

## Effects of Abscission Agents on Composition and Flavor of Cold-Pressed Orange Peel Oil

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Essential peel oils from Hamlin, Pineapple, and Valencia oranges treated with abscission agents were evaluated for flavor changes by comparison with oils from the untreated oranges. Fruit which had met minimum maturity standards (barely-mature) and well-mature oranges were studied. Each peel oil was added to a sample of high-quality "evaporator pumpout" containing no other flavoring fraction and was evaluated by a trained taste panel. Usually, the panel detected differences between oils from treated and control fruit and generally preferred the control oils. Seventeen predominant volatile constituents of the oils were isolated and identified. In the barely-mature fruit, quantitative differences in oil composition were observed and indicated an acceleration of maturity processes by the abscission agents; but in the well-mature fruit oil no marked quantitative differences were found. Oils from treated and control oranges did not differ qualitatively.

The most promising mechanical harvesting systems being tested require abscission-inducing chemicals to loosen the fruit. These compounds alter metabolic pathways within the fruit and thus could affect the flavor of the juices and peel oils. More than 90% of the 190 million boxes of oranges harvested annually in the United States are processed; and thus could be harvested mechanically. Although mechanical harvesting of citrus is at an experimental level, its anticipated commercial use suggests the need to assess any related effects on product quality. Thus, the effects of abscission chemicals on the chemical composition and/or flavor quality of expressed peel oil need to be evaluated.

In 1969 Cooper et al. reported on the potency of cycloheximide (Acti Aid) as an aid to abscission of citrus. Cooper and Henry (1973) found that as an abscission agent, cycloheximide could be used successfully for early- and mid-season fruit but not for late-season (Valencia) oranges because of excessive droppage of young fruit which the trees carry for the next season's crop. By 1974 two other abscission agents effective for citrus were developed and did not cause young Valencia oranges to drop. They were 5-chloro-3-methyl-4-nitro-1*H*-pyrazol (Release), reported by Wilson (1973) and Kenney et al. (1974), and glyoxal dioxime (Pik-Off), reported by Wilcox et al. (1974). The three agents damage the peel and the damage causes the release of ethylene, which promotes abscission. Ethephon, an abscission agent that does not injure rind, was also tested, and its effects on similar orange oils were compared with those of the above rind-injuring agents. These agents may cause other metabolic changes that could affect the qualitative and/or quantitative chemical composition or flavor quality of products obtained from treated fruit.

An earlier report (Moshonas et al., 1976) showed the effects of these agents on the flavor of processed juice. The current study was undertaken to evaluate the effects that abscission agents may have on the major volatile constituents and flavor quality of cold-pressed oils from early-, mid-, and late-season oranges at different stages of maturity. Although other abscission agents are being developed, those we used are the ones most commonly tested by the citrus industry.

### EXPERIMENTAL SECTION

**Sample Preparation.** Oranges treated with abscission chemicals and untreated control oranges were harvested for each evaluation from trees in the same area of the grove on similar rootstock that had been fertilized, sprayed, and irrigated the same way. The varieties were Hamlin, Pineapple, and Valencia. Oranges were thoroughly washed and processed with a commercial FMC In-Line Extractor. The crude oil emulsion that was extracted was put through a finisher with a 0.20-in. screen opening and a shaker screen (30 and 60 mesh) for removal of residual solid materials. The filtered emulsion was then placed in a holding tank for 4 h. The layers separated, and most of the water was removed. The oil-rich emulsion was then fed into a continuous-type centrifuge which was operated at about 30 000 rpm and which yielded the cold-pressed orange oil. The oil was stored at 0 °C until used.

**Quantitative and Qualitative Analysis.** Oil samples were analyzed on a Perkin-Elmer Model 900 gas chromatograph equipped with a thermal conductivity detector and connected to a computing integrator. Each sample (20  $\mu$ L) of cold-pressed orange oil was injected into a 0.10-in. i.d.  $\times$  20-ft column packed with 10% UCW-98 on 60-80 mesh Gas-Chrom P. Oven temperature was programmed from 90 to 110 °C at 1 °C/min, then to 230 °C at 4 °C/min with a helium flow of 30 mL/min. Injection temperature was 260 °C and detector temperature was 290 °C. After each oil analysis, the column was silanized with 5  $\mu$ L of Silyl 8 and held at 230 °C for 15 min so nonvolatile materials were removed and column resolution was restored (Shaw et al., 1971). Individual compounds were collected as they were eluted from the GLC and positively identified by comparison of infrared, mass spectra, and retention times with those for authentic samples.

**Flavor Evaluations.** Triangle and paired comparison tests were run as described by Boggs and Hanson (1949). In both tests, 12 trained panelists were each given two presentations. In the triangle tests, judges were asked to indicate which of the three samples had a different flavor. In the paired comparison tests they were asked to indicate the sample they preferred. For the preparation of each juice sample 296 g of 65°Brix mid-season orange pumpout, containing no added peel oil, essence, or other flavoring fractions, was placed in a 2-L beaker on a magnetic stirrer, and 326  $\mu$ L of peel oil was added dropwise. Stirring was continued for 15 min; then 1088 mL of water was added so the sample was brought to 12°Brix and contained 0.020 wt % oil by volume (w/v). Juice samples containing oil

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Table I. Flavor Evaluation of Cold-Pressed Oils from Early- (Hamlin), Mid- (Pineapple), and Late-Season (Valencia) Abscission Chemically Treated and Control Oranges

Harvest date	Abscission agent	Concentration of agent, ppm	Days on tree after spray	Flavor evaluation confidence level	
				Difference exptl vs. control	Preference for control
Hamlin oranges					
11-25-73	Acti Aid	20	7	0.01	0.05
01-04-74	Acti Aid	20	7	0.01	0.05
Pineapple oranges					
01-25-74	Acti Aid	20	6	0.001	0.05
03-21-74	Acti Aid	20	6	0.001	0.01
01-17-75	Acti Aid	10	6	0.01	0.01
03-11-75	Acti Aid	10	6	0.01	0.01
01-17-75	Ethephon	250	6	N.S. <sup>a</sup>	N.S. <sup>a</sup>
Valencia oranges					
03-28-74	Acti Aid	20	7	0.001	0.01
06-24-74	Acti Aid	20	7	0.01	0.01
04-02-75	Releases	250	5	0.001	0.01
06-03-75	Release	250	4	N.S. <sup>a</sup>	N.S. <sup>a</sup>
04-02-75	Pik-Off	300	5	0.05	0.05
06-03-75	Pik-Off	300	4	0.01	0.01

<sup>a</sup> Not significant at 0.05 confidence level or greater.

Table II. Quantitative Comparisons of Predominant Orange Peel Oil Volatiles Present in Abscission-Treated and Control Valencia Oranges that Were Barely-Mature

Compound	Identity	Concn in control, <sup>a</sup> %	Abscission agent and change in concn with treatment, <sup>b</sup> %		
			Acti Aid	Release	Pik-Off
$\alpha$ -Pinene	Ir, MS	0.44	N <sup>c</sup>	N	N
Myrcene	Ir, MS	2.8	N	N	N
<i>d</i> -Limonene	Ir, MS	95	+0.09	+0.09	-0.07
Linalool	Ir, MS	0.69	-32	-21	-10
Citronellal	Ir, MS	0.08	+20	+12	+12
Decanal	Ir, MS	0.51	+12	-7.7	-5.8
Neral	Ir, MS	0.08	+12	N	N
Geranial	Ir, MS	0.16	+5.9	N	+5.9
Perillaldehyde	Ir, MS	0.02	N	N	N
Dodecanal	Ir, MS	0.07	+25	+23	+23
$\beta$ -Elemene	Ir, rt	0.06	N	-17	-17
$\beta$ -Caryophellene					
+ $\beta$ -Copaene	Ir, rt	0.07	N	N	N
$\beta$ -Farnesene	Ir, rt	0.03	N	N	N
Valencene	Ir, rt	0.05	+33	+40	+40
$\beta$ -Sinensal	Ir, rt	0.07	N	N	N
$\alpha$ -Sinensal	Ir, rt	0.03	+33	+33	+20

<sup>a</sup> Based on total weight of the 17 predominant volatiles. <sup>b</sup> Change, based on the concentration of the particular constituent. <sup>c</sup> N = no change.

from experimental oranges sprayed with abscission agents were compared with those containing oil from control oranges of the same variety and maturity.

## RESULTS AND DISCUSSION

Results of the flavor evaluations are listed in Table I. Since Pineapple oranges require less Acti Aid than other varieties (Cooper and Henry, 1973), oil from fruit of this variety sprayed at half the normal level (10 ppm) was also evaluated. The table also lists types of abscission agents, levels used, and days the fruit were left on the tree after having been sprayed. In all but two evaluations, the trained taste panel distinguished a difference between experimental and control samples at a 95% confidence level or greater. Without exception, when a difference was detected, the panel preferred the juice with the control oil. Many panelists noted an overripe flavor in juice containing oil from treated fruit.

In one of the two tests that showed no difference or preference for either oil, the abscission chemical used was Ethephon. Ethephon is the only agent we have tested that breaks down chemically to release ethylene rather than injure the peel to cause the release of wound ethylene.

Thus, Ethephon might be expected to cause less metabolic and, therefore, less flavor changes than the rind-injuring agents. One major disadvantage of Ethephon as an abscission agent for citrus is that it causes excessive leaf drop. The other test that showed no difference between or preference for oil from control or treated fruit involved well-matured Valencia oranges and the use of 250 ppm Release. Since these tests were conducted, experimental field test data have shown that as much as 400 ppm or more Release may be needed to cause adequate loosening of well-matured Valencia for mechanical harvesting. Thus, the level used for this study may not have had a full effect on the flavor as was noted in the barely-mature Valencia sample.

Analyses revealed some quantitative differences between the predominant volatile constituents of the cold-pressed oils and their concentrations from treated and control oranges. Table II lists 17 predominant volatile compounds identified in oranges in the cold-pressed peel oils from treated and control Valencia oranges that were barely mature. Most of the values listed are the means of three analyses. Since response factors were not used, the values are estimates only, but adequate for comparison between

samples (Shaw and Coleman, 1974). The oils from all the chemically treated oranges contained less of the major terpene alcohol, linalool, and more of the aldehydes  $\alpha$ -sinensal, citronellal, and dodecanal and of the sesquiterpene hydrocarbon valencene than the oil from control fruit. An increase in  $\alpha$ -sinensal, which has an odor threshold in water of only 3.8 ppb (Ahmed and Dougherty, 1973), may be significant since its aroma resembles that of overripe citrus.

When control and treated oils from well-matured Valencia were compared, only minor quantitative differences in major constituents were observed. In quantitative composition the oils from control and treated, well-matured Valencia oranges were similar to the oil from the treated, barely-mature fruit. This finding suggests an acceleration of the maturing process when barely-mature oranges are sprayed with rind-injuring abscission agents, but not when well-matured fruit are similarly treated. Similar effects were observed in oils from early- and mid-season oranges.

The overripe flavor observed in oil from barely-mature oranges treated with rind-injuring abscission agents was probably not primarily due to an increase in  $\alpha$ -sinensal content since this flavor was also present in oils from well-mature treated fruit with the same concentration of  $\alpha$ -sinensal as the control oils. Other components must contribute to the overripe flavor, and their identities and flavor effects in juice and oil will have to be studied.

The flavor evaluations of this study were made only by a trained panel of expert tasters. One further important point that must be determined is whether the general consumer can detect a difference in juice flavor when oil

from treated fruit is used, and whether any difference that might be detected is objectionable.

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## Effects of Alkali on Glycoproteins. $\beta$ -Elimination and Nucleophilic Addition Reactions of Substituted Threonyl Residues of Antifreeze Glycoprotein

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A small molecular weight antifreeze glycoprotein (AFGP) was used as a model protein to study the effects of alkali on a glycoprotein containing glycosyl groups O-linked exclusively to threonyl residues. Further, this AFGP contains no phosphate, sulfhydryl, or disulfide groups. Base-catalyzed  $\beta$  elimination is involved in removal of the carbohydrate moieties from the protein resulting in formation of olefinic amino acid residues ( $\beta$ -methyldehydroalanine). The rate and extent of  $\beta$  elimination under various conditions of pH and temperature were monitored by absorbance at 241 nm or by loss of threonine or galactosamine. Initial rate data indicate that the  $\beta$ -elimination reaction is dependent on hydroxide ion concentration but not on AFGP concentration. Nucleophilic additions of sodium sulfite and *N* <sup>$\alpha$</sup> -acetyl-L-lysine to the unsaturated residues were studied. The addition products of *N* <sup>$\alpha$</sup> -acetyl-L-lysine were identified by periodate treatment and by combined GC/MS to be the D and L isomers of *N* <sup>$\alpha$</sup> -(1-methyl-2-amino-2-carboxyethyl)-L-lysine.

Glycoproteins serve a wide range of important biological functions and are widely distributed (Spiro, 1973). The two major types of bonds between the carbohydrate and the protein are easily distinguished by their different susceptibilities to alkaline and enzymatic cleavage, and a number of techniques for their identification have been developed (Downs and Pigman, 1976). When the car-

bohydrates are linked to the amide group of asparagine, the bond is relatively resistant to alkali. When the carbohydrates are attached by O-glycosidic linkages to seryl or threonyl residues, base-catalyzed  $\beta$  elimination is involved in the facile removal of carbohydrate chains from the protein core of the glycoprotein (Pigman and Moschera, 1973). Glycoproteins with base-labile O-glycosidic linkages occur mainly in animal protein sources (Spiro, 1973).

$\beta$  elimination of substituted seryl and threonyl residues has been described for a wide variety of complex proteins (Tanaka et al., 1964; Adams, 1965; Carubelli et al., 1965; Gottschalk, 1972; Simpson et al., 1972; Plantner and

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