

Historical Review of Citrus Flavor Research during the Past 100 Years

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Citrus juices are a complex mixture of flavor and taste components. Historically, the contributions of taste components such as sugar (sweet) and acid (sour) components were understood before impactful aroma volatiles because they existed at higher concentrations and could be measured with the technologies of the 1920s and 1930s. The advent of gas chromatography in the 1950s allowed citrus researchers to separate and tentatively identify the major citrus volatiles. Additional volatiles were identified when mass spectrometry was coupled to capillary GC. Unfortunately, the major citrus volatiles were not major influences of citrus flavor. The major aroma impact compounds were found at trace concentrations. With the advent of increasingly more sensitive instrumental techniques, juice sample size shrank from 2025 L in the 1920s to 10 mL today and detection limits fell from percent to micrograms per liter. Currently gas chromatography–olfactometry is the technique of choice to identify which volatiles in citrus juices possess aroma activity, determine their relative aroma strength, and characterize their aroma quality but does not indicate how they interact together or with the juice matrix. Flavor equations based primarily on nonvolatiles and other physical measurements have been largely unsuccessful. The most successful flavor prediction equations that employ instrumental concentration values are based on a combination of aroma active volatiles and degrees Brix (sugar) values.

KEYWORDS: GC-O; odor activity values; matrix effects; flavor models; flavor reconstitution; GC-MS

INTRODUCTION

Citrus juices are among the most heavily consumed fruit juices because of their combination of desirable flavor, appealing color, and health benefits. Citrus originated in Southeast Asia, and its beginning predates recorded history. It is thought that sweet oranges may have originated in India, the trifoliolate orange and mandarin in China, and acid citrus types in Malaysia. One of the earliest written references to citrus is found in ancient Chinese literature in a book titled “Yu Kung” or “Tribute of Yu” (Emperor Ta Yu, 2205–2197 B.C.), in which both oranges and pummelos (an ancient parent of grapefruit) were mentioned as part of the tribute to the emperor (1). The earliest mention of citrus in Europe was in 310 B.C. by Theophrastus, the father of botany, who provided a taxonomic description of the citron (2). Citrus was later spread throughout Asia, North Africa, and Europe through the conquests of Alexander’s and later Muslim armies as well as through trade. Citrus was brought to the Americas by explorers such as Christopher Columbus in the late 1400s to early 1500s. Ponce de Leon introduced the first orange trees in St. Augustine, FL, sometime between 1513 and 1565. Grapefruit was first established in Florida in 1823 by Don Philippe (a Spanish nobleman), who planted the first grapefruit

grove near Tampa, FL. During the many centuries of human cultivation, numerous citrus cultivars have been developed through selection of natural mutations as well as intended hybridizations to produce a wide range of citrus cultivars blurring the distinction between species.

Orange juice is the most popular juice in America, where on average, Americans consume more than 2.5 times more juice than its nearest competitor, apple juice (3). Orange juice consumption increased dramatically during the 1940s with the introduction of frozen concentrated juices. Grapefruit juice was the second most popular juice in 1999. Although consumption was not as high as it had been in the 1970s, it still accounted for 7% of all fruit juice consumption. The sale of grapefruit juice in not-from-concentrate form helped boost consumer demand at that time. However, grapefruit juice consumption has fallen in recent years due to grapefruit–drug interactions that affect those consumers over 55 years of age, the largest single demographic group of heavy grapefruit juice consumers. Lemon juice consumption declined in 1999, whereas lime juice consumption remained unchanged.

This study will examine the historical development in the understanding of citrus juice flavors. Orange juices will be discussed in the most detail because they are by far the most widely consumed citrus juice, and for that reason, most citrus flavor research has concentrated on orange juice flavor. Grapefruit juice flavor will also be discussed. Juice essential oils and peel

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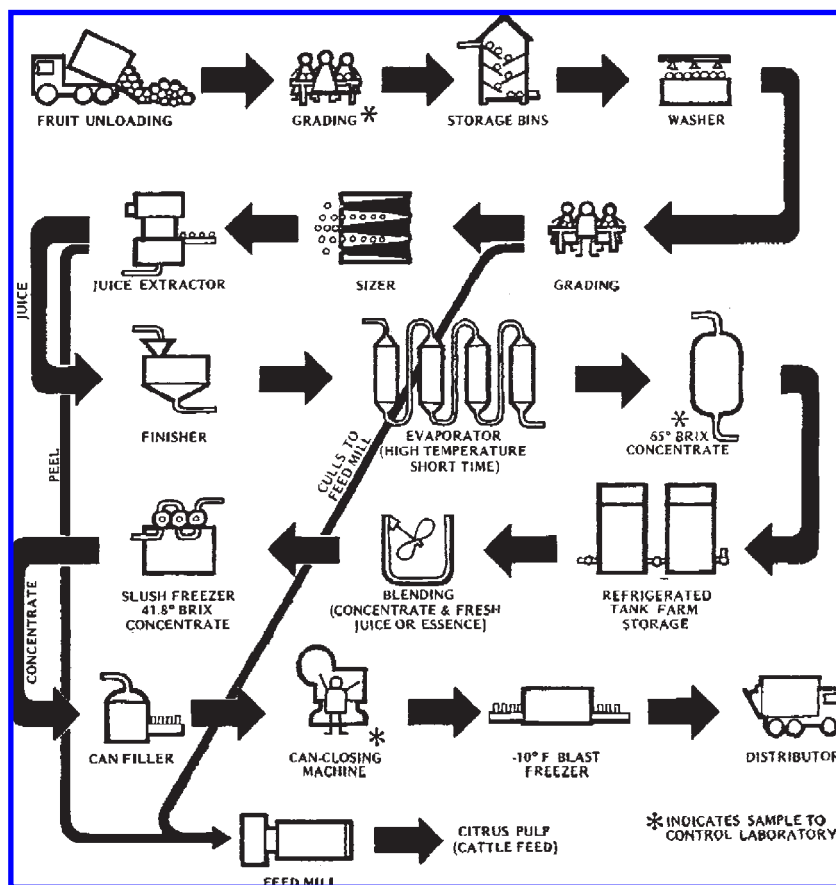


Figure 1. Schematic of how citrus fruit is processed into frozen concentrated juice. During the 1950s to the late 1980s most orange juice was sold as frozen concentrated juice. Today the majority of frozen concentrated juices are no longer canned, but shipped in bulk and reconstituted (and reheated) before being packaged into multilayer gable-top or PET containers. Reprinted from R. F. Mathews, UF/IFAS EDIS publication FS8, 1994. Copyright 2009 University of Florida.

oils will also be discussed only to the extent that they are components of juice flavor. The historical development in understanding citrus flavors will be discussed in terms of chemical methods of analysis, reconstructing citrus flavors, developing of juice flavor models, and sensory analysis. Current views of orange juice aroma volatiles have been recently reviewed for both fresh-squeezed (4) and processed orange juice (5) and will not be discussed here.

PROCESSED VERSUS FRESH-SQUEEZED FLAVOR

In a discussion of the flavor of citrus juices it is important to distinguish between fresh-squeezed and commercially processed juices. The flavor of fresh-squeezed citrus from sound, fully mature fruit is the standard by which all processed juices are judged. In the case of fresh-squeezed juice from fully mature fruit, all of the natural aroma and taste compounds are initially present in pleasing portions. However, the aroma quality of fresh-squeezed juice is unstable due to a combination of chemical, enzymatic, and possible microbial reactions. The flavor of fresh-squeezed juices changes rapidly with time, and steps must be taken to stabilize these juice systems. To study fresh juice volatiles, salt is added immediately after the juice is squeezed to inactivate enzymes without heating. Juices stabilized using this technique could not be used for sensory analysis other than aroma evaluations.

The pleasant odor of freshly squeezed orange juice is distinctly different from that of many commercial juices found in the marketplace. There is also a wide flavor range among the various types of commercial orange juices. Differences between commercially processed juices are due to the combined effects of fruit

cultivar and maturity, time–temperature conditions used to stabilize the juice, the number of times it has been heated, if the juice has been concentrated, and, if concentrated, how well the volatiles lost during concentration have been restored. In addition, storage time–temperature conditions and container type will have a profound impact on juice flavor at the time of consumption. Although alternate processes have been developed, almost all commercially produced orange juice is thermally processed because thermal processing is still the most cost-effective means to reduce microbial populations and enzyme activity. However, thermal processing will reduce the concentrations of some of the original juice volatiles as well as induce a complex series of chemical reactions that can ultimately produce odors foreign to freshly expressed juice (6).

The process by which juice is liberated from the fruit during juice extraction and converted to a commercial product is shown schematically in **Figure 1**. Citrus juice is a complex chemical mixture containing many compounds previously separated within the intact fruit but now free to interact. In addition, peel oil from the flavedo is commingled with the juice when the peel is broken during mechanical juice extraction. The juice also contains a natural indigenous amount of oil, which is slightly different in composition from that from peel (flavedo) oil. Some commercial extractors can induce larger amounts of peel oil than others because they macerate more of the peel in the juicing process. Therefore, the type of commercial extractor and extractor pressures will determine the relative levels of peel versus juice oils and the composition of the juice volatiles and, therefore, the overall flavor of the juice. Hand extraction is always the mildest extraction condition and will usually contain the least amount of

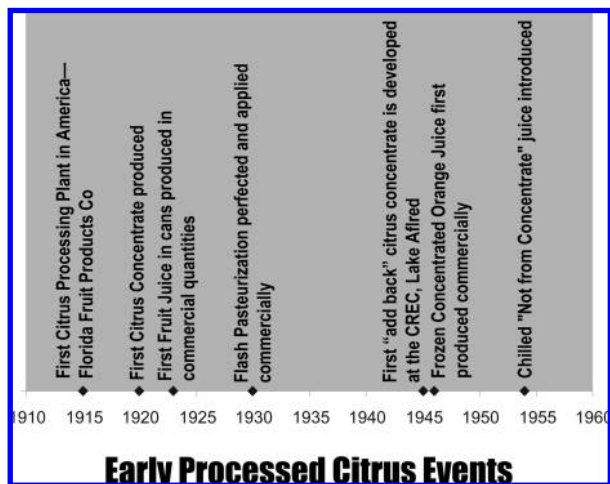


Figure 2. Time line of technological advances in processed citrus juices.

peel oil. However, it has been demonstrated that even hand extraction can introduce small amounts of peel oil components into the juice (7, 8). Mechanically squeezed orange juice contains higher concentration levels of certain aldehydes (octanal, nonanal, and decanal) and terpenes (mainly, limonene, myrcene, and linalool) than fresh hand-extracted juices, as they are also present in the peel oil (9, 10). Consequently, mechanically squeezed orange juices will have a sensory profile somewhat different from that of comparable hand-squeezed juices.

The flavor quality of commercial citrus juices has improved dramatically in the past few decades. Shown in **Figure 2** is a time line indicating when various citrus products were developed. Canned citrus juices were commercially produced in the 1920s. They were hot-filled to sterilize the container and air-cooled to prevent surface rusting. This prolonged time at high temperatures profoundly affected juice quality to the point that it barely resembles the flavor of the original juice. In the 1930s flash pasteurization reduced the time citrus juices were at high temperatures and preserved more of the initial flavor attributes. The first frozen concentrates were produced in the 1940s and became the flagship product of citrus juices as it greatly improved the flavor of the final juice because it stayed frozen until it was to be consumed. In the 1950s the first not-from-concentrate juice was developed. It possessed superior flavor quality because the initial fresh flavor components were little disturbed by the single heating and subsequent refrigerated storage. Most of the flavor volatiles were removed along with the water in the production of frozen concentrate, and not all commercial juice manufacturers went through the expense of fully restoring them.

EARLY STUDIES

As shown in **Figure 3**, the laboratory equipment and technology to analyze the flavor of citrus juices in the early part of the last century were limited to simple, straightforward analyses such as titrations to determine acid levels and hydrometers to determine soluble solids, which are primarily sugars (measured as degrees Brix). Equipment was limited to balances, burets, microscopes, and distillation glassware. Acid and sugar levels are important in determining the flavor balance between sweet and sour, which is one of the major taste attributes of citrus juices. The sugar acid ratio is also highly correlated to fruit maturity (11) and development of flavor volatiles. Flavor volatiles are secondary metabolites formed during normal ripening and maturation largely from fatty or amino acid precursors (12–14). Although the technology to determine sugar and acid levels is old, it is a simple, rugged



Figure 3. 1920s citrus research laboratory at University of Florida Citrus Research and Education Center in Lake Alfred, FL.

technology that is still employed in many citrus quality control laboratories as an initial determination of fruit quality (15–17).

Distillation is a separation process known to chemists since the middle ages. Shown in **Figure 4** is one of the early separation schemes to isolate the flavor volatiles in citrus juice (18). In the case shown, the separation is for Valencia orange juice, one of the most widely planted and highest quality orange cultivars. It is worth noting that because of limited analytical sensitivity, incredibly large volumes of the material of interest had to be used to concentrate materials of interest to levels at which they could be measured. In the 1925 study, over 2000 L (500 gal) was used as starting material. The juice was initially distilled under partial vacuum because it had been previously noted that most of the orange juice aroma was associated with the distillate. Separations achieved in **Figure 4** were based on boiling point differences, solubilities, and a process called cohobation. Cohobation is a term that was used in premodern chemistry and alchemy. It was the process of repeated distillation of the same sample, with the distillate being poured back again and again upon the matter left at the bottom of the flask. Because modern instrumental techniques such as NMR and MS were not available, final identifications were based upon melting point or boiling point values of derivatives of the final isolated fractions as well as carbon, hydrogen, and oxygen analyses, which provided molecular formulas but not structure. The technology at this time was extremely limited, requiring huge amounts of starting materials as well as exacting, time-consuming procedures. In the case shown in **Figure 4**, only 11 compounds were identified with limited certainty, and 2 alcohols (geraniol and terpineol) were tentatively identified. Using these techniques several major alcohols and esters in citrus juices were identified; however, identifications were not exact in many cases as, for example, one alcohol was reported as an "olefin alcohol, C₁₀H₁₈O, bp 92–3 Deg, n_D20 1.4650". Unfortunately, most of the important aroma compounds in citrus juices remained unidentified until gas chromatography was invented in the 1950s.

ANALYTICAL CHEMISTRY

Sample Size. The sensitivity of early chemical instrumentation required that large volumes of starting material be used to be able to measure compounds found at trace levels. Because most citrus aroma volatiles are found at trace ($\mu\text{g/mL}$) levels as much as 2025 L of juice was employed in early studies (18). As instrumental sensitivity improved, the required amounts of initial juice became smaller. Even after the advent of gas chromatography and mass spectrometry, 100 L of grapefruit juice was required to identify sulfur volatiles found at nanogram per milliliter levels (19). However, with further improvements in instrumentation,

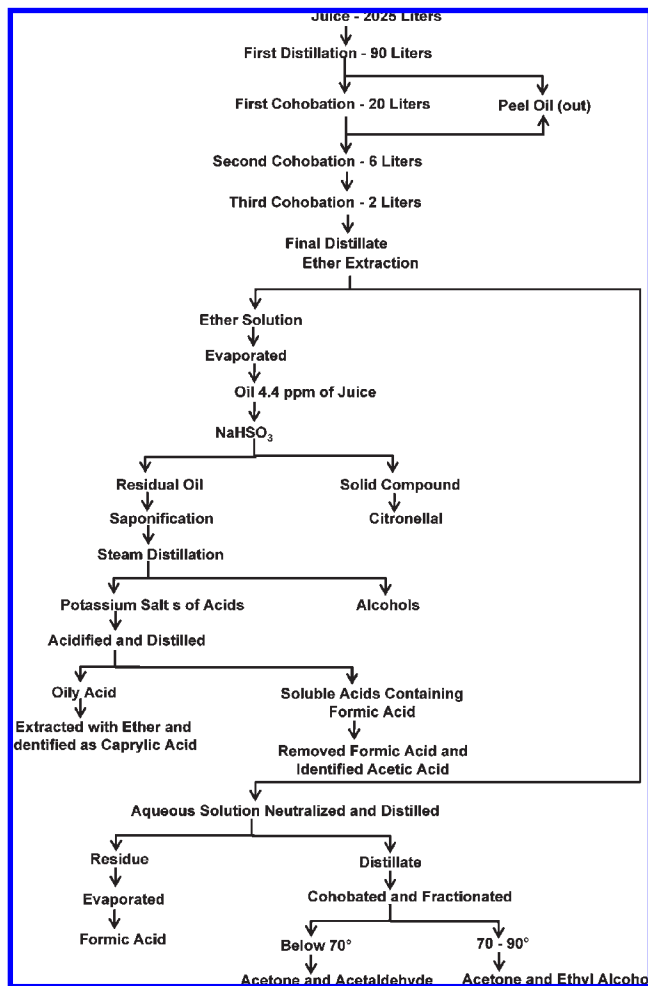


Figure 4. Example of an early study of the volatile constituents in Valencia orange juice. Adapted from ref 18.

these same volatiles can now be analyzed using as little as 10 mL of juice (20).

Importance of Trace Volatiles. Almost all of the early flavor research was focused on the idea of determining the chemical composition of specific juices. It had been reported in the early 1930s that flavor and chemical composition were related (21). Therefore, the thinking at the time was that if one could duplicate the chemical composition of the juice, one could duplicate its flavor. As a result, most research efforts were dedicated to determining the chemical composition of various citrus juices. The expectation was that once the compounds in the juices were quantified, then one could duplicate the flavor of these juices simply by reconstructing the major juice components in the proper concentrations.

Just as attempts to duplicate the complex aromas/flavors from rose oil (22) and bread (23) failed to duplicate the original aroma even when >90% of the compounds were quantified (24), citrus juice aromas could not be duplicated even though >98% of total juice volatiles were duplicated. Those compounds found in highest concentration such as limonene had little direct aroma impact but had been a required component in every successful flavor recombination study. It was the compounds found in low concentrations, particularly aldehydes and esters, that appeared to have the most impact on citrus flavors. For most food systems the flavor impact compounds are present only in trace amounts.

Chromatography. In the 1950s and 1960s the newly developed gas-liquid chromatography was employed to determine the

volatile composition of many juices (25–27). However, it was soon realized that the flavor of many citrus juices was associated with the peel oil or essential oil recovered from the concentration process. It was easier to analyze these concentrated flavor sources as the aroma compounds were present at higher concentrations. Therefore, many of the studies were directed at analyzing citrus peel oils (28–33) or essence oils (34–37). The chromatographic resolution of packed columns was relatively low, which limited the identification to as little as 31 volatiles (38). With the advent of open tubular capillary columns chromatographic column efficiency increased and the number of compounds that could be separated in citrus oils increased to over 100 compounds (39).

Coupled GC and MS. It must be kept in mind that gas chromatography, no matter how efficient, is only a separation technique. It does not directly identify the compounds that are separated. Possible or tentative chromatographic peak identifications can be made by determining retention index values based on a series of linear alkanes or ethyl esters using two or more dissimilar chromatographic materials. Volatile identifications were greatly facilitated when gas chromatography was coupled to mass spectrometry (40–43). Many forms of mass spectrometry can be employed for identification. Traditionally, the fragmentation pattern from an electron impact (EI) quadrupole MS is used for identification as most MS software libraries employ this type of MS. Ion trap MS fragmentation libraries are more limited in terms of numbers of volatiles in the library and are not employed as often in flavor research. Increased selectivity can be obtained using multidimensional MS as evidenced by the successful analysis of vanillin in a variety of citrus juices using this technique (44). Single ion monitoring has been employed in the analysis of trace aroma active compounds in orange juices (45), but identification using full spectra matching is no longer a possibility as only a single ion is monitored. This technique assumes that only the compound of interest elutes at the same time as the compound of interest.

NONVOLATILE FLAVORS

As previously discussed, the compounds responsible for the characteristic sweet-sour balance in citrus juices were understood to be due to sugar and acid levels by the 1930s. However, bitterness in citrus juices was understood much later. Although bitterness was observed in both orange and grapefruit juices, the major compounds responsible for bitterness were different in each case. The major bitter component in grapefruit was found to be naringin, a flavanone glycoside (46). However, bitterness in oranges was more difficult to understand as naringin was not present. Furthermore, the bitterness was not immediately apparent, but observed in only certain cultivars and was more pronounced in early-season fruit. This delayed bitterness was a puzzlement to citrus scientists for decades. The causative agent was established as limonin, a triterpenoid dilactone. Limonin was known to impart bitterness and was isolated from orange juice in 1938 (47). However, its complete structure was not known until 1960 (48). Limonin has been found in almost all citrus juices, but its concentration is below taste thresholds for most mature citrus cultivars of commercial interest. It is also found in grapefruit and sour/bitter orange, but is obscured by the more abundant bitter naringin. It was not until 1968 (49) that it was shown that the delayed bitterness was due to an acid-catalyzed conversion of the tasteless limonin A-ring lactone to the intensely bitter dilactone. Numerous analytical methods have been developed to determine concentrations of this important flavor component (50–53). The reported taste threshold of limonin in orange juice is 6.5 mg/kg (54) but can be altered by sugar, acid, and oil levels. Limonin concentrations generally decrease as the fruit becomes

more mature, apparently due to conversion of the A-ring lactone to the tasteless limonin glucoside (55).

HUMAN PERCEPTION/ THRESHOLD CONCEPTS

After attempts to reconstruct the flavor of several food systems based on analytical concentrations of major volatiles were unsuccessful, more emphasis was directed toward identifying just which compounds were aroma active. Food flavors were found to be almost exclusively from compounds found in trace quantities (56). However, aroma thresholds could be determined from human taste panel studies. Therefore, instrumental concentration values could be compared with human threshold values to determine if a particular volatile would be aroma active. To determine the relative intensities of those compounds found to have aroma activity, the concept of odor activity value (OAV) was developed (57). Compounds found to be several hundred times greater than their thresholds would be given values greater than those of compounds that barely exceeded their thresholds. Tamura and co-workers employed this concept to characterize orange juice (58). This approach has been successfully employed in a number of food systems. However, this approach suffers from several potential problems. First, most, if not all, aroma impact compounds must be known. Second, there must be a procedure to quantify each. These analyses are often difficult because many aroma active volatiles exist at nanogram per milliliter levels or lower. The threshold value can be difficult to determine because the matrix employed in diluting the compound of interest can profoundly affect the threshold value. Panelist training has also been shown to directly influence the threshold value. In general, thoroughly trained panels produce lower thresholds than panels with little or no training. An alternative to the OAV approach has been developed; this newer approach determines aroma activity directly by using a human assessor at the end of the chromatographic column. The human assessor then performs a bioassay by indicating when he or she smells something and describes the odor quality. This technique is called gas chromatography–olfactometry (GC-O).

GC-O. Many odor active compounds exist as low-level volatiles that are difficult or impossible to detect using typical FID or MS detectors. Thus, the chromatogram profile does not represent the aroma profile of the food; it represents only those volatiles found at higher concentrations. The human nose has a theoretical odor detection limit of about 10^{-19} mol (59) for some volatiles, making it a useful tool to detect odorants in a GC effluent. However, it is not a general detector as it cannot detect some volatiles such as CO_2 . Therefore, the human nose is a highly selective detector. GC-O is a technique designed to employ the enormous separation powers of capillary gas chromatography with the unique selectivity and sensitivity of the human nose. Various forms of GC-O have been developed but will not be discussed in this paper. Basically, GC-O indicates the number of aroma active compounds in a sample, their relative strengths, and their aroma quality. It does not identify the aroma active compounds directly. Possible identifications can be based on calculated retention index values using two or more dissimilar chromatographic columns. However, additional information is needed before identifications can be made with assurance. This technique has been used to identify aroma active compounds in both orange (60, 61) and grapefruit juices (62, 63) and in many subsequent citrus juice studies. GC-O has become the method of choice in studying the aroma of citrus juice, oil, and essence.

MATRIX EFFECTS/AROMA RELEASE

Citrus juices would be considerably easier to study if they were pure solutions consisting of dissolved solids in water. As it is,

citrus juices are liquid–solid suspensions. The solid material is a heterogeneous mixture of cell wall material, crystalline hesperidin, and embryonic seeds commonly called cloud or pulp (64–66). Particle size ranges widely. The effect of the pulp is to alter the mouthfeel of the juice as well as the headspace concentration of specific volatiles. It was first reported in the 1970s that most terpenes were adsorbed on the pulp but that most aldehydes and lower molecular weight esters were found almost exclusively in the serum (67). Subsequent studies of aroma volatile distributions have found that coarsest pulp (insoluble particles with a diameter of $>2\ \mu\text{m}$) retained large amounts of terpenes and aldehydes, whereas the finest insoluble particles (with a diameter of $<2\ \mu\text{m}$) also showed a strong retention effect on ethyl butanoate or hexanal (68). There is some disagreement in the findings between these two studies even though they both employed fresh-squeezed (unheated) juices, but the earlier study filtered the juice through cheesecloth before analysis, which might account for some differences. Nevertheless, there is common agreement that the amount of pulp/cloud in the juice will affect the juice's headspace volatiles and perceived aroma.

Using model solutions with thickeners such as sucrose, guar gum, and carboxymethylcellulose, it was found that headspace concentrations of highly volatile compounds decreased as viscosity increased (69). Furthermore, it was found that the type of thickening agent influenced headspace concentrations for equal viscosity solutions. For example, headspace concentrations of α -pinene, a terpene commonly found in citrus juices, were reduced by 36% compared to water when carboxymethylcellulose was used as the thickener. However, when sucrose was used to prepare a solution of equal viscosity, α -pinene headspace concentration decreased 86% compared to water. These findings indicate that both viscosity and chemical structure influence the degree to which volatiles will be retained or bound by particular foods. Thus, the sugars in citrus juices (typically 9–12%) will have a profound effect on the headspace concentrations of the more volatile juice components.

It has been suspected for some time that the sugars and suspended solids in citrus juices alter headspace concentrations and aroma thresholds of many citrus volatiles (70). Therefore, aroma thresholds for most volatiles were higher in citrus juices than in water (in which most aroma threshold values are evaluated) (71). It was also a factor in adjusting the flavor volatiles added to low-calorie citrus juice drinks (technically beverages) introduced in the 1990s, for which juice content ranged from 5 to 30%. Sugar substitutes and thickeners did not retain volatiles in the same manner or extent as 100% juice.

RECOMBINATION STUDIES

GC-O does not provide information about how an odor volatile interacts with the food matrix or with the other volatiles when combined in a food product. Recombination of odor active compounds in the food matrix to match the original aroma of the food and subsequent sensory analysis can be used to prove the correct selection of odor active compounds as final step in aroma analysis. Studies employed taste panels to test models against a reference juice. The idea is to formulate a synthetic blend (aroma model) that would be compared with a reference juice for evaluations for similarity or difference. This is a general technique employed by flavor scientists trying to duplicate the odor profile of a certain food based on key or essential ingredients. Another variation of flavor match is to ask the panel to express degrees of difference when one or more of the “key ingredients” is left out. These are called omission tests (72) and can be used to evaluate the relative value of ingredients thought to be essential in matching a juice flavor. There have been a few orange juice

reconstitution experiments to determine those components responsible for orange flavor. Early studies incorporated recombination of only a few compounds (73, 74). These authors concluded that deodorized orange juice base (sometimes called pump-out) added with acetaldehyde, citral (geranial and neral), ethyl butanoate, *d*-limonene, and octanal in the proper portions received the highest score by the sensory panel.

Tamura and co-workers analyzed orange juice volatiles and thresholds to reconstruct the orange juice aroma using 11 volatiles with the highest OAV values (limonene, linalool, octanal, decanal, dodecanal, geranial, neral, myrcene, α - and β -sinensal, and citronellal) and reported that this combination very closely duplicated the aroma of navel oranges (58).

In a more recent orange juice study, the aroma active volatiles were determined using aroma extract dilution analysis (AEDA) GC-O (45). Twenty-five odor active compounds were quantified using stable isotope dilution analysis. A total of 22 volatiles were employed to reconstruct orange aroma. Deodorized orange juice was not used as the juice matrix; rather, a synthetic mixture of sugars, acids, and sunflower oil with lecithin as an emulsifier was used to duplicate the juice matrix. Only when 0.1% fat (sunflower oil) was added did the model resemble the aroma of the original juice. The 22 volatiles employed were as follows: acetaldehyde, ethyl 2-methylpropanoate, (*R*)- α -pinene, ethyl butanoate, (*S*)-ethyl 2-methylbutanoate, hexanal, (*Z*)-hex-3-enal, myrcene, (*R*)-limonene, 3-methylbutanol, 2-methylbutanol, ethyl hexanoate, octanal, oct-1-en-3-one, nonanal, decanal, (*S*)-linalool, butanoic acid, (*R*)-ethyl 3-hydroxyhexanoate, (*E,E*)-deca-2,4-dienal, *trans*-4,5-epoxy-(*E*)-dec-2-enal, and vanillin. Noticeably missing were geranial, neral, and α - and β -sinensal used in the Tamura study, which may have been due to a different orange cultivar juice used or differences in sample preparation (volatile extraction).

FLAVOR MODELS

Juice flavor models from the 1970s were based on correlating analytical measurements with taste panel scores using various statistical techniques. Their objective was to employ chemical or physical measurements in an equation that would objectively predict a taste panel's preference score. One of the earlier reports attempted to develop an objective flavor prediction model using 24 chemical or physical measurements and hedonic sensory values from a large survey of Florida orange juices from the 1972–1973 and 1973–1974 seasons using an untrained but experienced 12-member taste panel (75). Their measurements included degrees Brix, percent acid, reflected color, HPLC limonin, total sugars, sucrose, pulp, oil, etc., but did not include GC volatile analyses. They applied multiple regression analysis on these measurements and could fit the data set fairly well if enough variables were employed in the regression equation. However, the equations did not predict flavor well when used on juices that were not part of the training set. Carter and co-workers (76) expanded their training set over four seasons of juice data using similar orange juice measurements but employing "forward selection" regression analysis resulting in different equations but with the same inability to accurately predict flavor of juices from outside the training set. With hindsight one can say that the problem was that their models were based on substances that correlated with taste only but little which would correlate with aroma.

In the 1980s Carpenter and co-workers examined the effect of juice volatiles by applying multivariate statistics to GC profile data to determine which peaks were correlated with juice quality (77). They examined differences in the volatile components (chromatographic peak areas) between three commercial juice

types. Not all of the peaks were identified, but identification was not required with their approach. Again, good correlations were obtained when the training set was modeled, but the equations developed were ineffective in modeling commercial juices not used in the training set.

Few studies have employed multivariate statistics on GC and sensory panel data. Pino examined GC profile data from heated and unheated orange juices and observed that seven volatiles were altered in the thermal process (78). Employing linear regressions, he found strong correlations between taste panel hedonic scores and myrcene, 2-hexanol, linalool, and α -terpineol and predicted they could be used to predict the sensory quality of orange juice. In the late 1990s, Petersen and co-workers employed partial least squares (PLS) to examine instrumental and sensory data from a storage time–temperature study of reconstituted orange juice (79). Eleven chromatographic peaks with high loadings were selected for the final PLS model. The 11 peaks were α -pinene, 2-methyl-3-buten-1-ol, octanal, 3-methyl-2-buten-1-ol, nonanal, acetic acid, furfural, linalool, *p*-terpineol, α -terpineol, and carveol. GC-sniffing was employed and identified α -pinene, octanal, acetic acid, furfural, linalool, and α -terpineol as being aroma active. Using the PLS equation it was reported that a trained panel's average score for orange taste, orange odor, oxidized taste, oxidized odor, and bitterness could be predicted with a high degree of certainty from measurement of these 11 volatiles. However, no juices other than those used in the training set were evaluated.

More recently, Elston employed a sensory-driven multivariate technique to evaluate the sensory quality of a wide range of commercial orange juices consisting of not from concentrate juices, refrigerated reconstituted from concentrate juices, and canned reconstituted from concentrate juices (80). Instrumental variables were limited almost exclusively to those that had demonstrated aroma or taste activity. A total of 49 aroma active components were observed using GC-O of orange juice ethyl ether extracts. Principal component analysis (PCA) loading values near zero were used to eliminate half of the aroma active compounds. Only 18 of these could be quantified. Results for these 18 variables in 24 juices were used to create a quality prediction equation, and 6 juices were held out to compare the predicted quality score and group versus the taste panel quality score and group. Shown in **Figure 5** is a plot of observed flavor score versus predicted flavor score for all 24 juices using the following equation: overall flavor quality = $-19.9 + 5.68 \times 10^{-4} \times \alpha$ -pinene + $5.52 \times 10^{-2} \times 1,8$ -cineole + $5.51 \times 10^{-3} \times$ nonanal – $2.04 \times 10^{-3} \times$ geraniol + $1.44 \times 10^{-2} \times \beta$ -ionone + $1.89 \times$ Brix. The equation correctly predicted a sensory quality score that corresponded to the correct sensory panel defined quality group (two good, two fair, two poor) for all six juices.

SENSORY ANALYSIS

Sensory analysis (which is defined as the analysis of food by the human senses) is the most reliable technique to evaluate the human perception of appearance, flavor, texture, and trigeminal attributes of any food product. Sensory analysis is used to describe or characterize a product (by trained panel) or to examine its acceptance by consumers (by consumer panel). A sensory profile is a descriptive analysis of a sample by a trained panel. Sensory profiling is based on the concept that the sensory impression made by the sample consists of a number of identified attributes (descriptors), each of which is present to a larger or smaller degree. The description of the sensory properties of a sample, comprising the sensory attributes in the order of perception and with assignment of an intensity value for each attribute, is the sensory profile (81).

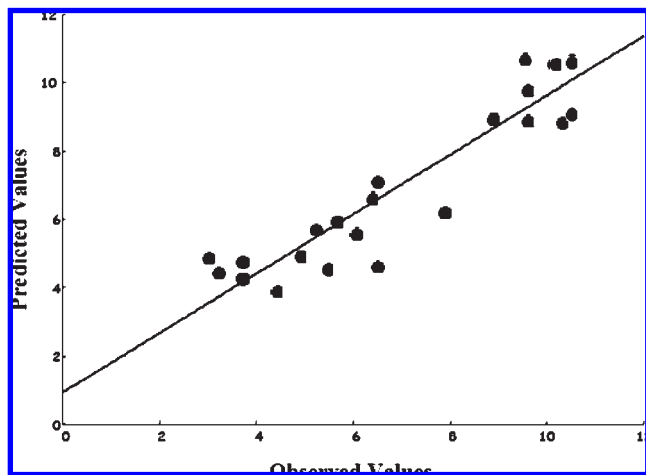


Figure 5. Comparison of actual taste panel score versus predicted flavor score from the work of Elston (80).

Relatively few orange juice sensory descriptive studies can be found in the literature. Initial studies primarily examined the basic tastes (acid, bitter, and sweet) with few odor attributes in refrigerated from concentrated and fresh-squeezed juices (79, 82–84). A few recent studies have employed sensory descriptive analysis to classify different categories of orange juices and to develop a sensory lexicon to describe these types of juices (4, 20, 85–88). Shown in **Figure 6** are the average aroma profile scores for four types of orange juice. Fresh hand-squeezed juices were described with strong citrus fruits, green, and floral attributes. An ideal orange juice processing technique would maintain these fresh orange sensory perceptions as much as possible. When the thermal processing is more severe or the storage conditions are inappropriate, these positive sensory descriptors are diminished or completely eliminated. At the same time new sensory attributes appear, which are not found in fresh-squeezed juices. As seen in **Figure 6**, the four thermally processed juices have very different flavor profiles. The refrigerated not from concentrate juice profile is closest to the fresh-squeezed juice, and the canned juice (which is hot-filled and most thermally abused) has a distinctly different aroma profile. Refrigerated from concentrate juices had intense orange peel oil, cooked, noncitrus fruits (passion fruit), chemical attributes (mainly, medicine), whereas canned from concentrate juices no longer tasted like orange juice and were characterized by tropical fruits, grapefruits, chemical (medicine), and moldy/musty. In addition, frozen concentrate juices were described with intense beany, cooked, and orange peel odor attributes.

GRAPEFRUIT JUICE FLAVOR

The unique flavor of grapefruit juice is due to a combination of sweet–tart and slight bitter taste with a characteristic aroma that differentiates this fruit from other members in the citrus family. A lower Brix/acid ratio, particularly in the early-season grapefruit, is related to increased tartness and decreased consumer acceptability. However, this ratio appears to play only a minor role in defining the overall flavor quality of grapefruit juice (89). Bitterness levels appear to be more important to consumer acceptance. Numerous attempts have been made to isolate, identify, and quantify the bitter principals in grapefruit juice. Naringin, a flavanone neohesperidoside, and limonoids such as limonin and nomilin are the major contributors to bitterness in grapefruit juice. Fruit maturity and juice extraction conditions have been shown to affect the bitterness level in grapefruit juice. For example, highest levels of nomilin were seen

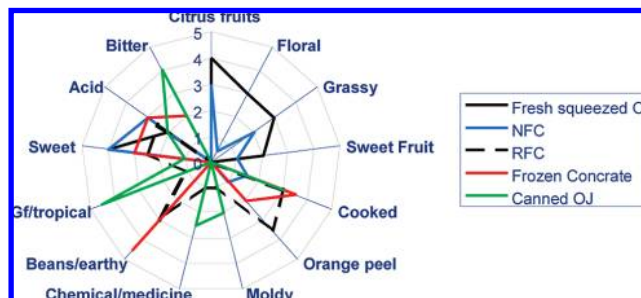


Figure 6. Aroma profiles various orange juice products consisting of average flavor scores for specific attributes. Reprinted from ref 20. Copyright 2007 American Chemical Society.

in the early-season grapefruit juices, and their concentration decreased dramatically with fruit maturity (90). Attaway (91) attributed the increased bitterness in commercial juices to high mechanical extraction pressure, extracting more limonoids from other fruit parts such as segment membrane, central core, and seeds in which limonoid concentrations are much greater than in juice. The author demonstrated that hard squeezing of grapefruit resulted in higher juice yields but also increased bitterness levels and elevated limonoids concentrations.

In general, the combination of low sugar acid ratios and high bitterness can overpower the pleasant aroma of grapefruit juice. Considerable efforts have been made to debitter grapefruit juices to increase consumer acceptance (92, 93). However, the consumer preference for grapefruit juice appears to be bimodal, with some liking the characteristic bitter character and others disliking it (94). According to one large USDA consumer study (95), 20% of the nonusers responded “we just don’t like them” referring to grapefruit juices. On the other hand, heavy grapefruit users seemed to expect the sweet, sour, bitter character of grapefruit juice.

Grapefruit juice’s volatile aroma composition and the changes related to processing and storage have been a subject of research since the early 1950s. The work of Kirchner and co-workers (96) revealed that canning and prolonged storage of canned juice increased volatile acids, methanol, and furfural levels in the water-soluble volatiles (essence) extracted from grapefruit juice. In the case of the essential oil extracted from these juices (96), they observed significant decrease in limonene levels and increase in linalool monoxide, α -terpineol, and furfural levels in the stored canned juice. These compounds when added to fresh juice contributed an undesirable flavor quality to juice. However, at the time (1953), no grapefruit character impact compound had been identified as citral had been for lemon.

In the 1960s nootkatone, a grapefruit peel oil constituent, was the first compound reported to possess a characteristic grapefruit aroma. This compound was initially detected as a prominent GC peak in highly flavored grapefruit oil but was less apparent in weak-flavored oils (97). Erdtman and co-workers (98) identified this peak as a sesquiterpene ketone, nootkatone, on the basis of the melting point and infrared spectrum. MacLeod and Buiges (99) further established nootkatone as a native component of grapefruit juice by analyzing juice that was carefully separated from its peel oil. The authors stated that the taste and odor of nootkatone were definitely recognizable as contributing to the distinctive grapefruit flavor and that its level in grapefruit juice was typically at or above the taste and odor thresholds as determined by them.

It was subsequently observed that the mother liquor from which nootkatone was crystallized retained much of the grapefruit flavor and at a higher intensity than that of pure nootkatone

itself (100). Subsequent taste and odor panel studies (101) further revealed that the level of nootkatone in grapefruit oil was more important to the aroma of the oil than to the flavor of the grapefruit juice containing the same oil. All of these studies led to the understanding that, in addition to nootkatone, there were other related components present in grapefruit that could contribute to its distinctive flavor. This hypothesis was later proved by the identification of 15 related sesquiterpene ketones from grapefruit juice using MS and NMR spectra. Among these, 8,9-didehydronootkatone was found to possess a more intense grapefruit aroma than nootkatone at equimolar concentrations. Another compound, 1,10-dihydronootkatone, was previously reported to be approximately 3.5 times more intense than pure nootkatone (100). However, their relative concentrations in grapefruit juice with respect to nootkatone were very small, and hence their actual contribution to grapefruit juice aroma remains to be established.

A new and probably more important class of chemicals in grapefruit juice aroma, to emerge in the early 1980s, were the sulfur-containing volatile compounds. Of particular significance was the identification of 1-*p*-menthene-8-thiol (PMT) by Demole and co-workers (19). The authors isolated this terpene thiol and its bicyclic epimer 2,8-epithio-*cis-p*-menthane (EPM) from a sulfurous heptane fraction from 100 L of canned grapefruit juice that had a characteristic fresh grapefruit smell. The authors reported PMT as the most potent aroma compound in nature with a taste threshold of 0.1 ng/L in water (the lowest known taste threshold at the time). EPM, on the other hand, was found to be a much less significant contributor to grapefruit aroma (with a taste threshold as high as 9 µg/L in water).

Recent studies employing high-resolution GC-O and CI-MS (102, 103) have afforded identification of a new mercaptan, 4-mercapto-4-methylpentan-2-one (4MMP), in grapefruit juice. This compound, which was originally detected in Sauvignon Blanc wine (104), is reminiscent of intense black currant or catty odor, but in combination with other odorous compounds in grapefruit, it yields an intense grapefruit-like aroma quality. With AEDA and reconstitution model studies, the authors suggested that the typical aroma of hand-squeezed grapefruit juice is due not only to 1-*p*-menthene-8-thiol but is evoked much more by 4-mercapto-4-methylpentan-2-one. Some other sulfur compounds to be variously reported as important odorants in grapefruit juice are H₂S, methyl sulfide, 4-mercapto-4-methyl-2-pentanol, 3-mercapto-1-hexan-1-ol, 3-mercaptohexyl acetate, and methional (105–108). However, comprehensive information on the relative concentrations of these potent aroma compounds in grapefruit juice is still lacking. The presence of these volatiles at nanogram per liter levels coupled with their highly reactive nature (easily oxidized, dimerized, or thermally/enzymatically altered) has made their extraction and analysis difficult and prone to artifact formation (109). By using a highly sensitive and specific sulfur analytical method, it may be possible to measure trace levels of these potent compounds in grapefruit juice and thereby establish their sensory significance to grapefruit juice aroma.

LITERATURE CITED

- Legge, J. The tribute of Yu. In *The Chinese Classics*; Trübner: London, U.K., 1865; Vol. III, Part 3, Book 1, pp 111–112.
- Webber, H. J. History and development of the citrus industry. In *The Citrus Industry. Vol. I. History, World Distribution, Botany and Varieties*; Reuther, W., Batchelor, L. D., Webber, H. J., Eds.; University of California: Riverside, CA, 1967; Vol. I, 611 pp.
- Pollack, S. L.; Lin, B.; Allshouse, J. *Characteristics of U. S. Orange Juice Consumption*; Economic Research Service, U.S. Department of Agriculture: Washington, DC, 2003.
- Ruiz Perez-Cacho, P.; Rouseff, R. Fresh squeezed orange juice odor: a review. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 681–695.
- Ruiz Perez-Cacho, M. P.; Rouseff, R. Processing and storage effects on orange juice aroma: a review. *J. Agric. Food Chem.* **2008**, *56*, 9785–9796.
- Bazemore, R.; Goodner, K.; Rouseff, R. Volatiles from unpasteurized and excessively heated orange juice analyzed with solid phase microextraction and GC–olfactometry. *J. Food Sci.* **1999**, *64* (5), 800–803.
- Nagy, S.; Shaw, P. E. Factors affecting the flavor of citrus fruit. In *Food Flavors: The Flavour of Fruits*; Morton, I. D., Macleod, A. J., Eds.; Elsevier: Amsterdam, The Netherlands, 1990; Vol. 3C, pp 93–124.
- Bazemore, R.; Rouseff, R.; Naim, M. Linalool in orange juice: origin and thermal stability. *J. Agric. Food Chem.* **2003**, *51*, 196–199.
- Moshonas, M. G.; Shaw, P. E. Quantitative determination of 46 volatile constituents in fresh, unpasteurized orange juices using dynamic headspace gas chromatography. *J. Agric. Food Chem.* **1994**, *42*, 1525–1528.
- Attaway, J. A.; Barron, R. W.; Blair, J. S.; Buslig, B. S.; Carter, R. D.; Dougherty, M. H.; Fellers, P. J.; Fisher, J. F.; Hill, E. C.; Huggart, R. L.; Maraulja, M. D.; Petrus, D. R.; Ting, S. V. New analytical indicators of processed orange juice quality, 1971–72. *Proc. Fla. State Hort. Soc.* **1973**, *85*, 192–203.
- Sinclair, W. B.; Ramsey, R. C. Changes in the organic-acid content of Valencia oranges during development. *Bot. Gaz.* **1944**, *106* (2), 140–148.
- Buttery, R. G. Vegetable and fruit flavours. In *Flavor Research—Recent Advances*; Teranishi, R., Flath, R. A., Sugisawa, H., Eds.; Dekker: New York, 1981; pp 175–216.
- Belitz, H.-D.; Grosch, W.; Schieberle, P. *Food Chemistry*, 3rd rev. ed.; Springer: Berlin, Germany, 2004.
- Peterson, D.; Reineccius, G. A. Biological pathways for the formation of oxygen-containing aroma compounds. In *Heteroatomic Aroma Compounds*; Reineccius, G. A., Reineccius, T. A., Eds.; American Chemical Society: Washington, DC, 2002; Vol. 826, pp 227–242.
- Attaway, J. A.; Carter, R. D. Some new analytical indicators of processed orange juice quality. *Proc. Fla. State Hort. Soc.* **1971**, *84*, 200–205.
- Veldhuis, M. K. In *Quality Parameters in Citrus Juices: Quality Control and Grade Inspection of Citrus Juices in the United States*, The quality and detection of adulteration in citrus juices, Valencia, Spain, Sept 30–Oct 2, 1974; International Union of Food Science and Technology: Valencia, Spain, 1976; pp 5–24.
- Kealey, K. S.; Kinsella, J. E. Orange juice quality with an emphasis on flavor components. *Crit. Rev. Food Sci. Nutr.* **1979**, *11* (1), 1–40.
- Hall, J. A.; Wilson, C. P. Volatile constituents of Valencia orange juice. *J. Am. Chem. Soc.* **1925**, *47*, 2575–2584.
- Demole, E.; Enggist, P.; Ohloff, G. 1-*p*-Menthene-8-thiol: a powerful flavor impact constituent of grapefruit juice (*Citrus paradisi* Macfayden). *Helv. Chim. Acta* **1982**, *65* (6), 1785–1794.
- Ruiz Perez-Cacho, P.; Mahattanatawee, K.; Smoot, J. M.; Rouseff, R. Identification of sulfur volatiles in canned orange juices lacking orange flavor. *J. Agric. Food Chem.* **2007**, *55*, 5761–5767.
- Fox, A. L. The relationship between chemical constitution and taste. *Proc. Natl. Acad. Sci. U.S.A.* **1932**, *18* (1), 115–120.
- Ohloff, G.; Demole, E. Importance of the odoriferous principle of Bulgarian rose oil in flavor and fragrance chemistry. *J. Chromatogr.*, **A** **1987**, *406*, 181–183.
- Schieberle, P.; Grosch, W. Evaluation of the flavor of wheat and rye bread crusts by aroma extract dilution analysis. *Z. Lebensm. Unters. Forsch.* **1987**, *185* (2), 111–113.
- Ohloff, G. Importance of minor components in flavors and fragrances. *Perfum. Flavor.* **1978**, *3* (1), 1–12, 14–16, 18–22.
- Miller, J. M.; Kirchner, J. G. Improvements in chromatographic techniques for terpenes. *Anal. Chem.* **1952**, *24*, 1480–1482.
- Wolford, R. W.; Atkins, C. D.; Alberding, G. E.; Attaway, J. A. Analysis of flavor and aroma constituents of Florida orange juices by gas chromatography. *J. Food Sci.* **1963**, *28* (3), 320–328.

- (27) Huet, R. The aromas of citrus juices. *Fruits* **1968**, *23* (9), 453–471.
- (28) Bernhard, R. A. Analysis and composition of oil of lemon by gas–liquid chromatography. *J. Chromatogr.* **1960**, *3* (5), 471–476.
- (29) Ikeda, R. M.; Vannier, S. H.; Stanley, W. L.; Rolle, L. A. Lemon oil composition— isolation and identification of aldehydes in cold-pressed lemon oil. *J. Agric. Food Chem.* **1962**, *10*, 98–102.
- (30) Hunter, G. L. K.; Brogden, W. B. Jr. Analysis of the terpene and sesquiterpene hydrocarbons in some citrus oils. *J. Food Sci.* **1965**, *30* (3), 383–387.
- (31) Yamanish, T.; Kobayash, A.; Mikumo, Y.; Nakasone, Y.; Kita, M.; Hattori, S. Composition of peel oil from citrus unshu. *Agric. Biol. Chem.* **1968**, *32* (5), 593.
- (32) Hendrickson, R.; Kesterson, J. W.; Ting, S. V. Peel oil content of Valencia oranges. *Proc. Fla. State Hortic. Soc.* **1969**, *82*, 192–196.
- (33) Shinoda, N.; Shiga, M.; Nishimura, K. Constituents of yuzu (*Citrus junos*) oil. *Agric. Biol. Chem.* **1970**, *34* (2), 234–242.
- (34) Bernhard, R. A. Citrus flavor. Volatile constituents of the essential oil of the orange. *J. Food Sci.* **1961**, *26*, 401–411.
- (35) Giacomo, A. D.; Rispoli, G.; Tracuzzi, M. L. Analysis of terpene constituents from citrus essential oils. *Riv. Ital. Essenze-Profumi Piante Offic.-Aromi-Sapono-Cosmet.* **1963**, *45* (6), 269–281.
- (36) Attaway, J. A.; Alberding, G. E.; Wolford, R. W.; Edwards, G. J. Orange essence analysis, identification of alcohols and volatile organic acids from natural orange essence. *J. Agric. Food Chem.* **1964**, *12*, 118–121.
- (37) Lijn, J. v. d. A chromatographic method for deterpenation of citrus essential oils. *Lebensm. Wiss. Technol.* **1969**, *2* (2), 39–43.
- (38) Moshonas, M. G.; Shaw, P. E.; Veldhuis, M. K. Analysis of volatile constituents from Meyer lemon oil. *J. Agric. Food Chem.* **1972**, *20*, 751–752.
- (39) Dugo, G.; Bartle, K. D.; Bonaccorsi, I.; Catalfamo, M.; Cotroneo, A.; Dugo, P.; Lamonica, G.; McNair, H.; Mondello, L.; Previti, P.; D'Alcontres, I. S.; Trozzi, A.; Verzera, A. Advanced analytical techniques for the analysis of citrus essential oils. *Essenze, Deriv. Agrum.* **1999**, *69* (3), 159–217.
- (40) Mazza, G. Identification of new compounds in citrus oils by GC/MS. *Essenze, Deriv. Agrum.* **1987**, *57* (1), 19–33.
- (41) Tajima, K.; Tanaka, S.; Yamaguchi, T.; Fujita, M. Analysis of green and yellow yuzu peel oils (*Citrus junos* Tanaka)—novel aldehyde components with remarkably low odor thresholds. *J. Agric. Food Chem.* **1990**, *38*, 1544–1548.
- (42) Sawamura, M.; Shichiri, K.; Ootani, Y.; Zheng, X. H. Volatile constituents of several varieties of pummelos and characteristics among citrus species. *Agric. Biol. Chem.* **1991**, *55* (10), 2571–2578.
- (43) Cadwallader, K. R.; Xu, Y. Analysis of volatile components in fresh grapefruit juice by purge and trap/gas chromatography. *J. Agric. Food Chem.* **1994**, *42*, 782–784.
- (44) Goodner, K. L.; Jella, P.; Rouseff, R. L. Determination of vanillin in orange, grapefruit, tangerine, lemon, and lime juices using GC–olfactometry and GC–MS/MS. *J. Agric. Food Chem.* **2000**, *48*, 2882–2886.
- (45) Buettner, A.; Schieberle, P. Evaluation of aroma differences between hand-squeezed juices from Valencia late and navel oranges by quantitation of key odorants and flavor reconstitution experiments. *J. Agric. Food Chem.* **2001**, *49*, 2387–2394.
- (46) Horowitz, R. M.; Gentili, B. Phenolic glycosides of grapefruit; a relation between bitterness and structure. *Arch. Biochem. Biophys.* **1961**, *92*, 191–192.
- (47) Higby, R. H. Bitter constituents of Navel and Valencia oranges. *J. Am. Chem. Soc.* **1938**, *60*, 3013–3018.
- (48) Arigoni, D.; Barton, D. H. R.; Corey, E. J.; Jeger, O.; Caglioti, L.; Dev, S.; Ferrini, P. G.; Glazier, E. R.; Melera, A.; Pradhan, S. K.; Schaffner, K.; Sternhell, S.; Templeton, J. F.; Tobinaga, S. Constitution of limonin. *Experientia* **1960**, *16*, 41–49.
- (49) Maier, V. P.; Beverly, G. D. Limonin monolactone, the nonbitter precursor responsible for delayed bitterness in certain citrus juices. *J. Food Sci.* **1968**, *33* (5), 488–492.
- (50) Tatum, J. H.; Berry, R. E. V. Method for determining naringin content in grapefruit juice. *J. Food Sci.* **1973**, *85*, 210–213.
- (51) Fisher, J. F. Quantitative determination of limonin in grapefruit juice by high-pressure liquid chromatography. *J. Agric. Food Chem.* **1975**, *23*, 1199–1201.
- (52) Mansell, R. L.; Weiler, E. W. Use of immunoassay in plant science. VIII. Radio-immunoassay for determination of limonin in *Citrus* [spp.]. *Phytochemistry* **1980**, *19* (7), 1403–1407.
- (53) Widmer, W.; Haun, C. A. Analysis of limonin and flavonoids in citrus juices and byproduct extracts by direct injection and in-line sample clean-up. In *Citrus Limonoids*; Berhow, M. A., Hasegawa, S., Manners, G. D., Eds.; American Chemical Society: Washington, DC, 2000; Vol. 758, pp 60–72.
- (54) Guadagni, D. G.; Maier, V. P.; Turnbaugh, J. G. Effect of some citrus juice constituents on taste thresholds for limonin and naringin bitterness. *J. Sci. Food Agric.* **1973**, *24* (10), 1277–1288.
- (55) Fong, C. H.; Hasegawa, S.; Herman, Z.; Ou, P. Limonoid glucosides in commercial citrus juices. *J. Food Sci.* **1989**, *54* (6), 1505–1506.
- (56) Grosch, W. In *Specificity of the Human Nose in Perceiving Food Odorants*, Proceedings of the Weurman Flavour Research Symposium, Freising, Germany, 1999; Schieberle, P., Engel, K., Eds.; Freising, Germany, 1999; pp 213–219.
- (57) Grosch, W. Determination of potent odourants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs). *Flavour Fragrance J.* **1994**, *9* (4), 147–158.
- (58) Tamura, H.; Fukuda, Y.; Padrayuttawat, A. Characterization of citrus aroma quality by odor threshold values. In *Biotechnol. Improved Food Flavors*; Takeoka, G., Teranishi, R., Williams, P. J., Kobayashi, A., Eds.; American Chemical Society: Washington, DC, 1996; Vol. 637, pp 282–294.
- (59) Reineccius, G. A.; Ward, F. M.; Whorton, C.; Andon, S. A. Developments in gum acacias for the encapsulation of flavors. *Encapsulation and Controlled Release of Food Ingredients*; ACS Symposium Series 590; American Chemical Society: Washington, DC, 1995; pp 161–168.
- (60) Marin, A. B.; Acree, T. E.; Hotchkiss, J. H.; Nagy, S. Gas chromatography–olfactometry of orange juice to assess the effects of plastic polymers on aroma character. *J. Agric. Food Chem.* **1992**, *40*, 650–654.
- (61) Hinterholzer, A.; Schieberle, P. Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by odour dilution techniques. *Flavour Fragrance J.* **1998**, *13* (1), 49–55.
- (62) Jella, P.; Rouseff, R.; Goodner, K.; Widmer, W. Determination of key flavor components in methylene chloride extracts from processed grapefruit juice. *J. Agric. Food Chem.* **1998**, *46*, 242–247.
- (63) Lin, J. M.; Jella, P.; Rouseff, R. L. Gas chromatography–olfactometry and chemilluminescence characterization of sulfur compounds in grapefruit juice. *Abstr. Pap. Am. Chem. Soc.* **2000**, *220*, U34–U34.
- (64) Scott, W. C.; Kew, T. J.; Veldhuis, M. K. Composition of orange juice cloud. *J. Food Sci.* **1965**, *30* (5), 833–837.
- (65) Jewell, G. G. An electron microscopy study of the particulate material in citrus fruits and beverages. *Dissertation Abstr. Int., C* **1976**, *37* (1), 100.
- (66) Beveridge, T. Opalescent and cloudy fruit juices: formation and particle stability. *Crit. Rev. Food Sci. Nutr.* **2002**, *42* (4), 317–337.
- (67) Radford, T.; Kawashima, K.; Friedel, P. K.; Pope, L. E.; Gianturco, M. A. Distribution of volatile compounds between the pulp and serum of some fruit juices. *J. Agric. Food Chem.* **1974**, *22*, 1066–1070.
- (68) Rega, B.; Fournier, N.; Nicklaus, S.; Guichard, E. Role of pulp in flavor release and sensory perception in orange juice. *J. Agric. Food Chem.* **2004**, *52*, 4204–4212.
- (69) Roberts, D. D.; Elmore, J. S.; Langley, K. R.; Bakker, J. Effects of sucrose, guar gum, and carboxymethylcellulose on the release of volatile flavor compounds under dynamic conditions. *J. Agric. Food Chem.* **1996**, *44*, 1321–1326.
- (70) Ahmed, E. M.; Dennison, R. A.; Dougherty, R. H.; Shaw, P. E. Effect of nonvolatile orange juice components, acid, sugar, and pectin on the flavor threshold of *d*-limonene in water. *J. Agric. Food Chem.* **1978**, *26*, 192–194.

- (71) Plotto, A.; Margaria, C. A.; Goodner, K. L.; Goodrich, R.; Baldwin, E. A. Odour and flavor thresholds for key aroma components in an orange juice matrix: terpenes and aldehydes. *Flavour Fragrance J.* **2004**, *19* (6), 491–498.
- (72) Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem. Senses* **2001**, *26*, 533–545.
- (73) Shaw, P. E.; Ahmed, E. M.; Dennison, R. A. Orange juice flavor: contribution of certain volatile components as evaluated by sensory panels. *Proc. Int. Soc. Citriculture* **1977**, *3*, 804–807.
- (74) Ahmed, E. M.; Dennison, R. A.; Shaw, P. E. Effect of selected oil and essence volatile components on flavor quality of pumpout orange juice. *J. Agric. Food Chem.* **1978**, *26*, 368–372.
- (75) Carter, R. D.; Buslig, B. S. Statistical inferences on processed orange juice quality from the 1972–73 and 1973–74 Juice Definition Program. *Proc. Fla. State Hort. Soc.* **1975**, *88*, 358–370.
- (76) Carter, R. D.; Cornell, J. A. Use of regression models in objective flavor evaluation of processed orange juice during four seasons. In *Flavor Quality: Objective Measurement*; Scanlan, R. A., Ed.; American Chemical Society: Washington, DC, 1977; Vol. 51, pp 104–109.
- (77) Carpenter, R. S.; Burgard, D. R.; Patton, D. R.; Zwerdling, S. S., Application of multivariate analysis to capillary GC profiles: comparison of the volatile fraction in processed orange juices. In *Instrumental Analysis of Foods: Recent Progress*; Charalambous, G., Inglett, G., Eds.; Academic Press: Orlando, FL, 1983; Vol. 2, pp 173–186.
- (78) Pino, J. Correlation between sensory and gas-chromatographic measurements on orange volatiles. *Acta Aliment.* **1982**, *11* (1), 1–9.
- (79) Petersen, M. A.; Tonder, D.; Poll, L. Comparison of normal and accelerated storage of commercial orange juice—changes in flavour and content of volatile compounds. *Food Qual. Pref.* **1998**, *9* (1–2), 43–51.
- (80) Elston, A. *Developing an objective flavor measurement tool for orange juice*. Ph.D. Dissertation, University of Florida, Gainesville, FL, 2005.
- (81) ISO International Standard 13299. Sensory analysis—Methodology—General guidance for establishing a sensory profile. *Ref. No. ISO 13299:2003 (E)*; International Organization for Standardization: Genève, Switzerland, 2003.
- (82) Spoto, M. H. F.; Domarco, R. E.; Walder, J. M. M.; Scarminio, I. S.; Bruns, R. E. Sensory evaluation of orange juice concentrate as affected by irradiation and storage. *J. Food Process. Preserv.* **1997**, *21* (3), 179–191.
- (83) Stampanoni, C. Influence of acid and sugar content on sweetness, sourness and the flavor profile of beverages and sherberts. *Food Qual. Pref.* **1993**, *4*, 169–176.
- (84) Petersen, M. A.; Tonder, D.; Poll, L. Comparison of normal and accelerated storage of commercial orange juice—changes in flavor and content of volatile compounds. *Food Qual. Pref.* **1998**, *9* (1–2), 43–51.
- (85) Lotong, V.; Chambers, E.; Chambers, D. H. Categorization of commercial orange juices based on flavor characteristics. *J. Food Sci.* **2003**, *68* (2), 722–725.
- (86) Lotong, V.; Chambers, D. H.; Dus, C.; Chambers, E.; Civille, G. V. Matching results of two independent highly trained sensory panels using different descriptive analysis methods. *J. Sens. Stud.* **2002**, *17* (5), 429–444.
- (87) Pérez-Cacho, P. R.; Galán Soldevilla, H.; Mahatanattawee, K.; Elston, A.; Rouseff, R. L. Sensory lexicon for fresh squeezed and processed orange juices. *Food Sci. Technol. Int.* **2008**, *14* (5), 131–142.
- (88) Ruiz Perez-Cacho, P.; Soldevilla, H. G.; Mahatanattawee, K.; Smoot, J.; Rouseff, R. Development of a flavor descriptive analysis procedure for orange juices by two trained panels in Spain and USA. *Chem. Senses* **2006**, *31* (8), E72–E73.
- (89) Fellers, P. J. The relationship between the ratio of degrees Brix to percent acid and sensory flavor in grapefruit juice. *Food Technol.* **1991**, *45* (7), 68.
- (90) Couture, R.; Rouseff, R. Debitting and deacidifying sour orange (*Citrus aurantium*) juice using neutral and anion-exchange resins. *J. Food Sci.* **1992**, *57* (2), 380–384.
- (91) Dougherty, M. H.; Ting, S. V.; Attaway, J. A.; Moore, E. L. Grapefruit juice quality improvement studies. Introduction and scope of the study, and the effect of processing variables, temperature and duration of storage on the quality of grapefruit juice. *Proc. Fla. State Hort. Soc.* **1977**, *90*, 165–167.
- (92) Fellers, P. J. A review of limonin in grapefruit (*Citrus paradisi*) juice, its relationship to flavour and efforts to reduce it. *J. Sci. Food Agric.* **1989**, *49* (4), 389–404.
- (93) Assmann, S.; Medeiros, D. M.; Chambers, E. Fortification with calcium citrate malate may not influence the sensory properties of an orange flavored beverage. *J. Food Qual.* **2003**, *26* (5), 395–407.
- (94) Drewnowski, A.; Henderson, S. A.; Shore, A. B. Taste responses to naringin, a flavonoid, and the acceptance of grapefruit juice are related to genetic sensitivity to 6-n-propylthiouracil. *Am. J. Clin. Nutr.* **1997**, *66* (2), 391–397.
- (95) USDA. *Homemakers Appraise Citrus Products, Avocados, Dates, And Raisins*; Market Research Report 243; Agricultural Marketing Service, Research Division, USDA: Washington, DC, 1958.
- (96) Attaway, J. A.; Edwards, G. J.; Wolford, R. W. Fruit flavors and odors— isolation and identification of some volatile carbonyl components from orange essence. *J. Agric. Food Chem.* **1962**, *10*, 102–104.
- (97) Beisel, C. G.; Johnson, V. E.; Kustel, P. A. *J. Food Sci.* **1964**, *29* (565).
- (98) Erdtman, H.; Hirose, Y. Chemistry of natural order cupressales. 46. Structure of nootkatone. *Acta Chem. Scand.* **1962**, *16* (6), 1311.
- (99) Macleod, W. D.; Buigues, N. M. Sesquiterpenes. 1. Nootkatone new grapefruit flavor constituent. *J. Food Sci.* **1964**, *29* (5), 565.
- (100) Stevens, K. L.; Guadagni, D. G.; Stern, D. J. Odour character and threshold values of nootkatone and related compounds. *J. Sci. Food Agric.* **1970**, *21* (11), 590.
- (101) Shaw, P. E.; Wilson, C. W. Importance of nootkatone to the aroma of grapefruit oil and the flavor of grapefruit juice. *J. Agric. Food Chem.* **1981**, *29*, 677–679.
- (102) Buettner, A.; Schieberle, P. Characterization of the most odor-active volatiles in fresh, hand-squeezed juice of grapefruit (*Citrus paradisi* Macfayden). *J. Agric. Food Chem.* **1999**, *47*, 5189–5193.
- (103) Buettner, A.; Schieberle, P. Evaluation of key aroma compounds in hand-squeezed grapefruit juice (*Citrus paradisi* Macfayden) by quantitation and flavor reconstitution experiments. *J. Agric. Food Chem.* **2001**, *49*, 1358–1363.
- (104) Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J.-N.; Dubourdieu, D. Identification of a powerful aromatic component of *Vitis vinifera* L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flavour Fragrance J.* **1995**, *10* (6), 385–392.
- (105) Kirchner, J. G.; Rice, R. G.; Miller, J. M.; Keller, G. J. The presence of hydrogen sulfide in citrus juices. *Arch. Biochem.* **1950**, *25* (1), 231–232.
- (106) Shaw, P. E.; Wilson, C. W. III. Importance of selected volatile components to natural orange, grapefruit, tangerine and mandarin flavors. *ACS Symp. Ser.* **1980**, *No. 143*, 167–190.
- (107) Lin, J.; Jella, P.; Rouseff, R. L. Gas chromatography—olfactometry and chemiluminescence characterization of grapefruit juice volatile sulfur compounds. In *Heteroatomic Aroma Compounds*; Reineccius, G., Reineccius, T., Eds.; American Chemical Society: Washington, DC, 2002; Vol. 826, 110–120.
- (108) Lin, J. M.; Rouseff, R. L.; Barros, S.; Naim, M. Aroma composition changes in early season grapefruit juice produced from thermal concentration. *J. Agric. Food Chem.* **2002**, *50*, 813–819.
- (109) Rouseff, R. L. Comparison of analytical procedures to measure volatile sulfur compounds in foods. In *Heteroatomic Flavor Chemistry*; Reineccius, G., Reineccius, T., Eds.; American Chemical Society: Washington, DC, 2002; Vol. 826, pp 2–24.