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Original Research Article

Phytochemical analysis of organic and conventionally cultivated Meyer lemons (*Citrus meyeri* Tan.) during refrigerated storage

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ABSTRACT

The levels of phytochemicals in organically and conventionally cultivated Meyer lemons (*Citrus meyeri* Tan.) are unknown. In this study, Meyer lemons grown in south Texas under similar climatic conditions, using organic and conventional cultivation practices, were evaluated for their levels of phytochemicals. Mature fruits were harvested in two seasons, stored at market-simulated post-harvest conditions for four weeks, and periodically evaluated for levels of phytochemicals, including flavonoids, amines, organic acids and minerals. Results indicate that organically grown lemons contain significantly ($P \leq 0.05$) higher levels of hesperidin, didymin and ascorbic acid than those cultivated in conventional system. Phenolic content was higher in organic lemons, whereas levels of citric acid and amines were higher in conventionally cultivated lemons. These results suggest that organically grown Meyer lemons are a good source of enhanced levels of flavonoids and ascorbic acid. Furthermore, storage of fruits at 10 °C up to four weeks helps maintain the levels of phytochemicals. To the best of our knowledge, this is the first report of phytochemicals evaluation of organic and conventionally grown Meyer lemons.

1. Introduction

Lemons are among the most commonly consumed citrus fruits in the world. The United States ranks fifth in world in lemon production (Perez and Pollack, 2003) with an estimated acreage of approximately 63,000 acres (Spreen, 2001). Among citrus fruits, lemons have high citric acid content, rendering them unpalatable (Penniston et al., 2008). Therefore, these fruits are primarily consumed fresh along with other food materials, used as garnishes, or juiced to make lemonades. Meyer lemon is considered as the hybrid of a true lemon and an unknown citrus species (*Citrus limon* × *Citrus sinensis*) (Uzun et al., 2014) and, unlike the more common lemon varieties, such as Lisbon or Eureka, Meyer lemon fruits have a sweeter, less acidic flavor. Meyer lemon is thornless, well adapted to warm climates and has the highest juice yield per box in comparison to several other common varieties of lemons (Lim, 2012; Moshonas et al., 1972). They also have high levels of both organic acids and health-promoting phytochemicals such as amines, flavonoids and limonoids (Del Río et al., 2004; Lario et al., 2004; Uckoo et al., 2011). These phytochemicals are associated with several health benefits, such as antioxidant, anti-proliferative and anti-inflammatory activities and also with the prevention of coronary heart disease (Benavente-García and Castillo, 2008; Kawaii et al., 1999; Patil et al., 2009). Furthermore, in human clinical trials, was implicated as a potential treatment for controlling bleeding from acute internal hemorrhoids (Misra and Parshad, 2000).

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Lemons also contain amines such as octopamine, synephrine and tyramine (Stewart and Wheaton, 1964). Metabolism of these amines forms epinephrine or norepinephrine; based on this, several formulations and extracts containing amines as the main ingredient have been promoted as weight-reducing dietary supplements (Stohs et al., 2011).

Consumer interest in the health benefits of phytochemicals has increased to significant proportion worldwide. Accumulating evidence on the health benefits of phytochemicals and increasing interest of consumers in health-promoting foods requires strategies to enhance the levels of phytochemicals. The content of phytochemicals depends both quantitatively and qualitatively on plant genotype (Bhattacharyya et al., 2014; Grusak et al., 1999) and on environmental factors including water and mineral nutrition (Mena et al., 2013; Uckoo et al., 2009); pre- and post-harvest factors can cause wide variation in the levels of phytochemicals. However, little information is available on the effect of pre-harvest factors on variation of phytochemicals in lemons. Cultivation practice is one of the major pre-harvest factors that could influence phytochemical contents (Goldman et al., 1999; Wang, 2006). In addition, evolving consumer preferences have driven a dramatic increase in organic cultivation (Willer and Lukas, 2009). Sales of organic citrus have increased at an annual rate of 20% since 1990 (Liu, 2003). Organic cultivation integrates basic agronomic practices such as crop rotation, green manure, compost, biological pest control and mechanical cultivation, to sustain productivity and control pests. Due to the lack of use of synthetic fertilizers, pesticides and growth regulators in organic cultivation, various biotic and abiotic stresses seems to enhance synthesis of polyphenolics to provide plant defenses (Luttikholt, 2007; Matern and Kneusel, 1988; Smith et al., 2014).

Currently, information related to the effect of organic cultivation on health-promoting phytochemicals in Mayer lemons is very limited. Also, the literature shows inconsistent findings on nutrient differences between organic *vs.* conventionally produced fruits and vegetables (Chassy et al., 2006). It is hypothesized that organically produced lemons could contain higher levels of phytochemicals than conventionally produced lemons. Determination of the levels of phytochemicals could help us to better understand the influence of cultivation systems and provide valuable information to consumers, enabling them to make better choices.

2. Materials and methods

2.1. Plant material and experimental design

During 2008–2010, a field experiment located in the Lower Rio Grande Valley of south Texas was conducted to evaluate the influence of organic and conventional cultivation practices on the levels of phytochemicals in Meyer lemon fruits. Two orchards were selected: the conventional citrus orchard was located at Texas A&M University-Kingsville, Citrus Center in Weslaco, and approximately 24 miles away, the organic orchard (certified by the Department of Agriculture (USDA)) located at Mission, TX. Both orchards used flood irrigation with a common irrigation source, the Rio Grande River. Trees were spaced 4.6 m × 7.3 m with a planting density of approximately 300 trees ha^{-1}. Five fruit trees were grouped as a replicate and three replications were used for each cultivation practices. Agronomic operations, nutrient management and weather data were monitored and recorded during the experiment period (Supplementary Tables S1 and S2). For phytochemical analysis, mature Meyer lemon fruits of uniform size and shape were harvested in November 2008 (early harvest) and February 2010 (late harvest) from both the experimental orchards for the harvest seasons of 2008 and 2009, respectively. The color of the fruit was considered as the criterion for maturity. Only completely yellow fruits were selected for harvest and considered mature. After harvesting, the fruits were washed with clean water, air dried and packed in cardboard boxes. The fruit boxes were stored at room temperature after harvest, for four days, and then moved to refrigerated storage, for both organic and conventionally grown lemons. After four days, the boxes were stored at the optimum temperature of 10 °C in an automated thermostat regulated refrigerator. Fruits were inspected every two days for any evidence of decay of fruits. For analysis of phytochemicals, 24 fruits from each replicate were randomly sampled at 4, 11, 18 and 25 days after storage.

2.2. Chemicals and reagents

(±)-Octopamine hydrochloride (≥98%), synephrine (≥95%), citric acid (≥99.5%), limonin (≥90%), meta-phosphoric acid (≥65%), Folin–Ciocalteu reagent (2N), glacial acetic acid (>99.5%) and high performance liquid chromatography (HPLC) grade phosphoric acid (≥85%), were purchased from Sigma–Aldrich (St. Louis, MO, USA). L-Ascorbic acid (>99.9) was purchased from Mallinckrodt (Paris, KY, USA). Authentic reference standards of narirutin (98%), didymin (98%) and hesperidin (90.8%), were purchased from ChromaDex Inc. (Irvine, CA, USA). Nanopure water (NANOpure, Barnstead/Thermolyne Corp., Dubuque, IA, USA) was used for sample preparation and HPLC analysis. Acetonitrile (HPLC grade, 99.9%) and N,N-dimethylformamide (HPLC grade, 99.7%) were purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.3. Quantification of amines and organic acids

The amines and organic acids were analyzed using the developed method reported earlier from our lab (Uckoo et al., 2011). Eight fruits were grouped as a subsample with three subsamples in each replication of individual treatment. Fruits from each subsample were peeled and blended using a household blender (Vita-prep, Cleveland, OH, USA). The blended juice was homogenized for 30 s using a Polytron homogenizer (Brinkmann Instruments Inc., Westbury, NY, USA). Three percent meta phosphoric acid (MPA) was used for extraction of amines and organic acids. In brief, 10 g of the homogenized juice sample was diluted with 30 mL of 3% MPA in a centrifuge tube and vigorously mixed. Three milliliters of sample mixture was filtered under vacuum using a 0.45 μm membrane filter (Millipore Corp., Bedford, MA, USA). The unfiltered juice residue was re-extracted with 3 mL of MPA in successive volumes of 1 mL each. All the extracts were pooled and 10 μL was injected into the HPLC for analysis. The HPLC system consisted of a Waters 1525 HPLC series (Milford, MA, USA) connected to a PDA detector. An Xbridge C18 column (3.54 μm, 4.6 mm × 150 mm i.d.) from Waters (Milford, MA, USA) was used for all the separations. Elution was carried out at ambient temperature using the mobile phase composed of 3 mM phosphoric acid under isocratic condition. The flow rate was set at 1.0 mL/min, and detection was set at dual wavelengths of λ_{223} nm and λ_{254} nm with a total analysis time of 10 min. Three injections were performed for each sample. Peaks were identified on the basis of comparing and matching the ultraviolet spectra as well as the retention time (RT) of the individual standards. The results were further validated by spiking the sample extracts with pure standards (Uckoo et al., 2011).

2.4. Analysis of flavonoids

Ten grams of blended juice sample were mixed with 10 mL of dimethyl formamide in a 50 mL centrifuge tube and homogenized for 30 s using a polytron homogenizer (Brinkmann Instruments Inc., Westbury, NY, USA). The homogenized juice was placed on a