Scientia Horticulturae 227 (2018) 244–254

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Evaluating effects of ellagic acid on the quality of kumquat fruits during storage

Yumin Liu[a,*], Yu Liu[b], Yamin Liu[b], Hongding Liu[b], Yanshuang Shang[b]

[a] Key Laboratory of the Three Gorges Reservoir Region's Eco-Environments, Southwestern University, 2 Tiansheng Road, Beibei, Chongqing 400716, PR China
[b] College of Resources and Environment, Southwestern University, PR China

ARTICLE INFO

Keywords:
Kumquat preservation
Liquidambar formosana hance
Ellagic acid
Physiological property
Principal component analysis

ABSTRACT

To assess the preservative effects of the active ingredients in the leaves of *Liquidambar formosana* Hance, the present study assessed the antioxidant activity of ellagic acid using various antioxidant assays; 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid, 1,1-diphenyl-2-picrylhydrazyl, superoxide anion radical scavenging activity and reducing power. The results of these assays were combined with principal component analysis to analyze the effect of ellagic acid treatment on the overall quality of kumquat. Whole kumquat fruit were immersed in different concentrations of ellagic acid (0, 100 and 300 mg L^{-1}) for 5 min, naturally dried then stored at room temperature (14–16 °C) for 18 days. During storage, the percentage weight loss and decay, firmness, total soluble solids, titratable acid and contents of malondialdehyde, superoxide dismutase and vitamin C were measured every 3 days. The results showed that, by 1,1-diphenyl-2-picrylhydrazyl scavenging activity and reducing power, the antioxidant activity of ellagic acid was more effective than butylated hydroxyanisole or ascorbic acid. Compared with untreated kumquat fruit, ellagic acid treatments can delay the decline in fruit firmness, total soluble solids, titratable acid and vitamin C contents, inhibit increases in malondialdehyde and enhance superoxide dismutase activity. The comprehensive evaluation of the results using principal component analysis was consistent with the experimental results, namely that ellagic acid treatment can significantly reduce the rate of deterioration in the quality of kumquat fruit, with the 300 mg L^{-1} treatment being better than the 100 mg L^{-1} treatment. The results show that ellagic acid has good prospects as a preservative treatment for kumquat fruit.

1. Introduction

Kumquats are native to Central China, belong to the Rutaceae family, and consist of oval or round-shaped fruits with a smooth, orange-yellow rind. The fruit is eaten whole with the peel and is rich in vitamins, carotene, pectin, calcium, phosphorus, iron and flavonoid compounds (Barreca et al., 2011). They have been used as a traditional folk medicine for removing phlegm, reducing alcohol intoxication, regulating qi-flowing for activating stagnancy and as an anti-depressant, so are good medicinally as well as being edible fruit (Peng et al., 2013; Lou et al., 2016; Zang, 2005). Recently, increasing attention has been paid by consumers to kumquats with the high annual output in China. However, owing to postharvest physiological deterioration, mechanical damage, infection by pathogens and other factors, the shelf life of kumquat is often reduced, leading to economic losses. Traditionally, postharvest preservation uses a chemical preservative, but these have many problems, such as residues, so should not be used for kumquat fruits which are eaten with the skin (Eckert and Brown, 1986; Li et al., 2011). Therefore, finding safe and effective natural compounds for preserving kumquat fruit is now an urgent task.

Some natural compounds have excellent antimicrobial and preservative properties, which can play an important role in the postharvest fruit production system (Burt, 2004). Natural ingredients derived from plant extracts containing active ingredients with antibacterial (Sarwar et al., 2015) and antioxidant activities (Wang et al., 2009; Weng and Yen, 2015) can inhibit or kill rot-causing fungi by removing active oxygen to prevent cell membrane lipid peroxidation, keep fruit fresh and prolong shelf-life (Womeni et al., 2016; Yuan et al., 2016; Chaikham, 2015). *Liquidambar formosana* Hance is a genus of *Hamamelidaceae Liquidambar* (State Administration of Traditional Chinese Medicine of the People's Republic of China, 1999). Its leaves are often used as a rice dye to prepare steamed glutinous rice by a small group of people in the south of China. The whole plant is used in traditional Chinese medicine for warming meridians and activating collaterals, expelling wind, removing dampness, detoxification and other functions (Yu et al., 2012). The leaves of *Liquidambar formosana* Hance

* Corresponding author.
E-mail addresses: yuminliu@163.com (Y. Liu), 13399802380@163.com (Y. Liu), 787057157@qq.com (Y. Liu), hongdingl@qq.com (H. Liu), 1061553130@qq.com (Y. Shang).

http://dx.doi.org/10.1016/j.scienta.2017.08.055
Received 2 June 2017; Received in revised form 28 August 2017; Accepted 30 August 2017

have been found to contain ellagic acid, α-shikimic acid, rutin, isoquercitrin, astragalin, hyperin, trifolin, monotropein, β-sitosterol and casuarinin (Hatano et al., 1986). The extract and isolate of its leaves have a strong antioxidant capacity (Wang et al., 2009) and effect on fruit preservation. Yan et al. (2013) have found that the extract of *Liquidambar formosana* Hance leaves can significantly improve the activity of antioxidant enzymes in fruit, delay fruit senescence and prolong the preservation period of plums (*Prunus salicina*) and was effective for keeping some fruits fresh. A similar effect was observed in loquat fruits by Ji et al. (2015), a result confirmed by Shang et al. (2015). This has further shown that the extract of its leaves is effective for preserving fruit. However, the identity of which specific active ingredient plays the leading role is still not clear. Ellagic acid, a polyphenolic compound found widely in all foods, has good antioxidant activity (Sepúlveda et al., 2011). Kasai et al. (2006) have reported that the daily consumption of a pomegranate extract containing 100 mg ellagic acid for 4 weeks could exert protective and ameliorative effects on pigmentation of the human skin after ultraviolet ray irradiation. Ellagic acid has also been used in food as an antioxidant in Japan since 1996 (Tasaki et al., 2008). So the present study aims to determine antioxidant ability of ellagic acid *in vitro* and its physiological effects on the quality of kumquats during storage. The mechanism of this preservative effect in kumquat fruit will also be explored to find new ways for comprehensively developing the resource of *Liquidambar formosana* and to provide a theoretical basis for finding natural plant preservatives for the kumquat.

Overall the purpose of the present study was to determine antioxidant activity of ellagic acid in *vitro* and the effects of it on the quality of kumquats stored at between 14 and 16 °C

2. Materials and methods

2.1. Plant materials

The test materials were kumquat fruits of uniform size, without disease, insect pests or mechanical damage at commercial harvest maturity(TSS:TA > 3). The fruits were washed with distilled water, then immersed in different concentrations of ellagic acid (Purchased from Xi'an Sen Zuo Biotechnology Co. Ltd; HPLC≧90%) solution (0, 100 and 300 mg L^{-1} (plus 5 mL 0.5 mol/l sodium hydroxide solution to help dissolution)) for 5 min. These fruits were labeled as CK, T1 and T2, respectively. Finally, the kumquats were dried at room temperature (14–16 °C), then put into polyethylene (PE) plastic bags (0.06 mm thick) for storage at room temperature (14–16 °C). The kumquats were randomly distributed for 3 treatments, and 15 fruits per treatment per replicate were randomly selected. During the storage period (up to 18 days), 3 replicates per treatment were randomly taken from storage every 3 days to determine the weight loss, firmness, vitamin C(VC), total soluble solid(TSS) and titratable acid(TA), malonic dialdehyde (MDA), superoxide dismutase(SOD). And three replicates were used individually for the determination of the decay rate.

2.2. Determination of antioxidant activity of ellagic acid

The DPPH free radical scavenging assay and The ferric reducing power (FRAP) assay was carried out according to the method of Zhou et al. (2011); The $ABTS^{\bullet+}$ method was conducted as described by Re et al. (1999); The ability of ellagic acid to scavenge superoxide radicals was determined according to the method of Wang et al. (2010).

2.3. Methods

2.3.1. Determination of percentage weight loss and decay and firmness

The percentage weight loss (W_w) determined using an electronic analytical balance (± 0.01 g) was defined as:

$$W_w = 100\ (W_i - W_f)/W_i,$$

where W_i is the initial sample weight and W_f the final weight.

The percentage decay was calculated as follows:

$$\text{Decay (\%)} = (\text{number of rotten fruit/total number of fruit}) \times 100$$

A rotten fruit was defined as one with an affected area of more than 20% of the surface.

The fruit firmness was measured at the equator of the fruit using a penetrometer (GY-2 fruit hardness tester, Yueqing Pet Si Instrument Co., Zhejiang, China) with a 3.5-mm diameter head. Measurements were made on opposite sides of each fruit after removing a 0.5 cm^2 area of peel. At least nine fruits were measured. The results were expressed as means ± SE.

2.3.2. Determination of total soluble solids (TSS), titratable acidity (TA) and vitamin C concentration, (VC)

TSS and TA were measured after completely mixing the kumquat juice. TSS, measured using a VBR90A handheld refractometer (Huier Instrument Equipment Co, Hangzhou, China), was expressed as a percentage. TA measurements were performed by titrating 20 mL of filtered kumquat juice with 0.1 N NaOH until attaining a pH of 8.1, with TA values being expressed as g citric acid per kg juice. The value of TA (X) was calculated as:

X = (C*V*K*F)/m*1000, C is concentration of NaOH (mol/L); V is the volume of consumption of NaOH (ml); K is conversion coefficient of citric acid; F is ratio of total volume to sample volume.

VC concentration was measured by the molybdenum blue spectrophotometry method (Gao, 2006) using different VC concentrations for the standard curve, and expressed as mg g^{-1} fresh weight.

2.3.3. Determination of malondialdehyde content (MDA) and SOD enzyme activity

The malondialdehyde (MDA) content was measured with thiobarbituric acid (TBA) chromatometry (Wang., 2006). Frozen kumquat fruit immediately are powdered after removal, powder (0.5 g) was homogenized in 5 mL of 5% (w/v) TCA. The homogenate was centrifuged at 3000 × *g* for 10 mins then 2 mL of the supernatant was added to 2 mL of 0.67% (w/v) TBA, incubated in boiling water for 30 min and cooled after centrifugation. The absorbance was measured at 450, 532 and 600 nm using an ultraviolet spectrophotometer (T6 new century UV visible spectrophotometer, Shanghai, China). The concentration of MDA (C) was calculated as:

$C\ (g\ mL^{-1}) = 6.45(A_{532} - A_{600}) - 0.56A_{450}$, and expressed as μmol g^{-1}.

The SOD enzyme activities were measured using the nitroblue tetrazolium (NBT) photochemical reduction method (Yu et al., 2008), one enzyme activity(U) is to increasing 50% of photochemical reduction on NBT. Enzyme activity was expressed as units (U) per mg protein.

2.4. Data analysis

Three replicates per treatments were done and the effect of ellagic acid on kumquat fruit preservation was analyzed using variance (ANOVA), with multiple comparisons made using the LSD method at a significance level of $p \leq 0.05$. The means ± S.E. (standard error) were used.

Principal component analysis was conducted using SPSS 17.0 (IBM, Chicago, USA) as the basis for establishing a comprehensive evaluation model (Patras et al., 2011; Peng et al., 2014). A cumulative variance contribution rate of > 85% was used as the basis for determining the number of principal components. To establish the comprehensive evaluation model, each component expression was obtained by multiplying each principal component feature vector by the normalized data;