THE UNIVERSITY OF MANITOBA

THE QUALITY AND STABILITY OF DILL WEED OIL DURING STORAGE

BY

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ABSTRACT

Dill weed oil is commercially produced in Manitoba for use in the pickling industry and accounts for one-sixth of the North American production. The large-scale production of this flavour oil requires some prolonged storage prior to sale. The quality of the oil is dependent on the dill maturity at harvest, the extraction procedure, the effect of weed contaminants and storage conditions. The objectives of this study were to examine the oxidative changes during storage and to investigate the effects of weed contaminants, headspace, storage temperature plus anti-oxidants on the overall oil stability. Gas chromatography and mass spectrometry were used to monitor changes in oil composition. These methods were complemented with sensory analysis.

Alpha-phellandrene, a hydrocarbon monoterpene proved most labile to oxidative degradation and shielded d-limonene from autoxidation until its levels decreased. The conversion of alpha-phellandrene and d-limonene to carvone exceeded 25% during storage without the use of anti-oxidants. With the phenolic anti-oxidant: BHA, the conversion ratio increased to 60% and oxidation rates dropped by eight-to-ten times compared to the control which was kept under the same experimental conditions. In comparison, alpha-tocopherol acetate was ineffective. The weed contamination studies showed that careful weed control would guarantee a low enough level of weed contamination such that

there would be no measureable impact on storage stability. Dill weed oil odour analysis emphasized the need to establish standards for the levels of alpha-phellandrene and d-limonene in addition to d-carvone. A compositional odour analysis indicated that increases in d-limonene could be more readily detected than increases in the other two terpene levels.

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1. INTRODUCTION

Dill weed oil production has been of considerable importance to the North American food industry since its commercial inception in 1932 in Ohio, Indiana and Michigan to meet the demands of the pickle and sauerkraut industries(Sievers, 1947). In 1970, the Mammoth Long Island strain of the Oregon variety of Anethum graveolens was for the first time successfully grown at the Morden Research Station in Manitoba(Chubey and Dorrell, 1976c). Initiation on a commercial basis began in 1974 with 20 hectares and by 1979 the commercial production of this spice exceeded 300 hectares(Stauffer and Chubey, 1979). This was equivalent to one-sixth of the North American requirements for dill weed oil in that year. The continued market success of the Manitoba oil is dependent on the maintenance of an acceptable quality product.

Since the present specifications for dill weed oil emphasize a particular range for carvone; one of the major components in the oil, dill weed must be harvested at the right stage to ensure good initial quality. During harvesting, other undesireable weed contaminants present in the dill fields can also be collected resulting in the contami-

nation of dill oils with non-dill distillates. These volatiles pose a potential threat to the quality of the dill weed oil. Because of the large-scale production of Manitoba dill weed oil, the storage of the oils prior to sale became necessary.

All three factors: maturity, weed contaminants and storage conditions were investigated. As well, the effects of the two anti-oxidants, alpha-tocopherol acetate and tert-butyl, hydroxy anisole on the quality of stored dill weed oil samples were also investigated. Since it was realized that the quality of the flavour oil was very much dependent on sensory characteristics, odour analysis involving a panel was conducted on dill oil samples that had been oxidized, contaminated with weed volatiles or compositionally altered.

2. REVIEW OF THE LITERATURE

2.1 Origin of Dill Worldwide

Dill weed belongs to the Umbelliferae family and consists of two major species: Anethum graveolens and Anethum sowa. Taxonomists regard Anethum graveolens as a distinctly different species from Anethum sowa due to both morphological and seed oil compositional differences.

Anethum graveolens has at least four known subspecies which are found in Europe, Asia, the Soviet Union, Africa and North America according to Baslas and Gupta (1971). Two types of oils are commercially obtained from Anethum graveolens: dill weed oil and dill seed oil. The entire herb, including the immature fruits, but excluding the roots is distilled to procure dill weed oil; whereas the mature fruits are distilled to produce dill seed oil. Dill weed oil is used for flavouring and seasoning purposes while dill seed oil is primarily used in the soap and perfumery industries as a replacement for caraway oil.

Sievers (1947) emphasized the considerable importance of dill weed oil to the North American food industry since

its commercial inception in 1932 in Ohio, Indiana and Michigan to meet the demands of the pickle and sauerkraut industries. The centre of production has gradually shifted westwards and currently Oregon is the most important area of production in North America.

The Mammoth Long Island strain of the Oregon variety of Anethum graveolens was successfully grown at the Morden Research Station in southern Manitoba in 1970(Chubey and Dorrell, 1976c) and the commercial harvesting of this spice exceeded 300 hectares in 1979, (Stauffer and Chubey, 1979). In this year about one-sixth of the North American requirements for dill weed oil was produced in Manitoba(Chubey, 1980).

Locally-produced dill weed oil is exported to the United States for use in the production of dill pickles. Over the years, industrial users have shifted their preference from using the whole plant to using just the flavour oils as a result of the lower storage space requirements and the absence of microbial or enzymatic material in the oils.

Compared to Anethum graveolens, the Anethum sowa varieties normally give a lower distillable oil value as these plants require an additional processing step to remove dillapiole, a toxic component with a density greater than that for water and described by Virmani and Datta(1970) and Shah et al.(1971). The extra processing step plus the typically lower d-carvone levels contribute to the reasons why sowa oil is not used as extensively as the graveolens oil. The

oil composition for the two varieties of dill are shown in Table I.

2.2 Composition and Properties of Dill Weed Oil

The composition of dill weed oil is presented in Table I while the structures of some of the terpenes are given in Figure 1. The major components: d-carvone, d-limonene and alpha-phellandrene constitute about 90% of the total oil mixture extracted from North American dill weed. The minor components normally comprise all but the final 1% of the volatiles. Apart from the terpenes listed in Figure 1, Embong et al. (1977) found that dihydrocarvone, p-cymene and beta-phellandrene can also occur as minor components in dill weed oil. Furthermore, these authors identified a minor component which had previously been reported as dill "ether" to be a benzofuran. This compound was confirmed by Chou and Iwamura (1979) to be a dimethyl tetrahydrocoumaran or more correctly: 3,9-epoxi-p-menth-1-ene, (Figure 1).

Dill oil also contains a host of trace components including trans-anethole, carvyl acetate, the cis and trans carveols, myristicin, apiole and dillapiole, (Figure 2). The last three compounds accumulate in very small amounts in the roots of the dill plant and were shown by Lichtenstein et al. (1974) to exhibit insecticidal properties against mosquitoes and houseflies. To a lesser degree carvone was

Table I. Dill Oil Composition

Component:	Anethum graveolens		Anethum sowa	
	Herb:[e]	Seed:	Herb:[d]	Seed:[b]
d-carvone	12.0-48.5% (c),(f)	39.0-63.0% (a),(c)	20.0-35.0% (a)	19.5-46.5%
d-limonene	26.0-40.8% (f)	NA	20.0-34.0%	9.0-30.0%
alpha-phellandrene	4.3-23.3%[f]	NA	NA	NA
dihydrocarvone	1.0-2.5%	NA	15.0-43.0%	7.2-9.0%
dill 'ether'	2.8-8.1%	NA	NA	NA
p-cymene	0.4-1.3%	NA	NA	NA
beta-phellandrene	1.4-7.2%	NA	NA	NA
alpha-pinene	0.2-1.2%	NA	NA	NA
beta-myrcene	0.5-1.2%	NA	NA	NA
dillapiole	trace	NA	12.0-13.0%	15.6-39.6% (c)

(a) Committee on Specifications(1972)

(b) Baslas and Gupta(1971)

(c) Virmani and Datta(1970)

(d) Shah et al.(1971)

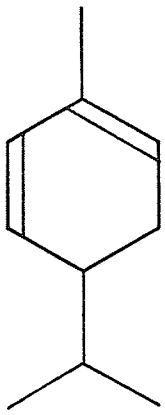
(e) Embong et al.(1977)

(f) Chubey and Dorrell(1976b)

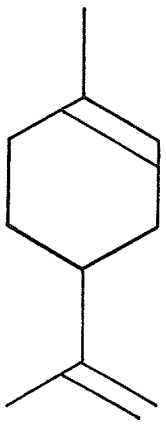
NA = not available

Figure 1. Major and Minor Components of Dill Weed Oil

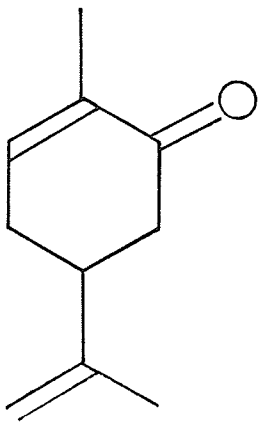
(A) Major:



alpha-phellandrene
(136 amu), 20-25%

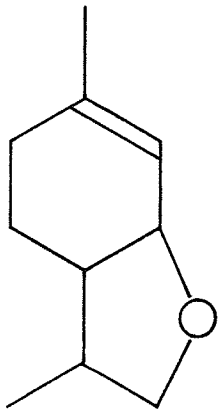


d-limonene
(136 amu), 30-35%



d-carvone
(150 amu), 30-40%

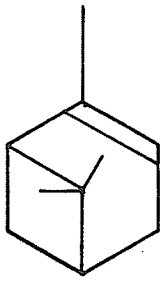
(B) Intermediate:



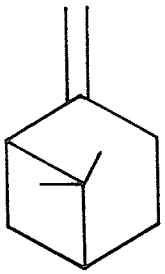
(152 amu), 4-7%

dill 'ether'
3,9-epoxi-p-menth-1-ene

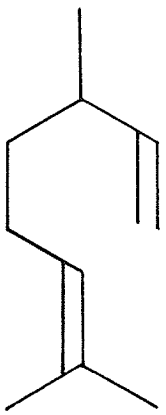
(C) Minor:



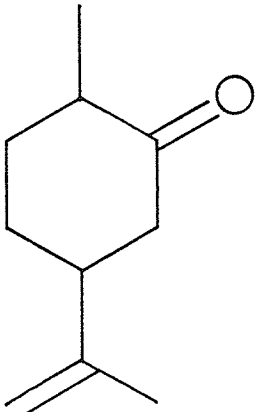
alpha-pinene
(136 amu),
0.5-1.0%



beta-pinene
(136 amu),
0.1-0.5%



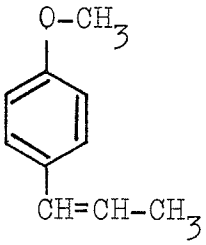
beta-myrcene
(136 amu), 0.5-1.0%



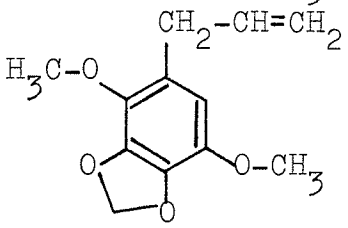
dihydro-
carvone
(152 amu),
0.5-1.5%

Figure 2. Trace Components Found in Dill Weed Oil

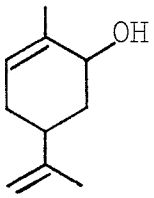
trans-Anethole: 148 amu



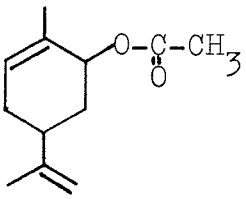
Apiole: (Toxic) 222 amu



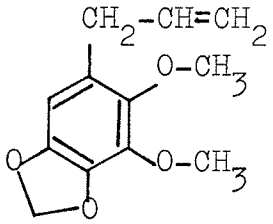
l-Carveol: 152 amu



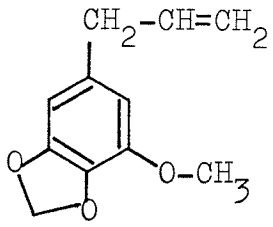
Carvyl Acetate: 194 amu



Dillapiole: (Toxic) 222 amu



Myristicin: 192 amu



also shown to have some effectiveness against these insects.

The lower molecular weight terpenes, particularly the monoterpenes are thought to play a number of minor functions in plants and their biological significance was discussed by Banthorpe et al. (1972) and Goodwin (1971). Specific monoterpenes act as growth, heat and transpiration regulators, participants in photosynthesis, tumor and oxidative phosphorylation inhibitors and stimulators of carotenogenesis. Other selected roles include attractants for pollinating insects and repellants for predatory birds, insects and herbivores(Goodwin, 1967).

At the microbial level, the terpenes in dill oil have shown some fungiostatic characteristics(Rao and Rao, 1972) and Europeans have recognized the medicinal qualities of dill weed since Biblical times, especially in the treatment of stomach ailments and infantile disorders, (Baslas et al., 1971).

2.3 Monoterpene Biosynthetic Pathway

The terpenes are thought to be derived from the Mevalonic Acid Pathway (MVA) according to Banthorpe et al. (1972) and Crouteau and Loomis (1975). The MVA pathway (Figures 3 and Figure 4) has definitely been established as the route for certain flower and iridoid monoterpenes(Francis, 1971) and it is generally believed that the pyrophosphates of

Figure 3. Terpene Mevalonic Acid Biosynthetic Pathway

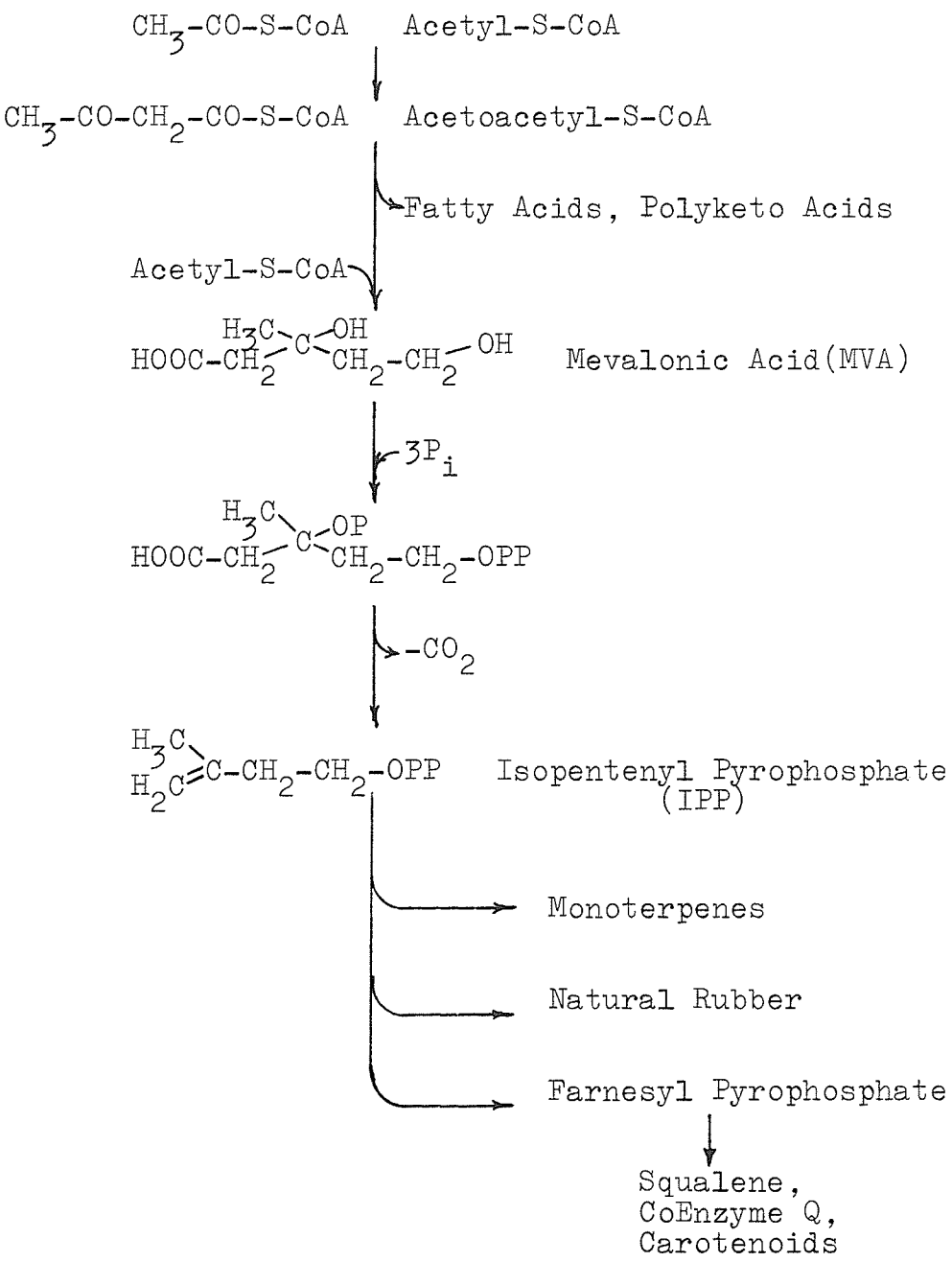
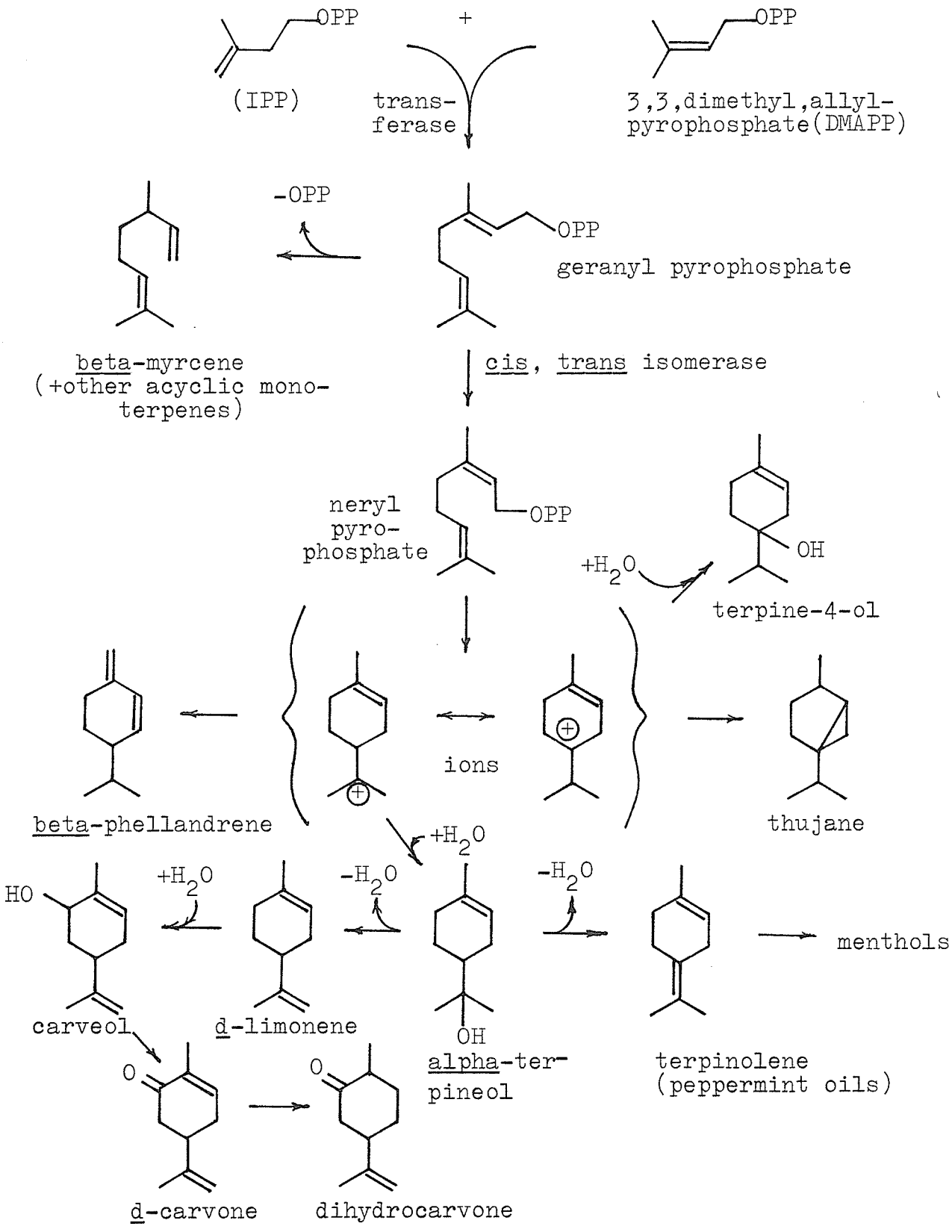


Figure 4. Monoterpene Biosynthetic Pathway



neryl, geranyl and linalyl compounds are the true precursors of the monoterpenes.

The formation of the terpene class of compounds follows the addition of isoprene units(C_5H_8) in a normally head-to-tail manner to form the larger chains. The $C_{10}H_{16}$ chains form the monoterpene group, $C_{15}H_{24}$ the sesquiterpenes, $C_{20}H_{32}$ the diterpenes, $C_{30}H_{48}$ the triterpenes and so on.

The sesquiterpenes and the higher terpenoids have not been reported in dill weed oil(Embong et al., 1977 and Schreier et al., 1981), and this limits further discussion to the monoterpenes. Of the monoterpenes that do occur in dill, Maarse(1974) has shed some light on the probable biosynthetic routes, especially for d-limonene and beta-myrcene which require a radical ion intermediate. The two proposed radical ions are illustrated in Figure 4 and d-carvone which is derived from d-limonene probably arises from the radical with the positive centre in the tail. The formation of alpha-phellandrene would probably arise from the second ionic radical as both double bonds for this terpene are located within the ring.

Once synthesized, the essential oils in plants are known to accumulate in specialized tissues known as oil glands or resin ducts(Banthorpe et al.,1972) and degenerate chloroplasts or plastids in neighbouring secretory cells are thought to be the actual sites of biosynthesis. Zlatev(1976a) reported that dill weed essential oils accumulate in the endogenous receptacles of the dill plant.

The oil content throughout herb or spice plants is unevenly distributed, and it has been suggested(Banthorpe et al., 1972) that the leaves have specialized glands that fill with oil at an early growth stage after which the necessary precursor molecules are made inaccessible to the leaves. In contrast, the flowering heads actively synthesize monoterpenes until full maturity. The overall composition of the oil is thought to be determined by the scion rather than the rhizome as the roots usually contain very little stored oil.

Banthorpe et al.(1972) explained that many of the terpenoid enzymes are not very substrate specific as liver dehydrogenase will oxidize geraniol and a host of other terpene alcohols.

Dill weed, among many other spice plants does not produce a wide variety of terpenes and Hefendehl and Murray(1976) proposed that the proliferation of only a few such compounds was indicative that different plants underwent duplication or deletion types of mutations which affected the final ratios of the essential oil products. They also postulated that the metabolism of terpenes was an isolated process from the rest of the plant metabolism as alterations in essential oil synthesis have not appeared to seriously affect overall plant vigour. Zavarin(1970) also noted that the proportional relationships between specific terpene products in plants could be used to determine the probable biosynthetic pathways.

2.4 Quality and Stability of Dill Weed Oil

2.4.1 Specifications of Dill Weed Oil and Quality

The Committee on Specifications(Food Chemicals Codex, 1972) has established that dill weed oil should not have less than 28% (V/V) nor more than 45% carvone for the oil to be acceptable in the spice trade. The angular rotation of the oil should lie between 84^{o} and 95^{o} and the refractive index between 1.480 and 1.485 at $20^{o}C$. The oil should be soluble in alcohol and have a specific gravity between Ø.884 and Ø.900Ø. Since d-carvone has a specific gravity of Ø.9645 while that for d-limonene is Ø.8411 and for alpha-phellandrene is Ø.8463(Weast and Astle, (eds.), 1980-81), the density specifications for dill weed oil cover the d-carvone concentration range from 28% to 45% but no standards are given for either d-limonene or alpha-phellandrene. The only other specification which the oil must meet is that heavy metals must not exceed 3 ppm for arsenic, 1Ø ppm for lead or 4Ø ppm for any combination.

Therefore, a typical dill weed oil sample is graded and priced on the basis of the d-carvone content. For both East Indian and North American dill weed oil, lower levels are

associated with lower prices, and the American pickle industry requires at least a 30% d-carvone level(Chubey and Dorrell, 1976b).

However, Guenther(1950) indicated that d-carvone levels below 20% gave a better approximation of the true aroma of dill herb. Georgiev et al.(1978) also concluded that the characteristic flavour of dill weed is retained only if the d-carvone levels do not exceed 37%. Heath(1978) referred to the milder and more pleasingly fresh qualities of dill weed oil which contained only 20% d-carvone, and the herby odour and flavour were attributed to alpha-phellandrene. Embong et al.(1977) noted that dill weed oils contained higher alpha-phellandrene levels and lower d-carvone levels than the seed oils and these differences were preferred by the spice industry. Furia(1980) implicated both alpha-phellandrene and d-carvone as the compounds responsible for the bitter and herb-like qualities of dill. Masada(1976) and Virmani and Datta(1970) also associated the typical odour and flavour of dill weed oil to the alpha-phellandrene content and they concluded that the oil approached the "seed" character at d-carvone levels above 35%. Finally, Schreier et al.(1981) associated the dill 'ether' component to the natural, herbal flavour of dill weed, but these authors worked with very immature dill samples and the dill 'ether' component was quite high. Given the divergence between the established standards and published, scientific opinion, a great deal needs to be studied to properly elucidate the

quality parameters of dill weed oil.

The stability of dill weed oil is dependent on four factors. The first two include the maturity of the plants at harvest and the oil extraction procedure. The last two represent the post-distillation factors and include the presence of weed contaminants and storage effects.

2.4.2 Effect of Different Stages of Maturity on Yield and Composition of Dill Weed Oil

Both the composition and the yield of dill weed oil are affected by growing conditions and the stage of maturity of the harvested plants. Guenther(1950) realized that alterations in the climate and habitat, as well as seasonal and diurnal variations all play a role in the development of the plant including essential oil accumulation.

In terms of composition, Betts(1965) observed a gradual decrease in d-limonene levels while the d-carvone levels increased in the developing fruits of Anethum graveolens. Zlatev(1975) showed that alpha-phellandrene levels dropped while d-carvone levels increased during the ripening process for Bulgarian dill weed. He found that the milk ripeness stage resulted in the highest oil yields for summer-sown dill weed but that the milk-wax ripeness stage had slightly higher yields for spring-sown fields. Zlatev(1976a,b) reported maximum yield values between 0.82-0.84% on a wet

plant basis. He also noted the greater abundance of the oils in the flowers, seeds and leaves compared to the stalks, ferns and roots, and that during maturity the stalks and leafy material dried out. Gradually this loss in plant weight was accompanied by a loss in oil levels as the mature seeds dropped from the flowering heads.

An investigation of the dynamics of essential oil accumulation in dill weed harvested over a 24 hour period(Zlatev, 1976a) revealed that alpha-phellandrene levels were the highest between 3 and 6 AM but decreased from 26.5% to 19.9% during the day. Limonene and d-carvone were synthesized in the daylight and decreased to 85-90% of the maximum levels in the evenings. Zlatev concluded that d-carvone was synthesized from d-limonene during the day as a result of the isomerization of alpha-phellandrene to d-limonene. This conclusion was inconsistent with information presented by Banthorpe et al.(1972). They explained that the level of monoterpenes in a herb or spice plant was controlled by two factors: photosynthesis in the daylight and the utilization of the photosynthate in the dark. Photosynthesis would provide respiratory substrate for the oil glands which could maintain the respiratory enzymes in a reduced state in the dark. The photosynthate would then be used via respiration to promote growth and this "evening effect" would be heightened during periods of high evening temperatures. This would result in the quick oxidation of hydrocarbon terpenes. Accordingly, d-carvone should build

up in the dark, yet Zlatev's data did not support this theory. Zlatev also indicated that field conditions for his study were quite variable and the observed compositional differences would not provide sufficient justification for harvesting the dill at a particular hour during the day.

Manitoba dill weed oil yields were investigated by Chubey and Dorrell(1976b) who concluded that the best oil composition and yields(70-80 kg/ha) could be obtained when the plants were harvested while the umbels were an amber colour and the seeds were soft and immature. Embong et al.(1977) reported lower yields for dill weed grown in Alberta and their study showed that the d-carvone levels increased from 26% to 38% at the expense of alpha-phellandrene as the plants matured.

Zlatev(1976b) conducted a three year study to investigate the meteorological influences on dill oil production. Wind speeds and daily temperatures proved to be quite variable for each season as were the average seasonal values for relative humidity, cloudiness and precipitation. Variation coefficients for the accumulation of oil were not significant given the considerable daily differences in the meteorological factors and Zlatev concluded that changes in oil composition and quantity were due more to the biological properties of the plant than to the meteorological conditions. However, given the shorter growing season in Manitoba, extremes in daily weather conditions at harvest could have a more pronounced effect on dill oil composition and

accumulation.

Photoperiodism also plays an important role in Northern climatic zones and Meyer et al.(1973) reported that the critical photoperiod for dill weed, a "long-day" plant was between 11 and 14 hours before the plant would flower. Dill weed also requires a photoinductive cycle of two-to-four days at the minimum photoperiod before the initiation of flowering. Fortunately, long, sunny days are typical during the growing season in southern Manitoba.

The value of dill weed to the local Manitoba growers is quite significant at the present time. With an average yield between 70-80 kg/ha, and a 1980 price of $19.65-27.50/kg, dill oil would give an expected return of $1375-2200/ha dependent on the d-carvone content. In comparison, the 1978 farm price value of other crops ranged from $135/ha for oats to $1637/ha for potatoes, (Manitoba Agriculture, 1978). Wheat, which represents the major crop grown in Manitoba had a 1978 average farm return of $283/ha, so that although there are risks involved in growing dill, the returns are of considerable value in comparison to other crops.

2.4.3 Commercial Extraction of Essential Oils

Steam distillation is the most common method for extracting essential oils from plant materials throughout the world, (Sterrett, 1962; Burchfield and Storrs, 1962;

Ames and Mathews, 1968 and Fennema, 1976). Oil changes can arise during the steam distillation process and chelating agents are occasionally used to remove trace metals and thus prevent post-distillation oxidation. It is therefore necessary to both understand and have control over the conditions of the distillation.

The energy of the steam can hydrolyze or polymerize labile terpenes. The decomposition of caryophyllene epoxide from clove oil has been reported(Fennema, 1976) and Furia(1980) indicated the loss of water soluble volatiles such as phenyl ethyl alcohol. Terpene alcohols such as linalool as well as various ester derivatives will undergo rearrangement and elimination reactions during steam distillation(Pickett et al., 1975). The water medium is normally in the acidic pH range and Francis(1971) reported a value as low as pH 2.8 for distillation mixtures.

In spite of certain inherent problems, steam distillation is regarded as a more gentle extraction process compared to water distillation. In physico-chemical terms, two volatile liquids which are mutually insoluble will boil together at a lower temperature than the boiling point for each compound(Johnson and Peterson, 1974). Since steam distillation reduces the presence of condensed water compared to water distillation, the oils are extracted at a faster rate and remain in contact with water and other polar catalysts for a much shorter period of time. This contact period is crucial since it will determine the extent of

isomerization or decomposition for the most labile terpenes.

Ames and Mathews(1968) indicated that water distillation can also result in uneven heating which may impart a burnt odour to the distillate. Also, water distillations require longer extraction times compared to steam distillations. Ames and Mathews concluded that steam stills produced the highest quality oil with a minimum in chemical alterations. They cautioned against using a very dry steam as the plant tissues would dehydrate very quickly, impeding the release of the oil fraction. They concluded that stainless steel, though expensive, provided the greatest resistance to acidic materials, especially when compared to galvanized steel.

Provatoroff(1972), also recommended the use of stainless steel for industrial distillation equipment. He emphasized that copper, aluminum and wood were undesirable materials from an oxidation and/or absorption standpoint.

Practical considerations were responsible for the decision to use steam distillation in the commercial plant near Morden, Manitoba. Since the rate and intensity of the distillation process are critical factors affecting the quality of the distillate, Chubey and Dorrell(1976a) investigated the changes in the composition of the dill oil during the steam distillation period. They discovered that when using either fresh or wilted dill weed, d-carvone distilled across very rapidly during the first hour, then dropped off while alpha-phellandrene distilled at a fairly constant rate for

the two hour distillation. The d-limonene levels gradually increased over this same period. Compared to the fresh dill, the wilted dill weed sample showed a slight increase in d-carvone content at the expense of d-limonene as would be expected from a biosynthetic viewpoint. These authors used a commercial still capable of holding up to 4.5 tonnes of material and a pilot-scale still with a capacity of 45 kg. Most of the d-carvone distilled across sooner for the pilot-scale still rather than for the commercial equipment. A follow-up laboratory distillation, lasting three and one-half hours and using wilted dill weed showed that almost 95% of the volatile oils could be extracted in just two and one-half hours. At present, the commercial production of Manitoba dill weed takes two and one-half hours at 6.8 atmospheres steam pressure per wagon load. Distillation beyond this time would not be justified in terms of energy input.

The practice of wilting dill weed prior to distillation was common at one time since it permitted some moisture loss to occur. Also, loss in tissue turgor allowed for a more efficient packing of the dill weed into the original distillation tanks. Currently, specially-designed dill wagons are used to transport the dill weed to the distillation plant and then act as the distillation vessel. This, coupled to the cooler temperatures and wetter climate in September have gradually reduced the time available to the farmers, and wilting has not remained a very common practice.

Historically, other extraction methods have been developed to obtain essential oils. Cold-pressing techniques have proven useful for citrus peels, cold-fat enfleurage for jasmine and rose blossoms and solvent extraction using dichloroethane and chloroform for various perfumery substances. Alcohol extraction has been used to concentrate oxygenated terpenes and can be used in combination with the vacuum distillation of apolar terpene hydrocarbons. The more labile, unsaturated hydrocarbons could then be treated separately from the more stable, oxygenated compounds and the two fractions could then be reformulated when needed.

The major advantage to vacuum distillation is that the flavour oils distil at a lower temperature than under standard conditions. The major drawback to the technique lies with the need for higher precision and more sophisticated technology than with a method like steam distillation. Ames and Mathews(1968) pointed out that steam distillation is much easier to carry out under field conditions since most herb and spice crops need to be distilled immediately after the harvest.

2.4.4 Storage Effects

During storage, gradual changes can occur in the dill weed oil which can be detrimental to the quality of the oil. The stability of Bulgarian dill oil during storage was studied by Georgiev et al. (1978) over a two year period.

They investigated the factors of light, temperature and headspace and chemical changes were most evident for oils kept in the light. Temperature differences appeared to be slight. Air increased the d-carvone content, the acidity of the samples, the resinification of the oils and deepened the yellow colouration during the study period. Light increased the amount of waxy residues, decreased the alcohol solubility and was responsible for a turpentine note in the oil odour. Filled vessels kept at refrigeration temperatures gave the least amount of change to the original samples. In summary, the factors of light and air resulted in the greatest changes to the stored samples. Autoxidation therefore played a significant role in decreasing the stability of the oils.

2.4.4.1 Oxidation by Singlet Oxygen

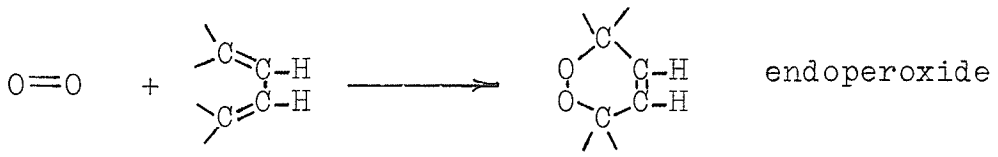
An understanding of the mechanisms involved in the autoxidation of dill oil monoterpenes would prove invaluable in deciding upon the best method for storing the oils. Wasserman and Murray(1979) described several mechanisms by which oxygen interacts with unsaturated hydrocarbons. Ground state oxygen exists as a triplet state, paramagnetic species which can be excited with ultra-violet (UV) or visible radiation to one of two possible singlet state entities; designated $^1\Sigma_g$ and $^1\Delta_g$, where $^1\Delta_g$ lies closest to the ground state molecule.

As the $^1\Delta_g$ singlet oxygen molecule exists for a longer period of time than $^1\Sigma_g$; in either the gaseous or liquid phase, this species is thought to be responsible for most natural oxidations of unsaturated hydrocarbons. The rate of autoxidation can be effectively controlled by using a quenching agent such as water, alcohols, ammonia or amine compounds. The hydrogen atoms in these molecules contain the appropriate vibrational frequencies to deactivate the singlet oxygen atom to the ground state. This occurs either through direct energy transfer or through charge transfer involving an electron-"rich" quencher donor such as a phenol.

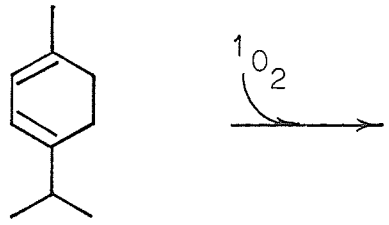
Three possible mechanisms have been proposed for the autoxidation of unsaturated hydrocarbons(Wasserman and Murray, 1979) and they are illustrated in Figure 5 and Figure 6. The Diels-Alder Reaction involves the addition of singlet oxygen to a molecule containing conjugated double bonds to form an endoperoxide which may undergo further chemical decomposition at room temperature. Terpinolene, a monoterpene, has been shown to oxidize to ascaridole following the 1,4 cyclo-addition mechanism. The "Ene" Reaction incorporates the addition of singlet oxygen to an unsaturated double bond with an available, allylic hydrogen atom. A peroxirane intermediate is formed, followed by the abstraction of the allylic hydrogen atom to give a hydroperoxide after the shift of the double bond over one carbon position. This appears to be the most common type of singlet oxygen autoxi-

Figure 5. Proposed Diels-Alder and 'Ene' Singlet Oxygen Reactions

Diels-Alder Reaction:



e.g.

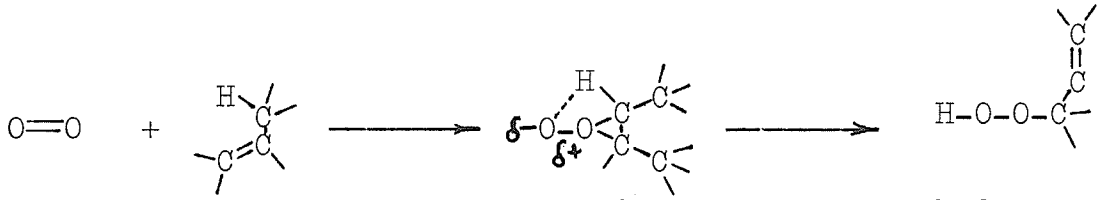


(1,4 cycloaddition of oxygen to conjugated dienes)

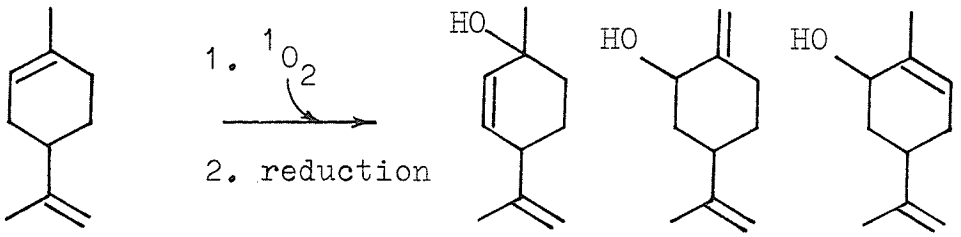
alpha-terpinene (136 amu)

ascaridole (168 amu)

'Ene' Reaction:



e.g.



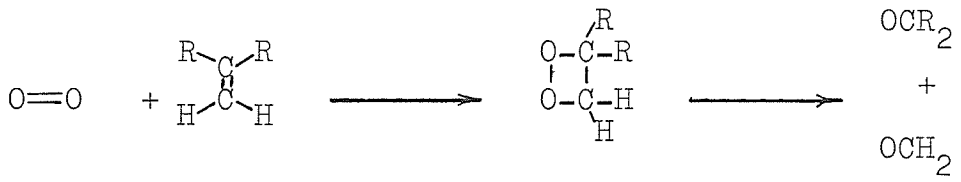
d-limonene (136 amu)

(42%) (46%) (12%)

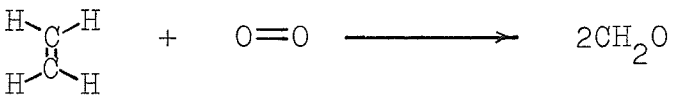
Described by Wasserman and Murray.(eds.) 1979.

Figure 6. Proposed Dioxetane Singlet Oxygen Reaction

Dioxetane Reaction: for olefins lacking an allylic hydrogen, 1,2 addition.



For ethylene:



Described by Wasserman and Murray.(eds.) 1979.

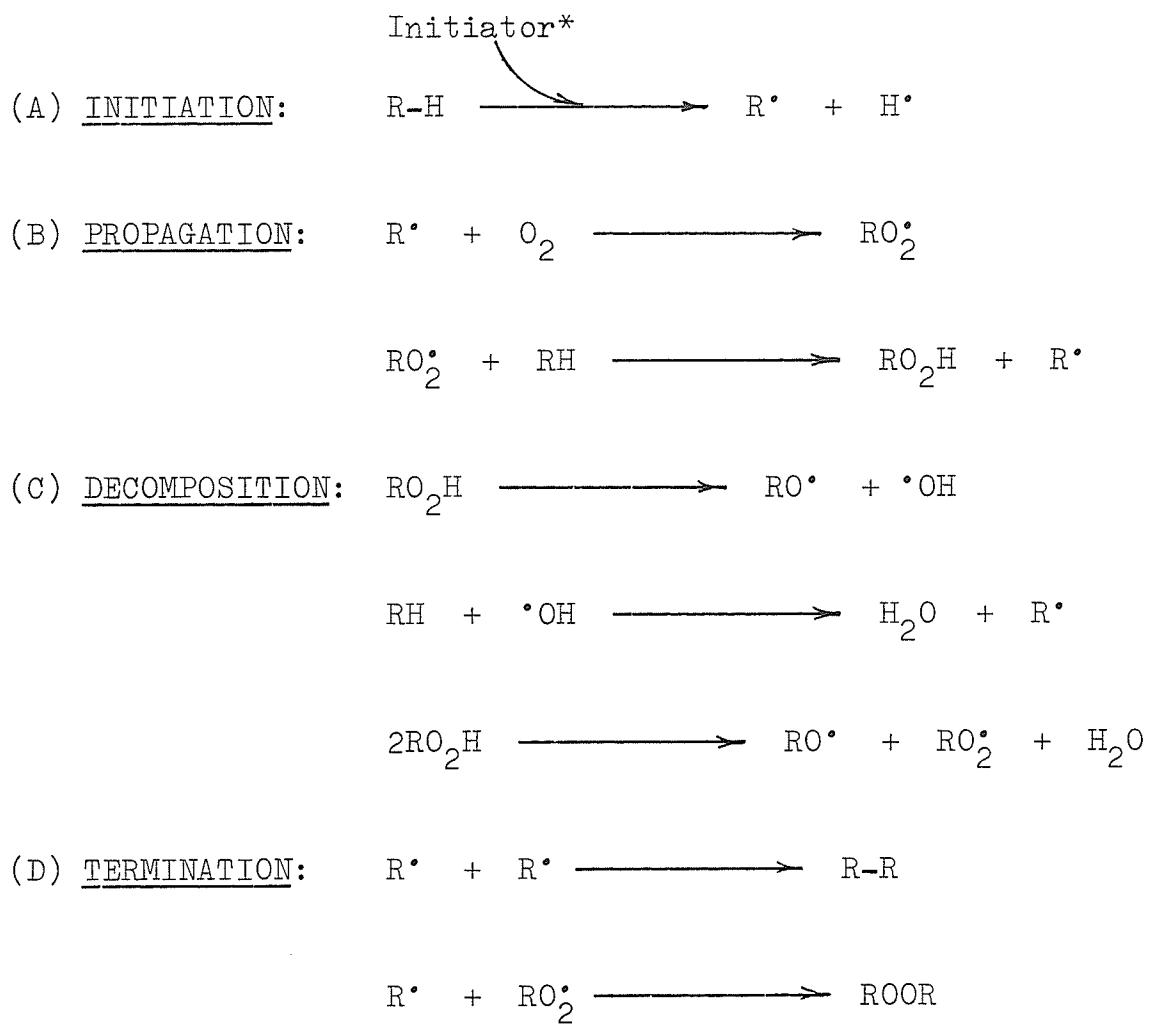
dation and it has been illustrated with many monoterpenes including d-limonene. The Dioxetane Reaction features the direct addition of singlet oxygen to an unsaturated double bond that does not have an available allylic hydrogen atom. The unstable dioxetane intermediate then decomposes to form two aldehyde products. This type of reaction appears to be uncommon with respect to monoterpenes while the Diels-Alder and "Ene" Reactions have been observed for numerous terpenes.

The important factor with respect to singlet oxygen reactions is that they only require the availability of light or heat and oxygen, without any additional catalysts. Unsaturated dill weed terpenes would therefore be quite susceptible to autoxidation dependent on the storage conditions used.

2.4.4.2 Anti-oxidant Theory in Preventing Autoxidation

Ingold(1961) summarized the theory for the anti-oxidant role of certain molecules with respect to unsaturated hydrocarbons. According to Sherwin(1978) and Ingold, fat or oil oxidation is initiated when a hydrogen atom departs from the alpha-carbon atom adjacent to a double bond on the molecule. This leaves a free radical which can undergo molecular oxygen attack to form an unstable peroxide free radical that acts as a propagator in further fat or oil oxidation(Figure

Figure 7. Proposed Mechanism of Fatty Acid Oxidation**



The termination step also results in a host of other compounds including aldehydes, ketones and alcohols.

* UV light, metal ions.

** Described by Ingold(1961).

7). Wasserman and Murray(1979) have indicated that autoxidation can also occur through molecular oxygen attack alone, without the radical formation. The end result of either reaction is the accumulation of aldehyde, ketone, alcohol and acid compounds which lower the sensory qualities of the flavour oils.

According to Ingold(1961), the role of synthetic, phenolic anti-oxidants such as tert-butyl hydroxy anisole(BHA) is to interfere in the free radical step by supplying a hydrogen atom. Such phenolics act as primary anti-oxidants and are not oxygen scavengers. Following the deactivation of the hydrocarbon free radical, the anti-oxidant is stabilized through resonance, (Figure 8). Ingold considered phenolics to be generally less effective in inhibiting peroxide radicals, and he concluded that a good anti-oxidant combination would consist of one agent that would inhibit free radical formation,(a phenolic) while a second component would decompose the peroxides already formed(a natural anti-oxidant).

Factors affecting phenolic anti-oxidant potency include solubility, storage conditions and the type of substituted group on the phenolic ring. Bulky alkyl groups have a positive inductive effect that prevents ion formation of the phenolic hydrogen atom, whereas groups with strong electron affinities, such as the halogens inhibit free radical formation and promote the acidity of the hydrogen atom. However, large side groups can lead to steric hindrance, affecting

Figure 8. Proposed Anti-Oxidant Mechanism*

$$R^{\bullet} + AH \longrightarrow RH + A^{\bullet}$$

$$RO^{\bullet} + AH \longrightarrow ROH + A^{\bullet}$$

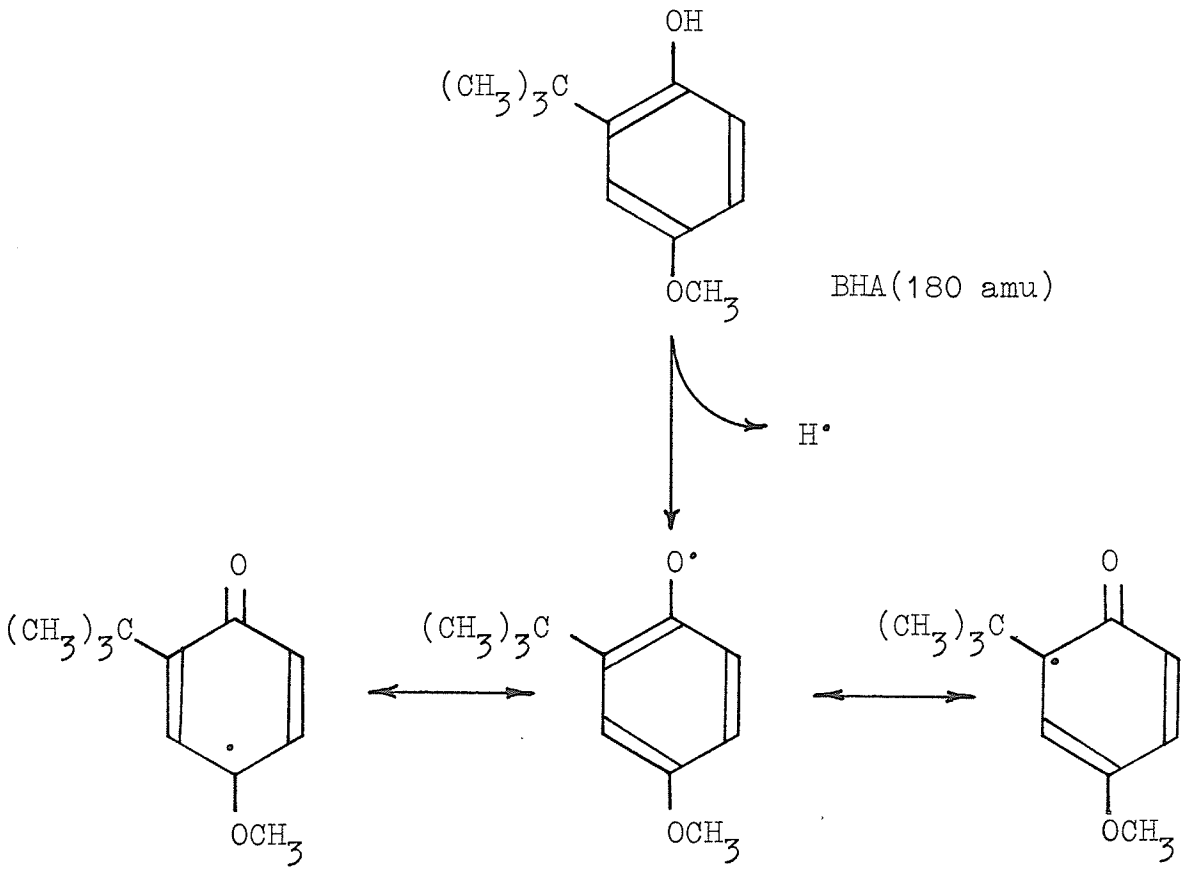
$$ROO^{\bullet} + AH \longrightarrow ROOH + A^{\bullet}$$

$$R^{\bullet} + A^{\bullet} \longrightarrow RA$$

$$RO^{\bullet} + A^{\bullet} \longrightarrow ROA$$

R = fatty acid; A = anti-oxidant molecule.

Resonance Stabilization of BHA:



*Described by Ingold(1961).

the electron-releasing mechanism for these types of anti-oxidants. High temperatures can also destabilize the phenolic radical and lower its usefulness in inhibiting autoxidation.

Natural anti-oxidants such as ascorbic acid and citric acid are usually ineffective inhibitors of oxidation for edible oils, but show pronounced synergistic effects when mixed with certain phenolics(Ingold, 1961). The tocopherols act as inhibitors of fat oxidation at low concentrations, but become pro-oxidants at higher levels. The exact role of phenolic or natural anti-oxidants in controlling autoxidation in monoterpenes has not been reviewed in the literature, and so their effectiveness can not be properly appraised at this time. One could expect that those natural anti-oxidants which act as oxygen scavengers might prove more effective than the phenolic inhibitors; although a combination of the two may be the most effective overall.

2.4.5 Undesirable Weed Contaminants

Weed contaminants pose a problem that is specific to the North American cultivation of spice crops such as dill weed. The large-scale farming operations can not rely on the labour-intensive farming techniques of the Third World countries where spice crops are also grown. In particular, the effect of weed contaminants on the quality of dill oil has not previously been investigated. Weed volatiles in

sufficient quantities would alter the sensory profiles of the commercial product, and these contaminants could also act as oxidative accelerants during the storage of the oils.

Herbicides have been used in the Morden area to control broadleaf weeds, but different weed varieties have been observed in and especially around the perimeters of dill fields. In recent years, these have included: green foxtail(Setaria viridis), Canada thistle(Cirsium arvense), kochia(Kochia scoparia), lamb's quarters(Chenopodium album), wormwood(Artemesia absinthium), redroot pigweed(Amaranthus retroflexus), wild barley(Hordeum jubateum) and common milkweed(Asclepias syriaca). The numbers of each variety of weed can vary within a field as well as within any given growing season, and a few species can attain dominance over the rest.

Previously, Chow and Lapka(1975) have revealed problems with weeds such as green foxtail in cereal grains during a dockage study undertaken in southern Manitoba. The problem of weed contaminants in grain seeds can oftentimes be eliminated through sifting whereas the removal of weed volatiles in dill oil can involve considerably more expense and expertise.

2.5 Analysis of Dill Weed Oil

2.5.1 Laboratory-Scale Distillations

Small-scale distillation apparatus used for obtaining essential oils from plant materials have been described by the American Spice Trade Association(1960), Naipawer et al.(1971) and Singh et al.(1971). Naipawer and his associates used a continuous vacuum steam distillation procedure to extract flavour volatiles from tomatoes. Vacuum stripping methods for citrus fruit aromas were previously described by Bomben et al.(1966,1969), but such techniques require considerable amounts of equipment and expertise. Singh et al.(1971) and the American Spice Trade Association(1960) recommended a much simpler apparatus requiring as little as 5g of sample. The oil distillate is collected in a graduated distillation receiver. This water distillation procedure requires that a pre-weighed sample is mixed with several volumes of water and heated in a round-bottom flask. This method gives a good indication of the essential oil content, but the quality of the oils can suffer from the direct exposure to the water and other polar catalysts.

The Romer and Renner(1974) method utilizes steam generated in a seperate distillation flask which passes through a condensate trap. Only the steam is permitted to come into

contact with the plant sample and the essential oils are distilled then condensed and trapped in an organic solvent. This method is suitable for the qualitative preparation of essential oils which can then be analyzed immediately by GLC. This method is not useful for yield determinations.

2.5.2 Gas Liquid Chromatography

Before the advent of gas liquid chromatography (GLC) flavour chemists relied exclusively on chemical analysis such as the Tilden procedure for preparing terpene crystals using nitrosochloride, or the identification of unsaturated bonds using either bromine or potassium permanganate(Simonsen, 1931). Chemical analysis was time-consuming and not always successful in the qualitative and/or quantitative analysis of terpene mixtures.

In comparison, GLC has proven to be a fast and reliable method for the analysis of terpene mixtures. Several of the advantages of using GLC include: short residence times and high separation efficiencies compared to the conditions encountered with fractional distillation techniques(Zubyk and Conner, 196Ø) as well as the requirement for very small sample sizes and the use of an inert carrier gas which does not affect the labile terpenes(Burchfield and Storrs, 1962).

The two major obstacles in terpene analysis; the very large number of possible terpene isomers and the acid-sensi-

tivity of many of the volatiles can largely be overcome through the proper selection of stationary phases, solid supports and operating temperatures(von Rudloff, 1974). Day and Miller(1962) observed the decomposition of alpha-terpineol and linalool when injection temperatures over 200°C were used and Mitzer(1964) suggested that the build-up of acidic materials in the injector could also decrease the stabilities of terpene alcohols.

Once the terpene sample has been separated in the column, the retention data can then be handled in several ways to properly identify each peak. Haken(1976) discussed three such methods but dismissed the use of specific retention volumes (Vg) as being very difficult to obtain. The relative retention index (RRI) method remains the most common procedure for reporting monoterpenes. The retention volume of a solute at its peak maximum is compared with an arbitrary standard to give an RRI value. In this method, the air peak volume is subtracted from the retention volume values to account for the deadspace of the column. A third method involving Kovat's Index compares the behavior of different volatiles to two, normal paraffins; one with the same number of carbon atoms as the sample solutes and the other with one additional carbon atom. The n-paraffins are given arbitrary values of 100 times their carbon number and the components are separated on the basis of slight polar differences. Kovats(1965) claimed that his system was not affected by temperature fluctuations and was therefore

superior to the RRI method.

With respect to column materials, Zubyk and Conner(1960) recommended that copper columns not be used due to the easy isomerization of labile terpenes such as the pinenes. Dickes and Nicholas(1976) indicated that aluminum columns led to similar problems. Von Rudloff(1974) strongly recommended the use of silanized glass or treated stainless steel columns due to their inert characteristics.

Generally, the smaller the inner diameter of the column, the greater the column efficiency(Scott, 1970), although small diameter columns have greater resistance to flow and can only handle very small sample amounts. Issenberg and Hornstein(1970) suggested high resolution columns(0.025 mm i.d.) for labile terpene analysis as the capillary columns have higher linear velocities than packed columns. This reduces the residence times for the different solutes. For analytical and preparative work, they recommended the use of a column with a diameter of 0.05-0.08 mm for capillary columns. Dickes and Nicholas(1976) recommended a 2 mm inner diameter column as the most efficient for a packed column up to 3 m in length. Rose(1959) suggested short 1-2 m columns for high boiling point (BP) compounds and longer column lengths for lower BP compounds.

To identify an unknown peak, Rose(1959) indicated that at least two columns with different liquid phase polarities should be used. In order to select the appropriate stationary phase, Rose divided the types of liquid phases into

three groups: nonpolar, (silicones), moderately polar, (high molecular weight (MW) alcohol esters) and polar, (esters of polyglycols). Dickes and Nicholas(1976) presented a general rule for stationary phase selection. If the sample components were structurally similar but had different BPs, then a nonpolar phase would work best; whereas if the compounds had different functional groups but similar BPs, then a polar phase would work best. With the extraordinary number of liquid phases now available, the proper selection requires a more specific procedure. With this goal in mind, Rohrschneider(1967) devised a polarity scale using two solutes: butadiene and n-butane and assigned different liquid phases a numerical value from Ø to 1ØØ to indicate the relative polarities of each. McReynolds(197Ø) extended this idea by characterizing the polarities of over 2ØØ stationary phases using ten different solute references. Currently most liquid phases are listed in order of increasing polarities using at least five solute references, so that far less quesswork is involved in the selection of the appropriate phase.

The theory of the retention behavior of terpenes has been mentioned previously(Klouwen and ter Heide, 1962 and Rohrschneider, 1967) and Scott(197Ø) discussed the dynamics of peak separation and bandwidth. Scott emphasized the need to optimize the support particle mesh size and the quantity of liquid phase added to the support as both factors influenced peak bandwidth and resolution. He also stressed the

importance of choosing the proper column temperature as peakwidth and resolution are affected by this factor.

The literature cites many different stationary phases for essential oil research. Carbowax 20M with a maximum loading of 15% is the most often recommended phase for general terpene analysis according to Georgiev et al.(1978), U-J. Salzer(1977), von Rudloff(1974), Luisetti and Yunes(1971) and Lawrence(1971). The Analytical Methods Committee(1980) indicated that its major disadvantages were solidification below 65°C and excessive phase bleeding above 225°C.

Other polar phases reported for essential oil research have included Carbowax 400(Burchfield and Storrs, 1962); cyanosilicone OV-225 (Analytical Methods Committee, 1978); ethylene glycol succinate (EGS)(Embong et al., 1977), butane diole succinate(Zlatev, 1976) and Quadrol, FFAP, polyethylene glycol (PEG)-600, PEG-400, DEGS, PDEAS, EGPN and PEG-1540(von Rudloff, 1974). New generation liquid phases include: poly-M-phenoxylene, polyamide and Durapak polymers(Dickes and Nicholas, 1976); and various polyesters evaluated by Bapat et al.(1976).

Nonpolar phases previously reported in the literature include didecyl phthalate(Haslam and Jeffs, 1962); Apiezon-L and silicone oils(von Rudloff, 1974). The Analytical Methods Committee (1981) found that the silicone polymers: OV-1, OV-101, SE-30 and SP-2100 were very stable up to 280°C with a maximum phase loading of 20%. The major disadvantage with

the methyl silicone polymers was their susceptibility to react with any available oxygen in the column.

Selective work on terpenes using a single liquid phase has been reported in the literature. Studies by Klouwen and ter Heide(1962) revealed that aliphatic terpenes with elongated, conjugated double bond systems were more polarizable and had stronger affinities for polar stationary phases than their more-saturated counterparts. Monocyclic terpenes showed a reversed trend as the conjugated dienes in the ring were less polar than isolated ethylenic linkages inside the ring. Also, external double bonds in the head or tail regions of the molecule enhanced the polarity of the terpene, and this exocyclic factor had a dominant polar influence in such bicyclic terpenes as the pinenes. Von Rudloff (1974) observed that the more compact bicyclic and tricyclic terpenes had shorter retention times than monocyclic terpenes, regardless of the liquid phase polarity. Burchfield and Storrs(1962) concluded that the smaller the size of the second ring in bicyclics, the greater the retention time on polar liquid phases. As a single stationary phase can prove ineffective in resolving all the peaks in a terpene mixture, von Rudloff(1974) suggested the use of mixed phases such as SE-30 and PEG-20M. The success of such mixtures has yet to be reviewed in the literature.

Mikkelson(1966) discussed some of the inherent advantages and disadvantages in using temperature-programmed gas chromatography. Careful temperature programming was shown

to lessen the problem of skewed peaks which occurred during isothermal runs. Before the introduction of the modern electronic integrator, asymmetrical peak areas were difficult to calculate and proper temperature programming eliminated this problem. Temperature programming can also be used to lessen downfield peak broadening and prevent the bunching of solutes at the start of the run.

The column support also affects peak symmetry according to Urone and Parcher(1968) depending on the number of acidic sites on the support. Firebrick C-22 was shown to isomerize pinenes(Zubyk and Conner, 1960), and von Rudloff(1974) recommended the preliminary alkali or acid washing of all Firebrick supports, or the silanization of active silanol sites with either hexamethyl disilazone (HMDS) or trimethyl chlorosilane (TMCS) to convert the active sites to silyl ethers.

The choice of support material not only depends on the terpene polarities anticipated, e.g. Chromosorb W HP for apolar compounds but also on the expected column pressures and the desired column efficiencies. The smaller the grain diameter, the greater the efficiency, but the higher the carrier flow restriction and risk of mechanical breakdown. Mesh range is equally important; as the wider the range the greater the peak broadening effect and the lower the column efficiency. The Analytical Methods Committee(1981) recommended the use of 80-100 mesh size support for good terpene analysis. The 100-120 mesh size would give higher efficien-

cies but lower mechanical strength, requiring more rigid control of the GLC operating conditions. Scott(1970) recommended the sieving of all support materials to remove "fines", and he suggested that the least amount of vibration be used in filling the columns to further limit the mechanical breakdown of the particles. Dickes and Nicholas(1976) recommended the use of a rotary evaporator with slow agitation rather than the slurry method of packing preparation, as the first method would ensure the even distribution of liquid phase onto the support. Once prepared, the columns then require a conditioning step. They are normally heated in a GLC for 24 hours at a temperature 20-30^{o}C higher than the expected operating temperature to remove residual solvent, water and contaminants. The carrier gas must be flowing through the column during this conditioning period.

Either helium or nitrogen are used in terpene analysis as the carrier gas because of their inertness. Burchfield and Storrs(1962) indicated that the best flow rate can be chosen by calculating the lowest Height Equivalent to the Theoretical Plate(HETP) value. For helium, this optimum usually occurs at a flowrate of 30 ml/min while for nitrogen the optimal flowrate is between 20-25 ml/min.

The flame ionization detector(FID) is widely used in terpene studies due to its high sensitivity, good thermal stability and linearity of response. Quantitative analysis is best performed on the FID using peak areas which are independent of most operating conditions. FID response is

affected by carrier flowrate and will give different responses for dissimilar terpenes. One report mentions lower responses for the alcohol derivatives of terpenes(Burchfield and Storrs, 1962). However, no systematic study of FID responses for different terpenes has been undertaken, partly because of the difficulties in obtaining pure standards.

2.5.3 Mass Spectrometry

Mass spectrometry(MS), as outlined by Budzikiewitz et al.(1967), Waller(1972), Beynon et al.(1968) and Pavia et al.(1979) has developed into an effective analytical tool in the identification of unknown organic compounds, including the terpenes. Where GLC has failed to give positive identification to a compound, MS can frequently be used to identify molecular isomers based only on the differences in the relative intensities of the fragmentation peaks. MS does not just provide the molecular weight(MW) of the compound, but through careful analysis of the fragmentation pattern, one can also obtain the probable chemical structure for the unknown.

The mass spectrometer can be a magnetic, quadrupole, monopole or a time-of-flight instrument(Waller, 1972), and the detector can either be a Faraday cup or an electron multiplier unit. Combined GLC/MS has been made possible through the use of an interface device such as a fritted

glass tube or a jet orifice. The interface is connected to a vacuum pump which selectively removes the lighter MW carrier gas. The solute molecules are therefore concentrated before entering the ionization chamber under reduced pressure. An electron beam with an energy of about 7Ø eV is produced by heating a fine wire of material such as rhenium. This electron beam bombards the incoming sample molecules and the electron impact dislodges a valence electron from each molecule the beam comes into contact with; resulting in the formation of positive ions. The ions are accelerated to the detector and the signal is then amplified.

Modern mass spectrometers only require ng amounts of sample and these instruments are capable of scanning a molecular range in less than a millisecond. However, the normal recording systems require up to 1Ø seconds to complete a printout of the scan.

The fragmentation pattern is characteristic for different organic molecules as only certain types of fragmentation are energetically probable. Cyclic organic compounds will lose external groups first as only a single bond needs to be broken(Pavia et al., 1979). Therefore, in terpene analysis, the loss of the menthadiene "tail",(C_3H_7, formula weight (FW)=43 amu) is quite common. The phellandrenes undergo the loss of this "tail" to give a dominant or base peak at 93 amu. The loss of a methyl group is also common for monoterpenes as the menthane skeleton has three available sites.

Double bond migrations within cyclic terpene rings are a probable occurance and would aid in the fragmentation mechanism(Beynon et al., 1968 and Budzikiewitz et al., 1967). Cyclohexene monoterpenes such as limonene are thought to undergo double bond rearrangement so that upon fragmentation, two isoprene units,(FW=68 amu) are formed. The base peak for limonene is in fact 68 amu.

Terpene alcohols and aldehydes will lose a water molecule and give a strong peak at 18 amu according to von Sydow(1964). The parent peak is usually very small as a result. Ketones differ from these compounds as they will undergo the loss of a carbon monoxide (CO) molecule instead of a water molecule. Terpene carboxylic acids will lose the carboxylic acid moiety during decomposition. Alcohols and esters both give strong isopentenyl (FW=69 amu) fragmentation peaks and bond breakage is generally favoured at strong, electronegative centres,(hydroxyl groups) which can stabilize the final products. Bicyclic monoterpenes such as the pinenes give very small intensity parent peaks as these compounds tend to be less stable than their monocyclic counterparts. A summary of the MS scans of the more common monoterpenes has been published in two different works and are edited by Stenhagen et al.(1969,1974). Gaps remain in both series of volumes, especially with respect to the oxygenated monoterpenes, but as long as the fragmentation rules are understood, an analyst can use the MS scan data to provide invaluable information on the identity of unknown ter-

penoid compounds.

2.5.4 Sensory Evaluation

Proper flavour profile analysis still requires sensory evaluation in spite of the sophistication of GLC and MS in analyzing spice volatiles. Only limited information is available at the present time concerning the sensory properties of dill weed oil. Furia(1980) described the sensory impressions of individual dill components and Heath(1978) described American dill oil as strongly aromatic, spicy, herbaceous, medicinal and with an aroma reminiscent of caraway. He remarked on the taste as pleasantly aromatic, fresh, green, herbaceous and with a slightly burning sensation. Overall, differences between dill weed and dill seed oils are attributed to the higher terpene hydrocarbon fraction, especially alpha-phellandrene, in the herb oil. Carvone levels contribute a caraway-like or minty flavour, yet a discrepency exists as to what levels give the best sensory quality to dill weed oil. The most recent information indicates that the American spice industry wants d-carvone levels above 30%(Chubey, 1981). Specific ranges need to be established for all three major components in dill weed oil.

The sensory analysis of flavour oils can deal with the odour of the oils, the taste, or both. Fennema(1976) indicated that studies have shown that human olfaction is more

sensitive than taste reception and certain volatiles can be detected in concentrations as low as 10^{-18} molar. An experienced judge can distinguish as many as 10,000 different odours at 20 different levels of intensity. Taste perception is lower and mixtures can be very confusing to the taste panelist.

In addition, olfaction is a distance receptor and the characteristic odour of dill weed would be recognized before its taste. Therefore, the odour evaluation of dill weed distillates would be of considerable importance to the sensory analysis of the sample. Odour analysis also requires very little sample, especially since many oxygenated terpenes exhibit strong odour impressions. One might also expect that a volatile contaminant could be noticed by the olfactory receptors before its recognition in the taste profile for the dill oil.

Sensory evaluation requires a trained group of panelists who are willing to participate and capable of distinguishing properties or differences in various samples. Flavour analysis could involve either attribute or difference testing methods. In attribute testing, specific standards are presented to each panelist, and the panelist is informed that each standard represents a particular flavour attribute such as mintiness. The panelist then compares this defined characteristic with various samples to detect their presence or absence. The attributes must be clearly understood and perceived by the panel before actual testing

begins. Difference testing does not necessarily focus on a single attribute, and therefore the panel do not require as extensive a preliminary training session. The test must be designed to enable the researcher to determine whether the panel can detect differences which do exist. Issenberg and Hornstein(1970) concluded that difference tests were the easiest measurements to determine and the simplest to interpret. Difference tests were used by Jones and Elliot(1975) and Leitereg et al.(1971) to verify the human olfactory descrimination of enantiomers of carvone and alpha-pinene, and could be used to detect contaminants or oxidation products in dill samples.

Amerine et al.(1965) discussed the traditional difference tests, including the triangle, duo-trio, paired comparison and the multi-sample tests. These tests were designed to determine whether a difference existed at a particular level of significance. The degree of difference, once established, must then be determined through additional testing, perhaps involving scaling methods. This route involves considerable time input and the heavy reliance on statistical tables. The large number of tests oftentimes used can add considerable systematic error to the results and the temptation can exist to replicate the tests until a significant difference is reached.

In comparison, a recent test based on signal detection theory has been devised and is called the Recognition Index(R-Index) Test, (O'Mahony, 1979,1981). This test can

be used simultaneously to determine whether a difference exists and the degree of the difference. The tests usually need to be given fewer times as the number of samples presented to each panelist at one time can be varied. The calculation of the probabilities of determining differences are easy to do(O'Mahony,1981), and the panelists have four choices instead of two. These include whether a given sample is: (1) the same as, (2) different from, (3) seems to be the same as, or (4) seems to be different from the reference.

Only if differences are shown to occur would it be necessary to undertake further sensory analysis to determine the magnitude and possibly the reasons for the existing differences. Magnitude Estimation(Moskowitz, 1974) is one such test that has proven useful in correlating changes between the concentration of a substance and the perceived intensity of either the odour or taste. Magnitude Estimation utilizes Steven's Power Function:

$$S=kC^n$$

where (S) is the sensory response, (C) the concentration, (k) a constant and (n) the indicator of sensory response to concentration. When the value of (n) is larger than one, the sensory response increases at a faster rate than the concentration of the substance. If (n) is less than one, then the opposite effect occurs. This particular sensory test would be of practical importance to the pickling industry in determining acceptable range limits for dill weed oil

use.

3 METHODS AND MATERIALS

3.1 Materials

A 1979 commercial dill weed oil sample was obtained from the Morden Research Station while 1980 commercial samples were acquired from the distillation plant near Morden. Dill weed samples of varying maturities were harvested from fields near Morden; courtesy of Peter George Dyck and Ike Dyck, two of the local farmers. Weed varieties, including kochia, wormwood, Canada thistle and lamb's quarters were cut from dill fields near Winkler in September, 1980.

Terpene standards used in this study are listed in Table II, and the GLC packing materials are shown in Table III. Stainless steel columns of 2 m and 4 m lengths(2.1 mm i.d.) were purchased from Chromatographic Specialties. The phenolic anti-oxidant: 3-tert-butyl-4-hydroxy-anisole was obtained from the Mande Scientific Company, dl-alpha-tocopherol acetate, (250 IU/g) from Eastman Kodak and mineral oil from Robinson and Webber Limited. Fisher Scientific supplied the pesticide grade n-pentane.

Table II. Terpene Standards Used in GLC Analysis

Compound:	Supplier:	Purity:
anethole	Eastman Kodak	MP range 2°C
(-) carveol (mixed isomers)	Aldrich Chemicals	99%
(+) d-carvone	Fluka AG	99+%(GLC)
(-) carvyl acetate (mixed isomers)	Aldrich Chemicals	98+%
1,8 cineole	BDH Chemicals	95%
p-cymene	Eastman Kodak	98%(GLC)
d-limonene	Sigma Chemicals	90-95%
beta-myrcene	Sigma Chemicals	90%
(-) alpha-phellandrene	Fluka AG	99+%(GLC)
alpha-pinene	K&K Chemicals	95%
beta-pinene	K&K Chemicals	90%
terpineols (mixed isomers)	BDH Chemicals	less than 4.0% distils below 214°C

Table III. GLC Packing Materials

Stationary Phase:	Company:
Carbowax 20M	Pye Unicam
diethylene glycol adipate(DEGA)	Chromatographic Specialties
EGSS-X(polyester)	Applied Scientific
SE-30(methyl silicone)	Pierce Chemical Company

GLC Packing:	Company:
5% SP-2100+0.1% SP-401 on Supelcoport, 100-120 mesh	Supelco Incorporated

Solid Support (other packings)	Company:
Chromosorb W HP, 80-100 mesh	Alltech Chemicals

Miscellaneous:	Company:
DMCS silanized wool	Alltech Chemicals
Stainless Steel Kleen	Alltech Chemicals

3.2 Methods

3.2.1 Field Sample Collection and Distillations

Dill weed samples were collected from two adjacent fields in southern Manitoba in the summer of 1980. Three rows from within each field were permanently selected for sampling for the duration of the maturity study. Samples were cut from each row at more than one spot to a depth of about 100 meters. Each dill plant was cut 8-10 cm above ground level, placed into poly bags and tagged. The samples were taken at the following stages of maturity: stalking and budding, initial flowering, mass flowering, milk ripeness and full seed ripeness. In total, samples were obtained on seven harvest dates from June 21 to September 9. All samples were kept frozen at -30°C until distilled.

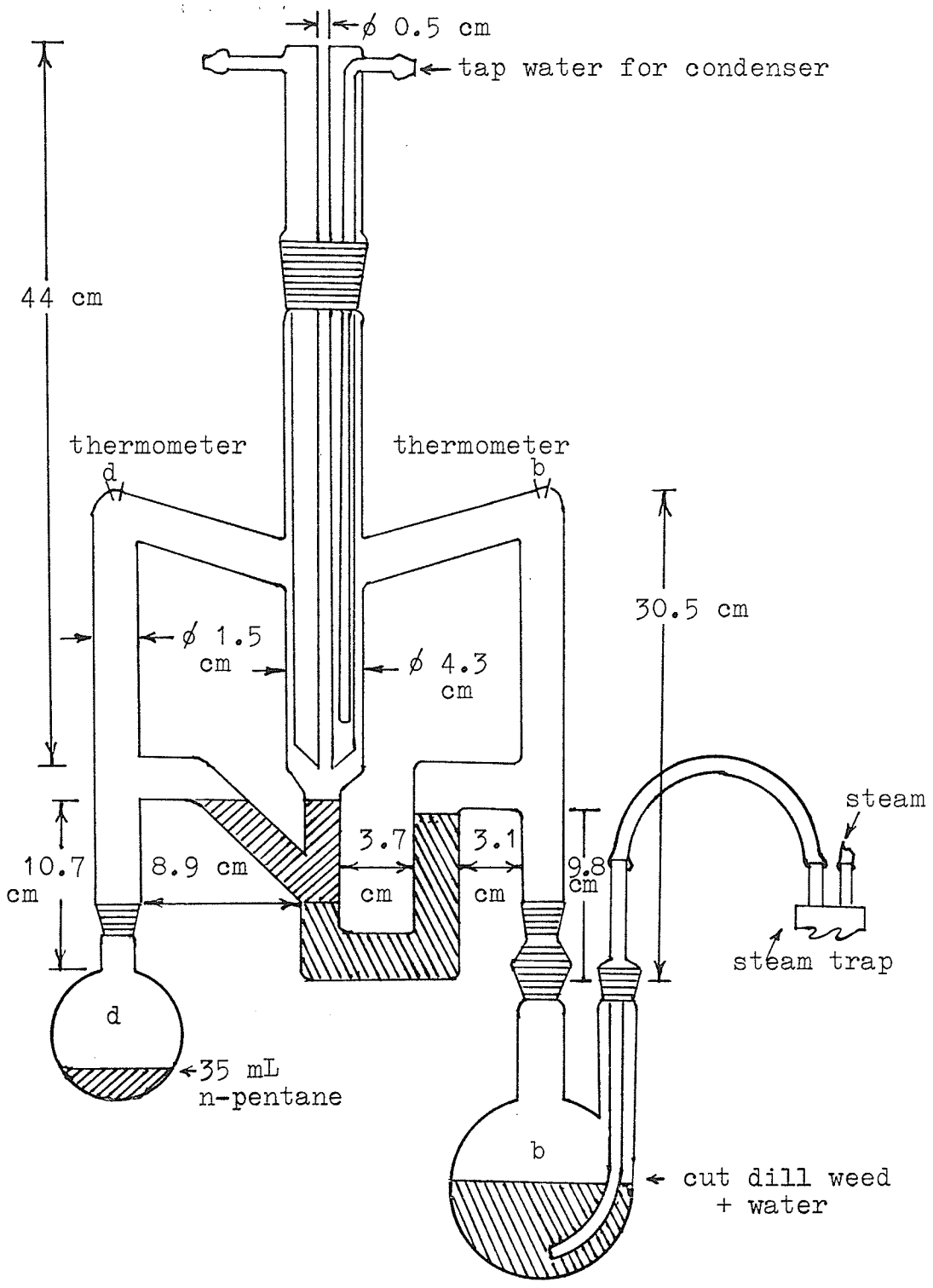
Additional dill weed samples, nearing maturity, were harvested in mid-September and distilled at the Morden Research Station after the removal of all contaminating plant material. During the same period, samples of four, different weed varieties were also distilled at Morden using pilot-scale equipment.

Two laboratory-scale distillation procedures were used in this study to extract dill oil from the plant materials.

The apparatus described by Romer and Renner(1974) is shown in Figure 9. Fresh dill samples were cut into 2-3 cm lengths, weighed and placed into a 2 liter round-bottom flask. Steam was generated under standard pressure in a 5 liter round-bottom flask by using a heating mantle. The steam passed through a condensate trap before entry into the sample flask. The steam temperature was monitored using a thermometer at 'b' and the distillation proceded for 45 minutes once the temperature reached 100°C. A 100 ml round-bottom flask containing 30 ml of n-pentane was connected to the 'a' side of the apparatus(Figure 9) and submerged into a water bath held above 50°C. The n-pentane boiled continuously, trapping the organic flavour volatiles during the phase change from gas to liquid. The water vapour condensed and gradually returned to the sample vessel. After the distillation, the n-pentane flask was allowed to cool and the sample was directly analyzed by GLC without further preparation.

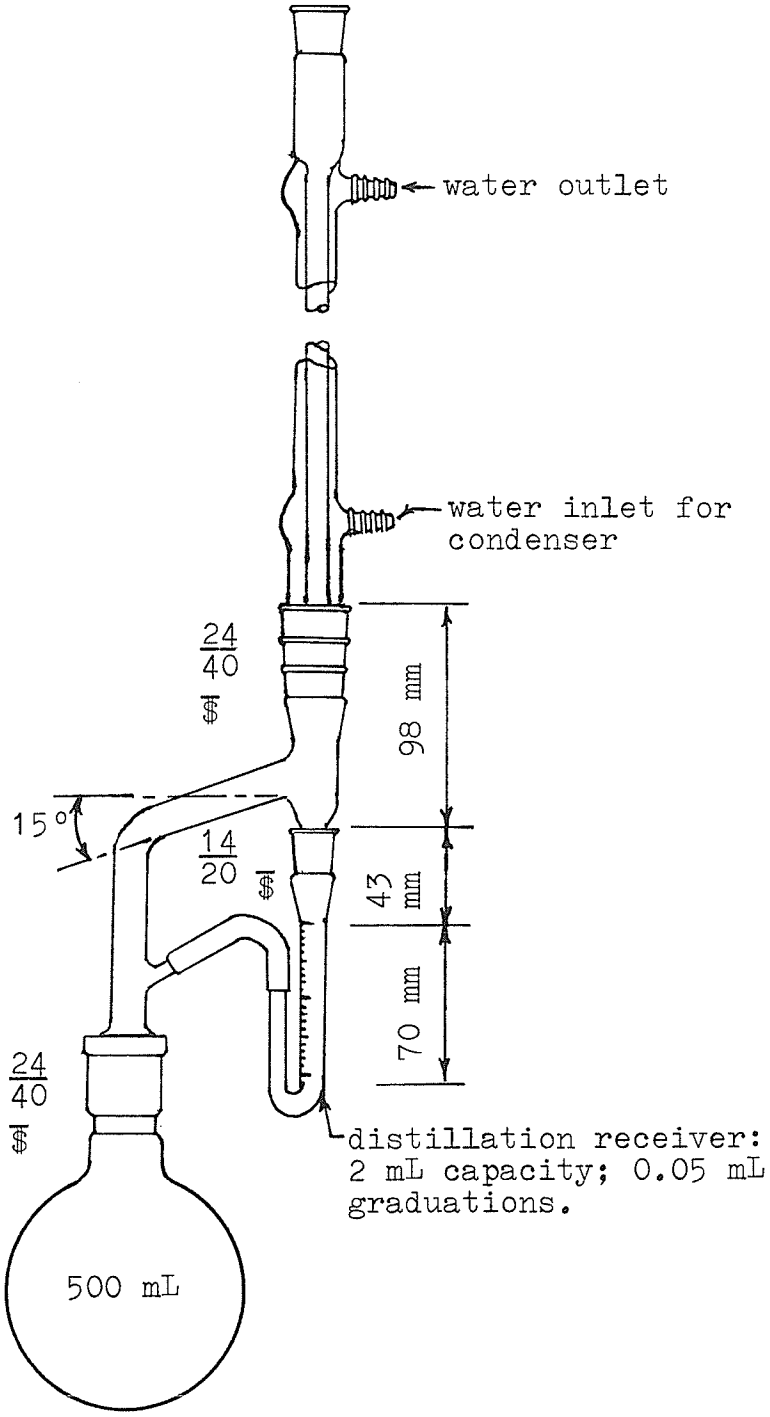
A second distillation procedure involved a modification of the Lee and Ogg apparatus(Figure 10) outlined by the American Spice Trade Association(1960). A stirring rod driven by an electric motor eliminated the need for a magnetic stirrer and stir bar. Fresh dill samples were cut into 2-3 cm length, then placed into a one liter double-neck flask containing 500 ml of water. A heating mantle, surrounding the sample flask was then turned on and the distillation continued for 45 minutes from the time when the water

Figure 9. Romer and Renner Distillation Apparatus



Described by Romer and Renner. 1974.

Figure 10. Lee and Ogg Distillation Apparatus



American Spice Trade Association. 1960.

vapour first condensed from the vertical condensers. The terpene fraction was collected on top of the water condensate in a calibrated distillation receiver and was allowed to cool before a reading was taken. The pure dill oil was then removed using a Pasteur pipette and diluted with n-pentane prior to further analysis.

All pilot-scale distillations were conducted at the Morden Research Station. Up to 30 kg of chopped plant material were placed into an upright stainless steel tank during each distillation. A steam hose was then connected to the bottom of the tank and steam at a boiler pressure of 5.1 atmospheres released into the vessel. As the steam rose through the plant layers, it evaporated the volatile compounds and the combined distillate then passed through a steel condenser coil into a 500 ml separatory funnel. The organic layer was removed at the end of each two hour distillation and the quantity recorded. Yields were calculated for each run. All samples were then analyzed by GLC to obtain the necessary qualitative information.

Commercial dill weed oil samples were obtained from the distillation plant near Morden. Each wagon load of dill was distilled for two and one-half hours under a steam pressure of 6.8 atmospheres. The volatiles passed through a condenser and the upper oil fraction was drained into stainless steel drums; each with a protective synthetic lining.

All the samples were kept in 100 ml amber glass bottles capped with screw tops complete with Teflon liners. The

bottles were labelled and kept at 2-4^{o}C until required for further analysis.

3.2.2 Accelerated Oxidation Apparatus and Experiments

A series of oxidation experiments were carried out using the apparatus illustrated in Figure 11. A preliminary study with glass sample vials containing a capillary tube bleed in the caps gave excessive evaporative losses. Subsequent studies were undertaken with sealed sample vials connected to the oxygen line with Pasteur pipettes. The tip of each pipette passed through a small hole drilled into each plastic cap, and the fit was sufficiently tight to prevent excessive evaporative losses. Tygon tubing with plastic connectors ensured equal oxygen pressure in each vial. Oxygen was added to the vials for five minutes each day at a flowrate of 20-25 ml/minute per vial to ensure oxygen excess. Oxidation rates were therefore independent of the oxygen concentration. The apparatus was placed inside an incubator to control the temperature factor. Sample weights were carefully monitored throughout each study. The initial terpene levels for the dill weed oil references used in the oxidation studies are given in Table IV.

The following oxidation studies were conducted:

Oxidation of Pure Dill Oil :One ml samples were added to

Figure 11. Dill Weed Oil Oxidation Apparatus

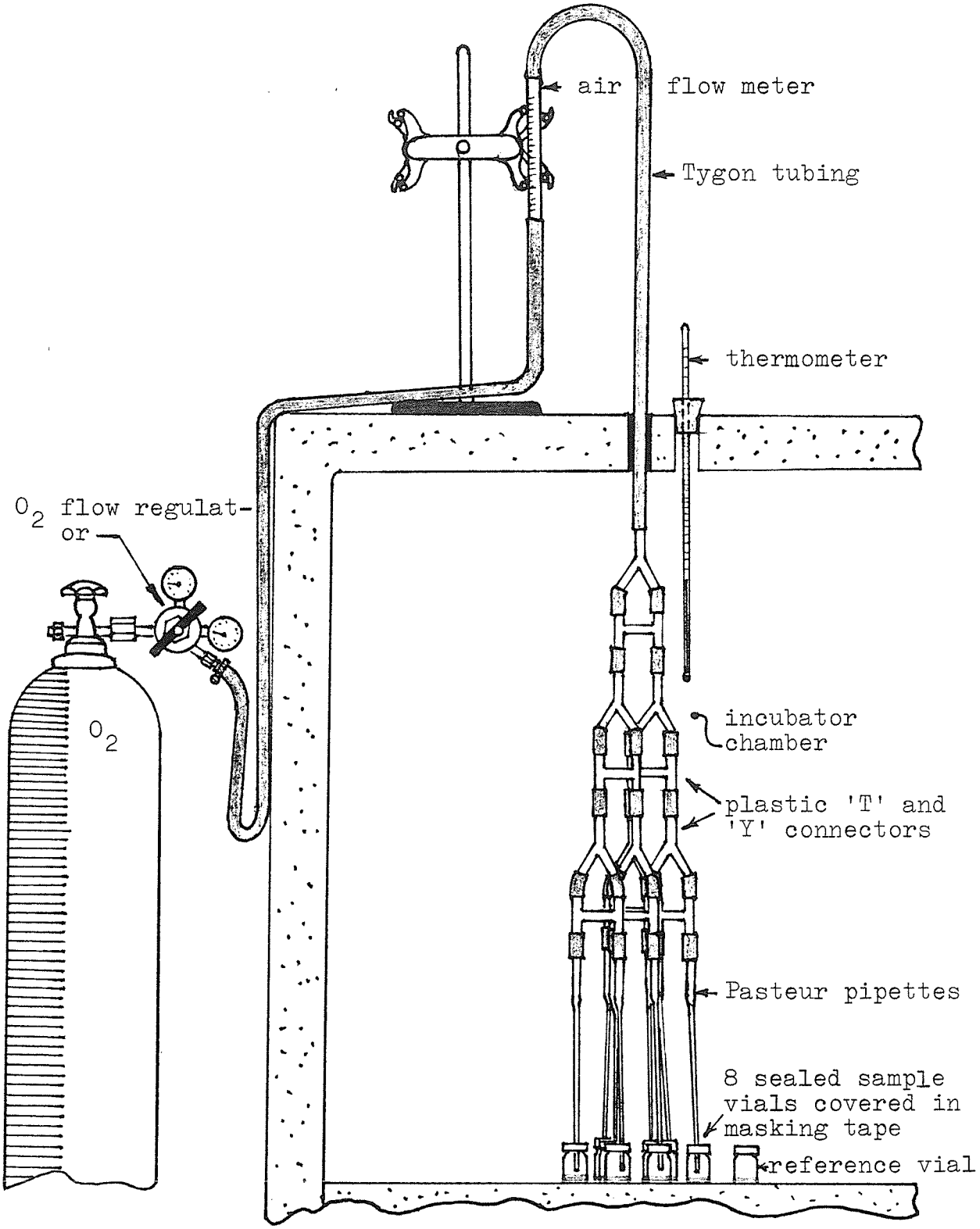


Table IV. Dill Weed Oil References Used in the Storage Studies

Component:	Reference:			
	(a)	(b)	(c)	(d)
alpha-pinene	0.8%	0.8%	0.8%	2.0%
beta-myrcene	0.5%	0.7%	0.4%	0.5%
alpha-phellandrene	24.2%	23.4%	21.2%	36.2%
intermediate isomer(136 amu)(2)	(1)	(1)	(1)	5.1%
d-limonene	33.2%	32.8%	30.6%	35.9%
dill 'ether'	4.3%	4.3%	4.2%	5.9%
dihydrocarvone	1.3%	1.3%	1.3%	0.4%
d-carvone	34.4%	34.0%	38.6%	13.8%
TOTAL:	98.7%	97.3%	97.1%	99.8%

(a) Pure Dill Weed Oil Storage Study

(b) Contaminated Dill Weed Oil Study

(c) 'Shelf-Life' Study

(d) Anti-Oxidant Study

(1) Inadequate separation from d-limonene for quantitative determination.

(2) Intermediate isomer tentatively identified as alpha-terpinene.

eight 1Ø mL glass vials which were attached to the accelerated oxidation apparatus. The incubator temperature was set at 26.Ø +/- Ø.5°C and the replicates were analyzed over a four month period using GLC. Initially the samples were analyzed every three days, but gradually the intervals were lengthened once the oxidation rates dropped off.

Contaminated Dill Oil - A Preliminary Study :wormwood oil, Artemesia absinthium was mixed with a pure dill oil reference in the ratio of 1:24 (V/V) to represent a moderately infested dill field, (1:4, wormwood:dill by plant weight). One ml samples were then added to 1Ø mL glass vials and the vials attached to the oxidation apparatus set at 26.Ø +/- Ø.5°C. The contaminated samples were monitored approximately every four days initially; followed by periodic analysis after the oxidation rates declined. The study covered four months in total.

Shelf-Life Study :The following contaminated dill oil samples were prepared: (1) 1:29(V/V) wormwood distillate:dill oil, (2) 1:149(V/V) Canada thistle distillate:dill oil, (3) 1:149(V/V) kochia distillate:dill oil, and (4) 1:149(V/V) lamb's quarters distillate:dill oil. One ml samples for each contaminated solution were pipetted into 2.5 mL glass vials and these vials were then capped and placed into a covered box along with reference samples. The box was left at room temperature for a period covering almost one year, (361 days). The contaminated samples and the references

were analyzed by GLC within one and one-half month periods for the first three times; then tested less frequently for the remainder of the year. Each sample vial was only analyzed once as a sufficient number of vials were prepared initially.

Oxidation of Major Terpenes Found in Dill Oil: One g samples of standards of alpha-pinene and d-carvone were placed into separate 10 mL vials, while 0.24 g of alpha-phellandrene and 0.86 g of d-limonene were placed into two other 10 mL vials. The four standards were then attached to the oxidation apparatus set at 36.0 +/- 0.5°C and analyzed over a two month period. All standards were analyzed each day for the first four days. The least oxidized samples were then analyzed twice at two week intervals and then at the end of the two month period to indicate long term storage effects.

Selected Anti-Oxidants Used in Dill Weed Oil: Two anti-oxidants were investigated in this study: alpha-tocopherol acetate, a natural anti-oxidant with oxygen scavenging properties, and tert-butyl hydroxy anisole(BHA), a phenolic inhibitor of hydrocarbon free radicals. A commercial dill oil sample was chosen as the reference which contained an abnormally high content of hydrocarbon terpenes. Three solutions were prepared: 0.125% BHA in dill oil, 0.125% alpha-tocopherol acetate in dill oil, and 0.0625% BHA + 0.0625% alpha-tocopherol in dill oil. The anti-oxidants were dissolved in a few drops of 95% ethanol before being

mixed with the dill standard. Two ml samples of the treated dill oil and the reference were then added to 10 ml vials and attached in duplicate to the oxidation apparatus. The incubator temperature was set at 35.0 +/- 0.5°C and each vial was analyzed once a day for the first eight days; plus the final analysis after 260 hours.

3.2.3.1 Preparation and Packing of Columns and GLC Conditions

The stationary phases were mixed onto the solid supports as outlined:

20% SE-30: 4.000g of SE-30 were dissolved in 80 ml of chloroform using gentle heat and hand stirring. 16.000g of Chromosorb W HP were added to the dissolved mixture and stirring was resumed for an additional 15 minutes. The mixture was then transferred to a round-bottom flask which was connected to a rotary evaporator. Most of the solvent was then evaporated and the semi-dry mixture placed onto aluminum foil and dried for 16 hours in an oven set at 100°C.

15% Carbowax 20M,(PEG-20M): 3.000g of Carbowax 20M were dissolved in 60 ml of chloroform and 17.000g of Chromosorb W HP were then added. The solvent removal and drying procedure were as outlined above.

1% DEGA + 4% Carbowax 20M: Ø.8ØØg of Carbowax 20M and Ø.2ØØg diethylene glycol adipate were dissolved in a 1:1 mixture of acetone and chloroform. Next, 19.ØØØg of Chromosorb W HP were added and the preparation dried as above.

3% EGSS-X: Ø.3ØØg of EGSS-X were dissolved in chloroform after which 9.7ØØg of Chromosorb W HP were added. The preparation was then dried as previously described.

5% SP-21ØØ + Ø.1% SP-4Ø1: This packing was purchased already prepared.

The stainless steel columns used in this study were deactivated with a Stainless Steel Kleen solution. This was followed by a rinsing in a weakly basic, phosphate solution; a flushing in distilled water; air drying with nitrogen gas; and pre-weighing. A Press-Pak 4ØØØ column(Altech Associates) was then used to fill each column. A vibrator facilitated the even packing of the columns and nitrogen air pressure was applied to a maximum of .68 atmospheres above the expected carrier gas inlet pressure. Each column was then reweighed and silanized glass wool was placed into both ends. The columns were then be conditioned for 18 hours at a temperature 2Ø^{o}C lower than the maximum recommended temperature for the liquid phase. Several blank runs were carried out before the columns were ready for use.

The GLC operating conditions for the Varian Aerograph 37ØØ are listed in Table V. A Varian Aerograph 477 integra-

Table V. GLC Run Parameters

Columns:	Solid Support:
1.) 1.82 m X 2.1 mm i.d. 20% SE-30	Chromosorb W HP, 80-100 mesh
2.) 1.85 m X 2.1 mm i.d. 15% Carbowax 20M	Chromosorb W HP, 80-100 mesh
3.) 3.76 m X 2.1 mm i.d. 4% Carbowax+1% DEGA	Chromosorb W HP, 80-100 mesh
4.) 1.88 m X 2.1 mm i.d. 5% SP-2100+0.1% SP-401	Supelcoport, 100-120 mesh
5.) 3.70 m X 2.1 mm i.d. 5% SP-2100+0.1% SP-401	Supelcoport, 100-120 mesh

Carrier gas:	nitrogen
Carrier flow rate:	20 mL/minute
Detector:	FID(Flame Ionization Detector)
Attenuation:	variable
Sensitivities:	10^{-11}amps/mV for standards diluted in n-pentane; 10^{-9}amps/mV for concentrated samples
Syringes:	1.0 microliter, 5 microliter, 10 microliter, SGE
Sample Size:	1.0 microliter for dilute samples and 0.4-0.6 microliters for concentrated samples
Chartspeed:	38 cm/hour for the Westronic MT-22 integrator; 0.5-1.0 cm/minute for the HP-3390A integrator

tor with a Victor Digit-matic printer and Westronics chart recorder were used for the preliminary studies, but were replaced with a Hewlett-Packard 3390A integrator unit for the major oxidation studies. The smaller dynamic range of the Varian integrator required sample dilution for optimal results whereas the Hewlett-Packard instrument could be used with concentrated samples at sub-microliter injection volumes. This permitted the direct injection of oxidized dill oil samples, without a dilution step.

3.2.3.2 Choice of Stationary Phase

Several liquid phases were investigated for dill oil analysis which had previously been reported for use in essential oil analysis. The proper selection of the liquid phase was complicated by the knowledge that both oxygenated(polar) and hydrocarbon(apolar) monoterpenes are present in dill weed oil. A comparison of several liquid phases is shown in Table VI. These phases cover a wide range of polarities as is evident from the summaries of the McReynold's Constants. This indicated that differences were to be expected in the separation of specific groups of terpenes.

The operating temperature range for each liquid phase is also compiled in Table VI. These values indicate that the most stable materials are the silicone polymers: SE-30

Table VI. GLC Liquid Phase Polarity Comparisons and Optimal Temperature Ranges

Liquid Phase:	Temperature Limits(°C):	McReynold's Constants: (1)	(2)	(3)	(4)	(5)	Total:
SE-30	50/300	015	053	044	064	041	217
SP-2100	0/350	017	057	045	067	043	229
Carbowax 20M	60/225	322	536	368	572	510	2308
DEGA	0/200	378	603	460	665	658	2764
EGSS-X	90/200	484	710	585	831	778	3388

1.) benzene

2.) 1-butanol

3.) 2-pentanone

4.) nitropropane

5.) pyridine

and SP-2100. By comparison, Carbowax 20M, DEGA and EGSS-X have lower operating temperature ranges which are indicative of lower thermal stabilities. These polar phases would therefore be expected to bleed at a lower temperature than the apolar phases.

Further evaluation of the liquid phases was based on theoretical plate determinations which give an indication of the efficiency of the phases. Since d-carvone was expected to have a similar polarity to the other oxidation products that could arise in the dill oil during storage, this terpene was chosen as an appropriate marker compound for the theoretical plate determinations. The results obtained on the different phases are summarized in Table VII. SP-2100 yielded the highest plate values while EGSS-X gave very low values. This was a good indicator that EGSS-X was not suitable for dill weed oil analysis and was eliminated from further evaluation.

A final step in the evaluation of the remaining stationary phases was based on the separation of selected terpenes which had previously been reported in dill weed oil. The standards and concentrations are listed in Table VIII. The evaluations were based on Relative Retention Indexes(RRI) and the calculation of the individual RRI values is shown in Table IX. Since the retention times for the major dill terpenes were found to increase from alpha-phellandrene to d-limonene to d-carvone, the central component: d-limonene was selected as the reference peak for the RRI

Table VII. Column Efficiencies in Terms of Theoretical Plates

Liquid Phase:	SP-2100+ SP-401	SE-30	PEG-20M+ DEGA	PEG-20M	EGSS-X
Solid Support:	Supelco-port	Chromo-sorb W	Chromo-sorb W	Chromo-sorb W	Chromo-sorb W
Mesh Size:	100/120	80/100	80/100	80/100	80/100
Column Length:	1.88 m	1.82 m	3.76 m	1.85 m	2.71 m
Theoretical Plate Value for 150°C Isothermal Run:	2520+/- 70	2080+/- 40	4200+/- 100	1300 +/- 100	700+/- 100
Theoretical Plates/m:	1340	1140	1100	700	300
Theoretical Plate Value for Programmed Run:	12,700+/- 200	11,280+/- 30	19,000+/- 200	2900+/- 100	1900+/- 200
Theoretical Plates/m:	6800	6200	5100	1600	700

Marker Terpene: d-carvone , PEG-20M = Carbowax 20M

Program Conditions: 75°C to 180°C at 4°C/minute

Sample: 1979 dill weed oil, 0.0657%(W/V) in n-pentane

Detector Temperature: 220°C

Injector Temperature: 180°C

Replicates: minimum of three per column

Integrator: HP-3390A

Table VIII. Terpene Standard Solutions for GLC Analysis

Compound:	g/100 mL with n-pentane:
anethole	0.0578
d-carvone	0.0697
carveols(cis,trans)	0.0173
carvyl acetate (mixed isomers)	0.0265
1,8 cineole	0.0618
p-cymene	0.0595
d-limonene	0.0474
beta-myrcene	0.0354
alpha-phellandrene	0.0530
alpha-pinene	0.0532
beta-pinene	0.0757
terpineols (mixed isomers)	0.0641

Table IX. Relative Retention Index Calculation

$$RRI_{\text{solute X}} = \frac{RT_X - RT_{\text{air peak}}*}{RT_{\text{limonene}} - RT_{\text{air peak}}}$$

RT = retention time for component, taken from the start of the run to the center of the peak maximum

*$RT_{\text{air peak}}$ values differ for the various columns used:

20% SE-30(1.82 m): 0.4 minutes,

15% Carbowax 20M(1.85 m): 0.4 minutes

5% SP-2100+0.1% SP-401(1.88 m): 0.5 minutes

1% DEGA+4% Carbowax 20M(3.76 m): 0.6 minutes

calculations. The airpeak times were subtracted from each retention time to account for the dead volume in each column.

The results showed that the RRI values for d-carvone on Carbowax 20M and the DEGA columns were 7.4 and 4.15 respectively. Although this indicated good separation from the hydrocarbon terpenes, the values also indicated excessively long runs would be required to obtain the d-carvone peak. The optimal conditions for the separation of the dill oil components required that the RRI values should be neither too low nor too high. The SE-30 column gave a low RRI value for d-carvone while SP-2100 gave a value that was between the low and high range. The SP-2100 column also gave better separation of the individual peaks, (as illustrated by the RRI values in Table X) compared to the SE-30 column.

In summary, the 1.88 m 5% SP-2100 + 0.1% SP-401 column was selected for use in this study as it performed well in all the different evaluation steps. During the oxidation studies it became necessary to use a 3.7 m column to enhance the separation of the oxidation peaks.

3.2.3.3 Flame Ionization Detector Response to Dill Terpenes

The flame ionization detector(FID) response differs for monoterpenes with dissimilar functional groups and possibly for isomers with different double bond positions. Func-

Table X. Relative Retention Index Summary

Compound:	Column: 1.82 m[a] SE-30	1.88 m[b] SP-2100+ SP-401	3.76 m[a] DEGA+ PEG-20M	1.85 m[a] PEG-20M
alpha-pinene	0.78	0.65	0.48	0.38
beta-pinene	NA	0.78	0.68	0.60
beta-myrcene	0.89	0.82	0.85	0.82
alpha-phell-andrene	0.94	0.89	0.88	0.83
p-cymene	0.96	0.95	1.36	1.44
1,8 cineole	0.99	0.98	1.12	1.17
d-limonene	1.00	1.00	1.00	1.00
dill 'ether'	1.39	1.71	2.79	4.40
dihydrocarvone	NA	1.78	3.45	5.60
d-carvone	1.50	2.00	4.15	7.40

Program Conditions: (a) 60°C to 200°C at 4°C/minute

(b) 75°C to 180°C at 4°C/minute

Sensitivities: 10^{-11} amps/mV

Detector Temperature: 200-210°C

Injector Temperature: 170-180°C

NA = not available

tional groups will affect the ratio of positive-to-negative ions produced in the hydrogen flame. This alters the specific response for each compound since only the positive ions produce a signal.

Three solutions containing known amounts of each of the major dill terpenes (alpha-phellandrene, d-limonene and d-carvone) plus two of the minor terpenes(alpha-pinene and beta-pinene) were prepared as shown in Appendix 1. The purity of each standard was checked using GLC and the same temperature conditions were used throughout the analysis, (Tables V and XI). Sample injections of one microliter were analyzed by GLC using the HP 3390A integrator for quantitative evaluation. Percentages of the integrator area values for each component were automatically calculated by the integrator. These values were then divided by the actual weight percent for each component and a summary of these ratios, i.e. FID responses, are given in Table XI. Limonene gave the closest approximation of the integrator values to the actual composition on a weight basis. Therefore this terpene was chosen as the standard for comparison by the other components and was assigned a response value of one. Correction factors were then determined for the other terpenes and are shown in Table XII. The d-carvone factor of 1.11 +/- 0.02 indicated the poor FID response per unit weight of this oxygenated monoterpene compared to the other hydrocarbon terpenes. Yet the deviations from the d-limonene standard for all the tested compounds were within +/-

Table XI. FID Response Factors for Dill Terpenes

Calculation: $$S_X = \frac{\text{Integrator \% for solute X}}{\text{Actual Weight \% for solute X}}$$

Compound:	Solution:* #1	#2	#3	$\bar{X}_3$
alpha-pinene	1.087	1.068	1.118	1.09+/-0.02
beta-pinene	1.074	1.033	1.087	1.06+/-0.03
alpha-phell-andrene	1.083	1.037	1.094	1.07+/-0.03
d-limonene	0.972	0.935	1.002	0.97+/-0.03
d-carvone	0.868	0.866	0.895	0.88+/-0.02

* mean values for five replicates

Note: GLC temperature program: 75-175°C @ 4°C/minute

Table XII. FID Correction Factors

Compound:	Correction Factor:*
alpha-pinene	0.89 +/- 0.01
beta-pinene	0.91 +/- 0.01
alpha-phellandrene	0.91 +/- 0.01
d-limonene	1.00
d-carvone	1.11 +/- 0.02

*Correction factors are compared to d-limonene which gave the best ratio of integrator % to the actual weight % of the terpenes investigated. A close approximation of the actual weight % of each major dill terpene can be determined by multiplying the integrator % by the correction factor for each compound.

11%.

Throughout the dill weed oil study, the relative changes in dill oil composition were of utmost importance and for this reason the GLC peak area ratios were considered adequate for evaluation. As well, standards were not available to determine the FID responses for all the minor dill components; in particular dill 'ether' and dihydrocarvone, and so the correction factors were not known for these compounds. However, in a test for specifications, correction factors would need to be used to obtain more accurate weight values for the major components.

3.2.4 Analysis by Mass Spectrometry

Dill weed essential oils, including some of the oxidized terpenes were analyzed using a mass spectrometer located in the Chemistry Department. A 3.7 m stainless steel column containing 5% SP-2100 + 0.1% SP-401 packing was installed into a Varian 1700 gas chromatograph. The GLC unit was connected to a Finnegan 1015 Quadrupole mass spectrometer,(1-700 amu) using a Biemann-Watson glass interface. The Varian 1700 contained an FID detector and helium was used as the carrier gas because of its low molecular weight. The electron impact type spectrometer produced 70 eV electrons that bombarded the sample molecules in the ionization chamber. An electron multiplier served as the fragment

detector and a mass recorder required 1Ø seconds scan time to reproduce the m/e peaks on Kodak processing paper.

Certain limitations were inherent to this type of combined GLC-MS analysis. Peaks at 27,28,29 and 32 amu appeared in all mass spectrograms, with the 28 amu peak considerably larger than the other three. This peak represented an accumulation of CO, C_{2H_4} fragments and possibly nitrogen which may have existed as an impurity in the helium carrier gas. As a result, these peaks were often represented as major peaks on the mass spectrograms. Consequently these peaks are not represented to full scale in the diagrams given in the Appendices.

An additional problem appeared during the MS analysis. The individual terpenes could not always be scanned at their GLC peak maxima for the required 1Ø seconds and this affected the relative heights of each m/e peak. If the scan occurred before the GLC peak maximum, then the heavier m/e fragments were over-represented in terms of relative abundance. If the scan took place after the GLC peak maximum, then the larger molecular weight fragments became diminished in size very quickly. Careful selection of the exact scanning period was required to reduce the effects caused by this problem.

Before any MS scans were made, all the major and minor peaks in an oxidized dill oil sample, (contaminated with wormwood oil) were numbered. The MS-derived formula weights were then assigned to the numbered peaks as shown in Figure

33.

3.2.5 Sensory Analysis

Sensory analysis was used to evaluate odour properties of essential oil samples. The relationship between concentration of dill oil and its odour intensity, the effect of oxidation, the effect of contamination and the effect of compositional changes on the odour compared to the odour of a standard sample were investigated. All samples were presented in 10 mL glass vials with plastic, snap-on caps. A small amount of cotton was added to each vial and 1.5 mL samples were then pipetted onto the cotton. The vials were wrapped in masking tape to prevent possible deterioration of the samples by light. However, it appeared that the tape adsorbed odour components which interfered with the sensory analysis. Therefore the masking tape was replaced with aluminum foil to alleviate this problem. Samples were coded with a three-digit number while the reference samples were denoted by the letter 'R'.

All panel sessions were conducted in a sensory panel room. Red light was used to reduce bias based on colour or other visual impressions. Smoking was not permitted in either the panel room or in the sample preparation area. Panelists were made aware of the difficulties that could be encountered with distracting odours such as perfumes and

colognes during sensory analysis. After each session the panelists were given a treat as a reward.

Sensory Study 1. Magnitude Estimation of Pure Dill Weed Oil

The purpose of this study was to determine the perceived intensity of different concentrations of dill weed oil. The study also provided the opportunity to familiarize the panelists with the odour of pure dill weed oil.

The pure standard was diluted with mineral oil to obtain the concentrations recorded in Appendix 2. The composition of the dill oil used in this study is also presented in Appendix 2. In a preliminary study, the Ø.1%(V/V) solution was selected as the reference for both sessions as the odour intensity for this sample was neither too weak nor too overpowering. The two sessions were given one week apart. In both trials, six samples were presented to the panelists. However, the Ø.Ø25%(V/V) solution was not presented in the second session as the majority of the panelists could not detect the odour. A 4%(V/V) solution was inserted in its place.

Each panelist was given a set of samples and a ballot (Appendix 3). To eliminate positional bias, the panelists were instructed to sample the vials in the sequence given on the ballot. As a further check against bias, two series of codes were utilized with each series of samples. Trays containing the sample sets were placed into the panel room immediately before each session and were arranged so that

neighbouring panelists did not have the same coded series.

The panelists were instructed to inhale several quick whiffs of the reference; wait for 20-30 seconds, then evaluate the first sample. After a further 20-30 seconds they were to resample the reference and then proceed to the second sample. They were expected to complete the analysis of all the samples using this procedure.

The impression of the strength of a sample was compared to the reference, then recorded on the ballot. If the panelist perceived a doubling in intensity, then a '2' was written in the 'Ratio Estimate' column of the ballot for that sample. This value was then multiplied by ten to obtain a 'Numerical Value'. If no odour could be detected, the panelists were asked to signify this with an 'np'. Space was provided on the ballot to allow the panelists to make suggestions, or to comment on the dill odour and they were encouraged to do so. The geometric means of the magnitude estimates of all panelists for each concentration were used in the calculation of the power function for the odour intensity of dill weed oil.

Sensory Study 2. Effect of Contamination on Dill Weed Oil Odour

The objective of this study was to determine the level at which a weed oil contaminant could be detected in dill oil. Wormwood, Artemesia absinthium was chosen as the weed contaminant since chemical studies involving contamination

showed that wormwood distillate would be potentially the most important of the four weeds investigated.

Wormwood distillate was mixed with pure dill weed oil in several concentrations as indicated in Appendix 4. The composition of the pure dill oil reference is also given in Appendix 4. The R-Index difference test described by O'Mahony(1979,1981) was used in this study. Panelists participated on the basis of their willingness and availability to complete the study. A preliminary session was held in which panelists were presented with a good dill oil standard. They were asked to describe the odour using their own descriptors. To facilitate this task, a list of descriptors obtained from Heath(1978) was given prior to the odour sampling. The results of this preliminary trial indicated that the panelists could readily identify the characteristic dill weed oil odour.

Nine panelists took part in the main sessions. In the first series, each panelist was asked to compare 16 coded samples with a reference. Eight of the samples were contaminated with wormwood oil while the other eight were coded references. They were instructed to sample the coded vials as outlined in Sensory Study 1. However, the samples were presented in a random fashion and the panelists were allowed to choose each sample in any order. The ballot illustrated in Appendix 5 was provided and the judges were instructed to record the codes for each sample and then decide whether the sample was: (1) different from, (2) the same as, (3) seemed

to be different from, or (4) seemed to be the same as the reference. The uncertainty was denoted on the ballot with a question mark after 'Different' and 'Reference'. If a sample was identified as being definitely different from the reference, then the panelist was instructed to comment on whether the odour appeared to be 'better' or 'worse' than the reference.

Sixteen samples were presented to each panelist for the first two series of trials and involved the 8.1% and 2.1% wormwood contaminated dill oil samples. Panelists experienced odour fatigue and the number of samples was reduced to 12 for the final series involving the 3.3% contamination samples. Altogether, two trials were conducted for each contaminated solution and the probability that a judge could correctly choose the different samples was calculated according to the method described by O'Mahony(1979,1981) and as shown in Appendix 6. The individual R-Index values were then collectively analyzed to determine possible significances between the different levels of contamination.

Sensory Study 3. Effect of Oxidation on Dill Weed Oil Odour

This study was undertaken to determine at what level of oxidation differences in dill weed oil odour could be perceived in comparison to a control. For this purpose dill oil was stored and the alpha-phellandrene levels monitored since decreases in alpha-phellandrene levels were shown to be a good indicator of the degree of oxidation. Four sam-

ples were prepared which differed in alpha-phellandrene content as shown in Appendix 7. The results of this study could then be used in conjunction with a shelf-life study to determine the expected storage life of dill oil on the basis of perceived odour differences.

The R-Index difference test was also used in this study and the sensory procedure used was as outlined in Sensory Study 2. Nine panelists participated in this study. During the first session, 15 coded samples were presented to each judge and consisted of five coded references, five coded samples with 17.9% alpha-phellandrene and five samples with 9.3% alpha-phellandrene. In subsequent trials, panelists were given only 12 samples consisting of six coded references and six coded dill oil samples at one level of oxidation. The least oxidized dill oil sample containing 17.9% alpha-phellandrene was presented to the panel only once while each of the other three samples were given to the panelists in two separate trials.

The ballot sheet(Appendix 5) and the calculations of the results were as previously described.

Sensory Study 4. Compositional Changes in Pure Dill Weed Oil

The purpose of this study was to determine which of the major terpenes in dill weed oil had the greatest impact on dill weed oil odour. Once again the R-Index test was used to determine differences between altered dill oil samples

and a commercially acceptable reference. The composition of the pure dill oil reference(Appendix 8) was altered through the addition of monoterpene standards. Each of the three major components: d-limonene, alpha-phellandrene and d-carvone were increased or decreased by approximately 50% with respect to the reference so that a total of six altered solutions were prepared as indicated in Appendix 8.

Eight panelists participated in these trials. Each judge was presented with 12 coded samples, including six altered and six reference samples. The ballot sheet remained the same as for the earlier R-Index tests(Appendix 5). Each altered sample was given to the panel in two separate trials and the results were evaluated as previously outlined.

3.2.6 Statistical Analysis

The two-way analysis of variance (ANOVA) was used to determine the significance of compositional changes of dill oil during the maturity study, storage study and for the three odour studies involving the R-Index difference test. Split-plot analysis of variance was used for the contaminated dill weed oil 'shelf-life' study to investigate the factors of oxidation time, treatments and replicates. A split-plot ANOVA was also used for the study involving the two anti-oxidants. Factors which were shown to be signifi-

cant from the ANOVAs were subjected to further analysis either by Least Significant Difference (LSD) or Duncan's New Multiple Range test to establish the significance between individual means at the 5% level of confidence. Linear regression and correlation analysis were used throughout the different storage studies to determine the order of oxidation kinetics involved. The odour study involving the R-Index difference testing of altered dill weed oil compositions was also analyzed by step-wise multiple regression to obtain a prediction equation for the three major dill components.

4. RESULTS AND DISCUSSION

4.1 Dill Weed Oil Composition and Content Versus Plant Maturity

The composition of dill weed oil samples distilled from plants at different stages of maturity was determined by gas chromatography.

Seven main peaks appeared in the chromatograms of all samples and are illustrated in Figure 12 with the example of oil distilled from dill in the late milk-wax ripeness stage. The identities of the components were determined on the basis of the comparison of their retention times(t_R) with those for reference compounds and by mass spectrometric analysis. The MS scans for the seven compounds are given in Appendices 9 to 12 and they were matched to the published data for these components(Stenhagen et al., 1969, von Sydow, 1964, Masada, 1976, Schreier et al., 1981, and Embong et al., 1977). The dill 'ether' compound was matched to data published by Schreier et al.(1981) which did not agree with the mass spectra of Embong et al.(1977). Dill 'ether': 3,9 epoxi-p-menth-1-ene along with several proposed peak fragments are shown in Figure 13. The simplest fragmentation

Figure 12. Gas Chromatogram of Pure Dill Weed Oil

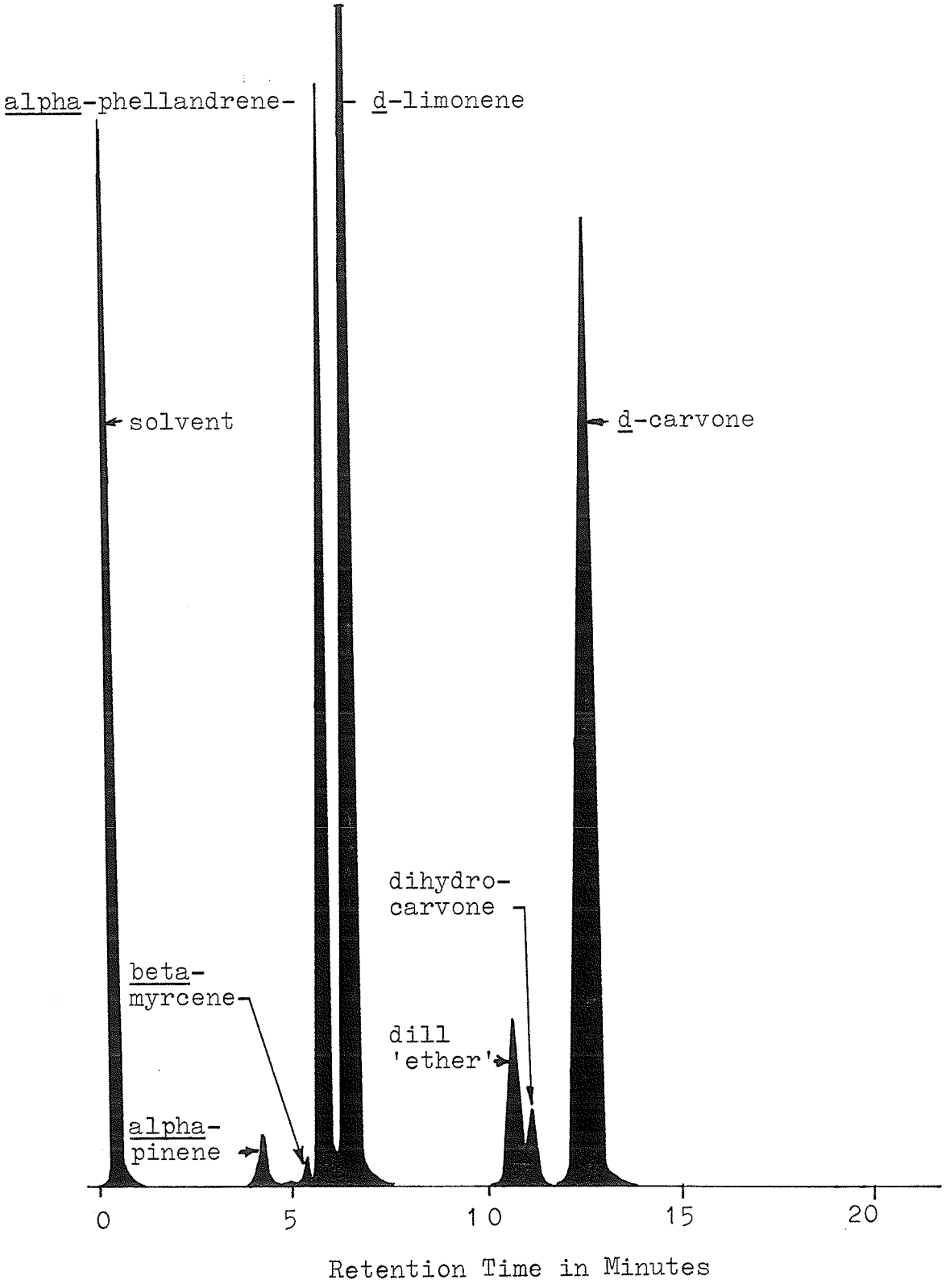
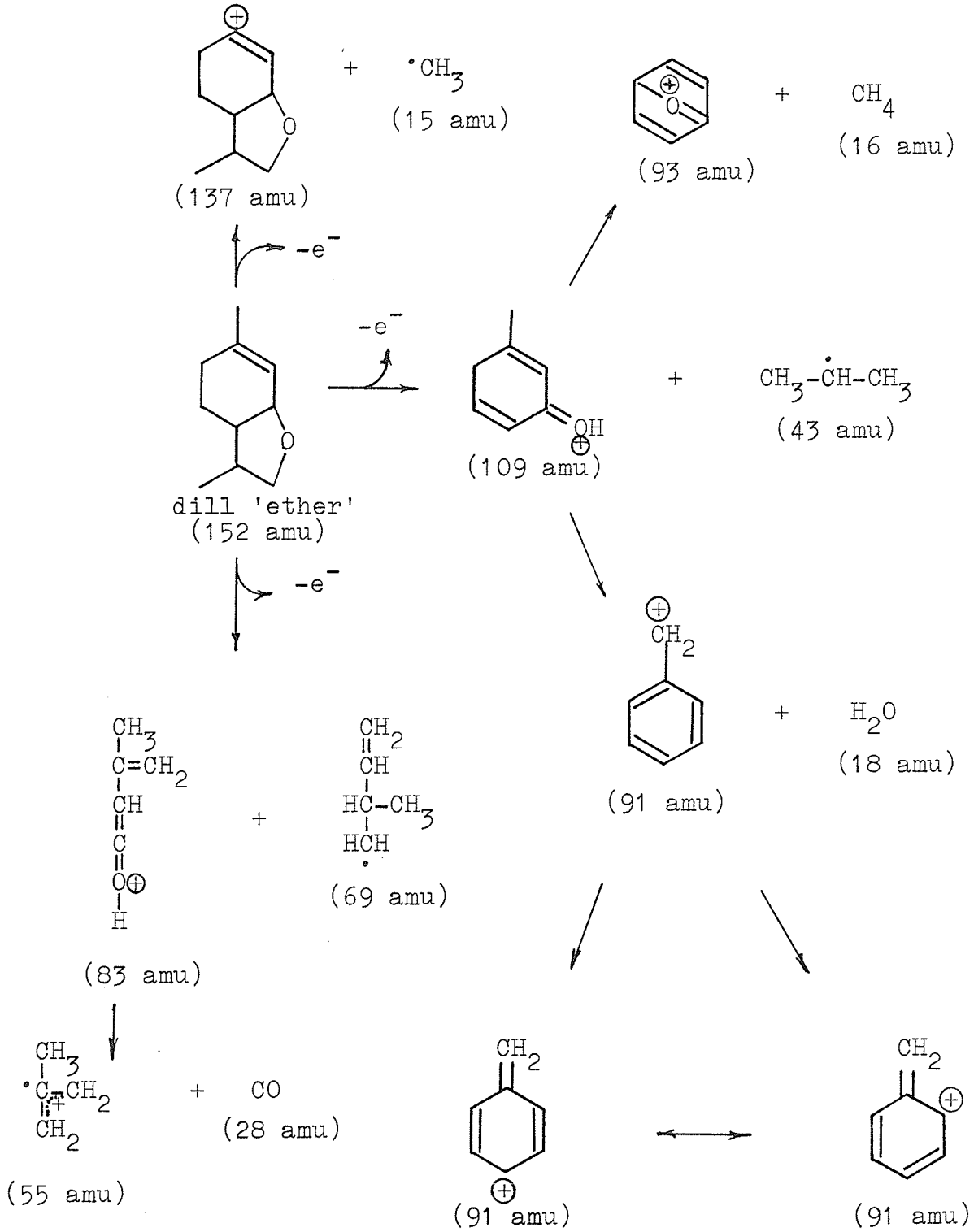


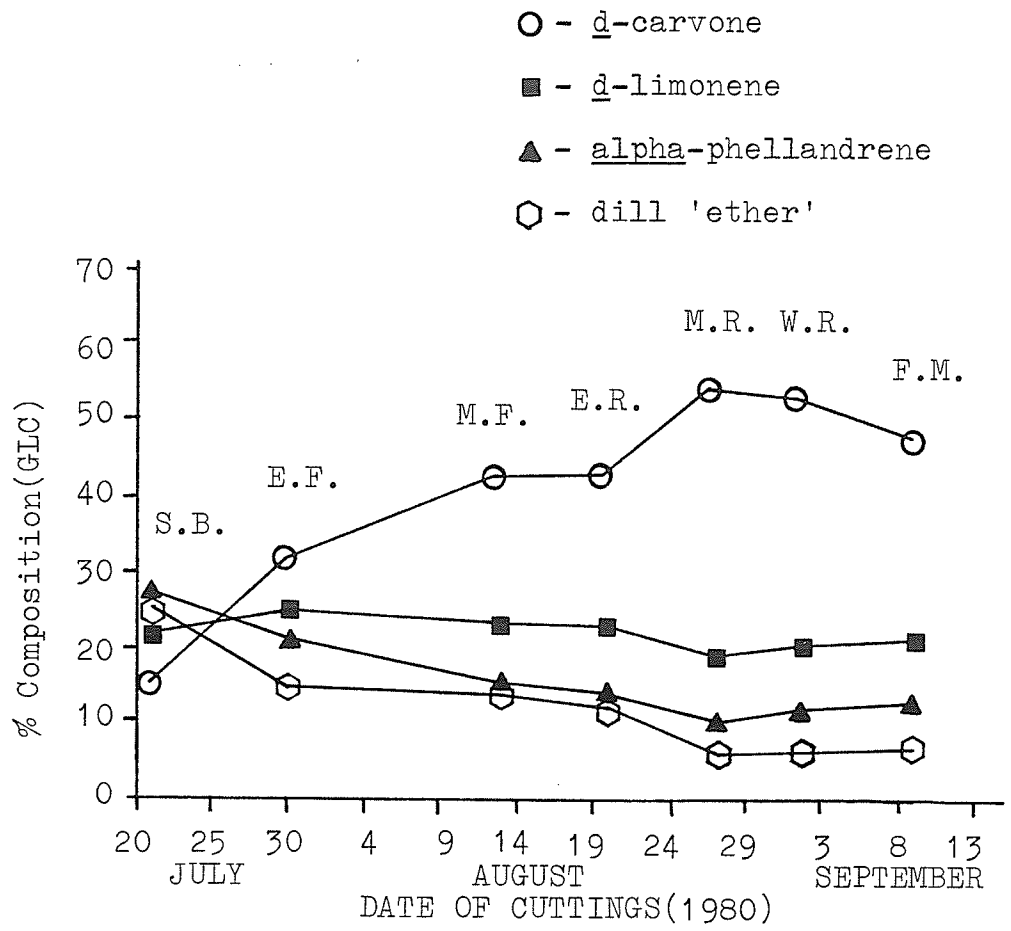
Figure 13. Possible Fragmentation of Dill 'Ether' in the Mass Spectrometer



would involve the loss of the methyl group in the head of the molecule to give a major peak at 137 amu, and this in fact forms the largest, or base peak in the dill 'ether' mass spectrogram obtained during this study(Appendix 12).

Evaluation of the gas chromatograms covering the ontogenetical development of dill weed revealed that compositional changes occurred. These changes are summarized in Figures 14 and 15 which depict the levels of the four major dill oil components during the different stages. The samples were taken from two adjacent fields, designated SE and SW. The SE dill field was planted two weeks earlier in the spring than the SW field, yet the dill oil compositions were similar after the initial cutting date. This emphasized the need to harvest the plant material at a particular stage of maturity to ensure the optimal oil composition. With respect to the current oil standards, the most significant change in oil composition was the increase in carvone from the stalking stage to the milk ripeness stage. After that, as indicated in Figures 15 and 16, d-carvone levels do not significantly change from the milk ripeness to milk-wax ripeness stage(Appendices 19,20,21 and 22). The alpha-phellandrene and dill 'ether' levels decreased slightly with the increase in maturity while the d-limonene levels remained quite constant throughout plant development. It was observed that after the milk-wax ripeness stage some of the mature seeds dropped from the plants. From data compiled in Table XIII, it was apparent that oil from the seeds con-

Figure 14. Maturity of Dill Weed and the Composition of its Oil(SE Plot)



S.B.: stalking and early budding stage

E.F.: early flowering stage

M.F.: mass flowering stage

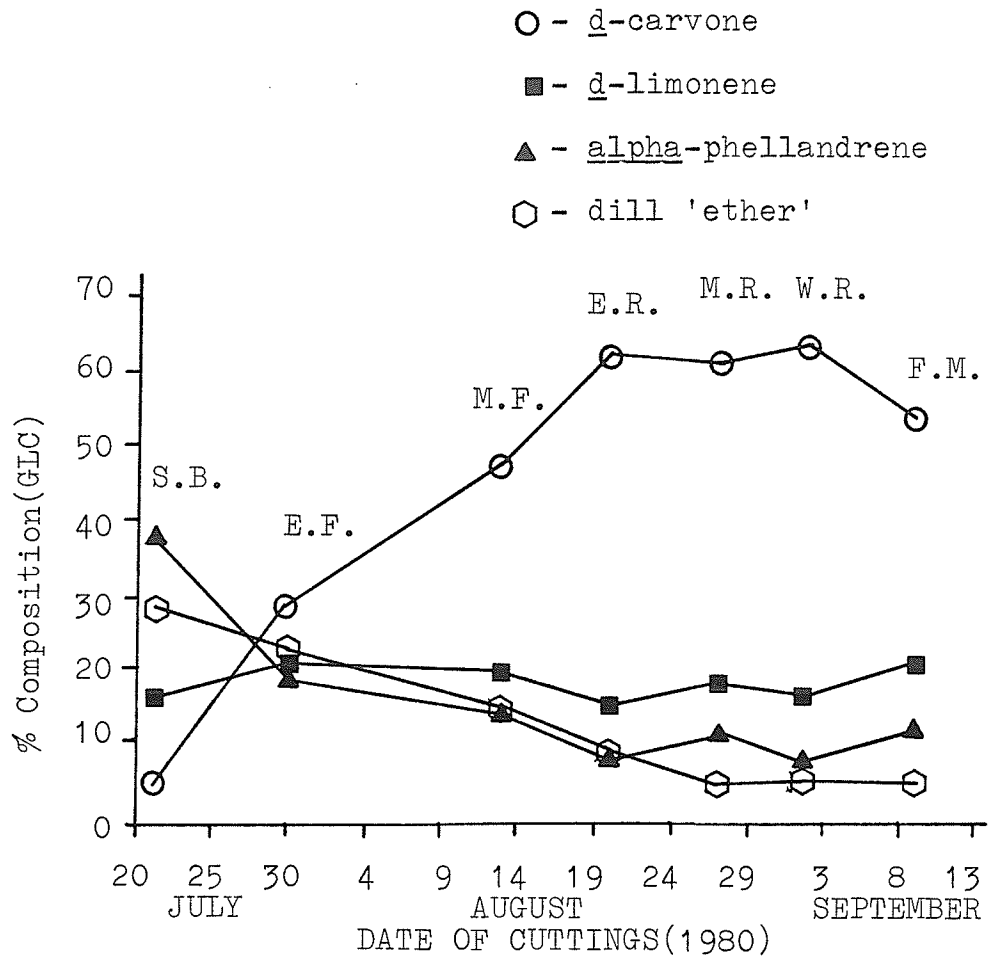
E.R.: early milk ripeness stage

M.R.: milk ripeness stage

W.R.: milk-wax ripeness stage

F.M.: full maturity stage

Figure 15. Maturity of Dill Weed and the Composition of its Oil(SW Plot)



S.B.: stalking and early budding stage

E.F.: early flowering stage

M.F.: mass flowering stage

E.R.: early milk ripeness stage

M.R.: milk ripeness stage

W.R.: milk-wax ripeness stage

F.M.: full maturity stage

Table XIII. Oil Composition from Different Parts of the Dill Plant

Plant Part:	%Composition:(1)			
	alpha-phellandrene	d-limonene	dill 'ether'	d-carvone
Entire Plant(3)	13.2 +/- 1.4	23.5 +/- 2.3	12.9 +/- 1.8	46.8 +/- 2.7
Umbel (2)	2.8 +/- 2.7	9.8 +/- 2.2	14.9 +/- 1.8	69.7 +/- 3.1
Seeds(2)	5.5 +/- 0.8	4.8 +/- 1.0	2.9 +/- 0.1	84.7 +/- 1.4

1.) Plants were harvested during the mass flowering stage.

2.) Means of four replicates.

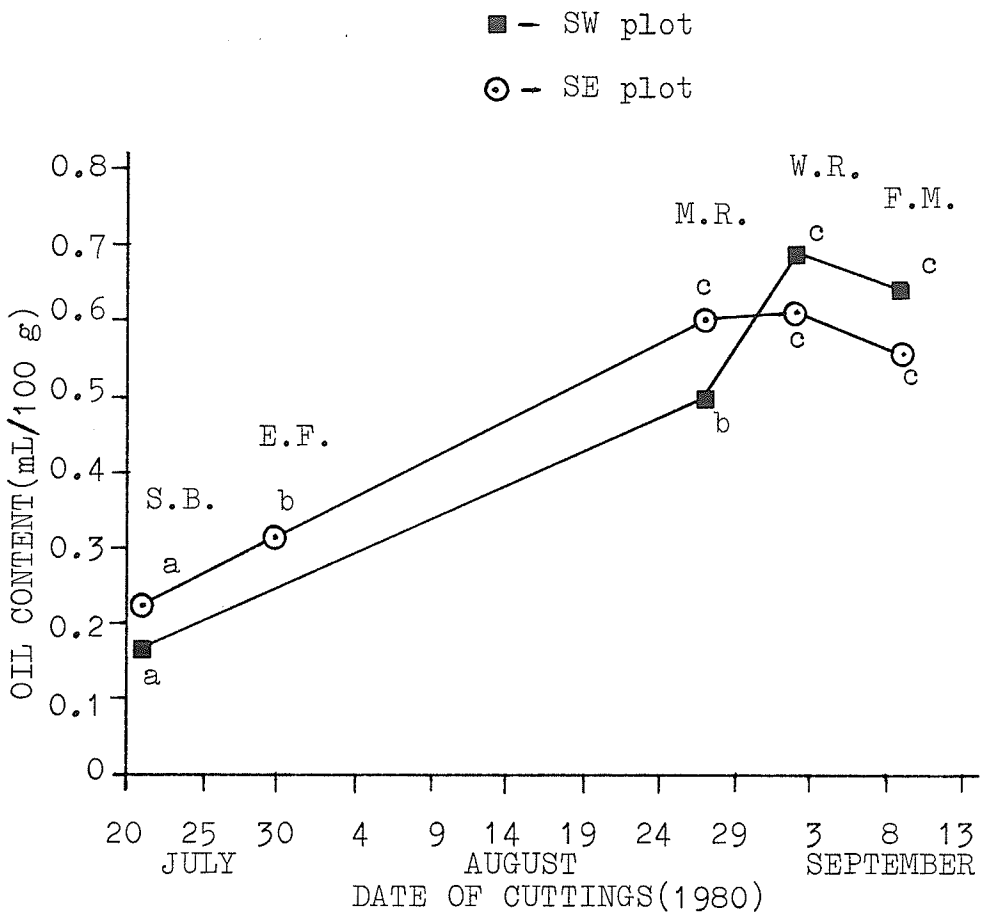
3.) Means of six replicates.

tained up to 85% d-carvone while oil from the whole plant contained only 46.8%. This indicated that the carvone levels in the stems and ferns were much lower than in the seeds and umbels. Therefore the loss of seeds would result in a drop in d-carvone levels and this fact may explain the observed decrease in d-carvone levels at full maturity compared to the level found at the milk-wax ripeness stage(Figures 14 and 15).

This study confirmed the findings of previous authors(Zlatev, 1975, 1976a,b; Embong et al., 1977) concerning the accumulation of dill oil during the physiological development of the plant as plotted in Figure 16. The absolute amounts of the different dill terpenes were then calculated based on the total oil content(Figure 16) and on the compositional information given in Figures 14 and 15.

Statistical analysis of these data(Appendices 23,24,25,26,27 and 28) revealed that the d-carvone content increased from the early stalking period to the milk-wax ripeness stage and then dropped slightly at full maturity(Figure 17). The d-limonene content also significantly increased from the stalking stage to full maturity, but at a lower rate than d-carvone(Figure 18). The Alpha-phellandrene content showed only a slight increase from the stalking period to full maturity, while the dill 'ether' content remained quite constant during maturation. Both d-limonene and d-carvone were synthesized throughout the physiological development of the plant to at least the milk ripeness

Figure 16. Maturity of Dill Weed Versus its Oil Content



S.B.: stalking and early budding stage

E.F.: early flowering stage

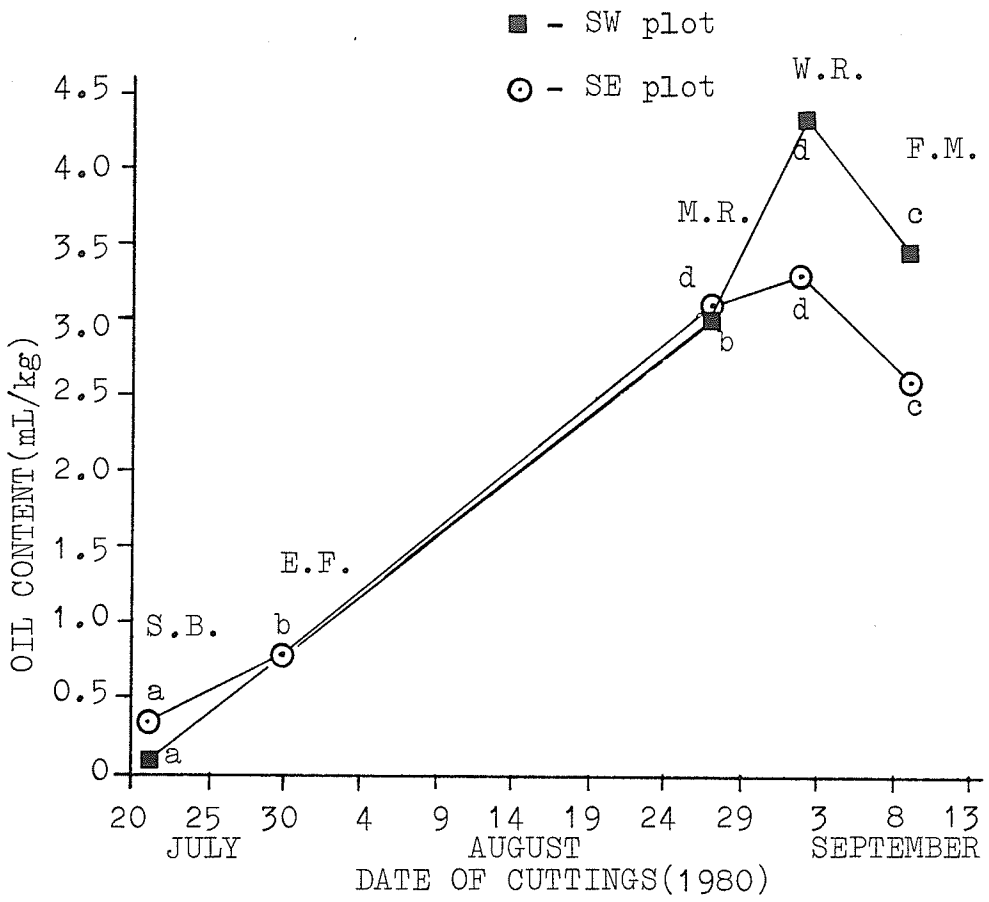
M.R.: milk ripeness stage

W.R.: milk-wax ripeness stage

F.M.: full maturity stage

Note: Values with the same letter designation are not significantly different at the 5% level of confidence.

Figure 17. Maturity of Dill Weed Versus its Carvone Content



S.B.: stalking and early budding stage

E.F.: early flowering stage

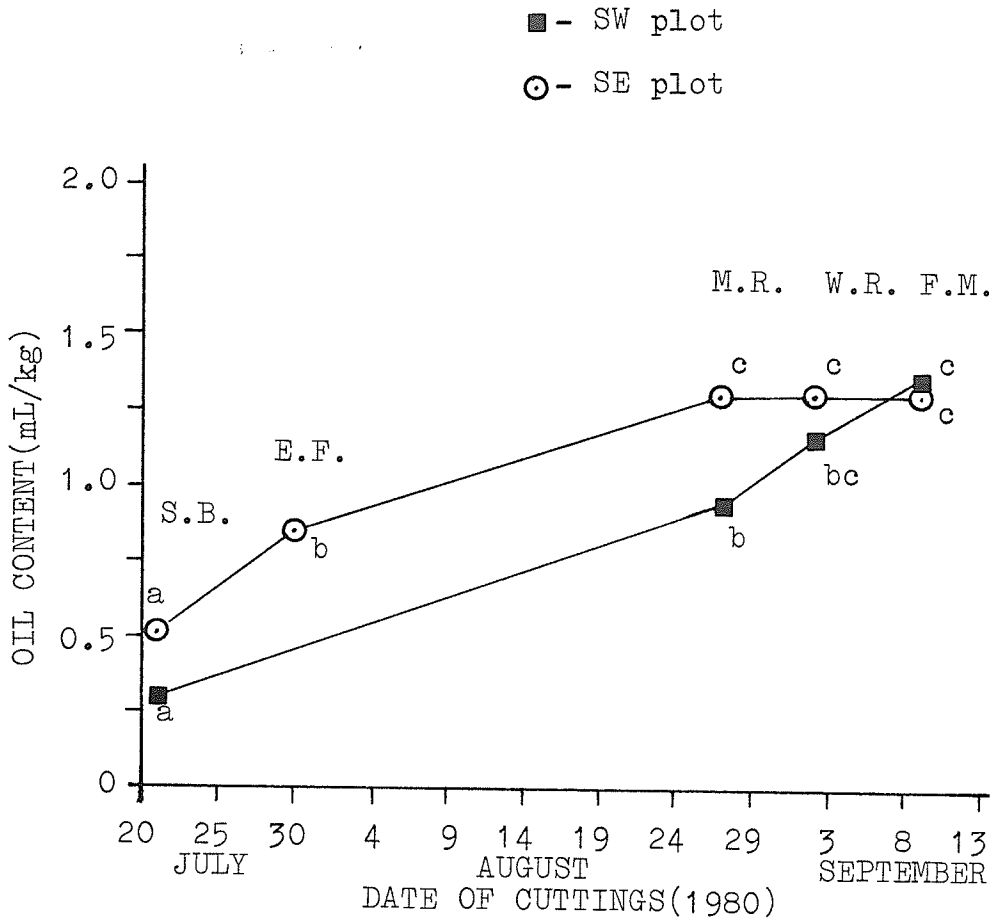
M.R.: milk ripeness stage

W.R.: milk-wax ripeness stage

F.M.: full maturity stage

Note: Values with the same letter designation are not significantly different at the 5% level of confidence.

Figure 18. Maturity of Dill Weed Versus its Limonene Content



S.B.: stalking and budding stage

E.F.: early flowering stage

M.R.: milk ripeness stage

W.R.: milk-wax ripeness stage

F.M.: full maturity stage

Note: Values with the same letter designation are not significantly different at the 5% level of confidence.

stage, and the lower accumulation of d-limonene provided support to the proposed enzymatic oxidation of d-limonene to d-carvone(Akhila et al., 1980, Crouteau and Loomis, 1975, and Banthorpe et al., 1972).

Furthermore, the nearly constant dill 'ether' and alpha-phellandrene contents throughout maturation implied that their biosynthesis occurred before the first samples were taken at the stalking stage. Consequently, the biosynthetic route for these terpenes was probably independent from that of d-limonene and d-carvone. The lower d-limonene levels in the umbel region of the plant compared to the ferns and stems seemed indicative of more active enzyme conversion of d-limonene to d-carvone in the generative organs. However, this hypothesis would require specific enzyme research of the different plant segments to provide the necessary confirmation.

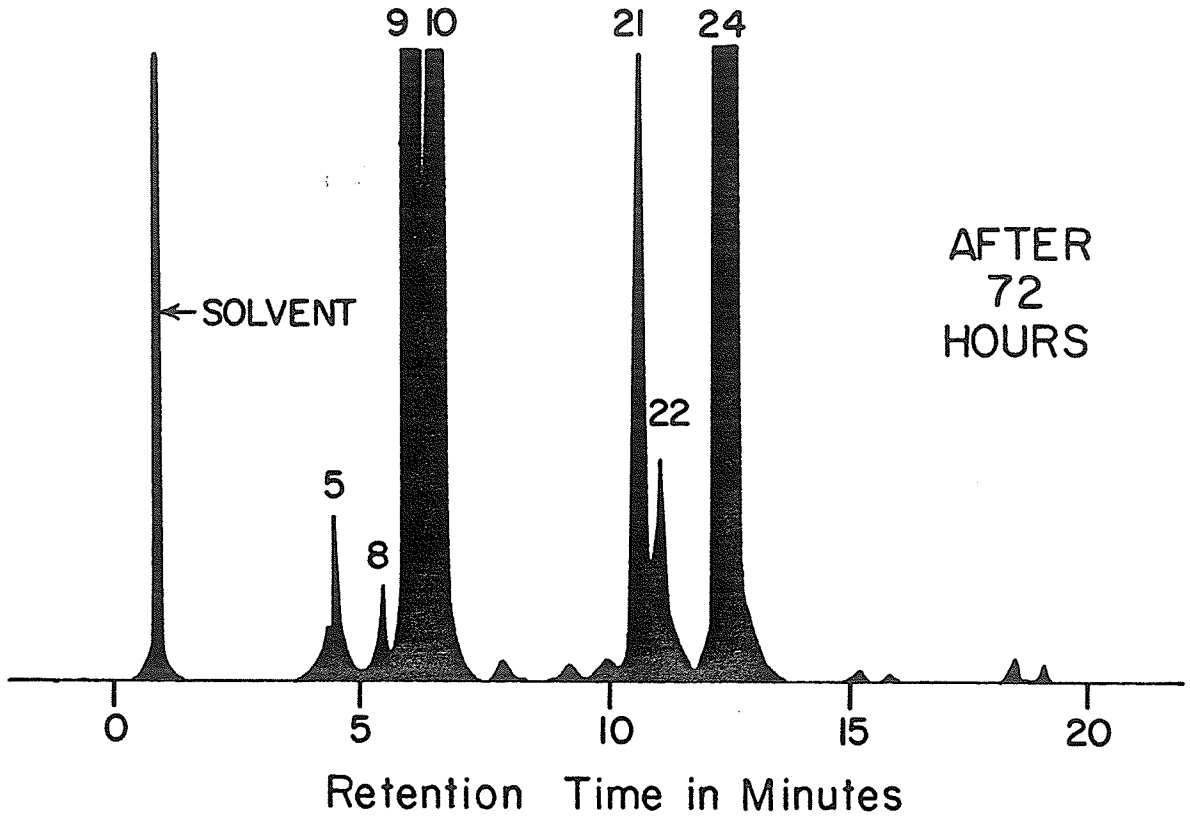
The results of this part of the study verified the need to harvest the dill at the proper stage of maturity. Considering distillation, the oil content study indicated that harvesting the dill weed immediately after the milk ripeness stage would permit the most efficient utilization of the distillation installations since the oil content was shown to reach the maximum level at this point. In addition, a desireable oil composition is reached by the milk-wax ripeness stage of maturity, also making this the most favourable time for harvesting the dill weed.

4.2 Oxidation of Dill Weed Terpenes

According to the singlet oxygen theory described by Wasserman and Murray(1979) every terpene with an unsaturated double bond can be expected to undergo autoxidation. All the major terpenes in dill oil contain at least one carbon-carbon double bond and could be expected to oxidize.

The first study was undertaken to monitor the changes of dill oil during storage. A saturated headspace was maintained for all the samples and changes were shown to occur at 26.0 +/- 0.5^{o}C as shown in Figure 19. A shift from the hydrocarbon terpenes, (peaks# 5,7,8,9,10 and 11) to oxygenated species, (peaks# 13-15, 17-19, 25-50) including carvone, (peak# 24) occurred between 72 hours and 2254 hours. Most of the compounds generated during storage eluted after carvone. This was indicative of their larger molecular weights and higher boiling points, resulting from the addition of oxygen. A summary of the changes which took place during storage is given in Figure 20. The hydrocarbon terpenes, (peaks# 5,7,8,9,10 and 11) decreased steadily from 59% of the total peak area at the beginning of the study to 20% after 2254 hours of storage, while the carvone levels increased from 34.5% to 46.5%. The oxidation products which eluted after carvone increased from 0.4% to 23.8%, while several minor products, (peaks# 13-15,17-20) eluting between

Figure 19. Gas Chromatograms of Stored Dill Weed Oil



See Table XIV for the identities of the peaks.

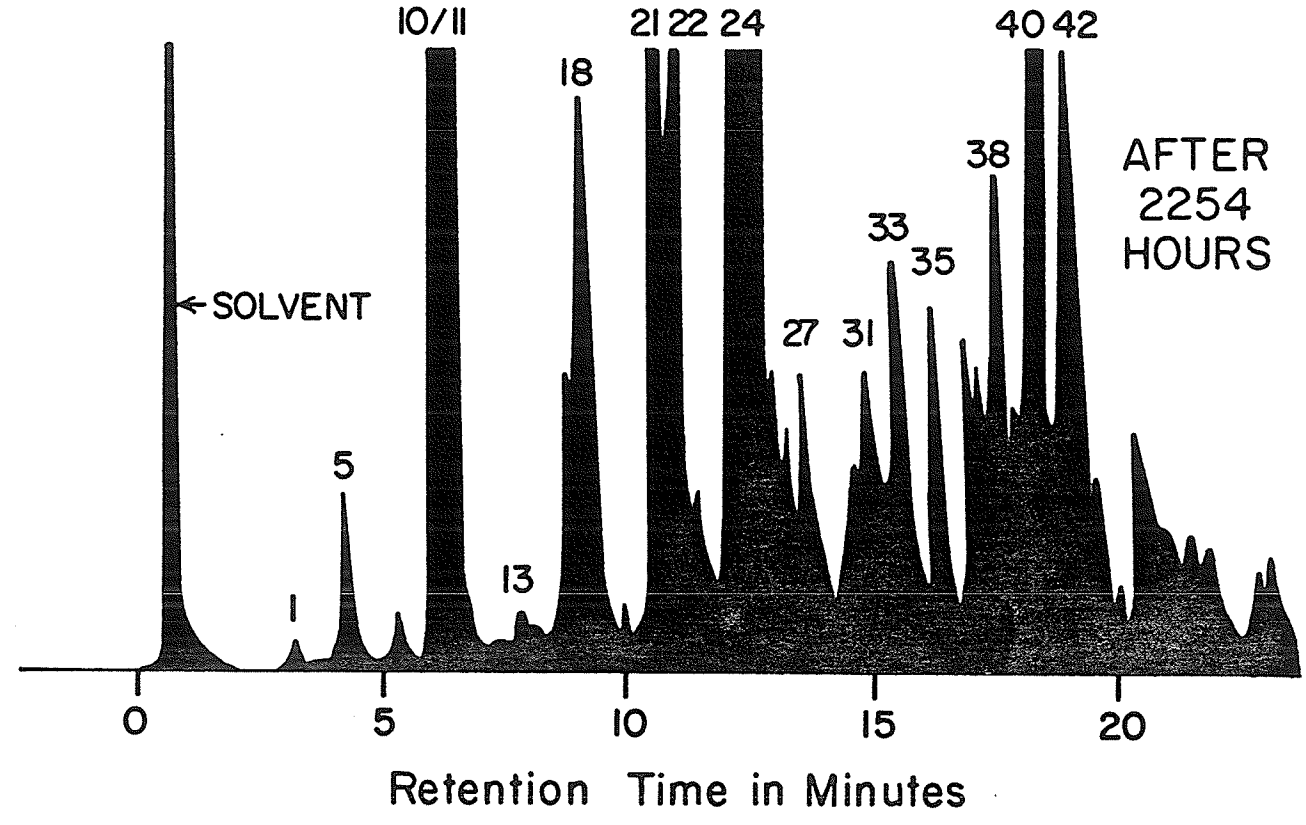
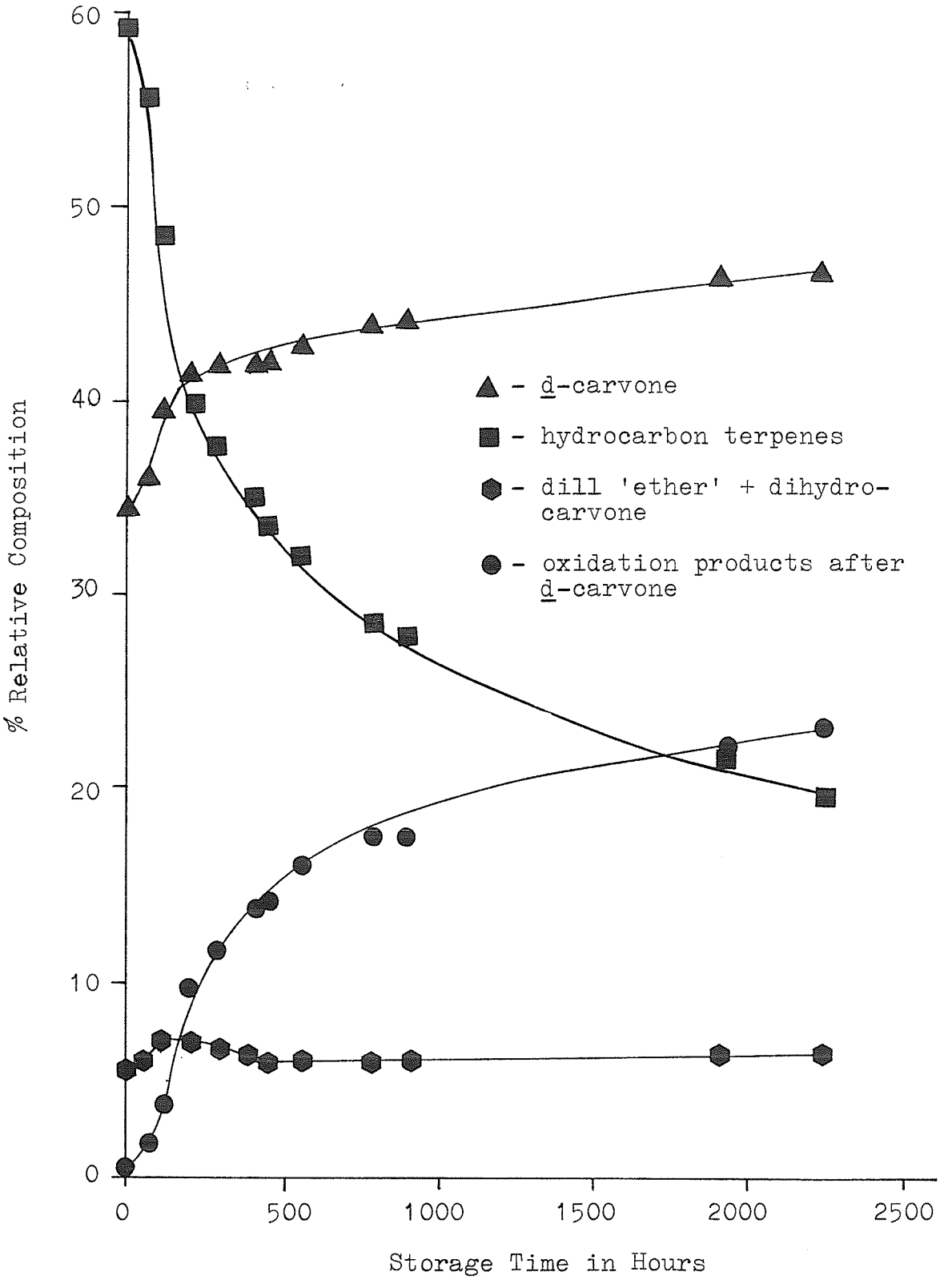


Figure 20. Oxidative Changes in Dill Weed Oil Composition

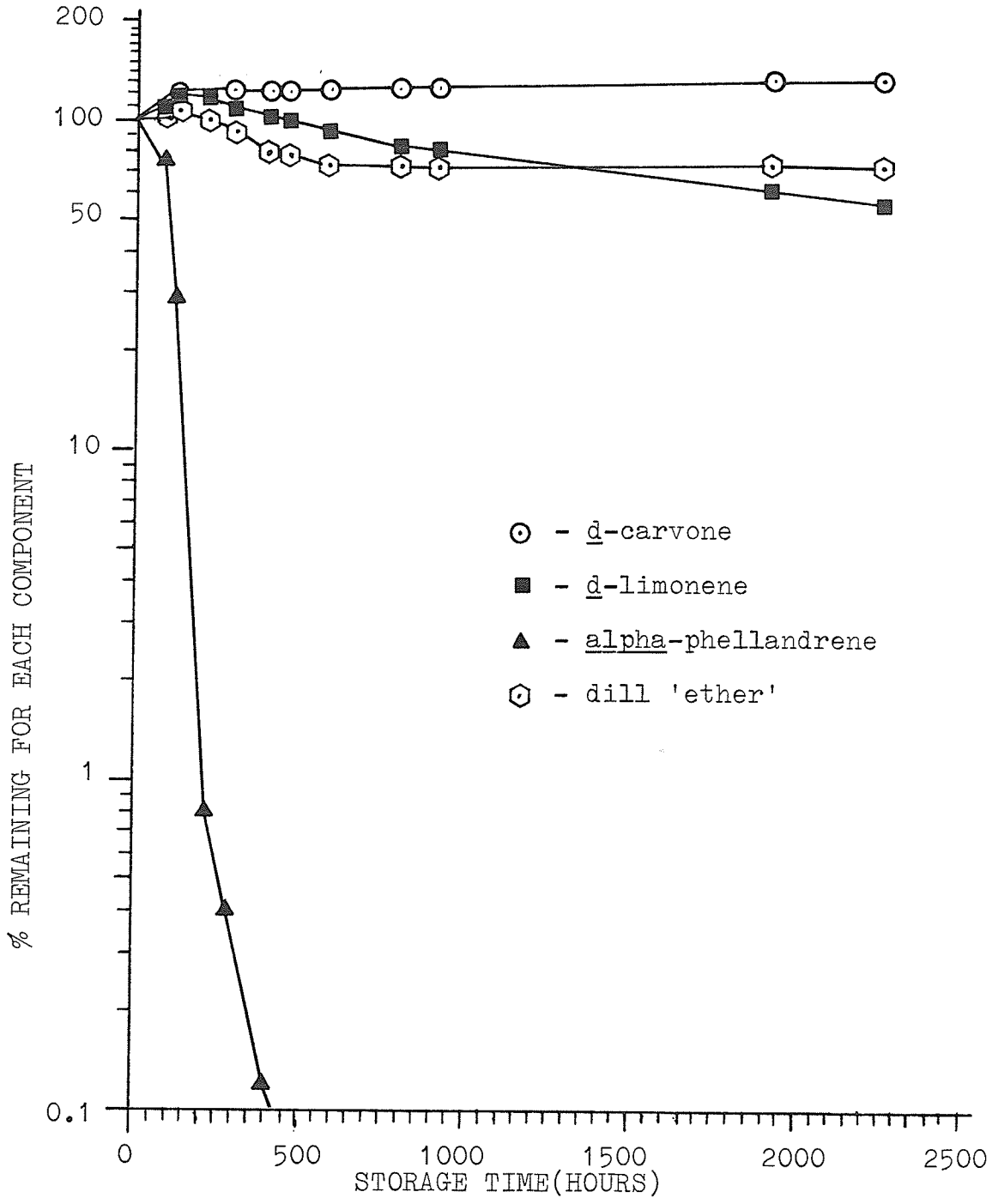


d-limonene, (peak# 11) and dill 'ether', (peak# 21) accounted for the remaining loss of less than 4%. These last-mentioned oxidation products, (peaks# 13-15,17-20) were not included in the summary of Figure 20 since their contribution to the oxidation profile of dill weed oil was very minor. The conversion of the hydrocarbons to carvone was about 30% while the oxidation products with longer retention times accounted for 60%.

The pattern of hydrocarbon decrease appeared to follow first order kinetics as suggested by the significant coefficient of correlation value (r= -0.911) obtained for the semi-log regression analysis of the data versus the direct linear regression analysis value of r= -0.828. The saturated oxygen headspace for the samples ensured that the oxidation reaction was independent of the concentration of available oxygen. The rise in the oxidation products which eluted after carvone appeared to follow pseudo-zero order kinetics (r_{linear} = 0.847) rather than first order kinetics ($r_{semi-log}$ = 0.616) which indicated that their formation was independent of the concentration of the reactant hydrocarbon molecules.

The quantitative change in the four major terpenes in dill oil during storage are shown in Figure 21. The pattern of alpha-phellandrene decrease was indicative of first order kinetics based on the significant semi-log correlation coefficient, r = -0.967; compared to the linear value of: r = -0.876. With the assumption of first order kinetics, the

Figure 21. Storage Changes to the Four Major Dill Oil Components



half-life time for alpha-phellandrene under the prescribed storage conditions was 62 hours. While this terpene was being oxidized, the apparent levels of d-limonene and carvone increased rapidly until most of the alpha-phellandrene had disappeared at the 215 hour point.

After this time, the limonene levels declined while the carvone content continued to increase at a slower rate. The eventual decrease in the d-limonene levels followed first order kinetics, ($r = -0.995$ for semi-log regression analysis), and the half-life time was calculated to be 2700 hours. The rise in d-carvone levels appeared to follow pseudo-first order kinetics ($r_{linear} = 0.824$) which indicated that the formation of carvone was independent of the concentration of the reactant hydrocarbons. The dill 'ether' content dropped initially, then remained at close to 75% of the original levels by the end of the study. This indicated the stability of this epoxide towards further oxidation.

The information obtained from this study was supportive of a 'cascade' effect in which alpha-phellandrene was isomerized to limonene, then oxidized to carvone. The build-up of the other oxidation products was accompanied after 2254 hours with an accumulation of resin in the bottom of the sample vials. This resinification made further GLC analysis very difficult since the resin tended to clog the syringe barrel. However, GLC analysis indicated the disappearance of the oxygenated compounds after carvone from the 2254 hour

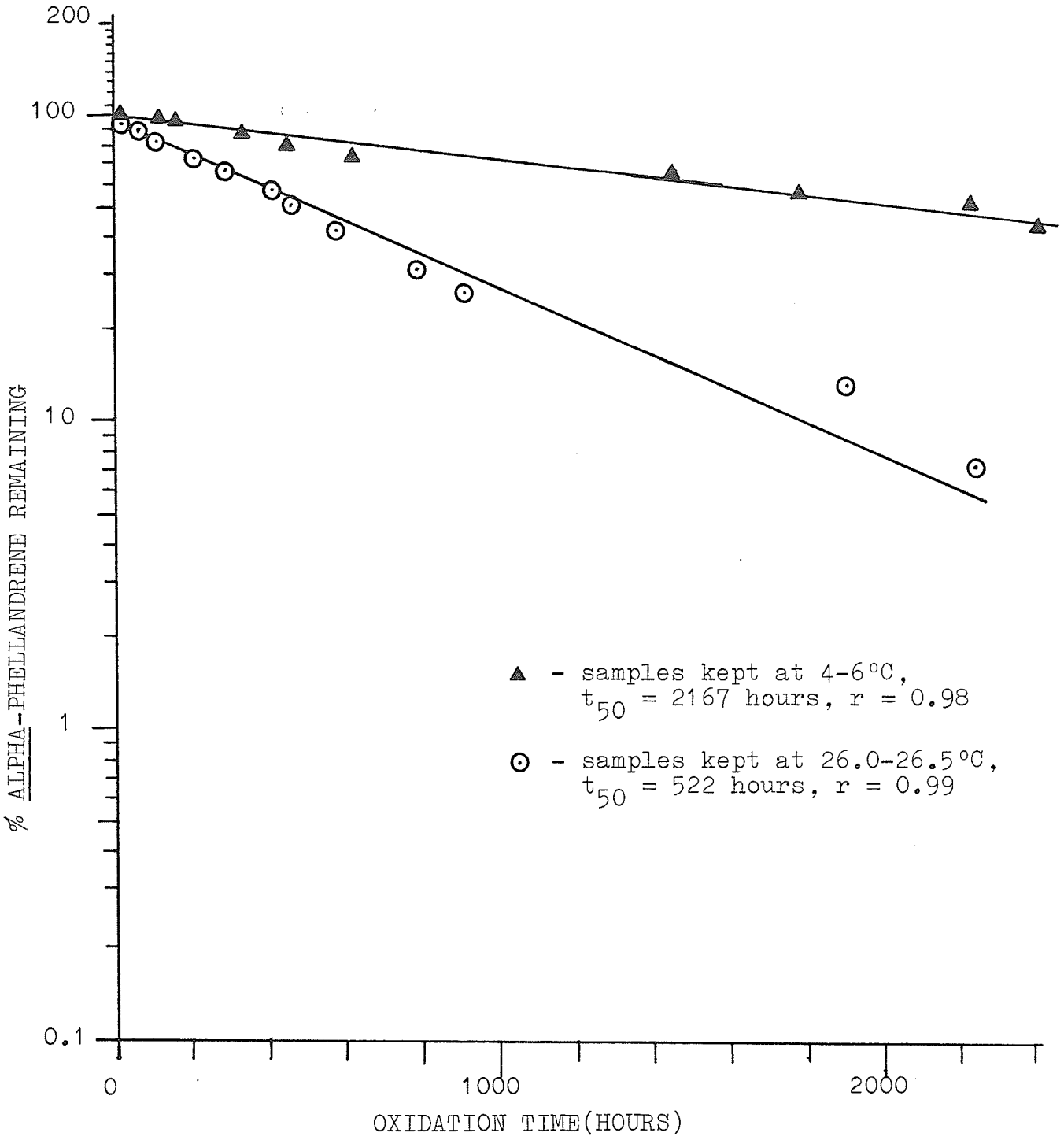
point. This disappearance suggested that these species were polymerized, resulting in the resin formation.

The effect of temperature on the oxidation of dill weed oil was demonstrated by the oxidative lability of alpha-phellandrene. The results, shown in Figure 22 indicated that the disappearance of alpha-phellandrene at room temperature, 26.0+/-0.5°C was four times that observed at refrigeration temperatures, 4-6°C. The practical implications were that every 10°C decrease in temperature would reduce oxidative changes of dill oil during storage by one-half. The decrease in alpha-phellandrene levels were again observed to follow first order kinetics, given the significant semi-log correlation coefficients, ($r = 0.98$-0.99).

The large number of oxidation products arising in stored dill oils prompted the analysis of some of the major products through the use of mass spectrometry. The MS spectrograms for some of these products, (peaks# 31,33,35,36,40 and 42) are presented in Appendices 13 to 17. Since it appeared that these mass spectra were not published, structures for these products were tentatively identified based on the MS fragmentation patterns.

Compounds with formula weights of 152 amu and 168 amu were indicative of the addition of one oxygen atom or molecule, respectively to an unsaturated hydrocarbon monoterpene such as d-limonene, alpha-phellandrene, alpha-pinene or beta-myrcene. These hydrocarbons all have formula weights of 136 amu.

Figure 22. Oxidation of Dill Weed Oil at Two Different Temperatures



Note: Dill oil samples kept in 10 mL vials with 9 mL headspaces.

The generation of these oxidation products during storage also prompted a further investigation into the specific terpene sources responsible for the major oxidation products. The terpene standards: alpha-phellandrene, d-limonene, d-carvone and alpha-pinene were stored for two months at 36.0+/-0.5°C and a saturated oxygen headspace was maintained during this time.

The decrease of these terpenes appeared to follow first order kinetics as shown by their highly significant correlation coefficients(Figure 23). The half-life of the least stable terpene: alpha-phellandrene was 18 hours, and was followed by d-limonene, (t_{50} = 622 hours), alpha-pinene (t_{50} = 1019 hours) and d-carvone (t_{50} = 6887 hours). The results suggested that the bicyclic terpene, alpha-pinene was more stable towards oxidation than d-limonene. Furthermore, d-carvone proved to be susceptible to oxidation, but at a far slower rate than for the other three hydrocarbon terpenes.

Gas chromatograms of the alpha-phellandrene standard before the initiation of the study and after 26 hours are shown in Figure 24. During the oxidation of this standard, a compound was formed which had a slightly shorter retention time than d-limonene. It appeared that this unknown compound was not resolved from the d-limonene in the earlier studies(Figure 21). Thus the apparent increase in the d-limonene levels during the storage experiments was due to the rise in levels of this unknown component. In the original

Figure 23. Oxidative Degradation of Selected Terpenes Found in Dill Weed Oil

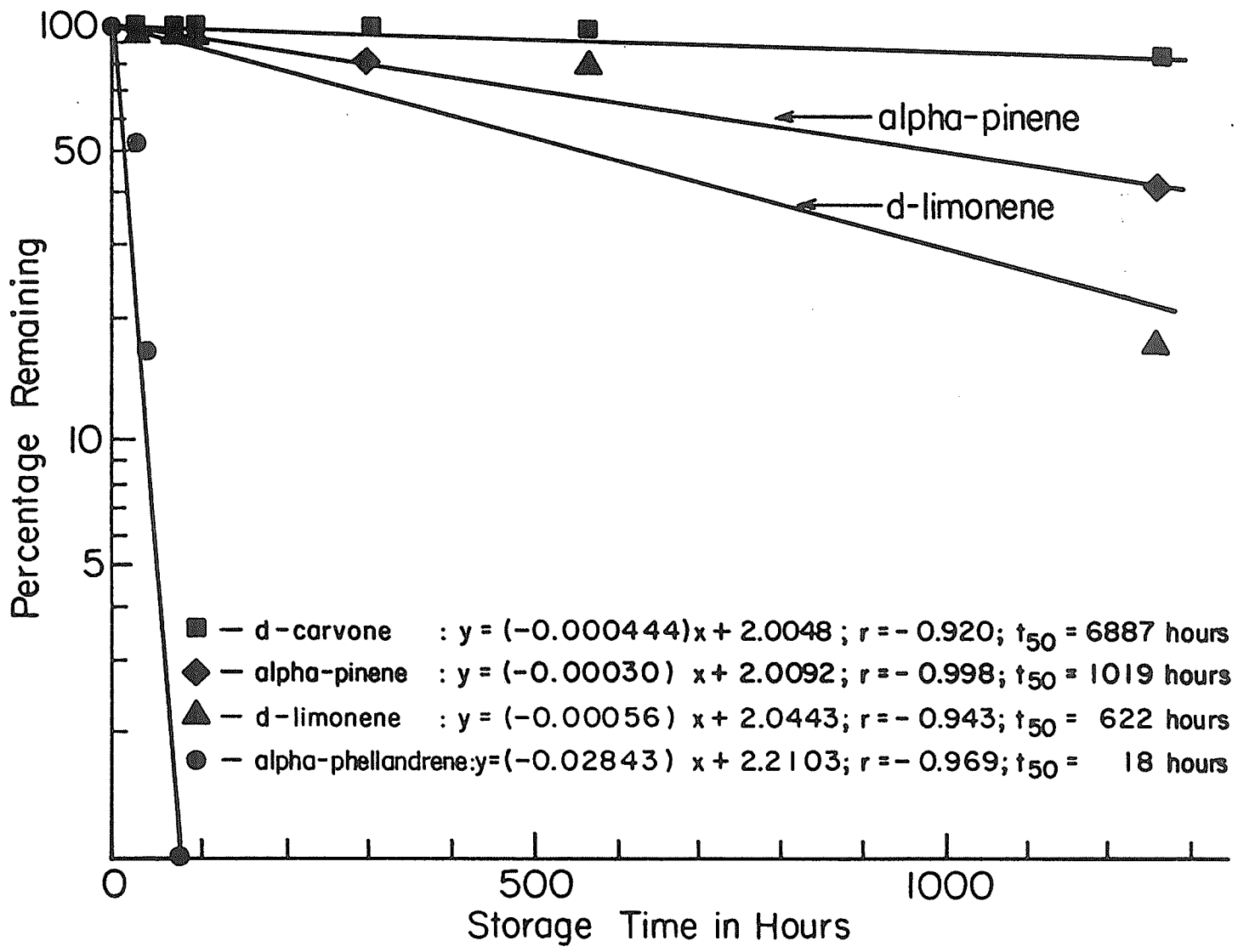
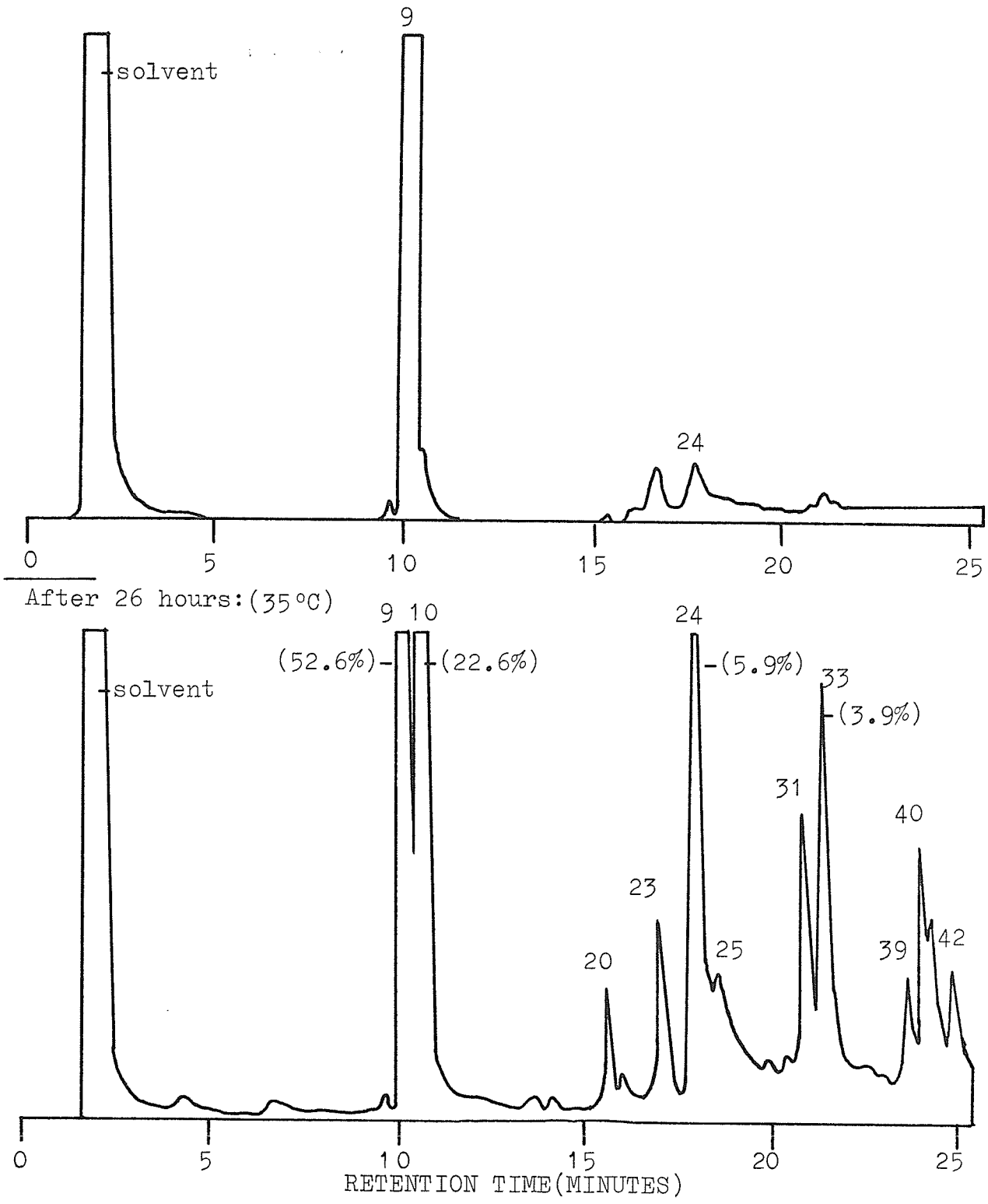


Figure 24. Gas Chromatograms of Alpha-Phellandrene

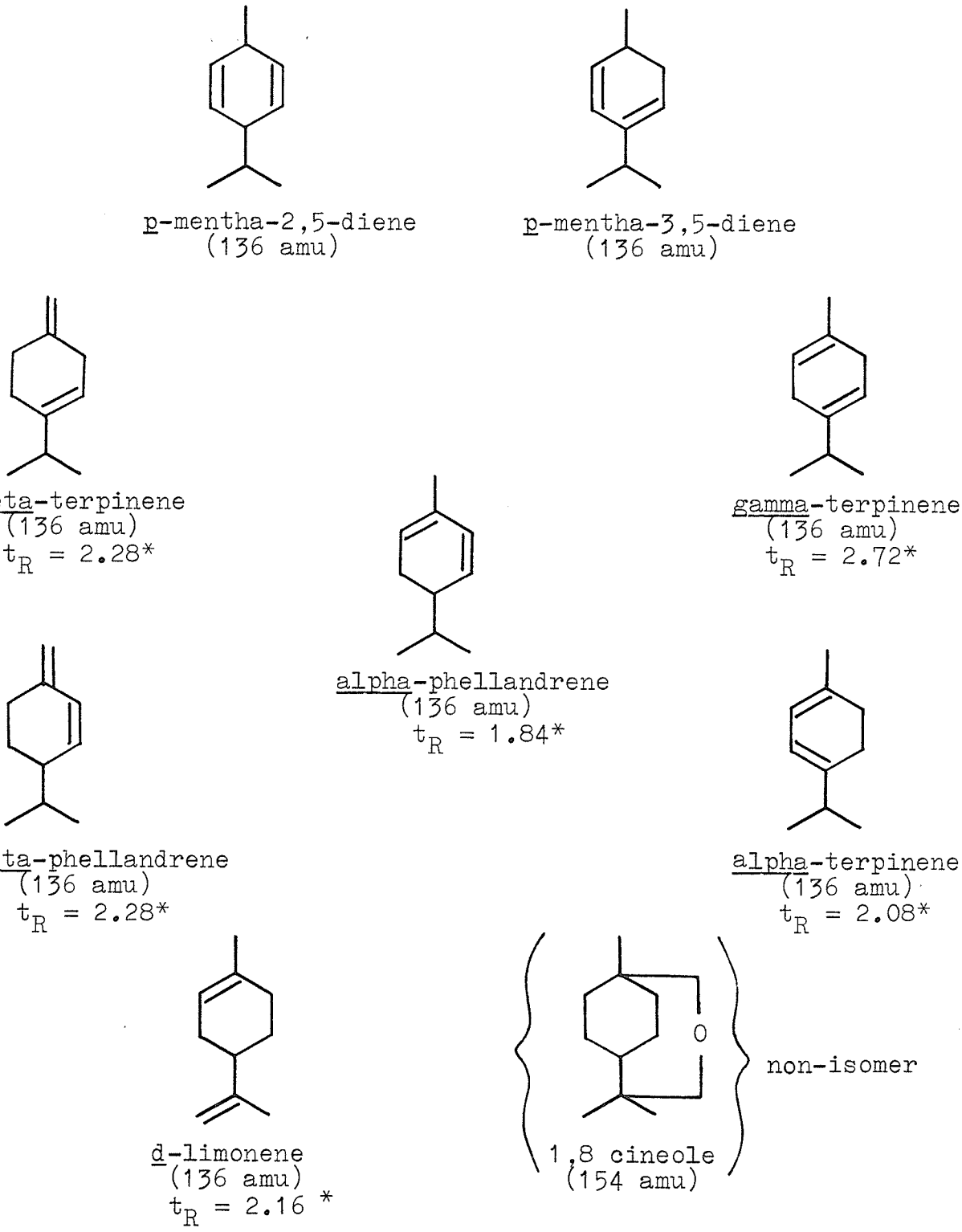


*See Table XIV for the identities of the peaks.

'cascade' hypothesis, the isomerization of alpha-phellandrene to d-limonene would have required the positional shift of a double bond from the ring to the tail section of the molecule. A shift of one of the double bonds to a different position within the ring, or to the methyl head of the molecule would have seemed a more probable consequence of an unsuccessful oxygen collision. An initial GLC investigation of some of the available terpene standards showed that 1,8 cineole(154 amu) had the same retention time as the unknown. However, mass spectrometric analysis of the unknown revealed a formula weight of 136 amu, which disallowed this possibility. The poor resolution of the unknown from d-limonene prevented the derivation of further structural data from the MS spectrogram, but the formula weight indicated that the unknown was an isomer of alpha-phellandrene and not an oxygenated product.

On the basis of this information, it was possible that an unsuccessful oxygen reaction with alpha-phellandrene would result in one of six hydrocarbon isomers proposed in Figure 25. Several of these terpenes were previously submitted to GLC analysis by Burchfield and Storrs(1962). These workers used a silicone oil, didecyl phthalate as the stationary phase. This compound has similar polarities to the methyl silicone stationary phase used in this study. The relative retention times obtained by these authors indicated that gamma-terpinene and beta-phellandrene eluted after d-limonene, making them unlikely candidates for the unknown

Figure 25. Possible Isomers Formed from Alpha-Phellandrene During Storage

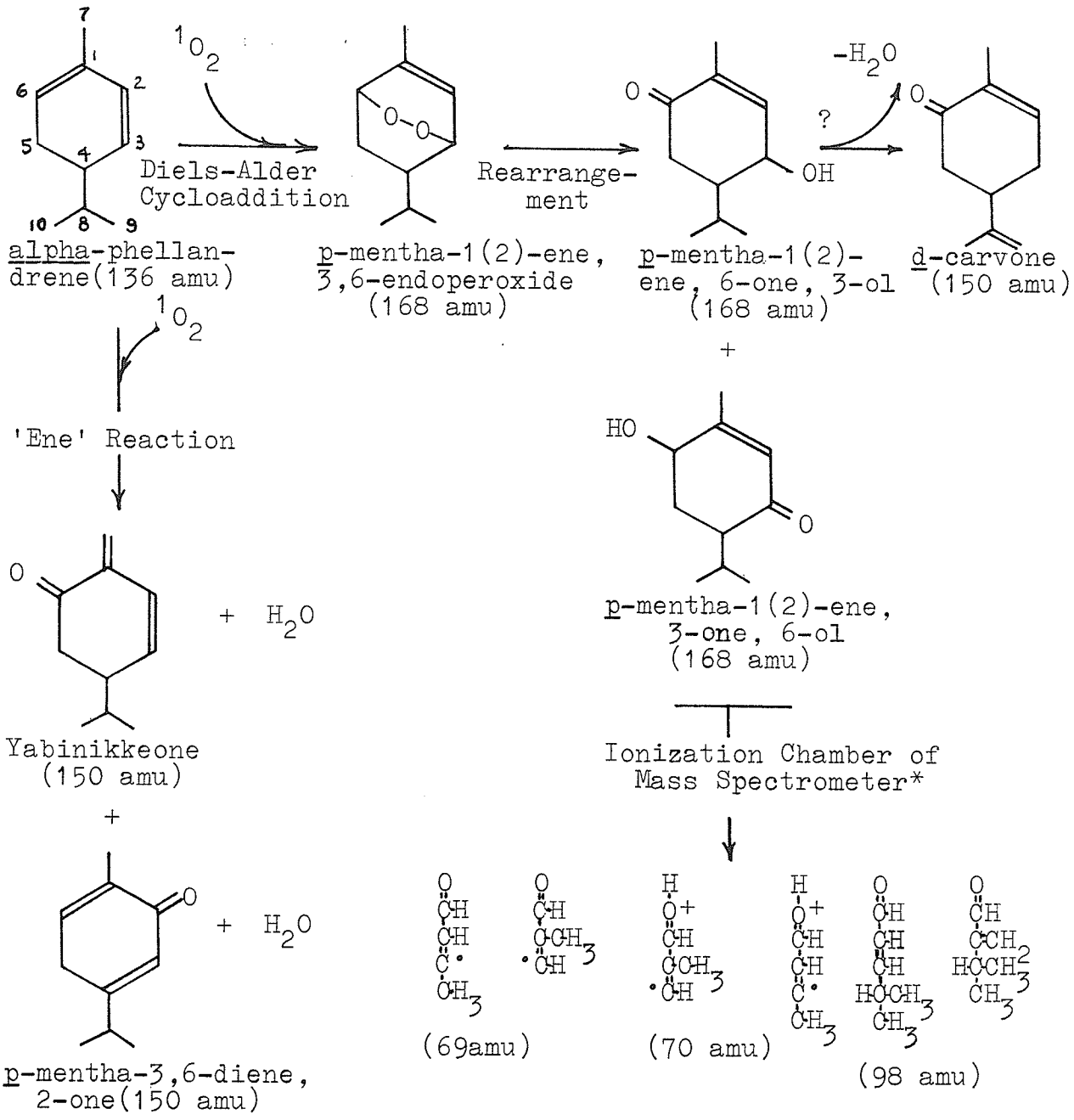


*Values obtained on didecyl pthalate by Burchfield and Storrs. 1962.

isomer. The differences in the retention times from d-limonene also suggested that they would be resolved better from d-limonene than the unknown. By comparison, alpha-terpinene eluted just before d-limonene and the small difference between the two relative retention times(Figure 25) implied that a strong overlap would occur with d-limonene. Added support for the possibility that alpha-terpinene was the identity of the unknown came from Simonsen(1931) who reported that alpha-terpinene was one of the oxidation products of alpha-phellandrene. Based on this information, an oxidized dill oil sample was spiked with alpha-terpinene and a perfect overlap resulted with the unknown peak. The identification of the unknown as alpha-terpinene remains only tentative and will require further confirmation.

The oxidation of alpha-phellandrene produced a series of other products which also appeared in the gas chromatograms of dill weed oil during storage. Carvone proved to be one of the major oxidation products of alpha-phellandrene. For four other products, structures were proposed based on mass spectrometry and singlet oxygen theory. As shown in Figure 26, the Diels-Alder oxidation of alpha-phellandrene would result in the formation of an endoperoxide which might then undergo rearrangement to two more stable structures. One of these rearrangement molecules might be converted to d-carvone following dehydration, although this would likely be a minor oxidation pathway. Both of these rearrangement products may have been present in the oxidized alpha-phel-

Figure 26. Possible Autoxidation of Alpha-Phellandrene



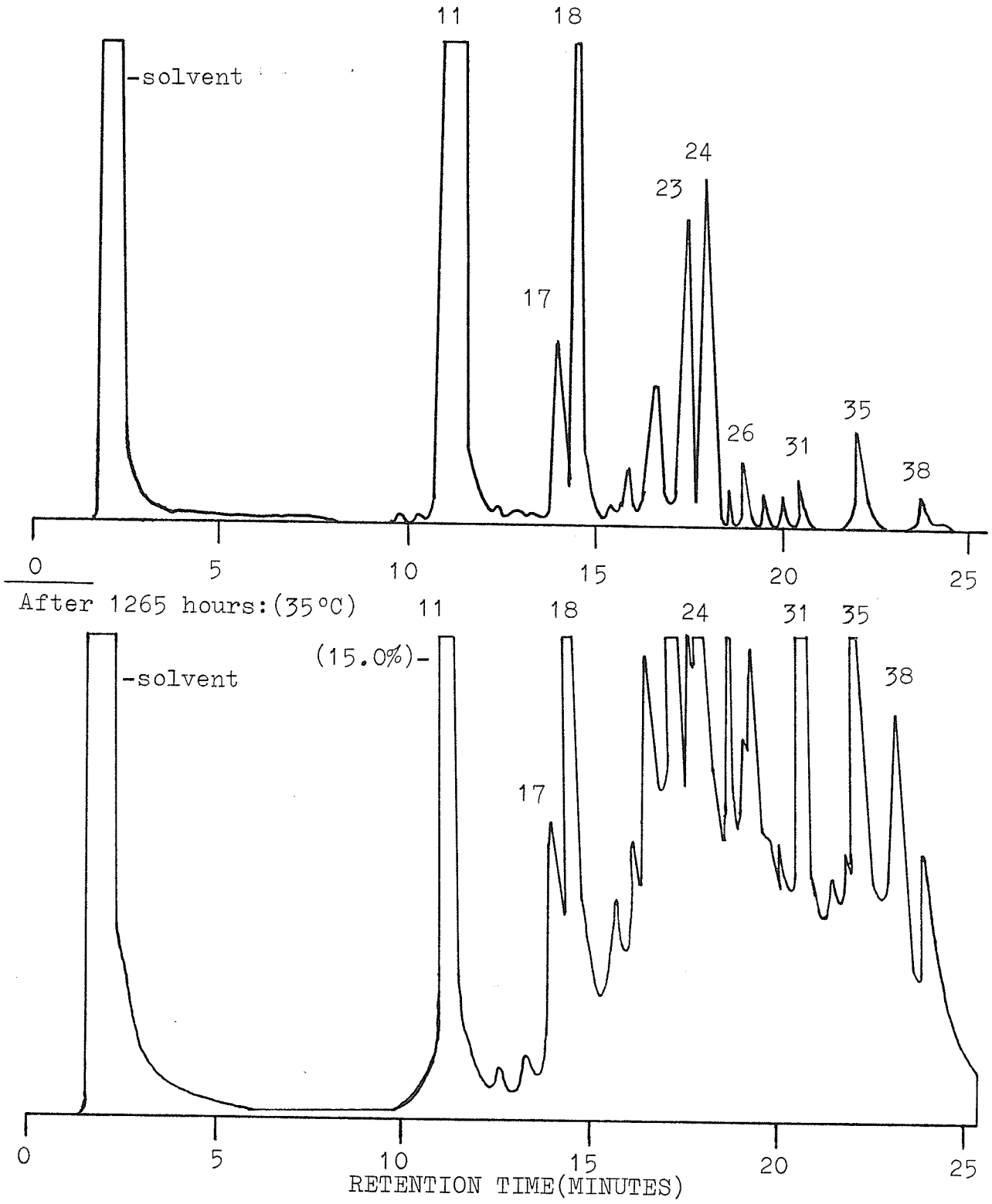
*GLC peaks #40 and #42 in oxidized dill weed oil each gave a molecular ion peak of 168 amu and major MS fragments at 69, 70 and 98 amu.

landrene as two peaks were found, (peaks# 40 and 42); each with a formula weight of 168 amu(Appendix 17). Their major fragmentation peaks, (69,70 and 98amu) corresponded to the possible breakdown products as outlined in Figure 26. The 'Ene' type singlet oxygen reactions also proposed in this figure may have been responsible for the presence of two additional compounds, (peaks# 31 and 33) found in the oxidized standard; each with a formula weight of 150 amu(Appendix 15). Altogether, the four oxidation products described above constituted about 40% of the total oxidative breakdown of alpha-phellandrene during storage.

The d-limonene used in this study was not as pure as the other standards as is evident from the gas chromatogram shown in Figure 27. The impurities appeared to be quite stable and were discounted from the oxidation profile of d-limonene.

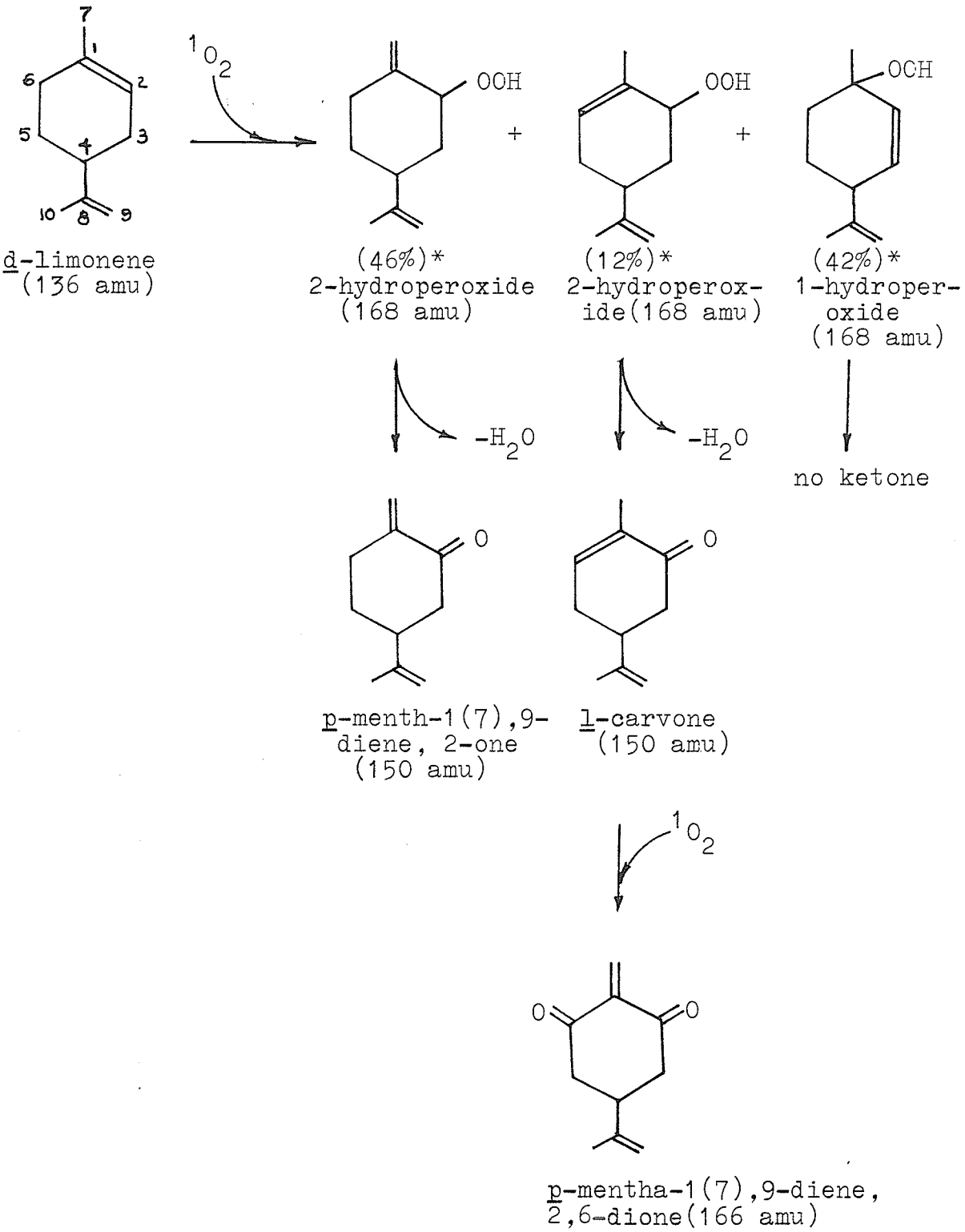
Products formed during storage were thereby considered to be oxidation products of d-limonene. One such product was identified as carvone while two other major products were matched to the following formula weights: 150 amu(peak# 27) and 166 amu(peak# 38). Reports in the literature indicated that d-limonene would undergo 'Ene' type oxidation reactions(Wasserman and Murray, 1979) and the structures of the possible hydroperoxide products in the absence of reducing agents are presented in Figure 28. Only two of the three hydroperoxides can form a ketone product and one of these is l-carvone. The second isomer, p-men-

Figure 27. Gas Chromatograms of d-Limonene



*See Table XIV for the identities of the peaks.

Figure 28. Possible Autoxidation of d-Limonene



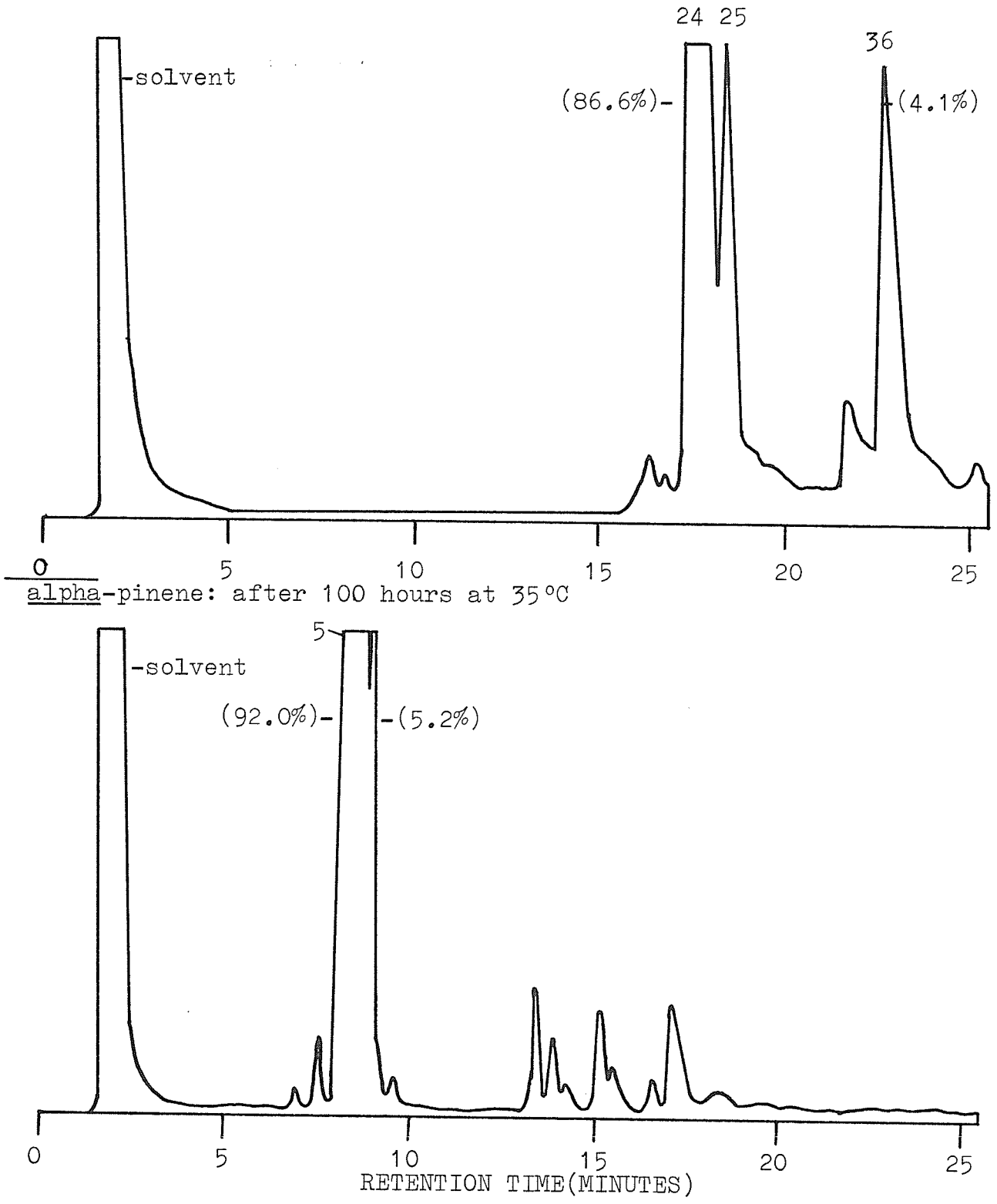
* Data from Wasserman and Murray.(eds.) 1979.

tha-1,(7),9-diene-2-one could be the second major oxidation product observed in the stored d-limonene standard. The 166 amu species may have formed from the further oxidation of l-carvone as shown in Figure 28.

Both of the d-carvone and alpha-pinene standards proved to be very resistant to oxidation and only a few products were formed during the study as shown in Figure 29. Two major compounds were generated from d-carvone and one was matched to a formula weight of 166 amu. This was equivalent to the addition of one oxygen atom to d-carvone, possibly via an 'Ene' reaction illustrated in Figure 30. The second peak was not clearly resolved from carvone and could not be analyzed by mass spectrometry. However, this peak was also identified in stored dill oils. The oxidation of alpha-pinene resulted in the formation of three major products which accounted for 63% of the compounds generated during storage. None of the oxidation products found in dill oil matched the components produced by the alpha-pinene standard. This came as no surprise since alpha-pinene normally accounted for less than 1% of the dill oil composition. One of the major products for alpha-pinene might have been pinocarvone as indicated in Figure 30. An overall summary of the findings for the terpene standard oxidation study is presented in Table XIV.

The dill oil storage studies revealed an order of oxidative stabilities for the unsaturated monoterpenes. The two double bonds within the alpha-phellandrene ring were

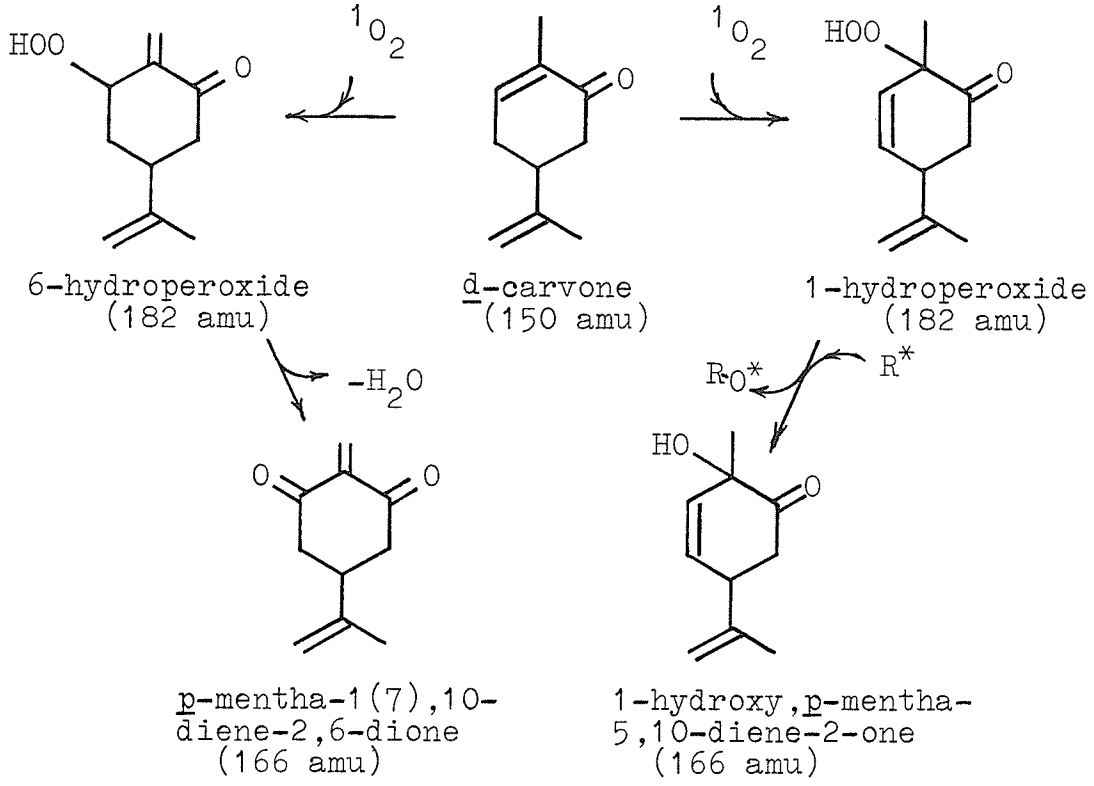
Figure 29. Gas Chromatograms of d-Carvone and Alpha-Pinene During Storage



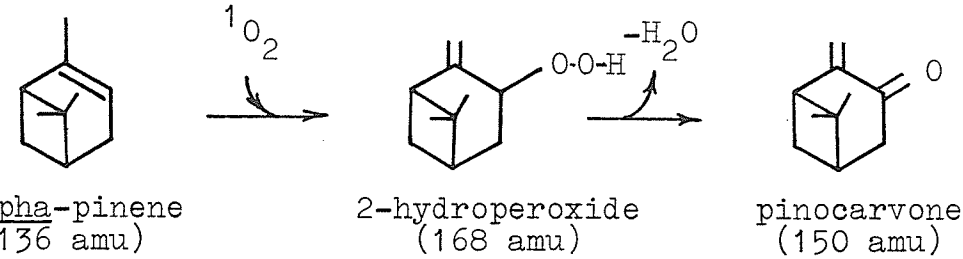
*See Table XIV for the identities of the peaks.

Figure 30. Possible 'Ene' Oxidation of d-Carvone and Alpha-Pinene

d-Carvone:



alpha-Pinene:



* R = reducing agent such as $LiAlH_4$

Table XIV. Summary of Components Found in Oxidized Dill Weed Oil

Peak Number: (1)	RRI: (2)	Formula (3) Weight:	Name:	Oxidized From:
(1)	0.36	142	unknown	unknown
5	0.72	136	alpha-pinene	NA
7	0.82	136	beta-pinene	NA
8	0.85	136	beta-myrcene	NA
9	0.92	136	alpha-phellandrene	NA
(10)	0.96	136	alpha-terpinene (4)	alpha-phellandrene
11	1.00	136	d-limonene	NA
(13)	1.15	150	unknown	unknown
(18)	1.30	152	unknown	d-limonene
(20)	1.40	152	unknown	alpha-phellandrene
21	1.45	152	dill 'ether'	unknown
22	1.49	152	dihydrocarvone	unknown
(23)	1.54	148	unknown	alpha-phellandrene
(24)	1.62	150	carvone	d-limonene + alpha-phellandrene
(25)	1.68	unknown	unknown	d-carvone
(27)	1.75	150	" "	d-limonene
(31)	1.85	150	" "	alpha-phellandrene
(33)	1.89	150	" "	alpha-phellandrene
(35)	1.97	152	" "	d-limonene
(36)	2.05	166	" "	d-carvone
(38)	2.08	166	" "	d-limonene
(39)	2.11	unknown	" "	alpha-phellandrene
(40)	2.14	168	" "	alpha-phellandrene
(41)	2.16	unknown	" "	alpha-phellandrene
(42)	2.21	168	" "	alpha-phellandrene
(43)	2.25	unknown	" "	alpha-phellandrene

(1) Peaks in brackets formed during storage; underlined peaks indicate dill weed oil components.
(2) d-Limonene = 1.00; all runs made on 3.70 m s.s. column.
(3) Based on MS analysis.
(4) Tentatively identified. NA = not applicable.

more susceptible to oxidation than for the single ring double bond in d-limonene and alpha-pinene. The fact that alpha-phellandrene could undergo Diels-Alder as well as 'Ene' type oxidation reactions may have contributed to this greater susceptibility to autoxidation. Although both d-limonene and alpha-phellandrene were partially converted to carvone, there were other oxidation products formed which could be detrimental to the quality of the oils. The deliberate aging of an immature oil to achieve a higher carvone content would also result in the build-up of these additional oxidation products. Furthermore, extended storage of the oils will result in physico-chemical changes including the formation of resins. These resins may arise from the polymerization of the oxidation products formed during storage which would be another important reason why dill oil should not be allowed to age. The resin build-up would require a removal stage, (rectification) before the oil could be used. The loss of product and increased costs due to rectification would lower the economic value of the oils.

4.3 Effect of Contaminants on the Quality and Stability of Dill Weed Oil

The objective of this section of the study was to determine the effect of weed contaminants on the initial

composition of dill oil as well as on the long-term stability of the oil. The approximate distillate yields of dill and four common weed varieties found in Manitoba are compiled in Table XV. The results showed that the distillate yields for kochia, lamb's quarters and Canada thistle were about 150 times lower than that for dill while the wormwood oil yield was only five times lower. The results suggested that only wormwood could significantly alter the initial composition of dill oil if large amounts of this weed were present in a dill field. Table XVI compares the degree of weed contamination in a hypothetical field with the contribution of the four different weed distillates to the dill oil composition. Again, only wormwood oil would significantly alter the initial dill oil composition if considerable amounts of this weed were present. However, the distillate yield information could provide no information on the long-term effects of the contaminants.

The four weed distillates were analyzed by gas chromatography. Canada thistle oil contained two prominent components which accounted for 50% of the total composition. The lamb's quarters and kochia oils contained numerous components but the identities of these compounds as well as those for Canada thistle were not determined as they did not match any of the 23 terpene references available. The only oil in which terpenes were identified was wormwood oil(Figure 31), which contained beta-pinene(3.4%), beta-myrcene(11.5%) and thujone(27.2%).

Table XV. Distillate Yields for Local Weed Varieties

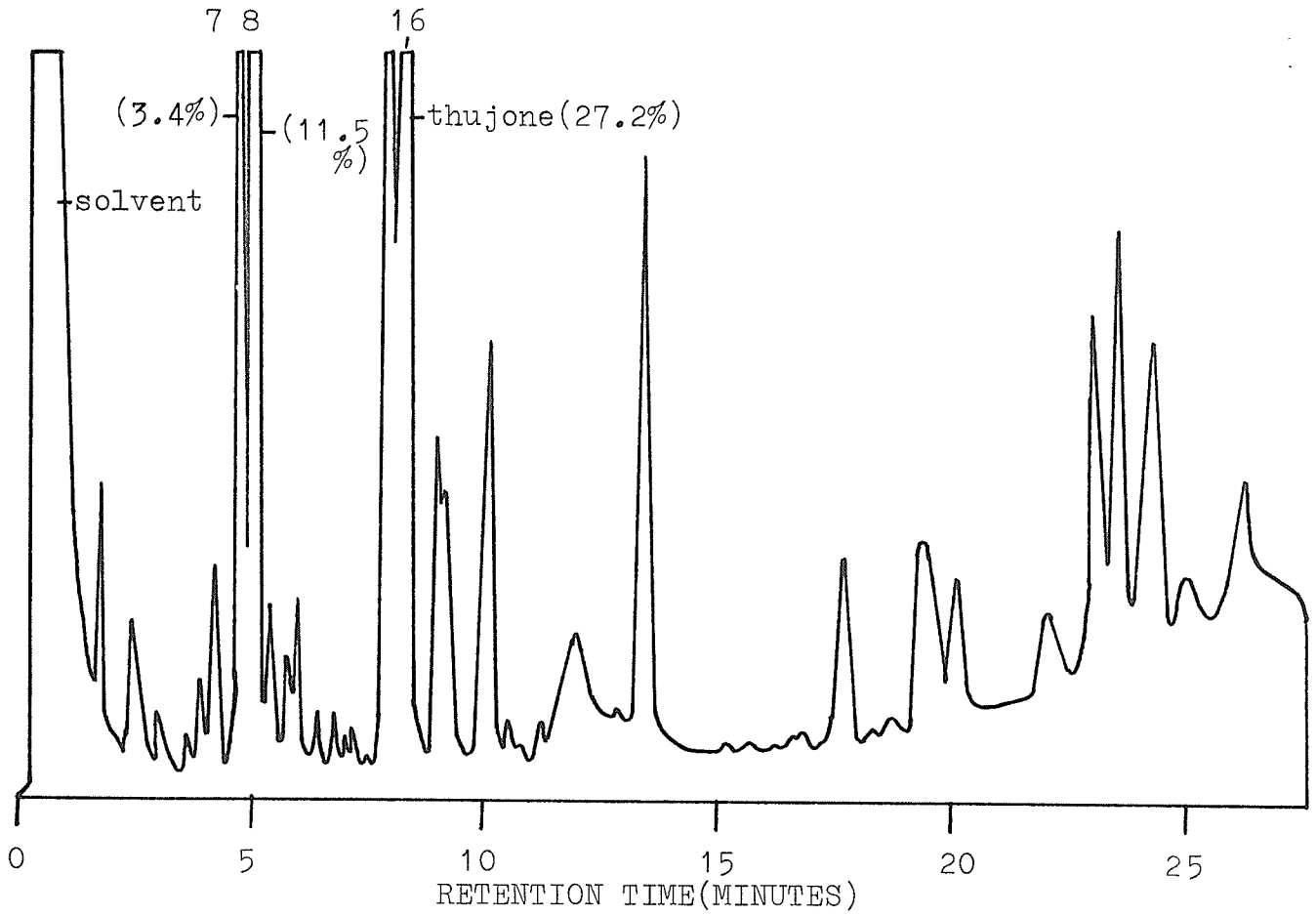
Weed Variety:	Distillate Yield(mL/ g X 100):
kochia(*Kochia scoparia*)	0.0025%
lamb's quarters (*Chenopodium album*)	0.0032%
Canada thistle (*Cirsium arvense*)	0.0030%
wormwood (*Artemesia absinthium*)	0.096%
dill weed (*Anethum graveolens*)	0.48%

Table XVI. Field Weed Infestation Versus Distillate Contamination Levels

Plant Mass Ratio:	% of Contaminant in Distillate(V/V):			
Dill:Contaminants	wormwood	kochia	lamb's quarters	Canada thistle
50/50	17%	0.52%	0.66%	0.62%
60/40	11%	0.35%	0.44%	0.41%
70/30	7.9%	0.22%	0.28%	0.27%
75/25	6.2%	0.17%	0.22%	0.21%
80/20	4.8%	0.13%	0.17%	0.16%
85/15	3.4%	0.092%	0.12%	0.11%
90/10	2.2%	0.058%	0.074%	0.069%
95/5	1.0%	0.027%	0.035%	0.033%
99/1	0.20%	0.0053%	0.0067%	0.0063%

Figure 31. Gas Chromatogram of Wormwood Distillate

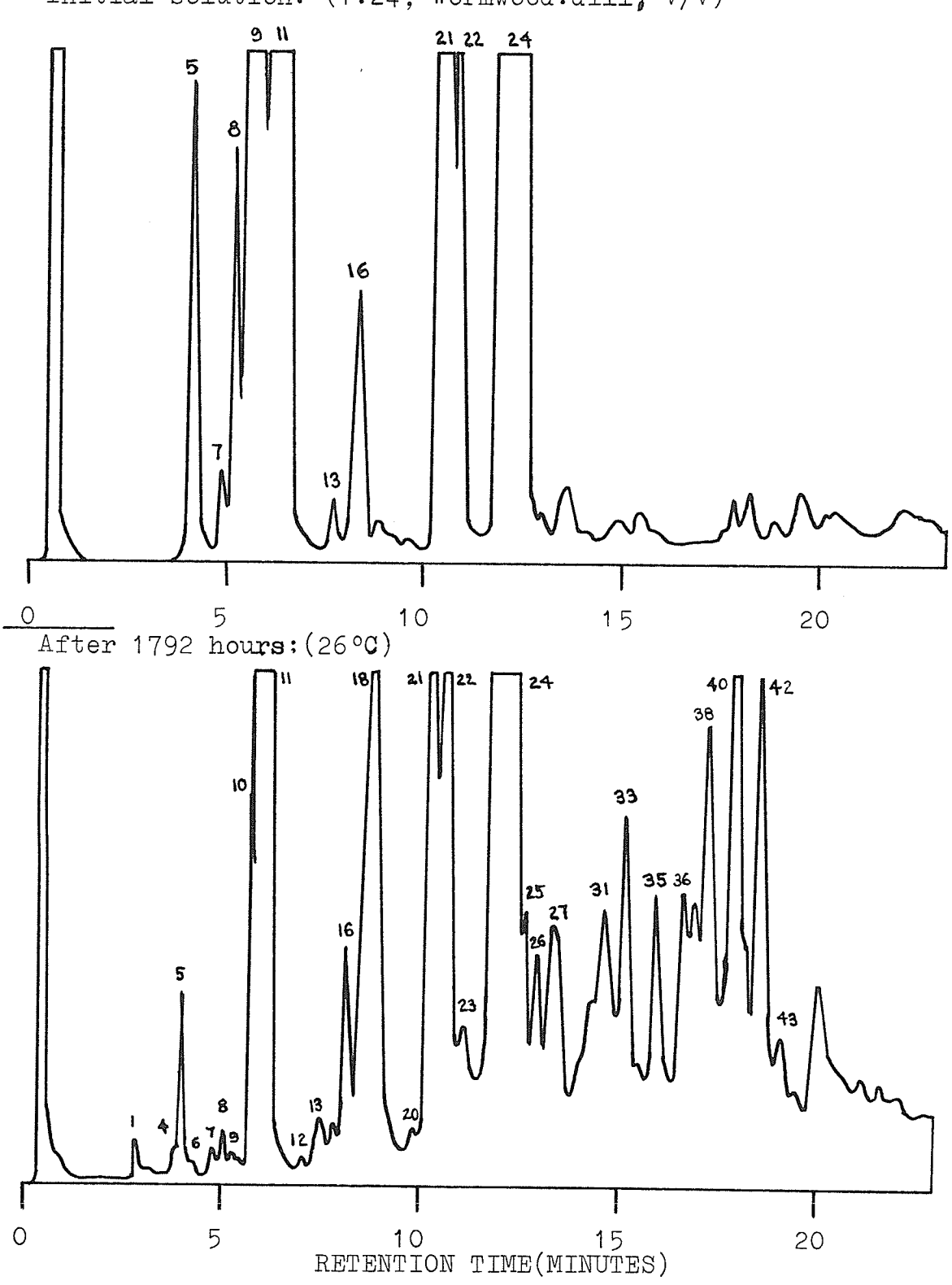
*See Table XIV for the identities of the peaks.



Thujone, a bicyclic monoterpene proved to be the major component in wormwood oil and was identified by mass spectrometry, (Appendix 18). This confirmed the findings reported by Masada(1976) that thujone was the major component in Artemesia absinthium distillate and could reach levels as high as 71%. Since this terpene is not found in dill oil, it is an excellent marker compound indicating the presence of wormwood oil in a contaminated sample of dill oil. The contamination level can be as low as 1%(V/V) for the thujone to be identified by gas chromatography. This would represent a fresh plant weight ratio of 19:1 in favour of dill. Wormwood oil also contains a green pigment which was shown to discolour dill oil samples in concentrations as low as 1%(V/V). In addition, wormwood oil imparts a strongly herbaceous odour, quite different from that of dill weed oil.

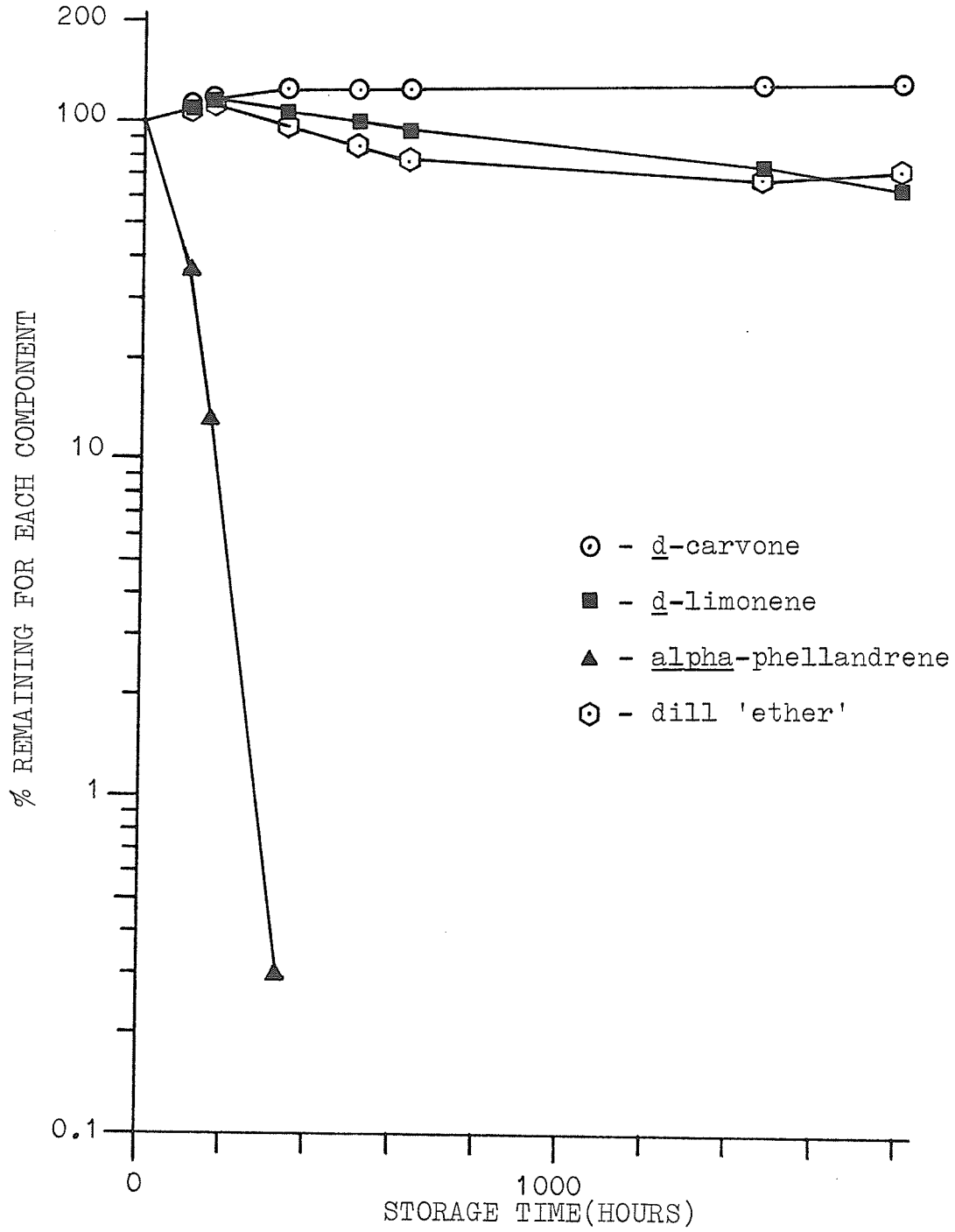
On the basis of the above information, a preliminary oxidation study was carried out using dill oil samples contaminated with wormwood oil. The concentration of the contaminant was 4%(V/V) which would be obtained from material harvested from a field with 20% wormwood infestation. Two gas chromatograms of the contaminated samples taken at different storage times are illustrated in Figure 32 and give a similar oxidation profile to that obtained with pure dill weed oil, (Figure 19). The changes to the four major dill terpenes in the contaminated samples, (Figure 33) were very similar to the results obtained for the pure dill oil study,

Figure 32. Gas Chromatograms of Dill Oil Contaminated with Wormwood Distillate



*See Table XIV for the identities of the peaks.

Figure 33. Storage Changes to the Four Major Dill Terpenes in Wormwood-Contaminated Dill Oil



(Figure 21). The oxidation rates appeared to follow first order kinetics and the half-life for alpha-phellandrene was calculated to be 66 hours for the contaminated samples compared to 62 hours for pure dill oil. The final dill 'ether' levels were 72% of the initial levels in the contamination study versus 75% for the pure dill oil. Initially, carvone levels increased rapidly while the alpha-phellandrene component oxidized, then increased at a slower rate while the d-limonene levels dropped. The decrease in d-limonene content beyond 160 hours seemed to follow first order kinetics as indicated by the highly significant semi-log correlation coefficient of 0.990. Assuming first order kinetics, the half-life for d-limonene was calculated to be 2700 hours, the same value obtained during the pure dill oil study.

Overall, the results obtained in this preliminary study were very similar to the data acquired in the pure dill oil study. This indicated that wormwood oil had no apparent catalytic effect on the oxidation of the labile dill hydrocarbons. However, the storage of the contaminated samples using saturated oxygen headspaces did not parallel the actual storage conditions under which the commercial oils are kept.

As a result, the main contaminant study was an attempt to approximate the actual storage conditions of dill oil on a small scale. The distillates for all four weed varieties, (kochia, lamb's quarters, Canada thistle and wormwood) were used in this study. The distillates were mixed with a pure

dill oil control and the treated solutions were prepared to reflect unusually heavy field contamination conditions. The wormwood concentration, 1:29(V/V) was analogous to a field infestation of 17% while the level of each of the other contaminants was 1:149(V/V), representative of a field contamination of 50% based on plant weight. The samples were kept in screw-top vials in the dark at room temperature, (20-30°C). Compositional weight changes of the four, major terpenes, (d-carvone, d-limonene, dill 'ether' and alpha-phellandrene) were calculated from the GLC data and the individual weights. Compositional values were used rather than just the GLC peak areas since weight analysis during the study showed that evaporative losses were quite significant by the end of the study.

The major dill terpene levels for the different treatments were analyzed separately using a split-plot analysis of variance(Appendix 29 and 30). The analysis revealed that the most significant factor affecting the individual terpene levels was storage time. Table XVII summarizes the individual treatment means for the four major terpenes. Only the d-limonene content was significantly lower for the contaminated samples compared to the control. However, the ANOVA summary for d-limonene(Appendix 29) indicated that the replicates also had significant differences. Moreover, the interaction between the treatments and storage times was not significant. In spite of this, the interaction means for d-limonene and d-carvone are shown in Table XVIII along with

Table XVII. 'Shelf-Life' Study: Treatment Means for the Four Major Dill Terpenes

Treatment:	Alpha-Phellandrene:	d-limonene:	d-carvone:	dill 'ether':
Control	75.5% A	86.1% A	102.9% A	91.0% AB
Wormwood	67.3% A	79.1% B	101.9% A	92.2% A
Lamb's Quarters	62.9% A	78.4% B	102.4% A	91.6% AB
Kochia	52.5% A	78.2% B	104.9% A	90.7% B
Canada Thistle	66.1% A	77.7% B	102.0% A	91.4% AB

Note: Means with the same letter designation are not significantly different at the 5% level of confidence.

Table XVIII. 'Shelf-Life' Study: Summary of d-Limonene and d-Carvone Levels for Different Treatments

1. d-Limonene*

TREATMENT:	TIME (DAYS)						
	0	40	87	141	195	272	361
Control	100.0 a	98.4 a	95.6 ab	88.7 abcde	78.4 cdefg	77.3 defg	64.3 gh
Wormwood	100.0 a	96.7 ab	96.8 ab	69.8 fgh	85.0 abcdef	70.2 fgh	35.0 j
Lamb's Quarters	100.0 a	95.0 abc	92.8 abcd	81.2 bcdefg	68.2 fgh	70.9 fgh	40.7 ij
Kochia	100.0 a	111.6 a'	99.4 a	75.1 efg	65.4 gh	54.3 hi	41.8 ij
Canada Thistle	100.0 a	97.8 ab	92.0 abcde	76.2 defg	67.4 gh	71.7 fg	38.8 i

2. d-Carvone

TREATMENT:	TIME (DAYS)						
	0	40	87	141	195	272	361
Control	100.0 a	102.2 abc	102.3 abc	103.8 abcd	106.4 abcd	103.2 abcd	102.7 abc
Wormwood	100.0 a	100.7 a	99.8 a'	102.0 ab	103.6 abcd	102.4 abc	104.8 abcd
Lamb's Quarters	100.0 a	100.3 a	98.7 a'	100.6 a	104.4 abcd	110.4 bcd	102.6 abc
Kochia	100.0 a	111.6 cd	112.5 d	100.6 a	104.2 abcd	101.1 ab	104.2 abcd
Canada Thistle	100.0 a	100.9 ab	99.6 a'	101.0 ab	105.1 abcd	103.2 abcd	104.2 abcd

*Means (of two replicates) represent % of original composition remaining after the given storage time. Means with the same letter grade are not significantly different at the 5% level of confidence. LSD test used to determine differences.

the significance between the means at the 5% level of confidence. All of the contaminated solutions gave lower d-limonene levels for the final analysis when compared to the control, yet the d-carvone levels had not significantly increased for these samples by this time. This required an investigation into the build-up of the other oxidation products for the different treatments. The results showed that by the end of the study these other oxidation products comprised less than 2% of the total composition for each of the samples. Therefore the total accumulation of oxidation products for both the control and contaminated samples were similar, and this suggested that the losses in the d-limonene levels were due to irregularities in evaporation rather than oxidation. As a result, the contaminants appeared to have little if any catalytic effect on the oxidation of the labile dill terpenes for the one year study period.

An analysis of the absolute weight losses for the control and contaminated samples showed that the evaporation rates were almost identical for all the samples, (Ø.9 mg/vial/day). The overall weight losses also corresponded very well to the calculated drops in hydrocarbon weights for the different treatments. The lower boiling point terpenes, (d-limonene, alpha-phellandrene, alpha-pinene and beta-myrcene) were therefore preferentially lost through evaporation around the screw-cap liners on each sample vial. The loss of these components also permitted air to permeate into each vial. By the end of the study the carvone levels for each

sample had increased by an average of 14 mg. This would have required the equivalent of 5.2 mL of air at 25°C and one atmosphere of pressure, (24.465 L/mole of air). The headspace in each vial was only about 1.4 mL, and so the amount of air required was 3.7 times the initial headspace value. With the assumption that the concentration of oxygen in each headspace remained the same at the end of the study, then the oxygen diffusion rate into each vial was at least 3.Ø microliters^3/vial/day to account for the carvone increase. The rise in levels of the other oxidation products would have meant that the actual diffusion rate was slightly higher and an estimation placed the actual diffusion rate at 4.Ø microliters^3/vial/day. The extent of oxidation was not limited to the oxygen in the headspace, but was dependent on the oxygen diffusion rate through each of the cap liners. This diffusion rate proved to be a critical factor in determining how much oxygen was available during storage.

The results confirmed the findings of the preliminary contamination study involving wormwood oil. In fact, none of the four weed distillates exerted any significant catalytic effect on the dill terpenes during storage. However, evaporative losses were observed as a result of the deterioration of the rubber cap liners used on the vials. Both the dill terpenes and the weed volatiles may have been responsible for this deterioration. Therefore, cap linings which may be used on commercial dill oil drums could also be sus-

ceptible to deterioration and should be examined periodically. Otherwise evaporative losses might increase, and this in turn could permit more oxygen to diffuse into the drums. This study also showed that evaporative losses preferentially involved the hydrocarbon terpenes; especially alpha-phellandrene and d-limonene. Heath(1978) and Guenther(1950) reported that the typical odour and flavour of dill weed oil is due to the content of alpha-phellandrene. Losses through evaporation would increase the 'seed' character of the oils which would lower the commercial value of the herb oil according to these authors.

4.4 Effect of Selected Anti-Oxidants on the Stability of Dill Weed Oil

The previous studies indicated that oxidation of dill weed oil during prolonged storage would occur. Anti-oxidants are permitted for use in essential oils and two anti-oxidants were investigated in a storage study. Alpha-tocopherol acetate, a Vitamin E derivative, and tert-butyl, hydroxy anisole(BHA) were added separately and in combination to pure dill weed oil to the maximum allowable concentration of 0.125% in accordance with the Canadian Food and Drug regulations. A dill oil reference was selected which contained high levels of unsaturated hydrocarbon ter-

penes(79.8%) and a low level of d-carvone(13.8%). Previous studies had shown that the hydrocarbon terpenes were prone to oxidation. The samples were stored with a saturated oxygen headspace at 36.Ø +/- Ø.5°C.

The study revealed that the alpha-tocopherol acetate was an ineffective oxidative inhibitor in dill weed oil, and may in fact have exerted a slight pro-oxidant role, (Table XIX, Appendices 31 and 32). In comparison, the addition of BHA resulted in eight-to-ten times lower oxidation rates for alpha-phellandrene under the same storage conditions as the other sample(Figure 34). The samples treated with BHA appeared to follow pseudo-zero order kinetics for the oxidation of alpha-phellandrene throughout the 26Ø hour study period. This was evident from the high correlation coefficient for the linear regression analysis of the decrease in alpha-phellandrene levels over time, (r = Ø.994). The rate of decrease therefore appeared to be independent of alpha-phellandrene concentration during the study period. The rate of decrease was calculated to be 11 mg/day for the Ø.125% BHA samples and 15 mg/day for the samples containing Ø.625% BHA and Ø.625% alpha-tocopherol acetate. BHA seemed to actively interfere in the oxidation mechanism for this hydrocarbon terpene.

Both the control and the samples containing Ø.125% alpha-tocopherol acetate exhibited pseudo-first order kinetics for the decrease in alpha-phellandrene according to their respective semi-log correlation coefficients, (r =

Table XIX. Anti-Oxidant Study: Treatment Means for the Four Major Dill Terpenes

Duncan's New Multiple Range Test[1]

Treatment:	COMPONENT:[4]			
	alpha-Phellandrene:	Intermediate:[5]	d-Limonene:	d-Carvone:
Control[2]	16.2% a	11.9% a	35.9% a	18.8% a
0.125% Toc[3]	14.4% a	12.3% a	35.0% b	18.7% a
0.125% BHA	32.6% b	5.6% b	34.4% c	15.4% b
0.062% Toc + 0.062% BHA	31.8% b	5.7% b	34.4% c	15.9% b

(1) Means with the same letter grade are not significantly different at the 5% level of confidence.

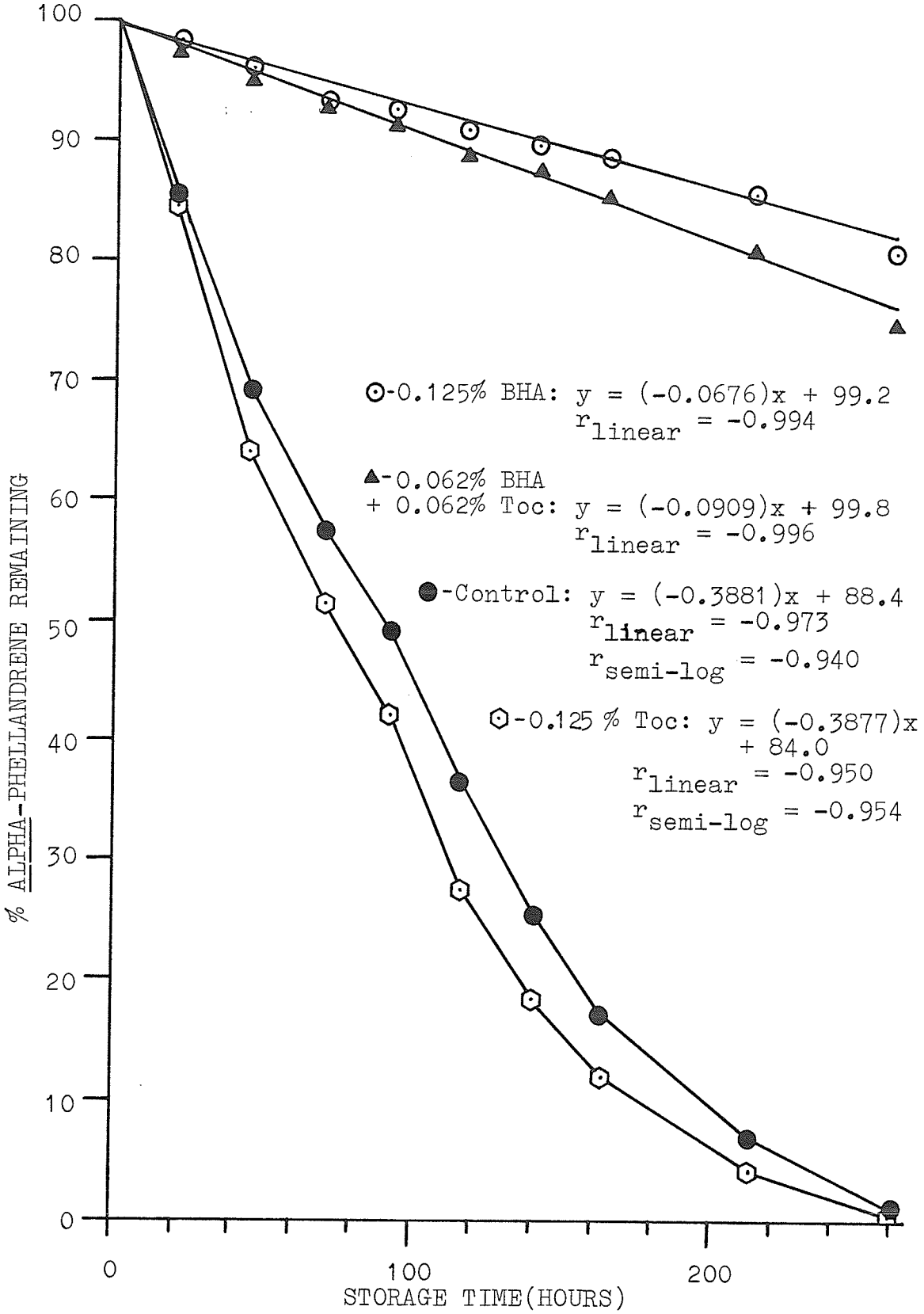
(2) Untreated, pure dill weed oil sample.

(3) Toc = alpha-tocopherol acetate.

(4) Mean values represent % of total GLC composition.

(5) Intermediate tentatively identified as alpha-terpinene.

Figure 34. Effect of Anti-Oxidants on Alpha-Phellandrene Levels in Stored Dill Weed Oil



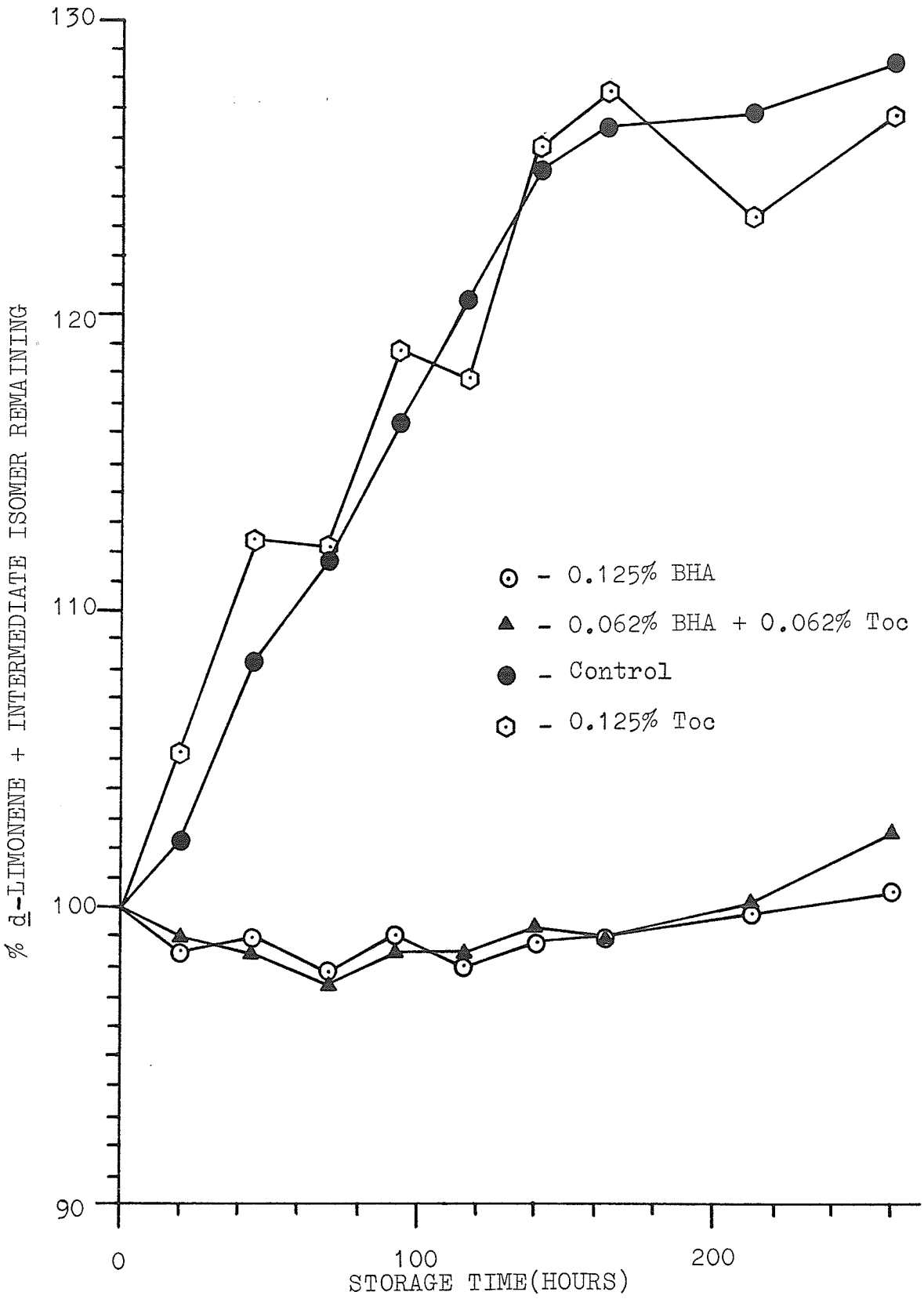
Note: Toc = alpha-tocopherol acetate; storage temperature: 35 °C

-Ø.954 for alpha-tocopherol acetate, r = -Ø.94Ø for the control). Therefore the rate of disappearance for these samples was dependent on the concentration of alpha-phellandrene. Based on this fact, the half-life for the control was calculated to be 73 hours while that for the Ø.125% alpha-tocopherol acetate samples was slightly less at 69 hours. The similarities between the half-lives and oxidation kinetics implied that alpha-tocopherol acetate did not actively participate in the oxidation mechanism for alpha-phellandrene.

The oxidation of alpha-phellandrene in all the samples followed the previously observed pattern of conversion into the intermediate isomer, tentatively identified as alpha-terpinene, (Section 4.2). As previously mentioned, the intermediate isomer was poorly resolved from d-limonene and therefore Figure 35 shows the combined levels of d-limonene and alpha-terpinene during storage. A large increase resulted for the control and samples containing only alpha-tocopherol acetate, while the samples containing BHA indicated no major differences. These results implied that BHA prevented the conversion of alpha-phellandrene to the intermediate isomer, but the oxidation trend for d-limonene needed to be evaluated to substantiate this hypothesis.

In spite of the poor resolution of alpha-terpinene and d-limonene, an attempt was made to estimate the levels of the two terpenes and the resulting data are listed in Table XX. For comparison, alpha-phellandrene and carvone levels

Figure 35. Effect of Anti-Oxidants on d-Limonene and Intermediate Isomer Levels in Stored Dill Weed Oil



Note: Toc = alpha-tocopherol acetate; storage temperature: 35°C

Table XX. Anti-Oxidant Study Summary: Intermediate Isomer and d-Limonene Levels for Different Treatments

1. Intermediate Isomer (Means of 2 reps of % composition (GLC)).

TREATMENT:	TIME (HOURS)									
	0	20	45	70	93	117	141	164	213	260
Control	5.1% ab	6.2% def	9.0% g	10.0% hi	11.2% j	12.8% l	14.6% m	15.6% n	16.8% p	17.9% q
0.125% Toc	5.0% a	6.9% f	9.4% gh	10.5% ij	12.0% k	13.2% l	15.0% mn	15.9% no	16.6% op	18.0% q
0.125% BHA	5.0% a	5.0% a	5.4% abc	5.2% abc	5.8% bcd	5.8% bcd	5.8 % bcd	5.8% bcd	5.9% cde	6.6% ef
Mixture	5.0% a	5.2% abc	5.2% abc	5.3% abc	5.5% abcd	5.8% bcd	5.8% bcd	6.2% def	6.6% ef	6.8% f

2. d-Limonene (Means with same letter are not significantly different, $LSD_{0.05}$).

TREATMENT:	TIME (HOURS)									
	0	20	45	70	93	117	141	164	213	260
Control	35.9% a	35.7% a	35.4% a	35.8% a	36.5% a	36.6% a	36.6% a	36.2% a	35.2% ab	34.8% abc
0.125% Toc	35.4% a	35.6% a	36.0% a	34.8% abc	36.0% a	34.4% abcd	35.8% a	35.6% a	33.2% d	33.2% d
0.125% BHA	35.4% a	34.8% abc	34.6% abc	34.3% abcd	34.2% abcd	33.8% cd	34.1% bcd	34.2% abcd	34.4% abcd	34.0% bcd
Mixture	35.4% a	34.8% abc	34.6% abc	34.1% bcd	34.3% abcd	34.0% bcd	34.3% abcd	33.8% cd	33.8% cd	34.6% abc

Table XXI. Anti-Oxidant Study Summary: Alpha-Phellandrene and d-Carvone Levels for Different Treatments

3. Alpha-Phellandrene (Means of 2 reps of % composition (GLC)).

TREATMENT:	TIME (HOURS)									
	0	20	45	70	93	117	141	164	213	260
Control	36.2% a	31.0% hi	25.0% k	20.8% m	17.8% n	13.2% o	9.2% p	6.2% qr	2.5% rs	0.3% t
0.125% Toc	35.6% ab	30.2% ij	22.8% l	18.2% n	15.0% o	9.8% p	6.6% q	4.3% r	1.5% st	0.2% t
0.125% BHA	35.6% ab	34.7% abc	34.3% abcd	33.3% cdef	33.0% cdefg	32.4% defgh	32.0% efghi	31.6% fghi	30.5% hij	28.8% j
Mixture	35.5% ab	34.7% abc	33.8% bcde	33.0% cdefg	32.4% defgh	31.6% fghi	31.2% ghi	30.4% ij	28.8% j	26.6% k

4. d-Carvone (Means with the same letter are not significantly different using LSD at the 5% level of confidence.)

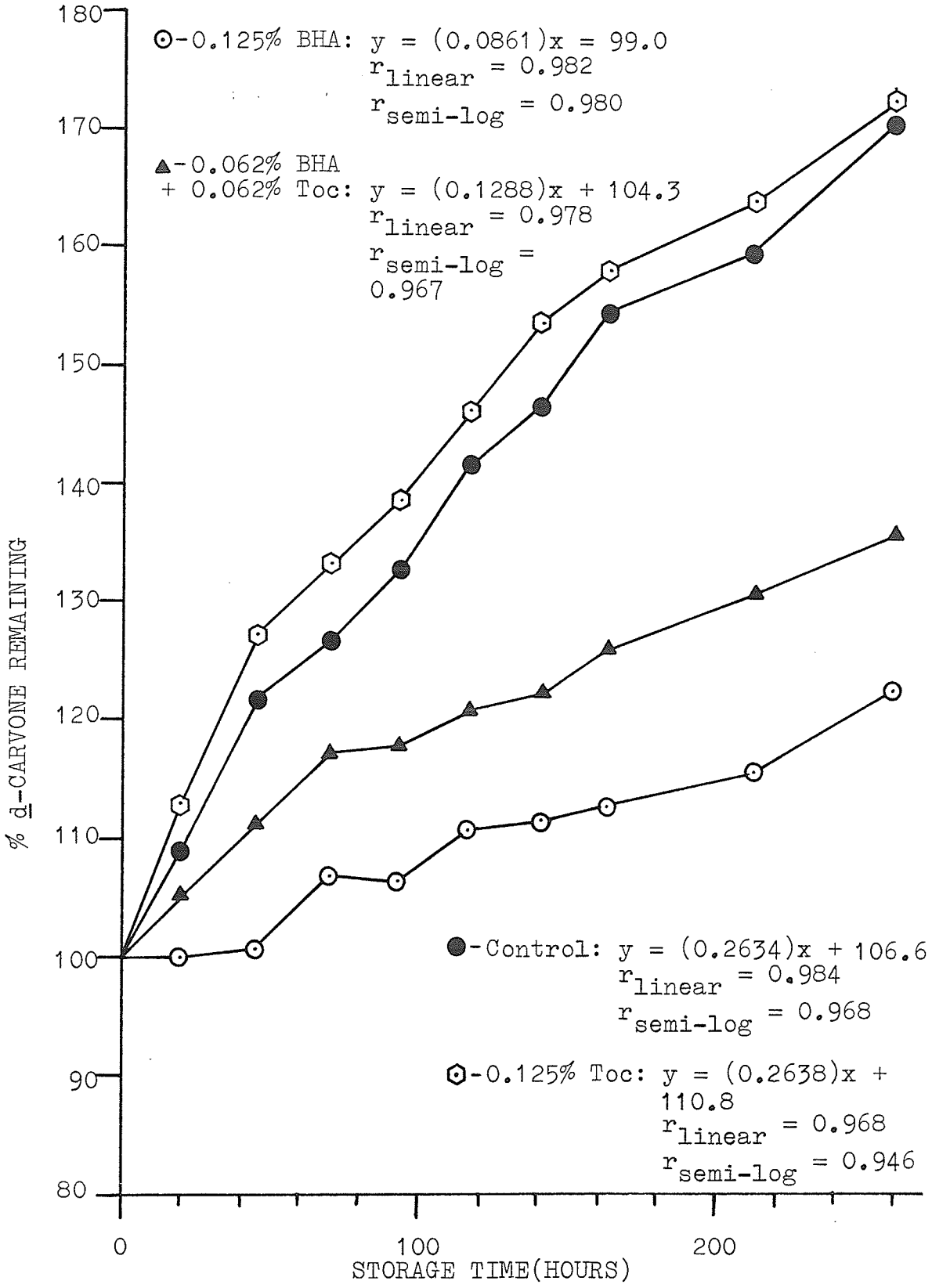
TREATMENT:	TIME (HOURS)									
	0	20	45	70	93	117	141	164	213	260
Control	13.8% a	15.0% bcde	16.8% hijk	17.5% kl	18.3% l	19.5% n	20.2% no	21.3% pq	22.0% qr	23.5% s
0.125% Toc	13.3% a	15.0% bcde	16.9% ijk	17.7% kl	18.4% lm	19.4% mn	20.4% nop	21.0% opq	21.8% q	22.9% rs
0.125% BHA	14.2% abc	14.2% abc	14.3% abc	15.2% cdef	15.1% bcde	15.7% defg	15.8% defgh	16.0% efghi	16.4% ghij	17.4% jkl
Mixture	13.4% a	14.1% ab	14.9% bcd	15.7% defg	15.8% defgh	16.2% fghi	16.4% ghij	16.9% ijk	17.5% kl	18.2% l

are included in Table XXI. From the available information it was apparent that the d-limonene levels generally did not undergo major changes during the experiment. Yet, major increases in the alpha-terpinene levels occurred for the control and samples containing only alpha-tocopherol acetate; whereas the levels in the samples containing BHA increased very slightly. Two conclusions were suggested. First, alpha-phellandrene appeared to have a protective effect on d-limonene as was pointed out earlier, (Section 4.2). Secondly, BHA actively inhibited the formation of the intermediate which represented the first major step in the oxidation of alpha-phellandrene.

Carvone was generated in all the samples as shown in Figure 36 and Table XXI. Significantly higher levels were found for the control and samples containing only alpha-tocopherol acetate. Both linear and semi-log regression analysis produced very significant correlation coefficients for all the samples($r>0.94$), but the linear correlation values were larger for all samples, and especially for those containing BHA. This insinuated that the build-up of carvone was independent of the reactant concentration.

If this was the case, then BHA was probably effective in controlling the further oxidation of alpha-terpinene to carvone, which was the second step in the oxidation of alpha-phellandrene. The other samples also appeared to generate carvone independently from the concentration of the hydrocarbon source, although the results, (Figure 36) were

Figure 36. Effect of Anti-Oxidants on d-Carvone Levels in Stored Dill Weed Oil



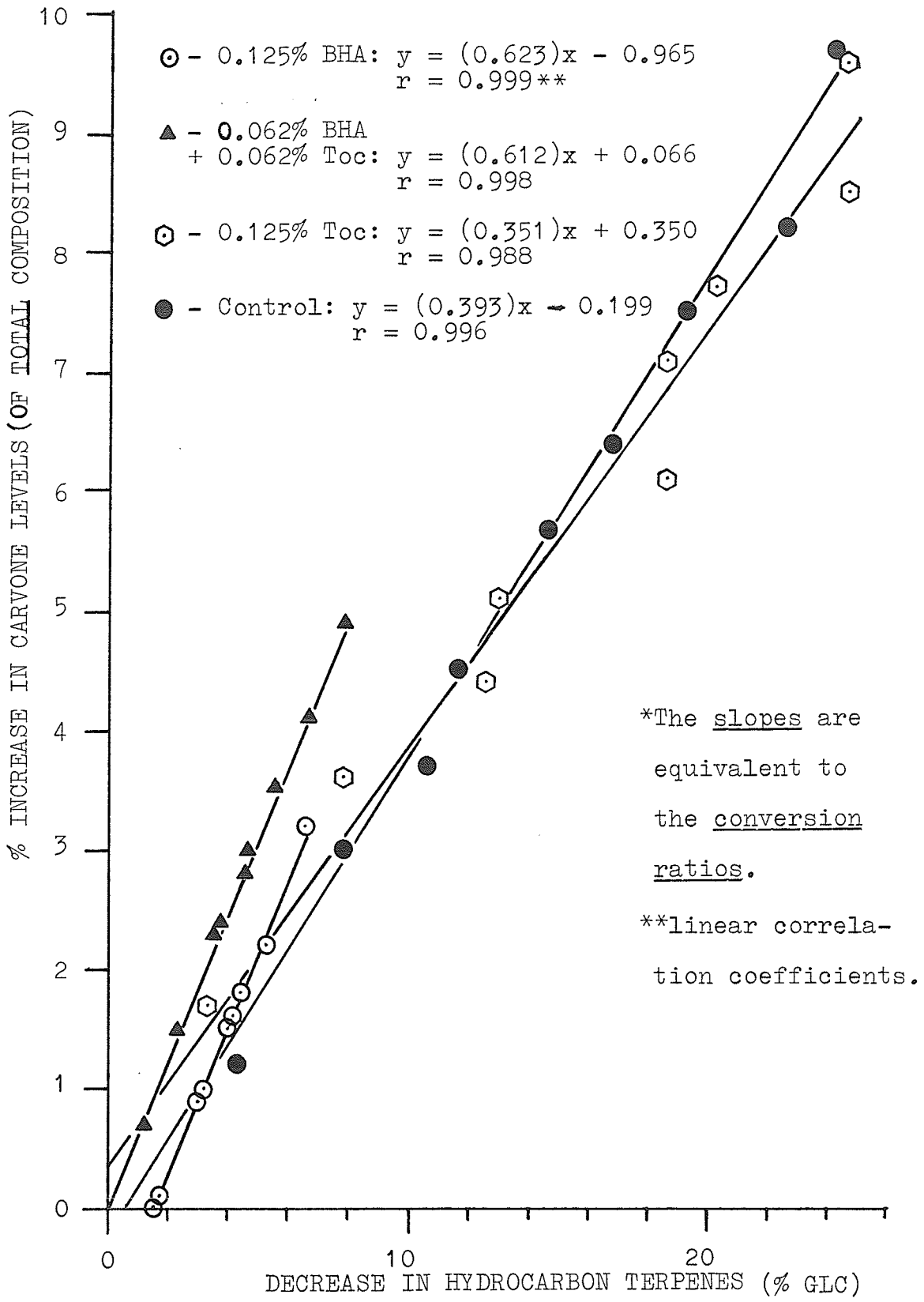
Note: Toc = alpha-tocopherol acetate; storage temperature: 35°C

not as linear as those for the BHA samples.

This pseudo-zero order behavior may have occurred because the alpha-phellandrene concentration was initially very high. If the study had been continued beyond 260 hours, then a leveling in the carvone levels would probably have been observed.

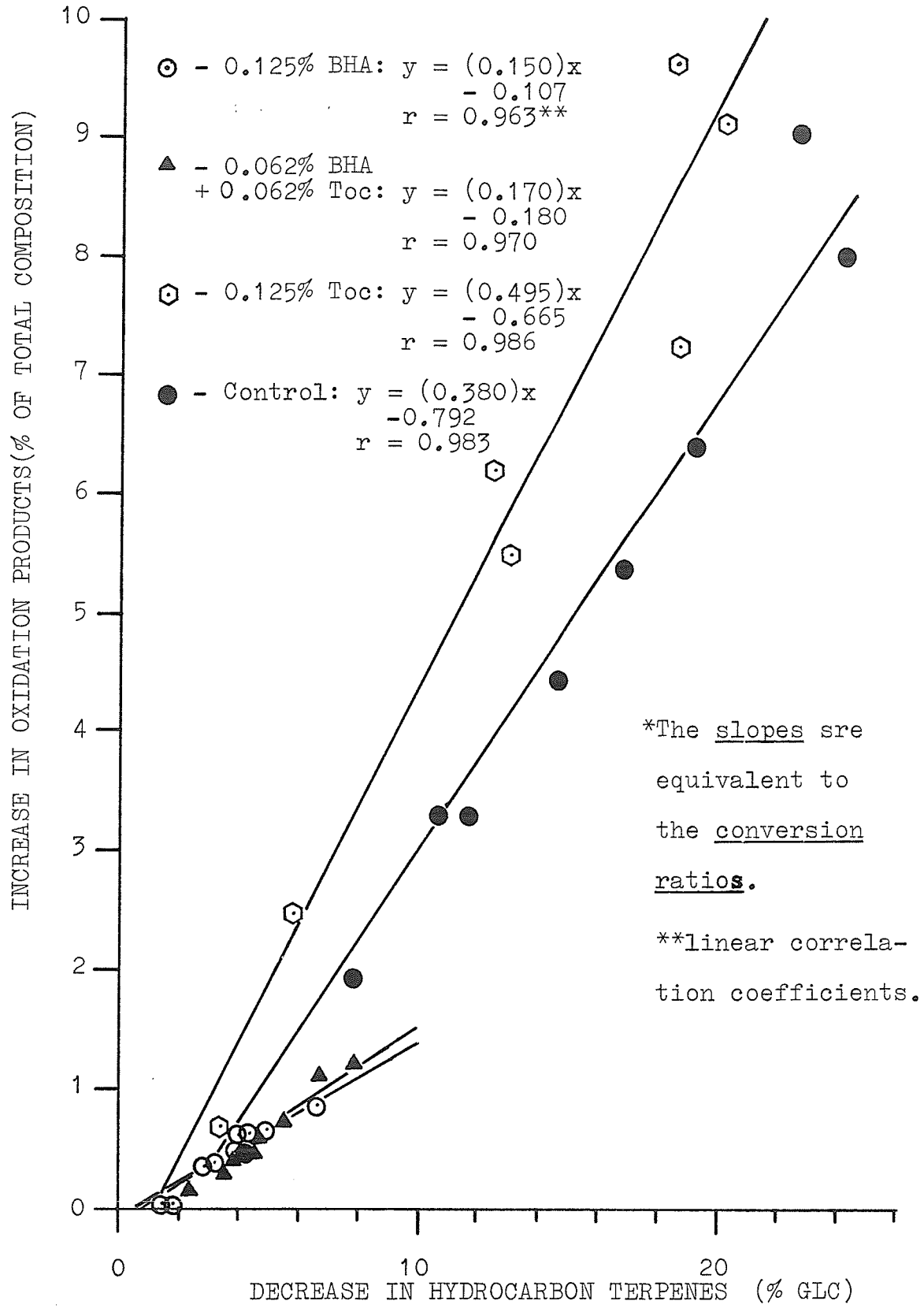
Further analysis showed that the conversion ratios of alpha-phellandrene to carvone in samples containing BHA were significantly higher than in the control and samples containing alpha-tocopherol acetate. This was evident from a comparison of the cumulative increases in carvone versus the cumulative decreases in the hydrocarbon levels, shown in Figure 37. The relationship between carvone increases and hydrocarbon decreases was highly linear in all cases. The slopes of these lines for the different treatments represented the conversion ratios of hydrocarbons to carvone. The ratio values for the samples containing BHA(61-62%) were almost double the values for the control(39%) and samples containing only alpha-tocopherol acetate(35%). A similar analysis of the increase in oxidation products which elute after carvone versus the decrease in hydrocarbon levels, (Figure 38) gave opposite results. The slopes of the curves again represented the conversion ratios of the hydrocarbons to the other oxidation compounds. However, this time the control and the Ø.125% alpha-tocopherol acetate samples had the highest conversion ratios, (38% and 50% respectively), while the samples containing BHA had very low values,

Figure 37. Conversion Ratios of Hydrocarbon Terpenes to Carvone*



Note: Toc = alpha-tocopherol acetate; storage temperature: 35 °C

Figure 38. Conversion Ratios of Hydrocarbon Terpenes to Oxidation Products with t_Rs Greater than t_R carvone*



*The slopes sre equivalent to the conversion ratios.

**linear correlation coefficients.

Note: Toc = alpha-tocopherol acetate; storage temperature: 35°C

(15-17%). Evidently, the BHA had a directional effect on the formation of the different oxidation products and this effect favoured the formation of carvone.

In summary, the alpha-tocopherol acetate proved to be an ineffective anti-oxidant in dill weed oil. This was surprising since this compound has been reported to have oxygen scavenging properties. The results of this study indicated that alpha-phellandrene, the intermediate isomer and most likely d-limonene showed higher affinities for oxygen than alpha-tocopherol acetate. Whether alpha-tocopherol would be a more powerful scavenger remains to be established. Even if it were proven effective, its usefulness from a commercial standpoint would be limited due to its high cost. BHA was shown to be effective at the permitted levels.

This anti-oxidant retarded the oxidation of the labile dill terpenes and directed the oxidation in favour of carvone. Given the commercial specifications for dill oil with respect to carvone, the use of BHA in dill oil with low carvone levels could improve the quality of the oil with time, although changes would have to be carefully monitored.

4.5 Odour Analysis of Dill Weed Oil

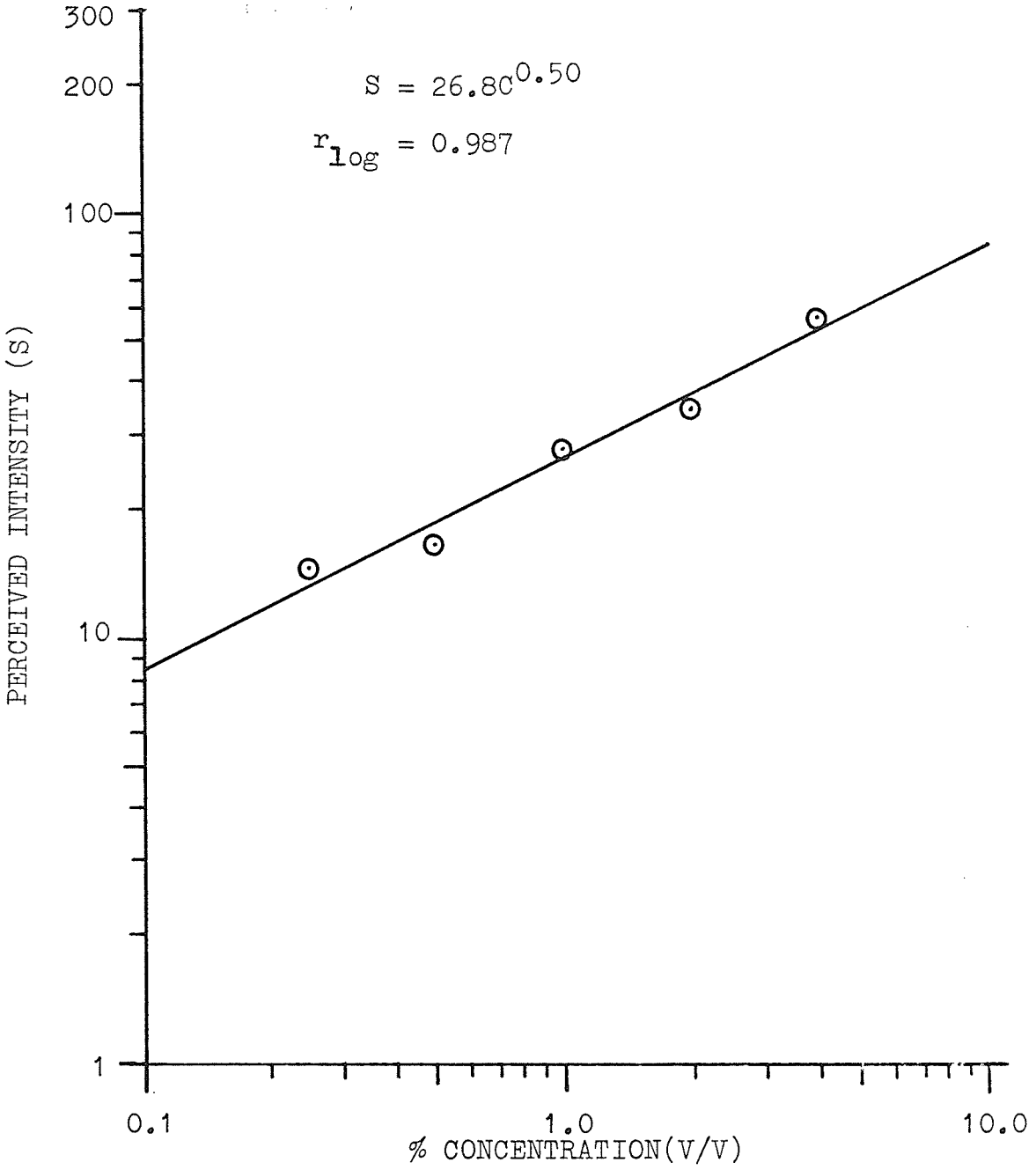
The first section of this study involved the monitoring by instrumental analysis possible compositional changes in

dill oil samples which had undergone storage, with and without weed distillate contamination. Since changes were observed, the next step was to assess the sensory impact of these altered samples through the use of a sensory panel. The analysis of odour rather than taste was chosen since this sensation would be detected before taste and human olfaction is more sensitive than taste reception(Fennema, 1976). The amount of sample required was also minimal, and problems associated with taste, such as the selection of a suitable carrier were avoided.

Since dill weed oil is prepared in an emulsion for use in the pickling industry, the first odour study involved the magnitude estimation of the perceived odour intensity of different concentrations of a commercially acceptable dill weed oil diluted in mineral oil. A summary of the analysis of the geometric means of the sensory values for the five highest concentrations is provided in Figure 39. The two, least concentrated samples, 0.025% and 0.050%(V/V) were excluded from the analysis since the odour for each solution was not detected by five or more of the panelists in each of the two sessions. The 0.050% solution may in fact have been the threshold limit for the odour of dill weed oil in mineral oil. The log of the perceived intensity values correlated very well with the log of the concentrations as shown by the high correlation coefficient, ($r = 0.987$). The resulting power function for dill weed oil was:

$$S = 26.8C^{0.50}$$

Figure 39. Magnitude Estimation of the Odour Intensity of Dill Weed Oil



Note: mineral oil used for dilution of dill oil. Values represent means obtained from 16 panelists.

This function showed that a doubling in dill oil concentration would result in an increase in the perceived odour intensity by 1.4 times. Therefore, if a dill pickle manufacturer changed the strength of his dill weed oil emulsion by 20%, the perceived odour would change by only 9.5%, assuming that the volatility characteristics of the dill oil in the emulsion were similar to those experienced when using mineral oil. Further studies would need to be done using actual dill oil emulsions to verify this fact. An investigation of the perceived changes in taste with changes in dill oil concentration also need to be undertaken to provide a more complete sensory analysis of different strengths of dill weed oil.

The effect of weed distillate contamination on dill oil odour was also investigated. Wormwood oil was selected as the contaminant based on its potentially adverse chemical properties. Different concentrations were prepared to reflect different levels of field contamination and were compared to a pure control by the panel. The calculated R-Index values for each panelist represented the probability that the panelist could differentiate the contaminated samples from the control. Table XXII includes a summary of the R-Index means for each of the three contaminated solutions. As expected, odour differences were more easily perceived as the concentration levels increased. The results indicated that in the case of wormwood contamination, instrumental analysis would be adequate to ensure the elimination of any

Table XXII. Odour Difference Test Summary for Wormwood-Contaminated Dill Oil

Wormwood Oil Concentration: (V/V)[1]	R-Index[2] Value:	Significance:[3] (Letter Grade)
2.1%	0.649	A
3.3%	0.824	B
8.3%	0.906	C

(1) Reference sample: 22.9% alpha-phellandrene; 35.0% d-limonene; 34.3% d-carvone.

(2) R-Index probability values obtained from the difference test described by O'Mahony(1979,1981).

(3) Means with the same letter grade are not significantly different at the 5% level of confidence.

dill oil samples with a significantly different odour profile. As an example, a dill oil sample containing 8.3%(V/V) wormwood oil produced a 90% probability of recognition, and this level of wormwood oil could easily be detected by gas chromatography.

In addition to contamination, alterations in the levels of the major dill components were also thought to influence the sensory qualities of the oil. Literature reports(Guenther, 1950) have referred to the importance of alpha-phellandrene to the odour profile of dill weed oil. Arctander(1969) referred to the odour of this terpene as pleasant and peppery-woody, and Guenther(1950) proposed that this terpene gave dill weed oil its herbaceous odour and taste. Since the storage studies revealed that alpha-phellandrene was very prone to oxidation, sensory tests were conducted on a commercially acceptable dill oil sample which was oxidized to obtain samples containing four different levels of alpha-phellandrene. The oxidized samples were then compared to the original, unoxidized standard.

Table XXIII provides a summary of the R-Index values as well as the significance between these values. Three of the four values follow a trend of increasing probabilities of recognition with decreasing alpha-phellandrene levels. The highest probability of 0.718 was derived for the samples containing only 5.1% alpha-phellandrene and oxidation species including higher levels of carvone may have contributed to the higher perceived odour differences by the panel.

Table XXIII. Odour Difference Test Summary for Oxidized Dill Oil Samples

Alpha-Phellandrene[1] Levels:	R-Index Value:	Significance:[2] (Letter Grade)
17.9%	0.598	AB
13.2%	0.695	B
9.3%	0.558	A
5.1%	0.718	B

(1) Reference sample: 22.9% alpha-phellandrene; 35.0% d-limonene; 34.3% d-carvone.

(2) Means with the same letter grade are not significantly different at the 5% level of confidence.

Therefore, even though the panel could detect odour differences in dill oil samples with lower alpha-phellandrene levels than the control, these differences may have been due at least in part to the build-up in oxidation products. These compounds could not be isolated from the samples in this study.

Therefore, a final series of odour tests was conducted to determine the significance of the three major dill terpenes to the odour profile of the oil. Alpha-phellandrene, d-limonene and d-carvone levels were individually increased or decreased by approximately 50% from that for a control sample. These samples were then subjected to an R-Index test by the panelists.

A summary of the resulting means is given in Table XXIV and the significance between the R-Index values is also shown. The results indicated that the panel was most capable of differentiating dill oil samples which contained high levels of d-limonene compared to the control, or low levels of either d-carvone or alpha-phellandrene. The decrease in d-carvone levels below 20% of the total oil composition gave the most significant R-Index value, confirming the need to maintain minimum standards for this component. In addition, the results implied that minimum standards should also be established for alpha-phellandrene and maximum standards should be established for d-limonene.

Additional analysis, involving stepwise multiple regression was used to compare the panel mean probabilities,

Table XXIV. Odour Difference Test Summary for Dill Samples with Altered Compositions

Altered Component:(1)	Increase/ Decrease: (%)	R-Index Value:	Significance:(2) (Letter Grade)
alpha-phellandrene	+60%	0.543	A
alpha-phellandrene	-52%	0.703	CD
d-limonene	+54%	0.741	CD
d-limonene	-53%	0.597	AB
d-carvone	+50%	0.681	BC
d-carvone	-50%	0.782	D

(1) Reference sample: 19.4% alpha-phellandrene; 35.9% d-limonene; 37.8% d-carvone.

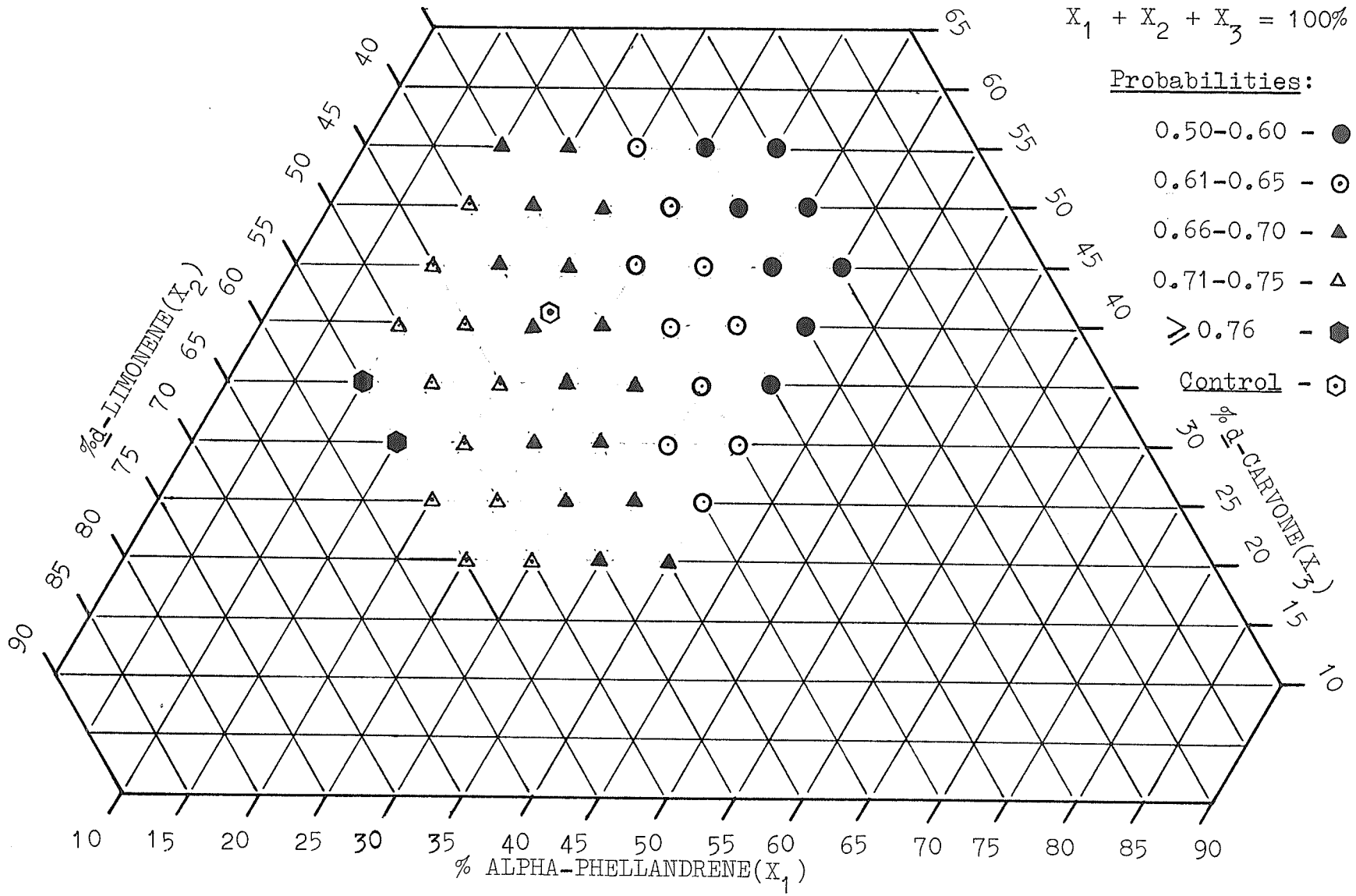
(2) Means with the same letter grade are not significantly different at the 5% level of confidence.

(dependent variable) with the concentration levels of each of the three terpenes, (independent variables). A prediction equation for the four variables was derived and the use of this equation is graphically presented in Figure 4Ø. Within the given compositional range, the equation can be used to predict whether dill samples with compositions different from the control would have a recognizably different odour from the control. The equation only takes into consideration the three major components in dill weed oil, and further odour analysis would need to be done to determine the effects of the minor components. To use the prediction equation, the content of each major terpene is only compared to the total content of the three major components. Therefore, a solution containing 5% alpha-phellandrene, 6Ø% d-limonene and 35% d-carvone would give a recognition probability of Ø.79. In comparison to the values obtained for the six test solutions, this value would be quite significant, indicating that the odour profile should be very different from the control. The available data can be updated with future results for other compositions of dill weed oil; allowing the range to be expanded. To expand on these results even more, work should be undertaken to derive a prediction equation for the taste of dill weed oil.

In summary, the sensory tests emphasized the need to control weed distillate contamination through proper weed control practices, and to establish specifications for the levels of all three major dill terpenes. A 5Ø% decrease in

Figure 40. Odour Prediction Equation for Dill Weed Oil*

Probability(Differentiation): $Y = -0.0168X_1 - 0.0113X_2 - 0.0151X_3 + 2.0850$, where:



*Derived from step-wise, multiple linear regression analysis.

carvone levels appeared to alter the odour profile of dill weed oil more than a 50% decrease in alpha-phellandrene levels compared to a control. This could reflect the more subtle herbaceous odour impression of alpha-phellandrene compared to the stronger spearminty characteristics of d-carvone. The increase in d-limonene levels produced a noticeable increase in the R-Index values which could be attributed to the sweet, citrusy or orange peel-like odour which has been associated with this terpene(Arctander, 1969).

5. CONCLUSIONS AND RECOMMENDATIONS

This study investigated several factors affecting the quality and stability of Manitoba dill weed oil. These included the effect of plant maturity on the quality and quantity of distillate obtained. In addition, post-distillation factors, including storage conditions, weed contaminants and the role of different anti-oxidants were also studied. Since it was realized before the study began that the quality of the dill oil was very dependent on its sensory properties, odour analysis was performed to evaluate possible sensory differences between a commercially acceptable control and samples which had been altered through oxidation, contamination or compositional modification.

The maturity study confirmed that the oil composition undergoes changes during the physiological development of the dill plant and that by the milk-wax ripeness stage the oil content is highest and the composition meets the current quality standards.

The oil yield per hectare and variances in oil content from different regions of the plant were not investigated. In order to make a more thorough recommendation, further studies in this area are required. The maturity study also

raised several questions about the enzymatic production of the dill terpenes. The biosynthesis of alpha-phellandrene and dill 'ether' appeared to follow an independent pathway from that for d-limonene and d-carvone, but currently, very little information is availbale on in vivo terpene biosynthesis. In order to clarify the questions raised, further enzyme research needs to be undertaken with spice crops such as dill, and the results could provide for the gradual development by plant breeders of better spice plant cultivars with faster terpene synthesis rates and/or higher oil contents.

Stored samples of dill weed oil were shown to undergo compositional changes. The unsaturated hydrocarbon terpenes proved to be susceptible to oxygen attack and an oxidative stability order was determined for the three, major dill components. Alpha-phellandrene, with two double bonds in the ring of the molecule was more labile to autoxidation than d-limonene. d-Limonene, in turn, was more vulnerable to oxidation than d-carvone. Data indicated that carvone was one of the major oxidation products for both d-limonene and alpha-phellandrene. The conversion ratios were in the 25-30% range at room temperature and under saturated oxygen conditions. The other oxidation products included four major components for alpha-phellandrene and two main products for each of: d-limonene and d-carvone. Prolonged storage of dill oil resulted in the formation of a resin which may have formed from the polymerization of the various oxi-

dation products. If stored oils with resinous material are used, they must first be rectified and this will lower their commercial value. Some of the stored dill oil samples in this study achieved carvone levels above 50%. This exceeded the maximum value of 45%(V/V) recommended by the Committee on Specifications(1972). Once levels go above the specified maximum, the dill weed oil acquires the dill 'seed' oil characteristics, resulting in a loss in the herbaceous qualities of the oil which is desired in the pickling industry. Consequently, the commercial value of the oil decreases.

Temperature had an important effect on the oxidation rates of the labile dill terpenes. Every 10^{o}C increase in storage temperatures resulted in a doubling in the rates. Therefore storage of the oils at refrigeration temperatures would considerably reduce oxidation during the harvest period when outside temperatures are normally higher. The container headspace also was shown to affect the oxidation rate. A reduction in the available headspace coupled to the careful sealing of the containers would prevent any serious oxidation from occurring during storage.

The investigation of the effects of the distillates of four weed varieties common to Manitoba on stored dill oil showed that only wormwood would pose the greatest immediate threat to the dill producer if field contamination were to exceed 5%. The low distillate yields for the other varieties, (kochia, lamb's quarters and Canada thistle) would require excessively high field infestations before they

would affect the initial quality of the oils. The storage studies involving the contamination of dill oil with the four weed distillates did not produce any significant oxidative catalytic effects. Careful weed control for these varieties would guarantee a low enough level of weed contamination such that there would be no measurable stability impact on dill oil during storage. Other weed varieties were observed in or near the perimeters of dill fields during this study but were not investigated. Some of these included foxtail, milkweed and redroot pigweed. Before all potentially adverse contaminant effects can be ruled out, these other varieties need to be assessed in terms of abundance, distillate yields and prolonged storage effects on dill oil.

Storage of dill oil is necessary since it is produced on a large scale and some oxidation will occur with time depending on the storage conditions. Drums which are poorly sealed will permit air to diffuse inside the containers. This will also allow the more volatile terpenes, such as alpha-phellandrene and d-limonene to escape through the seals, resulting in an increase in the d-carvone levels. The current study showed that BHA would be an effective anti-oxidant in dill weed oil. This compound reduced the rate of oxidation of the alpha-phellandrene by a factor of eight-to-ten times when compared to a control kept under the same conditions, and when used at the maximum permitted concentration of Ø.125%. In addition, the phenolic anti-oxi-

dant appeared to direct oxidation in favour of the formation of carvone at about twice the normal conversion rates for the two major hydrocarbon terpenes. This resulted in lower levels of the other oxidation products which may be responsible for the resin build-up observed in the different storage studies. Of further importance, BHA is quite soluble in dill weed oil. However, there are other phenolic anti-oxidants such as propyl gallate and tert-butyl, hydroxy toluene which may be more effective; singly or in combination in stored dill oil. Future work needs to be undertaken to determine an optimal mixture in terms of anti-oxidant behavior, strength and cost.

The dill oil magnitude estimation study correlated the perceived odour intensity with the concentration of the oil in mineral oil. The study showed that a doubling in the concentration would only increase the perceived intensity by 1.4 times. The results may reflect the same odour functionality of dill oil in a pickle emulsion containing Polysorbate(80), (polyoxyethylene(20) sorbitan monooleate) but this would require additional research to verify.

Other sensory results indicated that a very high d-limonene content with correspondingly low levels of d-carvone and alpha-phellandrene would give very significant differences in the dill weed oil odour profile compared to a commercial sample. This emphasized the need to establish specifications for d-limonene and alpha-phellandrene in addition to those already prescribed for d-carvone. The effects of

the minor components also have yet to be determined, and an overall odour and taste sensory profile for dill weed oil needs to be determined. Once this is accomplished, better specifications can then be devised for dill weed oil.

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Appendix 1. Solutions Used in FID Response Study

Compound:	Purity(GLC):	Solution #1:	Solution #2:	Solution #3:
alpha-pinene	94.2%	7.2%	10.6%	5.3%
beta-pinene	94.8%	3.6%	9.8%	4.8%
alpha-phellandrene	99.4%	24.3%	14.7%	19.5%
d-limonene	83.3%	24.7%	34.4%	19.5%
d-carvone	99.3%	40.3%	30.5%	50.8%
Total:	-	100.1%	100.0%	99.9%

Note: The actual weights for each component were multiplied by its GLC purity and the adjusted weights were then totaled so that each weight percentage value could be calculated.

e.g. $$\frac{\text{(weight of alpha-pinene)(GLC purity for alpha-pinene = 94.2\%)}}{\text{(adjusted weight total for all standards)(100)}}$$

Appendix 2. Solutions Used in Magnitude Estimation Study

Sample Concentration of Dill Oil(V/V):	
Trial#1	Trial#2
2.00%	4.00%
1.00%	2.00%
0.50%	1.00%
0.25%	0.50%
0.10%(R)	0.25%
0.05%	0.10%(R)
0.025%	0.05%

Dill Oil Composition:*

0.8%	alpha-pinene
0.5%	beta-myrcene
24.6%	alpha-phellandrene
33.1%	d-limonene
4.2%	dill 'ether'
34.6%	d-carvone
97.8%	Total

*average of two determinations.

(R) = reference concentration.

Appendix 3. Magnitude Estimation Ballot

Magnitude Estimation of the odour of dill weed oil in mineral oil at different concentration levels:

Use the first line to indicate your ratio estimate of the dill oil strength.

Let "np" denote the absence of the dill smell.

Sample #:	153	034	375	995	553	973	Standard: R
Ratio Estimate:							X1
Numerical Value:							X10

Description of Odour: _____

Other Comments: _____

_____

_____

Appendix 4. Contaminated Dill Weed Oil Samples

Sample#:	Wormwood Distillate/ Dill Oil(V/V):	Equivalent Field* Contamination:
1	8.3%	42%
2	3.3%	17%
3	2.1%	10%

* On a wet plant material basis; wormwood:dill.

Pure Dill Oil Composition:** 0.7% alpha-pinene
0.5% beta-myrcene
22.9% alpha-phellandrene
35.0% d-limonene
3.9% dill 'ether'
34.3% d-carvone
97.3% Total

** average of two determinations.

Appendix 5. R-Index Ballot

Dill Weed Odour Difference Tests

Sample the odour of the Reference(using three, quick whiffs), and evaluate the odour of the coded samples to determine whether each is the same or different than the Reference. Chose in randam number and record the three-digit code in the appropriate block.

If you are uncertain about a particular sample odour, then estimate whether it more closely resembles the Reference or whether it seems different than the Reference. In either case, use the appropriate block with the question mark indicating your uncertainty.

If you easily detect a very different sample, then asterisk(*) the particular coded number(s) and indicate whether you believe the odour is 'better' or 'worse' than the Reference, and if possible try to explain why in the Comments section.

CODE:	DIFFERENT:	DIFFERENT?:	REFERENCE?:	REFERENCE:

Comments: _____

Appendix 6. R-Index Calculation*

Actually:	Different	Different?	Reference?	Reference	
Different:	a	b	c	d	N_1
Reference:	e	f	g	h	N_2

(a-h): represent the number of observations per column.

N_1: total number of different samples.

N_2: total number of reference samples.

$$R = \text{probability} = \frac{a(f+g+h) + b(g+h) + ch + \frac{1}{2}(ae+bf+cg+dh)}{N_1 N_2}$$

*O'Mahony(1981).

Appendix 7. Oxidized Dill Weed Oil Samples

Sample#:	Level of Alpha-Phellandrene:(%)	% Alpha-Phellandrene Remaining (compared to Control):
Control	22.9%	100.0%
1	17.9%	78.4%
2	13.2%	57.6%
3	9.3%	40.7%
4	5.1%	22.2%

Control Dill Oil Composition:*

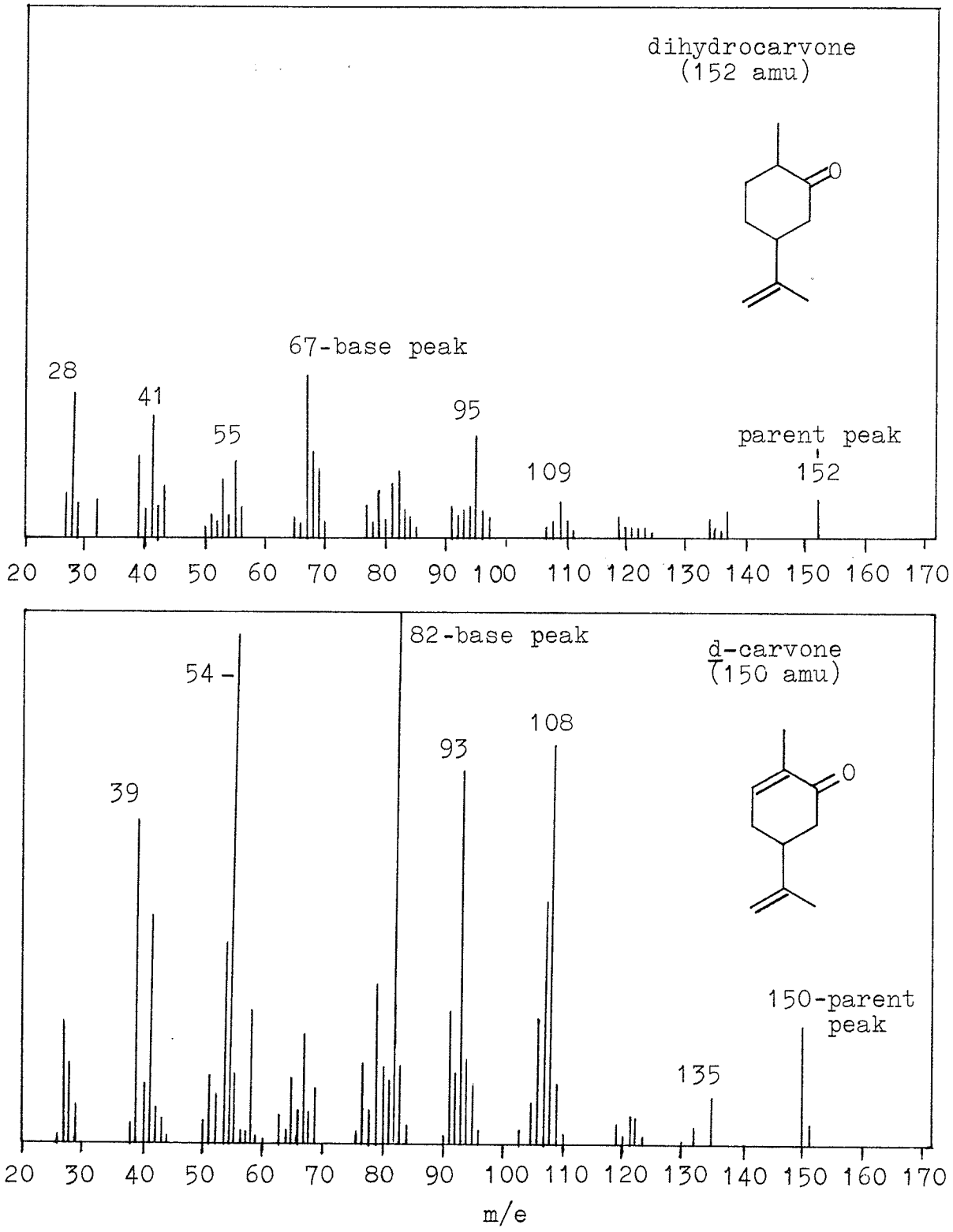
0.7%	alpha-pinene
0.5%	beta-myrcene
22.9%	alpha-phellandrene
35.0%	d-limonene
3.9%	dill 'ether'
34.3%	d-carvone
97.3%	Total

* average of two determinations.

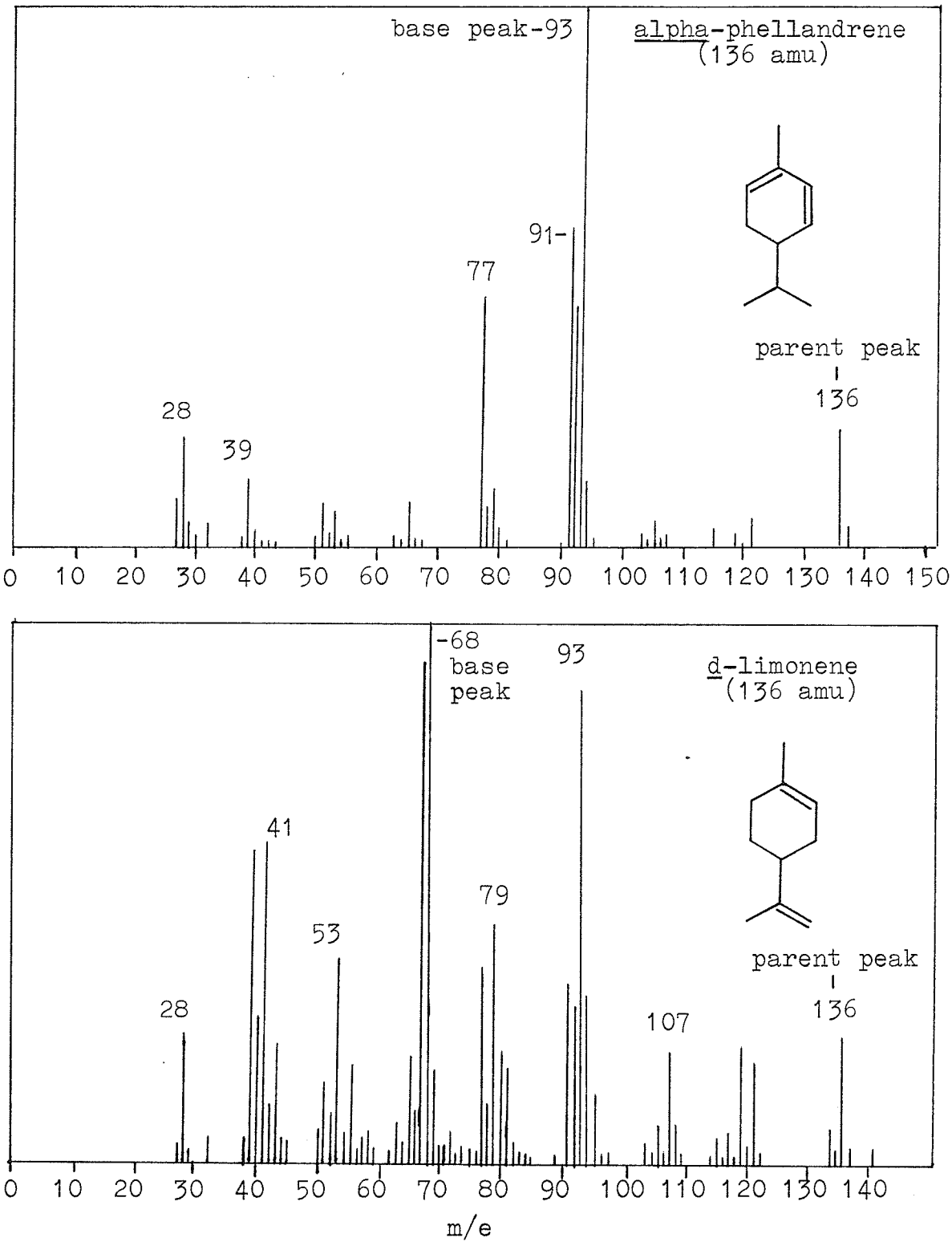
Appendix 8. Altered Dill Weed Oil Solutions

	% Composition(GLC):		
	alpha-phell-andrene:	d-limonene:	d-carvone:
Control	19.4%	35.9%	37.8%
Sample# 1	31.0%	29.9%	32.7%
Increase/ Decrease:	(+60.4%)	(-16.6%)	(-13.5%)
Sample# 2	9.3%	44.1%	41.8%
Increase/ Decrease:	(-52.1%)	(+22.9%)	(+10.5%)
Sample# 3	12.9%	55.2%	26.2%
Increase/ Decrease:	(-33.4%)	(+53.9%)	(-30.6%)
Sample# 4	37.2%	16.8%	41.9%
Increase/ Decrease:	(+92.0%)	(-53.0%)	(+10.8%)
Sample# 5	12.7%	25.4%	56.5%
Increase/ Decrease:	(-34.2%)	(-29.0%)	(+49.6%)
Sample# 6	29.1%	45.6%	20.4%
Increase/ Decrease:	(+50.6%)	(+27.2%)	(-46.0%)

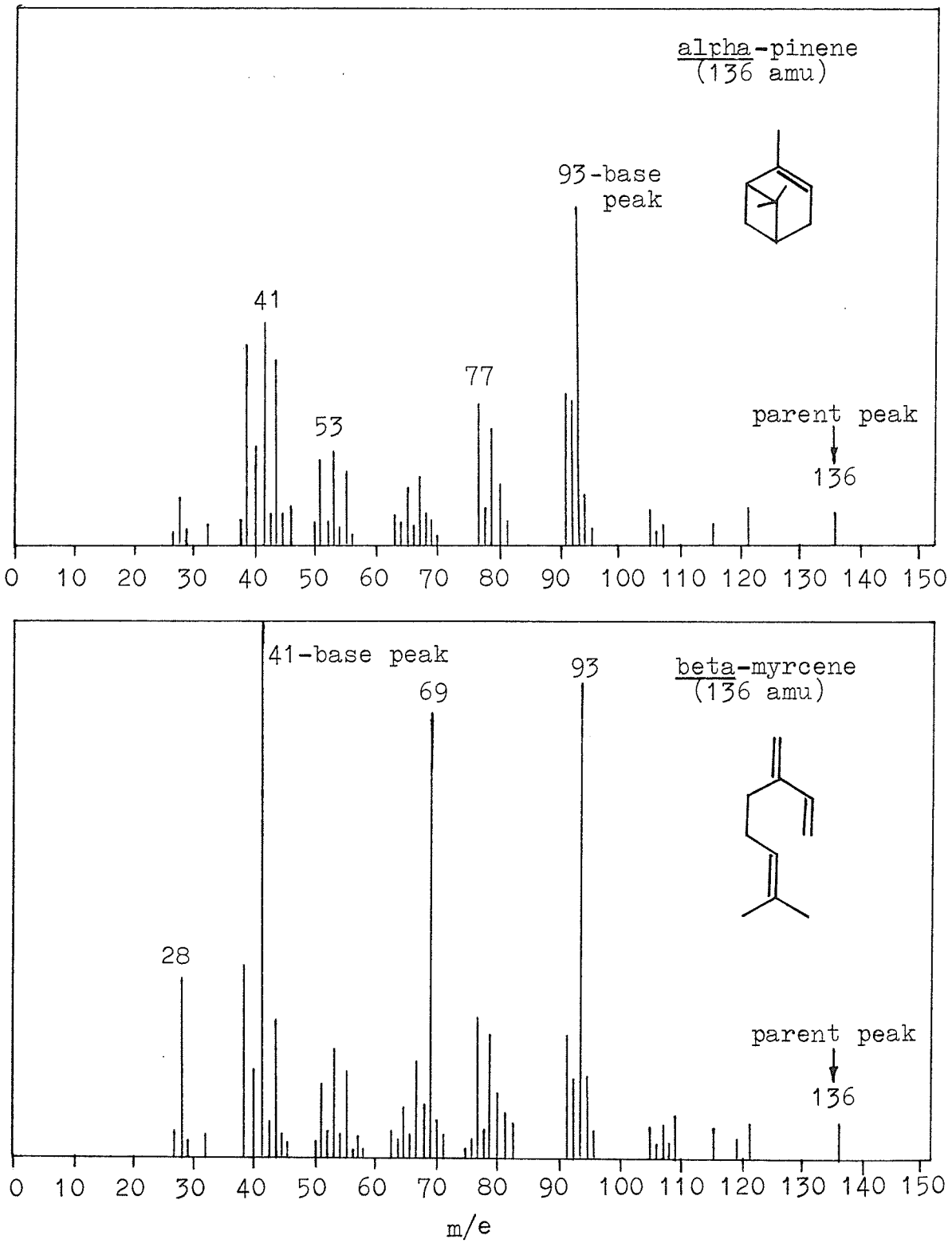
Appendix 9. Mass Spectrograms of Dihydrocarvone and d-Carvone



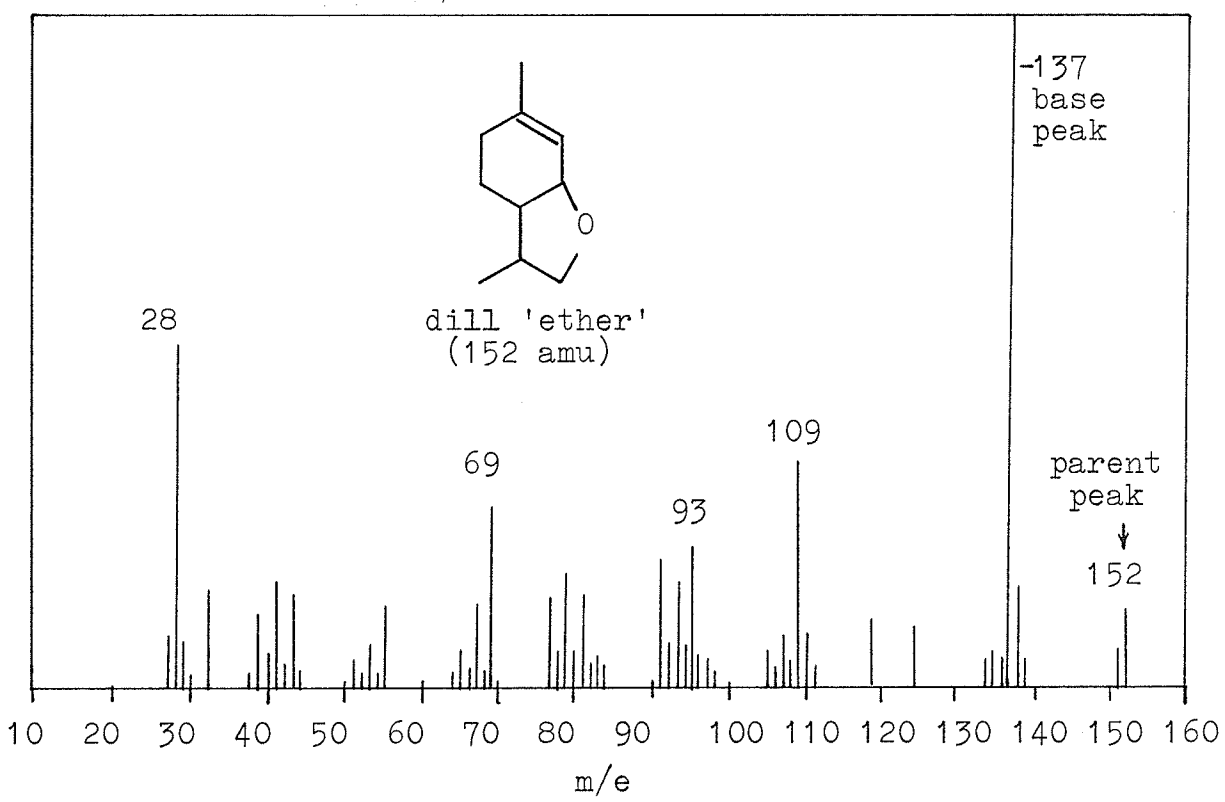
Appendix 10. Mass Spectrograms of Alpha-Phellandrene and d-Limonene



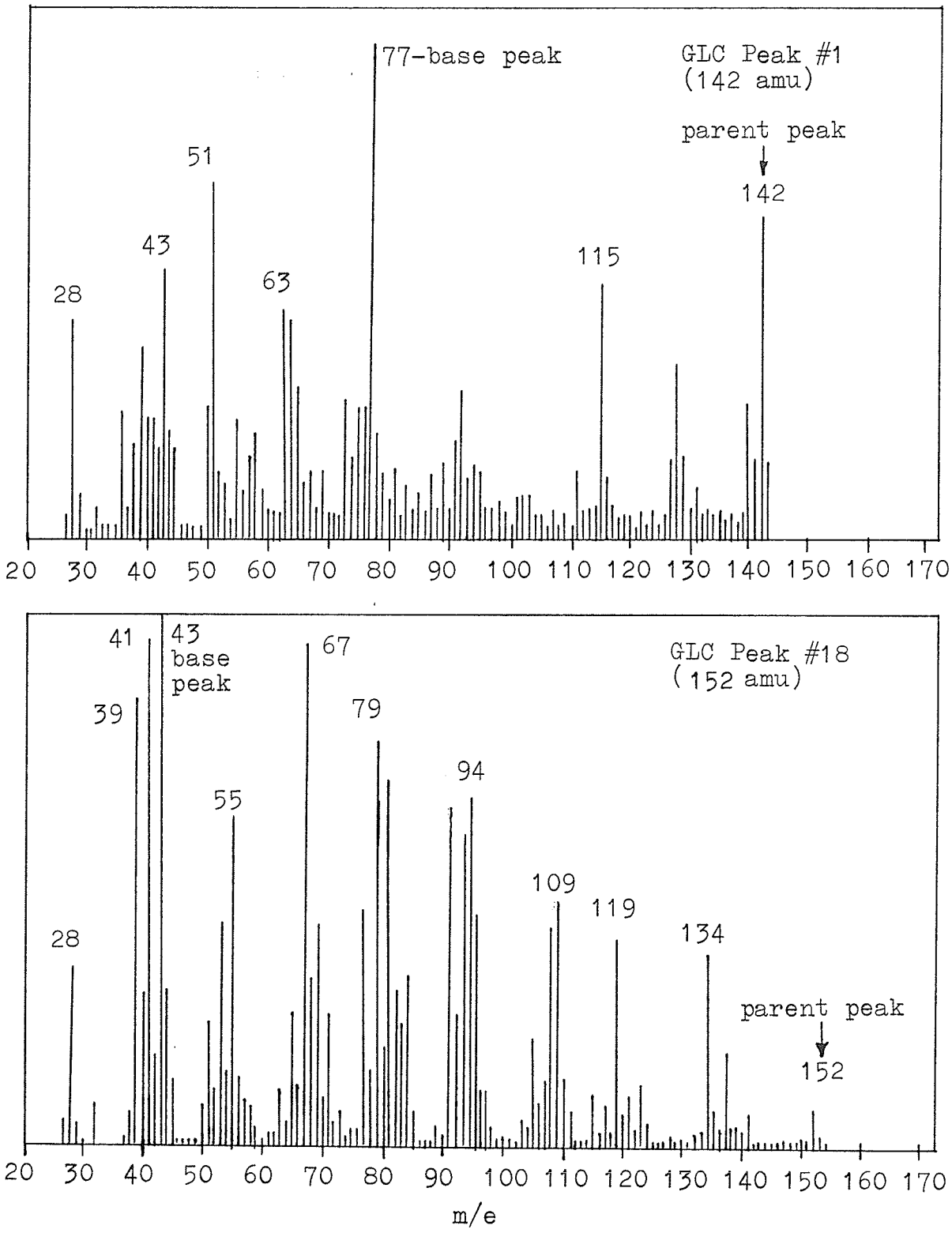
Appendix 11. Mass Spectrograms of Alpha-Pinene and Beta-Myrcene



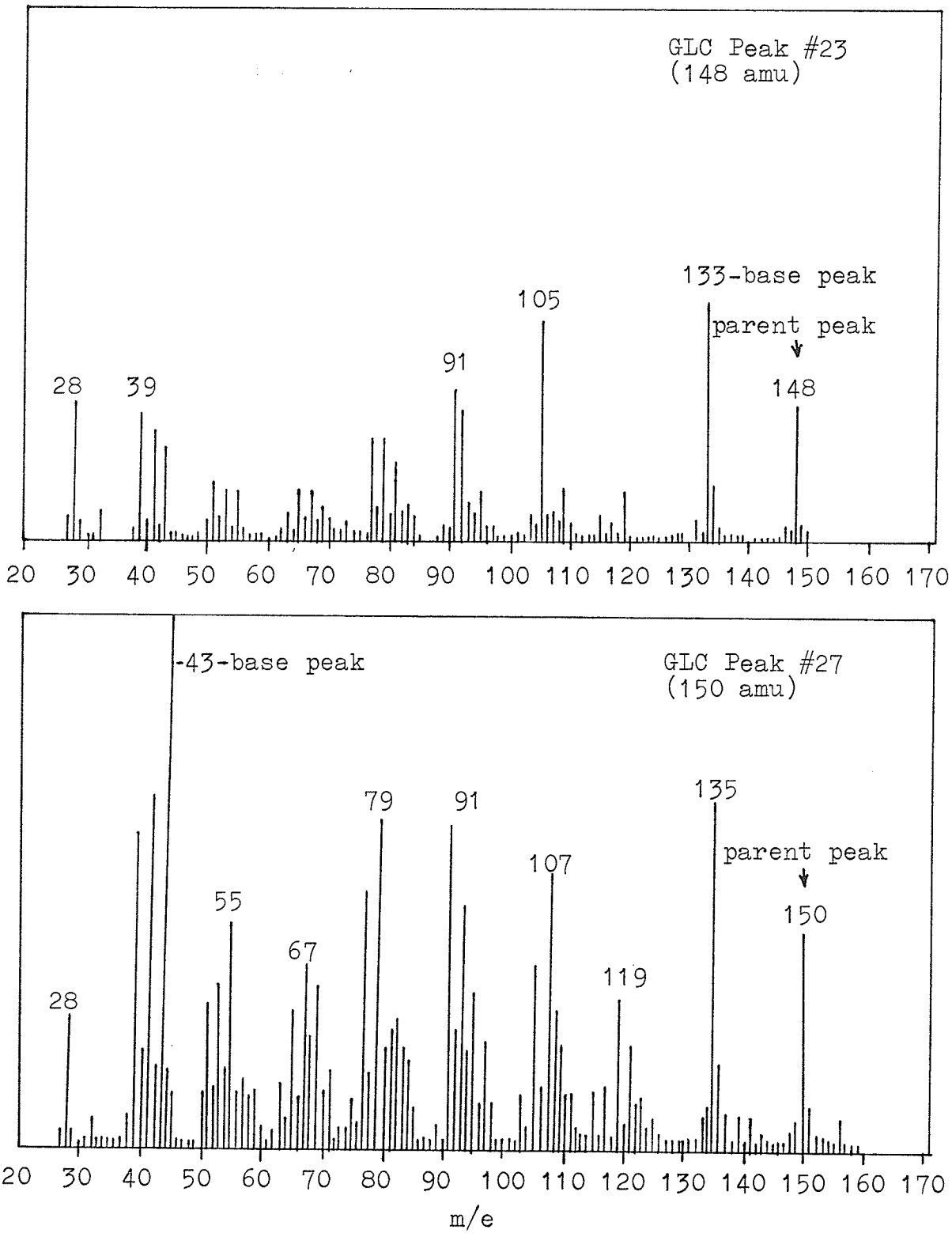
Appendix 12. Mass Spectrogram of Dill 'Ether'



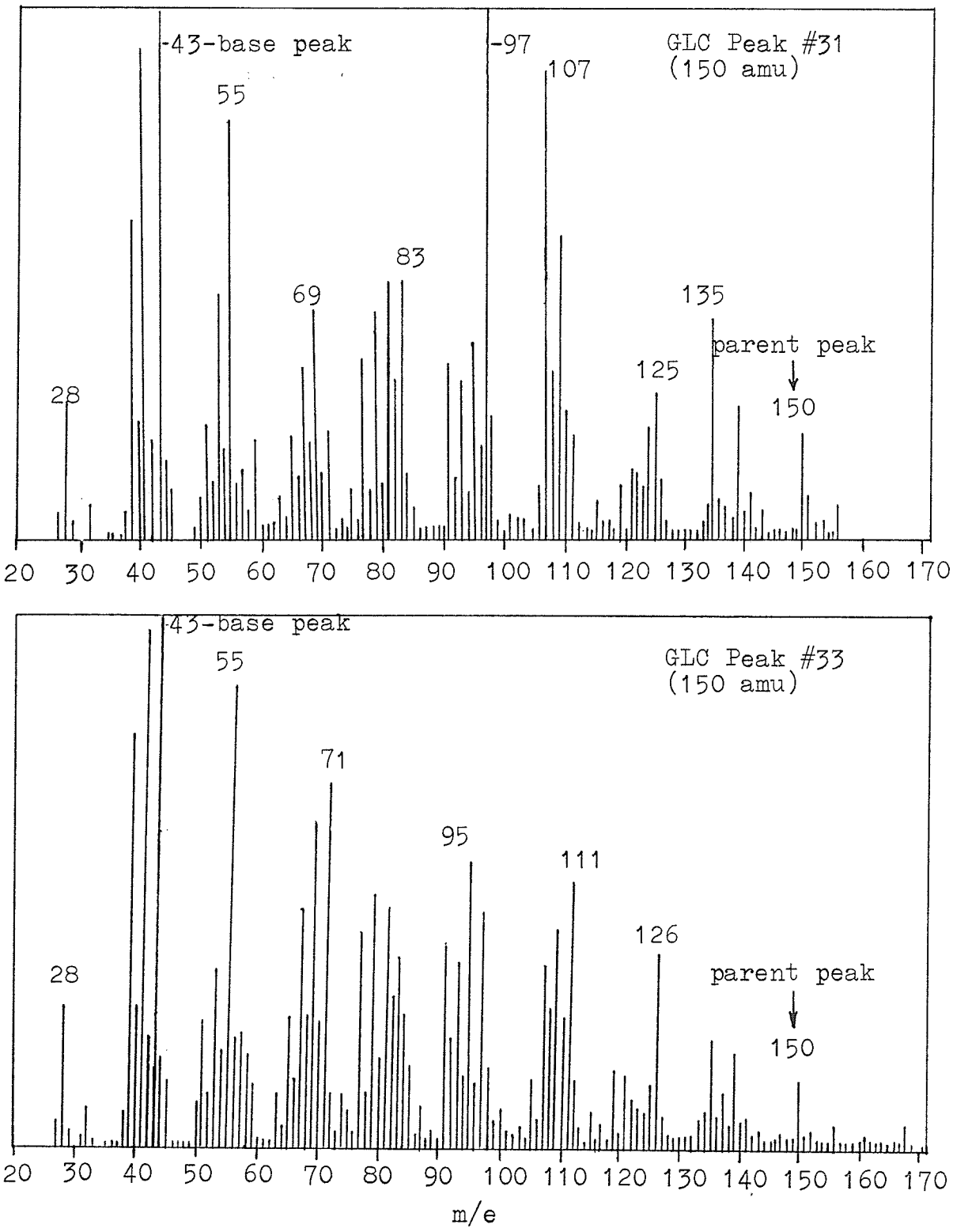
Appendix 13. Mass Spectrograms of GLC Peaks #1 and #18



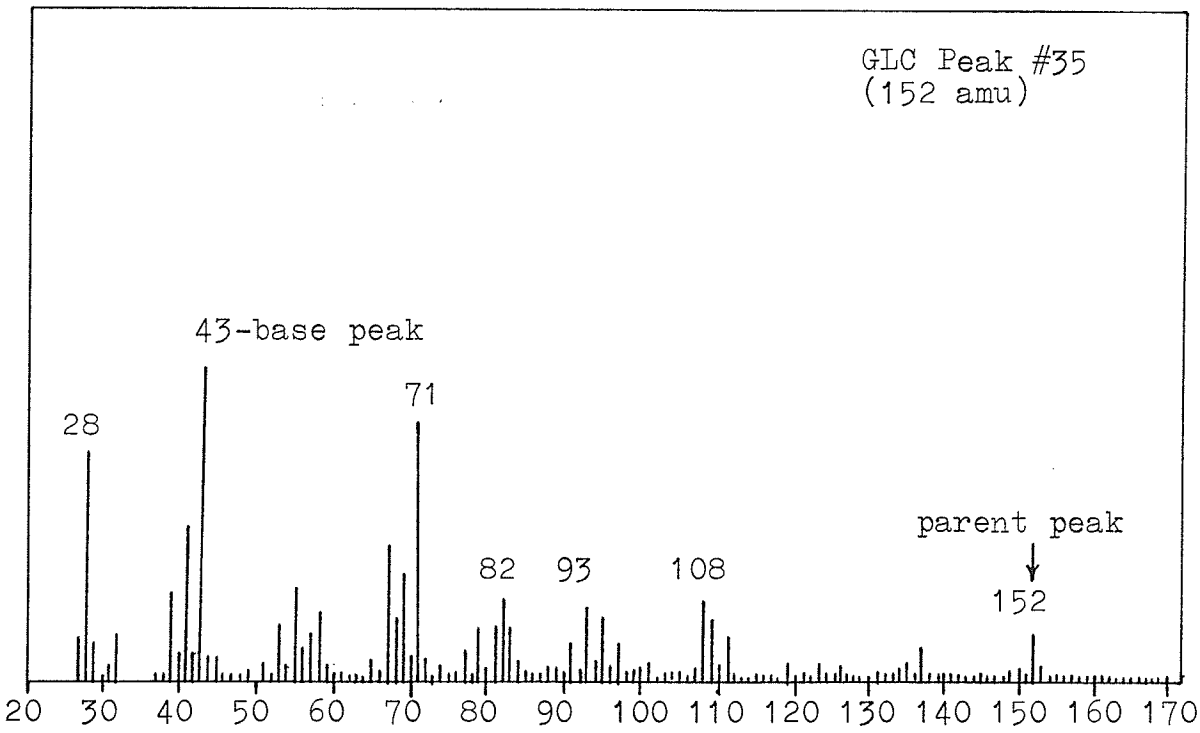
Appendix 14. Mass Spectrograms of GLC Peaks #23 and #27

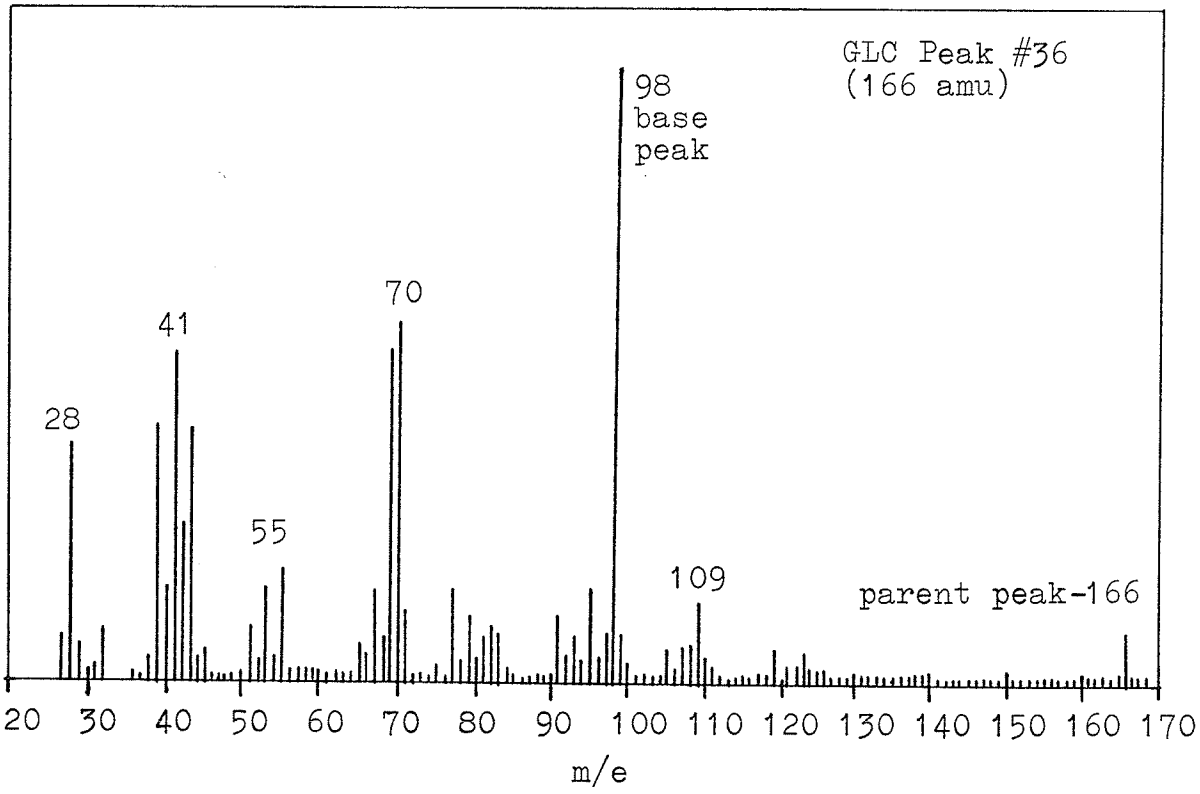


Appendix 15. Mass Spectrograms of GLC Peaks #31 and #33

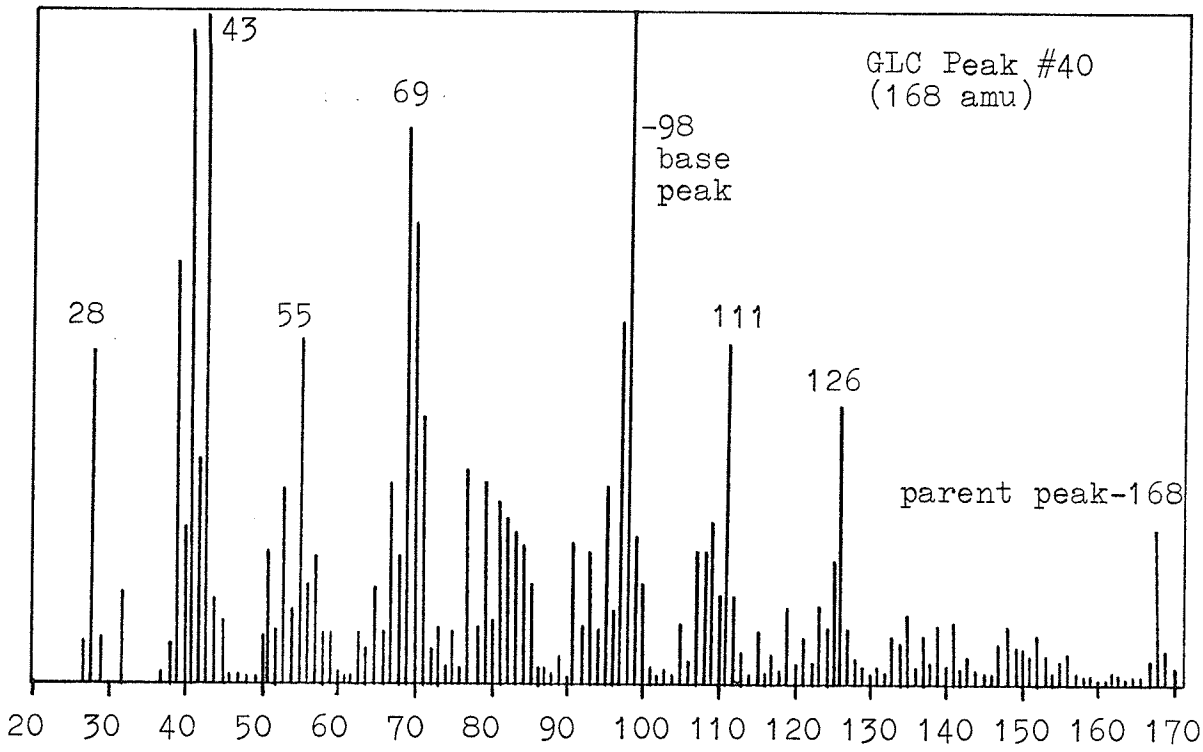


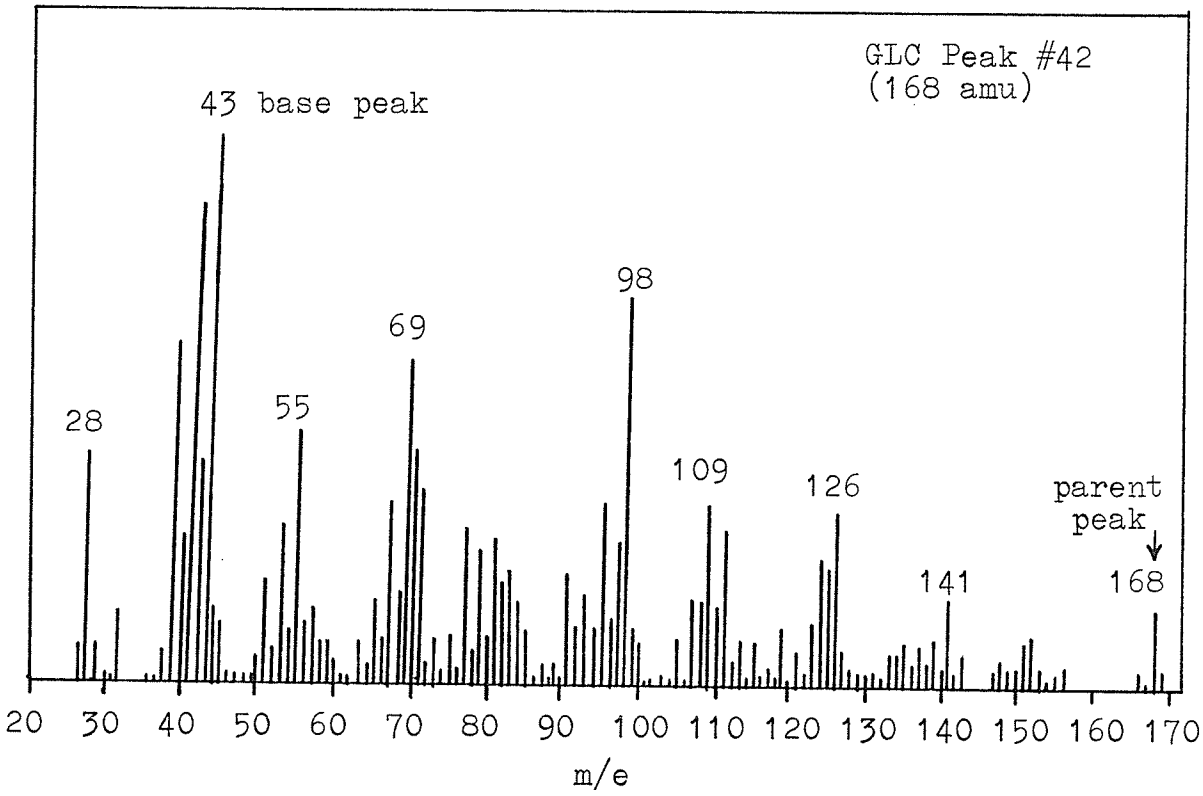
Appendix 16. Mass Spectrograms of GLC Peaks #35 and #36



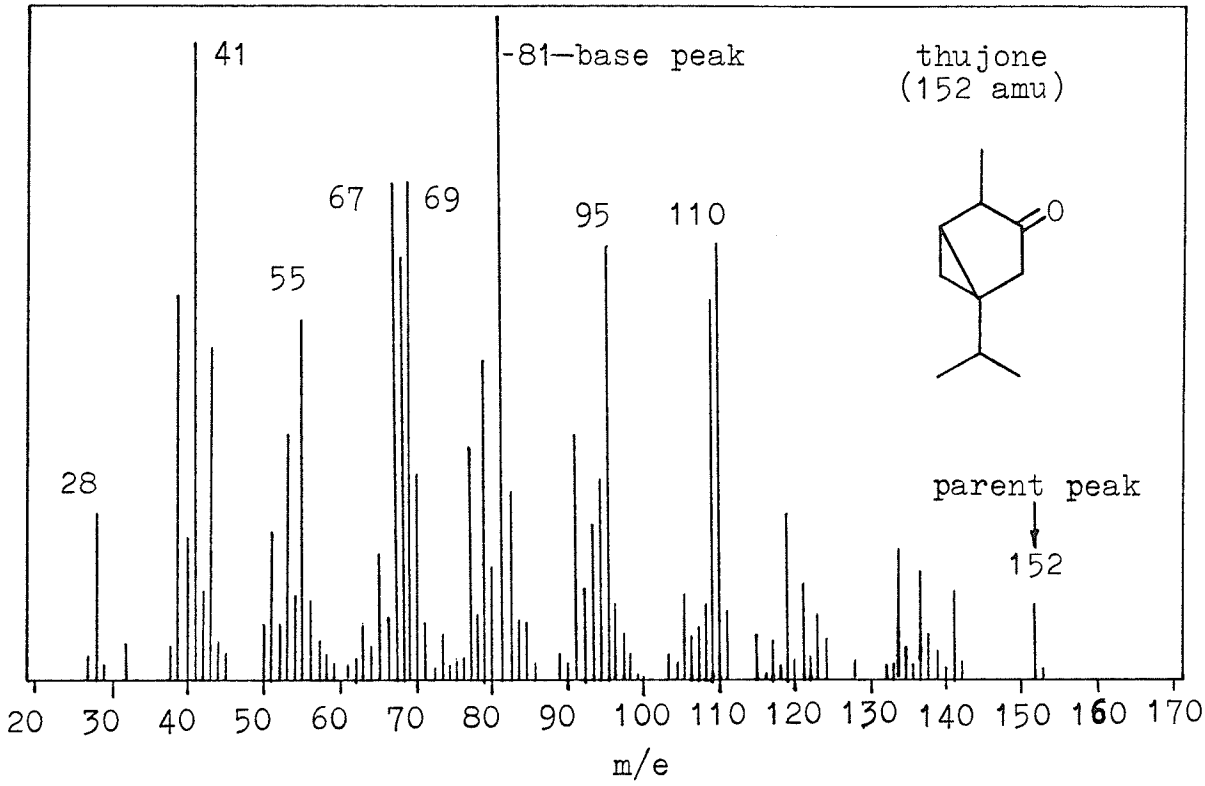


Appendix 17. Mass Spectrograms of GLC Peaks #40 and #42





Appendix 18. Mass Spectrogram of Thujone



Appendix 19. Analysis of Variance: d-Carvone and d-Limonene Composition During Maturation

d-Carvone (SE Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	199.1924	99.5962	1.33	ns
Harvest Times	6	3543.2124	590.5354	7.91	< 0.005
Error	12	895.3476	74.6123	-	-
Total	20	4637.7524	-	-	-

d-Carvone (SW Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	40.9914	20.3390	0.72	ns
Harvest Times	6	8414.2257	1402.3710	49.49	< 0.005
Error	12	340.0686	28.3390	-	-
Total	20	8795.2857	-	-	-

d-Limonene (SE Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	39.0438	19.5219	1.26	ns
Harvest Times	6	58.7067	9.7844	0.63	ns
Error	12	186.4162	15.5347	-	-
Total	20	284.1667	-	-	-

d-Limonene (SW Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	14.2410	7.1205	0.69	ns
Harvest Times	6	81.5029	13.5838	1.32	ns
Error	12	123.4257	10.2855	-	-
Total	20	219.1695	-	-	-

Appendix 20. Analysis of Variance: Alpha-Phellandrene and Dill 'Ether' Composition During Maturation

Alpha-Phellandrene (SE Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	29.4867	14.7433	1.15	ns
Harvest Times	6	567.7924	94.6321	7.36	< 0.005
Error	12	154.2533	12.8544	-	-
Total	20	751.5324	-	-	-

Alpha-Phellandrene (SW Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	2.2352	1.1176	0.06	ns
Harvest Times	6	1960.1790	326.6965	18.34	< 0.005
Error	12	213.7581	17.8132	-	-
Total	20	2176.1723	-	-	-

Dill 'Ether' (SE Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	12.7838	6.3919	0.39	ns
Harvest Times	6	604.0924	100.6821	6.15	< 0.005
Error	12	196.5362	16.3780	-	-
Total	20	813.4124	-	-	-

Dill 'Ether' (SW Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	21.1467	10.5733	3.90	0.05
Harvest Times	6	1699.9314	283.3219	104.42	< 0.005
Error	12	32.5600	2.7133	-	-
Total	20	1753.6381	-	-	-

Appendix 21. Least Significant Difference Analysis: d-Carvone and d-Limonene Composition During Maturation

d-Carvone

Harvest Time:	OIL COMPOSITION* (%GLC VALUES)	
	SE Plot	SW Plot
1. stalking and budding	15.6% a	4.9% a
2. early flowering	26.4% b	27.6% b
3. mass flowering	44.6% cd	46.6% c
4. early milk ripeness	43.9% c	60.5% e
5. milk ripeness	51.5% de	60.5% e
6. milk-wax ripeness	53.8% e	63.2% e
7. full maturity	46.7% cde	53.8% d

$LSD_{0.05}$ (SE plot) = 7.1% ; $LSD_{0.05}$ (SW plot) = 4.4%, where:

$LSD_{0.05} = t_{0.05} \ (MS_E / \#\ of\ reps)^{\frac{1}{2}}$

d-Limonene

Harvest Time:	OIL COMPOSITION* (%GLC VALUES)	
	SE Plot	SW Plot
1. stalking & budding	23.8% ab	16.7% a
2. early flowering	26.8% b	20.9% b
3. mass flowering	23.3% a	20.5% b
4. early milk ripeness	24.2% ab	16.2% a
5. milk ripeness	21.9% a	18.6% ab
6. milk-wax ripeness	21.1% a	17.0% a
7. full maturity	23.2% a	21.1% b

$LSD_{0.05}$ (SE plot) = 3.2% ; $LSD_{0.05}$ (SW plot) = 2.6%

* Mean values calculated from three replicates.

Appendix 22. Least Significant Difference Analysis: Alpha-Phellandrene and Dill 'Ether' Composition During Maturation

Alpha-Phellandrene

Harvest Time:	OIL COMPOSITION*(%GLC VALUES)			
	SE Plot		SW Plot	
1. stalking and budding	26.5%	a	38.3%	a
2. early flowering	23.3%	b	19.6%	b
3. mass flowering	14.4%	c	14.4%	c
4. early milk ripeness	14.8%	c	8.9%	d
5. milk ripeness	11.7%	d	11.4%	cd
6. milk-wax ripeness	12.8%	c	8.4%	d
7. full maturity	15.6%	c	12.7%	c

$LSD_{0.05}$ (SE plot) = 3.0% ; $LSD_{0.05}$ (SW plot) = 3.5%, where:

$LSD_{0.05} = t_{0.05} \ (MS_E / \#\ \text{of reps})^{\frac{1}{2}}$

Dill 'Ether'

Harvest Time:	OIL COMPOSITION*(%GLC VALUES)			
	SE Plot		SW Plot	
1. stalking and budding	23.9%	a	29.7%	a
2. early flowering	15.7%	b	23.7%	b
3. mass flowering	13.2%	b	15.0%	c
4. early milk ripeness	14.4%	b	9.6%	d
5. milk ripeness	8.3%	c	5.4%	e
6. milk-wax ripeness	7.4%	c	5.9%	e
7. full maturity	8.5%	c	5.8%	e

$LSD_{0.05}$ (SE plot) = 3.3% ; $LSD_{0.05}$ (SW plot) = 1.4%

* Mean values calculated from three replicates.

Appendix 23. Analysis of Variance: Dill Oil Content Versus Plant Maturity

1. SE Plot

Source of Variation:	df:	SS:	MS:	F:	Pr F:
Harvest Times	4	0.26644	0.06661	12.96	$\leqslant$0.05
Replicates	1	0.01369	0.01369	2.66	ns
Error	4	0.02056	0.00514	-	-
Total	9	0.30069	-	-	-

2. SW Plot

Source of Variation:	df:	SS:	MS:	F:	Pr F:
Harvest Times	3	0.31250	0.10417	37.65	$\leqslant$0.01
Replicates	1	0.02000	0.02000	7.23	ns
Error	3	0.00830	0.00277	-	-
Total	7	0.34080	-	-	-

ns = not significant at the 5% level of confidence.

Appendix 24. Least Significant Difference Analysis: Dill Oil Content Versus Plant Maturity

Harvest Times:	OIL CONTENT(mL/100g)*	
	SE Plot	SW Plot
1. stalking & budding stage	0.22 a	0.18 a
2. early flowering stage	0.31 b	NA
3. milk ripeness stage	0.60 c	0.50 b
4. milk-wax ripeness stage	0.61 c	0.69 c
5. full maturity	0.56 c	0.64 c

* Mean values for two replicates.

NA = not available.

$LSD_{0.05}$ (SE Plot) = 2.776 (0.00514/5)$^{\frac{1}{2}}$ = 0.089

$LSD_{0.05}$ (SW Plot) = 3.182 (0.00277/4)$^{\frac{1}{2}}$ = 0.084

where: $LSD_{0.05} = t_{0.05} \cdot (2s^2/\#\text{of reps})^{\frac{1}{2}}$; s^2 = sample variance = MS_E

Appendix 25. Analysis of Variance: d-Carvone and d-Limonene Oil Content Versus Plant Maturity

d-Carvone (SE Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	0.21625	0.10813	0.75	ns
Times	4	22.00596	5.50149	38.08*	< 0.005
Error	8	1.15588	0.14448	-	-
Total	14	23.37809	-	-	-

d-Carvone (SW Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	0.32082	0.16041	1.52	ns
Times	3	30.72289	10.24096	97.35*	< 0.005
Error	6	0.63118	0.10519	-	-
Total	11	31.67489	-	-	-

d-Limonene (SE Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	0.05601	0.02801	0.84	ns
Times	4	1.56151	0.39038	11.76*	< 0.005
Error	8	0.26565	0.03321	-	-
Total	14	1.88317	-	-	-

d-Limonene (SW Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	0.08622	0.04311	1.06	ns
Times	3	1.89190	0.63063	15.57*	< 0.005
Error	6	0.24305	0.04051	-	-
Total	11	2.22117	-	-	-

Appendix 26. Analysis of Variance: Alpha-Phellandrene and Dill 'Ether' Oil Content Versus Plant Maturity

Alpha-Phellandrene (SE Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	0.05049	0.02525	1.04	ns
Times	4	0.13904	0.03476	1.44	ns
Error	8	0.19364	0.02420	-	-
Total	14	0.38317	-	-	-

Alpha-Phellandrene (SW Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	0.00882	0.00441	0.22	ns
Times	3	0.11660	0.03887	1.95	ns
Error	6	0.11945	0.01991	-	-
Total	11	0.24487	-	-	-

Dill 'Ether' (SE Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	0.03700	0.01850	0.20	ns
Times	4	0.00964	0.00241	1.52	ns
Error	8	0.09760	0.01220	-	-
Total	14	0.14424	-	-	-

Dill 'Ether' (SW Plot):

Source of Variation:	df:	SS:	MS:	F:	Significance:
Replicates	2	0.00782	0.00391	2.52	ns
Times	3	0.10633	0.03544	22.83*	<0.005
Error	6	0.00932	0.00155	-	-
Total	11	0.12347	-	-	-

Appendix 27. Least Significant Difference Analysis: d-Carvone and d-Limonene Oil Content Versus Plant Maturity

d-Carvone

Harvest Times:	OIL CONTENT(mL/kg)*	
	SE Plot	SW Plot
1. stalking & budding	0.34 a	0.09 a
2. early flowering	0.82 b	NA
3. milk ripeness	3.09 d	3.02 b
4. milk-wax ripeness	3.28 d	4.36 d
5. full maturity	2.62 c	3.44 c

$LSD_{0.05} = 2.306\ (0.14448/5)^{\frac{1}{2}} = 0.39$ (SE Plot)

$LSD_{0.05} = 2.447\ (0.10519/4)^{\frac{1}{2}} = 0.40$ (SW Plot)

d-Limonene

Harvest Times:	OIL CONTENT(mL/kg)*	
	SE Plot	SW Plot
1. stalking & budding	0.52 a	0.30 a
2. early flowering	0.83 b	NA
3. milk ripeness	1.31 c	0.93 b
4. milk-wax ripeness	1.29 c	1.17 bc
5. full maturity	1.30 c	1.35 c

$LSD_{0.05} = 2.306 \times (0.03321/5)^{\frac{1}{2}} = 0.19$ (SE Plot)

$LSD_{0.05} = 2.447 \times (0.04051/4)^{\frac{1}{2}} = 0.25$ (SW Plot)

NA = not available. *Mean values of two replicates.

Appendix 28. Least Significant Difference Analysis: Alpha-Phellandrene and Dill 'Ether' Content Versus Plant Maturity

Alpha-Phellandrene

Harvest Times:	OIL CONTENT(mL/kg)*	
	SE Plot	SW Plot
1. stalking & budding	0.58 a	0.69 ab
2. early flowering	0.72 ab	NA
3. milk ripeness	0.70 ab	0.57 a
4. milk-wax ripeness	0.78 bc	0.58 a
5. full maturity	0.87 c	0.81 b

$LSD_{0.05} = 2.306 \times (0.02420/5)^{\frac{1}{2}} = 0.16$ (SE Plot)

$LSD_{0.05} = 2.447 \times (0.01991/4)^{\frac{1}{2}} = 0.17$ (SW Plot)

Dill 'Ether'

Harvest Times:	OIL CONTENT(mL/kg)*	
	SE Plot	SW Plot
1. stalking & budding	0.53 a	0.53 c
2. early flowering	0.49 a	NA
3. milk ripeness	0.50 a	0.27 a
4. milk-wax ripeness	0.45 a	0.41 b
5. full maturity	0.48 a	0.37 b

NA = not available. *Mean values of two replicates.

$LSD_{0.05}$ (SE) = 0.11; $LSD_{0.05}$ (SW) = 0.05

Appendix 29. Split-Plot Analysis of Variance: 'Shelf-Life' Study, Alpha-Phellandrene and d-Limonene Levels

1. Alpha-Phellandrene

Source of Variation:	df:	SS:	MS:	F:	Pr>F:
Replicates	1	26.169	26.169	0.05	0.8408
Treatments	4	3887.727	971.932	1.71	0.3089
R X Tr (Error A)	4	2280.021	570.005	-	-
Times	6	35520.909	5920.152	26.77	0.0001*
Ti X Tr	24	4699.165	195.799	0.89	0.6166
Error B	30	6635.300	221.177	-	-
Total	69	53049.291	-	-	-

2. d-Limonene

Source of Variation:	df:	SS:	MS:	F:	Pr>F:
Replicates	1	413.829	413.829	19.81	0.0112*
Treatments	4	686.029	171.507	8.21	0.0328*
R X Tr (Error A)	4	83.567	20.892	-	-
Times	6	24927.745	4154.624	61.04	0.0001*
Ti X Tr	24	2382.665	99.278	1.46	0.1626
Error B	30	2041.924	68.064	-	-
Total	69	30535.759	-	-	-

Appendix 30. Split-Plot Analysis of Variance: 'Shelf-Life' Study, d-Carvone and Dill 'Ether' Levels

3. d-Carvone

Source of Variation:	df:	SS:	MS:	F:	Pr > F:
Replicates	1	33.052	33.052	0.65	0.4649
Treatments	4	83.064	20.766	0.41	0.7960
R X Tr (Error A)	4	202.973	50.743	-	-
Times	6	155.396	25.899	1.20	0.3332
Ti X Tr	24	498.044	20.752	0.96	0.5340
Error B	30	647.340	21.578	-	-
Total	69	1619.869	-	-	-

4. Dill 'Ether'

Source of Variation:	df:	SS:	MS:	F:	Pr > F:
Replicates	1	20.956	20.956	15.46	0.0171*
Treatments	4	16.705	4.176	3.08	0.1507
R X Tr (Error A)	4	5.421	1.355	-	-
Times	6	4280.803	713.467	134.60	0.0001*
Ti X Tr	24	179.975	7.499	1.41	0.1826
Error B	30	159.019	5.301	-	-
Total	69	4662.878	-	-	-

Appendix 31. Split-Plot Analysis of Variance: Anti-Oxidant Study, Alpha-Phellandrene and Intermediate Isomer Levels

1. Alpha-Phellandrene

Source of Variation:	df:	SS:	MS:	F:	Pr>F:
Replicates	1	17.298	17.298	1.20	0.3537
Treatments	3	5745.356	1915.119	132.67	0.0011*
R X Tr (Error A)	3	43.305	14.435	-	-
Times	9	3740.362	415.596	501.27	0.0001*
Ti X Tr	27	1781.391	65.977	79.58	0.0001*
Error B	36	29.847	0.829	-	-
Total	79	11357.560	-	-	-

2. Intermediate Isomer(136 amu)

Source of Variation:	df:	SS:	MS:	F:	Pr>F:
Replicates	1	0.861	0.861	0.50	0.5322
Treatments	3	821.130	273.710	157.47	0.0008*
R X Tr (Error A)	3	5.214	1.738	-	-
Times	9	423.051	47.006	396.35	0.0001*
Ti X Tr	27	263.448	9.757	82.27	0.0001*
Error B	36	4.270	0.119	-	-
Total	79	1517.975	-	-	-

Appendix 32. Split-Plot Analysis of Variance: Anti-Oxidant Study, d-Limonene and d-Carvone Levels

3. d-Limonene

Source of Variation:	df:	SS:	MS:	F:	Pr>F:
Replicates	1	0.968	0.968	3.09	0.1769
Treatments	3	29.623	9.874	31.55	0.0091*
R X Tr (Error A)	3	0.939	0.313	-	-
Times	9	16.110	1.790	5.11	0.0002*
Ti X Tr	27	19.600	0.726	2.07	0.0208*
Error B	36	12.613	0.350	-	-
Total	79	79.852	-	-	-

4. d-Carvone

Source of Variation:	df:	SS:	MS:	F:	Pr>F:
Replicates	1	12.246	12.246	10.70	0.0467*
Treatments	3	190.038	63.346	55.35	0.0040*
R X Tr (Error A)	3	3.433	1.144	-	-
Times	9	332.428	36.937	156.52	0.0001*
Ti X Tr	27	65.065	2.410	10.21	0.0001*
Error B	36	8.496	0.236	-	-
Total	79	611.707	-	-	-

LIST OF ABBREVIATIONS

amu	atomic mass units
ANOVA	analysis of variance
BP	boiling point
BHA	butyl, hydroxy anisole
C	solute concentration
^{o}C	degrees Centigrade
C_2H_4	ethylene
$C_{10}H_{16}$	monoterpene
$C_{15}H_{24}$	sesquiterpene
$C_{20}H_{32}$	diterpene
$C_{30}H_{48}$	triterpene
Carbowax 20M	polyethylene glycol polmer (PEG-20M)
Chromosorb W HP	diatomaceous celite GLC packing support
cm	centimeter
CO	carbon monoxide
d	dextrorotatory
DEGA	diethylene glycol adipate
DEGS	diethylene glycol succinate
DMAPP	dimethyl allyl pyrophosphate
EGS	ethylene glycol succinate
EGSS-X	ethylene succinate-methyl silicone copolymer
eV	electron volt
EGPN	ethylene glycol pthalate
FFAP	silicone polymer
FID	flame ionization detector
FW	formula weight
g	gram
GLC	gas liquid chromatography
ha	hectare
HETP	height equivalent to the theoretical plate
HMDS	hexamethyl disilazone
i.d.	inner diameter
IPP	isopentenyl pyrophosphate
IU/g	International Units per gram
k	slope constant in Steven's Power Function
kg	kilogram
L	liter
l	levorotatory

LSD	Least Significant Difference test for means
m	meter
mg	milligram
m/e	mass per unit charge
mL	milliliter
mm	millimeter
MS	mass spectrometry
MVA	Mevalonic Acid Pathway
MW	molecular weight
n	power indicator of sensory response to concentration
ng	nanogram
np	no presence
OV-1	methyl silicone
OV-101	methyl silicone
OV-225	25% phenyl, 25% cyanopropyl, methyl silicone
PDEAS	polydiethanolamine succinate
PEG-20M	polyethylene glycol
ppm	part per million
r	coefficient of correlation
R	sensory panel code for reference samples
R-Index	Recognition Index difference test
RRI	Relative Retention Index used in GLC analysis
S	sensory panel response value
SE	south-east
SE-30	methyl silicone polymer
SP-401	methyl silicone polymer
SP-2100	methyl silicone polymer
SW	south-west
t_{50}	half-life time
TMCS	trimethyl chlorosilane
Toc	alpha-tocopherol acetate
t_R	retention time of solute molecule in GLC analysis
UV	ultra-violet
V	volume
V_g	specific retention volume used in GLC analysis