

**THERMAL REACTION OF ANISALDEHYDE IN THE PRESENCE OF L-CYSTEINE, A MODEL
REACTION OF CHINESE STEW MEAT FLAVOR GENERATION**

By

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ABSTRACT OF THE THESIS

THERMAL REACTION OF ANISALDEHYDE IN THE PRESENCE OF L-CYSTEINE, A MODEL REACTION OF CHINESE STEW MEAT FLAVOR GENERATION

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Chinese stew meat is very popular in China due to its special flavor generated during cooking process. However, how these flavors are produced has not been studied yet. Star anise is one of the indispensable ingredients in the formation of Chinese stewed meat flavor. One of the most important volatile components in star anise is anisaldehyde. Anisaldehyde may react with sulfur-containing constituents of meat such as cysteine to form components of sensory significant during the cooking of stewed meat. Cysteine is major precursor for meat flavor generation.

We present here results of a study that tested the thermal reaction of anisaldehyde in the presence of cysteine under certain conditions. Anisaldehyde was mixed with cysteine, then the mixture was heated to both 60 ° C, 80 ° C, 120 ° C and 150 ° C for two hours by oil bath. To determine both volatile and non-volatile compounds, products were separated into two parts. In order to detect non-volatile

compounds, one part of the products was extracted by ethyl acetate. One major non-volatile product was purified from the sample by HPLC. Mass spectrometry was used to determine the structure of that major non-volatile compound, which is identified as 4-methoxybenzothialdehyde. For volatile compound detection, the other part of the product was extracted by dichloromethane and the volatile compounds were then determined by using GC/MS. Several major sulfur containing aroma compounds were identified besides various products generated during cysteine thermal degradation. Detection for both volatile and non-volatile compounds indicates that anisaldehyde can react with cysteine and may influence the meat flavor generation during the Chinese stew meat cooking process.

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DEDICATION

I dedicate this work to my parents for their love and support.

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1. INTRODUCTION

Chinese stewing is a very popular cooking method throughout most of northern, eastern, and southeastern China. It usually requires a slow cook process which may take up to several hours and the cooked items must be submerged in the cooking liquid. A typical cooking liquid usually consists of soy sauce with various herbs, spices and seasonings. This cooking method can provide a dark red-brown color as well as unique flavors for Chinese stewing dishes. Among all the dishes, Chinese stewing pork is one of the most popular one. The flavor of Chinese stewing pork is characterized by the use of two indispensable ingredients: soy sauce and star anise. The influence of soy sauce on meat flavor generation has been well studied (Liu, 2001), while there are few researches on how star anise contributes to the flavor generation. List of Tables

Star anise is a small evergreen tree native to southwestern China and northern Vietnam (Editorial Board of Flora of China, 2004). The fruit is star-shaped and are harvested before they are ripe and will change color from green to dark brown on sun drying. It has a licorice-like, sweet and pungent flavor, similar to anise but slightly more bitter and pungent (Wang *et al.* 2011).

The fresh fruit pod yield 5% to 8% essential oil, and the dried fruit pod has 2.5% to 3.5% (Heath, 1981). The major components of the oil are typically *trans*-anethole and methyl chavicol. *P*-anisaldehyde is obtained when *trans*-anethole is heated in water during a typical Chinese stewing process (FIRDI, 1988). *P*-anisaldehyde is a

colorless to slightly yellowish liquid with a sweet, mimosa, hawthorn odor (Bauer *et al.* 2008). It is frequently used in sweet blossom compositions as well as in flavor compositions for confectioneries and beverages. It can be hydrogenated to anise alcohol and readily oxidizes to anisic acid when exposed to air (Ho *et al.*, 1999).

L-cysteine is a sulfur-containing amino acid that detected naturally in all high-protein foods: all meats and poultry, dairy and eggs. It is a well-known precursor of meat flavor (Mottram, 1991). Thermal degradation of L-cysteine leads to the formation of the corresponding amines and hydrogen sulfide via decarboxylation (Zhang and Ho, 1988). Mercapto acetaldehyde and acetaldehyde were identified as common products when L-cysteine was boiled with various carbonyl compounds (Zhang and Ho, 1988). The interaction of these products can form various types of heterocyclic compounds, such as thiazolidine, trithianes, trithiolanes and dithiazines which usually contribute to the characteristic food flavor especially meat flavor (Umano, 1995).

During the cooking of Chinese stew pork, *p*-anisaldehyde may react with the sulfur-containing components such as L-cysteine to generate aroma compounds which significantly influence the stew meat flavor.

In this research, we use *p*-anisaldehyde and L-cysteine to imitate the presence of star anise during the Chinese stew pork cooking process to understand how star anise influence the flavor generation.

The first objective of our research is to simulate cooking process of Chinese stew meat. Detect and identify non-volatile and volatile compounds produced during

thermal reaction

The second objective is to apply semi-quantitative analysis on the volatile compounds and study the flavor properties of those compounds.

2. LITERATURE REVIEW

2.1. Star Anise

Star anise is the star-shaped fruit of a small evergreen tree, *Illicium verum* Hook. F.. It is native to southwestern China and northern Vietnam. It is now cultivated in China, Vietnam, Laos, Philippines, India, Japan, and Korea. The fruits are harvested before they are ripe and will change color from green to dark brown on sun drying (Stobart, 2000). Star anise has a licorice-like, sweet and pungent flavor, similar to anise but slightly more bitter and pungent. The spicy, sweet flavor given by it become intense as the cooking process prolonged.

2.1.1 Botany and use of star anise

The plant is classified in the genus of the family Illiciaceae, order Illiciales, subclass Magnoliidae, and class Magnoliopsida, division Magnoliophyta (Editorial Board of Flora of China, 2004). It is a median-size evergreen tree reaching up to 15 meters in height. The trees have leathery to thickly leathery, aromatic leaves which are blade obovate-elliptic, oblanceolate, or elliptic. Flowers are pink to dark red, broadly elliptic to broadly ovate. The fruit is star-shaped, reddish-brown, made up to 1-seeded boat-shaped carpels, usually 8 in number, radiating from a central axis (Wang *et al.* 2011). This species is cultivated for perfume, medicines, and as a culinary spice in China, as well as in Vietnam.

The dried fruit is used as spice in China, its sweet and licorice flavor helps enhance the flavor of Chinese stewing dishes. It is also a dominant ingredient in

Chinese five-spice blend (Stobart, 2000). Chinese five-spice blend is a subtle Chinese spice mixture, consists of finely ground anise pepper, star anise, cassia, cloves and fennel seed, and can be used in marinades, barbecues, roasts, stews and soups that require long simmering.

In Southeast Asia, Vietnamese use star anise to formulate beef noodle soup called Pho (Chempakam *et al.*, 2008). The spice can also be traced in Thai, Malaysian and Singaporean cuisine. The flavor of star anise can be detected in some of the cooking of Kerala in southern India; in some dishes of north India it may be used as a cheaper substitute for anise seed (Uhl and Raghavan, 2000)

Star anise is said to have been first brought to Europe at the end of the sixteenth century. There is little used of the spice in western cooking. However, it is widely used as flavorings in commercial baked goods, confectionery, cough syrups, cough drops, oral care, chewing gum, tobacco and the licorice-flavored liqueurs such as pastis and anisette (Morton, 2004)

2.1.2. Chemistry of star anise

Volatile extraction techniques

A traditional extraction technique used in China is steam distillation. The cost of this process is quite low compared to any other methods, although thermal degradation, hydrolysis and low relative response ratio of water soluble fragrance constituents may occur during this steam distillation (Reverchon, 1997).

Volatile composition

Star anise oil is a colorless to pale yellow liquid, the color of the essential oil may differ from one to another due to different extraction techniques. It has a high

melting point and solidifies on cooling.

The fresh fruit pod yield 5% to 8% essential oil, and the dried fruit pod has 2.5% to 3.5% (Heath, 1981). The major components of the oil are typically *trans*-anethole (85 to 90%), which tastes like licorice and are used in anise liqueur, anise brandy, licorice sweets, and toothpaste, etc.; 2% methyl chavicol, related to chavicol, one of the chemicals that give black pepper its bite. It also contains various other aroma components such as Vanilla-flavored anisaldehyde; estragole, the main constituent of oil of tarragon; eucalyptol, a spicy cool chemical smells like camphor; and lemony, scented limonene (Rinzler, 1990). The thermal oxidation of *trans*-anethole has been studied. *P*-anisaldehyde was obtained when *trans*-anethole was heated in water (Ho *et al.*, 1999). Figure 1 shows the thermal oxidative decomposition products of *trans*-anethole.

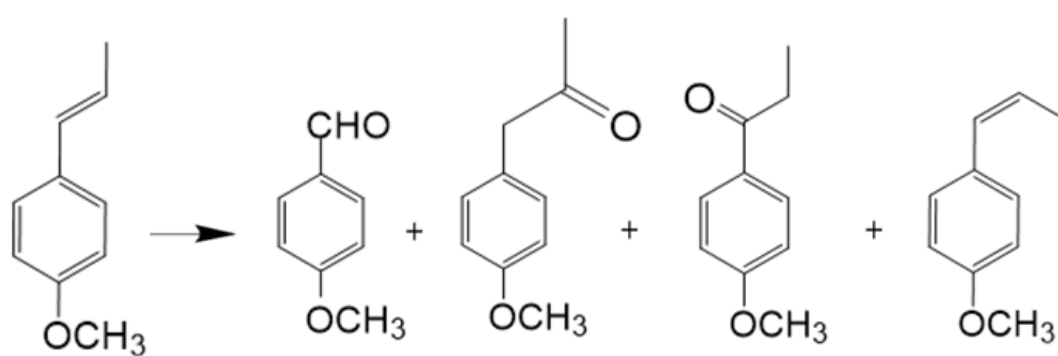


Figure 1. The thermal oxidative decomposition products of trans-anethole

2.1.3. *P*-anisaldehyde from star anise

P-anisaldehyde consists of a benzene ring substituted with an aldehyde and a methoxy group in para-substitution. It is a colorless to slightly yellowish liquid with a sweet, mimosa, hawthorn odor (Bauer, Garbe, *et al.* 2008). This component is frequently used in sweet blossom compositions as well as in flavor compositions for confectioneries and beverages. *P*-anisaldehyde can be hydrogenated to anise alcohol and readily oxidizes to anisic acid when exposed to air.

Previous study (Cortes, *et al.* 1987) shows the reaction between cysteine and aromatic aldehyde may generate certain type of thiazolidine, which is a group of well-known meat aroma compounds. Figure 2 is the overall reaction of cysteine and aromatic aldehyde under room temperature.

Work done by Huynh-Ba and Matthey-Doret (2003) indicates that multiple sulfur aroma compounds, such as benzylthiol, can be generated from the baker's yeast biotransformation of the cysteine-benzaldehyde conjugate. Table 1 shows the volatile compounds identified from the biotransformation of the cysteine-benzaldehyde conjugate.

During the cooking of Chinese stew pork, *p*-anisaldehyde may react with the sulfur-containing components such as l-cysteine or cystine to generate aroma compounds which significantly influence the stew meat flavor.

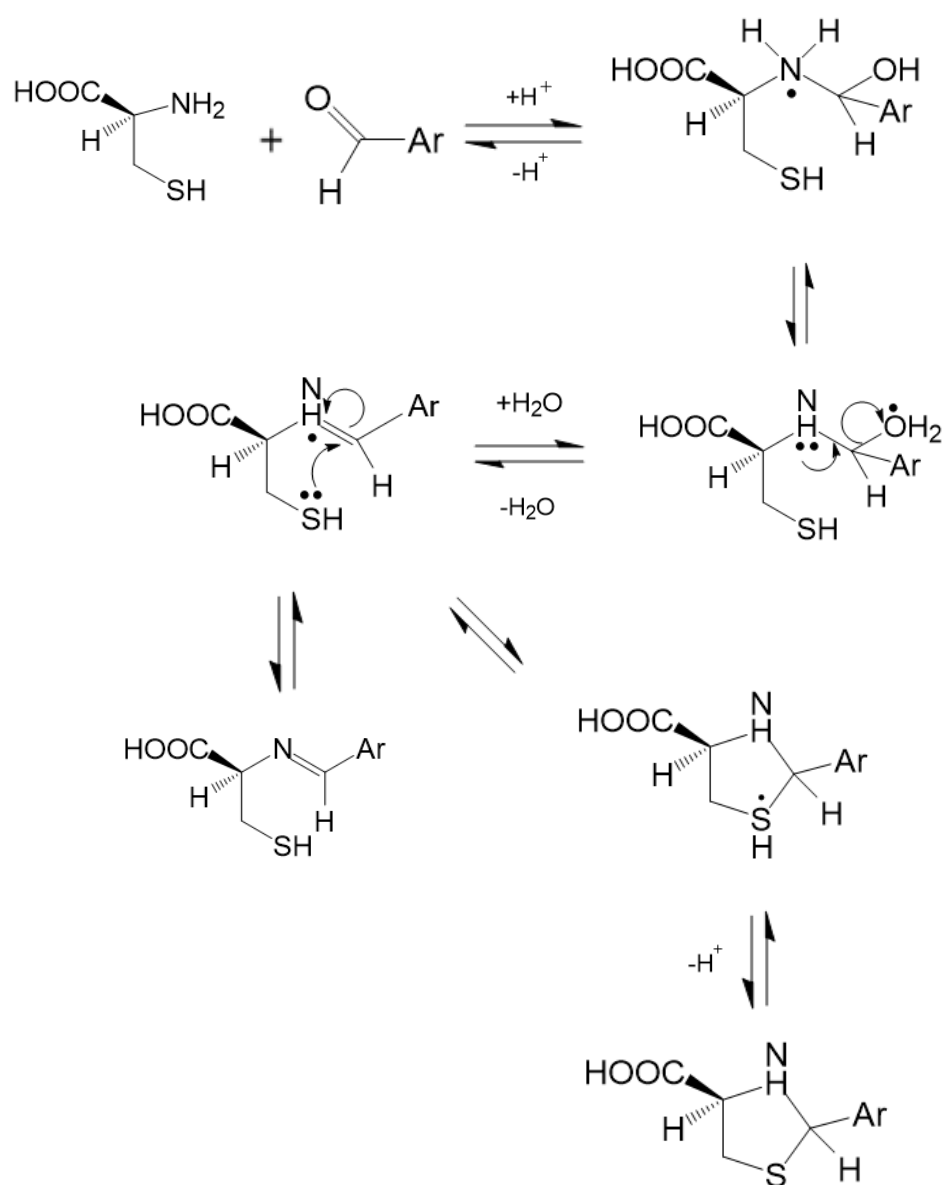


Figure 2. Overall reaction of cysteine and aromatic aldehyde under room temperature

Table 1. Volatile Compounds Identified from the Biotransformation of the Cysteine-Benzaldehyde Conjugate (Ba 2003)

compound	Aroma quality (GC-O)
S-methylthioacetate	cheese, eggy
thioacetic acid	sulfury, onion, garlic
acetic acid	acid, pungent
1-mercapto-2-propanol	
2-mercapto-1-ethanol	
benzaldehyde	almond, sweet aromatic
2-methylthio-1-ethanol	green, sulfury
propanoic acid	fruity, sour
benzylthiol	cabbage, roasted
benzylmethyl sulfide	
2-methylthio acetic acid	
benzy acetate	green, fresh, fruity
benzyl alcohol	balsamic, fruity
phenylethanol	fruity, sweet, floral
S-benzylthioacetate	
3-methylthiopropanoic acid	
4-methylthiobutanoic acid	
benzoic acid	

2.2. L-cysteine

Raw meat has little or no aroma and only bloody-like taste, in other words, meat flavor is thermal derived (Mottram, 1998). The volatile aroma compounds are formed by components of the raw meat. These components, or so called flavor precursors, react together to present volatiles responsible for certain types of meat flavors during cooking or thermal processing. A review of literature shows that more than 1000 volatile compounds found in meat have been identified. A large number of beef flavors have been identified compared with any other meats, although this may be due to a much larger number of publications related to beef than those focus on pork, poultry or fish meat. (Maarse and Visscher, 1996; Mottram, 1991)

The major meat flavor precursors can be divided into two parts: water soluble compounds and lipids. As sulfur-containing aroma compounds are major volatiles found in cooked meat, it has been accepted that sulfur-containing amino acids, such as cysteine and cystine, are indispensable for generating meat-like aroma during thermal processing (Gasser and Grosch, 1988; Farmer and Mottram, 1990; Block *et al.*, 1992). A removal of sulfur compounds would result in almost completely loss of meaty odor (Minor, 1965).

The main reactions result to meat flavors are the Maillard reaction between amino acids and reducing sugar, lipid degradation and fatty acid oxidation. Besides these, thermal degradation of certain components, such as sulfur-containing amino acids or peptides, has also been studied (Calkins, 2007).

2.2.1. Structure of cysteine

Cysteine is a semi-essential α -amino acid and has a thiol side chain. This sulfur-containing amino acid occurs naturally in foods and can also be manufactured by the body from other amino acid. L-cysteine in the form of cysteine presents in all high-protein foods: all meats and poultry, dairy and eggs, even many varieties of processed meats contain this amino acid. Two thiol substituted cysteine can be oxidized to form one cystine, and the disulfide bond of cystine cleaves under high temperature (Huang and Ho, 2001).

2.2.2. Maillard reaction

The Maillard reaction, or so called nonenzymatic browning, was reported by Louis Maillard in 1912 when he was studying the reaction between sugar and amino acids. Now Maillard reaction has been studied for a long time and has been considered as one of the most important reaction for quality of foods, especially thermal treated foods. It has a great impact on sensory properties such as aroma, taste and color by producing numerous products (Purlis, 2010). Through Maillard reaction, the nutrition value of food can be reduced by decrease of nutritional component or formation of toxic compounds (Gokmen, 2008a; Mottram, 2002; Stadler, 2002), but can also be increased by formation of antioxidative products (Morales, 2009; Yoshimura, 1997).

Chemical pathway

The Maillard reaction requires the presence of compound possessing a free amino group, reducing sugar and water. While in food, the amino-bearing compounds usually are proteins, peptides, amino acids, and other amine compounds.

Glucose, fructose and ribose are the most common reducing sugar in food, while disaccharides such as lactose, maltose and sucrose which can be cleaved into reducing saccharides also are important reactants for the Maillard reaction. Aldehydes and other carbonyls originate from sugar derivatives also participate in the browning reaction (Zdzistaw E et. al, 2008).

The first kinetic scheme of Maillard reaction was not proposed until 1953 by Hodge. Usually, this nonenzymic browning reaction can be divided into three stages. The first stage is the condensation between an amino group and a reducing sugar with the formation of glycosyl amine, followed by the Amadori/Heyns rearrangement. Second stage is the dehydration of the Amadori/Heyns product resulting sugar fragmentation and release free amino group. In the final stage, amino groups participate again and undergo dehydration, fragmentation, cyclization and polymerization reactions to form various melanoidins. Figure 3 is a general overview of flavor generation by the Maillard reaction proposed by Ho (1996)

Depending on reaction conditions, such as different reducing sugars and amines participate in the reaction, pH value, temperature, reaction time and water content, the products of Maillard reaction differ and therefore sensorially detectable or not detectable properties will differ (Zdzistaw E et. al, 2008).

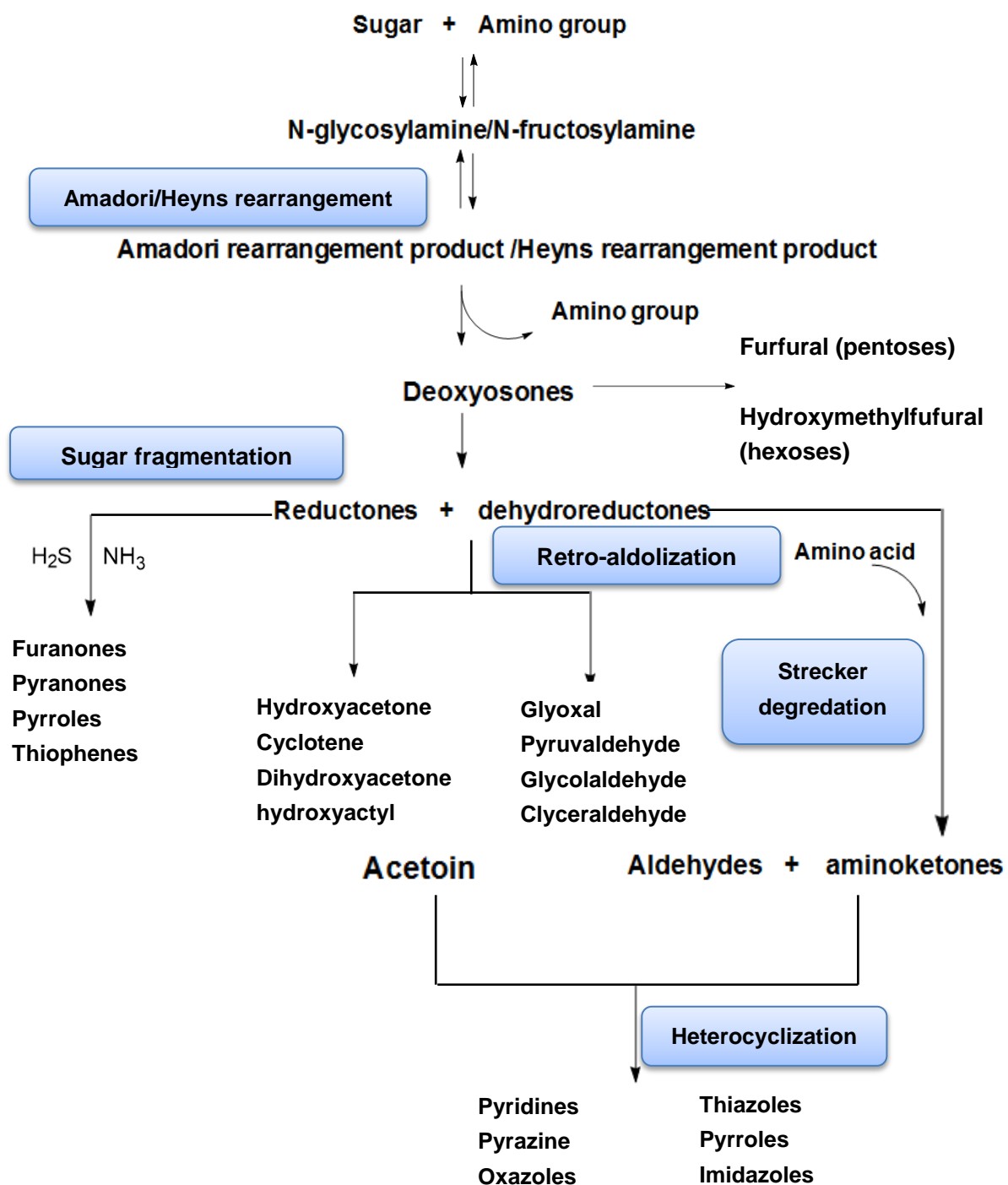


Figure 3. General overview of the maillard reaction showing flavor compounds as end products (Ho 1996.)

Parameters Affecting the Maillard Reaction

Composition and Concentrations

As reported by Maillard, sugar structure affects the browning outcomes in this reaction. Disaccharides were found to have less extent of browning than monosaccharide. Also amino acids are responsible for the formation of different products. Compared with reactions between sulfur-containing amino acids and sugar, there were less meat flavor compounds generated by non-sulfur-containing amino acids, which illustrated the importance of sulfur containing group for the generation of meat aroma compounds. However, browning is inhibited by sulfur-containing compounds as they react with sugar, aldehyde or ketone group and decrease their reactivity. The initial concentration and ratio of reactants influence the Maillard reaction as well. Kato (1969) observed that the browning activity of fructose was stronger than that of glucose at a low concentration of glycine. When in higher concentration of glycine, the result was contrary.

pH value

The Maillard reaction is highly effected by pH value. The rate of the Maillard reaction increases with increasing pH value, and it was generally reach its maximum at slightly alkaline medium. Research done by Nicoli, Anese, and Lericci (1993) shows that browning and reaction rate constant increase smoothly between pH 2 to pH 8, but they increase abruptly between pH 8 to pH 10. Also, pH value may have effect on which reaction route preferred and what kind of product to be generated. The degradation pathway of the Amodori, enolisation and retro-aldolisation, is pH

dependent (Huyghues-Despoints and Yaylayan, 1996). The amount of reactive unprotonated amino group increases with the increasing pH. Furthermore, the pH value has an effect on the reactant reducing sugar. The amount of open chain form of the sugar, which is considered to be reactive, increases with pH (Yaylayan, 1993). And ionization of sugar is rate-dependent, which means it will be effected by pH value of the reaction. In summary, the initial step of the Maillard reaction is highly influenced by the initial pH of the system.

The reaction temperature and time

Temperature affects both the rate and the mechanism and browning increases with temperature. Since the Maillard reaction is a multi-steps reaction, each step has different temperature sensitivity. Therefore, under different temperatures, reaction route of the whole browning reaction differs. Martins (2004) studied the influence of temperature on glucose/glycine Maillard reaction, the degradation steps in the glucose/glycine was shown to be Arrhenius-like. Martins and Van Boekel (2004) also reported that duration of the reaction is important for the Maillard reaction extent.

Water content

The water content has great influence on the activation energy. In the range of water activities between 0.3 and 0.7, the browning rate increases with the increasing temperature. At low water content, the reaction is slow; it increases with increasing water content until at a much higher level the concentration of reactants are influenced by the water solution, which leads to a decreasing of reaction rate again (Zdzistaw E et. al, 2008). Furthermore, research done by Hofmann and Schieberle

(1995, 1998) shows that under roasting conditions and aqueous conditions, volatiles obtained from cysteine and ribose, glucose or rhamnose differ. In all three reaction systems, the dry-heating process favoured the formation of the roasty, popcorn-like aromas 2-acetyl- and 2-propionyl-2-thiazoline as well as potato-chip-like 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine.

2.2.3. Strecker degradation

Strecker degradation was first discovered by A. Strecker in 1862 and was defined by Schonberg and Moubacher (1952) as degradations of alpha amino acids to corresponding alpha-amino ketones and aldehydes containing one less carbon atom, or strecker aldehyde. The Strecker degradation of amino acids in food is mostly induced by reactions between alpha amino acids and indigenous carbonyl compounds generated from Maillard reaction, leading to various flavor-significant aliphatic aldehydes. Recently, the Strecker degradation has also be studied for the formation food color compounds (Rizzi, 2006). An overall mechanism of Strecker degradation is show in Figure 4.

Cysteine has been associated with food flavor especially meat flavor due to the thiol side chain attached with it. Fujimaki and Kobayashi (1965) studied ninhydrin-induced strecker degradation of cysteine and identified hydrogen sulfide, acetaldehyde and mercaptoacetaldehyde as the reaction products.

Mulders (1973) also observed hydrogen sulfide, acetaldehyde and ammonia formed in a cysteine/cystine-ribose model system. An aroma compound, 2-acetylthiazole was isolated from that system. In order to understand the formation

mechanism, a cysteine/2-oxopropanal Strecker degradation system was used by the same research group. A Strecker intermediate, 2-acetylthiazolidine was formed and could form that 2-acetylthiazole via dehydrogenation.

It has now been accepted that products of cysteine Strecker degradation such as hydrogen sulfide and its reaction products aldehydes, furfurals, and furanones are important reactants for the formation of heterocyclic compounds in later reactions.

2.2.4. Cysteine thermal degradation

Thermal degradation of amino acid results to the formation of corresponding amines by decarboxylation. For sulfur-containing amino acids, additional reactive products generated during the thermal degradation. These reactive products have lots potential to react together and produce various types of heterocyclic compounds, which have been considered to be important for characteristic food flavors. Therefore, sulfur-containing amino acids are accepted as very important food flavor precursors.

The photolysis of cysteine was studied by Obata and Tanaka (1965a and 1965b) via an aqueous solution consists of cysteine and ribose. Hydrogen sulfide, ammonia, carbon dioxide and acetaldehyde were found during the research. They also observed a typical rice flavor formed in an aqueous mixture of hydrogen sulfide, ammonia and acetaldehyde.

In 1967, Merritt and Robertson analyzed 17 amino acids and 10 peptides by pyrolysis-gas chromatography and mass chromatography. They reported that a sulfur containing group in cysteine and cystine resulted in a more complicate pyrolysis

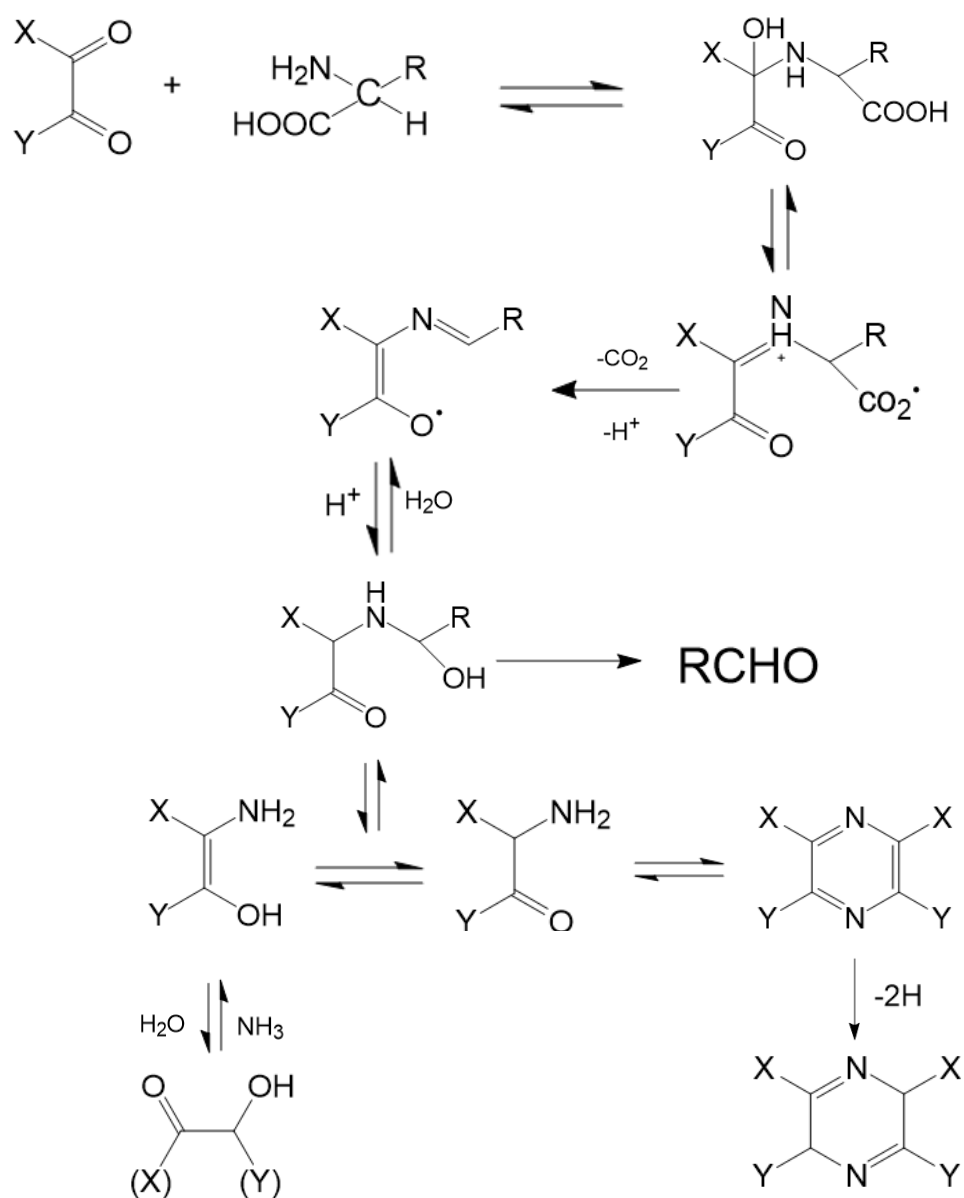


Figure 4. Mechanism for the Strecker degradation of α -amino acids Rizzi (1999)

production patterns than those produce by the simpler amino acids. Sulfur dioxide and carbon disulfide had also been detected and served as an indicator for the presence of sulfur-containing group in cystine. Methyl thiophene was a unique product among all 24 volatiles identified from cystine pyrolysis products.

In 1969, research on pyrolysis of sulfur-containing amino acids (By Masao Fujimai *et al.*) was conducted. Cysteine was heated at 270-300 ° C under reduced pressure of nitrogen. Several volatile compounds were identified including ethylamine, mercaptoethylamine, ammonia, hydrogen sulfide as well as 2-methylthiazolidine.

A possible formation pathway of 2-methylthiazolidine was also proposed. Mercaptoethylamine was generated from cysteine via decarboxylation and acetaldehyde was produced when hydrogen sulfide was lost before decarboxylation. They considered 2-methylthiazolidine to be the product of the interaction of acetaldehyde and mercaptethylamine.

Later in 1973, additional volatile compounds produced by pyrolysis of sulfur containing amino acids (Masao Fujimaki *et al*) had been reported. By using gas chromatography coupled mass chromatography, more volatile were identified. L-Cysteine was heated at high temperatures under nitrogen. Besides previously reported 2-methylthiazole, more thiazolidine, thiophenes, thiazoles and pyridines were identified including at least 11 thiophene compounds. Those new identified volatiles suggested degradation products such ammonia, acetaldehyde and cysteamine might be important as food flavor precursors.

Boelens (1974) studied the interactions between primary and secondary products of cysteine thermal degradation. After saturated aldehydes reacted with hydrogen sulfide at atmospheric pressure and room temperature, 2,4,6-trialkyl-1,3,5-dioxathianes, 4,6-trialkyl-1,3,5-oxadithianes, 2,4,6-trialkyl-1,3,5-trithiane were obtained. Interaction between acetaldehydes and hydrogen sulfide under enclosed condition formed 1,1-alkanedithiols and *bis*(1-mercaptoethyl) sulfides, which were easily oxidized to 3,5-dialkyl-1,2,4-trithiolanes. *Bis*(1-mercaptoalkyl) sulfides disproportionated to 2,4,6-trialkyl-1,3,5-trithianes when they were treated with acids. *Bis*(1-mercaptoalkyl) sulfides also reacted with ammonia to generate 2,4,6-trialkyldihydro-1,3,5-dithiazines and 2,4,6-trialkyltetrahydro-1,3,5-thiadiazines. Figure 5 shows the formation pathway of these sulfur-containing compounds.

Ledl (1976) studied the thermal degradation of cysteine in soybean oil other than aqueous solution and found different types of heterocyclic compounds including dithianes and trithiolanes.

Shu (1985) studied the pH effect on the thermal degradation of cysteine. In one research, cysteine in dilute aqueous was heated in closed model system under 160 ° C at pH 2.3 and pH 5.5. Three novel volatiles were found in the pH 5.5 volatile mixture: ethyl 1-(ethylthio)ethyl disulfide and the corresponding tri- and tetrasulfides. Only the disulfide was found in pH 2.3 mixture. A mechanism for the formation of these sulfides was proposed as well. Formation mechanism for these three compounds proposed by Shu is shown in Figure 6.

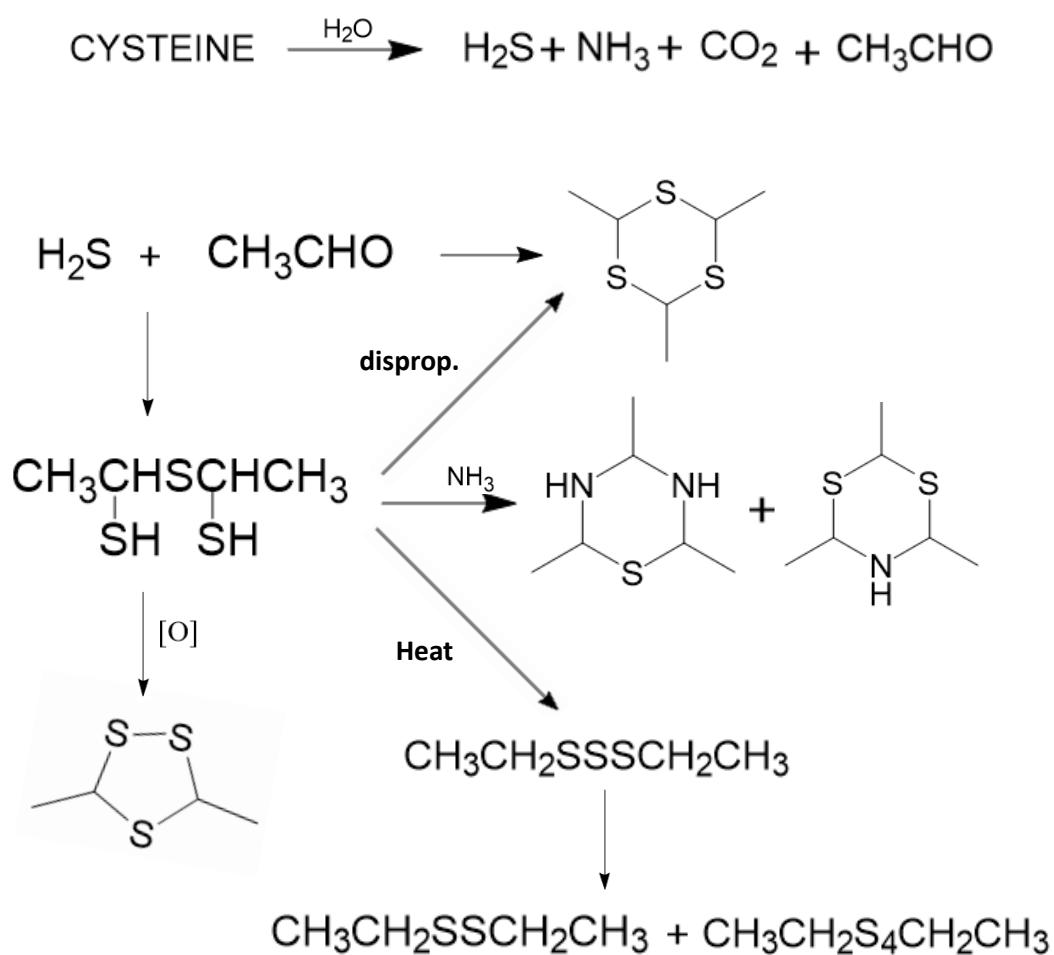


Figure 5. The formation pathway of ethyl 1-(ethylthio)ethyl disulfide and the corresponding tri- and tetrasulfides (Boelens 1974)

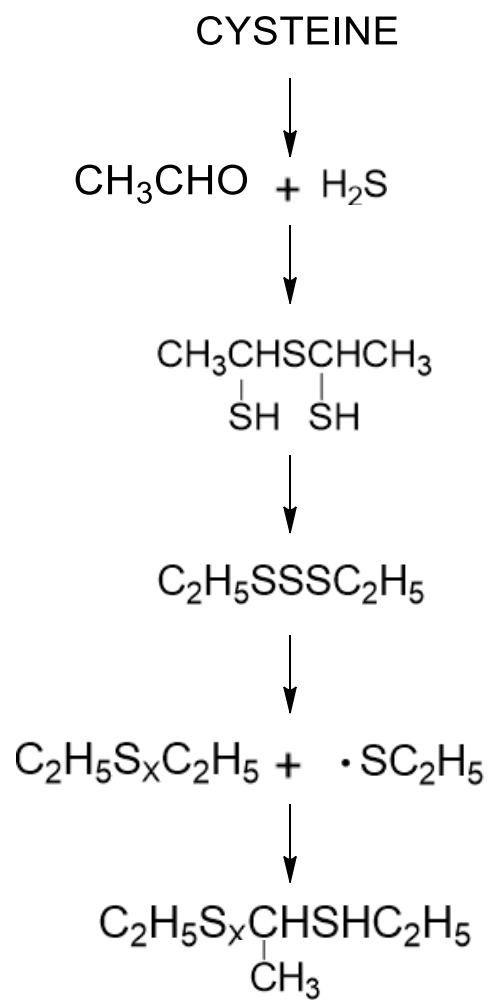


Figure 6. The formation pathway of ethyl l-(ethy1thio)ethyl disulfide and the corresponding tri- and tetrasulfides (Shu 1985)

In the following research, Shu (1985) studied the pH effect by conducting the thermal degradation at three pH values. pH 2.2, pH 5.1 and pH 7.1 were chosen to represent the pH values below, around, and above the isoelectric point of cysteine. At pH 2.2 the major components formed were 1,2,3-trithia-5-cycloheptene and 2-thiophenethiol. The trithiacycloheptene compound was found for the first time in a model system and had a roasted onion and roasted meat odor. A proposed formation mechanism is shown in Figure 7. Degradation occurred at pH 5.1 was vigorous while it was mild at pH 7.1.

The comparison of the thermal degradation volatile products of cysteine and glutathione has been studied by Zhang and Ho (1988) By imitating a frying temperature in water solution under 180 ° C at pH 7.5, 34 volatile compounds were identified including several newly identified components in the cysteine thermal degradation volatile products. One of those compounds, 2,4,6-trimethylperhydro-1,3,5-thiadiazine, was also observed by Boelens (1974) in a reaction mixture of acetaldehyde, hydrogen sulfide and ammonia (molar ratio 1:1:1) under enclosed condition. Effect of water contents on the thermal degradation was reported that between 20% and 80%, maximum volatiles were produced. Figure 8 is a possible mechanism of the formation of sulfur-containing compounds from cysteine degradation. Based on the literature cited, a summary of cysteine thermal degradation pathways are shown in Figure 9.

Umano and Shibamoto (1995) studied the volatile compounds released by cysteine when it was heated under 180 ° C alone in a glass tube. Fifty-two volatile

compounds were collected by three different traps and identified, most of which were pyridines, thiazoles, thiazolines, thiazolidines and thiophenes. This method was able to detect some of volatiles had not been reported in the cysteine degradation system before, which the researcher suggested as the advantage taken from the trap collection method. However, since the water activity influenced the thermal reaction a lot as we reviewed before, the identification of newly found volatiles may also due to the much lower water activity condition of this reaction compared with previous ones, which mostly were conducted under higher water activity condition or even in a cysteine water solution.

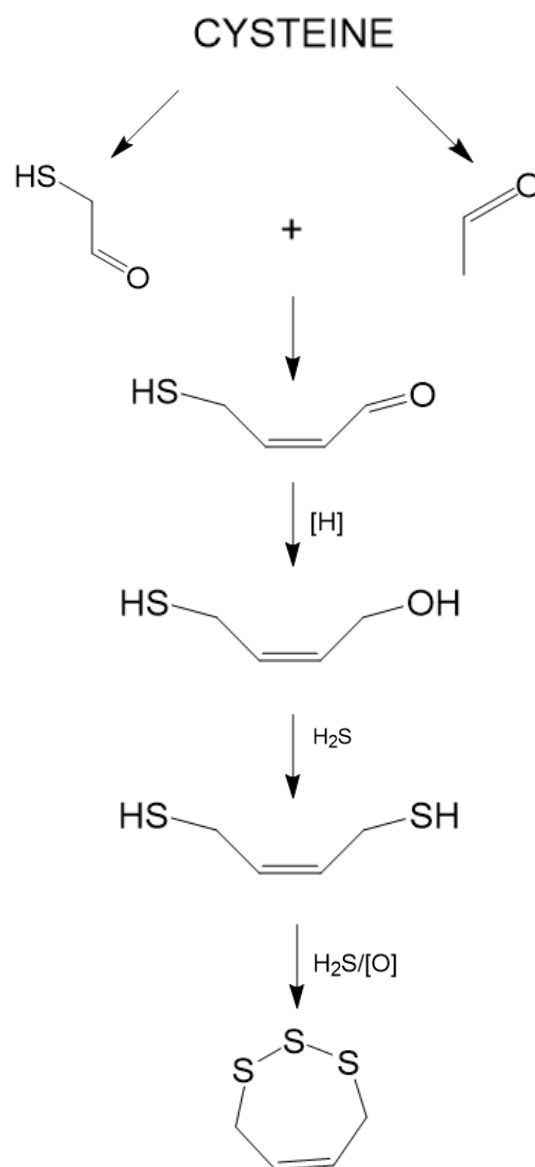


Figure 7. The formation pathway of 1,2,3-trithia-5-cycloheptene (Zhang and Ho 1988)

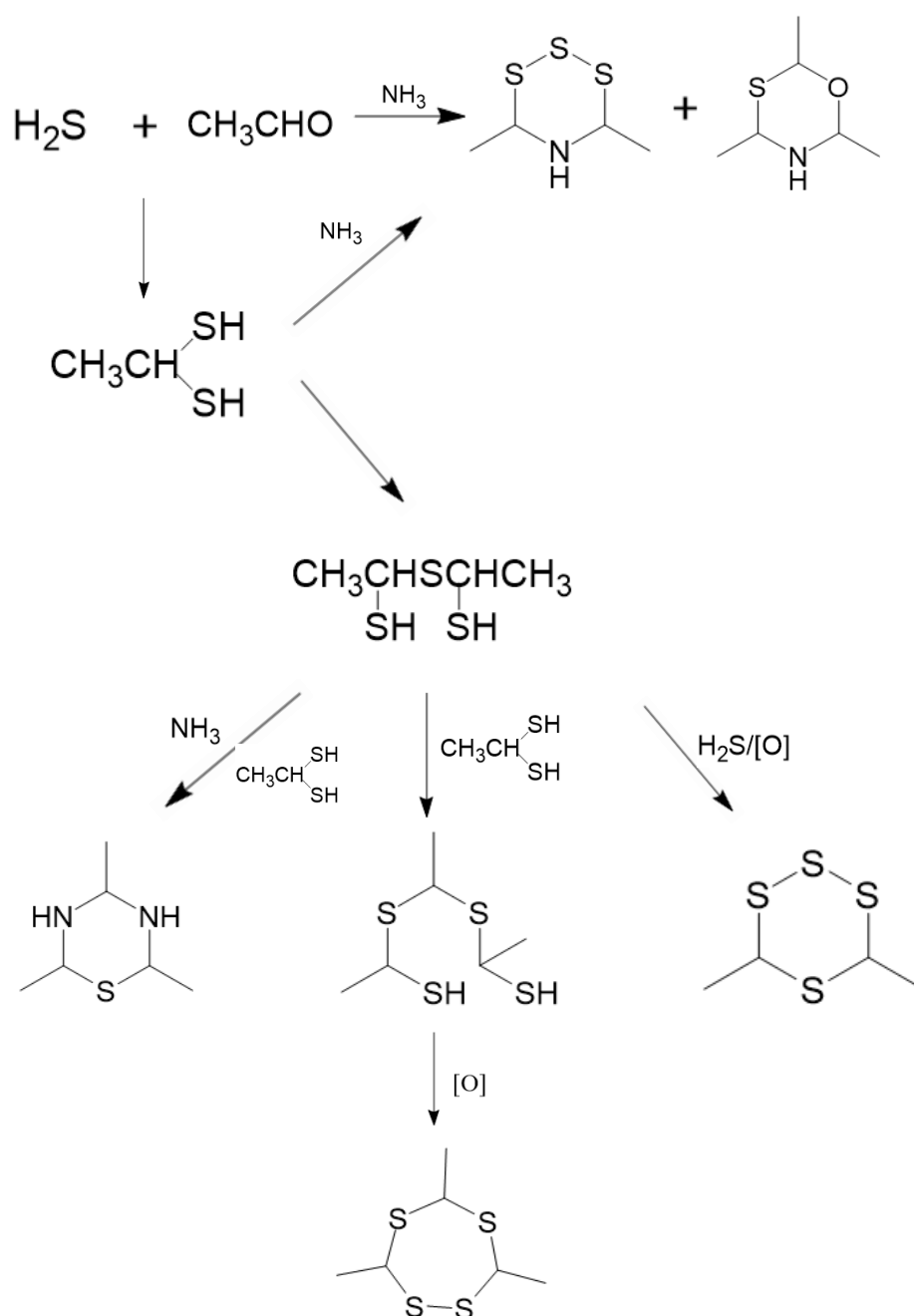


Figure 8. Possible mechanism of the formation of sulfur-containing compounds from cysteine degradation (Zhang and Ho 1988)

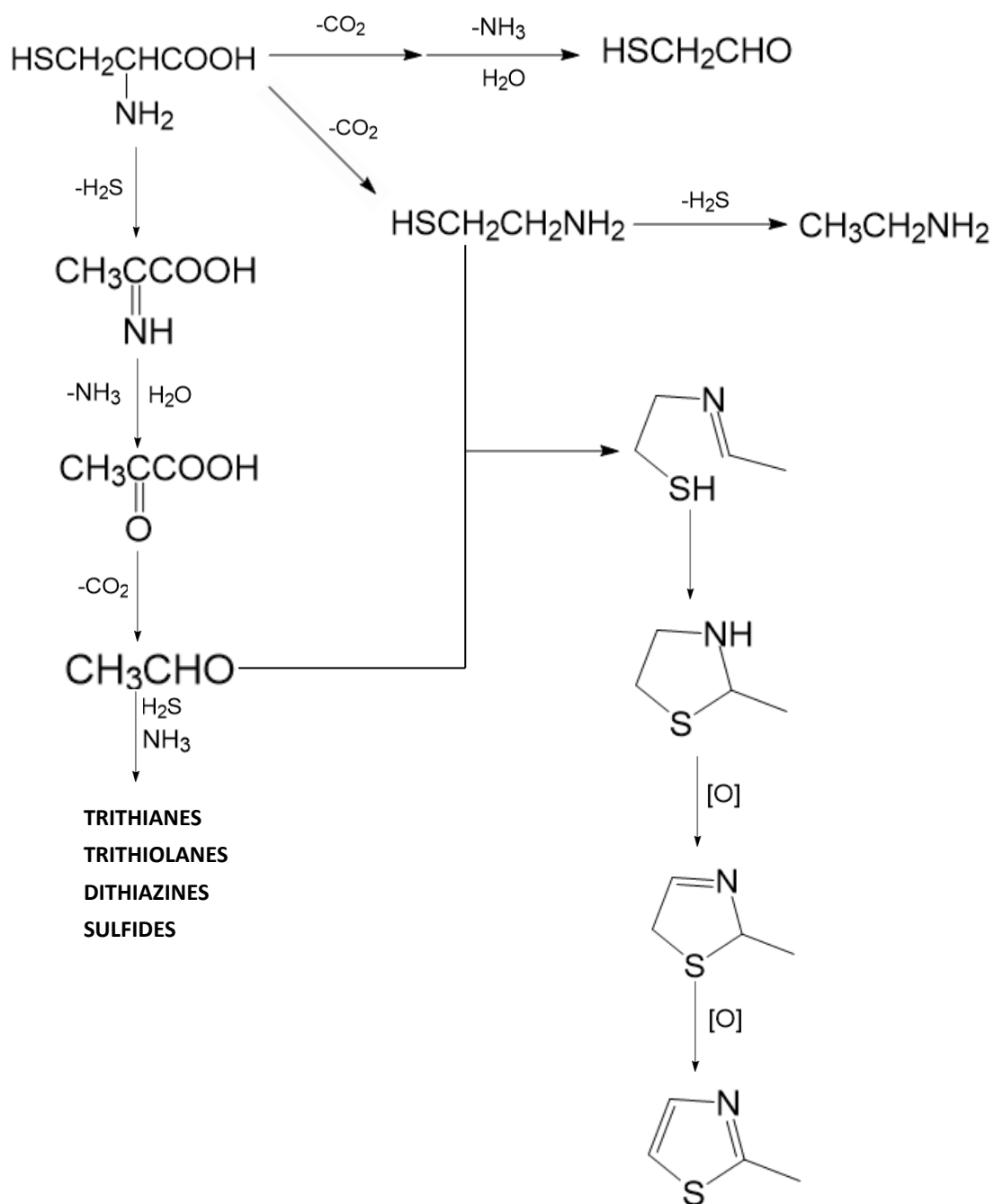


Figure 9. Thermal degradation of cysteine (Zhang and Ho 1988)

3. EXPERIMENTAL SECTION

3.1. Chemicals

Reactants:

L-cysteine (reagent grade, 99% pure) and *P*-anisaldehyde (FCC, 97.5+%) were purchased from Sigma-Aldrich Chemical Co., (St. Louis, MO). Sodium hydroxide (Certified ACS ≥ 97.0 % pure) was purchased from Fisher Scientific International, Inc.

Flavor standards

4-methoxy- α -toluenethiol was purchased from TCI AMERICA (Portland, OR). 2,3,5-Trimethylpyrazine was purchased from Sigma-Aldrich as the internal standard.

Solvents and other

Ethyl acetate (Reagent grade) and methanol (General use, HPLC-UV grade) were purchased from Pharmco-AAPER (Brookfield, CT). Dichloromethane (ACS, reagent grade, 99+ %) and Sodium sulfate (Granular, 99+%, ACS reagent) were purchased from Sigma-Aldrich.

3.2. Sample preparation

Enclosed reaction system:

For each sample, 0.01 mol/L *I*-anisaldehyde and 0.05 mol/L cysteine were dissolved in 75 mL DI water. The mixture pH was adjusted to 5.5 by using 10% NaOH and sealed. Then each of the mixture was heated in oil bath under 60 ° C, 80 ° C, 120 ° C and 150 ° C for two hours. After cooled to room temperature, a reaction mass was obtained. Duplicate reactions were conducted for the model system's accuracy.

Isolation of the non-volatiles:

Reaction mass was transferred to a separatory funnel. 50 mL of ethyl acetate was used to extract the organic compounds from the aqueous mixture, extracting separately in three times in the ratio of 2:2:1. The extractions were carefully separated by gently shaking the separatory funnel up and down 10 times, twice at each separate section. The extractions were collected and stored in a 250 mL Round-bottom flask before drying. Then we dried the ethyl acetate extractions by using rotary evaporator (BUCHI ROTAVAPOR, R-114). At last, dissolve the dry sample in 10 mL methanol. Then these 10 mL methanol solutions were stored in sample vials in a refrigerator.

Isolation of the volatiles:

Reaction mass was transfer to a separatory funnel. Fifty milliliters of dichloromethane was used to extract the organic compounds from the aqueous mixture, extracting separately in three times in the ratio of 2:2:1. Same extraction method was used as for the non-volatiles. 9.375×10^{-5} mol (approximate 11.2 μ L) 2,3,5-trimethylpyrazine used as internal standards was added to each cooled sample before the mixture was transferred. The extractions were carefully collected and stored in a 50 mL Round-bottom flask. 10 g of sodium sulfate was added to the flask earlier to eliminate water content in the volatiles extractions.

Concentration of the volatile:

After drying over sodium sulfate, the extractions were concentrated to 10 mL under a stream of nitrogen in the flask first. Then these semi-concentrated

extractions were transferred to a 10 mL insulated tip concentrator tube. Ten milliliters extractions were slowly further concentrated under nitrogen stream to a final volume of 1 mL. Each of the concentrations was stored in a sample vials in freezer before the GC-MS analyses.

3.3. Detection and purification

High Pressure Liquid Chromatography (HPLC) Analyses

A Dionex UltiMate 3000 LC Systems equipped with a reverse phase C-18 column (ODS HYPERSIL, Dim 256*4.6; Particle Sz. 5 μ m, Thermo Fisher Scientific Inc.). Mobile phases were water and acetonitrile. The operation programs were as follows: the percentage of acetonitrile was gradually increased from 50% to 90% in first 10 minutes, then stayed at 90% for 4 minutes, dropped back to 50% in one minute, kept that percentage till end. Detection UV wavelength was 214 nm and the flow rate was 1 mL/min.

Semi-Preparative High Pressure Liquid Chromatography Purification

A Gilson Semi-Preparative HPCL system was used for the purification of non-volatiles. The system includes Gilson 322 HPLC pumps, Gilson 156 UV/VIS detectors and Gilson GX-271 Liquid Handler. Column used in this process was a reverse phase C-18(2) column (150*21.20 nm i.d.; Particle Sz. 5 μ m; Pore Size 100 Å, Luna, Phenomenex Inc, Torrance, CA). Mobile phases were water and acetonitrile. The details of program were as follows: the initial percentage of acetonitrile was 30%, when the final was 70% under gradient-run. Retention time was 10 minutes and flow rate was 20 ml/min.

3.4. Identification

Liquid chromatography-electron spray ionization mass spectrometry (LC-ESIMS).

An HPLC-MS system was composed of an auto-sampler injector (Agilent Technologies, Santa Clara, CA), an HP 1090 system controller, with a variable UV wavelength 190-500 nm detector, an ELSD (Evaporizing Laser Scattered Deposition) detector and an ESI-MS detector from Micromass VG Platform II mass analyzer (Micromass, Beverly, MA). ESI-MS conditions were as following: acquisition mode, ESI-positive; mass scan range, 100-800 amu; scan rate, 0.4 sec; cone voltage, 25 volts; source temperature: 150 °C; probe temperature: 550 °C. Analytical HPLC conditions on HPLC-MS: column: Chromeabond WR C18, 3 mm, 120 Å; length and OD: 30 x 3.2 mm; injection volume, 15 µL; flow rate: 2 mL / min; run time: 3 min. Mobile phase consisted of acetonitrile and H₂O with 0.05% TFA, typical gradient of 10 - 90 % acetonitrile and the gradient varied.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS was obtained using an Agilent 6890 gas chromatography coupled to an Agilent 5973 network mass selective detector. A 14% cyanopropyl-phenyl methyl-polysiloxane column (19091S-436, Agilent Technologies, Santa Clara, CA; 60m * 25mm * 25µm) was used in the analysis. For each sample, 10 µL was injected into GC with a split ratio of 20:1. Temperature program was set as follow: initial column temperature 40 ° C, a temperature increase from 40 ° C to 280 ° C at a rate of 5 ° C /min and a running time of 60 minutes at the final temperature. The injector temperature was 250 ° C and detector temperature was 280 ° C. The helium carrier flow rate was 1.4 mL/min. The mass range of the mass spectrometer was

33-300 amu.

4. RESULTS AND DISCUSSION

Since dry star anise has a very strong flavor, although it is considered indispensable for Chinese stew meat, only a tiny amount of star anises are used compared with meat and other ingredients in the stew dish. It is difficult to identify trace constituents in cooked food, so we establish the model system to study how star anise influences the flavor generation of Chinese stew meat.

As reviewed before, various factors, such as pH and reaction temperature, may affect the reaction. In order to better understand what really happened during the cooking process, we set the reaction condition by simulating the stewing process. The reaction temperatures were 60 ° C, 80 ° C, 120 ° C and 150 ° C, representing the different periods of cooking process as well as the condition in a high pressure cooker. The reaction time was two hours, which is an average cooking time for a Chinese stew dish. pH value was adjusted to 5.5 by sodium hydroxide based the studies reviewed previously.

In this section, we will analyze both non-volatile and volatile products produced from this model reaction. Semi-quantitative studies on the volatile products were also conducted by using 2,3,5-trimethylpyrazine as internal standard.

4.1. Non-volatile Products

A major non-volatile compound was generated under 60 ° C and the reactant anisaldehyde was almost gone as shown from the HPLC profile. Several non-volatile compounds were generated under 150 ° C and the major non-volatile compound

from 60 ° C was disappear. The HPLC profiles are presented in Figure 10 and Figure 11. Both LC-ESIMS and LC-APCIMS profiles for the major non-volatile compound under 60 ° C are shown in Figure 12.

Both two LC-MS profiles show the molecular weight of the non-volatile is 152, and the structure was identified as 4-methoxybenzenecarbothialdehyde. We also proposed a possible pathway for the formation of this compound. Hydrogen sulfide is first added to the aldehyde group of anisaldehyde, then 4-methoxybenzenecarbothialdehyde formed by losing one water molecule. Both two reactions may be reversible considering the reaction conditions. The pathway is shown in Figure 13.

4.2. Volatile Products

A wide variety of volatiles products were obtained from the reaction under all the temperatures. Table 2 shows the major volatile compounds identified from the reaction. The gas chromatography-mass spectrometry chromatograms of products produced under each temperature are presented in Figure 14 to Figure 17. The mass spectrums of identified compounds are presented in the appendix.

Heterocyclic sulfur containing compounds produced from cysteine thermal degradation are expected to be observed based on the reaction conditions. As we reviewed before, this kind of sulfur containing volatiles are believed to contribute to the characteristic meat flavors.

Table 2. Volatile compounds identified from the anisaldehyde/cysteine thermal system

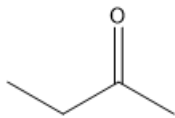
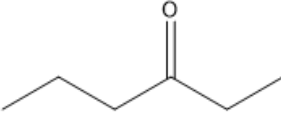
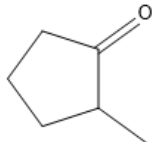
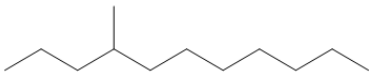
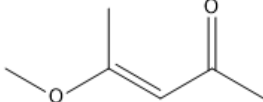
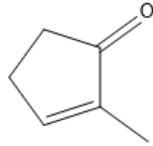
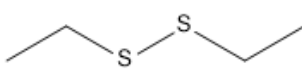
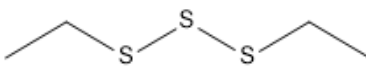
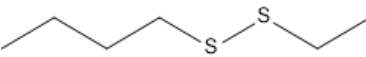
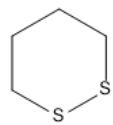
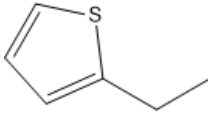
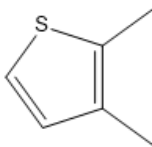
Compound	Structure	M.W.
Cysteine thermal degradation related compounds		
2-Butanone		72
3-Hexanone		100
2-Methylcyclopentanone		98
4-Methyl undecane		170
4-Methoxy-3-penten-2-one		114
2-Methyl-2-cyclopentenone		96
Sulfur containing compounds		
Diethyl disulfide		122
Diethyl trisulfide		154
Ethyl n-butyl disulfide		150
1,2-Dithiane		120
2-Ethylthiophene		112
2,3-Dimethylthiophene		112

Table 2. Volatile compounds identified from the anisaldehyde/cysteine thermal system (continued)

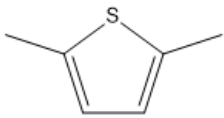
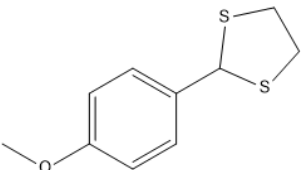
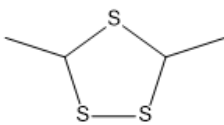
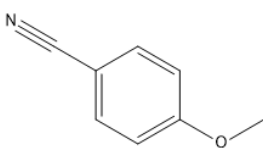
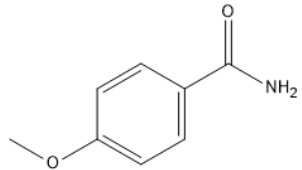
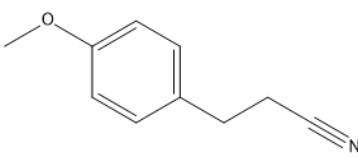
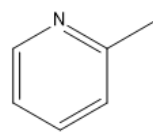
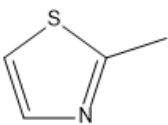
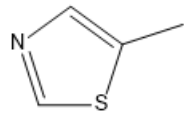
Compound	Structure	M.W.
2,5-Dimethylthiophene		112
2- (4-Methoxyphenyl)-1,3-dithiolane		212
3,5-Dimethyl-1,2,4-trithiolane		152
Nitrogen containing compounds		
<i>p</i> -Anisonitrile		133
<i>p</i> -Methoxybenzamide		151
3-(4-Methoxyphenyl)propionitrile		161
2-Methylpyridine		93
Nitrogen and sulfur containing compounds		
2-Methylthiazole		99
5-Methylthiazole		99

Table 2. Volatile compounds identified from the anisaldehyde/cysteine thermal system (continued)

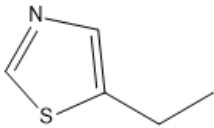
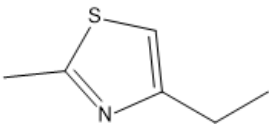
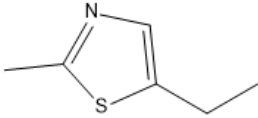
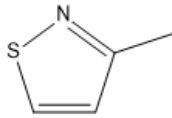
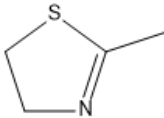
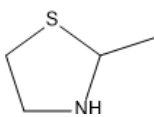
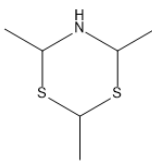
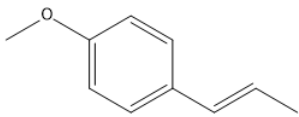
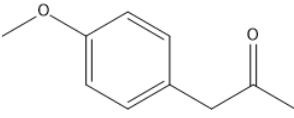
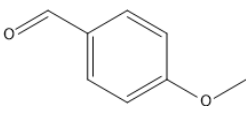
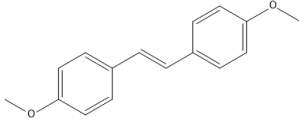
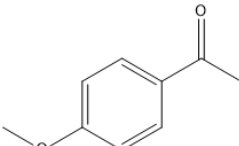
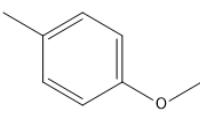
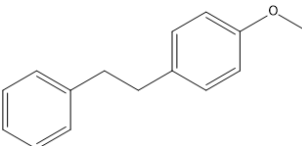
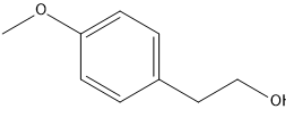
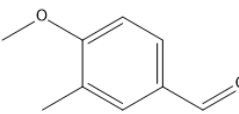
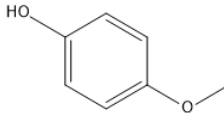
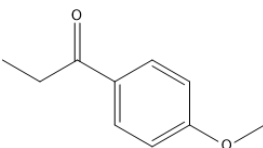
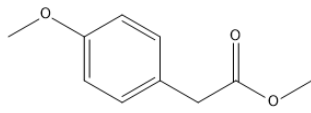
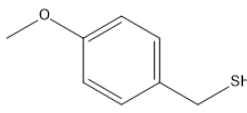
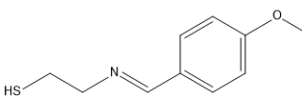
Compound	Structure	M.W.
5-Ethylthiazole		113
4-Ethyl-2-methylthiazole		127
5-Ethyl-2-methylthiazole		127
3-Methyl isothiazole		99
2-Methyl-2-thiazoline		101
2-Methylthiazolidine		103
2,4,6-Trimethylperhydro-1,3,5-dithiazine		163
Anisole related compounds		
Anethole		148
<i>p</i> -Acetonyl anisole		164
<i>p</i> -Anisaldehyde		136
Bianisal		240

Table 2. Volatile compounds identified from the anisaldehyde/cysteine thermal system (continued)

Compound	Structure	M.W.
<i>p</i> -Acetyl anisole		150
<i>p</i> -Methyl anisole		122
<i>p</i> -Phenethyl anisole		212
2-(4-Methoxyphenyl)ethanol		152
3-Methyl-4-anisaldehyde		150
4-Methoxyphenol		124
4'-Methoxy-propiophenone		164
Methyl (4-methoxyphenyl)acetate		180
4-Methoxy-α-toluenethiol		154
2-((4-Methoxybenzylidene)amino)ethanethiol		195

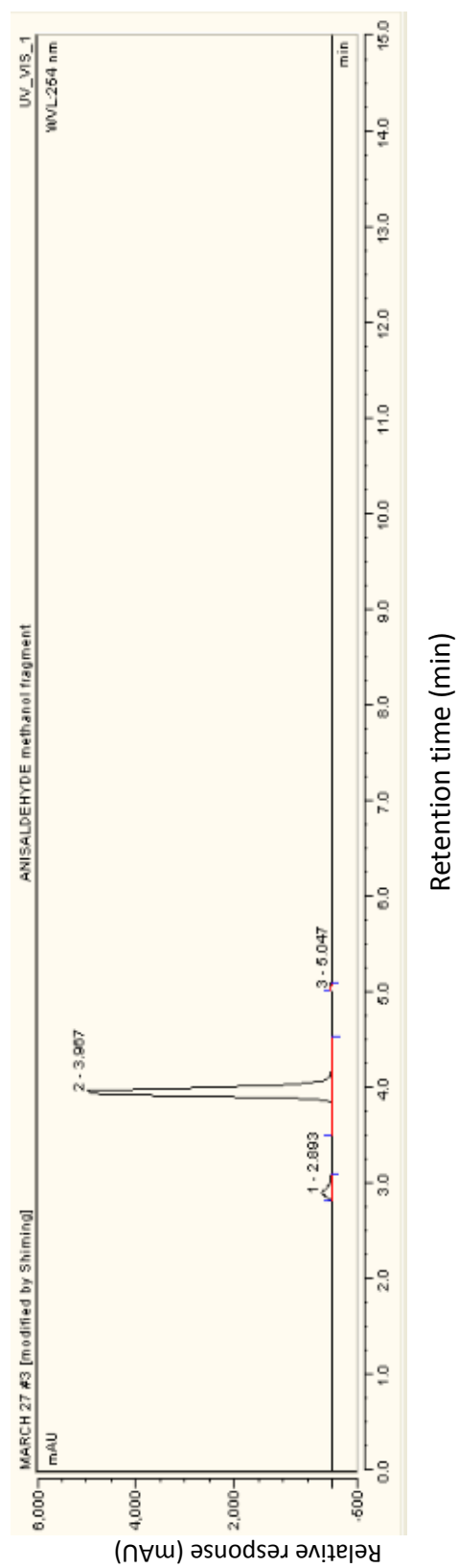


Figure 10. HPLC profile of the non-volatile products under 60 ° C

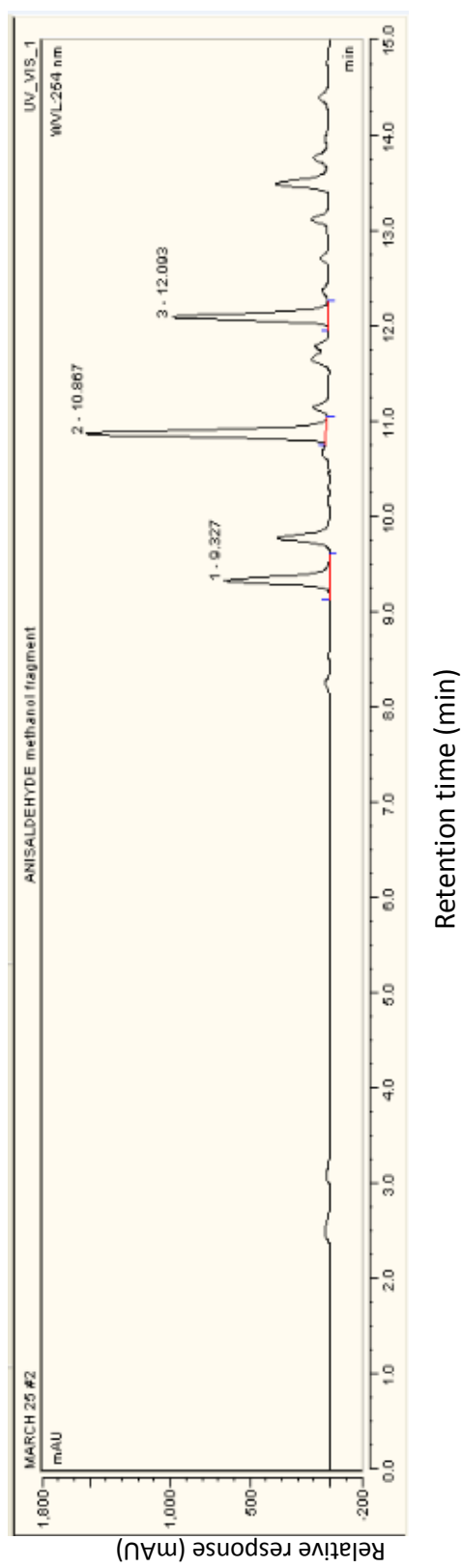


Figure 11. HPLC profile of the non-volatile products under 150 ° C

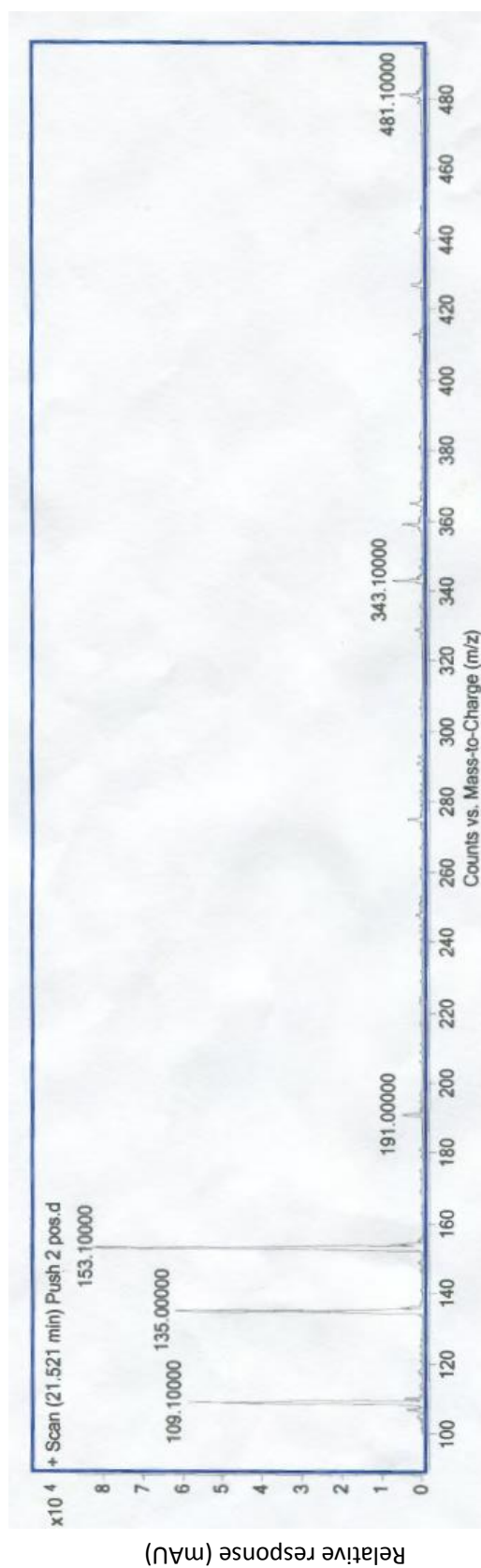


Figure 12. LC-ESIMS and LC-APCIMS profiles of the major non-volatile compounds under 60 ° C

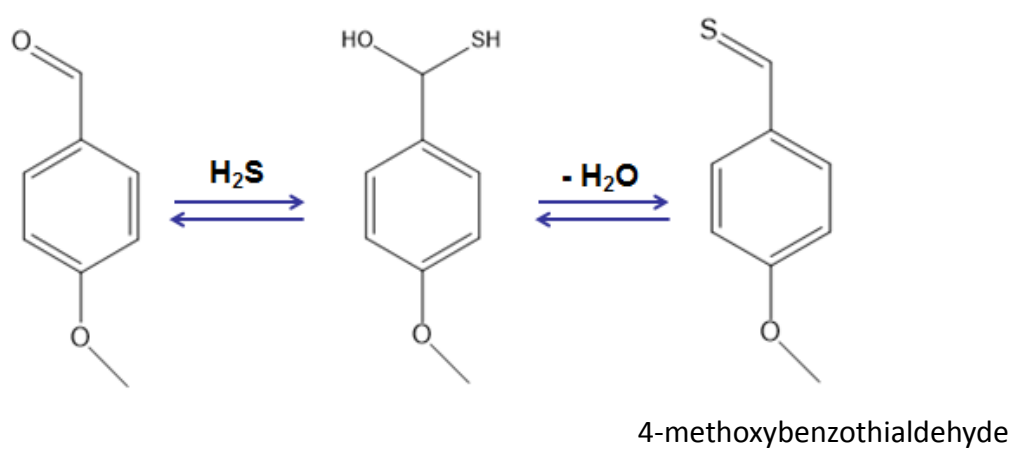


Figure 13. Formation pathway of 4-methoxybenzothialdehyde from anisaldehyde

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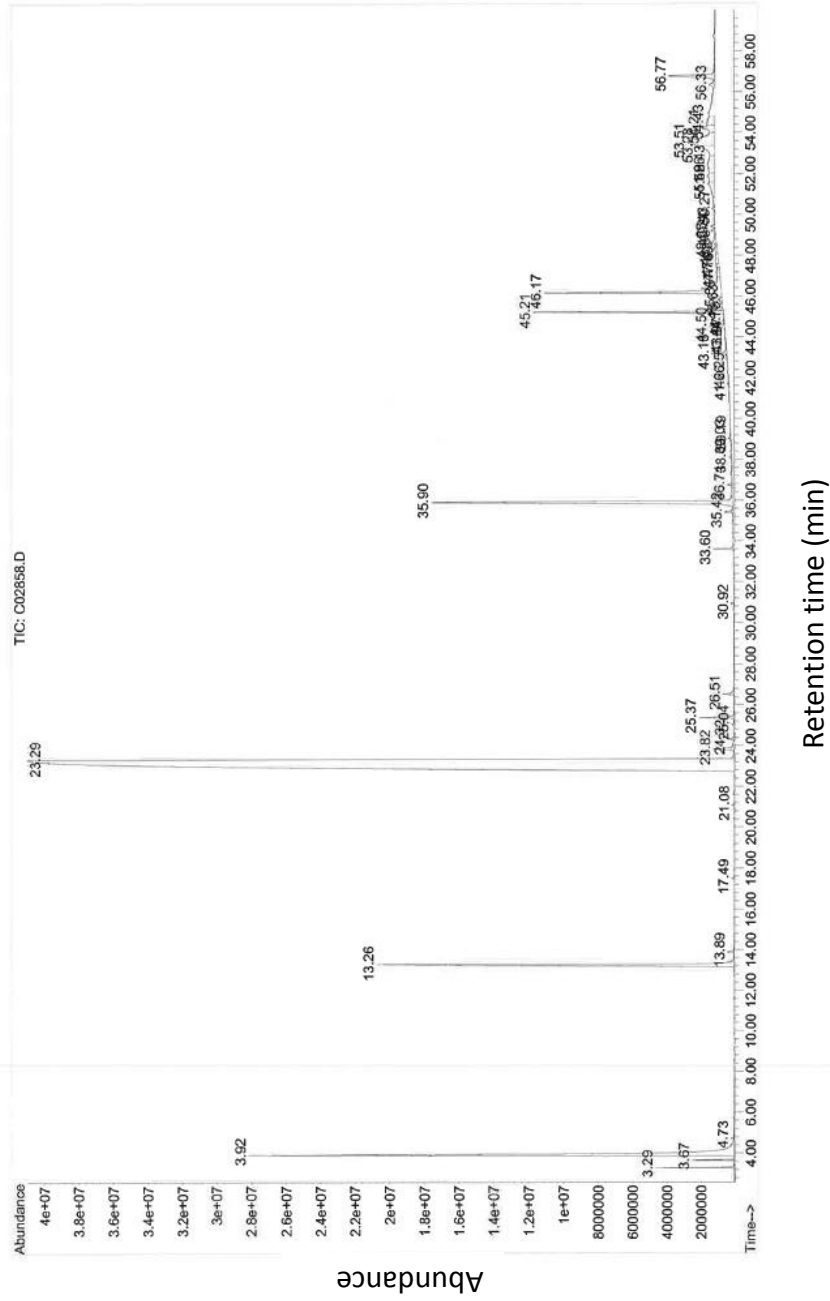


Figure 14. Gas chromatography-mass spectrometry chromatograms of products produced under 60 ° C

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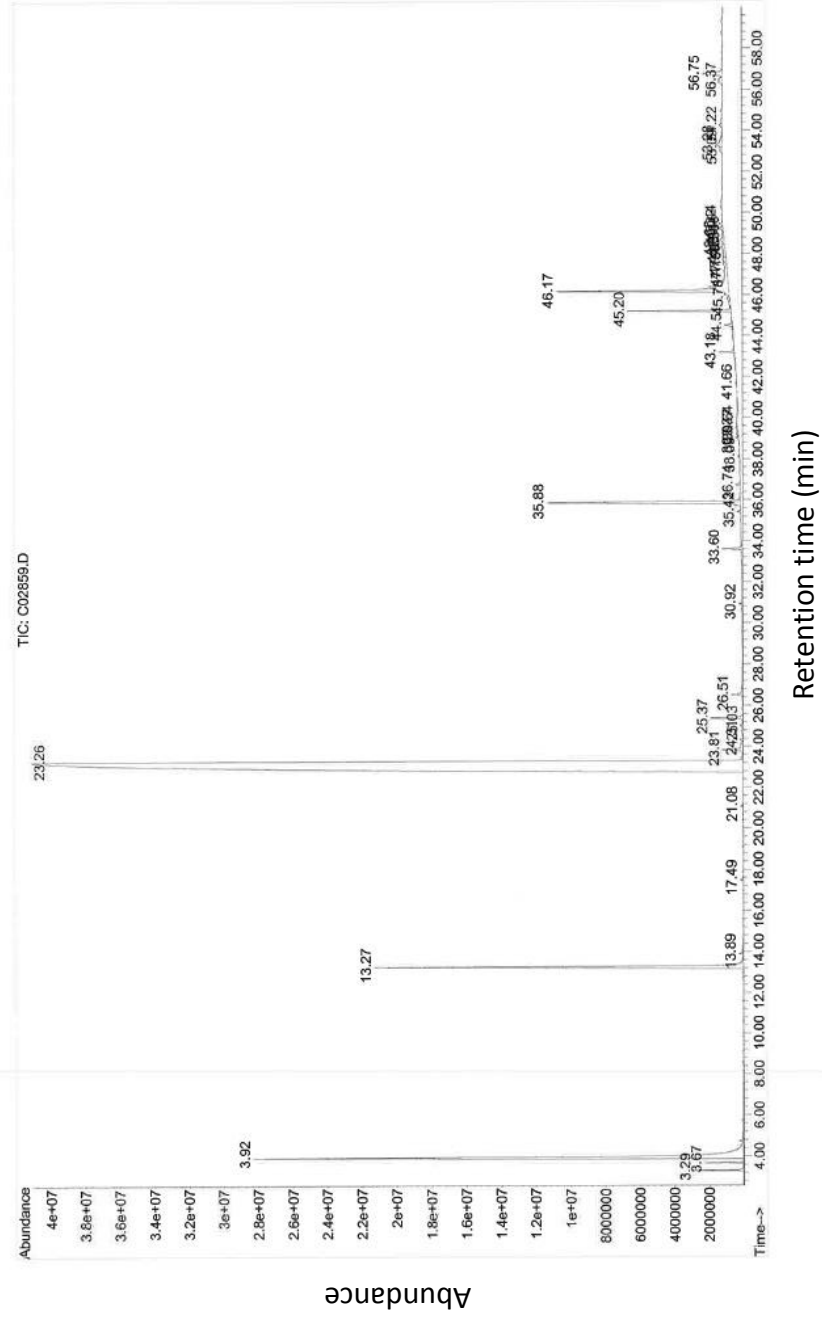


Figure 15. Gas chromatography-mass spectrometry chromatograms of products produced under 80 ° C

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 Vial Number: 13

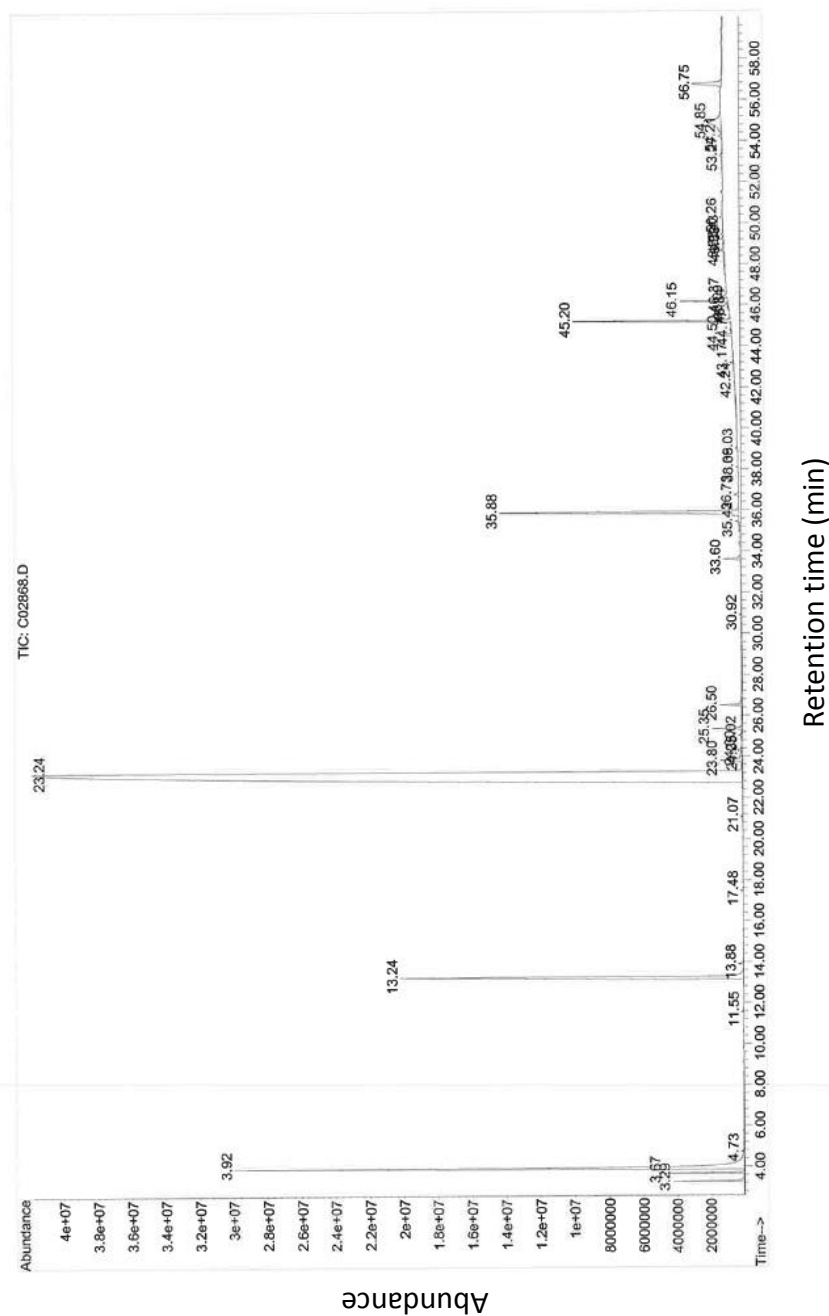


Figure 16. Gas chromatography-mass spectrometry chromatograms of products produced under 120 ° C

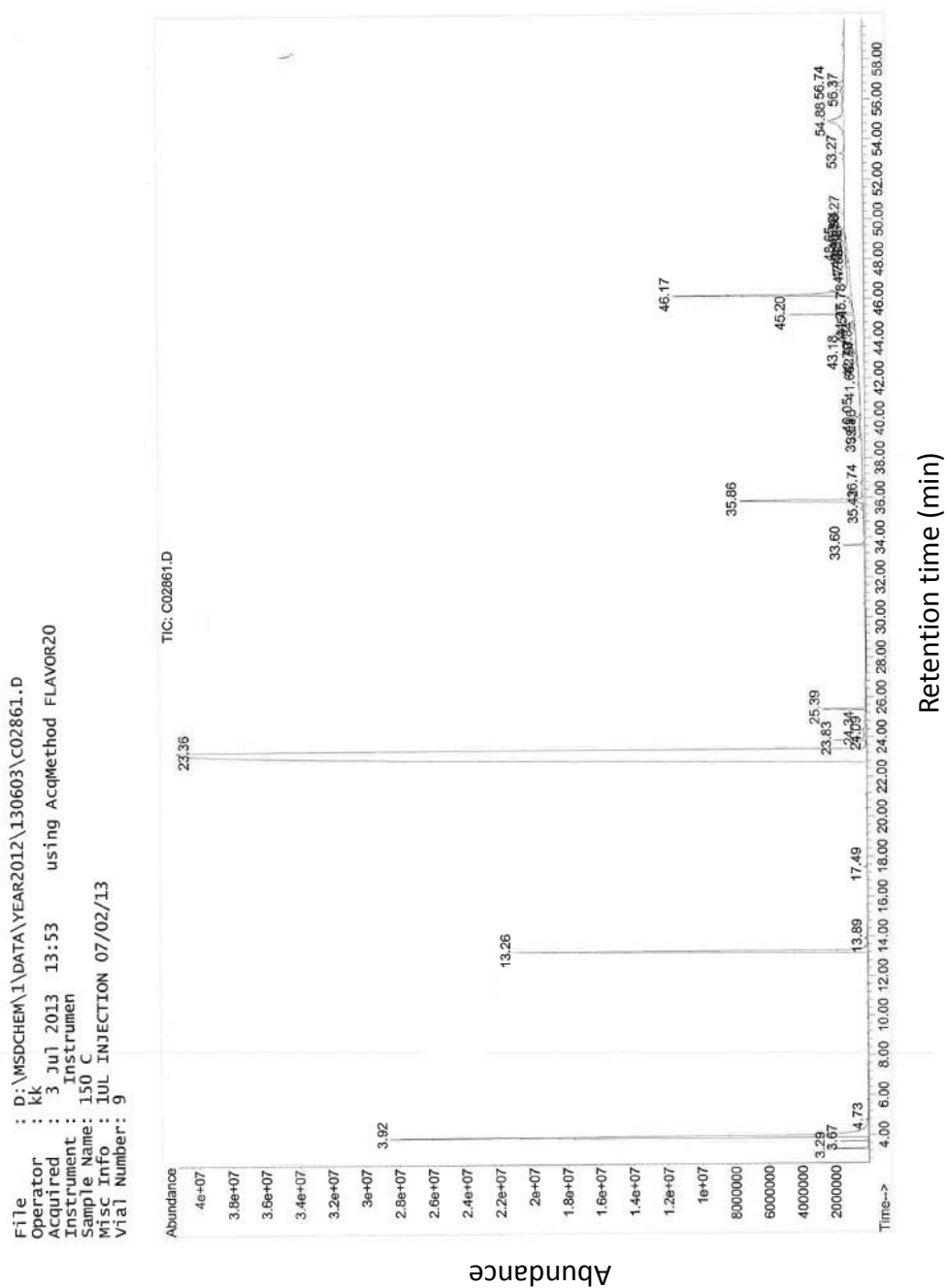


Figure 17. Gas chromatography-mass spectrometry chromatograms of products produced under 150 ° C

The early stage of cysteine thermal degradation is of great importance for this system, considering it provides the entire sulfur source by releasing hydrogen sulfide as well as other sulfur containing compounds derived from hydrogen sulfide. Numbers of heterocyclic sulfur containing products from cysteine thermal degradation were obtained with trace amount. Various thiazoles, thiophenes, thiazolidines, dithiazines, trithiolanes were identified, such as 2-methylthiazole, 3-methyl isothiazole, 2-ethylthiophene, 2-methylthiazolidine, 2,4,6-trimethyl-5-H-1,3,5-dithiazine and 3,5-dimethyl trithiolane. Other sulfur containing compounds identified as cysteine thermal degradation compounds are: diethyl disulfide, butyl ethyl disulfide and diethyl trisulfide. Some nitrogen containing compounds derived from the thermal degradation reactions were identified, 2-methylpyridine for example.

Sulfur containing aroma compounds generated from the system are of great interests, since these compounds indicate the reactions occur between anisaldehyde and cysteine. Several sulfur containing aroma compounds were detected and identified.

As Table 2 shows, we can divide all those volatile compounds into several classes: thiazoles, thiazolines, thiazolidines, thiolanes, thiazines, pyridines, thiophenes, anisole derivatives and other. Among all these compounds, sulfur-containing compounds were considered most important for the meat flavor properties. Although the volatile products were obtained from a single reaction conducted under certain condition, some of them were simply products from the

cysteine thermal degradation while others were produced from the reaction between anisaldehyde and cysteine or its thermal degradation products.

4.2.1. Cysteine Thermal Degradation Products

Cysteine thermal degradation products of trace amount were identified in our model reaction under 150 ° C. At lower temperature, such as 120 ° C and 80 ° C, less number of cysteine thermal degradation products were identified and no new volatile compound was identified. The amounts of those products were also of trace amount as produced under higher temperature.

Ketone Compounds

Several ketone compounds were identified as the cysteine thermal degradation products, including 3-hexanone and 2-methylcyclopentanone, which were reported in the earlier researches (Shu and Ho, 1985; Umano, 1995). 2-Butanone was found in nature cocoa aroma and had been isolated and identified from coffee aroma, heat-treated beef as well as processed pork and ham (Furia, 1975). 2-Methyl-2-cyclopenten-1-one was described having a coffee and smoky sausage aroma, and it can be generated from 2-methylcyclopentanone by oxidation or the other pathway.

Sulfur-Containing Compounds

2-Ethylthiophene was reported to occur naturally in beef by Stoffelsma (1968). It was identified as one of the volatile products from the reaction of D-glucose with hydrogen sulfide at pH 10 (Shibamoto and Sakaguchi, 1978), an alkaline environment not normally seen in meat processing. The flavor property of this compound was described as styrene-like. A trace amount of 2-ethylthiophene was also identified

from the well-done-grilled pork sample and was described as pork flavor (Mottram, 1985). Both 2-ethylthiophene and 5-Ethyl-2-methylthiophene were identified from the volatile compounds produced from the reaction of cysteine and methylglyoxal conducted under 160 ° C lasting for one hour, but neither of them was detected from the reaction of cystine and methylglyoxal under the same condition (Kato, 1973).

3,5-Dimethyl-1,2,4-trithiolane was first isolated and identified from boiled beef by Chang (1968). It was reported in previous studies on cysteine thermal degradation as well (Shu and Ho, 1985; Zhang and Ho, 1988; Umano, 1995). Together with 2,4,6-trimethyl-5-H-1,3,5-dithiazine, 3,5-dimethyl-1,2,4-trithiolane was the major volatile compound of fried chicken resulted from the thermal degradation of cysteine and glutathione (Shi and Ho, 1994). Proposed by Shu and Ho (1985), this compound may be also formed from the reaction of acetaldehyde and hydrogen sulfide.

Diethyl disulfide and Diethyl trisulfide were components with an odor like rotting meat. Study on off-flavor in skim milk (Jung, 1998) showed that quite amount of diethyl disulfide was produced in a cysteine/ riboflavin water solution when the solution was exposed to light which contributed to the off-flavor of skim milk. Diethyl trisulfide presents a sulfury, onion-like, alliaceous as well as sweet, typical durian odor and is widely distributed in foods and beverages such as cooked onion, leek, durian, milk, cheese, whiskey, beer, wine and stale Japanese sake (Isogai, 2008). The formation mechanism of both sulfur containing compounds in cysteine thermal degradation system was proposed by Shu and Ho (1985) when the thermal reaction

was conducted under 160 ° C at pH 5.5 for half an hour. Figure 6 shows how these two compounds were generated from cysteine.

Ethyl butyl disulfide was also identified in the cysteine thermal degradation system by Shu and Ho (1985). The existence of ethyl butyl disulfide in food material was first reported from durian in a GC-MS analysis of the fruit and the volatile accounted for 8% of the total volatiles in that study (Jiang and Choo, 1998). This asymmetric sulfur-containing compound may also be generated from the reaction of aldehyde and hydrogen sulfide, sharing the same formation mechanism with diethyl disulfide and diethyl trisulfide.

1,2-Dithiane was first described by Schoberl in 1958. It has a garlic character with slight metallic nuance and has a taste threshold value in 2 ppb. 1,2-Dithiane was valued suitable for stewed vegetable and horseradish flavor due to its flavor property. This compound was identified by GC-MS from a simulated meat flavor concentration fraction by Golovnya (1983) with a trace amount. Later, in a cysteine thermal degradation reaction conducted by Umano (1985), 1,2-dithiane was first identified as a cysteine thermal degradation product. Shibamoto and Chung (1994) reported that by heating and stirring peanut oil with different amount of cysteine under 200 ° C for 5 hours, 1,2-dithiane was identified with a very low concentration, though only in the high cysteine contained samples. No natural occurrence of 1,2-dithiane in food was reported before and it is the first time that 1,2-thiane was detected from a cysteine thermal degradation conducted in aqueous environment. The formation of this may be due to the presence of anisaldehyde, or it could also be the result of

some minor differences on the reaction conditions compared to the formation researches.

Nitrogen-Containing Compounds

2-Methylpyridine was the only nitrogen containing heterocyclic volatile compound identified in present model reaction. It was previously identified in cooked chicken (Horvat, 1976), roasted beef (Chang and Jin, 1983), fried chicken (Ho and Jin, 1983), fried bacon (Ho and Lee, 1983) and goat meat (Madruga, 2009). Described as astringent and hazelnut-like flavor, 2-methylpyridine is a widely found volatile compound in natural and processed food. Early study of cysteine degradation (Zhang and Ho, 1988; Umano, 1995) found it to be a major pyridine product generated from the thermal reaction. In a study on fish flavors, researcher (Horiuchi and Umano, 1998) found that 2-methylpyridine was found to increase when fish oil was heated with cysteine. Same increasing of 2-methylpyridine was observed when salmon was cooked with additional cysteine added the system (Methven and Mottram, 2007). In present model system, 2-methylpyridine may be derived from the reaction of acetaldehyde and ammonia, which were released from early step of cysteine thermal degradation.

Nitrogen- and Sulfur-Containing Compounds

Thiazole, thiazoline and thiazolidine are somewhat unique among the volatiles since they contain sulfur atom as well as nitrogen atom in the same ring. Thiazoles and thiazole derivatives play a key role in roasted flavors, particularly in meat products.

3-Methyl isothiazole, 2-methylthiazole, 5-methylthiazole, 5-ethylthiazole and

2,4,6-trimethyl-5-H-1,3,5-dithiazine were reported in Shu and Ho's (1985) study as cysteine thermal degradation volatile products produced under 160 ° C at pH 2.3 and pH 5.5 in a water solution. 2-Methylthiazole, 5-methylthiazole and 2,4,6-trimethyl-5-H-1,3,5-dithiazine were also identified in Zhang and Ho's (1988) study while cysteine thermal degradation was conducted under 180 ° C at pH 7.5 in water solution. At frying temperature (180 ° C) cysteine produces 2,4,6-trimethyl-5-H-1,3,5-dithiazine via thermal degradation and 2,4,6-trimethyl-5-H-1,3,5-dithiazine was also identified from cooked chicken (Shi and Ho, 1994). Former study (Kawai, 1985) also proposed that 2,4,6-trimethyl-5-H-1,3,5-dithiazine could be produced during storage of 2,4,6-trimethyl-5-H-1,3,5-thiadiazine, and the later can be synthesized from aldehyde, ammonia and hydrogen sulfide and was reported by Zhang and Ho (1988) as a newly found volatile product of cysteine thermal degradation. Under different pH value, 2,4,6-trimethyl-5-H-1,3,5-dithiazine was described as a medium roast shrimp (with slight sweetness) or weak edible mushroom shiitake.

2-Methylthiazole, 2-methyl-2-thiazoline and 2-methylthiazolidine were identified when cysteine was thermally treated with glass beads under 180 ° C for 2 hours and volatiles were collected by three traps of different temperatures (Umano, 1995). It gave mostly 2-methylthiazolidine and measurable amounts of 2-methylthiazole and 2-methyl-2-thiazoline among other heterocyclic compounds while only trace amount of these three volatiles were identified in our model reaction, which indicated a formation preference of these three compounds under

lower water activity environment. The formation pathway proposed by Zhang and Ho (1988) showed that cysteine generated cysteamine by decarboxylation and acetaldehyde was produced once hydrogen sulfide was lost before decarboxylation. Interaction of acetaldehyde and cysteamine led to the formation of 2-methylthiazolidine. Upon further oxidation, 2-methyl-2-thiazoline was generated from 2-methylthiazolidine. And 2-methylthiazole, the volatile compound widely reported can be considered a more oxidized product of 2-methylthiazolidine compared with 2-methyl-2-thiazoline.

4-Ethyl-2-methylthiazole and 5-ethyl-2-methylthiazole were not reported in previous studies on cysteine thermal degradation, but were reported as volatile compounds of cooked meat. Mottram (1985) reported that both trace amount of 4- and 5-ethyl-2-methylthiazole were detected from a well-done-grilled pork sample, but neither of them was detected under other conditions including medium or light grilled, roast and boiled. 4-ethyl-2-methylthiazole was also identified from the pressure-cooked ground beef (Wilson, 1973) while 5-ethyl-2-methylthiazole was identified from a cysteine/cystine-ribose browning system (Mulders, 1973). However, due to the very low amount occurrence in natural/processed food, both two thiazoles were not well studied and the formation pathway had not been proposed.

No oxazoles, imidazoles, pyrroles, pyrazines or furans was identified from our reaction as the products of cysteine thermal degradation. It may due to lack of nitrogen or oxygen containing compounds, which would react with anisaldehyde instead of going to the further stages of cysteine thermal degradation. The

concentration of these compounds may also be too low to be detected, leading to the absence of those heterocyclic volatile compounds in the model reactions. All the cysteine thermal degradation products occurred in our model reaction were at very low concentrations, mostly at trace amount. They were very potent contributors to the whole flavor profile because of their low odor thresholds.

4.2.2 Products from reactions between anisaldehyde and cysteine (or its thermal degradation products)

As Table 2 shows, all the aroma compounds we identified can be considered as anisole derivatives. Since anisaldehyde has both methoxy group and aldehyde group on para position, reactions on both functional groups were expected. However, from the identified products, only the aldehyde group reacted with cysteine or its thermal degradation products while methoxy group was still attached on the aroma ring. It reflected that in this thermal system, aldehyde group was more active compared to the methoxy group.

Several anisole ketones were identified but due to the lack of data we can hardly tell whether these anisole ketones were products from the thermal reaction system or simply were impurities from the anisaldehyde sample. However, the identification of 4-methoxyphenol, anisyl alcohol and 2-(4-methoxyphenyl)ethanol indicates the occurrence of reversible reduction/oxidation reaction between anisole ketones and anisole alcohols during the thermal reaction. Methyl (4-methoxyphenyl)acetate was also identified and can be considered as the product of a esterification reaction. Anisaldehyde condensation was observed for the

thermal reaction. Both bianisal and *p*-phenethyl anisole were identified as the condensation products. *P*-phenethyl anisole can be considered as the reduction reaction derivative of bianisal. Figure 18 shows the possible reactions occur between anisaldehyde, bianisal and *p*-phenethyl anisole.

Besides the volatile compounds discussed above, sulfur-containing and nitrogen-containing aroma compounds were also identified, which better proved the reaction between anisaldehyde and cysteine (or its thermal degradation products) since cysteine was the only sulfur and nitrogen atom source in the thermal reaction system.

2-(4-methoxyphenyl)-1,3-dithiolane was detected with a trace amount. A possible formation pathway is proposed in Figure 19. Cysteine can produce cysteamine during thermal degradation, then cysteamine reacts with hydrogen sulfide to produce 1,2-ethanedithiol (EDT). An addition reaction between anisaldehyde and EDT can explain the formation of 2-(4-methoxyphenyl)-1,3-dithiolane.

Figure 20 illustrate the main fragmentation pathway of 2-(4-methoxyphenyl)-1,3-dithiolane by EI-MS. The base peak ion m/z 151 appears to be formed by a ring cleavage reaction on the dithiolane group with a neutral loss of ethenethiol from the dithiolane group. Semi-quantitative analysis was not conducted on this compound due to its low concentration in the final reaction mixture.

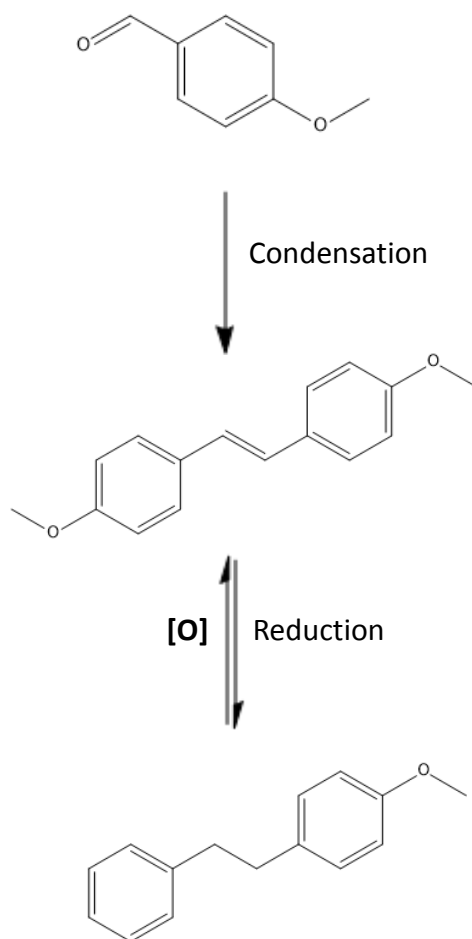


Figure 18. Possible reactions between anisaldehyde, bianisal and p-phenethyl anisole

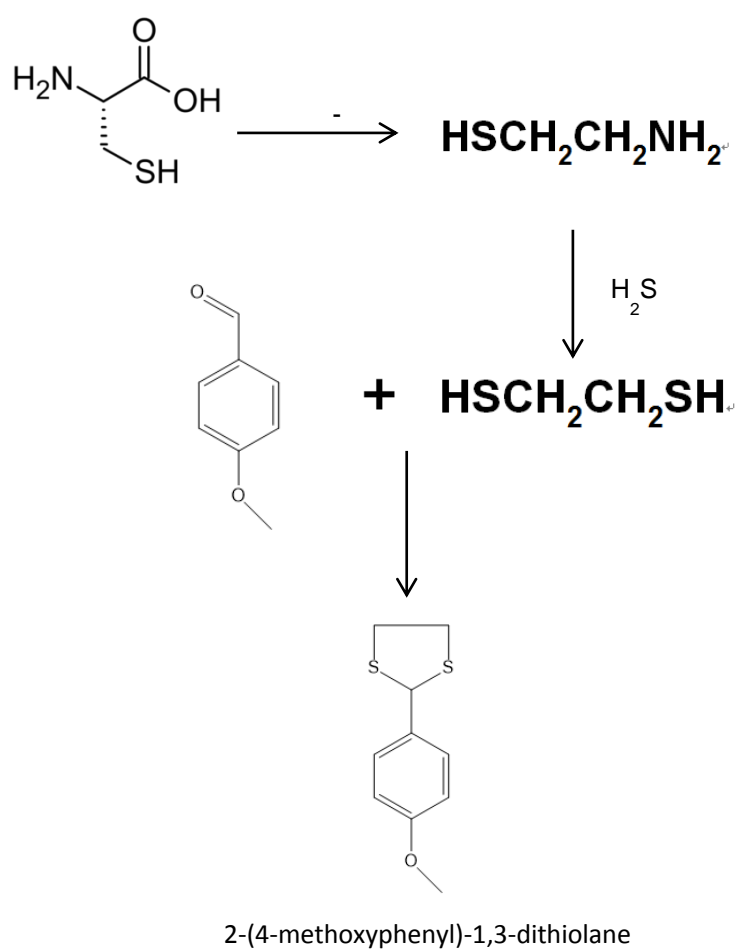


Figure 19. 2-(4-Methoxyphenyl)-1,3-dithiolane formation pathway

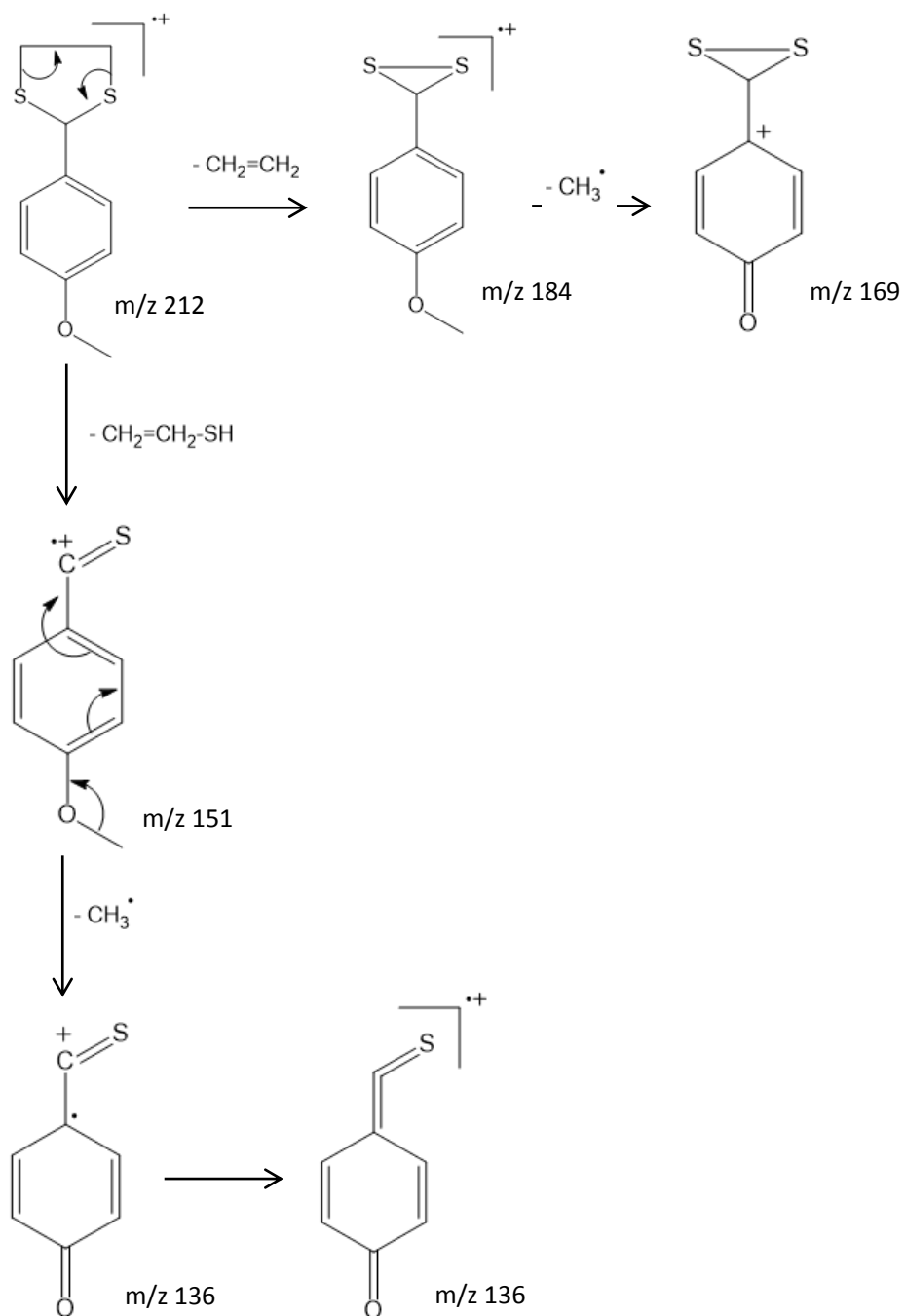


Figure 20. Fragmentation pathway of 2-(4-methoxyphenyl)-1,3-dithiolane

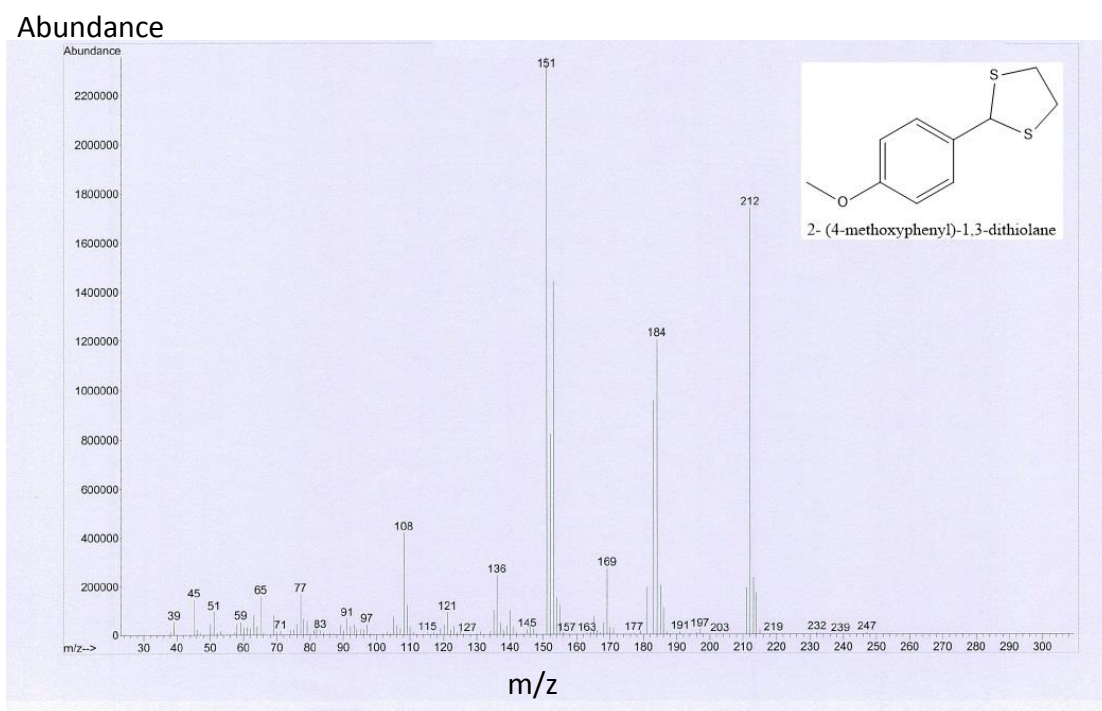


Figure 21. EI-MS Spectrum of 2- (4-methoxyphenyl)-1,3-dithiolane

4-Methoxy- α -toluenethiol was identified as one major product produced from this system (MS spectrum shown in Figure 24). The formation pathway (shown in Figure 22) is proposed to be the reduction of 4-methoxybenzothialdehyde, the major non-volatile we discussed before, under high temperature. Figure 23 illustrates the main fragmentation pathway of 4-methoxy- α -toluenethiol by EI-MS. The base peak ion m/z 154 appears to be formed by the loss of SH group from the methylene group. The aroma property of 4-methoxy- α -toluenethiol is described as coffee and sulfury. This compound was not reported previously in any food materials or as a flavor compound used in industry. Usually, 4-methoxy- α -toluenethiol was used as a building block to synthesize further sulfur-containing aroma compounds.

Semi-quantitative analyses were done for this compound under each temperature. Table 3 to Table 6 presents the peak height, corresponding peak area, corresponding percentage amount and amount of the total samples. We use equation one (Figure 25) to calculate the ratio of 4-methoxy- α -toluenethiol produced to the anisaldehyde left. A trend of 4-methoxy- α -toluenethiol relative response ratio is shown in Figure 26 based on the equation.

(Z)-2-((4-methoxybenzylidene)amino)ethanethiol was identified together with *p*-anisonitrile. MS spectrum of (Z)-2-((4-methoxybenzylidene)amino)ethanethiol is shown in Figure 29. This volatile compound was considered as a Schiff base product between anisaldehyde and cysteine while *p*-anisonitrile could be a further product of (Z)-2-((4-methoxybenzylidene)amino)ethanethiol under oxidation reaction. (Z)-2-((4-methoxybenzylidene)amino)ethanethiol was formed from the aliphatic

amine from the cysteine and the carbonyl group from anisaldehyde by nucleophilic addition forming a hemiaminal, followed by a dehydration to generate an imine. Then, (E)-2-((4-methoxybenzylidene)amino)ethanethiol was produced by losing one carbon dioxide from the carboxyl group. The formation pathway proposed is shown in Figure 27. Fragmentation mechanism is shown in Figure 28. The base peak is considered to be formed by losing one methylene thiol group.

Semi-quantitative analyses for each temperature were done by using the same equation as 4-methoxy- α -toluenethiol. A trend of (Z)-2-((4-methoxybenzylidene)amino)ethanethiol relative response ratio is shown in Figure 30 based on the equation. Table 7 shows trends of both 4-methoxy- α -toluenethiol and (Z)-2-((4-methoxybenzylidene)amino)ethanethiol relative response ratio in number format.

Although the anisaldehyde left from the reaction took a large percentage of the final reaction mixture, those anisole derivatives could also contribute to the overall anise like flavor profile based on their own flavor properties and low odor thresholds. Also, a lot of sulfur-containing aroma volatiles were produced. The semi-quantitative analyses for those volatiles showed a generally decreasing of relative response ratio was observed for (Z)-2-((4-methoxybenzylidene)amino)ethanethiol and 2-(4-methoxyphenyl)thiazole with increasing temperatures. In the case of 4-methoxy- α -toluenethiol, the relative response ratio was first decreased with increasing temperature, then it increased again when the reaction temperature reached 150 ° C. Temperature turning point of this compound would be

somewhere between 120 ° C and 150 ° C. Based on the semi-quantitative analyses, a lower temperature heating system was preferred for the generation of star anise influenced meat flavor generation, matching the fact that a slow simmer technique was also favored in cooking Chinese stew meat.

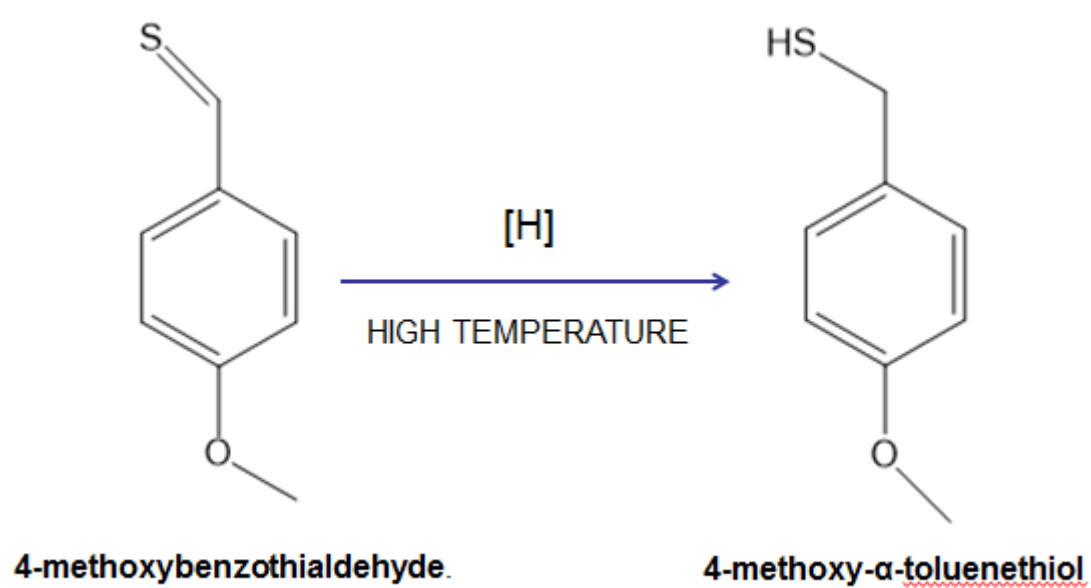


Figure 22. 4-Methoxy- α -toluenethiol formation pathway

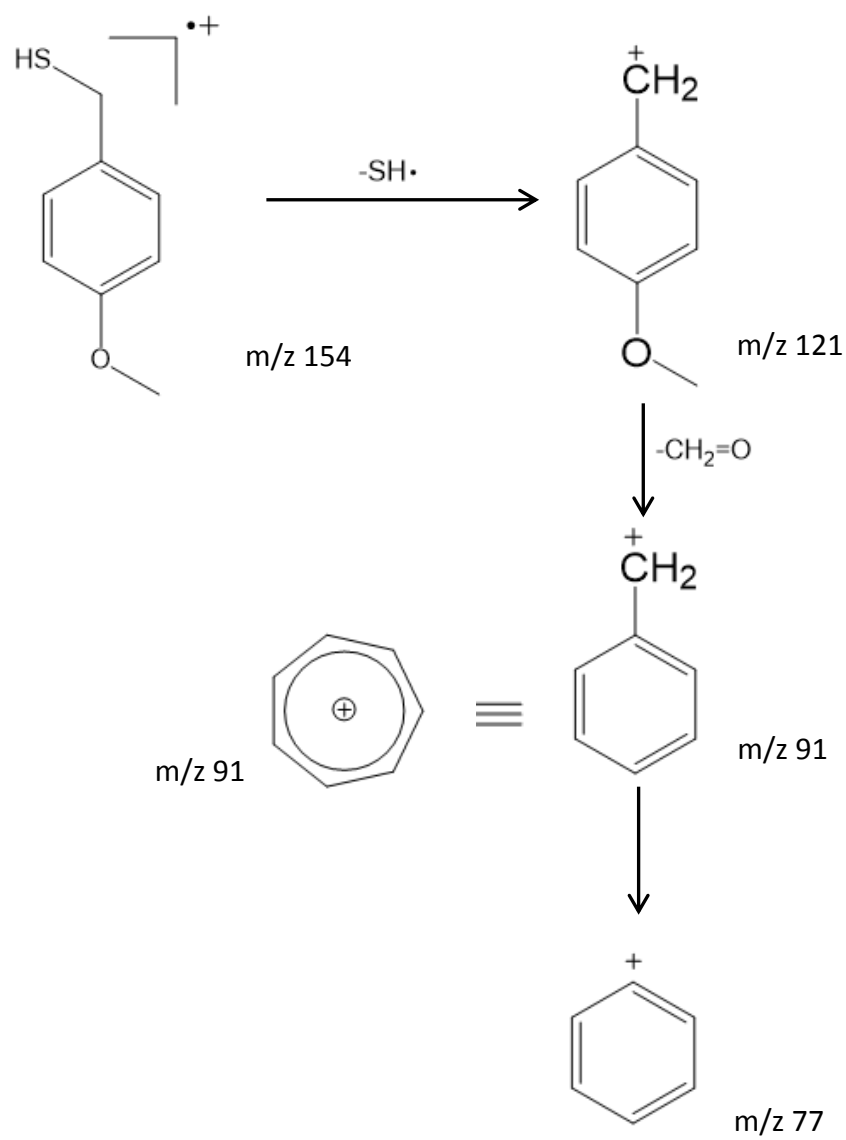


Figure 23. Fragmentation pathway of 4-methoxy- α -toluenethiol

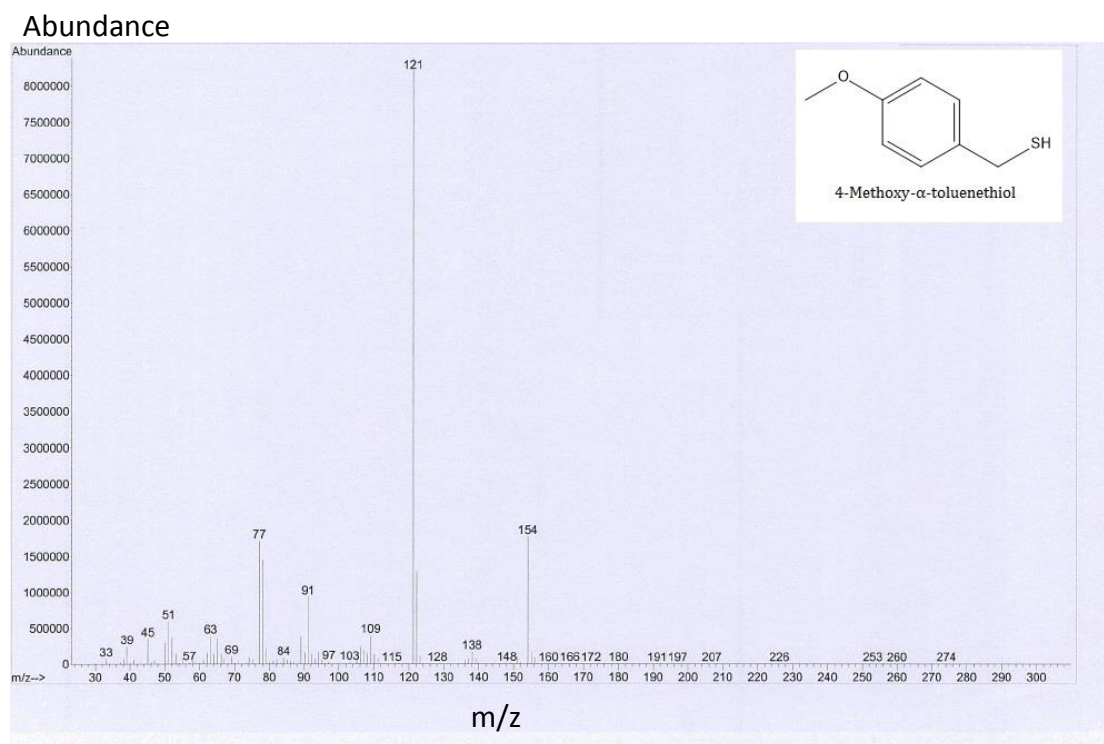


Figure 24. EI-MS Spectrum of 4-methoxy- α -toluenethiol

**Area ratio (r) = peak area of compound/
peak area of internal standard**

**Relative Response Ratio (R) =
r (volatile) / r (anisaldehyde)**

Both r(volatile) and r(anisaldehy) are calculated with average value of two reaction duplicates.

Figure 25. Semi-quantitative Analysis Equation

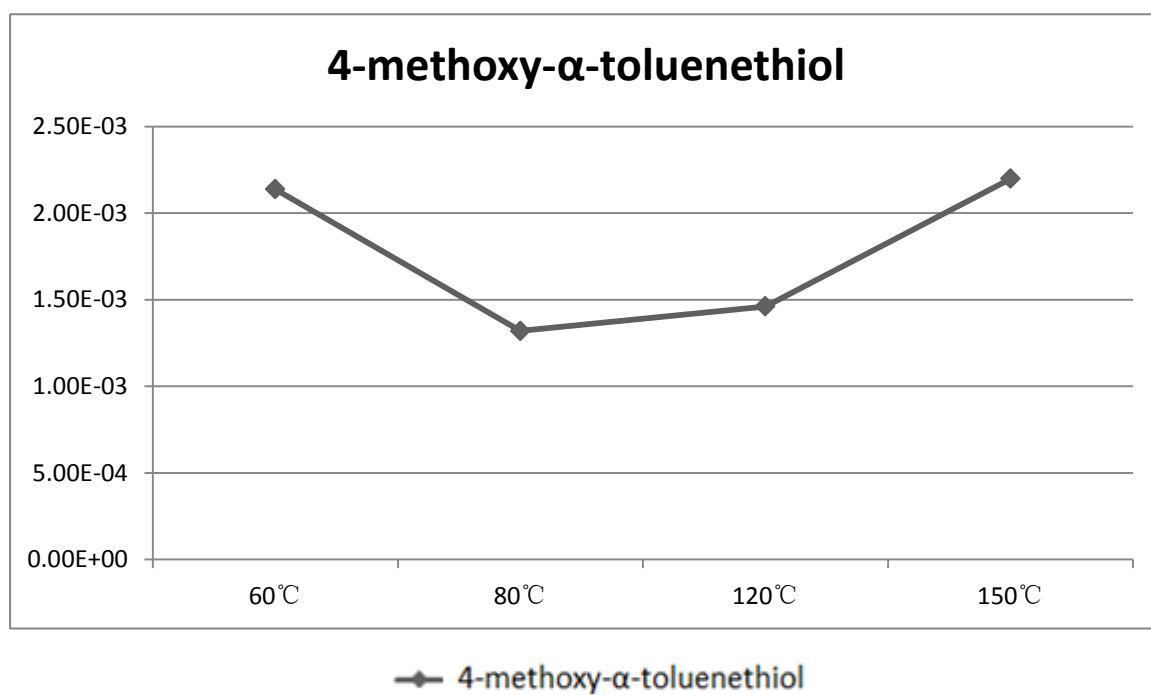


Figure 26. Trend of 4-methoxy- α -toluenethiol relative response ratio under four temperatures

Table 3. Peak Area Percent data of volatiles produced under 60 ° C

Compound name		Peak height	Corr. area	Corr. % of max	Percent age of total
1	2,3,5-trimethylpyrazine	18844289	1104269662	10.54	6.350
	Anisaldehyde	40761127	10479164684	100	60.264
	4-methoxy- α -toluenethiol	374361	24714834	0.24	0.142
	(Z)-2-((4-methoxybenzylidene)amino)ethanethiol	1183495	47328117	0.45	0.272
2	2,3,5-trimethylpyrazine	22533834	1472275447	13.27	7.551
	Anisaldehyde	42515090	11096499188	100	56.913
	4-methoxy- α -toluenethiol	625880	20651349	0.19	0.106
	(Z)-2-((4-methoxybenzylidene)amino)ethanethiol	2020944	78693303	0.71	0.404

Relative response ratio of each volatile:

4-methoxy- α -toluenethiol: 2.138E-3

(Z)-2-((4-methoxybenzylidene)amino)ethanethiol: 5.656E-3

Table 4. Peak Area Percent data of volatiles produced under 80 ° C

Compound name		Peak height	Corr. area	Corr. % of max	Percent age of total
1	2,3,5-trimethylpyrazine	193583 29	117467 5957	12.10	7.892
	Anisaldehyde	407401 37	970919 6713	100	65.234
	4-methoxy- α -toluenethiol	182143	164064 09	0.17	0.110
	(Z)-2-((4-methoxybenzylidene)amino)ethanethiol	107756 7	426704 66	0.44	0.287
2	2,3,5-trimethylpyrazine	215062 10	141669 1985	12.56	8.645
	Anisaldehyde	420290 76	112749 64353	100	68.806
	4-methoxy- α -toluenethiol	243508	105462 23	0.09	0.064
	(Z)-2-((4-methoxybenzylidene)amino)ethanethiol	953637	353890 01	0.31	0.216

Relative response ratio of each volatile:

4-methoxy- α -toluenethiol: 1.320E-3

(Z)-2-((4-methoxybenzylidene)amino)ethanethiol: 3.779E-3

Table 5. Peak Area Percent data of volatiles produced under 120 ° C

Compound name		Peak height	Corr. area	Corr. % of max	Percent age of total
1	2,3,5-trimethylpyrazine	18542466	1058392124	12.99	7.562
	Anisaldehyde	39753098	8146526750	100	58.208
	4-methoxy- α -toluenethiol	271089	13364721	0.16	0.095
	(Z)-2-((4-methoxybenzylidene)amino)ethanethiol	1274345	48693500	0.60	0.348
2	2,3,5-trimethylpyrazine	17977193	981888974	10.28	6.921
	Anisaldehyde	41531478	9549343104	100	67.310
	4-methoxy- α -toluenethiol	260198	12601273	0.13	0.089
	(Z)-2-((4-methoxybenzylidene)amino)ethanethiol	1036238	40506259	0.42	0.286

Relative response ratio of each volatile:

4-methoxy- α -toluenethiol: 1.461E-3

(Z)-2-((4-methoxybenzylidene)amino)ethanethiol: 3.377E-3

Table 6. Peak Area Percent data of volatiles produced under 150 ° C

Compound name		Peak height	Corr. area	Corr. % of max	Percent age of total
1	2,3,5-trimethylpyrazine	215783 49	135687 2686	10.04	7.621
	Anisaldehyde	420475 06	135129 62750	100	75.901
	4-methoxy- α -toluenethiol	710172	261480 57	0.19	0.147
	(Z)-2-((4-methoxybenzylidene)amino)ethanethiol	149540 1	548299 80	0.41	0.308
2	2,3,5-trimethylpyrazine	193971 36	112021 0593	9.08	6.489
	Anisaldehyde	411832 48	123407 72826	100	71.487
	4-methoxy- α -toluenethiol	573319	300903 68	0.24	0.174
	(Z)-2-((4-methoxybenzylidene)amino)ethanethiol	126231 8	481834 55	0.39	0.279

Relative response ratio of each volatile:

4-methoxy- α -toluenethiol: 2.199E-3

(Z)-2-((4-methoxybenzylidene)amino)ethanethiol: 3.977E-3

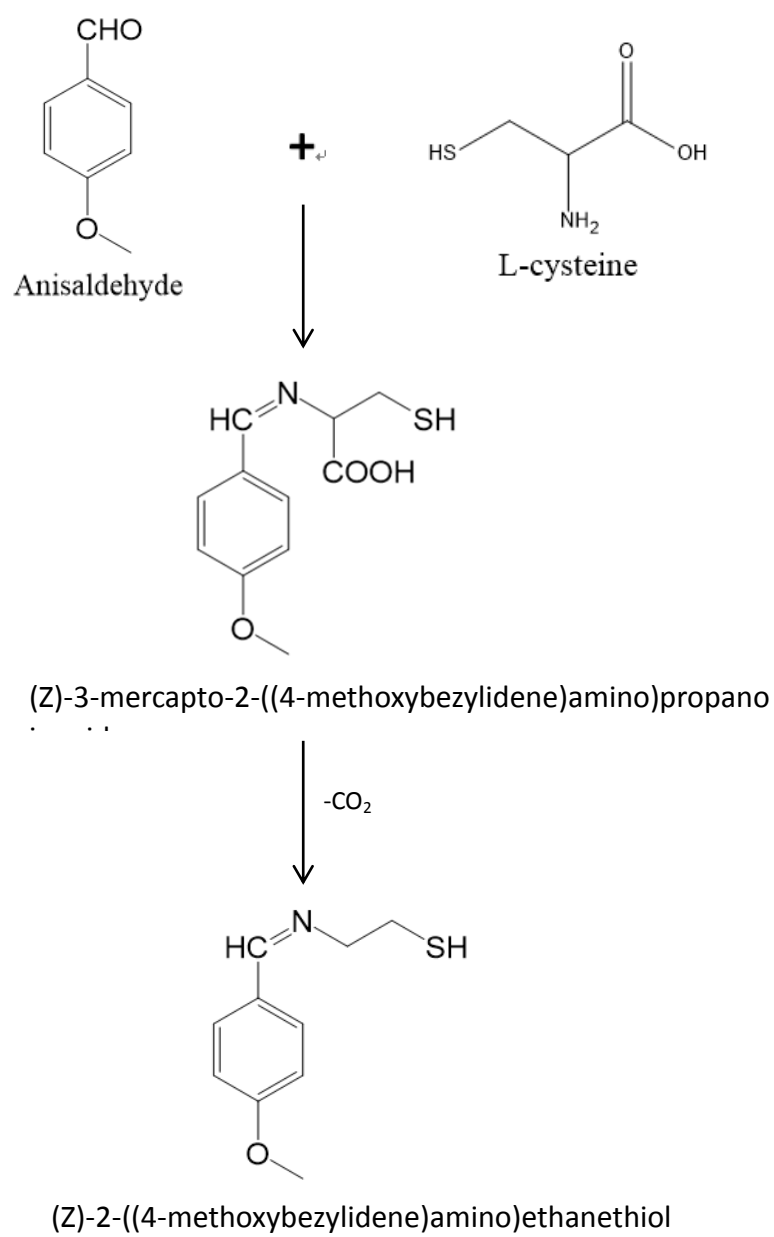


Figure 27. (Z)-2-((4-Methoxybenzylidene)amino)ethanethiol formation pathway

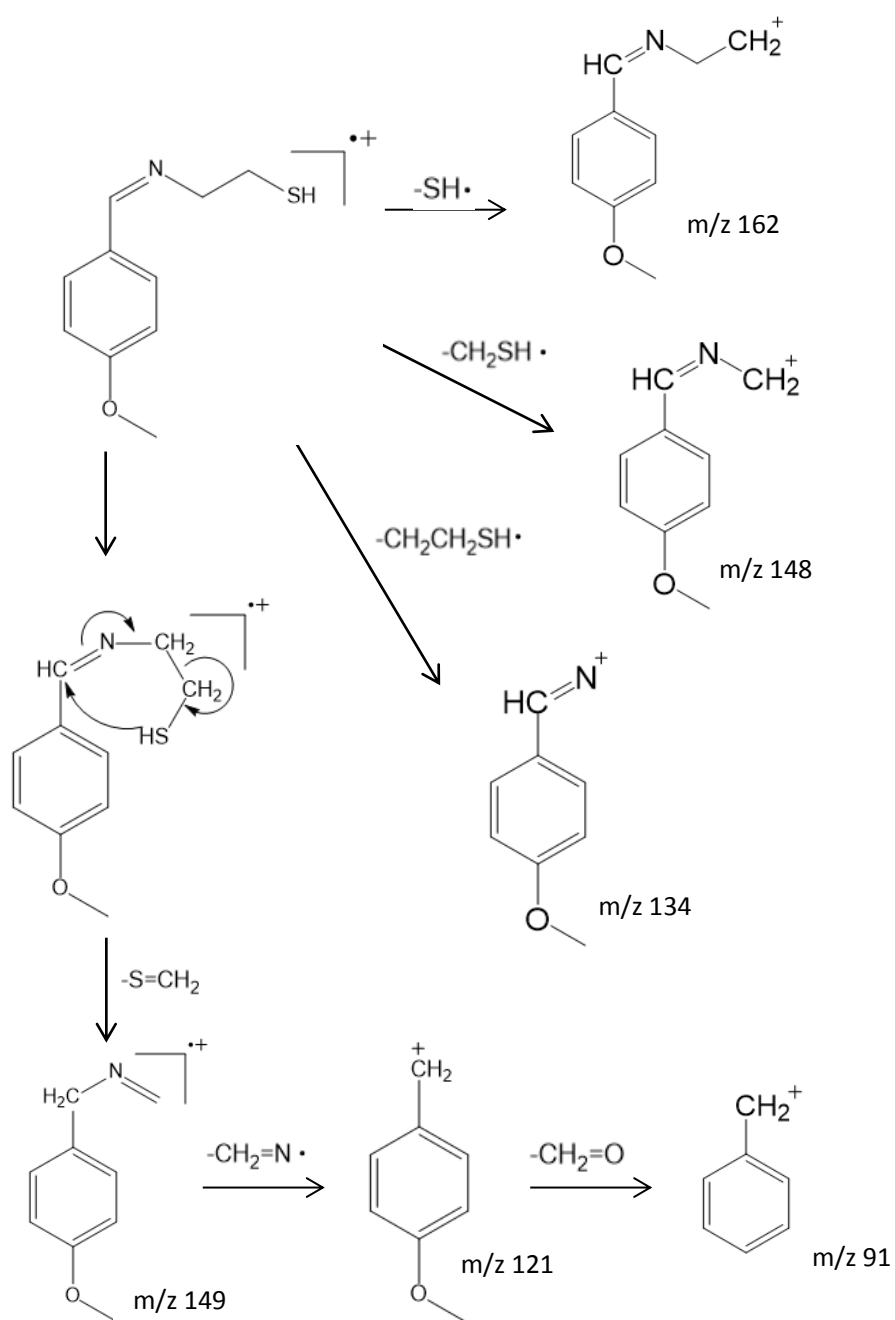


Figure 28. (Z)-2-((4-Methoxybenzylidene)amino)ethanethiol fragmentation pathway

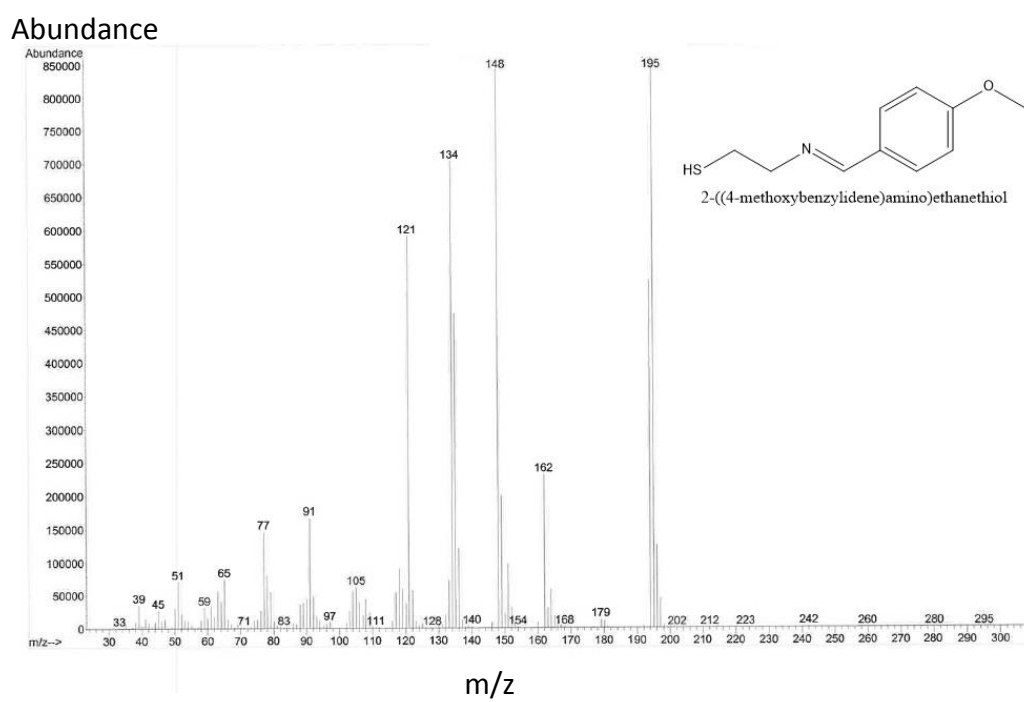


Figure 29. EI-MS Spectrum of 2-((4-methoxybenzylidene)amino)ethanethiol

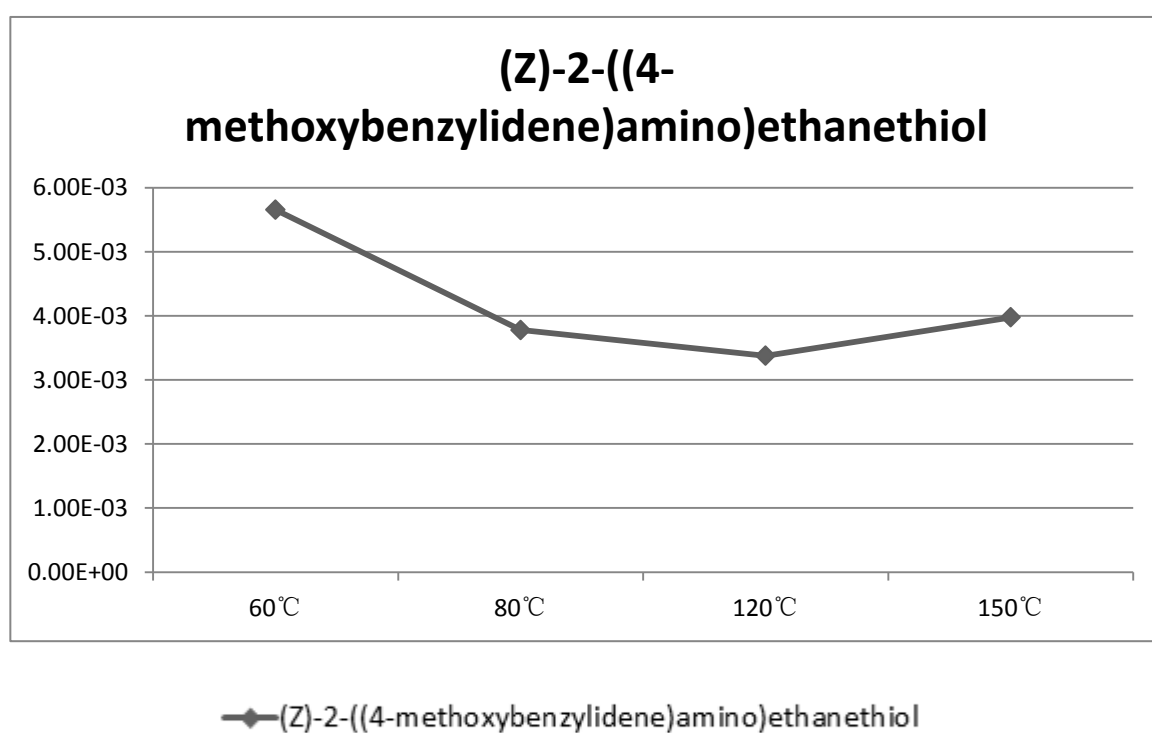


Figure 30. Trend of (Z)-2-((4-methoxybenzylidene)amino)ethanethiol relative response ratio under four temperatures

Table 7. Relative response ratio of two major volatile compounds under 60° C, 80° C, 120° C and 150 ° C

Compound Name	60° C	80° C	120° C	150° C
4-methoxy- α -toluenethiol	2.138E-3	1.320E-3	1.461E-3	2.199E-3
(Z)-2-((4-methoxybenzylidene)amino)ethanethiol	5.656E-3	3.779E-3	3.377E-3	3.977E-3

5. CONCLUSION

Star anise, a widely used spice, plays an important role in the flavor generation of Chinese stew meat. Anisaldehyde, the major component of star anise in water solution, was prepared to react with cysteine, a major amino acid in the meat and a well know meat flavor precursor. During the thermal process, several interesting sulfur-containing aroma compounds were generated and identified.

Various products were produced from cysteine thermal degradation. Among those degradation products, hydrogen sulfide was considered to be the most important one. By reaction between anisaldehyde and hydrogen sulfide, 4-methoxybenzenecarbothialdehyde, the major non-volatile compound was generated. Further reduction of this compound produced a major volatile product 4-methoxy- α -toluenethiol. The flavor property of this compound was described as coffee and sulfury.

A trace amount of 2-(4-methoxyphenyl)-1,3-dithiolane was detected and identified from the model cysteine. This compound was considered to be formed from the reaction of anisaldehyde and 1,2-ethanedithiol, another cysteine degradation products generated by the reaction between hydrogen sulfide and cysteamine. Both hydrogen sulfide and cysteamine were major first stage products in the cysteine thermal degradation reactions.

One other sulfur-containing aroma compounds were obtained and identified. It was proposed to be derived from the Schiff base product of cysteine and

anisaldehyde. (Z)-2-((4-methoxybenzylidene)amino)ethanethiol was first found and had not been reported before.

We proposed all the formation mechanisms for the compounds mentioned above as well as the fragmentation mechanisms under ESI-MS. Semi-quantitative analyses were conducted and it partially illustrated the trend of relative response ratio for each volatile in the model system as well as the overall star anise influenced flavor generation in the Chinese stew meat.

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7. APPENDIX

Abundance

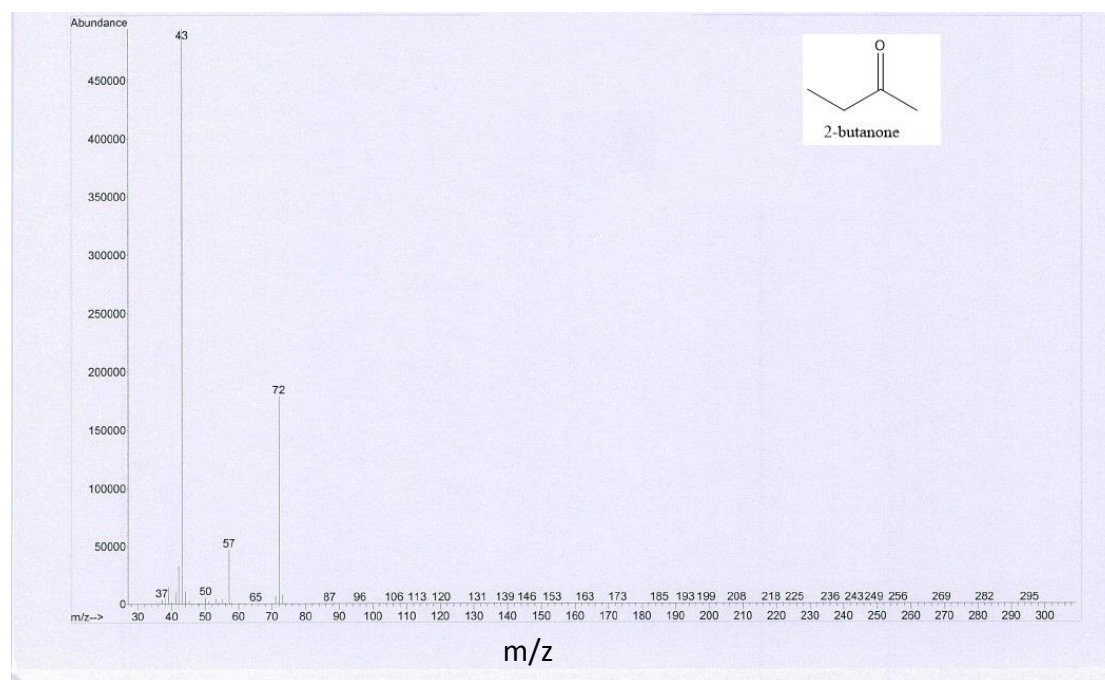


Figure 31. EI-MS Spectrum of 2-butanone

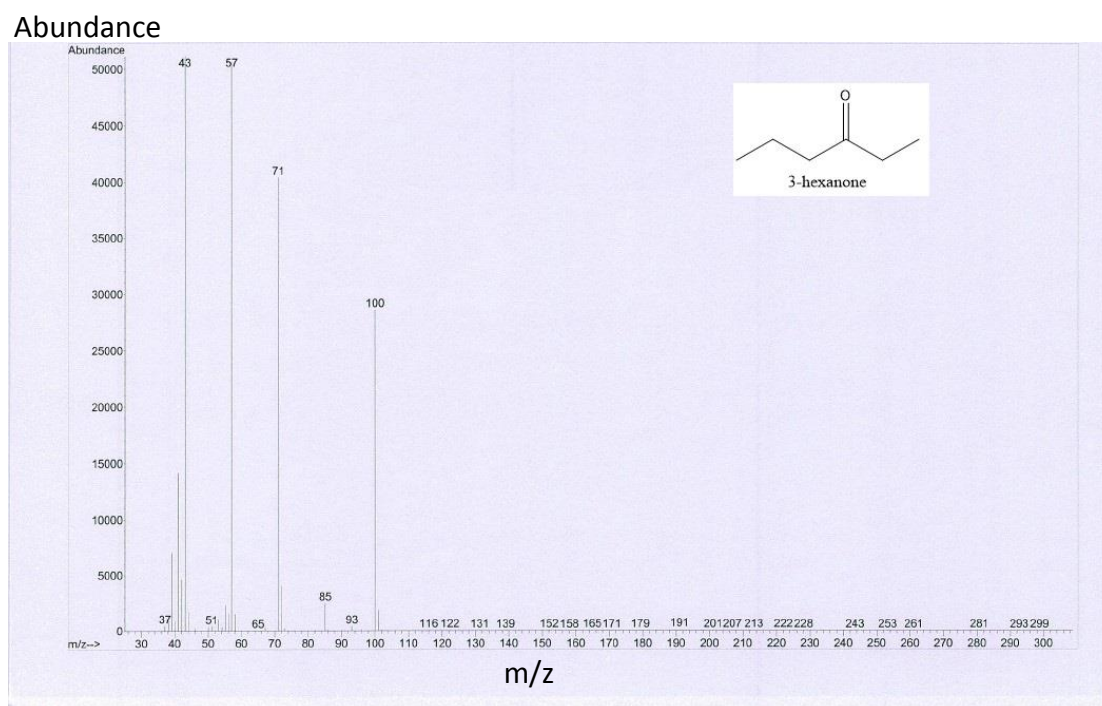


Figure 32. EI-MS Spectrum of 3-hexanone

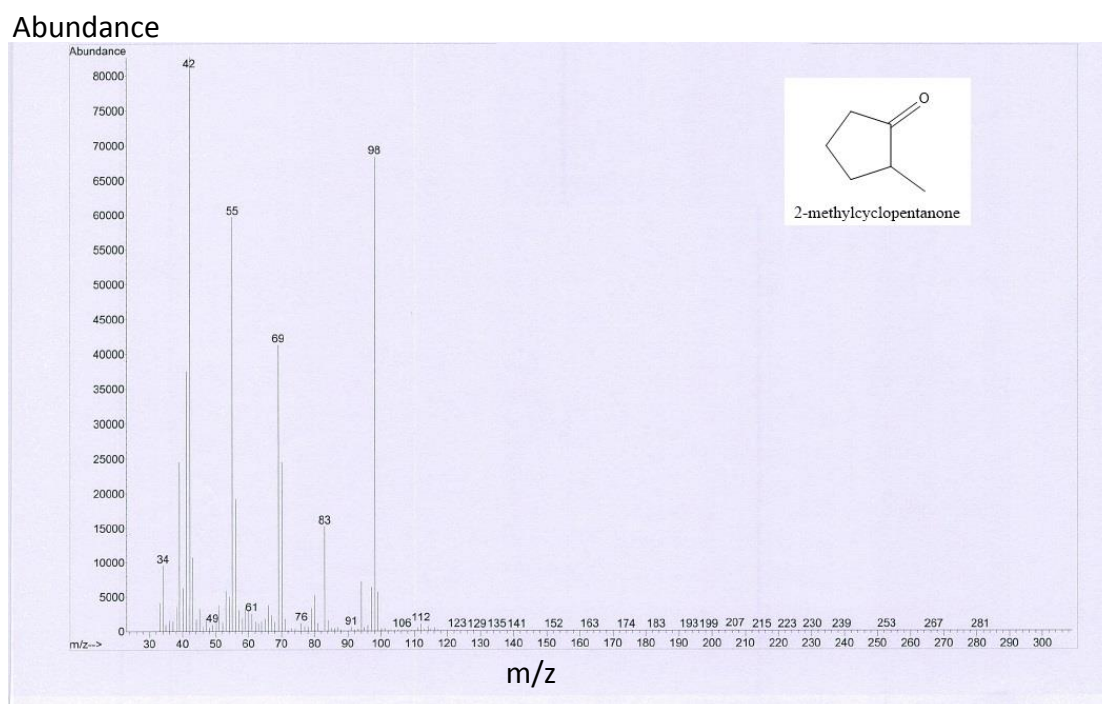


Figure 33. EI-MS Spectrum of 2-methylcyclopentanone

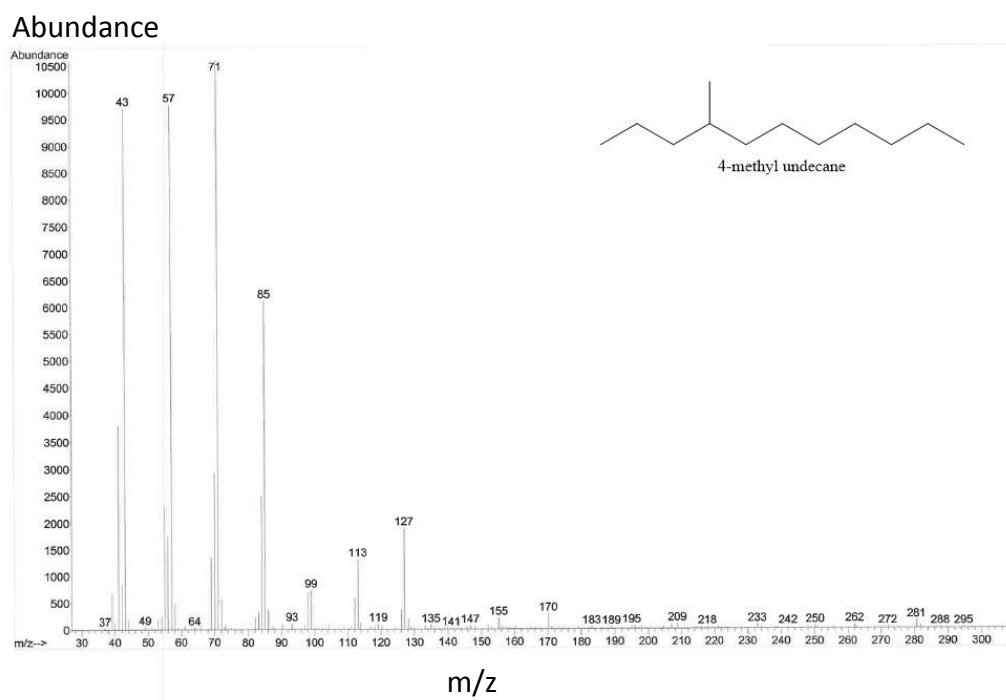


Figure 34. EI-MS Spectrum of 4-methyl undecane

Abundance

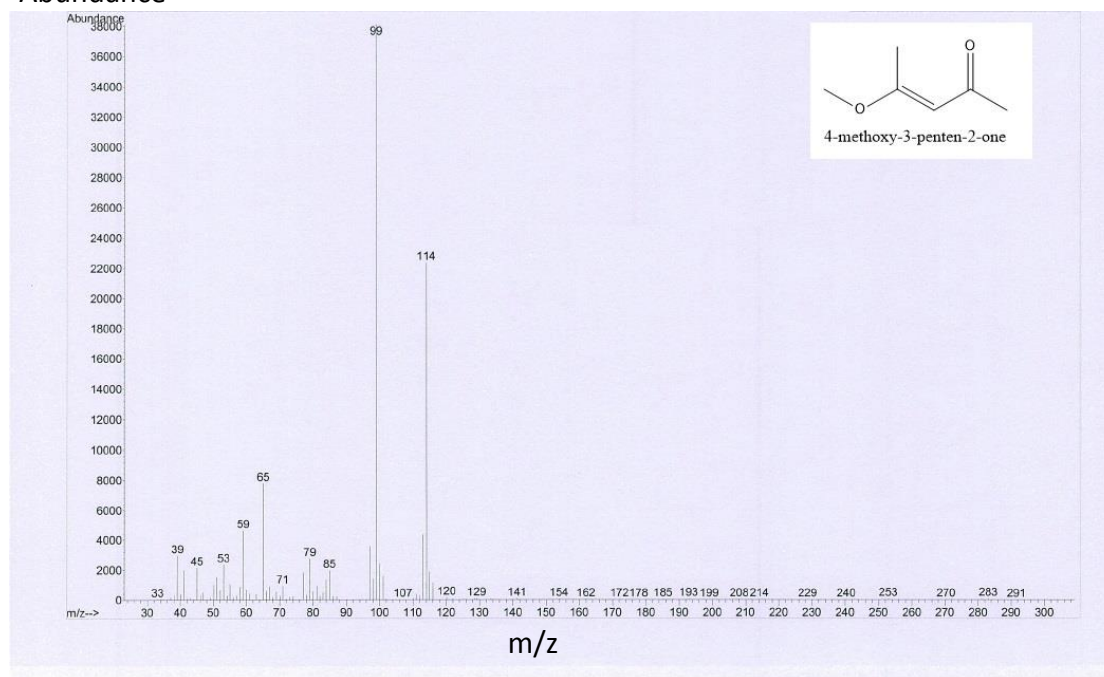


Figure 35. EI-MS Spectrum of 4-methoxy-3-penten-2-one

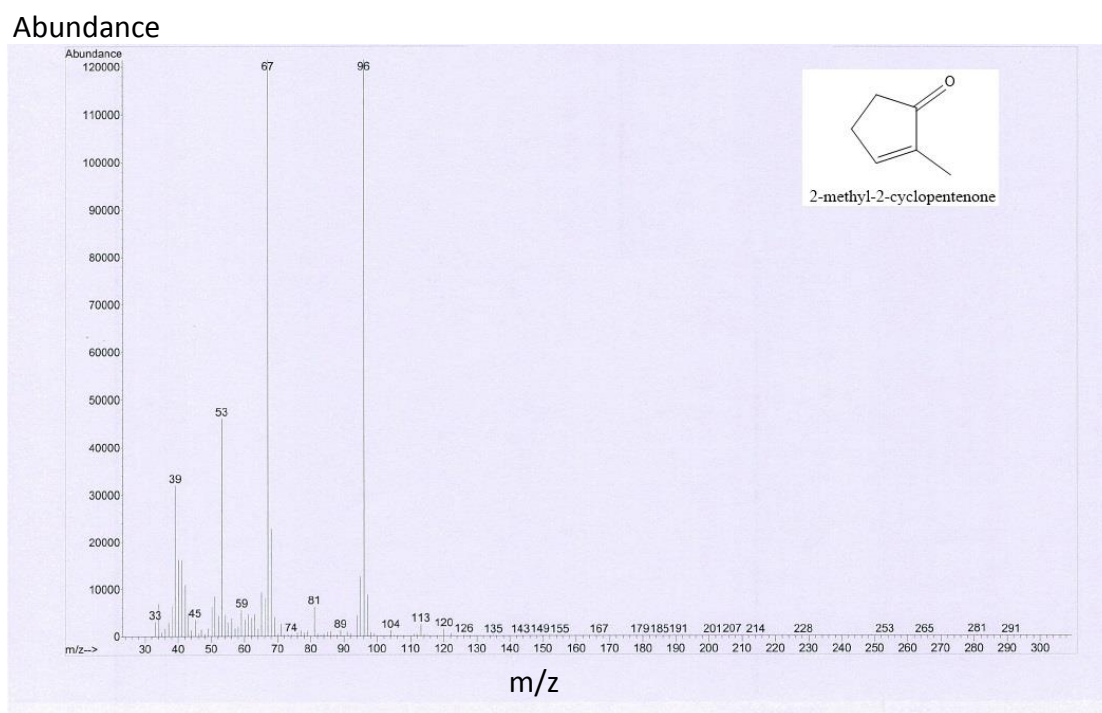


Figure 36. EI-MS Spectrum of 2-methyl-2-cyclopentenone

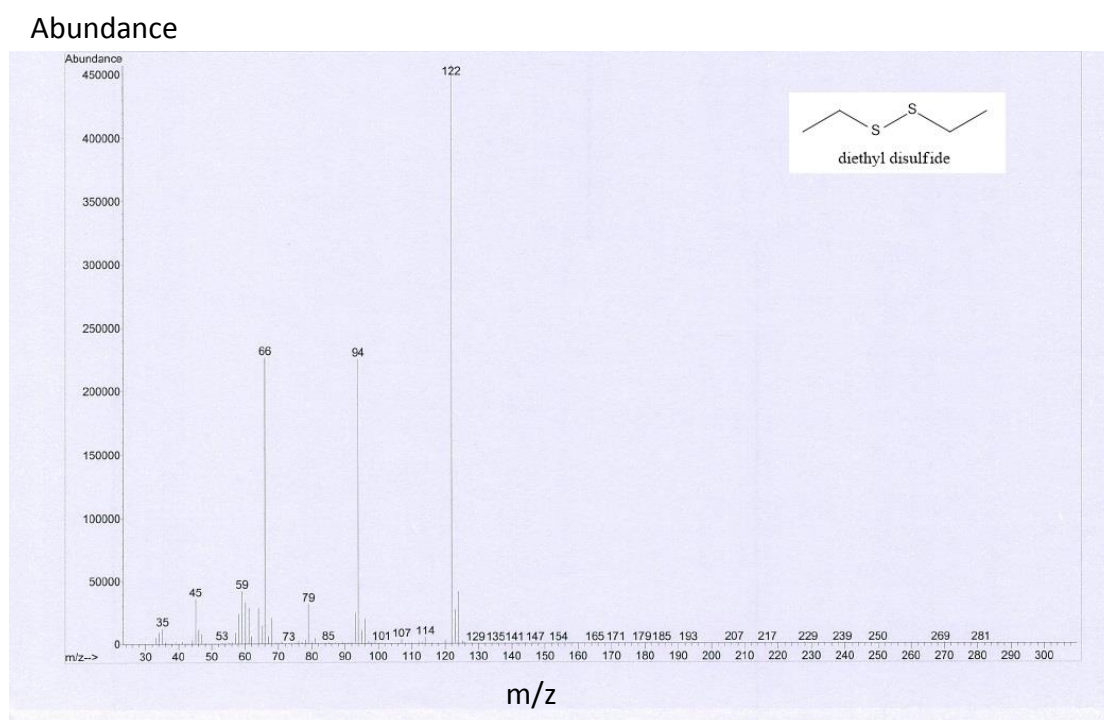


Figure 37. EI-MS Spectrum of diethyl disulfide

Abundance

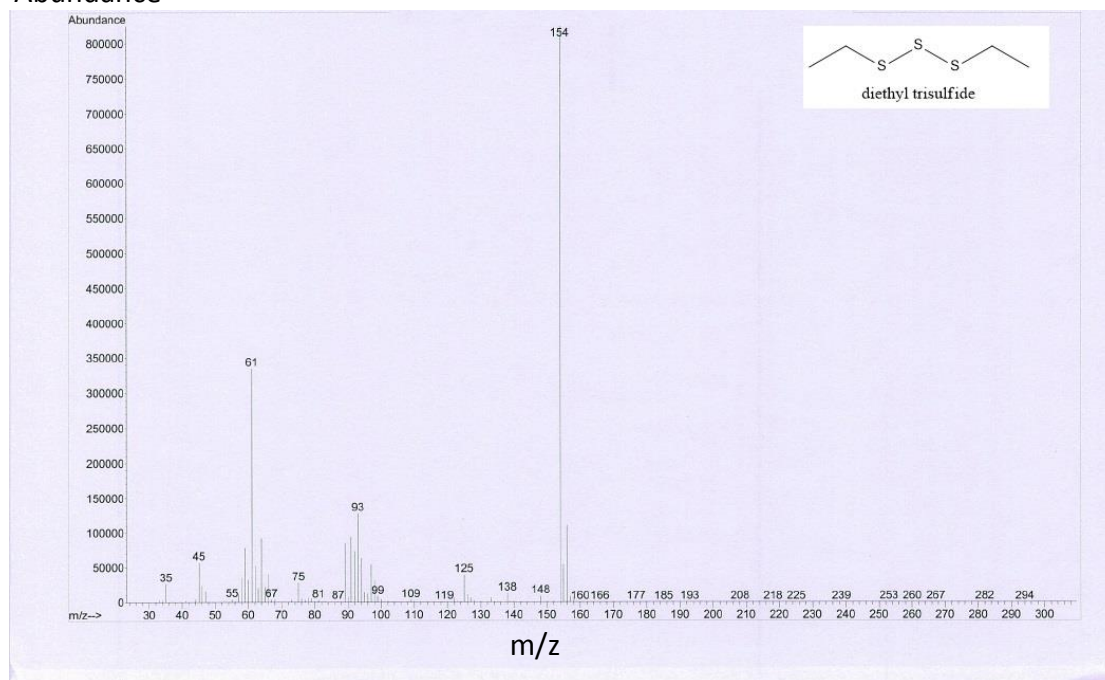


Figure 38. EI-MS Spectrum of diethyl trisulfide

Abundance

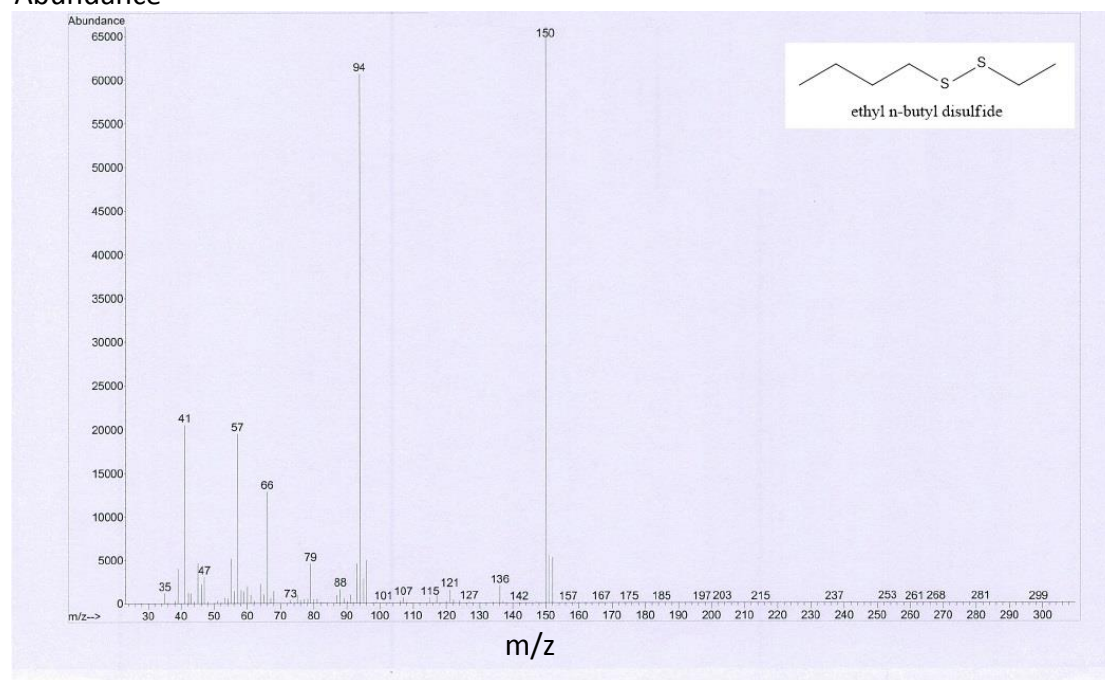


Figure 39. EI-MS Spectrum of ethyl n-butyl disulfide

Abundance

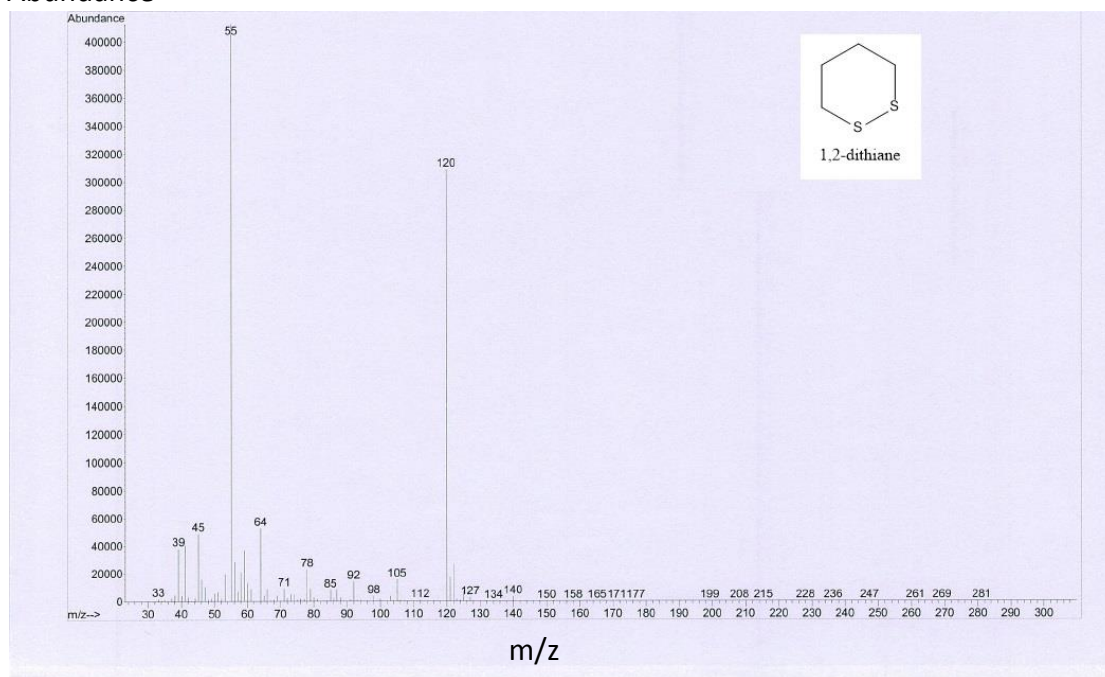


Figure 40. EI-MS Spectrum of 1,2-dithiane

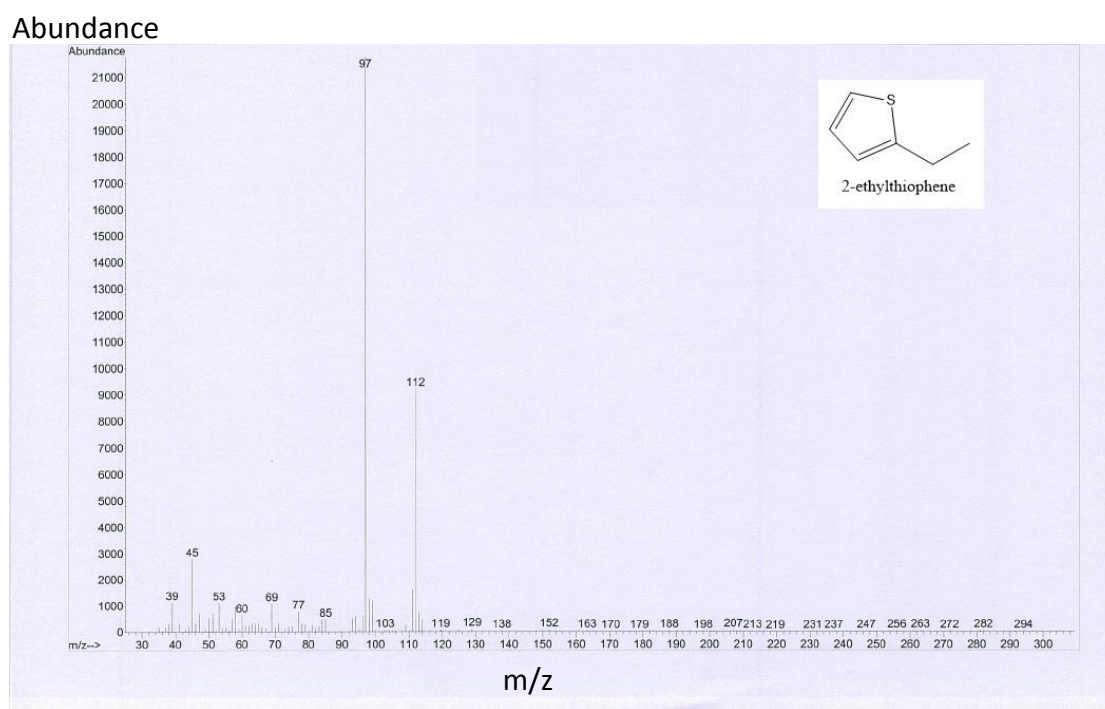


Figure 41. EI-MS Spectrum of 2-ethylthiophene

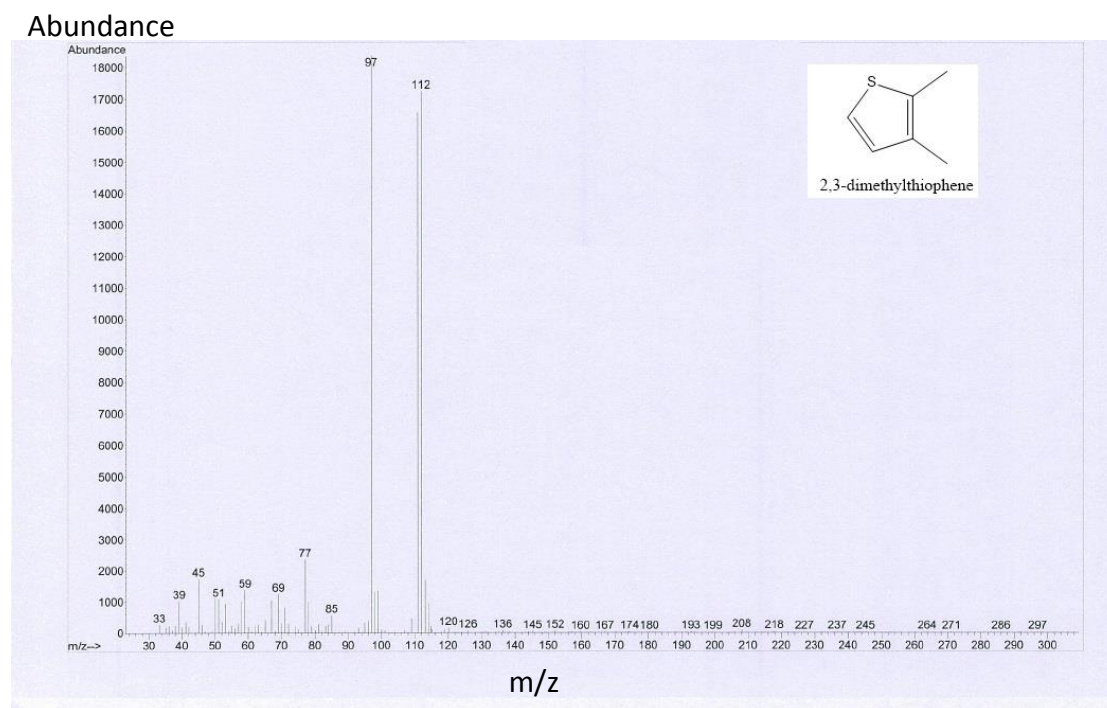


Figure 42. EI-MS Spectrum of 2,3-dimethylthiophene

Abundance

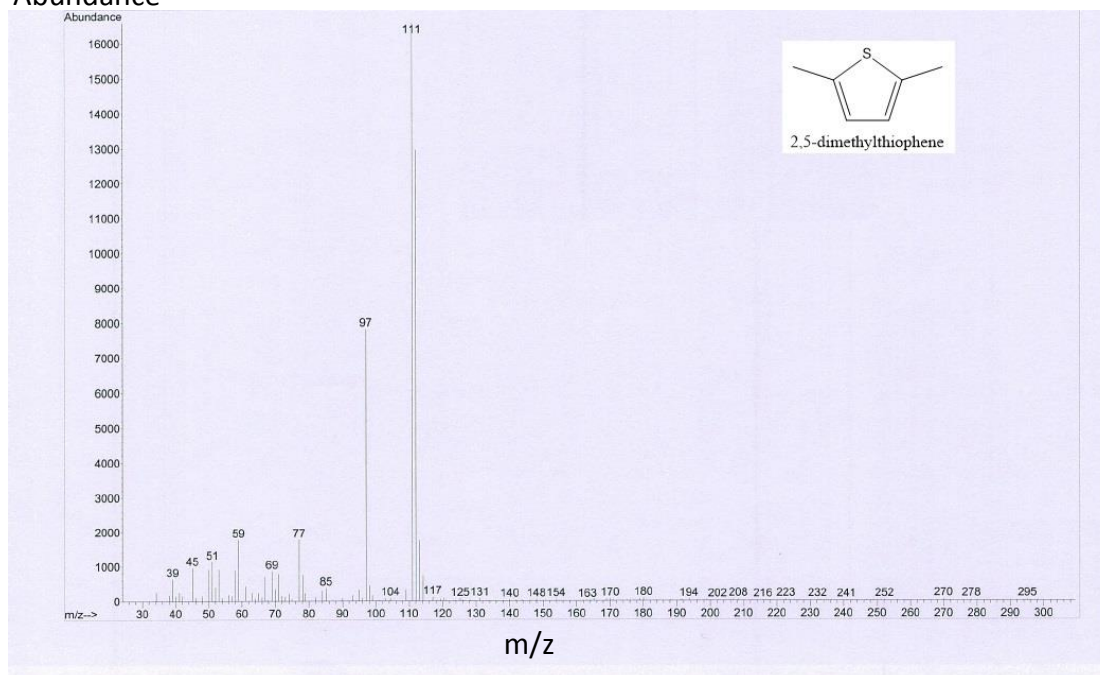


Figure 43. EI-MS Spectrum of 2,5-dimethylthiophene

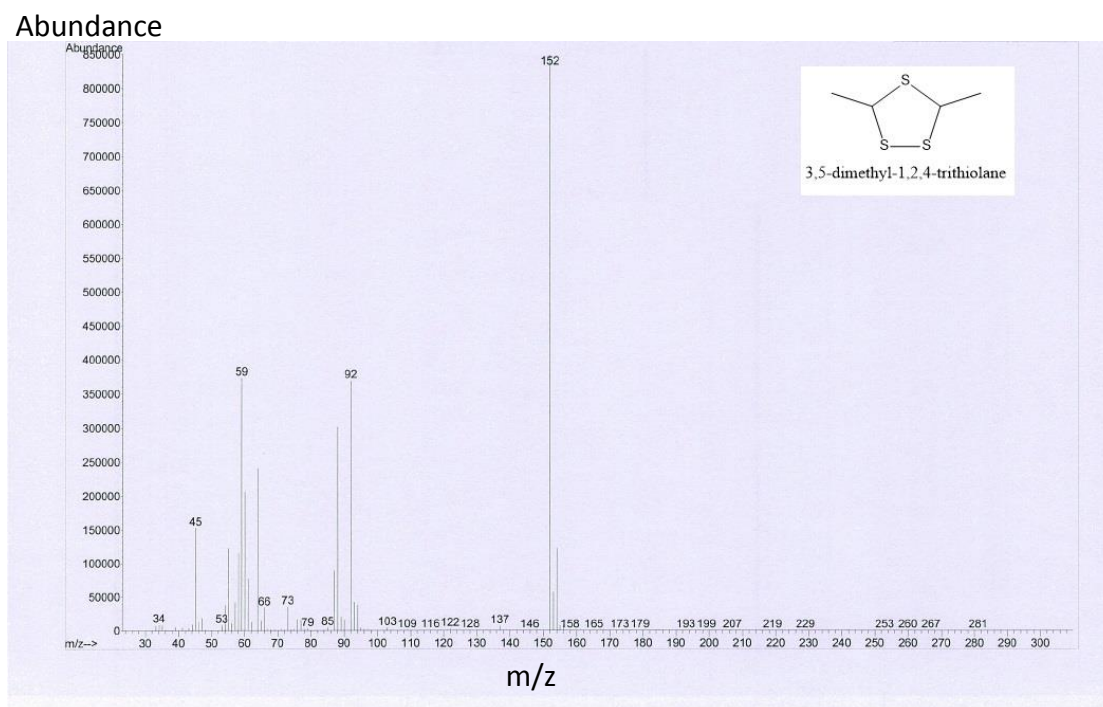


Figure 44. EI-MS Spectrum of 3,5-dimethyl-1,2,4-trithiolane

Abundance

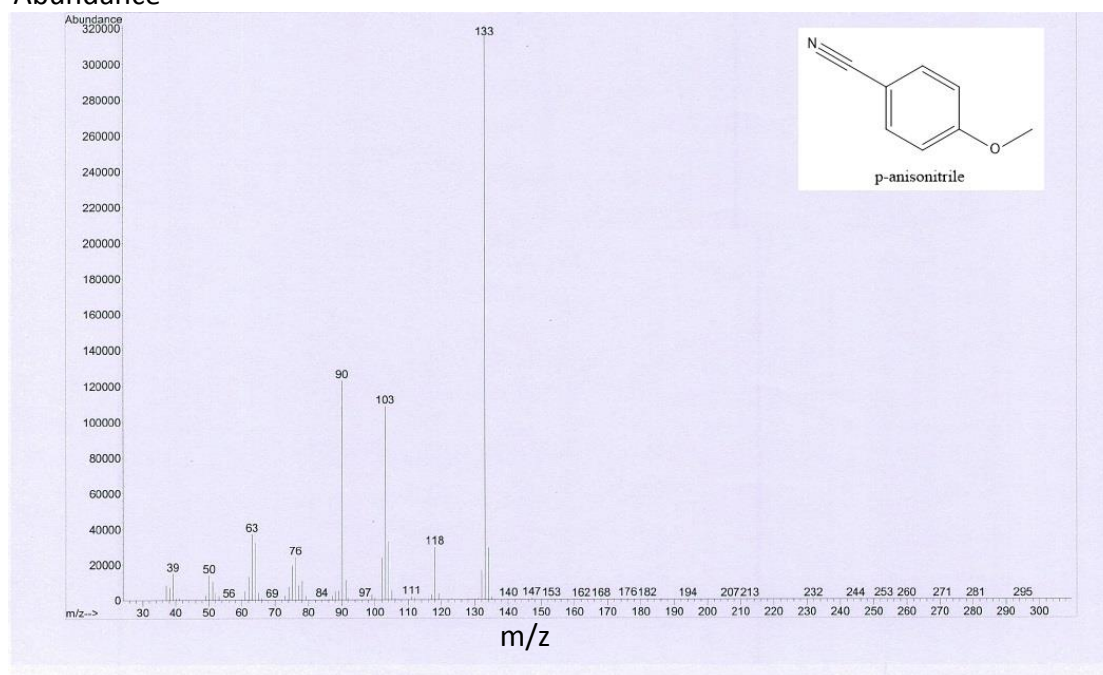


Figure 45. EI-MS Spectrum of p-anisonitrile

Abundance

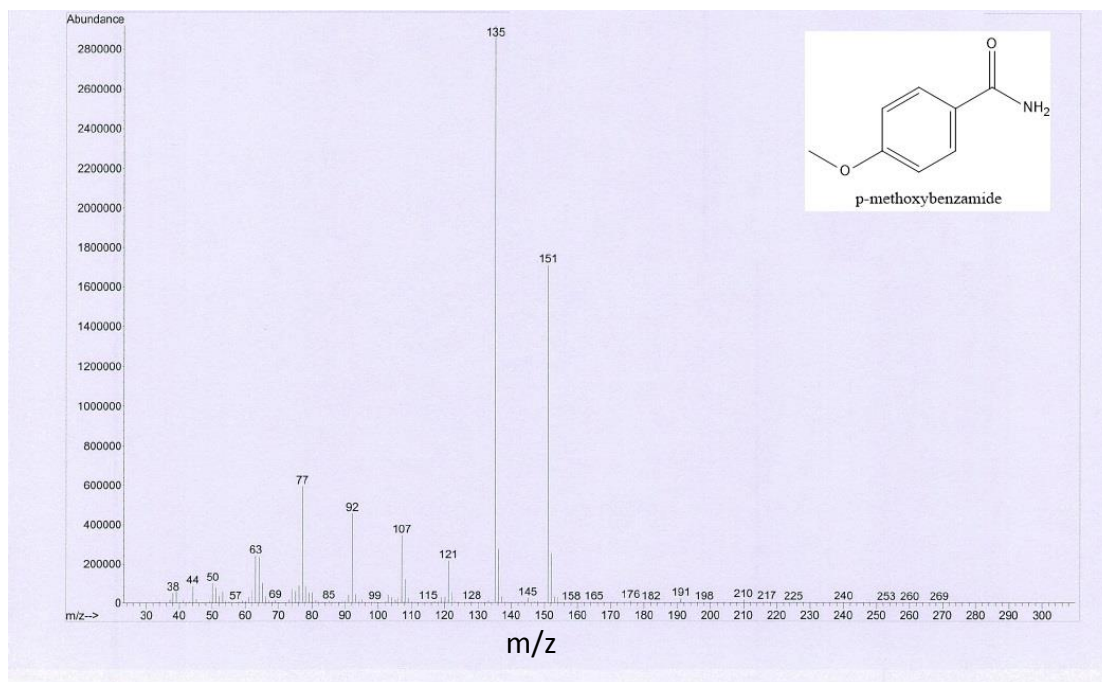


Figure 46. EI-MS Spectrum of p-methoxybenzamide

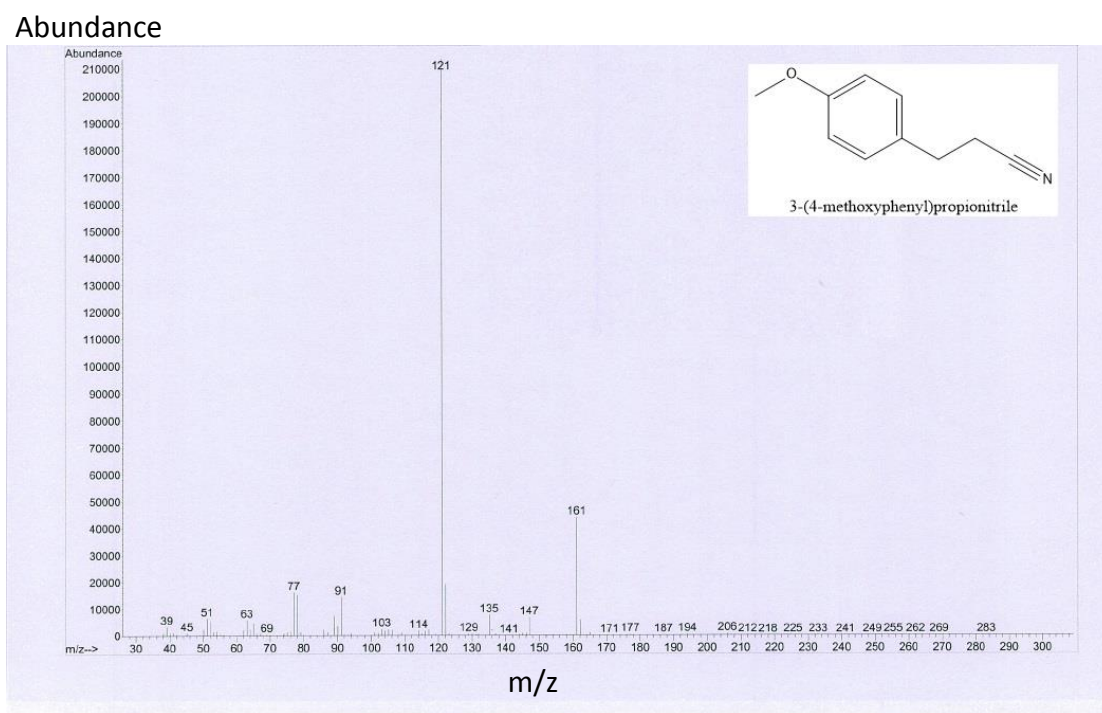


Figure 47. EI-MS Spectrum of 3-(4-methoxyphenyl)propionitrile

Abundance

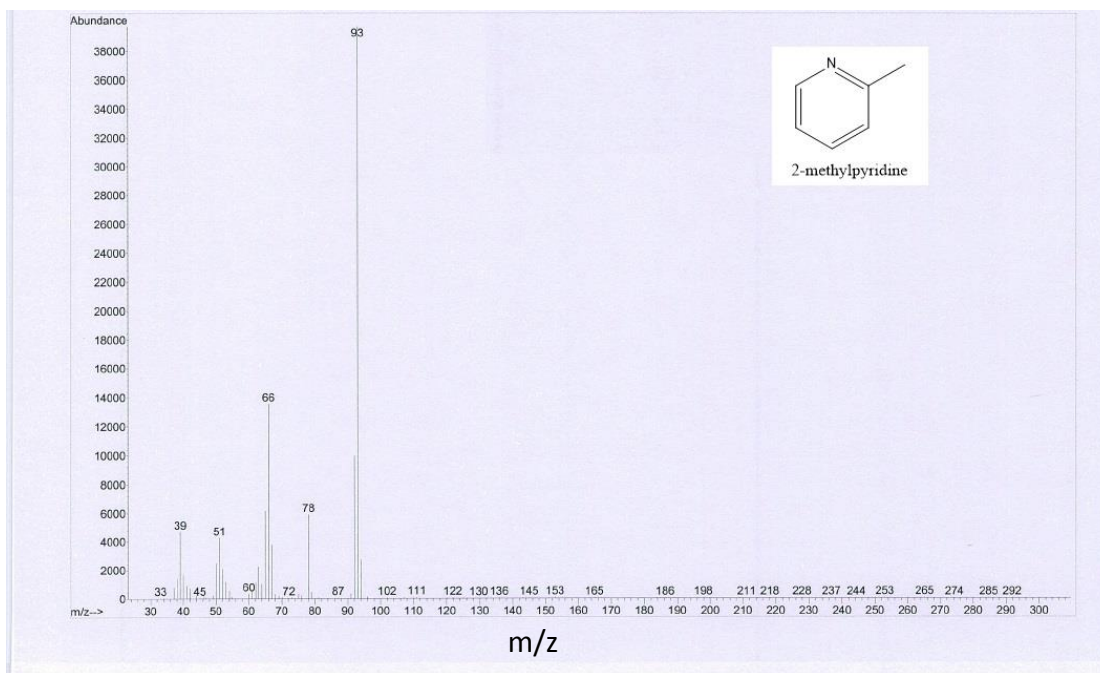


Figure 48. EI-MS Spectrum of 2-methylpyridine

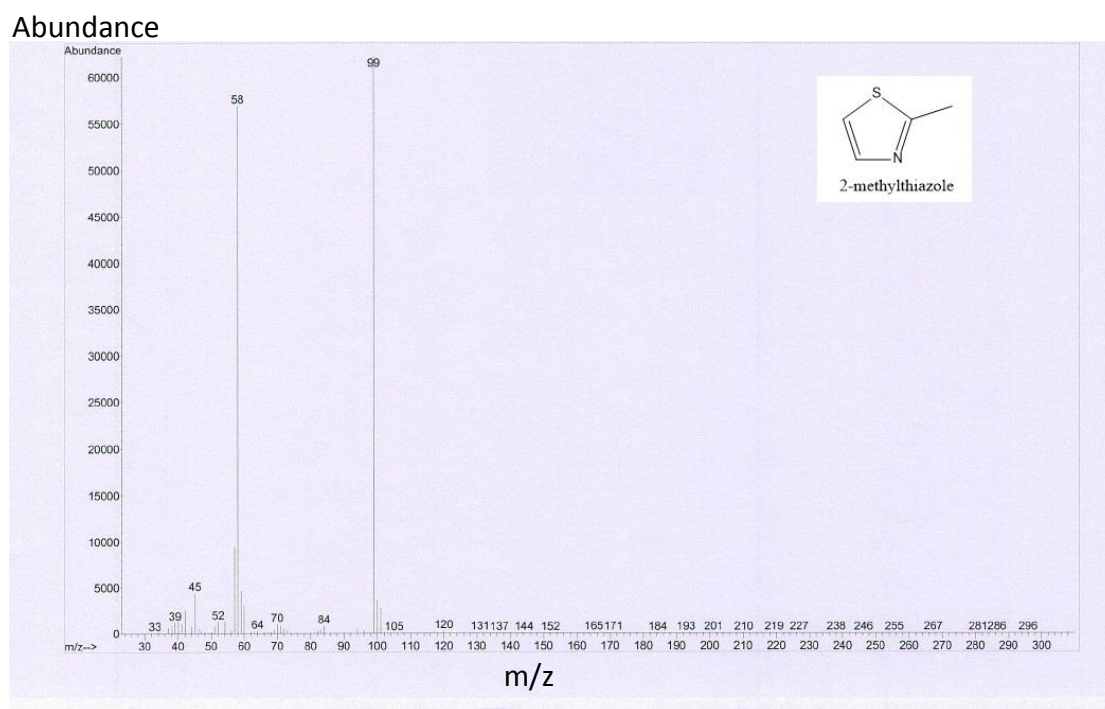


Figure 49. EI-MS Spectrum of 2-methylthiazole

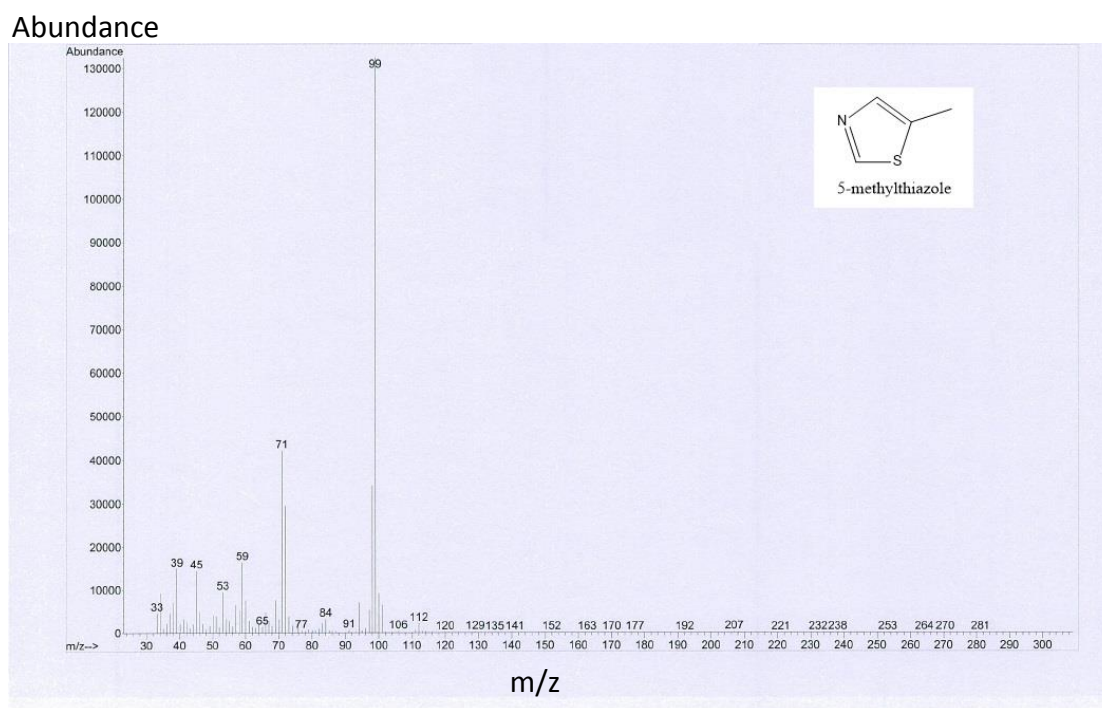


Figure 50. EI-MS Spectrum of 5-methylthiazole

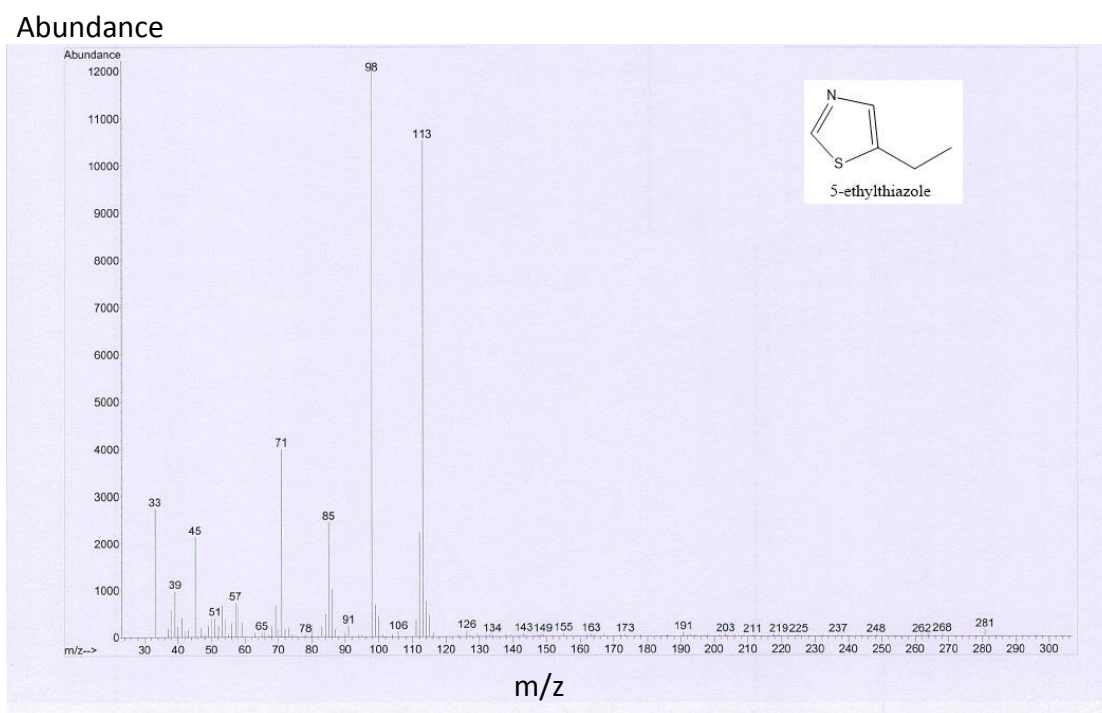


Figure 51. EI-MS Spectrum of 5-ethylthiazole

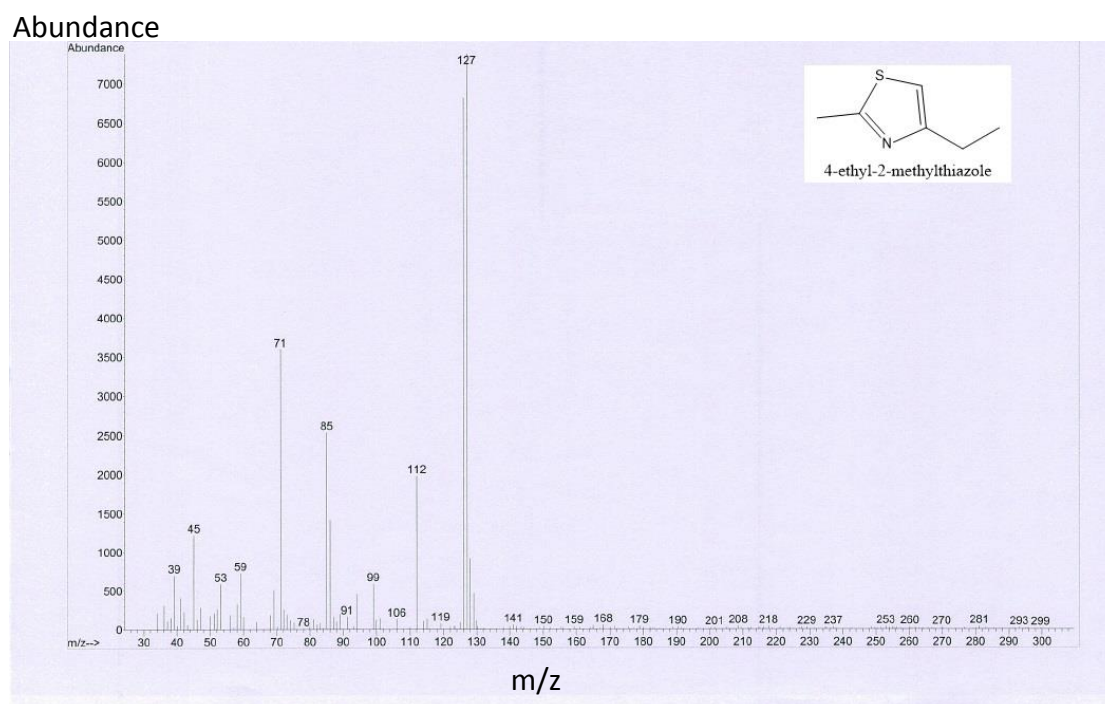


Figure 52. EI-MS Spectrum of 4-ethyl-2-methylthiazole

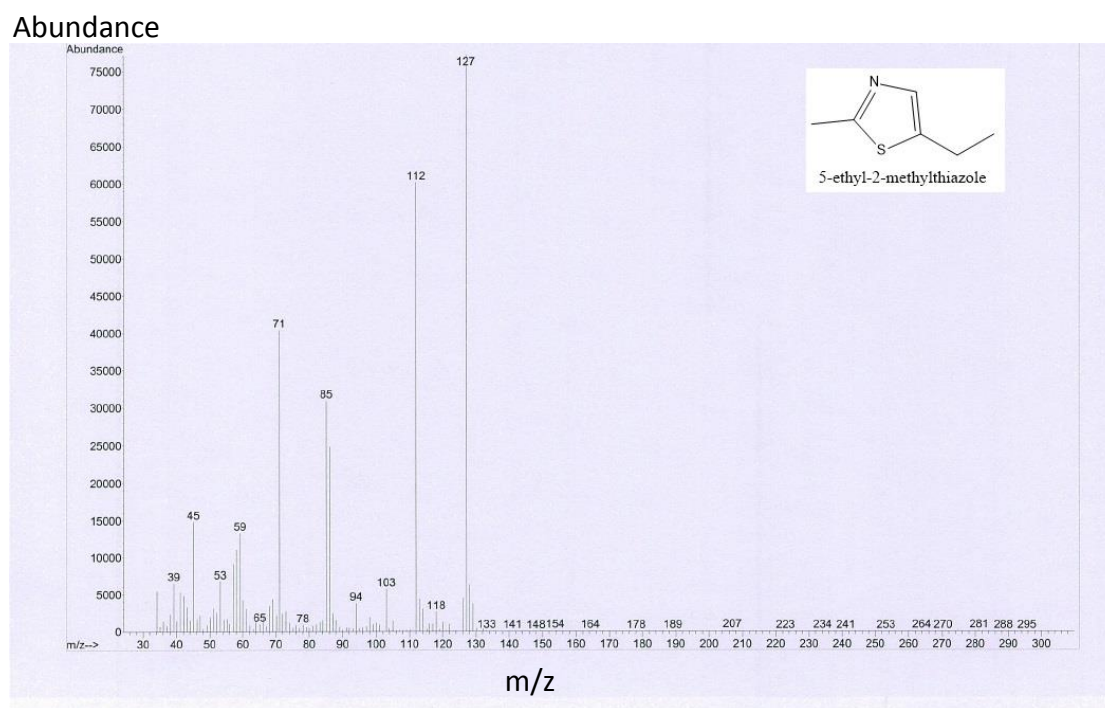


Figure 53. EI-MS Spectrum of 5-ethyl-2-methylthiazole

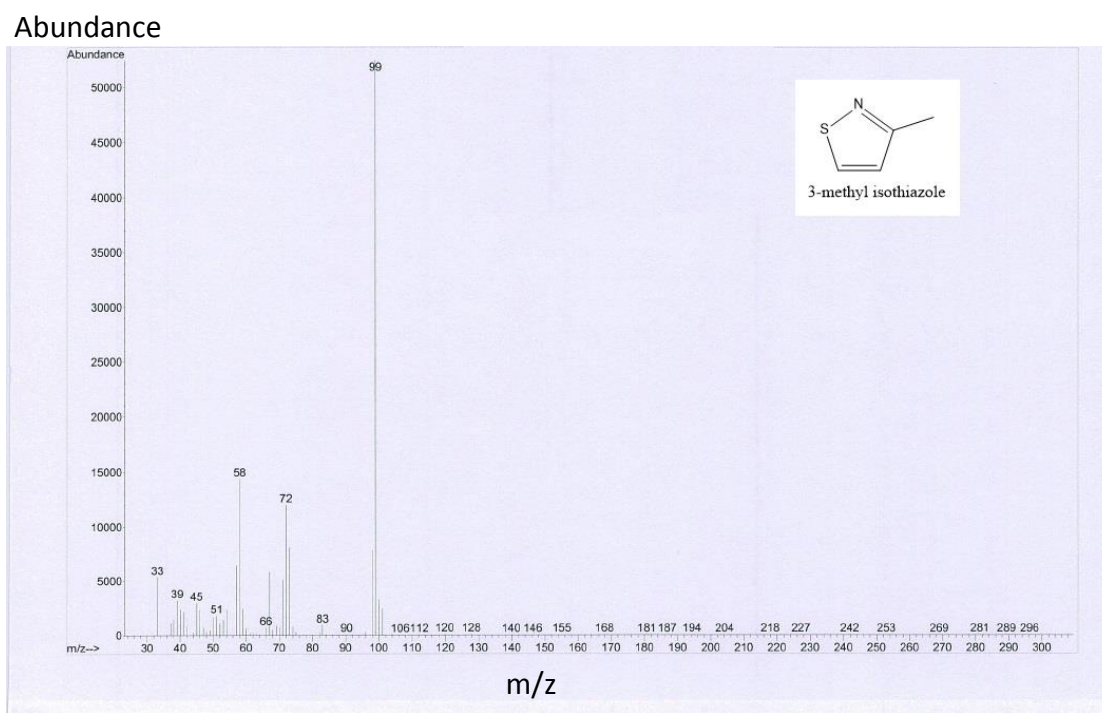


Figure 54. EI-MS Spectrum of 3-methyl isothiazole

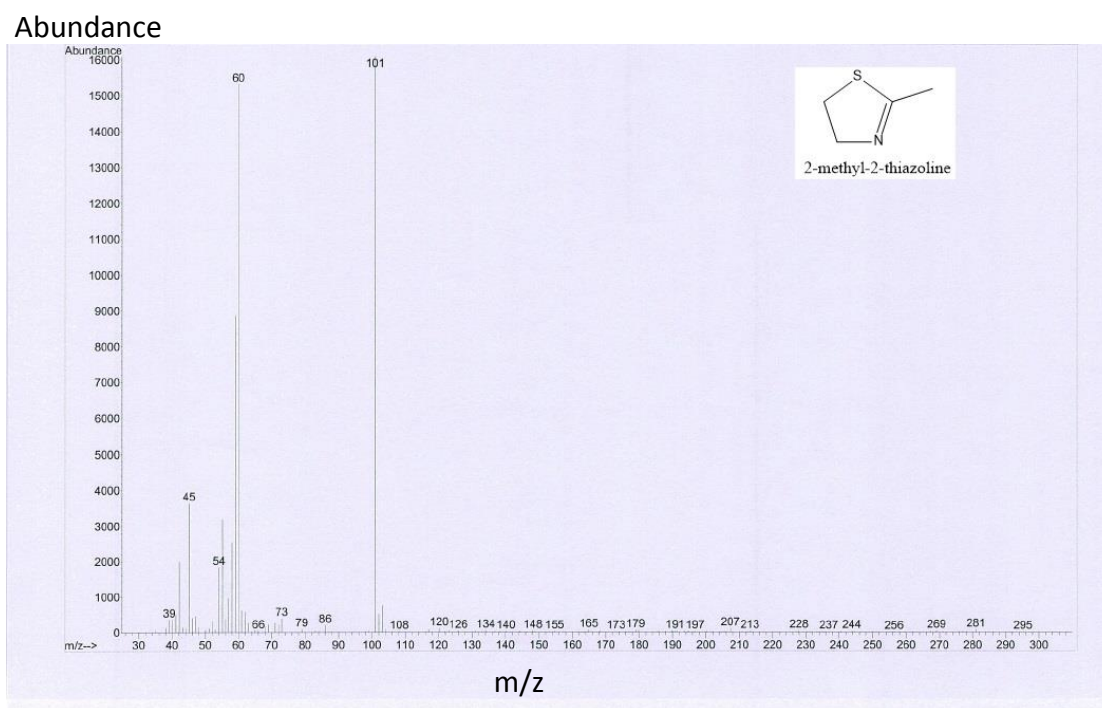


Figure 55. EI-MS Spectrum of 2-methyl-2-thiazoline

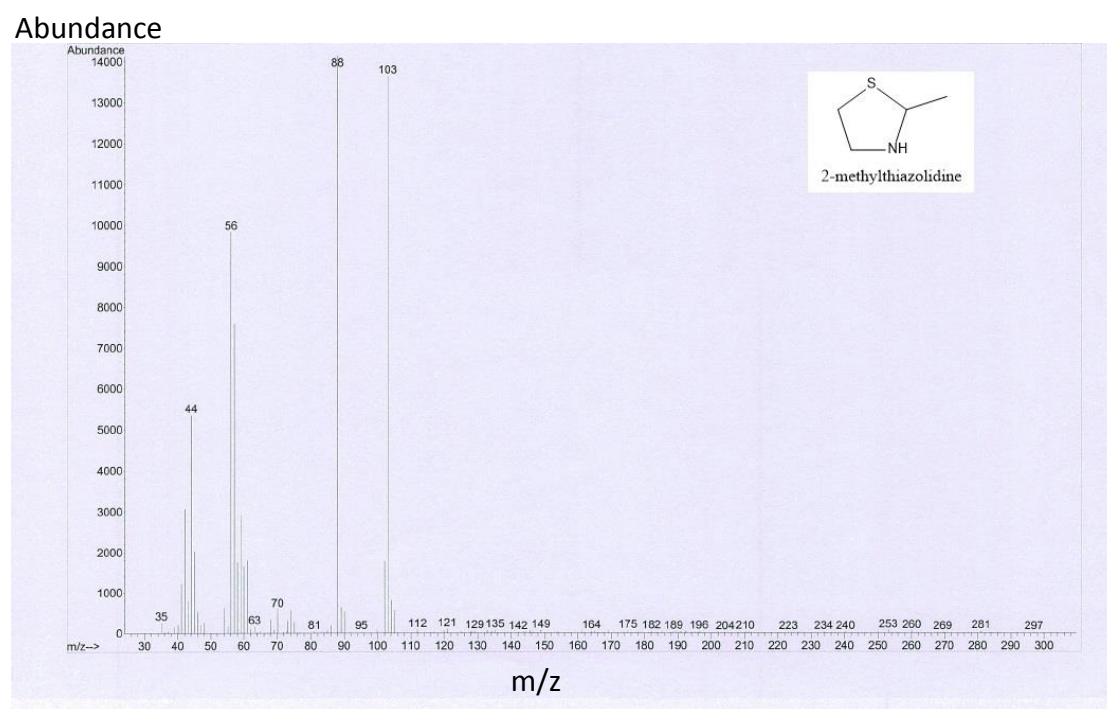


Figure 56. EI-MS Spectrum of 2-methylthiazolidine

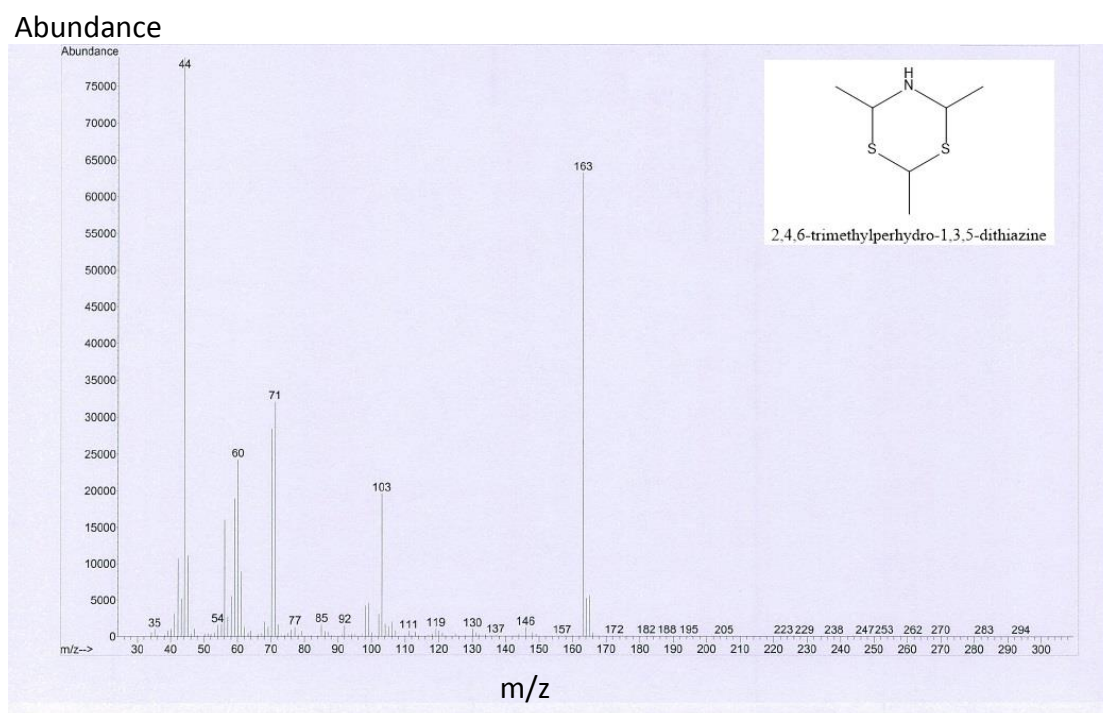


Figure 57. EI-MS Spectrum of 2,4,6-trimethylperhydro-1,3,5-dithiazine

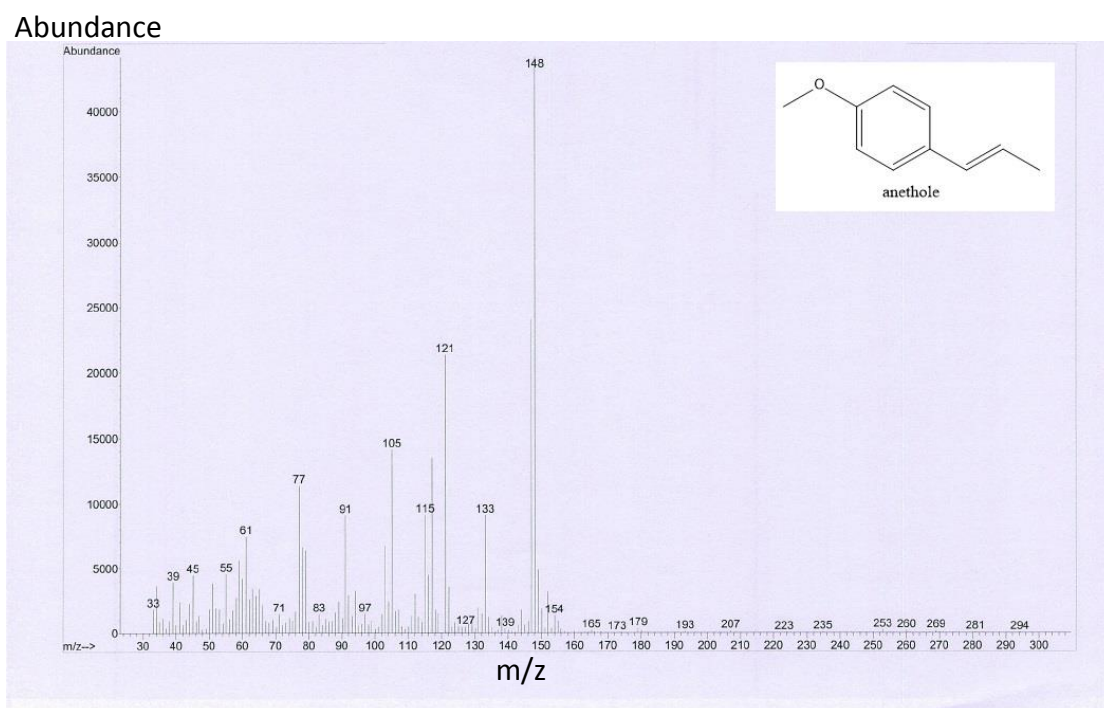


Figure 58. EI-MS Spectrum of anethole

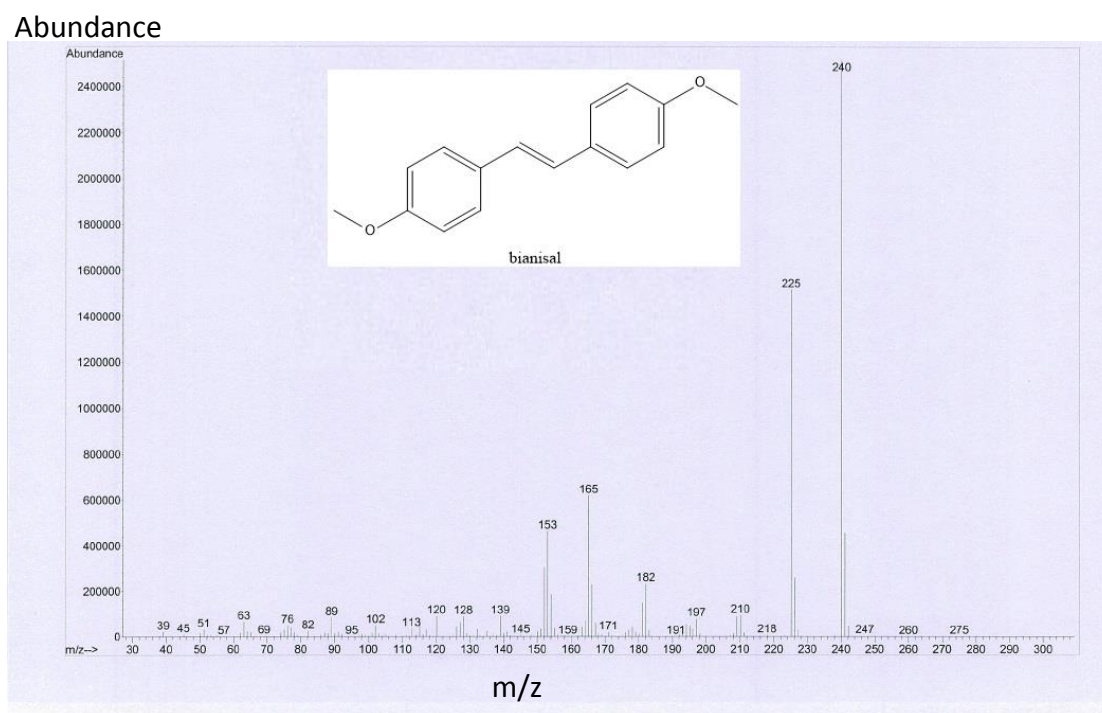


Figure 59. EI-MS Spectrum of bianisal

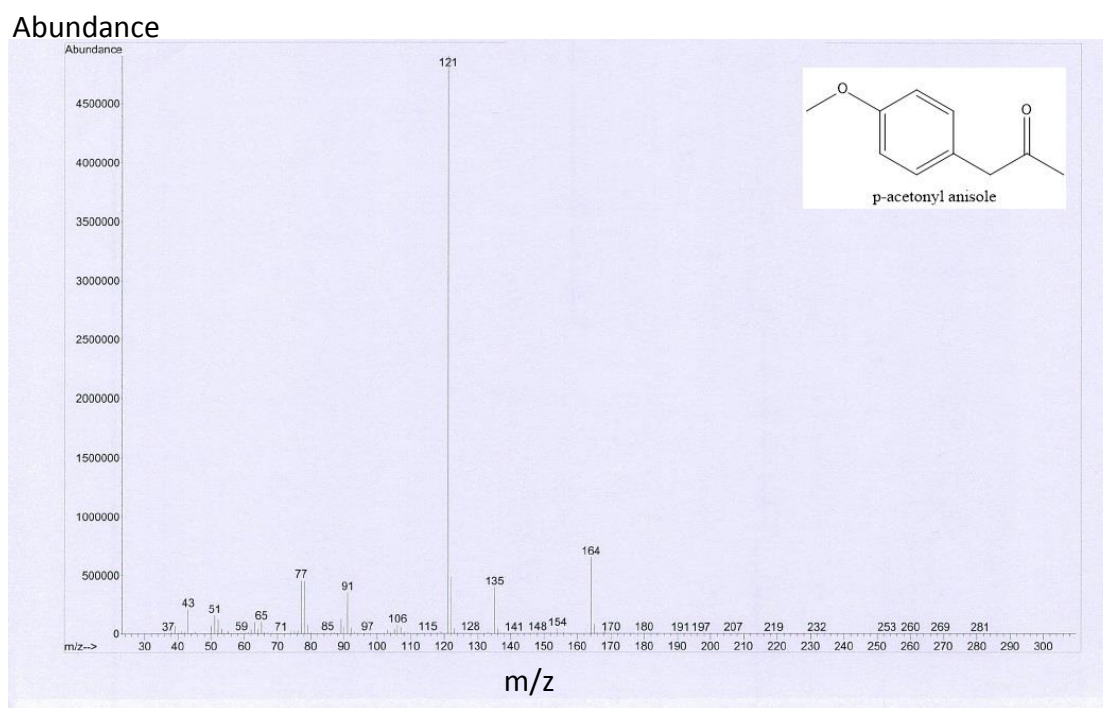


Figure 60. EI-MS Spectrum of p-acetonyl anisole

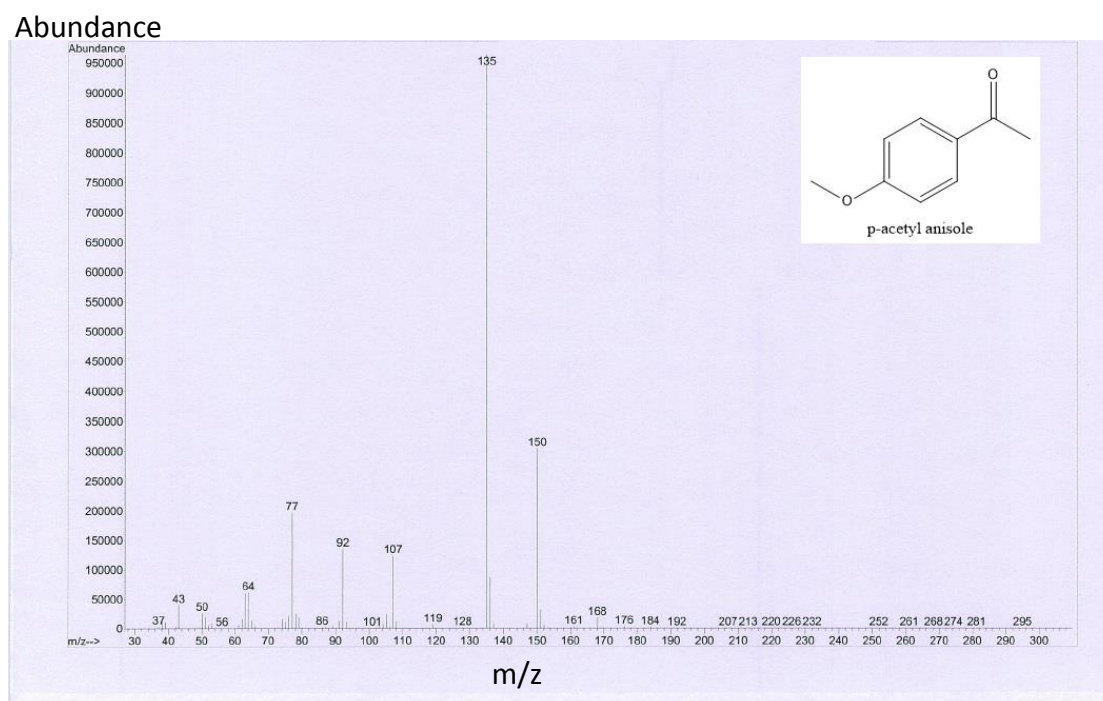


Figure 61. EI-MS Spectrum of p-acetyl anisole

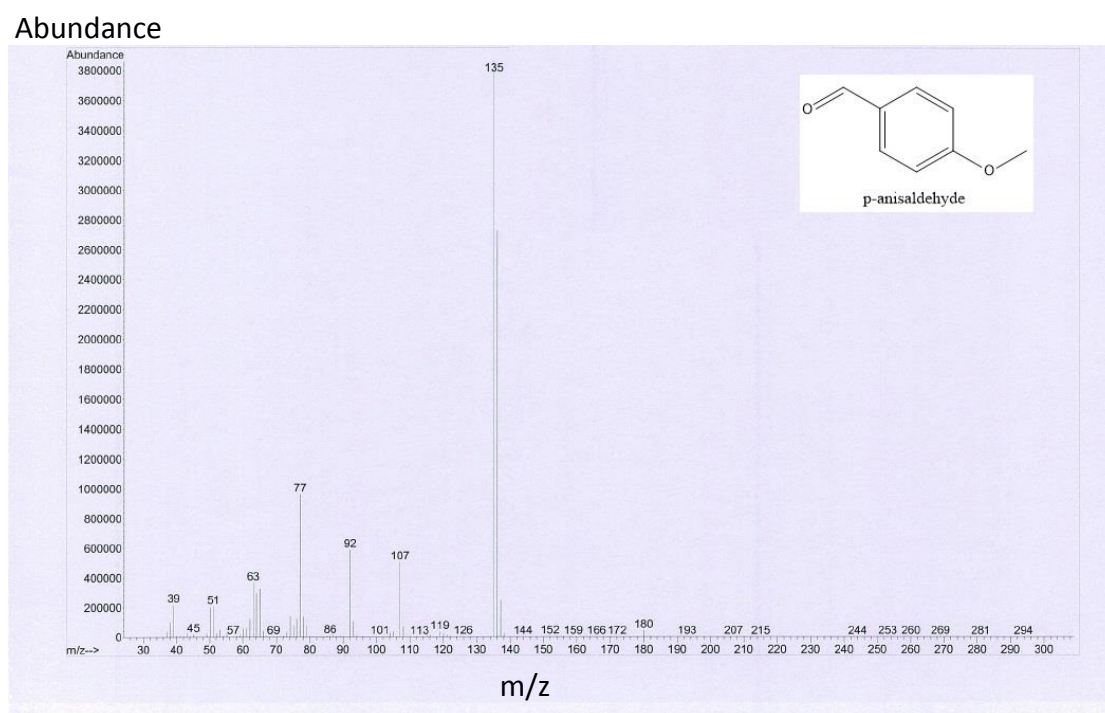


Figure 62. EI-MS Spectrum of p-anisaldehyde

Abundance

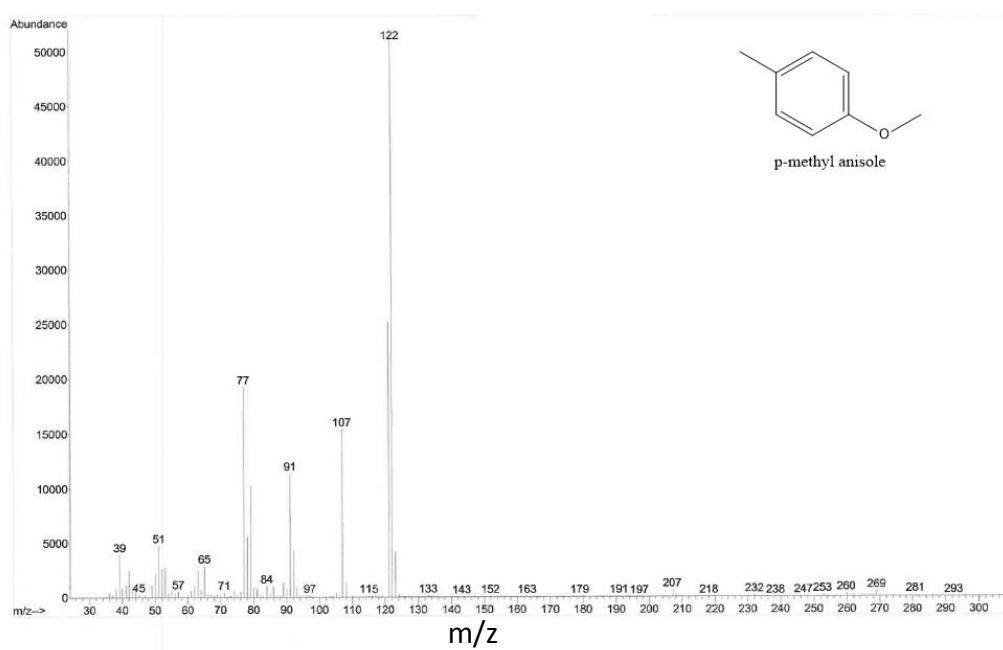


Figure 63. EI-MS Spectrum of p-methyl anisole

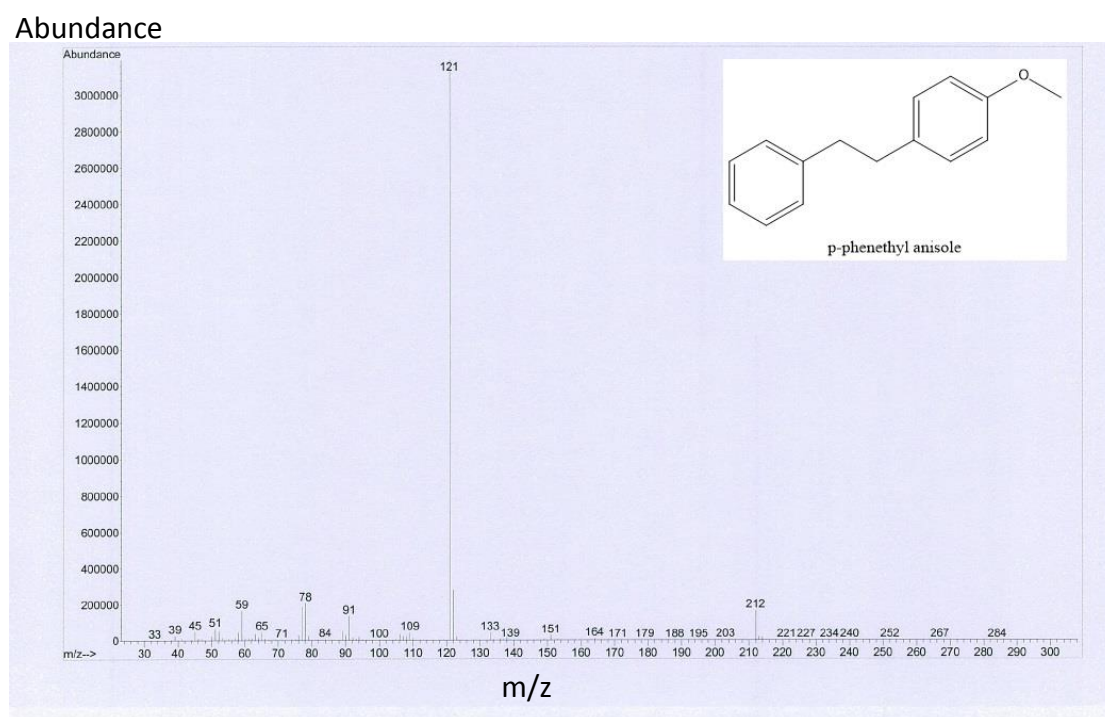


Figure 64. EI-MS Spectrum of p-phenethyl anisole

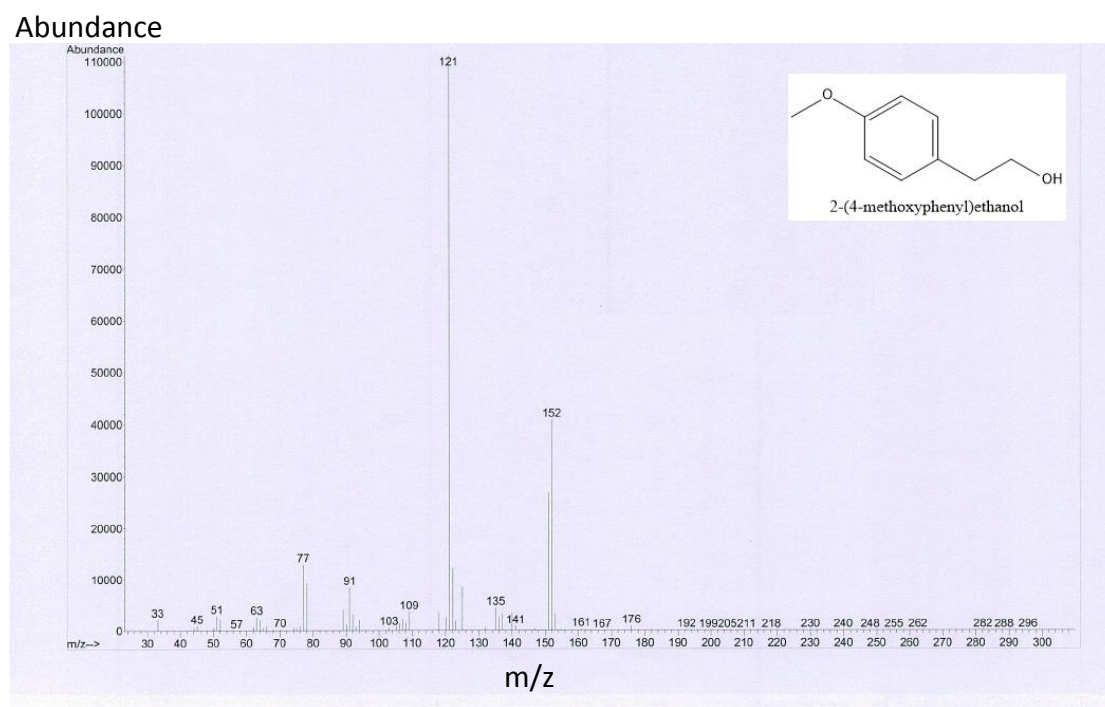


Figure 65. EI-MS Spectrum of 2-(4-methoxyphenyl)ethanol

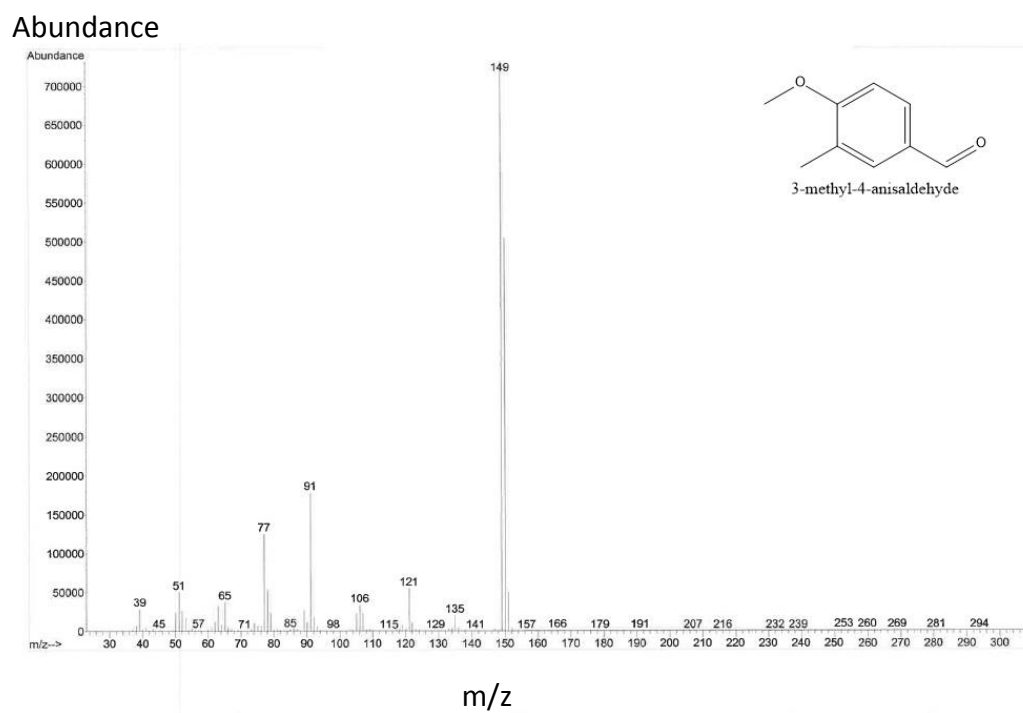


Figure 66. EI-MS Spectrum of 3-methyl-4-anisaldehyde

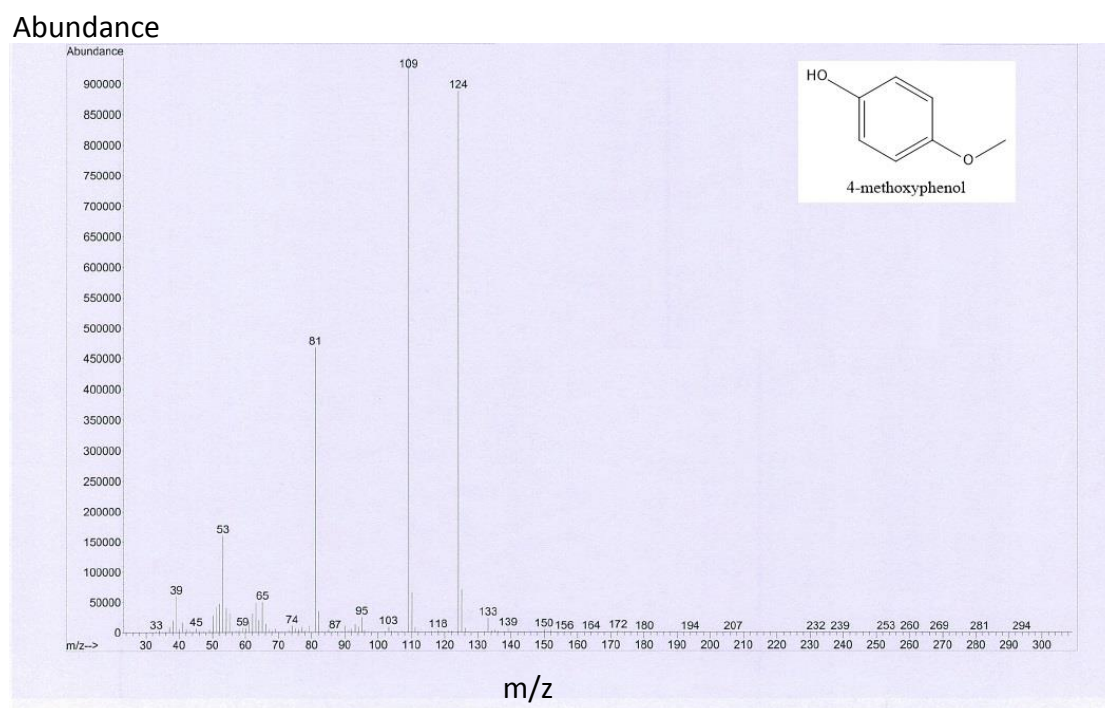


Figure 67. EI-MS Spectrum of 4-methoxyphenol

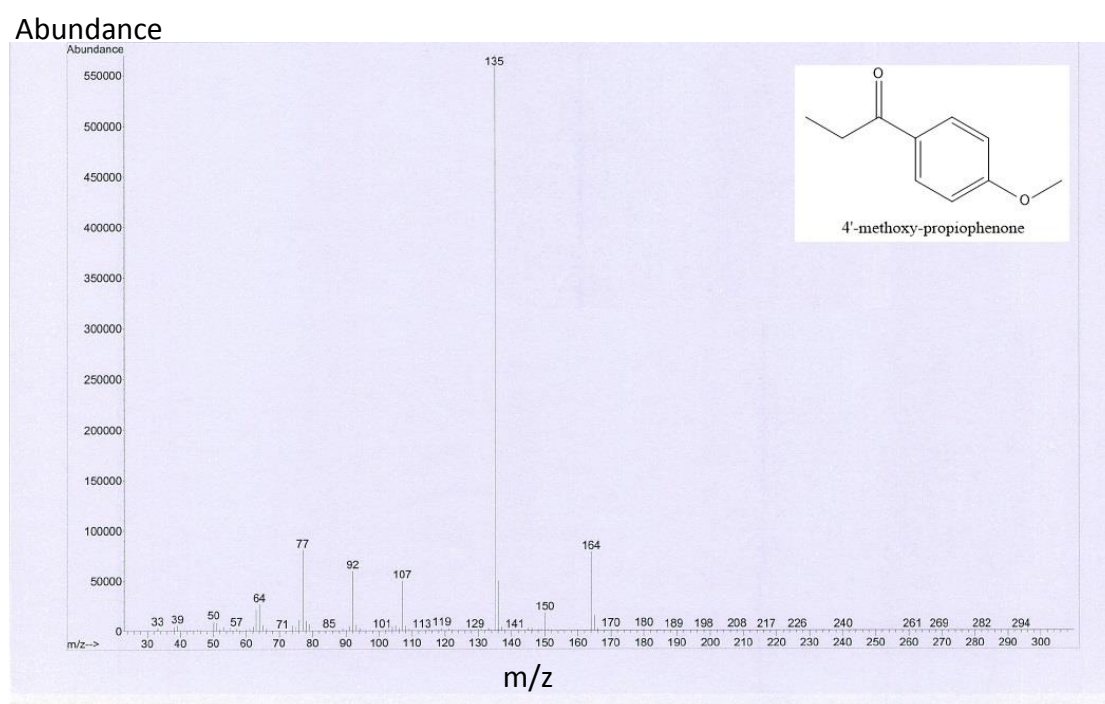


Figure 68. EI-MS Spectrum of 4'-methoxy-propiofenone

Figure 69. EI-MS Spectrum of methyl 2-(4-methoxyphenyl)acetate