# Nonvolatile Flavor Components of Peanuts

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The texture and sweet taste of raw peanuts are changed relatively little during roasting and they contribute significantly to the over-all flavor sensation obtained from roasted peanuts. Roasted-nutty character of roasted peanuts results largely from the reactions of reducing sugars, liberated from sucrose, with free amino acids. The majority of these amino acids are released from a large peptide during roasting. The biogenesis of this peptide and phenylalanine coincides with the maturation process which is necessary for development of good roasted peanut flavor. Biogenesis of sucrose and glutamic acid are positively correlated with maturation only in the latter half of the season. Work in model systems using $^{14}$C-glucose has shown that many of the pyrazines of roasted peanuts can arise from the glucose, fructose, and free amino acids found in raw peanuts.

In 1963 when the authors began work on roasted peanut flavor and its precursors, very little was known about the subject. Pickett and Holley (1943, 1952) had studied gases expelled during roasting and found that carbon dioxide and water were the major components expelled while ammonia, hydrogen sulfide, carbonyl compounds, and carbon monoxide were produced in smaller amounts. Hoffpauir (1953) reviewed what was known of raw peanut composition and speculated on the changes that might occur in raw peanuts during roasting. That starch, protein, and fat were important flavor precursors *per se* appears to have been over-speculation by Hoffpauir in light of present knowledge. The evidence for this conclusion is presented here in some detail.

Only a few comments about the flavor of raw peanuts are included in this report. The raw or "beany" nature of raw peanuts disappears upon roasting but the inherent "sweet" character of raw peanuts remains after roasting to contribute considerable sweetness to the integrated flavor response of roasted peanuts. Although raw peanuts are already brittle and "chewy" they become even more so upon roasting.

## EXPERIMENTAL PROCEDURES

Details of the procedures for extracting the raw peanuts have been published elsewhere (Newell *et al.*, 1967; Newell, 1967) along with methods for treating the extracts before amino acid, peptide, and sugar analyses were made.

Sugars were determined as their trimethyl-silyl ethers (Newell *et al.*, 1967) and amino acids and peptides were determined using the Beckman Model 120C amino acid analyzer. Peptides 1 and 2 were preparatively chromatographed and collected from the Model 120C analyzer. Larger amounts of peptide 2 were successfully prepared by placing the extracts on a Dowex-1-acetate column and washing the neutral and basic amino acids off the column with water. Elution with 2*N* acetic acid removed aspartic and glutamic acid quickly but the highly acidic peptide came off slowly with some difficulty. During this elution apparently some of peptide 2 was converted to peptide 1.

Hydrolysis of the peptides was carried out in 6*N* HCl in sealed borosilicate glass tubes at 110° C. for 12 hours and component amino acids were analyzed on the amino acid analyzer.

The amino acid, peptide, and sugar values reported here as a function of growth period were obtained by analyzing peanuts from plants grown in a randomized block design in a growth chamber. Two pots containing two plants each were selected at random from each of four blocks to provide material for each harvest date shown in Figures 1 and 2. The peanuts from all plants for each date were bulked and then divided into three age groups on the basis of seed, seed coat, and pod characteristics (Newell *et al.*, 1967); mature, intermediate, and immature. These divisions were necessary because the peanut is an indeterminate plant; plants will always bear peanuts in various stages of physiological development, regardless of the stage of growth at which the plants are harvested. The intermediate group represented peanuts from which the very mature and very immature peanuts had been removed. Since this was the most heterogeneous group, only the information on it is reported here.

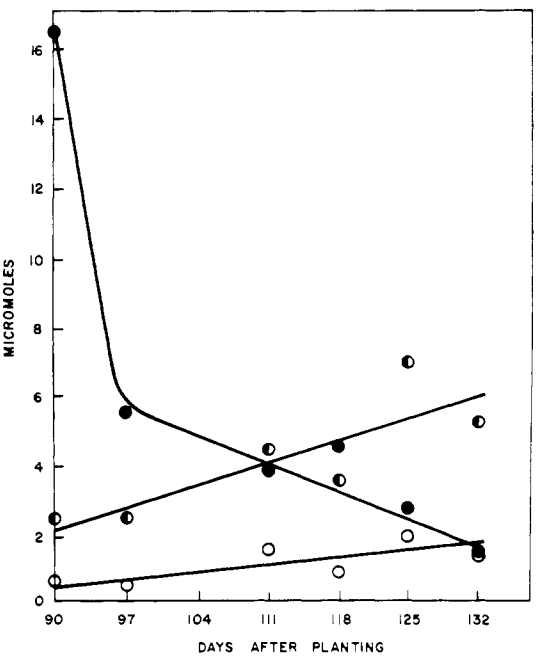


**Figure 1. Change in arginine, peptide 2, and phenylalanine content (μmoles/gm. fat-free meal) of Spanish peanut fruit as a function of maturation**

● Arginine
◐ Peptide 2
○ Phenylalanine

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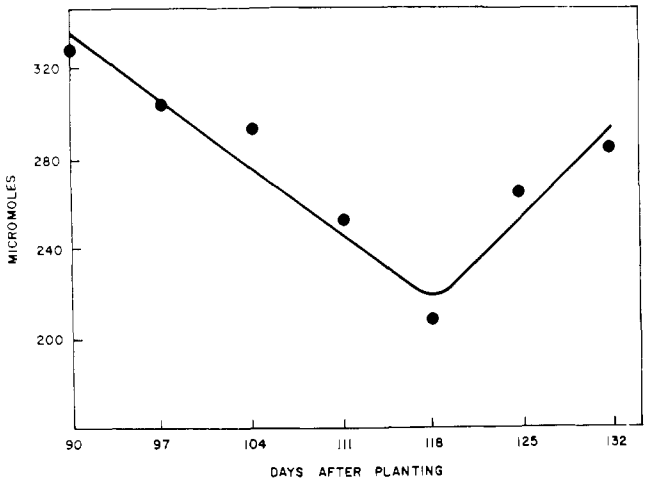


**Figure 2. Change in sucrose content (μmoles/gm. of fat-free meal) of Spanish peanut fruit as a function of maturation**

## NATURE OF THE SYSTEM

Morphology of peanuts was studied early by Woodroof (1940) and more recently by Diekert and coworkers (1962), Yatsu and Altschul (1963), and Jacks *et al.* (1967). Successful particulate fractionations of the starch grains, protein bodies, and spherosomes have been accomplished by this group. The accumulated information showed that parenchymal tissue of the peanut cotyledons is made up partially of particulate bodies, the integrity of which is maintained by permeable membranes and which house separately the starch, oil, and storage proteins of the cell. Endoplasmic reticulae, nuclei, and mitochondria are also present; vascular tissue permeates the intercellular spaces.

Peanuts which have been properly cured and stored contain about 6 to 8% moisture. Thus senescent peanuts represent highly compartmentalized, segregated, nonaqueous chemical systems which respire at a slow rate but which die readily if this meager respiration ceases. Such a system is certainly a far cry from that which can be described by classical solution chemistry. This realization prompted some of the authors' earlier experiments (Mason and Waller, 1964), the results of which are now briefly summarized.

Linear functions obtained by plotting roasting time *vs.* 1/T (reciprocal temperature) for several types of Spanish peanuts suggested to the authors that formation of peanut flavor obeyed the Arrhenius concept of temperature effects on reaction rates just as though solution chemistry principles applied. In view of the nonaqueous state of cured peanuts, these results suggested that either the precursors were physically in very close proximity or that intra- rather than intermolecular reactions were predominant in flavor formation. However, during roasting, oil exudes to the outer surface of the peanuts indicating that the heating process destroys the integrity of the spherosomes. Thus, during roasting, there is some nonaqueous fluidity within the cell structure. Starch grains ruptured during heating but the gross structure of the protein bodies changed very little (Mason and Waller, 1964).

## NATURE OF THE FLAVOR PRECURSORS

When fat-free peanut meal was extracted with 1*M* sodium chloride solution and the extract was filtered on Sephadex G-25, three fractions were obtained. The protein fraction (globulins) produced only slight browning and little flavor when heated in oil but the fraction containing amino acids, sugars, and other micromolecular species browned dramatically and

**Table I. Amino Acid Concentrations[a] in Spanish Peanuts Before and After Roasting**

| Amino Acid | Times Conc. Increased During Roasting | Mean Values for Four Determinations | |
|---|---|---|---|
| | | Raw | Roasted |
| Aspartic | 2 | 2.79 | 2.55 |
| Asparagine | 0 | 3.93 | 2.40 |
| Glutamic | 0 | 10.85 | 6.42 |
| Alanine | 1 | 1.35 | 1.26 |
| Phenylalanine | 4 | 2.24 | 2.73 |
| Peptide 1 | 3 | 0.17 | 0.21 |
| Peptide 2 | 0 | 2.34 | 1.08 |
| Ammonia | 3 | 1.59 | 2.69 |

[a] μmoles per gram of fat-free meal.

**Table II. Approximate Amino Acid Content[a] of Peptides 1 and 2**

| Amino Acid | Peptide 1 | Peptide 2 |
|---|---|---|
| Glutamic acid (glutamine) | 16 | 25 |
| Aspartic acid (asparagine) | 6 | 4 |
| Phenylalanine | 6 | 17 |
| Glycine | 14 | 11 |
| Serine | 11 | 6 |
| Alanine | 7 | 3 |
| Threonine | 1 | 2 |
| Leucine | 1 | 2 |
| Isoleucine | 1 | 1 |
| Valine | 1 | 1 |
| Tyrosine | 1 | 1 |

[a] Number of residues.

produced typical nutty aroma when heated in oil (Mason and Waller, 1964).

Newell *et al.* (1967) and Newell (1967) determined the amino acid content of extracted fat-free meal before and after roasting. Those amino acids destroyed to about the same extent in both good and off-flavored peanuts were judged to be precursors of typical flavor; those destroyed to a greater extent in off-flavored peanuts were judged to be precursors of off-flavor. Typical flavor precursors were aspartic acid, asparagine, glutamic acid, phenylalanine, and histidine; the first four of these five amino acids made up more than half the total free amino acids present.

Three of the peanut samples used by Newell were subjected to a normal roast but one was a light roast according to the panel. In the light roast sample, two of the five amino acids mentioned actually increased during roasting for some unexplained reason. More recently, a number of samples subjected to mild roasting consistently revealed the same phenomenon. Mean values for the most important amino acids of four of these samples are shown in Table I.

The data showed that it was not uncommon for some of the amino acids to increase during roasting; this was especially true with phenylalanine. Also, of the two peptides listed in the table, the most acidic one (peptide 1) tended to increase during roasting. These two compounds had not been characterized at the time Newell *et al.* (1967) published and peptide 2 was listed as an unknown.

Recently, peptides 1 and 2 were isolated, purified in small amounts, and the component amino acids of each were determined (Table II). The number of residues of each amino acid per peptide is only approximately correct because insufficient peptide was available to obtain accurate measurements of peak areas of the minor components. Evidence for proline and an unknown amino acid was found in the chroma-

**Table III. Sugar Content[a] of Spanish Peanuts Before and after Roasting**

| Sugar | Mean Values and Range for Five Samples | | | |
|---|---|---|---|---|
| | Raw | Range | Roasted | Range |
| Fructose and/or mannose (unresolved by GC) | 2.7 | 1.6–3.3 | 1.8 | 1.4–2.0 |
| Glucose | 1.9 | 1.7–2.1 | 1.3 | 0.9–1.5 |
| Inositol | 1.3 | 1.0–1.6 | 1.1 | 0.7–1.6 |
| Sucrose | 149.0 | 109 –197 | 125.3 | 107 –161 |

[a] Milligrams per gram of fat-free meal.

tograms for both peptides. Among the major amino acids of peptide 2 were the amino acids judged to be flavor precursors of typical roasted flavor. During the isolation and purification of peptide 2, some was converted to peptide 1 on the Dowex-1-acetate ion exchange column.

The close relationship between these two peptides and the knowledge of their component amino acids could explain the peculiar phenomenon just discussed: Peptide 2 was hydrolyzed to a considerable extent during roasting to its component amino acids and to a small extent it was converted to peptide 1. Only those amino acids which react slowly would show an increase after roasting. Preliminary evidence based on amino acid content indicated that both peptides were large. If true, the amount of peptide 2 lost during roasting could account for as much as 10 times more free amino acids than was present originally as free amino acids. Thus, peptide 2 may not only be the reservoir of amino acid precursors of typical roasted peanut flavor but its presence within protein bodies would insure a continuous supply of the proper concentration of the necessary amino acids for good flavor development within the narrow restricted environment. The presence of peptide 2 in isolated and purified protein bodies was clearly demonstrated in the authors' laboratories using the analytical procedures described herein.

In 1967, Newell reported the results of a study of the fate of reducing sugars and sucrose in saline extracts of raw and roasted peanuts. Newell's results, confirmed later by the present authors (Table III), indicated that sucrose was inverted during roasting. Thus, in some instances, glucose and fructose actually increased during roasting. This observation was most important in explaining how sufficient reducing sugars were produced to react with the considerable amount of amino acid that was being destroyed during roasting. The hydrolysis which occurred during the time peanuts were approaching roasting temperatures could be explained by invertase activity.

Table III shows mean sugar values for a number of samples analyzed recently. The data confirm the extremely low levels of reducing sugars relative to sucrose reported by Hoffpauir and the apparent destruction of sucrose during roasting (16%). Thus, the sugars which actually take part in browning during roasting are fructose and glucose for the most part. This was confirmed by Newell (1967) who found the same pyrazines produced from fructose and glucose in nearly the same quantities as those produced from sucrose in a model system.

## BIOGENESIS OF FLAVOR PRECURSORS

Figure 1 shows what is known about the biosynthesis of the amino acid and peptide precursors of typical flavor. Aspartic acid, asparagine, and histidine are not shown because their values became constant very early in the growing season and remained constant. Phenylalanine, an amino acid important to the development of roasted peanut flavor (Johnson, 1966; Mason *et al.*, 1967) and a major constituent of peptide 2, increased steadily throughout the growing season. Peptide 2 also increased during the growing season even more dramatically than phenylalanine. Changes in arginine were very dramatic and the authors have since shown that its concentration is inversely correlated with maturity. Thus, the nature of the arginine curve in Figure 1 shows that maturation of peanuts was indeed being measured in the experiments represented by this figure. Because glutamic acid decreased to mid-season and then increased from mid-season to late-season, its relation to maturation was in doubt.

Since proper flavor development in roasted peanuts is a very sensitive function of maturity and since peptide 2 concentration increased while other amino acids decreased or remained constant during maturation, the data suggested to the authors that peptide 2 is a "characteristic" precursor of typical roasted peanut flavor.

This suggestion was also supported by the fact that the peptide was destroyed to a greater extent than any amino acid, except glutamic, on roasting, and that the amino acids liberated would contribute mostly to those designated as good flavor precursors. Figure 2 shows that sucrose levels remain high during the same growth period shown for amino acid and peptide development, but a sharp decrease at mid-season similar to that observed for glutamic acid was not explained. Hoffpauir (1953) reported a mean value of 4.5% sucrose in raw peanuts, wet weight basis, and reducing sugars were reported to average only about 0.2%.

On a dry weight, fat-free, basis the peanuts used in these studies contained about 15% sucrose. Thus, the source of the sweetness of raw peanuts and fat-free peanut meal was obvious.

## RELATIONSHIP OF FLAVOR COMPONENTS TO FLAVOR PRECURSORS

Much of the evidence that flavor components formed during roasting arose from precursors released from peptide 2 and sucrose came from the knowledge of the structures of the flavor components themselves.

Carbonyl compounds from peanuts have been identified by Pickett and Holley (1952), Pattee *et al.* (1965), and Mason *et al.* (1967). The authors' work suggested that some very important multicarbonyl compounds remained to be identified (Johnson and Mason, 1967). The carbonyl compounds known to arise from Strecker degradation of their corresponding amino acids have been isolated from roasted peanuts: acetaldehyde, 2- and 3-methylbutanal, isobutyraldehyde, and phenylacetaldehyde (Mason *et al.*, 1967). All the corresponding amino acids were shown to be present in raw peanuts and were destroyed during roasting. Although the carbonyl compounds have never been quantitated successfully, we know that phenylacetaldehyde and acetaldehyde are produced in relatively large amounts during roasting; this would be expected from the large amounts of free and peptide bound alanine and phenylalanine present. A few pyrazines and a pyrrole have been reported in roasted peanuts (Mason *et al.*, 1966a). Although there were data suggesting the presence of many more pyrazines, several more were identified only recently by Johnson *et al.* (1968). Newell (1967) performed work in a nonaqueous model system with glucose and the free amino acids found in peanuts. When a mixture of all amino acids was used, he obtained amounts

of the major pyrazines and phenylacetaldehyde from the model system comparable to those obtained from roasted peanuts. Quantitative differences were noted when different individual amino acids were heated with glucose. The data suggested that the structure of the amino acid controlled quantitatively the pyrazines formed and that ammonia was not the common intermediate between amino acids and pyrazines.

Koehler *et al.* (1968) used $^{14}C$-labelled amino acids and glucose to show that the pyrazine carbon atoms came largely from glucose; amino acids contributed little to the pyrazine structure except for the nitrogen atoms. The data showed that glucose fragments going into pyrazines resulted largely from splitting between carbons 2 and 3 or between carbons 3 and 4 with the $C_2$-$C_3$ split predominating. Interestingly, glucose-1-$^{14}C$ and glucose-6-$^{14}C$ were incorporated into dimethylpyrazine to about the same extent, indicating that the two $C_3$ fragments resulting from the $C_3$-$C_4$ split must be equivalent, and that the six-carbon intermediate is symmetrical. However, labelling of methyl pyrazine by glucose-1-$^{14}C$ was higher than glucose-6-$^{14}C$ indicating the $C_2$-$C_3$ split takes place almost exclusively from the anomeric end of the molecule. This latter information suggested that the six-carbon intermediate leading to a $C_2$-$C_3$ split is dissymmetrical. Two different forms of diacetoformoin (Hodge, 1965; 1967), depending on the presence of polar or nonpolar solvents, might be good candidates for these intermediates.

A recent publication by Brown *et al.* (1968) reported the isolation of acids, phenols, and compounds that appeared to be lactones from whole roasted peanuts. Specific identities of some of the acids were reported. The nature of the methods used (boiling alcohol extraction followed by distillations) cast some doubts on the authenticity of some of the components identified. For example, the phenylacetic acid isolated by these workers may have resulted from oxidation of phenylacetaldehyde during isolation (Mason *et al.*, 1967). Nevertheless, the published information suggested the presence of classes of precursors not previously reported. The fact that the flavor precursors are relatively small water soluble compounds was emphasized by a patent recently issued to Proctor and Gamble Co. (Ince, 1968), in which a bland product was obtained by extracting whole raw peanuts with hot water and then drying them at roasting temperatures.

No one of the flavor compounds identified so far is entirely characteristic of roasted peanuts. Some of these compounds have been found in a number of roasted products including cocoa (Marion *et al.*, 1967; Rizzi, 1967; van Praag *et al.*, 1968), coffee (Bondarovich *et al.*, 1967; Goldman *et al.*, 1967), and potato chips (Deck and Chang, 1965). For that matter, the flavor precursors described are amazingly similar to those found in cocoa by Rohan and Stewart, (1966a; 1966b) and Pinto and Chichester (1966). Thus, even though we now have considerable information about compounds which contribute "nutty" or "roasted" character to several roasted foods, the characteristic peanut component or components and their precursors remain elusive.

## FLAVOR CONTRIBUTION OF SOME OTHER COMPONENTS OF PEANUTS

That peanut oil is not a flavor precursor *per se* is supported by several pieces of work. Mason and Waller (1964) found that oils other than peanut oil were effective in production of roasted peanut aroma from isolated protein bodies and precursor fractions on heating. Iverson *et al.* (1963) found no differences in the fatty acid content of peanut oil before and after roasting. Apparently, the medium need only be nonaqueous for the proper chemistry to occur during roasting. For example, the new low-fat peanuts developed at the Southern Utilization Research and Development Laboratories must retain 20 to 40% of their original oil to develop an acceptable flavor when roasted.

When raw peanuts were defatted with hexane and the pulverized fat-free powder was heated in various oils in the authors' laboratories, typical roasted peanut aroma developed, and pyrazines were among the major volatile products. However, when the same experiments were performed in water, carbonyl compounds were the major volatiles; pyrazines were much less conspicuous, and total aroma was not typical of roasted peanuts. Thus, the need for a nonaqueous medium was apparent, but whether it was needed as a medium to solubilize polar flavor substances or to insure the proper reaction kinetics is not known.

Protein bodies will develop typical peanut aroma, brown, and liberate considerable ammonia when heated in mineral oil or glycerol (Mason *et al.*, 1966b). These bodies contain considerable sucrose and much of the peptide and free amino acids of cured peanuts. As was previously stated, the large globular proteins which are also housed in the protein bodies apparently contribute very little to development of peanut flavor. The compartmentalized nature of peanuts and the highly specific nature of the peptides related to flavor production may provide the handle for performing very specific isotopic experiments in the future to prove unequivocally whether or not these precursors lead to flavor components and the mechanism by which the transformation takes place. Such experiments could lead us to the exciting possibilities of using chemical data on the specific precursors as criteria for genetic selection of superior flavor in plant breeding work.

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