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Effect of Ethylene Treatments on Limonin Reduction in Thai Pummelo (*Citrus grandis* (L.) Osbeck) Fruit

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ABSTRACT

Bitterness in the peel and juice of pummelo is mainly caused by limonin. This research studied different preparations of pummelo and used ethylene as fumigant at different concentrations in different treatments—namely, whole fruit (treatment1), pummelo fruit without the flavedo and albedo (treatment 2), pummelo fruit with only juice vesicle tissue (treatment 3) and pummelo juice (treatment 4). The limonin content in the flavedo, albedo, juice vesicle tissues and pummelo juice was significantly reduced following ethylene treatment. For effective limonin reduction in juice, the flavedo and albedo of pummelo fruit must be removed leaving only juice vesicle tissue (treatment 3) before treating with ethylene. The optimum treatment that reduced the limonin content by 78.38% was 200 ppm ethylene and 1.30 hr exposure. Treating pummelo with ethylene had no effect on nomilin, eriocitrin, neoeriocitrin and the antioxidant capacity of the juice; however, ethylene fumigation caused a slight decrease in the naringin content. Treating pummelo juice directly with ethylene had no effect on its limonin content.

Keywords: pummelo, limonin, ethylene

INTRODUCTION

In Thailand, pummelo (*Citrus grandis* (L.) Osbeck) is widely grown with many cultivars including Kao Yai, Kao Paen, Kao Nampheung, Kao Tanggkya, Kao Hom, Kao Phuang, Pattavee, Thongdee and Tha Khoi among others. In this study, Thongdee, the popular pummelo cultivar in Nakhon Pathom, was studied. Characteristics of Thongdee are its period of flowering in January and harvesting from August to September. The fruit shape is medium globose with a flattened bottom and pucker-head; fruit has a diameter 14–16 cm. The albedo color is pink and the juice color of the pummelo is also light pink. The taste of the juice sacs (called shrimp) is juicy and sweet (Department of Agriculture, 2002). Pummelo juice is a good source of ascorbic acid with a range from 37.03 to 57.59 mg/100 mL (Pichaiyongvongdee and Haruenkit, 2009a). However, the fruit juice also contains bitter substances such as limonoid with a range from 20.97 to 67.35 mg/L and flavanones (naringin, eriocitrin, neoeriocitrin, narirutin, neohesperidin and hesperidin) with ranges from 245.63 to 393.96 mg/L (Pichaiyongvongdee and Haruenkit, 2009b). The antioxidant capacity measured by 2, 2-diphenyl-1-1-picrylhydrazl (DPPH) and ferric reducing antioxidant power (FRAP) assays gave good correlations with the total polyphenol content measured as gallic acid equivalent in a fresh weight sample of 100 mg (GAE mg/100mg FW) ranging from 63.96 to 150.30 (Pichaiyongvongdee and Haruenkit, 2009b). Harborne and Williams

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(2000) and Silberberg *et al*. (2006) reported that citrus fruits all contain these substances and they have been proven beneficial to prevent diseases including prevention and control of coronary heart disease as well as having anti-inflammatory and antimicrobial activities.

However, in the pummelo juice or citrus juice industry, the bitter taste from limonin and naringin must be removed because the concentration of limonin is high as levels of 6 mg/L (Guadagni *et al*., 1973) or 9.78 mg/L in orange juice (Laohakunjit *et al*., 1997) were detected by panelists. Thus, the bitterness of pummelo juice is a problem for the juice industry and exceeds customer acceptance levels, as the content of limonin ranged from 10.07–29.62 mg/L (Pichaiyongvongdee and Haruenkit, 2009a). In contrast, the threshold level of naringin was as high as 800 mg/L (Rouseff *et al*., 1988) and was not a problem to panelists. The concentration of naringin in pummelo juice has been reported to range from 242.63 to 386.45 mg/L (Pichaiyongvongdee and Haruenkit, 2009a), which is lower than the threshold of 800 mg/L and consequently, it is not necessary to reduce naringin. Several processes have been reported to help reduce the bitterness in citrus juice, such as the enzyme technique, where *Pseudomonas* sp. 321-18 was extended to reduce the limonin content in grapefruit juice (Hasegawa and Herman, 1992) and *Pseudomonas putida* was found to reduce limonin by 64% (Sharma, 2005). Johnson and Chandler (1988) used cellulose acetate, a nylon membrane and ion exchangers in grapefruit juice to remove limonin. Caisawadi *et al*. (1998) applied Floricil to treat lime juice that reduced the limonin content by 51.55%. Laohakunjit *et al.* (1997) used natural egg shells to achieve a maximum reduction to 33.05% limonin, 17.85% naringin and 76.62% citrus acid. Deatcheewa and Yaowapha (2009) reported a method that used deacetylation to remove limonin (58.56%), naringin (22.26%) and cloudiness in juice (62.23%). However, all these methods have severe economic and technical limitations since they are non-specific in nature, alter the chemical composition of juices and affect their nutritional quality, texture, flavor, odor and stability (Puri *et al*., 1996). Another interesting method is to remove limonin by ethylene treatment because it has less effect on the chemical composition. Ethylene is recognized as a gaseous plant hormone whose role is essential at every phase of plant growth, particularly during the ripening phase. Ethylene can be internally synthesized in plants or externally applied. Vincent *et al*. (1973) reported that treating oranges for 3 hr with 20 ppm ethylene reduced the limonin by 38% more than the level in orange fruit that had not been treated with ethylene. Pichaiyongvongdee and Haruenkit (2001) also studied a 3-hour treatment of limes using 20 ppm ethylene and reported that the limonin was reduced by 56.59%. The ethylene treatment had no effect on the naringin and ascorbic acid content of the lime juice. In addition, the ethylene treatment was reported to reduce the bitterness in grapefruit**.** Thus, it is possible to apply an ethylene treatment as a tool for reducing the bitterness in pummelo juice in a way that has never been studied before. The aim of this research was to assess the effect of different ethylene levels on the reduction of bitter substances in the peel and juice of pummelo fruit.

MATERIALS AND METHODS

Materials

Samples of the pummelo cultivar Thongdee (diameter approximately 14.80 cm, weight approximately 1.550 kg) were collected from an orchard in Nakhon Pathom province. The fruits were prepared using four treatments (Figure 1).

Limonin, nomilin, and gallic acid monohydrate were purchased from Sigma-Aldrich (St Louis, USA). Naringin (naringenine-7-rhamnosido-glucoside, NAR), eriocitrin (eriodictyol 7-O-β-rutinoside), neoeriocitrin (eridictyol 7-o-neohesperidoside), DPPH (2,

Figure 1 Pummelo fruit samples showing the four treatments applied: a) Treatment 1 = whole fruit; b) Treatment 2 = peeled fruit (without flavedo); c) Treatment 3 = fruit segment (juice vesicles); d) fruit juice.

2-diphenyl-1-1-picrylhydrazl), TPTZ (2, 4, 6-tripyridyl-s-triazine) were purchased from Fluka (Buchs, USA). Ethylene gas was purchased from BOC-Scientific (Thailand). Other common reagents were purchased from Merch (Darmstadt, Germany).

Equipment used for analysis comprised: 1) a high performance liquid chromatography (HPLC) system with two hydraulic pumps (model Water 515, USA), an injection system (U6K), a Novapak C_{18} Column (3.9 × 150 mm, pore size 4 µm), a C_{18} guard column, a UV-VIS detector (model 2478), and a computerized recorder/ integrator (model Millennium 32); and 2) a gas chromatographer with flame ionization detector (GC-FID; Shimadzu GC-8A). In addition, PVC gas-tight tanks were used for the ethylene treatment and smaller 1500 mL as-tight jars were used for treatments (Figure 2).

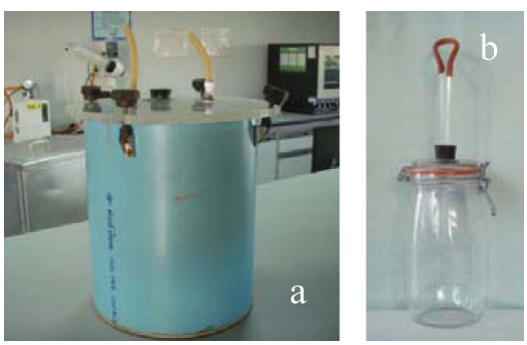

Figure 2 PVC gas-tight tanks for ethylene treatment (a) and 1500 mL gas-tight jars (b).

Methods

Effect of ethylene concentration and fumigation time on the limonin content in pummelo fruit (treatment 1)

Pummelo fruits (treatment 1, Figure 1(a)) were placed in a holding tank and then ethylene gas was used for fumigation at four different ethylene concentrations of 0, 50,100 and 200 ppm. The fruits remained in the tank for five different holding periods of 0, 12, 24, 36 and 48 hr. The treated pummelo fruits were taken out and separated by parts for analysis. The flavedo, albedo and juice vesicle tissues were dried and their limonin content determined. The juices were extracted and their limonin content determined.

Effect of ethylene concentration and fumigation time on the limonin content in juice from the peeled pummelo without flavedo and albedo (treatment 2)

Pummelo fruits were prepared by removing the flavedo and albedo leaving a thin layer of white spongy tissue (treatment 2, Figure 1(b)) and then placed in a holding tank. Ethylene gas was used for fumigation at four different ethylene concentrations of 0, 50,100 and 200 ppm. Fruits remained in the tank for five different holding periods of 0, 1, 2, 3 and 4 hr.

Effect of ethylene concentration and fumigation time on the limonin content in juice from the peeled fruit with only juice vesicle tissues (treatment 3)

The fruits were prepared by removing the flavedo and the albedo and leaving only juice

vesicle tissues (treatment 3, Figure 1(c)) and then were placed in a holding tank and ethylene gas was used for fumigation at four different ethylene concentrations of 0, 50,100 and 200 ppm. Fruits remained in the tank for five different holding periods of 0, 0.30, 1, 1.30 and 2 hr.

Effect of ethylene concentration and fumigation time on pummelo juices (treatment 4)

Samples of 500 mL each of pummelo juice (treatment 4, Figure 1(d)) were put into a gas-tight jar and then fumigated with ethylene gas. Three different ethylene concentrations of 0, 50 and 200 ppm were used. The juice samples remained in the gas-tight jars for five different holding periods of 0, 5, 10, 20 and 30 min.

To monitor the ethylene concentration in the tanks and gas-tight jars, gas samples were taken for ethylene analysis by GC-FID.

Effect of ethylene concentration and fumigation time on nomilin, flavanone and antioxidant capacity in pummelo juice

Samples of 500 mL each of pummelo juice were put into gas-tight jars and then fumigated with ethylene gas. Three different ethylene concentrations of 0, 50 and 200 ppm were used. The juice samples remained in the gas-tight jars for 30 min.

Statistical analysis

Data were analyzed by ANOVA using split plots in a completely randomized design, with ethylene concentration as a main plot and time of exposure as a subplot. Three replications of each experiment were performed. Means were compared by Duncan's multiple range test using a significance test level of $P < 0.05$.

Quantitative analysis of limonin, nomilin, naringin, eriocitrin, neoeriocitrin and antioxidant component

Sample preparation for analysis

The flavedo, albedo and juice vesicles were homogenized in a blender (Moulinex, Model A 327 R7, France) and dried at 40 °C in a freeze dryer (Heto model LyoPro 3000) for 12–15 hr.

Extraction of limonin and nomilin

To determine the limonin and nomilin content in the flavedo, albedo and juice vesicles, 5 g of fruit tissue was extracted with 20 mL of 100% methanol by shaking with a Vortex Mixer for 1 min and then centrifuging at 2,500×g for 10 min. The supernatant was filtered through a 0.22 µm nylon filter before analyzing. For juices, 10 mL of each sample was centrifuged at 2500×g for 10 min. The Millipore C18 Sep-pak cartridge was rinsed with 2 mL of 100% methanol and then 5 mL of deionized water before use and then1 mL of juice supernatant was filtered through the cartridge. The cartridge was rinsed with 5 mL of deionized water and limonin was slowly eluted from the cartridge with 1 mL of 100% methanol. The methanol effluent was filtered through a 0.22 µm nylon filter prior to HPLC analysis (Shaw and Wilson, 1984).

Extraction of naringin, eriocitrin and neoeriocitrin

To determine the naringin, eriocitrin and neoeriocitrin contents in juices, samples of 1–2 mL of each juice were extracted with 4 mL of 100% methanol by shaking for 1 min using a Vortex Mixer and then centrifuging at 2500×g for 10 min. Each supernatant was filtered through a 0.22 µm nylon filter prior to HPLC analysis (Rouseff, 1988).

Determination of limonin, nomilin, naringin, eriocitrin and neoeriocitrin contents

Each substance was determined by a reverse-phase HPLC method. For the analysis of limonin and nomilin, the mobile phase consisted of acetonitrile: deionised water (35:65) with a flow rate of 1 $mL.min^{-1}$. The injection volume of the samples was 20 µL. The detection wavelength was 210 nm. For naringin, eriocitrin and neoeriocitrin, the mobile phase consisted of acetonitrile and water which was varied in ratios according to the

individual standards; naringin (acetonitrile:DI water = 25:75); eriocitrin and neoeriocitrin (acetonitrile:DI water plus 1% acetic acid = 15:85) with a flow rate of 1 mL.min^{-1} and the injection volume of the samples was 20 µL. The detection wavelength was 280 nm.

Determination of total polyphenol content

The total polyphenol content in each pummelo juice sample was determined by the Folin-Ciocalteu method (Singleton *et al*., 1999). The juice (0.5 mL) was added to 2 mL of 10% Na_2CO_3. After 5 min the 25% Folin-Ciocalteu reagent (0.5 mL) was added to the mixture and allowed to stand for 10 min before measurement. The absorbance was measured at 760 nm using a UV–VIS spectrophotometer (Shimadzu 1601, Japan). The total polyphenol content was expressed as milligrams of gallic acid equivalent (GAE/100 mL).

Determination of antioxidant activity

Free radical scavenging assay (DPPH)

The free radical scavenging DPPH method of Shyu and Hwang (2002) was followed. A sample of 0.1 mL was added to 6 mL methanol followed by 0.6 mL of 0.8 mM solution of DPPH. The absorbance was read at 517 nm after 30 min of initial mixing. The same concentration of methanol (6 mL) was used as a control. The inhibitory percentage of DPPH was calculated using Equation 1:

$$\% \text{ inhibition} = [A0–A1/A0] \times 100 \quad (1)$$

where A0 is the absorbance of the control and A1 is the absorbance in the presence of the sample.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay method followed Benzie and Strain (1999). The FRAP reagent was composed of 0.1 M acetate buffer (pH 3.6): 40 mM TPTZ: 20 mM ferric chloride at the ratio of 10:1:1 by volume. A sample of 0.1 mL was added to 3 mL reagent and the absorbance was read at 593 nm. The result was expressed as milligrams of trolox equivalent (TE) per 100 mL.

RESULTS AND DISCUSSION

Effect of ethylene concentration and fumigation time on limonin content in pummelo fruits (treatment 1)

The pummelo fruits in treatment 1 were treated with ethylene gas. As shown in Table 1, the concentration of ethylene and the fumigation time affected significantly the limonin content in the flavedo, albedo and juice vesicles compared to the nontreated sample. The ethylene was absorbed by plant tissue from the outer to the inner part**.** Therefore, the thickness of each tissue will play a significant role in limonin reduction. The longer the fumigation time and the higher the ethylene concentration, the more effective was the reduction in limonin compared with shorter times and lower ethylene concentrations. No significant differences in limonin reduction were detected in the juice because the rind thickness of the pummelo ranged from 1.50 to 2.00 cm (Department of Agriculture, 2002), so that the flavedo and albedo consisted of oily, spongy tissue and large parenchyma cells. However, the spongy tissue allowed the ethylene to translocate more depending on the exposure time. Under this treatment, the limonin content in juice samples was nearly the same as the control. The limonin contents in the albedo, flavedo and juice were not effectively reduced to the acceptable threshold by the treatments.

Effect of ethylene concentration and fumigation time on limonin content in peeled pummelo without flavedo and albedo (treatment 2)

The peeled pummelo fruits without flavedo and albedo (treatment 2) but with the thin layer of white sponge membrane remaining were

treated with ethylene gas. As shown in Table 2, the reduction of limonin in the juice and juice vesicles was much greater than in the previous experiment. With conditions of 200 ppm after 4 hr, the maximum limonin content in the juice had decreased 81.12% from 25.37 to 4.79 ppm.

A good correlation of the fumigation time and the concentration of ethylene was evident from this treatment. The treatment of pummelo without the pericarp (albedo and flavedo) proved that reduction of limonin in the juice using ethylene was possible. However the pink membrane may block the ethylene penetrating to the juice. Therefore in experiment 3, pummelo fruits were prepared by removing all the white spongy membrane left with only the pummelo flesh. Such preparation removed the barrier to the ethylene resulting in a reduction in time for decreases in the limonin content. In terms of prevention from spoilage, the white spongy membrane can act as a guard layer to prevent microbial spoiling of the juice. Low temperature storage is needed to prolong the shelf life of peeled fruits.

Table 1 Average limonin content in various part of pummelo fruits (treatment 1) in a batch process using different ethylene concentrations.

Ethylene	Fumigation time (hr)				
(ppm)	0	12	24	36	48
		Limonin in flavedo (ppm, dry weight)			
0	264.11	252.91 (4.24%)	243.54 (7.79%)	255.85 (3.12%)	260.82 (1.24%)
50		219.29 (16.97%)	194.37 (26.40%)	160.09 (39.38%)	143.00 (45.85%)
100		211.34 (19.98%)	170.76 (35.34%)	150.73 (42.93%)	129.49 (50.97%)
200		199.66 (24.40%)	147.73 (44.06%)	97.21 (63.19%)	90.94 (65.57%)
		F-test (Ethylene* Time)		19.03*	
		Limonin in albedo (ppm, dry weight)			
0	423.90	412.59 (2.67%)	417.78 (1.44%)	414.84 (2.14%)	408.90 (3.54%)
50		410.33 (3.20%)	366.54 (13.53%)	321.08 (24.26%)	303.82 (28.33%)
100		403.69 (4.77%)	324.55 (23.44%)	282.72 (33.31%)	280.24 (33.89%)
200		401.30 (5.33%)	304.73 (28.11%)	271.56 (35.94%)	173.11 (59.16%)
		F-test (Ethylene* Time)		11.00*	
		Limonin in juice vesicles (ppm, dry weight)			
0	623.91	633.30 (0%)	637.44 (0%)	625.48 (0%)	632.88 (0%)
50		612.34 (1.85%)	546.90 (12.34%)	534.24 (14.37%)	507.16 (17.71%)
100		609.03 (2.38%)	521.42 (16.43%)	520.66 (16.55%)	468.85 (24.85%)
200		450.31 (27.82%)	446.97 (28.36%)	445.84 (28.54%)	407.14 (34.74%)
		F-test (Ethylene* Time)		18.99*	
		Limonin in juice (ppm, fresh weight)			
0	30.12	29.42 (2.32%)	28.50 (5.38%)	29.12 (3.32%)	29.93 (0.63%)
50		29.18 (3.12%)	28.63 (4.95%)	28.39 (5.74%)	29.38 (2.46%)
100		29.02 (3.65%)	28.56 (5.18%)	28.08 (6.77%)	27.83 (7.60%)
200		28.00 (7.04%)	27.03 (10.26%)	26.75 (11.19%)	26.18 (13.08%)
		F-test (Ethylene* Time)		0.29^{ns}	

Note : * = Significant difference tested at $P < 0.05$; ns = Not significant; ( ) = mean % of limonin reduction.

Effect of different ethylene concentration and fumigation time on limonin content in peeled pummelo with only juice vesicles (treatment 3)

In the fruit subjected to treatment 3, all obstacles to ethylene gas penetration into the vesicles had been removed. The juice vesicles and juice were determined for their limonin content. This experiment confirmed that removing all the ethylene obstacles could reduce the fumigation time and ethylene concentration with a significant difference in the limonin content in the juice vesicles and juice as shown in Table 3. The exposure time was reduced to 0.30–2 hr depending

Table 2 Average limonin content in various part of pummelo fruit without flavedo and albedo (treatment 2) in a batch process using different ethylene concentrations.

Ethylene	Fumigation time (hr)				
(ppm)	0	1	2	3	4
		Limonin in juice vesicles (ppm, dry weight)			
0	398.24	384.53 (3.44%)	423.71 (0%)	381.92 (4.10%)	399.92 (0%)
50		373.76 (6.15%)	338.91 (14.90%)	284.12 (28.66%)	201.78 (49.33%)
100		342.42 (14.02%)	251.12 (36.94%)	231.01 (41.99%)	175.30 (55.98%)
200		285.26 (28.37%)	174.77 (56.11%)	144.86 (63.62%)	105.38 (73.54%)
		F-test (Ethylene *Time)		41.52*	
		Limonin in juice (ppm, fresh weight)			
0	25.37	24.37 (3.94%)	23.49 (7.41%)	23.88 (5.87%)	24.08 (5.08%)
50		21.87 (13.80%)	20.62 (18.72%)	16.10 (36.54%)	11.12 (56.17%)
100		17.50 (31.02%)	14.86 (41.43%)	9.31 (63.30%)	8.32 (67.21%)
200		11.63 (54.16%)	7.83 (69.14%)	6.23 (75.44%)	4.79 (81.12%)
		F-test (Ethylene *Time)		143.37*	

Note : * = Significant difference tested at $P < 0.05$; ( ) = mean % of limonin reduction.

Table 3 Average limonin content in various part of pummelo fruit with only juice vesicles (treatment 3) in a batch process using different ethylene concentrations.

Ethylene	Fumigation time (hr)				
(ppm)	0	0.30	1.00	1.30	2.00
		Limonin in juice vesicles (ppm, dry weight)			
0	434.51	414.28 (4.66%)	433.24 (0.29%)	407.31 (6.29%)	414.85 (4.52%)
50		376.99 (13.24%)	308.87 (28.92%)	236.70 (45.52%)	206.24 (52.54%)
100		286.04 (34.17%)	249.26 (42.63%)	158.51 (63.52%)	153.15 (64.75%)
200		291.98 (32.80%)	183.04 (57.87%)	120.42 (72.29%)	110.84 (74.49%)
		F-test (Ethylene *Time)		27.09*	
		Limonin in juice (ppm, fresh weight)			
0	23.68	23.81 (0%)	24.00 (0%)	23.69 (0%)	22.49 (5.03%)
50		19.99 (15.58%)	14.80 (37.50%)	13.31 (43.79%)	10.68 (54.90%)
100		11.54 (51.27%)	9.60 (59.08%)	9.43 (60.18%)	8.91 (62.37%)
200		7.28 (69.26%)	6.90 (70.86%)	5.12 (78.38%)	4.62 (80.49%)
		F-test (Ethylene *Time)		55.03*	

Note : * = Significant difference tested at $P < 0.05$; ( ) = mean % of limonin reduction.

on the concentration used. To reduce the limonin content in pummelo juice to a lower concentration than the threshold of 6 ppm limonin reported for orange juice (Guadagni *et al*., 1973), the suggested choice of treatment is treating with 200 ppm ethylene gas for 1.30 hr (limonin content reduced to 5.12 ppm). This condition was more economical than that any mix of operational parameters in treatment 2.

Effect of ethylene concentration and exposure time on limonin content in pummelo juices (treatment 4)

In this treatment, the pummelo juice was directly fumigated with ethylene, which produced no significant change in the limonin content in the juice (Table 4). This was due to the fruit tissue becoming broken during juice extraction, thus allow the enzymatic process to occur. The limonin-A-ring lactone (LARL, non-bitter precursor) is found to be endogenously present in the cell cytoplasm of the membranous sacs at neutral or alkaline pH; when these sacs were ruptured, the precursor (LARL) encountered acidic pH and enzyme limonin-D-ring lactone hydrolase catalyzed the closure of the ring to form the limonin (Maier *et al.,* 1977). Therefore, an effective way is to prevent the change of LARL to form the limonin. Maier *et al.* (1973) also found that treating citrus fruit with 20 ppm ethylene for 3 hr resulted in loss of LARL and lowered the limonin content in fruit. Ethylene has a pronounced effect on physiological changes in climacteric fruits, including ripening, degradation of chlorophyll and volatile production. Pummelo is a non climacteric fruit, so the effect of ethylene on ripening was negligible. The ethylene may reduce or prevent the LARL forming limonin; however, the mechanism is not fully elucidated. If the limonin is already present in the juice, then the ethylene has no effect as was shown in the current study.

Table 4 Average limonin content in pummelo juice (treatment 4) in a batch process using different ethylene concentrations.

Ethylene	Fumigation time (min)				
(ppm)	0	5	10	15	20
0	26.48	26.84	25.88	25.68	26.53
50		25.84	26.23	26.28	28.93
200		27.13	26.55	26.83	26.68

Table 5 Change in nomilin, flavanones, total polyphenol content and antioxidant capacity of juice from different ethylene concentrations and fumigation time for 30 min of pummelo fruit with only juice vesicles (treatment 3) in a batch process.

	Control	Ethylene concentration		
		50 ppm	100 ppm	200 ppm
Nomilin (ppm)	21.90 ^{a}	21.69 ^{a}	21.27 ^{a}	20.83 ^{a}
Naringin (ppm)	383.92 ^{a}	367.87 ^{b}	356.05 ^{b}	325.76 ^{c}
Eriocitrin (ppm)	18.15 ^{a}	15.45 ^{b}	14.06 ^{b}	14.64 ^{b}
Neoeriocitrin (ppm)	25.36 ^{ab}	23.05 ^{ab}	21.66 ^{bc}	26.93 ^{a}
Total polyphenol (GAE mg /100 mL)	134.47 ^{a}	134.42 ^{a}	132.35 ^{a}	131.92 ^{a}
DPPH (%)	21.46 ^{a}	21.69 ^{a}	21.54 ^{a}	21.54 ^{a}
FRAP, mg TE/100mL	55.51 ^{a}	55.62 ^{a}	54.38 ^{a}	55.05 ^{a}

Note: Means in the same rows with different letters are significantly different at $P \geq 0.05$; DPHH = 2, 2-diphenyl-1-1-picrylhydrazl; FRAP = ferric reducing antioxidant power.

Effect of ethylene concentration and fumigation time on nomilin, naringin eriocitrin neoeriocitrin and antioxidant capacity in debittered pummelo juice

Treating the pummelo (Treatment 3) with ethylene had no effect on nomilin, eriocitrin and neoeriocitrin and the antioxidant capacity in the juice but ethylene had a slight effect on naringin by decreasing the naringin content in the juice at all tested ethylene levels (Table 4).

CONCLUSIONS

The effect of ethylene treatment on the reduction of bitter compounds in pummelo depended on the ethylene concentration and the duration of the fumigation. Ethylene fumigation reduced the limonin in the peel and the juice as it was translocated to layers of tissue in the peel and juice sacs. The limonin reduction in juice was most effective when the albedo and flavedo had been removed. Thus, treatment 3 for pummelo was proposed as the preferred treatment which involved treating with 200 ppm ethylene gas for 1.30 hr after which, the limonin content in the juice decreased 78.38% from 23.68 to 5.12 ppm. The limonin content in juice was not affected by the application of ethylene directly into the pummelo juice. Ethylene was proven effective in limonin reduction when the cells were not ruptured. Treating pummelo with ethylene had no effect on nomilin, eriocitrin, neoeriocitrin and the antioxidant capacity of the juice. However, ethylene fumigation caused a slight decrease in the naringin content. The quality of juice from the treated fruit in terms of health promotion was not altered, as the bioactive compounds and antioxidant activity were not affected.

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