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DEVELOPMENT AND CHARACTERIZATION OF A SYNTHETIC MEAT FLAVOR MIXTURE

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ABSTRACT

Development and Characterization of a Synthetic Meat Flavor Mixture

By

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A synthetic meat flavor model system was developed to determine the role and level of precursors contributing to meat aroma and taste. The model system was composed of reducing sugars, 5'-nucleotides, monosodium glutamate, glycoprotein, various amino acids, salt and fat. The levels of these components were evaluated by a taste panel, the the results were analyzed statistically by the surface response method in order to obtain optimum concentrations.

It was shown that certain precursor compounds, including simple sugars, 5'-nucleotides, glycoprotein, and sulfur containing amino acids (cysteine or methionine) were necessary for development of basic meat flavor. The initial concentration of these essential components was obtained from published data and their optimum levels were determined by the surface response method. Once the concentration of these precursors was determined, they were held constant while other amino acids were tested for their contribution to meat flavor.

Sensory evaluations were initially performed on individual amino acids at three different concentrations (low, medium, high). Panelists were asked to evaluate each sample for boiled, roast, sulfurous and overall odor and taste. Results were statistically analyzed using the surface response method, which predicted the maximum or minimum concentration. The best overall score was obtained by averaging the maximum values. Results showed that serine (21 mg/50 ml), alanine (23 mg), taurine (22 mg) and cysteine (20 mg) were necessary at higher concentrations than leucine (10 mg), methionine (11 mg), isoleucine (9 mg), valine (7 mg), arginine (6 mg) and glycine (8 mg). This indicated that the former group of amino acids contributed more toward the formation of meat flavor volatiles than the latter.

On testing combinations of amino acids in the model system, most amino acids had the same computer predicted level as when tested singly. However, isoleucine and arginine had lower predicted levels when combined with other amino acids probably due to inhibitory effects. Results also showed that the panelists were not able to differentiate between small changes in concentration.

Heating of either the gelatin-simple sugar mixture or the amino acid-simple sugar mixture caused most amino acids to decrease by 20-30%. However, the sulfur containing amino acids, methionine and cysteine, decreased by 58 and 95%, respectively. Amino acid analysis of the composite model

system showed that the amino acid losses during heating were the same as for the gelatin-simple sugar mixture. Results indicated that the rate of amino acid breakdown may be limited by the amount of available simple sugars to interact with amino acids and form nonenzymatic browning products.

The synthetic meat flavor model system was compared with some commercially available products for flavor, odor, mouthfeel and general acceptability. Results showed that the synthetic meat flavor system consistently received higher average panel scores than all commercial products, but were not rated as high as the authentic meat extract.

Several trapping apparatus for the analysis of meat volatiles were evaluated and compared. It was found that the Likens-Nickerson apparatus was the simplest and most reliable method for entrapping the meat volatiles. It simultaneously performed distillation and extraction, thus minimizing volatile losses that may occur during preparation and transferring of samples.

Volatiles isolated from the synthetic meat flavor model system were separated by GC-MS, and the unknown spectra were matched with the spectra of known compounds by Spectral-Search computer programs. Alcohols, hydrocarbons, aldehydes, thiophenes, furfurals and furans were tentatively identified as constituents of the synthetic meat flavor model system.

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INTRODUCTION

The characteristic aroma and flavor of meat have long interested researchers, but identification of the volatile flavor components in meats did not begin until about twenty years ago. Since meat flavor can not be attributed to any one key contributing component, it has been difficult to identify the major responsible constituents. With the development of new techniques and more sensitive instruments in recent years the detection and identification of meat flavor components has been gradually elucidated.

Generally, most flavor researchers believe that the major meat flavor components are located in the water soluble fraction of muscle (Kramlich and Pearson, 1958; Hornstein and Crowe, 1964). During cooking the flavor precursors interact with other substances or undergo degradation to produce volatile compounds, which are responsible for the characteristic meat flavor. Investigators have agreed that all meat contains similar water soluble components, such as carbohydrates, minerals, 5'-nucleotides, proteins and other amino compound (Batzer, 1961, 1963), whereas the characteristic species flavor of meat is derived from the fatty tissue (Hornstein and Crowe, 1960; Wasserman and Talley, 1968; Pepper and Pearson, 1971).

Meat flavor research has been approached in one of the following three ways: 1) to analyze the volatiles formed during cooking; 2) to study the reaction mechanisms of precursors which may be contributors to meat flavor and aroma; and 3) to use heated model systems to systematically evaluate the volatiles evolved from individual precursor components.

In recent years with the aid of gas chromatography-mass spectrometry (GC-MS), nearly 300 different compounds have been identified from cooked beef (MacLeod and Coppock, 1976; Chang and Peterson, 1977). In spite of the large number of compounds found in meat, Chang and Peterson (1977) stated that lactoses and acyclic sulfur compounds, containing S,N, and O, are probably the main contributors to meat flavor. They concluded, on the other hand, that aliphatic hydrocarbons, aromatic hydrocarbons, saturated alcohols, carboxylic acids, esters, ethers and carbonyl compounds are not primary contributors to meat flavor. Even with knowledge of the appropriate relative concentrations of these compounds, flavor chemists still have not been successful in reconstituting meat flavor. This clearly demonstrates the complexity of meat flavor.

Other investigators have approached the problem by studying the contributions of precursors in the development of meat flavor volatiles. It has been shown that the precursors of cooked meat flavor are water soluble, of low molecular weight, and when partially purified, are rather unstable. When subjected to heat, these components spontaneously undergo reactions to yield meaty odors.

Since none of the compounds identified in meat flavor concentrates have a distinctly meaty aroma, it is probable that a critically balanced mixture of several volatiles is responsible for meat flavor. The volatility and instability of some of these compounds make it very difficult to analyze or reconstitute meat flavor. Thus, flavor chemists have used model systems to study the contribution of individual precursors to flavor volatiles. It is questionable whether all compounds formed in the model systems are directly involved or found in meat flavor. Nevertheless, they have provided the flavor chemists much information about potential reactions that can occur in meat.

The purpose of the present research was to determine the role of various precursors to meat flavor by the following approaches:

1) To formulate synthetic meat flavor model system by using the principle precursors already found in beef concentrate;
2) To predict and test the best levels and combinations of the precursor compounds using taste panel evaluation and the surface response methodology for analyses;
3) To determine the contribution of individual amino acids to meat flavor by analyzing the amino acid content of model systems before and after heating;

4) To test suitable apparatus to trap the volatiles for GC-MS analysis;

and 5) To compare the GC-MS spectra between the synthetic meat model system and real beef concentrate, and to identify the major components found in both systems.

REVIEW OF LITERATURE

Effects of Cooking on Meat Flavor

The nature of cooked meat flavor depends upon the method of cooking. Raw meat in general has a salty, metallic, bloody taste and a sweet aroma resembling serum (Wasserman, 1972). When heated, however, it produces a complex mixture of volatile components from which the characteristic meat flavor is developed. Differences in flavor due to cooking are probably a direct function of temperature, time, degree of moisture and the method of cooking (Dwivedi, 1975). Thus, the quantity and quality of volatile components evolved during cooking depend upon the various cooking parameters (MacLeod and Coppock, 1976; 1977; Watanabe and Sato, 1971; 1972).

Wasserman (1972) concluded that boiled beef flavor is characteristically developed in water where the temperature does not exceed 100°C, whereas, roast beef flavor is produced under dry heating conditions in which the temperature exceeds 100°C. Hornstein and Crowe (1960b) and Batzer *et al*. (1961) demonstrated that an aroma reminiscent of roast meat was developed on heating a lyophilized powder from the water soluble fraction of beef extract, whereas, heating the same powder in water developed a brothy boiled meat aroma. Thus, meat roasted at 165°C tastes and smells different than that boiled at 100°C (Wasserman, 1978). Mabrouk (1976) stated that meat volatiles heated below 75°C are not particularly meaty or agreeable, but those obtained on boiling above 75°C are characteristically and pleasantly meaty. Similarly, beef cooked to an internal temperature of 82°C for 2 hours had a lower flavor rating than that which had been brought to the same internal temperature by heating for 30 min at 288°C (MacLeod and Coppock, 1977).

Lieblich _et al_. (1972) found that the major volatile components from roast beef and its drippings are alkanals (alk-2-enals and alka-2,4-dienals), 3-hydroxy-2-butanone and butyrolactone. Although many volatile compounds have been identified in boiled beef, none of the compounds have been shown to be responsible for the characteristic aroma (Hornstein, 1967). Recently some furanones (Hirai _et al_., 1973; Tonsbeek _et al_., 1968), 1-methylthio-ethanethiol (Brinkman _et al_., 1972), and thiophene-2-carboxyaldehyde (Hirai _et al_., 1973) were identified as characteristic compounds of boiled beef.

MacLeod and Coppock (1976; 1977) compared microwave to conventional cooking for beef and found that the latter method was preferred. Marshall (1960) and Law _et al_. (1967) attributed the poor acceptance of microwave cooked beef to the lack of flavor and stated it was due to a decrease in the nonenzymatic browning reaction. Recently, MacLeod and Coppock (1976) found that microwave boiled beef yielded a higher concentration of alkanes, alkenes and alcohols than conventional boiled beef. They concluded that these classes of compounds produce undesirable odors. Volatiles from conventionally boiled beef consisted of benzenoid compounds and furans, which they characterized as having a more desirable odor. In another study MacLeod and Coppock (1977) compared the volatiles evolved from conventional and microwave cooking in the presence and absence of water and for different periods of heating. Results indicated that certain carbonyl compounds, sulfides, pyrroles and pyridines are probably the most important contributors to roast beef aroma as opposed to the poorer qualities of boiled beef.

Watanabe and Sato (1971; 1972) found that the major contributors to pan fried beef are carbonyl compounds, esters, fatty acids, and a mixture of different compounds, including methional, 2-acetyl furan, 2-furfural, methyl ketone, 1-methyl-2-acetyl pyrrole and benzothiozole.

Luh _et al_. (1964) found a three fold increase in the concentration of hydrogen sulfide in conventionally canned meat as compared to that of high temperature-short time sterilized meat. They stated that the latter had more "raw" taste than the former, thus, it received a lower hedonic odor rating. Przezdziecka and Zoltowska (1967) investigated canned meat samples processed at different time-temperature relations. They found that temperature had a more pronounced effect on the sensory properties than processing time.

Ziemba and Malkki (1971) found that the concentration of hydrogen sulfide in canned beef was at its maximum between calculated F_c values of 5.2 and 34.5. They demonstrated that as the F_c values increased, the concentration of methanethiol and dimethyl disulfide paralleled off-odor development. They also found that the ammonia odor and meaty odor were least detectable when the hydrogen sulfide content was at its maximum.

Composition of Meat Flavor Precursors

The initial studies of Hornstein and Crowe (1960) and Hornstein et al. (1960; 1963a,b) investigated the localization of flavor precursors. The results of their experiments showed that meat flavor was really a blend of two separate basic flavor components: 1) a basic characteristic meaty flavor, which is common to all meat; and 2) a characteristic species flavor, which is unique to a particular type of meat. They concluded that the basic meaty flavor is derived from water soluble precursors, which originate in the fiber and are developed during cooking; whereas, the characteristic species flavor appears to originate in the fat during cooking.

Lean Tissue Fraction-Water Soluble

Crocker (1943) was one of the first flavor researchers to examine the components in meat responsible for flavor. He separated the juice from the fiber by pressing, and subsequently by leaching with water. On heating the meat fibers, a typical meat flavor was produced. Heating the press juice produced a nontypical, low intensity flavor. He demonstrated that bone contributed little to beef flavor, while marrow and fatty tissue supplied certain aroma notes, but contributed nothing to the development of the typical meaty flavor. Analysis of odor indicated the presence of free amines, ammonia, a fishy smelling amine, an "indole-like metallic odor" and a crackery derivative that suggested piperidine. Crocker (1943) concluded that the odor of cooked meat is due to a variety of chemical substances, presumably produced by fragmentation of amino acids, simultaneous with the breakdown of sulfur amino acids to yield hydrogen sulfide and propionic acid. Barylko-Pikielna (1957) confirmed these findings, but they concluded that all three fractions of cooked beef, i.e., meat fibers, denatured water-soluble proteins and extracted substances, participated in the development of a full meaty flavor.

Solamon (1943) attributed the flavor of cooked meat to disintegration of the proteins into cleavage products, such as proteases, peptones, peptides, and finally to the amino acids. He stated that glutamic acid was chiefly responsible for meat flavor.

Bouthilet (1951a,b) stated that the flavor of chicken is derived from substances which are attached to the fibers and cannot be removed by pressing. He also postulated that meat flavor is composed of at least two fractions, i.e., a sulfur-containing material, which is highly labile and gives off hydrogen sulfide on standing, and a characteristic chicken odor fraction, which is mainly composed of fatty acids.

Wood (1956) examined the chemical composition of cooked beef extract by removing the protein from the extract. Using paper chromatography, he identified several amino acids in the extract, but he did not detect any sugars or their derivatives.

Kramlich and Pearson (1958) were the first to demonstrate that meat flavor precursors are water soluble. Their results showed that cooking prior to extraction increased the flavor of meat, suggesting that full flavor development may be due to heating the juice and fibers together. Similarly, Hornstein and Crowe (1960) described a method of obtaining a flavor powder concentrate by lyophilyzing the water extract of ground muscle. On heating the dry powder, an odor reminiscent of roast meat was evolved, whereas, boiling produced the aroma of meat broth. They found that heating of the nondialyzable fraction, which is a mixture of the sarcoplasmic proteins, in a vacuum or in water solution did not yield meat-like aromas. Further separation of the diffusate by ion exchange chromatography into an amino acid fraction and a neutral fraction, containing reducing sugars, showed that neither of these fractions produced meat-like aromas upon heating. However, when these subfractions were recombined, typical meat aromas were obtained. Based upon these observations, they concluded that the flavor of lean beef was produced by an interaction between flavor precursors of low molecular weight and simple sugars, which undergo spontaneous Maillard-type reactions when heated.

It has been shown that extracts of lean meat contain a large variety of nonvolatile compounds exhibiting little flavor; these include amino acids, peptides, non-protein nitrogen compounds, 5'-nucleotides, carbohydrates, glycoproteins, vitamins and minerals (Herz and Chang, 1970; Dwivedi, 1975).

Amino acids, peptides, and non-protein nitrogen compounds. Macy et al. (1964a,b) have quantitatively determined the sugar and amino compounds present in heated and unheated lyophilized beef diffusate. They found that the total amino acid content in unheated beef extract amounted to 161 mg per 100 g of fresh tissue, but the total dropped to 70.5 mg upon heating. Anserine, carnosine, alanine and taurine made up the majority of the components in both unheated and heated beef extract. During cooking the loss of essential amino acids was approximately 55%. They also reported that beef had the largest quantity of amino acids (161 mg/100 g fresh weight), followed by lamb (130 mg/100 g) and pork (109 mg/100 g). The losses during cooking were greater from beef than from lamb or pork.

In a quantitative study of amino acids in raw and roasted beef, Macy et al. (1970c) found that most free amino acids increased in concentration by approximately 40% during cooking. However, threonine, serine, glutamic acid, histidine and arginine decreased in concentration. They found that the total amino acid level for raw beef was approximately 1260 mg/100 g of dry fat free tissue, but it increased to 1740 mg/100 g during cooking. They attributed the increase in amino acid content to hydrolysis of the protein. They concluded that cathepsins and other proteolytic enzymes in the tissue were probably involved, since they had shown previously that most of the free amino acids decreased upon heating of the protein dialysate (Macy _et al_., 1964a).

Jarboe and Mabrouk (1974) demonstrated that the sulfur-containing amino acid content in lamb was twice that of pork and three times the value for beef. The percentage of basic amino compounds in lamb was shown to be three times the value of pork and twice the value of beef. Lamb was also rich in acidic amino acids, being approximately two fold higher than either beef or pork. Macy _et al_. (1964a) have shown that glutathione can only be found in lamb, while cysteic acid and ornithine were only obtained from pork and lamb. Jarboe and Mabrouk (1974), thus concluded that a relationship may exist between the relative quantity of certain amino compounds and the amount of flavor evolved from the meat of different species.

Wasserman and Spinelli (1970) found that no aroma developed in a model system containing only amino acids upon drying at 125°C, and further reported that 80-90% of the amino acids remained unchanged. They concluded that pyrolysis occurred only when the roasting temperatures were above 300°C, which apparently can initiate pyrolytic decarboxylation and deamination of amino acids.

Fujimaki et al. (1969) studied the pyrolysis of cysteine, cystine and methionine. Although the conditions of the reactions are not representative of heating of natural products, some interesting volatiles were formed. For example, methanthiol and methional are pyrolytic products of methionine, while pyrolysis of cysteine and cystine yielded hydrogen sulfide, sulfur, cysteamine and 2-methylthiozoline.

Merritt and Robertson (1967) reported the major products derived from pyrolysis of 17 amino acids using gas chromatography and mass spectrometry. They found that 3-methyl-butanal and 2-methyl-butanal are derived from leucine and/or isoleucine. They stated that 2-methyl-propanal was derived from valine, while benzene, toluene, and ethylbenzene were obtained from phenylalanine. Such compounds have been identified in cooked meat volatiles, but may also arise by other reaction mechanisms. They also observed that the products formed during pyrolysis of dipeptides and dipeptide pairs depends on the sequence of amino acids. This may explain, in part, why some aromas derived by heating mixtures of amino acids do not reproduce the same kind of aroma as meat extracts containing similar precursors.

Lien and Nawar (1974a,b) also studied the thermal decomposition products of various amino acids at high temperatures (220°C). They concluded that a considerable number of the lower boiling volatiles found in cooked beef are derived from Strecker degradation products, whereby the α-dicarbonyls produced from sugars react with the deaminated and decarboxylated amino acids. For example, they pointed out that formaldehyde is derived from glycine, acetaldehyde from alanine, and propanal from α-aminobutyric acid. They also observed that olifins are produced by decarboxylation and deamination of aliphatic amino acids. They stated that primary amines are produced by amino acid decarboxylation, and aldehydes are derived from both deamination and decarboxylation.

Ballance (1961) investigated the Strecker degradation products of methionine. He suggested that methionine in meat can break down beyond methanal to produce the strongly odorous methyl mercaptans, and, thus contribute to the volatile sulfur compounds formed on cooking. Dimethyl sulfide is also produced in small amounts on heating aqueous solutions of methionine (Casey _et al._, 1965). It appears that during heating, destruction of methionine occurs and results in increased production of sulfur containing products (Kagan, 1961).

Macy _et al_. (1964a) showed that taurine (β-aminoethanesulfonic acid) is one of the principle amino compounds found in the lyophilized diffusate of meat. They found that the taurine concentration in beef decreased from 9.05 mg/100 g tissue in the unheated diffusate to 4.02 mg upon heating. Brewster (1953) postulated that taurine is formed by oxidation of the -SH group of cysteine followed by decarboxylation. However, Macy et al. (1964) did not find cysteine in the aqueous meat extract, indicating that oxidative reactions are occurring either before extraction of the water solubles or upon lyophilizing the extract. Since reduction does not occur during heating of precursor solutions, evidence for taurine as a source for hydrogen sulfide evolution is not convincing (Herz and Chang, 1970).

Macy et al. (1970c) have shown that free creatinine and creatine phosphate are found in large quantities in muscle, constituting about 0.5% of fresh muscle. They found that cooking increased free creatinine, but decreased creatine. However, total creatinine-creatine concentration increased with heating. They attributed the increase to the probable break down of creatine phosphate to creatine during heating. They further stated that the rate of dehydration of creatine to creatinine may be greater than the rate of formation of creatine from creatine phosphate. Although creatine and creatinine do not appreciably affect aroma, they may contribute to taste and mouthfeel (Wood and Bender, 1957; Zaika et al., 1968).

Carnosine, anserine, and glutathione are the only free peptides that have been isolated and identified in red meats (Macy et al., 1964a). Macy et al. (1970c) found that more than 60% of the diffusate consisted of histidine dipeptides, anserine, carnosine, and a small quantity of free histidine and methyl histidine. They also stated that heating greatly decreased these amino compounds. Although the contribution of these compounds to meat flavor is not known, carnosine and anserine have been commonly added to soup preparations to improve mouthfeel (Herz and Chang, 1970).

Bouthilet (1951b) could not positively identify glutathione as being a contributor to meat flavor, but he suggested that it is a major source of chicken flavor. He found heating glutathione in water produced an aroma reminiscent of meat. Furthermore, if the heated mixture was neutralized with sodium hydroxide, a taste resembling meat was developed.

Kirimura _et al_. (1969) characterized the taste of individual amino acids as being sweet, salty, sour, bitter or MSG-like, and described the various peptides as being sour, bitter or tasteless. Singly, alanine imparted a sweet taste to broth, and glutamic acid possessed a unique salty taste. Taurine has a serumy, somewhat astringent taste, and arginine tasted both bitter and sour. They concluded that glutamic acid enhances meaty flavors when combined with meat.

Kazeniac (1961) also compared the effect of several combinations of amino acids on mouth satisfaction. He found that glutamic acid gave the best mouthfeel when combined with lysine, carnosine and arginine. He further stated that a mixture of glutamic acid and histidine developed a sharp astringent taste. Combining glutamic acid with phenylalanine resulted in a sweet taste but on adding arginine to glutamic acid a meaty taste was developed.

Kirimura _et al_. (1969) summarized their findings on the contribution of amino acids and peptides to the taste of foods as follows: 1) Some amino acids contribute to the inherent tastes of foods themselves; 2) Some specific patterns of amino acid mixtures intensify the taste of foods and increase mouthfeel without losing the inherent taste; 3) The buffering action of amino acids can also contribute to the taste of foods; and 4) The peptides may contribute to both the complexity and favorable balance of taste.

Nucleotides and their degradation products. Inosinic acid is commonly accepted as an essential component for meat flavor development (Batzer _et al_., 1960; Shibamoto, 1964; Caul and Raymond, 1964). Shibamoto (1964) suggested that inosine-5'-monophosphate (IMP) is probably the most important single contributor to meat flavor, but this hypothesis has not found general support. Most studies (Caul and Raymond, 1964; Woscow, 1966; Rhodes, 1965) have shown that IMP and other nucleotides are flavor enhancers rather than flavorants. Caul and Raymond (1964) showed that inosinic acid blended flavor notes, thus enhancing the total flavor impression in soups. Woscow (1966) found that a mixture (1:1) of disodium inosinate and disodium guanylate consistently enhanced the saltiness of sodium chloride solutions and the sweetness of a sucrose solutions. He also suggested that 5'-nucleotides may mask or suppress sulfury, fatty, starchy, burnt or hydrolyzed vegetable protein flavors. However, Kuninaka (1967) showed that the basic tastes of sweet, salty, sour and bitter were not changed in any consistent manner.

IMP is a breakdown product of ATP during rigor mortis. Dannert and Pearson (1967) reported that IMP is the major 5'-nucleotide in beef, pork, and lamb. Caul (1957) showed that aging of meat decreased the IMP concentration. Moreover, Wismer-Pedersen (1966) found that roasting decreased 5'-nucleotides from 210 mg/100 g beef muscle to 160 mg. Rhodes (1965), on the other hand, found no correlation between nucleotide degradation and flavor changes in cooked ground meat to which IMP was added. He concluded that nucleotides probably do not play a major role in the formation of cooked meat flavor.

Like IMP, 5'-guanosine monophosphate (GMP) also has been shown to enhance meat flavor (Wagner _et_ _al_., 1963). Mabrouk (1976) stated that GMP was about four times stronger than IMP in aqueous solution, and it generally produced a broader and more harmonizing effect on overall flavor. However, Nakajima _et_ _al_. (1961) have shown that the concentration of GMP in meat is so small that it does not normally have any measurable effect on taste.

In studying the browning reaction of 5'-nucleotides with D-glucose, Fujimaki _et_ _al_. (1970) concluded that even though IMP is more stable, glucose accelerated the degradation of IMP more than GMP. Thus, a glucose-IMP solution resulted in more browning than a glucose-GMP solution.

Wasserman and Gray (1965) reported that hypoxanthine not inosinic acid is the predominant purine compound found in aged meat. Macy _et al_. (1970a,b) investigated the effects of cooking on nucleotides, nucleosides and bases in beef, pork and lamb. They reported that inosinic acid was the predominant nucleotide in all three species, but was degraded by heating. They further stated that even though the individual nucleotides decreased on heating, total purines, nucleosides and bases increased. They found that adenylic acid increased by a factor of two during cooking of meat from all three species, possibly due to the hydrolysis of adenosine di- and triphosphate and nucleic acids. Thus, the ratio of adenylic acid to inosinic acid increased with increasing cooking times. They concluded that cytidylic, uridylic and guanylic acid were present in relatively low concentrations in meat from all three species and changed little during cooking.

Carbohydrates. During aging, most of the glycogen in muscle is converted to lactic acid. Hornstein _et al_. (1963a) reported that lactic acid makes up 90% of the less volatile fraction of the lyophilized water extract. However, they concluded that lactic acid does not contribute to meat aroma. Kazeniac (1961) found lactic acid to be a major constituent of cooked chicken broth, and suggested that it may play a role in meat taste, but not in aroma.

According to Macy <u>et al</u>. (1964a,b), glucose, fructose and ribose are the principle monosaccharides present in muscle. The concentration of these sugars in beef, pork and lamb were quite similar, with glucose being the predominant sugar (44 mg/100 g), followed by fructose (3.56/100 g) and ribose (1.09 mg/100 g). They stated that fructose is most heat stable and is followed by glucose, about half of which is destroyed during heating. On the other hand, they showed that ribose is the most heat labile, with essentially all of it being destroyed during heating. Although the naturally occurring pentoses in beef are less than 0.1% on a wet weight basis, free ribose actually increases during storage (Fredholm, 1967). It becomes of greater importance because of its high reactivity in the carbonyl-amino reaction (Tarr, 1954). Tarr (1965) has shown that the postmortem pentoses probably originate from nucleotide and nucleoside breakdown rather than from carbohydrate metabolism. Jones (1969) has pointed out that ribose is particularly reactive with taurine, anserine and L-methyl histidine.

Wasserman and Spinelli (1970) found that upon boiling the diffusate from dialyzed meat extracts for 30 minutes, the concentration of ribose decreased by about 20%, whereas, only small changes occurred in the majority of amino acids. When the same diffusate was dried at 125°C for 15 min, meaty aroma and brown coloration developed rapidly, with nearly complete elimination of ribose, glucose and fructose, and a 40-60% reduction in most other amino acids.

Glycoproteins. Batzer et al. (1960) and Landmann and Batzer (1966) isolated a fraction from meat in which glycoprotein was the major constituent. A meaty flavor developed on heating the glycoprotein with inosinic acid, glucose and fat. On recovering the amino acids after hydrolysis of the glycoprotein and heating them with inosinic acid and glucose, they discovered that the aroma was quite different. They were unable to positively identify the glycoprotein, however, they determined its amino acids composition. It contained proline, isoleucine, leucine, alanine, valine, β-alanine, glycine, serine, glutamine and asparagine. They concluded that hydrolysis of the glycoprotein during cooking probably resulted in the formation of amino acids and sugars, which are important meat flavor precursors.

Hornstein (1967) used model systems to study the interactions of reducing sugars and water soluble proteins to produce meat-like aromas. On heating mixtures of gelatin and glucose, egg albumin and glucose, or the water soluble beef proteins and glucose at 100°C under vacuum, no meaty aromas were produced. They, thus, concluded that only low molecular weight precursor compounds are involved in the development of meaty flavor.

Minerals and vitamins. Herz and Chang (1970) postulated that a large number of ions in a solution containing flavor precursors may enhance the volatility of the more volatile compounds. Jarboe and Mabrouk (1974) also suggested that the presence of salts may retard oxidation of sulfhydryl groups, thereby enhancing the formation of the sulfur compounds, which play a role in the development of cooked meat aroma. Pepper and Pearson (1974) found that there was less breakdown of the proteins in salt-treated beef adipose tissue into their constituent amino acids than for non-treated beef adipose tissue proteins. These results suggested that salt may stabilize and inhibit adipose tissue protein breakdown.

On heating, thiamin produces a number of sulfur compounds, which have been widely used in development of synthetic meat-like flavor formulations (Dwivedi, 1975). Kiernat _et_ _al_. (1964) have shown that losses of thiamin during cooking falls in the range of 10 to 15%. Arnold (1968) found that heat degradation of thiamin produced compounds such as sulfur, hydrogen sulfide, 3-acetyl-3-mercaptopropanol, 3-mercaptopropanol and 4-methyl-5-vinyl thiazole, all of which are similar to some constituents found in boiled beef flavor concentrates.

Adipose Tissue Fraction

Howe and Barbella (1937) postulated that the species characteristic flavor of beef resides chiefly in the fatty tissues. Hofstrand and Jacobson (1960) did not find lamb fat to contribute to the taste of broths, but they concluded that it may contribute to the aroma of the broths. Pippen et al. (1954) and Peterson (1967) also reported that chicken fat had a negligible effect on flavor, but contributed to the aroma of broths.

Pearson et al. (1973) demonstrated that it is difficult to differentiate between the flavor of cooked beef and lamb, if only lean is used. However, on heating fatty tissue with the lean, they found that the characteristic species odors are evolved and identification became simpler. Wasserman and Talley (1968) also showed that addition of fat greatly improved the number of correct identifications for roast beef, veal, pork and lamb by taste panel members.

Hornstein and Crowe (1963b) heated lamb tissue in water at 100°C and noted a very strong mutton odor. They theorized that carbonyl compounds in the fat were primarily responsible for the mutton aroma. Wong et al. (1975) studied the composition of mutton fat and found that the branched chain unsaturated fatty acids having 8-10 carbon atoms contributed to the undesirable flavor of cooked mutton. Pippen et al. (1969) suggested that the aroma of chicken fat is due to the carbonyl compounds, which are derived from the lean and migrate into the fat during cooking.

Dwivedi (1975) summarized the possible contributions of fat to meat flavor as follows: 1) Fat contains precursors that liberate the species specific aroma on heating; 2) Fat may act as a reservoir for lipid soluble materials; 3) Fat may interact with compounds produced in the lean to give the desired aroma; and 4) Fat may also act as an apolar environment for flavor reactions which do not normally take place in the aqueous environment.

Water soluble fraction of adipose tissue. Pepper and Pearson (1971) found that the water soluble fraction from adipose tissue may make a distinct and characteristic contribution to meat flavor in addition to any effect from the lipids *per se*. Wasserman and Spinelli (1972) found that if fatty tissues are extracted and washed free of water soluble materials, the components involved in formation of typical meat odors also disappeared. They found that the water soluble fraction contained amino acids and glucose, which produced a nonspecific roast aroma when heated to dryness. They also reported that lamb had a higher concentration of sulfur-containing amino acids then beef or pork, whereas, beef had a lower concentration of taurine, asparagine, threonine, serine, proline, leucine and tyrosine than pork or lamb. Thus, they concluded that the amino acid composition of adipose tissue may contribute to the species specific aroma of meat.

Although amino acids, proteins and nucleic acids were found in beef adipose tissue (Pepper and Pearson, 1974), no nucleoproteins, glycoproteins or lipoproteins were detected (Pepper and Pearson, 1971). This may be due to inadequate detection techniques, since nucleic acids are essential metabolites and breakdown products, which are usually found in living cells (Dwivedi, 1975). Pepper and Pearson (1974), however, did confirm the presence of creatine, creatinine, creatine phosphate, cytosine, uracil and several flourescent and phosphate containing compounds in beef adipose tissue.

Fat soluble fraction of adipose tissue. Wasserman (1972) suggested that thermal autoxidation of lipids can occur at 60°C in the presence of free radicals, but pointed out that most of the degradation occurs at 200-300°C. He stated that the thermal oxidation products at the higher temperatures included lactones, alcohols, ketones, and fatty acids.

Siedler *et al*. (1964) studied the total fatty acid content of raw and cooked variety meats and concluded that the fatty acid distribution was not significantly changed by cooking. Similarly, Chang and Watts (1950) found only a slight loss of polyunsaturated fatty acids in glycerides from cooked meat. Hornstein and Crow (1960), on the other hand, found that the free fatty acid content increased from 1.5% to 3.7% in beef, from 2.9% to 5.5% in pork and from 0.3% to 0.4% for lamb. These results suggested that free fatty acids probably play a more important role in the development of the species flavors in beef and pork than in lamb.

Campbell and Turkki (1967) and Terrell *et al*. (1968) found that the phospholipid concentration was higher in cooked than in raw meat, whether expressed as a percentage of fat or as percentage of meat. They postulated that the increased phospholipid content upon cooking is probably related to the fact that phospholipids are an integral part of the muscle cell. Furthermore, Wilson *et al*. (1976) have shown that phospholipids may contribute to the development of "warmed over flavor".

Composition of Meat Flavor Volatiles

Although over three hundred volatile compounds have been identified as components of meat flavor (Dwivedi, 1975), much still remains to be learned about the mechanism of their formation and their contribution to flavor. On examining the compounds singly, many compounds had only a weak odor of a non-meaty nature, whereas, other compounds possess little or no meaty flavor. However, when these compounds are combined, they may react synergistically to produce meaty notes. This section will briefly examine the classes of volatile compounds that are primary and secondary contributors to meat flavor. The origin and mechanism of formation of some of the primary contributing compounds will also be examined through the model system studies.

Study of Meat Flavor Volatiles Using Model Systems

Although flavor chemists have learned much about meat flavor profiles from studying meat volatiles, nevertheless it is difficult to work with compounds of high volatility at low concentrations (Wasserman, 1978). Thus, many studies have been carried out with precursor compounds found in meat, and under conditions that presumably occur during cooking (Mulder, 1973; Shaw and Berry, 1977; Shibamoto and Russell, 1977). It is questionable whether all compounds formed in the model systems are found in meat aroma, but flavor chemists have learned much about the potential reactions from model system studies (Wasserman, 1978).

Wasserman and Spinelli (1970) showed that heating mixtures of amino acids in the absence of sugars at 125°C produced neither a meat flavor nor browning. However, they observed that addition of glucose to the initial solution resulted in large losses of amino acids and the development of meaty odors. They concluded that the volatiles evolved are products of Maillard-type browning reactions.

Hodge (1953; 1967) has extensively discussed the mechanisms of the Maillard or nonenzymatic browning reaction, which involves the interaction of an amino compound and a sugar. He found that the products of the Maillard reaction included furfural, furfural derivatives, ketone, and 1,2-dicarbonyl compounds. He also pointed out that Strecker degradation may be involved, in which aliphatic aldehydes are formed from amino acids that have been oxidized by diketones. For example, acetaldehyde is the Strecker degradation product of alanine and methional is the product of methionine.

Barnes and Kaufman (1947) examined the Maillard-type browning reaction using model systems. They detected aroma formation on heating an amino acid-glucose mixture. Pearson _et al_. (1962; 1966) studied browning development during heating of pork slurries to dryness. They found that the degree of browness was related to the level of reducing sugars present. They concluded that the majority of browning was due to the amino-sugar reaction, but some was the result of pyrolysis of the indigenous carbohydrates. Saisithi and Dollar (1966) substantiated these findings and reported that maximum browning occurred when various amino acids were heated with either glucose or ribose.

Although xylose is not an important sugar in meat, Rothe and Voigt (1963) found that on heating xylose with some amino acids there was considerable production of browning flavor compounds. They reported that alanine produced both intensive browning and considerable flavor, whereas isoleucine, leucine, valine, methionine and phenylalanine produced considerable flavor development but relatively little browning. They also found some amino acids did not significantly contribute to either browning or flavor development when combined with xylose. Rothe and Voigt (1963), thus, concluded that there is no direct relationship between the amount of brown pigment and the amount of flavor.

Casey et al. (1965) studied the interaction of amino acids with fructose. They found that methionine was degraded rapidly in the presence of fructose, whereas, little reaction occurred with glucose. In a study on the flavor of canned meat, Zoltowska (1967) showed that methionine had a greater sensory effect in Maillard-type reactions than any other amino acids.

Arroyo and Lillard (1970) identified some reaction products of the sulfur containing amino acids on heating with glucose and ribose. They identified methanethiol, ethanethiol, 1- and 2-propanethiol, pentanethiol, and hydrogen sulfide. However, the aroma obtained from these compounds was not meaty. They also observed that browning of amino acids increased with increasing pH.

More recently, Mulders (1973) heated cystine or cysteine with ribose at pH 5.6. He found that the volatile compounds formed included hydrogen sulfide, thiols, thiophenes, thiazoles, pyrroles, pyridines and furans.

Mussinan and Katz (1973) identified 24 reaction compounds from a model system composed of hydrolyzed vegetable protein (HVP), L-cysteine-HCl, D-xylose and water. In the absence of HVP, only 10 compounds were obtained, whereas, with HVP, 16 thiophenes and furfuryl mercaptans were identified. Kato et al. (1973) also detected a large variety of thiazoles and thiophenes on heating cysteine or cystine with glucose or pyruvaldehyde.

Frazen and Kinsella (1974) studied the binding of aldehydes and methyl ketones to various proteins. They found that the amount of flavor depended on the type, quantity and composition of the protein, and the type of solvent. They observed that addition of water decreased the volatility of the compounds and attributed it to the increased adsorption or solubilization of flavors by the protein-water mixture.

Primary Compounds Contributing to Meat Flavor

Chang and Patterson (1977) pointed out that lactones, acyclic sulfur containing compounds, nonaromatic heterocyclic compounds containing S,N and O, and aromatic heterocyclic compounds containing S,N and O, are important contributors to meat flavor.

Lactones. Various types of lactones have been found in both roast beef drippings and boiled beef (Hirai *et al*., 1973; Lieblich *et al*., 1972). Lactones may originate from protein fragments, or by the degradation of sugar molecules (Herz and Chang, 1970).

Watanabe and Sato (1968) isolated at least 19 lactone compounds from beef depot fat. They stated that the lactones are odorous compounds and may contribute either desirable or undesirable notes to meat aroma. They postulated that this class of compound originated from either the γ - or Δ-hydroxy fatty acids. In a later study, Watanabe and Sato (1969) also isolated lactones from heated pork fat.

α-hyxroxy-β-methyl-γ-hexenolactone has been confirmed as the bouillon-like flavoring compound developed by aging of α-ketobutyric acid (Sulser _et al_., 1967). Although α-ketobutyric acid is generally believed to be the principle flavoring compound in vegetable protein hydrolysates, recent research has shown that the flavor comes from dimerization, lactonization and decarboxylation of α-ketobutyric acid itself, and not from the 4-carbon acid (Herz and Chang, 1970).

Acyclic sulfur containing compounds. Hydrogen sulfide was one of the first basic sulfur compounds identified in flavor studies of meat isolates (Stahl, 1957; Yueh & Strong, 1960; Minor _et al_., 1976). Stahl (1957) isolated and identified some sulfur compounds, such as hydrogen sulfide, methane and ethanethiols in meat volatiles. Yueh and Strong (1960) further confirmed the presence of hydrogen sulfide. They concluded that hydrogen sulfide, liberated during cooking from odorless precursors, is one of the major components responsible for the characteristic aroma of cooked beef.

Minor _et al_. (1965) found that elimination of sulfur components from chicken broth resulted in a complete loss of meaty odor, while removal of the carbonyl compounds eliminated the chickeny odor, but intensified the meaty aroma. They, thus, concluded that sulfur compounds are important contributors to meaty odors. Similarily, Barylko-Pikielna _et al_. (1974) studied the effects of removing different classes of compounds on the aroma of beef extracts. They heated a model system composed of methionine and glucose at 120°C for 60 minutes at pH 6.5 to 7.0, and eliminated either the carbonyls, sulfides, or mercaptans in the trapped volatiles. They found that elimination of carbonyls emphasized the sulfury notes, while removal of sulfides eliminated the cabbage and onion-like aromas. When mercaptans were removed, the odors resembled bread crust, and boiled cabbage. When both sulfides and mercaptans were removed, the brothy odor disappeared. Thus, the authors concluded that the compounds responsible for the brothy aroma were bifunctional, containing both disulfide and SH groups.

Pepper and Pearson (1969) have shown that the water-soluble fraction of beef adipose tissue produced an appreciable quantity of hydrogen sulfide. They found that the water soluble fraction contributed about 71% of the hydrogen sulfide (17.6 μMoles/100 g), while the salt soluble and insoluble fractions contributed approximately 7% (1.7 μMoles) and 22% (5.5 μMoles), respectively. They, thus, concluded that the hydrogen sulfide evolved upon heating beef adipose tissue may contribute to the meaty aroma of cooked beef.

Boelens <u>et al</u>. (1974) have also examined some of the organic sulfur compounds formed by reactions with fatty aldehydes, hydrogen sulfide, thiols and ammonia, and their possible contribution to meat flavors. They demonstrated that saturated aldehydes react with hydrogen sulfide and thiols to produce a number of organic sulfur compounds, which are often found in the flavor volatiles of meat. Pippen and Mecchi (1969) demonstrated that heated chicken adipose tissue containing the natural polar compounds produced more aroma and higher concentrations of sulfur compounds than similar tissue from which the polar compounds were removed. They also showed that hydrogen sulfide reacted with acetaldehyde to form an odorous meaty compound, and suggested other components may be formed in a similar fashion.

Pippen and Mecchi (1969) found that freshly prepared chicken broth contained 35 ppb of hydrogen sulfide, but after simmering the level increased to 180-750 ppb. They found that roast and fried chicken contained hydrogen sulfide in excess of the 10 ppb, which is its odor threshold in water. Similarily, Parr and Levett (1969) also demonstrated that the amount of hydrogen sulfide present in freshly cooked chicken meat was 20 to 100 times higher than the odor threshold of hydrogen sulfide in aqueous solution. Therefore, the amount was sufficient to influence both aroma and flavor. They also stated that hydrogen sulfide liberated during heating may immediately react with other compounds, thereby making an indirect contribution to flavor.

Perrson and von Sydow (1973a) quantitatively measured the changes in the concentration of hydrogen sulfide, methanethiol and ethanthiol in cooked canned beef. As cooking time was increased from 15 to 75 minutes, they found that hydrogen sulfide increased more than 3-fold, whereas, methanethiol increased 4-fold, and ethanethiol went from 170 to 280 µg/liter. They also found that increasing the temperature resulted in less thiols, which indicates that thermal decomposition of the thiols was occurring.

Recently Shibamoto and Russell (1976) heated glucose with ammonia while simultaneously bubbling hydrogen sulfide through the solution. A beef-like odor was evolved. Their analysis of head gas vapor revealed that a number of thiophenes were present, indicating that thiophenes must play an important role in the development of meat flavor.

Nonaromatic heterocyclic compounds containing S,N and O. Furan compounds were isolated and identified in cooked beef by Herz and Chang (1970). They found that only those furan compounds containing sulfur groups in the side chain contribute to beef flavor. They isolated 2-pentylfuran from the boiled beef extract and stated that it may contribute significantly to the flavor of boiled beef. Nonaka et al. (1967) also found 2-pentylfuran in chicken broth and suggested that it may be an oxidation product of 2,4-decadienal.

Tonsbeek et al. (1968) found that the 4-hydroxy-2,5-dimethyl-3(2H)-furanone had a caramel odor, and the monomethyl analogue had a roast chicory-like odor. The furanone compounds were prepared by heating D-xylose (Severin and

Seilmeir, 1968), D-ribose (Peer *et al*., 1968a), D-ribose-5-phosphate (Peer *et al*., 1968b) and D-glucuronic acid (Hicks *et al*., 1974) with amine salts. The furanones are probably produced from condensation of an aldose and an amino to produce an Amadori product, which subsequently dehydrates with amine elimination to give the furanones (Tonsbeek *et al*., 1968; Peer *et al*., 1968a,b; Tonsbeek, 1969). Tonsbeek *et al*. (1968) have also shown that beef-like flavors are evolved on mixing hydroxyfuranones and hydrogen sulfide.

Aromatic heterocyclic compounds containing S,N and O. Maga and Sizer (1973) concluded that pyrazines are the most important group of flavor compounds, yet, few if any pyrazines have been reported to have a meat-like aroma. Pyrazines have been derived from Maillard reactions (Maga and Sizer, 1973), or by thermal treatment of aminohydroxy compounds (Wang and Odell, 1973). They have also been found in boiled (Lieblich *et al*., 1972) roasted (Flament and Ohloff, 1971), fried (Watanabe and Sato, 1971), and pressure cooked (Mussinan *et al*., 1973) beef.

Formation of pyrazine compounds have been shown to involve the combination of ammonia or amino compounds with sugars and/or other carbonyl substances (Newell *et al*., 1967; van Praag *et al*., 1968; Koehler *et al*., 1969). Newell *et al* (1967) and van Praag *et al*. (1968) reported that the same aklylpyrazines were obtained from different sugar-amino acid systems. van Praag (1968) theorized that ammonia was an intermediate, and that the composition of the pyrazine mixture did not depend upon any specific amino acid. Using radioisotope labeling techniques, however, Koehler _et_ _al_. (1969) concluded that the carbon came from the sugars and the nitrogen from the amino acids. They further reported that on reacting ammonium salts with sugars different pyrazines were produced than when amino acids were reacted with the same sugars.

Shibamoto and Bernhard (1976) conducted a series of studies on the formation of pyrazines and sulfur compounds in systems containing ammonia and hydrogen sulfide. They found that the distribution of pyrazine depended upon several factors, including: 1) the ratios of reactants; 2) the pH of the mixture; 3) the duration of heating; and 4) the temperature during heating. When they heated glucose with ammonia and hydrogen sulfide, they identified 23 compounds, which had previously been found in cooked meat - of these, 10 were pyrazines, and 5 were nitrogen-containing thiazoles. When they increased the concentration of ammonia, the total yield of pyrazines increased and the ratio of individual pyrazines changed. Increasing the pH caused greater fragmentation of the sugar, thus, producing more short chain carbon fragments and carbonyls, leading to increased formation of pyrazines.

Shibamoto and Bernhard (1976) stated that the normal pH of meat is approximately 5.5, which would favor the initial step in the carbonyl-amino reaction. They demonstrated that the liberation of ammonia during the reaction increased the pH of the meat, favoring the production of pyrazines. They also found that significant amounts of pyrazines appeared at 70°C and increased to an optimum at 120°C. Thus, pyrazines are likely be formed during surface browning of meat, and few would be expected in the interior, where the temperature is 70°C or less (Wasserman, 1978).

Specific Compounds from GC Fractions with Meaty Aroma/Flavor

Herz and Chang (1970) reported that certain compounds possess a distinct meaty aroma as they are eluted from the GC column. All of these compounds have five membered heterogeneous ring structures with the hetero atom being O, S or N.

3,5-dimethyl-1,2,4-trithiolane. Herz and Chang (1970) stated that 3,5-dimethyl-1,2,4-trithiolane is the most abundant five member sulfur-containing ring compound in the volatiles from cooked meat. Chang *et al*. (1968) initially concluded it was an important contributor to boiled beef aroma. However, careful examination of the pure compound later showed that it does not possess a beef-like odor (Chang and Peterson, 1977).

2,4,5-trimethyl-3-oxazoline. Chang *et al* (1968) also reported the volatiles from boiled beef contained oxazoline. They showed that oxazoline has a five member ring structure with two heteroatoms (N and O). Although they initially concluded that 3-oxazoline contributed to beef flavor, later they found the pure compound did not possess a beef-like aroma (Chang and Peterson, 1977).

Thiazolines and thiazoles. Asinger *et al*. (1964) prepared thiazolines by reacting ammonia with the appropriate aldehyde and mercapto ketone. Tonsbeek *et al* (1971) isolated 2-acetyl-2-thiazoline from beef broth and reported that it possessed the aroma of "freshly baked bread crust".

Asinger (1960) observed that 3-thiazoline can be thermally dehydrogenated in the presence of elemental sulfur to form thiazole. Pittet and Hruza (1974) described the di- and trialkylthiozoles as having nutty, roasted or meaty notes, whereas, Tonsbeek *et al* (1971) reported thiazole possessed a "cereal or popcorn-like aroma".

Mussinan *et al*. (1976) concluded that changing one of the hetero atoms of a compound has a much greater effect on its flavor than the degree of unsaturation it possesses. For example, the sulfur containing heterocyclic compounds (thiazolines) are "meaty and roast-like", whereas, most of the oxygenated compounds (oxazolines) are "sweet and green vegetable-like".

5-thiomethylfurfural and thiophencarboxy-2-aldehyde. The S-analogue of 5-hydroxymethylfurfural, a well known terminal Maillard-reaction product, is 5-thiomethylfurfural (Chang et al., 1968. Herz and Chang (1970) postulated that this compound is derived from methionine and possesses a meaty aroma when eluted from the GC column. They also pointed out that thiophencarboxy-2-aldehyde is another compound analogous to furfural and may originate from amino acids through browning.

1-methylthio-ethanethiol. Brinkman *et al*. (1972) isolated 1-methylthio-ethanethiol from the headspace of beef broth. Schutte and Koenders (1972) demonstrated that it is formed when acetaldehyde, methanethiol, and hydrogen sulfide are heated in aqueous solution at pH 6.0. They further demonstrated that these three immediate precursors are generated from alanine, methionine and cysteine in the presence of a Strecker degradation agent, such as pyruvaldehyde. Brinkman *et al*. (1972) found that very dilute aqueous solutions of 1-methythio-ethanethiol (1 to 5 µg/liter), has a meaty odor.

Thiladine. Thialdine is formed from ammonia, hydrogen sulfide and acetaldehyde (Brinkman *et al*., 1972). Since these compounds are found in the headspace of beef broth, Brinkman *et al*. (1972) concluded that thialdine is a contributor to beef broth flavor. They also found that thialdine may be a precursor of trithiolane by eliminating ammonia and acetalaldehyde in the presence of water and elemental sulfur. This observation was further substantiated by noting the decreasing levels of thialdine in the distillation traps during storage, and simultaneous increases in the amount of trithiolane.

Secondary Compounds Contributing to Meat Flavor

Chang and Peterson (1977) stated that the secondary compounds contributing to meat flavor include the aliphatic and aromatic hydrocarbons, alcohols, acids, esters, ethers and carbonyl compounds.

Aliphatic and aromatic hydrocarbons. Some aliphatic hydrocarbons have been found in raw meat, with high levels being present in the fatty tissue of irradiated beef (Merritt *et al.*, 1967; Wick *et al.*, 1967). Merritt (1966) stated that the saturated aliphatic compounds have no odors, while the odors of unsaturated compounds are neither pleasant nor meaty.

Merritt (1966) identified several aromatic hydrocarbons in flavor concentrates, but stated that none of them possessed a meaty note. They predicted that these aromatic compounds probably originated from amino acids such as phenylalanine, tyrosine and tryptophane.

Alcohols. Herz and Chang (1970) stated that considerable quantities of ethanol and propanol are found in cooked beef. They reported that other alcohols are present in small quantities, but their origin is unknown. They noted that somewhat large amounts of 1-octene-3-ol have been found in boiled beef flavor concentrate.

Acids, esters and ethers. Straight chain acids are found in muscle, due chiefly to anabolic or catabolic metabolism of lipids (Lehninger, 1975). Herz and Chang (1970) pointed out that high molecular weight fatty acids are not particularly volatile and are not found in meat flavor concentrates. They also stated that methyl formate and ethylacetate do not contribute to meat aroma. They were uncertain as to the contribution of esters and ethers to meat flavor.

Carbonyl compounds. Herz and Chang (1970) reported that carbonyl compounds are the most numerous members of any class identified in meat flavor concentrates. They arise principally from the lipids, and thus, are found in the volatiles from meat. Sink and Smith (1972) reported that the total carbonyl content in beef increased 2-fold when aged for 3 days. After 14 days, the total carbonyls had increased 2-fold, whereas, the monocarbonyls accounted for approximately 40% of the total carbonyls. They also found that the methyl ketones comprised the largest group of monocarbonyls, showing a 4-fold increase.

Since carbonyl compounds are oxidative degradation products of lipids, some researchers (Hornstein and Crowe, 1963a; Jacobson and Koehler, 1963; Watanabe and Sato, 1971; Wasserman, 1978) have concluded that they play an important role in meat flavor. It has been suggested that the species characteristic flavor resides in the qualitative and quantitative differences of the carbonyl compounds (Leibich et al., 1972; Wasserman, 1978). On the other hand, Chang and Peterson (1977) have shown that no odor or flavor reminiscent of meat is found in refined animal fats. Hirai et al. (1973) identified a limited number of carbonyl compounds in boiled beef flavor and found that none of them had meaty notes. Lieblich et al. (1972) on the other hand, showed that adipose tissue was the primary site for aldehydes and also proved that fatty tissues developed a stronger aroma during cooking than lean tissues. They identified 27 alkanals, 12 ketones, 12 alcohols and 10 aromatic compounds from roast beef and its drippings. Of the 9 lactones identified, they found that most were present in the fat drippings and were absent from the concentrate of lean meat.

Carbonyl compounds containing up to 4 carbon atoms may originate from sugars (Casey *et al.*, 1965) or from amino compounds by way of Strecker degradation and Amadori rearrangement (Hodge, 1953; Burton and McWeeny, 1964). The condensation of carbohydrates with amino acids during cooking leads to the formation of complex cyclic carbonyls, such as 4-hydroxy-5-methyl-3(2H)-furanones, which has been isolated from beef broth and is considered to play an important role in beef flavor (Tonsbeek *et al.*, 1968; 1969). van der Ouweland and Peer (1975) found that 5-methyl- and 2,4 dimethyl-4-hydroxy-3(2H)-furanones can act as intermediates to form other compounds with a roast meat-like flavor. They demonstrated that furanones may react with cysteine, which serves as a hydrogen donor to form mercapto compounds, and with several unidentified compounds posessing roast meat aromas.

Rao (1976) found that polyphosphates enhanced the meaty aroma of cooked chicken by lowering the carbonyl concentration in the polyphosphate treated meat. However, they discovered that the concentration of sulfur containing components increased.

Herz and Chang (1970) postulated that simple carbonyls, such as diacetyl or acetaldehyde, may react with hydrogen sulfide and/or ammonia to form meat flavor components, such as 3,5-dimethyl-1,2,4-trithiolane, 2,4,5-trimethyl-thiazoline, thialidine, 1-methylthio-ethanethiol, dithiazines, and tetrasulfides.

Patents on Synthetic Meat Flavors

With the advent of new isolation techniques and more sensitive instrumentation, flavor chemists have obtained a great amount of knowledge on the origin and chemistry of meat flavor (Wasserman, 1978). Such information has resulted in development of a variety of synthetic meat flavor additives.

Synthetic meat flavors were first formed by the blending of species, and the addition MSG or the 5'-nucleotides to increase the brothy taste (Kuninaka, 1966). May and Morton (1956) developed a meat flavor mixture by reacting an aldehyde with cysteine. May and Morton (1961) heated pentoses with cysteine in the presence of hydrolyzed vegetable protein (HVP) and produced a meat-like flavor. Morton _et al_. (1960) also reported that meat flavor can be produced by heating ribose with cysteine in excess water. They further stated that if other amino acids, such as glutamic acid, β-alanine and serine were added to the cysteine/ribose solution, flavors similar to beef, lamb and chicken could be produced.

In 1966, a patent issued to Unilever describes a method for preparing meat flavor compounds by heating a mixture of carbohydrates, and at least one amino acid in the presence of a fatty acid. The yellowish brown powder had the flavor and aroma of roast mutton. A similar Unilever (1968) patent described a mixture of amino acids, nucleotides, succinic acid and lactic acid and supposedly produced a pronounced meaty flavor.

Broderick and Marcus (1972) obtained a patent in which the ribose moeity of the ribonucleotide was reacted with cysteine or cystine in the presence of water to produce a meat-like flavor. Similarly, Thomas (1971) patented a preparation in which hexoses or pentoses, cystine or cysteine, and glycine were heated together. After cooling, MSG, a protein hydrolysate, sucrose, edible fat, ribonucleotide and hickory smoke flavor were added to the mixture to obtain a ham- and bacon-like flavor. Kyowa Fermentation Industry (1967) was also awarded a patent for a meat flavoring mixture prepared by applying the Maillard reaction to ribose phosphate, an amino acid (such as lysine), a hydrolyzed protein, or a casein hydrolyzate.

Many recent patents are based on the addition of chemical compounds that have been found in meat flavor extracts (Wasserman, 1978). Van der Ouweland and Peer (1970) used certain heterocyclic ketones (such as 5-methyl-2,3-dihydrofuran-3-one to react with inorganic or organic sulfur compounds to form flavor mixtures with an aroma similar to that of roasted meat.

Giacino (1968a,b) obtained two meat flavor patents assigned to International Flavors and Fragrances, in which thiamin was the basic component. One preparation (1968a) was developed by heating a mixture of taurine and thiamin and then adding it to a source of free amino acids. The other (1968b) was produced by heating thiamin with S-containing polypeptides and alkanones or hydroxyalkanones, then adding diacetyl and hexanal. International Flavors and Fragrances (1967) also patented a flavoring material with a strong meat flavor by merely react thiamin with an organic acid. Recently, it has been shown that thiamin would react with 2-aminoethane (Giacino, 1970) and sorbitan fatty acid esters (Hasegawa, 1972) to produce meat-like flavors.

Taste Panel Evaluations

Gordon (1972) pointed out that chemical compounds have different thresholds concentrations at which they can be detected or recognized. He further stated that the impact of a compound is not necessarily related to the concentration, since odor thresholds may be in the µg/kg range, so trace quantities of a compound may play an important role in the aroma. Wasserman (1978) stated that the threshold level depends on the solubility of the compound in the solvent.

Powers (1974) pointed out that lack of knowledge on the properties of the various flavor components make it difficult to formulate a synthetic flavor mixture. He further stated that the process of reconstituting flavors involves numerous trials. This is especially true in the formulation of synthetic meat flavors, since very few studies on meat flavor chemistry have been quantitative, thus, little is known about the concentrations of various components in meat aroma (Wasserman, 1978).

Although flavor evaluation involves subjective measurements, which lack consistency and accuracy, a person can be trained to perceive and recognize individual flavor characteristics by using appropriate training aids (Persson *et al*., 1973b; Powers, 1974; Powers and Quinlan, 1974; Parliment and Scarpellino, 1977; Horsfield and Taylor, 1976). However, Wasserman (1978) cautioned flavor chemists to consider each study on meat flavor on its own merits, since he found very little uniformity in the research reported. He also pointed out that it is very difficult to extrapolate from one study to another and to compare results from different studies, because many investigations involved subjective measurements.

Statistical Analysis of Sensory Evaluation

Since over three hundred different volatile compounds have been identified from cooked beef (MacLeod and Coppock, 1976), assessment of the relative importance of each component to meaty flavor is laborous and difficult. Persson _et al_. (1973b) applied a stepwise regression analysis to identify the relative contributions of several compounds to canned beef odor. Persson and von Sydow (1974a,b) using a similar method, predicted the concentration of four precursor components that yielded the minimum off-flavor development in canned beef.

Box and Wilson (1951) developed the surface response method to predict and plot the changes occurring when two or more variables are present. Chang _et al_. (1960) applied the surface response method to determine the best combination of four independent variables in order to get the maximum yield of 2,5 dimethylpiperazine. Pearson _et al_. (1962) first applied the surface response method to foods by predicting the best combination of sugar and salt to obtain maximum panel scores in cured ham. Powers and Quinlan (1974) also tested blueberry whey beverage using a regression computer program to calculate simple and multiple correlations between acceptability, appearance, flavor and texture. Recently Hutton and Campbell (1977) used the surface response method to investigate the protein solubility and water sorption properties of a soy concentrate and a soy isolate at different pH-temperature combinations.

Isolation and Identification of Flavor Compounds

Trapping of volatiles. In order to study the volatile compounds responsible for the flavor of meat, it is necessary to isolate the volatile flavor compounds without creating artifacts (Chang and Patterson, 1977). Herz and Chang (1966; 1970) have designed an apparatus to isolate meat flavor volatiles from meat extract. The method involves heating a meat slurry and pumping it into an evaporator where it undergoes flash evaporation. The volatile components and water evaporated are condensed in a series of traps and are cooled with dry ice or liquid nitrogen. The volatiles collected in the cold traps are then extracted with ethyl ether. The ether solution containing the meat flavor components is then concentrated to a volume suitable for GC analysis. Chang and Peterson (1977) recently have shown that this method of isolating of meat volatiles still develops artifacts. They, thus, proposed an improved method for isolating the volatiles under milder conditions. Although they found that the total number of volatiles was reduced, they reported that the aroma more closely resembled that of freshly cooked meat.

Likens and Nickerson (1964) designed a simple apparatus for simultaneously performing steam distillation and organic solvent extraction of the volatiles from foods. They pointed out many advantages of this method: 1) the desired substances were concentrated over a thousand fold; 2) a relatively small quantity of organic solvent was used; and 3) a relatively small amount of sample was required for extraction. Recently, MacLeod and Cave (1975) modified the Likens and Nickerson apparatus by including a double-surface water condenser and used it in their study of the volatile components of eggs and meat (MacLeod and Coppock, 1976;1977). Schultz _et al_. (1977) further modified the Likens and Nickerson apparatus and used it with model systems. McGill and Hardy (1976) showed that the artifacts produced by oxidation of the lipids can be minimized by using antioxidants and carrying out the extraction in the absence of oxygen.

Gas chromatography-mass spectrometry. Until recently, flavor chemists used gas chromatography as the major means of identifying the volatiles (Kramlich and Pearson, 1960; Tonsbeek _et al_., 1968; 1969; Minor _et al_., 1965; Pippen and Mecchi, 1969; Herz and Chang, 1970). With the development of elaborate systems of combination of GC-MS, many meat volatiles have been identified (Persson _et al_., 1973a; Watanabe and Sato, 1973; MacLeod and Coppock, 1976). The basic principle involved in GC-MS is the GC separation of the components followed by MS identification of each components according to its mass number and relative intensity (Merritt _et al_., 1974).

Materials and Methods

Synthetic Meat Flavor Model System

The model system was composed of simple sugars, various amino acids, 5'-nucleotides, glycoprotein, sodium chloride and other precursor compounds. Preliminary studies have indicated that certain basic ingredients shown in Table 1 are essential components to basic meat flavor. Thus, their predetermined levels remained constant, while various levels of amino acids were tested singly or in combination with other amino acids for their contribution to meat flavor.

After all precursor compounds were carefully weighed into a 125 ml Erlenmeyer flask and solubilized in 25 ml of water, the sample mixture was autoclaved at 17 psi for 90 min. at 121°C to develop the flavor. The autoclaved mixture was then diluted to 50 ml with deionized water before subjecting it to further analysis. The liquid product can be refrigerated at room temperature for 2-3 weeks without losing its meaty aroma or taste.

Freeze Drying the Model System

The final concentrations of the proposed meaty flavor model system is shown in Table 2. The liquid extract was then freeze-dried using a virtis Freeze Dryer Model No. 42WS. The shelf temperature was set at 38°C for drying and the

Table 1. Constituents of basic model meat flavor system

Classes and Individual Compounds	% of Dry Wt.
Simple Sugars	17.1
Glucose	6.4
Ribose	4.3
Xylose	6.4
Amino Acids*	6.7
L-Alanine	2.7
L-Glutamic (monosodium salt)	2.1
L-Cysteine (hydrochloride)	1.9
5'-Nucleotides	2.6
IMP (inosine-5'-monophosphate or GMP (guanosine-5-monophosphate)	2.6
Glycoprotein	53.0
Gelatin	59.0
Salt	11.0
NaCl	11.0
Fat	
Beef, pork or chicken (optional) (approximately 2.2g per 50 ml of mixture)	
Total	90.4

*Effects of other amino compounds were also studied, such as glycine, valine, serine, asparagine, leucine, isoleucine, methionine and taurine (<10%).

Table 2. Constituents and concentrations in the composite meat flavor model system

Classes and Individual Compounds	Weight (mg)/50 ml
Simple Sugars	
glucose	60
ribose	40
xylose	60
Amino Acids	
L-alanine	23
L-glutamic acid (monosodium salt)	20
L-cysteine (Hydrochloride)	20
L-isoleucine	9
L-methionine	11
Taurine	22
L-serine	21
L-leucine	10
Glycine	8
L-arginine	6
5'-Nucleotides	
IMP	25
GMP	optional
Glycoprotein	
Gelatin	500
Salt	
NaCl	100
Fat beef, pork, lamb	2.2 g
Total	935 mg (without fat)

condensor was maintained at -65°C. Samples were dried for 24 hours or until the vacuum reached 6 µ. The freeze dried samples were pulverized and vacuum sealed in a retortable aluminum foil bag and stored at -30°C. The freeze dried powder was easily rehydrated in water.

Preparation of Aqueous Beef Extract

Extra lean ground beef chuck was obtained from Michigan State University Food Stores, and divided into 350 g portions. It was stored in plastic bags and frozen at -30°C until prepared for further analysis. The aqueous beef extract was homogenized by adding 350 g of ground meat to 250 ml of deionized water in a Waring blendor. The beef slurry was then transferred to the extraction apparatus as described below.

Apparatus Used for Trapping of Volatiles

To isolate the volatiles from the meat and model systems a number of different apparatus were tested. Figure 1 shows a modified apparatus similar to one previously developed by Herz and Chang (1966). The beef slurry was heated in the extraction vessel (A) and was kept under constant agitation. The volatiles and water were evaporated into a second vessel (B), where they were further condensed under a vacuum in a series of traps (E-H) of decreasing temperatures, i.e., ice plus NaCl, dry ice, dry ice plus acetone, and liquid nitrogen.

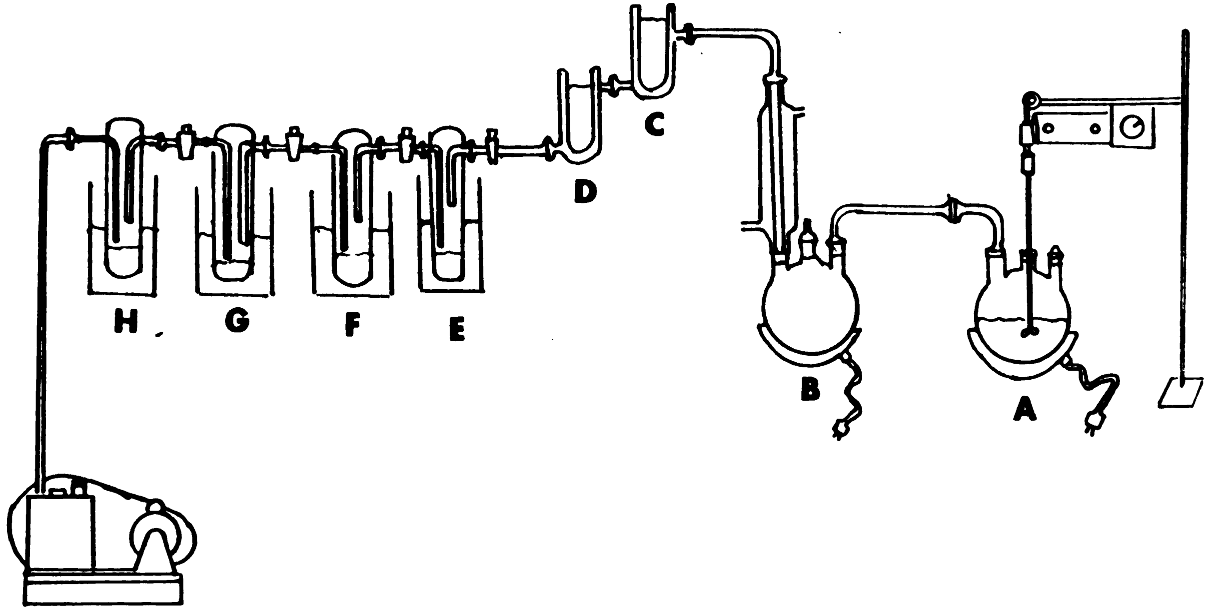

Fig. 1. A modified Herz and Chang Apparatus for the isolation of trace volatile constituents by vacuum steam distillation.

The volatiles from each trap were then individually extracted for 24 hours in the liquid-liquid extractor shown in Figure 2. The ether extract was dried over anhydrous Na_2SO_4 and concentrated to a volume of 10 ml by distillation in a Widmer column. Further concentration of the extract was accomplished by evaporating the sample under a stream of nitrogen until only 35-50 µl remained. The sample was then injected into a Beckman GC-4 gas chromatograph for separation and analysis of its volatiles.

A clear beef broth was also directly extracted with diethyl ether without initial trapping of the volatiles. A comparison of the GC spectra from the two methods of extraction was then made.

Preconcentration of Volatiles with Porapak

The volatiles from both the meat extract and the model meat flavor system were concentrated using the same porous polymer adsorbent shown in Figure 3 as described recently by Jennings and Filsoof (1977). In this study the porous polymer traps (C) were prepared by adding about 3 ml of 80-100 mesh Porapak QS (Waters Associates) between two silanized glass wool plugs in a silanized 12 ml micropipet. Prior to packing, the polymer adsorbent was preconditioned at 220°C for 24 hours with helium or nitrogen at a flow rate of 30 ml/min. The cold trap mixture (B) isolated with the Herz and Chang (1966) apparatus was heated and swept with nitrogen (purified by passage through freshly regenerated molecular sieve 5A and 13X shown in A) by means of a

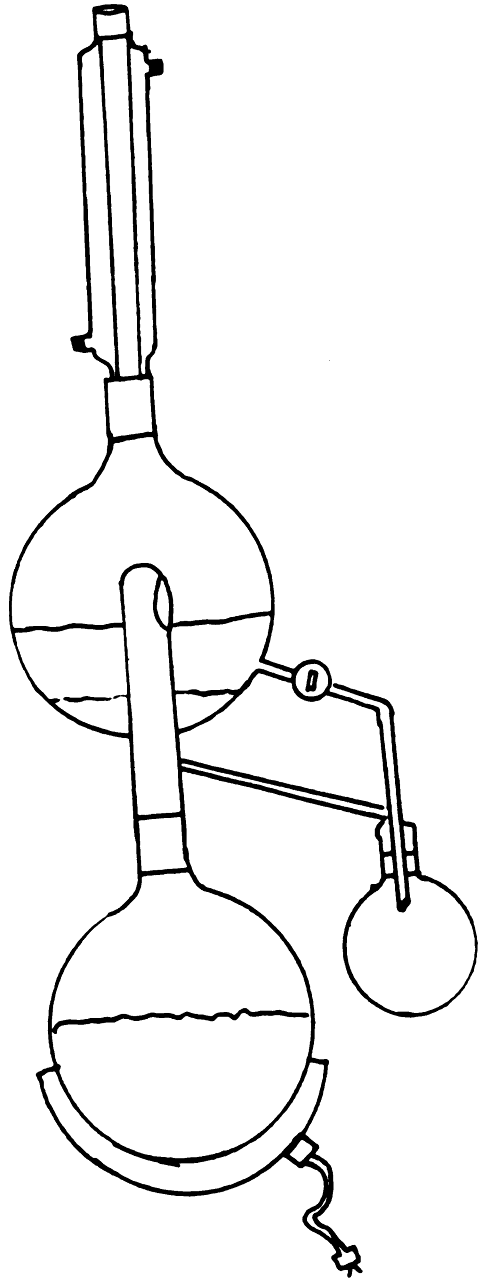

Fig. 2. A liquid-liquid extractor used for extraction of meat volatiles.

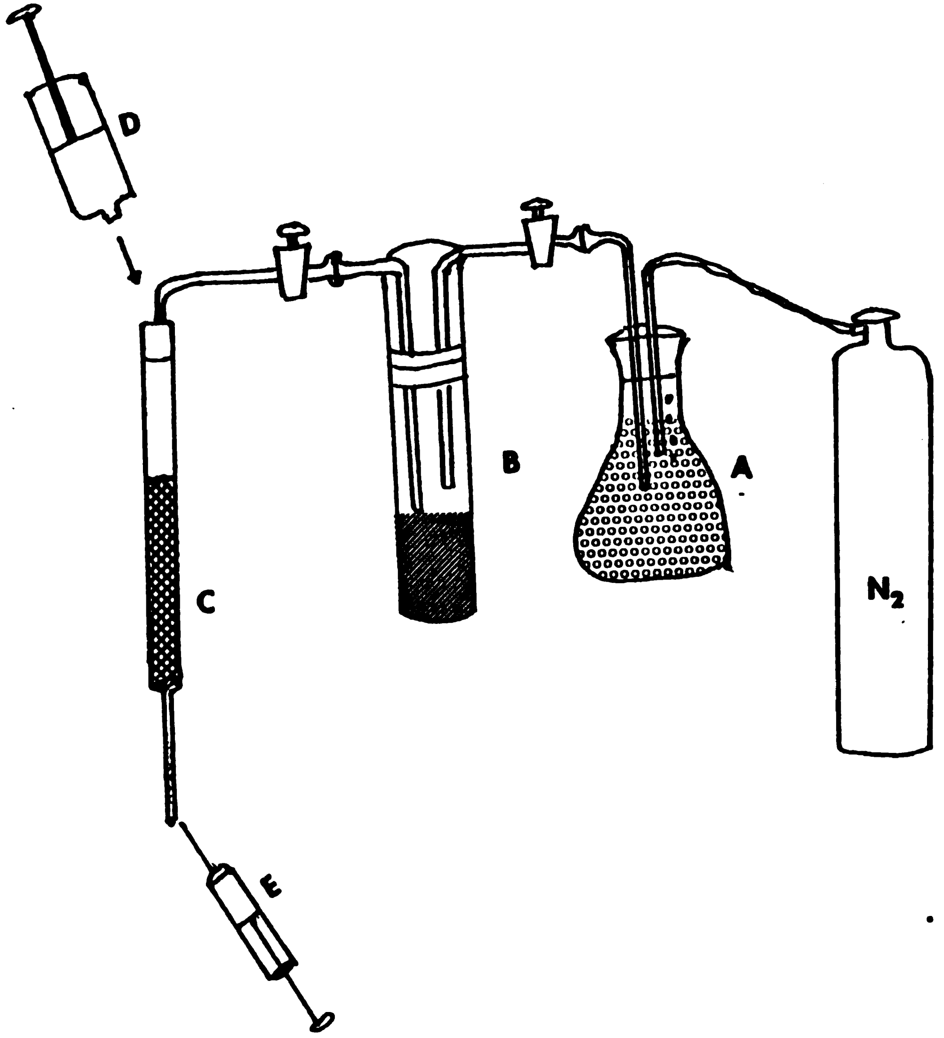

Fig. 3. Jennings and Filsoof apparatus for concentration of meat volatiles using a Porapak column.

glass frit sparger. The volatiles in the reaction mixture were adsorbed onto the Porapk QS and were removed by eluting the column with 250 µl of freshly redistilled ethyl ether. The pneumatic pressure from an empty syringe (D) was used to force the solvent through the porous polymer until a small amount collected on the capillary tip; this was then drawn into a microsyringe (E) and used for GC analysis.

Steam Distillation-Solvent Extraction

The apparatus finally adopted for the distillation-extraction of beef volatiles was the Likens and Nickerson apparatus (Kontes Glass, Vineland, N.J.) shown in Figure 4. The beef slurry (350 ml) was placed in a three-neck round bottom flask (E) and the mixture was constantly agitated during boiling. The solvent flask (F) contained 50 ml of redistilled ethyl ether, which was heated to 37^{o}C during the extraction. As the beef extract and ethyl ether were heated in their respective flasks, the solvent and the flavor volatiles ascended through the columns where they contacted the double surface water condensers (C and D). In this system maximum cooling of the volatiles resulted. The distillates were then returned to their distillation flasks through their respective return arms (A and B), i.e., the water phase through arm B and ethyl ether through arm A. A carbon dioxide-acetone condenser (G) was employed to prevent the loss of volatiles. After the mixture was extracted and distilled for 7 hours, the ether extract was

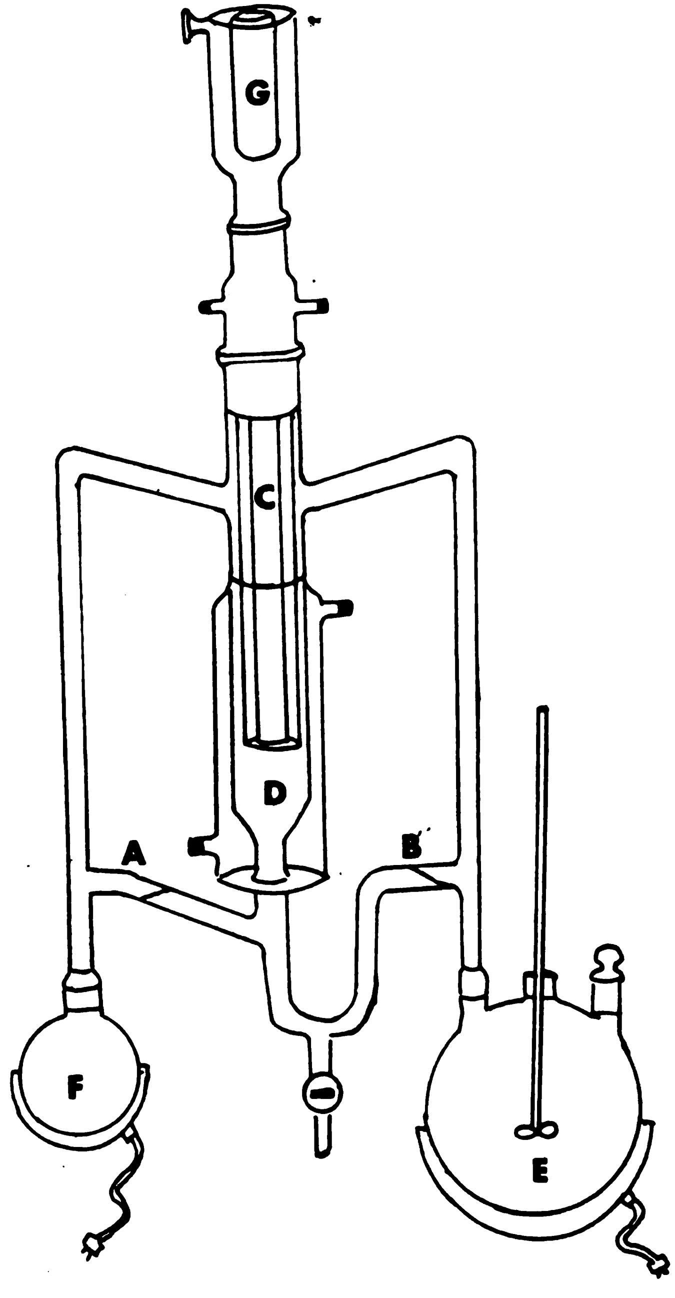

Fig. 4. Likens and Nickerson apparatus for steam distillation and solvent extraction of meat volatiles.

dried with anhydrous Na_2SO_4 and further concentrated by directing a stream of nitrogen over the surface at 600 ml/min. Samples were usually applied to the GC immediately, otherwise, they were stored at -30°C in a tightly closed container and analyzed within 10 days.

Volatiles from the beef model system were extracted and concentrated as described above. The GC spectra for the model system were compared to that of beef extract.

Gas Chromatographic Analysis

The ether extract containing the beef flavor volatiles was separated into its individual constituents using a Perkin-Elmer Model 900 gas chromatograph equipped with a flame-ionization detector. The column used was a 10 ft x 1/8 in glass column packed with 10% Carbowax-20M on 8-100 mesh acid washed Chromosorb W. The helium flow rate was 35 ml/min and the column temperature was held at 70°C for 5 min. The column temperature was then programmed to 200°C by raising the temperature 4°C per min. The injector port temperature was set at 220°C and the detector temperature was maintained at 240°C.

Gas Chromatography-Mass Spectrometry

A combination gas chromatograph-mass spectrometer, LKB Model 9000, was used to separate and identify the volatiles. The concentrate was analyzed under the same conditions as were used in the initial GC separation. Mass spectrometer operating conditions were as follows: ion accelerating voltage, 3555 V; ionizing voltage, 70 eV; ion source temperature, 290; molecular separator temperature, 250°C; scan rate, 6 sec between m/e 10-400. The output routine was obtained with a Digital Equipment Computor Model PDP A-E connected to a Tektronic Hard Copy Model No. 4610.

Amino Acid Analysis

The various model systems were subjected to amino acid analyses to determine the contribution of the individual amino acids to meat flavor. The following model systems were used:

I. Free amino acids + simple sugars (See Table 2) - without heat.
II. Free amino acids + simple sugars - heated for 90 min at 121°C.
III. Gelatin + simple sugars (See Table 2) - without heat.
IV. Gelatin + simple sugars - heated for 90 min at 121°C.
V. Gelatin + simple sugars + free amino acids (See Table 2) - without heat.
VI. Gelatin + simple sugars + free amino acids - with heat.

All model systems (I through VI) were dissolved in 25 ml of deionized water before heating. Samples II, IV and VI were autoclaved for 90 min at 17 psi before amino acid analysis, whereas, samples I, III, and V were heated only for a few minutes on top of a steam bath to dissolved the samples.

Amino acid analyses were performed according to the procedure described by Moore *et al*. (1958). A total of 4 mg of protein (dry weight basis) was transferred into 10 ml ampoule. The final mixture was diluted to 5 ml with concentrated HCl so that concentration of the acid was 6N. The content of the ampoules were frozen in a dry ice-ethanol bath. As the frozen sample was thawed slowly at room temperature, the dissolved gas within the sample was evacuated by a vacuum pump. The sample was refrozen and the ampoule was sealed with a propane flame. The sealed ampoule was placed in an oil bath and hydrolyzed at 110°C for either 24 or 72 hour.

The ampoule was broken and 1 ml of norleucine (2.5 µmoles/ml) was added to the ampoule as a standard to account for transfer losses. The content of the ampoule was then quantitatively transferred to an evaporating flask to remove the HCl from the sample. The dried sample was washed with a small amount of deionized water and again taken to dryness. This was repeated three times to remove residual HCl. The acid free hydrolysate was transferred to a 5 ml volumetric flask and diluted to volume with citrate-HCl buffer (pH 2.2). A 0.2 aliquot of the hydrolysate was applied to the Beckman Spinco Model 120-C amino acid analyzer. The chromatograms were quantitated by peak integration using a Spectra Physics Autolab System AA.

Methionine and cystine analyses. For analysis of methionine and cystine, the methods of Schram et al. (1954) and Lewis (1966) were used. The methods involve performic acid oxidation of methionine and cystine to form methionine sulfone and cysteic acid, respectively. Exactly 4 mg of protein (dry weight basis) were transferred to a 24 ml pear-shaped flask. The protein was oxidized for 15 hr with 10 ml of performic acid at $4^{o}C$. After oxidation, 1 ml of norleucine (2.5 μmoles/ml) was added to the mixture, and the performic acid was removed in a rotatory evaporator. The dried sample was quantitatively transferred to a 10 ml ampoule using 5 ml of 6 N HCl. Hydrolysis and amino acid analyses were performed as previously described.

Sensory Analyses

Panelists were initially trained to detect both the aroma and taste of meat. This was accomplished by preliminary tasting of beef samples cooked conventionally ahd by microwave radiation for different periods of time as described by Bodrero and Pearson (1978). A scoring system was developed during training of the panelists and is shown in Appendix Table 1.

The experiment was designed so that each variable was tested at 3 different concentrations (high, medium and low). If 2 variables were used for each test period, a total of 9 samples would need to be evaluated by each panelist.

However, results from preliminary tests showed that the maximum number of samples which a panelist could accurately evaluate for taste and aroma at one setting was 6 samples. Thus, an incomplete block design was used so that each panelist evaluated only 6 out of 9 samples at one time (See Appendix Table 2).

To evaluate the odor and flavor of each model system, the panelists were presented with 20 ml of each heated sample in individual plastic cups. Samples were coded with 2 digit random numbers. Each judge evaluated 6 samples 3 times a week for 10 weeks. To evaluate odor, the panelists were asked to smell the sample and then circle the appropriate category (ies) best describing the odor. To evaluate flavor, the panelists were asked to place a few drops of the sample in their mouths using the disposable micropipet provided and then to circle the appropriate category (ies) best describing the flavor. Panelists were allowed to expectorate the sample into an empty paper cup and rinse their mouth thoroughly with water between samples.

All of the odor and flavor qualities were expressed on the following intensity scale: (0) no flavor or odor; (1) faint; (2) slight; (3) moderate; (4) strong; and (5) very strong. Overall odor and flavor were rated on a hedonic scale of 9, in which 1 = extremely dislike, and 9 = extremely like (Peryam and Pilgrim, 1957). Reference samples were provided during each test session to acquaint judges with the odor/flavor characteristics.

Statistical Analyses

Results from the taste panel evaluation were statistically analyzed using the surface response method. A multiple regression program (M.S.U. Stat System Version 4.4) was used to predict the best combination of one or more variables that may contribute to meat aroma and flavor. This method was also capable of predicting the maximum levels of precursors required to produce the maximum panel scores.

After the maximum concentration of each amino acid had been predicted by the surface response method, it was then added to the basic model system (Table 1) and remained as a constant, while a different amino acid(s) was tested. The entire procedure then was repeated again for each amino acid tested.

Comparison of Model System and Commercial Beef Flavor Products

The final model system developed was compared with existing commercial products. The panelists were asked to evaluate the model system in comparison to the following commercial beef flavor products: Herbox (The Pure Food Company), Maggi (The Nestle Company), Wyler's (Borden Inc.), Ajimate (Ajinomoto Company) and Knorr-Swiss (Knorr Food Products). All commercial products were prepared according to the instructions on the package. Spices were added to the model systems to resemble those in the commercial products. All samples were coded and were presented to the panelists without revealing brand names. Samples were evaluated according to flavor, odor, mouthfeel and generally acceptability as presented in Appendix Table 3.

RESULTS AND DISCUSSION

Development of a Synthetic Meat Flavor Model System

The synthetic meat flavor mixture was developed by using the principle flavor precursors previously identified by other investigators (Batzer et al., 1960; Macy et al., 1964a,b; Wasserman and Spinelli, 1970; Herz and Chang, 1970). Selection of the essential precursors for the model system was based on the theory that the majority of meat flavor was developed from nonvolatile compounds, which upon heating undergo spontaneous Maillard-type reactions. These precursors included simple sugars, glycoprotein, 5'-nucleotides, amino acids and non-protein nitrogen compounds in the proportions given in Table 1.

Simple Sugars

The simple sugars used in the model system were taken from the data presented by Macy et al. (1964a), in which the carbohydrate concentration in the lyophilized diffusate from beef before and after heating was reported. Since reducing sugars are required for nonenzymatic browning reactions, ribose and glucose were selected as the carbohydrate components for the model system. Fructose, which is also a reducing sugar, decreased very little during heating with other components of the meat hydrolysate. Thus, it probably does not play an important role in development of meat flavor, and was not used in the model system.

Although xylose does not occur in meat naturally, it was added to the model system because it reacts with cysteine-HCl to form thiophene compounds, which are believed to play an important role in meat flavor (Mussinan and Katz, 1968). Furthermore, Rothe and Voight (1963) showed that certain amino acids, such as serine, lysine, threonine, glycine, glutamic acid, proline, arginine and histidine produced more browning substances than other amino compounds on reacting with xylose. On the other hand, reacting xylose with isoleucine, leucine, valine, methionine, and phenylalanine produced a considerable amount of flavor compounds, but contributed relatively little to browning.

Glycoprotein (Gelatin)

During formulation of the model system, it was noted that the synthetic meat flavor mixture containing only amino acids and simple sugars produced a sharp and pungent odor, but lacked a brothy taste. However, addition of gelatin, which comprises a major portion of the meat proteins, greatly improved the overall mouthfeel and increased the brothiness of the synthetic meat mixture. The idea of using gelatin in the model system was derived from the observation that all cooked meat broths gelled upon refrigeration. Furthermore, Batzer *et al*. (1960) have shown that an unidentified glycoprotein is a major precursor component of meat flavor. Since gelatin is a glycoprotein and has an amino acid profile very similar to that of the unknown glycoprotein isolated by Batzer et al. (1960), it was chosen as a major constituent of the model system.

The optimum amount of gelatin was determined by adding different levels to the model system and using sensory evaluation to determine its effects upon mouthfeel and flavor. Results showed that 0.5 g of gelatin in 50 ml gave the best acceptability.

In order to determine the role of gelatin in the synthetic meat flavor mixture, amino acid analyses was performed on the gelatin and simple sugar mixtures before and after heating. The results of the amino acid analysis are shown in Table 3.

When gelatin was heated at 121°C for 90 minutes, it underwent hydrolysis to form peptides and amino acids, which are important flavor precursors of meat. Amino acid analysis revealed that all of the amino acids present decreased by 20-30% during heat treatment. The losses of amino acids are undoubtedly in part due to destruction during heating. However, the decrease is mainly due to the interaction of amino acids with the simple sugars to form nonenzymatic browning products. Observation of the solution following heat treatment indicated that nonenzymatic browning had indeed occurred. The heated mixture lacked a meaty flavor, although it did possess a brothy taste. This may be due to the lack of sulfur containing amino acids in gelatin (Leninger, 1975), since addition of sulfur amino acids to

Table 3. Amino acid analysis of gelatin - simple sugar model system before and after heating

Amino Acids	Gram of Amino Acids per 100 g Sample		
	Before Heating	After Heating	% Loss
L-Lysine	5.87	4.16	29.1
L-Histidine	2.15	1.36	36.7
NH_3	1.27	1.11	12.6
L-Arginine	9.54	6.39	33.0
L-Aspartic Acid	8.80	6.10	31.6
L-Threonine	2.09	1.51	27.8
L-Serine	4.04	2.88	28.7
L-Glutamic Acid	11.98	8.40	29.9
L-Proline	17.08	11.65	31.8
Glycine	23.76	17.21	27.6
L-Alanine	10.15	6.80	32.8
L-Cysteine	0.0	0.0	0.0
L-Valine	3.42	2.39	30.1
L-Methionine	0.0	0.0	0.0
L-Isoleucine	1.55	1.05	32.3
L-Leucine	3.58	2.41	32.7
L-Tyrosine	0.90	0.38	57.8
L-Phenylalanine	2.63	1.59	39.5
L-Tryptophane	0.0	0.0	0.0

the model system produced a meaty odor. Thus, the function of gelatin in the model system is probably three-fold: (1) It contributed brothiness to the synthetic meat mixture; (2) It provided the synthetic model system with additional amino acids and sugars through hydrolytic breakdown to form nonenzymatic browning products during heating; and (3) It tended to mask the strong sulfury odor due to the presence of sulfur amino acids in the model system.

Kjeldahl analysis of the gelatin and simple sugar mixture showed that the percent of total extractable nitrogen in the heated sample decreased from 20.8 to 14.5%. This indicated that the amino acids or peptides derived from the gelatin underwent breakdown or interacted with other constituents to form completely different compounds, in which ammonia was liberated or volatilized during heating. This is further verified by the results of the amino acid analysis of the gelatin and simple sugar mixture, which showed that ammonia decreased following heating. This is also in agreement with the result of Shibamoto and Russell (1976) who showed that thiophenes, which are found in beef volatiles, are produced on heating ammonia with glucose and hydrogen sulfide. Ammonia has also been shown to be involved in the formation of pyrazine compounds by combining with sugars and/or carbonyl substances (Newell _et al_., 1967; Koehler _et al_., 1969). Thus, ammonia liberated from the reaction mixture during heating may interact with other components to form important volatiles that may contribute to meat flavor.

5'nucleotides.

Preliminary studies showed that 5'nucleotides did not contribute to the basic meat flavor, but on addition to the model system enhanced and blended the flavor notes. The role of nucleotides in development of meat flavor was pointed out earlier by Caul and Raymond (1964), who demonstrated that inosine monophosphate (IMP) and other nucleotides are merely flavor enhancers, not flavorants. More recently, however, Fujimake *et al*. (1970) have shown that IMP forms browning products on reacting with glucose. They also demonstrated that a glucose-IMP solution produced more browning than a glucose-GMP (guanosine monophosphate) solution. Thus, IMP probably contributes more to overall meat flavor in the model system than GMP.

Since IMP is a breakdown product of AMP, which occurs naturally in meat, it was selected as the nucleotide to be added to the model system. GMP was also examined, but was not used in the model system, because its concentration in meat was so small that it did not have any measurable effect on taste. The amount of IMP used (25 mg/50 ml) was based on the concentration of IMP found in beef tissue by Wismer-Pedersen (1966). The level used in the model system was determined by the surface response method.

Monosodium Glutamate (MSG).

Another essential component of the model system is MSG, which is also a meat flavor enhancer. Kuninaka (1967) has shown that a strong synergistic relationship exists between nucleotides and MSG, so that blending of the two components produces more flavor than either compound alone. Kazeniac (1961) also demonstrated that certain amino acids when combined with glutamic acid improved mouth satisfaction. Thus, MSG was added to the model system to further improve mouthfeel and enhance the meaty flavor. Its concentration was determined by the surface response method, with 20 mg/50 ml finally being added to the model system.

Amino Acids.

During the formulation of the model system, it was found that certain amino acids were essential to the development of meat flavor, especially the sulfur containing amino acids, cysteine, cystine and/or methionine. In their absence, no meat-like flavor was evolved. Thus, it was necessary to first test the levels of these amino acids with the remaining components (simple sugars, glycoprotein, 5'-nucleotides and MSG).

Cysteine and cystine were first tested in the model system and a meat-like odor was detected following heating of the mixture for 90 min at 17 psi. However, the mixture had a strong sulfury odor and flavor, which was quite objectionable to many panelists. Later it was found that if only cysteine-HCl was used in combination with alanine, a significant decrease in the sulfury odor and taste occurred without any loss of meaty flavor. Thus, different levels of cysteine and alanine were tested simultaneously using the surface response method and the results are shown in Table 4. The computer predicted that cysteine and alanine at a

Table 4. Computer predictions of maximum or minimum levels of L-cysteine and L-alanine using the surface response method

	L-Cysteine[a]	L-Alanine[b]
Boiled odor	26 (min)	43 (min)
Boiled taste	34 (min)	18 (max)
Roast odor	24 (max)	25 (max)
Roast taste	20 (max)	19 (min)
Sulfur odor	18 (max)	17 (min)
Sulfur taste	19 (max)	25 (max)
Overall odor	16 (max)	22 (max)
Overall taste	21 (max)	28 (min)
Average maximum	20 (max)	23 (max)
Average minimum	30 (min)	27 (min)

a) L-Cysteine was tested at 10, 20 and 30 mg/50 ml.

b) L-alanine was tested at 15, 25 and 35 mg/50 ml.

concentration of 20 and 23 mg/50 ml sample, respectively, would produce the maximum panel score.

Since cysteine and alanine were essential ingredients for the development of meat flavor in the model system, it was necessary to keep their concentration constant while other amino acids were individually tested. The concentration of the other amino acids used in the model system were taken from the work of Macy _et al_. (1964a; 1970c), who reported the concentration of various amino acids in beef, pork and lamb hydrolysates before and after heating. Results of the taste panel evaluation for the different amino acids and the computer prediction for their maximum levels are shown in Tables 5 through 20.

Methionine. Table 5 presents the panel evaluation of methionine at concentrations of 6, 12 and 20 mg/50 ml and gives the maximum levels predicted by the computer. Results showed that as the concentration of methionine increased up to 12 mg/50 ml sample, maximum panel scores were obtained for both boiled odor and flavor, and for roast odor and flavor. Further increases in the concentration (up to 20 mg/50 ml) caused a decrease in panel score. This is probably due to the increased release of sulfur compounds as the concentration of methionine increased. Further confirmation of the negative influence of high levels of methionine was obtained from the results of taste panel evaluation for sulfur odor and taste. It was shown that taste panel scores decreased as the concentration of methionine increased.

Table 5. Average panel scores of L-methionine at concentrations of 6, 12 and 20 mg/50 ml and its computer predicted levels

	Computer predicted levels	Average panel scores (1-5)		
	(mg/50 ml)	6	12	20 (mg/50 ml)
Boiled odor	11 (max)	1.4	1.7	0.9
Boiled taste	15 (max)	1.5	1.8	1.1
Roast odor	13 (max)	1.5	1.9	1.5
Roast taste	12 (max)	1.6	2.3	1.4
Sulfur odor	25 (max)	0.5	1.0	1.4
Sulfur taste	13 (max)	0.5	1.0	0.5
Overall odor[1]	6 (max)	5.1	4.6	4.0
Overall taste[1]	10 (max)	5.4	5.4	4.6
Average maximum	12 (max)			
Average minimum	---			

[1] Overall odor and taste scores ranged 1-9

Similarly, overall odor and taste were inversely correlated with increasing amounts of sulfur production, i.e. higher concentrations of methionine were related to higher sulfur production and resulted in lower overall panel scores.

The predicted surface response values correlated well with actual taste panel scores. For example, the computer predicted that a methionine concentration of 12 mg/50 ml should give the maximum panel score. Actual taste panel evaluation showed that the sample containing 12 mg/50 ml did indeed give the highest panel score. In order to obtain the best overall score, an average of all the maximum values was used. The average maximum value was then used as the level for testing of methionine with other amino acids in all subsequent computer predictions.

Table 6 shows the computer prediction for maximum and minimum levels of methionine in combination with the other amino acids. When methionine was tested singly at concentration ranges of 6, 12 and 20 mg/50 ml, the average maximum was 12. When methionine was combined with other amino acids, the range in concentration was arbitrarily decreased to 5, 9 and 14 mg/50 ml for isoleucine and to 9, 12 and 15 mg/50 ml with leucine. The narrowing of the concentration range provided more accurate predictions. Results showed that when methionine was combined with other amino acids, such as isoleucine and leucine, its average maximum concentration was not

Table 6. Computer predictions of maximum or minimum levels for L-methionine in basic model system or in combination with other amino acids

	Computer predicted levels (mg/50 ml)		
	L-methionine[a]	Meth. vs Ile[b]	Meth. vs. Leu[c]
Boiled odor	11 (max)	9 (min)	15 (min)
Boiled taste	15 (min)	11 (max)	14 (min)
Roast odor	13 (max)	11 (max)	12 (max)
Roast taste	12 (max)	13 (max)	12 (max)
Sulfur odor	25 (max)	11 (max)	12 (min)
Sulfur taste	13 (max)	8 (max)	14 (min)
Overall odor	6 (max)	11 (min)	10 (max)
Overall taste	10 (max)	11 (min)	12 (max)
Average maximum	12 (max)	12 (max)	11 (max)
Average minimum	15 (min)	11 (min)	14 (min)

a) L-methionine was tested singly at 6, 12 and 20 mg/50 ml.

b) L-methionine was tested with L-isoleucine at 5, 9 and 14 mg/50 ml

c) L-methionine was tested with L-leucine at 9, 12 and 15 mg/50 ml) affected and remained at 12 mg/50 ml. On comparing the maximum concentration for methionine with that of cysteine, it was found that less methionine (12 mg/50 ml) than cysteine (20 mg/50 ml) was required (Table 4).

Taurine. Average panel scores of taurine at concentrations of 9, 18 and 30 mg/50 ml were predicted by the computer, and the results are shown in Table 7. Results indicated that as taurine concentration increased, panel scores for boiled odor and taste decreased. Thus, the computer predicted that a minimum score would occur for boiled odor and flavor at concentrations of 29 and 27 mg/50 ml, respectively. On the other hand, the average panel scores for roast odor and taste increased with increasing concentrations. Thus, the computer predicted that the maximum panel scores for roast odor and taste would occur at levels of 25 and 20 mg of taurine/50 ml, respectively.

Since taurine (β-amino-ethanesulfonic acid) is a sulfur containing amino compound, it was expected that sulfur production would increase directly with increasing concentration and result in lower panel scores. However, results showed that as the concentration of taurine increased, the panel scores also increased. This indicated that taurine probably did not release appreciable quantities of sulfur-containing compounds. This is further confirmed by the findings of Herz and Chang (1970), who stated that taurine is not a major source for hydrogen sulfide evolution as reduction does not occur during heating. The computer

Table 7. Average panel scores of taurine at concentrations of 9, 18 and 30 mg/50 ml and its computer predicted levels

	Computer predicted levels (mg/50 ml)	Average panel scores (1-5)		
		9	18	30 (mg 50 ml)
Boiled odor	29 (min)	1.2	0.8	0.5
Boiled taste	27 (min)	1.5	1.0	1.0
Roast odor	25 (max)	1.7	2.3	2.7
Roast taste	20 (max)	1.2	1.8	1.5
Sulfur odor	18 (max)	0.5	0.6	0.8
Sulfur taste	18 (min)	0.3	0.3	0.8
Overall odor[1]	20 (max)	4.7	6.0	5.8
Overall taste[1]	22 (max)	4.3	5.8	5.7
Average maximum	21 (max)			
Average minimum	25 (min)			

[1] Overall odor and taste scores ranged 1-9.

predicted that concentrations of 20 and 21 mg/50 ml would give maximum panel scores for overall odor and taste, respectively.

Table 8 shows the maximum computer prediction for taurine when combined with other amino acids. Results indicated that as taurine was tested with leucine at concentrations of 20, 22 and 25 mg/50 ml, the number of maximum predictions decreased. Similarly, on testing taurine with methionine at the same concentration range, the maximum predictions were similar to the minimum predictions. This can be clearly explained by plotting the panel scores for roast odor against the concentration of taurine (Fig. 5). The plot shows that as the concentration range was narrowed (20-25 mg/50 ml), any change in concentration within that range caused only a minor alteration in panel score. Results from the taste panel evaluation indicated that panelists were not able to differentiate between such small changes in concentration. Thus, the similarities in scores by varying the concentration within such a narrow range resulted in poor computer predictions, i.e. minimum and maximum predictions were similar. This is evident in the case of taurine vs. methionine, in which the maximum and minimum predictions were 23 and 22 mg/50 ml, respectively.

Serine. Average panel scores of serine at concentrations of 12, 30 and 38 mg/50 ml and the computer predicted levels are shown in Table 9. Results showed that serine contributed little toward boiled odor or taste as shown by panel

Table 8. Computer predictions of maximum or minimum levels for taurine in basic model system or in combination with other amino acids

	Computer Predicted Levels (mg/50 ml)		
	Taurine[a]	Tau. vs. Leu[b]	Tau. vs. Meth.[c]
Boiled odor	29 (min)	22 (max)	22 (max)
Boiled taste	27 (min)	18 (min)	22 (max)
Roast odor	25 (max)	26 (min)	23 (max)
Roast taste	20 (max)	23 (min)	22 (max)
Sulfur odor	18 (max)	22 (max)	22 (min)
Sulfur taste	18 (min)	22 (max)	23 (max)
Overall odor	20 (max)	23 (max)	22 (max)
Overall taste	22 (max)	22 (min)	27 (max)
Average maximum	21 (max)	22 (max)	23 (max)
Average minimum	25 (min)	22 (min)	22 (min)

a) Taurine was tested singly at 9, 18 and 30 mg/50 ml

b) Taurine was tested with L-leucine at 20, 22 and 25 mg/50 ml

c) Taurine was tested with L-methionine at 20, 22 and 25 mg/50 ml

Table 9. Average panel scores for L-serine at concentrations of 12, 20 and 28 mg/50 ml and its computer predicted levels

	Computer predicted levels	Average panel scores (1-5)		
	(mg/50 ml)	12	20	28 mg/50 ml)
Boiled odor	24 (max)	0.5	0.7	0.7
Boiled taste	15 (min)	0.6	0.7	2.0
Roast odor	24 (max)	1.6	1.7	1.8
Roast taste	20 (max)	1.8	2.3	1.9
Sulfur odor	20 (max)	0.9	1.0	0.3
Sulfur taste	30 (min)	0.8	0.4	0.3
Overall odor[1]	20 (max)	4.9	5.0	4.9
Overall taste[1]	19 (max)	5.1	5.4	5.0
Average maximum	21 (max)			
Average minimum	23 (min)			

1) Overall odor and taste scores ranged 1-9.

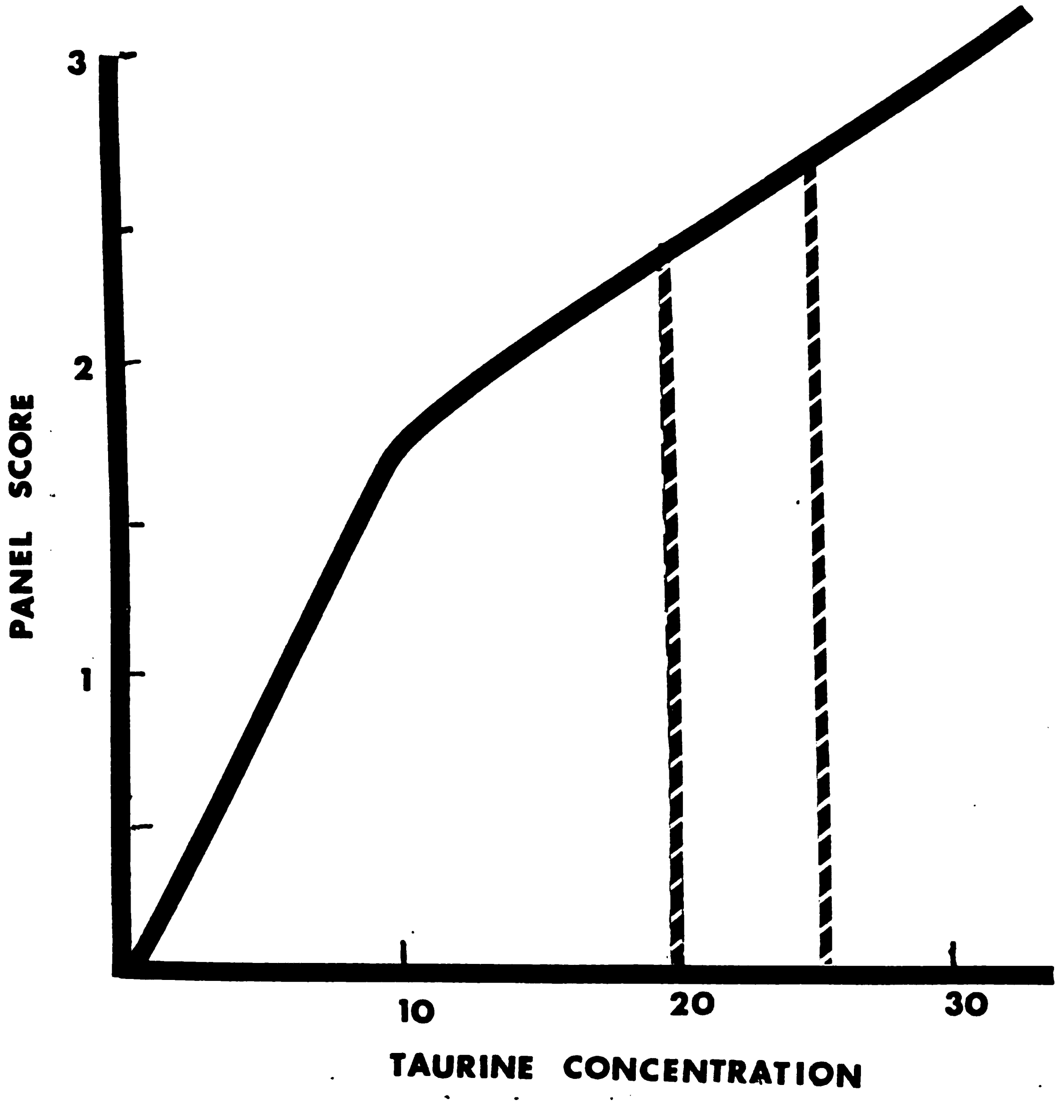

Fig. 5. Concentrations of taurine vs. panel scores for evaluation of roast odor.

scores of <1 based on a hedonic scale of 1-5. It did, however, contribute toward roast odor and taste since the panel scores increased directly with concentration. Since serine is not a sulfur containing amino acid, increasing its concentration would not be expected to have any direct effect on sulfur odor or taste. However, results showed that as serine concentration increased sulfur taste decreased. This may be due to a sulfur masking effect, since serine has a natural MSG-like taste by itself. Thus, it probably enhanced the meaty flavor in the model system. The optimum level of serine for producing maximum panel score was predicted to be 20 and 19 mg/50 ml for overa-l odor and taste.

The computer prediction for serine in combination with other amino acids is shown in Table 10. Results indicated that as the concentration range of serine was narrowed from 12-28 to 20-25 mg/50 ml, the minimum and maximum predictions became similar. This indicated that the concentration range of 20-25 mg/50 ml was too narrow and did not differentiate between predictions of minimum and maximum concentrations.

<u>Leucine</u>. Average panel scores for leucine at concentrations of 4, 9 and 15 mg/50 ml and the optimal levels predicted by the computer are presented in Table 11. The taste panel results were very similar to that of serine, except that the predicted concentration (10 mg/50 ml) was approximately half that of serine (21 mg/50 ml). Overall odor and taste scores were higher than those for most other amino acids. This indicates an important role for leucine

Table 10. Computer predictions of maximum or minimum levels for L-serine in basic model system or in combination with other amino acids

	Computer Predicted Levels (mg/50 ml)		
	L-Serine[a]	Ser. Vs. Val[b]	Ser. Vs. Tau[c]
Boiled odor	24 (max)	22 (min)	19 (min)
Boiled taste	15 (min)	12 (min)	20 (min)
Roast odor	24 (max)	20 (min)	16 (max)
Roast taste	20 (max)	22 (min)	20 (max)
Sulfur odor	20 (max)	36 (min)	20 (max)
Sulfur taste	30 (min)	21 (max)	24 (max)
Overall odor[1]	20 (max)	21 (max)	18 (min)
Overall taste[1]	19 (max)	20 (max)	20 (min)
Avg. max.	21 (max)	21 (max)	20 (max)
Avg. min.	23 (min)	22 (min)	19 (min)

a) Serine was tested singly at 9, 18, 30 mg/50 ml

b) Serine was tested with L-valine at 20, 22, 25 mg/50 ml

c) Serine was tested with Taurine at 18, 20, 22 mg/50 ml

1) Overall odor and taste scores ranged 1-9

Table 11. Average panel scores of L-leucine at concentrationf of 4, 9 and 15 mg/50 ml and its computer predicted levels

	Computer predicted levels	Average panel scores (1-5)		
	(mg/50 ml)	4	9	15 (mg/50 ml)
Boiled odor	8 (max)	0,6	1,0	1,3
Boiled taste	13 (max)	0.6	1.1	2.2
Roast odor	11 (max)	2,0	2.2	2.5
Roast taste	9 (max)	2.3	2.7	2.5
Sulfur odor	7 (min)	0.6	0.7	0.3
Sulfur taste	13 (max)	0.0	0.5	0.7
Overall odor[1]	9 (min)	5.8	5.2	5.5
Overall taste[1]	7 (max)	6.2	6.2	6.7
Average max	10 (max)			

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1) Overall odor and taste scores ranged 1-9

in the production of meat flavor.

Computer predictions giving maximum and minimum levels of leucine combined with other amino acids are presented in Table 12. Results indicated that there was no synergistic effect upon combining leucine with other amino acids, since the predicted concentration for maximum scores remained the same.

Isoleucine. Average panel scores for isoleucine at concentrations of 6, 12 and 20 mg/50 ml are presented in Table 13. Taste panel scores decreased in almost every category as the concentration increased. This is especially evident for overall odor and taste. The decrease may be related to an increase in the sulfurous taste of the mixture as the concentration of isoleucine was raised. Although isoleucine, which has the same molecular formula as leucine, does not have a sulfurous group, it may increase the sulfurous taste and suppress other flavors. The fact that the computer analyses gave many minimal predictions suggested that the range of concentration (6-20 mg/50 ml) was probably too high and a lower concentration should have been used. Another possible explanation for the poor prediction on adding isoleucine may be that it plays only a minor part in the flavor/aroma of meats.

When isoleucine was combined with other amino acids at a narrower concentration range at 6-16 mg/50ml (Table 14). the computer predicted 9 mg/50 ml as producing the optimal panel scores. When isoleucine was combined with valine at

Table 12. Computer predictions of maximum or minimum levels of L-leucine in basic model system or in combination with other amino acids

	Computer Predicted Levels (mg/50 ml)		
	L-leucine[a]	leu vs. meth[b]	leu vs. taur[c]
Boiled odor	8 (max)	5 (min)	9 (min)
Boiled taste	13 (max)	12 (max)	9 (max)
Roast odor	11 (max)	8 (max)	11 (max)
Roast taste	9 (max)	9 (max)	9 (max)
Sulfur odor	7 (min)	11 (max)	10 (min)
Sulfur taste	13 (max)	12 (max)	10 (min)
Overall odor	19 (min)	10 (min)	10 (max)
Overall taste	7 (max)	6 (min)	10 (max)
Average maximum	10 (max)	10 (max)	10 (max)
Average minimum	13 (min)	7 (min)	9 (min)

a) L-leucine was test singly at 4, 9 and 15 mg/50 ml

b) L-leucine was test with L-methionine at 6, 9 and 13 mg/50 ml

c) L-leucine was tested with taurine at 6, 9 and 15 mg/50 ml.

Table 13. Average panel scores of L-isoleucine at concentrations of 6, 12 and 20 mg/50 ml and its computer predicted levels

	Computer Predicted Levels (mg/50 ml)	Average panel scores (1-5)		
		6	12	20 (mg/50 ml)
Boiled odor	16 (min)	1.9	1.1	1.1
Boiled taste	20 (min)	1.6	1.5	0.9
Roast odor	7 (max)	1.9	2.0	1.3
Roast taste	20 (min)	1.9	1.8	1.6
Sulfur odor	13 (max)	0.6	0.8	0.8
Sulfur taste	12 (min)	0.6	0.6	1.1
Overall odor[1]	12 (min)	5.4	4.1	4.8
Overall taste[1]	18 (min)	4.9	4.8	4.3
Average maximum	11 (max)			
Average minimum	15 (min)			

1) Overall odor and taste scores ranged 1-9

Table 14. Computer predictions of maximum or minimum levels for L-isoleucine in basic model system or in combination with other amino acids

	Computer Predicted Levels (mg/50 ml)		
	L-Isoleucine[a]	Ile vs Meth[b]	Isl vs Val[c]
Boil odor	16 (min)	10 (min)	5 (max)
Boil taste	20 (min)	5 (max)	5 (max)
Roast odor	7 (max)	14 (max)	7 (max)
Roast taste	20 (min)	8 (max)	9 (max)
Sulfur odor	13 (max)	8 (max)	5 (max)
Sulfur taste	12 (min)	3 (min)	6 (max)
Overall odor	12 (min)	8 (min)	6 (max)
Overall taste	18 (min)	10 (max)	6 (max)
Average maximum	11 (max)	9 (max)	6 (max)
Average minimum	15 (max)	7 (min)	-

a) L-isoleucine was tested singly at 6, 12 and 20 mg/50 ml

b) L-isoleucine was tested with L-methionine at 6, 21 and 26 mg/50 ml

c) L-isoleucine was tested with L-valine at 3, 6 and 9 mg/ 50 ml a still lower concentration range (3, 6 and 9 mg/50 ml), maximum predictions at the level of 6 mg/50 ml were obtained for all categories. This indicated that lower concentrations of isoleucine improved panel scores.

Valine. The average panel scores and the computer predicted levels for valine at concentrations 3, 8 and 15 mg/50 ml is presented in Table 15. Results showed that a lower concentration range should have been used as panel scores decreased with increasing concentrations. The computer predictions for maximum concentration also showed poor predictions. Thus, a lower concentration range (4-9 mg/50 ml) was used when valine was tested with serine (Table 16). Results showed that the computer produced more maximum predictions than minimum predictions. However, when valine was tested with isoleucine at a slightly higher concentration range (5-12 mg/50 ml), poor predictions again resulted. This may be due to an inhibitory effect of isoleucine on production of flavor volatiles.

Glycine. Evaluation of glycine at concentrations of 5, 8 and 10 mg/50 ml by the panelists is presented in Table 17. Results showed that the highest panel score occurred at 8 mg/50 ml, which is also the value predicted by the computer. Panel evaluation also showed that higher panel scores were obtained for boiled odor and taste than roast odor and taste. This was quite different from all other amino acids tested, which always received a higher panel rating for roast than boiled odor and taste. Thus, results indicated that glycine

Table 15. Average panel scores of valine at concentrations of 3, 8 and 15 mg/50 ml and its computer predicted levels

	Computer predicted levels	Average panel scores (1-5)		
	(mg/50 ml)	3	8	15 (mg/50 ml)
Boiled odor	6 (max)	0.7	0.7	0.3
Boiled taste	11 (min)	1.2	0.7	0.8
Roast odor	7 (max)	2.0	2.4	1.0
Roast taste	9 (max)	1.5	2.7	1.3
Sulfur odor	1 (min)	0.7	0.9	1.5
Sulfur taste	6 (min)	0.3	0.3	1.2
Overall odor[1]	9 (min)	5.5	4.9	4.3
Overall taste[1]	5 (max)	6.0	5.7	4.8
Average maximum	7 (max)			
Average minimum	9 (max)			

1) Overall odor and taste scores ranged 1-9

Table 16. Computer predictions of maximum or minimum levels for valine in basic model system or in combination with other amino acids

	Computer Predicted Levels (mg/50 ml)		
	L-Valine[a]	Val vs. Ser[b]	Val vs Ile[c]
Boiled odor	6 (max)	7 (max)	10 (min)
Boiled taste	11 (min)	6 (max)	9 (min)
Roast odor	7 (max)	6 (max)	14 (min)
Roast taste	9 (max)	6 (max)	6 (min)
Sulfur odor	1 (min)	10 (min)	9 (min)
Sulfur taste	6 (min)	8 (min)	9 (max)
Overall odor	19 (min)	6 (max)	7 (max)
Overall taste	5 (max)	7 (max)	12 (min)
Average maximum	7 (max)	6 (max)	8 (max)
Average minimum	9 (min)	9 (min)	8 (min)

a) L-Valine was tested singly at 3, 8 and 15 mg/50 ml

b) L-Valine was tested with L-serine at 4, 7 and 9 mg/50 ml

c) L-valine was tested with L-isoleucine at 5, 8 and 12 mg/50 ml

Table 17. Average panel scores of glycine at concentrations of 5, 8 and 10 mg/50 ml and its computer predicted levels

	Computer predicted levels	Average panel scores (1-5)		
	(mg/50 ml)	5	8	10 (mg/50 ml)
Boiled odor	7 (max)	1.7	2.2	2.4
Boiled taste	8 (max)	2.3	2.7	1.5
Roast odor	7 (max)	1.7	1.8	1.8
Roast taste	8 (min)	1.7	1.3	2.2
Sulfur odor	7 (max)	2.0	2.3	2.0
Sulfur taste	7 (max)	1.0	1.2	1.4
Overall odor	7 (max)	4.8	5.7	5.4
Overall taste	8 (min)	6.8	6.2	5.0
Average maximum	7 (max)			
Average minimum	8 (min)			

1) Overall odor and taste scores ranged 1-9

probably contributed more toward boiled than roast flavor.

When glycine was reacted at a concentration range of 5-10 mg/50 ml with leucine (Table 18), the same concentration was predicted as when it was tested singly (8 mg/50 ml). However, when glycine was tested at a slightly higher concentration range (8-14 mg/50 ml), a maximum level of 10 mg/50 ml was predicted. This suggests that the maximum predicted concentration tended to be near the middle of the range.

Arginine. Average panel scores for arginine at concentrations of 8, 12 and 15 mg/50 ml and its computer predicted levels are presented in Table 19. Results showed that average panel scores decreased with increasing concentrations. This resulted in minimum computer predictions for all but 3 of the categories evaluated. In order to improve the computer predictions, a lower concentration range (3-9 mg/50 ml) was used when arginine was tested with valine (Table 20). Results showed that the average maximum level predicted for arginine (6 mg/50 ml) was approximately half of the concentration predicted when it was tested singly (13 mg/50 ml). A slightly higher prediction (9 mg/50 ml) was obtained when arginine was tested with glycine. This decrease in computer predicted level suggested that arginine probably exerted an inhibitory effect upon flavor development.

Amino acid analysis of the amino acid-sugar model system. In order to examine the contribution of individual amino acids to the development of meat flavor, amino acid analyses were performed on the amino acid-simple sugar

Table 18. Computer predictions of maximum or minimum levels for glycine in basic model system or in combination with other amino acids

	Computer Predicted Levels (mg/50 ml)		
	Glycine[a]	Gly. vs. Leu[b]	Gly vs. Arg.[c]
Boiled odor	7 (max)	6 (max)	10 (max)
Boiled taste	8 (max)	9 (max)	11 (max)
Roast odor	7 (max)	6 (min)	2 (min)
Roast taste	8 (min)	6 (min)	10 (max)
Sulfur odor	7 (max)	4 (min)	9 (max)
Sulfur taste	7 (max)	6 (min)	9 (max)
Overall odor	7 (max)	7 (max)	11 (max)
Overall taste	8 (min)	8 (max)	12 (max)
Average maximum	7 (max)	8 (max)	10 (max)
Average minimum	8 (min)	6 (min)	12 (min)

a) Glycine was tested singly with 5, 8 and 10 mg/50 ml

b) Glycine was tested with L-leucine at 5, 8 and 10 mg/50 ml

c) Glycine was tested with L-arginine at 8, 10 and 14 mg/50 ml

Table 19. Average panel scores of L-arginine at concentrations of 8, 12 and 15 mg/50 ml and its computer predicted levels

	Computer predicted levels	Average panel scores (1-5)		
	(mg/50 ml)	8	12	15 (mg/50 ml)
Boiled odor	12 (min)	3,0	1.5	2.2
Boiled taste	13 (min)	2,7	2.0	1.4
Roast odor	11 (min)	1,7	1,0	2.0
Roast taste	11 (min)	1,7	1,0	1.8
Sulfur odor	13 (max)	1,0	1.5	1.4
Sulfur taste	11 (max)	0,8	1,8	1.0
Overall odor	13 (max)	4,8	5.8	6.0
Overall taste	11 (min)	5,8	5.8	6.0
Average maximum	13 (max)			
Average minimum	11 (min)			

1) Overall odor and taste scores ranged 1-9

Table 20. Computer predictions of maximum or minimum levels for L-arginine in basic model system or in combination with other amino acids

	L-arginine[a]	Arg. vs. Gly.[b]	Arg. vs. valine[c]
Boiled odor	12 (min)	9 (max)	6 (max)
Boiled taste	13 (min)	10 (max)	6 (max)
Roast odor	11 (min)	11 (min)	6 (max)
Roast taste	11 (min)	6 (max)	11 (min)
Sulfur odor	13 (max)	11 (min)	5 (min)
Sulfur taste	11 (min)	11 (min)	5 (max)
Overall odor	13 (max)	10 (max)	6 (min)
Overall taste	11 (min)	10 (max)	6 (min)
Average maximum	13 (max)	9 (max)	6 (max)
Average minimum	11 (min)	11 (min)	7 (min)

a) L-arginine was tested singly at 8, 12 and 15 mg/50 ml

b) L-arginine was tested with glycine at 6, 10 and 14 mg/50 ml

c) L-arginine was tested with L-valine at 3, 6 and 9 mg/50 ml system before and after heating (Table 21). Results showed that the sulfur containing amino acids, cysteine and methionine, had the greatest losses during heating (95, 58%, respectively), whereas most other amino acids decreased by 20-30%. Decreases in the amino acids during heating were similar to those in the gelatin-simple sugar mixture except for arginine and valine, which suffered higher losses in the latter system. This could be due to higher concentrations of arginine and valine in the gelatin which would provide more substrate for interaction with the simple sugars to form nonenzymatic browning products.

Amino acid analysis of composite model system. Amino analyses were also performed on the synthetic meat flavor model system (gelatin, amino acids and simple sugars) before and after heating. Results presented in Table 22 reveal that the majority of the amino acids decreased approximately the same amount as in the heated gelatin-simple sugar mixture. Exceptions to this occurred in the case of histidine and lysine, which had higher values, and for threonine, valine and tyrosine, which had lower values than for the heated gelatin-sugar mixture. Despite the additional free amino acids present in the composite model system, the amount of amino acid losses remained essentially the same. Thus, the rate of breakdown may be limited by the amount of available simple sugars, since additional amounts of free amino acids in the model system did not cause further breakdown. Results also demonstrated that there is minimal destruction of amino

Table 21. Amino acid analysis of amino acid - simple sugar model system before and after heating

Amino Acids	Grams of Amino Acids per 100 g Sample		% Loss
	Before Heating	After Heating	
NH_3	1.65	1.57	4.8
L-Arginine	5.46	4.27	21.8
L-Serine	13.64	9.36	31.4
L-Glutamic Acid	11.81	8.71	26.3
Glycine	8.62	6.27	27.3
L-Alanine	17.34	12.22	29.5
L-Cysteine	10.57	0.55	94.8
L-Methionine	7.84	3.30	51.9
L-Isoleucine	7.95	4.98	37.4
L-Leucine	8.37	5.15	38.5
Valine	1.06	0.86	18.9

Table 22. Amino acid analysis of the composite model system (gelatin, amino acids and simple sugars) before and after heating

Amino Acids	Grams of Amino Acids per 100 g Sample		% Loss
	Before Heating	After Heating	
L-Lysine	4.21	2.72	35.4
L-Histidine	1.21	0.55	54.5
NH_3	1.41	1.20	14.9
L-Arginine	9.15	6.64	27.4
L-Aspartic Acid	7.12	5.08	28.6
L-Threonine	1.73	1.39	19.7
L-Serine	6.73	4.88	27.5
L-Glutamic Acid	12.22	9.46	22.6
L-Proline	13.27	9.54	28.1
Glycine	22.45	16.72	25.6
L-Alanine	12.21	8.99	26.4
L-Cysteine	0	0	0
L-Valine	2.90	2.21	23.8
L-Methionine	1.73	0.70	59.5
L-Isoleucine	3.47	2.27	34.6
L-Leucine	50.2	3.45	31.3
L-Tyrosine	0.56	0.38	32.1
L-Phenylalanine	1.71	1.29	24.6

acids by heating unless excess simple sugars are also present.

Fat.

An experiment was designed to test the influence of the type of fat upon aroma and taste in the model system. Results showed that out of 90 samples evaluated by the panels, approximately 46% were correctly identified as to the source (species) of fat added to the model system. However, there was considerable variation among individual panelists. Results showed that some of the panelists were able to correctly identify 80% of the samples, while others were unable to make any correct identifications. Out of the three types of fat tested, beef was most easily identified followed by chicken and pork.

The taste panel results are in agreement with those of Wasserman and Talley (1968), who showed that only one third of all panelists could correctly identify beef, veal, pork and lamb. It also agrees with results reported by the same workers showing that addition of fat greatly improved the number of correct identifications by panel members.

The contribution of fat to species flavor in meat is still not completely understood by meat scientists. No particular component of fat has been isolated that imparts the characteristic species flavor. In this study, addition of fat definitely improved the overall odor of the synthetic meat flavor mixture. This may be explained by the findings of Pippen et al. (1969), who reported that fat may dissolve and retain the aroma components formed during cooking. They indicated that the sulfur compounds in particular were trapped and retained by the fat.

Various levels of fat were tested in the model system to ascertain the best level to enhance flavor. It was found that 2.2 g/50 ml gave the best score. This value is similar to the amount of intramuscular fat (4.57 g/100 g tissue) reported in beef by Hornstein *et al*. (1961).

Salt.

Sodium chloride (100 mg/50 ml) was added to the model system, mainly for taste. Pepper and Pearson (1974) showed that beef adipose tissue treated with salt had less protein breakdown than identical but non-treated tissue. However, it is questionable if the level of fat (4.4%) added to the model system would be affected by the small amount of added salt.

Packaging and Storage of the Model System.

After each individual component was carefully tested and evaluated by the taste panel, all components were combined at the optimum levels determined by the surface response method (Table 2). Various tests were then performed on the shelf life of the model system using different methods of packaging. It was observed that the synthetic meat extract could be refrigerated for 3-4 weeks without losing its meaty flavor. For long term storage, it was found that the sample was stable if freeze-dried and stored under vacuum. The freeze-dried sample had the advantage of being easily rehydrated at low concentrations (2%) yet still possessed a strong meaty odor/taste.

Comparison of the Composite Model System with Commercial Products.

The composite model system was evaluated and compared to some commercially available products. Taste panel results are presented in Table 23. All samples were compared with the model meat system for flavor, odor, mouthfeel and general acceptability. Results showed that the synthetic meat flavor was rated higher than all of the commercial products in every category, but did not receive a score as high as the authentic meat extract.

On evaluating flavor, the synthetic beef model system had a panel score of 4.8 on a hedonic scale of 1-7. Statistical analysis using Dunnett's test (Dunnett, 1964) revealed that the synthetic beef flavor model system was rated significantly higher than commercial sample no. 2 ($P=<0.05$), but was not significantly different from the real beef extract or the remaining commercial products.

On comparing the aroma with the commercial products, it was found that the beef flavor system developed in this study was scored significantly higher (5.0) than commercial sample no. 1 (3.3) and 2 (2.6). However, it was not significantly different from the authentic beef extract (5.5).

On evaluating mouthfeel, the beef flavor model system had a slightly higher score (5.5) than real beef extract (5.3), although the difference was not significant ($P=<0.05$). On the other hand, the beef flavor model system was rated significantly higher ($P=<.05$) than sample no. 2, but was not significantly different from the other commercial

Table 23. Taste panel evaluation of synthetic meat flavor model system and commercial beef flavor preparations

Sample No.	Average Panel Scores (1-7)			
	Flavor	Odor	Mouthfeel	General Acceptability
Sample 1	4.0 ± 1.9	3.3 ± 1.5*	4.5 ± 1.6	4.0 ± 1.4
Sample 2	2.4 ± 1.3*	2.6 ± 1.7*	2.8 ± 1.3*	2.4 ± 1.5*
Sample 3	3.0 ± 1.9	3.5 ± 1.5	3.8 ± 1.4	3.4 ± 1.3
Syn. beef flav. sys.	4.8 ± 1.8	5.0 ± 1.1	5.5 ± 1.7	4.6 ± 1.9
Sample 4	3.4 ± 1.7	3.5 ± 1.6	3.9 ± 2.0	3.4 ± 1.8
Sample 5	3.6 ± 1.4	4.3 ± 1.3	4.5 ± 1.9	3.9 ± 1.6
Authentic beef extract	5.8 ± 1.2	5.5 ± 1.6	5.3 ± 1.8	5.1 ± 1.6

* Significantly different from synthetic beef model system ($p = <0.05$)

a-e The commercial available meat flavor systems were coded as follows: a=Maggi; b=Campbells; c=Wylers; d=Ajimate; e=Herbox.

products.

On evaluating general acceptability, the synthetic beef flavor model system received an average score of 4.6, which was higher than all of the commercial products. However, it was only significantly different (P=<.05) from sample no. 2. Thus, results showed that the model beef flavor system developed by using the surface response method was equal to or superior to the commercial products on the market in all categories.

Analysis of the Meat Volatiles

Analyses of volatiles were also performed on authentic cooked meat extract and the composite synthetic meat model system. The gas chromatography-mass spectrometry (GC-MS) spectra of the two systems were compared in order to determine the contribution of various compounds to meat flavor.

Evaluation of Various Trapping Apparatus.

To insure that all volatiles were extracted from the meat mixture, several different trapping procedures were compared. The Herz and Chang (1966) method was found to have several disadvantages: 1) Large amounts of sample were required for analysis; 2) Too many steps were involved in transferring the samples and could result in losses of the highly volatile components; 3) Too much solvent and meat extract were required during the extraction procedure, which made it difficult to concentrate for GC analysis; and 4) The extraction and concentration time was too long and involved (24 hr and

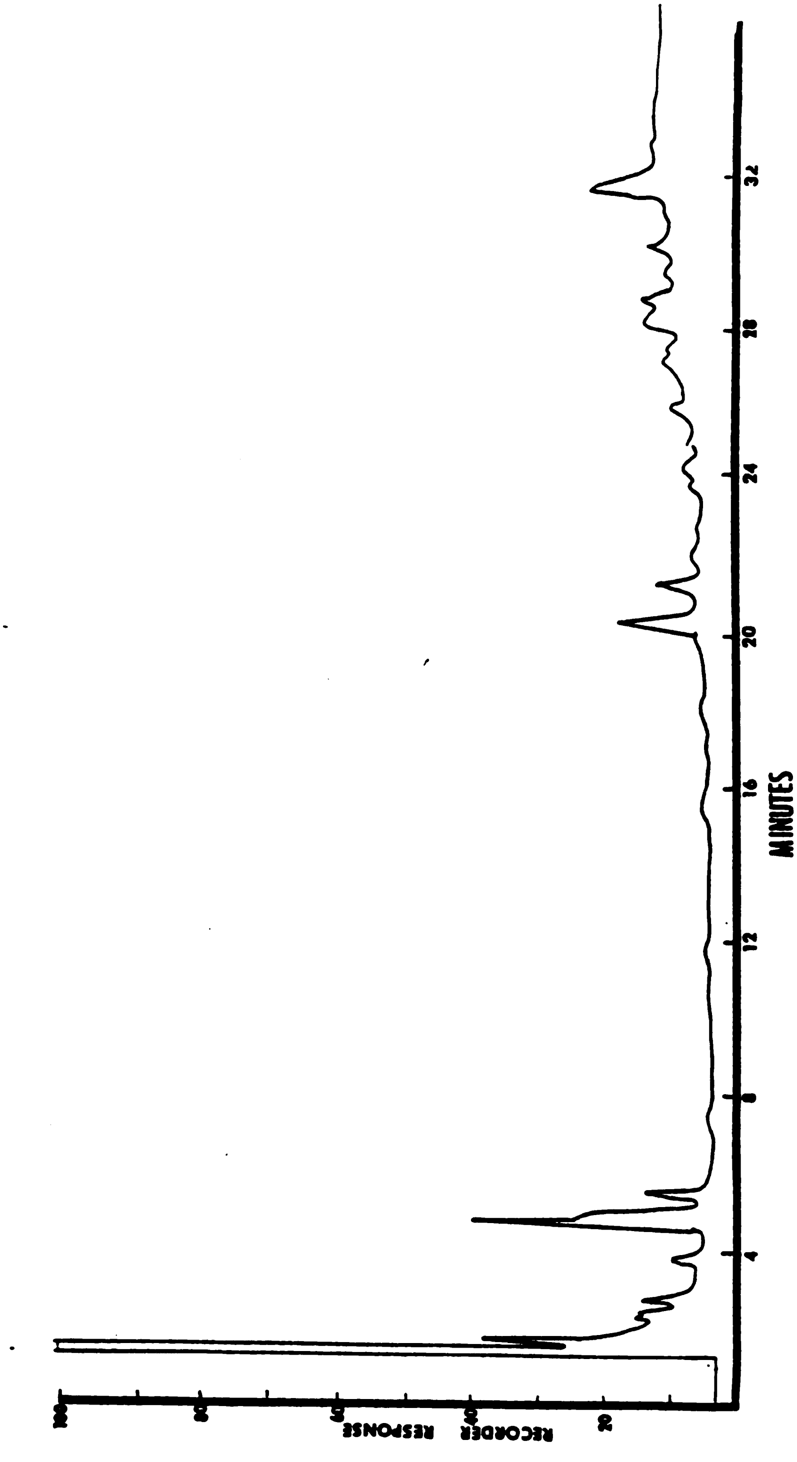
8 hr, respectively).

The recently proposed method of Jennings and Filsoof (1977) for concentrating flavor volatiles using a porous polymer adsorbent was also tested. The method was found to be unacceptable due to a large number of contaminants isolated from the blank sample. GC analysis of the blanks (Fig. 6) showed that several peaks were detected when the column temperature was programed from 70 to 200°C. These peaks were inherent in the blank and were probably derived from the adsorbent. Although the column adsorbent was washed repeatedly with diethyl ether and preconditioned at 200°C for 24 hours, the blank samples continued to show contamination, so the method was abandoned.

The Likens and Nickerson apparatus (Likens and Nickerson, 1964) was finally adopted for this study. The apparatus allowed simultaneous condensation of the steam distillate with the volatiles being trapped in the immiscible extracting solvent, ethyl ether. This method has several very desirable features: (1) Only a small amount of solvent is needed (50 ml) and can be used repeatedly; (2) Only a small amount of sample is required; (3) It has a short extraction period (7 hr); (4) There is very little loss of sample due to transferring; and (5) The total sample preparation time is 1 day compared to 3 days for the Herz and Chang (1966) method.

Several methods were tested for concentrating the ether extract containing the volatile flavor compounds. Removal

Fig. 6. Gas chromatogram of a blank sample obtained from elution of ethyl ether through Porapak adsorbent column. Beckman GC4.5 µl, a 16'x ¼"OD, SS, Carbowax 20 M, 80-100 mesh 80°C to 210°C @ 4°C/min. He 33 CC/min. FID.


MINUTES
4
8
12
16
20
24
28
32
RECORDER RESPONSE
20
40
60
80
100

of ethyl ether from the volatile sample using a Widmer or a Vigreux column required heating of the ether mixture to 37°C. This could result in the loss of some volatiles during the concentration process. The concentration method finally adopted was to evaporate the sample under a slow stream of nitrogen. As the solvent was evaporated, the heat of vaporization lowered the sample temperature, thus minimizing volatile losses.

Gas Chromatography-Mass Spectrometry Analysis (GC-MS).

Volatiles from the synthetic meat flavor model system and the authentic meat extract were initially separated using gas chromatography (Perkin-Elmer GC Model 900). The chromatograms are shown in Fig. 7 and 8, respectively. Results showed that the synthetic beef flavor model system had a very similar GC pattern to that of authentic beef extract.

As over 300 compounds have been identified in beef extract (Dwivedi, 1975), it is vitrually impossible to identify these compounds by means of GC using known retention times. Thus, GC-MS was applied to identify the volatiles isolated from the synthetic meat flavor model system. Even with the aid of MS, however, the identification process was still laborous and difficult. Fortunately, a Spectra-Search computer program was recently set up to aid in identification of various spectra. The Spectra-Search system contains MS spectra for over 70,000 known compounds. By giving the two

Fig. 7. Gas chromatogram of the volatile constituents from boiled beef extract. Perkin-Elmer 900. 3 µl, 10' x 1/8" OD SS. Carbowax 20 MonChromosorb W 80-100 mesh, 70°C isothermally for first 2 min. then programmed 70°C - 200°C @ 2.5°C/min. He 35 CC/min FID.

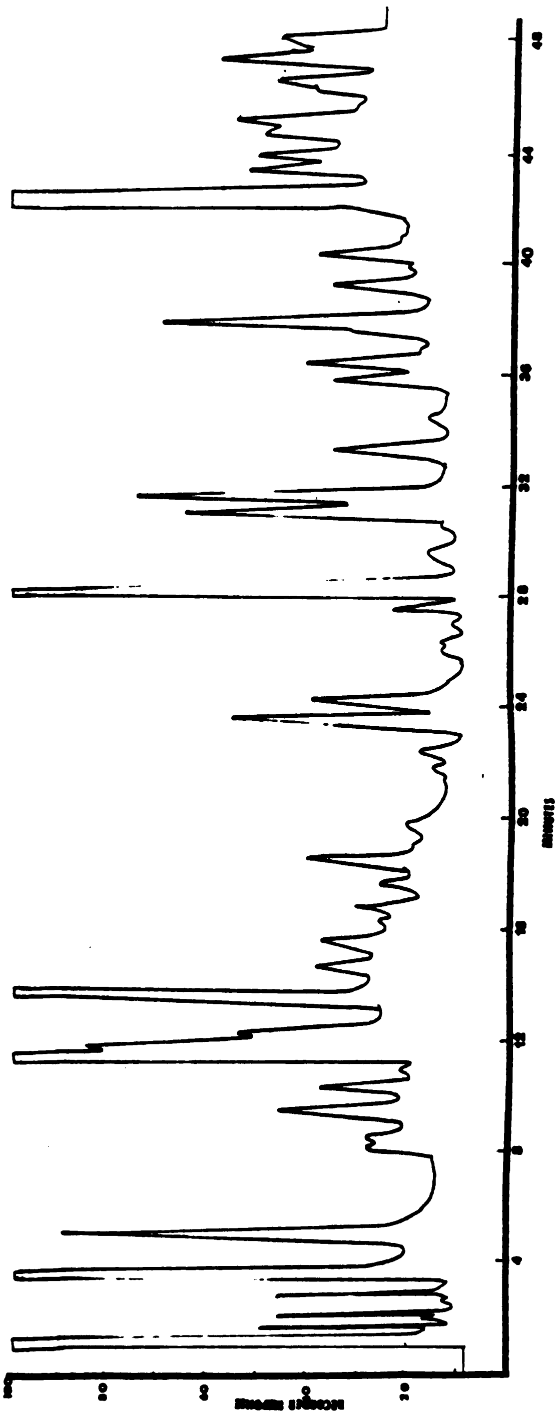
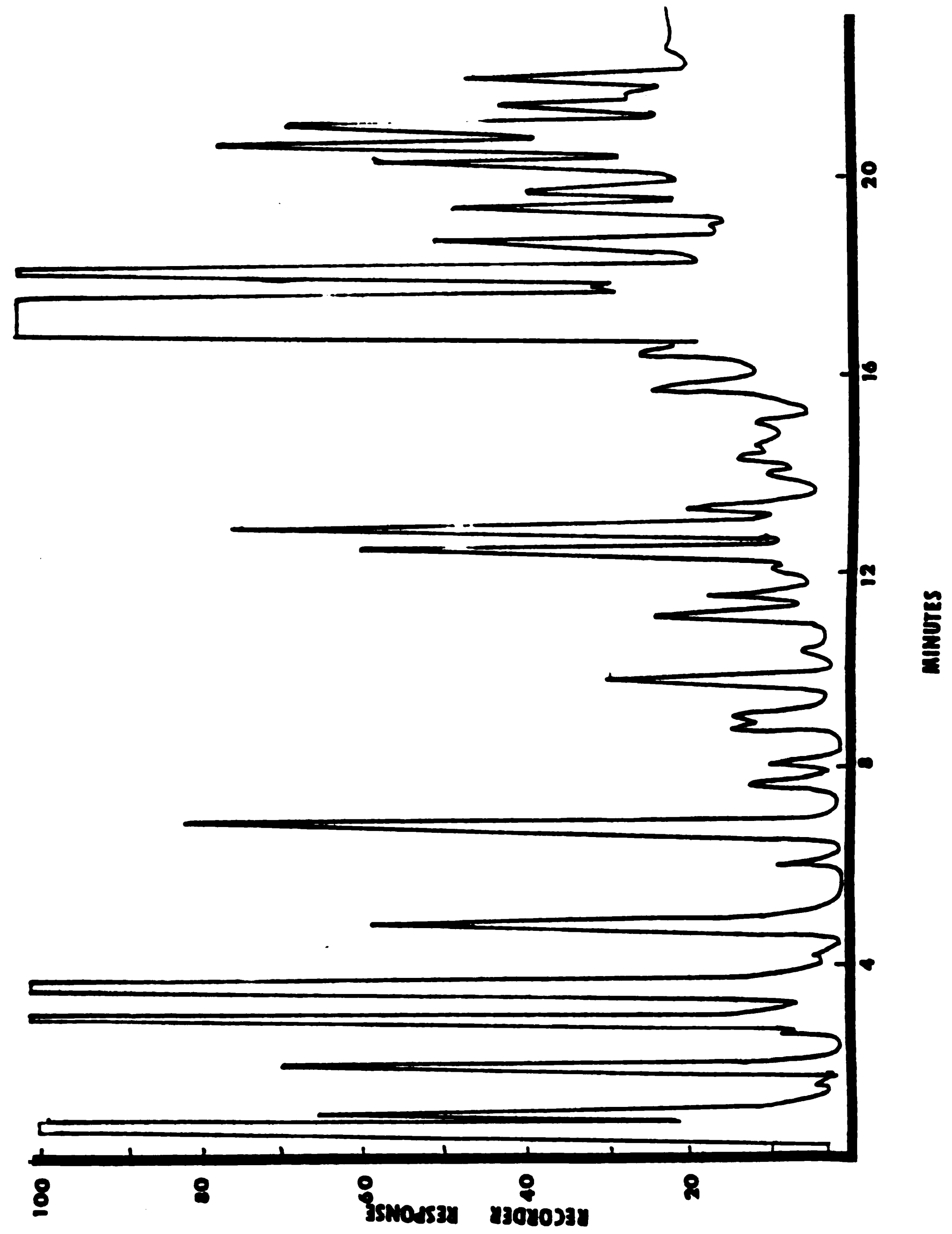

100
80
60
40
20
RECORDER RESPONSE
4
8
12
16
20
24
28
32
36
40
44
48
MINUTES

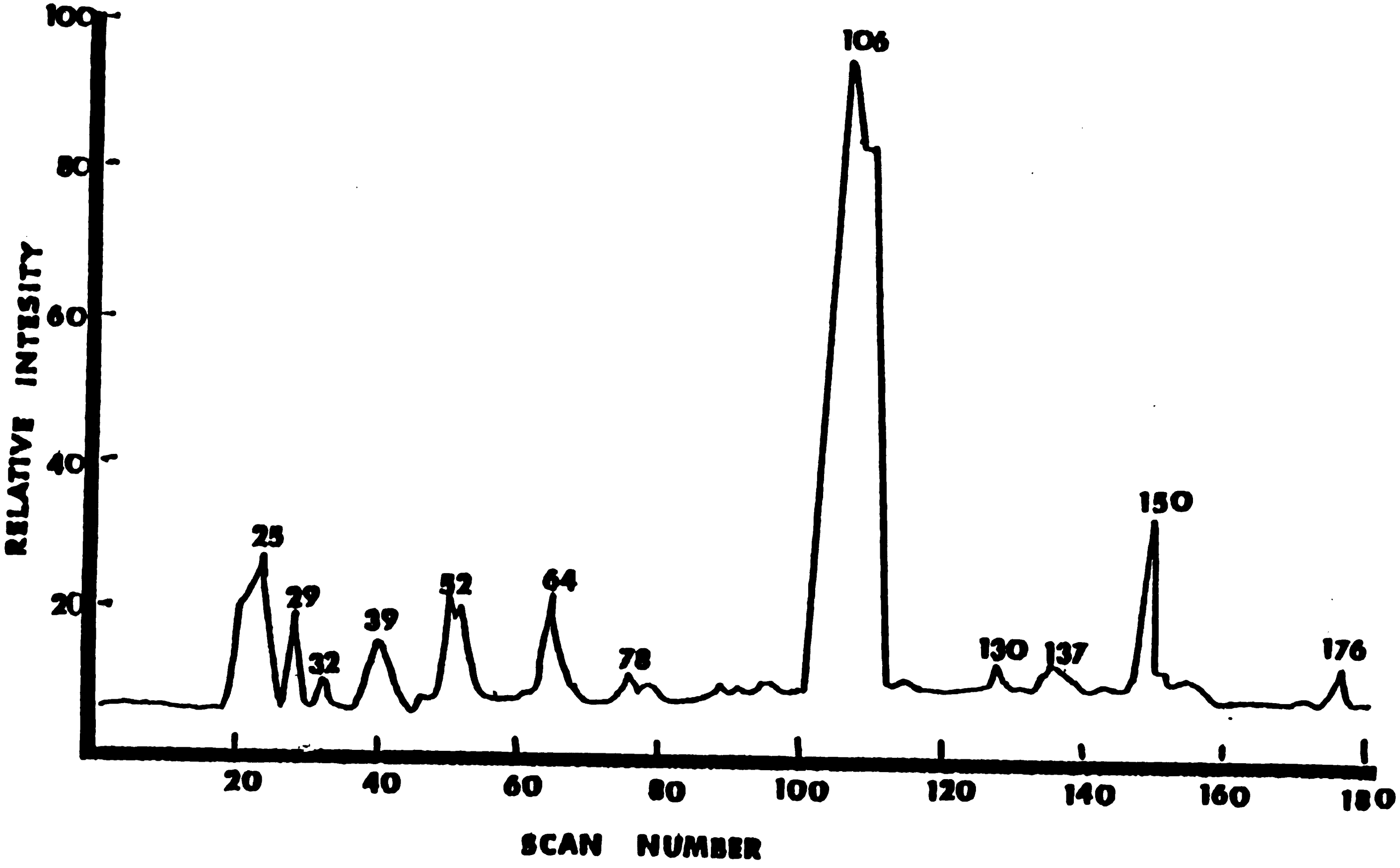
Fig. 8. Gas chromatogram of the volatile constituents from synthetic meat flavor model system. Perkin-Elmer 900.3 µl.10'x1/8" OD SS, Carbowad 20M on Chromosorb W 8-100 mesh, 70°C isothermally for first 2 min. then programmed 70°C @ 6.5°C/min. He 35 CC/min FID.


100
80
60
40
20
RECORDER RESPONSE
4
8
12
16
20
MINUTES

largest intensities for every 14 mass units of the unknown, the computer is able to match the spectral pattern of the unknown with known compounds in the Spectra-Search program. For each unknown, the ten best matching compounds and the relative correlation for each match to that of the unknown is given. The matching program was of tremendous help in identifying unknown compounds in the synthetic meat flavor system. However, it does have its drawbacks. First, it is not known whether all of the compounds found in beef volatiles are among the 70,000 cataloged compounds in the Spectra-Search system. Thus, some compounds may be missing from the system. Second, many of the 70,000 compounds are duplicates of the same compounds because of slight differences in fragmentation patterns. Most of these problems are in the process of being corrected, but at this time all corrections have not yet been entered into the system.

The GC chromatogram obtained from the LKB GC-MS (Model 9000) lacked the resolution of that obtained from the Perkin Elmer GC (Model 900) as shown in Fig. 8 and 9, respectively. The poor resolution from the LKB chromatogram could have been due to several factors: (1) The column diameter (¼ in) used in the LKB was twice as large as that of Perkin Elmer GC (1/8 in), thus, decreasing the ability to resolve the spectra; (2) The flow rate was not properly adjusted so that maximum output could be obtained; and (3) Initiation of sample analysis was delayed too long after collection of the sample, thus many volatiles may have escaped or interacted

Fig. 9. Gas chromatogram of the volatile constituents of the synthetic meat flavor model system using GC-MS. LKB 9000, 3 µl, 10'x1/4", Carbowax 20 M on Chrom W. 80-100 mesh. 70°C isothermally for first 2 min. then programmed 70° - 200°C @ 6.5 C/min. FID.


RELATIVE INTESITY
100
80
60
40
20
SCAN NUMBER
20
40
60
80
100
120
140
160
180
25
29
32
39
52
64
78
106
130
137
150
176

before analysis.

The background was subtracted from each spectrum before submitting it to Spectral-Search. However, identification of any poorly resolved peaks was still difficult. This could be due to: (1) the presence of a distorted fragmentation pattern resulting from sample carry over of previous compounds; (2) the presence of two or more compounds in an unresolved major peak; and (3) the presence of compounds derived from column bleeding. Feeding a distorted fragmentation pattern to the computer for Spectral-Search may have resulted in poor correlations with known compounds. It seems probable that the poor separation of the unknown volatiles submitted for Spectral-Search occurred since poor correlations with the unknown and known spectra were obtained.

Although the original plan called for a comparison of the spectra from the synthetic beef model system and that of authentic beef extract, the spectral analysis of the beef extract was accidentally erased from the computer, and was not available for comparison. However, the spectra of the volatiles from the model meat system were compared to the beef volatiles reported earlier by Dwivedi (1975).

The results of the Spectral-Search of the meat flavor model system are presented in Table 24. Results showed that several classes of compounds that have been previously identified as contributors to beef flavor are probably present in the synthetic meat flavor volatiles. Classes of compounds

Table 24. Spectral-Search of volatiles isolated from synthetic meat flavor model system

Spectra No.[1]	Tentative Compounds	Classes of Compound
25	2,4-dimethyl-undecane	hydrocarbon
29	2-butyl-1-octanol	alcohol
32	2,2,5,5-tetramethyl tetrahydrofuran	furan
39	2,2,4 trimethyl-heptane	hydrocarbon
52	1-undecene	hydrocarbon
64	methylethylether	ether
78	acetyl pyrrolidine	pyrrolidine
106	furan	furan
130	5-methyl-2-furfural	furfural
137	2,2-dimethyl-3,4-pentadienal	aldehyde
150	2-methyl-thiophene	thiophene
176	2,4-bis (methyl-butyl)-phenol	phenol

1) Spectra no. correlated with those shown in Fig. 9.

2) Relative correlation of the unknown compounds to the known compounds

tentatively identified as being present included hydrocarbons, alcohols, ethers, aldehydes, furans, furfurals, and thiophenes.

Three hydrocarbons were tentatively identified in the synthetic beef flavor volatiles (Table 24). These included 1-undecene, 2,4-dimethyl-undecane, 2,2,4-trimethyl heptane. Undecene, undecane and heptane have been reported to be contributors to boiled, roast and canned beef flavor by McLeod and Coppock (1976), Merritt (1966) and Lieblich _et al_. (1972), respectively. Since the model system was formulated under pressure and heated at high temperatures, methylation of the hydrocarbons may have occurred. Merritt (1966) stated that the saturated aliphatic compounds have no odors, while the odors of unsaturated compounds are neither pleasant or meaty.

Examination of Table 24 also showed that low molecular weight alcohols, acids, ketones and aldehydes were not identified in the volatiles of the synthetic meat flavor model system. Due to the volatility of these compounds, they were probably eluted at the beginning of the GC separation and were not identified. Failure to find these low molecular weight compounds in the scan may be due to the fact that the spectral scans were not commenced until 2 minutes after sample injection. Earlier reports (Herz and Chang, 1970; Dwivedi, 1975; and Chang and Peterson, 1977) have indicated that the low molecular weight compounds probably contribute little to meat flavor.

Only one alcohol (2-butyl-1-octanol) with a molecular weight of 226 was tentatively identified in the model system. Octanol has been reported in the volatiles from boiled and roast beef by Lieblich _et al_. (1972) and Watanabe and Sato (1972). According to Herz and Chang (1970), the majority of alcohols present in beef extract are of low molecular weight and are present in only small amounts, which makes detection difficult.

2,2-dimethyl-3,4-pentadienal was the only aldehyde tentatively identified in the model system. It has not been previously reported to be a constituent of meat flavor volatiles.

The detection of methylethylether in the model system probably came from the solvent, since only pentyl ether has been previously isolated from beef flavor volatiles.

2,4 bis(methyl-butyl)-phenol has not been previously reported as a constituent of meat flavor volatiles. Since it is not certain whether all compounds found in meat volatiles are among the 70,000 known compounds, 2,4 bis (methylbutyl) phenol could be due to a mismatched spectrum.

Acetyl pyrrolidine was also tentatively identified in the model meat system. It has not been previously reported in meat flavor volatiles, but may be derived from breakdown of hydroxyproline, which is a major constituent of gelatin. However, pyrroles have been reported in roast beef flavor volatiles by Lieblich _et al_. (1972).

5-methyl-2-fufural, 2,2,5,5-tetramethyl tetrahydrofuran, and furan were tentatively identified in the volatiles from the model system. They are probably products of nonenzymatic browning. Furan appear to be present in large amounts as indicated by the large broad peak at scan 106 on the CG chromatogram (Fig. 8). Herz and Chang (1970) have suggested that furans and furfurals may plan an important role in the development of meat flavor.

In addition, 2-methyl-thiophene was tentatively detected in the synthetic meat flavor model system. It was probably derived from the interaction of xylose and cysteine HCl as has been reported previously by Mussinan and Katz (1968). They have suggested that thiophenes may play an important role in meat flavor.

SUMMARY

A synthetic meat flavor model system was developed by using the principle flavor precursors previously identified by other investigators. Selection of the basic components for the model system was based on the theory that the majority of the meat flavor was developed from interaction of simple sugars (glucose, ribose, xylose) with sulfur containing amino acids (cysteine or methionine) to form non-enzymatic browning products. Glycoprotein was also incorporated into the model system to improve mouthfeel and to mask the harsh sulfury taste derived from the breakdown of sulfur-containing amino acids. In addition, 5'-nucleotides were added to the system to enhance meat flavor, whereas, fat was added to give the model system a meaty and brothy taste. The levels of the components were elevated by a taste panel, and the results were analyzed statistically by the surface response method in order to obtain optimum concentrations.

The contribution of individual amino acids to the development of meat flavor within the model system was evaluated by the taste panel at wide concentration ranges. The surface response method showed that certain amino acids (serine, alanine, taurine, and cysteine) were required at concentrations approximately twice as high as others (leucine, methionine, isoleucine, valine, arginine and glycine). This suggested that the amino acids needed at high concentrations probably contributed more toward meat flavor than those required at lower levels.

On testing combinations of amino acids in the model system most amino acids had approximately the same computer predicted level as when tested singly. The similarity in concentration on testing singly or in combination with other amino acids indicated that there is probably little synergistic or inhibitory effect among majority of the amino acids. On the other hand, isoleucine and arginine, had lower predicted levels when combined with other amino acids, which suggests that they exerted an inhibitory effect upon flavor development of other amino acids.

Taste panel evaluations showed that the surface response method is an excellent procedure for predicting the optimum level of components necessary to produce maximum acceptability. However, at lower concentration ranges, panelists were not able to differentiate between small changes in concentration, thus lowering the sensitivity of the method.

Amino acid analyses showed that majority of amino acids in the gelatin-simple sugar mixture and the amino acid-simple sugar mixture decreased by 20-30% during heating. However, the sulfur containing amino acids methionine and cysteine decreased by 58 and 95%, respectively. Amino acid analysis of the composite model system exhibited the similar loss following heating. Results indicated that simple sugars appear to be the limiting factor, since 70-80% of amino acids remained following heat treatment.

The synthetic meat flavor model system was compared with some commercially available products for flavor, odor, mouthfeel and general acceptability. Results showed that the synthetic meat flavor system consistently received higher average panel scores than all commercial products, but was not scored as high as the authentic meat extract.

Evaluation of several trapping apparatus for the isolation of meat volatiles showed that the Likens and Nickerson apparatus was the best method for trapping meat volatiles. The method involved simultaneous extraction and steam distillation of the meat flavor volatiles, thus minimizing volatile losses occurring during isolation and concentration.

Volatiles isolated from the synthetic meat flavor model system were separated by GC-MS. By feeding the spectral pattern of the unknown volatiles isolated from the synthetic meat flavor model system to a Spectral-Search computer program composed of the spectra for over 70,000 known compounds, the unknowns were tentatively identified. Results showed that the synthetic meat flavor model system contained several classes of compounds, which included hydrocarbons, alcohols, ethers, aldehydes, furans, furfurals and thiophenes. These classes of compounds have been previously identified as constituents of meat flavor volatiles.

APPENDIX

Appendix Table 1. Taste panel score card for evaluation of synthetic meat flavor model system

Directions: Evaluate the sample by first sniffing the sample and then circle the value for each category which best describes the level of the odor.

Then place a few drops of the sample in your mouth using the micropipet provided. Circle the value which best describes the taste for each category. Do Not Swallow the Sample. Expectorate into the cup provided. Please rinse your mouth thoroughly with water between samples.

Please use the standards provided to reacquaint yourself with the odor characteristics. Feel free to sniff and taste the standards as many time as you like. If you run out of standards, please let me know.

SAMPLE NO.______

Boiled Meat Flavor			Burn Roast-Like			Sulfurous		
Odor		Taste	Odor		Taste	Odor		Taste
0	No boiled meat flav.	0	0	No rst. flavor	0	0	No sulfur	0
1	Faint boiled mt.	1	1	Faint rst flav.	1	1	Faint sulfur	1
2	Slight boiled mt.	2	2	Slight rst flav.	2	2	Slight sulfur	2
3	Moderate boiled mt.	3	3	Moderate rst flav.	3	3	Moderate sulfur	3
4	Strong boiled mt.	4	4	Strong rst flav.	4	4	Strong sulfur	4
5	V. strong boiled mt.	5	5	V. strong rst flav.	5	5	V. strong rst. flav.	5

Others, specify: sharp, bloody, potato-water, garlic, onion, sour, sweet, bitter, fatty

Overall odor and flavor:	Odor[a]	1	2	3	4	5	6	7	8	9
	Flav.[a]	1	2	3	4	5	6	7	8	9

a) Odor and flavor were evaluated on a hedonic scale of 1-9 where

1 = dislike extremely
2 = dislike a lot
3 = dislike
4 = slightly dislike
5 = neigher
6 = slightly like
7 = like
8 = like a lot
9 = like extremely

Appendix Table 2. Incomplete block design used for taste panel evaluation of meat model system

Block reps I and II							Reps III and IV						
(1)	1	2	4	5	7	8	(4)	1	2	5	6	7	9
(2)	2	3	5	6	8	9	(5)	1	3	4	5	8	9
(3)	1	3	4	6	7	9	(6)	2	3	4	6	7	8
Reps V and VI							Reps VII and VIII						
(7)	1	3	5	6	7	8	(10)	4	5	6	7	8	9
(8)	1	2	4	6	8	9	(11)	1	2	3	4	5	6
(9)	2	3	4	5	7	9	(12)	1	2	3	7	8	9

Conditions for the block design can be expressed by the following:

$t=9$, $k=6$, $r=8$, $b=12$, $\gamma=5$, $e=0.94$

Appendix Table 3. Score sheet for evaluation of synthetic meat flavor model system and commercial beef flavor preparations

Please use the following scales to evaluate the samples:

	Flavor	Odor	Mouth-feel	General Acceptability
7	Meaty, pleasantly spiced	Meaty	Brothy	Excellent
6				
5	Slightly off; slightly bland or slightly too much spice	Mod. meaty	Mod. brothy	Good
4				
3	Mod. off; mod. bland of mod. spiced	Slightly meaty	Slightly brothy	Poor
2				
1	Off flavor; very bland or overly spiced	Not meaty	Not brothy at all	Unacceptable

Sample No.	Flavor	Odor	Mouthfeel	General Acceptability

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