

AN ABSTRACT OF THE THESIS OF

Carlos Sanz for the degree of Master of Science in Plant Physiology presented on November 12, 1993.

Title: Furaneol Flavor Compounds in Ripening Strawberry

(*Fragaria x ananassa* Duch.).

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Abstract approved: \_\_\_\_\_

*J*

\_\_\_\_\_ Daryl G. Richardson

Among the main flavor components of strawberry now considered by many researchers are: 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol), 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane), and the flavor precursor 2,5-dimethyl-4-hydroxy-3(2H)-furanone- $\beta$ -D-glucopyranoside (furaneol glucoside). However, reports on the quantitative analytical procedures are limited because of the instability of these compounds. The interest in a better understanding of the roles of furaneol, mesifurane and furaneol glucoside in strawberry flavor, and in other fruits that reportedly contain these compounds, or their biosynthesis during fruit ripening, initiated this study.

A rapid methodology is described for the extraction and quantitative analysis of furaneol, mesifurane and furaneol glucoside in strawberries. This method takes less than 90 min to complete the quantitation of these

compounds, compared to previous time consuming liquid-liquid or distillation extractions. The aqueous extraction procedure gives an average recovery of these compounds from strawberries greater than 90%, with detection limits of 0.14, 0.36 and 0.05  $\mu\text{g/mL}$  for furaneol, mesifurane and furaneol glucoside, respectively. These compounds were resolved by HPLC with a RP C18 column, using acetate buffer and methanol as binary mobile phase and detection at 280 nm. Compound identities were confirmed by GC-MS analysis after their isolation by combining C18 cartridge fractionation and ethyl acetate extraction.

Concentration of furaneol and derivatives were assessed during ripening in seven strawberry varieties: Chandler, Parker, Douglas, Pajaro, Redcrest, Benton and Totem. In general, these compounds sharply increased with fruit ripening, attaining maximum values at the overripe stage. The largest amount of furaneol, mesifurane and furaneol glucoside were found in overripe strawberries of cultivars Douglas (22.89  $\mu\text{g/g FW}$ ), Pajaro (39.13  $\mu\text{g/g FW}$ ), and Totem (16.51  $\mu\text{g/g FW}$ ), respectively. These concentrations are the highest values so far reported in strawberry, and they were expected since this was the first non-gas chromatographic analysis of these compounds in this fruit. Results obtained showed quantitative differences among varieties that could be related to their organoleptic properties. Extracts from each strawberry variety at different maturity stages were evaluated for strawberry and overall aroma intensity. The concentration of furaneol and mesifurane correlated better to strawberry aroma than to overall aroma intensity, and

furaneol concentration correlated better with both attributes than did mesifurane. The best correlation values between furaneol and strawberry aroma were found for Parker ( $r = 0.741$ ) and Benton ( $r = 0.733$ ).

Furaneol Flavor Compounds in Ripening  
Strawberry (*Fragaria x ananassa* Duch.)

by

Carlos Sanz

A THESIS

submitted to

Oregon State University

in partial fulfillment of  
the requirements for the  
degree of

Master of Science

Completed November 12, 1993

Commencement June 1994

APPROVED:

Redacted for Privacy

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Date thesis is presented November 12, 1993

Typed by Carlos Sanz for Carlos Sanz

This thesis is dedicated to **Ana**

## **ACKNOWLEDGEMENT**

I would like to express my sincere thanks to Dr. Daryl G. Richardson, my major professor, for his support, hospitality and friendship. Thanks are also due to my M.S. program committee members: Patrick J. Breen, Ronald E. Wrolstad and my graduate representative, Marcos Kogan. Their valued comments and constructive suggestions have been very helpful in writing this thesis.

Thanks go to all people I have had the opportunity to work with, especially those in Daryl's lab, and to all my friends for their encouragement, help throughout my studies, and the friendships which have developed.

I would also like to thank my sponsor Ministerio de Educación y Ciencia of Spain for my Postdoctoral M.E.C./Fulbright grant and financial support for my studies.

Finally, I would like to thank my wife Ana for her understanding, encouragement, and for making my life even more enjoyable.

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# FURANEOL FLAVOR COMPOUNDS IN RIPENING STRAWBERRY

*(Fragaria x ananassa Duch.)*

## CHAPTER 1

### INTRODUCTION AND LITERATURE REVIEW

Although strawberry species have existed for an estimated 50 million years, and their use by man has been dated to the bronze age, only after the 14th century A.D. were strawberry plants gathered from the wild and grown in gardens. These first cultivated strawberry plants were grown for both ornamental and medicinal purposes (Otterbacher and Skirvin, 1978). Today, strawberry is among the first of the fresh fruits on the market in spring, and they are grown extensively both for fresh and processed use. The cultivated strawberry (*Fragaria x ananassa Duch.*) is an American fruit that originated by hybridization from wild species of Eastern North America and South America. The berries, which are rich in vitamin C, have a unique and attractive flavor that, together with color and textural features, make them highly valued as dessert fruit.

This chapter is dedicated to review general aspects of fruit flavor, with emphasis on strawberry flavor and in particular three components: furaneol, mesifurane and furaneol glucoside, that are major contributors to the flavor of this fruit.

## FRUIT FLAVOR

### Flavor

Flavor is a complex sensation which is experienced when a food or beverage is placed in the mouth. Different authors have tried to make precise definitions of flavor, but none has completely succeeded. Flavor was defined by Hall (1968) as "the sum of those characteristics of any material taken in the mouth, perceived principally by the senses of taste and smell and also by the general pain and tactile receptors in the mouth, as received and interpreted by the brain". The senses of odor and taste are the primary components of flavor, but odor is generally the most important component of flavor and by far the most difficult to describe and define. This predominance of one sensation over the other usually leads to an incorrect utilization of the term flavor, that is identified with odor instead of its general meaning, a combination of odor and taste.

### Fruit aroma

The odors of fruits are often described as aromas, because this term is normally associated with a pleasant sensation not necessarily true of odor. In general terms, fruit aromas are derived from minute quantities of volatile substances such as esters, aldehydes, ketones, alcohols, hydrocarbons and other compounds that are often concentrated in the peel.

### **Aroma and quality**

Fruit quality is the combination of the inherent properties or attributes of a fruit which determine its relative degree of excellence. Aroma is only one of several sensory properties which affect the acceptability of a fruit; appearance, flavor, texture, and possibly sound (e.g. 'crunchy') are also important. Appearance is probably the first factor considered in relation to quality for consumer purchase, but flavor is usually the most important property when fruit quality is assessed. In this sense, Williams (1979) described the existence of complex interactions among the attributes that determine fruit quality. According to this author, the contribution of a particular attribute is variable along the hedonic scale, and it exerts its main effect only over a particular quality range. Above or below this range, previously less significant characters may play a dominant role.

In many instances the characteristic aroma of a particular fruit species has been associated with a single compound or class of compounds with similar structure (Williams, 1979). However, despite the organoleptic importance of some compounds, most fruit aromas can not be clearly identified with just one character-impact compound. That is the case, for example, of peach (Schlich and Guichard, 1989), pineapple (Takeoka et al., 1989) or pear (Russell et al., 1981). In some fruits such as the strawberry, it is even more difficult to assign compounds of high sensorial impact due to the extreme complexity of their aromas.

There are several works relating the range of acceptability of different fruit varieties as a function of their aromatic characteristics. Paillard (1975) studied the volatile composition of different apple varieties, and this work was later continued by Williams and Carter (1977), who tried to justify the higher acceptability of Cox Orange Pippin apple by its aroma composition. More recently, Dirinck et al. (1989) have studied the aromatic quality of 25 different varieties of apple.

On the other hand, the study of fruit aroma composition can provide information about possible physiological problems. For instance, the production of  $\alpha$ -farnesene in pears and apples is related to a physiological disorder known as scald (Huelin and Coggiola, 1968, 1970; Chen et al., 1990).

### **Aroma and ripening**

The typical aroma of fruits such as bananas, peaches, pears, and cherries is not present during early fruit formation but develops during a rather brief period, late in ripening (Paillard, 1981). This aroma development period, or ripening, begins during the climateric rise in respiration. The rate of aroma production reaches a maximum after the climateric ripening phase. At these stages, metabolism of the fruit changes to mainly catabolic pathways and aroma formation begins. Minute quantities of lipids, carbohydrates, proteins and amino acids are enzymatically converted to volatile compounds. In this sense, metabolites such as the fatty acids, usually in very low concentration

in the free form, increase drastically due to phospholipid catabolism (Mazliak, 1967) and they are converted into an array of alcohols and volatile esters (Jennings and Tressl, 1974; Paillard, 1979a, b). Tressl and Drawert (1973) described the relationship between the increase in free amino acids and the increase in aroma production during the final stage in banana ripening. This increase in the level of precursors and volatile compounds biogenesis is determined by a general increase in enzymatic activities during this period (Hulme et al., 1971).

The relationship between aroma formation and ripening is so close that some authors proposed volatile production as an index of fruit maturity (Biale and Young, 1981), or to make prediction of picking dates (Dirinck et al., 1989).

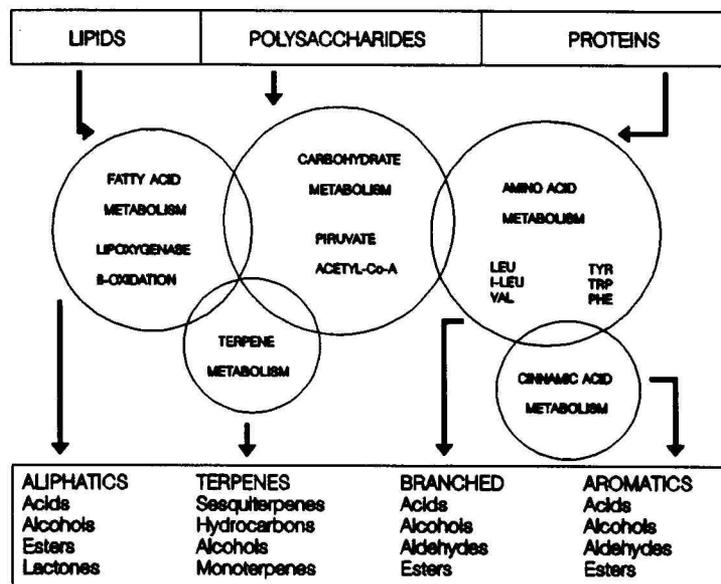
### **Primary and secondary aroma**

A distinction can be made between the primary aroma components, which are biosynthesized by the whole fruit, and the secondary aroma compounds (e.g. hexanal, 2-hexenal), formed after disruption of the cells during processing or chewing (Drawert et al., 1976). The first ones derive from the enzymatically controlled metabolism of the plant, described by Schreier (1986) as intracellular pathways. The secondary aroma components appear when metabolites and enzymes, previously separated in different cell organelles, meet after cellular disruption. The sensory contribution of such 'secondary' volatiles can be favourable or detrimental depending on their

concentrations, composition and type of plant material. Thus, they can accumulate in concentrations to cause an off-flavor (Buttery et al., 1987), but, on the other hand, there are examples (e.g. cucumber, melon) where the typical aroma is characterized by these 'secondary' volatiles (Engel et al., 1990).

### Biogenesis of fruit aroma

Aromas are formed from major plant constituents (e.g., carbohydrates, lipids and proteins) under genetic control. Each metabolic pathway is connected to other metabolic pathways as is shown below in an overall view:



Volatile compounds are produced as direct products of a metabolic pathway or as a result of interactions between pathways or end products.

**Aromas from fatty acid metabolism.-** Volatile flavor compounds may be formed from lipids via several different pathways. These include lipoxygenase pathway,  $\beta$ -oxidation and hydroxyacid cleavage (lactone formation). While the primary products of these pathways are aldehydes and ketones, various oxidations, reductions and esterifications also yield substantial quantities of acids, alcohols, lactones and esters.

The widest variety of flavor compounds formed from lipids arises via the lipoxygenase pathway (Forss, 1973; Grosh, 1982). Many of the aliphatic esters, alcohols, acids and carbonyls found in fruits are derived from the oxidative degradation of linoleic and linolenic acids. Studies on the generation of flavor in banana (Tressl and Drawert, 1973), tomato (Galliard and Matthew, 1977; Schreier and Lorenz, 1981), apple (Schreier and Lorenz, 1982), and strawberry (Gorst-Allman and Spiteller, 1988) elucidate and point out the importance of lipoxygenase activity to flavor development in fruit. The main products of the lipoxygenase pathway are  $C_6$ -compounds that are themselves mainly responsible for the characteristic 'green grassy' odor notes in the aroma of various fruits. The actual mechanism of this pathway consists of four sequential enzymic steps: acyl hydrolysis of lipids by lipases or phospholipases; peroxidation of the free polyunsaturated fatty acids by means of the lipoxygenase, producing 9- and 13-hydroperoxides; cleavage of fatty acid hydroperoxides by a lyase; and reduction of the resulting aldehydes to

alcohols by alcohol hydrogenase, followed by isomerization of the products (Olías et al., 1993a).

The formation of aromas via  $\beta$ -oxidation is exemplified by considering aroma formation in pears. The decadienoate esters are generally considered carriers of the aroma of Barlett type pears (Jennings and Tressl, 1974). These esters are formed via  $\beta$ -oxidation of linoleic acid. This compound is metabolized, two carbons at a time, to shorter chain CoA derivatives which may react with alcohols to yield esters. During this process, isomerizations may occur to yield the *trans-cis* isomers. It is interesting that the  $\beta$ -oxidation pathway can account for all of the volatiles which have been identified in pear. This pathway has been demonstrated to be also operative in apple (Paillard, 1979a), banana and strawberry (Tressl and Albrecht, 1986).

The production of lactones presents a third means of obtaining aroma compounds from lipid sources. Lactones are very important to the flavor of peach, apricot or coconut. Numerous investigators have proposed oxidative pathways which would result in lactone formation (Maga, 1976). A major pathway involving lipoxygenase action on linoleic acid has been recently reported (Albrecht et al., 1992).

**Aromas from amino acid metabolism.-** Amino acid metabolism generates aliphatic and branched chain alcohols, carbonyls and esters which are important to the flavor of fruits. Yu et al. (1967) demonstrated that valine, leucine, alanine and aspartic acid could be converted to short chain carbonyls by tomato extracts. Studies with banana tissue slices have shown

that leucine and valine concentrations increase about threefold following the climateric rise in respiration (Tressl and Drawert, 1973). Radioactive labeling studies have shown that these amino acids are transformed into branched chain aroma compounds, such as isoamyl acetate, which are essential to banana aroma. The initial step in the metabolic pathway is the deamination of the amino acid by an aminotransferase, followed by decarboxylation. Various reductions and esterifications then lead to a number of volatiles which are significant to fruit flavor. Aromatic aminoacids also may serve as important precursors to fruit flavor. Some of the aromatic aroma compounds have been shown to come from tyrosine and phenylalanine (Tressl and Albrecht, 1986). Odors characterized as 'phenolic' or 'spicy' could arise via this pathway. Pérez et al. (1992) have recently demonstrated a relationship between the increase in ethyl esters and the decrease in alanine concentration during strawberry ripening. Alanine is the major free amino acid in Chandler strawberries, and can be transformed into ethanol by the pathway described above.

**Aromas from carbohydrate metabolism.-** One may state that nearly all of the plant flavors come indirectly from carbohydrate metabolism since all of the flavor precursors are derived from carbohydrate metabolism through the photosynthetic pathway. However, there are some aroma constituents that come more directly from carbohydrates, such as the terpenes and furane derivatives.

Terpenes may arise from either carbohydrate or lipid metabolism. Citrus products obtain their characteristic aroma from terpenes. Limonene, a monoterpene hydrocarbon possessing little odor, is the major terpene in most citrus oils often accounting for nearly 90% of the oil. The oxygenated terpenes, often comprising less than 5% of the oil, generally provide the characteristic flavor of different citrus species. For example, citral is considered the flavor impact component of lemon oil yet is unusual to find more than 2% citral in the oil (Mussinan et al., 1981). The metabolic pathways leading to terpene formation are only partially known in fruits. While it is postulated that terpene formation involves mevalonic acid, the mechanism of mevalonic acid synthesis is not well characterized. Most plants synthesize mevalonic acid from acetate, however key enzymes leading from acetate to mevalonic acid have not been found in citrus fruit (Potty, 1969). The mechanism of terpene formation consists of five steps (Erman, 1985): the first step is the formation of mevalonic acid from acetate; this acid is converted into the isoprene units, isopentenyl pyrophosphate and dimethylallyl pyrophosphate; the third step is a condensation of two isoprene units to yield pyrophosphorylated terpenic alcohols; they are then hydrolyzed to cyclic or acyclic monoterpenic alcohols; finally these compounds are transformed into different terpenic derivatives through not well known reactions of oxidation, hydrogenation, dehydrogenation or hydroxylation (Engel and Tressl, 1983; Winterhalter et al., 1986).

Interest has focused during recent years on odorous furane compounds. This is true particularly for two furane derivatives, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) and 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane), which have been identified among the volatile constituents of certain fruits such as arctic bramble, strawberry, raspberry, pineapple, mango or grapefruit (Hunter et al., 1974; Pickenhagen et al., 1981; Kallio et al., 1984; Lee and Nagy, 1987; Pabst et al., 1991). The biosynthesis of furaneol and mesifurane has not been fully elucidated. The hypothesis has been advanced that furaneol is derived from fructose through hydrogen transfer and water elimination, and that the mesifurane is then formed from furaneol through methylation. There is some evidence for the presence of enzymic systems in arctic bramble berries which can carry out the conversion of sugar into furanone, since a homogenate of these berries converted all-carbon labelled fructose into a labelled compound that migrated in thin-layer chromatography identically with synthetic furaneol (Kallio, 1975).

### **Factors determining fruit aroma**

Aroma development in fruits occurs during ripening and it is conditioned by a number of factors. Paillard (1981) distinguished external and internal factors influencing aroma formation in fruits. The first ones associated with the culture of the plant and post-harvest treatments, the second are in connection with the metabolic regulation of the fruit and ripening.

**External factors.**- Plant culture and fruit handling may have an effect on fruit aroma.

Preharvest factors.- Culture conditions as soils, fertilizers, climate, irrigation influence aroma development. High nitrogen treatments of apple trees produce an increase in volatile content in fruits (Somogyi et al., 1964), and phosphorous fertilization has been observed to increase the rate of production of 3 major volatile esters in Golden Delicious apples (Brown et al., 1968). More recently, Nagy and Shaw (1990) have shown the influence of fertilization, climate, rootstock, maturity and spray material on the flavor of citrus fruits. Drought stress promotes the production of smaller fruits but high in flavor, because of the higher concentration of metabolites such as sugars, organic acids and amino acids, precursors for aroma biosynthesis (Freeman, 1979).

Picking date or stage of maturity.- A number of fruits are harvested just before full ripening in order to extend their shelf-life. However, if the fruit is picked too early its development and nutrition are interrupted and it may never develop its full flavor. Late picked apples show an immediate aroma development and reach a considerably higher volatile maximum compared to early picked apples (Dirinck et al., 1984, 1989). In citrus, early harvest produces fruits with scarce flavor where the 'green grassy' odor notes are predominant (Fellers, 1985).

Post-harvest treatments.- A systematic study of temperature effects on ripening and volatile emission has been done with banana (Mattei, 1973).

The volatile production is an exponential function of temperature from 5°C to 25°C, considering 12°C as optimal temperature for storage and transport. The aroma is developed during ripening but depends on the duration of the storage at 12°C (Mattei and Paillard, 1973). In general, any kind of storage or refrigeration process of fruits promotes losses in their aromatic quality (Buttery et al., 1987). However, for some fruits stored for long period, such as apples, it is possible to determine temperatures and storage times which significantly minimize aroma losses (Paillard and Mattei, 1971).

Modified atmospheres containing higher carbon dioxide and lower oxygen concentration than air, used in storage to delay fruit ripening, usually have a negative effect on volatile production (Patzold, 1984). Nevertheless, for some fruits such as kiwi (Harman and McDonald, 1983) or peach (Brecht et al., 1982) controlled atmosphere (CA) may be acceptable from an organoleptic point of view. The apple is the fruit more extensively studied regarding its behaviour under CA. Short CA storage of apples (up to 3 months) does not affect volatile biosynthesis (Lidster et al., 1983a, b). However, long term CA storage irreversibly reduces fruit aroma production (Streif and Bangerth, 1988; Olías et al., 1992), especially when ultralow oxygen concentrations are used (Brackmann et al., 1993). In more delicate fruits such as strawberries, CO<sub>2</sub> concentrations equal or greater than 15% promote off-flavors (El-Kazzaz et al., 1983).

Storage of fruits in air at lower relative humidities increases both the rate of water loss and emission of volatiles (Ohloff et al., 1976; Wahlberg et

al., 1979). Illumination of apples with fluorescent lights during storage at 5°C also affects some fruit characteristics. There are changes in pigment content and an increase in volatile production, which may be due to an increase in membrane lipid peroxidation that would promote an increase in precursors for volatile production (Knee et al., 1979).

**Internal factors.-** Some biological factors related to the structure and metabolism of the fruit have an effect on aroma formation.

It is well known that the ability to produce volatile compounds is not uniform in all cells or tissues of the fruit, with clear differences among the different parts. For instance, in the citrus fruits the composition and biosynthesis of essential oils are different in the glandular cells of exocarp and in the juice vesicles of the endocarp (Peleg et al., 1991). Skin is the most active part in the aroma biosynthesis in most fruits. Studies on flavor location in the Muscat grape berry showed that the major part of volatile components proceeds from the grape-skin and the cellular residues of the pulp (Bayonove et al., 1974). This may be explained by important enzyme activities in the peel and outer flesh, and the abundance of fatty acids which are precursors of alcohols and esters.

There are several studies related to the distribution of precursors in the fruit and its aromatic composition. For example, the differences in aroma of two varieties of mango, Alphonso and Totapuri, were related to the different content in triglycerides, compounds that are the source of the main precursors for mango aroma (Gholap and Bandyopadhyay, 1975). Studies on specificity

of the enzymatic systems implicated in aroma formation have been done in few fruits. The work by Paillard (1979b) on apples, studying varietal differences related to volatile precursor metabolism, is probably the clearest approach.

## THE STRAWBERRY

### Fruit origin and distribution

The strawberry fruit is not a true fruit in that the fleshy edible portion is the enlarged receptacle on which the achenes are borne externally. It consists of a fleshy pith at the center, next a ring of vascular bundles branching out into the achenes. Each achene contains a single seed that is not surrounded by fleshy mesocarp (Darrow, 1966).

Strawberries belong to the genus *Fragaria* (*Rosaceae*). Along with many other genera of plants, the genus *Fragaria* probably has its origin in the regions of the Himalayas and Southeast Asia (Darrow, 1966; Scott and Lawrence, 1975). A relationship between three residing Asian species, *F. daltoniana* J. Gay, *F. nubicola* Lindl. and *F. nilgerrensis* Schlecht., and the species growing in Europe, Asia and America, has been demonstrated. During the period of distribution, the continent of Eurasia was connected to North America. Over this long period, the chromosome number of the original diploid species ( $2n = 14$ ) was multiplied by 2, 3 or 4, so that American *Fragaria* species are octaploid ( $2n = 56$ ).

Nowadays, *F. vesca* L. has the widest area of distribution, including Europe, Asia and North America. Some variant forms of *F. vesca* are also known, for example *F. vesca alba*, which has white or pale yellow berries, and *F. vesca semperflorens*, which is octaploid and it is best known by the name Rügen. Other wild *Fragaria* species, for example *F. moschata* Duch. and *F. viridis* Duch., are native to Europe; *F. nubicola* Lindl., *F. nilgerrensis* Schlecht., *F. orientalis* Losinsk., *F. moupinensis* Franch. and *F. daltoniana* J. Gay to Asia; *F. ovalis* Lehm. and *F. virginiana* Duch. to North America; and *F. chiloensis* Duch. to South America.

Modern commercial strawberry plants with large fruits are the result of intercrosses of *F. chiloensis* and *F. virginiana*. The large strawberry *F. chiloensis* was imported to Europe by the navy skipper François Amédeé Frézier from Concepción (Chile), where it was cultivated by the Indians. Frézier came back to Marseille in 1714 bringing five plants of *F. chiloensis* that were distributed among several European botanic gardens. Those plants had only female flowers, and they were pollinated in Bretagne with pollen parent from *F. virginiana* from the North American Atlantic Seaboard, which had been introduced into Europe at least one century earlier (Wilhelm and Sagen, 1974). The region of Plougastel, near Brest, France, around 1750 became the European center of strawberry production, supplying Paris and London. Strawberry production was so important that in 1850, during the peak of harvest, twenty sailing-ships and a steamboat were required in Brest to load all the freight (Wilhelm, 1974).

Cultivated strawberries, all octaploids, have been regrouped as *Fragaria x ananassa* Duch. (Staudt, 1961; Otterbacher and Skirvin, 1978) in recognition of Duchesne's reports in 1766. The descendants of the original hybrids were very heterogeneous and produced cultivars with either one or two annual blossoms or perpetual blossoms. Due to their hybrid origins, strawberries are adapted to many different climates: moderate, mediterranean, subtropical, and even tropical if grown at high altitudes. Most cultivars are propagated vegetatively. There is a demand for new strawberry cultivars with the pleasant herbaceous aroma typical of wild strawberries. In the hope of obtaining such new cultivars, *F. vesca semperflorens* and *F. virginiana*, which both have the same chromosome number ( $2n = 56$ ) as *F. x ananassa*, have been cross-bred with cultivated strawberry varieties such as Valentine and Senga Sengana, respectively (Brooks and Olmo, 1968).

Strawberry production has increased consistently since 1945. However, further development will probably proceed most rapidly in less developed countries, where labor is less expensive. About 75% of the production cost of strawberries is due to labor of which 50% is required for the harvest. This labor cost explains the current interest in mechanical harvesting.

World production of strawberries is about 2.36 million tons (FAO, 1990). The main production centers are all located in the Northern Hemisphere. Europe produces 50.2% of the world's strawberries, and North America produces 27.9%. Most of the strawberry fields in North America are

found in California. Asia accounts for 14.2% of world production, mainly in Japan. The most productive countries in descending order of importance are the United States (22.8%), Poland (11.4%) and Spain (9.6%).

### **Aroma composition**

The volatile aroma compounds of cultivated strawberries have been extensively studied by many authors during the past 30 years. Winter and Willhalm (1964) identified over 60 compounds in the cultivar Surprise des Halles, including *trans*-2-hexenol, 2-ethylhexanol, ethyl butanoate, hexyl acetate, *trans*-2-hexenyl acetate, ethyl acetoacetate and  $\alpha$ -terpineol. Later, Willhalm et al. (1966) reported that the main volatile acids of strawberries consisted of 2-methylpropanoic, 2-methylbutanoic, and hexanoic acids. McFadden et al. (1965) reported the identification of *cis*- and *trans*-3-hexenyl hexanoates, *trans*-2-hexenyl hexanoates, linalool and several other compounds not reported previously as constituents of strawberry aroma. Tressl et al. (1969) identified about 200 compounds in the berries of the cultivar Revata. The main components included previously unidentified compounds such as octyl butanoate, octyl 2-methylbutanoate, octyl hexanoate and  $\gamma$ -dodecalactone. Stoltz et al. (1970) identified 1,2-dihydro-1,1,6-trimethylnaphthalene in strawberry oil, and Mussinan and Walradt (1975) found over 20 volatile acids which had not previously been reported in strawberries. Dirinck et al. (1977, 1981) used headspace concentration on Tenax to evaluate the aroma quality of cultivated strawberries. They

identified about 30 new volatiles, among them, sulfur-containing compounds were identified for the first time in strawberry aroma. Schreier (1980) compared the volatile constituents of three fresh and deep-frozen strawberry cultivars, Senga Sengana, Senga Litessa and Senga Gourmella, and identified 74 volatile components, of which 43 were esters. Hirvi (1983) has studied the aromas of eight fresh and deep-frozen strawberry cultivars by mass spectrometry and sensory evaluation. The concentrations of 17 major components of Senga Sengana were determined in each cultivar. Intensity of odor, character of odor, overall impression of odor, sweetness, overall impression of taste, sourness, off-odors and off-tastes were evaluated using a graphical scale method. The concentrations of the most important aroma compounds, ethyl hexanoate, ethyl butanoate, *trans*-2-hexenal, mesifurane and linalool, were highest among cultivars Senga Sengana, Kristina and Lihama x Senga Sengana. These three were rated best with regard to character of odor and overall impression of odor. Pérez et al. (1992) using activated charcoal for headspace concentration found that ethyl esters, mainly ethyl butanoate and ethyl hexanoate, were the main volatile compounds during the ripening of Chandler strawberries.

Other studies have emphasized more the analysis of wild strawberry aroma. Drawert et al. (1973) and Staudt et al. (1975) compared the volatile constituents of some wild *Fragaria* species (*F. vesca*, *F. moschata*, *F. chiloensis* and *F. virginiana*) with those of the cultivar Revata. They found that *F. vesca* and *F. moschata* have the same main flavor compounds.

Typical for these berries were high concentrations of 2-alkanones and 2-alkanols, especially 2-tridecanone and 2-tridecanol. On the other hand, *F. chiloensis* contained neither 2-alkanones nor 2-alkanols. The presence of ethyl esters of C<sub>6</sub>-C<sub>12</sub> acids is characteristic for *F. chiloensis*. *F. virginiana* has the strongest aroma of all these four species. It also gives 2-alkanones, and ethyl and methyl esters of C<sub>6</sub>-C<sub>12</sub> acids. The high concentrations of ethyl and methyl cinnamates and  $\gamma$ -decalactone are also characteristic of *F. virginiana*. The volatile constituents of *F. nilgerrensis* differ clearly from those of *F. virginiana*. Typical compounds from *F. nilgerrensis* are octyl and decyl acetates, methyl benzoate and benzyl acetate. Pyysalo et al. (1979) quantified the volatile constituents of wild strawberry (*F. vesca*) and compared them with the cultivar Senga Sengana, and identified a total of 87 volatile components. They found that the main volatile compound in the press juice of *F. vesca* was mesifurane. The cultivated Senga Sengana strawberries were also shown to contain this compound, although at a considerably lower concentration. On the other hand, the corresponding hydroxy compound, furaneol, which had previously been identified in strawberries (Sundt, 1970) was not found in either the wild or cultivated berry studies (Pyysalo et al., 1979). Other major neutral volatiles identified in the wild berries included *trans*-2-hexenol, methyl butanoate, hexanol, 2-pentadecanone, benzyl alcohol, ethyl butanoate, 2-heptanone, 2-pentadecanol and eugenol. Typical for the flavor of *F. vesca* were also methyl anthranilate, methyl nicotinate, carvyl acetate, verbenone, citronellol and myrtenol, which had not previously been

identified as strawberry volatiles. Hirvi and Honkanen (1982) studied the volatiles of two new strawberry cultivars, Annelie and Alaska Pioneer, obtained by backcrossing cultivated strawberries with wild strawberries, *F. vesca semperflorens* Rügen and *F. virginiana*. The flavor of the berries of both cultivars resembled that of the fresh wild strawberry more closely than that of the cultivated strawberry. The main volatile compound of wild strawberry (*F. vesca*), mesifurane, was also an important constituent of the aromas of both berries. The concentration of this compound was 0.8 and 0.45 mg/kg in Annelie and Alaska Pioneer cultivars, respectively. These values are somewhat lower than those reported for *F. vesca* (1.7 mg/kg) by Pyysalo et al. (1979). Only traces of the corresponding hydroxy compound, furaneol, were found in both berries. In general, the aroma of both berries consists of the same compounds, the concentrations of which are higher in the cultivar Annelie, however.

All esters, alcohols and carbonyl compounds identified in these studies seem to contribute, with more or less strength, to the 'estery' and 'green grassy' notes of strawberry aroma, but they can hardly be considered to include a character-impact compound or compounds. Sundt (1970), however, reported the identification of furaneol in strawberries, and considered this compound the most important aroma constituent. Later on, other studies have shown that furaneol, ethyl butanoate and ethyl hexanoate contribute mainly to the aroma because of their high concentrations and their very low threshold values. Further, mesifurane, linalool, C<sub>6</sub>-aldehydes, responsible for

the 'green grassy' odor notes, such as 2-hexenal, and sulfur-containing compounds seem to be also very important aroma constituents (Hannover, 1991).

Besides the contribution to the strawberry aroma by these free form compounds, the conjugate forms, mainly glycosides (Wintoch et al., 1991), could also be important as added flavor developing on chewing in the mouth through their hydrolysis by the hydrolytic enzymes of saliva. It might explain the differences found in aftertaste or persistence of strawberry flavor in the mouth among strawberry varieties (D.G. Richardson, personal communication).

## **FURANEOL AND DERIVATIVES**

Furaneol (structure I, p. 24) is a registered name for 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Firmenich, Inc., Switzerland). In the flavor industry, this compound is very important due to its pleasant organoleptic character and has been used extensively for flavoring jams, jellies, beverages, ice creams, alcoholic drinks, and sweets (Hirvi et al., 1980). Furaneol aroma is described as burnt sugar-like or caramel-like at high concentrations, and as strawberry-like flavor at low concentrations.

This compound was first reported in pineapple by Rodin et al. (1965), and was described as being burnt pineapple-like and the major character-impact compound in pineapple fruit concentrate. Later, Sundt (1970) reported

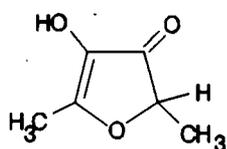
the presence of furaneol in strawberry aroma, and was considered the most important aroma constituent of strawberries so far reported. It has also been reported in arctic bramble (Kallio et al., 1984), raspberry (Honkanen et al., 1980), grapefruit (Lee and Nagy, 1987), as an off-flavor in aged orange juice (Tatum et al., 1975), and in experimental hybrids of German wine (Rapp et al., 1980).

In the aroma profiles of heat-processed foods containing carbohydrates, caramel-like odor notes frequently contribute to the overall flavor impressions. Among the flavor compounds exhibiting caramel-like odors, furaneol is of special interest because of its relatively low threshold of 0.03 ppb (Honkanen et al., 1980). Furaneol was found in beef broth (Tonsbeek et al., 1968), roasted almonds (Takei and Yamanishi, 1974), roasted filberts (Sheldon et al., 1972), and coffee (Tressl et al., 1978). Model experiments such as the degradation of fructose (Shaw et al., 1968), pyrolysis of D-glucose (Johnson et al., 1969), and roasting of alanine and rhamnose (Shaw and Berry, 1977) have revealed that furaneol is formed in heat-processed foods by a thermal degradation of 6-deoxy sugars in the presence of amines or amino acids.

The furaneol methyl-ether derivative, 2,5-dimethyl-4-methoxy-3(2H)-furanone (structure II, p. 24), also called mesifurane after Kallio (1976a), has a more sherry-like aroma than furaneol. Mesifurane was first reported in canned mangoes (Hunter et al., 1974). Kallio (1976a, b) showed that this compound was the major component in arctic bramble aroma (30% of total volatiles), and the one responsible for its flavor. Three years later, Pyysalo et

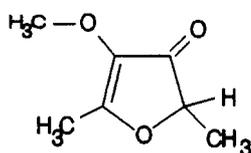
al. (1979) described the presence of mesifurane in the aroma of wild and cultivated strawberries, and was considered to contribute to the characteristic sweet aroma of overripe strawberries, showing a threshold of 0.02 ppm in distilled water.

Due to the important role of glycosidically bound aroma compounds as flavor precursors (Williams et al., 1989), recently, studies of glycoconjugates of fruit volatiles have been carried out. The  $\beta$ -D-glucoside derivative of furaneol, 2,5-dimethyl-4-hydroxy-3(2H)-furanone- $\beta$ -D-glucopyranoside (structure III, below), was first reported in strawberries (Mayerl et al., 1989). Pabst et al. (1991) also found furaneol glucoside in the composition of glycosidically bound aroma fraction of raspberry fruit pulp, and Wu et al. (1990, 1991) reported that this compound is the most abundant aroma glycoside in pineapple juice.



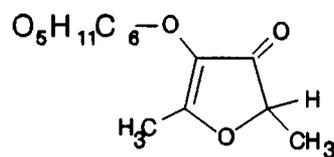
(I)

Furaneol



(II)

Mesifurane



(III)

Furaneol glucoside

A literature survey about the presence of furaneol and mesifurane in strawberries shows that mesifurane was found in the aroma composition of

both wild and cultivated strawberries, showing different concentrations among varieties (Pyysalo et al., 1979; Schreier, 1980; Pickenhagen et al., 1981; Hirvi and Honkanen, 1982; Hirvi, 1983; Douillard and Guichard, 1989, 1990). In the case of furaneol, the story is different. Before 1980 and with the exception of the work by Sundt (1970), there was no report describing the presence of this compound in strawberry aroma. However, after 1980, some studies reported the presence of this compound in the aroma of this fruit (Pickenhagen et al., 1981; Douillard and Guichard, 1989, 1990). Similar results can be found in studies done on the aroma composition in other fruits such as pineapple, arctic bramble or raspberry.

Although furaneol and mesifurane are considered by most of the authors as the main aroma constituents of strawberries, furaneol was rarely quantified. Furaneol has proved difficult to isolate due to its water-soluble nature and that it does not steam distil. Those studies where the distillation technique was used in order to obtain the aroma extract found no furaneol in the aroma analysis. That is the case of Kallio (1976a) analyzing arctic bramble aroma, and of Schreier (1980) studying the aroma of cultivated strawberries. By contrast, most studies where aroma extraction was accomplished by organic solvent extraction have found furaneol. For instance, Pickenhagen et al. (1981) reported the presence of both furaneol and mesifurane in different varieties of strawberry and pineapple. Moreover, when switching to organic solvent extraction Kallio et al. (1984) found high concentrations of these compounds in the aroma of the same varieties of

arctic bramble used in his 1976 work (Kallio, 1976a), where furaneol was reported only as traces.

Another important factor that could well account for the failure of some authors to detect furaneol is the fact that this compound can only be analyzed by gas chromatography (GC) on fused silica capillary columns (Williams and Mottram, 1981). Flath and Forrey (1970) suggested that the presence of active sites on other types of GC columns and the high temperatures used in the analysis would promote degradation of this compound. In this context, Pickenhagen et al. (1981) warned about the blind use of the GC analysis because it may lead to erroneous results and misinterpretations of results. These authors demonstrated, by using a fused silica capillary column in the GC analysis, the presence of furaneol in strawberries, contrary to the results published by Pyysalo et al. (1979) and Schreier (1980). In the latter reports glass capillary columns were used for the GC analysis.

## **RESEARCH OBJECTIVES**

Furaneol and derivatives are now considered by most authors to be among the main flavor components of strawberries. However, no study has focussed on the relation of these compounds to the ripening of this fruit, or to the actual contribution of these compounds to strawberry flavor. One reason for this has been the lack of a reliable quantitative method of analysis. Besides, in the case of furaneol, time consuming liquid-liquid extraction has

been the only possible approach in sample preparation since this compound is highly oxygenated and does not distil. Since we have been studying the composition and biogenesis of strawberry aroma previously (Pérez et al., 1992, 1993a, b; Olías et al., 1993b), and because we were not able to identify any of these furaneol compounds with our current analytical conditions, the major objectives of this thesis are:

- Development of a rapid and reliable procedure for simultaneous extraction and quantification of furaneol, mesifurane and furaneol glucoside from strawberry fruits. (This paper has been accepted for publication in the Journal of Food Science).
  
- Study of the biosynthesis of these three compounds during strawberry ripening, and their relation to the aroma of this fruit. (Part of this paper has been submitted for inclusion in the American Chemical Society Monograph "Advances in Fruit Flavor").

**CHAPTER 2**

**SIMULTANEOUS HPLC DETERMINATION OF 2,5-DIMETHYL-  
4-HYDROXY-3(2H)-FURANONE AND RELATED FLAVOR  
COMPOUNDS IN STRAWBERRIES**

**ABSTRACT**

A rapid methodology (less than 90 min) is described for the extraction and quantitative analysis of 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furanol), furaneol glucoside, and 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane) in strawberries. These compounds were resolved by HPLC with a RP C18 column, using acetate buffer and methanol as binary mobile phase and detection at 280 nm, and confirmed by GC-MS. The aqueous extraction procedure gave an average recovery of these compounds from strawberries greater than 90%, with detection limits of 0.14, 0.05 and 0.36  $\mu\text{g/mL}$  for furaneol, furaneol glucoside and mesifurane, respectively.

## INTRODUCTION

Flavor of cultivated strawberries is mainly determined by a complex mixture of esters, aldehydes, alcohols and sulfur compounds which have been extensively studied during the last 30 years (Mc Fadden et al., 1965; Dirinck et al., 1977, 1981; Schreier, 1980; Hirvi and Honkanen, 1982; Pérez et al., 1992). Among the most important aroma compounds reported in strawberries are 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol, Trade Mark from Firmenich, Inc., Switzerland) (Re et al., 1973; Pickenhagen et al., 1981) and its methyl ether, 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane) (Pyysalo et al., 1979). Glycosidically bound aroma compounds have been recently identified as important flavor precursors (Williams et al., 1989). Mayerl et al. (1989) isolated and identified the  $\beta$ -D-glucoside of furaneol from strawberry juice. More recently, Wintoch et al. (1991) have isolated glycosidically bond aroma compounds from two strawberry fruit species, among which furaneol glucoside showed to be a major component.

Furaneol and mesifurane are considered important compounds related to the organoleptic properties of cultivated strawberries (Hirvi, 1983; Douillard and Guichard, 1989, 1990; Hannover, 1991). However, reports on quantitation and analytical procedures are limited because of the instability of these compounds. Furaneol has proved difficult to isolate by organic solvent extraction due to its water-soluble nature, and also difficult to analyze because it thermally degrades under normal GC conditions (Flath and Forrey,

1970; Shu et al., 1985). Hirvi et al. (1980) studied its stability in aqueous buffer solutions and Pickenhagen et al. (1981) showed that furaneol does not steam distil, but can be detected after extraction with ethyl ether. Williams and Mottram (1981) reported that furaneol can only be analyzed on fused silica capillary columns. When this kind of column was first introduced in 1979 it was suggested that the inertness of fused silica, due to the absence of metal ions, made it an ideal material for chromatography (Dandeneau et al., 1979). The results obtained by Williams and Mottram (1981) clearly illustrate the advantage of fused silica capillary columns for the analysis of potentially unstable compounds, such as furaneol. They further suggested that it is the presence of active sites on other types of columns rather than heat alone which causes problems in the analysis of this compound. Such variability between different columns types offers a possible explanation of why furaneol has been reported by some researchers but not others as an aroma component of strawberries.

HPLC methods should avoid some of the risks of thermal and oxidative decomposition inherent in GC analysis. While mesifurane has never been analyzed by HPLC before, furaneol was quantified by HPLC in pineapple and grapefruit juices (Lee and Nagy, 1987; Wu et al., 1991). Furaneol glucoside content in pineapple juice was also determined by HPLC by an indirect procedure, involving hydrolysis by  $\beta$ -glucosidase and measuring the increase in furaneol content (Wu et al., 1990, 1991).

Since furaneol, its glucoside, and mesifurane are flavor characteristic compounds of high importance in strawberry flavor, reliable methods to determine and quantify them are necessary so that relationships between their concentrations in the fruit and different aspects of strawberry flavor quality can be properly studied. Thus, the objective of this work was to develop a rapid extraction and HPLC analytical method to simultaneously measure the content of furaneol and its derivatives in strawberry fruits.

## MATERIALS AND METHODS

### **Fruits**

Fresh strawberries (*Fragaria x ananassa* Duch., cv. Totem), from the O.S.U. horticultural research fields in Corvallis (OR, USA), were harvested at the firm overripe stage, immediately frozen, and kept at -25°C until used for analysis.

### **Reagents and standards**

Furaneol was obtained from Aldrich Chemical Co. (Milwaukee, WI). Almond  $\beta$ -glucosidase was from Sigma Chemical Co. (St. Louis, MO). Mesifurane was synthesized by the reaction of diazald with furaneol in methanol, according to the method of Willhalm and Thomas (1969), slightly modified. The reaction yield was 91.5%. Carrez clarification reagents (Wallrauch, 1984) consisted of two solutions: Carrez I, 15% (w/v) potassium ferrocyanide, and Carrez II, 30% (w/v) zinc sulfate in distilled water. Other chemicals were reagent grade.

### **Preparation of samples for HPLC**

Strawberries were cut symmetrically into 4 pieces. Four pieces, from four different fruits ( $\approx 15$  g), were thawed and macerated in a Sorvall Omni-mixer with 15 mL of distilled water. Celite (1.5 g) was added and mixed with the homogenate, and allowed to stand at room temperature for 5 min. This

mixture was vacuum filtered through Whatman No. 1 filter paper (Whatman Int. Ltd., Maidstone, UK), and the residue washed 3 times with 5 mL distilled water. Five mL of this filtered extract were clarified by first adding 0.25 mL of Carrez I solution, and then 0.25 mL of Carrez II solution slowly with gentle mixing, according to Wallrauch (1984). After standing for 5 min, the mixture was centrifuged at 2500 x g for 5 min. The supernatant was filtered through a 0.2  $\mu\text{m}$  nylon membrane (Alltech Associates, Inc., Deerfield, IL) before HPLC analysis.

### **HPLC analysis**

Quantitative HPLC analysis of strawberry extracts was accomplished with a Beckman model 334 liquid chromatograph (Beckman Instruments Inc., Berkeley, CA), Hitachi 100-10 detector (Hitachi Ltd., Tokyo, Japan), and Shimadzu C-R3A integrator (Shimadzu Co., Kyoto, Japan). Analysis was carried out using a reverse phase Econosil C18 column (25 cm x 4.6 mm, 10  $\mu\text{m}$ , Alltech) coupled to a ODS-5S guard column (3.0 cm x 4.6 mm, Bio-Rad, Richmond, CA). The mobile phase consisted of: A) 0.2M sodium acetate/acetic acid (pH 4) buffer (acetate buffer), and B) methanol, with the following chromatographic conditions: 0-2 min, isocratic 10% methanol; 2-18 min, gradient 10-12% methanol; 18-36 min, isocratic 12% methanol; flow rate, starting 1.5 mL/min and at 18 min increased to 2.0 mL/min; detector, UV 280 nm; and injection volume, 20  $\mu\text{L}$ . Further confirmation of peak purity and molar extinction coefficients were assessed utilizing a Hewlett-Packard

model 1050 HPLC equipped with a 1040M photodiode array detector, and the same column and gradient conditions.

### **Compound isolation**

Strawberry extract (7 mL) was loaded onto acetate buffer conditioned Sep-Pak C18 cartridge (1 mL, part no. 51910, Waters Associates, Milford, MA) and eluted with 4 mL of acetate buffer (fraction 1) to eliminate the most polar compounds. Elution with 4 mL acetate buffer:methanol (90:10, v/v), gave fraction 2 which contained most of the furaneol and furaneol glucoside from the extract. Final elution of the column with 4 mL acetate buffer:methanol (85:15, v/v) gave fraction 3, with most of the mesifurane. Furaneol was obtained from fraction 2 after evaporation of the methanol in this fraction under a N<sub>2</sub> stream, and extraction with 2 x 3 mL ethyl acetate. The organic phase was dried over anhydrous sodium sulfate and concentrated by N<sub>2</sub> in order to be analyzed by GC-MS. The aqueous phase from fraction 2 after furaneol isolation was concentrated from 3.5 to 0.3 mL in a N<sub>2</sub> stream at 50°C, and furaneol glucoside was collected from this solution by HPLC, carried out under the conditions described, but with a 50 µL sample loop. The separated furaneol glucoside fraction was concentrated from 15 to 4 mL under N<sub>2</sub> stream at 50°C, and 2 mL incubated with 5 mg almond β-glucosidase at 37°C for 1 hr in N<sub>2</sub> atmosphere, according to Wu et al. (1990), slightly modified. After hydrolysis, the aglycone was extracted and concentrated as described for furaneol. Mesifurane was isolated by ethyl

acetate extraction (2 x 3 mL) of fraction 3. The organic phase was dried over anhydrous sodium sulfate, concentrated under N<sub>2</sub>, and analyzed by GC-MS.

### **GC-MS analysis**

GC-MS analyses were performed with a HP5890-Series II gas chromatograph (Hewlett-Packard Co., USA), equipped with a Carbowax 20M capillary column (25 m x 0.2 mm (i.d.), 0.2 μm coating; Hewlett-Packard Co., USA), and coupled to a HP-5971 mass selective detector (Hewlett-Packard Co., USA). Ionization potential was 70 eV, with a source temperature of 180°C. The carrier gas (He) flow was 1 mL/min, the injector port was held at 150°C; GC-MS interface at 280°C; and the oven temperature was isothermal at 50°C for 3 min, then increased from 50 to 190°C at 3°C/min.

## RESULTS AND DISCUSSION

Analytical methodology for quantitation of furaneol and mesifurane is currently carried out by GC, although this technique has shown to be inadequate for this purpose, especially in the case of furaneol. HPLC analysis seems to be a more suitable technique to determine the actual content of these compounds in fruits, allowing also the quantitation of the flavor precursor furaneol glucoside. Furaneol, furaneol glucoside and peak 1 (Fig. 2.1) can be resolved by HPLC in acetate buffer:methanol mixture intervals from 95:5 to 85:15, and flows ranging 0.5 to 2 mL/min, in the HPLC conditions described above. On the contrary, only the combination of a very smooth increasing methanol gradient in the mobile phase and a higher flow at 18 min allowed a complete separation of mesifurane and peak 2, while still allowing resolution of furaneol glucoside, furaneol and peak 1. The result is the typical HPLC chromatogram from strawberry extracts shown in Figure 2.1. Furaneol glucoside, furaneol and mesifurane were resolved into unique peaks with retention times (Rt) of 10.0, 12.2 and 30.3 min, respectively. Peak 2 (Rt = 31.7 min) corresponded to an unknown compound that proved not to be extractable with organic solvents, and remained inalterable after incubation with  $\beta$ -glucosidase for 18 hr. On the other hand, peak 1 (Rt = 13.5 min) was an unknown compound, not extractable with low polar organic solvents, that was 70% hydrolyzed with  $\beta$ -glucosidase in 30 min (decrease in peak area),

and completely hydrolyzed after 18 hr of incubation in the conditions described above.

The analytical methodology described in this work allowed identification and accurate quantitation of these 3 key strawberry flavor compounds in a single injection within 90 min, including extraction. Traditional methods involving liquid-liquid extraction of volatiles require from 8 to 20 hr (Pyysalo et al., 1979; Schreier, 1980; Pickenhagen et al., 1981), with an additional 12-72 hr of hydrolysis for the glycosides (Wintoch et al., 1991; Wu et al., 1991).

Linear responses ( $r = 0.999$ ) were obtained for furaneol and mesifurane in the concentration range 0.5-60  $\mu\text{g/mL}$  in distilled  $\text{H}_2\text{O}$ . Lower detection limits (3 x baseline noise) were 0.14  $\mu\text{g/mL}$  for furaneol, 0.05  $\mu\text{g/mL}$  for furaneol glucoside and 0.36  $\mu\text{g/mL}$  for mesifurane. Furaneol glucoside content in the extracts was determined based on a molar extinction coefficient for furaneol glucoside 2.66 times greater than furaneol at 280 nm. This was calculated by comparison of peak areas before and after complete hydrolysis with almond  $\beta$ -glucosidase of isolated furaneol glucoside, as described. We found that overripe Totem strawberries contained  $21.7 \pm 1.2 \mu\text{g/g}$  FW of furaneol,  $16.5 \pm 2.1 \mu\text{g/g}$  FW of furaneol glucoside, and  $20.1 \pm 0.9 \mu\text{g/g}$  FW of mesifurane (means of 6 analyses).

The presence of furaneol and furaneol derivatives in the HPLC analysis were confirmed by comparison of  $R_t$  and by adding commercial furaneol, the isolated furaneol glucoside, and synthesized mesifurane. Strawberry extract furaneol compounds were separated by HPLC and assessed for peak purity

with a photodiode array detector, by comparing spectra at the beginning, apex, and end of each peak. Analyses indicated virtually no coelution by other compounds. Furaneol standard in H<sub>2</sub>O had maximum absorbance at 285.7 nm with  $\epsilon_{285.7\text{nm}} = 7128 \text{ M}^{-1}$ , compared to  $\epsilon_{289\text{nm}} = 6700 \text{ M}^{-1}$  in methanol reported by Rodin et al. (1965). For mesifurane in H<sub>2</sub>O we measured  $\epsilon_{277.3\text{nm}} = 9114 \text{ M}^{-1}$ , compared to  $\epsilon_{278.8\text{nm}} = 7510 \text{ M}^{-1}$  in ethanol as reported by Willhalm et al. (1965). The furaneol glucoside had maximum absorbance at 276 nm, and we calculated  $\epsilon_{276\text{nm}} = 18606 \text{ M}^{-1}$ . However, for simultaneous HPLC analysis of all 3 furaneol compounds, absorbance at 280 nm was chosen as an optimum compromise. The molar extinction coefficients at this wavelength were furaneol 6809, furaneol-glucoside 18141, and mesifurane 8597 M<sup>-1</sup> in distilled water.

In order to further prove the identity of these peaks, presumed furaneol and mesifurane were isolated directly from the strawberry extract after fractionation in C18 Sep-Pack cartridges as described (Fig. 2.2 and 2.3), and analyzed by GC-MS. Injector temperature for the GC-MS analysis was set at 150°C in order to avoid decomposition of furaneol observed at 220°C. At this temperature, around 35% of standard furaneol was thermally degraded to compounds such as 2-oxo-propanoate and 2-hydroxy-propanoate methyl esters. Similar structures were found by Shu et al. (1985) among the components of thermal degradation of furaneol at different pHs. Furaneol had a  $R_t = 33.5$  min in GC-MS analysis and showed a fragmentation pattern (Fig. 2.4-A) quite similar to published data (Tonsbeek et al., 1968) and to

commercial furaneol, with  $M^+$  (m/e 128) as a base peak, and m/e 43 ( $CO-CH_3^+$ ) as the second most abundant fragment. The mass spectrum of mesifurane (Fig. 2.4-B),  $R_t = 19.0$  min in GC-MS analysis, had base peak  $M^+$  (m/e 142) and m/e 43 as the second most abundant fragment, although in this compound this fragment was much more characteristic than in furaneol. This spectrum was corroborated with synthesized mesifurane and its published spectrum (Hunter et al., 1974). Almond  $\beta$ -glucosidase hydrolyzed the putative furaneol glucoside, liberating glucose and an aglycone that showed the same  $R_t$  as furaneol by HPLC analysis (12.2 min) and by GC-MS analysis (33.5 min). It had the same fragmentation pattern (Fig. 2.4-C) as that described for furaneol (Fig. 2.4-A).

The aqueous extraction method of strawberry fruits and use of Carrez clarification reagents were tested for recovery of furaneol and mesifurane by HPLC. Three different amounts of furaneol (0.23, 0.58 and 1.10 mg) and mesifurane (0.47, 1.13 and 1.88 mg) were injected into the fruit pieces ( $\approx 15$  g) prior to extraction and compared to the blank (opposite halves of fruit without additional furaneol and mesifurane). The mean recovery found for furaneol was 97.5% ( $\pm 1.6\%$ ), and for mesifurane 91.7% ( $\pm 2.7\%$ ), slightly lower than furaneol attributable to the less polar methoxy group. Although we did not have a pure standard, furaneol glucoside can be assumed to be extracted at least at the same rate as furaneol since the glucose moiety is highly soluble in aqueous solutions.

According to Schreier (1980), deep-freezing of the fruit resulted in lower concentration of most of the aroma substances except for mesifurane, while Hirvi (1983) found the opposite, especially in Senga Sengana strawberries. In the present study no influence of freezing (-25°C) on the content recorded for furaneol and its derivatives was observed when compared to fresh strawberries. These results agree with the work by Douillard and Guichard (1990), where 6 strawberry varieties showed no changes in furaneol content after freezing. Similarly, strawberry extracts prepared as described in Materials and Methods proved to have good stability, since no significant decomposition of the three compounds under investigation was observed during 3 months at - 25°C. On the other hand, while clarification with Carrez reagents removed pulp, oils, protein and carotenoids from the extract, there was no effect on final concentration of furaneol compounds.

In conclusion, a procedure was developed to assess the content of strawberry flavor impact compounds, furaneol, furaneol glucoside and mesifurane. It could provide a simple and rapid tool to relate concentration of these compounds with flavor intensity values in strawberry flavor panels, and may also prove applicable to analyses of furaneol compounds from other sources.

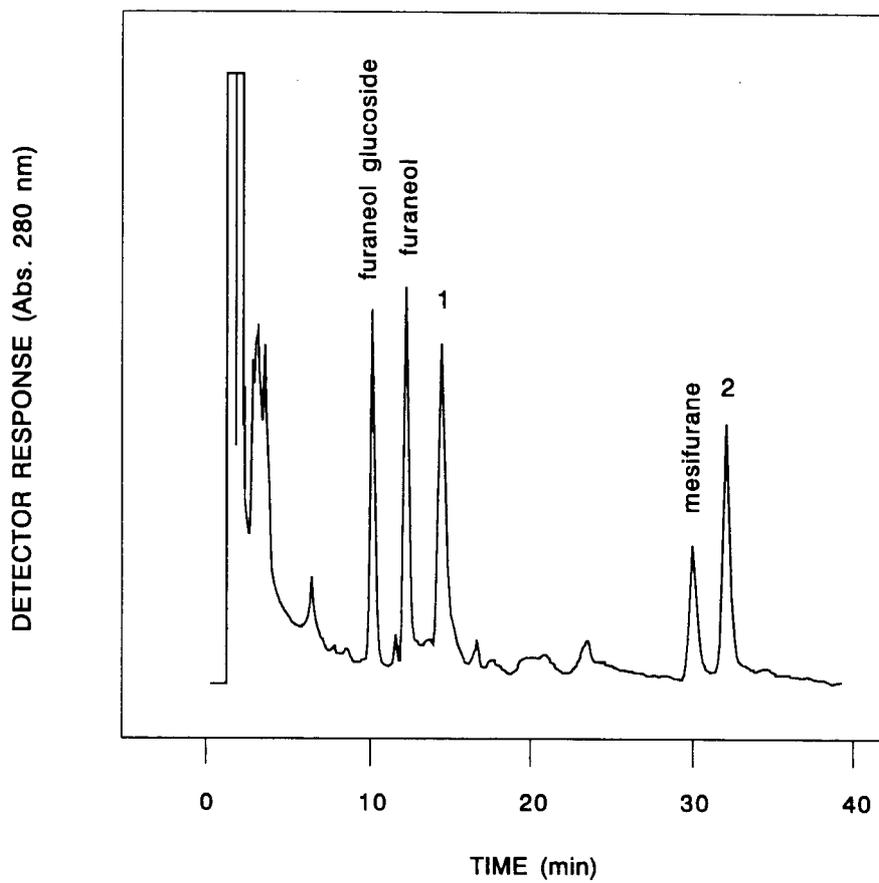


Figure 2.1. Typical HPLC analysis of a strawberry extract (overripe Totem).

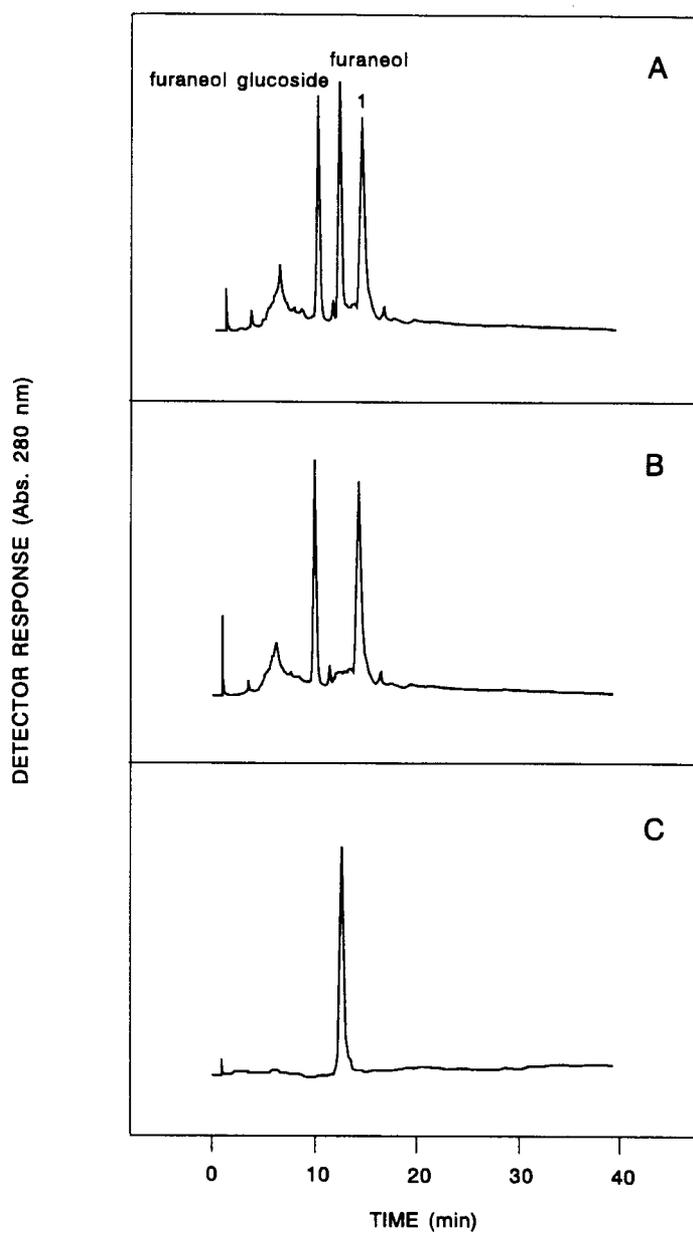


Figure 2.2. HPLC analyses of: (A) Sep-Pack C18 cartridge fraction 2; (B) fraction 2 after ethyl acetate extraction of furaneol; (C) extracted furaneol after evaporation and redissolved in acetate buffer (0.2M, pH 4).

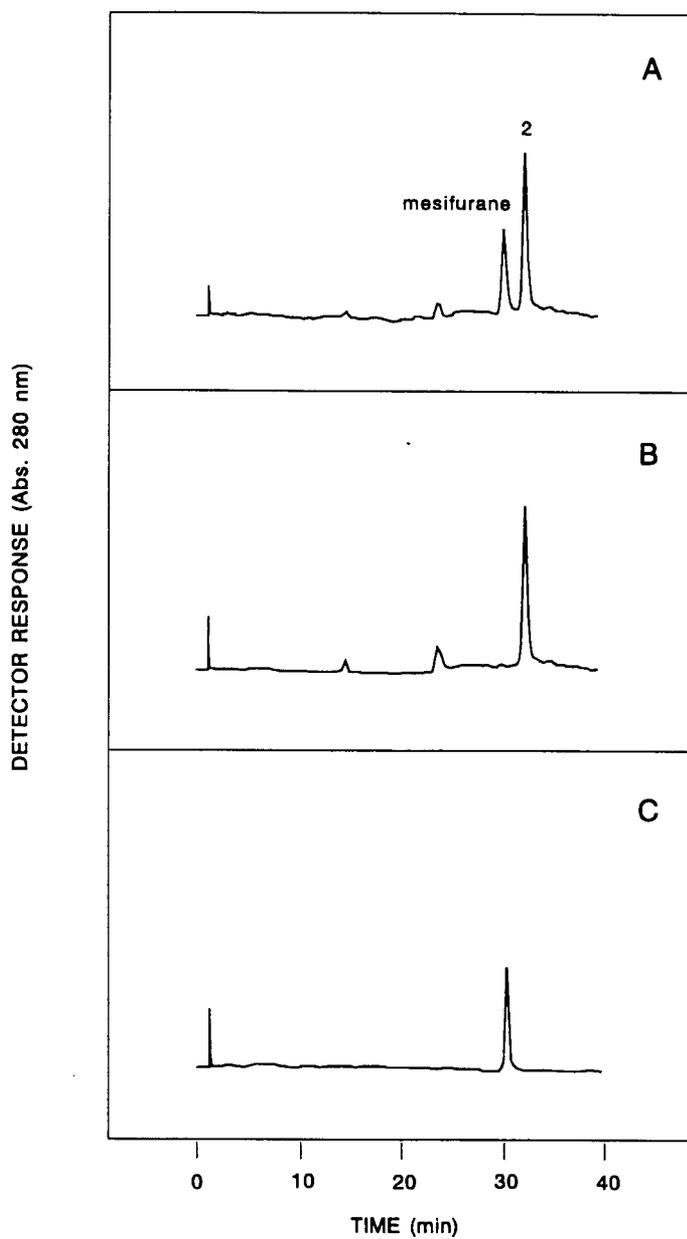


Figure 2.3. HPLC analyses of: (A) Sep-Pack C18 cartridge fraction 3; (B) fraction 3 after ethyl acetate extraction of mesifurane; (C) extracted mesifurane after evaporation and redissolved in acetate buffer (0.2 M, pH 4).

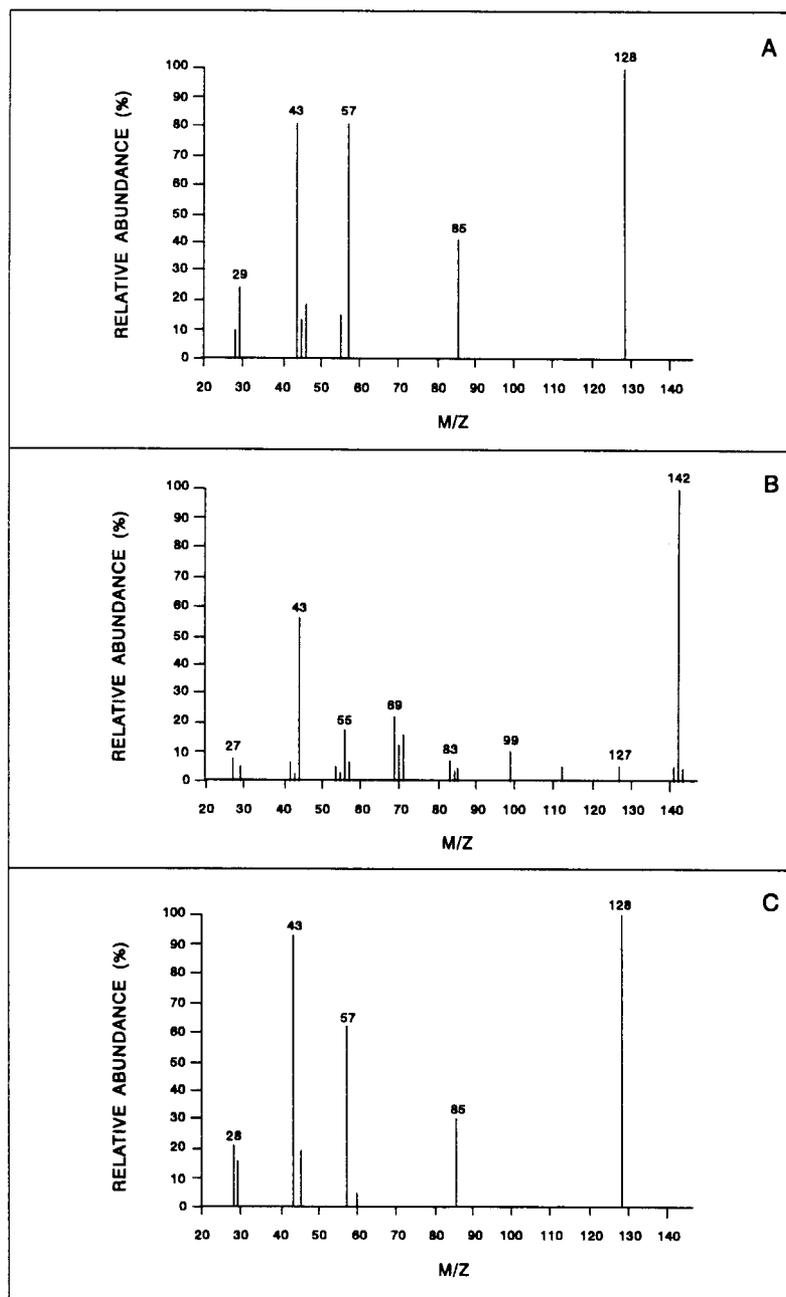


Figure 2.4. Mass spectra found for furaneol (A), mesifurane (B) and furaneol from  $\beta$ -glucosidase hydrolyzed furaneol glucoside (C).

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**CHAPTER 3**  
**FURANEOL AND DERIVATIVES CONTENT IN STRAWBERRIES**  
**DURING RIPENING**

**ABSTRACT**

Concentration of furaneol and derivatives in seven strawberry varieties were assessed during ripening. In most cases, these compounds sharply increased with fruit ripening, attaining maximum values at the overripe stage. The largest amount of furaneol, mesifurane and furaneol glucoside were found in overripe strawberries of cultivars Douglas (22.89  $\mu\text{g/g}$  FW), Pajaro (39.13  $\mu\text{g/g}$  FW), and Totem (16.51  $\mu\text{g/g}$  FW), respectively. Results obtained showed quantitative differences among varieties that could be related to their organoleptic properties. The best correlation values between furaneol and strawberry aroma were found for Parker ( $r=0.741$ ) and Benton ( $r=0.733$ ) strawberries.

## INTRODUCTION

It seems to be a general phenomenon, that increase in berry size, obtained by breeding, has inexorably lead to a decrease in the aroma of berries. Thus, the pleasant and aromatic characteristic of wild strawberries is only faintly detected in most cultivated varieties.

Strawberry aroma is mainly determined by a complex mixture of esters, aldehydes, alcohols and sulfur compounds which have been extensively studied (Mc Fadden et al., 1965; Drawert et al., 1973; Dirinck et al., 1977; Dirinck et al., 1981; Pérez et al., 1992). More recently, two compounds: 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) (Sundt et al., 1970; Pickenhagen et al., 1981) and 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane) (Pyysalo et al., 1979) are considered to be among the most important volatiles reported in wild strawberries. Although furaneol and mesifurane are regarded by most authors as the main aroma constituents of strawberries from an organoleptic point of view, these two compounds were not originally found (analytical problems) in all cultivated varieties. Douillard and Guichard (1989), studying the volatile components of fourteen strawberry varieties, found large amounts of furaneol and mesifurane in some varieties such as Confitura, Jesco and Tioga, however, varieties such as Cambridge and Elvira did not contain mesifurane, and Cardinal had no furaneol. Factors such as furaneol's water-soluble nature (Hirvi et al., 1980; Pickenhagen et al., 1981), low concentration, and thermal instability (Flath and Forrey, 1970; Shu

et al., 1985), could well account for the failure of some authors to detect furaneol.

Both furaneol and mesifurane have very strong, sweet and pleasant odors. Furaneol at high concentrations imparts caramel burnt sugar notes, but at lower concentrations becomes fruity, strawberry-like (Re et al., 1973), while mesifurane is described as having a more sherry-like aroma (Hunter et al., 1974). Kallio et al. (1984) found in arctic bramble berries good correlations between content of furaneol and the intensity of odor ( $r=0.867$ ), character of odor ( $r=0.821$ ), and the overall impression of odor ( $r=0.751$ ). Contrary to previous results, Kallio (1976a) could not find good correlations between the content of mesifurane and the odor evaluations. However, some dependence of the overall impression of the taste of mesifurane seems evident ( $r=0.662$ ).

There are several studies which have identified the presence of furaneol, mesifurane (Pickenhagen et al., 1981; Hirvi and Honkanen, 1982; Douillard and Guichard, 1989, 1990) and 2,5-dimethyl-4-hydroxy-3(2H)-furanone- $\beta$ -D-glucopyranoside (furaneol glucoside)(Mayerl et al., 1989; Wintoch et al., 1991) in strawberries. These studies have always been carried out at the fully ripe stage of the fruit. Since the flavor quality of the strawberry changes quickly in a few days before and after this stage, it would be interesting to know how the concentration of these three flavor compounds changes during this period of time. We have previously studied changes in the aroma components, mainly esters, of Chandler strawberries at

different ripening stages (Pérez et al., 1992). However, no study has focused on the production of furaneol, mesifurane and furaneol glucoside during the ripening of this fruit, and due to the lack of a reliable quantitative analytical method of analysis their actual contribution to strawberry aroma is still not well characterized.

In this work a new less destructive analytical procedure, involving HPLC separation and quantitation of furaneol, furaneol glucoside and mesifurane, was developed to determine the amount of these three compounds in seven strawberry cultivars during ripening, and to assess the relationship of furaneol and mesifurane concentrations to aroma evaluations.

## MATERIALS AND METHODS

### Fruits

Fruits from seven strawberry (*Fragaria x ananassa* Duch.) cultivars, Chandler, Parker, Douglas, Pajaro, Benton, Redcrest and Totem, grown at the O.S.U. horticultural research fields in Corvallis (OR, USA), were used in this study. Strawberries were harvested at four ripening stages: white (I), pink (II), bright-red (III, ripe) and dark-red fruits (IV, overripe), and immediately frozen, and kept at -25°C.

### Preparation of samples for HPLC

Strawberries (still frozen) were cut symmetrically in four pieces. Four pieces from four different fruits, approximately 15 g, were thawed and homogenized in a Sorval Omni-mixer with 15 mL of distilled water. Celite (1.5 g) was added and mixed with the homogenate, then allowed to stand for 5 min. This mixture was vacuum filtered through Whatman No. 1 filter paper (Whatman Int. Ltd., Maidstone, UK), and the residue washed three times with 5 mL distilled water. Five mL of this filtered extract was clarified (removing pulp, fat, protein, and carotenoids) by first adding 0.25 mL of Carrez I solution, and then 0.25 mL of Carrez II solution added slowly with gentle mixing, according to Wallrauch (1984). After standing for 5 min, the mixture was centrifuged at 2500 x g for 5 min. The supernatant was filtered through

a 0.2  $\mu\text{m}$  nylon membrane (Alltech Associates, Inc., Deerfield, IL) before HPLC analysis.

### **HPLC analysis**

Quantitative HPLC analysis of strawberry extracts was accomplished with a Beckman model 334 liquid chromatograph (Beckman Instruments Inc., Berkeley, CA), Hitachi 100-10 detector (Hitachi Ltd., Tokyo, Japan), and Shimadzu C-R3A integrator (Shimadzu Co., Kyoto, Japan). Analysis was carried out using a reverse phase Econosil C18 column (25 cm x 4.6 mm, 10  $\mu\text{m}$ , Alltech) coupled to a ODS-5S guard column (3.0 cm x 4.6 mm, Bio-Rad, Richmond, CA). The mobile phase consisted of: A) 0.2 M sodium acetate/acetic acid (pH 4) buffer (acetate buffer) and B) methanol, with the following chromatographic conditions: 0-2 min, isocratic 10% methanol; 2-18 min, gradient 10-12% methanol; 18-36 min, isocratic 12% methanol; flow rate, starting 1.5 mL/min and increased at 18 min to 2.0 mL/min; detector, UV 280 nm; and injection volume, 20  $\mu\text{L}$ .

Calibration curves ( $r = 0.999$ ) were obtained for furaneol and mesifurane in the concentration range 0.5-60  $\mu\text{g/mL}$  in distilled  $\text{H}_2\text{O}$ . Furaneol glucoside content in the extracts was determined based on a molar extinction coefficient for furaneol glucoside 2.66 times greater than furaneol at 280 nm. This was calculated by comparison of peak areas before and after complete hydrolysis with almond  $\beta$ -glucosidase of isolated furaneol glucoside.

### **Sensory evaluation**

Extracts from each strawberry variety at different maturity stages were evaluated by means of a 16-point scale for strawberry aroma and overall aroma intensity. Samples were assessed by 18 judges at room temperature. A random three-digit code was given to each sample, and they were served in a random presentation, either three or four samples at a time. Each sample, 25 mL of strawberry extract, was presented in a 75 mL odorless glass jar covered with black paper. Results were evaluated by Analysis of Variance and Least Significant Difference (LSD) means separation tests, and linear regression coefficients were calculated for the organoleptic characters in relation to the concentration of furaneol compounds.

## RESULTS AND DISCUSSION

### Furaneol and derivatives during strawberry ripening

Gas liquid chromatography is the most frequently used method for the separation of aroma compounds of strawberry. However, this technique has been shown to be inadequate for the determination of compounds such as furaneol and mesifurane, due to the thermal instability of these compounds under normal GC conditions (Williams and Mottram, 1981; Shu et al., 1985). This may also explain the low reproducibility of the results found in early reports on furaneol content in fruits (Pyysalo et al., 1979; Schreier, 1980; Pickenhagen et al., 1981).

HPLC analysis seems to be a gentler and more suitable technique to determine the actual content of furaneol, mesifurane and furaneol glucoside in fruits, as it has been shown for furaneol in recent studies on grapefruit and pineapple (Lee and Nagy, 1987; Wu et al., 1990). Nevertheless, no method for the determination of the three compounds in strawberry has been reported.

The analytical methodology used in this work is much faster than traditional methods involving liquid-liquid extraction (Pyysalo et al., 1979; Schreier, 1980; Pickenhagen, 1981), and avoids any kind of concentration procedure which could cause alterations in the aroma composition. Thus, furaneol, mesifurane and furaneol glucoside concentration of seven strawberry varieties during ripening were determined by HPLC. Results are shown in

Figures 3.1-3.3 (formation of the three compounds during ripening for each variety is presented in Appendices Figure A.1-A.7).

In order to understand the aroma of a fruit it is necessary to know not only the nature of constituents, but how the significant components change in kind and quantity during the development of the fruit. In all varieties studied only when fruits reached a certain degree of ripeness was the biosynthesis of the three compounds enhanced. In general, furaneol, furaneol glucoside and mesifurane concentrations sharply increased at the last ripening stage. Mesifurane was identified as the major volatile component of arctic bramble, and one of only 11 volatiles that underwent an increase in concentration until the over-ripe stage during the fruit ripening (Kallio, 1976b). These results also agree with those reported on the formation of methyl and ethyl esters during strawberry ripening (Ito et al., 1991; Pérez et al., 1992). However, there are other groups of volatile compounds such as amyl, isoamyl and hexyl esters whose contents decrease at the last maturity stages, as reported in Chandler strawberries (Pérez et al., 1992).

Although the biosynthetic pathway of furaneol is still unknown, the rationale for the presence of this compound and derivatives only at late ripening stages could be due to the lack of the forming enzyme activity in unripened fruits, as proposed by Yamashita et al. (1979) for volatile esters. In this sense, experiments were carried out according to Kallio (1975) and Forney and Breen (1986), in an attempt to find the natural precursor of furaneol in strawberry fruits. Fully ripe strawberries, either fruit slices or

whole fruits, infiltrated with different sugars (rhamnose, glucose, fructose, sorbitol), sugar phosphates (glucose-1-phosphate, fructose-6-phosphate), or combinations of rhamnose and alanine solutions, after the work by Shaw and Berry (1977), and incubated for up to 55 hr at 21 °C, showed no significant differences from controls in the concentration of furaneol, furaneol glucoside and mesifurane (data not shown).

Among the seven strawberry cultivars studied, four were developed in California: Chandler, Parker, Douglas and Pajaro, and three in the Pacific Northwest: Redcrest, Benton and Totem. Most studies dealing with the quantitative comparison of volatiles, have found great differences among cultivars (Dirinck et al., 1981; Hirvi, 1983; Douillard and Guichard, 1989, 1990). In this work, a different pattern for the formation of furaneol was found among the strawberry cultivars under investigation (Fig. 3.1). In the Californian cultivars, low amounts of furaneol were found in the fruits in early ripening stages (I and II), while in the Pacific Northwest cultivars it was almost totally absent. Chandler, Parker and Benton strawberries could be grouped as cultivars in which there is an earlier production of furaneol, increasing the concentration drastically at the commercial fresh market maturity (stage III), and practically unchanged in concentration at the last maturity stage. On the other hand, Douglas, Pajaro and Totem strawberries showed a more steady increase in concentration of this compound during ripening, reaching the highest values for furaneol in Douglas (22.89  $\mu\text{g/g}$  FW) and Totem (21.61  $\mu\text{g/g}$  FW). Redcrest showed a distinct evolution in the production of furaneol,

with a maximum at stage III and decreasing at stage IV, accounting for the lowest concentration of furaneol at the last ripening stage found among the different varieties under study.

The production of mesifurane followed also a different pattern for each of these seven strawberry varieties (Fig. 3.2). While Pacific Northwest strawberries lacked this compound at the early maturity stages (I and II), among the Californian cultivars, Chandler and Pajaro showed a certain amount of mesifurane, and in the case of Douglas and Parker, it was detected as traces. In general, there was an increase in the concentration of mesifurane during ripening with maximum values clearly at the last maturity stage (IV), ranging from 13 to 20  $\mu\text{g/g}$  FW, except for Redcrest, which showed again the lowest concentration among the strawberry varieties studied, and Pajaro, which showed the highest concentration of mesifurane at the last maturity stage (39.13  $\mu\text{g/g}$  FW).

Figure 3.3 shows the production of furaneol glucoside found for the seven strawberry varieties under investigation. Strawberries from California showed a similar profile of concentrations of this compound during ripening, with a steady increase from maturity stage II to stage IV, reaching concentrations at the latter stage around 10  $\mu\text{g/g}$  FW. On the contrary, Pacific Northwest strawberries showed a dissimilar evolution of furaneol glucoside production. Strawberries from these cultivars lacked furaneol glucoside in the early maturity stages (I and II), and rapidly increased at stages III and IV. Although Redcrest strawberries showed a relatively high

concentration of this compound at stage III, they had the lowest content of furaneol glucoside at stage IV ( $4.05 \mu\text{g/g}$  FW). Totem strawberries showed the highest concentration of this compound among the seven strawberry varieties studied in this work,  $16.51 \mu\text{g/g}$  FW.

The concentration of furaneol in Douglas strawberries, mesifurane in Pajaro strawberries, and furaneol glucoside in Totem strawberries found in this study are the highest values so far reported in this fruit, only comparable to values described for cultivar Confitura by Douillard and Guichard (1989). These results were expected, since this is the first non-gas chromatographic analysis of these compounds in strawberries. Similar differences are found when HPLC quantitation data of furaneol in pineapple (Pickenhagen et al., 1981; Wu et al., 1990) are compared to those obtained by GC analysis (Wu et al., 1991).

### **Sensory evaluation**

Tables 3.1-3.4 summarize the results of aroma evaluations from the seven strawberries varieties at each maturity stage using the ballot shown in Appendix Figure A.8. According to these results, in general there is an increase in the contribution of strawberry aroma to the overall aroma intensity during the fruit ripening, from an average value of approximately 26% at stage I (Table 3.1) to 78% at stage IV (Table 3.4). Maturity stages I and II (Tables 3.1 and 3.2) showed lower levels of strawberry aroma, probably due to the lower content of furaneol and mesifurane. At the ripe maturity stage

(III)(Table 3.3), there are no statistical differences between Chandler, Parker, Redcrest and Totem strawberries for overall aroma intensity and strawberry aroma. Parker overripe strawberries stage IV (Table 3.4) had the highest strawberry aroma with the highest overall aroma intensity. Totem had the second highest score for strawberry aroma. The excellent organoleptic quality of this strawberry cultivar was reported in a previous study (Skrede, 1980).

Data from the four maturity stages were pooled in order to carry out regression analysis of furaneol and mesifurane concentrations against odor panel values for strawberry aroma and overall aroma intensity. Table 3.5 shows the correlation coefficients (*r*-values) obtained for linear regression studies, that best described the relationships. In general terms, the amount of furaneol and mesifurane positively correlated with the desirable strawberry aroma and overall aroma intensity, and the concentration of furaneol and mesifurane correlates better to strawberry aroma than to overall aroma intensity. Furaneol concentration correlates better with both attributes than does mesifurane, with the exception of cultivars Parker (strawberry aroma and overall intensity) and Benton (strawberry aroma). This could be explained because furaneol has a considerably lower threshold concentration (0.03 ppb) than mesifurane (10 ppb)(Honkanen et al., 1980), so the former compound may contribute much more to the typical aroma of strawberries. Kallio et al. (1984) also found better correlations for furaneol content than for mesifurane versus character of odor in the sensory analysis of different varieties of arctic bramble. In this work, the best correlation values between furaneol content

and strawberry aroma were found for Parker ( $r = 0.741$ ) and Benton ( $r = 0.733$ ) strawberries, which also showed the best correlations for mesifurane content (0.746 and 0.736, respectively). Correlation coefficients obtained for furaneol and mesifurane are comparatively good if compared to those reported by Shamaila et al. (1992) when studying the relationship between odor attributes and different aroma components in Chandler strawberries. The highest value found by these authors was between methyl butanoate and strawberry odor ( $r = 0.41$ ), which could suggest the major involvement of furaneol and mesifurane in the strawberry aroma.

## VARIETY/STAGE

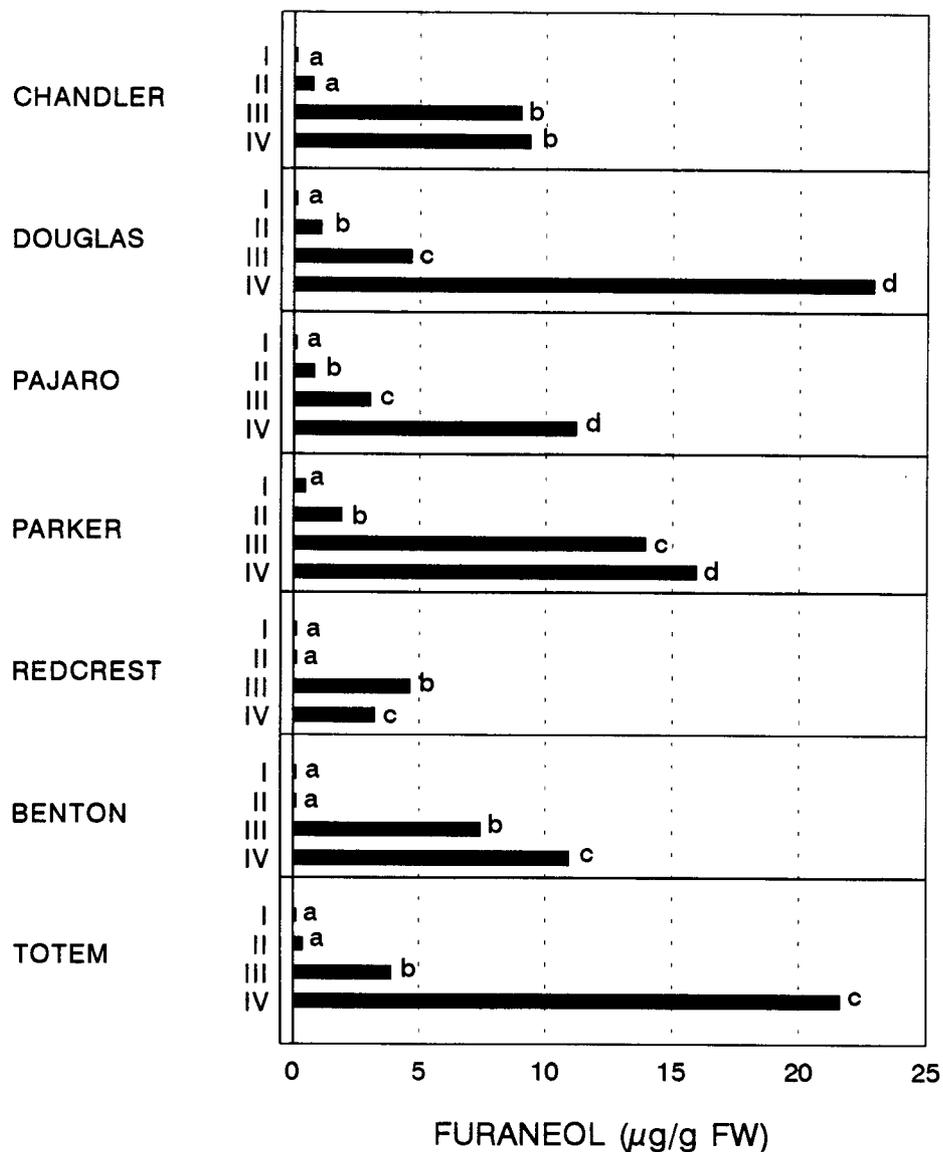


Figure 3.1. Concentration of furaneol at different maturity stages during ripening of seven strawberry varieties. Each bar represents the mean of 6 analyses. Means within the same variety with the same letter are not statistically different at significance level  $p=0.05$ .

## VARIETY/STAGE

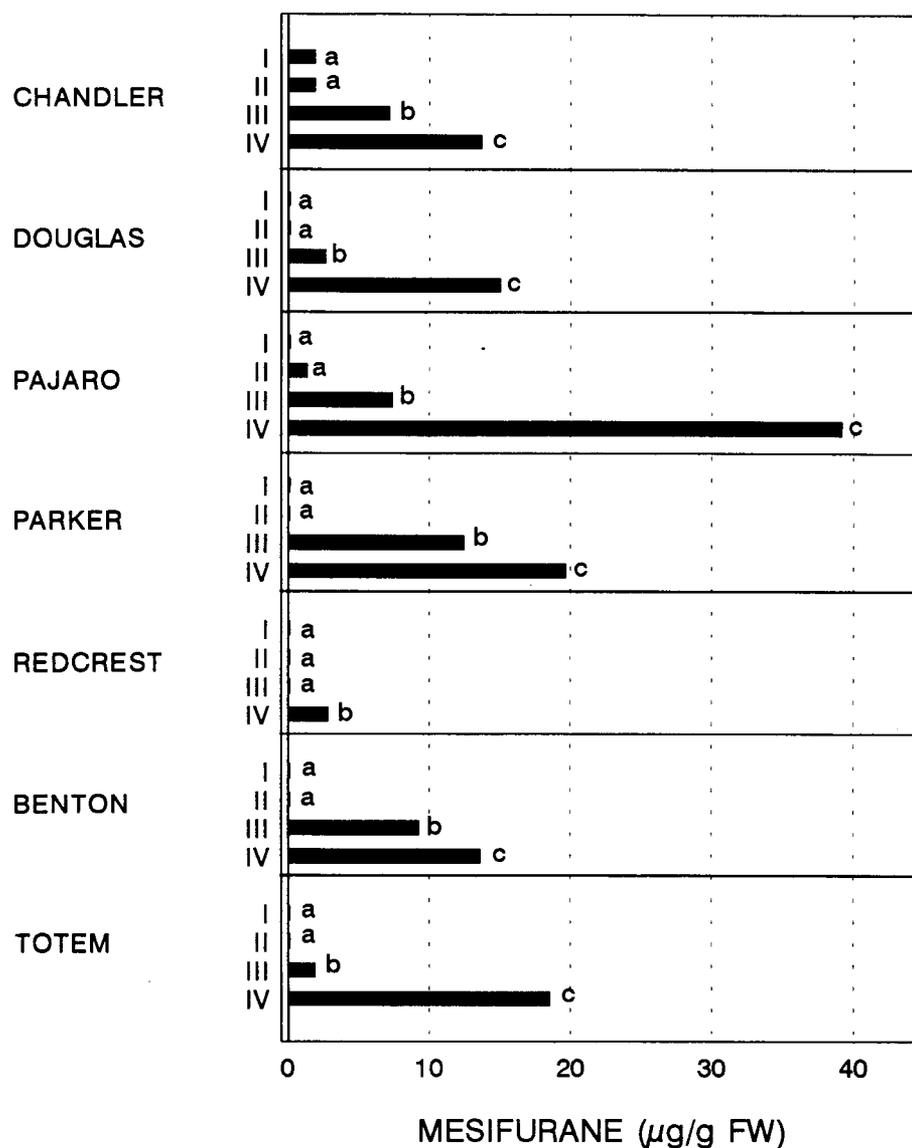


Figure 3.2. Concentration of mesifurane at different maturity stages during ripening of seven strawberry varieties. Each bar represents the mean of 6 analyses. Means within the same variety with the same letter are not statistically different at significance level  $p=0.05$ .

## VARIETY/STAGE

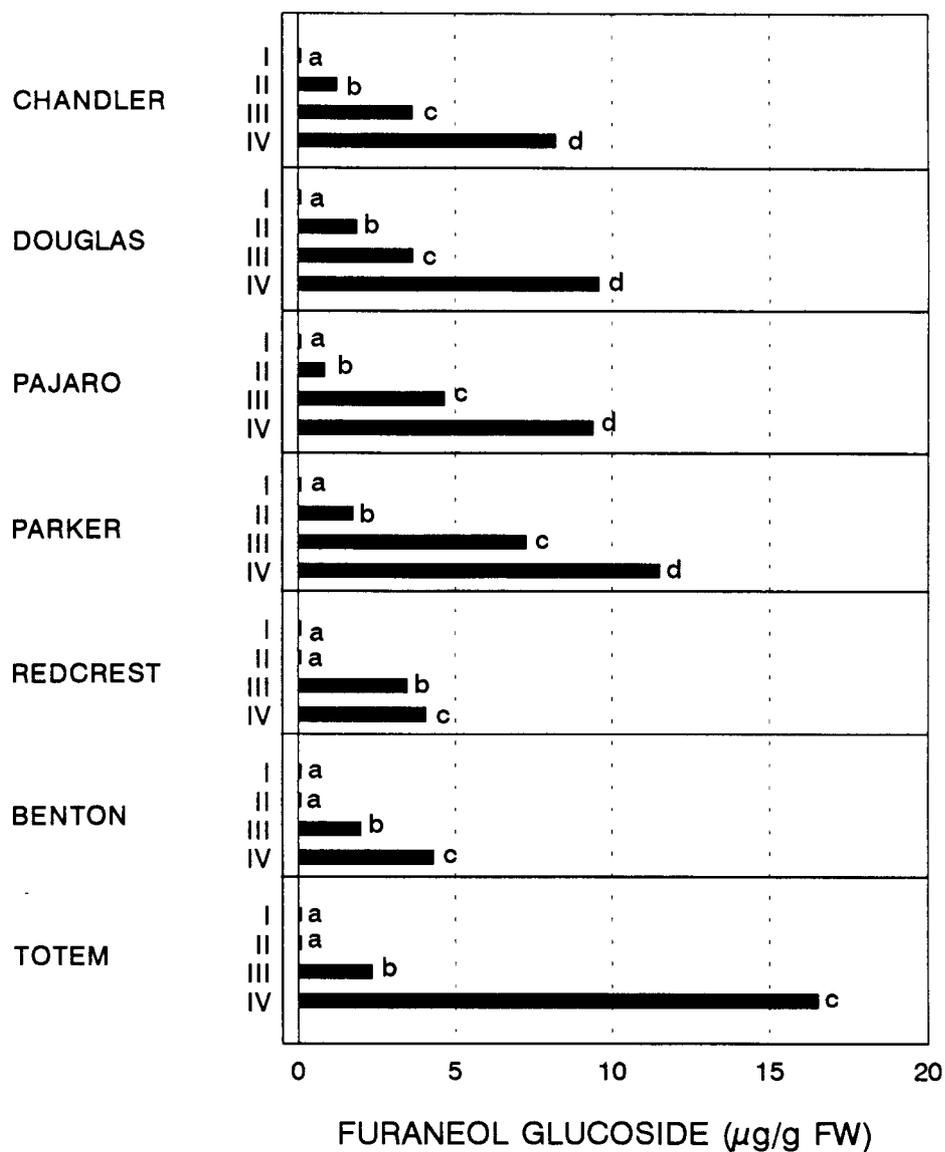


Figure 3.3. Concentration of furaneol glucoside at different maturity stages during ripening of seven strawberry varieties. Each bar represents the mean of 6 analyses. Means within the same variety with the same letter are not statistically different at significance level  $p = 0.05$ .

Table 3.1. Means<sup>1</sup>, standard deviations<sup>2</sup>, and Least Significant Difference (LSD) for strawberry aroma and overall aroma intensity for each strawberry variety at maturity stage I.

Strawberry variety	Strawberry aroma		Overall intensity	
Chandler	<sup>1</sup> 1.50 ab	<sup>2</sup> (0.86)	4.50 a	(2.12)
Douglas	0.77 a	(1.00)	4.33 a	(2.11)
Pajaro	1.22 ab	(1.59)	4.67 a	(2.77)
Parker	1.61 ab	(1.61)	5.72 ab	(2.27)
Redcrest	1.78 b	(1.66)	6.44 b	(2.26)
Benton	1.67 ab	(1.55)	6.39 b	(2.00)
Totem	1.56 ab	(1.34)	6.72 b	(2.16)
Mean	1.44	(1.37)	5.54	(2.24)
LSD	0.93		1.49	

Means within a column followed by the same letter are not different, significance level  $p = 0.05$ . Response range for each attribute was from 0 to 15 ( $n = 18$  for each treatment mean).

Table 3.2. Means<sup>1</sup>, standard deviations<sup>2</sup>, and Least Significant Difference (LSD) for strawberry aroma and overall aroma intensity for each strawberry variety at maturity stage II.

Strawberry variety	Strawberry aroma		Overall intensity	
Chandler	<sup>1</sup> 4.44 a	<sup>2</sup> (2.23)	6.00 a	(1.37)
Douglas	1.83 b	(2.01)	4.83 b	(2.04)
Pajaro	1.72 b	(1.18)	5.78 ab	(1.66)
Parker	4.22 ac	(1.90)	5.89 ab	(1.13)
Redcrest	3.11 c	(1.60)	6.50 a	(2.09)
Benton	1.56 b	(1.38)	5.89 ab	(1.71)
Totem	1.44 b	(1.46)	5.89 ab	(1.87)
Mean	2.62	(1.68)	5.83	(1.70)
LSD	1.13		1.14	

Means within a column followed by the same letter are not different, significance level  $p=0.05$ . Response range for each attribute was from 0 to 15 ( $n=18$  for each treatment mean).

Table 3.3. Means<sup>1</sup>, standard deviations<sup>2</sup>, and Least Significant Difference (LSD) for strawberry aroma and overall aroma intensity for each strawberry variety at maturity stage III.

Strawberry variety	Strawberry aroma		Overall intensity	
Chandler	17.39 a	<sup>2</sup> (2.77)	8.56 a	(2.33)
Douglas	4.39 b	(1.97)	7.22 ab	(2.69)
Pajaro	2.39 c	(2.33)	5.67 bc	(2.52)
Parker	6.28 a	(2.97)	7.72 a	(2.63)
Redcrest	6.00 ab	(2.45)	8.00 a	(2.57)
Benton	4.28 b	(1.93)	5.28 c	(1.84)
Totem	4.39 ab	(3.91)	7.72 a	(1.96)
Mean	5.02	(2.62)	7.17	(2.36)
LSD	1.78		1.57	

Means within a column followed by the same letter are not different, significance level  $p = 0.05$ . Response range for each attribute was from 0 to 15 ( $n = 18$  for each treatment mean).

Table 3.4 Means<sup>1</sup>, standard deviations<sup>2</sup>, and Least Significant Difference (LSD) for strawberry aroma and overall aroma intensity for each strawberry variety at maturity stage IV.

Strawberry variety	Strawberry aroma		Overall intensity	
Chandler	5.11 a	<sup>2</sup> (1.97)	7.11 ab	(2.49)
Douglas	5.22 a	(2.18)	7.61 ab	(1.91)
Pajaro	4.94 a	(2.41)	7.89 ab	(2.05)
Parker	9.78 b	(2.10)	11.00 c	(2.06)
Redcrest	6.06 a	(2.53)	8.28 a	(3.08)
Benton	5.44 a	(1.65)	6.50 b	(2.28)
Totem	7.72 c	(2.91)	8.61 a	(1.82)
Mean	6.32	(2.25)	8.14	(2.24)
LSD	1.51		1.50	

Means within a column followed by the same letter are not different, significance level  $p = 0.05$ . Response range for each attribute was from 0 to 15 ( $n = 18$  for each treatment mean).

Table 3.5. Correlation coefficients (r-values) of furaneol and mesifurane concentration with strawberry aroma and overall aroma intensity.

Strawberry variety	Attribute	Furaneol	Mesifurane
Chandler	Strawberry aroma	0.580***	0.375**
	Overall intensity	0.488***	0.358**
Douglas	Strawberry aroma	0.575***	0.541***
	Overall intensity	0.428***	0.369**
Pajaro	Strawberry aroma	0.600***	0.597***
	Overall intensity	0.432***	0.427***
Parker	Strawberry aroma	0.741***	0.746***
	Overall intensity	0.623***	0.653***
Redcrest	Strawberry aroma	0.634***	0.381**
	Overall intensity	ns	ns
Benton	Strawberry aroma	0.733***	0.736***
	Overall intensity	ns	ns
Totem	Strawberry aroma	0.626***	0.602***
	Overall intensity	0.414***	0.395**

ns, \*\*, \*\*\*, non significant or significant at  $P < 0.01$  and  $P < 0.001$ , respectively.

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## **APPENDIX**

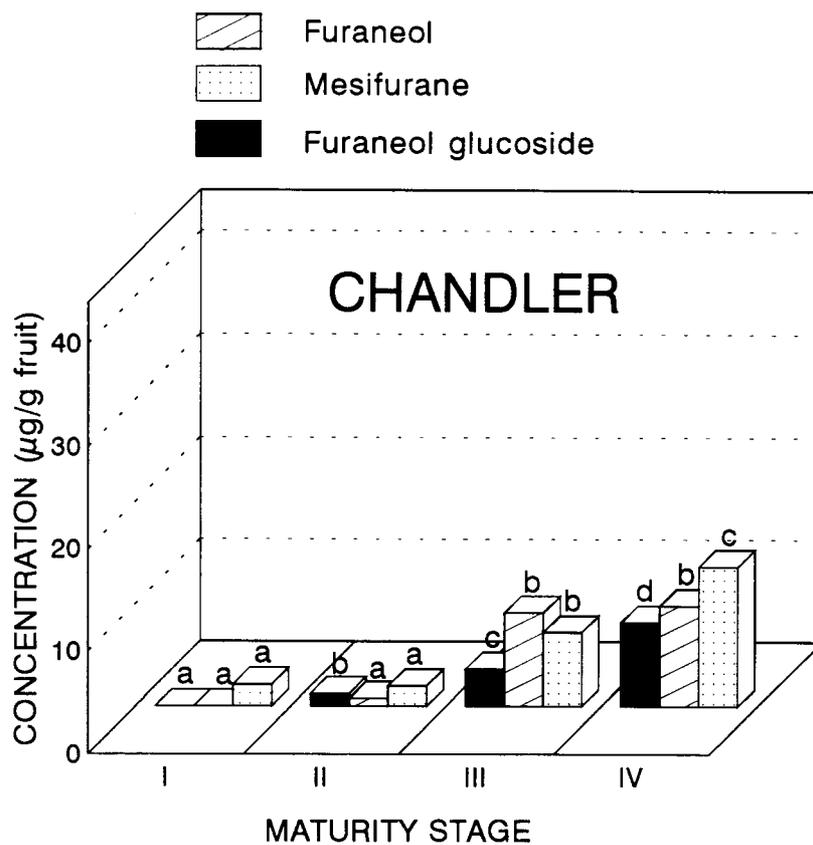


Figure A.1. Concentration of furaneol, mesifurane and furaneol glucoside during ripening of Chandler strawberries. Each bar represents the mean of 6 analyses. Means for the same compound with the same letter are not statistically different at significance level  $p=0.05$ .

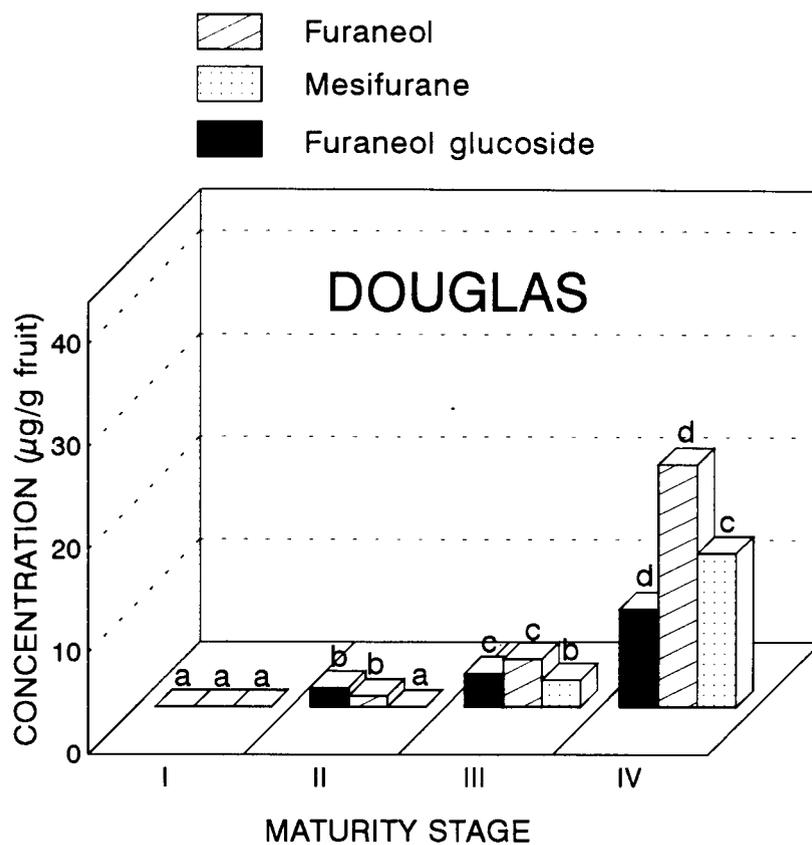


Figure A.2. Concentration of furaneol, mesifurane and furaneol glucoside during ripening of Douglas strawberries. Each bar represents the mean of 6 analyses. Means for the same compound with the same letter are not statistically different at significance level  $p=0.05$ .

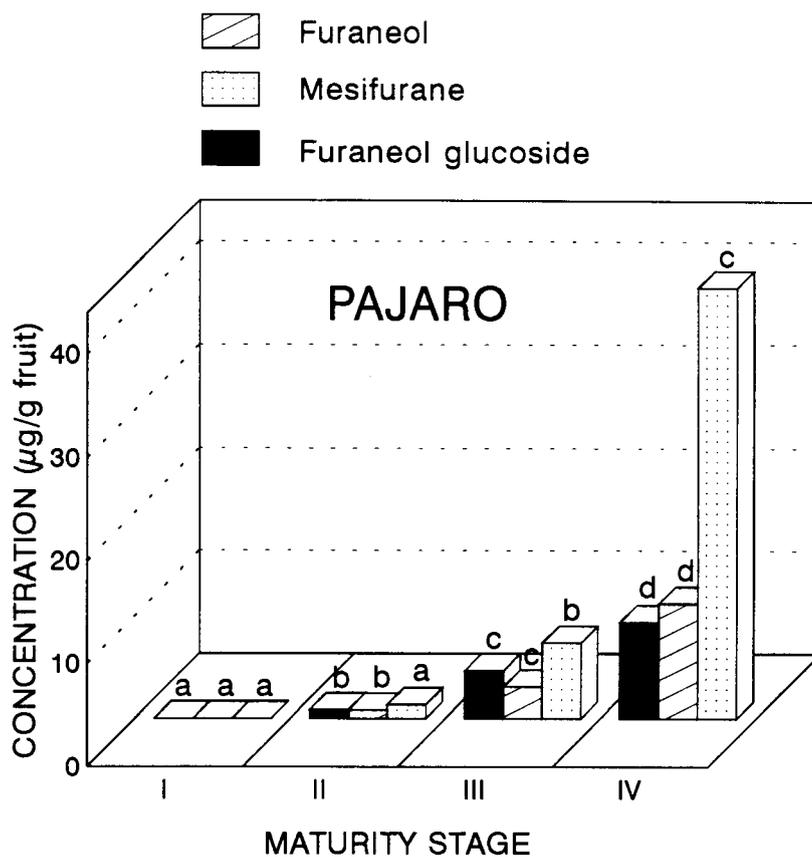


Figure A.3. Concentration of furaneol, mesifurane and furaneol glucoside during ripening of Pajaro strawberries. Each bar represents the mean of 6 analyses. Means for the same compound with the same letter are not statistically different at significance level  $p=0.05$ .

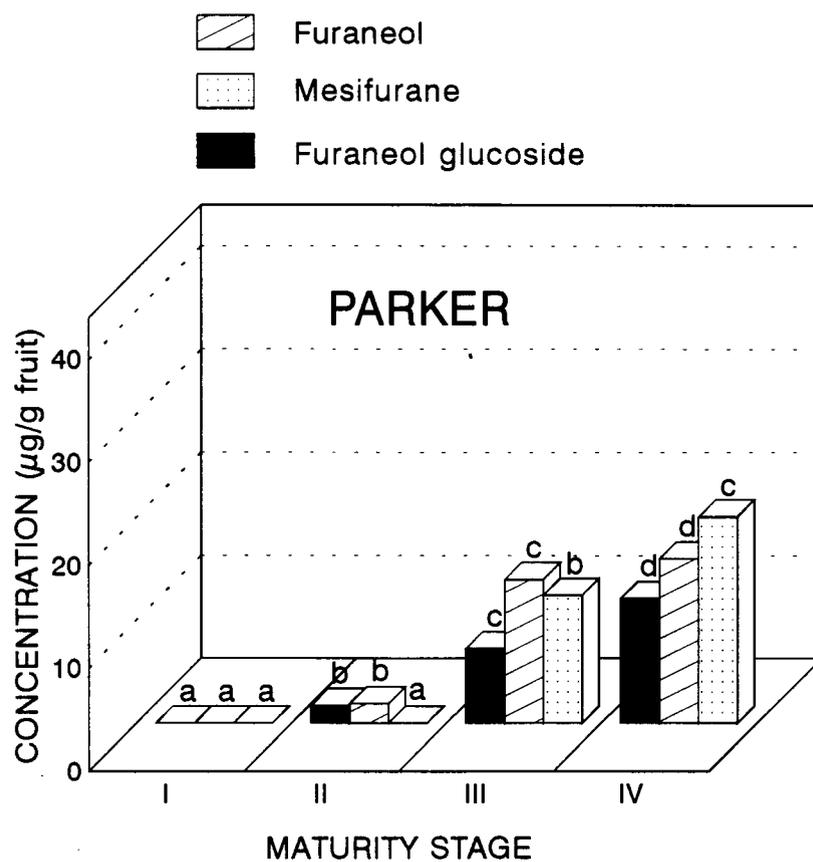


Figure A.4. Concentration of furaneol, mesifurane and furaneol glucoside during ripening of Parker strawberries. Each bar represents the mean of 6 analyses. Means for the same compound with the same letter are not statistically different at significance level  $p=0.05$ .

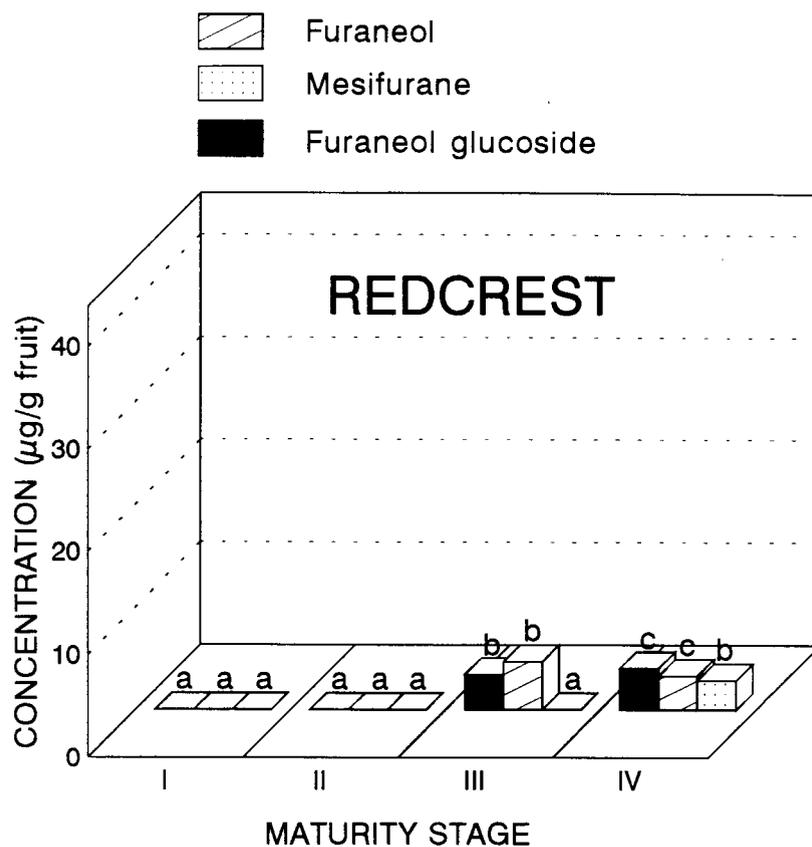


Figure A.5. Concentration of furaneol, mesifurane and furaneol glucoside during ripening of Redcrest strawberries. Each bar represents the mean of 6 analyses. Means for the same compound with the same letter are not statistically different at significance level  $p=0.05$ .

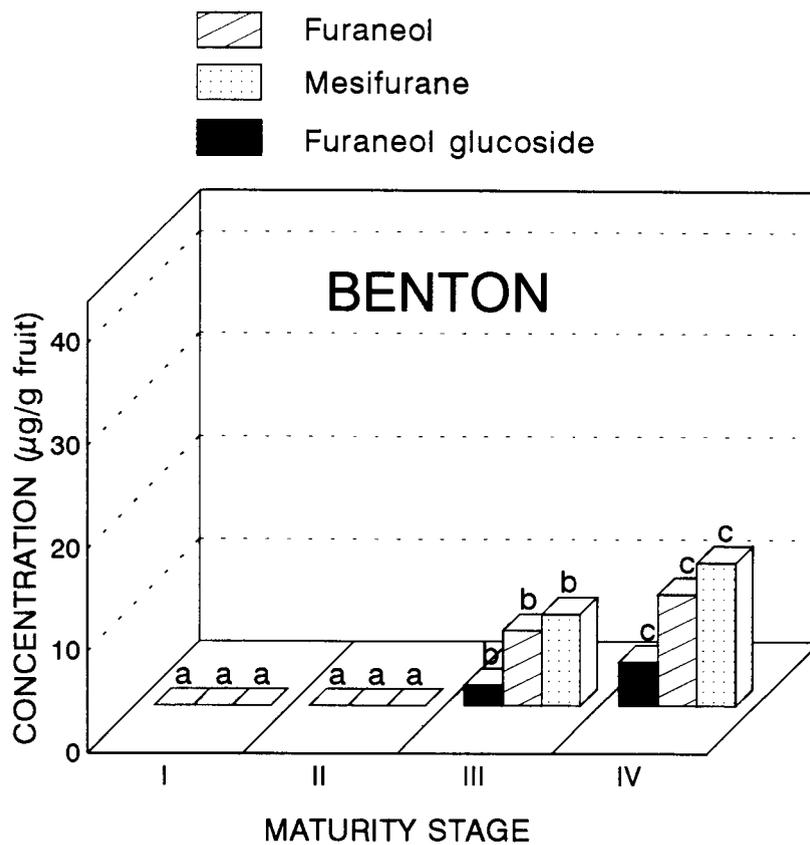


Figure A.6. Concentration of furaneol, mesifurane and furaneol glucoside during ripening of Benton strawberries. Each bar represents the mean of 6 analyses. Means for the same compound with the same letter are not statistically different at significance level  $p = 0.05$ .

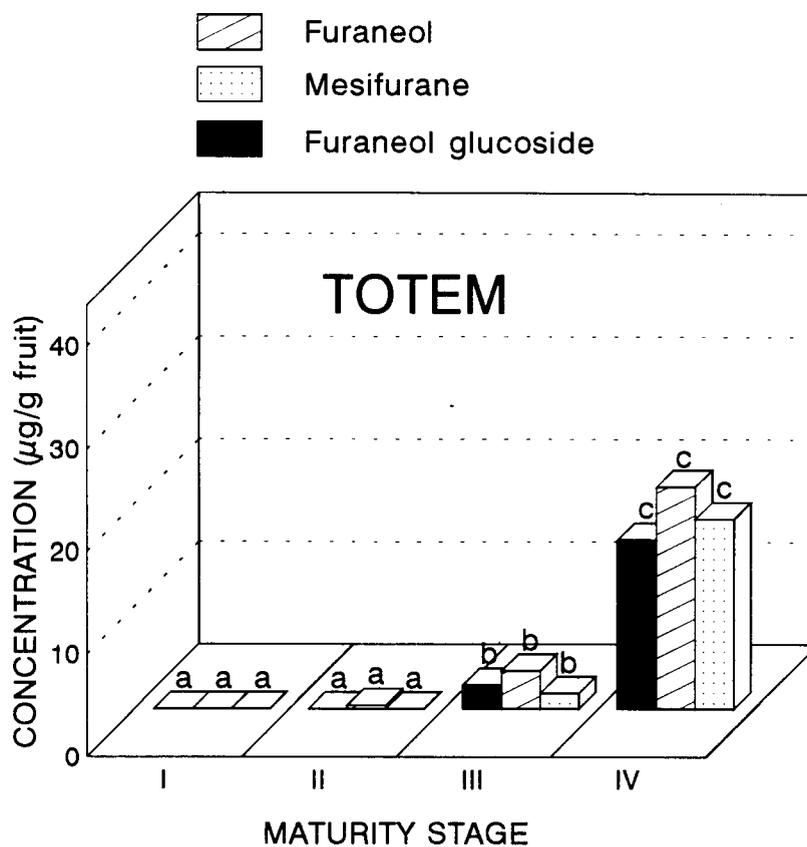


Figure A.7. Concentration of furaneol, mesifurane and furaneol glucoside during ripening of Totem strawberries. Each bar represents the mean of 6 analyses. Means for the same compound with the same letter are not statistically different at significance level  $p=0.05$ .

## STRAWBERRY AROMA TESTING BALLOT

- Please fill in the code number of the samples from left to right as they appear.
- Remove the lid from the container and evaluate the aroma of the strawberry juice by taking 3 quick sniffs and rate the overall intensity and strawberry aroma by placing a scale number which best describes the sample.

0= None	8=
1= Just detectable	9= Moderate to large
2=	10=
3= Slight	11= Large
4=	12=
5= Slight to moderate	13= Large to extreme
6=	14=
7= Moderate	15= Extreme

<b>SAMPLE #</b>	_____	_____	_____	_____	_____	_____	_____
<b>Overall Intensity</b>	_____	_____	_____	_____	_____	_____	_____
<b>Strawberry</b>	_____	_____	_____	_____	_____	_____	_____

Figure A.8. 16-Point intensity scale ballot used for sensory evaluation of strawberry extracts.