Development of citrus wine with green tea

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Abstract: Citrus and green tea are dominant sources of phenolic compounds contributed to improving human health. This study evaluated the potential for the development of wine using citrus and green tea as a raw material. The ethanol concentration of citrus wine with/without green tea was 13.4–15% W/V after fermention for 16 days at 20ºC. The contents of total sugar, free sugar (fructose and glucose) and organic acids (fumaric acid and malonic acid) were slightly decreased in citrus wine after addition with green tea, while purine alkaloids and catechins were increased.
[Jung Hyun Kim, Min Young Kim. **Development of citrus wine with green tea.** *Life Sci J* 2013;10(1):1176-1180] (ISSN: 1097-8135). http://www.lifesciencesite.com. 181

Keywords: citrus; green tea; wine; physicochemical and bioactive components

1. Introduction

In general, wine is the alcoholic beverage made by the alcoholic fermentation of sound ripe grape. Citrus fruit, such as orange, mandarin and grapefruit, are also used for wine production (Selli et al., 2002; Selli et al., 2003; Selli et al., 2004; Ogunjobi and Ogunwolu, 2010). In Korea, Jeju island is well known for its important production of citrus fruits. Nearly 60% of total quantity produced is exported. The reminder is locally consumed, with the exception of a percentage which is not marketable due to its appearance or its being in excess of demand. To exploit this surplus of non-attractive, small-sized citrus fruit, there is the possibility of wine production, which is applied in this research study.

Green tea is consumed worldwide, especially in Ease Asian countries. Green tea contains caffeine and polyphenolic compounds known as catechins, which has been suggested to be responsible for many of the potential health effects including heart stimulation, anti-oxidation, anticancer, or antibiotics effects (Cooper et al., 2005a; Cooper et al., 2005b). Green tea powder (or leaves) can contribute to the development of some valued added products, which would be acceptable consumers. Therefore, this study intends to develop a new wine product using citrus and green tea supplements, and then evaluate its physicochemical and bioactive components.

2. Material and Methods

2.1. Plant materials and microorganism

Citrus fruits (Citrus *unshiu* Marc) and fresh young tea leaves (Yabukita cultivar) were collected from Jeju island in Korea. Tea leaves were given a roasting process for 5 min at 300 ºC, followed by cooling and rolling, three cycles of hand-rolling for 3 min at 250 ºC and shaking and ash, and then roasting for 90 min. Green tea powder was obtained from a local market in Jeju, Korea.

Saccaromyces cerevisiae IFO 2363 used to prepare the mash was obtained from NIHHS (National Institute of Horticultural and Herbal Science, Jeju, Korea).

2.2. Preparation of mash and fermentation process

Fresh matured citrus fruits were cleaned by washing in tap water and the pulp was then crushed with water (1:1, w/v) in a grinder. Citrus juice was extracted with the aid of a juice squeezer, passed through the strainer to remove the pulp, and treated with sucrose solution and calcium carbonate to attain 25 °Brix (pH 4.5). The powder and leaves of green tea were then added with different concentrations (1-3%, w/v), respectively. The must prepared above was inoculated with 2% starter culture of *Saccharomyces cerevisiae* IFO 2363. After fermentation for 16 days at 20 ºC, the wine sample was prepared by using ultrafiltration system (model SKUF10-312, SK Chemical, Korea) with a porous membrane and allowed to mature for one month. All the samples were prepared in triplicates.

2.3. Standard chemical analysis and color measurement

Apparent total sugar concentration was tested using a Brix Saccharo meter (model LH-T20, Atago, Japan). The total titratable acidity was assessed by titration with sodium hydroxide (0.1 N) and expressed as percent citric acid. The ethanol concentration of the wine was determined by measuring the specific gravity of the distillate

according to the procedure described previously (Swain et al., 2007).

The color was determined using a color difference meter (model ZE2000, Nippon Denshoku, Japan) and is described as the L value (lightness), a value (redness), and b value (yellowness). A penetrable whiteboard (x, 93.18; y, 95.18; z, 112.18) was used as the standard.

2.4. Free sugar analysis

The samples (100 mL) were condensed by a vaccum rotatory evaporator (model R101, Buchi, Germany) at 50 ºC, filled up to a total volume of 20 mL with ultra pure water, centrifuged at 3,000 × g for 10 min, and then filtered through a 0.45 µm membrane filter (Whatman, NJ, US) and the Sep-pak C 19 Cartridge. Aliquot of the resultant supernatant (5 µL) was injected in a HPLC system (Waters 600S controller, Waters 626 pump, Waters, Mildford, MA, US) with high performance carbohydrate column (3.9 mm × 300 mm i.d., Waters, Mildford, MA, US) coupled to a refractive index (RI) detector (Waters, Mildford, MA, US). The analysis was carried out at 33 at a flow rate of 1.6 mL/min with acetonitrite/water (83:17, v:v) as the mobile phase. Sugars present in each sample were identified and quantified using external standards.

2.5. Volatile component analysis

The extract was prepared according to the method described in previous papers (Selli, 2007; Selli et al., 2003) with minor modification. Briefly, 50 µg/L of 4-nonanol (internal standard) was added to wine sample (100 mL) before extraction, and then mixed with 50 mL of dichloromethane. The organic phase was recovered and extracted twice with centrifugation. The combined extracts were dried over anhydrous sodium sulphate, reduced in volume to 20 µL in a vaccum rotatory evaporator (model R101, Buchi, Germany), and then evaporated by a gentle stream of nitrogen.

Volatile components were quantified with gas chromatography with a HP-5890 (Hewlett Packard Corp., US) chromatograph equipped with a flame ionization detector (FID) and 30 m × 0.32 mm I.D. HP-Supercowax 10 column (0.5 µm). The chromatographic conditions were as follows: initial temperature, 40 ºC (10 min); 2 ºC /min ramp to 200 ºC; and 15 min hold at 200 ºC. The injector and flame ionization detector temperature were both 280 ºC and nitrogen was used as the carrier gas, at a flow-rate of 0.6 mL/min. Compound quantification was based on the international standard method. The efficacy of the method was verified from the analysis performed on standard solutions of the components, and with the aid of an HP-5979 mass spectrometer linked to the chromatograph. The variance of the method was determined by the analysis of three replicates of each sample.

2.6. Organic acid analysis

Organic acids were determined by a high performance liquid chromatography using the method described previously (Frayne, 1986; Herjavec et al., 2003). After the sample was centrifuged at 12,500 × g for 10 min, the supernatant was filtered through a 0.45 µm membrane filter (Whatman, NJ, US). Twenty microliters were directly injected in a HPLC system (1050 series, Hewlett Packard Corp., US) comprising an Aminex HPX-87H organic acid analysis cation exchange column (7.8 × 300 mm i.d., Bio-Rad Laboratories, VA, US) and a Variable Wavelength detector linked to a HP 3395 integrator with solvent delivery systems. The content of organic acids was determined by measuring the absorbance at 210 nm. Organic acids were identified and quantified by comparison of their retention time and peak area with standard solutions of known organic acids.

2.7. Purine alkaloids and catechins analysis

Phenolic compounds were determined using a modification of the procedure described by Yang *et al* (Yang et al., 2007). Ten milliliters of sample was extracted thrice with 10 mL of ethyl acetate. Fractions were pooled and evaporated to dryness and the residue was dissolved in 1 mL of HPLC grade methanol. The resultant solution was filtered through a 0.45 µm membrane filter (Whatman, NJ, US) prior to HPLC analysis. The filtrate (10 µL) was injected onto a C-18 symmetry (5 µm, 3.9 mm × 150 mm i.d.) column of the Waters HPLC system equipped with a 626 pump, a 486 UV detector fixed at 230 nm plus autosampler (Waters, Milford, MA) and eluted with a linear gradient starting at a proportion of 90:10 of 5 % AcOH/H_2O/0.1 % AcOH/MeOH for 10 min and then changing to 80:20 in 20 min and to 90:10 in 15 min. The flow rate was 1 mL/min.

2.8. Statistical analysis

Results are presented as means ± standard deviation. One-way non-parametric ANOVA, Kruskal–Wallis tests were used to compare the difference between control and treated groups, and the Mann-Whitney test for comparing two independent samples, and values were set as significant when $p < 0.05$. Data were analyzed by using a statistical software package (SPSS for Windows, 12.0, SPSS Inc. Chicago, IL, USA).

3. Results and Discussion

They physicochemical characteristic of wine prepared from citrus fruit and/or green tea powder (or leaves) is presented in Table 1. The percentage of ethanol produced in the citrus wine with/without green tea was between 13.4 and 15% w/v. Another parameter, which highly influences the quality of wine is acidity. The acidity of citrus wine samples was 0.8% (v/v) (as citric acid) (Table 1). Both parameters of citrus wine were not affected by green tea supplementations in our study.

Table 1. Chemical composition of citrus wine with/without green tea

Sample	Total sugar (°Brix)	Total acidity (% citric acid)	Ethanol (%)
Citrus	10.4±0.21[a]	0.8±0.01	14.3±0.42
+ tea powder, 1%	10.2±0.51[a]	0.8±0.01	13.4±0.40
+ tea powder, 2%	9.8±0.35[a]	0.8±0.02	14.1±0.66
+ tea powder, 3%	8.6±0.40[b]	0.8±0.03	15.0±0.60
+ tea leaves, 1%	8.8±0.25[b]	0.8±0.03	14.5±0.70
+ tea leaves, 2%	8.6±0.60[b]	0.8±0.04	14.9±0.95
+ tea leaves, 3%	8.8±0.45[b]	0.8±0.02	14.7±0.62

[a~d] Values with different superscripts in a row are significantly different ($p < 0.05$).

Total sugar content of citrus wine was 10.4 °Brix, but it was significantly decreased in the presence of tea power (8.6 °Brix, 3%) and leaves (8.6-8.8 °Brix, 1-3%) (Table 1). By HPLC analysis, sucrose, the major type in concentration being fructose (Table 2). As expected, fructose and glucose contents of citrus wine decreased in the presence of green tea supplements (Table 2), which it was found that the sugar types in citrus wine with/without green tea were fructose, glucose and yield reduced total sugar concentrations (Table 1).

Table 2. Free sugars in citrus wine with/without green tea

Sample	Compounds (mg/L)		
	Fructose	Glucose	Sucrose
Citrus	767.7±5.08[a]	150.4±6.75[a]	276.6±5.26[a]
+ tea powder, 1%	824.8±14.75[b]	169.6±10.57[b]	245.9±2.52[b]
+ tea powder, 2%	687.2±8.58[c]	172.7±3.81[b]	319.5±7.71[c]
+ tea powder, 3%	590.8±8.91[d]	121.9±8.28[c]	391.9±6.54[d]
+ tea leaves, 1%	513.7±11.35[e]	100.8±6.25[d]	371.5±4.85[e]
+ tea leaves, 2%	694.9±7.30[c]	107.6±2.40[d]	288.4±2.59[f]
+ tea leaves, 3%	713.3±9.31[f]	109.8±7.44[cd]	288.8±4.39[f]

[a~f] Values with different superscripts in a row are significantly different ($p < 0.05$).

Glucose has been associated with bitter flavor, and the taste attribute of sweetness decreased with increasing content of bitter glucose, influencing consumer acceptance (Baik et al., 2003). The type of sugar has an effect on flavor in addition to sweetness: Fructose is 5 times sweeter than maltose (Biester et al. 1925). Fructose is 5 times sweeter than maltose (Biester et al., 1925).

The color and brightness of alcoholic beverage was determined by the Hunter value (Table 3). The Hunter a and b value is measured for red and yellow color respectively. The degree of brightness is indicated by L value. Hunter a value of citrus wine was -3.4 and redness developed with tea powder (-2.8–2.4) and leaves (-3.1–2.3) (Table 3). The Hunter b value of citrus wine was 11.6 and slightly increased in the presence of green tea leaves, while brightness (the Hunter L) showed similar values in all test groups.

Table 3. Color coordinates of citrus wine with/without green tea

Sample	Hunter color value		
	L-value	a-value	b-value
Citrus	95.4±0.07[a]	-3.4±0.11[a]	11.6±0.24
+ tea powder, 1%	95.2±0.07[a]	-2.7±0.33[bcd]	10.8±1.31
+ tea powder, 2%	94.9±0.34[b]	-2.4±0.20[cd]	10.6±0.51
+ tea powder, 3%	94.5±0.07[c]	-2.8±0.12[bcd]	12.0±0.42
+ tea leaves, 1%	95.3±0.04[a]	-3.0±0.43[bc]	11.5±1.51
+ tea leaves, 2%	95.1±0.08[ab]	-3.1±0.16[a b]	12.8±0.51
+ tea leaves, 3%	94.5±0.27[c]	-2.3±0.63[cd]	12.1±1.86

[a~d] Values with different superscripts in a row are significantly different ($p < 0.05$).
L-value: Degree of lightness (white +100 ↔ 0 black).
a-value: Degree of redness (red +60 ↔ -70 green).
b-value: Degree of yellowness (yellow +60 ↔ -70 blue).

Table 4 shows the volatile compounds of citrus wine with/without green tea, expressed by mean (mg/L), which correspond to the three analytical replicates. In the present study, in wine produced from citrus with/without green tea, the content of isoamyl alcohol varied between 362 and 396 mg/L (Table 4). The other higher alcohols like propanol and isobutyl alcohol concentrations were in the medium range (57.3–66.2 and 82.7–89.4 mg/L, respectively). These are much responsible for wine quality in terms of aroma. Polyols and isobutyl alcohol are also known for their stability to bacterial attack (Reddy and Reddy, 2005). The acetaldehyde content in wine produced from grapes is usually in the range of 13–30 mg/L (Longo et al., 1992), while acetaldehyde concentrations of citrus wine were between 36.4 and 43.3 mg/L (Table 4). The methanol ranged between 30.3 and 35.3 mg/L. In the present experiment, volatile compound contents of citrus wine were not affected by green tea supplements (Table 4).

Organic acids can be one of the compounds studies for its potential as flavor enhancers. Organic acids not only elicit sourness but also contribute to

bitter and astringent taste quality (Thomas and Lawless, 1995; Kang et al., 2007). Therefore, we investigated the contents of organic acids in citrus wine with/without green tea in the following experiments (Table 5). These include malic acid (141.4–193.6), malonic acid (70.8–123.2 mg/L), succinic acid (20.8–52.6 mg/L), Levulinic acid (12.3–54.5 mg/L), citric acid (13.6–33.2 mg/L), oxalic acid (23.7–30.9 mg/L), fumaric acid (7.7–19.0 mg/L) and glutaric acid (1.2–2.9 mg/L). The results showed that malic acid is the major organic acid found in citrus wine with/without green tea. The contents of fumaric, malonic, succinic, levulinic, glutaric and malic acid were significantly lower in the citrus wine with tea powders than those in citrus wine alone ($p < 0.05$) (Table 5). In contrast, citrus wine with tea leaves show more succinic (45.3–52.6 mg/L), levulinic (29.8–54.5 mg/L) and glutaric (2.3 mg/L) contents than those of citrus wine (29.3, 23.8 and 1.7 mg/L), respectively ($p < 0.05$). However, no significant difference in oxalic acid content between citrus wine alone and citrus wine with green tea was observed in this study (Table 5).

amounts of all alkaloids and catechin were increased in citrus wine with green tea than those of citrus wine alone. The amount of catechins in all samples showed the order: (-)-epigallocatechin (EGC) > (-)-epicatechin (EC) > (-)-epicatechin gallate (GCG) > (-)-epicatechin-3-gallate (ECG) > (-)-epigallocatechin gallate (EGCG) > epigallocatechin (C) ≒ theobromine (Tb) ≒ (-)-gallocatechin (GC) ≒ caffeine (Caff) (Table 6). This could be due to the supplements of green tea. The polyphenols are generally considered to be the most important elements of normal green tea, with the catechins being the most important polyphenols. The primary catechins in green tea are EC, ECG, EGC and EGCG (Ahmad and Mukhtar, 1999). In addition, Tb, Caff, theophylline, and phenolic acids, such as gallic acid, are also present as minor constituents of green tea (Ahmad and Mukhtar, 1999).

As citrus and green tea are grown very widely as choicest fruit and plant, their use in wine production would go a long way in contributing considerably to the economy of international citrus and green tea producers. Large-scale production of citrus wine with green tea needs further research in screening of other variety of citrus and green tea, suitable yeast strain and fermentation methods.

Table 4. Volatile compounds in citrus wine with/without green tea

Sample	Compound (mg/L)				
	Acetaldehyde	Methanol	Propanol	Isobutyl alcohol	Isoamyl alcohol
Citrus	39.4 ± 2.14	30.3 ± 3.31	61.4 ± 5.69	89.4 ± 1.16	374.8 ± 6.67^{ab}
+ tea powder, 1%	36.4 ± 3.67	31.1 ± 2.28	58.3 ± 3.52	82.7 ± 3.21	385.3 ± 8.15^{bc}
+ tea powder, 2%	38.2 ± 1.59	34.3 ± 5.15	60.4 ± 2.76	86.1 ± 4.18	366.7 ± 11.04^{a}
+ tea powder, 3%	42.7 ± 4.48	33.7 ± 2.11	66.2 ± 5.39	85.6 ± 8.49	362.1 ± 9.94^{a}
+ tea leaves, 1%	41.1 ± 3.95	33.1 ± 4.71	60.9 ± 2.95	85.1 ± 3.14	375.5 ± 5.05^{ab}
+ tea leaves, 2%	36.4 ± 5.86	34.3 ± 3.81	57.3 ± 4.46	83.3 ± 4.29	396.1 ± 4.14^{c}
+ tea leaves, 3%	43.3 ± 3.54	35.3 ± 4.32	62.5 ± 3.70	86.8 ± 4.48	386.2 ± 3.14^{bc}

$^{a\sim d}$ Values with different superscripts in a row are significantly different ($p < 0.05$).

Table 5. Organic acids in citrus wine with/without green tea

Organic acid (mg/L)	Sample						
	Citrus	+ tea powder, 1%	+ tea powder, 2%	+ tea powder, 3%	+ tea leaves, 1%	+ tea leaves, 2%	+ tea leaves, 3%
Oxalic	29.5±1.31	27.6±2.20	30.9±3.37	28.4±5.65	23.7±4.16	24.8 ± 1.18	27.2±3.96
Fumaric	19.0±2.53^{a}	17.7±1.11^{a}	11.5±0.83^{b}	8.8±0.94^{b}	20.3±5.88^{a}	7.7 ± 1.91^{b}	7.8±1.59^{b}
Malonic	116.5±9.71^{ae}	96.4±4.70^{b}	80.3±3.18^{c}	76.7±4.12^{cd}	70.8±3.28^{d}	123.2 ± 4.32^{e}	113.3±1.71^{a}
Succinic	29.3±2.18^{a}	25.8±1.53^{a}	31.8±2.11^{a}	20.8±0.74^{b}	52.6±3.51^{c}	45.3 ± 4.32^{d}	46.8±5.87^{cd}
Levulinic	23.8±3.39^{a}	18.9±0.97^{ab}	12.3±1.62^{c}	22.3±1.09^{a}	54.5±5.31^{d}	15.9 ± 1.23^{bc}	29.8±2.05^{e}
Glutaric	1.7±0.02^{ab}	1.2±0.12^{a}	1.9±0.31^{bc}	2.9±0.43^{d}	2.3±0.42^{c}	1.7 ± 0.32^{ab}	1.8±0.31^{bc}
Malic	187.7±8.64^{a}	168.9±2.30^{bc}	157.2±9.26^{b}	141.4±8.28^{d}	180.9±6.17^{ac}	185.1 ± 2.41^{a}	193.6±8.05^{a}
Citric	26.7±4.24^{ab}	15.8±3.41^{cd}	13.6±3.99^{c}	33.2±4.77^{a}	32.8±2.54^{a}	22.9 ± 5.55^{bd}	27.1±4.15^{ab}

$^{a\sim e}$ Values with different superscripts in a line are significantly different ($p < 0.05$).

Table 6 shows purine alkaloids and catechins of citrus wine with/without green tea. The

Table 6 Purine alkaloids and catechins in citrus wine with/without green tea

Compound[a] (mg/L)	Sample						
	Citrus	+ tea powder, 1%	+ tea powder, 2%	+ tea powder, 3%	+ tea leaves, 1%	+ tea leaves, 2%	+ tea leaves, 3%
Tb	1.6±0.22^{a}	1.6±0.20^{a}	3.3±0.23^{bc}	3.5±0.30^{bc}	3.3±0.11^{bc}	3.2±0.48^{b}	3.8±0.13^{c}
GC	1.6±0.26^{a}	1.3±0.18^{a}	2.3±0.37^{b}	4.5±0.28^{c}	2.4±0.14^{b}	4.4±0.19^{c}	3.4±0.40^{d}
EGC	15.7±3.63^{a}	22.0±1.96^{a}	50.4±2.79^{b}	62.2±2.87^{c}	17.8±1.29^{a}	53.1±7.41^{b}	52.7±4.46^{b}
C	2.7±0.31^{a}	2.1±0.16^{a}	5.1±0.23^{bc}	6.4±1.76^{b}	2.9±0.29^{ad}	2.5±0.45^{a}	4.1±0.66^{cd}
EC	4.2±1.12^{a}	5.2±1.56^{a}	14.9±2.34^{b}	26.3±3.57^{c}	4.5±0.40^{a}	15.2±0.88^{b}	23.8±5.54^{c}
EGCG	2.1±0.62^{a}	2.3±0.14^{a}	4.2±0.98^{b}	4.9±0.43^{b}	1.7±0.21^{a}	2.7±0.19^{a}	6.5±0.69^{c}
GCG	3.2±0.45^{a}	4.8±0.29^{bc}	4.3±0.21^{b}	6.4±0.73^{d}	5.0±0.13^{c}	9.9±0.29^{e}	12.1±0.27^{f}
ECG	2.1±0.28^{a}	2.8±0.34^{a}	7.0±0.81^{b}	9.7±1.19^{c}	2.5±0.28^{a}	4.1±0.33^{d}	9.8±0.94^{c}
Caff	1.3±0.27^{a}	1.2±0.20^{a}	2.3±0.12^{b}	2.9±0.47^{c}	1.2±0.16^{a}	2.3±0.44^{b}	3.4±0.45^{c}

TB, theobromine; GC, gallocatechin; EGC, epigallocatechin; C, catechin; EC, epicatechin; EGCG, epigallocatechin gallate; GCG, gallocatechin gallate; ECG, epicatechin gallate; Caff, caffeine.

[a~g] Values with different superscripts in a line are significantly different ($p < 0.05$).

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Acknowledgement
This research was supported by the 2013 scientific promotion program funded by Jeju National University

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1/15/2013