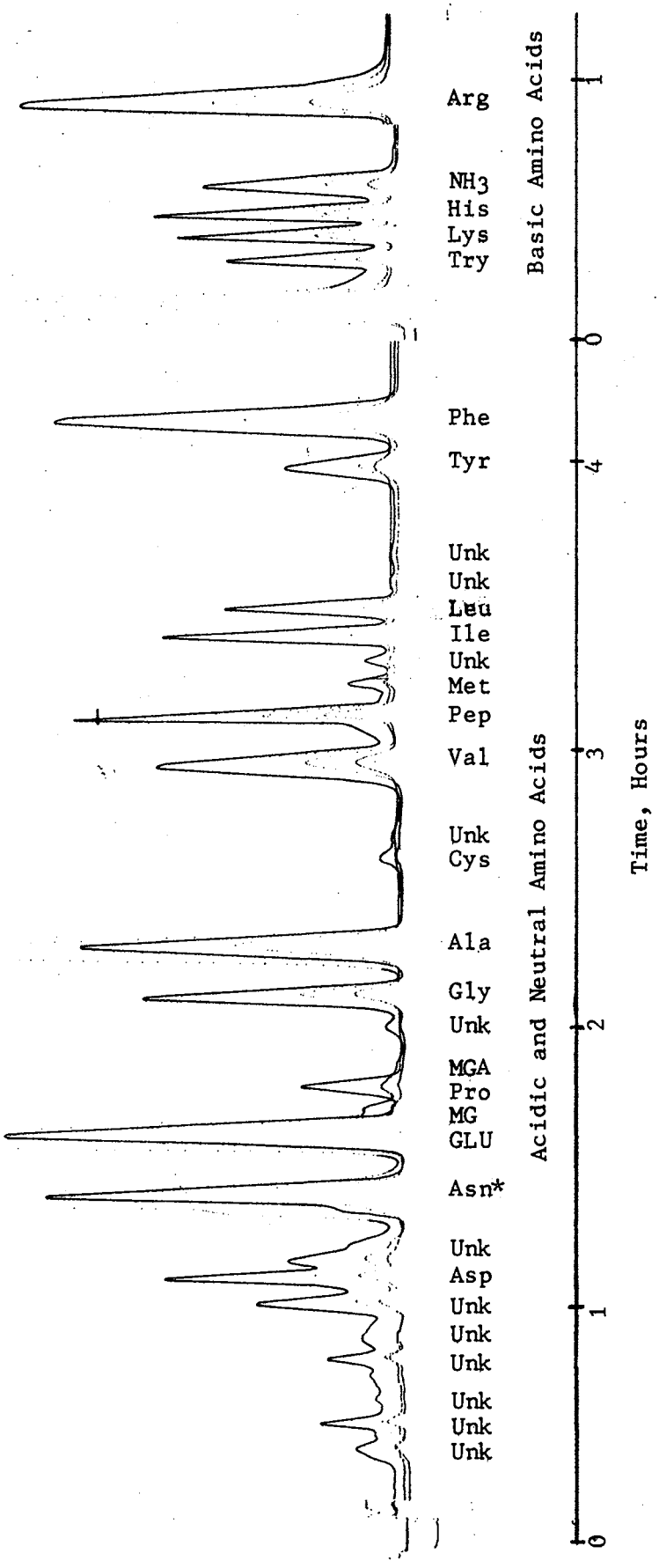

Arg
NH3
His
Lys
Try
Basic Amino Acids
Phe
Tyr
Unk
Unk
Leu
Ile
Unk
Met
Pep
Val
Unk
Cys
Ala
Gly
Unk
MGA
Pro
MG
GLU
Asn*
Unk
Asp
Unk
Unk
Unk
Unk
Unk
Unk
Acidic and Neutral Amino Acids
0
1
0
4
3
2
1
0
Time, Hours

Figure 23. Chromatogram of Free Amino Acids from mmature Peanuts, Variety Argentine P-2. onditions were as follows:

Harvested - 10/8/68 - 141 days

Concentration of Sample - 10 gm peanuts/20 ml of pH 2.2 itrate Buffer

	Acidic & Neutral Amino Acids	Basic Amino Acids
Resin	PA-28	PA-35
Buffer Flow	50 ml/hr	68 ml/hr
Ninhydrin Flow	25 ml/hr	25 ml/hr
Sample Applied	0.1 ml	0.1 ml

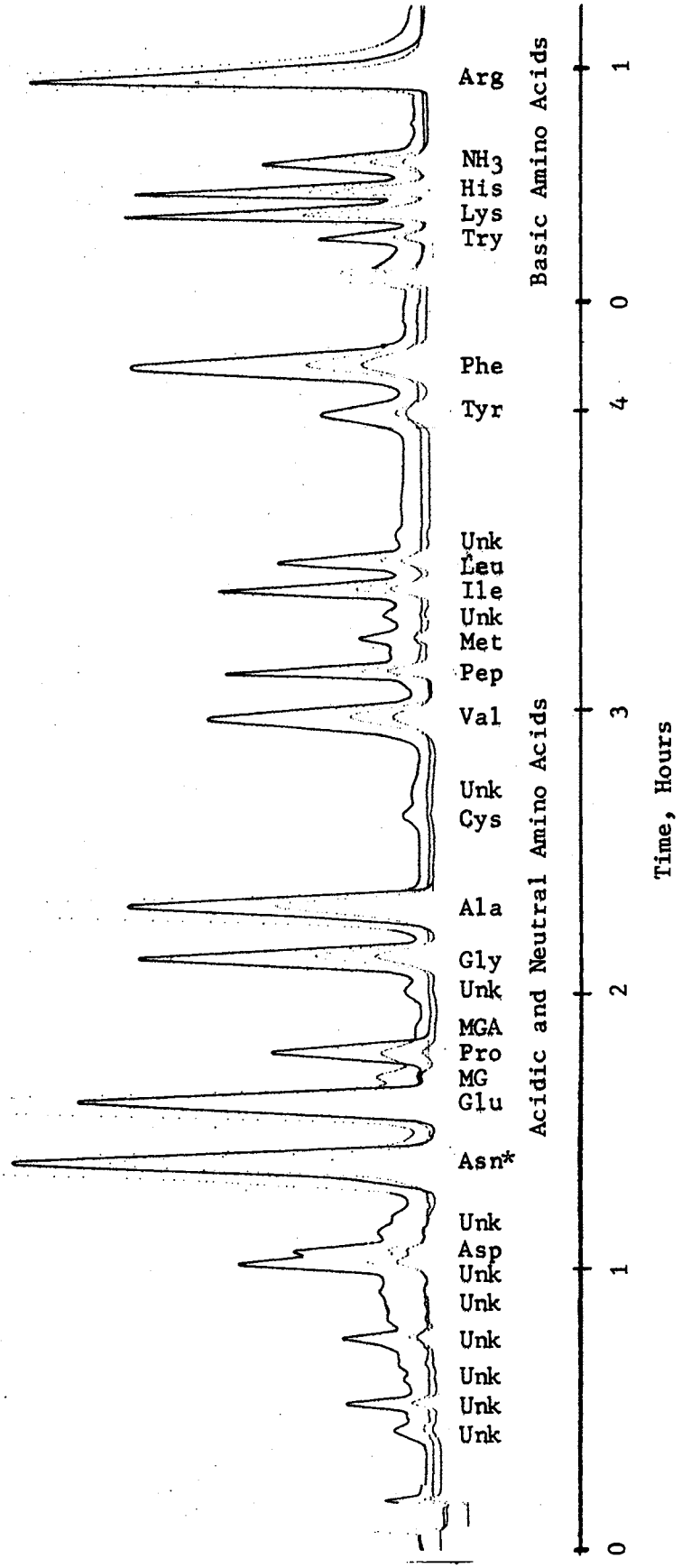
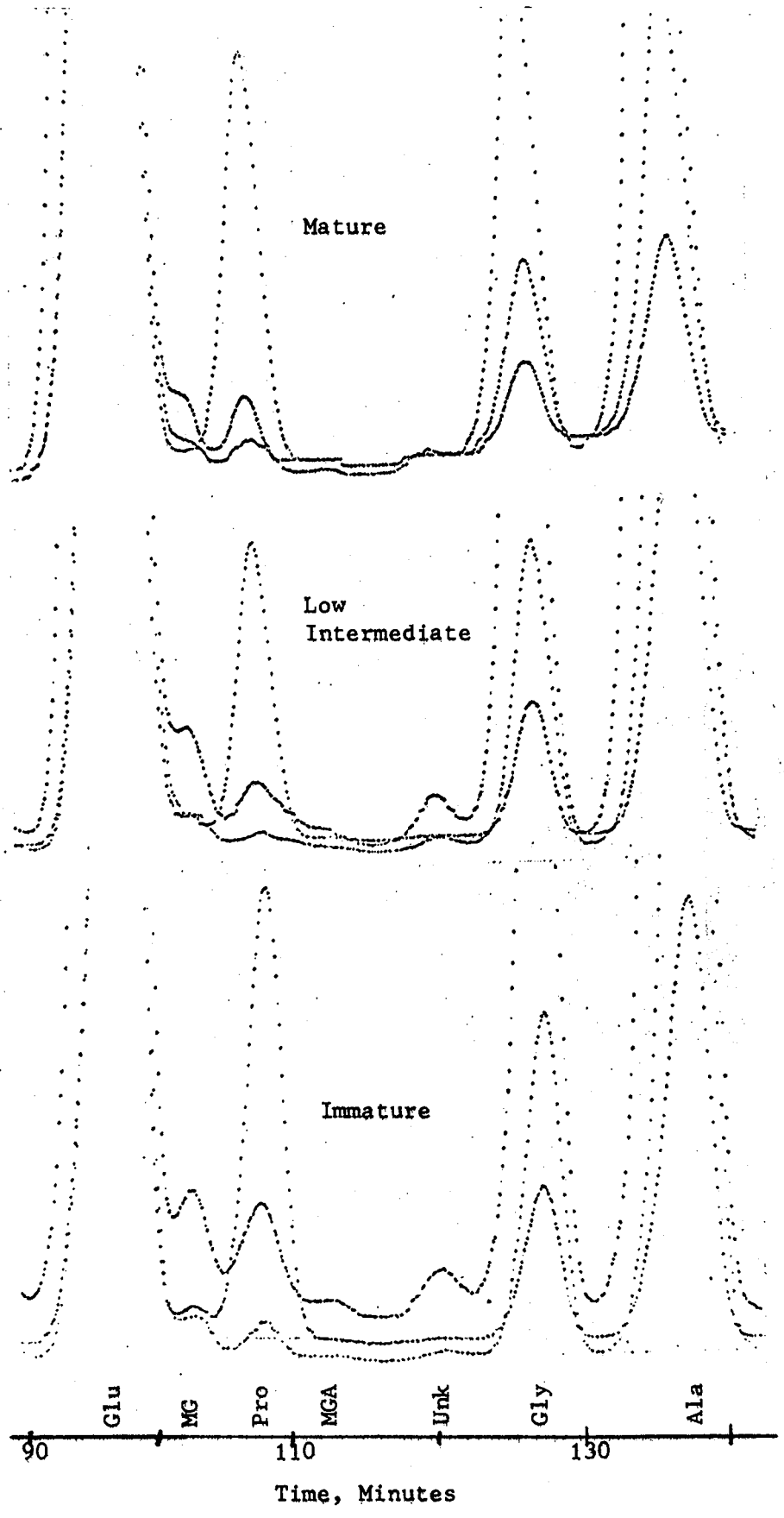

Arg
NH3
His
Lys
Try
Phe
Tyr
Unk
Leu
Ile
Unk
Met
Pep
Val
Unk
Cys
Ala
Gly
Unk
MGA
Pro
MG
Glu
Asn*
Unk
Asp
Unk
Unk
Unk
Unk
Unk
Unk
Basic Amino Acids
Acidic and Neutral Amino Acids
0
1
2
3
4
0
1
Time, Hours

Figure 24. A Portion of the Chromatogram (Figures 21, and 23) near Proline to show the Retention Time of MG and ., and to show the Effect on Maturity on MG and MGA.


Mature
Low
Intermediate
Immature
Glu
MG
Pro
MGA
Unk
Gly
Ala
90
110
130
Time, Minutes

CHAPTER IX

ISOLATION AND PARTIAL CHARACTERIZATION OF AN ACIDIC PEPTIDE

Introduction

An unidentified peak called an unknown amino acid was described by Newell (21) to be related to maturity and to produce atypical flav in roasted peanuts. Later it was found not to be amino acid but was peptide or small protein and was considered to have typical flavor (1 As it eluted with the amino acids, it was suspected to be a peptide but was later found to contain approximately 80 amino acid units (2). Recently Mason, et al. (1, 2) proposed that the majority of the amino acids that react with the reducing sugars were released from a large peptide during roasting. The concentration of this peptide was shown to increase from two to four micromoles in fat-free peanut meal with increasing maturity (21, 1, 2) a fact also substantiated by this dissertation (Chapter VIII, page 129).

The peptide, which was probably a misnomer, described in this chapter is the same as the one labeled peptide 2 by Mason et al. (1, 2) Chapter II, page 8 . The present study was a continuation of the above research initiated by Mason, et al. and was an attempt to obta a sufficient quantity of highly purified peptide suitable for characterization and for testing its role as a flavor precursor in a model system (21, 76).

Attempts at purification and characterization are described. A ew method, partially evaluated, is proposed for the isolation of the cidic peptide.

This study was conducted with a good flavored selection (P-74) hich had been found to be high in peptide content.

Apparatus and Reagents

pparatus

Peptide analyses were made using the ion-exchange column chromato- raphy technique of Spackman, et al. (16) on a Beckman Model 120-C mino Acid Analyzer. A 54 cm column with a buffer flow rate of 50 ml/ our containing the PA-28 resin was used for the monitoring of the cidic peptides presence.

eagents

Bio-Gel P-2 (BIO-RAD Laboratories).

Dowex AG 1-X2 (BIO-RAD Laboratories).

QAE Sephadex A-50 (Pharmacia Fine Chemicals, Inc.).

Reagent grade chemicals were used.

For buffers used in ion-exchange chromatography on the analyzer, he Beckman Procedure Manual of instructions was followed (63).

Procedures

urification of an Acidic Peptide as Reported by Mason et al. (2).

Raw peanuts were extracted by the methods of Newell et al. (85) nd Newell (21). The extract was placed on a Dowex -1-acetate column

nd the neutral and basic amino acids washed from the column with wate
he peptide was eluted with 2 N acetic acid. The peptide was prepara-
ively chromatographed and collected from the amino acid analyzer
olumn.

lution of the Peptide with Sodium Chloride

The above method of Mason et al. (2) was repeated using a 2.2 x
.0 cm Dowex AG1-X2-acetate column except elution was with a one per-
ent sodium chloride solution for 60 tubes followed by a two percent
odium chloride solution for the remainder of the elution steps.
pproximately 15 ml per tube were collected and the 280 nm absorbance
as measured on every second or third tube.

lution of Peptide with an Acetic Acid-Ammonium Hydroxide Mixture

Using the same resin material (Dowex-1-acetate) as used by Mason,
et al. (2), a 2.2 x 10 cm column was prepared. His procedure was
followed except the peptide was eluted using a mixture of acetic acid
and ammonium hydroxide consisting of 3 ml of glacial acetic acid and
5.1 ml of concentrated ammonium hydroxide diluted to a 500 ml volume
with water. The peaks were detected using a combination of ultra
violet absorbance at 280 nm, spotting on paper to detect (a weak rose
colored spot) and checking the apparent peaks on the Beckman model
120-C amino acid analyzer. It should be pointed out that we had no
positive method of assay for the peptide except with the amino acid
analyzer.

Partial Desalting of the Peptide

The peptide solution obtained when the peptide was eluted with twc ercent sodium chloride solution, was passed through a 2.2 X 28 cm io Gel P-2 column and 10 ml fractions collected using a ISCO model UA- ltraviolet analyzer.

xtraction of Peptide From Raw Peanuts

Methanol was used to extract the peptide (as compared to per- hloric acid or sodium chloride solution used in the above method) rom fat-free peanut meal. The extract was evaporated to an oily resi- ue and this crude preparation, which gave an excellent peanut aroma hen heated in an oven, was used for further isolation and purificatio ith most of the tests described in the chapter, a crude preparation repared as above was processed from 200 pounds of peanuts (P-74).

etection of Peptide

At present the only positive method of identification of the pep- ide is by ion-exchange column chromatography. With flow rates of 8 ml/hour, the peptide was not detected, therefore flow rates of 50 l/hour were used on a 54 cm PA-28 ion-exchange column. If the peptid as present in high enough concentration and relatively pure, it pro- uced a faint rose colored spot when reacted with ninhydrin solution o aper. It was assumed to have a 280 nm absorption as it contained an romatic amino acid although the pure peptide has not been obtained an ested for maximum absorbance.

reparative chromatography on amino acid analyzer

The peptide had been isolated from 200 pounds of peanuts (P-74).

had been partially purified on a 2.2 x 10 cm Dowex AG1-X2 acetate
lumn. This partially purified peptide when examined by ion-exchange
lumn chromatography appeared relatively pure in small quantities, but
is quite impure when larger amounts were examined using the amino acid
alyzer. The peptide was prepared on the analyzer column by repeated
ins on the 54 cm PA-28 ion-exchange column and collecting as it eluted
om the column in pH 3.250 citrate buffer. The peptide preparation
is rechromatographed to determine the purity of the peptide.

drolysis of Peptide with 6 N HCL

The method (Chapter VI, page 85) previously described was used
n this study.

Results and Discussion

This discussion is essentially a progress report of efforts over
early three years to isolate a larger quantity of purified peptide
uitable for determining its amino acid sequence, molecular weight,
iological role and suspected role as a flavor precursor. It is now
nought that most of the problems of isolation were due to the highly
cidic nature of the peptide (about one-third of the amino acid resi-
ues in the peptide were aspartic and glutamic acids) which caused
t to attract and carry along the impurities. But first a naive
pproach to the problem follows.

The loss of peptide in solutions of trichloroacetic acid and acetic
cid even when kept at -20° C was a problem, whereas water or sodium
hloride solutions seemed to have no effect on stability. Only recentl
as it realized that a rather pure peptide could be stored in pH 3.250

.trate buffer at 4° C for as much as three months. The peptide
)pears to hydrolyze very easily, thus adding to the problems of iso-
ıtion.

The following results are typical of those experienced with the
:tempts at isolation and purification of the acidic peptide. Mason,
: al. (2) found the peptide difficult to elute with 2 N acetic acid.
:cause of difficulty with elution and stability of the peptide in
:etic acid, a one percent sodium chloride solution (followed by a two
:rcent sodium chloride solution) was used to elute the peptide. A
:acing of the spectra is shown in Figure 25. There were four major
:aks and several minor peaks present. Tubes 25 (which produced a good
)lor with ninhydrin in a spot test), 45 (which produced a very good
)ot test) and 67 and 115 (which gave a faint spot test) were analyzed
/ ion-exchange chromatography.

Figure 26 shows the chromatogram produced when tube 25 was analyze
:ing the amino acid analyzer. At least four unknown substances were
:tected and aspartic acid, glutamic acid, proline, asparagine, gluta-
ıne, trace of peptide and possibly hydroxy-proline were detected.
ıe presence of the neutral amino acids was not expected since the pro-
:dure using a thorough washing with water as described by Mason et al.
2) was followed.

The chromatogram obtained on tube 45 (Figure 27) shows four uni-
:ntified substances, glutamic acid and larger amounts of peptide. The
nalysis of tube 67 (Figure 28) revealed four unidentified substances,
sparatic acid, glutamic acid, glycine and a good size peptide peak.
astly, Figure 29 shows the chromatogram from the analysis of tube 115.
hus far, this tube showed the least amount of contamination of the pep

ie peak. It contained aspartic acid, phenylalanine and two unidenfied peaks. Because of the many impurities in the peptide solutions, ne of these fractions were considered pure enough for a characteriion of the peptide.

Attempts to desalt the above peptide fractions using a P-2 resin re not successful. On a P-2 resin the peptide appeared both before d after the salt peak. The first peak was the largest and contained ly small amounts of free amino acids whereas the second peak had rger amounts of free amino acids.

When the peptide was eluted from the Dowex-1-acetate column (using e same resin as used by Mason, et al. (2) with the acetic acid-ammonm hydroxide mixture, nine peaks were observed by ultra violet absorbce. These peaks were examined using the amino acid analyzer. The sults are somewhat similar to those obtained with the sodium loride elution as discussed above. The first two peaks were, as pected, aspartic acid and glutamic acid. The peak containing the ptide also contained several amino acids. Before and after the pepde peak were several peaks that absorbed light at 280 nm but were not tected by the amino acid analyzer. This indicated that this preparaon might not be pure and the impurities were not alpha amino acids.

Another anion exchange resin (QAE A-50) was utilized and was found t to be satisfactory. This was due to the large volume changes of ie resin that occurred with changes in ionic strength of the solutions

Dialysis also was considered as a possible purification tool. ius, dialysis, using a three-fourth inch tubing treated with EDTA and zine and washed throughly, was attempted on another crude preparation. ie peptide dialyzed through the membrane but some residual material

emained in the tubing. A Biomed Microconcentrator which should con-
entrate small peptides, was used. The peptide passed through the mem-
rane along with the amino acids.

When analyzing for free amino acids and peptide (Chapter VIII), it as known that the pH of the first buffer (pH 3.250 $\pm$ 0.005) and time hange to the second buffer was important to obtain a symmetrical peak hat could be quantitatively measured (Chapter VIII, Figure 21). Using he peptide solution previously eluted from the Dowex AG 1X2-acetate olumn with sodium chloride, it was decided to use the amino acid nalyzer system for the preparation of larger quantities of the peptide. he collected peptide fraction in pH 3.250 citrate buffer solution was hecked for purity and the chromatogram is shown in Figure 30. Only hree very small peaks were seen as compared with the large broad pep-ide peak which was estimated to be about 99 percent pure.

Hydrolysis of this purified peptide (for 10, 20 or 30 hours) gave mall amounts of most all acidic and neutral amino acids (Figure 31). henylalanine and glutamic acid were the major amino acids present. able XLIII shows a tabulation of this analysis and is compared with he published figures of Mason et al. (2).

An examination of the data of Mason, et al. (2) on the peptide solated from a Dowex column revealed small peaks which had not been onsidered by those authors. The amount of peptide available for heir analysis was small. Additional studies must be made before an ccurate composition of the peptide can be reported.

Using the amount of peptide before acid hydrolysis as a base nd the amount of amino acids present after hydrolysis, calcula-

ons[1] were made and showed that the peptide was 50 percent hydrolyzed th our analytical method when it passed through the coils of the amin id analyzer. Therefore the values for the peptide concentration re- rded in this dissertation should be only twice as large and not proximately 80 times as was assumed in all previous work. An accurat amination of Mason's data (2) is not possible.

It would appear that too much importance has been attached to the le of the peptide in roasted peanut flavor. Examination of the data Chapter VIII indicated that the peptide was usually about third in nk of amount in raw peanuts (assuming that the peptide value should twice as large) following glutamic acid and asparagine*.

The final experiment employed the following method and is now commended for extraction and purification of the peptide. The pea- ts were extracted 48 hours with methanol by constant stirring of the xture. The mixture was filtered. The filtrate was passed through a wex AG1-X2-acetate column previously equilibrated with methanol. ution was accomplished by increasing amounts of ammonium acetate. en the solution containing the peptide peak was evaporated to near yness on a rotary evaporator at 40° C. The residue was lyophilized remove the ammonium acetate. Further purification using the amino id analyzer was the next step planned.

Summary

---

[1]One ml of peptide solution (Figure 30) has a HW value of 27.7. n ml were taken and hydrolyzed and 0.5 ml of the 2.5 ml of pH 2.2 trate buffer was analyzed and gave a 113 17 HW value for the amino ids (Figure 31). Thus 10 X 27.7 + 277 and 5X 113 + 565 is 277/565 = 49 percent.

Various attempts to isolate and purify the acidic peptide found in nuts from the contaminating amino acids and other ninhydrin positive stances were described.

Larger amounts of the peptide were prepared using the amino acid lyzer and one preparation appeared to be nearly pure (99 percent). no acid composition of the purified peptide was different from the lished results.

Because the peptide was partially hydrolyzed in the reaction coils the amino acid analyzer, the amount present in peanuts was not nearly high as previously assumed. Although still important, the role of peptide as a flavor precursor of roasted peanuts may have been over-hasized.

An improved method of isolation and purification of the peptide proposed.

TABLE XLIII

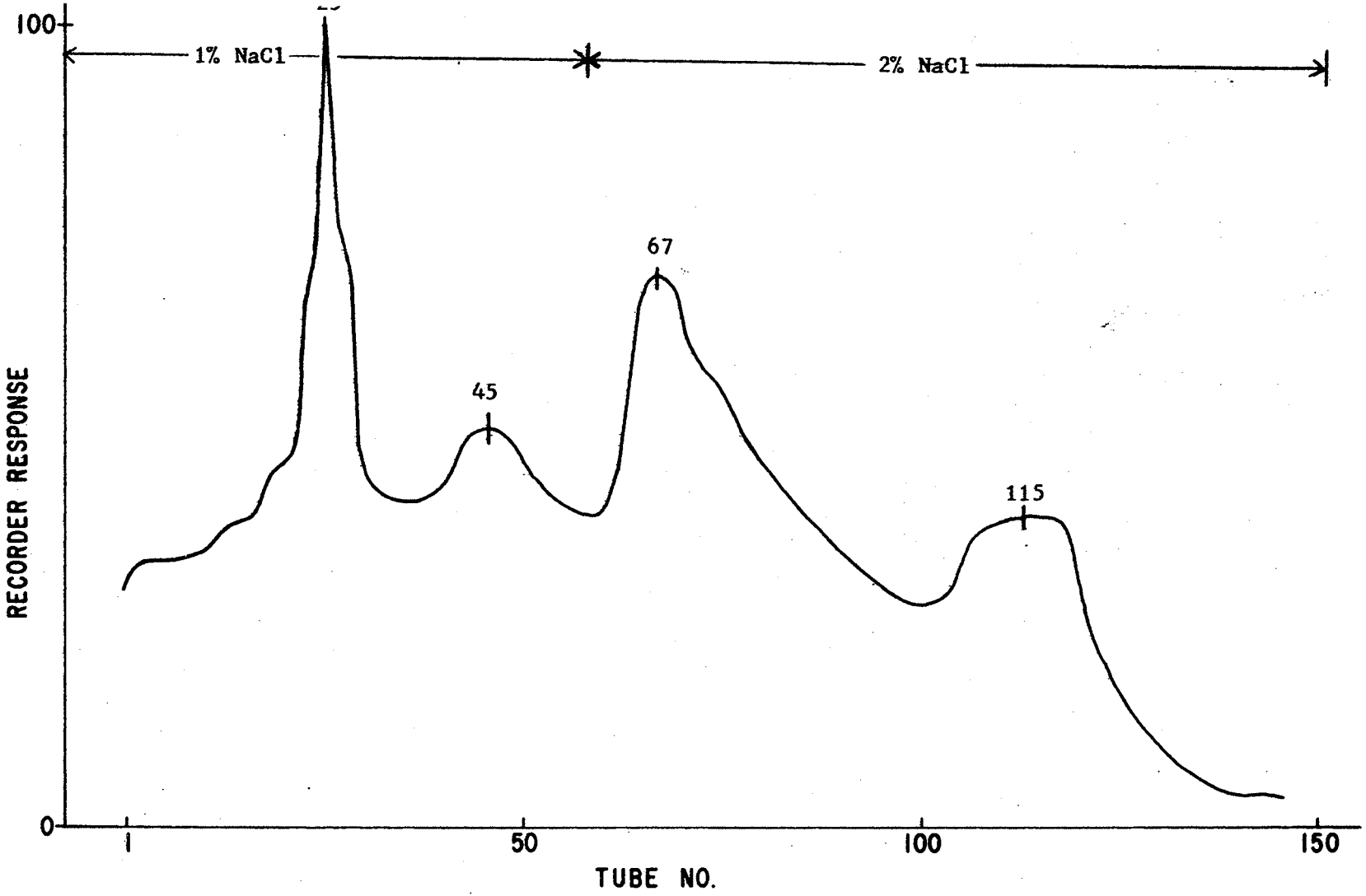
COMPARISON OF THE AMINO ACID COMPOSITION[a] OF A PARTIALLY PURIFIED PEPTIDE WITH MASON'S (2) PEPTIDE 2

	Peptide 2	Partially Purifed Peptide
Glutamic Acid (+ Gln)	25	50
Asparatic Acid (+ Asn)	4	1
Phenylalanine	17	58
Glycine	11	*
Serine	6	*
Alanine	3	1
Threonine	2	*
Leucine	2	*
Isoleucine	1	*
Valine	1	1
Tyrosone	1	1
Unknown		1
Hydroxy-Proline		1

[a] Number of residues

* Detected but in very small amount

Figure 25. Elution Profile of the Peptide from a 2.2 x 1(cm BioRad Dowex AG 1-X2 - Acetate Column.


100
0
1% NaCl
2% NaCl
67
45
115
RECORDER RESPONSE
1
50
100
150
TUBE NO.

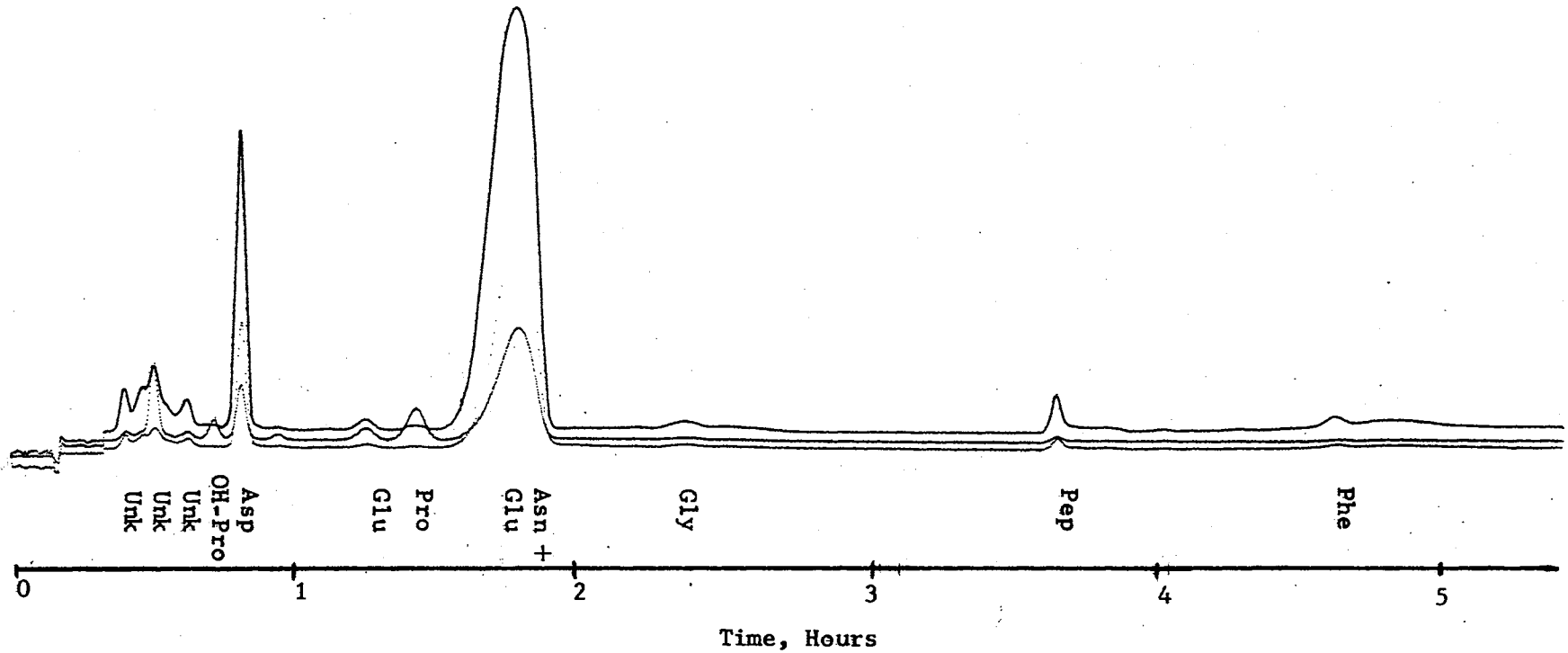


Figure 26. Chromatogram of Fraction No. 25 from the Dowex AG 1-X2 Acetate Column (see Figure 25).

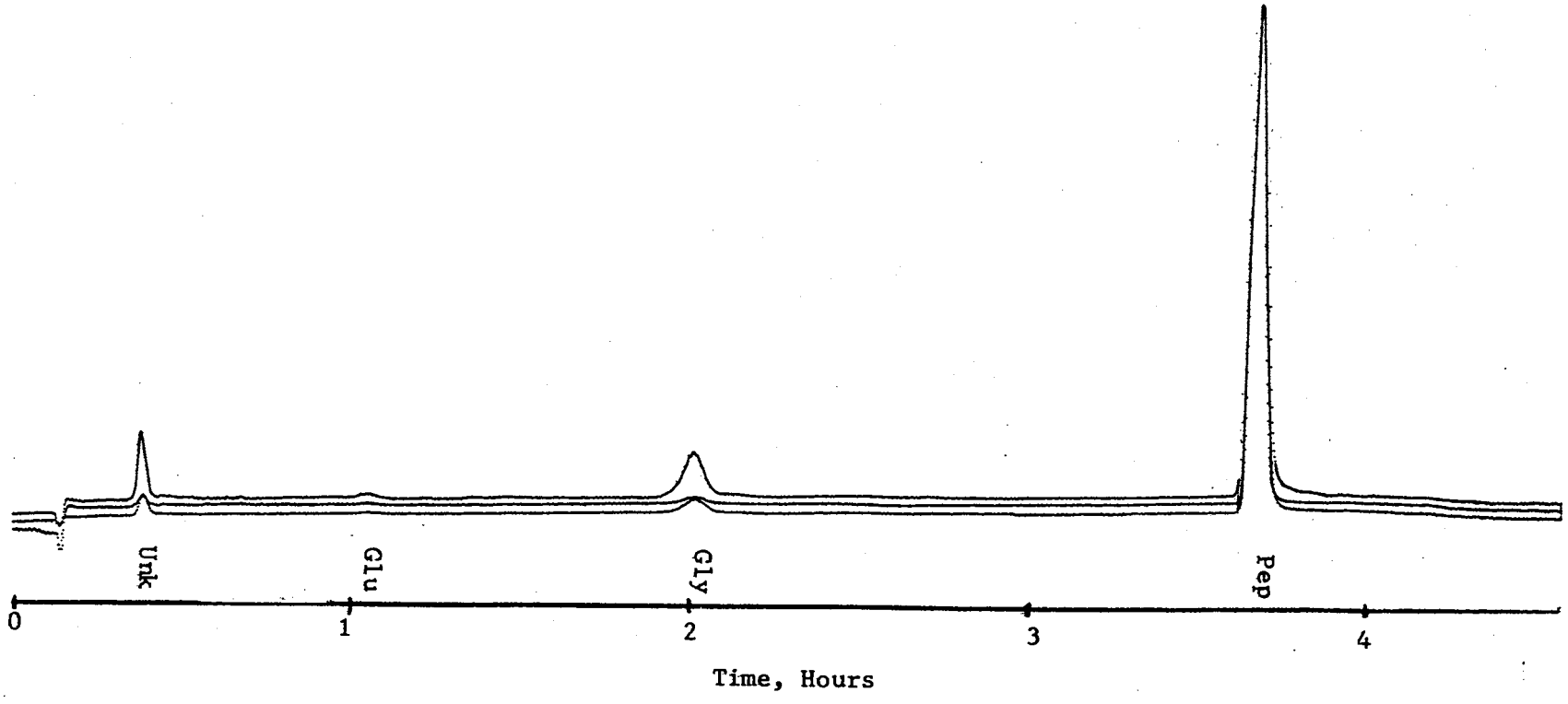


Figure 27. Chromatogram of Fraction No. 45 from the Dowex AG 1-X2 Acetate Column (see Figure 25).

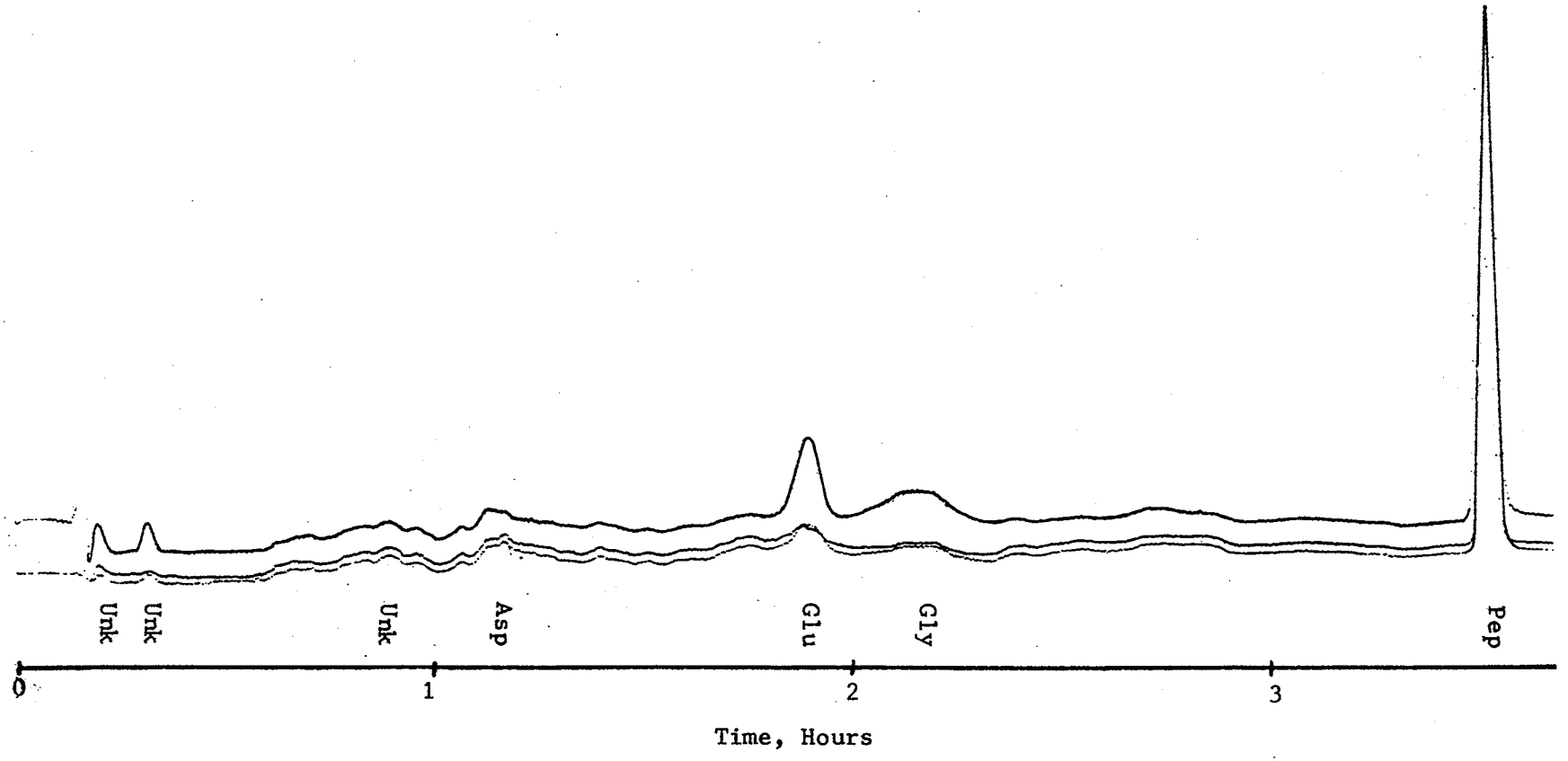


Figure 28. Chromatogram of Fraction No. 67 from the Dowex AG 1-X2 Acetate Column (see Figure 25).

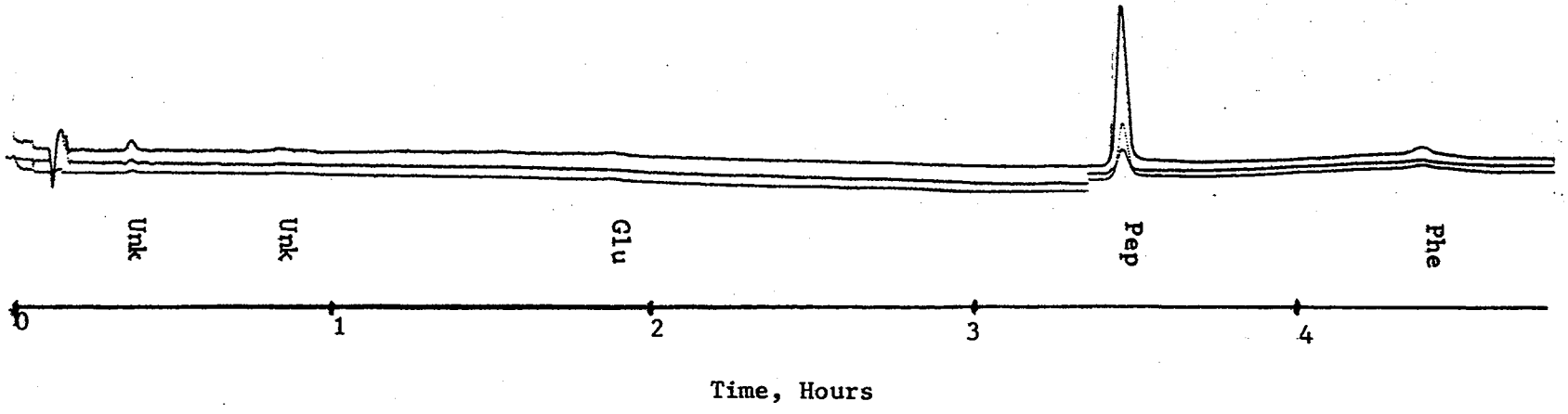


Figure 29. Chromatogram of Fraction No. 115 from the Dowex AG 1-X2 Acetate Column (see Figure 25).

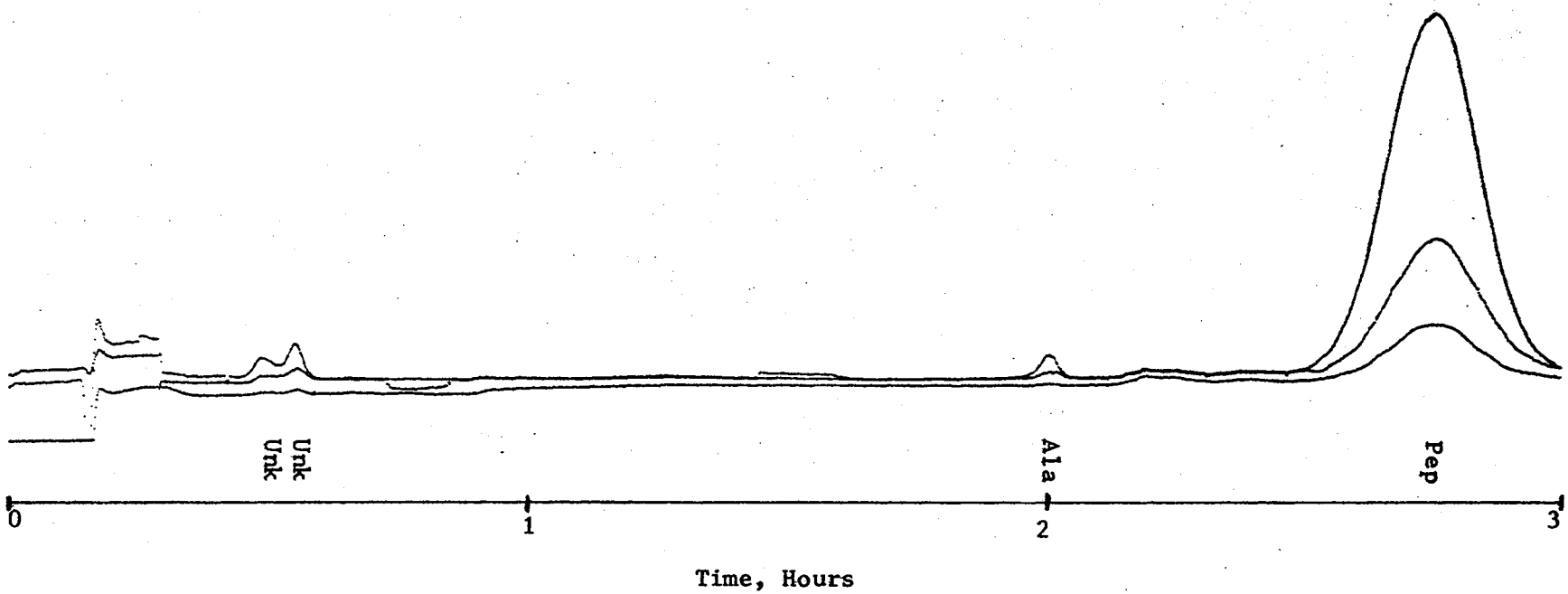


Figure 30. Chromatogram of the Partial Purfied Peptide, after Preping on the Amino Acid Analyzer.

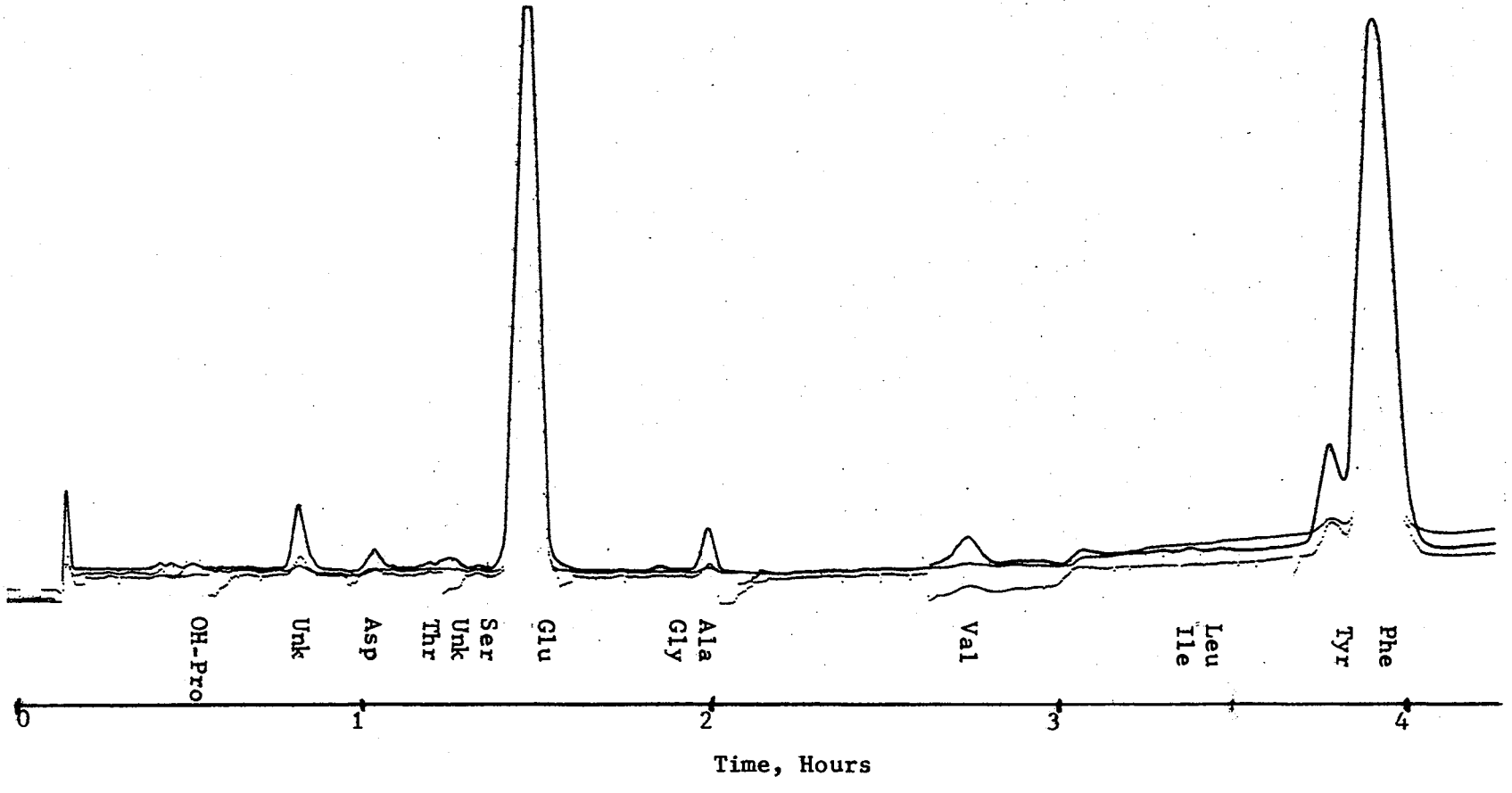


Figure 31. Chromatogram of the Hydrolyzate of the Partially Purified Peptide (see Figure 30).

CHAPTER X

Summary

A better knowledge and measurement of the contribution and effect of variety, maturity, irrigation and planting location on chemical constituents such as flavor precursors, fatty acids and amino acids were desired for the improvement of flavor and other quality factors of roasted peanuts and peanut products.

This dissertation was divided into three broad classifications and the following findings are reported.

A. Fatty Acids

A rapid biochemical microanalytical technique is described wherel a portion of a peanut kernel was analyzed for oleic acid/linoleic acic (methylester) ratio with the remainder of the kernel being planted to obtain genetic information. A description of the preparation of the methyl esters was reported. Analysis of the methyl esters by gas liquid chromatography required two to four minutes per sample dependi upon the equipment. The analytical techniques were evaluated for pre cision using 100 peanut varieties. This methodology is now in use to aid the breeder in the rapid selection and screening of genetic material and to date approximately 2,200 selected peanuts have been analyzed. Factors which influence O/L ratios are reported. The described procedures should speed the development of new and improved peanut varieties and perhaps other oilseed crops. The composition of

tty acids were reported for eight varieties each harvested at two-
ek intervals and classified into three maturity groups. Mature pea-
ts usually contain relatively higher amounts of oleic (18:1) and
earic (18:0) acids and less linoleic acid (18:2) and other fatty
ids when compared with immature peanuts. Behenic (22:0) and arachidic
0:0) acids which were recently implicated in heart disease were lower
the mature peanuts. Another study on nine varieties showed that
ate (Georgia vs Oklahoma), treatment (irrigation vs nonirrigation)
d variety had a significant effect (per cent distribution) on all
the fatty acids with the exceptions of behenic and arachidic. Sol-
nt extracted oils from Georgia had greater stability than those oils
om Oklahoma while there were no differences for the hydraulic pressed
ls. Possible correlation of fatty acid composition and organoleptic
ta were not possible because the size of samples were not sufficient
replicate the organoleptic tests.

B. Amino Acids

A protein hydrolyzate procedure with a precision and accuracy of
2.74 percent is described. The procedure is used to show large var-
ations in the amino acid composition of 16 varieties of peanut meal,
elected because of their relatively wide variation in protein content
24-30 percent). Variations of approximately two-fold for the limit-
ng essential amino acids (lysine, methionine, isoleucine and threonin
ere found which had not previously been reported.

An improved method for the extraction of free amino acids and the
eptide from peanuts with a methanol, chloroform and water mixture
as described. The effect of variety, maturity and harvest date on
ree amino acids and the peptide content was also determined. Gluta-

ic acid and asparagine (includes glutamine, threonine and serine) were resent in highest concentration in the mature and low intermediate eanuts. Arginine was the highest in immature peanuts. Two nonprotein nino acids, γ-methylene glutamin (MG) and γ-methylene glutamic acid MGA) were identified in mature peanut kernels and found to increase ith immaturity. Asparagine, glutamine and most of the peptide dis-ppeared in shelled peanuts stored six months at 34° F and 60 percent elative himidity. The effect of state, irrigation and variety on free nino acid content under the above storage conditions was evaluated tatistically. The analyses of variance were made and the statistical ignificance was summarized in tabular form.

Arginine content was evaluated for measuring the degree of imma-urity in freshly harvested or cured peanuts. Precision and accuracy f the modified Sakaguchi method was reported. Calibration curves to redict the degree of immaturity in peanuts were formulated. Lastly, ne modified Sakaguchi method for arginine was tested under field onditions and found to be an accurate measure of immaturity.

C. Isolation and Partial Characterization of an Acidic Peptide

Various attempts to isolate and purify the acidic peptide from ne contaminating amino acids and other ninhydrin positive substances ere described. Larger amounts of the peptide were prepared using ne amino acid analyzer and appeared to be quite pure (99 percent). ne amino acid composition of the hydrolyzate of the purified peptide as different from the published results. Because the peptide was only artially hydrolyzed in the reaction coils of the amino acid analyzer, ne amount present in peanuts was not nearly as high as previously ssumed. Although still important, the role of the peptide in flavor

f roasted peanuts may have been over-emphasized. A method of isolation and purification of the peptide was proposed.

A SELECTED BIBLIOGRAPHY

Mason, M. E., Newell, J., Johnson, B., Koehler, P., Waller, G. and Young, C., 156th Annual Meeting American Chemical Society, Sept., 1968, Div. of Agr. and Food Chem. Abstract 069.

Mason, M. E., Newell, J. A., Johnson, B. R., Koehler, P. E. and Waller, G. R., J. Agr. Food Chem., 17, 728 (1969).

Higgins, B. B. in The Peanut the Unpredictable Legume, The National Fertilizer Association, Washington, D. C. (1951) p. 18.

Chevalier, A., Rev. Bot. Appl. and d'Agr. Trop., 13, 722-752 (1933).

J. of Commerce, Norfolk, Va., Nov. 5, 1898.

Sessions, L. H., "The Problem of the Peanut Butter Industry", Natl. Peanut Council Yearbook, Washington, D. C. (1942) p. 29.

Graham, S. and Lipscomb, G. D., George Washington Carver, Scientist Julian Messner, Inc., New York, 1944.

Brakman, P., Sjoelin, K. E. and Astrup, T., Thromb. Diath. Haemorrhag 8, 442 (1962).

Hoffpauir, C. L., J. Agr. and Food Chem., 1, 668 (1953).

James, A. T. and Martin, A. J. P., Biochem. J., 50, 679 (1952).

James, A. T. and Martin, A. J. P., Analyst, 77, 915 (1952).

Mason, M. E., "Procedures in Studying and Factors Influencing the Quality and Flavor of Roasted Peanuts," (Ph.D. dissertation, Oklahoma State University, 1963).

Mason, M. E. and Waller, G. R., Anal. Chem., 36, 583 (1964).

Jellium, M. D. and Worthington, R. E., Crop Sci., 6, 251 (1966).

Waller, G. R., Proc. Okla. Acad. Sci., 47, 271 (1968).

Spackman, D. H., Stein, W. H. and Moore, S., Anal. Chem., 30, 1185 (1958).

Matlock, R. S., Proc. 5th Natl. Peanut Research Conf., 41 (1968).

Sexton, E. L., Emery, D. A., Perry, A. and Golumbic, C., Proc. 4th

Natl. Peanut Research Conf., 98, (1966).

Sexton, E. L., Proc. Peanut Improvement Working Group, 60, (1963).

Thomas, M. C., Food Tech, 22, 1442 (1968).

Newell, J. A., "Precursors of Typical and Atypical Roasted Peanut Flavor", (Ph.D. Dissertation, Oklahoma State University, 1967)

Young, C. T., Mason, M. E. and Matlock, R. S., A Peanut Progress Report, Oklahoma Agricultural Experiment Station Mimeograph Paper, Research and Education, 25 (1967).

Pickett, T. A. and Holley, K. T., Georgia Expt. Sta. Tech. Bull. No. 1, 1952.

McOsker, D. E., J. Nutr., 76, 453 (1962).

Cobb, W. Y., in The Unpredictible Legume, in press.

Woodroof, J. G., Production Peanut Processing Products, The AVI Publishing Co., Inc., Westport, Conn., 1966.

Rosen, G. D., A. M. Altschul, ed., Processed Plant Protein Foodstuffs, Academic Press, New York, 1958, p. 419.

Knowles, P. F., Econ. Botany, 19, 53 (1965).

Mason, M. E. and Matlock, R. S., Progress Report VIII on Agronomics Organoleptic, and Biochemical Study of Factors Responsible for the Flavor of Peanut Butter and Roasted Peanuts, Biochemistry Department, Oklahoma State University, 1968.

Holley, K. T. and Young, C. T., Proc. Peanut Improvement Working Group, 52 (1963).

Young, C. T. and Holley, K. T., Univ. of Georgia Expt. Sta. Tech. Bull. No. 41, 1965.

Pang, Li-Sung, "The Influence of Maturity and Time of Harvesting Spanish Peanuts on Peanut Butter Quality", (M.S. Thesis, Oklahoma State University, 1967).

Young, C. T. and Waller, G. R., American Chemical Society Southwest Regional Meeting, Tulsa, Okla., Dec. 4-5, 1969, Abstract B 10-11.

Kartha, A. R. S., J. Sci. Food Agric., 14, 515 (1963).

Holley, K. T. and Hammons, R. O., Univ. of Georgia Research Bulleti No. 32, 1968.

. Pickett, T. A. and Holley, K. T., Georgia Exp. Sta. Mimeo Series No 90, 1960.

. Prasad, B. G. and Diswas, B., J. Oil Tech. Assoc., (India), 12, 23 (1956).

. Schenk, R. U., Univ. of Georgia Tech. Bull. No. 22, 1961.

. Yermanos, D. M., California Agriculture, 15 (1968).

. Worthington, R. E., Proc. 5th Natl. Peanut Research Conf., 87 (1968)

. Sweeley, C. C., Elliott, W., Fries, I. and Ryhage, R., Anal. Chem. 38, 1549 (1966).

. Waller, G. R., Odell, G., Matlock, R. and Kirby, J., Progress Report IX on Biochemical, Agronomic and Organoleptic Study of Factors Responsible for the Flavor of Peanut Butter and Roaste Peanuts, Biochemistry Department, Oklahoma State University, 1969.

. Stokes, W. E. and Hull, F. H., J. Amer. Soc. of Agron., 22, 1004 (1930).

. John, C. M., Venkatanarayana, G. and Seshadri, C. R., Indian J. Agr. Sci., 14, 159 (1954).

. Tripp, L. D., "Germ Plasm Evaluation and Inheritance Studies in Peanuts" (Ph.D. dissertation, Oklahoma State University, 1968).

. Worthington, R. E. and Holley, K. T., J. Amer. Oil Chem. Soc., 44, 515 (1967).

. Olcott, H. S. and Einset, E., J. Amer. Oil Chem. Soc., 35, 161 (1958

. Roberson, E., Marion, S. E. and Woodroof, J. G., J. Amer. Diet. Assoc., 49 208 (1966).

. Howell, R. W., Proc. 5th Natl. Peanut Research Conf., p. 27, 1968.

. Snedecor, G. W., Statistical Methods, 4th ed., The Iowa State College Press, 1950.

. Lancaster, A. M., National Observer, December 22, 1969.

. Holley, K. T., Personal Communication, 1964.

. Kirby, J. S., Choate, V., and Collins, J., "Peanut Quality Evaluation Laboratory Procedures", Mimeographed Circular, Agronomy Department, Oklahoma State University, 1969.

. Matlock, R. S. and Waller, G. R., Progress Report I on Agronomic, Organoleptic and Biochemical Study of Factors Responsible for

the Flavor of Peanut Butter and Roasted Peanuts, Department of Biochemistry, Oklahoma State University, 1961.

. Cecil, S. R. and McWatters, K., J. Amer. Peanut Res. and Educ. Assoc., Proc. of Meeting, 1, 1969.

. Beasley, E. O. and Dickens, J. W., Proceeding of Peanut Improvement Working Group, 1963.

. Young, C. T., Manuscript in Preparation.

. Mitra, C. R. and Misra, P. S., J. Agr. and Food Chem., 15, 697 (1967).

. Altman, P. L. and Dittman, D. S., eds., Metabolism, Fed. of Amer. Soc. for Exp. Biology, Bethesda, Maryland, 1968.

. Evans, R. J. and Bandimer, S. L., Cereal Chem., 44, 417 (1967).

. Chopra, A. K. and Sidhu, G. S., Br. J. Nutr., 21, 519 (1967).

. Tkachuk, R. and Irvine, G. N., Cereal Chem., 46, 206 (1969).

. Beckman Model 120 C Amino Acid Analyzer Instruction Manual, Pub. by Beckman Instruments, Inc., Palo Alto, Calif., June, 1965.

. American Oil Chemists Society, Chicago, Illinois, Official and Tentative Methods, 1951.

. Guthrie, J. D., et al., A Literature Review, Southern Reg. Res. Lab., Agric. Res. Ser., U. S. Dept. Agric., AIC-61, 50-78, 1949.

. Pickett, T. A., Plant Physiol., 25, 210 (1950).

. Grau, C. R., J. Nutr., 32, 303 (1946).

. Izumi, Y., Anal. Biochem., 10, 218 (1965).

. Sturkie, D. G. and Williamson, J. T., in The Peanut, The Unpredictable Legume, The National Fertilizer Association, Washington D. C., 1951, p. 173.

. Barrs, H. D., Aust. J. Exp. Agr. Anim. Husband, 2, 106 (1962).

. Emery, D. A., Gupton, G. L., and Hehem, R. O., Proc. Peanut Improvment Working Group, 25, 1966.

2. Mills, W. T., Amer. Soc. Agri. Eng. Mtg. 1 (1961).

3. Teter, N. C., Proc. Peanut Research Conference, 54, (1966).

74. Toole, V. K., Baily, W. K., and Toole, E. H., Plant Physiol., 39, 822 (1964).

Izum, Y., Anal. Biochem., 12, 17 (1965).

Koehler, P. E., "Formation of Alkylpyrazine Compounds and Their Role in the Flavor of Roasted Foods", (Ph.D. dissertation, Oklahoma State University, 1969).

Bieleski R. L. and Turner, N. A., Anal. Biochem., 17, 278 (1966).

Nowacki, E., Z. Pflanzenzuehtg, 61, 232 (1969).

Done, J. and Fowden, L., Biochem. J., 51, 451 (1952).

Conkerton, E. J. and Neucere, N. J., Anal. Biochem., 32, 512 (1969).

Fowden, L., Personal Communication, 1970.

Mason, M. E. and Matlock, R. S., Contract Research Project Final Report to U. S. Dept. of Agriculture from the Biochemistry Dept., Oklahoma State University, June 24, 1964.

Prentice, N., Burger, W. C., Kastenschmidt, J. and Moeller, M., Phytochem., 9, 41 (1970).

Burger, W. C., Prentice, N., Moeller, M. and Robbins, G. S., Phytochem., 9, 49 (1970).

Newell, J. A., Mason, M. E. and Matlock, R. S., J. Agr. Food Chem., 15, 767 (1967).

Bailey, W. K., Proc. 5th Natl. Peanut Research Conf., 1, 1968.

VITA

Clyde Thomas Young

Candidate for the Degree of

Doctor of Philosophy

sis: BIOCHEMICAL STUDIES OF PEANUT (ARACHIS HYPOGAEA L.) QUALITY

or Field: Food Science

graphical:

Personal Data: Born in Durham, North Carolina, August 22, 1930, the son of Charles C. and Effie J. Young. Married Mary Catherine Seawell of Highfalls, North Carolina in Highfalls, North Carolina, December 24, 1955; five children.

Education: Graduate from Chapel Hill High School, Chapel Hill, North Carolina, in 1948; received Bachelor of Science degree in Animal Science from North Carolina State University, Raleigh, North Carolina, in 1952; received the Master of Science degree in Animal Science from North Carolina State University, Raleigh, North Carolina, in 1955. Completed the requirements for the Doctor of Philosophy degree in July, 1970.

Professional Experience: Instructor (Chemistry and Mathematics), North Carolina State University, Raleigh, North Carolina, from September, 1954 to June, 1956; Assistant Chemist, Georgia Institute of Technology, Atlanta, Georgia, from February, 1959 to October, 1960; Assistant Chemist, Georgia Experiment Station, Experiment, Georgia, since November, 1960. On sabbatical leave from the Georgia Experiment Station during the academic years of 1967-68, 1968-69 and 1969-70 in the Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma.

Professional and Honorary Societies: Member of Alpha Zeta, Phi Lambda Upsilon, Sigma Xi, The American Oil Chemists' Society, American Chemical Society, Institute of Food Technologists, American Peanut Research and Education Association and Farm Bureau.

Awards: Recipient of the 1967-68 Anderson Clayton and Company Fellowship.