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Effects of Orange Juice Fortification with Thiols on *p*-Vinylguaiacol Formation, Ascorbic-Acid Degradation, Browning, and Acceptance during Pasteurization and Storage under Moderate Conditions

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Pasteurization of orange juice produced sub-taste-threshold levels of *p*-vinylguaiacol (PVG) and induced ascorbic-acid degradation but had almost no effect on browning. Fortification with glutathione, L-cysteine, or *N*-acetyl-L-cysteine at concentrations below 4.0 mM had no effect on PVG formation and browning but inhibited ascorbic-acid degradation during pasteurization and improved juice acceptance. Storing the orange juice for 12 weeks at 25 °C resulted in a PVG content that reached its taste threshold; fortification with the above concentrations of thiols reduced PVG formation and browning. Storage at 35 °C resulted in a PVG level about 10-fold above its taste threshold, 25% degradation of ascorbic acid, and significant browning. Fortification with thiols at concentrations below 4 mM reduced PVG formation, ascorbic-acid degradation, and browning. Sensory evaluation tests indicated increased hedonic scores due to thiol fortification during pasteurization and storage (though not statistically significant for the latter), and aroma-similarity tests performed on juice stored at 35 °C indicated that fortification with 1.0 mM glutathione results in an aroma similar to that of the control juice stored at 4 °C.

Keywords: *Orange juice; glutathione; L-cysteine; N-acetyl-L-cysteine; p-vinylguaiacol (PVG); ascorbic acid; browning; acceptance*

INTRODUCTION

Thiol compounds have been proposed to inhibit nonenzymic browning in fruit juices (Molnar Perl and Friedman, 1990) and off-flavor formation in citrus juice (Naim et al., 1993b). Past suggestions for using L-cysteine to inhibit nonenzymic browning (Arnold, 1969; Montgomery, 1983) did not attract further research as cysteine is a flavor source in some foods (Hurell, 1982; Shu et al., 1985) which can be objectionable in fruit juice (Molnar Perl and Friedman, 1990). Nevertheless, some thiol compounds are natural components of human diets and play significant physiological roles in vivo as nucleophiles and scavengers of free radicals (Friedman, 1991). As such, they may be appropriate additives for preventing the deterioration of fruit juices.

In stored canned orange juice, *p*-vinylguaiacol (PVG), α-terpineol, and 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone (furaneol, DMHF) have been proposed to be the principal detrimental, off-flavor compounds (Tatum et al., 1975), which reach their taste-threshold levels under typical processing and storage conditions (Handwerk and Coleman, 1988). Of these three compounds, PVG is considered to be the most detrimental. PVG has been proposed to be formed in citrus products from free ferulic acid due to nonenzymic decarboxylation following the ferulic acid's release from bound forms (Peleg et al., 1988, 1992). Although ferulic acid occurs mainly in bound form, 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). PVG formation was found to increase under practical storage conditions for orange juice (Naim et al., 1988; Lee and Nagy, 1990), and this formation was accelerated when the juice was fortified with free ferulic acid, resulting in inferior aroma quality (Naim et al., 1988).

Accelerated storage of citrus juice under laboratory conditions (up to 14 days at 45 °C) has shown that fortification with L-cysteine, and to a lesser extent with *N*-acetyl-L-cysteine (at concentrations below 5.0 mM), reduces DMHF and PVG content, as well as ascorbic-acid degradation in orange juice without significant production of sulfur-containing off-flavors (Naim et al., 1993a.b). The present study was designed to further investigate the effect of three thiols (L-cysteine, *N*-acetyl-L-cysteine, and glutathione) on PVG formation, ascorbic-acid degradation, browning, and acceptance of orange juice pasteurized in a pilot plant and stored under practical conditions designed to represent those in the marketplace. Three main experiments were performed. In the first, commercial orange juice was used to determine the never before investigated effects of glutathione fortification on off-flavor formation. In the second and third experiments, fresh orange juice was used to investigate the effect of fortification with low concentrations of the three thiols during pasteurization and commercially simulated storage conditions.

MATERIALS AND METHODS

Materials. L-Cysteine, *N*-acetyl-L-cysteine, and reduced glutathione were purchased from Sigma Chemical Co. (St. Louis, MO). PVG was purchased from Lancaster Synthesis (U.K.). Commercial single-strength orange juice (SSOJ) (11.5–11.8 °Bx; acidity, 0.81–0.85%, pH, 3.50–3.52) was purchased from Rimon, Givaat Brenner, Israel. Fresh juice for the pasteurization experiment (12.1 °Bx; acidity, 0.79%; pH, 3.57) and for the storage experiment under simulated industrial

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conditions (13.0 °Bx; acidity, 0.91%; pH, 3.44) consisted of Shamuti orange juice from Prigat, Givaat Haim, Israel.

Preparation and storage of SSOJ samples under accelerated conditions: Commercial SSOJ (1-L bottles) was preserved with 500 ppm sodium benzoate. SSOJ samples (50 mL) fortified with either 1.0, 2.0, 3.0, 4.0, or 7.0 mM glutathione; with either 2.5, 5.0, 10.0, 15.0, or 25.0 mM *N*-acetyl-L-cysteine; with 0.5 mM L-cysteine; and without addition of any thiol were transferred to 64-mL glass vials and sealed with a septum. Nitrogen was bubbled for 1 min via a small needle inserted into the sealed vials equipped also with another needle as an outlet. Sealed vials were then stored for 2 weeks at 45 °C. Control (unadulterated) samples were stored at 4 °C.

Pasteurization Experiments. Fresh orange juice was fortified with either L-cysteine (0.5 or 1.0 mM), *N*-acetyl-L-cysteine, or glutathione (both at 2.0, 3.0 or 4.0 mM), pasteurized in a pilot plant (90−92 °C, 30 s), hot filled into 250-mL glass bottles, and immediately cooled to room temperature in a water bath (200-fold volume). These samples were kept at −18 °C and then subjected to chemical analyses with no prior storage.

Storage under Simulated Commercial Conditions. Fresh orange juice was fortified with either L-cysteine (0.5, 0.75 mM), *N*-acetyl-L-cysteine (2.5, 3.5 mM), or glutathione (1.0, 1.5 mM), pasteurized (95 °C, 36 s), hot filled into 250-mL glass bottles, and immediately cooled to room temperature in a water bath (200-fold volume). Bottles were then stored for 12 weeks at 25 or 35 °C. Control samples were stored at 4 °C.

Chemical Analyses. Brix (°Bx) was measured by a refractometer (PR-100 Atago), and acidity was determined according to Ting and Rouseff (1986). Resultant sterility after pasteurization was verified according to Hays (1951) and Murdock and Folinazzo (1952) with the following modifications: 10 mL of juice were added to 90 mL of enrichment media (orange serum broth, Difco, Detroit, MI) and incubated for 48 h at 30 °C. A large loopful of the enrichment culture was then plated on orange serum agar and incubated for 48 h at 30 °C. No observable growth indicated that the culture was negative.

PVG extraction from orange-juice samples and concentration determinations were performed according to Lee and Nagy (1990) with the following modifications: juice samples were centrifuged (10 min, 14000*g*, 4 °C). Supernatant aliquots (1 mL) were applied to C-18 Sep-Pak cartridges (Waters Associates, Medford, MA) pre-conditioned with 3 mL of methanol and 5 mL of water. Cartridges were then washed with 1 mL of water followed by 2 mL of hexane. The hexane fraction was filtered before being applied to the HPLC equipped with a Lichrosorb RP-18 column (7 μm, 250 mm × 4 mm, Merck) with a Lichrosphere 100 RP-18 precolumn (25 mm × 4 mm, Merck). PVG was analyzed and quantified according to Lee and Nagy (1990). A 100-μL aliquot of each extract was injected, and the column was eluted isocratically with 1.5% acetic acid in water/methanol (60:40 v/v) at a flow rate of 1 mL/min, at room temperature. The separated chromatographic peaks were identified and quantified by F-1050 Merck-Hitachi fluorescent detector (excitation 300 nm, emission 340 nm). PVG could be clearly detected at concentrations of 1 ppb and above (RT = 14.45 ± 0.25 min). Authentic samples of PVG were used as external standards for identification and to obtain calibration curves during quantification of the separated chromatographic peak. The recovered yield of authentic PVG sample (0.25 μg) via this procedure was 89%.

Browning was determined in 10 mL of stored SSOJ after centrifugation (4 °C, 10 min, 12000*g*) and ethanol extraction of the supernatant based on the procedure by Meydav and Berk (1978). The absorbance (OD) at 420 nm was measured. Ascorbic-acid content was determined potentiometrically (Spaeth et al., 1962). The following ascorbic-acid concentrations were used for the calibration curve: 230, 250, 300, 330, 350, 370, and 400 mg/L, resulting in $R^2 = 0.97$, indicating high correlation between ascorbic-acid concentration and the potentiometric titration. The possiblity that the presence of the three thiols added to the juice could affect the ascorbic-acid determination in the potentiometric method was tested on untreated juice samples and samples fortified either by 1.0, 2.0, or 3.0 mM cysteine, by 2.0, 4.0, or 8.0 mM glutathione, or by 5.0, 10.0, or 25.0 mM *N*-acetyl-L-cysteine. The content of ascorbic acid in the control juice and juice samples fortified by the indicated concentrations of the three thiols varied between 410 and 430 mg/L regardless of the thiol type or the concentration. The general mean ± SEM value of the tested samples was 421 ± 1.7, indicating that the added thiols had no effect on the potentiometric procedure under the experimental conditions. L-Cysteine, *N*-acetyl-L-cysteine, and reduced glutathione were determined in the fortified juices according to Ellman (1959) with slight modifications. Juice samples were centrifuged for 10 min (4 °C, 14000*g*). Phosphate buffer (10 mL) was added to 0.05−1 mL juice supernatant, and the solution was adjusted to pH 8.3 with 1.0 N NaOH. Then 1 mL of 0.05 N Ellman reagent was added and the absorbance at 412 nm was determined. An unadulterated juice sample was used as a reference.

Sensory Analysis. Because L-cysteine may contribute to the food's flavor, its odor threshold was determined in commercial orange juice fortified with different concentrations of L-cysteine using the forced-choice, up−down procedure (Bartoshuk, 1978).

The significance of thiols to juice taste or aroma acceptance was evaluated by hedonic tests (1−9 scale, 1 for very aversive, 9 for very appealing, and other ratings between) were conducted (Moskowitz, 1977) using untrained, 25−35-year-old male and female subjects.

In addition, aroma characteristics were determined in fortified and unadulterated juice samples after storage using aroma similarity-cluster analysis (Everitt, 1974; Sattath and Tversky, 1977; Naim et al., 1993a). Briefly, nine students, 20−25 years of age, were trained to evaluate the aroma similarity of eight orange-juice treatments. Vials containing the various juice samples were wrapped in aluminum foil so that panelists could not see the color of the tested samples, which were brought to room temperature prior to each aroma test. During a 2-h aroma session, four 15-min aroma similarity tests were conducted. In each test, on a verbal signal from the experimenter, a panelist opened the vials (after shaking) of two samples at 15-s intervals, smelled them, and was requested to rate the similarity level of their aromas on a scale of 1 to 20 (1 for no similarity, 20 for identical). Eight sample pairs were presented in coded, randomized order per test, resulting in 32 pairs tested during each aroma session. Two sessions were conducted with at least 4 h between, so that each panelist rated the similarity levels of 64 treatment combinations.

Data Analyses. Results of chemical analyses and sensory hedonic tests were tested by one- or two-way analyses of variance using SAS-statistical-package programs. A Duncan multiple range test was performed for comparisons among the means. When SEM values differed among treatments, variance was determined on the natural logarithm of the raw data.

A data matrix representing the aroma similarity results was obtained, in which each cell in the matrix represented the mean similarity for all panelists for the corresponding comparison. This proximity matrix was then analyzed by the clustering program ADDTREE (Sattath and Tversky, 1977), yielding a tree structure of branches and subdivisions with aromas located at the ends of the branches. Sets of aromas connected to the same node at relatively short (horizontal) distances from each other were highly similar. The same data were also analyzed by a multidimensional scaling (MDS) procedure (Schiffman et al., 1981).

RESULTS AND DISCUSSION

Effects of Glutathione on PVG Formation, Ascorbic-Acid Degradation, Browning, and Aroma in SSOJ Stored under Accelerated Conditions. Previous findings indicated that fortification of SSOJ with L-cysteine or *N*-acetyl-L-cysteine reduced PVG formation (Naim et al., 1993b). Unadulterated SSOJ stored at 4 °C (used as a control) contained 34 μg/L PVG, a level below the reported 50 μg/L sensory threshold of this compound (Tatum et al., 1975). Storage of SSOJ for 14

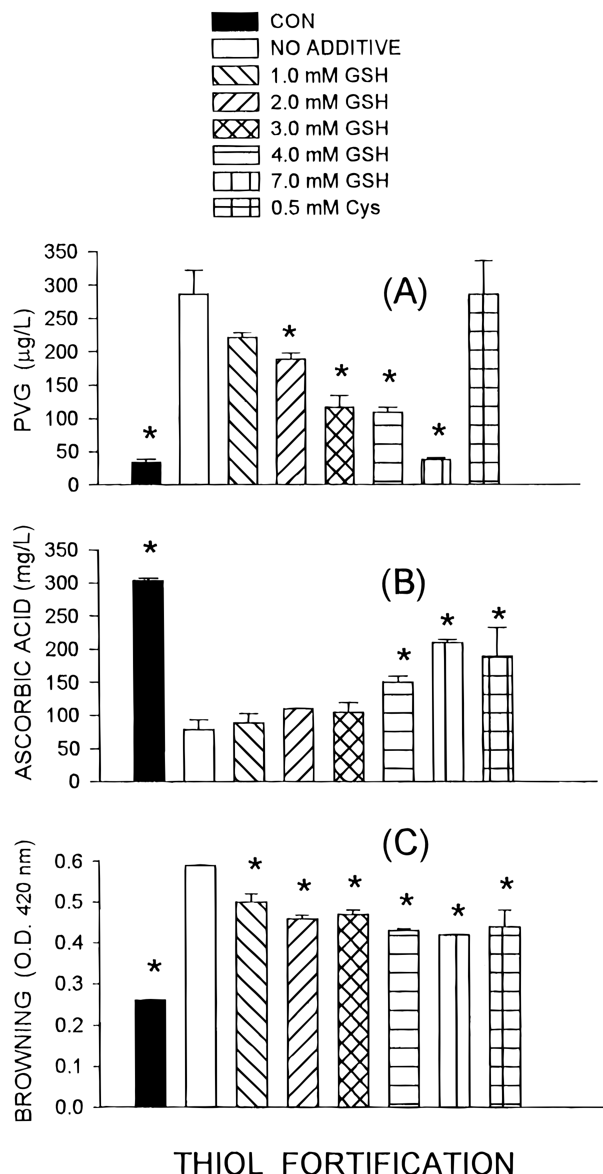

Figure 1. Effect of glutathione (GSH) fortification on PVG formation (A), ascorbic acid degradation (B), and browning (OD 420 nm) (C) of SSOJ stored at 45 °C for 2 weeks. Values are the mean and SEM of three replicates, each tested twice by HPLC (for PVG), potentiometry (for ascorbic acid), and spectrophotometry (for browning). Unadulterated control (CON) juice was kept at 4 °C. L-Cysteine was used as a reference. * indicates values which are significantly ($p < 0.05$) different from those of the unadulterated (no additive) stored juice.

days at 45 °C increased the PVG level to five times its threshold (Figure 1A), a result which was almost identical to those obtained previously (Naim et al., 1993b). Although fortification with 0.5 mM L-cysteine (used here as a reference) previously inhibited PVG formation (Naim et al., 1993b), this concentration was shown to be ineffective in present experiments. Glutathione, never tested before, inhibited PVG formation during storage in a concentration-dependent manner. The fact that the pasteurized juice already contained some PVG before storage led us to conclude that fortification with 7.0 mM glutathione abolishes PVG formation under accelerated storage conditions (Figure 1A). Glutathione probably acts like cysteine, i.e., by shifting ferulic-acid degradation to vanillin formation through a retro-aldol reaction, rather than being decarboxylated to PVG (Peleg et al., 1992; Naim et al., 1993b). In the presence of a thiol (RSH), the conversion of ferulic acid to vanillin, as opposed to decarboxylation, is expected to be easier since the nucleophilic attack by the RSH group is by far more efficient than that by water. The levels of vanillin formation under these conditions (Naim et al., 1993b) are expected to be much below this compound's high taste threshold (100 ppm) (Amerine et al., 1965).

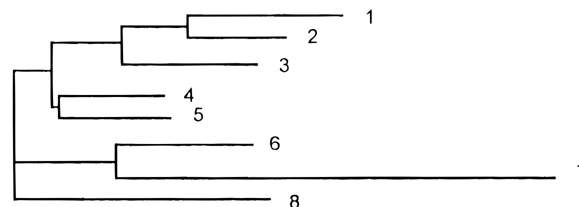

Figure 2. Cluster analysis by ADDTREE of aroma similarity values. Branches: 1, SSOJ stored at 45 °C for 14 days; 2, fortified with 0.5 mM L-cysteine; 3, with 1.0 mM glutathione; 4, with 2.0 mM glutathione; 5, with 3.0 mM glutathione; 6, with 4.0 mM glutathione; 7, with 7.0 mM glutathione; 8, control SSOJ, stored at 4 °C. Each value in the original matrix represents the mean of eight panelists (each tested twice).

About 75% of the ascorbic acid was degraded in SSOJ stored under accelerated conditions (Figure 1B). Glutathione inhibited ascorbic-acid degradation in a concentration-dependent manner, albeit less effectively than cysteine: only the high concentration of 7.0 mM glutathione reached the inhibition level of about 40% achieved by 0.5 mM cysteine. Thiols may reduce ascorbic-acid degradation by complexing with metal ions such as Cu^{2+} (Russo and Bump, 1988) and by reducing dehydroascorbic acid to ascorbic acid (Fasman, 1976). L-Cysteine's more efficient inhibition of ascorbic-acid degradation relative to *N*-acetyl-L-cysteine and glutathione may be explained by the fact that thiols, being in great excess relative to Cu^{2+}, are expected to reduce Cu^{2+} to Cu^{+}, which in turn forms complexes that are particularly stable with L-cysteine (Levitski, 1964). Glutathione moderately inhibited browning with almost no dependence on concentration (Figure 1C).

With respect to thiol degradation, in contrast to L-cysteine and *N*-acetyl-L-cysteine fortification where significant amounts (above 50%) remained after storage, almost all of the fortified glutathione was lost under the accelerated conditions used in the present experiments. The reasons for this is unknown.

In the aroma-similarity space diagram (Figure 2), no glutathione-fortification treatment resulted in an aroma connected to the same branch as that of the control juice (branch 8) kept at 4 °C. This suggests no significant improvement in juice-aroma quality due to glutathione fortification under accelerated storage conditions. Sets of aromas that are connected to the same node at relatively short (horizontal) distances from each other represent high similarity. The proportion of variance explained was 90.9% ($r^2 = 0.96$), indicating that distances in the clustering structure are highly correlated with the original aroma similarity. Nevertheless, the raw similarity data and the MDS analysis (not shown) indicated that the aroma of 1.0 mM glutathione-fortified, stored SSOJ was closest in distance to the control juice (branch 8) in the space diagram, relative to other treatments.

Since in previous studies the effect of *N*-acetyl-L-cysteine on PVG formation, ascorbic-acid degradation, and browning during storage of SSOJ under accelerated conditions was tested at concentrations of 2.5 mM and below (Naim et al., 1993a,b), a complementary experiment has been conducted employing higher concentra-

Table 1. Effect of L-Cysteine (Cys), *N*-Acetyl-L-cysteine (AcCys), and Glutathione (GSH) Fortification on PVG Formation, Ascorbic-Acid Degradation, Browning, and Acceptance of Orange Juice during Pasteurization[a]

additive (mM)	PVG (μg/L)	ascorbic acid (mg/L)	browning (OD 420 nm)	acceptance (1−9 scale)
AcCys (4.0)	8.4 ± 1.9	411 ± 3[ab]	0.17 ± 0.001	5.42 ± 1.9[a]
AcCys (3.0)	14.6 ± 3.4	397 ± 16[bcd]	0.17 ± 0.001	5.38 ± 1.9[ab]
GSH (2.0)	16.8 ± 2.5	413 ± 2[a]	0.18 ± 0.002	5.19 ± 2.1[ab]
GSH (3.0)	19.3 ± 0.2	407 ± 11[abc]	0.18 ± 0.001	5.02 ± 2.0[ab]
AcCys (2.0)	20.2 ± 4.1	397 ± 8[cd]	0.17 ± 0.002	4.96 ± 2.2[ab]
Cys (1.0)	10.5 ± 1.5	385 ± 9[d]	0.18 ± 0.002	4.75 ± 2.2[ab]
GSH (4.0)	15.6 ± 0.8	411 ± 5[ab]	0.17 ± 0.002	4.65 ± 2.0[ab]
Cys (0.5)	14.9 ± 2.5	408 ± 14[abc]	0.17 ± 0.001	4.58 ± 2.2[b]
no additive	8.1 ± 0.9	367 ± 6[e]	0.18 ± 0.001	3.70 ± 2.5[c]

[a] Values are the means ± SEM of three replicates, each tested twice by HPLC (for PVG), by potentiometry (for ascorbic acid) and by spectrophotometry (for browning).Values for the hedonic taste test are the means ± SEM of 24 subjects. Values not sharing the same superscript letter are different at $p < 0.05$.

tions of this thiol. Fortification of SSOJ by 2.5, 5, 10, 15, and 25 mM *N*-acetyl-L-cysteine resulted in significant ($p < 0.05$) 28, 25, 20, 23, and 33% inhibition of PVG formation, respectively. The same fortifications resulted in 4, 16, 30, 37, and 55% inhibition of ascorbic-acid degradation and in 23, 36, 34, 45, and 42% inhibition of browning, respectively. In the sensory-similarity experiments (cluster analysis by ADDTREE), fortification by 5 mM *N*-acetyl-L-cysteine resulted in aroma connected to the same branch in the space diagram as that of the control juice kept at 4 °C. The aroma of SSOJ fortified by *N*-acetyl-L-cysteine at concentrations higher than 5 mM, grouped together in a different location of the space diagram whereas the aroma of concentrations lower than 5 mM, including the unadulterated juice, also grouped together in an additional different location. These results suggest, therefore, that fortification of SSOJ by 5 mM *N*-acetyl-L-cysteine produced a desirable aroma, closer to that of the control juice than other treatments under accelerated storage conditions.

Effects of Thiol Fortification on PVG Formation, Ascorbic-Acid Degradation, Browning, and Acceptance of Orange Juice Following Pasteurization. Although pasteurization stimulated PVG formation, its level did not reach the 50 μg/L taste threshold (Table 1). Nevertheless, pasteurization of orange juice has been found to release ferulic acid from its bound forms (Naim et al., 1988), thereby increasing the precursor content for PVG formation during storage. Orange-juice fortification with L-cysteine, *N*-acetyl-L-cysteine, or glutathione had no effect on PVG formation or on browning during pasteurization. However, thiol fortification had a significant protective effect on ascorbic-acid degradation and significantly improved juice acceptance (Table 1), with *N*-acetyl-L-cysteine and glutathione appearing to be the most effective at both. It should be noted that, in previous studies (Naim et al., 1993a,b), L-cysteine was added to SSOJ after pasteurization. Thus, any adverse effects of cysteine on juice acceptance produced during pasteurization could not be detected under those conditions. Cysteine may create off-flavors (Molnar Perl and Friedman, 1990), and in fact, the present experiment (involving 16 taste panelists) indicated that the odor threshold for L-cysteine in orange juice, prior to any processing or storage, is 1.8 mM. Nevertheless, juice acceptance during pasteurization was improved by thiol fortification under conditions where PVG content was below its taste threshold,

Table 2. Remaining Thiols in Fortified Orange Juice Following Storage for 12 weeks at 25 and 35 °C[a]

additive (mM)	remaining thiols (mM) 25 °C	remaining thiols (mM) 35 °C
Cys (0.5)	0.30 ± 0.05 (61)	0.23 ± 0.03 (45)
Cys (0.75)	0.36 ± 0.02 (48)	0.32 ± 0.02 (43)
GSH (1.0)	0.45 ± 0.04 (45)	0.39 ± 0.02 (39)
GSH (1.5)	0.86 ± 0.10 (57)	0.68 ± 0.05 (45)
AcCys (2.5)	1.05 ± 0.09 (42)	0.91 ± 0.07 (33)
AcCys (3.5)	1.30 ± 0.11 (37)	1.20 ± 0.16 (34)

[a] Values are the means ± SEM of three replications for each data point. Numbers in parentheses indicate the remaining percentage. Abbreviations are as in Table 1.

suggesting that during pasteurization thiols inhibit the formation or induce the masking of off-flavors other than PVG.

Effects of Thiol Fortification on PVG Formation, Ascorbic-Acid Degradation, Browning, and Acceptance of Orange Juice during Storage for 12 Weeks at 25 and 35 °C. The pasteurization and storage conditions chosen here were designed to approximate those in the marketplace. The control juice stored at 4 °C contained 3.6 μg/L PVG. Juice stored at 25 °C contained 75 μg/L PVG (Figure 3A), above the 50 μg/L taste threshold (Tatum et al., 1975). Thus, PVG content was increased by 20-fold during storage at 25 °C, reaching a level that could reduce juice quality. Its use as an indicator for quality deterioration under normal storage conditions is therefore suggested. Similar storage at 35 °C elevated PVG level about 10-fold above taste threshold (Figure 3D), undoubtedly contributing significantly to reduced quality.

Thiol concentrations in these experiments were selected to be below those found in previous experiments to form detectable, objectionable odors. The low cysteine levels did not significantly inhibit PVG formation in juice stored at 25 °C, but reduced PVG content by 20% in juice stored at 35 °C. *N*-Acetyl-L-cysteine and glutathione, which can be used at higher concentrations without forming sulfur off-flavors, were more effective, reducing PVG formation by up to 50% as compared to the unadulterated juice. Glutathione (1.0 mM) was, as with storage under accelerated conditions, a potent inhibitor of PVG formation.

About 15% degradation of ascorbic acid was noted in juice stored at 25 °C (Figure 3B). Fortification with 0.75 mM L-cysteine prevented this degradation. About 25% of ascorbic-acid degradation was found in juice stored at 35 °C (Figure 3E), and except for 0.5 mM cysteine, fortification with the three thiols at all concentrations tested inhibited this degradation to a similar extent. Browning was, as expected, elevated when juice was stored at both 25 and 35 °C (Figure 3C,F). In general, thiol fortification reduced browning by 25−50% in juice stored at either temperature, probably due to their SH groups which are nucleophiles and scavengers of free radicals (Friedman, 1991). Interestingly, in contrast to *N*-acetyl-L-cysteine, storing juices fortified with 0.5 mM cysteine or 1.0 mM glutathione at 35 °C inhibited browning more than the correspondingly higher concentrations of 0.75 and 1.5 mM cysteine and glutathione, respectively. Since these two thiols bear a free amino group, it is possible that the higher concentrations of these two thiols may form brown pigments of their own concomitantly with inhibition of browning due to ascorbic-acid degradation and other processes. It should also be noted that storing juice fortified with 3.5 mM *N*-acetyl-L-cysteine at 25 °C resulted in lower

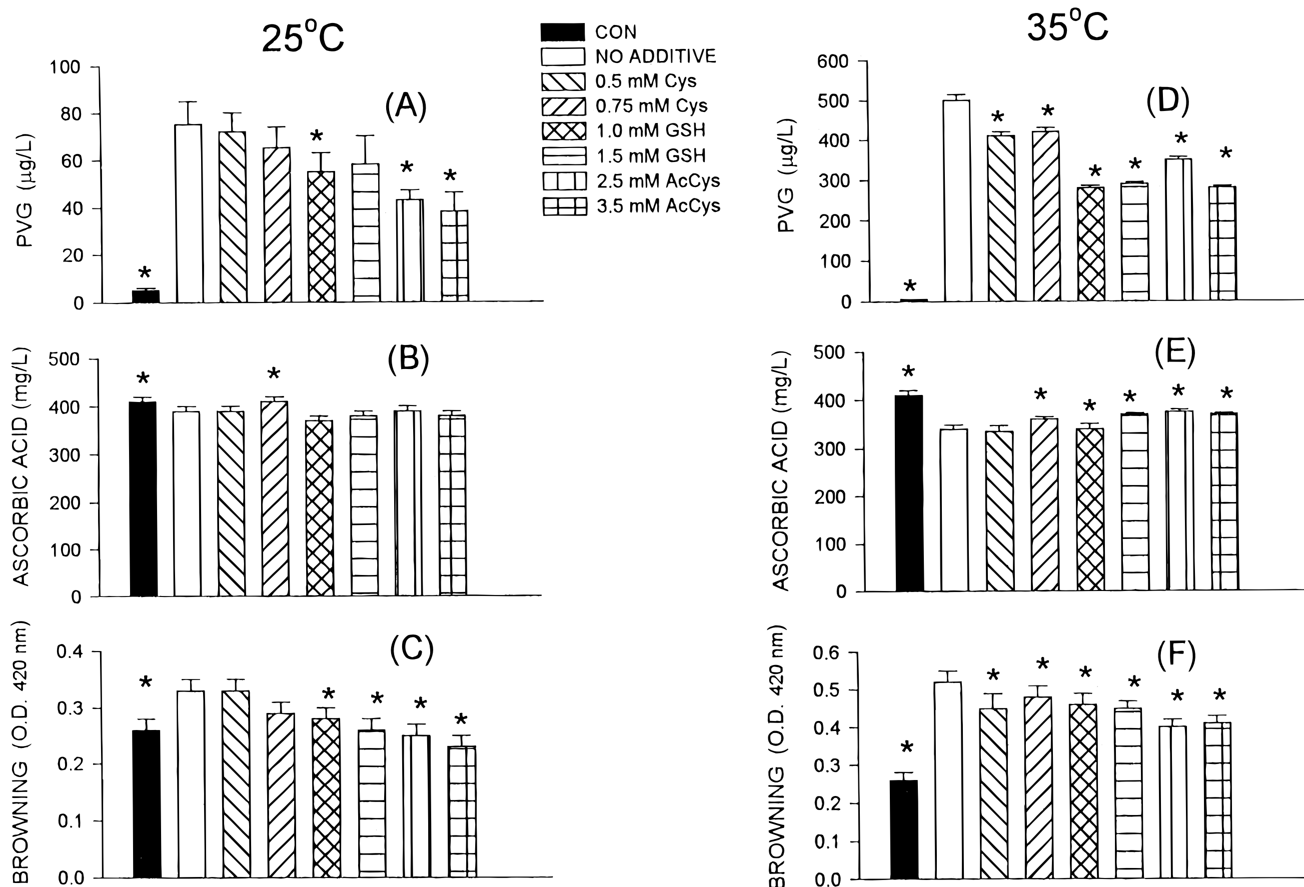

Figure 3. Effect of L-cysteine (Cys), glutathione (GSH), and *N*-acetyl-L-cysteine (AcCys) fortification on PVG formation (A, D), ascorbic-acid degradation (B, E), and browning (C, F) of orange juice stored at 25 or 35 °C for 12 weeks. Values are the mean and SEM of three replicates, each tested twice by HPLC (for PVG), potentiometry (for ascorbic acid), and spectrophotometry (for browning). Unadulterated control (CON) juice was kept at 4 °C. L-Cysteine was used as a reference. * indicates significant ($p <$ 0.05) different value from that of the unadulterated (no additive) stored juice.

Table 3. Effect of Thiol Fortification on Taste Acceptance of Juice Stored at 25 °C and Aroma Acceptance of Juice Stored at 35 °C for 12 weeks[a]

	acceptance (1−9 hedonic scale)	
additive (mM)	25 °C	35 °C
Cys (0.5)	5.16 ± 0.24	5.05 ± 0.22
GSH (1.0)	4.94 ± 0.28	5.35 ± 0.28
GSH (1.5)	4.93 ± 0.32	4.98 ± 0.21
AcCys (2.5)	4.79 ± 0.30	4.89 ± 0.22
Cys (0.75)	4.75 ± 0.33	5.00 ± 0.25
no additive	4.71 ± 0.27	4.75 ± 0.30
AcCys (3.5)	4.67 ± 0.32	4.94 ± 0.26

[a] Values are the means ± SEM of 29 subjects for the hedonic taste test (25 °C) and 26 for the aroma hedonic test (35 °C). Control values (4 °C) indicated acceptance levels of 5.19 ± 0.29 and 6.20 ± 0.31 for the 25 and 35 °C storage experiments, respectively. Abbreviations are as in Table 1.

browning than that found in the control (unadulterated) sample stored at 4 °C. This was probably due to the inhibitory effect of this thiol during pasteurization.

The content of the thiols remaining after storage is shown in Table 2. The minimum remaining percentage of thiols after storage at 25 and 35 °C was about 33% and, as expected, it was higher after storage at 25 °C as compared to 35 °C. The loss of the various thiols during storage did not vary much among the treatments and did not exceed the 70% level.

No significant improvement in juice acceptance due to fortification with the low thiol concentrations was found at either 25 or 35 °C (Table 3). Nevertheless, in both sensory tests, scores given to the unadulterated, stored juice were lower than those given to the thiol-fortified juices. Fortification with 1.0 mM glutathione and 0.5 mM L-cysteine resulted in the highest scores. Concomitantly, in the aroma similarity, 35 °C storage experiment (Figure 4), the aroma of juice fortified with 1.0 mM glutathione (branch 4) was connected to the same node as the control juice (branch 8) kept at 4 °C (r^2 = 0.98), indicating that both exhibited a similar aroma. This suggests that fortification with 1.0 mM

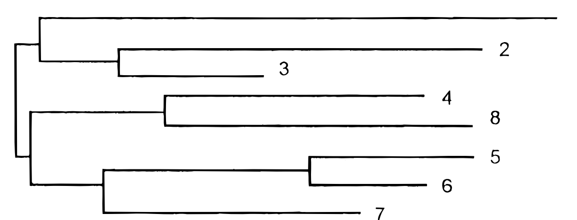

Figure 4. Cluster analysis by ADDTREE of aroma-similarity values of orange juice stored at 35 °C for 12 weeks. Branches: 1, juice stored at 35 °C for 12 weeks; 2, fortified with 0.5 mM L-cysteine; 3, with 0.75 mM L-cysteine; 4, with 1.0 mM glutathione; 5, with 1.5 mM glutathione; 6, with 2.5 mM *N*-acetyl-L-cysteine; 7, with 3.5 mM *N*-acetyl-L-cysteine; 8, control juice stored after pasteurization at 4 °C. Each value in the original matrix represents the mean of eight panelists (each tested twice).

glutathione is most effective at retaining the aroma of the original juice. Unadulterated stored juice (branch 1) and L-cysteine- and *N*-acetyl-L-cysteine-fortified juices produced different aromas.

CONCLUSIONS

Pasteurization of orange juice stimulated PVG formation to a level below its taste threshold. Fortification with low concentrations (0.5−4.0 mM) of L-cysteine, *N*-acetyl-L-cysteine, or glutathione during pasteurization improved juice acceptance regardless of PVG content. Thus, unlike the storage conditions where thiol fortification inhibited PVG formation in accordance with improvement of aroma quality, in the pasteurization process, thiols inhibit the formation of off-flavors other than PVG. Ascorbic-acid degradation due to pasteurization (about 11%) may account for almost all of the ascorbic-acid degradation found in juice stored at 25 °C for 12 weeks and almost 50% of that found in juice stored at 35 °C. Thiol fortification (especially glutathione) improved ascorbic-acid retention during pasteurization but had no effect on the slight occurrence of browning. Storage of orange juice under accelerated conditions (45 °C for 2 weeks) resulted in PVG formation and browning levels similar to those observed in juice stored for 12 weeks at 35 °C. Thiol fortification also exerted similar effects on PVG formation and browning under these conditions. However, the magnitude of ascorbic-acid degradation was significantly higher in juice stored under the accelerated conditions than in that stored for 12 weeks at 35 °C. Glutathione was a very effective inhibitor of PVG formation in juice stored under simulated industrial conditions: about 50% of it inhibited by 1.0 mM glutathione. This concentration of glutathione reduced the PVG level of juice stored at 25 °C to its taste-threshold level.

In line with the chemical results, the aroma-similarity experiments indicated that fortification with 1.0 mM glutathione results in an aroma similar to that of control juice stored at 4 °C. Fortification of orange juice with this concentration of glutathione, as well as with 0.5 and 0.75 mM L-cysteine or up to 3.0 mM *N*-acetyl-L-cysteine, is proposed to improve the flavor and reduce ascorbic-acid degradation and browning, thereby improving the shelf life of citrus products. Finally, it is suggested that fortification of orange juice with L-cysteine and glutathione may be regarded as an enrichment rather than as adulteration since small endogenous amounts (albeit below 0.1 mM) of these thiols have been found in the juice (Saetre and Rabenstein, 1978; Tabachnic-Ma'ayan and Fuchs, 1982).

ABBREVIATIONS USED

SSOJ, single-strength orange juice; PVG, *p*-vinylguaiacol; DMHF (furaneol), 2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone; MDS, multiple dimensional scaling.

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