Changes in Volatile Flavor Components of Pineapple Orange Juice As Influenced by the Application of Lipid and Composite Films

Myrna O. Nisperos-Carriedo,* Philip E. Shaw, and E. A. Baldwin

U.S. Citrus and Subtropical Products Laboratory, South Atlantic Area, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 1909, Winter Haven, Florida 33883-1909

Five lipid and composite films were tested for their ability to retain volatile flavor components in Pineapple oranges during storage at 21 °C. By use of a headspace analysis technique, 15 components were detected and quantified in juice from both coated and uncoated stored fruits. Uncoated fruits showed minor increases in ethanol, methanol, acetaldehyde, hexanol, and *cis*-3-hexenol during storage for 2 days. The coated fruits showed significant increases in components considered important to fresh orange flavor (acetaldehyde, ethyl acetate, ethyl butyrate, and methyl butyrate). Use of beeswax emulsion and TAL Pro-long alone or in combination was the most effective coating in retaining or increasing volatile components.

The application of protective coatings on citrus fruits is not new. Although various types of coatings have been developed for extending fruit storage life, there is little descriptive information and quantitative data concerning the effect of films on volatile flavor components.

Citrus fruits, once harvested, are subject to desiccation and progressive deteriorations as a result of bacterial and fungal action. Such deteriorations greatly impair flavor, aroma, and appearance of the product and are responsible for substantial economic loss. Several methods have been investigated to prevent these deteriorative changes among which are controlled-atmosphere (CA) storage and hypobaric storage (Grierson and Ben-Yehoshua, 1986). These methods, however, are capital intensive and costly to carry out.

An alternative system is the use of waxes and other protective films and coatings (Kester and Fennema, 1986). Hot-melt paraffin waxes and carnauba wax oil-in-water emulsions have been used in citrus fruits as early as 1930 (Kaplan, 1986). Other waxes like beeswax, candelilla, and rice bran wax were also used to prevent desiccation and provide an attractive gloss to oranges, limes, and grapefruit (Lawrence and Iyengar, 1983; Lakshminarayana et al., 1974; Paredes-Lopez et al., 1974). Ukai et al. (1976) used hydrophobic emulsions to coat oranges for prolonged freshness. Tal-Chemicals Co. produced a coating composition, TAL Pro-long, consisting of sucrose esters of fatty acids, (carboxymethyl)cellulose sodium salt, and mono- and diglycerides of fatty acids that has proven effective in extending the shelf life of bananas (Banks, 1984), mangoes (Dhalla and Hanson, 1988), apples (Banks, 1985; Smith and Stow, 1984), and limes (Motlagh and Quantick, 1988).

Most of these processes are successful in providing

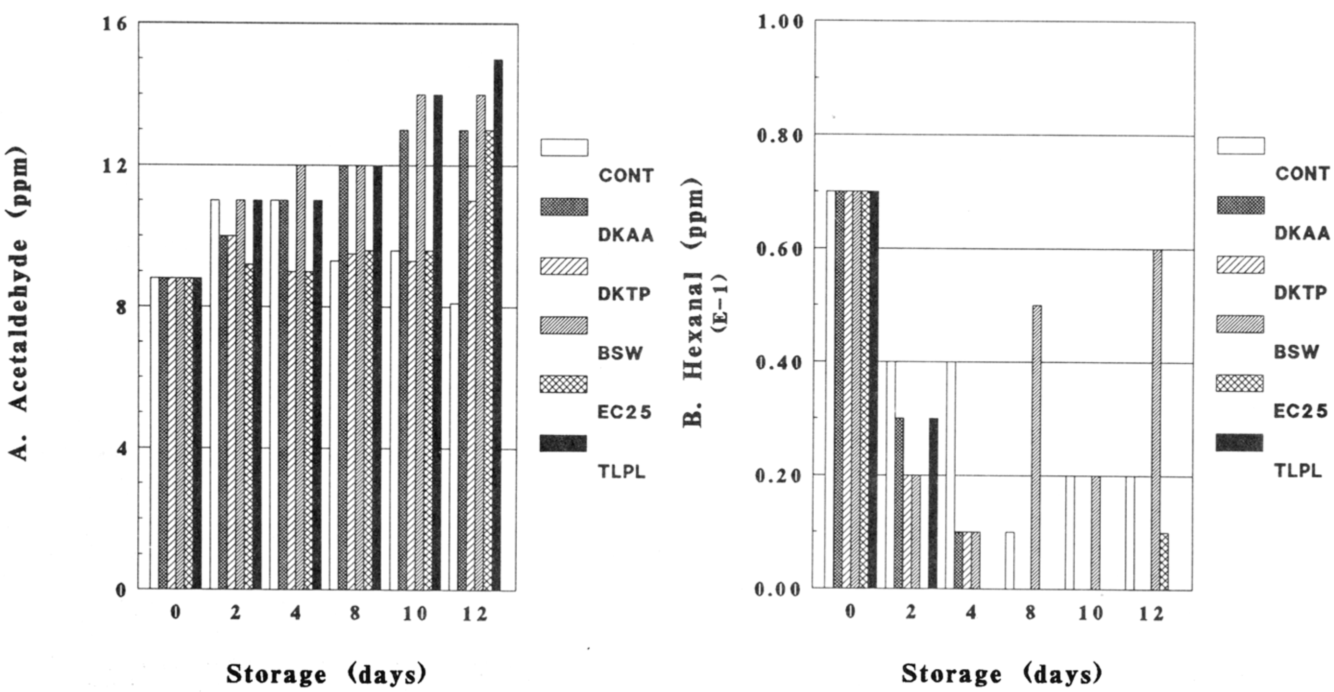

Figure 1. Changes in (A) acetaldehyde and (B) hexanal levels in juice from Pineapple oranges with various lipid and composite films during storage at 21 °C.

J. Agric. Food Chem., Vol. 38, No. 6, 1990 1383

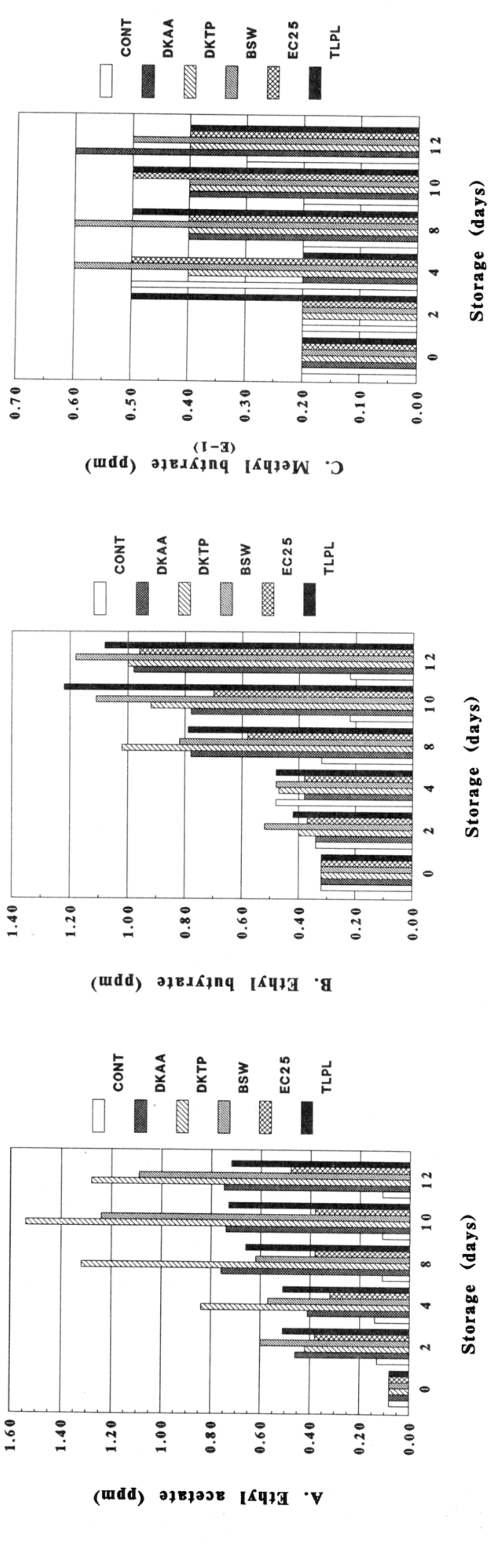

Figure 2. Changes in (A) ethyl acetate, (B) ethyl butyrate, and (C) methyl butyrate levels in juice from Pineapple oranges with various lipid and composite films during storage at 21 °C.

attractive gloss and preventing desiccation, color change, and incidence of peel injuries. Their effects on flavor, however, have not been fully investigated. Preservation or improvement of flavor is an important goal and should be thoroughly studied to evaluate the performance of the different postharvest storage techniques.

The present study was designed to determine the influence of five lipid and composite films on the volatile flavor components of Pineapple oranges during relatively short term storage at ambient temperature. Volatile components from juice of coated and uncoated oranges were identified and quantified, and the compositional changes during storage were also determined. These changes would be most significant when the fruit is consumed within a few days or is processed into juice products after several days of storage at ambient temperature.

MATERIALS AND METHODS

Fruits. Mature Pineapple oranges [*Citrus sinensis* (L.) Osbeck] obtained from a local grove were used in this study. After washing, fruits were carefully selected for uniformity in maturity, size, color, and absence of physical damage. Fruits were randomly divided into groups of 200 for the different treatments. The first group consisted of control (uncoated) samples. Treatments consisted of the different lipid and composite coatings described below.

figure legend	film
CONT	control, uncoated samples
DKAA	100 g of Durkex 500 and 20 g of EC-25 (both supplied by Durkee Industrial Foods Corp., Louisville, KY) were mixed at 70 °C. Durkex 500 is a partially hydrogenated vegetable oil, while EC25 is an emulsifier consisting of propylene glycol esters of fats and fatty acids. The hot lipid mixture was added to 500 mL of a 5% ascorbic acid palmitate solution at 70 °C in a beaker equipped with a stirrer. Stirring continued for 1 h after which time the solution was left to cool at ambient temperature.
DKTP	100 g of Durkex 500 and 20 g of EC25 were mixed at 70 °C. The hot lipid mixture was added to 500 mL of 2% TAL Pro-long (Courtaulds Group, London) and was prepared as described for DKAA.
BSW	100 g of beeswax, 20 g of ethanolamine, and 16 g of oleic acid were mixed at 70 °C. The hot lipid mixture was added to 500 mL of distilled water at 70 °C and was prepared as described for DKAA.
EC25	200 g of EC25 was dissolved at 70 °C and then left to cool at ambient temperature.
TLPL	10 g of TAL Pro-long was added to 500 mL of distilled water with sufficient agitation until a smooth suspension was formed.

These hydrophobic coatings are comparable to commercially available lipid-based coatings except for differences in permeability characteristics. All chemicals used in formulations unless mentioned otherwise were supplied by Sigma Chemical Co.

Fruit Coating and Storage. Pineapple oranges were coated with the prepared lipid or composite films by using a domestic paint brush. Fruits were allowed to dry at ambient temperature prior to storage at 21 °C. Control fruits were similarly stored at 21 °C.

Analyses of Volatile Flavor Components. During sampling and testing, 20 fruits were obtained from each group and the juice was hand-extracted by using a domestic mixer fitted with a reamer. The extracted juice was screened to separate the seeds and pulp from the juice. Two milliliters of juice was transferred to 10-mL vials equipped with crimp-top caps and seals. Volatile flavor components were determined by using a Perkin-Elmer Model 8500 gas chromatograph with a Model HS-6 headspace sampler and a flame ionization detector (FID). A

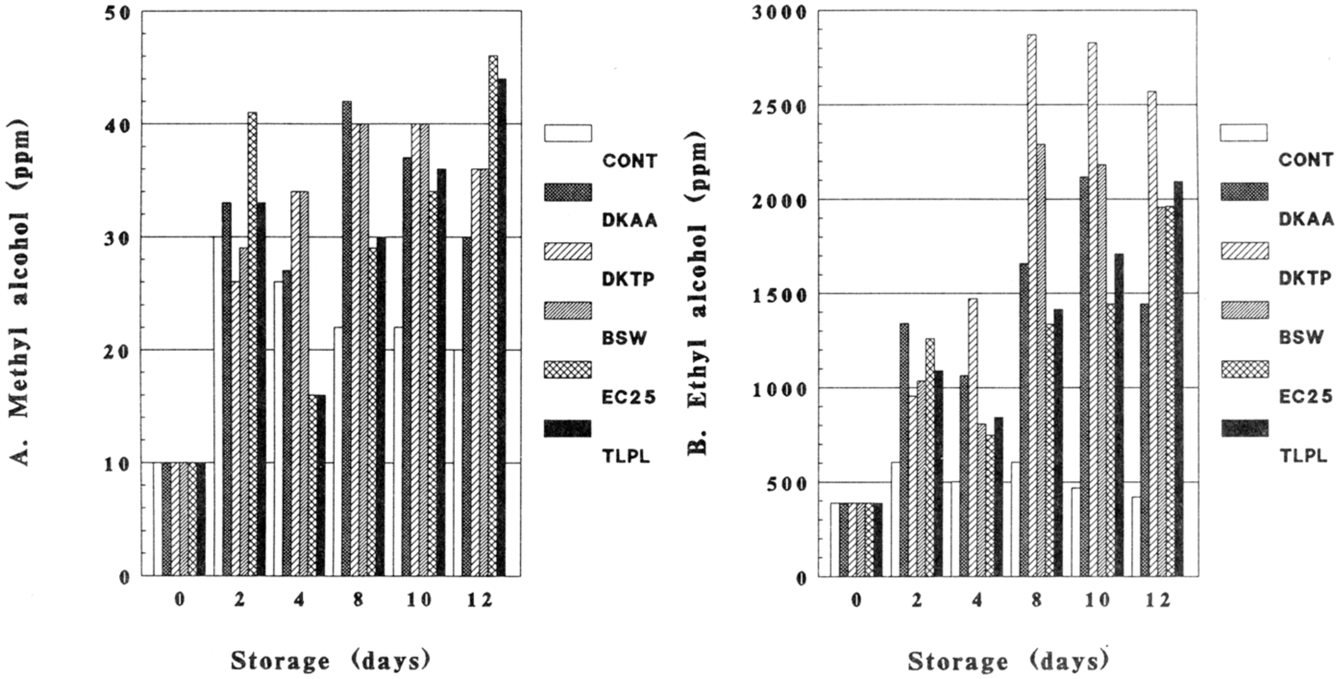

Figure 3. Changes in (A) methanol and (B) ethanol levels in juices from Pineapple oranges with various lipid and composite films during storage at 21 °C.

0.53 mm × 30 m polar Durowax column (1.0-μm film thickness) (J & W Scientific, Folson, CA) was used with 6.0 psi of helium head pressure (81 cm/s linear gas velocity). Juice samples were equilibrated in the headspace sampler for 15 min at 80 °C prior to injection. Injection parameters for the headspace sampler were 0.5-min vial pressurization time followed by 0.02-min injection time. These parameters were shown earlier to afford reproducible results with fresh orange juice (Nisperos-Carriedo and Shaw, 1990). Column oven temperature programming was 40 °C for 6 min, raised at 6 °C/min to 180 °C. The FID detector amplifier range setting was for high sensitivity, and the detector temperature was 250 °C. The different components were identified by comparison of retention times with those of standards and by enrichment of juice with authentic samples. Concentrations were calculated by using regression equations, determined by injection five different concentrations of each standard to obtain a peak height calibration curve. The standard solutions were prepared by addition of 4 μL of an aqueous ethanolic solution of standards to 2 mL of bland juice prepared by dilution of concentrated orange juice containing no added volatile components (evaporator pumpout) to 11.9° Brix. All determinations were done in triplicate.

Analysis of Internal CO_2 and O_2. Analysis of internal atmosphere was done by inserting a hypodermic needle at the stem end into the core of the fruit prior to immersion in water. Two milliliters of the internal atmosphere was withdrawn by holding the syringe for 10 s. A 0.5-mL sample was then injected and analyzed for CO_2 and O_2 on a gas chromatograph (Perkin-Elmer, 8500) equipped with Poropac S and molecular sieve columns and a thermal conductivity detector, operating at 10 mL/min with helium as carrier gas. Detector temperature was 80 °C. Gas levels were identified and quantified by comparison of retention times and peak areas with those of standards. Determinations were done on four fruits per treatment.

Statistical Analysis. Data for the different components were analyzed by Analysis of Variance using the General Linear Model (GLM) procedure, a package program of the Statistical Analysis System (SAS Institute Inc., Cary, NC). Experimental design involved a completely randomized design (CRD) with a split-plot treatment arrangement. Specific differences were determined by least significant difference (LSD). All comparisons were made at a 5% level of significance.

RESULTS AND DISCUSSION

Changes were observed in specific aldehyde, ester, alcohol, and hydrocarbon constituents of Pineapple oranges uncoated and coated with edible films after storage at 21 °C for up to 12 days.

Acetaldehyde is the major volatile aldehyde in orange juice (Kirchner and Miller, 1957) and accounts for most of the aldehyde increase (Figure 1A). This component increased by the second day of storage for control fruits but declined throughout the remaining storage period. For coated fruits, a progressive increase in this component was observed during storage except for film DKTP, where an increase was noted only during the 12th day of storage. Fruits coated with TAL Pro-long (TLPL) exhibited the highest increase in acetaldehyde, which occurred on the 12th day of storage. Acetaldehyde is considered an important contributor to fresh orange flavor (Boelens and van Gemert, 1987; Ahmed et al., 1978a; Bruemmer, 1975; Arctander, 1969). The other aldehyde quantified, hexanal, showed varying changes (Figure 1B). A decrease with prolonged storage was observed in the control fruits. The levels in the coated samples were generally lower except for those with the beeswax formulation (BSW), where the concentrations remained relatively high during the 8th and 10th days of storage. Hexanal is not considered important to fresh orange flavor, except for some possible contribution to a green flavor note (Arctander, 1969). Octanal was quantified in only a few samples, where it was found at ≤0.03 ppm. This component has been reported to be important to fresh orange flavor (Arctander, 1969; Boelens and van Gemert, 1987).

Volatile esters are important contributors to the fruity "top-note" of fresh orange juice, and any changes in their concentrations during storage could affect fruit flavor. The three esters quantified are known to contribute to the top-note of fruit flavors including citrus (Arctander, 1969), with ethyl butyrate being the major volatile ester in orange juices (Ahmed et al., 1978a). Ethyl acetate levels in control fruits did not change significantly during storage (Figure 2A). The application of films, however, increased the levels by 4.6–14 times, depending on the storage period. The highest increase was noted in fruits coated with formulation DKTP during the 10th day of storage. Ethyl butyrate increased in control fruits after 4 days of storage and then declined during the latter part

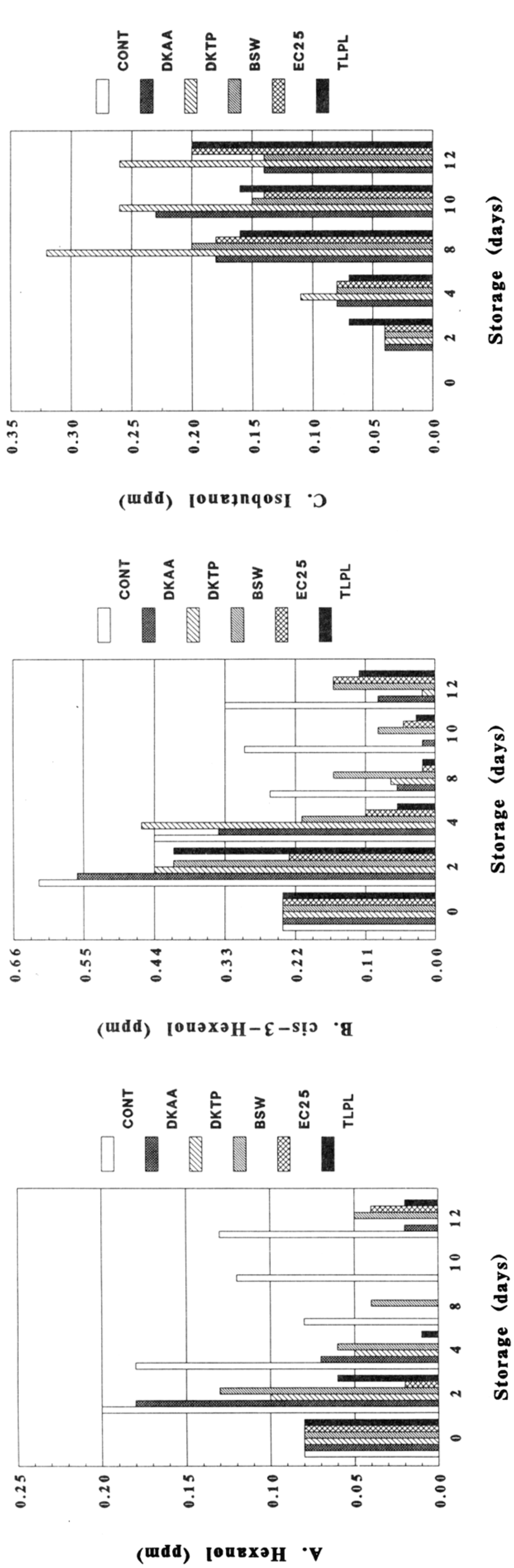

Figure 4. Changes in (A) hexanol, (B) *cis*-3-hexenol, and (C) isobutyl alcohol levels in juices from Pineapple oranges with various lipid and composite films during storage at 21 °C.

of the storage period (Figure 2B). The beeswax formulation (BSW) caused a significant increase in this component on the 2nd day, and the increase continued until the 12th day of storage. The highest level of ethyl butyrate was noted in fruit coated with TAL Pro-long (TLPL) and stored for 10 days. The films DKTP and BSW also caused great increases in this component, occurring mainly during 8 and 12 days of storage, respectively. The methyl butyrate level in coated fruits was basically unchanged after 4 days (Figure 2C) except for films EC25 and TLPL, where relatively lower levels were observed. Increases in this component were generally observed after 8–10 days when films were applied, especially when BSW was used.

Quantities of five alcohols were followed during storage. Most of these alcohols probably are not important to orange aroma except for *cis*-3-hexenol, which contributes to the green, leafy top-note in fresh orange flavor (Arctander, 1969). The two major alcohols present, methanol and ethanol, showed similar changes, but the changes in these two alcohols during storage of uncoated fruits were not significant (Figure 3). Coating the fruits caused increases in methanol by over 2-fold and in ethanol by up to 6-fold. EC25 and DKTP caused the highest increases in ethanol and methanol in fruits stored for 12 and 8 days, respectively. Levels of two other alcohols in the uncoated samples (hexanol and *cis*-3-hexenol) showed increases on the second day of storage followed by a decline on the eigth day and then an increase toward the end of the storage period (Figure 4A,B). For coated fruits, these two alcohols generally decreased after 2 days of storage. Isobutyl alcohol was not detected in uncoated fruits but was detected in levels varying from 0.04 to 0.32 ppm in stored coated fruits (Figure 4C). The level was highest in fruits coated with DKTP and stored for 4–12 days. *trans*-2-Hexenol was found in a few samples at ≤0.04 ppm, but was not detected in most samples.

Changes in five hydrocarbon components, limonene, valencene, α-pinene, sabinene, and γ-terpinene, were monitored during this study. Levels of limonene, the major volatile hydrocarbon present, were fairly constant in the control juice samples during storage (Figure 5A). There was a general decrease in limonene content in stored, coated samples, except for those with formulation DKTP. Some peel oil, which contains greater than 95% limonene, can be introduced into the juice during hand extraction of fruit (Rice et al., 1952) and add to the limonene content normally present from juice oil. Thus, any changes in limonene content should be considered trends rather than significant changes. Other peel oil hydrocarbons, α-pinene, sabinene, and γ-terpinene, showed variable changes with type of film and length of storage (data not presented). The fifth hydrocarbon quantified, valencene (Figure 5B), occurs mainly in juice oil rather than peel oil. Its amounts should not be affected by any peel oil introduced during juice extraction. Its level was highly variable also, and no consistent pattern was established for any treatment used. Thus, the hydrocarbons were the least useful group of components monitored for detecting effects due to film treatments.

A few of the changes noted in this study (increased acetaldehyde and/or ethanol) have been observed in oranges stored in low oxygen or high carbon dioxide atmospheres (Bruemmer and Roe, 1969) and in waxed fruit stored for 6 weeks (Davis, 1970). The films used in this study have simulated controlled atmosphere conditions, as evidenced by low oxygen and high carbon dioxide levels obtained in the internal atmosphere of the fruits stored for 10 days (Figure 6). The changes observed in the vol-

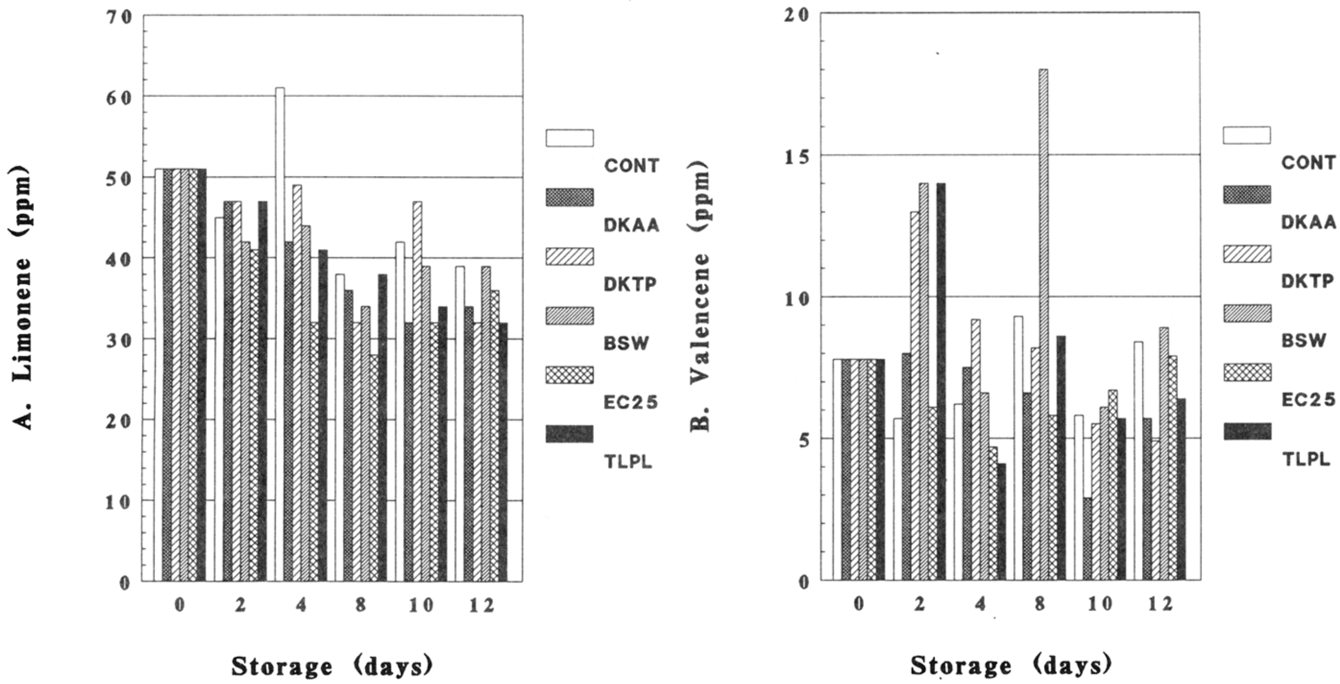

Figure 5. Changes in (A) limonene and (B) valencene in juices from Pineapple oranges with various lipid and composite films during storage at 21 °C.

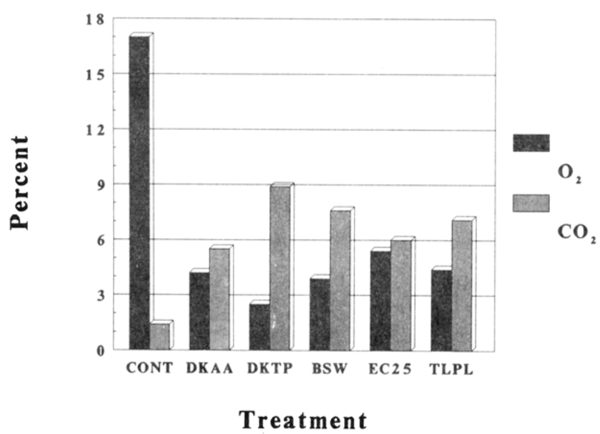

Figure 6. Comparison of the internal O_2 and CO_2 levels of Pineapple oranges with various lipid and composite films during storage for 8 days at 21 °C.

atile flavor components of the coated fruits probably resulted from the differential permeability of the coatings to O_2 and CO_2 which reduced the metabolic rate and altered metabolic pathways.

In conclusion, use of edible film coatings on oranges prior to storage at 21 °C generally increased the levels of the volatile components acetaldehyde, ethyl acetate, methyl butyrate, ethyl butyrate, methanol, and ethanol during storage at 21 °C for up to 12 days. Use of beeswax emulsion (BSW) and TAL Pro-long alone (TLPL) or in combination (DKTP) generally caused the greatest changes. The increases in the methyl and ethyl esters may be related to the increases in methanol and ethanol, since these alcohols are considered to be precursors of the corresponding esters in fruit (Croteau, 1979). Acetaldehyde, ethyl acetate, and methyl and ethyl butyrate are used in synthetic flavors to contribute to the fruity top-notes (Arctander, 1969); acetaldehyde and ethyl butyrate were shown also to be important to a good orange flavor in juice (Ahmed et al., 1978). Thus, increases in these four carbonyl compounds could add to the fruity top-notes of orange flavor. Whether these changes are large enough to affect significantly the flavor of stored, treated fruit has not been determined. However, in informal tasting during sampling periods by two laboratory personnel, no detectable off-flavors were noticed in any of the treated samples.

LITERATURE CITED

Ahmed, E. M.; Dennison, R. A.; Shaw, P. E. Effect of selected oil and essence volatile components on flavor quality of pumpout orange juice. *J. Agric. Food Chem.* **1978a**, *26*, 368–372.

Arctander, S. *Perfume and Flavor Chemicals*; Arctander: Montclair, NJ, 1969; Vol. I, II.

Banks, N. H. Some Effects of TAL Pro-long Coating on Ripening Bananas. *J. Exp. Bot.* **1984**, *35*, 127.

Banks, N. H. Internal Atmosphere Modification in Pro-long Coated Apples. *Acta Hortic.* **1985**, *157*, 105.

Boelens, M. H.; van Gemert, L. J. Organoleptic properties of aliphatic aldehydes. *Perfum. Flavor.* **1987**, *12*, 31–43.

Bruemmer, J. H. Aroma Substances of Citrus Fruits and Their Biogenesis. In *Symposium on Fragrance and Flavour Substances*; Drawert, F. Ed.; Verlag Hans Carl: Nurnberg, Germany, 1975; pp 167–176.

Bruemmer, J. H.; Roe, B. Post-harvest treatment of citrus fruit to increase Brix/acid ratio. *Proc. Fla. State Hortic. Soc.* **1969**, *82*, 212–215.

Croteau, R. Biogenesis of flavor components: volatile carbonyl compounds and monoterpenes. In *Postharvest Biology and Technology*; Hultin, H. O., Milner, M., Eds.; Food and Nutrition Press: Westport, CT, 1979; p 408.

Davis, P. L. Relation of Ethanol Content of Citrus Fruits to Maturity and to Storage Conditions. *Proc. Fla. State Hortic. Soc.* **1970**, *83*, 294–298.

Dhalla, R.; Hanson, S. W. Effect of Permeable Coatings on the Storage Life of Fruits. II. Pro-long Treatment of Mangoes (Mangifera indica L. cv. Julie). *Int. J. Food Sci. Technol.* **1988**, *23*, 107–112.

Grierson, W.; Ben-Yehoshua, S. Storage of citrus fruits. In *Fresh Citrus Fruits*; Wardowski, W. F., Nagy, S., Grierson, S., Eds.; AVI: Westport, CT, 1986; pp 479–501.

Kaplan, H. J. Washing, Waxing and Color-adding. In *Fresh Citrus Fruits*; Wardowski, W. F., Nagy, S., Grierson, W., Eds.; AVI: Westport, CT, 1986; p 379.

Kefford, J. F. The Chemical Constituents of Citrus Fruits. *Adv. Food Res.* **1959**, *9*, 285–372.

Kester, J. J.; Fennema, O. R. Edible Films and Coatings: A Review. *Food Technol.* **1986**, *40* (12), 47-59.
Kirchner, J. C.; Miller, J. M. Volatile Water-Soluble and Oil Constituents of Valencia Orange Juice. *J. Agric. Food Chem.* **1957**, *5*, 283.
Lakshminarayana, S.; Luis-Samimento, L.; Isabel-Ortiz, R. J.; Siade, G. Extension of Storage Life of Citrus Fruits by Application of Candelilla Wax Emulsions and Comparison of its Efficiency with Tag and Flavorseal. *Proc. Fla. State Hortic. Soc.* **1974**, *87*, 325-330.
Lawrence, J. F.; Iyengar, J. R. Determination of Paraffin Wax and Mineral Oil on Fresh Fruits and Vegetables by High Temperature Chromatography. *J. Food Saf.* **1983**, *5*, 119.
Motlagh, H. F.; Quantick, P. Effect of Permeable Coatings on the Storage Life of Fruits. I. Pro-long Treatment of Limes (Citrus aurantifolia cv. Persian). *Int. J. Food Sci. Technol.* **1988**, *23*, 99-105.
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.
Paredes-Lopez, O.; Camargo-Rubio, E.; Gallardo-Navarro, Y. Use of Coating of Candelilla Wax for the Preservation of Limes. *J. Sci. Food Agric.* **1974**, *25*, 1207-1210.
Rice, R. G.; Keller, G. J.; Beavens, E. A. Flavor fortification of California frozen orange concentrate. *Food Technol.* **1952**, *6*, 35-39.
Shaw, P. E. Essential Oils. In *Citrus Science and Technology*; Nagy, S., Shaw, P. E., Veldhuis, M. K., Eds.; AVI: Westport, CT, 1977; Vol. 1, pp 463-478.
Shaw, P. E. Review of Quantitative Analyses of Citrus Essential Oils. *J. Agric. Food Chem.* **1979**, *27*, 246-257.
Shaw, P. E. The Flavor of non-alcoholic fruit beverages. In *Food Flavours. Part B. The Flavour of Beverages*; Morton, I. D., Macleod, A. J., Eds.; Elsevier Science: Amsterdam, 1986; pp 337-368.
Smith, S. M.; Stow, J. R. The Potential of a Sucrose Ester Coating Material for Improving the Storage and Shelf-life Quantities of Cox's Orange Pippin Apples. *Ann. Appl. Biol.* **1984**, *104*, 383.
Ukai, N.; Ishibashi, S.; Tsutsumi, T.; Marakami, K. Preservation of Agricultural Products. U.S. Patent 3,997,674, 1976.

Received for review October 6, 1989. Accepted March 5, 1990. Mention of a trademark of proprietary product is for identification only and does not imply a warranty or guarantee of the product by the U.S. Department of Agriculture over other products which may also be suitable.

Registry No. Acetaldehyde, 75-07-0; hexanal, 66-25-1; ethyl acetate, 141-78-6; ethyl butyrate, 105-54-4; methyl butyrate, 623-42-7; methanol, 67-56-1; ethanol, 64-17-5; hexanol, 111-27-3; *cis*-3-hexenol, 928-96-1; isobutyl alcohol, 78-83-1; limonene, 138-86-3; valencene, 4630-07-3; octanal, 124-13-0; *trans*-2-hexenol, 928-95-0; carbon dioxide, 124-38-9; oxygen, 7782-44-7; EC-25, 125622-48-2; TAL Pro-long, 89468-99-5.

Immobilized Endo-β-glucosidase Enriches Flavor of Wine and Passion Fruit Juice

Oded Shoseyov,[‡,§] Ben Ami Bravdo,[*,‡] Dan Siegel,[‡] Alexander Goldman,[∥] Shlomo Cohen,[⊥] Lisa Shoseyov,[‡] and Raphael Ikan[#]

Department of Horticulture, Faculty of Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot, Israel, Department of Organic Chemistry, Faculty of Natural Sciences, The Hebrew University of Jerusalem, Givat Ram Jerusalem, Israel, and Israel Wine Institute, Rehovot, Israel

Aspergillus niger endo-β-glucosidase was immobilized to acrylic beads as well as to corn stover cellulose particles. The efficiency of the immobilization at pH 6.5 to the acrylic beads, with respect to activity, was higher than that to the cellulose but still low (10%). However, higher immobilization efficiency for cellulose (30%) was achieved at pH 4.5. The thermal stability of the enzyme was improved by its immobilization in both methods. Free and immobilized endo-β-glucosidases were used to treat Muscat Roy wine and passion fruit juice (pH 2.45). GC-MS analysis as well as sensory evaluation indicated a significant increase in flavor compounds, monoterpene alcohols, and linalool oxides in the wine and linalool, benzyl alcohol, and benzaldehyde in the passion fruit juice. The precursor of the benzaldehyde is a cyanogenic glycoside as evident from the evolution of cyanide followed by the enzymatic treatment.

Enzymatic hydrolysis of flavor precursors attracts many researchers (Schreier, 1988). Cordonnier and Bayonove (1974) were the first to suggest that must of Muscat of Alexandria (*Vitis vinifera* L.) contains nonvolatile (flavorless) forms of monoterpene glycosides. Williams et al. (1982) identified a series of glycosides, such as β-rutinosides (6-*O*-α-rhamnopyranosyl-β-D-glucopyranosides) and 6-*O*-α-arabinofuranosyl-β-D-glucopyranosides of monoterpene alcohols and β-glycosides of benzyl alcohol and 2-phenylethanol. The glycosides were found in must as well as in wine, indicating that there is no naturally occurring enzymatic hydrolysis during the fermentation that can hydrolyze the glycosides. The importance of the monoterpenes to must and wine flavor has been extensively reviewed (Marais, 1983; Rapp et al., 1984; Strauss et al., 1986). Many fruits, such as passion fruit (Engel and Tressl, 1988), prunes (Chet et al., 1986), apples

* To whom correspondence should be addressed.
‡ Department of Horticulture, The Hebrew University of Jerusalem.
§ Present address: Department of Biochemistry and Biophysics, University of California at Davis, Davis, CA 95616.
∥ Deceased 1988.
⊥ Israel Wine Institute.
Department of Organic Chemistry, The Hebrew University of Jerusalem.

0021-8561/90/1438-1387$02.50/0