DETERMINATION OF KEY AROMA ACTIVE COMPOUNDS IN RAW AND ROASTED LILY BULBS (BAI HE) – AN INGREDIENT IN CHINESE CUISINE

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ABSTRACT OF THE THESIS

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Lily bulbs, known as 'Bai He' in Chinese, are a popular ingredient used in Asian cooking, primarily in stir-fried dishes, soups, and stews. Despite their popularity, little is known about the odorants responsible for their pleasant aroma. The key aroma active compounds present in both raw and roasted lily bulbs (*Lilium longiflorum* Thunb.) were isolated by solvent extraction followed by solvent-assisted flavor evaporation (SAFE) and gas chromatography/Olfactometry (GC/O) analysis. The results of a comparative aroma extract dilution analysis (cAEDA) in the flavor dilution (FD) factor range from ≥ 1 to 1024 resulted in 48 aroma-active compounds, all of which were reported in *L. longiflorum* bulbs for the first time. The highest FD factors in raw lily bulbs were determined for (E)-hex-3-enal (green, FD 1024) and 3-(methylsulfanyl)propanal (methional) (cooked potato-like, FD 1024) followed by 1,8-cineole (eucalyptol) (eucalyptus-like, FD 256) and 2-phenylacetaldehyde (floral, FD 256). After thermal treatment, the odorants with high FDs in raw lily bulbs decreased in intensity. As a result of the roasting process, an increase in and generation of 34 aroma-active compounds was found in the roasted lily bulbs. The

aroma-active compounds with FD factors ≥ 1024 in roasted lily bulbs were identified as 2-acetyl-1-pyrroline (roasty); 3-(methylsulfanyl)propanal (methional) (cooked potato-like); 2-ethyl-3,5-dimethylpyrazine (earthy); 2,3-diethyl-5-methylpyrazine (earthy); 3-hydroxy-4,5-dimethylfuran-2(*5H*)-one (sotolon) (maple-like); 5-ethyl-3-hydroxy-4-methyl-5H-furan-2-one (abhexon) (maple-like) and 2-methoxy-4-[(E)-prop-1-enyl]phenol ((E)-isoeugenol) (clove-like). In summary, most of the aroma-active compounds with high FD factors in raw lily bulbs decreased significantly as a result of thermal treatment and a new pool of aroma-active compounds were formed during the roasting process.

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DEDICATION

I dedicate this work to my husband, Jeff, and two daughters, Josie and Evalyn, for their constant love and support.

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1 INTRODUCTION

The Easter lily (*Lilium longiflorum* Thunb., family Liliaceae), is a flowering plant with long prominent leaves and large, white trumpet-like flowers. Although it is most commonly grown as an ornamental plant because of its attractive appearance and fragrant aroma, lily bulbs are frequently consumed as food, such as stews, stir fries and soups, and as traditional medicine in many Asian cultures [1-3]. Despite the fact that they are consumed on a regular basis in Asia, little is known about their flavor.

To date, there have been no reports on the key aroma active compounds that contribute to the flavor of raw or roasted lily bulbs. The application of solvent-assisted flavor evaporation (SAFE), an efficient and versatile high vacuum distillation technique, has been used to carefully and directly isolate aroma volatiles from complex foods. Unlike other distillation techniques, SAFE has high thermal control, reducing the possibility of compound degradation and the generation of artifacts within the aroma extract. This isolation technique also produces aroma extracts that best represent the organoleptic properties of the original food. Comparative aroma extract dilution analysis (cAEDA) is a valuable tool for the identification of differences in the aroma-active compounds.

The first objective of this research was to characterize the key aroma active compounds in raw and roasted lily bulbs, specifically the Nellie White cultivar, by the application of a comparative aroma extract dilution analysis (cAEDA) on isolates generated by the solvent-assisted flavor evaporation (SAFE) distillation [4]. Comparison of the odorant profile of raw vs. roasted lily bulbs will help to provide new insights into our knowledge of which odorants are transferred from raw bulb to roasted bulb, which odorants are lost from the raw bulb during roasting, and which odorants are formed during thermal

treatment from flavor precursors present in raw bulbs. The second objective of this research was to generate quantitative descriptive data on both the raw and roasted lily bulbs to help us better understand what sensory attributes are complementing the key odorants of both types but more specifically the roasted as it is frequently used in Asian cooking.

2 LITERATURE REVIEW

2.1 Botany

L. longiflorum is native to the Ryukyu Islands of Japan. They belong to the class Liliopsida, order Liliales, family Liliaceae and genus *Lilium*. In addition to being called Easter lily, they are also commonly termed Bermuda lily, trumpet lily and white trumpet lily.

The botanical description of *L. longiflorum* consists of the following organs: bulb, stem, leaves and flowers. The average bulb below ground is roughly an inch and a half in diameter with roots stemming from the bottom as shown in Figure 1 and 2. Above ground, *L. longiflorum* has an unbranched stem that is roughly 16-38 inches tall, with glossy green leaves attached to it as shown in Figure 3. The leaves are roughly seven inches long and one inch wide. The white trumpet-like flower consists of six tepals and six stamens with yellow anthers stemming from the center as shown in Figure 4. The anthers are roughly seven inches long and often range in quantity of two to six per flower.

Figure 1. Bulb of *Lilium longiflorum* (root side down).

Figure 2. Bulb of *Lilium longiflorum* (root side up).

Figure 3. Glossy green leaves of *Lilium longiflorum*[5].

Figure 4. White trumpet-like flowers of *Lilium longiflorum*[6].

2.2 History in Food and Medicine

In Asia, lily bulbs are often utilized for consumption and medicinal purposes. In Chinese and Japanese cuisines, the bulbs of diverse lily species including *L. longiflorum*, *L. brownii* F. E. Br. ex Miellez var. viridulum Baker, *L. pensylvanicum* Ker Gawl., and *L. pumilum* DC [1, 2], are roasted or boiled in similar ways as potatoes (*Solanum tuberosum*) [7] and used in recipes such as soups and stir-fries. Lily bulbs are a source of carbohydrate and protein, and are low in fat. In addition to their nutritional value, lily bulbs are enjoyed in recipes due to their rich flavor. In Asia, there are various cultivars of edible lilies that exhibit different flavor profiles. For example, both 'culinary' and 'medicinal' varieties of *L. brownii* are commercially available. The 'culinary' lily bulb varieties are considered to have a more attractive flavor profile than their medicinal counterparts [8]. Despite the fact that lily bulbs are consumed on a regular basis and are in important food in the Asian diet, little is known about their flavor composition.

In addition to culinary uses, lily bulbs species, *L. brownii* F. E. Br. exMiellez var. *viridulum* Baker, *L. longiflorum*, *L. pumilum* DC, and *L. pensylvanicum* Ker Gawl, are frequently consumed for their purported medicinal and health promoting properties, including the treatment of inflammation and lung ailments, such as coughs [2, 3, 9]. For these purposes, the bulbs are usually taken in the form of 'medicinal' soups, in combination with other Traditional Chinese Medicine (TCM) botanicals, and as an ingredient in shelf-stable liquid preparations such as cough syrups. Because of their long history of traditional use, bulbs of numerous *Lilium* species have been increasingly the subject matter of scientific probing into their chemistry and biological activities

with respect to potential health promoting properties. These studies have led to the discovery of numerous natural products, especially steroidal glycosides [10].

2.3 Chemistry and Bioactivity

L. longiflorum is composed of many natural products such as phenolic glycosides [11], flavonoids [12], carotenoids [13], sterols [13], steroidal saponins [10, 14], steroidal glycoalkaloids [10] and phenylpropanoid glycerol glucosides [15]. Some of these have been reported to have bioactivity. Despite the frequent medicinal usage of *L. longiflorum*, the bioactive components and mechanisms accountable for their purported medicinal properties still remains obscure [15].

Steroidal glycosides, a class of compound that include saponins and glycoalkaloids, are well known for their putative biological activities such as anticancer [16-19], antiviral [20], anti-inflammatory [21], cholesterol-lowering [22], antihypertensive [23], antidiabetic [24], anticholinergic [25], platelet aggregation inhibition [26, 27] and antifungal [28, 29]. In *L. longiflorum*, it was reported that six spirostanol saponins and three furostanol saponins exhibited antitumor activity [30]. It has also been reported that steroidal glycosides in *L. longiflorum* could possibly be a factor in the healing process of wounds. Reports that steroidal glycosides promote dermal fibroblast migration *in vitro* suggest that these compounds in *L. longiflorum* may enhance wound healing [31]. Given these observations, steroidal glycosides, other bioactive components and their mechanisms of action certainly merit further exploration.

2.4 Key Aroma-active Compounds in Liliopsida

The class Liliopsida is composed of a variety of genera including, but not limited to, *Allium*, *Asparagus* and *Fritillaria* whose aroma profiles have been investigated. The

genus *Allium* includes garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) which have been consumed in both raw and cooked form since ancient times. Previous studies on the flavor profile of raw onions show that sulfur containing compounds such as S-(E)-1-propenyl-L-cysteine S-oxides, S-methyl-L-cysteine S-oxides and S-propyl-L-cysteine S-oxides are the key contributors to the sharp pungent and eye-stinging sensation of freshly cut onions [32]. The aroma profile of onions that have endured thermal treatment is more complex as compared to its raw counterpart. Heating of onions gives rise to new volatiles as a result of autoxidation, thermal degradation and Maillard reaction between amino acids and reducing sugars. As a result, the key odorants contributing to the flavor profile of cooked onions were primarily cyclic sulfur compounds and thiols, followed by aldehydes, carboxylic acids, ketones, hydrocarbons, furans, pyrroles and alcohols [33].

The genus *Asparagus* is both consumed as a food and utilized for ornamental purposes. *Asparagus officinalis* L. is the edible species that is widely cultivated and consumed. Unlike *A. sativum* and *A. cepa*, *Asparagus officinalis* L. is predominantly eaten when cooked. The aroma profile of raw asparagus is largely composed of sulfur-containing carboxylic acids and esters such as methyl-1,2-dithio-lane-4-carboxylate and 1,2-dithiolane-4-carboxylic acid (asparagusic acid) [34]. Thermal treatment of the asparagus resulted in dimethyl sulfide as the key component contributing to the cooked asparagus aroma followed by 3-(methylthio)propanal (methional), 2-sec-butyl-3-methoxypyrazine, 1-octen-3-one, 2,3-butanedione (diacetyl) and 2-acetylpyrroline [35].

Fritillaria is mainly known for their bell-shaped flowers that are used for ornamental purposes. Certain *Fritillaria* species including *F. imperialis* and *F. agrestis*,

are also known for emitting unpleasant scents that can be described as phenolic, putrid sulfurous, sweaty and skunky [36]. Investigation of the aroma profile of the bulbs of six *Fritillaria* species and cultivars concluded that the major volatile component contributing to the foul, skunky aroma is 3-methyl-2-butene-1-thiol [36].

The commonality of the key aroma-active compounds of the genera mentioned above is that they contain sulfur compounds in the raw and/or cooked form. Similar to *Allium*, *Asparagus* and even *Fritillaria*, the *Lilium* species, specifically *L. longiflorum*, also contains sulfur-containing aroma compounds in both its raw and roasted aroma profiles. These findings will be discussed in the present work.

2.5 Roasting Process

The roasting process is a key factor in generating the characteristic aroma of the roasted foods. The process allows one to better interpret the flavor profile of the roasted food by understanding which odorants are passed on from the original product to the roasted product, which odorants are removed from the initial product, and which odorants are generated from the flavor precursors present in the initial product.

The volatiles produced during the roasting can be mainly attributed to the Maillard reaction between amino acids and reducing sugars within the food. The chemical pathway of the Maillard reaction can be visually explained by Hodge's model in three essential phases (Figure 5). The first phase is the reaction between an amino group and the carbonyl group of the reducing sugar, resulting in the production of water and a glycosylamine. Due to the instability of the glycosylamine, Amadori or Heyns rearrangement typically follows. The second phase is the dehydration and de-amination of the Amadori or Heyns product. At a pH $\leq$ 7, 1,2-enolization will occur

and give rise to hydroxymethylfurfural (HMF) or furfural. If the system exhibits a pH > 7, 2,3-enolization will occur producing reactive fission products such as carbonyls or α-dicarbonyl compounds and reductones such as deoxyosones. In the last phase of the Maillard reaction, the deoxyosones undergo further rearrangements and polymerizations to form a large number of volatiles and melanoidins responsible for the aroma, color and texture of thermally processed foods.

A common degradation reaction that often follows the Maillard reaction is Strecker degradation. This reaction occurs when dicarbonyl compounds from fission products or deoxyosones interact with free amino acids forming aldehydes and aminoketones (Figure 6) which often possess strong odors. Common Strecker aldehydes include 3-methyl(sulfanyl)propanal (methional) (cooked potato-like), 2- and 3-methylbutanal (malty) and 2-phenylacetaldehyde (floral, honey-like).

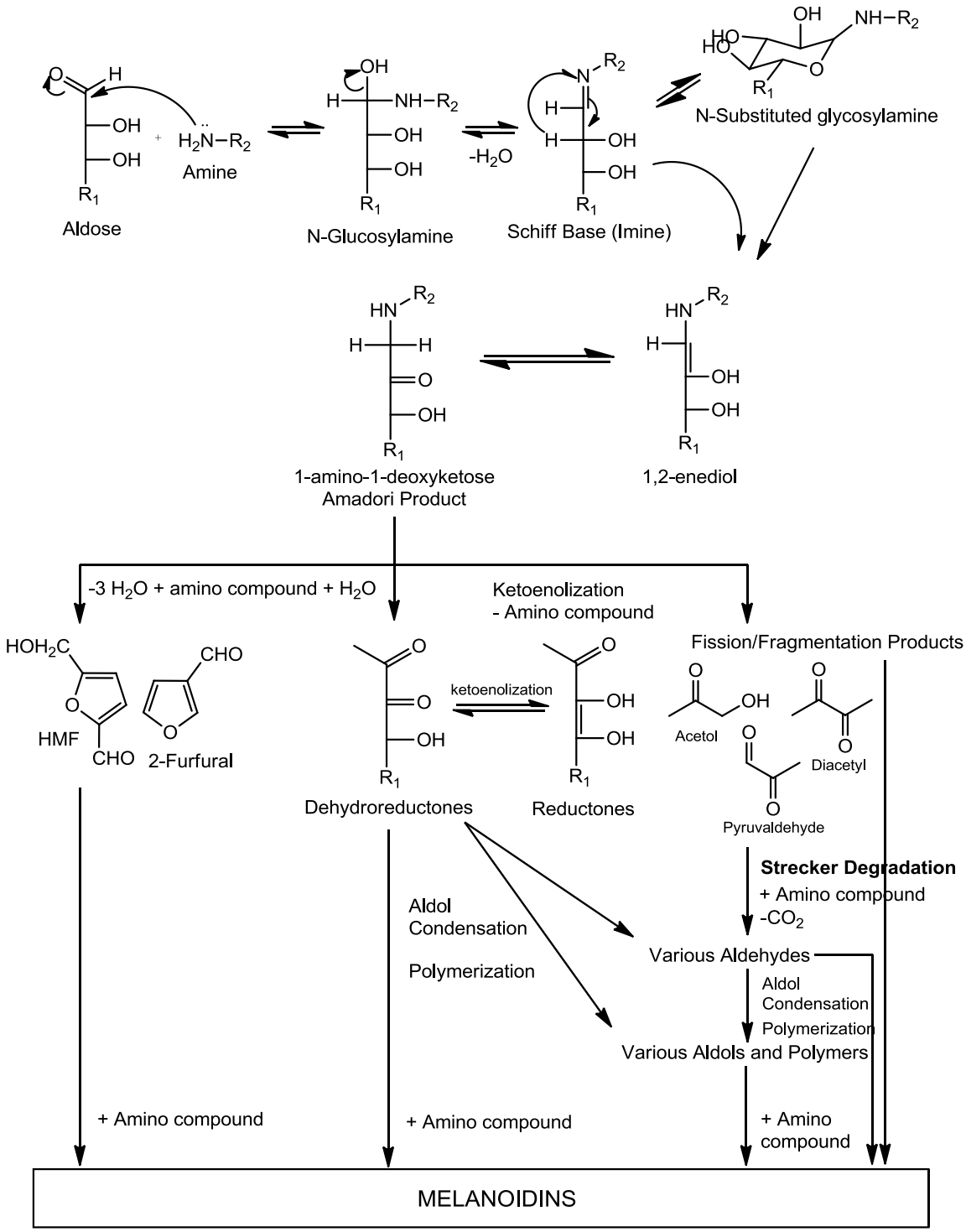


Figure 5. Maillard Reaction according to Hodge's Scheme[37].

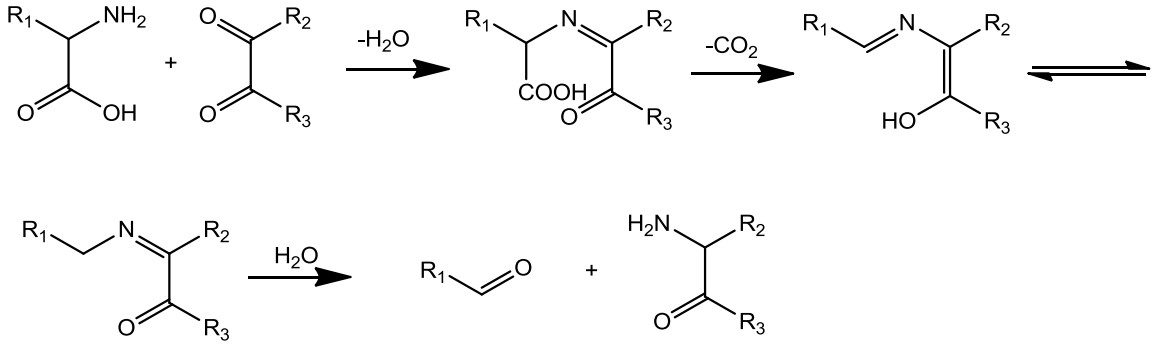


Figure 6. Strecker Degradation Mechanism[38].

3 MATERIALS AND METHODS

The objectives of this research was to determine the key aroma active compounds in the raw and roasted lily bulbs and to also obtain the quantitative descriptive data of both types in order to gain insight on the sensory characteristics that are complementing the key odorants. Two approaches, analytical and sensory, were taken in order to carry out the objectives (Figure 7). The analytical approach consisted of performing a series of analytical techniques such as solvent extraction and solvent assisted flavor evaporation (SAFE) in order to obtain the aroma volatiles. Once isolated from the non-volatiles, three techniques namely, comparative aroma extract dilution analysis (cAEDA) followed by gas chromatography/Olfactometry (GC/O), solid phase extraction (SPE) followed by gas chromatography/mass spectrometry (GC/MS), and gas-chromatography/mass spectrometry, were implemented. cAEDA was performed on the volatiles in order to determine the potency of the aroma-active compounds. The volatiles were also analyzed via GC/MS to aid in the identification of the aroma-active compounds. SPE, a sample preparation process used to separate compounds according to their physical and chemical properties, was also implemented on the volatiles to assist in the identification of the aroma-active compounds. The sensory approach comprised of two analyses, free choice profiling (FCP) and quantitative descriptive analysis (QDA), which were crucial in the determination of the critical attributes in both the raw and roasted lily bulbs.

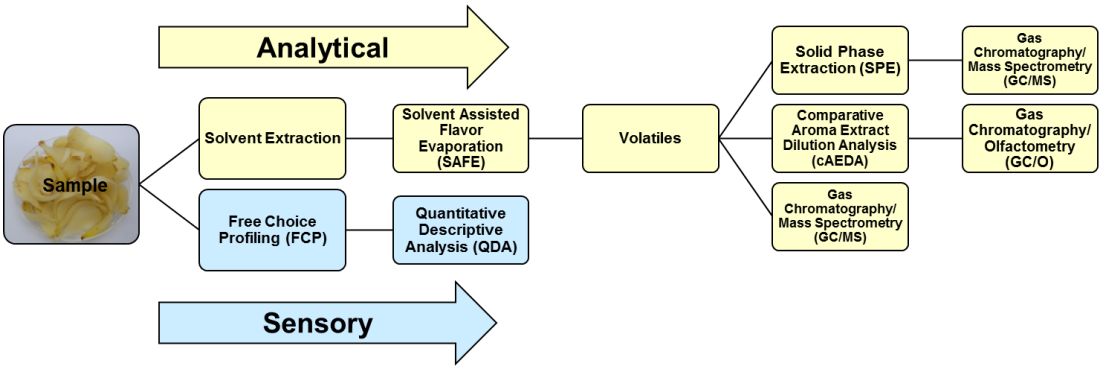


Figure 7. Experimental Work Flow.

3.1 Lily Bulbs

Lily bulbs cultivar 'Nellie White' were obtained from Kurt Weiss Greenhouses, Inc (Center Moriches, NY, USA).

3.2 Chemicals

Unstabilized diethyl ether was obtained from Burdick & Jackson (Court Zelienople, PA, USA). Pentane was obtained from Fisher Scientific (Pittsburgh, PA, USA). The ether and pentane were of chromatographic grade and were freshly distilled in-house prior to use. Anhydrous sodium sulfate was obtained from Fisher Scientific. Deionized (DI) water (18 MΩ cm) was prepared in house using a Milli-Q-water purification system (Millipore, Bedford, MA, USA).

3.3 Reference Standards

The following reference standards shown in Figure 8 were obtained from commercial suppliers given in parentheses: **1 – 10, 12 – 18, 20, 22 – 27, 30 – 34, 36 – 48** (Sigma Aldrich, St. Louis, MO, USA); **28** (Penta Manufacturing Company, Livingston, NJ, USA). Compounds **11** [39], **19** [40], **21** [41], **29** [42] and **35** [43] were synthesized in-house (Mars, Inc., Hackettstown, NJ, USA) according to its corresponding literature.

no.	Odorant
1	2-and 3- methylbutanal
2	butane-2,3-dione[g]
3	Ethyl 2-methylbutanoate
4	2,3-pentanedione
5	hexanal
6	(3E)-hex-3-enal
7	(3Z)-hex-3-enal
8	1,8-cineole
9	oct-1-en-3-one
10	2-acetyl-1-pyrroline
11	(5Z)-octa-1,5-dien-3-one
12	dimethyl trisulfide
13	4-methyl-4-sulfanylpentan-2-one
14	2-methoxy-3-propan-2-ylpyrazine
15	acetic acid
16	3-(methylsulfanyl)propanal
17	2-ethyl-3,5-dimethylpyrazine
18	2,3- diethyl-5-methylpyrazine
19	(2Z)-non-2-enal
20	(2E)-non-2-enal
21	2-ethenyl-3,5-dimethylpyrazine
22	(2E,6Z)-nona-2,6-dienal
23	butanoic acid
24	1-(1,3-thiazol-2-yl)ethanone

no.	Odorant
25	2-phenylacetaldehyde
26	2- and 3- methylbutanoic acid
27	(2E,4E)-nona-2,4-dienal
28	3-methylnonane-2,4-dione
29	dimethyl tetrasulfide
30	1-(4,5-dihydro-1,3-thiazol-2-yl)ethanone
31	2-hydroxy-3-methyl-2-cyclopenten-1-one
32	2-methoxyphenol
33	2-phenylethanol
34	3-hydroxy-2-methyl-4*H*-pyran-4-one
35	trans-4,5-epoxy-(E)-2-decenal
36	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF)
37	γ-nonalactone
38	3-methylphenol
39	ethyl cinnamate
40	4-allyl-2-methoxyphenol
41	3-ethylphenol
42	sotolon
43	2-methoxy-4-[(Z)-prop-1-enyl]phenol
44	5-ethyl-3-hydroxy-4-methyl-5H-furan-2-one
45	1,3-dimethoxy-2-hydroxybenzene
46	2-methoxy-4-[(E)-prop-1-enyl]phenol
47	3-methylindole
48	4-hydroxy-3-methoxy-benzaldehyde

Figure 8. Reference Standards used for the Identification of Aroma-active Compounds in Raw and Roasted Lily Bulbs.

3.4 Sample Preparation

3.4.1 Preparation of Raw Bulb Scales

Raw lily bulbs were separated into individual scales (Figure 9) and washed with deionized (DI) water.

3.4.2 Preparation of Roasted Bulb Scales

The same approach was taken for the roasted bulb scales in terms of washing and separating the raw scales. In order to obtain the appropriate amount of roasted product for analysis, three batches (40 g per batch) of raw scales were used. Each batch of raw scales were evenly distributed on a 12 cm x 12 cm piece of aluminum foil and enclosed. Thirty-five holes were made to the top of each aluminum piece of foil using a metal needle (Figure 10). The three batches were placed on an aluminum tray and roasted in a conventional oven at 400°C for 45 min then removed from the oven and cooled prior to analysis (Figure 11). It was confirmed that three batches of raw scales equates to roughly 57 g of roasted product.

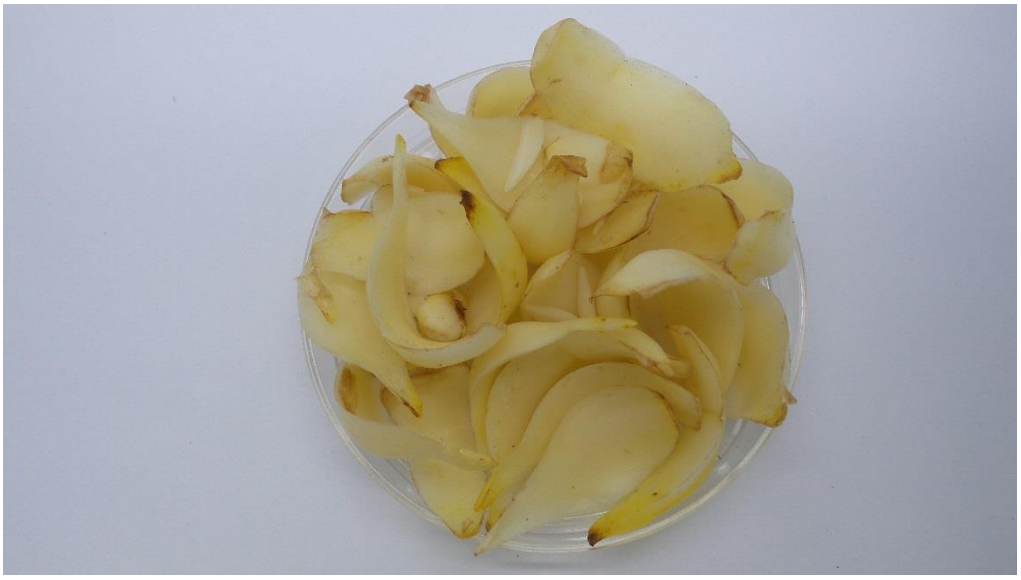


Figure 9. Raw Lily Bulbs.

Figure 10. Raw Lily Bulbs Wrapped in Aluminum Foil Prior to Thermal Treatment.

Figure 11. Roasted Lily Bulbs.

3.4.3 Isolation of the Volatiles

For aroma isolation, 55 g of raw and roasted lily bulb scales were frozen separately with liquid nitrogen (LN_2) and ground into a fine powder using a coffee grinder. 50 g of each type were weighed into individual centrifuge tubes. 100 mL of redistilled ether was added to each frozen sample. Both samples were shaken for 15 min. and centrifuged at 4500 RPM for 10 min. The volatiles were isolated by solvent-assisted flavor evaporation (SAFE) at 40 ºC under high vacuum (10^{-3} mbar). Due to water present in the raw sample, liquid-liquid extraction was performed on the sample prior to isolation. A separatory funnel was employed to separate the lower layer water from the diethyl ether phase in the raw lily bulb sample, and the supernatant then decanted into the dropping funnel of the SAFE apparatus (Figure 12). Next, the residue was re-extracted with 50 mL of redistilled ether and treated again as described above. The distillation of the aroma volatiles was initiated by slowly opening the valve to allow a small portion of the supernatant at a time to enter the distillation chamber. Once in the chamber, the volatiles from the extract was evaporated and condensed along the walls of the collecting vessel, which was submerged under LN_2 while the non-volatiles remained in the distillation chamber. At the completion of the vacuum distillation, the vacuum was broken and the collection flask removed. The samples were then thawed at room temperature and dried over anhydrous sodium sulfate, concentrated to approximately 2 mL with a Vigreux column (50 x 1 cm) held at 45 ºC, and finally concentrated under a gentle stream of nitrogen to approximately 200 µL.

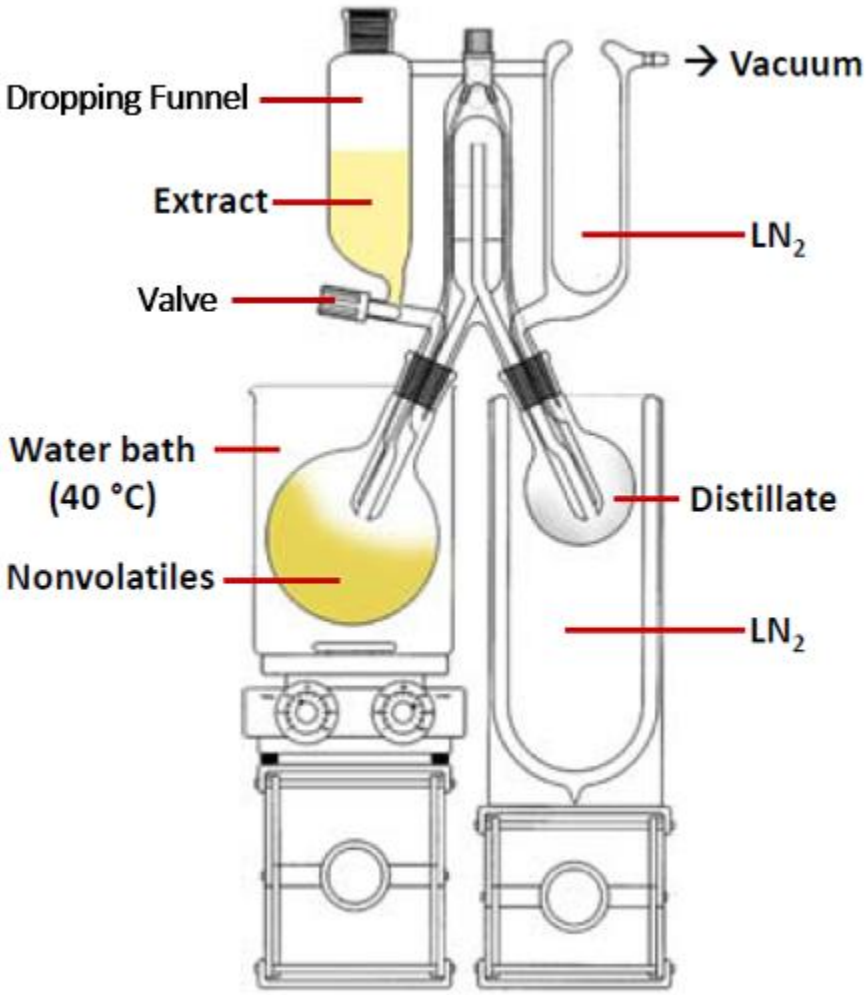


Figure 12. Solvent Assisted Flavor Evaporation Apparatus used to isolate Aroma Volatiles from Food Extract[44].

3.4.4 Comparative Aroma Extract Dilution Analysis (cAEDA)

The aroma volatiles were diluted to obtain serial dilutions of 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256; 1:512 and 1:1024 of the stock aroma distillates (Figure 13). Each dilution was analyzed by GC/O utilizing a capillary FFAP column, according to the conditions described in the *Detection and Identification: Gas-Chromatography/Olfactometry (GC/O)* section below. The aroma-active regions were identified in the chromatograms and each detected aroma assigned a flavor dilution (FD) factor corresponding to the highest dilution in which the aroma was noticeable.

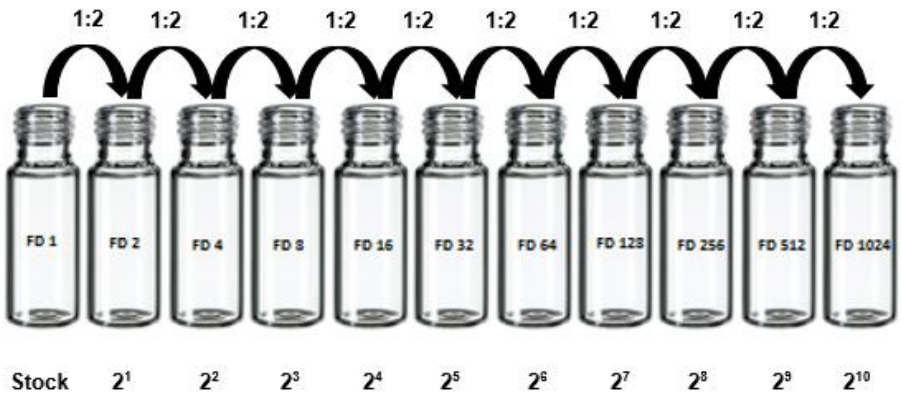


Figure 13. Comparative Aroma Extract Dilution Analysis (cAEDA) used to determine Flavor Dilution Factor of each Odorant.

3.4.5 Fractionation of the Volatile Distillates

Aroma distillates, prepared as described above, were fractionated via solid phase extraction (SPE) to aid in the identification of odorants. Prior to fractionation, the SPE cartridge (55 μm, 70A, 1 g/12 mL; Phenomenex, Torrance, CA, USA) was sequentially conditioned with 5 mL of pentane, 5 mL of diethyl ether and 5 mL of pentane. Elution of each sample was performed with the following solvents respectively: 5 mL of pentane (fraction A), 5 mL of pentane/diethyl ether (98:2 v/v; fraction B), 5 mL of pentane/diethyl ether (95:5 v/v; fraction C), 5 mL of pentane/diethyl ether (9:1 v/v; fraction D), 5 mL of pentane/diethyl ether (1:1 v/v; fraction E) and 5 mL of diethyl ether (fraction F). The six fractions collected were then concentrated to 200 μL under a gentle stream of nitrogen prior to GC/MS analysis.

3.5 Detection and Identification

Aroma-active compounds in raw and roasted lily bulbs were isolated by SAFE and detected and identified via GC/O and GC/MS. SAFE was utilized to carefully and directly isolate aroma volatiles from the lily bulbs. As opposed to other distillation techniques, SAFE produces aroma extracts that best represent the organoleptic properties of the original food. It uses a low boiling solvent, diethyl ether, to extract the volatiles from the food extract. Its only drawback is that odorants with the same or lower boiling point as the ether may potentially get lost during the concentration of the extract. To overcome these limitations, other forms of detection, such as headspace analysis, will be explored in the near future.

3.5.1 Gas Chromatography-Olfactometry (GC/O)

An Agilent 6890 series GC system was employed for GC/O analysis. A capillary column HP-FFAP (30 m × 0.32 mm, 0.25 μm film thickness) was used for the chromatographic separation. The samples were injected via an on-column technique at 35 °C, with helium as the carrier gas. The flow rate was set to 3.6 mL/min. The temperature program was as follows: 35 °C for 1 minute, increased at 60 °C/min to 60 °C, then increased at 6 ºC/min to 230 ºC and held for 10 minutes. The effluent was split 1:1 by volume at the end of the capillary by a Y-type splitter into two sections of deactivated fused silica capillaries. One section was directed to the flame ionization detector (FID) held at 250 °C, and the other to a heated sniffing-port held at 250 °C.

3.5.2 Gas Chromatography-Mass Spectrometry (GC/MS)

An Agilent 6890 series GC system coupled to an Agilent 5973 mass spec (MS) detector was used for GC/MS analysis. A capillary column HP-FFAP (30 m × 0.25 mm, 0.25 μm film thickness) was used for the chromatographic separation. The temperature program was the same as that described above. Samples were injected on-column and heated at 250 °C, with helium as the carrier gas with a constant flow of 1 mL/min. MS parameters were as follows: operation was in electron impact (EI) ionization mode at 70 eV; scan range, m/z 50-550 for identification experiments. The transfer line was maintained at 250 °C. Calculation of linear retention indices (RI) was achieved by using a series of adjacently eluting *n*-alkanes with increasing aliphatic chain length. The formula used to calculate the RI is as followed: $I = 100 \times \left[n + (N - n)\frac{t_{r,a} - t_{r,n}}{t_{r,N} - t_{r,n}}\right]$ where I is the retention index (RI), n is the number of carbon

atoms in the alkane with the lower retention time, *N* is the number of carbon atoms in the alkane with the higher retention time, *a* is the analyte and *t* is the retention time.

3.6 Sensory Analyses

Raw and roasted lily bulb scales were placed into two separate 20 mL borosilicate glass scintillation vials (Thermo Fisher Scientific, Fair Lawn, NJ, USA). A spatula was used to break down each sample so that ample aroma may be perceived during evaluation. Each sample was evaluated by eight trained sensory panelists via free choice profiling (FCP) followed by quantitative descriptive analysis (QDA).

3.6.1 Free Choice Profiling (FCP)

Eight trained sensory panelists conducted this study by smelling each sample and assigning no more than eight attributes that they thought best described the sample's aroma. Data was collected and descriptors were grouped into categories of similar attributes (Figure 14). For example, french fries and baked potato were two similar terms organized under the category, "cooked potato-like". The number in parentheses represents the number of panelists who gave the sample that particular attribute. The critical descriptors that appeared most frequently in the panelists' ballots (bolded in Figure 14) and subsequently utilized in the quantitative descriptive analysis (QDA) were grassy, fruity, cooked potato-like, medicinal, metallic, smoky, caramel-like, maple-like, deep-fried, roasty, mushroom-like, meaty and toasted.

Raw	Roasted
fruity (4)	**cooked potato-like (6)**
green/grassy (8)	**green/grassy (2)**
cooked potato-like (2)	**smoky/burnt (6)**
medicinal (2)	**caramel-like (3)**
metallic	**roasty/toasty (3)**
sweaty, cheesy, rancid	**meaty (2)**
woody	**maple-like**
hay-like	**mushroom-like**
floral	wheaty/grainy
dirty/earthy	

fried potatoes
fried potatoes
fried potatoes
french fries
raw potatoes
uncooked potato

Figure 14. List of Attributes determined via Free Choice Profiling (FCP) describing Raw and Roasted Lily Bulbs.

3.6.2 Quantitative Descriptive Analysis (QDA)

The reference compound for each descriptor in the QDA was dissolved in water at a concentration 100 times above threshold value. Reference odorants used were (Z)-hex-3-enal (green, grassy); ethyl 2-methylbutanoate (fruity); 3-(methylsulfanyl)propanal (methional) (cooked potato-like); 3-methylphenol (*m*-cresol) (medicinal, phenolic); trans-4,5-epoxy-(E)-2-decenal (metallic); 2-methoxyphenol (guaiacol) (smoky); 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) (caramel-like); 3-hydroxy-4,5-dimethylfuran-2(*5H*)-one (sotolon) (maple-like); (2E,4E)-deca-2,4-dienal (deep fried); 2-ethyl-3,5-dimethylpyrazine (roasty); oct-1-en-3-one (mushroom-like); 2-methyl-3-methyldisulfanylfuran (meaty) and 1-(1,3-thiazol-2-yl)ethanone (2-acetylthiazole) (toasted). Each panelist rated each descriptor for both the raw and roasted samples on a seven-point scale that ranged from 0 to 3 in 0.5 increments; 0 = not detectable, 1 = weak, 2 = moderate and 3 = strong. The QDA ballot used by the trained panelists (Figure 15).

Name: _____

Aroma profile of raw & roasted lily bulbs.
Please smell the samples and evaluate the intensity of the given odor qualities.
Please use the range between 0 – 3.

0 = not observable, **1** = weakly observable, **2** = moderately observable, **3** = strong observable
(**0.5** steps are allowed)

	Odorant	**Odor quality**	**Raw**	**Roasted**
1	(Z)-Hex-3-enal	green, grassy		
2	Ethyl-2-methylbutanoate	fruity		
3	3-(Methylsulfanyl)propanal (Methional)	cooked potato		
4	3-Methylphenol (*m*-Cresol)	medicinal, phenolic		
5	Trans-4,5-(E)-epoxy-2-decenal	metallic		
6	2-Methoxyphenol (Guaiacol)	smoky, burnt		
7	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF)	caramel, burnt sugar		
8	3-Hydroxy-4,5-dimethylfuran-2(*5H*)-one (Sotolon)	maple syrup		
9	(E, E)-Deca-2,4-dienal	deep fried		
10	2-Ethyl-3,5-dimethylpyrazine	roasty		
11	Oct-1-en-3-one	mushroom-like		
12	2-Methyl-3-methyldisulfanylfuran	meaty		
13	1-(1,3-Thiazol-2-yl)ethanone (2-Acetylthiazole)	toasty, tortilla		

Figure 15. Quantitative Descriptive Analysis (QDA) Ballot.

4 RESULTS AND DISCUSSION

Odorant profiles of the raw and roasted lily bulbs will be discussed; specifically focusing on which odorants were transferred from the raw to the roasted, which ones were lost from the raw during roasting and which ones were generated during thermal treatment. The quantitative descriptive analysis of the raw and roasted lily bulbs will also be discussed to help get a better understanding as to what sensory descriptors are analogous to the key odorants of both types.

A total of 48 aroma active compounds were identified in the raw and roasted lily bulbs (Figure 16). In the raw bulbs, 25 odorants were detected in the FD factor range from 2 to 1024, whereas, in the roasted bulbs, 42 odorants were detected in the FD factor range from 2 to 1024. The key odorants in both the raw and roasted lily bulbs can be visually depicted in the flavor dilution chromatogram shown in Figure 14 where the RIs versus FD factor of each aroma compound are plotted against each other. Only odorants with FD ≥ 256 are labeled with its corresponding number.

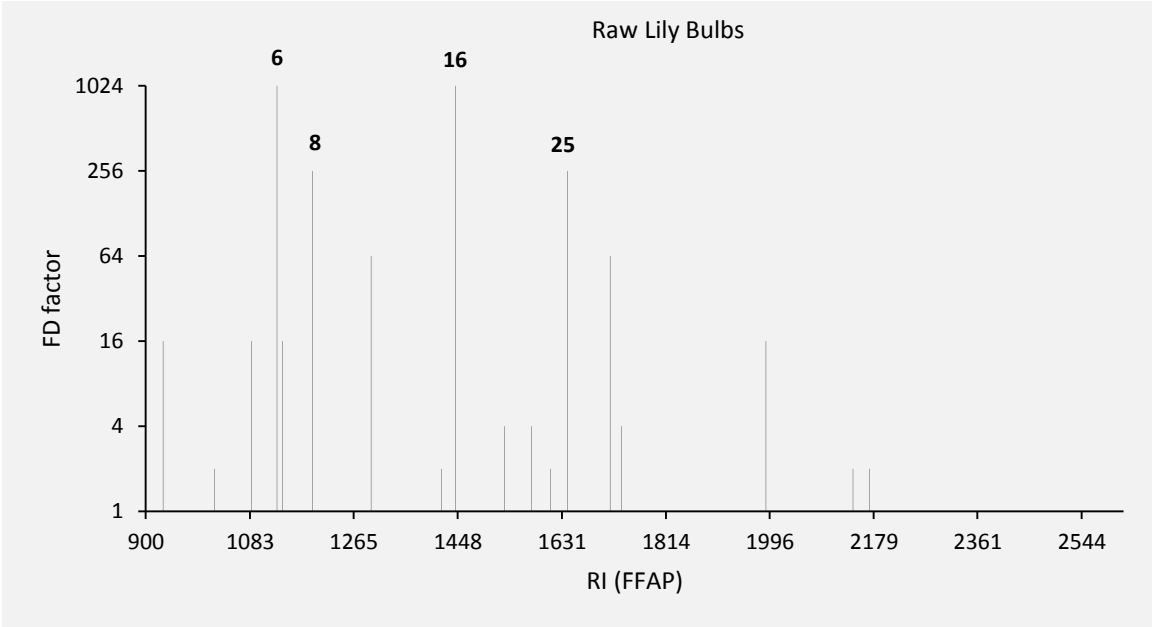
To identify the aroma-active compounds in the aroma isolates, the retention indices (RIs) of the aroma compounds, aroma characteristics and mass spectra were evaluated and compared to the reference standards. Aroma-active compounds were further separated from the other compounds with similar retention times by subjecting the original distillates to SPE. As a result, six fractions (A-F) for each distillate were generated in order to assist in the identification of the aroma-active compounds via GC-MS. Out of the 48 compounds identified in the raw and roasted bulbs, 16 of them were detected in fractions D (pentane/diethyl ether (9:1 v/v)), E (pentane/diethyl ether (1:1 v/v)) and F (diethyl ether).

no.[a]	odorant[b]	odor quality[c]	RI[d] on		FD[e] factor		fraction[f]
			FFAP	DB-5	Raw	Roasted	
1	2-and 3- methylbutanal	malty	930	668	16	256	E
2	butane-2,3-dione (diacetyl)[g]	buttery	985	<600		64	
3	ethyl-2-methylbutanoate	fruity	1020	851	2	1	
4	2,3-pentanedione (acetylpropionyl)	buttery	1053	700		4	
5	hexanal	green	1085	802	16		
6	(3E)-hex-3-enal[g]	green	1130	800	1024	2	
7	(3Z)-hex-3-enal	green	1140	790	16		
8	1,8-cineole (eucalyptol)	eucalyptus-like	1193	1031	256	4	
9	oct-1-en-3-one	mushroom	1295	975	64	4	F
10	2-acetyl-1-pyrroline	roasty	1322	920		1024	F
11	(5Z)-octa-1,5-dien-3-one	geranium	1345	988	1		
12	dimethyl trisulfide[g]	sulfurous	1365	968		4	
13	4-methyl-4-sulfanylpentan-2-one	catty	1377	942	1	16	E
14	2-methoxy-3-propan-2-ylpyrazine[g]	earthy, beany	1419	1091	2		
15	acetic acid	vinegar	1439	600		64	F
16	3-(methylsulfanyl)propanal (methional)	cooked potato	1443	902	1024	1024	
17	2-ethyl-3,5-dimethylpyrazine	earthy	1450	1095		1024	F
18	2,3- diethyl-5-methylpyrazine	earthy	1480	1158		1024	
19	(Z)-non-2-enal	green, fatty	1496	1149		4	
20	(E)-non-2-enal	green	1530	1161	4	64	
21	2-ethenyl-3,5-dimethylpyrazine	roasty	1556	1078		4	
22	(E,Z)-nona-2,6-dienal	cucumber	1577	1150	4	16	
23	butanoic acid	sweaty, rancid	1610	820	2	16	F
24	1-(1,3-thiazol-2-yl)ethanone (2-acetylthiazole)	roasty	1624	1018		256	

Figure 16. Aroma-Active Compounds (FD ≥ 1) found in Raw and Roasted Lily Bulbs. [a]Number reflects the order in which the odorants appear on the FFAP column. [b]Aroma-active compounds were identified by comparing odor quality and intensity, mass spectra, and RI on FFAP and DB-5 columns in comparison to an authentic reference standard. [c]Odor quality of aroma-active compounds determined by GC-O. [d]Linear retention index. [e]Flavor dilution factor. [f]Fraction in which the odorant was identified. [g]Mass spectra could not be acquired and the identification was based on the remained criteria described above. [h]Odorants were not resolved on either the FFAP or DB-5 column.

no.[a]	odorant[b]	odor quality[c]	RI[d] on		FD[e] factor		fraction[f]
			FFAP	DB-5	Raw	Roasted	
25	2-phenylacetaldehyde	floral, honey	1640	1045	256	64	D
26	2- and 3- methylbutanoic acid	sweaty, rancid	1660	885	1	4	F
27	(E, E)-nona-2,4-dienal[g]	fatty	1697	1212		4	
28	3-methylnonane-2,4-dione	anise/hay	1715	1246	64	64	E
29	dimethyl tetrasulfide	sulfurous	1735	1192	4	2	
30	1-(4,5-dihydro-1,3-thiazol-2-yl)ethanone (2-acetyl-2-thiazoline)[g]	roasty	1743	1106		4	
31	2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene)	caramel	1850	1021		256	E
32	2-methoxyphenol (guaiacol)	smoky	1860	1187	1	64	E
33	2-phenylethanol	rose	1909	1117	1		
34	3-hydroxy-2-methyl-4*H*-pyran-4-one (maltol)	caramel	1973	1108		1	F
35	trans-4,5-epoxy-(E)-2-decenal	metallic	1989	1380	16	64	
36	4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF)[g]	caramel	2006	1080		256	
37	γ-nonalactone	coconut	2013	1361	1	2	F
38	3-methylphenol (*m*-cresol)	phenolic	2091	1076		4	
39	ethyl cinnamate	cinnamon	2109	1460	1		
40	4-allyl-2-methoxyphenol (eugenol)	clove	2142	1359	2	16	
41	3-ethylphenol	leather	2156	1173		4	
42	3-Hydroxy-4,5-dimethylfuran-2(5*H*)-one (sotolon)[g]	maple	2171	1135	2	1024	F
43	2-methoxy-4-[(Z)-prop-1-enyl]phenol ((Z)-isoeugenol)[g]	clove	2231	1407		2	
44	5-ethyl-3-hydroxy-4-methyl-5H-furan-2-one (abhexon)	maple	2238	1184		1024	
45	1,3-dimethoxy-2-hydroxybenzene (2,6-Dimethoxyphenol)	smoky	2240	1349		2	
46	2-methoxy-4-[(E)-prop-1-enyl]phenol ((E)-isoeugenol)[g]	clove	2323	1451		1024	
47	3-methylindole (skatole)	fecal	2485	1383		2	
48	4-hydroxy-3-methoxy-benzaldehyde (vanillin)	vanilla-like	2600	1411		4	E

Figure 16. Continued.



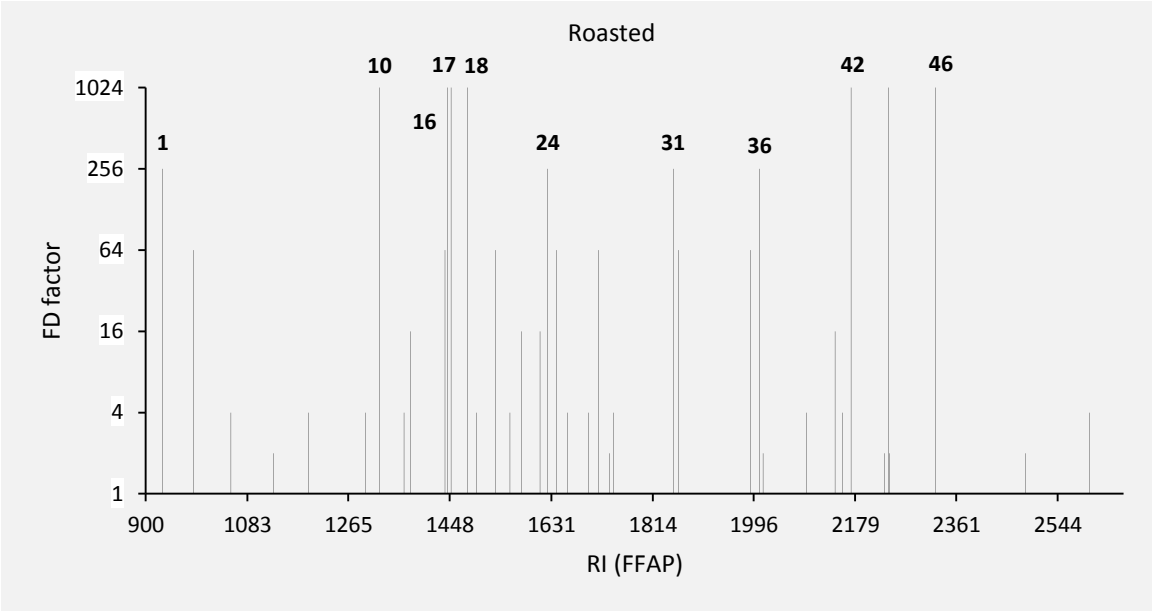


Figure 17. Flavor Dilution (FD) Chromatograms of the Aroma Distillates isolated from Raw and Roasted Lily Bulbs (Compound Numbers as in Figure 16).

4.1 Sensory Evaluation of Raw and Roasted Lily Bulbs

Raw and roasted lily bulb scales were orthonasally analyzed by eight trained panelists using free-choice profiling followed by QDA. Results displayed noticeable differences between the two types of lily bulbs (Figure 18). The aroma of the raw lily bulbs was predominantly green and grassy with some cooked potato and metallic notes. The aroma of the roasted lily bulbs was weak in green and grassy notes but strong in cooked potato, toasted, roasty, deep fried, smoky, caramel, and maple-like notes.

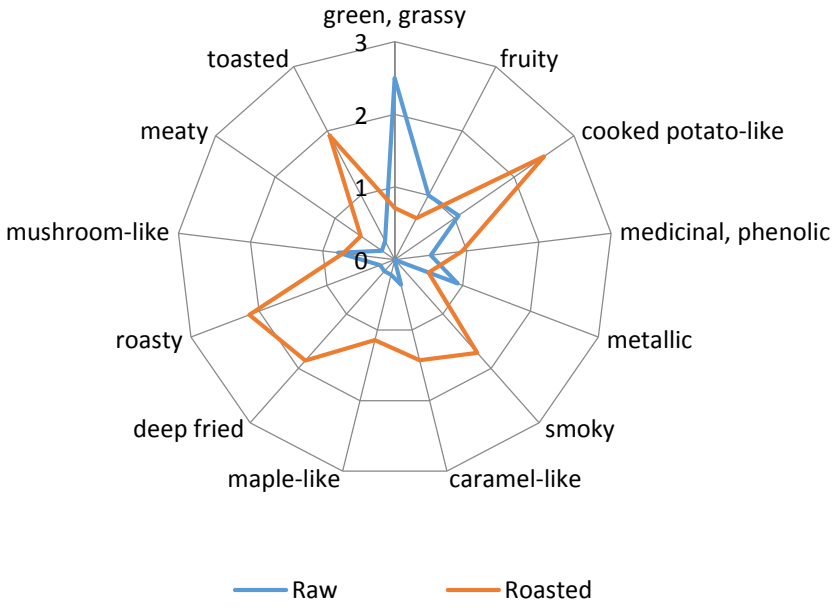


Figure 18. Flavor Profiles of Raw and Roasted Lily Bulbs generated via QDA. 13 attributes were evaluated by trained panelists using a scale from 0 to 3 in 0.5 increments; 0 = not detectable, 1 = weak, 2 = moderate and 3 = strong.

4.2 Key Odorants in Raw Lily Bulbs

The key aroma-active compounds in the raw lily bulbs shown in Figure 19 were determined after performing serial dilutions in the cAEDA. The results indicated that green compound, (E)-hex-3-enal (**6**) had the highest FD factor of 1024 along with cooked potato-like compound, 3-(methylsulfanyl)propanal (methional) (**16**). Other odorants that contributed to the aroma impression of the raw lily bulbs, with an FD factor of 256, were eucalyptus-like compound, 1,8-cineole (eucalyptol) (**8**) and floral, honey-like compound, 2-phenylacetaldehyde (**25**), followed by mushroom-like compound, oct-1-en-3-one (**9**) and hay-like compound, 3-methylnonane-2,4-dione (**28**) with an FD factor of 64. All of this information can be visually captured in the flavor dilution chromatogram (Figure 20) where the RIs versus FD factor of each aroma compound in the raw lily bulbs are plotted against each other. Only odorants with FD $\geq$ 256 are labeled with its corresponding number. The mass spectra of the key odorants found in raw lily bulbs are also depicted in Figures 21 – 26.

The sensory profile of the raw lily bulbs indicated strong green and grassy notes that may be explained by green smelling compounds (E)-hex-3-enal (**6**, FD 1024) and (Z)-hex-3-enal (**7**, FD 16). The cooked potato note in the sensory profile may be explained by the potato-like smelling compound, 3-(methylsulfanyl)propanal (methional) (**16**, FD 1024), and the metallic note in the raw bulbs may be due to the metallic smelling compound, trans-4,5-epoxy-(E)-2-decenal (**35**, FD 16). In addition, the mushroom note and fruity notes may result from the mushroom-like smelling compound, oct-1-en-3-one (**9**, FD 64) and the fruity smelling compound, ethyl-2-methylbutanoate (**3**, FD 2), respectively.

No.	Odorant	Odor Quality	FD Factor
6	(E)-hex-3-enal	green, grassy	1024
16	3-(methylsulfanyl)propanal (methional)	cooked potato	1024
8	1,8-cineole (eucalyptol)	eucalyptus-like	256
25	2-phenylacetaldehyde	floral, honey	256
9	oct-1-en-3-one	mushroom	64
28	3-methylnonane-2,4-dione	anise/hay	64

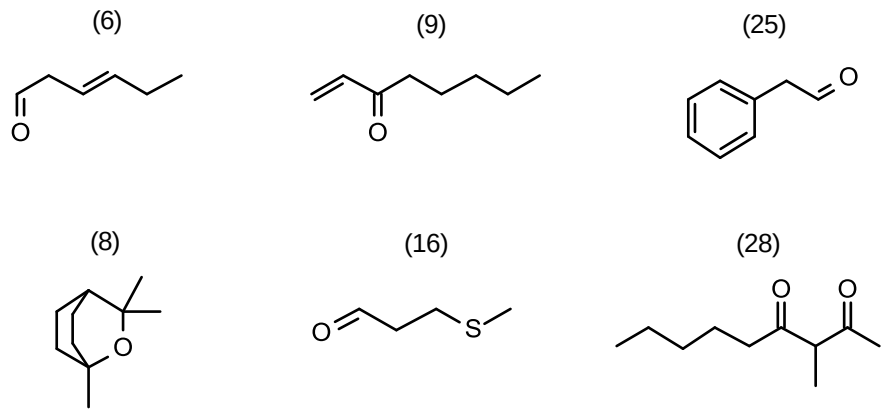


Figure 19. Key Odorants in Raw Lily Bulbs.

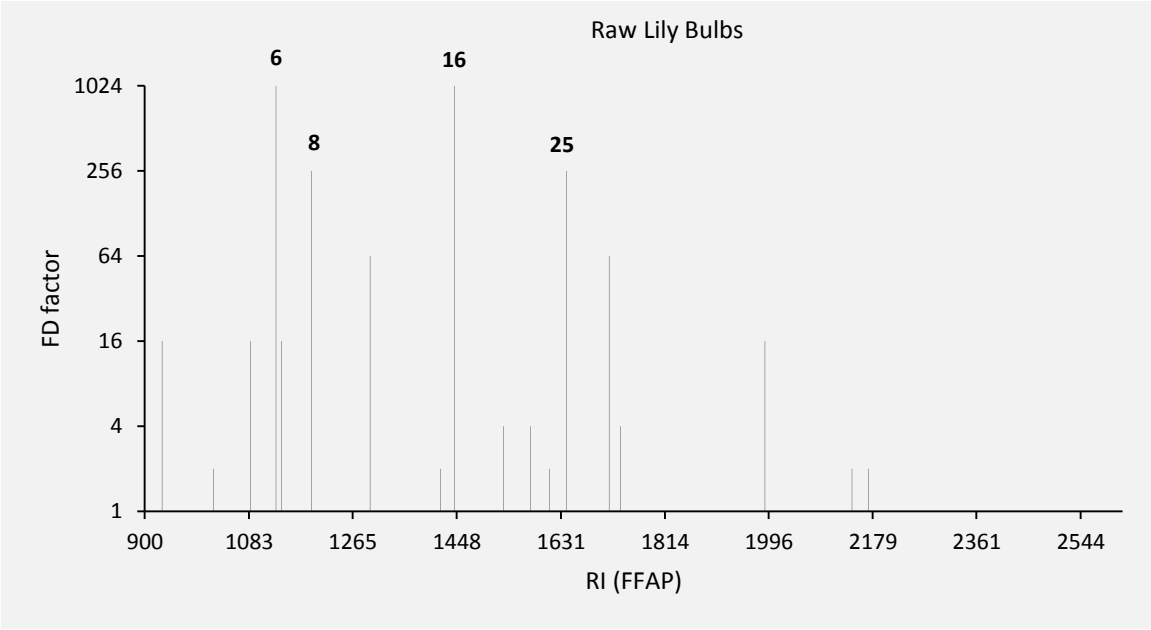


Figure 20. Flavor Dilution (FD) Chromatogram of the Aroma Distillate isolated from Raw Lily Bulbs (Compound Numbers as in Figure 16).

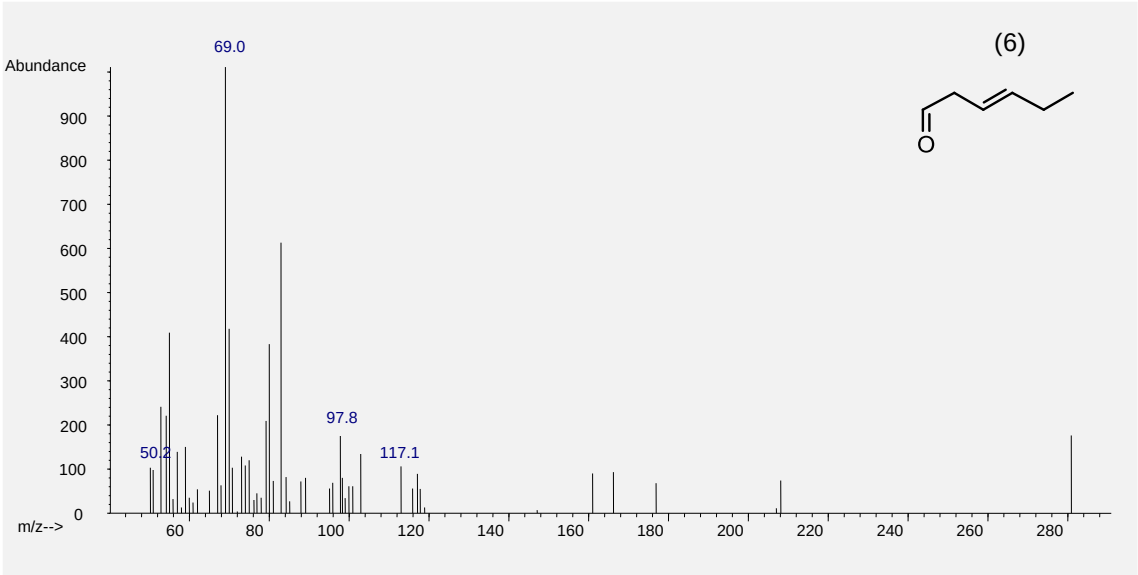


Figure 21. Mass Spectrum of (E)-Hex-3-enal.

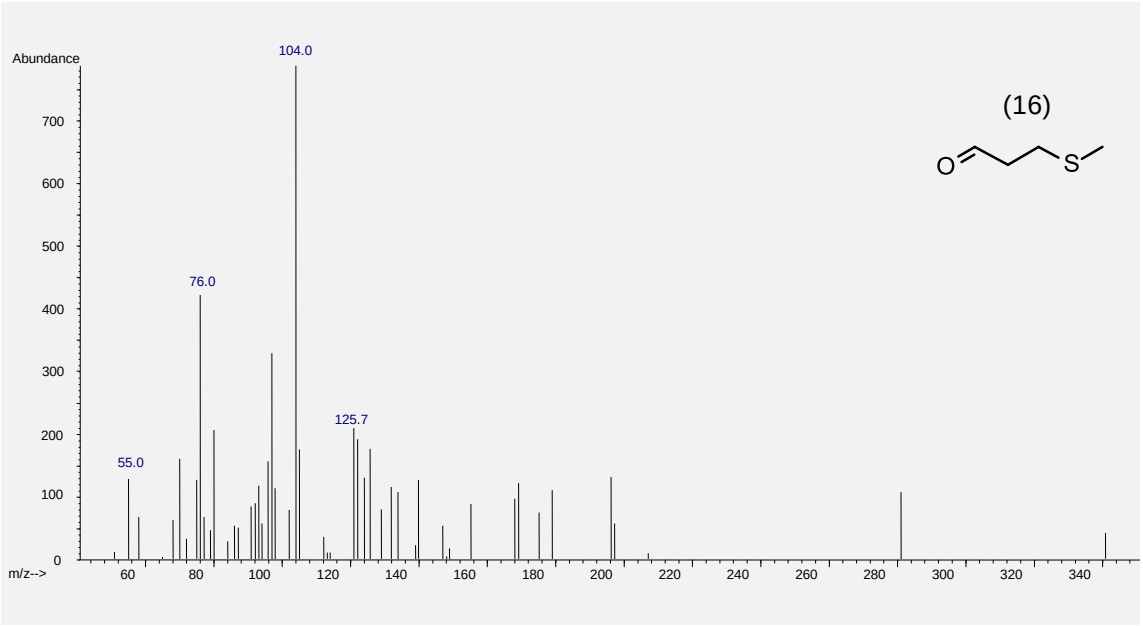


Figure 22. Mass Spectrum of 3-(Methylsulfanyl)propanal (Methional).

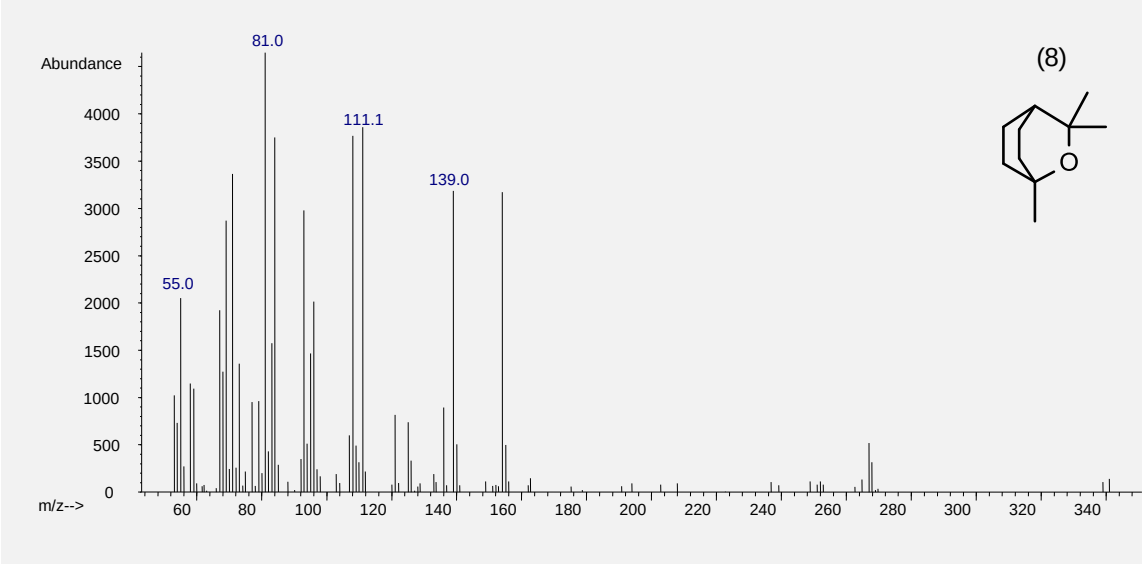


Figure 23. Mass Spectrum of 1,8-Cineole (Eucalyptol).

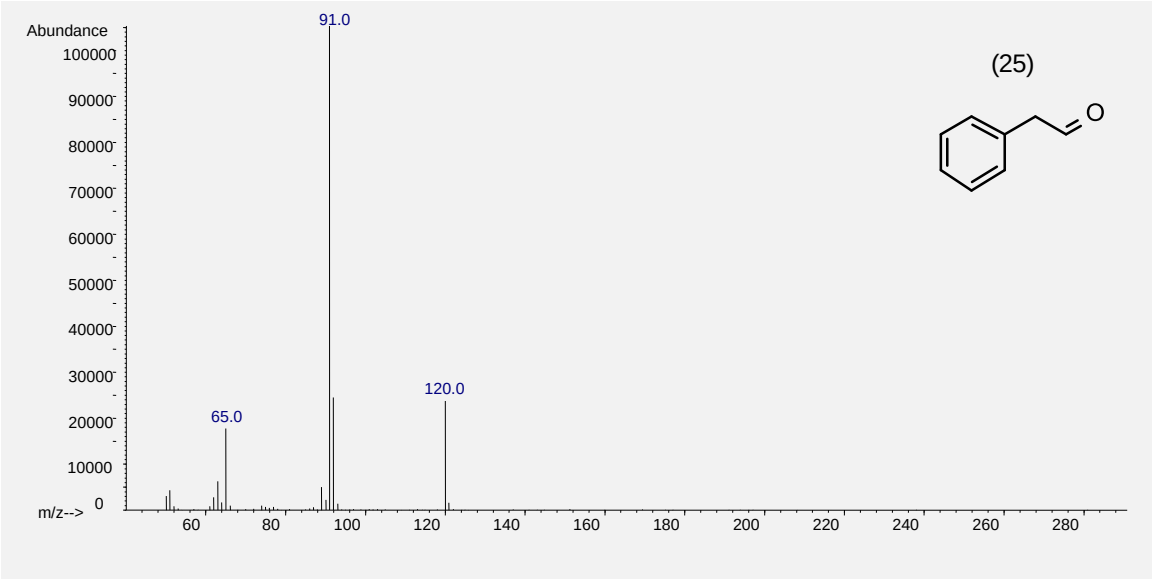


Figure 24. Mass Spectrum of 2-Phenylacetaldehyde.

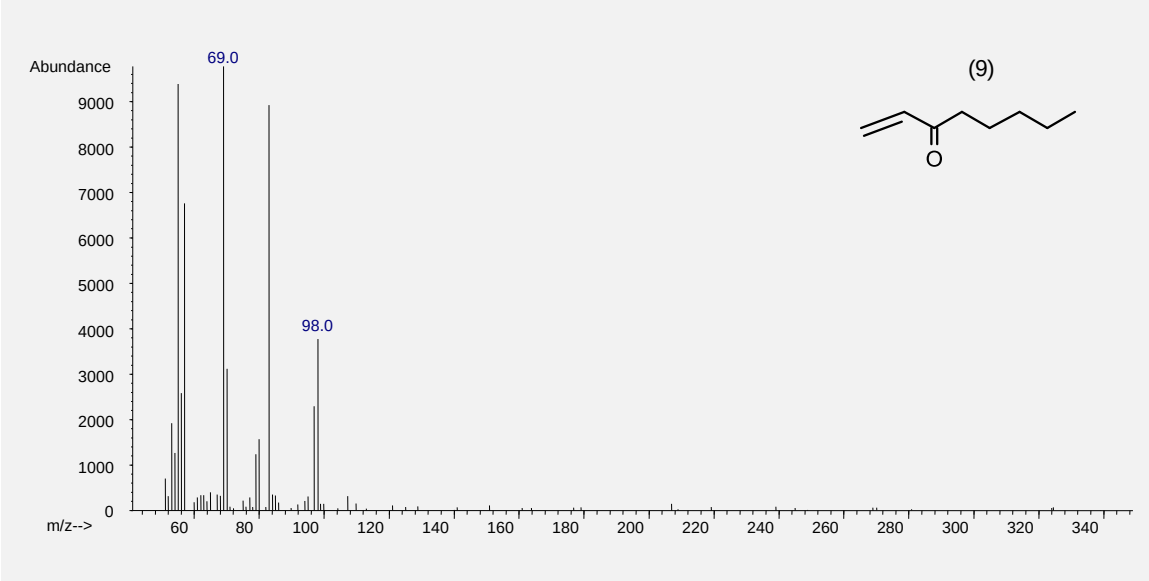


Figure 25. Mass Spectrum of Oct-1-en-3-one.

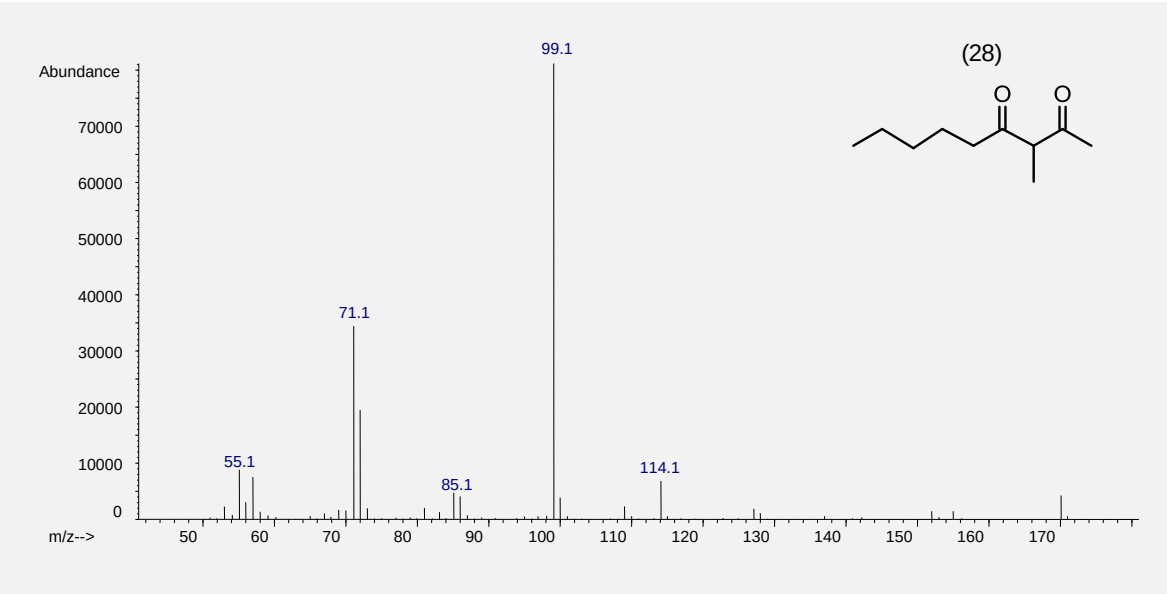


Figure 26. Mass Spectrum of 3-Methylnonane-2,4-dione.

4.3 Additional Odorants in Raw Lily Bulbs

Odorants with an FD factor of 16 shown in Figure 27 that also contributed to the aroma profile of the raw lily bulbs were as followed: 2-and 3-methylbutanal (**1**, malty), hexanal (**5**, green), (Z)-hex-3-enal (**7**, green) and trans-4,5-epoxy-E-2-decenal (**35**, metallic). The mass spectra of these odorants are presented in Figures 28 – 31.

No.	Odorant	Odor Quality	FD Factor
1	2-and 3- methylbutanal	malty	16
5	hexanal	green	16
7	(Z)-hex-3-enal	green	16
35	trans-4,5-epoxy-(E)-2-decenal	metallic	16

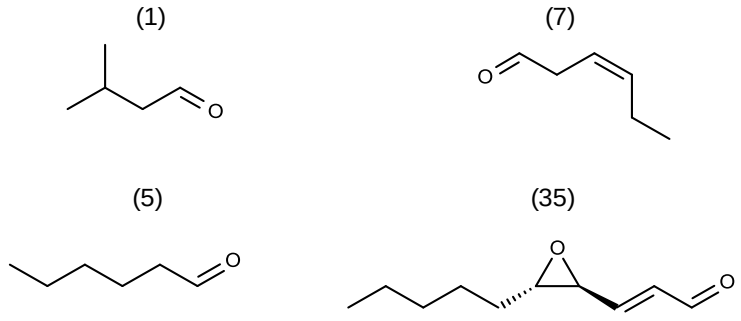


Figure 27. Additional Odorants in Raw Lily Bulbs with FD 16.

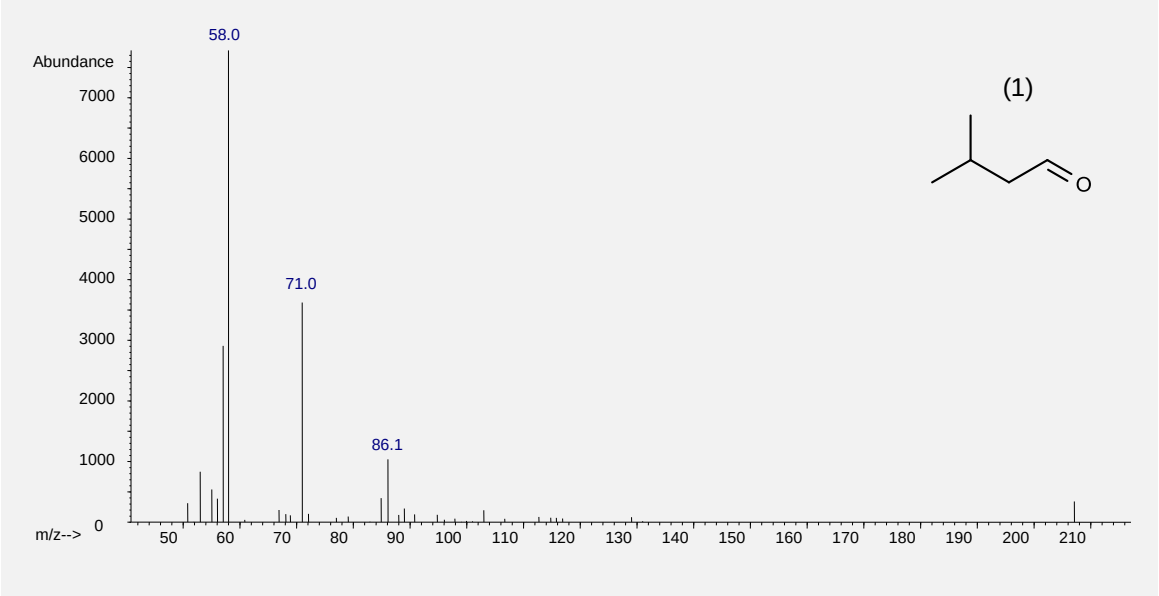


Figure 28. Mass Spectrum of 2-and 3-Methylbutanal.

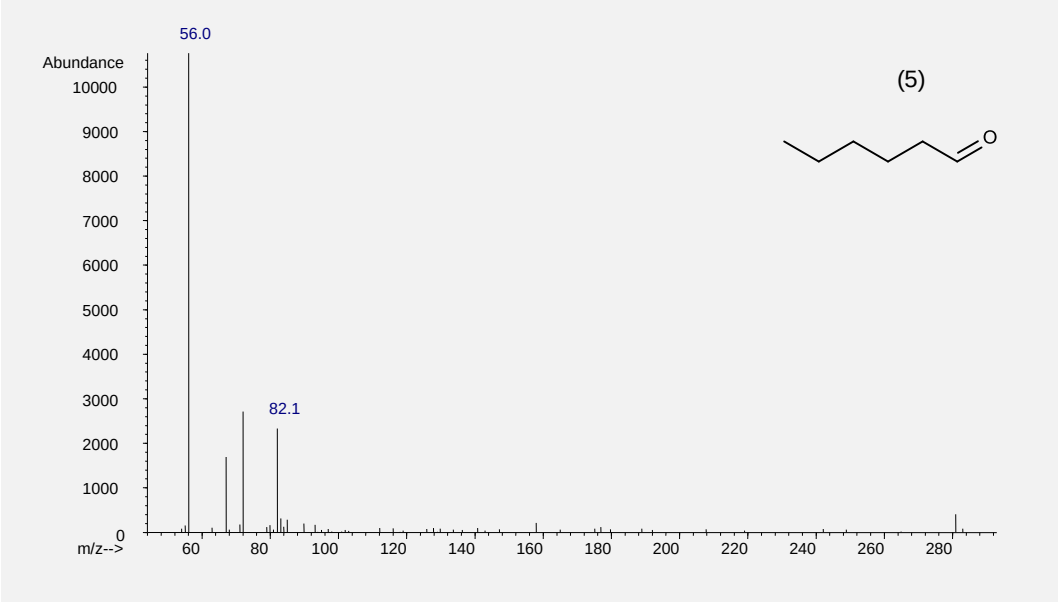


Figure 29. Mass Spectrum of Hexanal.

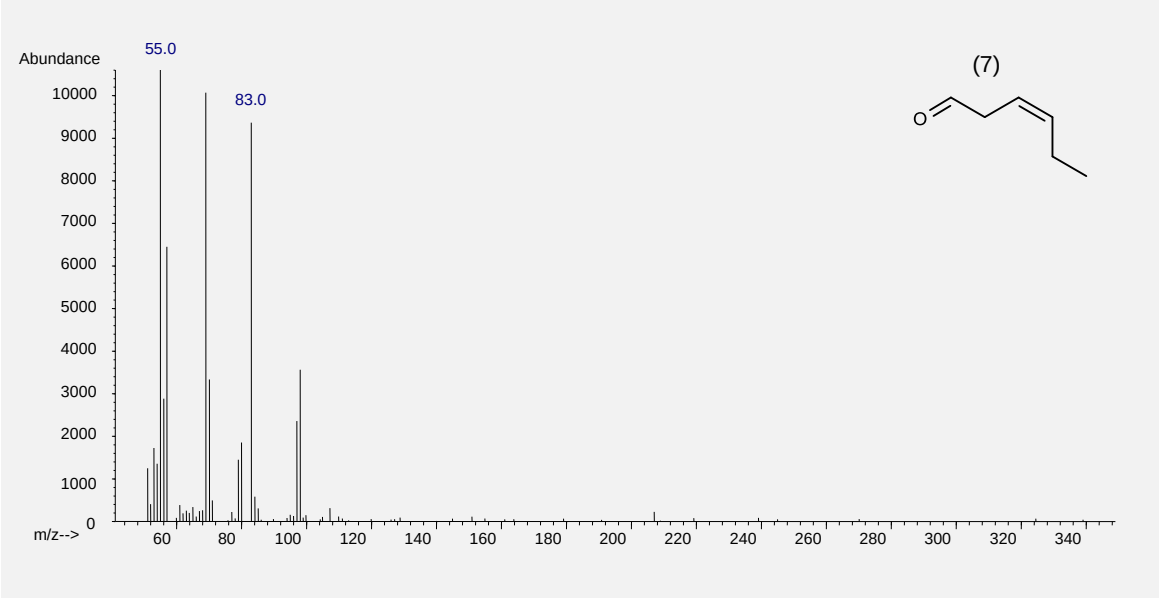


Figure 30. Mass Spectrum of (Z)-Hex-3-enal.

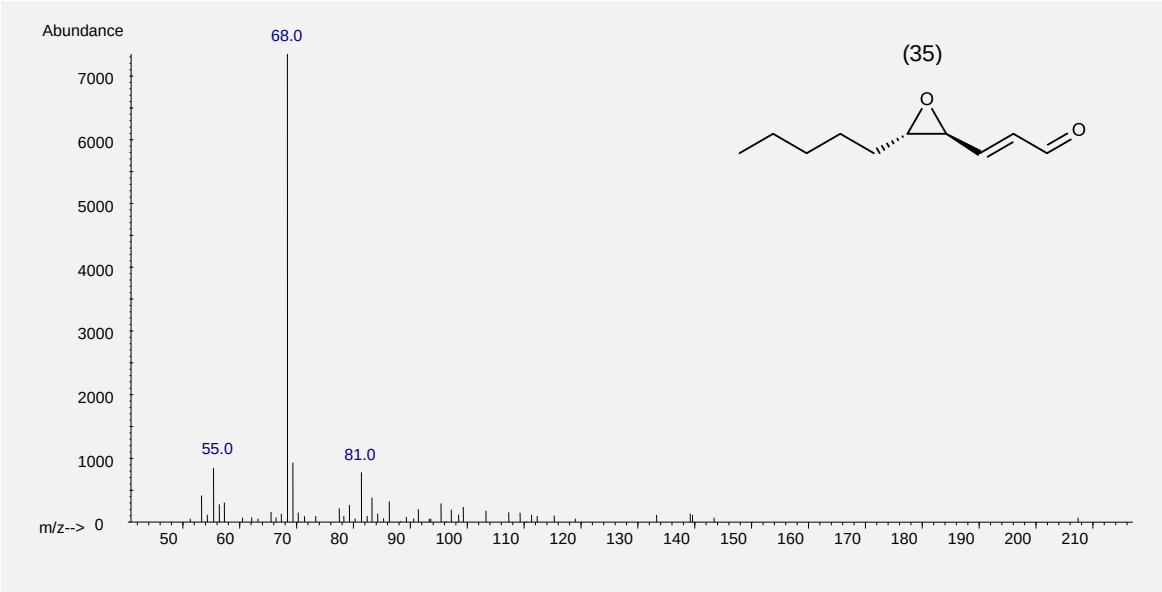


Figure 31. Mass Spectrum of Trans-4,5-epoxy-(E)-2-decenal.

Fifteen additional odorants with an FD factor ≤ 4, namely, green-like compound, (E)-non-2-enal (**20**, FD 4); cucumber-like compound, (E,Z)-nona-2,6-dienal (**22**, FD 4); sulfurous compound, dimethyl tetrasulfide (**29**, FD 4); fruity compound, ethyl-2-methylbutanoate (**3**, FD 2); earthy, beany compound, 2-methoxy-3-propan-2-ylpyrazine (**14**, FD 2); sweaty, rancid compound, butanoic acid (**23**, FD 2); clove-like compound, 4-allyl-2-methoxyphenol (eugenol) (**40**, FD 2); maple-like compound, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (sotolon) (42, FD 2); geranium-like compound, (5Z)- octa-1,5-dien-3-one (**11**, FD 1); catty compound, 4-methyl-4-sulfanylpentan-2-one (**13**, FD 1); sweaty, rancid compound, 2- and 3- methylbutanoic acid (**26**, FD 1); smoky compound, 2-methoxyphenol (guaiacol) (**32**, FD 1); rosy compound, 2-phenylethanol (**33**, FD 1); coconut-like compound, γ-nonalactone (**37**, FD 1) and cinnamon-like compound, ethyl cinnamate (**39**, FD 1) (Figure 32) were also identified in the raw bulbs. To the best of our knowledge, all of these compounds were identified and reported in raw bulbs of *L. longiflorum* for the first time.

No.	Odorant	Odor Quality	FD Factor
20	(E)-non-2-enal	green	4
22	(E,Z)-nona-2,6-dienal	cucumber	4
29	dimethyl tetrasulfide	sulfurous	4
3	ethyl-2-methylbutanoate	fruity	2
14	2-methoxy-3-propan-2-ylpyrazine	earthy, beany	2
23	butanoic acid	sweaty, rancid	2
40	4-allyl-2-methoxyphenol (eugenol)	clove	2
42	3-Hydroxy-4,5-dimethylfuran-2(*5H*)-one (sotolon)	maple	2
11	(5Z)-octa-1,5-dien-3-one	geranium	1
13	4-methyl-4-sulfanylpentan-2-one	catty	1
26	2- and 3- methylbutanoic acid	sweaty, rancid	1
32	2-methoxyphenol (guaiacol)	smoky	1
33	2-phenylethanol	rose	1
37	γ-nonalactone	coconut	1
39	ethyl cinnamate	cinnamon	1

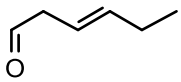
Figure 32. Additional Odorants in Raw Lily Bulbs with FD ≤ 4.

4.4 Decrease of Key Odorants in Raw Lily Bulbs after Thermal Treatment

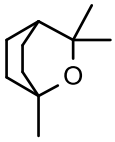
A few aroma active compounds that were present with relatively high FD factors in the raw lily bulbs but decreased post roasting can be seen in Figure 33. The FD factor of green compound, (E)-hex-3-enal (**6**) decreased from 1024 to 2. Similarly, both the eucalyptus-like compound, 1,8-cineole (eucalyptol) (**8**) and the mushroom-like compound, oct-1-en-3-one (**9**), decreased in FD factor from 256 and 64, respectively, to 4. These observations seemed to be consistent with the sensory profile.

No.	Odorant	Odor Quality	FD Factor	
			Raw	Roasted
6	(E)-hex-3-enal	green, grassy	1024	2
8	1,8-cineole (eucalyptol)	eucalyptus-like	256	64
9	oct-1-en-3-one	mushroom	64	4

(6)



(8)



(9)

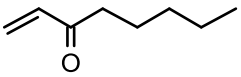


Figure 33. Decrease of Key Odorants in Raw Lily Bulbs after Thermal Treatment.

4.5 Sulfur-Containing Compounds in Raw Lily Bulbs

Three aroma-active compounds containing sulfur were detected in the raw lily bulb and they were as followed: cooked potato-like compound, 3-methyl(sulfanyl)propanal (methional) (**16**, FD 1024); sulfurous compound, dimethyl tetrasulfide (**29**, FD 4) and catty compound, 4-methyl-4-sulfanylpentan-2-one (**13**, FD 1) (Figure 34). As noted in the introduction, sulfur-containing compounds were also identified in the aroma profile of other species in the Liliopsida class, namely, *Allium*, *Asparagus* and *Fritillaria*. Sulfur compounds were also key contributors to the aroma profiles of raw onions, raw asparagus and *Fritillaria*. Analogous to its counterparts, *Longiflorum* also contained a sulfur compound, 3-methyl(sulfanyl)propanal (methional), that played a key role in the aroma of raw lily bulbs.

No.	Odorant	Odor Quality	FD Factor
16	3-(methylsulfanyl)propanal (methional)	cooked potato	1024
29	dimethyl tetrasulfide	sulfurous	4
13	4-methyl-4-sulfanylpentan-2-one	catty	1

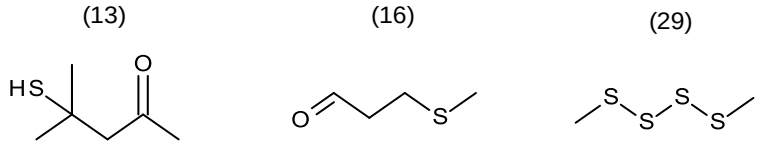


Figure 34. Sulfur-containing Compounds in Raw Lily Bulbs.

4.6 Key Odorants in Roasted Lily Bulbs

Key odorants of roasted lily bulbs can be characterized into three categories: generation of key odorants after thermal treatment, an increase of key odorants after roasting and key odorants that displayed no change after thermal treatment.

4.6.1 Generation of Key Odorants after Thermal Treatment

In the roasted lily bulbs, key odorants that were generated with an FD ≥ 64 post roasting were roasty, popcorn-like-compound, 2-acetyl-1-pyrroline (**10**, FD 1024); earthy compound, 2-ethyl-3, 5-dimethylpyrazine (**17**, FD 1024); earthy compound, 2, 3-diethyl-5-methylpyrazine (**18**, FD 1024); maple-like-compound, 5-ethyl-3-hydroxy-4-methyl-5H-furan-2-one (abhexon) (**44**, FD 1024); clove-like-compound, 2-methoxy-4-[(E)-prop-1-enyl]phenol ((E)-isoeugenol) (**46**, FD 1024); roasty-compound, 1-(1,3-thiazol-2-yl)ethanone (2-acetylthiazole) (**24**, FD 256); caramel-like-compounds, 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene) (**31**, FD 256) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF) (**36**, FD 256); buttery compound, butane-2,3-dione (diacetyl) (**2**, FD 64) and vinegar-like compound, acetic acid (**15**, FD 64) (Figure 35). The mass spectra of the key odorants generated after thermal treatment are depicted in Figures 36 – 46.

No.	Odorant	Odor Quality	FD Factor
10	2-acetyl-1-pyrroline	roasty	1024
17	2-ethyl-3,5-dimethylpyrazine	earthy	1024
18	2,3- diethyl-5-methylpyrazine	earthy	1024
44	5-ethyl-3-hydroxy-4-methyl-5H-furan-2-one (abhexon)	maple	1024
46	2-methoxy-4-[(E)-prop-1-enyl]phenol ((E)-isoeugenol)	clove	1024
24	1-(1,3-thiazol-2-yl)ethanone (2-acetylthiazole)	roasty	256
31	2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene)	caramel	256
36	4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF)	caramel	256
2	butane-2,3-dione (diacetyl)	buttery	64
15	acetic acid	vinegar	64

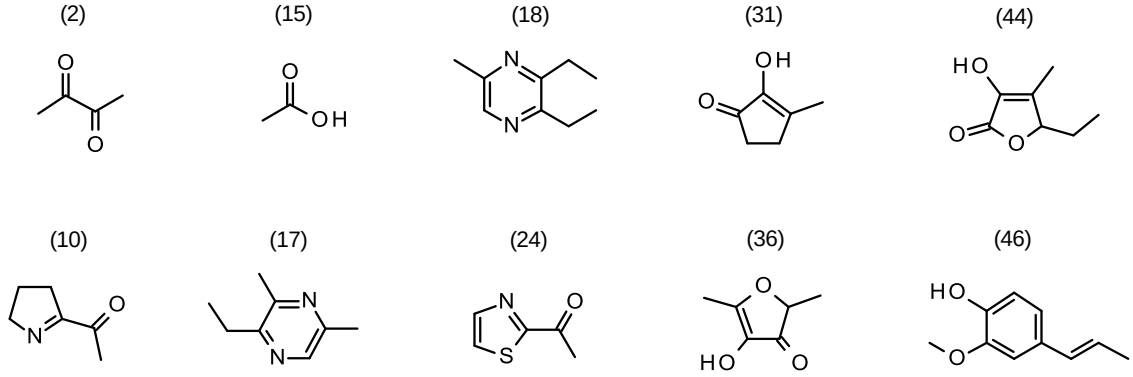


Figure 35. Generation of Key Odorants after Thermal Treatment.

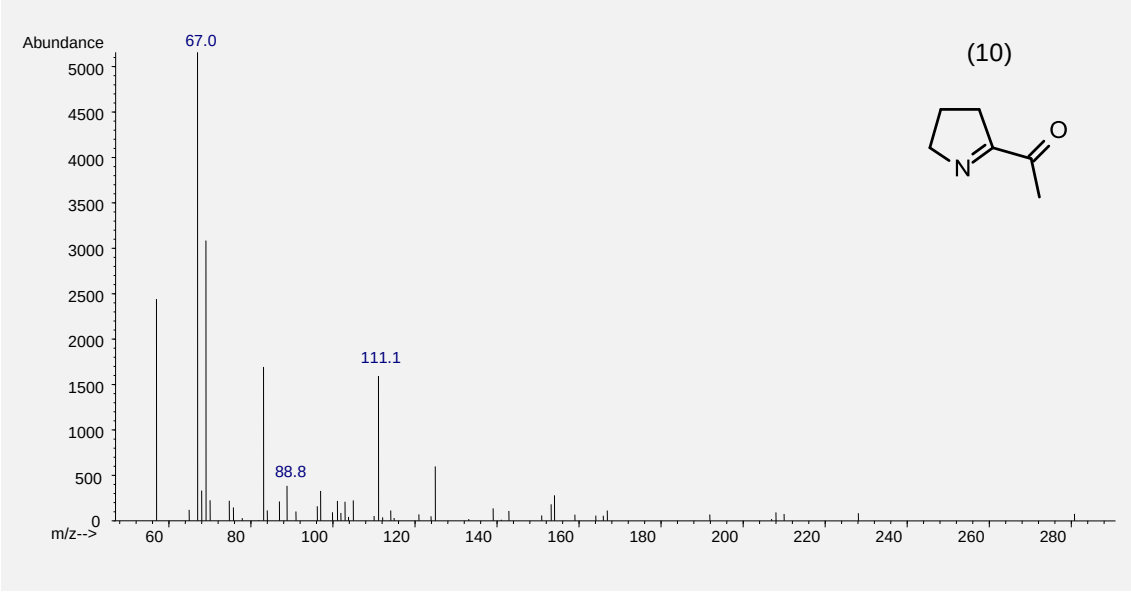


Figure 36. Mass Spectrum of 2-Acetyl-pyrroline.

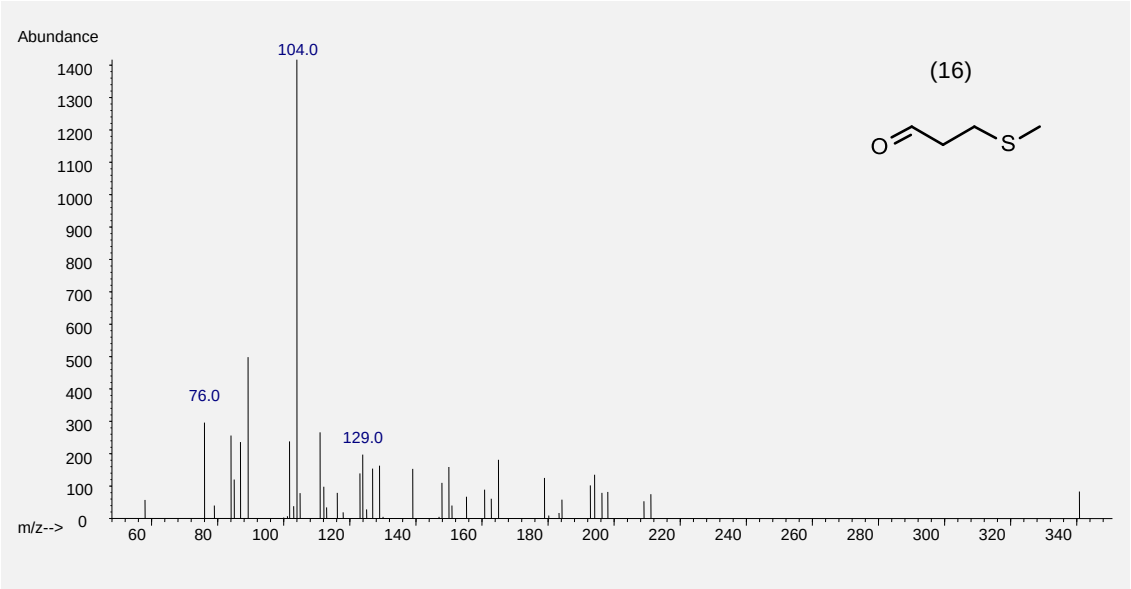


Figure 37. Mass Spectrum of 3-(Methylsulfanyl)propanal (Methional).

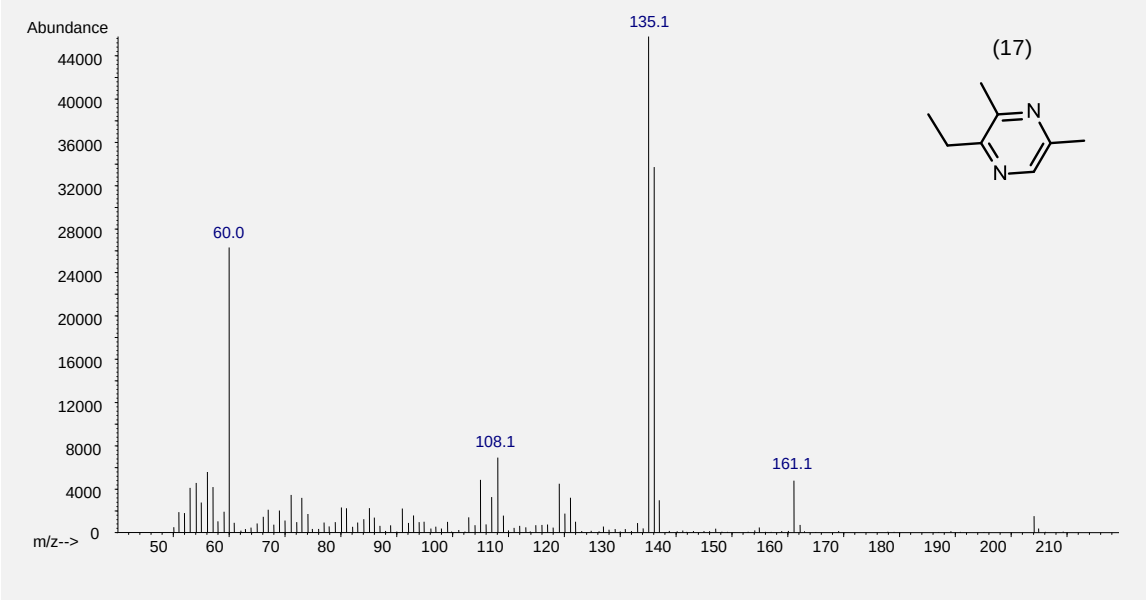


Figure 38. Mass Spectrum of 2-Ethyl-3,5-dimethylpyrazine.

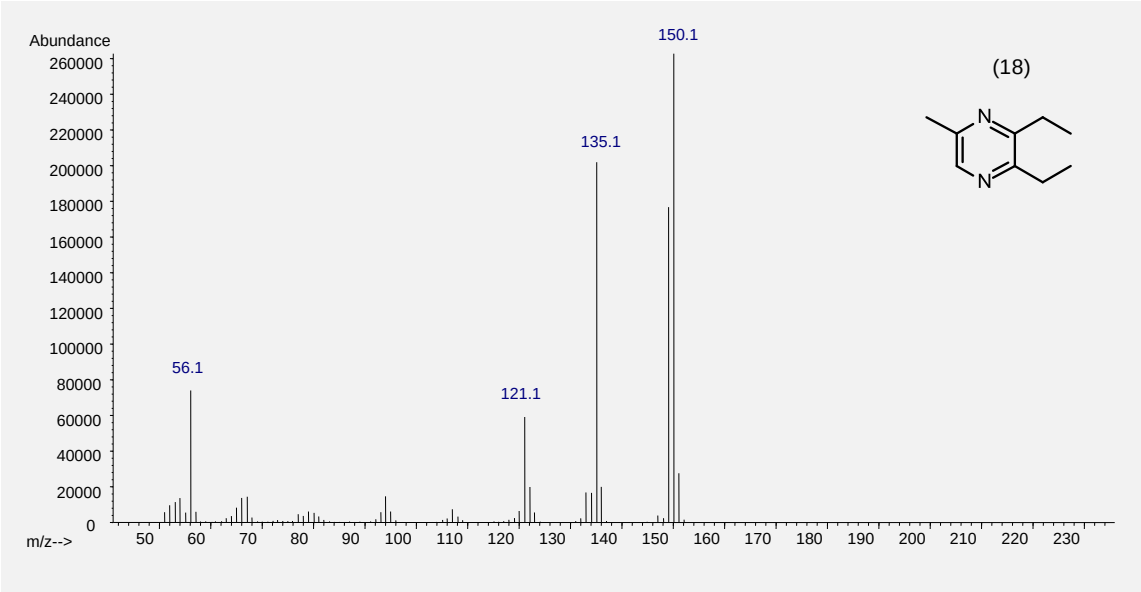


Figure 39. Mass Spectrum of 2,3- Diethyl-5-methylpyrazine.

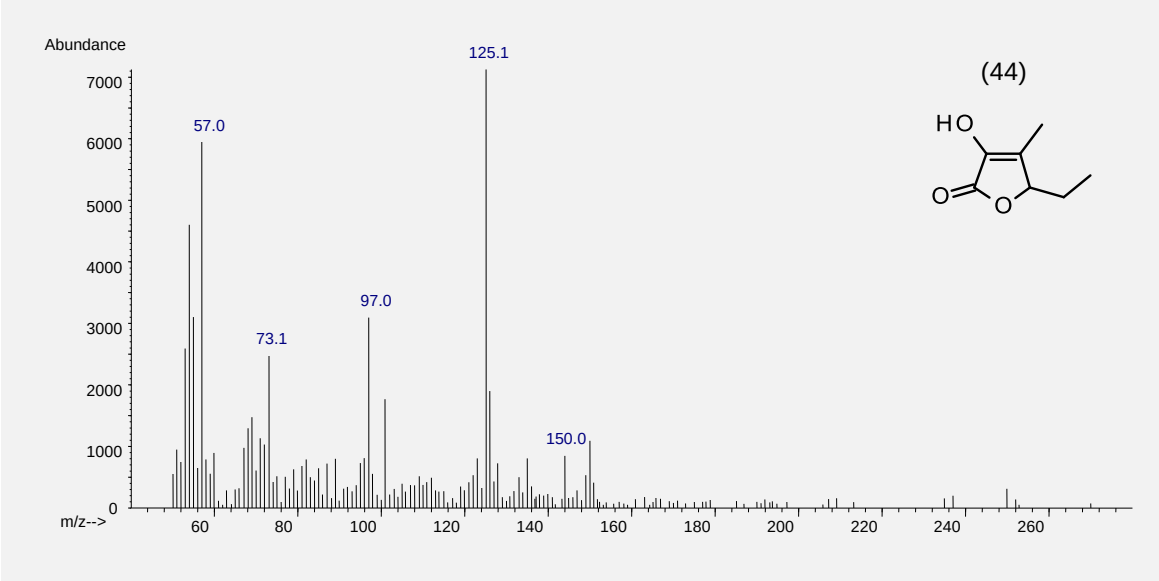


Figure 40. Mass Spectrum of 5-Ethyl-3-hydroxy-4-methyl-5H-furan-2-one (Abhexon).

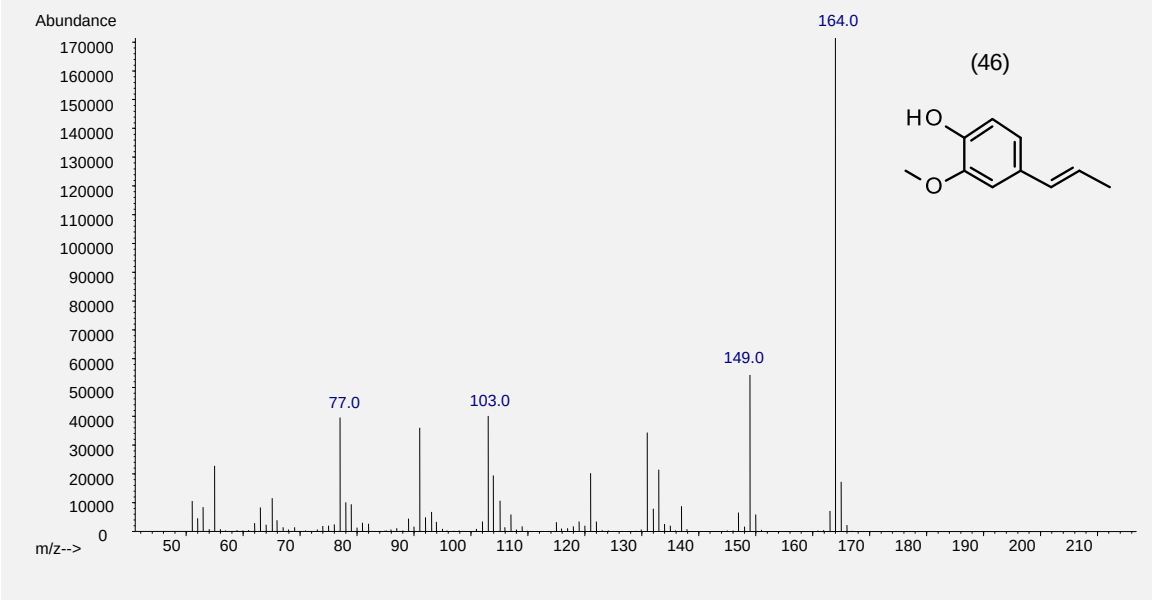


Figure 41. Mass Spectrum of 2-methoxy-4-[(E)-prop-1-enyl]phenol ((E)-Isoeugenol).

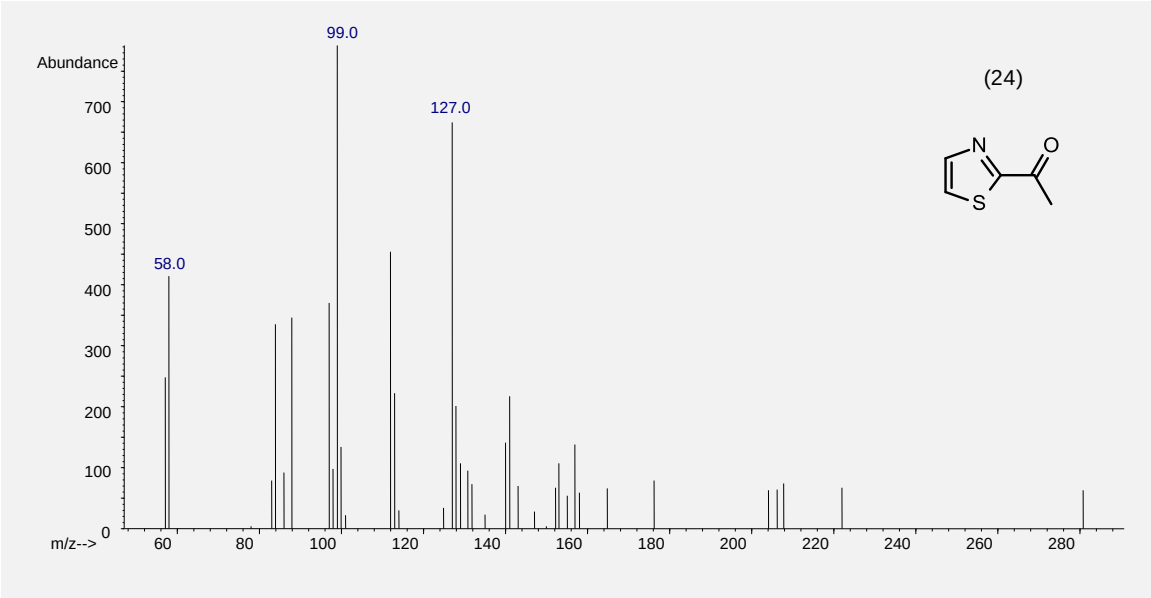


Figure 42. Mass Spectrum of 1-(1,3-thiazol-2-yl)ethanone (2-Acetylthiazole).

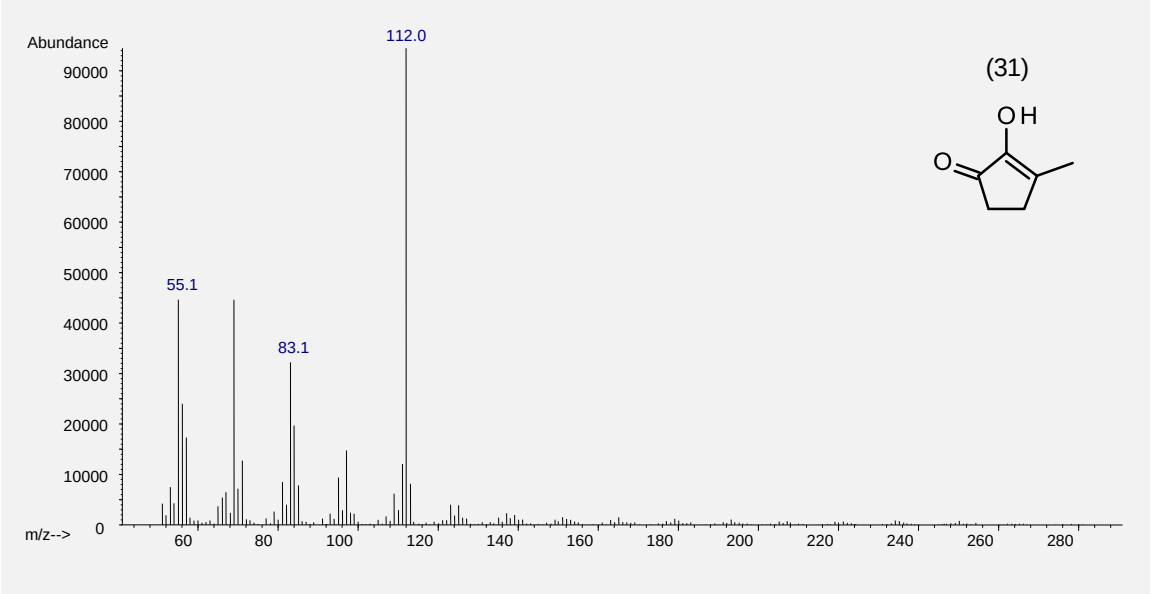


Figure 43. Mass Spectrum of 2-hydroxy-3-methyl-2-cyclopenten-1-one (Cyclotene).

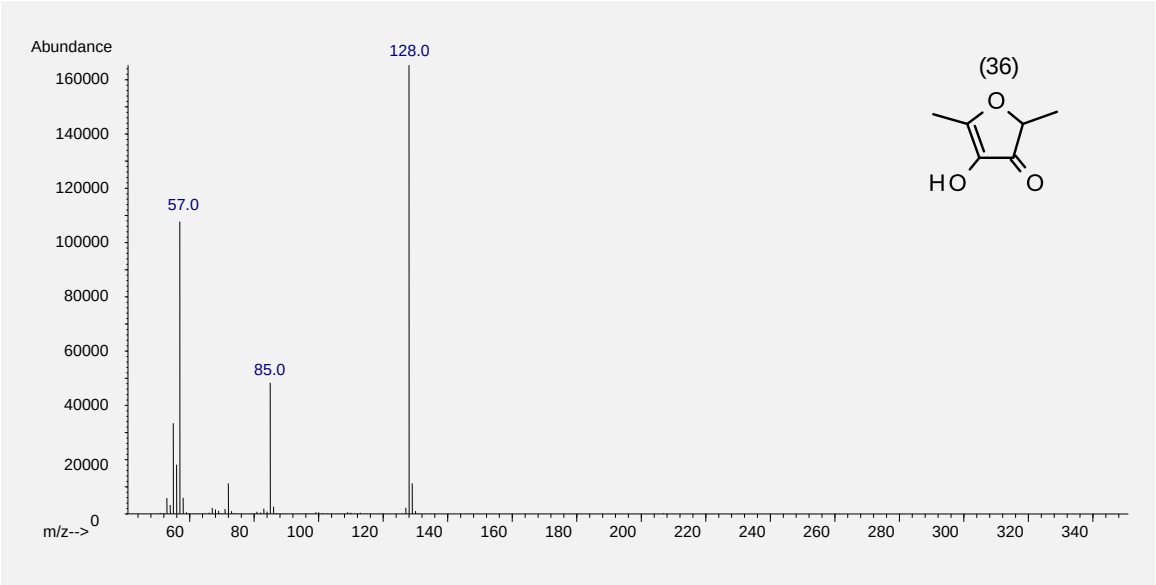


Figure 44. Mass Spectrum of 4-Hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF).

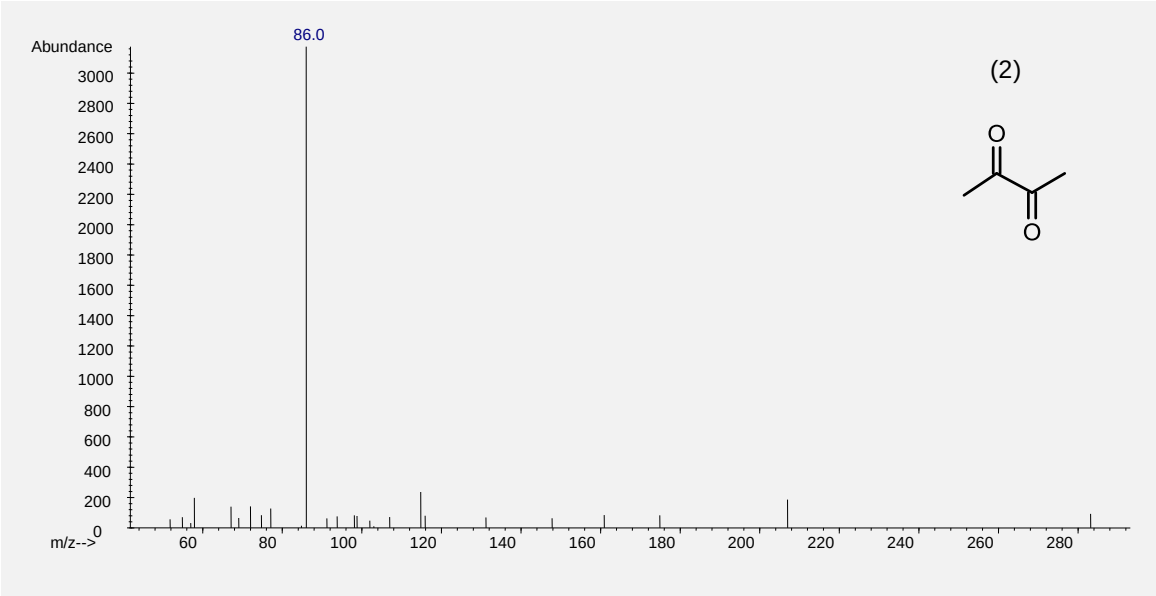


Figure 45. Mass Spectrum of Butane-2,3-dione (Diacetyl).

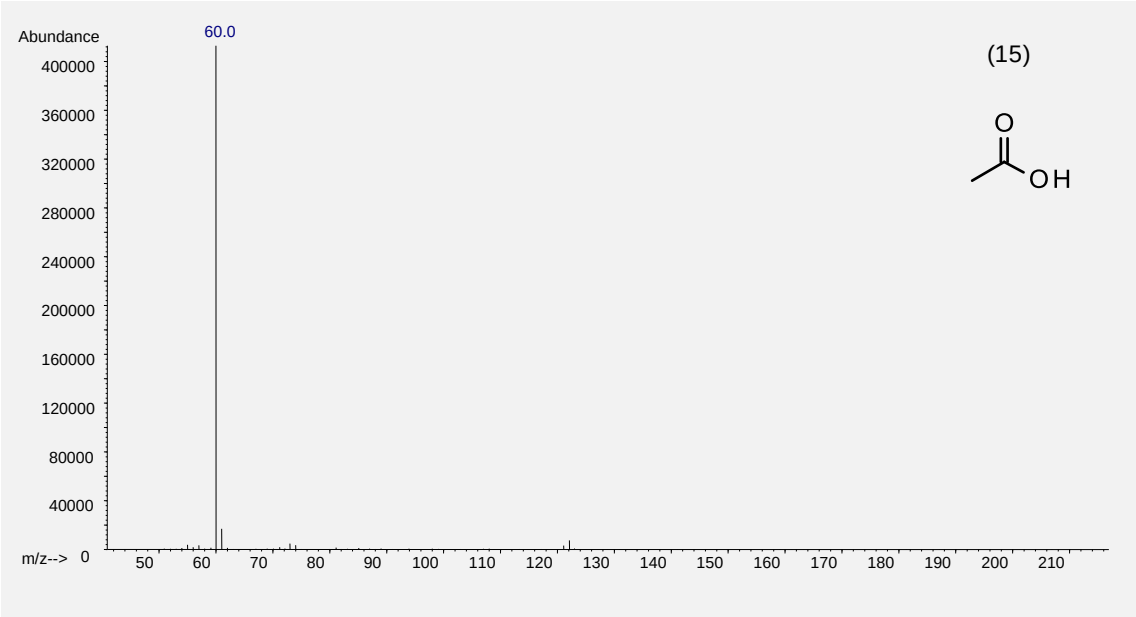


Figure 46. Mass Spectrum of Acetic Acid.

4.6.2 Increase of Key Odorants after Thermal Treatment

Odorants with FD values that were low in the raw lily bulbs (FD 1–16) but increased in potency to ≥64 after roasting included maple-like compound, 3-hydroxy-4,5-dimethylfuran-2(*5H*)-one (sotolon) (**42**, FD 1024); malty compound, 2- and 3-methylbutanal (**1**, FD 256); green compound, (E)-non-2-enal (**20**, FD 64); smoky compound, 2-methoxyphenol (guaiacol) (**32**, FD 64) and hay-like compound, 3-methyl-2,4-nonanedione (**28**, FD 64) (Figure 47). These aroma active compounds along with the new aroma compounds generated after thermal treatment were determined to be key contributors of the overall scent of the roasted lily bulbs. The flavor profile of the roasted lily bulbs is depicted in the aromagram shown in Figure 48 where the RIs versus FD factor of each odorant are plotted against each other. Only aroma compounds with FD ≥ 256 are labeled with its corresponding number. The mass spectra of the key odorants generated and increased after thermal treatment are also depicted in Figures 49 - 53.

No.	Odorant	Odor Quality	FD Factor	
42	3-hydroxy-4,5-dimethylfuran-2(*5H*)-one (sotolon)	maple	2	1024
1	2-and 3- methylbutanal	malty	16	256
20	(E)-non-2-enal	green	4	64
32	2-methoxyphenol (guaiacol)	smoky	1	64
35	trans-4,5-epoxy-(E)-2-decenal	metallic	16	64

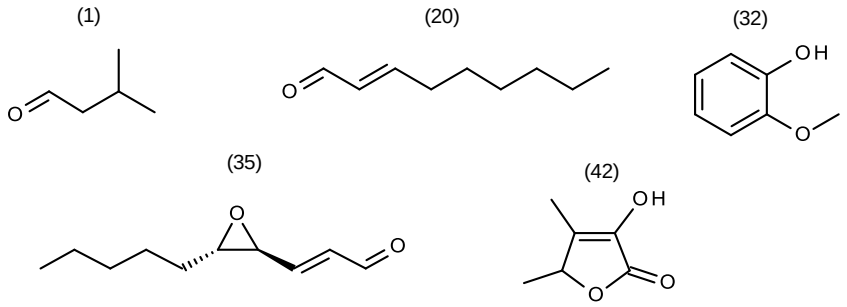


Figure 47. Increase of Key Odorants after Thermal Treatment.

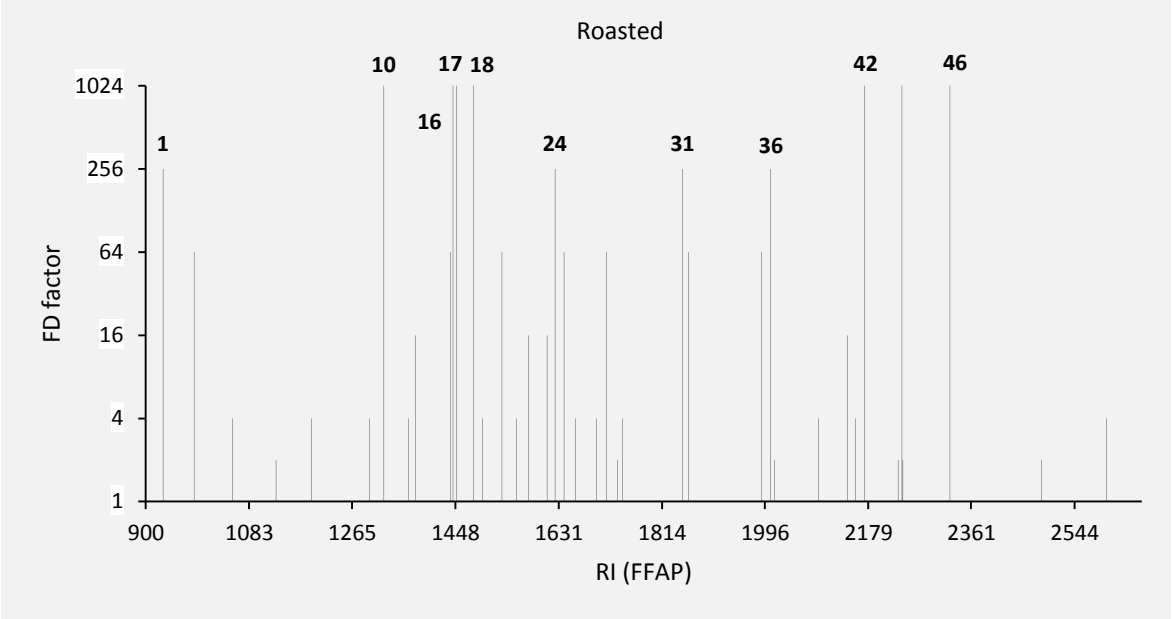


Figure 48. Flavor Dilution (FD) Chromatogram of the Aroma Distillate isolated from Roasted Lily Bulbs (Compound Numbers as in Figure 16).

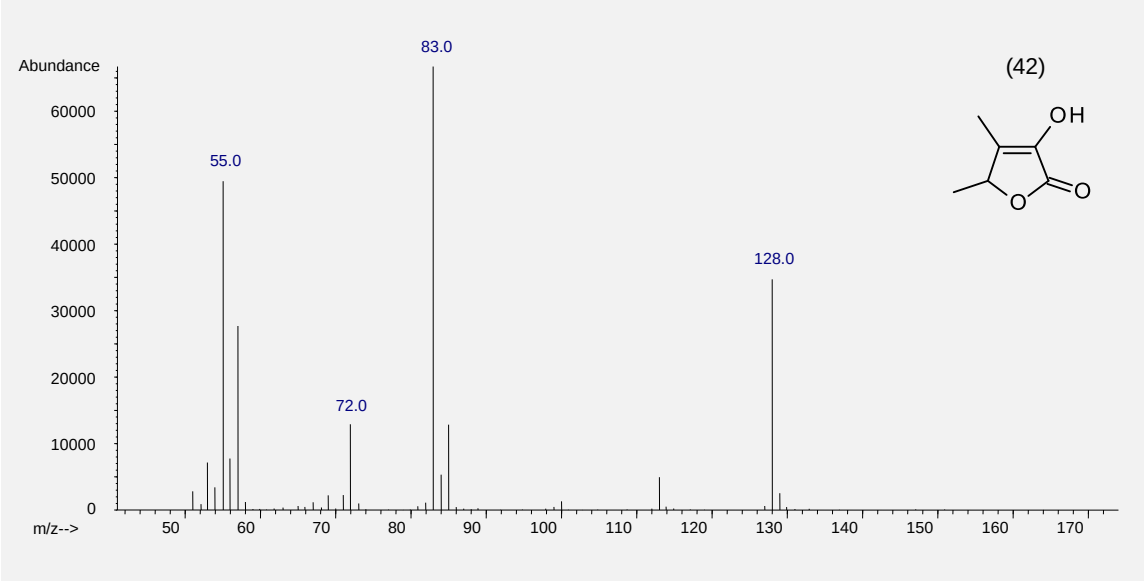


Figure 49. Mass Spectrum of 3-Hydroxy-4,5-dimethylfuran-2(*5H*)-one (Sotolon).

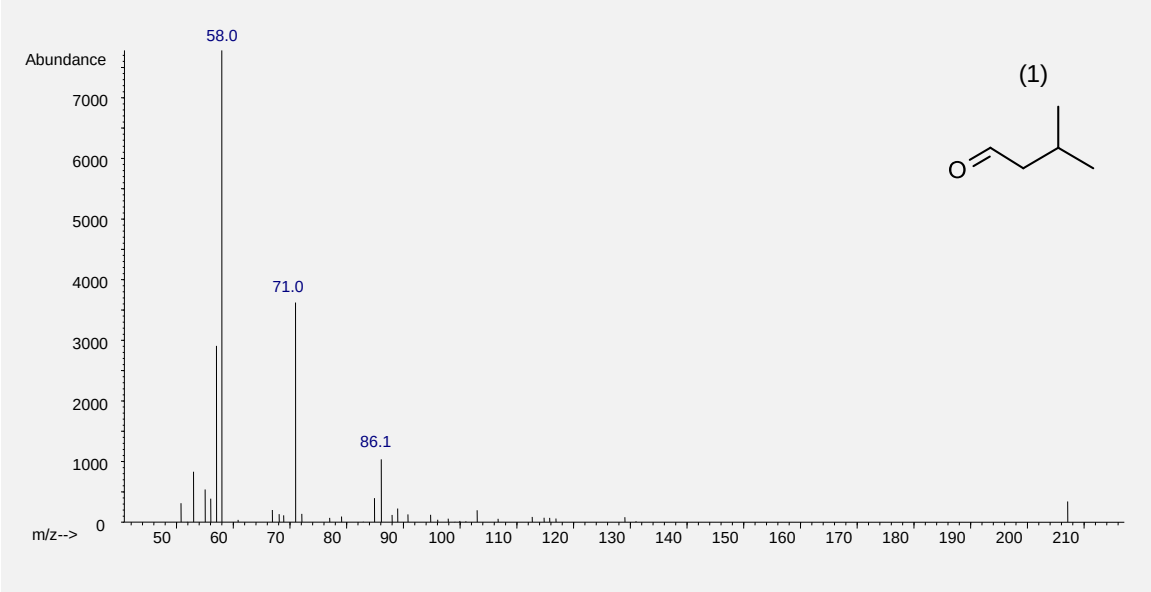


Figure 50. Mass Spectrum of 2-and 3-Methylbutanal.

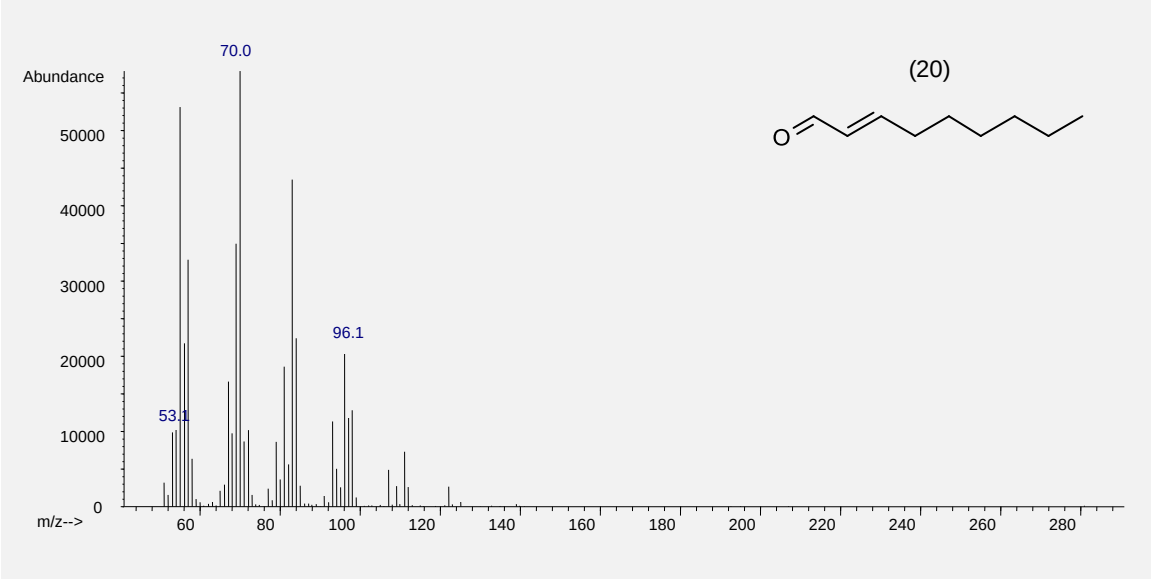


Figure 51. Mass Spectrum of (2E)-Non-2-enal.

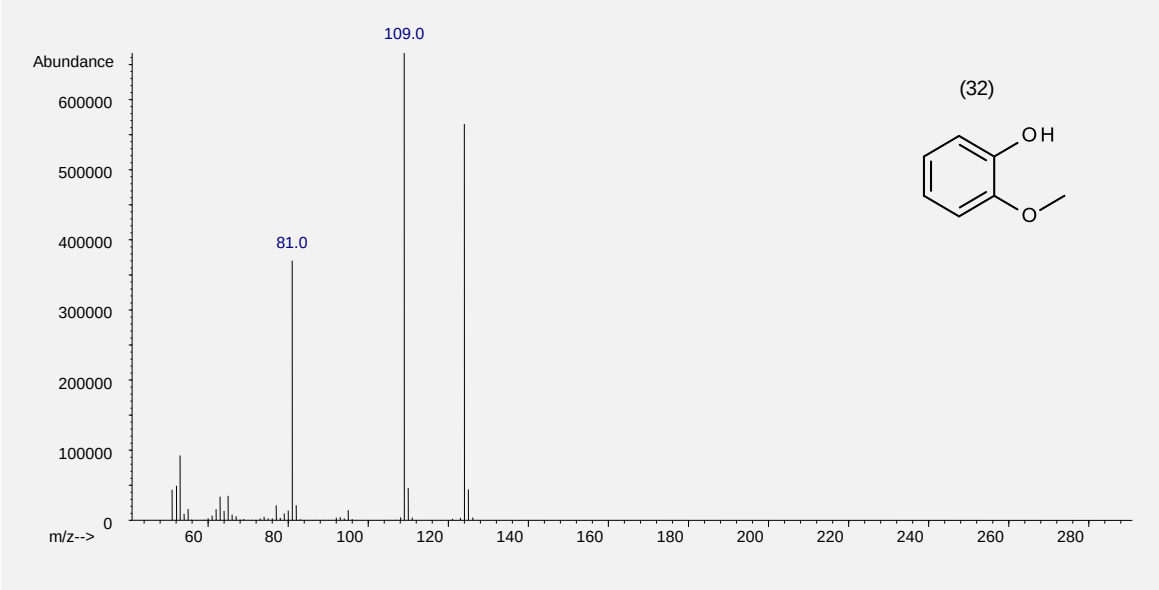


Figure 52. Mass Spectrum of 2-Methoxyphenol (Guaiacol).

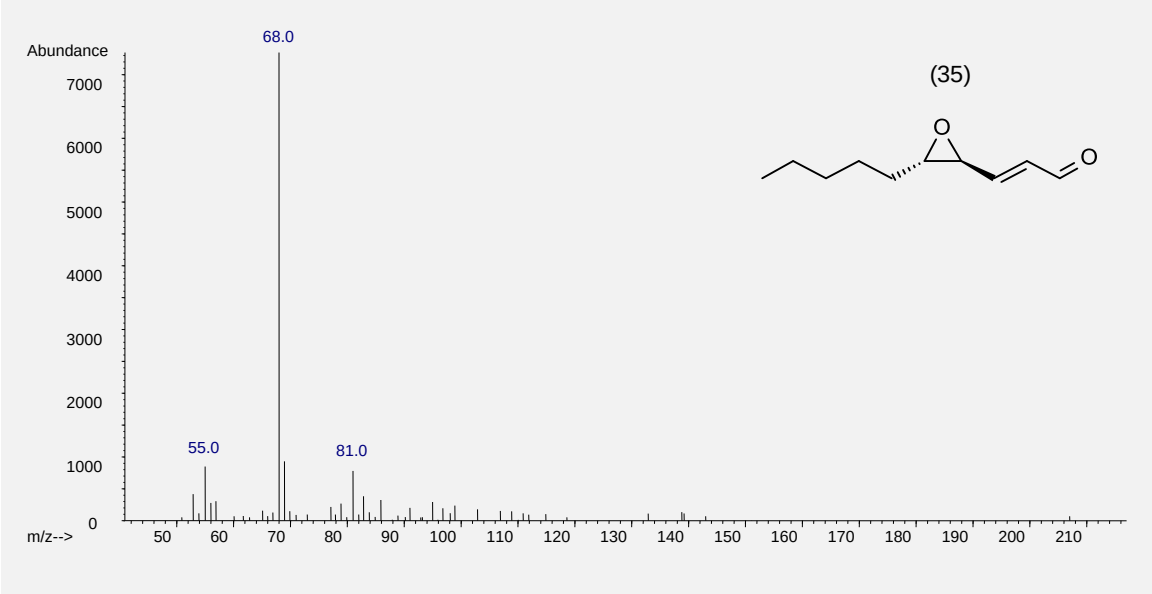


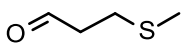
Figure 53. Mass Spectrum of Trans-4,5-epoxy-(E)-2-decenal.

4.6.3 Key Odorants that Showed no Change after Thermal Treatment

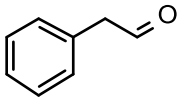
The key aroma-active compounds in the raw lily bulbs that remained the same after roasting were cooked potato-like compound, 3-(methylsulfanyl)propanal (methional) (**16**, FD 1024); floral, honey-like compound, 2-phenylacetaldehyde (**25**, FD 64) and anise/hay-like compound, 3-methylnonane-2,4-dione (**28**, FD 64) (Figure 54). According to the sensory results, the cooked potato-like attribute was perceived more strongly in the roasted sample than in the raw, so there is a possibility that the FD of the cooked potato-like compound, 3-(methylsulfanyl)propanal (methional) in the roasted sample was actually much higher than the 1024 limit measured in this study. Quantification of these aroma compounds via stable isotope dilution analysis (SIDA) will be the next step in determining the concentration of each compound in the raw and roasted lily bulbs. The mass spectra of the odorants that remained the same in potency after thermal treatment are presented in Figures 55 – 57.

No.	Odorant	Odor Quality	FD Factor
16	3-(methylsulfanyl)propanal (methional)	cooked potato	1024
25	2-phenylacetaldehyde	floral, honey	64
28	3-methylnonane-2,4-dione	anise/hay	64

(16)



(25)



(28)

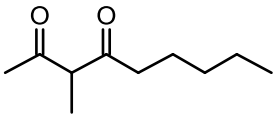


Figure 54. No Change in Key Odorants after Thermal Treatment.

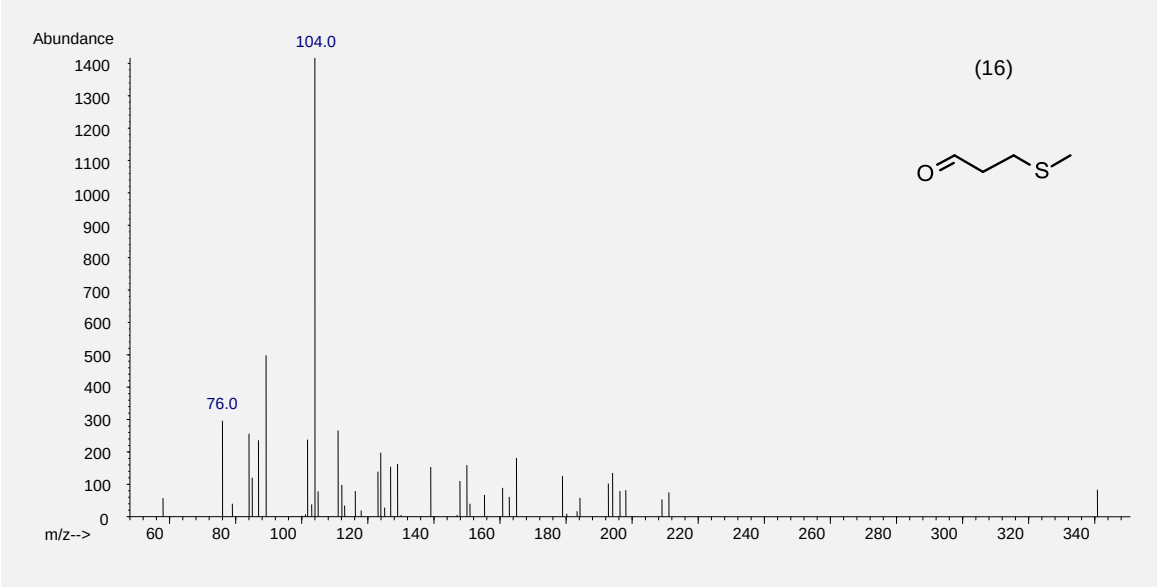


Figure 55. Mass Spectrum of 3-(Methylsulfanyl)propanal (Methional).

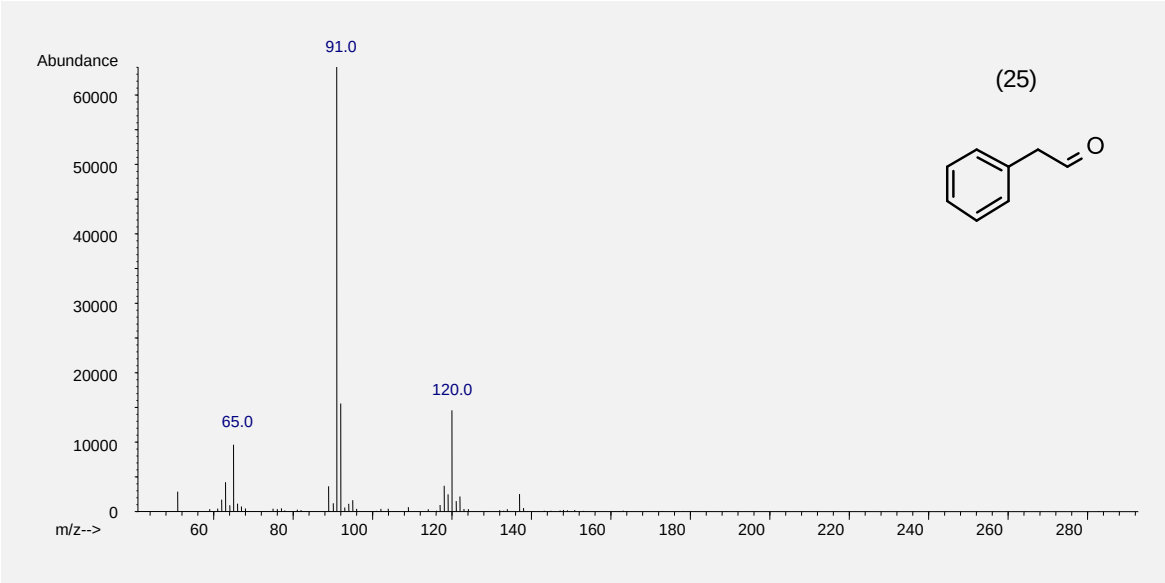


Figure 56. Mass Spectrum of 2-Phenylacetaldehyde.

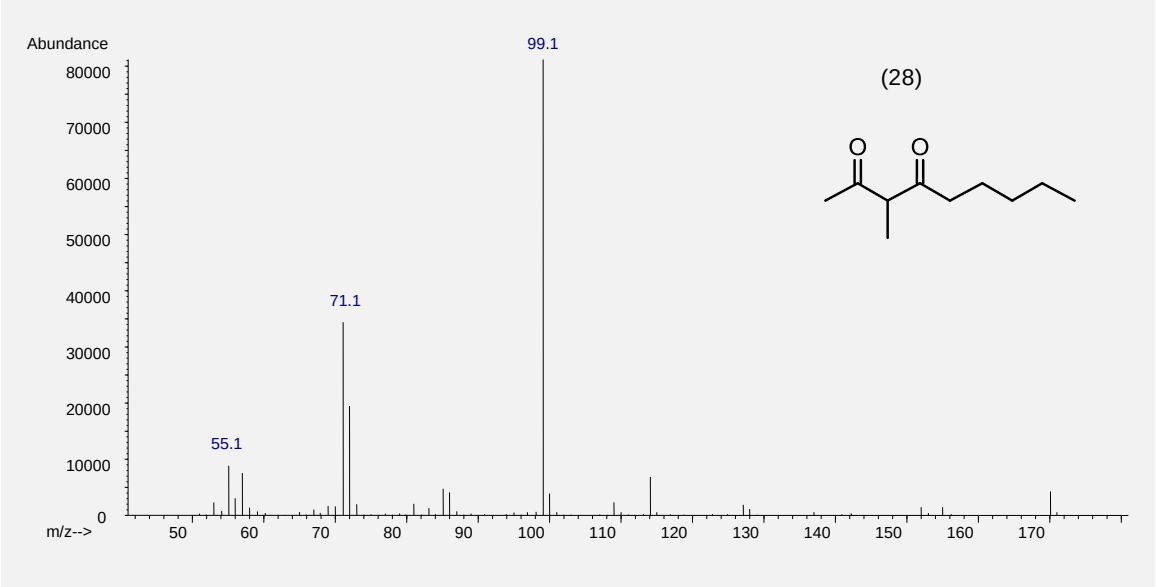


Figure 57. Mass Spectrum of 3-Methylnonane-2,4-dione.

The sensory profile of the roasted lily bulbs indicated a strong cooked potato note that may be explained by potato-like smelling compound, 3-(methylsulfanyl)propanal (methional) (**16**, FD 1024). The toasted and roasty notes also detectable notable may be explained by the roasty smelling compounds, 2-acetyl-1-pyrroline (**10**, FD 1024) and 1-(1,3-thiazol-2-yl)ethanone (2-acetylthiazole) (**24**, FD 256). The deep fried note in the roasted bulbs may be due to the fatty smelling compound, (E, E)-nona-2,4-dienal (**27**, FD 4) while the smoky note can be attributed to the smoky smelling compound, 2-methoxyphenol (guaiacol) (**32**, FD 64). In addition, the caramel and maple-like notes in the roasted bulbs can be explained by caramel-like smelling compounds, 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene) (**31**, FD 256) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF) (**36**, FD 256) and maple-like smelling compounds, 3-hydroxy-4,5-dimethylfuran-2(*5H*)-one (sotolon) (**42**, FD 1024) and 5-ethyl-3-hydroxy-4-methyl-5H-furan-2-one (abhexon) (**44**, FD 1024) respectively.

4.7 Additional Odorants in Roasted Lily Bulbs

Odorants with an FD factor of 16 that also contributed to the aroma profile of the roasted lily bulbs included 4-methyl-4-sulfanylpentan-2-one (**13**, catty), (E, Z)-nona-2,6-dienal (**22**, cucumber), butanoic acid (**23**, sweaty, rancid) and 4-allyl-2-methoxyphenol (eugenol) (**40**, clove) (Figure 58). The mass spectra of these odorants are depicted in Figures 59 - 62.

No.	Odorant	Odor Quality	FD Factor
13	4-methyl-4-sulfanylpentan-2-one	catty	16
22	(E,Z)-nona-2,6-dienal	cucumber	16
23	butanoic acid	sweaty, rancid	16
40	4-allyl-2-methoxyphenol (eugenol)	clove	16

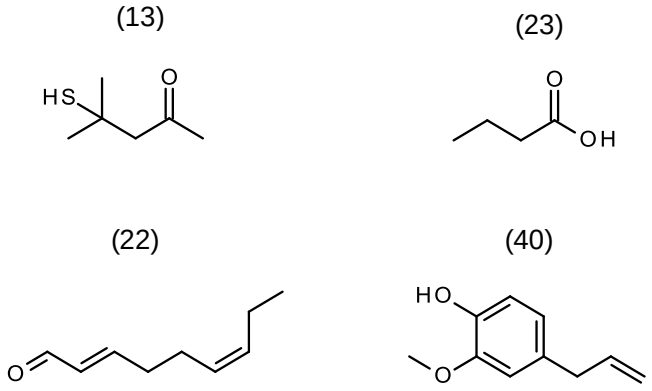


Figure 58. Additional Odorants in Roasted Lily Bulbs with FD 16.

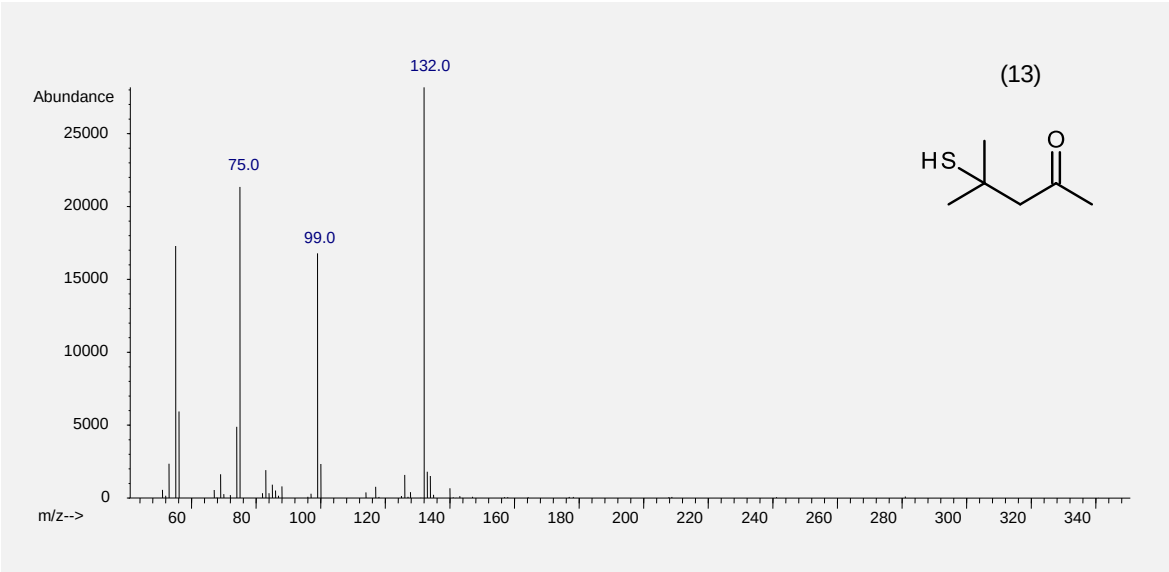


Figure 59. Mass Spectrum of 4-Methyl-4-sulfanylpentan-2-one.

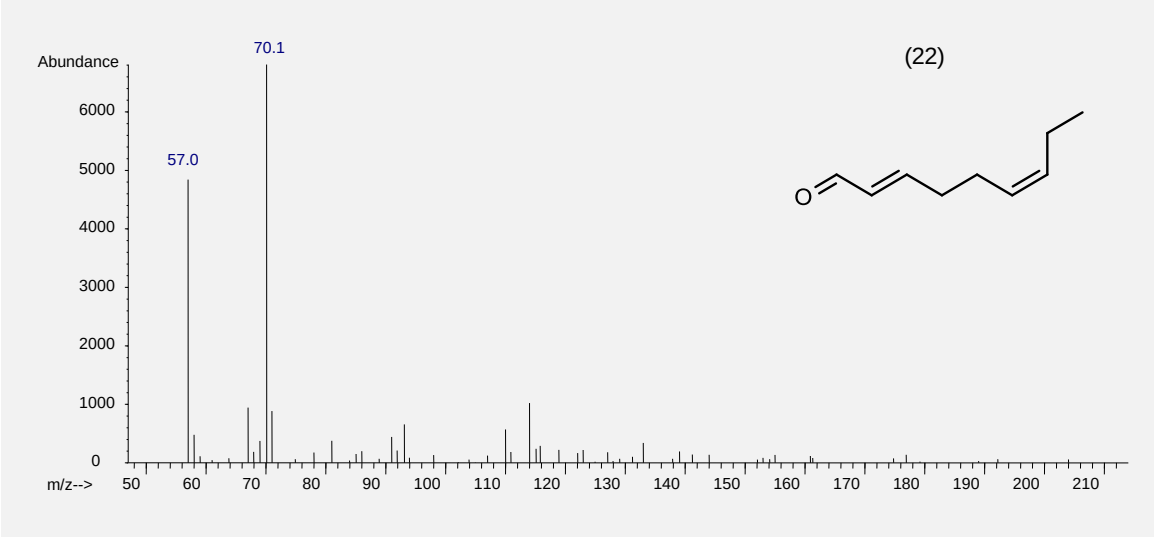


Figure 60. Mass Spectrum of (E,Z)-Nona-2,6-dienal.

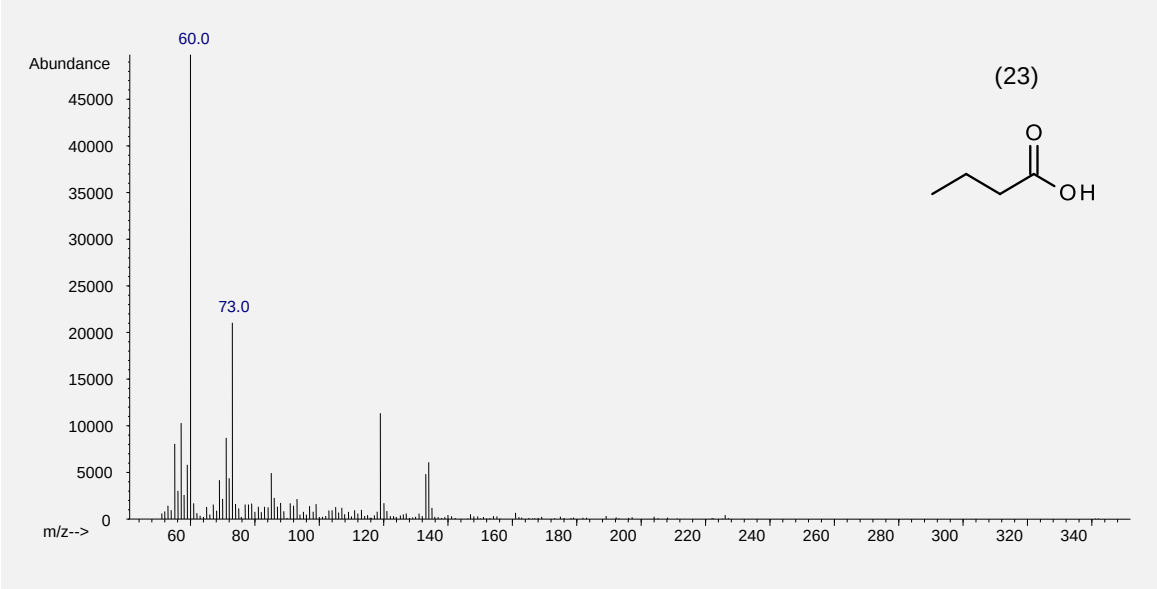


Figure 61. Mass Spectrum of Butanoic Acid.

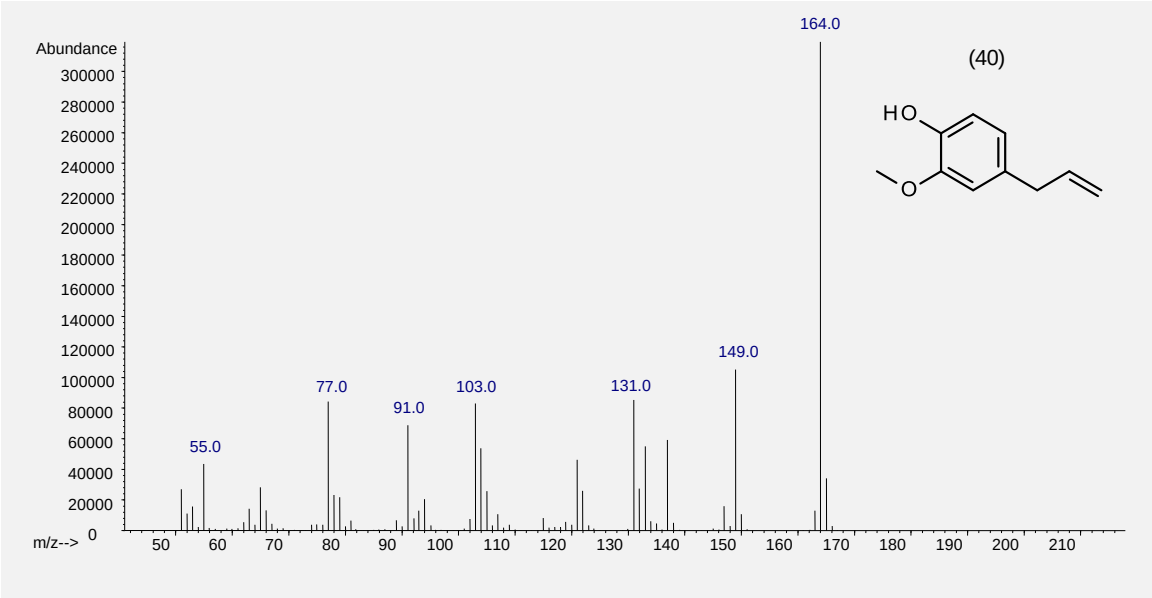


Figure 62. Mass Spectrum of 4-Allyl-2-methoxyphenol (Eugenol)

Twenty additional odorants present in the dilutions with an FD factor ≤ 4, namely, buttery compound, 2,3-pentanedione (acetylpropionyl) (**4**, FD 4); eucalyptus-like compound, 1,8-cineole (eucalyptol) (8, FD 4); mushroom-like compound, oct-1-en-3-one (**9**, FD 4); sulfurous compound, dimethyl trisulfide (**12**, FD 4); green, fatty compound, (Z)-non-2-enal (**19**, FD 4); roasty compound, 2-ethenyl-3,5-dimethylpyrazine (**21**, FD 4); sweaty, rancid compound, 2- and 3- methylbutanoic acid (**26**, FD 4); fatty compound, (E, E)-nona-2,4-dienal (**27**, FD 4); roasty compound, 1-(4,5-dihydro-1,3-thiazol-2-yl)ethanone (2-acetyl-2-thiazoline) (**30**, FD 4); phenolic compound, 3-methylphenol (*m*-cresol) (**38**, FD 4); leather-like compound, 3-ethylphenol (**41**, FD 4); vanilla-like compound, 4-hydroxy-3-methoxy-benzaldehyde (vanillin) (**48**, FD 4); green-like compound, (E)-hex-3-enal (**6**, FD 2); sulfurous compound, dimethyl tetrasulfide (**29**, FD 2); coconut-like compound, γ-nonalactone (**37**, FD 2); clove-like compound, 2-methoxy-4-[(Z)-prop-1-enyl]phenol ((Z)-isoeugenol) (**43**, FD 2); smoky compound, 1,3-dimethoxy-2-hydroxybenzene (2,6-dimethoxyphenol) (**45**, FD 2); fecal compound, 3-methylindole (skatole) (**47**, FD 2); fruity compound, ethyl-2-methylbutanoate (3, FD 1) and caramel-like compound, 3-hydroxy-2-methyl-4*H*-pyran-4-one (maltol) (**34**, FD 1) (Figure 63) were also identified in the roasted lily bulbs, all of which to the best of our knowledge were reported in the bulbs of *L. longiflorum* for the first time.

No.	Odorant	Odor Quality	FD Factor
4	2,3-pentanedione (acetylpropionyl)	buttery	4
8	1,8-cineole (eucalyptol)	eucalyptus-like	4
9	oct-1-en-3-one	mushroom	4
12	dimethyl trisulfide	sulfurous	4
19	(Z)-non-2-enal	green, fatty	4
21	2-ethenyl-3,5-dimethylpyrazine	roasty	4
26	2- and 3- methylbutanoic acid	sweaty, rancid	4
27	(E,E)-nona-2,4-dienal	fatty	4
30	1-(4,5-dihydro-1,3-thiazol-2-yl)ethanone (2-acetyl-2-thiazoline)	roasty	4
38	3-methylphenol (*m*-cresol)	phenolic	4
41	3-ethylphenol	leather	4
48	4-hydroxy-3-methoxy-benzaldehyde (vanillin)	vanilla-like	4
6	(E)-hex-3-enal	green	2
29	dimethyl tetrasulfide	sulfurous	2
37	γ-nonalactone	coconut	2
43	2-methoxy-4-[(Z)-prop-1-enyl]phenol ((Z)-isoeugenol)	clove	2
45	1,3-dimethoxy-2-hydroxybenzene (2,6-dimethoxyphenol)	smoky	2
47	3-methylindole (skatole)	fecal	2
3	ethyl-2-methylbutanoate	fruity	1
34	3-hydroxy-2-methyl-4*H*-pyran-4-one (maltol)	caramel	1

Figure 63. Additional Odorants in Roasted Lily Bulbs with FD ≤ 4.

4.8 Formation of Maillard-derived Products

Twelve Maillard-derived products were generated during roasting and they were as followed: 2- and 3- methylbutanal (**1**, malty); butane-2,3-dione (diacetyl) (**2**, buttery); 2,3-pentanedione (acetylpropionyl) (**4**, buttery); 2-acetyl-1-pyrroline (**10**, roasty); 3-(methylsulfanyl)propanal (methional) (**16**, cooked potato); 2-ethyl-3,5-dimethylpyrazine (**17**, earthy); 2,3- diethyl-5-methylpyrazine (**18** earthy); 2-ethenyl-3,5-dimethylpyrazine (**21**, roasty); 1-(1,3-thiazol-2-yl)ethanone (2-acetylthiazole) (**24**, roasty); 1-(4,5-dihydro-1,3-thiazol-2-yl)ethanone (2-acetyl-2-thiazoline) (**30**, roasty); 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene) (**31**, caramel); 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF) (**36**, caramel); 3-hydroxy-4,5-dimethylfuran-2(*5H*)-one (sotolon) (**42**, maple) and 5-ethyl-3-hydroxy-4-methyl-5H-furan-2-one (abhexon) (**44**, maple). Another three compounds detected at major levels in the roasted bulbs namely, 2-and 3- methylbutanal (**1**), 3-(methylsulfanyl)propanal (methional) (**16**) and 3-hydroxy-4,5-dimethylfuran-2(*5H*)-one (sotolon) (**42**) (Figure 64), were present in the raw bulbs and either remained the same or increased in potency after roasting.

The generation of these odorants can be attributed to the Maillard reaction. Since the chemical composition of the lily bulbs is poorly defined, the paths of formation of these compounds can only be speculated, based on assumptions about composition. M 2-and 3- methylbutanal (**1**) and 3-(methylsulfanyl)propanal (methional) (**16**) are Strecker aldehydes formed from reacting their parent free amino acids, isoleucine, leucine and methione respectively, with α-dicarbonyl compounds that were previously generated from carbohydrate degradation [45]. Butane-2,3-dione (diacetyl) (**2**) can be

formed by the aldol condensation of formaldehyde and hydroxypropanone also formed during carbohydrate degradation [45]. The formation pathway of 2,3-pentanedione (acetylpropionyl) (**4**) is similar to that of butane-2,3-dione (diacetyl) except that acetaldehyde, formed by the Strecker degradation of alanine, is the aldehyde involved in the aldol condensation with hydroxypropanone [45]. 2-Acetyl-1-pyrroline (**10**) can be assumed to be the degradation product of proline when heated in the presence of carbohydrates [45]. In this reaction, 1-pyrroline, which is formed from proline via Strecker degradation, reacts with 2-oxopropanal to form 2-acetylpyrrolidine which then oxidizes, rearranges and oxidizes once more prior to the formation of 2-acetyl-1-pyrroline [46] (Figure 65). 3-Hydroxy-4,5-dimethylfuran-2(*5H*)-one (sotolon) (**42**) and 5-ethyl-3-hydroxy-4-methyl-5H-furan-2-one (abhexon) (**44**) can be explained by aldol condensation of sugar fragments[47]. 4-Hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF) (**36**) can be formed with precursors, glucose and glycine. The path of formation is by way of 1-deoxyglycosone and 2,4-diydroxy-2,5-dimethyl-3(2H)-furanone (acethylformoine) [48] (Figure 66). Pyrazines can be formed through several pathways along with a variety of precursors. For example, 2-ethyl-3,5-dimethylpyrazine (**17**) is formed with precursors, alanine and 2-oxopropanal [41]. These two precursors undergo Strecker degradation yielding aminoacetone, 2-aminopropanal and acetaldehyde. 2-ethyl-3,5-dimethylpyrazine is then formed by the condensation of the aminoacetone with 2-aminopropanal [49]. 1-(1,3-thiazol-2-yl)ethanone (2-acetylthiazole) (**24**) and 1-(4,5-dihydro-1,3-thiazol-2-yl)ethanone (2-acetyl-2-thiazoline) (**30**) can be formed with precursors, L- cysteine, and 2-oxopropanal. 2-oxopropanal condenses with L-cysteine forming a Schiff base which then undergoes decarboxylation and cyclization to form,

intermediate, 2-acetylthaizolidine [50]. The oxidation of 2-acetylthaizolidine gives way to the formation of 1-(1,3-thiazol-2-yl)ethanone (2-acetylthiazole) and 1-(4,5-dihydro-1,3-thiazol-2-yl)ethanone (2-acetyl-2-thiazoline) (Figure 67).

No.	Odorant	Odor Quality	FD Factor	
			Raw	Roasted
1	2-and 3- methylbutanal	malty	16	256
2	butane-2,3-dione (diacetyl)	buttery		64
4	2,3-pentanedione (acetylpropionyl)	buttery		4
10	2-acetyl-1-pyrroline	roasty		1024
16	3-(methylsulfanyl)propanal (methional)	cooked potato	1024	1024
17	2-ethyl-3,5-dimethylpyrazine	earthy		1024
18	2,3- diethyl-5-methylpyrazine	earthy		1024
21	2-ethenyl-3,5-dimethylpyrazine	roasty		4
24	1-(1,3-thiazol-2-yl)ethanone (2-acetylthiazole)	roasty		256
30	1-(4,5-dihydro-1,3-thiazol-2-yl)ethanone (2-acetyl-2-thiazoline)	roasty		4
31	2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene)	caramel		256
34	3-hydroxy-2-methyl-4*H*-pyran-4-one (maltol)	caramel		1
36	4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF)	caramel		256
42	3-hydroxy-4,5-dimethylfuran-2(*5H*)-one (sotolon)	maple	2	1024
44	5-ethyl-3-hydroxy-4-methyl-5H-furan-2-one (abhexon)	maple		1024

Figure 64. Maillard-derived Products generated after Thermal Treatment.

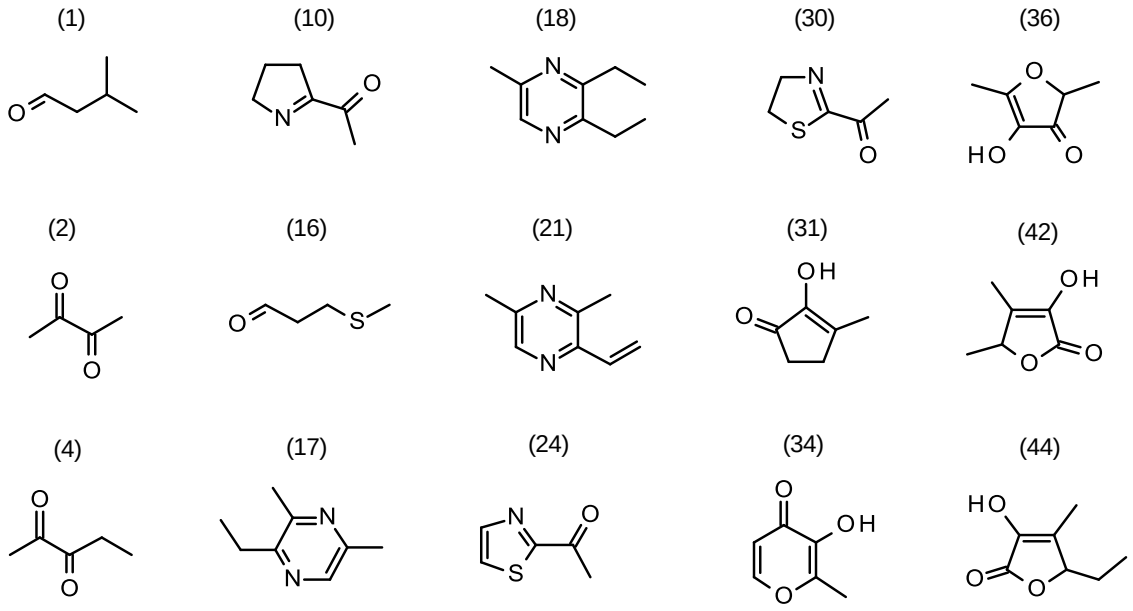


Figure 64. Continued.

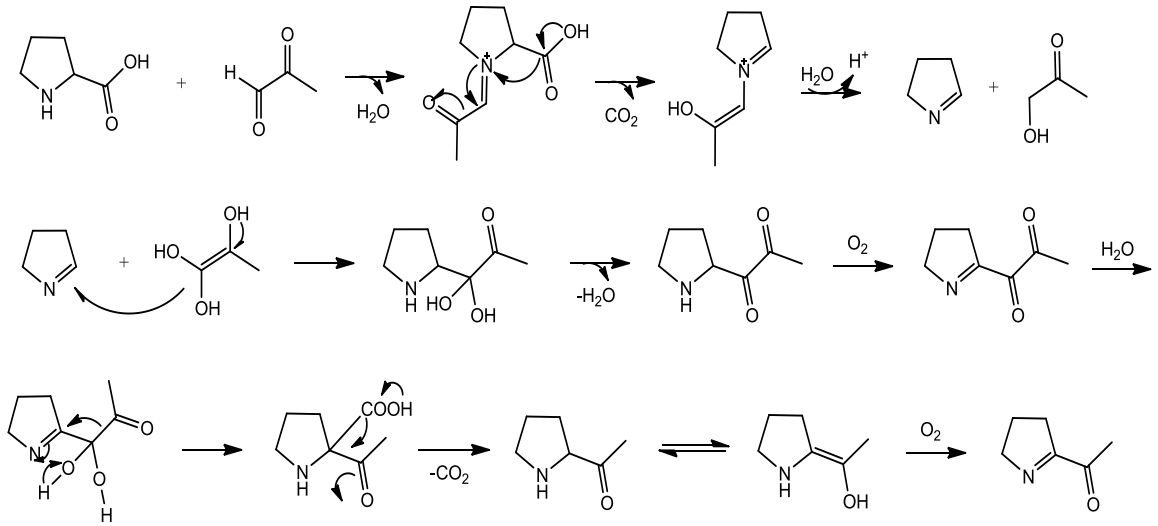


Figure 65. Potential Formation Pathway of 2-Acetyl-1-pyrroline[46].

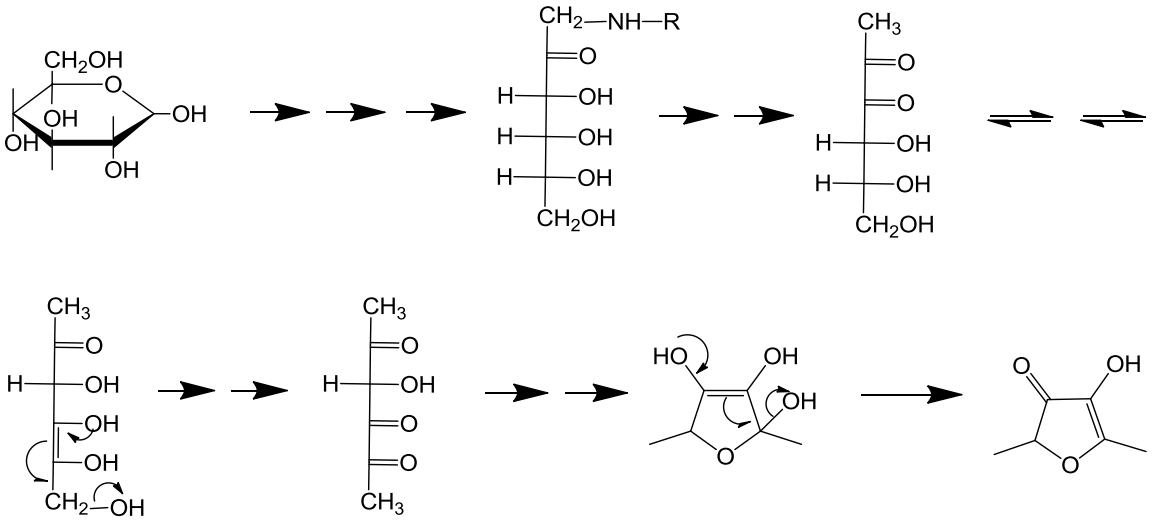


Figure 66. Potential Formation Pathway of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF)[48].

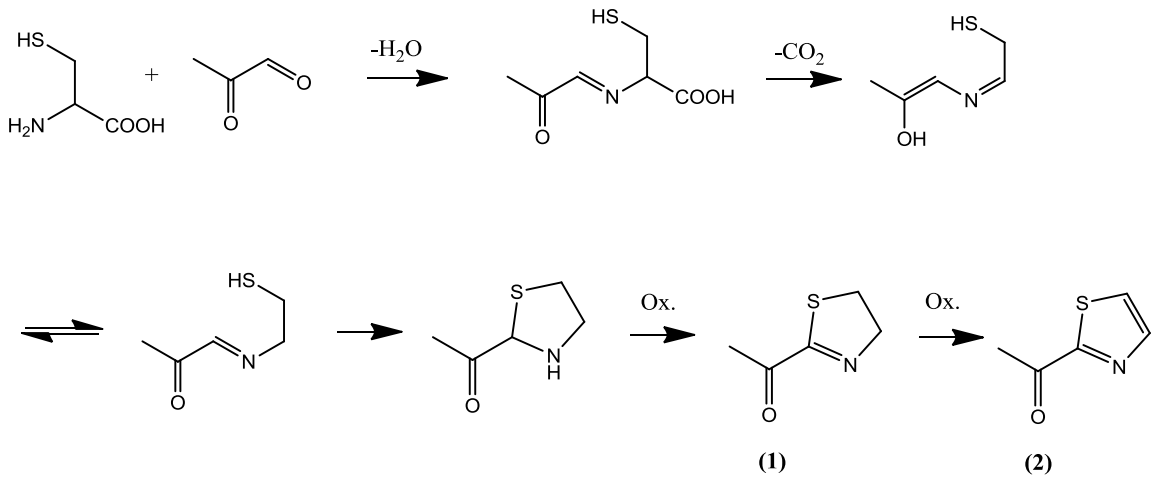


Figure 67. Potential Formation Pathway of 1-(4,5-dihydro-1,3-thiazol-2-yl)ethanone (2-Acetyl-2-thiazoline) (1) and 1-(1,3-thiazol-2-yl)ethanone (2-Acetylthiazole) (2)[50].

4.9 Sulfur-Containing Compounds in Roasted Lily Bulbs

Six aroma-active compounds containing sulfur shown were detected in the roasted lily bulb and they were as follows: cooked potato-like compound, 3-methyl(sulfanyl)propanal (methional) (**16**, FD 1024); roasty compound, 1-(1,3-thiazol-2-yl)ethanone (2-acetylthiazole) (**24**, FD 256); catty compound, 4-methyl-4-sulfanylpentan-2-one (**13**, FD 16); sulfurous compound, dimethyl trisulfide (**12**, FD 4); roasty compound, 1-(4,5-dihydro-1,3-thiazol-2-yl)ethanone (2-acetyl-2-thiazoline) (**30**, FD 4); and dimethyl tetrasulfide (**29**, FD 2) (Figure 68). Cooked garlic, onions and asparagus also contained of sulfur compounds that play major roles and were considered to be key odorants in their aroma profiles. Sulfur compounds with strongest impact on the aroma of the roasted lily bulbs were 3-methyl(sulfanyl)propanal (methional) and 1-(1,3-thiazol-2-yl)ethanone (2-acetylthiazole) with an FD $\geq$ 256.

No.	Odorant	Odor Quality	FD Factor
16	3-(methylsulfanyl)propanal (methional)	cooked potato	1024
24	1-(1,3-thiazol-2-yl)ethanone (2-acetylthiazole)	roasty	256
13	4-methyl-4-sulfanylpentan-2-one	catty	16
12	dimethyl trisulfide	sulfurous	4
30	1-(4,5-dihydro-1,3-thiazol-2-yl)ethanone (2-acetyl-2-thiazoline)	roasty	4
29	dimethyl tetrasulfide	sulfurous	2

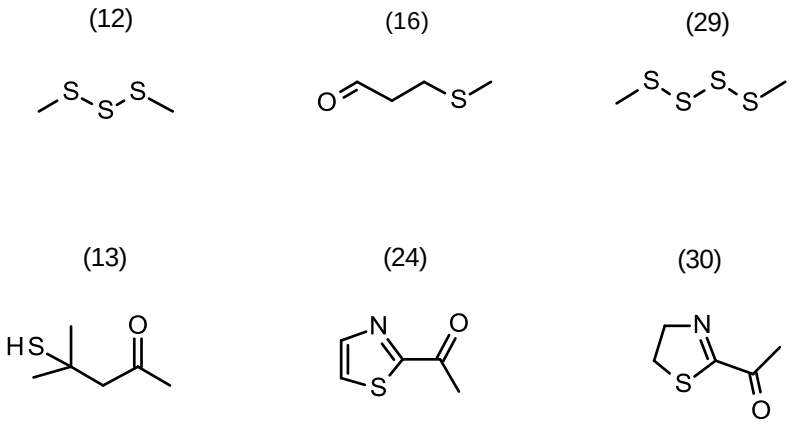


Figure 68. Sulfur-containing Compounds in Roasted Lily Bulbs.

5 CONCLUSION

SAFE isolation of volatile compounds from raw and roasted lily bulbs, combined with cAEDA, GC/O, and MS instrumental analyses of the volatiles, identified 48 aroma-active compounds, all of which to the best of our knowledge were reported for the first time. Determination of the flavor dilution (FD) factors identified the aroma-active compounds that were most significant in both the raw and roasted bulbs and sensory profile analysis connected these compounds to its specific aroma characteristics.

For the raw bulbs, the aroma-active compounds contributing to the overall aroma with an FD factor ≥256 were as follows: green compound, (E)-hex-3-enal (FD 1024); cooked potato-like compound, 3-(methylsulfanyl)propanal (methional) (FD 1024); eucalyptus-like compound, 1,8-cineole (eucalyptol) (FD 256) and floral, honey-like compound, 2-phenylacetaldehyde (FD 256).

Aroma-active compounds that made a huge impact on the flavor profile of the raw bulbs but decreased significantly after thermal treatment were as followed: green compound, (E)-hex-3-enal FD factor of 1024 decreased to an FD factor of 2, mushroom-like compound, oct-1-en-3-one and eucalyptus-like compound, 1,8-cineole (eucalyptol) with an FD factor of 256 and 64 respectively, both decreased to an FD factor of 4.

The formation of odor-active compounds from the flavor precursors of the roasted bulbs were as follows: roasty, popcorn-like-compound, 2-acetyl-1-pyrroline (FD 1024); earthy compound, 2-ethyl-3,5-dimethylpyrazine (FD 1024); earthy compound 2, 3-diethyl-5-methylpyrazine (FD 1024); maple-like-compound 5-ethyl-3-hydroxy-4-methyl-5H-furan-2-one (abhexon) (FD 1024); clove-like-compound, 2-methoxy-4-[(E)-prop-1-enyl]phenol ((E)-isoeugenol) (FD 1024); roasty-compound, 1-(1,3-thiazol-2-yl)ethanone

(2-acetylthiazole) (FD 256); caramel-like-compounds, 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene) (FD 256) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF) (FD 256).

Ultimately, the identification of the aroma-active compounds in the bulbs of *L. longiflorum* will help build a clearer and deeper perspective on the critical odorants contributing to the sensory attributes of both raw and roasted lily bulbs, specifically the roasted bulbs as commonly used in Chinese cooking.

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