Inhibition by Thiol Compounds of Off-Flavor Formation in Stored Orange Juice. 2. Effect of L-Cysteine and *N*-Acetyl-L-cysteine on *p*-Vinylguaiacol Formation†

Michael Naim,*,‡ Inbar Zuker,‡ Uri Zehavi,‡ and Russell L. Rouseff§

Department of Biochemistry, Food Science and Nutrition, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot 76-100, Israel, and Citrus Research and Education Center, University of Florida, Lake Alfred, Florida 33850

The formation of *p*-vinylguaiacol (PVG), a major off-flavor in citrus products, was reduced by 30–80% in model solutions of orange juice (MOJ) and in single-strength orange juice (SSOJ) fortified by L-cysteine and stored at 45 °C. The fortification by 2.5 mM *N*-acetyl-L-cysteine was also effective in reducing PVG content. Experiments with MOJ samples indicated that, concomitant with the inhibition of PVG formation due to the fortification by L-cysteine, vanillin production and ferulic acid degradation were accelerated. It is concluded that although off-flavors in citrus products such as PVG and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone are formed by different routes, their production may be reduced by a common approach, namely application of minor amounts of naturally occurring thiol compounds such as L-cysteine.

INTRODUCTION

In stored orange and grapefruit juices, *p*-vinylguaiacol (PVG) is a major detrimental off-flavor with a taste threshold of 0.075 ppm contributing "old fruit" or "rotten" flavor to the juice (Tatum et al., 1975). It has been proposed that PVG is formed in citrus products from free ferulic acid due to nonenzymic decarboxylation following release from bound forms (Peleg et al., 1988, 1992). PVG formation increased under practical storage conditions of orange juice (Naim et al., 1988; Lee and Nagy 1990), and this formation is accelerated if the juice is fortified by free ferulic acid, resulting in inferior aroma quality (Naim et al., 1988). Although ferulic acid occurs mainly in bound forms, the amount of free ferulic acid present in citrus fruit before processing exceeds the amount needed to form an above-taste threshold level of PVG during processing and storage (Peleg et al., 1991).

L-Cysteine inhibits heat-induced food browning (Arnold, 1969; Montgomery, 1983), and due to the undesired use of sodium sulfite (Brown, 1985; Taylor and Bush, 1986), natural thiols have recently been proposed to inhibit browning in fruit juices (Molnar-Perl and Friedman, 1990a,b). Cysteine itself is, however, a source for flavors in some foods (Hurrell, 1982) and may be objectional in fruit juice (Molnar-Perl and Friedman, 1990a). In the preceding paper (Naim et al., 1993) we have shown that fortification of orange juice during storage by small amounts of L-cysteine can effectively reduce browning, ascorbic acid degradation, and formation of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), which is known to be an undesired flavor in citrus products. Since free ferulic acid, the precursor of PVG, contains an activated double bond conjugated to a carboxylic acid and to a phenyl ring, we hypothesized that it may also be sensitive to nucleophilic attack by thiols, thereby inhibiting PVG formation. The present study was therefore undertaken to reveal the effects of L-cysteine and *N*-acetyl-L-cysteine on PVG formation during accelerated nonenzymic browning in both orange juice and model systems.

MATERIALS AND METHODS

Materials. L-Cysteine, *N*-acetyl-L-cysteine, and ferulic acid were purchased from Sigma. PVG was purchased from Lancaster Synthesis (U.K.). Vanillin was a product of BDH Chemicals. Commercial single-strength orange juice (SSOJ) was purchased from Rimon, Givaat Brener, Israel.

Preparation and Storage of Fortified Synthetic Mixtures of Orange Juice and SSOJ Samples. A model solution of orange juice (MOJ) of pH 3.8 was prepared according to the methods of Curl (1949) and Clegg (1966), with modifications (Peleg et al., 1992). MOJ solutions were modified by the addition of ferulic acid (10 mg/L) and were further fortified by either 2.5 or 8.25 mM L-cysteine. Twenty-milliliter aliquots of modified and nonmodified MOJ solutions were transferred to 20-mL vials, sealed, and stored at 45 °C for either 6 or 12 days. Pasteurized SSOJ (1-L bottles) was preserved with 500 ppm of sodium benzoate. SSOJ samples were fortified with 0.5, 1.0, and 2.5 mM L-cysteine or *N*-acetyl-L-cysteine. Forty-milliliter aliquots of SSOJ samples with and without added thiol compounds were transferred to 40-mL glass vials and stored at 45 °C for 14 days.

Chemical Analyses. Following the storage of MOJ samples, ferulic acid, PVG, and vanillin contents were extracted and analyzed according to the procedure of Peleg et al. (1992) by HPLC equipped with a LiChrospher 100 RP-18 column (5 μm, 250 mm × 4 mm, Merck) with a RP-18 precolumn (25 × 4 mm, Merck). The column was eluted isocratically with 1.5% acetic acid in water/methanol (70:30) at a flow rate of 1 mL/min, at room temperature. Each sample (20 μL) was injected twice. The separated chromatographic peaks were identified and quantified by HPLC equipped with a Chrom-A-Scope UV–visible rapid-scanning detector (Barspec, Rehovot, Israel) set for PVG at 256 nm. The detection threshold of PVG by this detector was 0.05 μg. Authentic samples of ferulic acid, PVG, and vanillin were used as external standards for identification and quantification of the separated chromatographic peaks. Frequently, internal standards were co-injected with samples.

Extraction of PVG from the incubated SSOJ samples was performed according to the method of Lee and Nagy (1990) with the following modifications: The incubated SSOJ samples were centrifuged (10 min, 10000*g*, 4 °C). Supernatant aliquots (10 mL) were applied to C-18 Sep-Pak cartridges (Waters Associates, Milford, MA) which had been preconditioned with methanol (3 mL) and water (5 mL). Cartridges were then washed with water

* Author to whom correspondence should be addressed.

† This research was supported by Grant I-1967-91 from BARD, The United States–Israel Binational Agricultural Research and Development Fund.

‡ The Hebrew University of Jerusalem.

§ University of Florida.

0021-8561/93/1441-1359$04.00/0

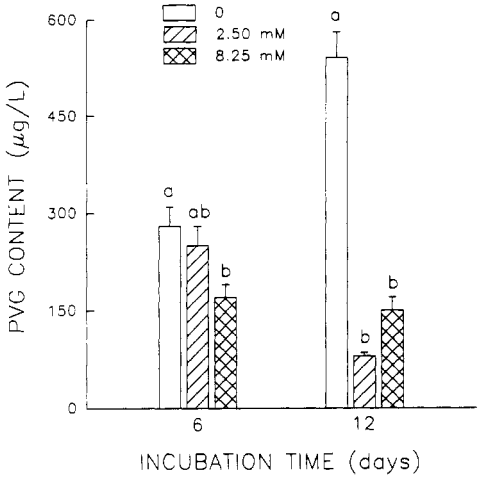


Figure 1. Inhibition of PVG formation from ferulic acid by L-cysteine in MOJ solutions stored for 6 and 12 days at 45 °C. Values are the mean and SEM of three samples, each analyzed twice by HPLC. Bars within each time period not sharing the same superscript letter are different at least at the $p < 0.05$ level.

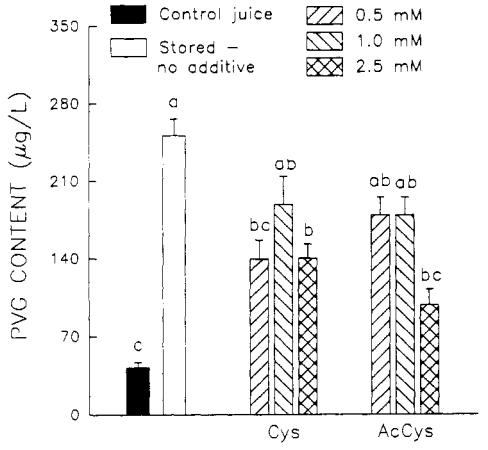


Figure 2. Effects of low doses of L-cysteine (Cys) and *N*-acetyl-L-cysteine (AcCys) on PVG formation in SSOJ samples stored for 14 days at 45 °C. Control juice was stored at 4 °C. Values are the mean and SEM of three samples, each analyzed three times by HPLC. Bars not sharing the same superscript letter are different at least at the $p < 0.05$ level.

(1 mL). Hexane (2 mL) was then sequentially used for elution of three Sep-Pak cartridges. The recovery yield of authentic PVG samples (0.25 μg) in this procedure was 89%. Samples were analyzed for PVG by HPLC as described above for MOJ samples except that the injected volume was 50 μL rather than 20 μL. Each sample was injected three times.

Data Analyses. Results were tested by one- and two-way analyses of variance using SAS statistical package programs. Duncan multiple-range test was performed for comparisons among the means.

RESULTS AND DISCUSSION

PVG Formation. The presence of L-cysteine significantly ($p < 0.01$) reduced the formation of PVG (RT in HPLC = 31.06 ± 0.13 min) from ferulic acid during storage of model solutions of orange juice (MOJ; Figure 1) and reduced PVG content in stored orange juice kept at 45 °C (Figure 2). When a higher range of L-cysteine concentrations was used in MOJ solutions (Figure 1), the effect of cysteine was clearly dose-dependent. It is pertinent to note that these levels of L-cysteine were recently shown to reduce browning in stored SSOJ samples (Molnar-Perl and Friedman, 1990a; Naim et al., 1993). Although high doses of L-cysteine may, in themselves, produce off-flavors

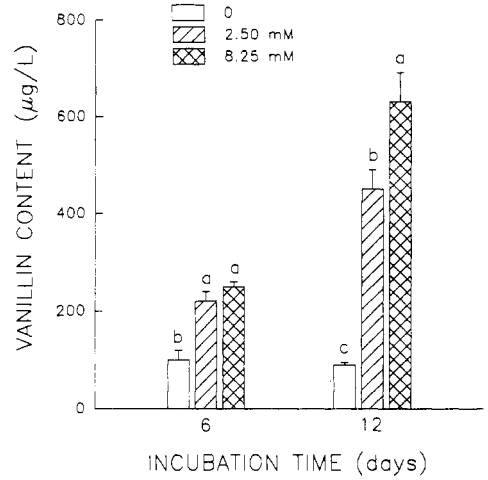


Figure 3. Effect of L-cysteine on vanillin formation in MOJ solutions stored for 6 and 12 days at 45 °C. Values are the mean and SEM of three samples, each analyzed twice by HPLC. Bars within each time period not sharing the same superscript letter are different at least at the $p < 0.05$ level.

(Molnar-Perl and Friedman, 1990a), our recent sensory experiments (Naim et al., 1993) indicated that L-cysteine at a concentration of 0.5 mM when added to stored SSOJ was able either to retain the aroma of original SSOJ juice or to reduce off-flavor formation during storage. Indeed, here we show that fortification of SSOJ by 0.5 mM L-cysteine (Figure 2) reduced the PVG accumulation following storage by 50%, close to its taste threshold level. *N*-Acetyl-L-cysteine (2.5 mM) was also effective in reducing PVG level in stored SSOJ. Thus, our studies with juice and model systems (Naim et al., 1993; and present results) suggest that under present experimental conditions the formation of two major off-flavors in citrus products, PVG and DMHF, can be significantly reduced by low doses (0.5 mM) of L-cysteine, an amino acid found in almost all proteins. These results call for further consideration of the practical use of L-cysteine as an inhibitor of off-flavor formation in citrus products. It is proposed that although PVG and DMHF are formed by different pathways, objectional aroma may be reduced by a common approach—using naturally occurring thiols such as L-cysteine.

Vanillin Formation. The exact mechanism by which thiol compounds reduced PVG formation is not clear. MOJ experiments (Figure 3) indicated that, concomitantly with the inhibition of PVG formation due to the addition of L-cysteine, vanillin production (RT in HPLC = 7.4 ± 0.04 min) was significantly accelerated. The formation of vanillin in stored orange juice has been recently reported (Martin et al., 1991). We (Peleg et al., 1992) proposed that vanillin is probably produced directly from ferulic acid (retro-aldol) rather than via the oxidation of PVG. Thus, rather than being decarboxylated to PVG, part of the ferulic acid was converted to vanillin, and this shift in ferulic acid degradation pathways was stimulated due to the fortification by L-cysteine.

Ferulic Acid Degradation. The fortification of MOJ by L-cysteine accelerated the degradation of free ferulic acid ($p < 0.05$, RT in HPLC = 11.1 ± 0.09 min) (Figure 4). When a thiol compound is present, a retro-aldol mechanism of converting ferulic acid to vanillin, as opposed to decarboxylation of ferulic acid to PVG, may become easier since the nucleophilic attack by the RSH group (Figure 5) is by far more efficient than that of water (Peleg et al., 1992). The obtained vanillin may then be further stabilized by the presence of RSH (a scavenger of free radicals). Thus, the presence of a thiol group may, on the

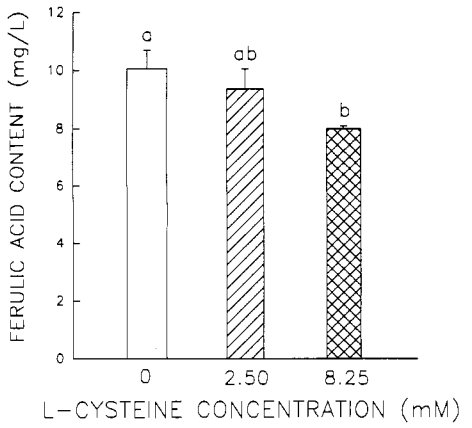
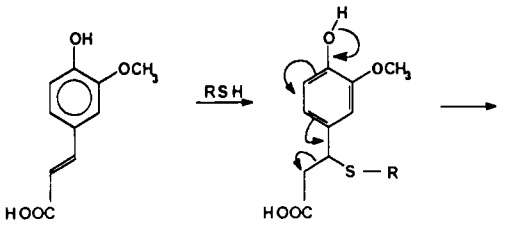
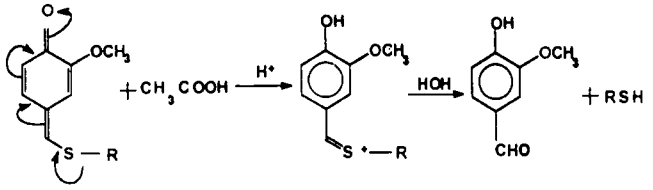


Figure 4. Degradation of ferulic acid in MOJ solutions fortified with L-cysteine and stored for 14 days at 45 °C. Values are the mean and SEM of three samples, each analyzed twice by HPLC. Bars not sharing the same superscript letter are different at least at the $p < 0.05$ level.



FERULIC ACID



VANILLIN

Figure 5. Possible pathway for L-cysteine-stimulated vanillin formation from ferulic acid.

one hand, reduce the extent of ferulic acid conversion to PVG and, on the other, increase vanillin formation. The formation of vanillin which is stimulated by cysteine should have little effect on citrus flavor profile since the taste-threshold level for vanillin (100 ppm; Amerine et al., 1965) is much greater than that expected to occur due to the fortification by a small amount of L-cysteine.

ACKNOWLEDGMENT

We thank Dr. H. Peleg and Ms. S. Wainish for helpful comments. We also thank Dr. H. Voet for statistical advice and Dr. S. Nagy for his encouragement. We greatly appreciate the excellent technical assistance of Mrs. M. Levinson and Ms. O. Schutz.

LITERATURE CITED

Amerine, M. A.; Pangborn, R. M.; Roessler, E. B. *Principles of sensory evaluation of food*; Academic Press: New York, 1965; p 109.

Arnold, R. G. Inhibition of heated-induced browning of milk by L-cysteine. *J. Dairy Sci.* **1969**, *52*, 1857–1859.

Brown, J. L. Sulfite sensitivity. *J. Am. Med. Assoc.* **1985**, *254*, 825.

Clegg, K. M. Citric acid and the browning of solutions containing ascorbic acid. *J. Sci. Food Agric.* **1966**, *17*, 546–549.

Curl, A. L. Ascorbic acid losses and darkening on storage at 49 °C (120 °F) of synthetic mixtures analogous to orange juice. *J. Food Sci.* **1949**, *14*, 9–14.

Hurrell, R. F. Maillard reaction in flavour. In *Food Flavors Part A, Introduction*; Morton, I. D., Macload, A. J., Eds.; Elsevier Publishing: Amsterdam, 1982; pp 399–437.

Lee, H. S.; Nagy, S. Formation of 4-vinyl guaiacol in adversely stored orange juice as measured by an improved HPLC method. *J. Food Sci.* **1990**, *55*, 162–163, 166.

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

Molnar-Perl, I.; Friedman, M. Inhibition of browning by sulfur amino acids. 2. Fruit juices and protein-containing foods. *J. Agric. Food Chem.* **1990a**, *38*, 1648–1651.

Molnar-Perl, I.; Friedman, M. Inhibition of browning by sulfur amino acids. 3. Apples and potatoes. *J. Agric. Food Chem.* **1990b**, *38*, 1652–1656.

Montgomery, M. W. Cysteine as an inhibitor of browning in pear juice concentrate. *J. Food Sci.* **1983**, *48*, 951–952.

Naim, M.; Striem, B. J.; Kanner, J.; Peleg, H. Potential of ferulic acid as a precursor to off-flavors in stored orange juice. *J. Food Sci.* **1988**, *53*, 500–503, 512.

Naim, M.; Wainish, S.; Zehavi, U.; Peleg, H.; Rouseff, R. L.; Nagy, S. Inhibition by thiol compounds of off-flavor formation in stored orange juice. 1. Effect of L-cysteine and N-acetyl-L-cysteine on 2,5-dimethyl-4-hydroxy-3(2H)-furanone formation. *J. Agric. Food Chem.* **1993**, preceding paper in this issue.

Peleg, H.; Striem, B. J.; Naim, M.; Zehavi, U. Phenolic compounds contributing to off-flavors in citrus products. In *Proceedings, International Citrus Congress*; Goren, R., Mendel, K., Eds.; Balaban: Rehovot, 1988; Vol. 4, pp 1743–1748.

Peleg, H.; Naim, M.; Rouseff, R. L.; Zehavi, U. Distribution of bound and free phenolic acids in oranges (*Citrus sinensis*) and grapefruits (*Citrus paradisi*). *J. Sci. Food Agric.* **1991**, *57*, 417–426.

Peleg, H.; Naim, M.; Zehavi, U.; Rouseff, R. L.; Nagy, S. Pathways of 4-vinylguaiacol formation from ferulic acid in model solutions of orange juice. *J. Agric. Food Chem.* **1992**, *40*, 764–767.

Tatum, J. H.; Nagy, S.; Berry, R. E. Degradation products formed in canned single-strength orange juice during storage. *J. Food Sci.* **1975**, *40*, 707–709.

Taylor, S. L.; Bush, R. K. Sulfites as food ingredients. *Food Technol.* **1986**, *40*, 47–52.

Received for review January 11, 1993. Accepted May 24, 1993.