JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Synephrine Content of Juice from Satsuma Mandarins (*Citrus unshiu* Marcovitch)

KLAUS DRAGULL, ANDREW P. BREKSA III,* AND BRIAN CAIN

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710

Synephrine, the main protoalkaloid in *Citrus* species, is commonly analyzed as the active component in citrus peel-containing herbal supplements, but the edible parts of mandarins have been largely ignored. The synephrine concentration has been determined in the juices of *Citrus unshiu* mandarins harvested from 10 different groves located in a major growing region in California. For comparison, the physicochemical properties of the juices, including pH, conductivity, soluble solids content, and titratable acidity, were also measured. The synephrine values among 10 groves ranged from 73.3 to 158.1 mg L^{-1}. Repeat sampling of fruit from the 10 locations showed that the intragrove variability in synephrine concentrations ranged from 1.0 to 27.7% CV and was grove dependent. Among the physicochemical properties, titratable acidity weakly correlated with synephrine, and for one sample a low maturity index was linked to high synephrine content. The overall mean synephrine concentration of 92.8 mg L^{-1} is up to 6-fold higher than values previously determined for orange juices and suggests that mandarin juice could constitute a significant dietary source of synephrine. Furthermore, the results suggest that grove location and maturity affect synephrine content.

KEYWORDS: *Citrus*; juice; synephrine; *Citrus unshiu*; *Citrus reticulata*; mandarin; tangerine; Satsuma; variability

INTRODUCTION

Synephrine (**Figure 1**) is a phenolic amine that was initially isolated as synthetic product and used pharmacologically as a vascoconstrictor and bronchiectatic agent (*1*). The presence of synephrine in citrus leaves was first reported in 1964 by Stewart et al. (*2*), who in the course of evaluating the micronutrient contents of several hundred varieties determined that the greatest synephrine concentrations were found in mandarin and orange leaves. No synephrine was detected in the leaves from grapefruit, pummelo, or shaddock trees; neither was it detected in roots of any of the citrus varieties tested or in leaf samples taken from trees suffering from Mn deficiencies (*2*). Results obtained in a follow-up study examining the juice concentrations of synephrine corresponded well with those observed for leaves, but were at concentrations 10−20 times less than those found in leaves (*3, 4*). A subsequent analysis of the phenolic amine content of *Citrus* and another 187 plant species demonstrated that *Citrus* possessed the greatest concentrations of synephrine and that the highest concentrations in citrus were at least 10-fold higher than those in the other plants evaluated (*5*). *Citrus* spp. are the only known plants having synephrine in the edible portions.

It was not until recently, when sour orange (*Citrus aurantium* L.) derived botanicals and extracts were introduced to consumers as alternatives to the FDA-banned ephedrine-containing products, that the presence of synephrine in citrus fruits and products became of interest (*6*−*13*). With the resurgence in the interest in synephrine, multiple analytical methods for the quantitation of synephrine have been reported, and a review of these methods was recently completed by Pellati et al. (*14*). A direct comparison of the synephrine concentrations reported among all these papers is difficult due to the ambiguous sample descriptions found in some and differences in sample preparation. However, as a whole, they support the original observations made by Stewart et al. (*2*) and have additionally contributed by identifying sour orange as a citrus with synephrine concentrations comparable to those found in mandarins (*Citrus reticulata* Blanco, *Citrus unshiu* Marcovitch).

As indicated above, the literature contains a number of papers detailing the development and demonstration of analytical methods for determining synephrine concentrations. The emphasis on method development has resulted in a wide breadth of *Citrus* samples analyzed and within those samples the identification of those cultivars with the highest synephrine concentrations. However, the variability in synephrine concentrations within the cultivars tested is still largely unknown because in most cases only single representative samples were used. The main objective of this study was to close this gap. An understanding of the variability in concentration, and eventually the effects of environment and agricultural practices, will be essential if citrus growers and processors are to capitalize on the economic potential of

* Corresponding author [telephone (510) 559-5898; fax (510) 559-5849; e-mail andrew.breksa@ars.usda.gov.

10.1021/jf801225n
Published on Web 09/05/2008

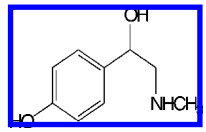

Figure 1. Chemical structure of synephrine.

delivering dietary levels of synephrine through their fresh fruits and products. Tangerine or mandarin oranges are ideally suited to be utilized in this way because they already possess many properties important to consumers (e.g., easy peeling, seedless, etc.) and have some of the highest reported synephrine concentrations (i.e., Cleopatra, 280 mg L^{-1}) (*3*). For this study, we chose to characterize the synephrine concentrations found in peeled fruit from commercial groves located in Placer County (California) of the Owari selection of Satsuma mandarin (*C. unshiu* Marcovitch). Placer County is a major mandarin-producing region in the state. To gain better insight into the variations in synephrine concentration found within and across the groves, mandarins were obtained from 10 different groves. Physicochemical properties of the harvested fruits were measured in addition to the synephrine concentrations to facilitate comparison of the samples.

MATERIALS AND METHODS

Materials and Chemicals. ACN (HPLC grade), ammonium acetate (enzyme grade), and water (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ). Alternatively, water was deionized to ≥18.1 MΩ/cm resistance using a Barnstead NANOpure Deionization System (Dubuque, IA) and filtered through a 0.45 μm type HA membrane filter (Millipore, Billerica, MA) prior to use. The (±)-synephrine standard was purchased from ChromaDex (Santa Ana, CA).

Plant Materials. Fruits [Owari selection of Satsuma mandarin (*C. unshiu* Marcovitch)] were harvested from each of 10 different groves located in Placer County, CA. Harvesters were instructed to pick mature fruits of random sizes from throughout the entire grove. Fruits were harvested in mid-November 2007 and stored for <1 week at 5 °C until sampling.

Sample Preparation and Measurement of Physicochemical Properties. For each location, healthy and undamaged fruits were randomly divided into three groups. For each group, 10 fruits were used for the analysis. Because the peels of mandarin fruits are known to contain synephrine (*9*), the use of a juice reamer was ruled out to avoid potentially contaminating the juice with synephrine derived from the peel. Fruits were therefore peeled manually (the peelers wore nitrile gloves) and then blended in an Osterizer Classic mixer (now Jarden Consumer Solutions, Boca Raton, FL) until no chunks of pulp remained (10−20 s). The resulting juice was clarified by centrifugation at 4700 rpm for 4 min using a Marathon 8K from Fisher Scientific Ltd. (Waltham, MA). The clarified juice was vacuum filtered through Whatman no. 1 filter paper (Clifton, NJ). The pH was measured using a Beckmann Instruments model 34 pH-meter (Fullerton, CA) standardized to pH 4 and 7. A YSI model 32 conductance meter (Yellow Springs Instrument Co., Yellow Springs, OH) was used for conductivity measurements and expressed in mS. Soluble solids were measured using an Atago PR-100 digital refractometer (Tokyo, Japan) and expressed in °Brix. Total titratable acidity was determined with a Brinkmann 682 Titroprocessor (Westbury, NY) used in conjunction with a 665 Dosimat and an E 649 stirrer. The titroprocessor was calibrated with pH 7 and 10 buffers. The juice sample was diluted 10:1 with water and 100 mL of the test solution titrated to a pH of 8.2 using NaOH (0.156 M). The acid level was calculated from the amount of NaOH (mL) × 0.1 and expressed as percent citric acid.

Synephrine Analysis. Synephrine concentration determinations were conducted utilizing a Discovery HS F5 pentafluorophenyl column (150 × 4.6 mm i.d., 5 μm) and guard purchased from Supelco (Bellefonte, PA). Prior to injection, clarified juice samples obtained following centrifugation were filtered through a 25 mm diameter, 0.45 μm pore size Whatman GD/X nylon syringe filter. The (±)-synephrine standard was dissolved in deionized water, diluted to volume with the same, and stored at 4 °C until analysis.

Samples and standard (10 μL) were analyzed under isocratic conditions (*9*) at 25 °C on a HPLC system consisting of a Waters 2695 (Milford, MA) coupled to a SpectraSystem UV6000LP photodiode array detector (Thermo Separation Products, San Jose, CA) set to scan from 190 to 450 nm. The mobile phase, ACN/H_2O (90:10 v/v) with ammonium acetate at 10 mM concentration, was filtered through a 0.45 μm Teflon filter prior to use. The flow was 1 mL min^{-1}, and the wavelength used for quantification was 225 nm. Injections were performed in triplicate. Four-point calibration curves were established with the synephrine standard covering 22.4−240 μg mL^{-1}, and each level was injected before and after each sample set as standard brackets. R^2 values were typically 0.997 or greater. After 12 sample injections, the column was washed with 90% ACN (aq) for 15 min at 1 mL min^{-1}, followed by 100% ACN for 15 min at 1 mL min^{-1}.

Samples were typically analyzed within 24 h. In some instances, a white precipitate formed, presumably pectins, in samples that were stored for several hours under refrigeration prior to analysis. These samples were reclarified by centrifugation to protect the HPLC column. Initial tests had shown that neither storage (up to 1 week) nor recentrifugation had any measurable effect on quantification results.

Table 1. Summary of Physicochemical Properties and Synephrine Concentrations (n= 30)

	pH	conductivity, mS	soluble solids content (SSC), °Brix	total titratable acidity (TTA), % (w/v)	maturity index (SSC/TTA)	synephine, mg L^{-1}
low	3.12	1.89	10.8	0.84	8.95	54.5
high	3.59	4.57	15.6	1.74	14.93	160.2
average	3.43	2.73	12.7	1.02	12.65	98.1
median	3.44	2.70	12.5	0.96	12.48	92.8
% CV	3.21	19.84	8.66	19.60	12.51	28.10

RESULTS AND DISCUSSION

From among the available methods for quantifying synephrine we selected Pellati et al.'s recently reported method based upon a pentafluorophenyl stationary phase (*9*) because of its analysis time and direct detection of synephrine by UV. This method, and a version modified for detection by mass spectrometry put forth by Nelson et al. (*10*), were developed for the analysis of extracts derived from dried fruit materials, dietary supplements, and standard reference materials. In contrast, we chose clarified juice as our intended target to reduce the number of sample preparation steps. However, in applying clarified mandarin juices to the pentafluorophenyl stationary phase, we observed the following that we attributed to matrix effects: synephrine in samples eluted later than synephrine prepared in water alone; the retention time of synephrine in both samples and standards increased with each sample injection; and peak resolution gradually deteriorated with successive sample injections. Spiking experiments were used to address the difference in retention times. The difference in retention time is not totally unexpected considering that Pellati et al. (*9*) reported that retention of synephrine on the pentafluorophenyl stationary phase was directly dependent upon the ammonium acetate concentration in the mobile phase and that ion concentrations of the juice samples (see **Table 1**, conductivity measurements) are significantly higher than those of the synephrine standards prepared in water. The remaining issues were alleviated when after 9−12 sample injections the column was regenerated as described in the Materials and Methods section.

Table 1 summarizes the physicochemical properties and synephrine levels measured for a total of 30 sample juices tested, 3 sample sets taken from each of 10 locations. The synephrine

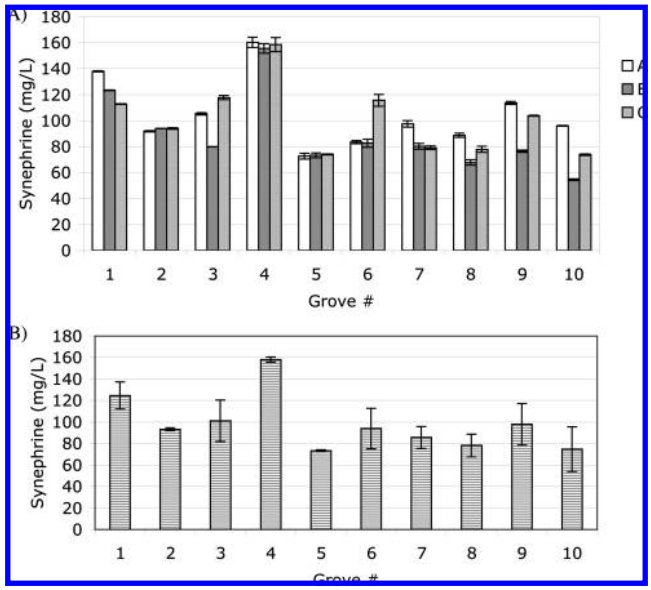

Figure 2. Variability of synephrine concentration in mandarin juices (*Citrus unshiu* cv. Owari Satsuma) from different groves in Placer County, CA: (**A**) mean ($n = 3$) of each of the 3 test groups, designated A−C, prepared individually from 10 peeled fruits. HPLC analyses were conducted in triplicate; (**B**) mean ($n = 9$) resulting from three test groups for each location. Mean ± SD is displayed in each case.

concentrations ranged from 54.5 to 160.2 mg L^{-1}. The mean calculated from this 3-fold difference in concentrations is 92.8 mg L^{-1} and is 5.8 times higher than the level reported for Brazilian orange juices, which had a mean of 16 mg L^{-1} (*7*). However, the synephrine level in orange juice may occasionally reach to 61 mg L^{-1} (*15*), which is close to the lower end of our presently determined range. Wheaton and Stewart (*3*) found in one sample of Cleopatra mandarin juice a concentration of 280 mg L^{-1} synephrine, whereas the concentration they reported for Dancy tangerines (125 mg L^{-1}) is more closely aligned with the results from our present study.

On the basis of the mean for our samples, mandarin juice could constitute a significant dietary source of synephrine. Haaz et al. (*16*) has suggested that doses in the range of 32 mg day^{-1} are required for weight loss, and a dose at this level would potentially correspond to the consumption of 344 mL of mandarin juice, which is less than the typical volume of a canned beverage sold in the United States. In this light, it would be interesting to analyze and compare mandarin juices taken from geographical regions where mandarins are most commonly consumed, such as Japan or Israel. In the United States, mandarin juice is routinely mixed with orange or tangelo juices prior to commercial distribution. Synephrine concentrations in tangelos have not been reported, other than a brief mentioning of its presence (*2*).

Results for the synephrine analysis of each juice sample tested are shown in **Figure 2A**. For each location, three groups of fruit were harvested for examination to assess the heterogeneity among the fruits within each grove. The three independent results are labeled A, B and C in **Figure 2A**, and in **Figure 2B** is presented the mean of the three experiments. Beginning with **Figure 2A**, error bars in the figure represent the standard deviation of the triplicate analysis for each sample. Rep-to-rep variability was low (% CV < 3.6%) and attests that the initial issues with chromatography were successfully resolved. Among the 10 locations, groves 2, 4, and 5 exhibited the least variability and are clearly distinguishable from one another possessing synephrine concentrations of 93.2 ± 1.2, 158.1 ± 2.4, and 73.3 ± 0.7 mg L^{-1}, respectively. The remaining groves possessed increased heterogeneity and could not be differentiated from one another. Intragrove variability represented as % CV ranged from 1.0% (grove 5) to as high as 27.7% (grove 10). Nevertheless, the data collected provide a valuable characterization of the variability that can be expected from commercially available mandarins from this particular region.

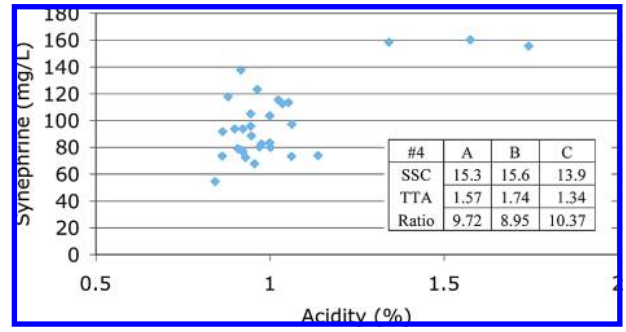

Figure 3. Correlation between juice synephrine and acid concentrations. Methods for measuring synephrine and total titratable acid concentrations are detailed under Materials and Methods. The three values (A−C) in the upper right were obtained from fruits harvested from grove 4.

Although subconscious preferences for selecting only particular types of fruits (e.g., color, size) cannot be totally excluded, each grove consisted of the same cultivar and harvesters were instructed to harvest fruits with regard to maturity across the entire grove rather than only a few trees. Therefore, the observed intra- and intergrove differences in synephrine concentrations described above are not expected to be due to genetic differences, but rather appear to be the result of location or environmental factors. The location or environmental factors that lead to a "grove effect" may be due to a wide variety of causes. One of the most obvious possible effectors is that of plant nutrients and their available supply. One of the earliest observations made by Stewart et al. (*2*) was that citrus plants suffering from Mn deficiency were devoid of synephrine, and more recently a positive correlation between soil nitrogen levels and synephrine concentrations in orange fruits was reported (*17*). Other perceivable nongenetic factors that may cause differences in synephrine concentrations include soil, light, temperature, and external stresses resulting from pests, etc., because they are known to have effects on similar phytochemicals in other plants such as barley (*18*−*20*).

A standard method for assessing the maturity of citrus is captured through the calculation of a maturity index, a value calculated from the ratio of soluble solids content (SSC), typically measured in °Brix, to the percent of total titratable acidity (TTA). During maturation, SSC increases while TTA decreases, resulting in a net increase in the maturity index value. Physicochemical characteristics including the maturity index are summarized in **Table 1**. Physicochemical values from all samples were plotted (data not shown) against their corresponding synephrine concentrations to determine if any correlations existed and with the initial exception of total titratable acidity (Pearson correlation = 0.69), no significant correlations were found. **Figure 3**, representing TTA plotted versus synephrine concentration, points out that the result of the Pearson calculation was greatly influenced by the three points in the upper right of the plot. When these three points are omitted from the calculation, the correlation between TTA and synephrine is extinguished.

Comparisons of physicochemical properties with synephrine concentrations found within the literature are limited. One study

conducted by Vieira et al. (*7*) reported a positive correlation (Pearson = 0.83) between TTA and synephrine concentration when seven Brazilian commercial orange juices were compared. However, this observation is potentially suspect considering that TTA and synephrine values observed by Vieira et al. spanned only narrow ranges, 0.61−0.81% for TTA and 10.1−21.8 mg L^{-1} for synephrine, and, additionally, we were unable to arrive at a Pearson value at or near the one reported by the authors using the data they provided in their paper. In light of this and our results as reported above, it is fairly unlikely that for mature fruit there is any reliable correlation between synephrine and TTA or any of the other physicochemical properties measured. This does not eliminate the possibility that a correlation between synephrine content and physicochemical properties may exist during fruit maturation as has been suggested by Cancalon (*21*). Further investigation targeting changes during fruit formation and maturation would be required to adequately address this question.

Returning to **Figure 3**, the three points in the upper right of the plot belong to repeats A, B, and C of fruits harvested from grove 4. Individual TTA and SSC results and the calculated maturity indices for each of the sample groups from grove 4 are shown in **Figure 3**. In relation to the maturity indices of the other samples (mean = 12.65) these fruits from grove 4 would be considered to be less mature. Although maturity index is generally a good indicator of maturity among samples, closer examination of the values obtained for samples from location 4 show that the lower maturity indices obtained are more a result of an elevated level of acidity than of a low SSC value. In fact, the sample (see also **Table 1**) that exhibited the lowest maturity index (8.95) also possessed the highest TTA (1.742%) and SSC (15.6 °Brix) values of all the samples tested. Whether the low maturity index is really a result of immaturity and immaturity is the *cause* for the high synephrine content in grove 4 cannot be decided on the basis of mere correlation. All fruits were of commercial maturity. Rather, it is perceivable that, like synephrine, SSC, and TTA, the maturity index may also be grove specific. The mean °Brix value for grove 4 was determined to be 14.93 ± 0.91. The fruits from grove 4 with their high sugar content should not be considered particularly immature in comparison to fruits from the other groves in this study. Alternatively, these results may be due to a particular unidentified environmental condition at this orchard, which enhanced biosynthetic activity, sugar, acid, and synephrine concentrations together at the point of commercial maturity. The agronomical and environmental conditions in this grove are therefore of particular interest and should be investigated through further study.

In conclusion, we determined by HPLC the synephrine concentrations found in the juices of Satsuma mandarin fruits obtained from 10 different groves located in Placer County, California. The HPLC analyses for synephrine were compared with physicochemical characteristics of the juices. Synephrine concentrations found in Satsuma mandarins were on average higher than those reported for sweet and sour oranges and in a range similar to those of other mandarin and tangerine fruits. These results suggest that mandarin juice would be a significant dietary source of synephrine. The present data also reveal an up to 2-fold difference among groves in the same county, suggesting that factors including microclimate or localized agricultural practices may influence synephrine concentrations.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; SSC, soluble solids content; TTA, total titratable acidity.

ACKNOWLEDGMENT

We thank Joanne Neft of PlacerGROWN for coordinating fruit harvest and Tracy Kahn, Principal Museum Scientist of the Citrus Variety Collection (UC Riverside), for helpful and insightful suggestions. We also thank Dominic Wong (USDA, ARS) and Tom McKeon (USDA, ARS) for their careful reading of the manuscript.

LITERATURE CITED

(1) Takei, H.; Hirabuki, M.; Yoshizaki, F. Analysis of synephrine in the peel of citrus fruit, immature citrus fruit and decoctions of Chinese medicinal prescriptions containing these crude drugs by capillary electrophoresis. *Anal. Sci.* **1999**, *15*, 1017–1020.

(2) Stewart, I.; Newhall, W. F.; Edwards, G. J. The isolation and indentification of *l*-synephrine in the leaves and fruit of citrus. *J. Biol. Chem.* **1964**, *239*, 930–932.

(3) Wheaton, T. A.; Stewart, I. Quantitative analysis of phenolic amines using ion-exchange chromatography. *Anal. Biochem.* **1965**, *12*, 585–592.

(4) Wheaton, T. A.; Stewart, I. Biosynthesis of synephrine in citrus. *Phytochemistry* **1969**, *8*, 85–92.

(5) Wheaton, T. A.; Stewart, I. The distribution of tyramine, *N*-methyltyramine, hordenine, octopamine, and synephrine in higher plants. *Lloydia* **1970**, *33*, 244–254.

(6) Avula, B.; Upparapalli, S. K.; Navarrete, A.; Khan, I. A. Simultaneous quantification of adrenergic amines and flavonoids in *C. aurantium*, various *Citrus* species, and dietary supplements by liquid chromatography. *J. AOAC Int.* **2005**, *88*, 1593–1606.

(7) Vieira, S. M.; Theodoro, K. H.; Glória, M. B. A. Profile and levels of bioactive amines in orange juice and orange soft drink. *Food Chem.* **2007**, *100*, 895–903.

(8) Niemann, R. A.; Gay, M. L. Determination of ephedrine alkaloids and synephrine in dietary supplements by column-switching cation exchange high-performance liquid chromatography with scanning-wavelength ultraviolet and fluorescence detection. *J. Agric. Food Chem.* **2003**, *51*, 5630–5638.

(9) Pellati, F.; Benvenuti, S. Fast high-performance liquid chromatography analysis of phenethylamine alkaloids in *Citrus* natural products on a pentafluorophenylpropyl stationary phase. *J. Chromatogr., A* **2007**, *1165*, 58–66.

(10) Nelson, B. C.; Putzbach, K.; Sharpless, K. E.; Sander, L. C. Mass spectrometric determination of the predominant adrenergic protoalkaloids in bitter orange (*Citrus aurantium*). *J. Agric. Food Chem.* **2007**, *55*, 9769–9775.

(11) Pellati, F.; Benvenuti, S.; Melegari, M.; Firenzuoli, F. Determination of adrenergic agonists from extracts and herbal products of *Citrus aurantium* L. var. *amara* by LC. *J. Pharm. Biomed. Anal* **2002**, *29*, 1113–1119.

(12) Avula, B.; Upparapalli, S. K.; Khan, I. A. Enantiomeric separation of adrenergic amines in citrus species, related genera and dietary supplements by capillary electrophoresis. *Chromatographia* **2005**, *62*, 151–157.

(13) Mattoli, L.; Cangi, F.; Maidecchi, A.; Ghiara, C.; Tubaro, M.; Traldi, P. A rapid liquid chromatography electrospray ionization mass spectrometry method for evaluation of synephrine in *Citrus aurantium* L. samples. *J. Agric. Food Chem.* **2005**, *53*, 9860–9866.

(14) Pellati, F.; Benvenuti, S. Chromatographic and electrophoretic methods for the analysis of phenetylamine alkaloids in *Citrus aurantium*. *J. Chromatogr., A* **2007**, *1161*, 71–88.

(15) Avula, B.; Upparapalli, S. K.; Khan, I. A. Simultaneous analysis of adrenergic amines and flavonoids in *Citrus* peel jams and fruit juices by liquid chromatography: Part 2. *J. AOAC Int.* **2007**, *90*, 633–640.

(16) Haaz, S.; Fontaine, K. R.; Cutter, G.; Limdi, N.; Perumean-Chaney, S.; Allison, D. B. *Citrus aurantium* and synephrine alkaloids in the treatment of overweight and obesity: an update. *Obes. Rev.* **2006**, *7*, 79–88.

(17) Rapisarda, P.; Calabretta, M. L.; Romano, G.; Intrigliolo, F. Nitrogen metabolism components as a tool to discriminate between organic and conventional citrus fruits. *J. Agric. Food Chem.* **2005**, *53*, 2664–2669.

(18) Mann, J. D.; Steinhart, C. E.; Mudd, S. H. Alkaloids and plant metabolism. V. The distribution and formation of tyramine methylpherase during germination of barley. *J. Biol. Chem.* **1963**, *238*, 676–681.

(19) Hanson, A. D.; Ditz, K. M.; Singletary, G. W.; Leland, T. J. Gramine accumulation in leaves of barley grown under high temperature stress. *Plant Physiol.* **1983**, *71*, 896–904.

(20) Leland, T. J.; Hanson, A. D. Induction of a specific *N*-methyltransferase enzyme by long-term heat stress during barley leaf growth. *Plant Physiol.* **1985**, *79*, 451–457.

(21) Cancalon, P. F. Analytical monitoring of citrus juices by using capillary electrophoresis. *J. AOAC Int.* **1999**, *82*, 95–106.

Received for review April 17, 2008. Revised manuscript received July 15, 2008. Accepted August 2, 2008. This work was supported through a grant from the High Sierra Resource Conservation and Development Program.

JF801225N