# Linalool in Orange Juice:  Origin and Thermal Stability

RUSSELL BAZEMORE,[†,§] RUSSELL ROUSEFF,[*,†] AND MICHAEL NAIM[‡]

University of Florida Citrus Research and Education Center, 700 Experiment Station Road,
Lake Alfred, Florida 33850, and Institute of Biochemistry, Food Science and Nutrition, Faculty of
Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem,
P.O. Box 12, Rehovot 76-100, Israel

Linalool concentrations were determined in juice from three groups of 60 Valencia oranges using pentane:ether extraction and high-resolution capillary GC. The outer peel (flavedo) was removed from one group. The other two groups retained their peel intact. Juice was extricated from the halved fruits of the flavedo-less group and from one of the peel-intact groups using a hand reamer. A peel-cutting/macerating juice extractor was used for the other peel-intact group. Linalool concentrations were 0.004 mg/L in peeled fruit juice and 0.020 and 0.106 mg/L for hand-reamed and mechanically extracted peel-intact juice, respectively. Juice from peeled fruit contained significantly ($P$ < 0.05) less linalool than peel-intact juice. Approximately 80% of the total juice linalool content was associated with peel using reamer design, and 96% was associated with peel-cutting/macerating design. Linalool increased with increasing peel oil levels; however, the increases were not proportionate. Since all commercial juices are mechanically extracted, the vast majority of linalool in commercial orange juice originates from the peel and not from the juice vesicle cytoplasm. Juice from peel-macerated, mechanically extracted fruit increased from 0.106 to 0.134 mg/kg after thermal processing, whereas juice from reamer extraction was essentially unchanged.

**KEYWORDS:  Pasteurization; flavor changes; aroma impact; citrus; flavedo; orange peel**

## INTRODUCTION

Linalool, a terpene alcohol with a floral aroma, is an important contributor to the flavor and aroma of numerous foods, flavors, and fragrances, including tomato (*1*), grape and wine (*2*, *3*), mango (*4*, *5*), lemon oil (*6*, *7*), lime oil (*8*), apricots (*9*, *10*), tea (*11*, *12*), carrots (*13*), basil (*14*), and perfumes (*15*). It has also been reported to be the most potent flavor impact component in commercial orange juice (*16*). However, literature reports of concentrations and stability of linalool in orange juice prepared, processed, and stored in different manners vary widely. Marin et al. (*16*) could not detect linalool in unpasteurized, hand-squeezed juice, whereas others (*17*) have reported linalool concentrations ranging from trace amounts to 5.3 mg/L in orange juices. The lowest levels were reported for fresh (unpasteurized) samples and two pasteurized juices. The highest levels of linalool were observed in juices that had been concentrated. Recent studies employing isotope dilution analysis (*18*) found 0.081 and 0.073 mg/kg linalool in unpasteurized, hand-squeezed Valencia and navel orange juices.

The relationship between heat treatment (pasteurization) and linalool concentration has not been clearly established, even though some juices are heated once during pasteurization and others twice, once during thermal concentration and again after reconstitution from concentrate. Askar et al. (*19*) reported losses of linalool and limonene after pasteurization and storage for up to 6 months. Schreier (*20*) reported the concentration of linalool to be greater in fresh juice (unpasteurized) than in pasteurized juice. Marin and co-workers, using GC−O, could not detect linalool in hand-squeezed, unpasteurized juices but found it to be the major flavor impact compound in juice from the same lot of oranges that had been mechanically squeezed and pasteurized. It was unclear if linalool concentrations changed due to pasteurization or to method of juicing. A more recent GC−O study (*21*) reported that linalool was aroma-active in hand-squeezed orange juice but was not one of the most potent aromas. The primary objective of this study was to determine whether the majority of juice linalool originates from macerated peel or is endemic in juice cytoplasm. A secondary objective was to determine whether linalool levels are altered as a result of thermal processing.

## MATERIALS AND METHODS

**Juice Preparation.** Mature Valencia oranges harvested in March 1998 from University of Florida, CREC groves were divided into three equal groups of 60 fruit. The outer portion of the peel containing the oil glands (flavedo) was mechanically shaved off from one group prior to juicing (to ensure that the juice was essentially free of peel oil), sliced equatorially, and juiced with a mechanical reamer. The white

* Corresponding author [phone (941) 956-1151; fax (941) 956-4631; E-mail rlr@lal.ufl.edu].
§ Current address: Department of Food Science and Technology, Mississippi State University, Mississippi State, MS 39762.
† University of Florida Citrus Research and Education Center.
‡ The Hebrew University of Jerusalem.

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pithy portion of the peel remained on the fruit. Fruit from a second group (peel intact) was sliced equatorially and juiced in the same manner. The third group (peel intact) was juiced with an FMC single-head juice extractor, which macerates peel during juicing. Brix and acid values from a representative juice were 12.6° and 0.65%, respectively. To measure the effects of heat processing, half of each juice treatment was pasteurized at 92 °C for 30 s with a Microthermic UHT/HTST pasteurizing system, model 35 (Microthermic Corp., Raleigh, NC). The other half of each juice treatment was immediately extracted with pentane:ether.

**Extraction of Juice Volatiles.** Pentane:diethyl ether extraction was conducted in the manner of Jella and co-workers (*24*) by mixing 5 mL of *n*-pentane and 5 mL of diethyl ether with 16 mL of juice using two 50-mL syringes. The subsequent emulsion (maintained at 2 °C) was broken by centrifuging at 2500 rpm (750*g*). The upper solvent layers from two extractions were combined and concentrated under nitrogen to yield 100 $\mu$L of extract. Propyl benzene was added to juice prior to extraction at a final concentration of 10 mg/L as an internal standard. The linalool peak was identified using mass spectrometry and Kovat's retention values as well as co-injection (stacked injection) of standard linalool with the sample. Linalool concentrations were determined using GC FID peak area and separate response factors for linalool and propyl benzene.

**Chromatographic Conditions.** GC analysis was conducted with an HP 5890 equipped with a 30-m × 0.25-mm-i.d. DB5 capillary column (J&W Scientific, Folsom, CA). Operating conditions were as follows: column temperature was held at 32 °C for 3 min and then increased at a rate of 6 °C/min to 200 °C; injection and detection port temperatures were 250 °C; helium carrier gas linear velocity was 29 cm/s.

**GC−MS Analysis.** A Thermo Finnigan GCQ Plus system (Finnigan Corp., San Jose, CA) was used to separate and identify the major chromatographic peaks. Chromatographic separation was achieved using a J&W Scientific DB-5 column (60 m × 0.25 mm i.d. × 0.25 $\mu$m film thickness). The initial column oven temperature was 40 °C and was increased at 7 °C/min to a final temperature of 275 °C. The injector temperature was 200 °C. The transfer line temperature was maintained at 250 °C. Injection was in the splitless mode. MS (electron impact ionization) conditions were as follow: ionization energy, 70 eV; mass range, 40−300 amu; scan rate, 2 scans/s; electron multiplier voltage, 1050 V. Compounds were identified by comparing the mass spectra at the peak apex with those in computerized libraries (Wiley and Adams) along with those of authentic standards. Background subtraction was employed before searching. Peak purity was evaluated by comparing the spectrum obtained at half peak height from both the leading and trailing portions of the peak.

**Statistical Analyses.** Linalool values were obtained in quadruplicate, averaged, and analyzed for differences between means using the ANOVA and LSD programs in SAS.

**Oil Measurements.** The amount of oil in each sample was determined using the classic Scott oil procedure (*22*, *23*).

## RESULTS AND DISCUSSION

**Source of Juice Linalool.** As shown in **Figure 1**, juice linalool concentrations were significantly different between the various juice extraction conditions. The lowest linalool levels (0.004 mg/L) were observed for juice that was hand-squeezed (reamer) with the flavedo removed. Linalool levels increased significantly to 0.028 mg/L when the fruit was prepared in the same manner but with the peel left intact. This 7-fold increase suggests that the flavedo was the source of the majority of the linalool observed in this juice. Hand juicing of fruit using a reamer is generally considered to be a milder juicing condition (lower juice recovery) than the mechanical reamers used commercially. The mechanical pressure of the reamer against the peel is generally higher in commercial extraction compared to hand extraction. Nisperos-Carriedo and Shaw (*17*) examined a range of commercial and hand-squeezed orange juices and found only trace linalool levels in hand-squeezed juices and
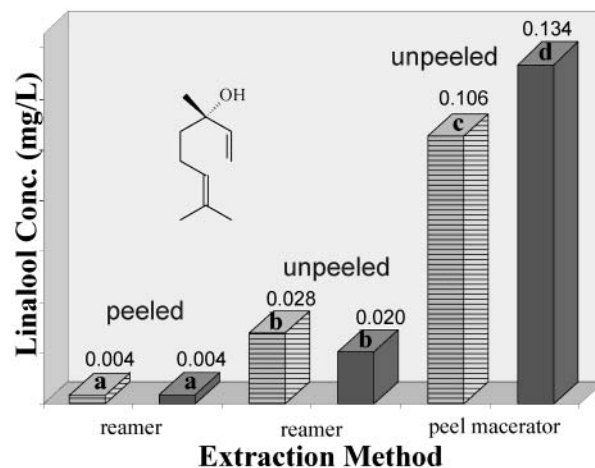


**Figure 1.** Comparison of linalool levels in flavedo-removed (peeled) and peel-intact (unpeeled) Valencia orange juices. The significant increase of juice linalool levels from juice prepared using a hand reamer compared to that obtained with a mechanical, peel-macerating juicing device is noteworthy. Crosshatched bars represent average values for unheated juices, and dark solid bars represent corresponding values for heated juices. Juices with different labels a–d are significantly different ($p < 0.05$).

values from 0.04 to 0.63 mg/L linalool in commercial pasteurized juice. It is worth noting that their lowest value for peel-intact fruit was 10 times greater than the peeled, hand-extracted juice value observed in the present study. Their commercial pasteurized linalool values would be best compared to the 0.134 mg/L observed from the peel-macerated, mechanically extracted juice in the present study, as this juicing technique is used by the majority of the citrus industry in Florida. Our results are also similar to those obtained by Marin and co-workers (*16*), who concluded that linalool was absent or barely detected in hand-squeezed, unpasteurized juice but was the most important aroma contributor in pasteurized, mechanically squeezed orange juice.

**Peel and Peel Oil.** Linalool is found in relatively high concentrations in cold-pressed orange oil. In surveying the data on citrus oils, Shaw (*24*) found that linalool was the component in highest concentration after the major terpene, limonene. It is therefore not surprising that if peel oil is introduced into the juice during juice extraction, overall linalool levels will be elevated. Scott oil values for the juices in this study were 0.006% in the hand-reamed flavedo-removed fruit, 0.008% from hand-reamed flavedo-intact fruit, and 0.018% in the peel-macerated, mechanically extracted fruit. These values mimic the linalool values observed in this study. However, the oil values are not exactly proportional to the linalool concentrations; for example, the highest oil content is only 3 times greater than the lowest juice oil content, whereas the highest linalool concentration is over 20 times greater than the lowest concentration. Part of this quantitative discrepancy may be due to the fact that the Scott oil test does not measure polar terpene alcohols such as linalool; rather, it measures terpenes, primarily limonene. In an earlier study comparing terpene levels, Ohta and co-workers (*25*) found higher levels of limonene and other terpenes in mandarin juice from a peel-macerating extractor (FMC type) than from a new screw-press extractor that extracts peeled fruit. Terpene differences were attributed to differences in oil levels as well.

**Thermal Stability.** It has been known for some time that linalool is a fairly reactive molecule. Baxter and co-workers (*26*) found that 56% linalool degraded in 20 days in model solutions containing 0.025 M aqueous citric acid at 24 °C,

producing 31% α-terpineol and 17% 3,7-dimethylact-1-ene-3,7-diol. Most accounts of linalool thermal instability in the acidic matrix of orange juice (pH 3.8) are from elevated temperature storage studies lasting as long as 6 months. There is little information concerning the effects of high-temperature, short-time thermal processes currently used to stabilize commercial citrus juices. Older reports of linalool thermal stability can be found from workers modeling the "hot fill" process that is used for a decreasingly small percent of citrus juices produced in the United States. For example, Askar and co-workers (*27*) heated orange juice to 80 °C for 10 min before cooling and storing at 4 and 30 °C and reported that linalool decreased during storage, but they did not indicate how linalool changed as the result of initial heat treatment alone. Linalool model studies from the same workers (*19*) indicate that linalool degrades primarily to α-terpineol but also forms 1,8-terpine, geraniol, and nerol during storage at 4 and 30 °C. In another study (*28*), when ultra-high-temperature conditions were applied to a 10% orange juice drink, levels of both linalool and limonene diminished as a result of thermal treatment.

In the current study, there were significant differences in linalool levels in juices produced by hand reaming of peel-intact fruit and hand reaming of peeled fruit. There were also significant differences in linalool levels found in juices from peel-intact fruit extricated by a hand reamer and peel-intact fruit extricated by a macerating mechanical extractor (**Figure 1**). However, the effects of heating on linalool concentrations were mixed. Juices produced from hand reaming showed no significant differences in linalool concentrations due to heat treatment. However, thermally treated juice from the peel-macerating, mechanical juice extractor contained significantly greater concentrations of linalool than the identical unheated juice. Somewhat similar results were observed in a recent study employing dynamic headspace (*29*). Linalool concentrations were reported to be 0.79 mg/kg in fresh squeezed juice, 1.56 mg/kg in a juice heated to 98 °C for 11 s, and 0.91 mg/kg in a juice heated to 98 °C for 37 s. External standards were employed for quantification. The juices were processed in a commercial citrus plant, but the type of extractor was not described, nor were oil levels measured. In their survey of commercial orange juices, Nisperos-Carriedo and Shaw (*17*) found wide ranges in juice linalool concentrations in pasteurized orange juice from concentrate (0.38–5.3 mg/L) and high-quality pasteurized juice (0.04–0.63 mg/L). The differences in linalool concentration observed in this study are due to the combination of heating, juice extraction, and cultivar differences between the samples. Oil levels, type of extractor, and cultivar composition information was not provided, so it was not possible to determine the effect of heating alone.

Glycosides of linalool have been reported in grape, passion fruit, and apricot fruit (*30*). Furthermore, free linalool has been shown to be liberated from the glycoside through heating (*31*). Since many of these glycosides are associated with fruit peel and skin, and since mechanical extraction procedures typically contain increased peel components because of increased extraction pressure, the presence of a linalool glycoside in orange peel would explain the increased levels of linalool found in mechanically extracted juice. However, we were unable to confirm the presence of linalool glycoside in orange juice using the method employed by Wu et al. (*32*) when they determined glycosidically bound volatiles in pineapple juice. The inability to detect the linalool glycoside may be due to the exceptionally high concentrations of flavanone glycosides (*33*) found in citrus

juices, which would make it difficult to separate total glycosides using traditional XAD resins.

## LITERATURE CITED

(1) Buttery, R. G.; Seifert, R. M.; Guadagni, D. G.; Ling, L. C. Characterization of additional volatile components of tomato. *J. Agric. Food Chem.* **1971**, *19*, 524–529.

(2) Simpson, R. F.; Miller, G. C. Aroma composition of Chardonnay wine. *Vitis* **1984**, *23*, 143–158.

(3) Williams, P. J.; Strauss, C. R.; Wilson, B. New linalool derivatives in Muscat of Alexandria grapes and wines. *Phytochemistry* **1980**, *19*, 1137–1139.

(4) Abd El Baki, M. M.; Askar, A.; El Samahy, S. K.; Abd El Fadeel, M. G. Studies on mango flavor. *Dtsch. Lebensm.-Rundsch.* **1981**, *77*, 139–142.

(5) Sakho, M.; Chassagne, D.; Crouzet, J. African mango glycosidically bound volatile compounds. *J. Agric. Food Chem.* **1997**, *45*, 883–888.

(6) Dugo, G.; Ragonese, C.; Licandro, G. Essential oil of lemon from Argentine Mesopotamia. *Essenze, Deriv. Agrum.* **1977**, *47*, 503–514.

(7) Schieberle, P.; Grosch, W. Quantitative analysis of important volatile flavour compounds in fresh and stored lemon oil/citric acid emulsions. *Lebensm. Wiss. Technol.* **1988**, *21*, 158–162.

(8) Tamura, H.; Yang, R. H.; Sugisawa, H. Aroma Profiles of Peel Oils of Acid Citrus Lemon and Lime Citrus Essential Oils—Analysis and Organoleptic Evaluation. In *Bioactive Compounds from Plants*; Teranishi, R., Buttery, R., Sugisawa, H., Eds.; American Chemical Society: Washington, DC, 1993; pp 121–136.

(9) Issanchou, S.; Schlich, P.; Guichard, E. Odour profiling of apricot aroma volatiles. Description correspondence analysis. *Sci. Aliments* **1989**, *9*, 351–369.

(10) Rhoades, J. W.; Register, J. W., Jr.; Millar, J. D. Apricot flavour enhancement. U.S. Patent 3634098, 1972.

(11) Kita, Y.; Nakatani, Y.; Kobayashi, A.; Yamanishi, T. Composition of peel oil from Citrus unshiu. *Agric. Biol. Chem.* **1969**, *33*, 1559–1565.

(12) Godwin, D. R. Relationships between sensory response and chemical composition of tea. *Diss. Abstr. Int., B* **1984**, *45*, 3801.

(13) Alabran, D. M.; Moskowitz, H. R.; Mabrouk, A. F. Carrot-root oil components and their dimensional characterization of aroma. *J. Agric. Food Chem.* **1975**, *23*, 229–232.

(14) Akguel, A. Volatile oil composition of sweet basil (Ocimum basilicum L.) cultivated in Turkey. *Nahrung* **1989**, *33*, 87–88.

(15) Bedoukian, P. Z. Perfumery and flavor materials. *Perfum. Flavor.* **1981**, *6*, 3–6.

(16) Marin, A. B.; Acree, T. E.; Hotchkiss, J. H.; Nagy, S. Gas chromatography–olfactometry of orange juice to assess the effects of plastic polymers on aroma character. *J. Agric. Food Chem.* **1992**, *40*, 650–654.

(17) Nisperos-Carriedo, M. O.; Shaw, P. E. Comparison of volatile flavor components in fresh and processed orange juices. *J. Agric. Food Chem.* **1990**, *38*, 1048–1052.

(18) Buettner, A.; Schieberle, P. Evaluation of aroma differences between hand-squeezed juices from valencia late and navel oranges by quantitation of key odorants and flavor reconstitution experiments. *J. Agric. Food Chem.* **2001**, *49*, 2387–2394.

(19) Askar, A.; Bielig, H. J.; Treptow, H. Aroma changes in orange juice. III. Model studies on the losses of linalool and limonene during storage in bottles and cans. *Dtsch. Lebensm.-Rundsch.* **1973**, *69*, 360–364.

(20) Schreier, P. Changes of Flavour Compounds During the Processing of Fruit Juices. *Proc. LongAshton Symp.* **1981**, *7*, 355–371.

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(21) Hinterholzer, A.; Schieberle, P. Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by odour dilution techniques. *Flavour Frag. J.* **1998**, *13*, 49−55.

(22) Ting, S. V.; Rouseff, R. L. *Citrus fruits and their products: analysis, technology*; Marcel Dekker: New York, 1986; p 293.

(23) Kimball, D. A. *Citrus Processing: Quality Control and Technology*; Van Nostrand Reinhold: New York, 1991; p 473.

(24) Jella, P.; Rouseff, R.; Goodner, K.; Widmer, W. Determination of key flavor components in methylene chloride extracts from processed grapefruit juice. *J. Agric. Food Chem.* **1998**, *46*, 242−247.

(25) Shaw, P. E. Review of quantitative analyses of citrus essential oils. *J. Agric. Food Chem.* **1979**, *27*, 246−257.

(26) Ohta, H.; Tonohara, K.; Watanabe, A.; Iino, K.; Kimura, S. Flavor specificities of Satsuma mandarin juice extracted by a new-type screw press extraction system. *Agric. Biol. Chem.* **1982**, *46*, 1385−1386.

(27) Baxter, R. L.; Laurie, W. A.; McHale, D. Transformations of monoterpenoids in aqueous acids. The reactions of linalool, geraniol, nerol and their acetates in aqueous citric acid. *Tetrahedron* **1978**, *34*, 2195−2199.

(28) Askar, A.; Bielig, H. J.; Treptow, H. Aroma changes in orange juice. II. Aroma changes during manufacture and storage of bottled orange juice. *Dtsch. Lebensm.-Rundsch.* **1973**, *69*, 162−167.

(29) Bertels, J. R.; Potter, R. H.; Sinki, G. Stability of citrus flavors in aseptic packaging. *Beverages* **1987**, *52*, 4−5.

(30) Moshonas, M. G.; Shaw, P. E. Flavor and chemical comparison of pasteurized and fresh Valencia orange juices. *J. Food Qual.* **1997**, *20*, 31−40.

(31) Salles, C.; Jallageas, J. C.; Crouzet, J. C. HPLC separation of fruit diastereoisomeric monoterpenyl glycosides. *J. Essen. Oil Res.* **1993**, *5*, 381−390.

(32) Porto, C.; Sensidoni, A.; Battistutta, F. Composition and flavour of Muscat of Canelli grape distillates obtained using different oenological techniques and unconventional distillation processes. *Ital. J. Food Sci.* **1995**, *7*, 47−56.

(33) Wu, P.; Kuo, M. C.; Hartman, T. G.; Rosen, R. T.; Ho, C. T. Free and glycosidically bound aroma compounds in pineapple (*Ananas comosus* L. Merr.). *J. Agric. Food Chem.* **1991**, *39*, 170−172.

(34) Rouseff, R. L.; Martin, S. F.; Youtsey, C. O. Quantitative survey of narirutin, naringin, hesperidin, and neohesperidin in citrus. *J. Agric. Food Chem* **1987**, *35*, 1027−1030.