

- J. Agric. Food Chem. 1977, 25, 446-449.
- Still, W. C.; Kahn, M.; Mitra, A. "Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution". *J. Org. Chem.* 1978, 43, 2923-2925.
- Takeo, T. "Production of Linalool and Geraniol by Hydrolytic Breakdown of Bound Forms in Disrupted Tea Shoots". *Phytochemistry* 1981, 20, 2145-2147.
- Taskinen, J. "Composition of the Essential Oil of Sweet Marjoram Obtained by Distillation with Steam and by Extraction and Distillation with Alcohol-Water Mixture". *Acta Chem Scand., Ser. B* 1974, B28, 1121-1128.
- Taskinen, J. "The Acid Catalyzed Reaction of some Monoterpene Alcohols in Aqueous Ethanol". *Int. Flavours Food Addit.* 1976, 7, 235-236.
- Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, R. A. "Use of C₁₈ Reversed-Phase Liquid Chromatography for the Isolation of Monoterpene Glycosides and Norisoprenoid Precursors from Grape Juice and Wine". *J. Chromatogr.* 1982, 235, 471-480.
- Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, R. A. "Glycosides of 2-Phenylethanol and Benzyl Alcohol in *Vitis vinifera* Grapes". *Phytochemistry* 1983, 22, 2039-2041.
- Wilson, B.; Strauss, C. R.; Williams, P. J. "Changes in Free and Glycosidically Bound Monoterpenes in Developing Muscat Grapes". *J. Agric. Food Chem.* 1984, 32, 919-924.

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Investigation of Volatile Constituents in Nectarines. 2. Changes in Aroma Composition during Nectarine Maturation

Karl-Heinz Engel,*¹ David W. Ramming, Robert A. Flath, and Roy Teranishi

Changes in the distribution of volatile constituents during the maturation of nectarines (*Prunus persica* L., Batsch var. *nucipersica* Schneid) were investigated by means of capillary gas chromatography. C₆ aldehydes and alcohols are the major components isolated from "green" fruit; in the course of fruit maturation, the concentrations of these C₆ components decrease and lactones become prominent constituents. γ - and δ -lactones develop differently depending upon their chain lengths. The last period of nectarine maturation, from "shipping-ripe" to "tree-ripe" fruit, is characterized by a significant increase in the amounts of aroma-contributing lactones having longer aliphatic side chains.

In the first part of our investigation of volatile constituents in nectarines (*Prunus persica* L., Batsch var. *nucipersica* Schneid), we characterized aroma patterns in different cultivars (Engel et al., 1988). Qualitative and quantitative distributions of volatiles were determined. Saturated and unsaturated γ - and δ -lactones were shown to be major aroma-contributing constituents in "tree-ripe" fruits.

The formation of flavor and aroma compounds in fruits is a dynamic process. Concentrations of volatiles cannot be seen as "fixed values", but they are subject to many variations; the degree of maturity or ripeness of the fruit plays a major role. Do et al. (1969) and Bayonove (1973) reported changes in the composition of volatiles during maturation and ripening of peaches. The knowledge about this phenomenon in nectarines is limited to the findings of Lim and Romani (1964). They demonstrated by means of gas chromatography an increase in the concentration of several volatile fractions during ripening of nectarines, but they did not identify individual components.

The objective of this study was to correlate the stages of nectarine fruit maturity with analytically detectable differences in the qualitative and quantitative composition of volatiles.

Western Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, 800 Buchanan Street, Albany, California 94710 (K.-H.E., R.A.F., R.T.), and Horticultural Crops Research Laboratory, U.S. Department of Agriculture—Agricultural Research Service, 2021 South Peach Avenue, Fresno, California 93727 (D.W.R.).

¹Present address: Technische Universität Berlin, Seestr. 13, D-1000 Berlin 65, FRG.

EXPERIMENTAL SECTION

Materials. A white-flesh (P 89-56) and two yellow-flesh (Flavortop, P 62-27) nectarine cultivars were obtained from orchards of the Horticultural Crops Research Laboratory, U.S. Department of Agriculture, Fresno, CA. Flavortop is a commercially grown cultivar; P 89-56 and P 62-27 are presently being tested by the USDA in Fresno, CA. The fruits were harvested at four different degrees of maturity, which were determined according to size, color, and firmness (Haller, 1952). The selected degrees were classified as "green", "green-red", "shipping-ripe", and "tree-ripe"; they are shown on a growth curve of nectarines in Figure 1. Green-red and shipping-ripe are equivalent to the terms "immature" and "firm-mature", respectively, as they were defined on the maturity scale for peaches by Haller (1952).

Isolation of Volatiles. At each stage of maturity volatiles were isolated from 500 g of homogenized fruit pulp by liquid-liquid extraction with ether following the previously described procedure (Engel et al., 1988). (S)-(+)-Octanol-2 was used as standard.

Capillary Gas Chromatography—Mass Spectrometry (GC-MS). For capillary GC and GC-MS investigations a DB-Wax column (J&W Scientific, Folsom, CA; 60 m \times 0.32 mm; 0.25- μ m film thickness) was used. Instruments and conditions were as described previously (Engel et al., 1988).

RESULTS AND DISCUSSION

The maturation of nectarines can be divided into several stages. Figure 1 presents a growth curve typical for the cultivar Flavortop, showing the increase in fruit diameter over the season. Rapid development in stage I (increase of the pericarp) is followed by a rest period in stage II (hardening of the stone and development of the embryo) before the fruit finally reaches its maximal size and ma-

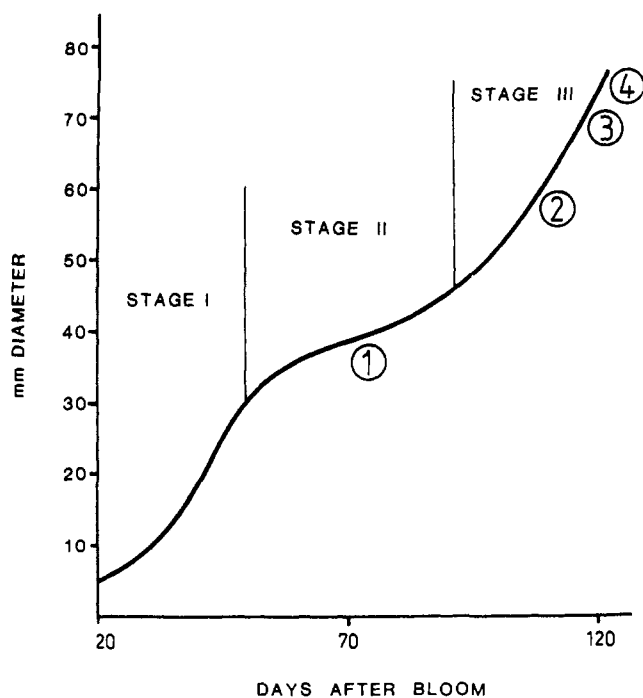


Figure 1. Degrees of maturity investigated in this study shown on a growth curve of the nectarine cultivar Flavortop: 1 = green; 2 = green-red; 3 = shipping-ripe; 4 = tree-ripe.

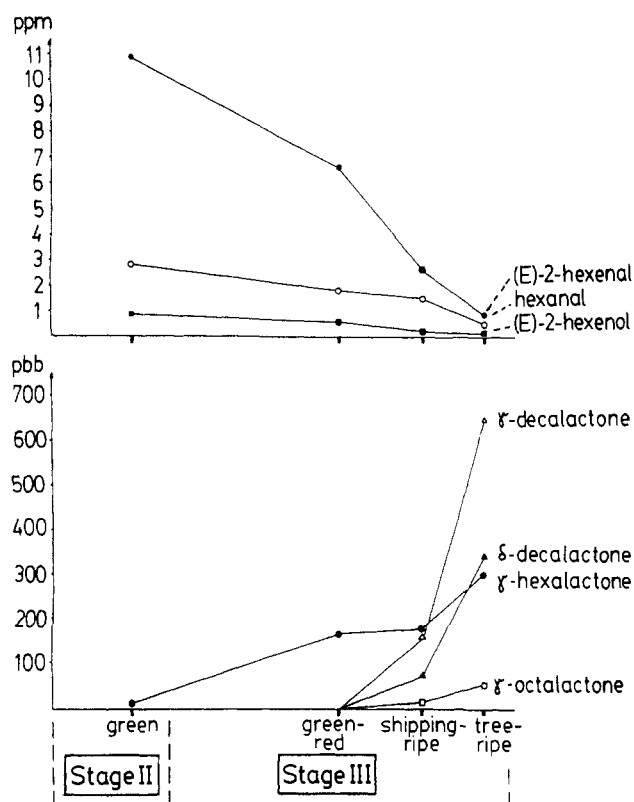


Figure 2. Changes in the concentrations of volatiles isolated from nectarines (P 62-27; yellow-flesh) at different stages of maturity.

turity in stage III (increase of the flesh). Analogous growth curves are obtained for other cultivars and have been described for peaches (Lilleland, 1932). Flavor and aroma qualities become sensorially apparent during stage III. We followed the volatile compositions at different degrees of maturity ranging from green fruits in stage II over green-red and shipping-ripe to tree-ripe fruits in stage III (Figure 1).

Table I. Changes in the Concentrations of Volatiles during Maturation of Nectarine Cultivar P 62-27

component	concentration, ppb			
	stage II: green	stage III green-red	stage III shipping-ripe	stage III tree-ripe
Lactones				
γ -hexalactone	10	160	180	300
γ -heptalactone	— ^a	—	10	30
γ -octalactone	—	—	10	50
δ -octalactone	—	—	10	30
γ -nonalactone	—	—	—	+ ^b
γ -decalactone	—	—	160	650
6-pentyl- α -pyrone	—	—	20	120
δ -decalactone	—	—	60	340
(Z)-dec-7-en-5-olide	—	—	+	30
γ -dodecalactone	—	—	—	+
C ₆ Components				
hexanal	2900	1900	1500	600
(Z)-3-hexenal	20	10	—	—
(Z)-2-hexenal	100	60	30	10
(E)-2-hexenal	10900	6600	2700	1000
hexanol	500	300	100	50
(Z)-3-hexenol	70	80	50	30
(E)-2-hexenol	1000	700	100	30
Others				
benzaldehyde	30	60	40	90
linalool	—	10	10	10
hotrienol	—	50	40	20

^a Not detected. ^b Concentration less than 10 ppb.

Table II. Changes in the Concentrations of Volatiles during Maturation of Nectarine Cultivar Flavortop

component	stage III concentration, ppb		
	green-red	shipping-ripe	tree-ripe
Lactones			
γ -hexalactone	100	150	270
γ -heptalactone	— ^a	+ ^b	10
γ -octalactone	—	+	10
δ -octalactone	—	—	+
γ -nonalactone	—	—	+
γ -decalactone	20	60	220
6-pentyl- α -pyrone	—	+	70
δ -decalactone	+	20	190
(Z)-dec-7-en-5-olide	—	+	30
γ -dodecalactone	—	—	+
C ₆ Components			
hexanal	3500	2400	1950
(Z)-3-hexenal	100	50	40
(Z)-2-hexenal	170	120	40
(E)-2-hexenal	12800	5800	3950
hexanol	750	200	150
(E)-2-hexenol	2400	200	170
Others			
benzaldehyde	20	30	30
linalool	160	200	500
hotrienol	50	70	90

^a Not detected. ^b Concentration less than 10 ppb.

The homogenized fruits were subjected to liquid-liquid extraction with ether. The extracts were investigated by capillary GC, and the identities of the components were determined by capillary GC-MS and Kovats index comparisons. Quantification of the compounds was based upon octanol-2 as internal standard. The results are summarized in Table I and II.

As demonstrated in Figure 2 for the cultivar P 62-27, the maturation of nectarines is characterized by opposite changes in two major groups of volatiles: C₆ components and γ - and δ -lactones. C₆ aldehydes and alcohols, products of enzyme-catalyzed breakdown of unsaturated fatty acids

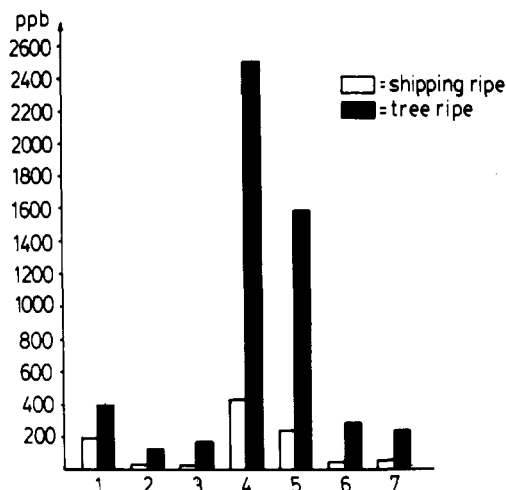


Figure 3. Concentrations of lactones in shipping-ripe and tree-ripe nectarines of the white-flesh cultivar P 89-56: 1 = γ -hexalactone; 2 = γ -heptalactone; 3 = γ -octalactone; 4 = γ -decalactone; 5 = δ -decalactone; 6 = (*Z*)-dec-7-en-5-olide; 7 = γ -dodecalactone.

(Schreier, 1984), are the main components isolated from green nectarines. With increasing maturity of the fruit however, the concentrations of these so-called green components decrease drastically. For the cultivar P 62-27 the total amount of C_6 compounds in tree-ripe fruits constitutes only 10% of the concentrations determined in green fruits during stage II.

Enzyme activities were not inhibited during the isolation of volatiles; therefore, the concentrations of C_6 components listed in Tables I and II comprise amounts present in the intact fruit and those formed after disruption of the cell tissue. Control experiments using enzyme deactivation with saturated $CaCl_2$ solution (Buttery et al., 1987) showed a 14-fold increase of the concentration of (*E*)-2-hexenal (from 0.25 to 3.5 ppm) during homogenization of green-red fruits for 30 s. During the following 24-h extraction period further enzymatic changes of the initially formed C_6 volatiles by lipoxygenase-, isomerase-, and oxidoreductase-catalyzed reactions (Tressl et al., 1981) take place. Therefore, the actual concentrations of C_6 components in nectarines and their contribution to nectarine aroma have not been established in this study. However, as all isolations were carried out under the same conditions, especially duration of blending and extraction, the amounts of isolated C_6 components can be compared and used as measurement for lipoxygenase activities at different stages of maturity.

With decreasing concentrations of C_6 components, lactones become prominent volatiles in nectarines. The results in this study show that the development of lactones during the maturation of nectarines differs, depending upon their chain lengths. γ -Hexalactone is present already in green fruits; its concentration increases steadily during the season. On the other hand, lactones with longer side chains, such as γ - and δ -decalactone, were not detected before the fruit reached green-red (Flavortop) and shipping-ripe (P 62-87) stages, respectively. Their concentrations, however, increase significantly in the last stages, and they become major constituents in tree-ripe fruits. This indicates either different pathways and enzymes or different precursors are involved in formation of lactones in nectarines.

The analytical data correlate with the sensory observation that the nectarines did not show "lactonic" aroma before they reached the final stages of maturity. According

to its odor threshold of 1600 ppb (Engel et al., 1988), γ -hexalactone has no significance for the nectarine aroma. On the other hand, the concentrations of the more important lactones with longer side chains, such as γ -decalactone (T_c in H_2O : 11 ppb), do not exceed their threshold levels before the fruits are green-red (Flavortop) or shipping-ripe (P 62-27), respectively.

An aspect that might be interesting for commercial nectarine growers are the significant differences in the lactone concentrations between shipping-ripe and tree-ripe fruits. At the shipping-ripe stage commercially sold nectarines are usually picked and sent to the stores. As shown in Tables I and II the amount of total lactones is up to 3 times higher in tree-ripe than in shipping-ripe fruits. These results are similar to findings of Do et al. (1969), who demonstrated that tree-ripe peaches contain 4 times more lactones than firm-mature fruits.

An impressive example for this phenomenon in nectarines is the concentrations of lactones in the white-flesh cultivar P 89-56. As shown in Figure 3 tree-ripe fruits contain up to 6 times higher amounts of γ - and δ -decalactone than shipping-ripe nectarines. The last period of the maturation process seems to be characterized by highly active lactone metabolism. Fruits harvested at this stage should be used in future experiments to study enzymes active in the biosynthesis of lactones.

The final quality of fruits picked at shipping-ripe stage is determined by changes of the flavor and aroma composition during subsequent artificial ripening. Changes in the content of aroma constituents during this period have been described for peaches (Do et al., 1969; Bayonove, 1973). The influence of artificial ripening under controlled conditions on the aroma components of nectarines will be the subject of further investigations. The findings of Lim and Romani (1964), however, indicated that the final amounts of volatiles in artificially ripened nectarines markedly depend upon maturity and content of volatiles at harvest. More information on the kinetics of volatile accumulation during ripening and senescence is needed, but the results of this study should help to provide first objective data for nectarine growers to find the time to pick fruits with optimum flavor and aroma qualities.

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Registry No. (*E*)-2-Hexenal, 6728-26-3; hexanal, 66-25-1; (*E*)-2-hexenal, 928-95-0; γ -hexalactone, 895-06-7; γ -heptalactone, 105-21-5; γ -octalactone, 104-50-7; δ -octalactone, 698-76-0; γ -nonalactone, 82373-92-0; γ -decalactone, 706-14-9; 6-pentyl- α -pyrone, 27593-23-3; δ -decalactone, 705-86-2; (*Z*)-dec-7-en-5-olide, 100428-67-9; γ -dodecalactone, 2305-05-7; (*Z*)-3-hexenal, 6789-80-6; (*Z*)-2-hexenal, 16635-54-4.

LITERATURE CITED

- Bayonove, C. "Recherches Sur L'Arome De La Peche". *Ann. Technol. Agric.* 1973, 22, 35-44.
- Buttery, R. G.; Teranishi, R.; Ling, L. C. "Fresh Tomato Aroma Volatiles: A Quantitative Study". *J. Agric. Food Chem.* 1987, 35, 540-544.
- Do, J. Y.; Salunkhe, D. K.; Olson, L. E. "Isolation, Identification and Comparison of the Volatiles of Peach Fruit as Related to Harvest Maturity and Artificial Ripening". *J. Food Sci.* 1969, 34, 618-621.
- Engel, K.-H.; Flath, R. A.; Buttery, R. G.; Mon, T. R.; Ramming, D. W.; Teranishi, R. "Investigation of Volatile Constituents in Nectarines I. Analytical and Sensory Characterization of Aroma Components in Some Nectarine Cultivars". *J. Agric. Food Chem.* 1988, 36, 549-553.

Haller, M. H. "Handling, Transportation, Storage and Marketing of Peaches". USDA Bibliographical Bulletin No. 21; GPO: Washington, DC, 1952.

Lilleland, O. "Growth of Peach Fruit". *Proc. Am. Soc. Hortic. Sci.* 1932, 29, 8-12.

Lim, L.; Romani, R. J. "Volatiles and the Harvest Maturity of Peaches and Nectarines". *J. Food. Sci.* 1964, 29, 246-253.

Schreier, P. "Chromatographic Studies of Biogenesis of Plant Volatiles". Bertsch, W., Jennings, W. G., Kaiser, R. E., Eds.; Dr. Alfred Hüthig Verlag: Heidelberg, Basel, New York, 1984

Tressl, R.; Bahri, D.; Engel, K.-H. "Lipid Oxidation in Fruits and Vegetables". In *Quality of Selected Fruits and Vegetables of North America*; Teranishi, R., Barrera-Benitez, H., Eds.; American Chemical Society: Washington, DC, 1981; pp 213-232.

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Contribution of Volatiles to Rice Aroma

Ron G. Buttery,* Jean G. Turnbaugh, and Louisa C. Ling

The odor thresholds in water solution of 64 known rice volatiles are listed and compared. Compounds with the lowest odor thresholds (T) include (E,E)-2,4-decadienal ($T = 0.07$ ppb), (E)-2-nonenal ($T = 0.08$ ppb), and 2-acetyl-1-pyrroline ($T = 0.1$ ppb). Data from quantitative analysis (using a Tenax trap isolation method) of the major volatiles in cooked California long-grain rice were used to determine the ratio of each compound's concentration in the cooked rice to its odor threshold (odor unit value). This indicated that the probable major contributors to the Californian long-grain cooked rice odor included 2-acetyl-1-pyrroline, (E,E)-2,4-decadienal, nonanal, hexanal, (E)-2-nonenal, octanal, decanal, 4-vinylguaiacol, and 4-vinylphenol.

More than 100 volatile aroma components have been identified in cooked rice. These have been covered in some recent reviews (Maga, 1984; Tsugita, 1985-1986) and publications (Tsugita et al., 1980; Yajima et al., 1978). However, there has been little scientific information reported on the relative importance of these compounds to total rice aroma. The present work was carried out in order to compare the odor potencies of various major rice components and to make some estimate of their probable relative contribution to the total rice aroma. A number of odor thresholds on rice components had been determined by some of the authors in previous studies of other foods (Buttery et al., 1971; Guadagni et al., 1966; Guadagni and Turnbaugh, 1980). Other odor thresholds were determined during the present work.

EXPERIMENTAL SECTION

Materials. California long-grain rice (variety L-202) was purchased from local retail markets and stored at room temperature in the dark. It was used within a few weeks. Chemical compounds were obtained from reliable commercial sources or synthesized by established methods. All compounds were purified by gas-liquid chromatography (GLC) separation before use and their identities verified by spectral (MS or IR) methods.

Quantitative Analysis of Volatiles in Cooked Rice. Rice (California long grain, variety L-202) was cooked in the normal way (500 g of rice was added to 1 L of water and the mixture brought to a boil and held at 100 °C for 20 min). The freshly cooked rice (300 g) was immediately placed in a 2-L round-bottom flask (containing an efficient magnetic stirrer) with 300 mL of odor-free (20 °C) water. A quantity (10.0 mL) of a standard solution of 50.0 ppm 2-octanone (internal standard) in water was then added. The volatiles were then isolated by a Tenax trap procedure similar to that previously described by Buttery et al.

(1987). This was done as follows. A Tenax trap (a Pyrex tube containing a 14 cm × 2.2 cm column of 60-80-mesh Tenax) and air inlet head were attached to the neck of the flask. The mixture was vigorously stirred, and purified air (3 L/min) was passed over the cooked rice slurry and led out of the flask through the Tenax trap. The isolation was carried out for 60 min; the trap was then removed and eluted with freshly distilled diethyl ether (50 mL). The ether extract was concentrated to ca. 10 μ L with a warm water bath and Vigreux distillation column. This concentrate was used for the quantitative GLC analysis.

GLC Analysis. A commercially obtained 60-m length × 0.32-mm i.d. DB-wax wall coated fused silica capillary column was used in a HP5880 gas chromatograph with electronic peak integration. The column was temperature programmed by holding the column at 30 °C for the first 5 min and then increasing the temperature at 4 °C/min until 170 °C where it was held for another 30 min. Injector temperature was 150 °C and He flow velocity 32 cm/s.

Odor Threshold Determinations. Odor thresholds of GLC-purified compounds were determined as described previously (Guadagni and Buttery, 1978) using an experienced panel of 16-20 members. Odor-free Teflon squeeze bottles and tubes were used to contain the solutions, and the judging room was supplied with a slight positive pressure of odor-free air (purified by passage through activated charcoal).

RESULTS AND DISCUSSION

The odor thresholds (T) found for a number of major volatile rice components are listed in Table I in parts (mL) of compound per billion (10^9) parts (mL) of water. Most of these had been determined in the authors' laboratory either in the present study or in other food volatile studies over the last 20 years (Buttery et al., 1971). A few figures were obtained from data published by other laboratories, and these are indicated by a footnote in Table I. Most of the compounds in Table I had been identified in rice by the authors listed in the reviews cited above.

Of the compounds listed in Table I the aldehydes (E)-2-nonenal ($T = 0.08$ ppb) and (E,E)-2,4-decadienal (T

Western Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, Albany, California 94710.