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**Bound and Free Phenolic Acids as Precursors
to Objectional Aroma in Citrus Products**

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Title:

**BOUND AND FREE PHENOLIC ACIDS AS PRECURSORS
TO OBJECTIONAL AROMA IN CITRUS PRODUCTS**

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C. ABSTRACT

p-Vinyl guaiacol (PVG) is believed to be an important detrimental off-flavor in stored orange and grapefruit juices, possessing a taste threshold level of 0.075 ppm and contributing "old fruit" or "rotten" odor to the juice. The present joint research was conducted to test the hypothesis that PVG is formed from free ferulic acid in the following sequence: Bound Forms of Ferulic Acid -> Free Ferulic Acid --> PVG. The release of ferulic acid from bound forms may be a key step in a process which eventually leads to the potent aroma of PVG. Solvent optimization techniques were developed and UV photodiode array and fluorescence detection procedures for HPLC were elaborated for the separation of PVG, bound and free phenolic (hydroxycinnamic) acids in citrus fruit, juice and in model systems. The evidence that PVG is formed from free ferulic acid (probably by ionic mechanisms) in citrus juices was provided in a model system of orange juice. The formation of PVG and vanillin (another product of ferulic acid degradation) was already observed during storage at room temperature. Different factors affect PVG formation and sugar degradation during storage. In all fruit parts, only minor amounts of cinnamic acids occur in the free state while most is present in bound forms. A concentration gradient of phenolic acids appears to exist from the flavedo towards the juice vesicles suggesting that processing technologies that minimize juice contact with peel components during juice extraction will reduce the amount of phenolic acids transferred into the juice. The content of free ferulic acid in citrus fruit decreased during the season. However, the amount present at the end of the season still exceeded the amount needed to produce PVG at levels above its taste threshold during juice processing and storage.

D) OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

In our original research plan we proposed to identify precursors to objectional aroma and explore the mechanisms by which they are converted to final concentrations of malodorous compounds in citrus juices. Since previous published and early experiments at our laboratory suggested that free ferulic acid may be the precursor for the potent off-flavor PVG, and since ferulic acid appeared to occur mainly in bound forms, we proposed the following sequence as our major working hypothesis:

The release of ferulic acid from bound forms was proposed to be a key step in a process which eventually leads to the potent aroma component of p-vinyl guaiacol (PVG). To accomplish these goals we originally proposed five experimental groups:

- Isolation, characterization and quantification of the bound forms of ferulic in orange juice, grapefruit juice, and in individual fruit parts.
- Determine the contribution of each form of bound and free ferulic acid has to aroma quality and verify the conversion of ferulic acid to PVG.
- Develop HPLC and capillary GC methods to determine volatile aroma storage off flavors.
- Investigate the role of fruit cultivar, maturity, processing equipment, essence, oil and storage on the release of ferulic acid from bound forms and in the production of PVG and other components of objectional aroma.
- The role other bound and free phenolic acids such as sinapic, coumaric and caffeic acids contribute to storage off flavors.

Due to the reduced budget that was made available for our project, new priorities were established in order to achieve most of the original goals. The experimental approach was directed to the developments of advanced analytical tools to monitor off-flavor precursors (Group C and part of A), quantify precursors in fruit parts (Group D) and study mechanistic aspects of off-flavor formation (Group A, and part of group B). Our quantification studies demonstrated (see part E for details) that although the content of free ferulic acid in both oranges and grapefruit juice decreased during the season, there was sufficient "free" ferulic acid present at the end of the season to produce super-threshold levels of PVG. Therefore, determination of contribution of each bound form to aroma quality (the first part of group B) was given a low priority in the experimental design. Group E experiments were originally proposed only if time and funding permitted. These experiments were not carried out due to reduced funding.

E) BODY OF THE REPORT

INTRODUCTION

The aroma of citrus is extremely complex in composition. Components of aroma originate principally from the contents of juice vesicles but also from peel oil (Kealey and Kinsella, 1979). Color deterioration and appearance of undesirable volatile compounds in citrus products during storage is well documented, and the sensory impact of these compounds on quality is profoundly negative (Dinsmore and Nagy, 1971; Handwerk and Coleman, 1988; Meydav and Berk, 1978; Naim et al., 1988). Flavor usually becomes unacceptable before color becomes objectional (Shaw et al., 1977). Some flavor degradation, along with a complete loss of volatile compounds, occurs when citrus juice is concentrated. Aqueous essence, essence oil and cold pressed peel oil are added to restore the original flavor and to mask off-flavors.

Flavor degradation in citrus juice has been correlated with furfural production (Maraulja et al., 1973; Nagy and Randall, 1973). Furfural and 5-hydroxy methyl furfural (HMF), can only be useful as indicators as they are not present in sufficient quantity to cause the off-flavors observed after storage (Lee and Nagy, 1988). Limited information is available on the nature and mechanisms of off-flavors produced during citrus juice processing and storage. Some of the objectional volatiles can significantly affect acceptance since they reach their taste threshold levels under typical processing and storage conditions while other volatiles may be irrelevant as off-

flavors (Handwerk and Coleman, 1988). In stored orange and grapefruit juices, α -terpinol, 2,5 - dimethyl -4-hydroxy -3-(2H)-furanone (DMHF) and p-vinyl guaiacol (PVG) are believed to be the main detrimental off-flavors with taste threshold levels of 2.5, 0.1 and 0.075 ppm, respectively (Tatum et al., 1975). PVG contributes "old fruit" or "rotten" flavor to the juice, DMHF produces a pineapple-like odor typical of aged juice and α -terpinol imparts a stale, musty or piney odor. When the above three compounds were added collectively to freshly processed juice they gave an aged, off-flavor aroma, similar to that observed in stored juice (Tatum et al., 1975). PVG formation was found to be accelerated during storage of orange juice adulterated by ferulic acid (Naim et al., 1988). This juice had greatly inferior aroma quality when compared to controls.

The demand for fresh tasting (premium) citrus juices has increased significantly in recent years. Currently, it is the fastest growing sector of the citrus juice market. Since chemical rather than enzymic processes are usually responsible for reduced quality of stored citrus products, storage at low temperature and the exclusion of oxygen and light are critical factors. Various juice extraction and concentration techniques along with diverse container and storage condition combinations have been evaluated in order to maximize fresh flavor retention and minimize off-flavors. The most effective packaging materials and low temperature storage are economically prohibitive except for premium juices. Therefore, most current commercial juices represent some compromise either in packaging or storage. The following research project was undertaken to explore the role of bound forms of ferulic acid and free ferulic acid as precursors to the objectional aroma of PVG and explore the mechanisms by which they are converted to final concentrations of malodorous compounds in citrus juices. We proposed that PVG is formed in the following sequence: Bound Forms of Ferulic Acid ---> Free Ferulic Acid ---> PVG. The release of ferulic acid from bound forms may be a key step in a process which eventually leads to the potent aroma component of PVG.

STUDY I: ISOLATION AND IDENTIFICATION OF BOUND FORMS OF FERULIC ACID AND FACTORS AFFECTING THE RELEASE OF FERULIC ACID

Potential sources of free ferulic acid in oranges and grapefruits are bound forms of the acid, five of them are currently known: feruloylputrescine, feruloylglucose, feruloylglucaric, diferuloylglucaric and feruloylgalactaric acids (Figure 1; Wheaton and Stewart, 1965; Reschke and Herrmann, 1981; Risch et al., 1987; Risch et al., 1988). As indicated before (Peleg et al., 1988), however, bound or conjugated forms of ferulic acid, depending on the type of each linkage with ferulic acid, may differ significantly in their ability to serve as precursors for ferulic acid and the resulting objectional aroma of PVG.

Figure 1: Possible pathways for the release of ferulic acid from its bound forms in citrus juice and the degradation to either PVG or vanillin. Open arrows indicate hypothetical pathways. * Could not be detected in our experiments.

Experiment 1: Isolation of bound forms of ferulic acid by conventional chromatography

The following experiment was conducted to elaborate a procedure for the isolation of bound forms of ferulic acid from orange juice.

Experimental, Results and Discussion

Based on our initial studies (Peleg et al., 1988), the following procedure was elaborated (Figure 2). This procedure applies to 4 bound forms of ferulic acid. Orange juice (1000 mL) was centrifuged (15000 X g, 20 min, 4°C). The supernatant was extracted with ethylacetate (4 X 600 ml) and water phase (fraction c) was kept for a later isolation of feruloylputrescine (FP). The organic phase was combined and evaporated under reduced pressure to a small volume (about 50 ml). Four such organic extractions originated from 4000 mL orange juice were combined and evaporated to dryness. The residue was dissolved in a small volume of methanol (10-20 ml) and then resuspended in a solution of NaHCO_3 (50 mL, 5% w/v). This suspension was extracted by ethylacetate (50 mL X 6) and the organic phase (fraction a) was kept for the isolation of feruloylglucose (FG). The water phase (fraction b) was acidified (concentrated HCl) to pH 3-5 and then extracted with ethylacetate (50 mL X 6), dried (Na_2SO_4), evaporated under reduced pressure to 5 mL, adsorbed onto silica gel (2 gr, 70-230 mesh) and applied to a silica gel column (160 gr, 65 X 3 cm). The column was first washed with methanol-chloroform (1200-1500 ml, 1:5 v/v) in order to remove residues of FG and this first elute was combined with fraction B. The elution was continued with acetic acid - water - ethyl acetate (5 L, 1:1:14, V/V). Following TLC (acetic acid - water - ethylacetate, 1:1:7, V/V), the fraction of $R_f = 0.18$ was collected, evaporated to 8 mL and separated by preparative HPLC using 80% of solvent A (1.5% acetic acid in water) and 20% of solvent B (methanol). Two fractions of RT = 69 (feruloylglucaric acid - FGA) and 119 min (diferuloylglucaric acid - DFGA) were collected (see Figure 3). The purity of these fractions was determined after evaporation by analytical HPLC using solvent A (90%) and B (10%). RT values for FGA and DFGA being 20.8 and 28.3 respectively. Samples of fractions from last purification were subjected to basic hydrolysis, and the presence of ferulic acid was demonstrated by TLC and analytical HPLC.

Figure 2: Isolation of bound forms of ferulic acid; a flow-sheet depicting the isolation of feruloylglucose (FG), feruloylputrescine (FP), feruloylglucaric acid (FGA) and diferuloylglucaric acid (DFGA).

The final purification of FP was performed according to Wheaton and Stewart (1965), see left column of Figure 2 and the characterization of FP was described before (Peleg et al., 1988). FG was purified as follows: Fraction a was evaporated under reduced pressure to a small volume and adsorbed onto silica (2 g, 230-400 mesh) and then separated on a silica gel column (80 g, 65 X 1.5 cm, i.d.). The elution was done by MeOH - CHCl₃ (1200-1500 mL, 1:4, V/V). Following identification by TLC (MeOH - CHCl₃, 1:3, V/V), the fraction of R_f = 0.53 was collected, evaporated under reduced pressure (see right column, Figure 2) and characterized as FG (Peleg et al., 1988).

Partial characterization of FGA and DFGA was carried out as follows: Following basic hydrolysis, ferulic acid was identified by TLC and HPLC as described before (compare Peleg et al., 1988) in the organic phase. The aqueous phase was evaporated and silylated using 0.3 mL (BSA - TMCS, 1:20, V/V) and shaking; 10 min at room temperature. Identification was performed by GLC using a 4% SE 30 column according to Risch et al., (1987; 1988). A peak corresponding to a silylated marker of glucaric acid was identified.

Figure 3: Isolation of feruloylglucaric acid (FGA) and diferuloylglucaric acid (DFGA) from preparative HPLC using 90% of solvent A (1.5% acetic acid in water) and 10% of solvent B (methanol).

Experiment 2: Isolation of bound forms by a reverse phase gradient system and factors affecting the release of ferulic acid

Abstract

A reverse phase gradient system employing MeOH and water (2% acetic acid) has been developed to identify the bound forms of ferulic acid in orange juice so that the effects of thermal processing, storage and juice extractor pressure can be studied. Ferulic acid, feruloylglucose and feruloylputrescine concentrations were determined in juices stored at 10, 20, and 30°C. Feruloylglucose concentrations decreased with increasing storage time at 30°C with a concomitant increase in ferulic acid concentration. Little change was observed at lower temperatures. Identical lots of Florida Valencia orange juice were extracted with varying extractor pressure. Feruloylputrescine concentrations increased with increasing extractor pressure whereas feruloylglucose was independent of extractor pressure. Concentrations of feruloylputrescine, feruloylglucose or sinapylglucose were unchanged after boiling fresh juice for up to 15 min.

Introduction

Although there is a small amount free ferulic acid in orange juice, the vast majority is bound in more complex forms. Bound forms are the source of free ferulic acid and ferulic acid was suggested to be the source of the highly malodorous PVG in orange juice (Naim, et al., 1988). Production of PVG from ferulic acid have also been noted in beer (Narziss, et al., 1990) and in distilled grain fermentation products (Thurston and Tubb, 1981). Five of bound forms of ferulic acid are currently known (see Exp. 1, Figure 1). As indicated before (Peleg et al., 1988), bound or conjugated forms of ferulic acid, depending on the type of each linkage with ferulic acid, may differ significantly in their ability to serve as precursors for ferulic acid and the resulting objectional aroma of PVG. Feruloylputrescine, has been identified and quantified in orange juice for some time (Wheaton and Stewart, 1965). Other forms of ferulic acid such as feruloylglucose, (E)-2-O-feruloylgalactaric, (E)-2-O-feruloylglucaric and (E,E)-2,4-O-diferuloylglucaric acids have been identified only in recent years (Reschke and Herrmann, 1981; Risch and Herrmann, 1988; Risch, et al., 1988). Naim and coworkers (Naim, et al., 1988) found the concentration of free ferulic acid in orange juice doubled after laboratory pasteurization. They also found considerably higher amounts of free ferulic acid in commercial orange juice which they attributed to processing effects. This study was undertaken to improve the methodology available to isolate bound forms

of phenolic acids from citrus juice. Since feruloylglucose is the predominant bound form of ferulic acid (Risch and Herrmann, 1988), the effects of processing and storage on feruloylglucose and other ferulic acid precursors in orange juice was also investigated.

Materials and Methods

Reagents and Standards - Optima® grade organic solvents from Fisher Chemical (Fairlawn, NJ, USA) and doubly distilled water were used. Ferulic, caffeic, coumaric acids and β -glucosidase were obtained from Sigma Chemical Co. (St. Louis, MO, USA), sinapic acid was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Both feruloylglucose and feruloylputrescine were purified using preparative HPLC.

Instrumentation - A Perkin-Elmer (Norwalk, CT, USA) model 410 quaternary gradient pump was used with a Hewlett-Packard (Waldbronn, FDR) series 1050 autosampler. Detection was accomplished with a Waters (Milford, MA, USA) model 490E multiwavelength detector (280 and 324nm) in series with a Perkin-Elmer LC-240 Fluorescence Detector (EX=335nm, EM=435nm). Chromatographic peak spectra were obtained using a Waters 990+ UV-visible photodiode array detector. Chromatographic data were obtained using a Spectra-Physics SP 4290 Integrator (Freemont, CA) in combination with an Epson Equity I+ XT computer used for data storage and retrieval.

Chromatography - An Analytichem (Harbor City, CA, USA) 5 μ C-18 column, 250mm x 4.6mm id was used for analytical separations. Solvent flow rate was 1 mL/min. Injection volume was usually 20 μ L. Initial mobile phase solvent composition consisted of 10% MeOH: 90% water (with 2% acetic acid). Four minutes after injection a linear gradient over two minutes was initiated, final concentration 18% MeOH. This concentration was maintained for 17 minutes. Then the amount of MeOH was increased linearly to 50% over the next 32 min. This concentration was maintained for 10 minutes before the MeOH was stepped to 100% MeOH and held for 5 min. Initial conditions were re-established using a 5 min. linear gradient.

Juice Extractor Pressure and Storage Study - Juice was extracted from the fresh Valencia oranges using commercial Food Machinery Corporation extractors. Extractor pressures were adjusted to produce a low yield "soft" squeeze, a typical commercial squeeze and a high yield "hard" squeeze. Commercially extracted Valencia orange juice was hot filled (91°C) into 12 oz tin plated

steel cans and cooled immediately with ambient temperature water before being placed into storage chambers set at 10, 20 and 30°C.

Sample preparation - The primary procedure was simply to centrifuge and filter (0.45μ) prior to injection. Glucosidase treated juice was adjusted with NaOH to pH 5 before the enzyme was added and incubated at 37°C for 3 hr. For the heating/ pasteurization experiments, clarified juice samples were placed in a beaker of boiling water for 15 min. after the juice reached 98°C. Samples were filtered before injection.

Results and Discussion

In an earlier study, Winter and Herrmann (Winter and Herrmann, 1984) developed a polyamide open column procedure to quantitatively separate plant extracts into fractions containing either the hydroxycinnamic acid derivatives of glucose or the corresponding esters with quinic acid. This procedure was subsequently used to determine the hydroxycinnamic esters in orange peel and whole fruit (Risch and Herrmann, 1988; Risch, et al., 1988; Risch, et al., 1987). We attempted to use this procedure as it would isolate the compounds of interest from the complex matrix of orange juice, thus simplifying subsequent chromatography. Using domestic polyamide material we were not able to duplicate their work. Therefore, it became necessary to modify the chromatographic conditions to separate the additional materials that would be present in a direct injection of the juice. To compensate for the additional material, fluorescence detection was utilized as it would be more specific for the compounds of interest. The resulting chromatographic separation is shown in the top two traces in Figure 4.

As with most amines, feruloylputrescine tails severely. The flavanone glycosides narirutin and hesperidin have their maximum UV absorbance at approximately 280 nm, but have some absorbance at 324 nm. The fluorescence chromatogram from the same juice treated with β -glucosidase is shown on the lower trace in Figure 4. It should be noted that the feruloylglucose peak disappears completely with a concomitant increase in free ferulic acid. Similarly, the sinapylglucose peak is greatly diminished and a corresponding increase in free sinapic is observed.

The many small peaks between 14 and 20 minutes shown in Figure 4 appear to be bound forms of ferulic and coumaric acids. This deduction was reached from analyzing the results of several independent experiments. First, when the alkaline hydrolysis of these peaks were reinjected and

Figure 4: Chromatograms of fresh Valencia orange juice before and after treatment with β -glucosidase.

only ferulic and coumaric acid peaks were observed. Secondly, the spectral characteristics of these peaks were determined using a photodiode array detector; the overall shape of the absorbance spectrum and the position of maximum absorbance was consistent with what one might expect for bound forms of ferulic and coumaric acids. As noted in Figure 4, these peaks are not effected by β -glucosidase, indicating that they are not glycosides. Since (E)-2-O-feruloylgalactaric acid, (E)-2-O-feruloylglucaric acid and (E,E)-2,4-O-diferuloylglucaric acid had been recently identified by Herrmann and coworkers (Risch, et al., 1988; Risch, et al., 1987) but were not identified in the chromatogram (Figure 4), these peaks may be due to these compounds. However the extremely low concentrations reported for these compounds suggest there may be additional bound forms of ferulic acid which have yet to be characterized.

Heating/ Pasteurization

Peleg and coworkers (Peleg, et al., 1988) studied the stability of feruloylglucose and feruloylputrescine in model orange juice solutions at 75°C for up to three hours. They detected ferulic acid within 30 minutes of heating the feruloylglucose solution but the feruloylputrescine solution required 180 min. of heating before any ferulic acid was observed. This suggests that feruloylputrescine has greater thermal stability than feruloylglucose. As shown in Figure 5, no difference in feruloylputrescine, feruloylglucose or sinapylglucose peaks was observed as a result of boiling fresh juice for up to 15 min.

Figure 5: Fresh (raw) Valencia orange juice before and after heating for 15 min.

This suggests that these compounds are relatively heat stable and are unlikely to decompose during typical industrial pasteurization conditions. It should be noted that there were changes in the peaks labelled 1-3.

Figure 6: Effect of increasing pressure of juice extractor on bound forms of ferulic acid.

Extractor Pressure Study

As shown in Figure 6, increasing extractor pressures increased the concentrations of feruloylputrescine only. Feruloylglucose concentration was not effected by extractor pressure. Feruloylputrescine concentrations increased from 8 ppm with the soft squeeze to 13 ppm for the hard squeeze. Commercial extraction conditions produced a juice with 10 ppm. Feruloylglucose concentrations averaged 10 ppm with all extractor pressures.

Storage Studies

Concentration changes versus storage time at 30°C are shown in Figure 7. Feruloylglucose concentrations appear to decrease slightly but erratically with increasing storage time. Ferulic acid concentrations are much lower, increasing from 0.15 ppm to 0.9 ppm after 12 weeks storage. Since feruloylglucose appears to decrease as ferulic acid increases, it is likely that feruloylglucose is a probable source for ferulic acid during long term storage. There was no discernable change in either ferulic acid or feruloylglucose in juices stored at 10 and 20°C for the same period of time.

Figure 7: Florida Valencia orange juice stored in cans.

Conclusions

Feruloylglucose and feruloylputrescine (the precursors to ferulic acid and hence PVG) are heat stable. Normal commercial pasteurization will not produce increased levels of ferulic acid from the thermal decomposition of these compounds. Concentrations of feruloylputrescine can be increased by increasing extractor pressure whereas feruloylglucose concentrations are independent of extractor pressure. Feruloylglucose concentrations did not change during 12 weeks storage at 10 and 20 °C but at 30°C feruloylglucose decreased slightly and ferulic acid increased slowly. This suggests that ferulic acid accumulation and the accompanying formation of PVG can be minimized by storing juices at or below 20°C.

STUDY II: SEPARATION AND DETERMINATION OF HYDROXYCINNAMIC ACIDS AND p-VINYLGUAIACOL BY HPLC

Early studies (Heimann, et al., 1971; Schmidlein and Herrmann, 1975) employed TLC to separate and quantify hydroxycinnamic acids. Spots were scraped off the plates and hydroxycinnamic acids quantified using spectrophotometric techniques. Later, GLC procedures were developed (Schulz and Herrmann, 1980), but required the acids to be derivitized into more volatile forms. HPLC is the current procedure of choice because it requires minimal sample preparation, is relatively rapid and allows easy quantitation. HPLC procedures have been developed to determine hydroxycinnamic acids and/or hydroxycinnamic acid esters in orange and grapefruit juices and peels (Naim, et al., 1988; Winter and Herrmann, 1984), wine (Baranowski and Nagel, 1981; Wulf and Nagel, 1976), beer (Kenyhercz and Kissinger, 1977), tea (Bailey, et al., 1990) and cereal grains (Hahn, et al., 1983; Pussayanawin and Wetzal, 1987). Since PVG is such a potent deleterious compound in citrus juices and ferulic acid is its putative precursor, a procedure to quantify ferulic acid would be a useful quality control tool. Juices found to be high in ferulic acid could then be channelled into products unlikely to be stored under non-refrigerated conditions.

The purpose of Study II was to optimize the HPLC separation of the cinnamic acids and PVG in citrus juice, thus reducing overall analysis time. The experimental approach, results and discussion are detailed in the following three reprints (Rouseff et al., in: *Flavour Science and Technology*, 1990; Rouseff et al., *J. Agric. Food Chem.*, 1992; Rouseff et al., *J. Chromatog. Sci.*, 1992). Of all the factors that influence chromatographic resolution, solvent selectivity is the single most influential feature. Procedures have been developed to optimize solvent selectivity (Glajch, et al., 1980; Lehrer, 1981) based on the separations observed from binary, ternary and quaternary mixtures of solvents composed of widely differing solvents. Here we apply solvent optimizing techniques to select the best solvent or combination of solvents which would improve the separation of the four hydroxycinnamic acids which are found in orange juice. Solvent optimization techniques were used to maximize chromatographic resolution between sinapic, coumaric, ferulic and caffeic acids and components in orange juice. Various reverse phase solvent systems consisting of binary, ternary and quaternary combinations of methanol, acetonitrile and tetrahydrofuran (THF) with water were evaluated. Greatest chromatographic resolution was realized from binary mobile phases consisting of THF and water and ternary solvents containing THF, methanol and water. Resolution between the least resolved peak pair was improved 46%

using the binary THF mobile phase compared to the best previously reported mobile phase. The THF, methanol and water mobile phase produced the greatest resolution between the four acids of interest.

As indicated before, the taste threshold of PVG in orange juice is 50 ppb (Tatum, et al., 1975). Concentrations several times greater than this have been found in stored orange juices (Lee and Nagy, 1990; Naim et al., 1988; Tatum, et al., 1975). As it is reasonably volatile and found in low concentrations, PVG was usually determined by GLC with flame ionization detection. For greater sensitivity, it can be derivitized and measured with electron capture detection (Villarreal, et al., 1986). Recently, it has been shown that PVG can be determined by HPLC with fluorescence detection (Lee and Nagy, 1990). Reported detection limits were 10 ppb PVG. Since it would be advantageous to evaluate both present and potential deleterious aroma in orange juice, we also develop (Rouseff et al., J. Chromatog. Sci., 1992) a rapid solid phase extraction and subsequent HPLC separation to quantify both ferulic acid and PVG.

A simplified, rapid method to determine both immediate and potential off odors due to PVG and its odorless precursor ferulic acid was developed. Orange juice sample preparation consists of a simple, C₁₈ solid phase extraction. Utilizing a 5 μ , 25 cm, C18 column, both compounds can be separated within 40 min using a one step linear gradient beginning with an aqueous 12% THF/5% acetonitrile mixture and ending with 35% aqueous THF. Fluorescence detection with wavelength programming offers optimal sensitivity and selectivity. Recoveries of PVG and ferulic acid range from 90 to 103%. Detection limits are 1 ppm and 5 ppb for ferulic acid and PVG, respectively. Other hydroxycinnamic acids such as coumaric, sinapic and caffeic acids may also be determined from the same chromatogram.