

Pathways of 4-Vinylguaiacol Formation from Ferulic Acid in Model Solutions of Orange Juice[†]

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4-Vinylguaiacol (PVG), a major off-flavor in citrus products, was detected in stored model solutions of orange juice (MOJ) containing ferulic acid, and its amount increased with time and temperature. PVG was not found in MOJ incubated without ferulic acid. Vanillin, another ferulic acid degradation product, was also detected in MOJ containing ferulic acid after incubation at 35 and 45 °C, but only minute amounts occurred at 25 °C. Vanillin was not produced, however, in MOJ incubated with added PVG but which did not contain ferulic acid. Incubation of MOJ under nitrogen atmosphere rather than air or including butylated hydroxytoluene did not affect PVG levels even though nonenzymic browning products such as 5-(hydroxymethyl)furfural and furfural, and optical density values were reduced. Cu ions accelerated browning but decreased PVG levels. It appears that different factors affect PVG formation and sugar degradation.

INTRODUCTION

4-Vinylguaiacol (PVG) and other vinyl phenols are produced from free phenolic acids and contribute to either desirable or objectional aroma of important food products such as fried soy products, cauliflower, cooked asparagus, tomato, roasted peanuts, cooked corn, and dried mushrooms [see Maga (1978) for detailed references]. In stored orange and grapefruit juices, PVG is a major detrimental off-flavor with a taste threshold of 0.075 ppm, which in combination with α -terpineol and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) contributes "old fruit" or "rotten" flavor to the juice (Tatum et al., 1975). We have previously hypothesized that PVG is formed in citrus products from free ferulic acid (FA; Naim et al., 1988). Indeed, PVG formation increased during storage of orange juice (Naim et al., 1988; Lee and Nagy, 1990), and this formation is accelerated if the juice is fortified with free FA (Naim et al., 1988). The resultant juice had greatly inferior aroma quality when compared to controls.

Reported potential sources of free FA in oranges and grapefruits are five bound forms, namely, feruloylputrescine, feruloylglucose, and feruloylglucaric, diferuloylglucaric, and feruloylgalactaric acids (Reschke and Herrmann, 1981; Risch et al., 1987, 1988; Wheaton and Stewart, 1965; see Figure 1). Only minute amounts of FA occur in a free form (Peleg et al., 1991). Bound or conjugated forms of FA, depending on the type of each linkage with FA, differ significantly in their ability to serve as precursors for FA and the resulting objectional aroma of PVG (Peleg et al., 1988). A mechanism by which cinnamic acids are decarboxylated to vinylphenols has been proposed (Pyysalo et al., 1977). The current investigation was undertaken to monitor the formation of PVG from FA in a model system of orange juice and to evaluate factors

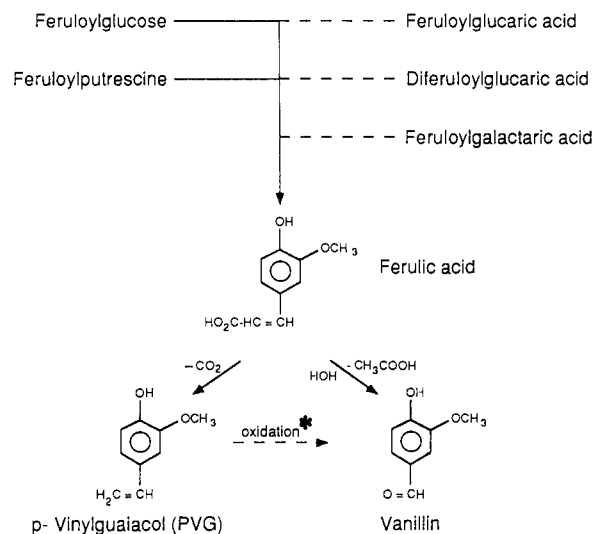


Figure 1. Possible pathways for the release of FA from its bound forms in citrus juice and the degradation to either PVG or vanillin. Dotted lines indicate hypothetical pathways. (*) Not demonstrated in our MOJ experiments.

that may inhibit or accelerate the formation of PVG, thereby suggesting whether ionic or radical mechanisms [e.g., Fiddler et al. (1967)] are responsible for PVG accumulation.

MATERIALS AND METHODS

Materials. Ferulic acid (FA), 5-(hydroxymethyl)furfural (HMF), and butylated hydroxytoluene (BHT) were purchased from Sigma. PVG was either synthesized (Klaren-Dewite et al., 1971; Naim et al., 1988) or purchased from Lancaster Synthesis (U.K.). Vanillin was a product of BDH Chemicals, furfural was purchased from Aldrich, and CuSO_4 was from Mallinckrodt.

Preparation and Storage of Synthetic Mixtures of Orange Juice. 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., 1988). One hundred grams of MOJ contained (w/w) the following components: glucose, 2.5; fructose, 2.5; sucrose, 5.0; citric acid, 1.2; tripotassium citrate, 0.6; ascorbic acid, 0.06; γ -aminobutyric acid, 0.3; L-arginine, 0.3; water (double distilled), 87.54. MOJ solutions were modified by the addition of FA (10 mg/L) or PVG (1 mg/L). Twenty-milliliter

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[†] Preliminary results of this study have been presented.

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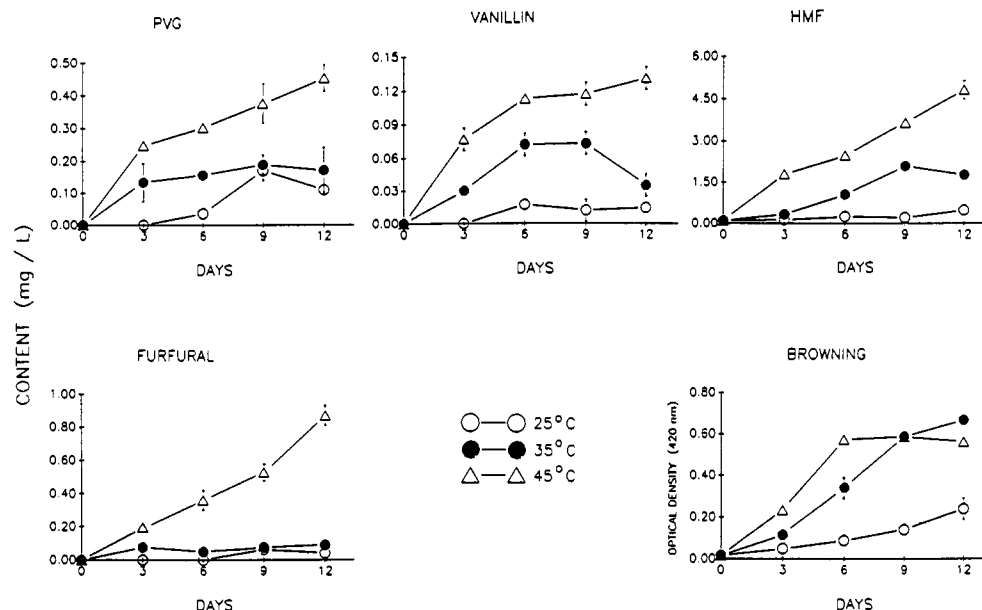


Figure 2. Effects of temperature and time of incubation on the accumulation of PVG, vanillin, HMF, furfural, and browning in MOJ solutions containing free FA. Values are the mean and SEM of three different samples for browning and the mean and SEM of three samples (each analyzed twice by HPLC) for other variables. In cases where SEM bars are missing, their values were too small to be shown.

aliquots of modified and nonmodified MOJ solutions were transferred to 28-mL vials and incubated at either 25, 35, or 45 °C for different time periods for up to 12 days. Further modifications in the MOJ system were as follows: nitrogen atmosphere was produced by nitrogen flowing into the MOJ-containing vials (each sealed with a septum) before the incubation was started. An addition of either CuSO_4 (2 ppm) or butylated hydroxytoluene (45 ppm) to MOJ solutions was also performed. Following the incubation of MOJ samples, absorption at 420 nm was determined to indicate the browning level (Clegg, 1966). The incubated solutions (10 mL each) were applied to a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA) which had been preconditioned with methanol, water, and 0.01 N HCl (5 mL each), washed with water (1 mL), and eluted with methanol (2×3 mL). The combined methanolic fractions were concentrated under nitrogen to 1 mL, and the samples were analyzed by HPLC equipped with a LiChrospher 100 RP-18 column (5 μm , 250 mm \times 4 mm, Merck) with a RP-18 precolumn (25 mm \times 4 mm, Merck). The mobile phase was 1.5% acetic acid in water/methanol (70:30). Operating conditions were as follows: flow rate, 1 mL/min; injected volume, 20 μL ; room temperature. Each sample was injected twice. The separated chromatographic peaks were identified and quantitated by a Chrom-A-Scope UV rapid-scanning detector (Barspec, Israel).

Data Analyses. Results were analyzed by one- and two-way analyses of variance using SAS statistical package programs. The Duncan multiple-range test was performed for comparisons among the means. Regression analyses (linear model) were performed to reveal possible effects of temperatures on the rate of accumulation of various products.

RESULTS AND DISCUSSION

The present study utilizing a model system of orange juice (MOJ) provides the evidence that PVG is indeed formed from FA in citrus juices (Figure 2). The accumulation of PVG (RT in HPLC = 31.1 ± 0.13 min) in MOJ containing FA was temperature and time dependent; its content increased at elevated temperatures ($p < 0.005$) and with extending time periods ($p < 0.0001$). In the MOJ solution, PVG content reaches taste threshold level (Tatum et al., 1975) after 9 days of incubation even at 25 °C. The accumulation of PVG in MOJ after 12 days of incubation (150–450 $\mu\text{g/L}$) was comparable to that (300–600 $\mu\text{g/L}$) formed in single-strength orange juice stored for 14 days under similar conditions (Naim et al.,

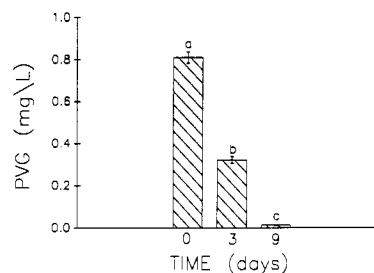


Figure 3. Degradation of PVG in MOJ during incubation at 45 °C. PVG (1 mg/L) was added to MOJ solutions incubated for 3 and 9 days. Values are the mean and SEM of three samples (each analyzed twice by HPLC). Bars not sharing the same superscript letter are different at least at the $p < 0.05$ level.

1988). Following incubation, vanillin (RT = 7.4 ± 0.04 min), another possible product of FA degradation (see Figure 1), was found, and its accumulation was also affected by temperature ($p < 0.01$; Figure 2). A trend for time effect was also observed ($p < 0.07$). Neither PVG nor vanillin could be found in MOJ incubated without the addition of FA. A recent study (Marin et al., 1992) confirmed the presence of vanillin also in processed orange juice.

Regression analyses (linear model) were performed to obtain estimated reaction order values for PVG and vanillin. The kinetics, however, could not unequivocally be determined for PVG and vanillin formation ($R^2 = 0.834$ and 0.839 for zero order and 0.845 and 0.666 for first order, respectively), which together with the shape of the accumulation curve suggest a complex mechanism.

It has been suggested (Fiddler et al., 1967) that, upon the decarboxylation of FA, the resulting PVG is oxidized to vanillin (Figure 1). However, since vanillin was not found in MOJ incubated with PVG and which did not contain FA (Figure 3), one may hypothesize that FA could be converted to vanillin via a direct route (retro-aldol) (Figure 4a) without PVG formation. Alternatively, and especially in the absence of OH^- ions, due to the presence of a free OH group at C-4 of FA, the following mechanism (Figure 4b) is feasible.

Although PVG was not oxidized to vanillin in stored MOJ, it is evident that in the absence of its precursor (FA)

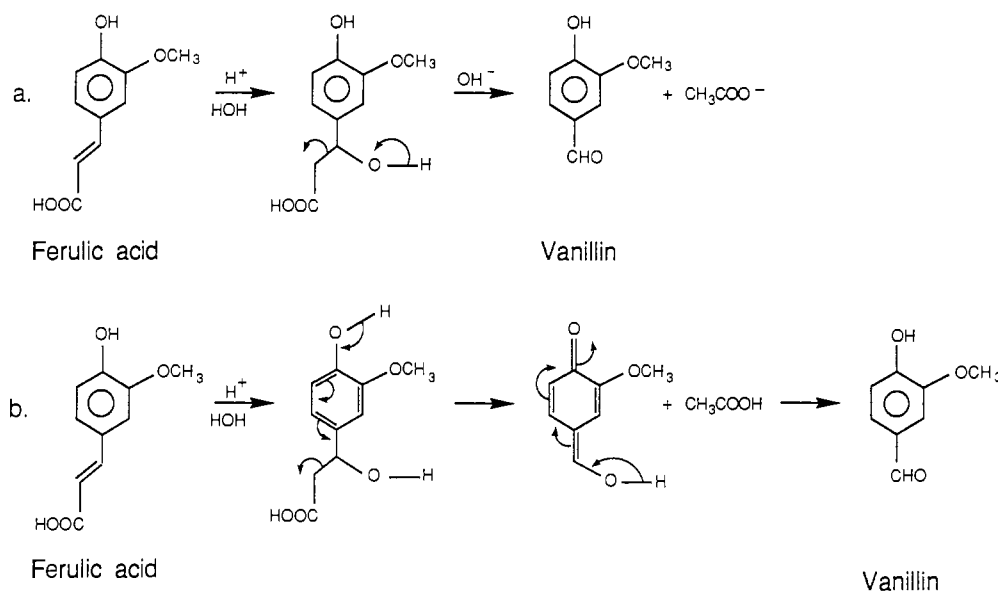


Figure 4. Proposed mechanisms for the formation of vanillin from FA in MOJ solutions.

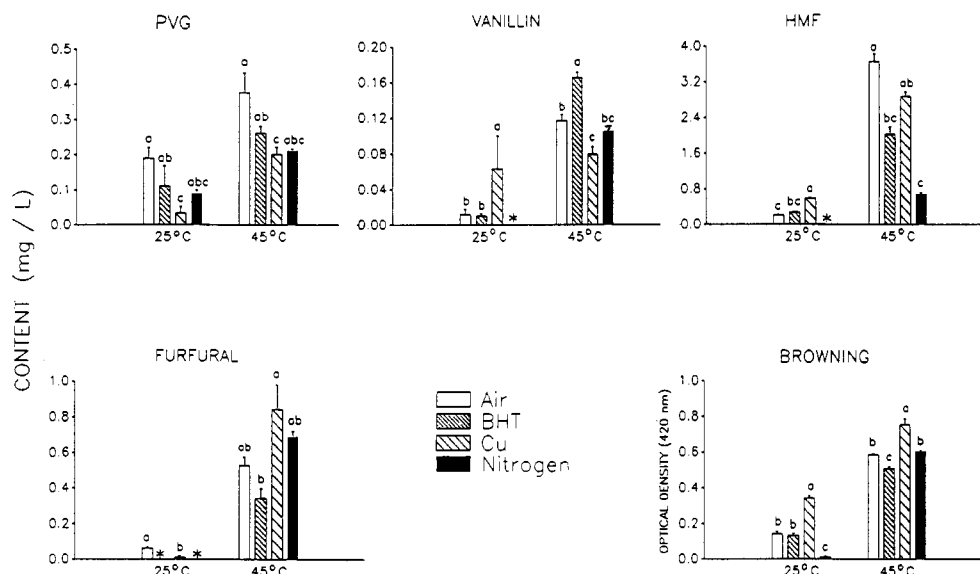


Figure 5. Effects of BHT, Cu^{2+} , and nitrogen atmosphere on PVG, vanillin, HMF, furfural, and browning formation in MOJ solutions containing free FA. Solutions were incubated at 25 and 45 °C for 9 days. Values are the mean and SEM of three different samples for browning and the mean and SEM of three samples (each analyzed twice by HPLC) for other variables. Bars not sharing the same superscript letter within each temperature group are different at least at the $p < 0.05$ level. (*) Not detected under present experimental conditions.

its content is reduced ($p < 0.001$), indicating that it undergoes accelerated degradation (Figure 3). Any kinetic calculations as to formation rates of PVG may, therefore, be questionable. It is possible that the accelerated non-enzymic browning which occurs in MOJ during incubation transformed the produced PVG into its dimer or trimer forms (Klaren-Dewite et al., 1971). The accumulation of PVG in stored orange juice was also reduced when the temperature was high (Lee and Nagy, 1990). Nevertheless, in the presence of free FA, the rate of formation of PVG evidently exceeds its degradation in our MOJ system (Figure 2).

Previous studies (Shaw et al., 1977) suggested that flavor becomes unacceptable before color becomes objectional and that furfural and 5-(hydroxymethyl)furfural (HMF) can only be useful as indicators to the extent that they are not present in sufficient quantity to cause the off-flavors observed after storage (Lee and Nagy, 1988). In line with these results, it is evident that, at 25 °C, above taste threshold levels of PVG were observed in MOJ between 6 and 9 days of incubation, whereas the formation of fur-

fural ($\text{RT} = 4.3 \pm 0.02$ min) and HMF ($\text{RT} = 3.0 \pm 0.01$ min) was not stimulated at this temperature (Figure 2). It appears that HMF formation preceded that of furfural, which was evident only in MOJ incubated at 45 °C. The rate of HMF accumulation increased as temperature was elevated ($p < 0.01$). The rates of furfural accumulation were not statistically different between 25 and 35 °C. The rate at 45 °C, however, was significantly higher ($p < 0.001$) than those at lower temperatures. The accumulation of furfural and HMF in MOJ at 45 °C was 2–4-fold higher than that reported for orange (Nagy and Dinsmore, 1974) and grapefruit juices (Lee and Nagy, 1988) stored at similar temperatures. The overall rate of browning was higher ($p < 0.01$) at 35 and 45 °C when compared with that at 25 °C. The reaction order for furfural accumulation appeared to fit a zero kinetic ($R^2 = 0.953$), while the accumulation of HMF and browning could fit either zero- or first-order kinetics in the present MOJ model. Nevertheless, depending on experimental condition, HMF formation can also fit a second-order reaction (Shallenberger and Mattick, 1983). The results of furfural formation in MOJ are

in agreement with furfural formation in orange concentrate rediluted by water (Herrmann and Partassidou, 1979).

The effects of nitrogen atmosphere, BHT, and Cu^{2+} on the formation of PVG, vanillin, browning, and browning products during incubation of MOJ containing FA are shown in Figure 5. There was a major qualitative difference between the effects observed at 25 °C and those found following the incubation at 45 °C. Although the accumulation of PVG was reduced under nitrogen atmosphere, this reduction did not reach a statistical significance. The present results (Figure 5) are in line with those of Fiddler et al. (1967), who found that PVG can be formed from FA under nitrogen atmosphere. Under nitrogen and at the present experimental conditions, however, at 25 °C (and also at 35 °C; data are not shown) the accumulations of vanillin, HMF, furfural, and browning were either stopped or significantly reduced (Figure 5). Compared to the air atmosphere control, nitrogen did not inhibit (except in the case of HMF) the formation of these parameters when the same MOJ mixtures were incubated at 45 °C.

Cu^{2+} , as expected, stimulated browning and HMF formation at both temperatures probably and primarily due to ascorbic acid degradation (Khan and Martell, 1967). The presence of Cu^{2+} was especially relevant to the degradation of FA to either PVG or vanillin. The presence of Cu^{2+} in the incubated MOJ significantly reduced the content of PVG at 25 and 45 °C. In the presence of Cu^{2+} , on the other hand, the content of vanillin increased significantly when MOJ was incubated at 25 °C. It is thus possible that Cu^{2+} may catalyze and, therefore, shift the degradation of FA to vanillin rather than to PVG. Alternatively, perhaps due to a possible free-radical mechanism that may be operative in the presence of Cu^{2+} , PVG content is reduced since it may be transformed to dimer and trimer forms (Klaren-Dewite et al., 1971). Therefore, the balance between PVG formation vs its conversion to other products may be shifted to the latter possibility when Cu^{2+} is present.

The presence of BHT resulted in reduced browning and HMF formation in MOJ incubated at an elevated temperature but had no such effect at 25 °C. It had no significant effect on PVG accumulation, but it stimulated vanillin formation when MOJ was incubated at 45 °C.

In conclusion, since nitrogen atmosphere and BHT (a well-known free-radical terminator in food systems; Lindsay, 1985) did not appear to significantly inhibit PVG accumulation and the presence of Cu^{2+} did not stimulate but rather inhibited PVG accumulation, one may hypothesize that PVG was mainly formed in the present model solutions by ionic mechanisms. The stimulation of vanillin accumulation, on the other hand, by Cu^{2+} at 25 °C and by BHT at 45 °C may suggest the involvement of radical mechanisms.

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Registry No. PVG, 7786-61-0; Cu, 7440-50-8; FA, 1135-24-6; HMF, 67-47-0; vanillin, 121-33-5; furfural, 98-01-1.