PRECURSORS OF TYPICAL AND ATYPICAL

ROASTED PEANUT FLAVOR

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ROASTED PEANUT FLAVOR

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CHAPTER I

INTRODUCTION

A quotation from a recent article of Lepkovsky (1) says, "An adequate diet comprises the essential nutrients plus sensory stimuli with sensory properties being treated collectively as an essential nutrient." Indeed sensory depreivation may lead to neurosis and psychosis. Flavors of food may become an important source of sensory stimuli (2).

Flavor is a nebulous term the definition of which no two authors agree upon completely. The presently accepted definition of flavor which was advanced by Moncrieff (3) includes the sensations of taste (sweet, sour, bitter, and salty), odor (via olfactory receptors), tactual response (via pain receptors), hotness or coldness, and pungency or blandness. Of these qualities, odor and taste are the main contributors to flavor. Yet if one's sense of smell is deadened, most of the flavor disappears. Taste remains but, without odor, an apple and an onion taste alike. Few foods have a characteristic flavor which can be described by one of the basic taste responses alone. In order for a chemical compound to play a significant role as a food aroma, it is evident that it must have significant volatility since without a reasonably high vapor pressure a compound is unable to reach the locus of the odor response. Much of the modern work in the chemistry of flavor is based upon this reasoning. In other words, it is assumed that the odor of a food product rather than its taste or feel actually defines the typical

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flavor of that food.

If this reasoning is valid, then it should be possible to reproduce a flavor by adding compounds which elicit the odor response of a given food to a medium having the correct tactual and taste response. That this is in fact possible has been shown by recent work on the production of artificial pork from soybean meal. If the typical pork flavor compounds are added to or produced in this high protein (therefore meatlike) medium, the product is nearly indistinguishable from natural pork (4).

Several foods such as peanuts, cocoa beans, and coffee require a process in which they are roasted at high temperature to induce chemical reactions which in turn produce volatile compounds which give the roasted product its typical flavor. Since peanuts are such a pyrrolytically processed food product, they represent an excellent system in which to study the compounds of low volatility which are converted to more volatile ones during heating and the manner in which this conversion takes place.

A study of the flavor precursors of peanuts was initiated for the following reasons:

1) Most importantly, to accumulate basic knowledge concerning the kinds of compounds that serve as flavor precursors in foods.

2) To determine if there exists a correlation between flavor measured subjectively and flavor precursor concentration. If such a correlation could be demonstrated, it would be possible to predict the flavor of a roasted peanut product by means of a few objective measurements made on raw peanuts.

3) Knowledge of the flavor precursors will allow one to take a scientific approach to the off-flavor problem which arises from either

improperly cured or very immature peanuts. Even correctly cured immature peanuts possess a high degree of off-flavor. This fact means that immature peanuts either contain high concentrations of flavor precursors which are converted to compounds associated with atypical flavor during roasting or that they lack precursors of typical flavor. Therefore it becomes important to study the formation of peanut flavor precursors as a function of growing season to determine if a correlation exists between flavor precursor concentration and age of the peanut fruit.

The objectives of the work reported in this thesis were fourfold:

1) To develop methods of extraction and quantitative estimation of peanut flavor precursors.

2) To determine the nature and identity of the precursors of typical peanut flavor.

3) To quantitatively measure peanut flavor precursor concentration with respect to flavor measured subjectively and peanut age.

4) To investigate pyrolytic reactions of the flavor precursors in model systems to determine:

   a) If the so-called flavor precursors when heated can give rise to the same volatile compounds which have been isolated from normally roasted peanuts.

   b) The likely mechanism(s) of formation of these volatile flavor components in peanut-like model systems.

CHAPTER II

LITERATURE REVIEW

Introduction

With the exception of sweetness, raw peanuts exhibit none of the
flavor usually associated with roasted peanut products such as peanut
butter. Since this is the case, it is obvious that raw peanuts contain
compounds (flavor precursors) which are converted to new compounds
(flavor components) which give roasted peanuts their characteristic
flavor.

Roasting of peanuts represents an unusual system in that the inter-
nal temperature of the peanut cotyledon reaches 130-150°C during the
process (5). The internal temperature of food products such as meat and
vegetables seldom reach temperatures greater than 100°C since they have
a very high initial and final water content. Peanuts and other food
products such as coffee and cocoa which have low moisture content there-
fore represent a system in which to study the chemical reactions which
can occur between the constituents of the food at higher temperatures.
The nature of these pyrrolytic reactions and their reactants are of ex-
treme interest collectively since they must dictate the nature of the
products produced which in turn determine the flavor of the finished
product.

A knowledge of the exact mechanisms of production of flavor in such
pyrrolytically processed foods would allow the duplication of a natural

flavor in an artificial system or the modification of the flavor of a natural food product.

In this review, the author will discuss what was known concerning the precursors of peanut flavor prior to the work described in this dissertation. Research on flavor precursors of other pyrolytically processed foods, especially cocoa, will be discussed along with a review of the relationship between the Maillard browning reaction and production of volatile flavor components.

Precursors of Peanut Flavor

In 1952, Pickett and Holley (5), of the University of Georgia, published a bulletin, later reviewed by Hoffpauir (6), which contained a summary of their studies on the process of peanut roasting. A significant portion of this work was concerned with the changes in concentration of various compounds (possible flavor precursors) during roasting. Their investigation will be discussed in some detail since it represented the state of knowledge up to 1964 when a study of peanut flavor precursors was initiated by Mason and Waller (7).

Pickett and Holley noted prior to 1952 that during roasting peanut proteins were denatured (8) but that their nutritive value was unchanged (9). The oil and starch remained unchanged (8), while total sugars decreased.

Volatile components associated with the gases over roasted peanuts were found to consist of 98 percent of carbon dioxide and lesser quantities of aldehydes, furfurals, ammonia, hydrogen sulfide, diacetyl and volatile sulfur compounds. These workers (5) concerned themselves with determining the origin of these volatile substances.

It was found that peanut protein heated at 150°C gave hydrogen

sulfide but no carbon dioxide. A 50 percent acetone extract of raw peanuts when dried and roasted, browned and evolved hydrogen sulfide and carbon dioxide. By fractionation of the acetone extract it was found that browning and carbon dioxide evolution arose from nonenzymatic browning reactions between amino acids and water soluble carbohydrates. After it was established that the Maillard reaction (nonenzymatic browning reaction between amines and carbohydrates) was important in peanut roasting, total free amino nitrogen was measured during the course of an ordinary roast and was found to decrease significantly. However it was shown that total nitrogen did not decrease significantly. Sucrose concentration decreased when peanuts were heated for prolonged periods of time (5).

These authors went on to study the Maillard reaction between sucrose and amino acids in aqueous solutions at normal roasting temperatures. Alanine and sucrose when heated together in aqueous solution browned and produced carbon dioxide; in the dry state no browning or carbon dioxide evolution was observed. During the reaction, production of unfermentable reducing substances was noted. It was concluded that either sucrose was not hydrolyzed prior to its participation in browning or that the hydrolysis products were immediately consumed by the amino acid in the system since using their methods, no glucose or fructose could be detected during the course of the reaction.

A number of amino acids including aspartic acid, asparagine, glutamic acid, serine, glycine, threonine, alanine, alpha amino-n-butyric acid, histidine, arginine, lysine, methionine, leucine or isoleucine, phenylalanine, tyrosine and tryptophan were detected on paper chromatograms of 70 percent ethanol extracts of raw peanuts. Cystine, proline,

glutamine and glucosamine were tentitively identified. Extracts of roasted peanuts contained the same amino acids but at lower concentrations than in the raw nuts. Pickett and Holley also demonstrated that of the isomeric alanines, only alpha alanine produced carbon dioxide when heated with sucrose at $140^\circ C$; either alpha- or beta-alanine gave brown products (5). From this evidence it was proposed that browning during roasting of peanuts might arise via three routes: from caramelization, Maillard reaction or reaction of carbohydrates with free nonterminal amino groups in protein.

Raw peanuts were soaked in solutions of amino acids and reducing sugars and after drying it was observed that a normal roast could be obtained at a much lower temperature while the color and aroma of the nuts were nearly normal (5).

The work of Pickett and Holley (5), while limited by the experimental tools available in 1952, is consistent with results to be discussed in this thesis. One criticism of their work is that a decrease in amino acid and carbohydrate concentration during roasting was only circumstantial proof that these compounds were flavor precursors. Flavor precursors must be shown to be reactants in the processes which produce volatile flavor components. However at the time of Pickett and Holley's work the flavor components of peanuts were largely unknown since modern methods were not available for the identification of trace amounts of volatile compounds. Since the Georgia workers demonstrated that amino acids and sucrose could give rise to Maillard browning and since browning might serve as a marker of formation of flavor components (7), the observed browning could be taken as somewhat better than circumstantial evidence that the reactants were indeed flavor precursors.

Another criticism is that these workers used aqueous model systems. Since peanuts contain only 4-6 percent water (5) the aqueous system is not a valid model for roasting peanuts.

Nothing concerning peanut flavor precursors appeared until 1964 when Mason and Waller (7) from this laboratory published a paper concerning the isolation and localization of the precursors of roasted peanut flavor. Ground defatted peanuts which characteristically contain large amounts of globular protein were stirred with 1M sodium chloride. After removal of the insoluble material by centrifugation the soluble material (45 percent of the original dry weight of the fat-free peanuts) was lyophilized to dryness and roasted. Since the roasted material had the aroma of roasted peanuts it was evident that flavor precursors were contained in this fraction. The components of the unheated soluble extract were separated by gel filtration on Sephadex G-25. Each individual peak from this column was lyophilized and roasted. Only the fraction (the flavor precursor fraction) which contained molecules of low molecular weight gave rise to peanut aroma upon heating. The very large amount of protein isolated produced little or no aroma or browning upon heating in the dry state. Paper chromatography of the flavor precursor fraction indicated the presence of two ninhydrin positive components and one spot which was highly basic and exhibited a yellow ninhydrin reaction.

Linear Arrhenius type plots of the log of the time required to roast a series of peanut samples plotted versus reciprocal temperature of roasting were interpreted to mean that the rate limiting processes occurring during peanut roasting were unimolecular decompositions. This could have meant that production of flavor resulted from decomposition

of a single preformed flavor precursor although this mechanism seems unlikely in view of results to be discussed in this thesis.

Mason and Waller (7) also found that a single subcellular fraction of raw peanuts, the aleurone grain-protein body fraction, produced typical peanut flavor when roasted; starch grains or vascular material exhibited little typical roasted peanut aroma upon heating.

The work of Mason and Waller (7) does not completely rule out the possibility that protein could be a precursor of peanut flavor since it was not heated with a carbohydrate which was indicated to be a necessary reactant in the reactions to produce flavor by the work of Pickett and Holley (5). During roasting of coffee protein bound arginine was extensively degraded, cystine, lysine, serine and threonine were slightly degraded while other protein amino acids remained unchanged during roasting (10).

Peanut Flavor Components

Any mechanism to explain the decomposition of flavor precursors must take into account the volatile flavor components produced during the reactions involving the precursors.

As previously mentioned, Pickett and Holley (5) demonstrated the presence of carbon dioxide, aldehydes, ammonia and sulfur compounds. More recently Pattee et al. (11) found by means of gas liquid chromatographic retention times and functional group tests, formaldehyde, acetaldehyde, ethanol, acetone, isobuteraldehyde, 2-methyl valeraldehyde, methyl butyl ketone, hexaldehyde, 2-or 3-methyl butanol (tentatively) and furfural in raw, high temperature cured, off-flavored peanuts. Recently Young and Holley (12) showed that roasted off-flavored peanuts contained unusually large amounts of volatile carbonyl compounds.

Mason et al. (13) characterized by nuclear magnetic resonance spectrometry, ultraviolet spectroscopy and mass spectrometry 2-methylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine, 2-methyl-5-ethylpyrazine, 2,5-dimethyl-3-ethylpyrazine and N-methylpyrrole from peanuts having normal flavor. In a later paper, the same group (14) identified acetaldehyde, isobuteraldehyde, benzaldehyde, phenylacetaldehyde and tentatively 2- and 3-methylbutanals and 3-methyl-2-butanone by use of gas liquid chromatography in conjunction with a rapid scanning mass spectrometer, thin layer chromatography of 2,4-dinitrophenylhydrazone derivatives along with ultraviolet spectra and regeneration of the derivatives (15). Mason (14) believed that phenylacetaldehyde contributed to the sweet bouquet of roasted peanuts; low molecular weight aldehydes gave the harsh note to freshly roasted peanuts. Pyrazines were apparently the compounds responsible for the roasted-nutty aroma of peanuts (13). However Deck and Chang (16) reported aroma responses of "earthy-raw potato" for 2,5-dimethylpyrazine.

From this flavor component work taken as a whole, one might conclude that pyrazine compounds give peanuts their nut-like aroma while aldehydes play a contributory role. Aldehydes in high concentrations might be responsible for atypical- or off-flavor.

Precursors of Chocolate Aroma

Cocoa beans, like peanuts, require roasting to develop their typical flavor. Rohan (17) found that cocoa flavor precursors were water soluble, methanol soluble, dialyzable compounds. The flavor precursor fraction of fermented cocoa beans was composed of 35 percent sugars, 25 percent amino acids and 30 percent flavonoids. These extracted precursors, which accounted for 5 percent of the shell-free bean, produced

typical chocolate aroma upon heating for 8 minutes at 130°C. This observation held in fermented beans but not in unfermented ones (18). The main groups of compounds in the cocoa flavor precursor fraction were separated into flavonoids, amino acids and carbohydrates by lead acetate precipitation and cation exchange chromatography (18). Fermented beans contained lower concentrations of carbohydrates and higher concentrations of amino acids than unfermented ones. Unfermented beans contained only sucrose while fermented ones contained glucose and fructose in addition. All amino acids except histidine increased in concentration during fermentation. The flavonoids and phenolic acids contained in the flavor precursor fraction of cocoa did not appear to be essential to production of chocolate aroma (19).

Later the same group (20) showed that different varities of cocoa had different amino acid concentrations and it was postulated that the differences in flavor between varieties arose from the variability in amino acid levels in the fermented bean.

Rohan and Stewart (21) determined the concentration of groups of amino acids during roasting of Accra cocoa beans at 182°C. The results indicated that destruction of amino acids was a linear function of time of roasting and that only about 50 percent of the total amino acids were destroyed during the course of an ordinary 28 minute roast. Different groups of amino acids reacted at variable rates. Total nitrogen in the beans was unaltered during roasting just as was observed in the case of peanuts (5). A mechanism employing the ideas of Schonberg et al. (22, 23) concerning the mechanism of Strecker degradation, was proposed to explain the decrease in amino acid concentration during roasting of cocoa beans.

In some particularly elegant work, Pinto and Chichester (24) determined individual amino acids and total reducing sugars utilized during roasting of cocoa. In addition, they followed the production of individual aldehydes by gas-liquid chromatography. They found that all amino acids were destroyed during roasting with the exception of methionine and that glutamic acid, leucine, phenylalanine and an unknown amino acid decreased markedly in concentration. Ammonia increased during roasting (a phenomenon noted during roasting of peanuts--Chapter IV). A very large increase in concentration of isovaleraldehyde was noted along with smaller increases in concentration of propionaldehyde and isobutyraldehyde. Volatile aldehydes identified in addition to those already mentioned were acetaldehyde and diacetyl. It was interesting to note that the concentration of acetaldehyde initially increased and then decreased during the final stages of roasting. The significance of this observation was not discussed.

Both Pinto and Chichester (24) and Rohan and Stewart (25) found nearly complete destruction of reducing sugars during cocoa roasting. Further, Rohan and Stewart's results (25) suggested destruction of sucrose during roasting presumably by hydrolysis to glucose and fructose. They explained the incomplete destruction of amino acids during roasting by the fact that the degrading reagents, reducing sugars, were completely utilized during the process (25).

The overall conclusion was that amino acids were converted to aldehydes by Strecker degradation which occurred during roasting. The evidence of Pinto and Chichester (24) concerning the origin of volatile flavor components seems to be irrefutable since a large decrease in leucine concentration and a concomitant increase in its Strecker aldehyde,

isovaleraldehyde, was demonstrated. This evidence is all the more convincing since Herz and Shallenberger (26) produced chocolate aroma by heating leucine, glutamine and threonine at $100^\circ C$ and valine at $180^\circ C$ with glucose.

Considering the striking morphological similarities of peanuts and cocoa beans (27) and the similarity of the roasting process to which each is subjected, it is not surprising that these two foods have the same flavor precursors (amino acids and carbohydrates). It is surprising that simple alkylated pyrazines have not been isolated from roasted cocoa beans since they have been characterized in peanuts (13), coffee (28, 29) and heated carbohydrate-amino acid model systems (30, Chapter VI). This author suggests that pyrazine compounds will prove to be quantitatively important in the volatile components of roasted cocoa beans. However it is impossible to predict the significance of pyrazines in the flavor of chocolate.

Chemistry of Nonenzymatic Browning

Evidently, considering what has previously been discussed, one of the primary events occurring during roasting of peanuts or cocoa beans is the Maillard reaction between amino acids and reducing carbohydrates. Since this is true, a brief description of the reactions occurring during the Maillard reaction, taken from the volumes of literature available on this reaction, is in order since a mechanism based upon the Maillard reaction is proposed in this thesis to account for the formation of flavor compounds during roasting of peanuts.

The Maillard reaction is very complex and, as a result, most of the knowledge concerning the reaction has been derived from work in simplified model systems which contain a single amino acid and carbohydrate.

Information concerning the chemistry of nonenzymatic browning reactions was reviewed by Heyns and Paulsen (31), Danehy and Pigman (32), Reynolds (33,34), Ellis (35), Anet (36) and Hodge (37). The very detailed reviews of Reynolds (33,34) are an excellent guide for those interested in the preparation and properties of intermediates in the Maillard reaction; the review of Hodge (37) is concerned with the chemistry of browning from the standpoint of the food or flavor chemist. Hodge's review (37) presents the Maillard reaction in an extremely well organized manner and was used as an outline for the review presented here.

Hodge (37) divided the browning reaction into three stages. The initial colorless stage involves sugar-amine condensation and Amadori rearrangement. In the intermediate (colorless or yellow) stage, sugar dehydration, sugar fragmentation and amino acid degradation occur. In the highly colored final stages, aldol condensation, aldehyde-amine polymerization and formation of heterocyclic nitrogen compounds occurs.

Initial Stage--Sugar Amine Condensation

The initial stages of the Maillard reaction between glucose and glycine have recently been investigated by Song and Chichester (38) and Song et al. (39). Song et al. (39) studied the kinetics of formation of brown polymers during the Maillard reaction and observed a pronounced lag phase prior to the steady phase formation of melanoidins (brown polymeric materials). The lag phase was interpreted to mean that certain colorless intermediates were formed prior to the steady phase formation of melanoidins. They prepared N-D-glucosylglycine and compared its chromatographic properties with a product from a heated aqueous glucose-glycine model system (38). The coincidence of the synethtic material with the compound from the model system was interpreted to mean that

N-D-glucosylglycine was one of the primary products formed in this Maillard reaction. Qualitative tests on the isolated material also indicated that it was N-D-glucosylglycine. That the material from the model system was composed of glucose and glycine was shown by the fact that it incorporated label from either ^{14}C glucose or ^{14}C glycine to the same extent.

Song and Chichester (38) determined the induction period for a number of different sugar-amino acid reactions. A short induction period meant that the rate of reaction for one of the components was faster than in the glucose-glycine system. In other words, a short induction time meant that a rate limiting reaction had been overcome. A fructose-glycine mixture had an induction time of 2.3×10^3 minutes while the glucose-glycine system had an induction period of 6.9×10^3 minutes. This observation was explained by the increased reactivity of the carbonyl group of fructose toward nucleophylic attack by glycine. In comparison, α-methyl-D-glucoside which has a stable hemiacetal ring had an induction time of 13×10^3 minutes while arabinose which possesses an unstable hemiacetal had an induction time of only 1.1×10^3 minutes. On the basis of this evidence, the open chain form of glucose was judged the reactive species of the carbohydrate in the Maillard reaction. As the pH of the reaction mixture approached pK_2 of glycine, the velocity of browning increased markedly. Thus the conjugate base of the glycine amino group, rather than the conjugate acid, must have been the reactive species of the amino acid. On the basis of Song and Chichester's evidence (38) nucleophylic attack of the conjugate base of the glycine amino group on the carbonyl group of glucose in its open chain form was the primary event in the Maillard reaction.

Initial Stage--Reaction of N-Substituted Carbohydrate

Song and Chichester (38) detected another compound in their work with the glucose-glycine system which contained glucose and glycine in equimolar quantities. They felt that it was formed by consecutive reaction of N-D-glucosylglycine, and thus represented the product of the second step of the Maillard reaction. The fact that the compound gave a positive test with m-phenylenediamine and decolorized bromine indicated to them that the compound was probably an α,β-unsaturated enolic aldimine in equilibrium with its keto form. This unsaturated compound was isolated from the glucose-glycine reaction mixture by adsorption onto aluminum oxide. When the material, which had an ultraviolet spectrum characteristic of a semicarbazone, was eluted from the adsorbant with ether, it browned rapidly at room temperature. This fact substantiates the conclusion that the unsaturated compound was indeed an intermediate in the browning reaction.

2-propenal was used as a model of a compound having a structure more like the postulated α,β-unsaturated aldimine intermediate and it was found that the 2-propenal-glycine model system had no induction period. The induction period when 2-amino-D-glucose was heated alone at pH 6.0 was 150 times less than in the glucose-glycine system. These induction time data point to the fact that during the induction period in the glucose-glycine reaction nitrogen was incorporated into the carbohydrate and unsaturation was produced. In the kinetic studies of Katchalsky and Sharon (40), the formation of the N-D-glucosylamino acid was assumed to be the rate determining step in the Maillard reaction. The work of Song and Chichester (38) and Song et al. (39) bears out this assumption since substitution of a substrate which was more reactive to

nucleophylic attack by the amino acid amino group exhibited a reduced induction period (38,39).

Because they isolated a reactive α,β-unsaturated aldimine, Song and Chichester suggested that N-D-glucosylglycine lost the elements of water to give the Amadori rearrangement product keto, 1-amino-1-deoxy-glycino-D-fructose in equilibrium with its enol. This enol lost water at C-3 to yield the α,β-unsaturated aldimine. Hodge (37) pointed out that Amadori rearrangement (conversion of N-substituted aldosylamines to 1-amino-1-deoxy-2-ketoses) was suspected to occur during the Maillard reaction, but it was not until 1952 that the Amadori product was isolated by Hodge (41) and Gottschalk (42). That the Amadori product was an intermediate in nonenzymatic browning was demonstrated by the fact that the N-substituted glucosylamine browned at room temperature and from the browned material could be isolated the Amadori rearrangement product in 30-50 percent yield. However, if the C-2 hydroxyl of the N-substituted glucosylamine was substituted (2-0-methyl-D-glycosylpiperidine) then the derivative remained white and stable for over two years at 25°C (41,43). The carbohydrate which participated in the formation of the N-substituted glucosylamine could not be isolated after acid hydrolysis of the Amadori product (44). This is an indication that reactions occurred within the carbohydrate moiety of the glucosylamine during the Amadori rearrangement.

Other workers proposed that furans play an important role as intermediates in the Maillard reaction (35,45). However, Song and Chichester (38) showed that addition of hydroxymethyl furfural (HMF) did not eliminate the induction period in the browning reaction. It was reasoned that if HMF were an intermediate it would reach its maximum concentration

near the end of the lag phase of the reaction, however the experimental evidence indicated that HMF was accumulated throughout the reaction rather than incorporated into the steady phase of the reaction. Also these authors pointed out that **trioses and tetroses could undergo browning** but could not form HMF. From the data and these lines of reasoning, HMF was concluded not to be an important intermediate in Maillard browning.

Intermediate Stage of Browning--Dehydration of Sugar Moiety

The intermediate stage of the Maillard reaction is characterized by appearance of absorbancy in the region from 277-285 mμ. Dehydration of the sugar by loss of the elements of three molecules of water and cyclyzation gives a furfural. As was mentioned earlier, furfurals are not important intermediates in browning (39). Hodge and Rist (43) showed that after Amadori rearrangement the sugar moiety can lose two molecules of water to give a reductone, $\overset{\text{OH}}{-\text{C}}=(\text{CH}-\text{CH})_n=\overset{\text{CHO}}{\text{C}-\text{C}}-$ (n=0 or 1). The reductone browned in aqueous solution at pH 6.0, more rapidly in the presence of an amino acid. The reductone described by Hodge and Rist is very similar to the dehydration product postulated to arise from the α,β-unsaturated aldimine by Song and Chichester (38).

Thus in the dehydration stage of the Maillard reaction, two primary events apparently occur: loss of three molecules of water to give furfurals or loss of two molecules of water from the carbohydrate moiety to give a reductone or a reductone-like product containing nitrogen in the case of dehydration of an α,β-unsaturated aldimine.

Intermediate Stage of Browning--Fragmentation of Sugar Moiety

Among the known sugar fission products are glycolaldehyde,

glyceraldehyde, pyruvaldehyde, dihydroxyacetone, acetoin, diacetyl, acetaldehyde, aldol, triose reductone and propionaldehyde (37,46). Many of these compounds, especially with the α-hydroxycarbonyl grouping, can undergo browning and many have characteristic aromas which could be of importance in the flavor of heated foods which contain carbohydrates. The accepted mechanism for sugar fragmentation according to Hodge (37) is a reverse aldol condensation (dealdolization). Since the aldol condensation is catalyzed by amines and their salts, these same reagents will also catalyze the reverse reaction. The dealdolization of diacetone alcohol was catalyzed by amines but not bases in general (47). To explain dealdolization, Hodge (37) postulated that the 1,2-enol or 2-ketose configuration of the Amadori rearrangement product had a weakened C-C bond α,β to the carbonyl group.

Intermediate Stage of Maillard Browning--Strecker Degradation

Strecker degradation is the conversion of α-amino acids to aldehydes containing one less carbon atom (Strecker aldehydes) and carbon dioxide. The importance of this reaction to the formation of flavor components (e.g. 3-methylbutanal from leucine during roasting of cocoa beans and phenylacetaldehyde from phenylalanine during peanut roasting) has already been noted in this literature review. Strecker degradation was reviewed by Schonberg and Moubasher (22) and Schonberg et al. (23). It was found that only carbonyl compounds containing the structure $\overset{O}{\overset{\|}{C}}-(-C=C-)_n-\overset{O}{\overset{\|}{C}}-(n=0,1,2...)$ are capable of initiating Strecker degradation. It can be seen that the type of molecule reactive in Strecker degradation could be formed by dehydrogenation of a reductone $(-\overset{OH}{\overset{|}{C}}=(CH-CH)_n=\overset{OH}{\overset{|}{C}}-\overset{O}{\overset{\|}{C}}-)$ formed during the Maillard reaction. Transamination of the amino group of the amino acid to the degrading molecule could be important in the

incorporation of nitrogen into Melanoidin pigments. The fact that little ammonia is released during Strecker degradation has been demonstrated (48).

Stadtman et al. (49) have shown by the use of ^{14}C labelled glucose and glycine that over 80 percent of the carbon dioxide generated during Strecker degradation arises from the amino acid carboxyl group; ten percent arises from glucose. The fact that Maillard browning, carbon dioxide production and production of Strecker aldehydes are nearly parallel reactions has been pointed out (37). The aldehydes produced during Strecker degradation, especially acetaldehyde, can undergo browning in the presence of amino acids (50). However, this process is probably of little importance as a color producing reaction.

In summary, the intermediate stage of Maillard browning is characterized by dehydration reactions to give furfurals and/or reductones, production of sugar degradation products, and Strecker degradation.

The Final Stages of Browning

In the final stages of browning, the compounds formed during the intermediate stage are thought to polymerize to unsaturated, colored, nitrogen containing polymers. The chief reactions, on the basis of inhibition studies, were aldol condensations, aldehyde-amine polymerization and formation of heterocyclic nitrogen compounds such as pyrazines, pyrroles, pyridines and imidazoles (37). Song and Chichester (38) postulated that anionic polymerization occurred by nucleophylic attack of electrons from highly unsaturated nitrogen containing carbohydrate-like molecules on carbonyl groups. The structure of their proposed intermediates is in accord with the evidence of other workers who found that the backbone of the brown pigments apparently corresponded to

carbohydrates less three molecules of water (51). Ultraviolet spectra of the brown polymers from a glucose-glycine model system appeared to be due to π→π* transitions as was predicted for the postulated polymeric materials.

Flavor Components Produced by Sugar-Amine Reactions

Certain volatile compounds that might be important to flavor are produced during the Maillard reaction. That this is the case has already been discussed in this review in connection with production of flavor components during roasting of cocoa beans or peanuts.

As has previously been pointed out, many of these compounds originate by Strecker degradation of α-amino acids or sugar fragmentation during the Maillard reaction.

The formation of volatile pyrazine compounds during roasting of peanuts and coffee has been discussed previously but nothing was mentioned concerning the origin of this class of compounds.

Only scattered reports concerning formation of pyrazines during the Maillard reaction are available. Etard (52) and Stoehr (53) demonstrated formation of 2,5-dimethylpyrazine and traces of 2,5-dimethyl-3-ethylpyrazine by distillation of glycerol with a mixture of ammonium chloride and ammonium phosphate. Formation of acrolein from glycerol with concomitant combination of two molecules of ammonia was the mechanism postulated to explain the formation of the dimethyl derivative. Stoehr (54) observed that addition of acetaldehyde increased the yield of 2,5-dimethyl-3-ethylpyrazine in this reaction. Other workers (55,56, 57) detected pyrazine, 2-methylpyrazine and 2,6-dimethylpyrazine when glucose and ammonium hydroxide were heated at 100°C. Wiggins (58) treated inverted molasses for two hours at 100-120°C with anhydrous

ammonia at 60 pounds per square inch. From the reaction mixture were
isolated a number of imidazole compounds (10% yield) and pyrazine deriv-
atives (20% yield) which included 2,6-dimethylpyrazine, 2-hydroxymethyl-
pyrazine, 5-hydroxymethyl-2-methylpyrazine, 2-methyl-5-arabotetrahydroxy-
butylpyrazine and 2-methyl-6-arabotetrahydroxybutylpyrazine. Stiles (59)
found that acid or invertase treated molasses incorporated nearly twice
as much ammonia as did non-inverted molasses. This information pointed
out the importance of monosaccharides to the formation of pyrazine com-
pounds. Hough et al. (60) reacted glucose and aqueous ammonia at 37°C
for two weeks and isolated 2-methyl-5-(tetrahydroxybutyl) pyrazine and
2-methyl-6-(tetrahydroxybutyl) pyrazine. These derivatives were found
to occur in the D-arabo configuration based upon molecular rotation evi-
dence. Reaction of glucose or fructose with alcoholic ammonia yielded
fructoazine (2,5-bistetrahydroxybutylpyrazine) (61,62).

The mechanism of formation of simple volatile alkyl pyrazines is at
present unknown. In a recently published paper, Dawes and Edwards (30)
identified 2,5-dimethylpyrazine and trimethylpyrazine from aqueous
aldose-amino acid solutions refluxed for two hours at pH 9.0. 2,5-
dimethylpyrazine was isolated from both L-phenylalanine-D-fructose and
glycine-D-fructose model systems; trimethylpyrazine was formed only in
the glycine system. The formation of these compounds was explained in
the following manner: Pyruvaldehyde was assumed to be generated by
degradation of the carbohydrate. Amino acetone or 2-amino-propanal was
postulated to be formed from the reaction of the amino acid and pyruv-
aldehyde. Condensation of these α-amino carbonyl compounds was postu-
lated to occur to give a dihydropyrazine which underwent oxidation to
2,5-dimethylpyrazine. In like manner, trimethylpyrazine was assumed to

arise by condensation of 2-amino-propanal or amino acetone with the α-amino ketone derived from diacetyl. The suggested mechanism is attractive since Hurd (63) demonstrated the formation of 2,5-dimethylpyrazine from a model system containing acetol and alanine. It is possible that simple alkyl substituted pyrazines could arise from the easily formed fructosazine by removal of portions of the tetrahydroxybutyl side chains. However this kind of reaction is difficult to rationalize; the former one is simple to rationalize in terms of known reactions of Maillard browning.

CHAPTER III

SOME PROPERTIES OF THE FLAVOR PRECURSOR FRACTION

OF SPANISH PEANUTS

Introduction

Mason and Waller (7) described the isolation of a soluble fraction of raw peanuts which produced typical peanut aroma when heated in the dry state. This soluble fraction was called the flavor precursor fraction. Since the flavor precursor fraction evidently contained all of the compounds necessary to produce peanut aroma during heating, the nature of the compounds in this fraction was of great interest.

Work described in this chapter was aimed at the qualitative determination of the nature of the components in the flavor precursor fraction, the nature of the brown pigment formed when the flavor precursor fraction was heated and a comparison of volatile compounds formed when the flavor precursor fraction was heated with the volatile compounds isolated from normally roasted peanuts and heated defatted peanut meal.

Reagents

Ion retardation resin AG-11A8 (50-100 mesh) and Aminex-MS cation exchange resin (blend Q-50) were purchased from the Biorad Corporation, Richmond, California; Sephadex G-25 (course grade) was purchased from the Pharmacia Chemical Company, Uppsala, Sweden. Apiezon-L, SE-52, and the Chromosorb W (60-80 mesh) were obtained from the Wilkins Instrument Company, Walnut Creek, California. Hexamethyldisilazane and

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trimethylchlorosilane were purchased from Applied Science Laboratories, Inc., State College, Pennsylvania. Elemental analyses were performed by Midwest Microlab, Inc., Indianapolis, Indiana.

Procedure*

Preparation of the Flavor Precursor Fraction

Ten pounds of raw peanuts with hearts and testa removed were ground with a food grinder and defatted by soxhlet extraction with redistilled n-hexane for 48 hours. After removal of the hexane by evaporation at room temperature, the fat-free material was homogenized at one-half speed with 1M NaCl solution (7.5 liters per kg of fat free meal) in a Servall Omnimixer for 2 minutes. The resulting suspension was stirred in a plastic bucket for 24 hours at 5°C. The insoluble peanut meal was allowed to settle and the supernatent suspension was centrifuged for 15 minutes at 5000 x g. The clear supernatent solutions from the centrifuge bottles were combined and the extract was reduced to 1 liter by rotary evaporation. All of the evaporated material was placed on a Sephadex G-25 column (10.2 x 132 cm). The column was developed with 1M NaCl solution at a flow rate of 35 ml per min; 100 ml fractions were collected. The peaks corresponding to the flavor precursor fraction (fractions 65-115) described by Mason and Waller (7) were collected. The flavor precursor fraction contained 0.43 gm per ml of total organic solids as determined by the procedure of Johnson (64).

A portion of the flavor precursor fraction was placed on a 4.9 x 36 cm column of Biorad AG-11A8 ion retardation resin. The column was developed with deionized water at a flow rate of 10 ml per minute. The

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*Abbreviations used throughout this dissertation are those suggested in the Journal of Biological Chemistry 242, 1 (1967).

salt free fractions were combined; fractions containing both organic
material and NaCl were combined, evaporated to 70 ml, and recycled.
This procedure gave a salt free preparation suitable for qualitative
analysis.

Comparison of the Yield of Various Ultraviolet Absorbing Fractions from Whole Peanut Cotyledons and Peanut Aleurone Grain-Protein Body Fractions

Fifty gram samples of the aleurone grain-protein body fraction of
peanuts prepared as described by Mason and Waller (7) and defatted pea-
nut cotyledons were extracted with 1M NaCl solution as described in the
section entitled "Preparation of the Flavor Precursor Fraction." Sepha-
dex gel filtration curves were prepared in the manner shown by Mason and
Waller (7) for extracts of peanuts and the aleurone grain-protein body
fraction.

The absorbancy of each peak in the elution pattern at its absorp-
tion maximum and the width of each peak in ml was determined from the
elution pattern. The product of the peak height and peak width repre-
sented the amount of ultraviolet absorbing material (in arbitrary units)
in that peak.

Determination of Neutral Amino Acids in the Flavor Precursor Fraction

Neutral amino acids in the flavor precursor fraction were deter-
mined qualitatively using procedures similar to those outlined by Hunter
et al. (65) in which aldehydes having one less carbon atom than their
parent amino acids were prepared by the use of the ninhydrin reaction.

Two hundred mg of ninhydrin and 100 mg of a mixture containing
19.15 gm of citric acid and 2.06 gm of tripotassium citrate were mixed
with 5 ml of flavor precursor fraction in saturated NaCl solution which

was obtained by evaporation of the fraction collected from the Sephadex G-25 column. After refluxing the mixture for 30 min, it was steam distilled until 10 ml of distillate were collected. Aldehydes present in the distillates were determined in the manner to be described in the section entitled "Gas Chromatography." Standard amino acids were treated in the same manner and served as standards for the identification of amino acids present in the flavor precursor fraction.

All neutral amino acids except tryptophan, proline, hydroxyproline, and cystine could be detected in artificial mixtures using this procedure.

Separation of Anionic Constituents of the Flavor Precursor Fraction

A 30 ml portion of the desalted flavor precursor fraction was placed on a 0.9 x 45 cm column of Dowex 1 in the formate form. A gradient elution, similar to that described by Wilken and Hansen (66), in which the eluant was delivered to a 500 ml round bottomed mixing flask and then to the column was used. Ten ml fractions were collected. Deionized water was delivered to the mixing flask which also initially contained deionized water for the elution of fractions 1-85. Four normal formic acid was delivered from a reservoir to the mixing volume for the elution of fractions 86-240. In like manner, 4N formic acid which was 0.2N in ammonium formate was used for the elution of fractions 241-410; 4N formic acid 0.4N in ammonium formate eluted fractions 411-500.

Determination of Amino Acids in the Flavor Precursor Fraction Using Ion Exchange and Paper Chromatography

Basic and neutral amino acids were eluted from the Dowex-1 column just described in fraction 1-30 (fraction I). Most of the water was

removed from this fraction by lyophilization and the basic amino acids contained in it were determined using the procedure of Spackman et al. (67), buffers described by Moore et al. (68), and the ninhydrin reagent developed by Moore and Stein (69). A 1.0 x 50 cm column of Aminex-MS cation exchange resin (blend Q-50) was employed for the separation of basic amino acids of the flavor precursor fraction. One ml of ninhydrin reagent was added to the 2 ml fractions collected from the column. The test tubes were covered and the mixture was heated on a boiling water bath for 30 min to develop color in the tubes which contained ninhydrin positive material. After cooling, the absorbancy at 570 mμ was determined on each tube using a Beckman DU spectrophotometer. Elution patterns were prepared by plotting A_{570} vs tube number. Amino acids were identified by comparing the elution volume of peaks from the flavor precursor fraction with those of standards determined on the same column.

Acidic amino acids were eluted from the Dowex-1 column in fractions 100-106 and paper chromatographed along with standards in a solvent composed of isobutyric acid, concentrated NH_4OH, and water 66/1/33 by volume. The paper was sprayed with 0.5% ninhydrin in butanol and heated to 100°C for 10 minutes to reveal ninhydrin positive compounds.

Gas Chromatography

Steam distillates from heated defatted peanuts, methylene chloride extracts of the steam distillates from the flavor precursor fraction, molecular distillates of roasted peanuts, aldehydes produced by the ninhydrin reaction on the flavor precursor fraction, and carbonyl compounds regenerated from their 2,4-dinitrophenylhydrazones were all gas chromatographed on a Perkin-Elmer Model 800 gas chromatograph equipped with a dual hydrogen flame ionization detector. In each case, the components

were separated on a 20 foot x 1/8 in o.d. (0.062 in i.d.) stainless steel column packed with 20% w/w Apiezon L on Chromosorb W (60-80 mesh). A nitrogen flow rate of 36 ml per minute was used; the temperature was held at 100°C for 2 min and then temperature programmed to 200°C at 6° per minute. Retention times of the peaks were normalized by setting that of n-butanal equal to 1.00.

Trimethylsilyl (TMS) ethers of carbohydrates from the flavor precursor fraction were separated using the same gas chromatograph as described above. A 5% w/w SE-52 on 60-80 mesh Chromosorb W column (8 feet x 1/4 inch o.d.) operated at a nitrogen flow rate of 85 ml per min and temperature programmed from 125-260°C at 4° per minute was employed for these samples. Quantitation was accomplished by comparing peak areas of the sugar TMS ethers in the samples to those of standards prepared and chromatographed in the same manner.

Regeneration of Carbonyls from their 2,4-Dinitrophenylhydrazones

Regeneration of 2,4-dinitrophenylhydrazones was accomplished using the procedure of Ralls (70). For gas chromatography, 6-20 mg of a mixture of 2,4-dinitrophenylhydrazones were mixed with three times their weight of α-ketoglutaric acid and heated to 250°C for 30 sec in a glass tube sealed with a rubber septum. A sample was removed with a gas tight syringe and immediately introduced onto the gas chromatographic column.

Procedures for the Generation and Identification of Volatile Compounds from Peanuts, Defatted Peanuts, and the Flavor Precursor Fraction

Finely divided defatted peanut meal was suspended in sodium chloride solution (saturated at room temperature) at a ratio of 1 pound of meal per 2 liters of salt solution. The suspension was stirred and

refluxed at 105°C in a roundbottomed flask for 12 hr or until consider-
able browning was evident. The refluxed material was steam distilled
until no more than a faint caramel aroma existed in the reflux flask.
These distillates were sufficiently concentrated that they could be gas
chromatographed without further manipulation.

A portion of the flavor precursor fraction isolated from the large
Sephadex G-25 column which was saturated with sodium chloride at room
temperature and contained a total of 13.5 gm of organic solids was re-
fluxed as previously described for defatted peanuts. After steam dis-
tillation of the heated flavor precursor fraction, the 500 ml of steam
distillate was extracted 6 times with 20 ml portions of redistilled
methylene chloride. Rotary evaporation of the bulk of the methylene
chloride gave a material sufficiently concentrated for gas chromatography.

The volatile constituents from normally roasted whole peanuts were
isolated by homogenizing the peanuts in an equal weight of heavy mineral
oil and then passing this material across a 98°C falling film molecular
still attached to a vacuum manifold. A vacuum of 10^{-4} mm of Hg was
maintained by a three stage oil diffusion pump in line with a high ca-
pacity roughing pump. Volatile components were collected in a series of
two traps cooled to -196°C with liquid nitrogen. The trapped volatiles
were cryogenically pumped to a detachable bulb on the vacuum manifold.
After the bulb was detached from the vacuum manifold, the contents were
allowed to warm until they became liquid. Aliquots of the resulting
milky suspension were removed for gas-liquid chromatography.

Preparation of Browning Pigments

Fifty ml of the flavor precursor fraction described in the section
entitled "Preparation of the Flavor Precursor Fraction" were saturated

(at room temperature) with NaCl and refluxed at 106°C for 4 hr. The
resulting material was dialyzed against four changes of deionized water.
The material inside the dialysis tubing was evaporated to a small volume
and then subjected to gel filtration on a 2 x 30 cm column of Sephadex
G-25. The brown material which was completely excluded from the gel was
collected and lyophilized to dryness. The dry brown powder was examined
by infrared and nuclear magnetic resonance spectrometry, and analyzed
for carbon, hydrogen, and nitrogen. NMR spectra were run in D_2O solu-
tion using a Varian Model A-60 spectrometer; infrared spectra were taken
on KBr pellets using a Beckman IR5-A infrared spectrophotometer.

Browning Reactions in Modified Flavor Precursor Fractions

Neutral compounds were removed from a portion of the desalted fla-
vor precursor fraction as described in the section entitled "Separation
and Determination of Carbohydrates." Amino acids and other cationic
species were eluted from the cation exchange resin with 5N NH_4OH in a
manner described by Furuholmen et al. (72). After evaporation of the
amino acid fraction to one-fifth its original volume, it was saturated
with NaCl and adjusted to pH 6.5. Five ml of this amino acid fraction
was refluxed for 10 hr at 105°C in the presence and absence of added
carbohydrates. Coagulated material was filtered from the heated solu-
tion and absorbancy at 440mμ was determined on the filtrate. The amount
of brown pigment formed was determined by comparison of absorbancies of
experimental tubes with tubes containing known weights of the brown pig-
ment isolated as described in the section entitled "Preparation of
Browning Pigments."

Results and Discussion

The relative amounts of ultraviolet absorbing material in NaCl extracts of equivalent amounts of the aleurone grain-protein body fraction of peanuts and defatted peanut cotyledons is shown in Table I. Since the yield of the aleurone grain-protein body fraction of peanuts is about 30% of the dry fat-free weight of the peanut, the amount of ultraviolet absorbing material in 50 gm of the aleurone grain-protein body fraction was multiplied by a factor of 0.3 to obtain the amount of absorbing material that could be extracted from the weight of the aleurone grain-protein body fraction contained in 50 gm of dry, defatted peanuts.

If a given absorbing peak originated exclusively from the aleurone grain-protein body fraction, then the amount of absorbing material isolated from this fraction would be the same as that isolated from an equivalent amount of defatted peanut cotyledons.

The results indicated that the 308 mμ absorbing material arose almost exclusively from outside the aleurone grain-protein body fraction; the 262mμ absorbing material appeared to be distributed throughout the particulates of the parenchyma cells of the cotyledons and the 254 mμ absorbing material seemed to be concentrated somewhat in the aleurone grain-protein body fraction.

Mason and Waller (7) demonstrated that the aleurone grain-protein body fraction produced typical peanut aroma upon roasting. Since they also showed that material in the peaks absorbing at 262 mμ gave peanut aroma upon heating in the dry state, it seems evident that precursors of peanut flavor, even though they reside in part in the aleurone grain-protein body fraction, are also distributed with nearly equal frequency

TABLE I

YIELD OF ULTRAVIOLET ABSORBING MATERIALS
IN DEFATTED PEANUT COTYLEDONS AND
ALEURONE GRAIN-PROTEIN
BODY FRACTIONS

Starting Material	Units of Ultraviolet Absorbing Material at λ Max		
	254 mμ	262 mμ	308 mμ
Aleurone Grain-Protein Body Fraction (50 gm)	290	405	290
Aleurone Grain-Protein Body Fraction Equivalent to 50 gm of Defatted Peanut Cotyledons	87	122	87
Defatted Peanut Cotyledons (50 gm)	160	790	5490

in other locations in the cotyledon.

Aldehydes were generated from neutral amino acids in the flavor
precursor fraction and their identity determined as described in the sec-
tion entitled "Determination of Neutral Amino Acids in the Flavor Pre-
cursor Fraction."

The results indicated that the flavor precursor fraction contained
isoleucine, phenylalanine, and valine along with smaller quantities of
alanine, leucine, tyrosine, and α-amino-n-butyric acid.

Anionic constituents of the flavor precursor fraction were separated
on a Dowex-1 formate column as described in the section entitled "Sepa-
ration of Anionic Constituents of the Flavor Precursor Fraction." The
elution pattern of this column is shown in Figure 1. Peak I was nin-
hydrin positive and probably contained neutral and basic amino acids as
well as other neutral components such as carbohydrates. Fraction II was
also ninhydrin positive and paper chromatography showed it to contain
glutamic acid, aspartic acid, NAD^+ and two unknown ninhydrin positive
compounds having R_f values of 0.18 and 0.77 respectively in the solvent
described in the section entitled "Determination of Amino Acids in the
Flavor Precursor Fraction Using Ion Exchange and Paper Chromatography."
The identity of the other peaks eluted from this column are unknown but
their ultraviolet spectra in acidic, basic and neutral media are similar
to the spectra of nucleotides (λ max in the area of 260 mμ). No peaks
were detected after peak VIII.

Fraction I from the Dowex-1 column was chromatographed on the 50 cm
Aminex-MS column which was described in the section entitled "Determina-
tion of Amino Acids in the Flavor Precursor Fraction Using Ion Exchange
and Paper Chromatography." Amino Acids contained in the flavor precursor
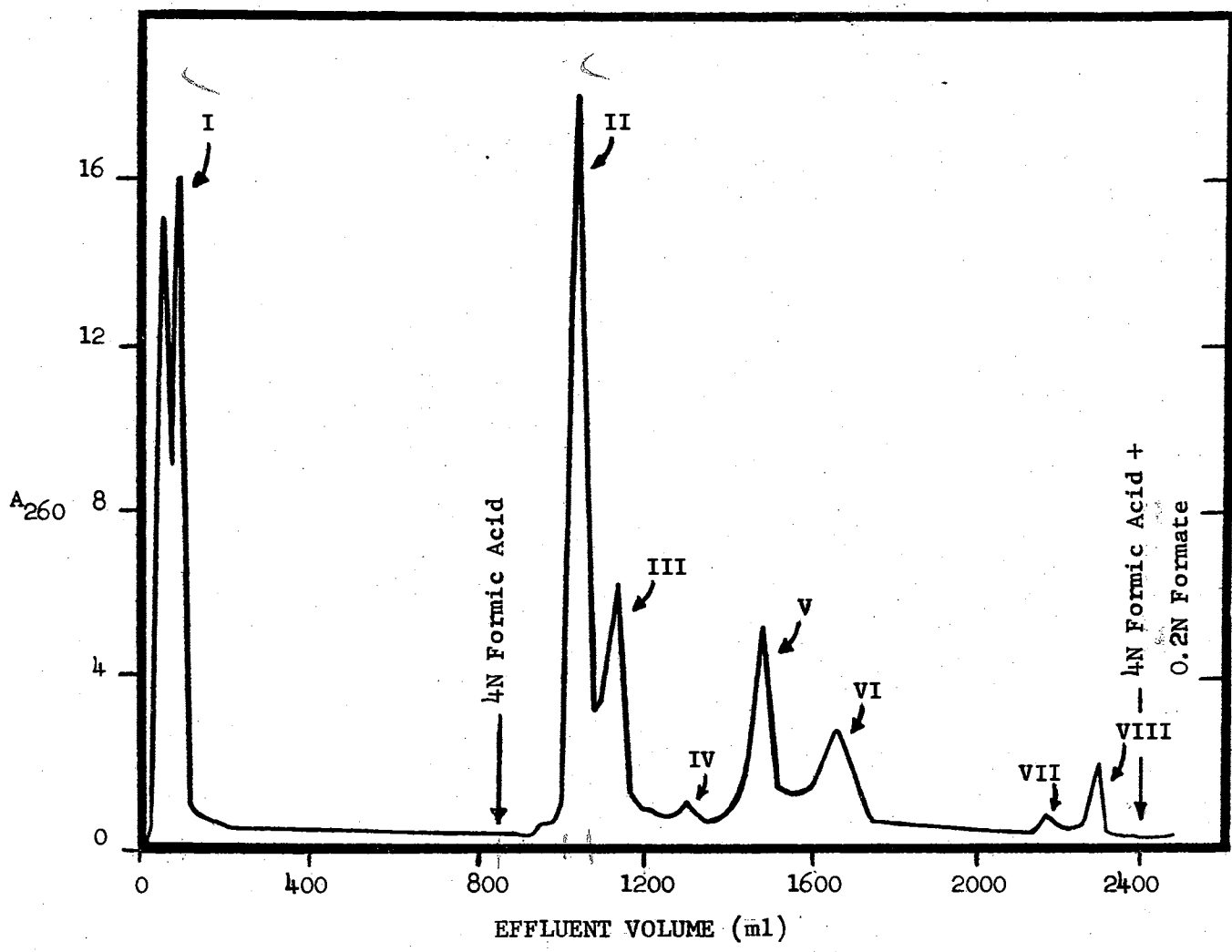
Figure 1.   Elution Pattern of Dowex-1 Formate Column

fraction identified using this procedure were tryosine and/or phenyl-
alanine, lysine, tryptophan, histidine, and ammonia. No arginine was
detected although it has been shown to occur at relatively high concen-
trations in raw peanuts (Chapter IV). Arginine probably was present in
the flavor precursor fraction but was not detected since the arginine
was probably eluted from the column as a broad diffuse peak that could
not be distinguished from the background ninhydrin color.

Table II contains a comparison of volatile constituents from heated
defatted peanut meal, the heated flavor precursor fraction, and a molec-
ular distillate of normally roasted peanuts. Gas chromatographic char-
acteristics are given for regenerated carbonyl compounds as well as
distillates. Because condensates from the flavor precursor fraction had
to be concentrated by extraction into methylene chloride, the solvent
peak obliterated the peaks representing lighter components. For this
reason these were listed as "not analyzed" in Table II.

The data of Table II show: a) In each of the three cases, defat-
ted peanuts, flavor precursor fraction, and roasted peanuts, many of the
components were carbonyl compounds; b) Relative retention data which
indicated that many of the same components were present in all three
cases. The exceptions included the presence of the component from dis-
tillates of defatted peanuts having a relative retention of 1.01 which
was not present in the other two cases and the 4.56 component which was
not detected in the distillates from the flavor precursor fraction but
which probably occurred in the distillates of roasted peanuts (the 4.68
component). The regenerated carbonyls of the precursor fraction con-
tained a component having a relative retention of 5.72 which was detect-
ed in distillates of roasted peanuts but not in distillates from

TABLE II

RELATIVE RETENTIONS OF COMPONENTS ISOLATED
FROM ROASTED PEANUTS, DEFATTED PEANUTS
AND THE FLAVOR PRECURSOR FRACTION

Defatted Peanuts		Precursor Fraction		Roasted Peanuts	
Steam distillate (Condensate)	Regenerated Carbonyls	Steam distillate (Condensate)	Regenerated Carbonyls	Molecular Distillate	Regenerated Carbonyls
0.56	0.53		----	0.59	0.58
0.65	0.68	Not	0.73	----	0.66
0.84	----		----	0.82	0.89
1.01	0.97	Analyzed	0.98	----	----
1.39	----		1.35	1.39	----
1.50	1.45		1.49	----	1.52
1.81	1.89	1.85	----	1.81	----
2.17	----	----	----	2.24	----
2.45	----	----	----	----	2.46
2.69	2.70	2.68	2.86	2.81	----
----	----	----	----	2.99	2.96
3.46	3.48	3.38	----	3.44	3.51
3.64	3.62	3.70	----	3.73	----
4.18	----	4.00	4.17	4.10	----
----	4.46	4.38	4.35	4.35	----
----	4.56	----	----	4.68	----
4.96	4.80	4.78	4.90	----	4.81
5.12	5.10	5.17	5.24	5.15	5.13
----	----	----	5.72	5.68	5.56
5.80	5.88	----	----	5.95	5.98
6.25	----	6.14	6.07	6.11	6.22
6.43	6.45	6.49	6.38	6.40	----
6.65	----	----	----	6.65	6.74
6.92	7.00	7.08	----	6.94	6.92
----	----	----	----	----	7.29
7.55	7.49	----	7.69	7.51	7.52
8.00	----	----	----	----	7.90
9.04	----	9.13	----	----	9.04
----	9.89	10.04	----	----	9.96

defatted peanuts. Compounds having relative retentions of 2.99 and 7.29 were present in distillates and/or regenerated carbonyls of roasted peanuts but were absent in the other two cases.

It is possible that some components were present in the various systems but did not occur in sufficient quantities to allow their detection as distinct peaks or shoulders in the gas chromatograms. Thus, since the relative retentions of the individual peaks compared in Table II generally agreed within 2-3%, the major differences in components of the various systems were probably quantitative rather than qualitative.

Since neutral lipid was absent in the defatted peanuts and in the flavor precursor fraction and since these fractions produced many of the same volatile components as conventionally roasted peanuts, it was concluded that neutral lipids have little importance as precursors of peanut flavor. This was in accordance with the findings of Iverson et al. (73) that there was no difference in fatty acid content of peanut triglycerides before and after roasting. However, Casey et al. (74) showed that the nature of the medium in which flavor producing reactions occurred was important in the quantitative distribution of low boiling volatiles produced during heating of many foods.

Thus it was concluded that the qualitative nature of the volatiles produced from roasted peanuts, heated defatted peanuts or the heated flavor precursor fraction was similar and that the quantitative differences of volatile components arose primarily from differences in the reaction medium.

Table III gives the identity of carbohydrates identified in the flavor precursor fraction using methods described in the section entitled "Separation and Determination of Carbohydrates." Unidentified

TABLE III

SUGARS FOUND IN THE FLAVOR PRECURSOR
FRACTION OF ARGENTINE PEANUTS AS
DETERMINED BY GAS CHROMATOGRAPHY
OF THEIR TRIMETHYLSILYL ETHERS

Component	Identity	Relative Retention	μg Sugar/gm Organic Solids in Precursor Fraction
1	Unknown	0.41	3.9
2	Arabinose	0.50	Trace
3	Unknown	0.55	Trace
4	β-Xylose	0.65	8.7
5	Fructose	0.70	15.3
6	α-galactose	0.75	Trace
7	α-glucose	0.78	Trace
8	β-glucose	0.82	Trace
9	N-acetyl-galactosamine or β-galactose	0.87	100.4
10	Sucrose	1.17	161.8
11	Unknown	1.28	5.5
12	Unknown	1.53	25.8

sugars were present in trace amounts.

The brown pigment isolated as described in the section entitled "Preparation of Browning Pigments" had an elemental analysis of C 37.86%, H 5.01%, N 3.97%, ash 12.70%, and O (by difference) 40.46%. The emperical formula was $C_{11}H_{18}O_9N$. The fact that most of the pigment was excluded from Sephadex G-25 indicated an average molecular weight in excess of 3000.

NMR spectra of the isolated pigment in D_2O (Figure 2) showed that about 8 of the 18 protons exchanged instantly with D_2O. This fact coupled with the strong absorption between 3500 and 3700 cm^{-1} in the infrared spectrum (Figure 3) was interpreted to mean that these protons were hydroxyl or enolic. The strong absorption between 1500 and 1700 cm^{-1} was thought to arise from carbonyl groups conjugated with double bonds. However, since evidence for protons of the $-\overset{\text{(H)}}{C}=C-$ type was lacking in the NMR spectrum, it was postulated that the double bonds indicated in the infrared spectrum were enolic and that the protons had exchanged with D_2O. Absorption in the NMR spectrum between 3.8 and 4.8 ppm indicated approximately 8 protons of the $\text{(H)}-C-OH$ type. The remaining protons were probably of the type $H-C-\text{(H)}$ as indicated by weak absorption at 0.5 ppm.

These spectral data coupled with elemental analysis indicated that the brown polymer was probably composed of residues from both carbohydrates and amino acids at a frequency of about two, six carbon sugars to one amino acid nitrogen atom. Oxidation or losses of the elements of water along with facile rearrangements could account for the presence of enolic protons.

Neutral and cationic species in the desalted flavor precursor fraction were separated and treated as described in the section entitled
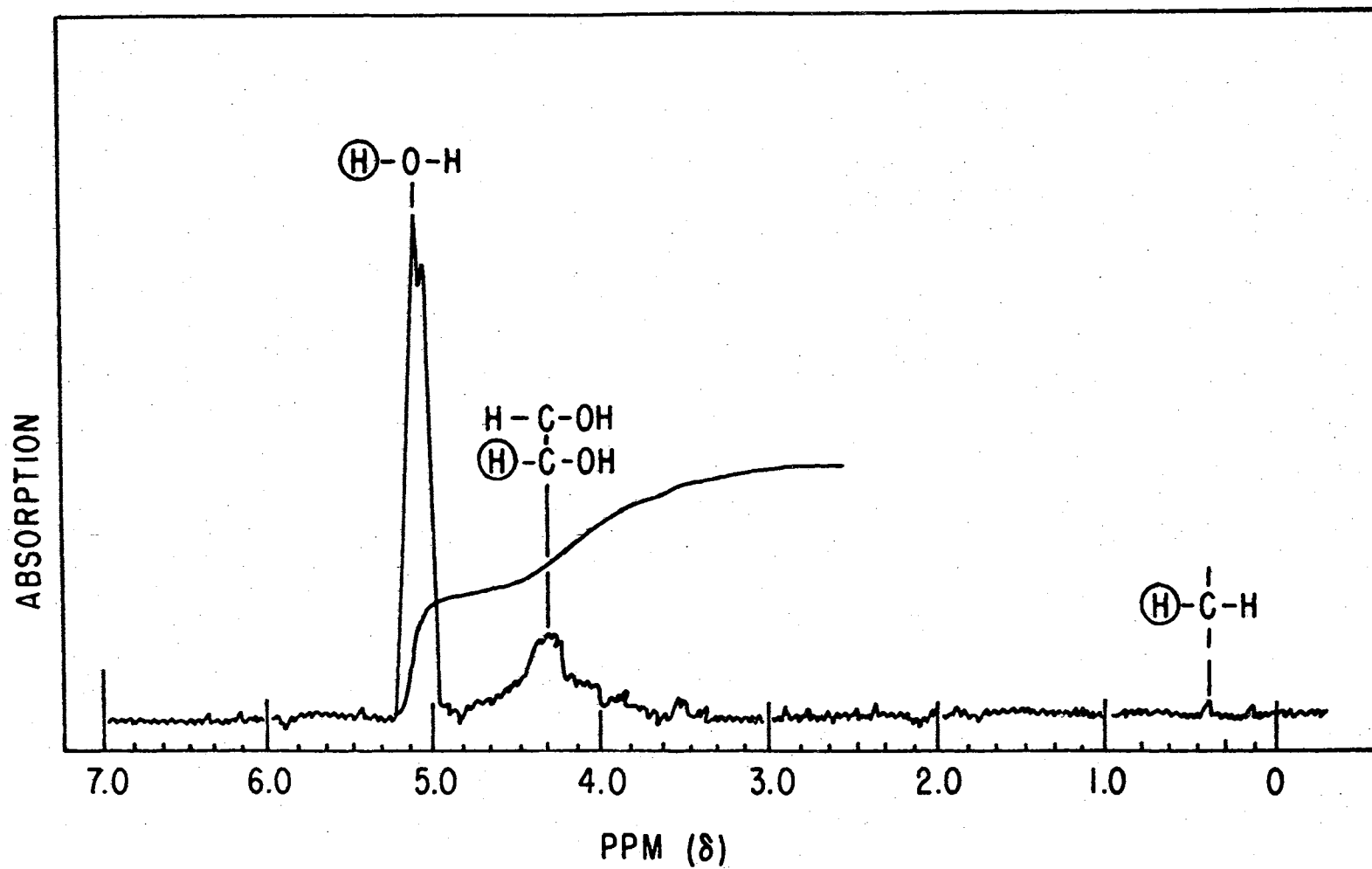
Figure 2. NMR Spectrum of the Isolated Brown Pigment in D_2O
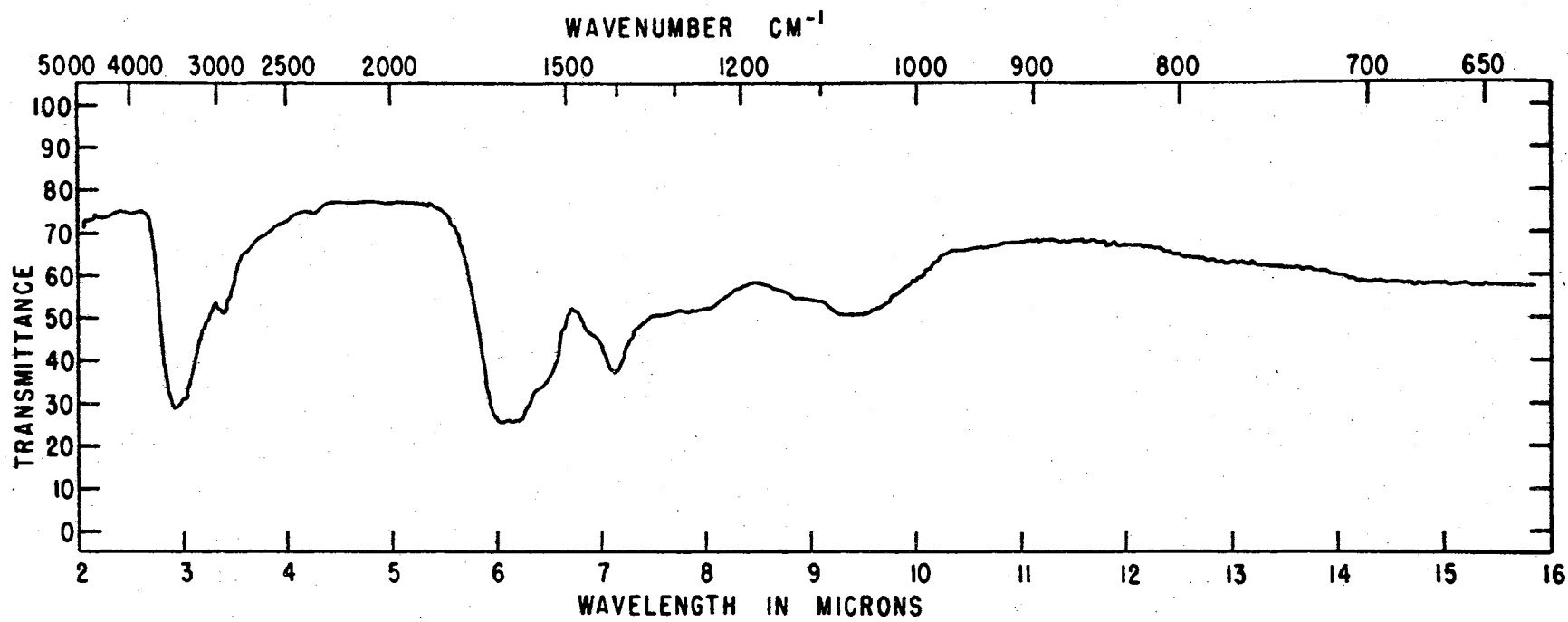
Figure 3.   Infrared Spectrum of the Isolated Brown Pigment
on a KBr Disc

"Browning Reactions in Modified Flavor Precursor Fractions." Table IV shows that significant browning occurred in the cationic fraction only in the presence of added carbohydrates. Fructose was a more efficient reactant in the browning reaction than glucose or sucrose at the same concentration. Maximum browning occurred in the reaction that contained glucose and fructose. The reaction mixture containing sucrose did not brown readily which was the expected result if browning was dependent on the presence of a reducing group. Song and Chichester (38) demonstrated that a reducing group is required for significant browning.

Since the reaction mixture containing both glucose and fructose did not produce as much brown pigment as would have been expected from the sum of the pigment produced from glucose and fructose alone, the amount of cationic constituents in the mixture containing both glucose and fructose probably was the limiting reactant.

To the extent that non-enzymatic browning indicated the formation of compounds which may play a role in the flavor of heated foods (Chapter II) amino acids and/or other cationic constituents must be important in the production of typical peanut flavor.

TABLE IV

BROWNING OF CATIONIC CONSTITUENTS OF THE
FLAVOR PRECURSOR FRACTION IN THE
PRESENCE AND ABSENCE OF
ADDED CARBOHYDRATES

Reactant Added*	mg pigment formed in 10 hours heating
No additions**	0.00
1 ml 0.5M fructose	1.54
1 ml 0.5M glucose	1.39
1 ml 0.5M sucrose	0.68
glucose and fructose	1.85
sucrose and fructose	1.64
sucrose and glucose	1.48
sucrose, glucose and fructose	1.63

*All reaction mixtures contained 5 ml of the cationic fraction from the Dowex-50 column as described in the procedure.

**This mixture browned slightly and was used as a blank.

CHAPTER IV

PRECURSORS OF TYPICAL AND ATYPICAL

ROASTED PEANUT FLAVOR

Introduction

Earlier work from this laboratory described the isolation of a fla-

vor precursor fraction of raw peanuts which consisted of low molecular

weight compounds rather than proteins or polysaccharides. Paper chroma-

tography of the fraction containing the flavor precursors indicated

further the presence of ninhydrin positive and basic compounds (7).

Any compound that is a flavor precursor must, by nature, be a sub-

stance of low volatility which can be converted to a more volatile one

during heating. The authors' approach to the peanut flavor precursor

problem was to analyze the heated and unheated flavor precursor fraction

or extracts of raw and roasted peanuts. Knowing the volatile compounds

produced (11,13,14,75) and the non-volatile material degraded allowed

the postulation of a mechanism to explain the decrease in flavor pre-

cursor concentration during roasting and the production of compounds

which exhibit typical roasted peanut flavor. A similar approach has

been employed in cocoa flavor precursor work (21,24,25).

Procedures

Determination of Peanut Maturity

Peanuts having dark colored interior pericarp surfaces and thin

pink colored testa were classed as mature; those having light colored

interior pericarp surfaces and fleshly white testa were classed as im-
mature. Peanuts falling between these extremes were classified as inter-
mediate in maturity. The peanuts from the maturity classes used in the
work presented here were large enough to be retained on a 15/64 x 3/4-
inch screen.

Subjective Analysis of Peanut Flavor

A flavor panel consiting of five members was instructed to rate the
flavor of four coded samples of ground, roasted plus coded and uncoded
standards of Argentine Spanish peanuts. The Argentine Spanish standard
has consistently proven to have superior organoleptic properties over
several years of testing. The organoleptic evaluation was reported on
rating sheets like that shown in Figure 4. Mean preference rank was ob-
tained by taking the average of the preference rank given by each panel
member to a particular sample. The mean preference rank is an estimate
of peanut flavor in numerical terms as compared to a standard. The mean
preference ranks reported in this thesis represent an average of two
separate determinations. In each replication, the sample in question
was roasted immediately before it was presented to the panel. Due to
variations in the roasting process and the inability of the panel to
detect subtle flavor differences, the two replications of the mean pref-
erence rank were seldom exactly the same. For example, sample 2 (Table
VI) had a mean preference rank of 2.4; sample 1 had a mean preference
rank of 2.2. The former sample was considered to have better flavor
because the panel rated it, with respect to flavor, superior--or equal--
to the standard (Figure 4) more consistently than the latter one.

According to taste panel results, samples 1, 3 and 5 had flavor
somewhat inferior to samples 2, 4 and 6. The difference in flavor of

Date _____ Taster _____ Description _____

CODE NO.	ODOR			FLAVOR			COMMENTS					
	Superior to Standard	Equal to Standard	Inferior to Standard	Better than Standard	Equal to Standard	Poorer than Standard	ODOR	FLAVOR	TASTE	ROAST	DRY-NESS	Pref. Rank No. One Best
1												
2												
3												
4												
5												

	Odor		Flavor		Taste		Roast		Dryness
1.	None	1.	Lacks	1.	Rancid	1.	Under	1.	Very Dry
2.	Weak	2.	Low	2.	Bitter	2.	Good	2.	Moderate
3.	Moderate	3.	Undesirable	3.	Salty	3.	Excellent	3.	Moist
4.	Strong	4.	Good	4.	Sweet	4.	Over	4.	Oily

Figure 4.   Form Used by Each Panel Member
in Organoleptic Evaluation

the peanut samples used in this work was due to the degree of maturity of the samples. All samples were of the same variety, grown at the same location, and processed in the same manner. Samples 1, 3 and 5 were harvested September 18, 1965, and were more immature; samples 2, 4 and 6 were harvested on October 9, 1965. The mean preference ranks for samples 1, 2, 3 and 4 were 2.2, 2.4, 3.6 and 2.4 respectively.

Preparation of Peanuts for NaCl or Perchloric Acid Extraction

Raw peanuts ground with a food grinder or ground roasted peanuts were extracted with redistilled n-hexane in a soxhlet extractor. After the solvent was removed by evaporation, the dry peanuts were ground to a fine powder and the fat-free meal was stored in tightly stoppered bottles at room temperature prior to further extraction.

Preparation of a Peanut Flavor Precursor Fraction

The flavor precursor fraction from 2 kg of raw fat-free peanuts was obtained by extraction with 15 liters of 1M NaCl solution. The extract was concentrated to 1 liter by rotary evaporation and the evaporated extract was subjected to gel filtration on a 10.8 liter column of Sephadex G-25. From the Sephadex column was collected the flavor precursor fraction described by Mason and Waller (7).

Perchloric Acid Extraction

Ground fat-free meal was stirred with 3N perchloric acid (25 ml of 3N $HClO_4$ per gm of fat-free meal) on a magnetic stirrer for 1 hour at $4°C$. The acidic extract was centrifuged at 10,000 x g in the cold to remove insoluble material. The pH of the supernatant liquid was adjusted to 7.0 with saturated KOH; the $KClO_4$ formed was removed by high speed centrifugation at $4°C$. The clear supernatent liquid was lyophilized to

dryness and redissolved in a minimum volume of 0.2 N citrate buffer at pH 2.2. Hydrolysis of amide nitrogen was not a problem as judged by the absence of hydrolysis of glutamine and asparagine standards which were treated in the same manner.

Preparation of Samples for Carbohydrate Analysis

Fat-free meal (0.5 gram) was extracted for 24 hours with 100 ml of 80% ethanol in a soxhlet extractor. The volume of the extract was reduced to about 15 ml by rotary evaporation and placed on a 0.8 x 20 cm column of Amberlite CG-120 (100-200 mesh) in the sodium form. Neutral Na^+ compounds were eluted from the column with 50 ml of deionized water and the eluate was lyophilized to a solid residue. The residue was dissolved in 2.0 ml of N,N-dimethylformamide and allowed to stand at room temperature for about one hour to achieve dissolution. The following additions were made to the dimethylformamide solution in the order: 0.5 ml of pyridine, 0.4 ml of hexamethyldisilazane and 0.2 ml of trimethylchlorosilane. One ml of a standard solution containing 8 μmole each of ribose, fructose, glucose, inositol, and sucrose was treated in the same manner as the samples from peanuts. Sugar concentrations were calculated by comparison of sample peak areas with corresponding peak areas of the standard.

Gas Chromatography of Trimethylsilyl Ethers

Gas chromatography was conducted on a Perkin-Elmer model 801 gas chromatograph equipped with a hydrogen flame ionization detector. A 6 foot x 1/4 inch o.d. glass column packed with 5% (w/w) SE-52 on 100-110 mesh Gas Chrom Q operated at a nitrogen flow rate of 60 ml per minute and temperature programmed from 120-250°C was used to separate

the trimethylsilyl derivatives.

Amino Acid Analyses

Amino acid contents of perchloric acid extracts obtained as pre-
viously described were determined with a Beckman 120-C Amino Acid
Analyzer using Beckman PA-28 and PA-35 resins respectively.

Gross Changes in the Flavor Precursor Fraction During Heating

A NaCl solution (saturated at room temperature) of the flavor pre-
cursor fraction obtained in the manner previously described which con-
tained 13.5 grams of total organic solids was refluxed for 15 hours at
105°C. Total ninhydrin positive material (76), pentoses (77), carbohy-
drates (78) reducing sugars (79), and organic solids (64) were determined
before and after refluxing using published procedures.

Results and Discussion

The Effect of Heating on the Flavor Precursor Fraction

The heated and unheated flavor precursor fractions were analyzed as
previously described. Table V shows that total carbohydrates and amino
acids decreased, pentose sugars and total organic solids remained rela-
tively constant, and reducing activity increased sharply during reflux-
ing. The biuret test (80) indicated that the flavor precursor fraction
contained only traces of peptides after residual protein was precipitated.

The results of the analysis for ninhydrin positive material and
total sugars indicated that amino acids and carbohydrates had reacted in
some manner and that both were modified during the process. The ob-
served increase in reducing power was explained by hydrolysis of sucrose
or by a process in which carbohydrates were converted to reductones (37).
The fact that total organic solids decreased only slightly during the

TABLE V

CHANGES IN COMPOSITION OF THE FLAVOR PRECURSOR
FRACTION DURING REFLUXING

Treatment	Total in Milligrams			Percent Change
	Before Heating	After Heating	Change During Heating	
Ninhydrin (as leucine)	517	263	- 254	49.1
Orcinol (as ribose)	16	15	- 1	6.3
Anthrone (as glucose)	8915	6360	-2555	28.6
Alkaline copper (as glucose)	470	6400	+5930	1261.9
Total Organic Solids	13500	12750	- 750	5.6

heating process meant that most of the material produced during heating was of low volatility.

The formation of large amounts of polymeric material was evidenced by formation of considerable insoluble dark colored material in the flavor precursor fraction during heating. When a portion of the heated saline solution was dialyzed against deionized water, the brown material remained inside the dialysis tubing. The nondialyzable material was lyophilized to dryness, redissolved in a minimum of water, and subjected to gel filtration on a 20 x 3 cm column of Sephadex G-25. The brown material which was completely excluded from the gel was collected, lyophilized and analyzed for carbon, nitrogen, hydrogen, and ash. The analysis (C 37.86, H 5.01, N 3.97, ash 12.70, and O by difference 40.46) corresponded to an empirical formula of $C_{11}H_{18}O_9N$. The results of these experiments indicated that amino acids and carbohydrates of the flavor precursor fraction were undergoing chemical changes.

Changes in Individual Amino Acid Concentrations During Roasting

Amino acids can give rise to aldehydes by Strecker degradation and can serve as the source of nitrogen for the formation of pyrazine compounds (30,37). That these compounds are produced during roasting of peanuts was indicated by recently published results (13,14,75) in which pyrazines and aldehydes were shown to represent the major classes of organic compounds evolved from peanuts during roasting. Data presented in Tables VI and VII indicated very strongly that amino acids and sugars were the precursors of these volatile flavor components in roasted peanuts.

Table VI shows amino acid concentrations (expressed in μmoles per gm of fat-free peanut meal) in raw and roasted peanuts and the ratio:

TABLE VI

AMINO ACID CONCENTRATIONS (μMOLES/GRAM OF FAT-FREE MEAL) AND RAW/ROASTED RATIOS IN FULLY MATURE AND INTERMEDIATE MATURITY PEANUTS

Amino Acid	FULLY MATURE						INTERMEDIATE MATURITY					
	Sample 1			Sample 2			Sample 3			Sample 4		
	Raw	Roasted	Ratio	Raw	Roasted	Ratio	Raw	Roasted	Ratio	Raw	Roasted	Ratio
Aspartic acid	0.58	0.73	0.79	0.88	0.58	1.52	0.58	0.44	1.33	1.02	0.88	1.16
Threonine	0.73	0.58	1.26	0.29	0.12	2.42	0.88	0.58	1.52	0.44	0.29	1.52
Serine	1.17	0.88	1.33	0.58	0.44	1.32	1.61	1.02	1.58	1.75	0.88	1.99
Asparagine and Glutamine	2.19	1.32	1.66	2.92	0.88	3.32	2.49	1.02	2.44	4.53	1.46	3.10
Proline	1.32	1.17	1.13	1.02	0.58	1.76	1.02	0.88	1.17	2.19	1.02	1.98
Glutamic Acid	7.02	3.95	1.78	7.89	2.34	3.37	7.02	3.36	2.09	11.26	3.07	3.67
Glycine	1.17	1.02	1.15	0.44	0.44	1.00	1.75	1.17	1.50	1.61	0.73	2.21
Alanine	4.68	3.22	1.45	2.05	1.32	1.55	8.19	5.41	1.51	5.56	3.36	1.65
Valine	1.32	1.02	1.29	0.73	0.29	2.52	1.75	1.02	1.72	1.90	0.88	2.16
Unknown	2.92	1.32	2.21	4.09	0.44	9.30	1.90	0.73	2.60	1.46	0.29	5.03
Methionine	0.29	0.15	1.93	0.29	0.15	1.93	0.15	0.15	1.00	0.29	0.15	1.93
Isoleucine	0.73	0.58	1.27	0.44	0.29	1.52	0.88	0.44	2.00	0.88	0.44	2.00
Leucine	0.58	0.44	1.32	0.44	0.15	2.93	0.73	0.44	1.66	0.73	0.44	1.66
Tyrosine	0.44	0.44	1.00	0.15	0.15	1.00	0.73	0.44	1.66	0.29	0.15	1.93
Phenylalanine	2.19	2.63	0.83	2.34	1.61	1.46	2.05	1.32	1.55	1.75	1.46	1.20
β-alanine	trace	trace	--	0.15	trace	--	trace	trace	--	trace	0.15	--
Lysine	0.88	0.58	1.52	0.44	0.15	2.93	0.88	0.73	1.21	0.73	0.29	2.52
Histidine	0.44	0.29	1.52	0.29	0.15	1.93	0.58	0.29	2.00	0.58	0.29	2.00
Ammonia	1.75	4.09	0.43	0.73	0.58	1.26	2.34	4.97	0.47	2.63	6.43	0.41
Arginine	1.46	1.17	1.25	1.46	1.32	1.11	1.32	2.19	0.60	1.61	1.17	1.38
Tryptophan	0.29	0.29	1.00	0.44	0.29	1.52	0.73	0.44	1.66	0.58	0.29	2.00

μmoles per gm raw / μmoles per gm roasted. Several conclusions were drawn from examination of these data. First, regardless of the maturity category studied, glutamic acid, asparagine-glutamine, the unknown amino acid, phenylalanine, and alanine made up most of the total free amino acids and probably were the most important as flavor precursors. Secondly, with the exception of the under-roasted sample (Sample No. 1) the raw/roasted ratios for a given amino acid in different samples were fairly consistent. The raw/roasted ratios of the under-roasted sample were consistently lower than the average of the other three samples with the exception of the very minor components: methionine, lysine, and arginine. This result would be predicted if destruction of amino acids were a function of the duration of roasting.

These two facts show that free amino acids were destroyed during roasting to a degree approximately proportional to their original concentration. Further, although initial concentrations of some free amino acids appeared to be a function of maturity, the proportion of each degraded during heating was fairly constant from sample to sample.

The observations prompted the authors to consider kinetic control as the control mechanism in the conversion of amino acids to flavor compounds during roasting. If kinetic control were independently operative (i.e., if available energy were not a limiting factor) one would expect the raw/roasted ratios for a given amino acid in different samples to remain relatively constant provided the samples were roasted to the same extent.

Breakdown of all carbohydrates in the better flavored peanuts (Table VII) in each maturity class was of the order of 4 to 10 μmoles per gm, assuming that all sucrose lost was converted to fructose and

TABLE VII

GAS CHROMATOGRAPHIC ANALYSIS OF TRIMETHYLSILYL
ETHERS OF CARBOHYDRATES FROM RAW
AND ROASTED PEANUTS

Sample Designation	Carbohydrate	Raw μmole/ gm	Roasted, μmole/ gm	Change, μmole/ gm	% Change
MATURE					
Sample 1	fructose	6.22	11.42	+ 5.20	45.5
	glucose	6.90	15.72	+ 8.82	56.1
	unknown	5.87	2.96	- 2.91	49.6
	inositol	2.48	3.34	+ .86	25.7
	sucrose	312.00	262.00	- 50.00	16.0
Sample 2	fructose	4.18	4.62	+ .44	9.5
	glucose	4.00	5.72	+ 1.72	31.1
	unknown	3.36	1.22	- 2.14	63.7
	inositol	1.78	1.44	- .34	19.1
	sucrose	266.00	266.00	0.00	00.0
INTERMEDIATE					
Sample 3	fructose	4.38	4.76	+ .38	8.0
	glucose	3.27	3.64	+ .37	10.2
	unknown	1.39	1.18	- .21	15.1
	inositol	5.30	3.98	- 1.32	24.9
	sucrose	299.00	188.00	-111.00	37.1
Sample 4	fructose	6.24	5.36	- .88	14.0
	glucose	3.82	5.74	+ 1.92	33.4
	unknown	2.36	1.08	- 1.28	54.2
	inositol	3.02	2.46	- .56	18.5
	sucrose	236.00	232.00	- 4.00	1.7

glucose; breakdown of amino acids was roughly twice that of the carbohydrates. This fact suggested that these two classes of compounds reacted in a two to one stoichiometric ratio during roasting. However, under the conditions of roasting ($210^{\circ}C$ in a non-aqueous medium) glutamine and glutamic acid can readily self condense to form pyrolidone carboxylic acid (81) and thus may not have reacted in the same manner as other amino acids.

With the thought in mind that the initial concentration of each amino acid controlled the extent to which it was degraded during roasting (kinetic control), the authors derived an equation which related the concentration of amino acid flavor precursor in the raw peanut with subjectively measured flavor. As a first approximation, based on the evidence, the assumption was made that typical flavor was directly proportional to the concentration of each amino acid flavor precursor, Flavor α Cp, where Cp was the measured flavor precursor concentration in the raw peanut. Since mean preference rank (M) decreased as typical flavor increased (i.e., M was a smaller number for a better tasting sample or Flavor $\alpha \frac{1}{M}$) it followed from these proportionalities that another could be written, Cp $\alpha \frac{1}{M}$ or M $\alpha \frac{1}{Cp}$. Insertion of the constant K_f (the flavor function constant) into the second proportionality yielded:

$$M = K_f/Cp \tag{1}$$

which was rearranged to give:

$$K_f = MCp \tag{2}$$

If the assumption that typical flavor was proportional to flavor precursor concentration was justified, the proportionality constant K_f, for any given precursor in one peanut sample, should be equal within experimental error of determining M and Cp, to K_f for that precursor in any

other sample regardless of the flavor of the two samples. If, however, the flavor precursor in question produced compounds associated with atypical or off-flavor then K_f values for a given precursor in two different samples would not agree since the assumption that typical flavor was proportional to flavor precursor concentration would not be satisfied. These statements would apply only to those samples which were roasted to the same extent. Since the raw/roasted ratio would increase with heaviness of roast and would decrease with lighter roasting, the inclusion of the raw/roasted ratio (R) in Equation 2 reduced all samples to an identical heaviness of roast. Division of the right side of Equation 2 by R yielded:

$$K_f = MCp/R \hspace{4cm} (3)$$

K_f values calculated from Equation 3 using the data in Table VI, ratios of K_f values, and average K_f ratios for the amino acids occurring in peanuts of two maturity classes were determined. The results are tabulated in Table VIII. Ratios near 1.0 indicated those amino acids which contributed to typical flavor; those which departed sharply from 1.0 indicated precursors of atypical or off-flavor. The amino acid concentration data are much more precise by nature than the mean preference rank data. Therefore the agreement of the K_f values can be no better than the precision of values for the least precise measurement; namely, the mean preference rank. The arbitrary limits set for average K_f values of precursors of typical flavor were $K_f = 1.0 - 1.7$; K_f values greater than 2.5 were considered to indicate that the amino acid was a precursor of atypical flavor.

On the basis of these calculations, aspartic acid, asparagine-glutamine, glutamic acid, phenylalanine, and histidine were considered

TABLE VIII

K_f VALUES, RATIO OF K_f VALUES, AND AVERAGE RATIOS OF K_f VALUES IN INTERMEDIATE MATURITY AND FULLY MATURE PEANUTS

Amino Acid	Mature Sample 1	Sample 2	Ratio	Intermediate Sample 3	Sample 4	Ratio	Average Ratio ± Std. Deviation	Interpretation
Aspartic Acid	1.6	1.4	1.1	1.6	2.1	1.3	1.2 ± 0.1	T
Threonine	1.3	0.3	4.3	2.1	0.7	3.0	3.7 ± 0.7	A
Serine	1.9	1.1	1.7	3.7	2.1	1.8	1.8 ± 0.1	X
Asparagine and Glutamine	2.9	2.1	1.4	3.7	3.5	1.1	1.3 ± 0.1	T
Proline	2.6	1.4	1.9	3.1	2.4	1.3	1.6 ± 0.2	*
Glutamic Acid	8.7	5.6	1.6	12.1	7.4	1.6	1.6 ± 0.0	T
Glycine	2.2	1.1	2.0	4.2	1.7	2.5	2.3 ± 0.2	X
Alanine	7.1	3.2	2.2	19.5	8.1	2.4	2.3 ± 0.1	X
Valine	2.2	0.7	3.1	3.7	2.1	1.8	2.5 ± 0.6	*
Unknown	2.9	1.1	2.6	2.6	0.7	3.7	3.2 ± 0.5	A
Isoleucine	1.3	0.7	1.9	1.6	1.1	1.5	1.7 ± 0.2	X
Leucine	1.0	0.4	2.5	1.6	1.1	1.5	2.0 ± 0.5	*
Tyrosine	1.0	0.4	2.5	1.6	0.4	4.0	3.3 ± 0.7	A
Phenylalanine	5.8	3.9	1.5	4.8	3.5	1.4	1.5 ± 0.1	T
Lysine	1.3	0.4	3.3	2.6	0.7	3.7	3.5 ± 0.2	A
Histidine	0.6	0.4	1.5	1.0	0.7	1.4	1.5 ± 0.1	T
Ammonia	9.0	1.4	6.4	17.9	15.4	1.2	3.8 ± 2.6	*
Arginine	2.6	3.2	1.2	7.9	2.8	2.8	2.0 ± 0.8	*
Tryptophan	0.6	0.7	1.2	1.6	0.7	2.3	1.8 ± 0.3	X

T Precursor of typical flavor
A Precursor of atypical flavor
X Intermediate ratio, no prediction
* Anomolous ratios

to be precursors of typical peanut flavor; threonine, the unknown, tyrosine, and lysine were precursors of off-flavor. In Table VIII, precursors of typical flavor were designated as T whereas precursors of atypical flavor were designated as A. A few average K_f value ratios in Table VIII were anomolous in that they had large standard deviations and could have been classified as either precursors of typical or atypical flavor. Some of the average K_f values in Table VIII fell between the arbitrary limits set for precursors of typical flavor and precursors of atypical flavor. No predictions were made conerning these amino acids.

K_f values calculated from Equation 3 and ratios of K_f values for immature peanuts are given in Table IX. The ratio of K_f values indicated that glutamic acid and phenylalanine, as in mature and intermediate maturity peanuts, were precursors of typical roasted peanut flavor. However, the results indicated, in contrast to the results shown in Table VIII for mature and intermediate maturity peanuts, that serine, proline, alanine, valine, leucine, and isoleucine were precursors of typical flavor; asparagine-glutamine and aspartic acid were precursors of atypical flavor.

The differences in interpretation between Tables VIII and IX probably arose from the fact that Sample 5 had a very high mean preference rank (M = 4.0). In peanuts of such poor flavor, the mean preference rank must have deviated considerably from a linear function of roasted peanut flavor. This in turn caused anomolous results in K_f values since it was assumed in the derivation of Equation 3 that typical peanut fla- increased in a linear fashion with decreasing mean preference rank.

Equation 3 predicts that K_f values for precursors of atypical flavor should be higher in poorer flavored peanuts than the corresponding

TABLE IX

K_f VALUES AND RATIO OF K_f VALUES IN
IMMATURE PEANUTS

| Amino Acid | K_f | | Ratio | Inter-pretation |
	Sample 5 M=4.0	Sample 6 M=3.2		
Aspartic Acid	2.08	5.25	2.52	A
Threonine	2.08	1.05	1.98	X
Serine	3.76	4.20	1.12	T
Asparagine and Glutamine	3.36	9.45	2.82	A
Proline	4.60	5.63	1.22	T
Glutamic Acid	10.00	14.33	1.43	T
Glycine	6.70	2.46	2.72	A
Alanine	19.70	13.00	1.52	T
Valine	3.76	3.84	1.02	T
Isoleucine	1.68	1.76	1.05	T
Leucine	1.68	1.76	1.05	T
Tyrosine	1.68	----	----	-
Phenylalanine	4.20	5.60	1.33	T

value in good flavored peanuts since for a given M, Cp should be higher
in poorer flavored peanuts. Table VIII shows that K_f values for threo-
nine, the unknown, tyrosine, and lysine were indeed higher in peanuts
which had poorer flavor. The fact that in very immature peanuts, which
have a very high level of off-flavor, the amino acids which were pre-
dicted to be precursors of atypical flavor occur at very high concen-
trations, tends to support the conclusions drawn from K_f values
concerning precursors of atypical flavor.

Similarly, the amino acids which contributed to typical peanut
flavor according to calculation of K_f values were the same ones which
made up about 50% of the total free amino acids in the fully mature
peanuts.

Changes in Carbohydrates During Roasting

The data in Table VII showed that sucrose underwent only small
decreases in concentration during roasting of peanuts having good flavor
(Samples 2 and 4). The results might have been interpreted to mean that
sucrose per se was not involved in the production of flavor. This inter-
pretation agreed with the results of El'ode et al. (82) who showed that
sucrose was much less reactive in the browning reaction than monosac-
charides.

Glucose and fructose must have been consumed during roasting since
the increase in concentration of these sugars was less than the amount
which could have been produced from the hydrolysis of sucrose.

Evidently, fructose was utilized in the reactions to produce vola-
tile compounds to a greater extent than glucose since the increase in
fructose concentration during roasting of peanuts was less than that of
glucose. This result was reasonable in light of the work of

Casey et al. (74) who showed that fructose exhibited much higher rates
of production of volatiles than glucose in model hexose-amino acid
systems.

Monosaccharides are extremely important in the formation of pyra-
zine compounds which have been implicated as the character impact com-
pounds of roasted peanuts (7). Wiggins (58) observed that ammonia
heated with an acid hydrolyzed (inverted sucrose) sample of molasses
gave a variety of pyrazine compounds; unhydrolyzed molasses took up only
about one-half as much ammonia. Evidence discussed in Chapter VI with
model glucose-amino acid systems showed that qualitatively the same
volatile pyrazine compounds were produced regardless of the amino acid
employed as the nitrogen source.

Hypothetical Mechanism for the Conversion of Amino Acids and Carbohy-drates to Volatile Compounds

Consideration of the results obtained in this work led the authors'
to postulate a plausible mechanism, shown in Figure 5, for the conver-
sion of amino acids and sugars to volatile compounds associated with
peanut flavor. The mechanism, which includes ideas set forth by Hodge
(37,46) and Rohan and Stewart (21), involves the initial addition of an
amino acid to the anomeric carbon atom of an aldose followed by dehydra-
tion to the 1,2-eneaminol (I) and elimination of hydroxyl ion to give
the Schiff base cation (II). The Schiff base cation can undergo hydrol-
ysis to an α-dicarbonyl compound (III) which is converted to browning
pigments by a series of steps. Alternatively, the Schiff base cation
(II) could decarboxylate to the imine (IV) which would rapidly hydrolyze
to yield an aldehyde and a dieneamine (V). Enolization of the 1,2
double bond and migration of the 3,4 double bond yields the unsaturated
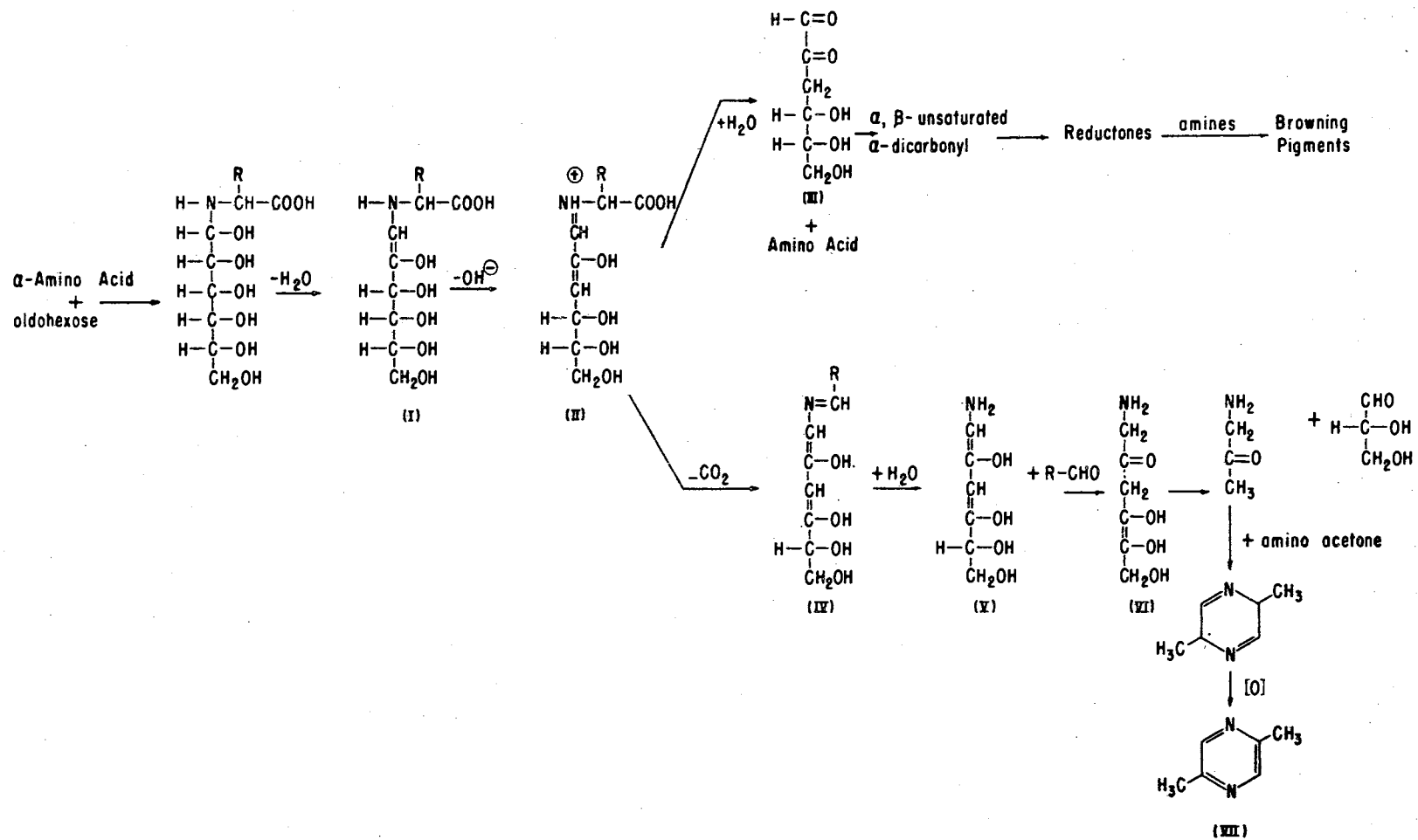
Figure 5. A Postulated Mechanism for the Conversion of Amino Acids
and Sugars to Volatile Compounds

ketoamine (VI). This compound then could undergo retro-aldol condensation to yield amino acetone and glyceraldehyde. Condensation of two molecules of amino acetone yields 2,5-dimethylpyrazine (VII) which was one of the major pyrazine compounds found in roasted peanuts (13,75).

CHAPTER V

PRODUCTION OF PRECURSORS OF TYPICAL AND ATYPICAL

PEANUT FLAVOR IN MATURING PEANUTS

Introduction

Immature peanuts, even when correctly cured, possess a high level
of off-flavor. It was suggested in Chapter IV that amino acids and car-
bohydrates are the precursors of the compounds that give roasted peanuts
their flavor. Further, it was suggested that aspartic acid, glutamic
acid, glutamine-asparagine, histidine, and phenylalanine were the pre-
cursors of typical peanut flavor; threonine, tyrosine, lysine, and an
unknown amino acid were considered to be precursors of atypical flavor.

If the previous suggestions that certain amino acids are required
for the production of typical peanut flavor while others produce atypi-
cal- or off-flavor are true, then one would expect that one or both of
two events would occur: Precursors of typical peanut flavor would in-
crease in concentration during maturation or precursors of atypical
flavor would decrease in concentration as maturity was approached.

Procedure

Collection of Samples

Argentine-Spanish peanuts, which were planted on the same date and
grown in pots in a plant growth chamber in a randomized block design
were harvested periodically by removing two pots (replicates) from each
block. During the maturation of the plant, pegs were dated at the time

of their appearance and at each harvest date the peanuts were divided into three groups. Peanuts in group A were those whose pegs appeared earliest of those harvested; group B represented peanuts of intermediate age and group C contained peanuts harvested from the most recent pegs. Therefore in group A were peanuts having a long growing season and group C peanuts were those having the shortest growing season. In addition, peanuts within each harvest date were classified as mature, intermediate in maturity, or immature according to external seed characteristics as described in the section of Chapter IV entitled "Determination of Peanut Maturity."

Extraction and Determination of Individual Amino Acids and Carbohydrates

Flavor precursors were extracted and estimated using procedures outlined in Chapter IV in the sections entitled "Preparation of Peanuts for NaCl or Perchloric Acid Extraction," "Perchloric Acid Extraction," "Preparation of Samples for Carbohydrate Analysis," "Gas Chromatography of Trimethylsilyl Ethers," and "Amino Acid Analyses."

Results and Discussion

In a preliminary experiment, the replicates from each harvest date from the mature peanuts of group A were combined and in like manner, the immature peanuts from each harvest date of group C were combined.

Amino acid analyses were performed on these samples as described in the "Procedure" section of this chapter. The concentration of individual amino acids in these samples is presented in Table X. Proline and serine values from group C harvest 7/2/66 were not calculated due to malfunction of the amino acid analyzer. There were no group A peanuts available from the 5/8/66 harvest date (i.e., there were no peanuts

TABLE X

AMINO ACID CONTENT (μMOLES/GM FAT-FREE MEAL) OF PEANUTS CLASSIFIED
INTO THREE GROUPS ACCORDING TO AGE WITHIN HARVEST DATE

	Oldest (Group A)						Youngest (Group C)			
Harvest Date	6/5	6/11	6/11	6/25	7/2	7/9	5/28	6/4	6/11	7/2
Sample No.	2	3	4	5	6	7	1	2	3	6
Aspartic	1.65	1.54	1.78	1.58	1.98	1.39	2.48	4.05	1.44	8.89
Threonine	0.24	0.24	0.40	0.40	0.40	0.40	0.88	1.19	0.67	2.37
Serine	0.71	1.07	0.99	0.79	0.99	0.40	0.73	1.19	0.58	-
Asparagine and Glutamine	4.73	3.33	2.38	2.18	3.37	2.18	6.72	10.48	7.40	11.66
Proline	1.18	2.61	0.79	0.40	0.59	-	2.19	1.90	2.79	-
Glutamic	11.58	3.92	1.58	1.58	20.59	13.66	8.47	16.43	3.75	26.48
Glycine	0.71	0.95	0.79	0.99	0.79	0.59	0.58	0.71	0.38	1.98
Alanine	1.42	1.43	1.19	1.19	1.39	1.19	2.04	2.86	1.44	5.34
Valine	0.71	0.83	0.59	0.59	0.79	0.59	0.44	0.71	0.29	1.19
Half-Cystine[*]	3.31	3.92	8.12	9.90	14.45	9.90	2.34	4.29	0.77	5.73
Isoleucine	0.24	0.36	0.40	0.40	0.59	0.40	0.15	0.24	0.10	0.59
Leucine	0.47	0.36	0.20	0.20	0.20	0.20	0.29	0.24	0.10	0.59
Tyrosine	0.05	0.12	trace	0.20	0.20	0.20	0.15	0.14	0.06	0.20
Phenylalanine	0.71	0.83	2.77	3.76	5.35	2.57	0.29	0.71	0.29	1.19
Lysine	0.30	0.14	0.17	0.25	0.20	0.20	1.75	1.67	3.83	2.47
Histidine	0.02	0.07	0.14	0.12	0.25	0.20	0.44	0.71	0.42	0.74
Ammonia	0.89	0.71	3.11	1.49	1.73	7.92	0.73	0.95	0.42	0.74
Arginine	1.18	0.48	0.45	0.50	0.50	0.59	21.46	18.33	12.08	33.33

[*] Recently found not to be cysteine

having mature seed characteristics in this early harvest date.)

Certain conclusions of considerable interest were drawn from the data. First, in group A, the concentration of aspartic acid, glutamic acid, glutamine-asparagine, phenylalanine and histidine (i.e., the amino acids implicated in the production of typical peanut flavor in Chapter IV) either remained relatively constant or tended to increase with age. Next, the concentration of threonine, tyrosine, and lysine (amino acids implicated in the production of atypical peanut flavor in Chapter IV) remained relatively constant or decreased with age. The unknown amino acid which was thought to be a precursor of atypical flavor increased significantly with age however. When the data of Table X were plotted (harvest date vs. amino acid concentration) a large amount of scattering of points was observed. This was attributed to sampling error and the inability of personnel to separate nature and immature nuts on the basis of seed characteristics alone.

In an effort to minimize sampling error, the replicates of group B (peanuts of intermediate age) from each harvest date were combined. These samples contained peanuts of all maturity groups on the basis of seed characteristics. Table XII gives percent by weight of the various maturity groups within group B. Samples from the early harvest date contained more immaturity than samples from later harvest dates which was expected.

Table XI gives amino acid contents in peanuts from various harvest dates; Figure 6 shows the same information in graphic form. Apparently sampling error was still prevalent but the conclusions drawn in Table XI and Figure 6 were the same as those drawn from analyses of the older and younger peanuts (Table X). An interesting point brought out by the data

TABLE XI

SYNTHESIS OF AMINO ACID FLAVOR PRECURSORS--AMINO ACID CONTENT (μMOLES/GM FAT-FREE MEAL) OF INTERMEDIATE AGE PEANUTS FROM EACH HARVEST DATE

| Harvest Date	5/28	6/4	6/11	6/18	6/25	7/2	7/9
Sample Number	1	2	3	4	5	6	7
Aspartic Acid	4.5	1.9	1.8	1.1	0.8	1.9	0.8
Threonine	0.8	0.3	0.5	0.2	0.2	0.3	0.1
Serine	0.8	0.5	0.7	0.3	0.2	0.3	0.2
Asparagine + Glutamine	7.2	3.4	3.9	2.1	1.2	2.5	1.1
Proline	2.3	0.9	1.5	0.8	0.6	1.0	0.4
Glutamic Acid	15.3	9.8	12.4	10.4	6.8	15.8	8.7
Glycine	1.1	0.4	0.9	0.5	0.4	0.7	0.5
Alanine	3.4	1.5	1.8	1.0	0.7	1.6	0.7
Valine	0.9	0.5	0.9	0.6	0.4	0.7	0.4
Unknown	2.5	2.4	-	4.4	3.1	7.1	5.3
Isoleucine	0.2	0.1	0.3	0.3	0.2	0.3	0.2
Leucine	0.4	0.2	0.3	0.2	0.1	0.2	0.2
Tyrosine	0.1	0.1	0.2	0.1	0.1	0.1	0.1
Phenylalanine	0.6	0.4	0.9	1.5	0.9	2.0	1.2
Lysine	1.4	0.6	-	0.3	0.3	0.2	0.2
Histidine	0.5	0.2	-	0.2	0.2	0.2	0.2
Ammonia	1.1	4.2	-	1.4	0.9	0.7	0.6
Arginine	16.4	5.5	-	3.8	4.6	2.7	1.1
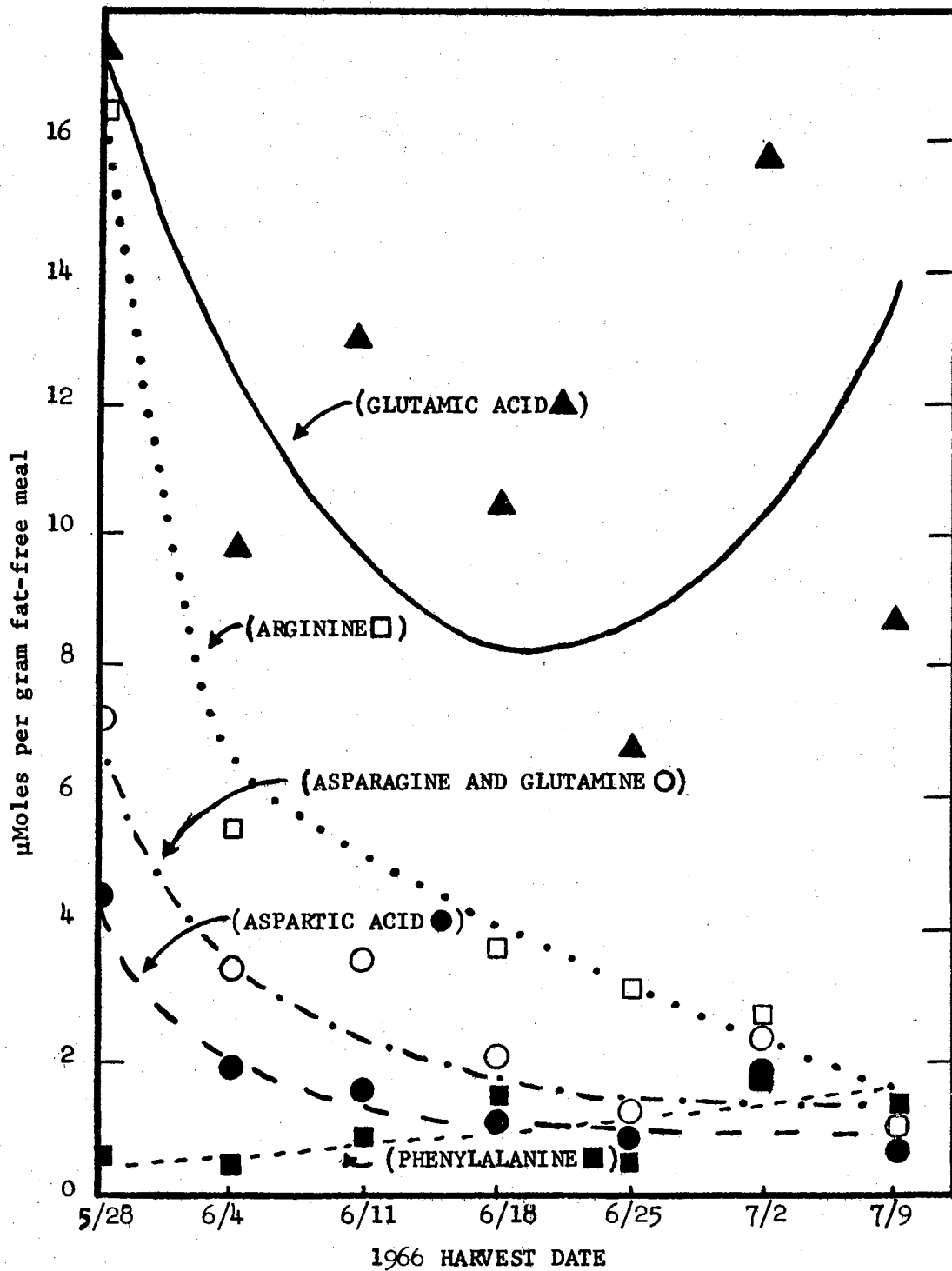
Figure 6. Amino Acid Concentration in Intermediate Age
Peanuts as a Function of Harvest Date

in Figure 6 was the sharp drop in glutamic acid concentration near the middle of the growing season followed by a sharp increase during the latter stages of the growing season. This observation may be significant in view of results presented in Chapter VI which show that the major portion of volatile pyrazine compounds in roasted peanuts probably arise from glutamic acid.

The data of Figure 6 and Tables X and XI also indicated that the difference in arginine concentration between immature and mature peanuts was on the order of 20 to 40 fold showing that a chemical measurement of arginine content holds some promise as a sensitive method for the evaluation of immaturity in certain lots of peanuts.

Carbohydrate concentrations in the peanut samples used for the amino acid analyses given in Table XI and Figure 6 were determined as described in the "Procedures" section of this chapter. The results, which are given in Table XII and Figure 7, were not as dramatic as those of the amino acid analyses but certain conclusions could be drawn. Total sugars decreased with peanut age but increased rather sharply at the latter stages of development. This trend was due largely to changes in sucrose concentration, but fructose seemed to follow the same trend. Glucose concentration dropped off and then rose slightly at later harvest dates; inositol concentration tended to decrease with age.

Percent immaturity by weight was determined in these peanuts before analysis (Table XII) so that any variation in maturity might be accounted for in interpreting the results. Sample 6 contained a higher percentage of immature and intermediate maturity peanuts which probably accounted for values for amino acid and carbohydrate concentrations which were out-of-line with surrounding values (Figure 6 and Table XII).

TABLE XII

SYNTHESIS OF CARBOHYDRATE FLAVOR PRECURSORS--SUGAR CONTENT OF FAT-FREE MEAL
FROM INTERMEDIATE AGE PEANUTS FROM EACH HARVEST DATE

|	Harvest Date		μMoles/gm fat-free Meal					Percent by Weight			
Sample No.	Month	Day	Fructose	Glucose	Inositol	Sucrose	Total	Mature	Intermediate	Mature + Intermediate	Immature
1	5	28	7.0	6.8	6.0	328.3	348.1	33	36	69	31
2	6	4	9.3	5.9	4.7	301.6	321.5	54	34	88	12
3	6	11	4.3	3.8	3.4	293.6	305.1	63	26	89	11
4	6	18	3.4	3.8	2.3	250.9	260.4	43	46	89	11
5	6	25	7.1	4.4	2.9	206.0	220.4	50	38	88	11
6	7	2	10.1	7.3	5.0	308.9	331.3	29	56	85	15
7	7	9	7.8	4.3	2.1	284.0	298.3	45	45	90	10
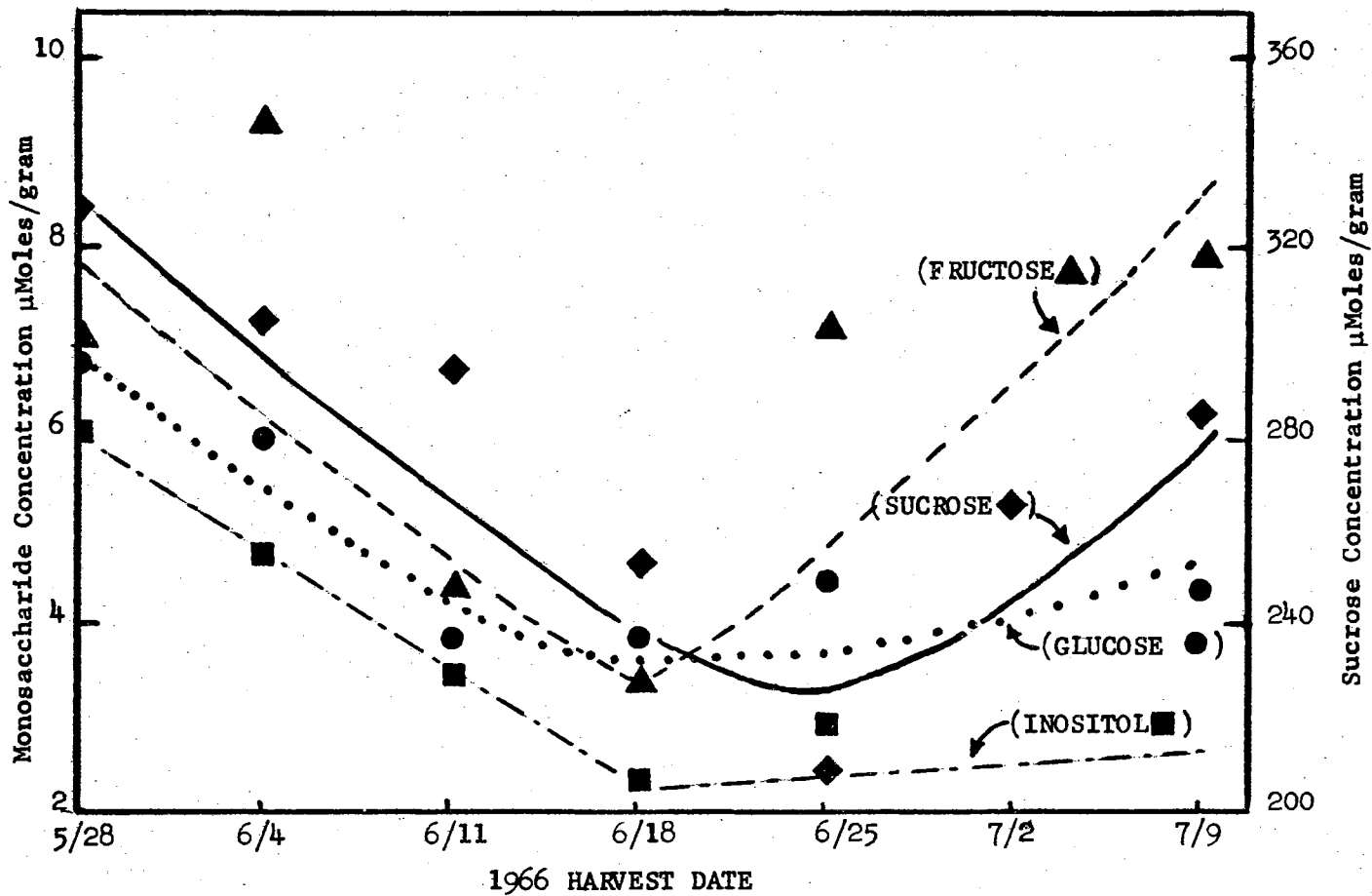
Figure 7. Carbohydrate Concentration in Intermediate Age
Peanuts as a Function of Harvest Date

Since immature peanuts have poorer flavor than mature ones, one would predict that precursors of typical peanut flavor would increase in concentration during maturation or that precursors of atypical flavor would decrease in concentration during the same period or that both events would occur.

The results presented in this chapter indicated that both events occurred during maturation of peanuts. The only exception was that the concentration of the unknown amino acid increased in concentration with increasing age whereas its concentration was predicted to decrease with age.

CHAPTER VI

FORMATION OF PYRAZINE COMPOUNDS

IN MODEL SUGAR-AMINE SYSTEMS

Introduction

Mason et al. (13) showed that a number of simple alkylated pyra-

zines were contained in the volatile compounds obtained by high vacuum

outgassing of roasted peanuts. This group suggested that some of these

pyrazine compounds were responsible for the roasted-nutty aroma of

roasted peanuts. Deck and Chang (16) isolated 2,5-dimethylpyrazine from

potato chips; Reichstein and Staudinger (28) and Goldman et al. (29)

found pyrazines in roasted coffee. It was suggested in Chapter III that

roasted cocoa beans might also contain pyrazine compounds.

In Chapter IV it was proposed that amino acids and carbohydrates

were the precursors of volatile compounds responsible for the typical

flavor of roasted peanuts. Since this was suggested, it was important

to demonstrate that the peanut flavor precursors (amino acids and sugars)

could give rise to peanut flavor compounds (pyrazines).

The work of Etard (52), Stoehr (53), Brandes and Stoehr (55),

Stolte (56), Tanret (57), Wiggins (58), Hough et al. (60), Hurd (63)

and Dawes and Edwards (30) demonstrated that pyrazines were indeed

formed in heated aqueous carbohydrate-amine or carbohydrate-ammonium

ion systems. The preceding references were discussed in greater detail

in Chapter II.

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The early work concerning the formation of pyrazine compounds was carried out in aqueous model systems which were not a good representation of a peanut-like situation.

The objectives of work presented in this chapter were to develop a model sugar-amino acid system which was more peanut-like than the aqueous ones used by earlier workers, to demonstrate the formation of peanut flavor components in this system and to try to gain some insight into the mechanism of formation of pyrazine compounds in amino acid carbohydrate systems of low water content. A further objective was to show that a model system containing a mixture of carbohydrates and amino acids qualitatively and quantitatively like that of peanuts could give the same distribution of volatile pyrazine compounds as that produced during normal roasting of peanuts.

Procedure

Materials--DL-aspartic acid (A grade), L-glutamine (A grade) and D-asparagine hydrate were obtained from Calbiochem, Los Angeles, California; DL-glutamic acid-H_2O and DL-phenylalanine were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio; diethylene glycol (histological) and sucrose were purchased from Fisher Chemical Company, Fair Lawn, New Jersey; D-glucose (A.R.) was obtained from the Mallinckrodt Chemical Company, St. Louis, Missouri; fructose (C.P.) was a product of Pfanstiehl Laboratories, Inc., Waukegan, Illinois; and methylene chloride was purchased from the Aldrich Chemical Company, Milwaukee, Wisconsin. Pyrazine, 2-methylpyrazine, 2,5-dimethylpyrazine, 2-methyl-6-ethylpyrazine, 2-n-pentylpyrazine, and 2-methyl-5-n-pentyl-pyrazine were gifts from the Wyandotte Chemicals Corporation, Wyandotte, Michigan. Trimethylpyrazine, 2,6-dimethyl-3-ethylpyrazine, and

2,5-dimethyl-3-ethylpyrazine were synthesized by alkylation of corresponding dimethyl compounds with ethyl lithium or methyl lithium. The last three compounds were synthesized by Mr. Philip Koehler in our laboratory. The Thermocap relay was a product of the Niagra Electron Laboratories, Andover, New York.

Methods--Model systems were routinely prepared by dissolving 10 mMoles each of the carbohydrate and amino acid in 20 ml of deionized water. The water solution of the reactants was transferred to 200 ml of diethylene glycol maintained at 120-123°C in a 250 ml round bottomed flask immersed in a mineral oil bath. The temperature of the mineral oil bath was maintained with a Thermocap relay connected to a 1000 watt immersion heater; both the oil bath and the reaction mixture were stirred with a magnetic stirrer.

After heating had continued for 26 hours, the reaction mixture was transferred to a 250 ml separatory funnel connected to the inlet of a falling film evaporator. The falling film evaporator used in these studies was similar to that described by Herz and Chang (83) except that volatiles were trapped in a single cold finger trap at -196°C, the column was heated to 98°C, and the sample was introduced onto the column from a separatory funnel. A smaller trap was placed between the vacuum pump and the larger trap to prevent volatile material from the vacuum or oil diffusion pumps from contaminating the volatiles produced during heating of the reaction mixture. The material obtained after heating the reaction mixture was passed over the evaporator three times at a rate of about 400 ml per hour to remove volatiles as completely as possible. The material collected in the large cold finger trap was transferred under vacuum to a detachable 100 ml flask connected to the

bottom of the cold finger trap by evaporating the liquid nitrogen in the trap with a stream of air. After the liquid nitrogen had evaporated, warm water was poured into the cold finger trap to cause the material contained in it to drop to the liquid nitrogen cooled 100 ml flask attached to the cold finger trap. After the transfer was complete, the small flask was removed from the cold finger trap and stored at -20°C prior to further steps.

The collected material was allowed to warm to room temperature after which ultraviolet spectra were obtained on appropriate water dilutions of the falling film distillate. The material from the trap was extracted 5 times with 5 ml portions of redistilled methylene chloride. The combined methylene chloride extracts were dried over 2 gm of sodium sulfate after which the extract was reduced to a small volume by rotary evaporation. The evaporated extract was transferred quantitatively to a 3 ml volumetric flask and the contents were made up to volume with redistilled methylene chloride. Aliquots of this material were analyzed by gas liquid chromatography on a Perkin-Elmer Model 801 dual hydrogen flame gas chromatograph using the following conditions: Column--20 ft x ¼ inch o.d. glass column containing 15% w/w Carbowax 20-M on Gas Chrom Q; flow rate--48 ml per minute of nitrogen; temperature--linear program from 75-190°C at 4°C per minute, injector temperature 220°C.

Gas chromatographic peaks were quantitated by comparison of peak areas from samples with those of known weights of standards chromatographed the same day under the same conditions. Duplicate analyses of the standard and samples were conducted and the results averaged to obtain the data presented in this chapter.

A prototype of the LKB 9000 combination mass spectrometer-gas

chromatograph (MS-GLC) was used for mass spectral investigations. The conditions were as follows: Column and conditions--24 ft x ¼ inch o.d. glass column containing 5% w/w Carbowax 20-M on Gas Chrom Q operated isothermally at 120°C at a helium flow rate of 40 ml per minute; mass spectrometer--electron energy 70 ev, trap current 38 µamps, accelerating voltage 3.5 kv, multiplier voltage 2.1 kv, filter 120 cps, and molecule separator temperature 280°C.

Results and Discussion

A mixture containing about 0.21 gm of 2,5-dimethylpyrazine in 50 ml of diethylene glycol was passed over the falling film evaporator. Quantitative ultraviolet spectra were taken on the material which did not distill to the cold finger trap. The results showed that 95.1% of the original absorbancy was removed after one pass over the evaporator; 99.3% after two passes.

A solution of 10 µl of 2-methylpyrazine and 10 µl of 2-n-pentyl-pyrazine in 50 ml of water was prepared. Sixteen ml aliquots of this solution were placed into separatory funnels and each aliquot was extracted 3 times with 5 ml of n-pentane, diethyl ether, or methylene chloride. It was found that after the third extraction about 4% of the original absorbancy remained in the aqueous layer when extraction was carried out with methylene chloride while diethyl ether or n-pentane failed to extract 26% and 61% of the original absorbancy respectively.

Diethylene glycol (100 ml) was heated with 50 mMoles of ammonium hydroxide in water for 30 hours at 120°C. The material was passed over the falling film evaporator three times, extracted with methylene chloride and subjected to gas-liquid chromatography as described in the "Procedure" section of this chapter. No pyrazine compounds were

detected by ultraviolet spectrometry of the falling film distillate or by gas chromatography of methylene chloride extracts of the distillate.

A standard solution containing pyrazine, 2-methylpyrazine, and 2,5-dimethylpyrazine in 25 ml of methylene chloride was subjected to rotary evaporation as described in the "procedure" section of this chapter. Gas-liquid chromatograms of the solution were obtained before and after evaporation. It was found that about 83% of the pyrazine was recovered after evaporation; 92% and 96% of 2-methylpyrazine and 2,5-dimethyl-pyrazine respectively were recovered.

This work showed that the solvent (diethylene glycol) when heated with a nitrogen source produced no pyrazine compounds and that pyrazine compounds (except pyrazine itself) which were produced in the sugar amino acid model systems could be recovered from the diethylene glycol reaction medium in nearly quantitative yield.

The retention times of compounds producted in the model systems were compared with standards run under the same conditions. Retention times relative to that of 2-methylpyrazine were also calculated for each significant peak derived from the model systems (Table XIII). Equality of a relative retention time of a sample peak with that of a standard one was routinely used for the identification of peaks from the various model systems. Mass spectra of peaks from the asparagine-glucose model system were identical with standards having the same relative retention time. This fact indicated that it was valid to identify peaks from the model systems on the basis of their relative retention times.

Table XIV shows the total yield of pyrazine compounds (measured by total ultraviolet absorbancy at the absorption maximum in the aqueous falling film distillates) from several of the model systems studied.

TABLE XIII

RETENTION TIMES AND RETENTION TIMES RELATIVE
TO 2-METHYLPYRAZINE OF GAS CHROMATOGRAPHIC
PEAKS FROM THE ASPARAGINE-GLUCOSE
MODEL SYSTEM

COMPOUND	MODEL SYSTEM		STANDARD	
	Retention Time (min)	Relative Retention	Retention Time (min)	Relative Retention
pyrazine	13.65	0.88	13.65	0.88
2-methylpyrazine	15.50	1.00	15.55	1.00
2,5-dimethylpyrazine	17.60	1.14	17.55	1.13
2-methyl-6-ethylpyrazine	19.70	1.28	19.70	1.27
trimethylpyrazine	20.30	1.31	20.30	1.31
2,5-dimethyl-3-ethylpyrazine	21.55	1.39	21.60	1.39
2,6-dimethyl-3-ethylpyrazine	22.20	1.43	22.20	1.43
2-n-pentylpyrazine	27.70	1.79	27.70	1.78
2-methyl-5-n-pentylpyrazine	29.90	1.93	29.50	1.90

TABLE XIV

TOTAL YIELD OF PYRAZINES PRODUCED FROM
HEATED SUGAR-AMINO ACID OR SUGAR-
AMMONIUM CHLORIDE MODEL SYSTEMS

MODEL SYSTEM	TOTAL YIELD (μMoles)	ABSORPTION MAXIMUM (mμ)
Asparagine-glucose	411	272
Glutamine-glucose	205	272
Aspartic acid-glucose	198	274
Glutamic acid-glucose	114	274
NH_4Cl-glucose	59	262
NH_4Cl-fructose	195	280
Asparagine-sucrose	268	274
Asparagine-glucose-acetaldehyde	11643	280

The results indicated that the absorption maximum was near that expected for a mixture of pyrazines (λ max 2-methylpyrazine = 278 mμ). Asparagine gave the highest yields of pyrazines when heated with glucose; glutamic acid the lowest. The table also shows that the yield for aspartic acid is nearly one-half that of asparagine suggesting that both nitrogens of asparagine were incorporated into pyrazines. The same was true when the glutamine-glucose system was compared with the glutamic acid-glucose system. The overall yield of pyrazines in the asparagine-glucose system was about 4% on the basis of 10 mMoles of glucose used as a reactant; the yield from glutamic acid and glucose was about 1%.

One mechanism which could be postulated for the formation of pyrazines involves the addition of ammonia, produced from the amino acid, to sugar degradation products to give reactive compounds, such as α-amino carbonyl compounds, which could form pyrazines readily (Chapter II). Another possible mechanism involves the initial rate limiting nucleophylic attack of the amino acid nitrogen on the carbonyl group of the sugar (38). In the first mechanism, the amino group of each amino acid would ultimately be converted to ammonia and thus each amino acid would give the same distribution of pyrazine compounds and also the same product distribution as an ammonium salt heated with a carbohydrate. In the second mechanism, the ease of the initial nucleophylic attack would be influenced by the nature of the amino acid reactant and each amino acid would give different product distributions and product distributions different from an ammonium salt heated with glucose.

Figure 8 shows the product distribution for a number of amino acids and ammonium chloride heated with glucose in the model system. The results clearly showed that amino acids gave product distributions very
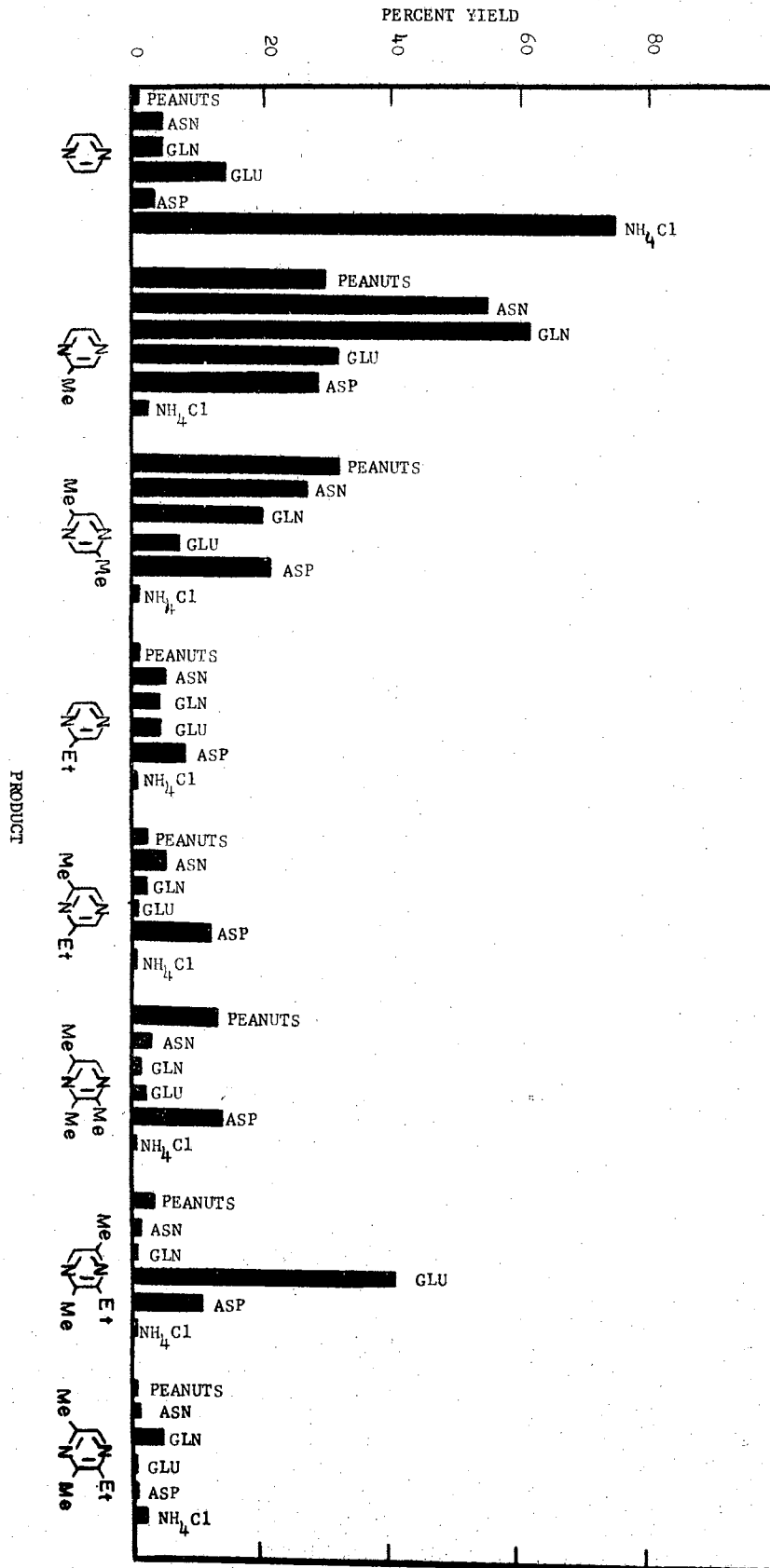
Figure 8. Distribution of Pyrazine Compounds Produced by Heating Glucose with Various Amino Acids

different from an ammonium salt when the nitrogen-containing reactant
was heated with glucose. Amino acids gave 2-methylpyrazine as a major
product; ammonium chloride gave more pyrazine. These facts ruled out
the first mechanism for the production of pyrazines proposed above. The
facts that the yield of 2,5-dimethylpyrazine was lower and that the yield
of dimethylethylpyrazines was higher in the glutamic acid-glucose sys-
tem than in the other systems studied indicated to the author that a
two carbon fragment might have alkylated 2,5-dimethylpyrazine to increase
the yield of the dimethylethyl derivative. This phenomenon was also sug-
gested by the work of Stoehr (54). To determine if a two carbon frag-
ment could effect the proposed alkylation reaction, a model system
containing 10 mMoles of glucose, 10 mMoles of asparagine and a large
excess of acetaldehyde was prepared. The total yield of pyrazines from
this system is given in Table XIV. The product distribution shown in
Figure 9 indicated that larger amounts of pyrazine and 2-methylpyrazine
were formed in comparison to the product distribution in the absence of
acetaldehyde. This was expected since all carbons of pyrazine and car-
bons 5 and 6 of 2-methylpyrazine probably can arise from two carbon
fragments. Mass action would tend to increase the yield of these prod-
ucts in the presence of excess acetaldehyde. Figure 9 showed that the
yield of 2-methyl-5-ethylpyrazine was increased many fold in the presence
of added acetaldehyde; 2,6-dimethyl-3-ethylpryazine also increased but
not as dramatically as the former derivative. These results strongly
suggested the participation of a two carbon fragment, such as acetalde-
hyde, in the formation of ethyl substituted pyrazine compounds. It was
impossible to determine from the data if the acetaldehyde added to the
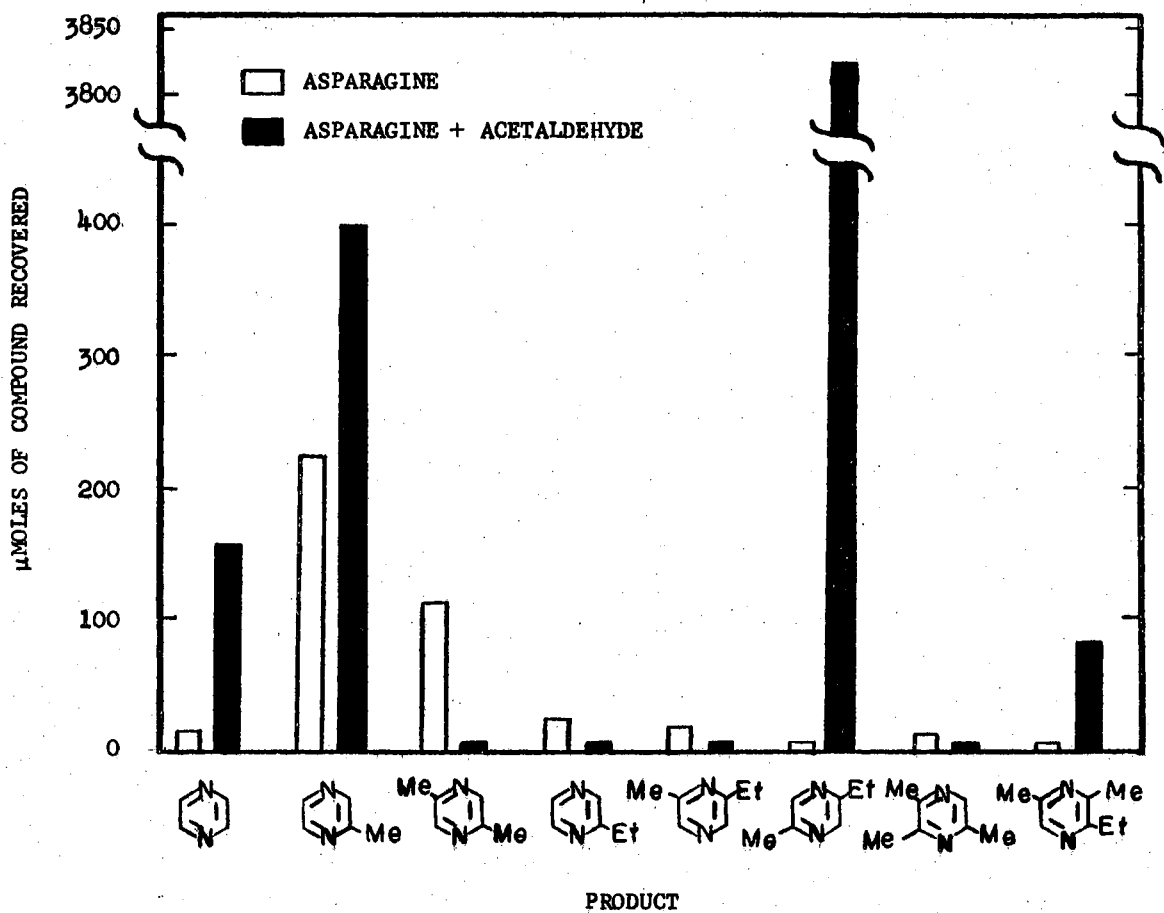pyrazine or to a pyrazine precursor.

Figure 9. Effect of Adding Acetaldehyde to Model System
Containing Glucose and Asparagine

Sucrose is the major carbohydrate in peanut cotyledons (Chapter IV). The reaction of sucrose (10 mMoles) with asparagine (10 mMoles) was studied to determine if the sugar could serve as a reactant in the formation of pyrazines. Table XIV shows that sucrose gave a significant amount of ultraviolet absorbing material when heated with asparagine but not as much as did glucose under the same conditions. Figure 10 gives the product distribution for asparagine heated in the presence of glucose, sucrose, and fructose plus glucose in three separate experiments. The glucose and sucrose gave significantly different product distributions; fructose plus glucose gave nearly the same product distribution as sucrose, especially for the major products 2-methylpyrazine and 2,5-dimethylpyrazine. The results suggested that sucrose was hydrolyzed to glucose and fructose prior to reaction with the amino acid to give pyrazines. The hydrolysis must have been incomplete since Table XIV shows that sucrose gave only about one-half as much ultraviolet absorbing material as an equimolar quantity of glucose. If 10 mMoles of sucrose were completely hydrolyzed, one would expect to obtain nearly twice as much ultraviolet absorbing material as with an equimolar quantity of glucose heated with asparagine.

Data in Figure 8 shows that no single amino acid when heated with glucose gave a product distribution the same as that from a falling film distillate of roasted peanuts. However, a mixture of amino acids (glutamic acid, aspartic acid glutamine, asparagine, and phenylalanine) and monosaccharides (glucose and fructose) which closely approximated the amino acid and carbohydrate analysis of good flavored raw peanuts, gave a distribution of products similar to roasted peanuts (Figure 11). The agreement was particularly good for 2-methylpyrazine,
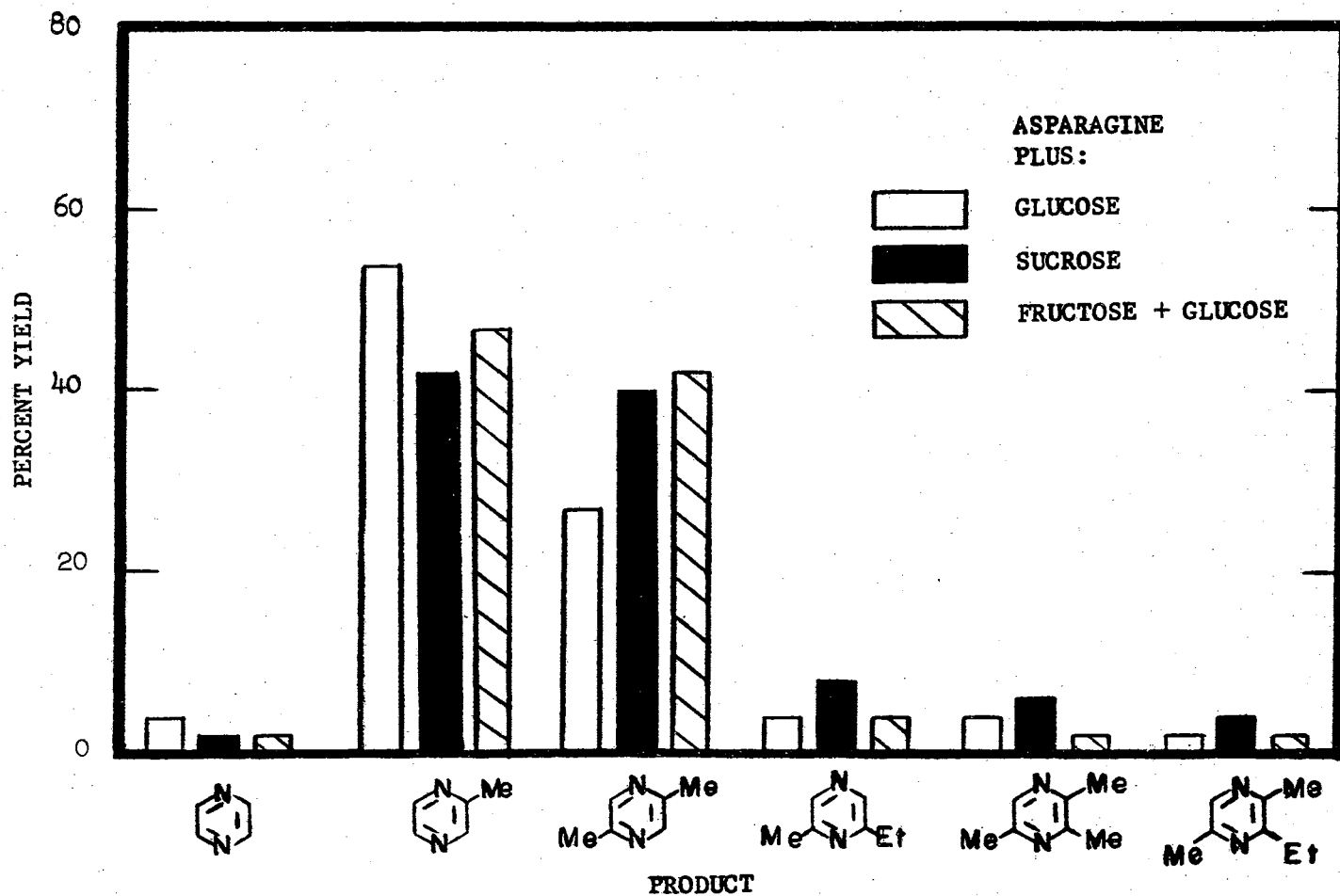
Figure 10. Distribution of Pyrazine Compounds Produced by
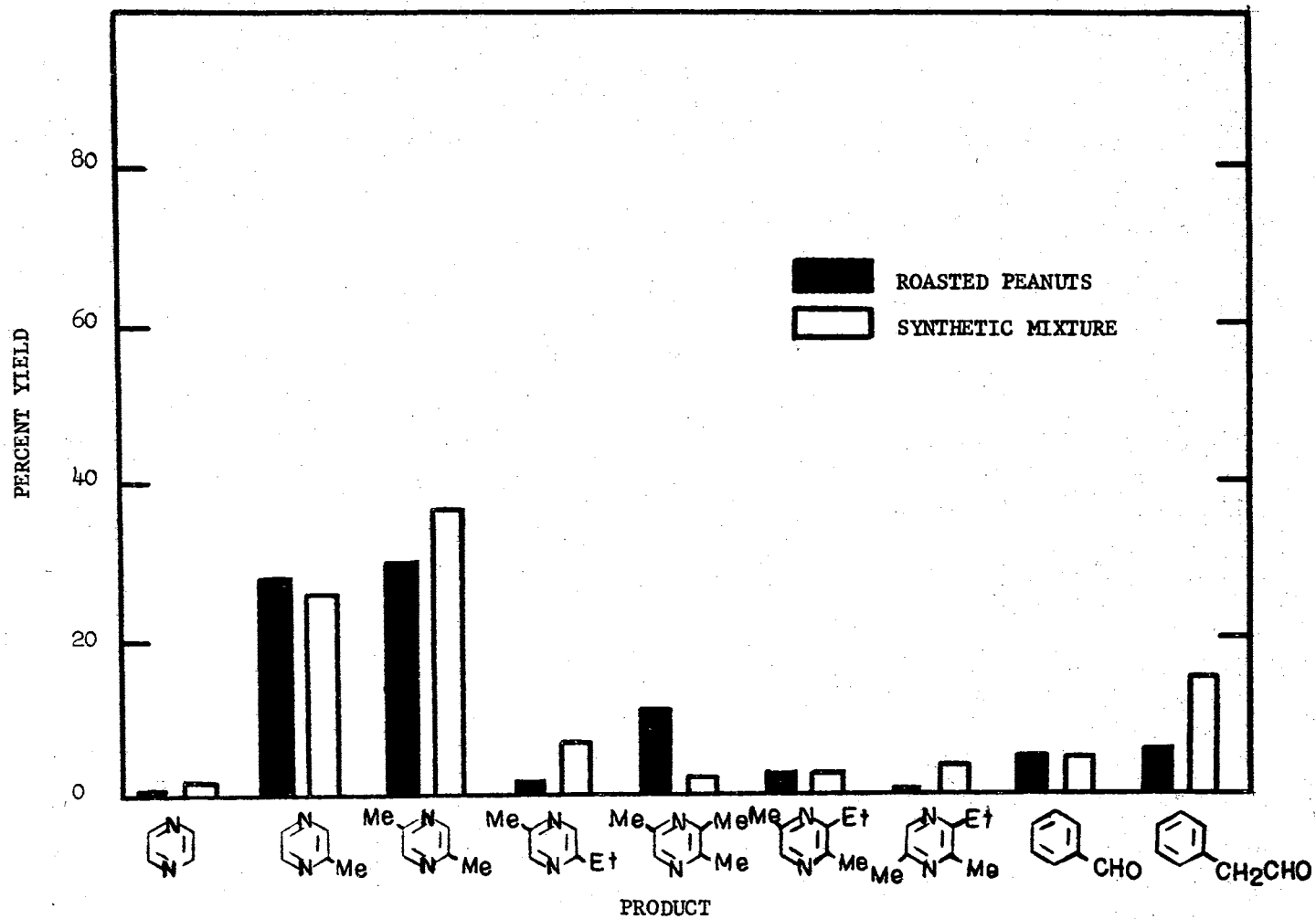Heating Asparagine with Various Carbohydrates

Figure 11. Pyrazines Produced from a Mixture of Amino Acids
and Reducing Sugars which are Precursors of
Typical Flavor

2,5-dimethylpyrazine, 2,5-dimethyl-3-ethylpyrazine, and benzaldehyde.
It was assumed in preparing the peanut-like mixture of amino acids and
monosaccharides that the ratio of aspartic acid to glutamic acid was the
same as the ratio of asparagine to glutamine. This assumption was nec-
essary since asparagine and glutamine could not be separated on the
amino acid analyzer and thus could not be exactly quantitated.

Although glutamic acid forms pyrazines more slowly than the other
amino acids studied, its contribution to the pyrazines of roasted pea-
nuts is considerable since its concentration in raw peanuts is high.
Table XV gives μMoles per pound of some precursors of typical peanut
flavor and the μMoles of total pyrazines formed per hour in model sys-
tems containing the amino acid and glucose. The results indicated that
the reaction of glutamic acid and glutamine with monosaccharides could
account for nearly 87% of the volatile pyrazines formed during roasting
of peanuts.

Results presented in this chapter showed that amino acids and
sugars when heated together can form volatile pyrazine compounds. A
peanut-like mixture of amino acids and monosaccharides produced pyra-
zines having a distribution similar to that found in roasted peanuts.

However, none of the mixtures of pyrazines isolated from the model
systems had a truly characteristic roasted peanut aroma but rather had
a roasted-nutty aroma. Raw peanuts must contain one or a series of as
yet unknown flavor precursors which are responsible for the formation of
volatile compounds which give roasted peanuts their characteristic
"peanutty" aroma.

Since one can supress the peanut-like aroma of a distillate of
roasted peanuts by acidification of the mixture, the compounds

TABLE XV

APPARENT CONTRIBUTION OF VARIOUS AMINO
ACIDS TO TOTAL PYRAZINES FORMED
DURING ROASTING OF PEANUTS

Compound	Amino Acid μmoles/lb	μMoles Pyrazines formed/hour/μmole Amino Acid	Contribution	
Glutamic acid	3180	4.4	13,992	57.4%
Aspartic acid	263	7.6	1,999	8.2%
Glutamine	915	7.8	7,137	29.3%
Asparagine	78	15.8	1,232	5.1%

responsible for the "peanutty" aroma of roasted peanuts must be basic
compounds not unlike the pyrazines.

Amino acids and carbohydrates are among the precursors of roasted
peanut flavor since, when heated together in a system of low water con-
tent, they produce many of the same volatile carbonyl and pyrazine
compounds that are produced during normal roasting of peanuts.

CHAPTER VII

SUMMARY

Peanuts require a high temperature roasting process in order to develop the typical flavor associated with roasted peanuts. This fact implies that raw peanuts contain certain compounds of low volatility (flavor precursors) which are converted, during roasting, to other more volatile compounds (flavor components) which are responsible for the flavor of roasted peanuts.

Raw and roasted peanuts or heated and unheated soluble extracts of raw peanuts were analyzed for various classes of compounds. Compounds which decreased in concentration during heating were implicated as precursors of peanut flavor.

Only amino acids and carbohydrates were observed to decrease significantly in concentration during roasting of peanuts. Analysis of peanuts of known flavor for individual amino acids and carbohydrates resulted in the derivation of an equation which related amino acid flavor precursor concentration to subjectively measured flavor of roasted peanuts. Calculations on the basis of this equation indicated that aspartic acid, glutamic acid, glutamine, asparagine, histidine and phenylalanine were associated with the production of typical peanut flavor; threonine, tyrosine, lysine and an unknown amino acid were considered to be precursors of atypical flavor.

The concentration of precursors of typical flavor increased during

maturation; precursors of atypical flavor decreased in concentration during the same period.

Amino acids associated with the production of typical peanut flavor were heated with carbohydrates in a nearly non-aqueous model system. The same volatile compounds produced during roasting of peanuts were produced in the model systems.

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