Research Article

Effect of Thermal Treatment on Synephrine, Ascorbic Acid and Sugar Content of *Citrus aurantium* (Bitter Orange) Juice

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ABSTRACT

Objective / Purpose: The aim of this study was to understand the change of synephrine, ascorbic acid and sugar contents after thermal treatment of peeled and grated *Citrus aurantium* (Bitter Orange) Juice. **Material and Methods**: *Citrus aurantium* was extracted either by peeling or grating methods. Clarification of juice was achieved by a pectic enzyme. After filtration, juices were thermally treated using rotary evaporator until 67°Brix. Synephrine, sugar and ascorbic acid analysis were performed with a HPLC system. **Results:** All compounds' amounts, except synephrine, changed significantly after thermal treatment. The results, given as dry matter based, were as below. Synephrine contents of fruit juice and concentrated juice of grated bitter oranges were 14.15 g/L and 12.88 g/L, respectively. Ascorbic acid contents of fruit juice and concentrated juices of grated sample were 6.33 and 5.85 g/L, respectively. Synephrine content of peeled fruit juice and concentrated samples were 12.84 and 10.64 g/L, respectively. Ascorbic acid content of peeled fruit juice and concentrated samples were 5.28 and 4.31 g/L, respectively. **Conclusion:** Thermal treatment has no significant effect on synephrine content of fruit juices. Ascorbic acid content decreased significantly after thermal treatment. Moreover, glucose and fructose content of samples increased and sucrose content diminished significantly.

Key words: Bitter (Sour) Orange, *Citrus aurantium*, Ascorbic Acid, Synephrine, Thermal Treatment.

INTRODUCTION

Citrus aurantium L. var. *amara* is also called sour orange, Seville orange and bitter orange. It is a plant belonging to the Rutaceae family.[1] The origin of the plant is East Africa and Tropical Asia. It is used hundreds of years to remedy headaches, indigestion, to aid weight loss and for treatment of cancer.[2-4] It is also used pharmacologically as a vaso-constrictor.[5] Primary constituents of the plant are adrenergic amines, which mimic like endogenous agonists of nervous system and stimulate thermogenesis and conduce to lipolysis in the body.[6] *P*-synephrine is the most important alkaloid of the bitter orange. After banning of ephedrine in US market, because of increasing blood pressure and heart rate and some cardiovascular abnormalities, synephrine has become best alternative. Some studies, being carried out to determine the adverse effects of synephrine, show that synephrine doesn't lead any side effect in the limits of daily dosages. These limits were defined by several studies. According to Hansen *et al.*[7] doses of up to 100 mg of synephrine per kg body weight did not show any toxicity on embryo development. Kaats *et al.*[8] reported that 98 mg/day usage of synephrine appeared to be without adverse effects. Ascorbic acid (Vitamin C) is a very well-known and easily accessible anti-oxidant.[9-12] Citrus fruits are good sources of ascorbic acid.[13, 14] Sugar are the major

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component of all citrus species. Bitter orange has glucose, fructose and sucrose, which are compose almost 80% of all carbohydrate constituents.[15] Citrus fruits can be consumed both as raw and after processing. Thermal treatment can usually be applied for longer shelf lives of fresh fruits. Most studies showed that after heat processing of fresh fruit, some bioactive ingredients are degraded. However, there are some exception that heat treatment can increase bioactivity positively. Improvement of lycopene and β-carotene bioactivity after cooking process of tomato and carrots are good examples for this case.[16]

The aim of this study was to understand the change of synephrine, ascorbic acid and sugar content after thermal treatment of peeled and grated Seville (bitter) orange juice.

MATERIAL AND METHODS

Bitter oranges were collected from garden of Alata Horticultural Research Institute, Mersin, Turkey. The time of harvesting was decided according to maturity index ratio of fruit (°Brix/acidity). Samples were harvested on January and March. All chemicals were of analytical grade

Thermal treatment

Bitter oranges were cleaned in water and divided in two for peeling and grating extraction processes. After extraction and finishing at 1000 rpm, juice were pasteurized at 85℃ for 3 min. Pasteurized fruit juices were clarified with a pectic enzyme, Pectinex Ultra clear, Novozyme (Denmark). Juices were filtrated with rough filter paper under vacuum. Filtrated samples were aliquoted and stored at -20℃ until analyzed. Thermal treatment was held using rotary evaporator (Heidolph, Germany) up to 67°Brix.

Synephrine, ascorbic acid and sugar analysis

Synephrine, ascorbic acid, sugar analysis were performed using a Shimadzu 20 AD (Japan) HPLC system consisting of a diode array detector (DAD) and refractive index detector (RID). All Samples were diluted to ~5°Brix with water, centrifuged at 12,000*g* and filtered through 0.45 µm nylon syringe filter.

Synephrine were determined according to modified method defined by Dragull *et al.*[17] 10 µL of samples were separated by Inertsil ODS3 (4,6x150 mm 5µm) column. Isocratic flow of acetonitrile/H_2O [90:10 (v/v) with 10 mM ammonium acetate] was finished after 15 min of elution. Flow rate was constantly 1 mL/min. Ascorbic acid content were found according to modified method of Ozkan *et al.*[18] 20 µL of samples were injected to HPLC system with diode array detector. Detection was at 254 nm. Separation was achieved at Inertsil ODS3 (4.6x250 mm 5µm) column. 0.2 M potassium dehydrogenase phosphate (KH_2PO_4) solution (adjusted to pH 2.4 with H_3PO_4) was eluted isocratic ally as mobile phase at a flow rate of 0.5 mL/min. Sugar contents of samples were analyzed according to modified method of Mendes *et al.*[19] Sugars were separated by Shim-pack NH_2 column (4.6 x 250 mm 5 µm) at 25℃ and detected by refractive index detector at 225 nm. Mobile phase of ACN: H_2O (80/20) was eluted isocratically at a rate of 1.3 mL/min. 20 µL of samples were injected.

Statistical analysis

Statistical analysis were performed by IBM-SPSS 20. Means were analyzed by one way ANOVA followed by post-hoc Tukey's test. Statistical significance was defined as a two-sided $\alpha<0.05$.

RESULTS & DISCUSSION

Synephrine, ascorbic acid, reducing sugars and sucrose contents of thermally processed samples were compared with control sample (fruit juice) on wet basis and dry matter basis. Results are shown in Table 1.

Wet basis results clearly indicate that all compound's amounts increased significantly ($p<0.05$). Synephrine contents of grated and peeled fruit juice were found as 1.16 g/L and 1.10 g/L respectively. The results were compatible with some studies. According to Sun *et al.*[20] synephrine content of bitter orange was 2.25 g/kg. Pellati *et al.*[21] reported that synephrine percentages of fruit juice and dry extract of bitter orange were 0.1-0.35% and 3.5-3.08%, respectively.

Ascorbic acid contents of grated and peeled fruit juices were 0.57 and 0.48 g/L, respectively. Ascorbic acid content of blood orange was between 0.318 and 0.416 g/L.[13] According to Gazdik *et al.*,[22] ascorbic acid amount of bitter orange was between 0.30 to 0.56 g/kg fresh weight. In another study, ascorbic acid contents of peeled lemon juice and peel of lemon fruit were 0.48 and 0.60 g/L, respectively. However that of grapefruits were 0.35 and 0.44 g/L.[23] Ascorbic acid content of conventionally pasteurized grape juice decreased from 0.36 g/kg to 0.34 g/kg.[24] This results are compatible with present study.

In order to understand the effect of thermal treatment on control sample, the results were converted to dry matter basis. According to Table 1, except for synephrine, all compounds' contents changed significantly after thermal treatment ($p<0.05$).

Table1: Synephrine, sugar and ascorbic acid content of thermal treated and control samples

		Wet Basis			Dry Basis		
	Compound	Fruit Juice (Control)	Thermal Treated	*p* Value	Fruit Juice (Control)	Thermal Treated	*p* Value
Grating	Synephrine[1]	1.16 ± 0.09	7.21 ± 0.20	0.001	14.15 ± 0.84	12.88 ± 0.44	0.200
	Ascorbic Acid[1]	0.57 ± 0.05	3.29 ± 0.35	<0.001	6.33 ± 0.07	5.85 ± 0.53	0.013
	Fructose[2]	1.86 ± 0.04	14.87 ± 0.09	<0.001	23.76 ± 0.44	26.58 ± 0.36	0.020
	Glucose[2]	1.81 ± 0.17	16.33 ± 0.72	0.001	22.82 ± 0.41	28.61 ± 0.53	0.007
	Sucrose[2]	1.20 ± 0.07	7.56 ± 0.06	<0.001	14.87 ± 0.23	8.78 ± 0.41	0.003
Peeling	Synephrine[1]	1.10 ± 0.11	5.47 ± 0.25	0.002	12.84 ± 0.55	10.64 ± 0.48	0.051
	Ascorbic Acid[1]	0.48 ± 0.07	2.32 ± 0.07	0.002	5.28 ± 0.09	4.31 ± 0.04	0.006
	Fructose[2]	2.19 ± 0.07	16.17 ± 0.27	<0.001	24.84 ± 0.24	27.51 ± 0.42	0.017
	Glucose[2]	2.39 ± 0.06	19.17 ± 0.89	0.001	26.30± 0.21	30.96 ± 1.18	0.018
	Sucrose[2]	1.39 ± 0.02	6.16 ± 0.12	<0.001	17.21 ± 0.28	10.13 ± 0.25	0.001

[1] :units as g/L , [2] :units as %

Synephrine content of grated fruit juice was 14.15 g/L and this value decreased to 12.88 g/L at the end of thermal treatment. This decreasing were statistically insignificant (p=0.2). There are some similar results that synephrine and other amines were resistant to thermal degradation during concentration processing.[25] Ascorbic acid content was one of the heat damage indicator of thermal processing.[24,26] Therefore, decreasing of ascorbic acid content after thermal treatment was an expected result. In present study, ascorbic acid content of control sample (6.33 g/L) diminished to 5.85 g/L after heat treatment. The results were comparable with previous studies. After 30 min of heating processing of tomato juice, ascorbic acid content decreased from 0.76 μmol/g to 0.54 μmol/g.[27] Although fructose and glucose content increased 11% and 25% respectively, sucrose content decreased 40% after thermal treatment. The reason of this decreasing in sucrose was due to hydrolysis of sucrose into glucose and fructose during thermal treatment in order to yield hydroxyl methyl furfural (HMF).[28] Synephrine content of thermal treated and peeled sample decreased also from 12.84 to 10.64 g/L. Despite to this decreasing, there were no statistically differences between thermally treated and control sample (p=0.051). Synephrine and ascorbic contents of peeled samples were slightly less than grated ones (12.85 g/L and 5.28 g/L respectively). However, fructose, glucose and sucrose content of peeled fruit juice was higher than that of grated samples (24.84, 26.30 and 17.21%, respectively).

CONCLUSION

The results show that thermal treatment of bitter orange doesn't have significant effect on synephrine content of fruit juices. However, ascorbic acid content of grated and peeled bitter orange juice significantly diminished. After thermal treatment, while glucose and fructose content of samples increased, sucrose content diminished significantly.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

None

ABBREVIATION USED

DAD: Diode array detector; **RID:** Refractive index detector; **HMF:** Hydroxyl methyl furfural.

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PICTORIAL ABSTRACT

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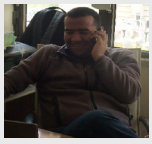
[1] :units as g/L , [2] :units as %

SUMMARY

- Thermal treatment has no significant effect on synephrine content of bitter orange juices.
- Although, ascorbic acid and sucrose contents decreased, glucose and fructose contents increased significantly after thermal treatment.

About Authors



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