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PROLONGING OF ORANGE JUICE SHELF LIFE VIA GREEN TEA ADDITION

Leila M.A. Taghian[1*], S.H. Mosilhey[2], M.A.S. Abdel-Samie[2] and Lobna A.M. Hareedy[1]

1. Dept. Hort. Crops Res., Inst. Food Technol. Res., Cent. Agric. Res., Giza, Egypt.

2. Dept. Food and Dairy Sci. and Techol., Fac. Environ. Agric. Sci., Arish Univ., Egypt.

ABSTRACT

Fruit Juice especially orange (*Citrus Sinensis*) juice is very desirable for the consumer because of its fresh aroma and flavor, but its shelf life is less than 6 days at 4°C in best conditions during preparation, packaging, storage, handling and distribution. Microbiological quality is the bottleneck for safety and shelf life .So, this study aimed to extend the shelf life of orange juice by using green tea extracts as natural preservatives. The effect of adding green tea extracts by 0.1%, 0.2%, 0.3% to orange juice on the physical, microbial, chemical and sensory properties during storage up to 6 months were evaluated. The most important findings showed that the highest content of antioxidants, phenols and flavonoids for juice with green tea extracts (0.1%, 0.2%, and 0.3%) compared to control sample. While, green tea extracts as the best way to minimize the total number of bacteria in orange juice samples compared with control during storage period up to 6 months. Generally, it could be conducted that using of green tea water and alcoholic extracts may extend the shelf life and raise the nutritional and health value of orange juice**.**

Key words: Green tea, water and alcoholic extract, orange juice, bioactive compounds, antioxidant activity, antimicrobial, HPLC analysis.

INTRODUCTION

Orange juice is the most predominant juice manufactured by the beverage processing industry worldwide with a share of about 50% of the total fruit juice market according to Food and Agriculture Organization (FAO) **(Navarro *et al*., 2011).** Orange juice is considered as an excellent source of vitamin C and is a desired product by many consumers who are interested in maintaining healthy diet. Fruit juices contain important nutrients (i.e., vitamin C, potassium, folate, magnesium, and ß-carotene) and flavonoids, and are an important contributor to total fruit intake **(Aschoff *et al*., 2015**). The importance of orange juice consumption has long been established. It is a source of vitamin C, flavonoids, and carotenoids and also contains folic acid, potassium, and fibers **(Stella *et al*., 2011; Stinco *et al*., 2012).** Freshly squeezed, un-pasteurized orange juice is very desirable for the consumer because of its fresh aroma and flavor, but the shelf life is less than 20 days at 1ºC as it is highly susceptible to microbial spoilage. The manufacturing operations from fruit washing to packaging must be exceptionally clean to minimize product spoilage. Pectin esterase activity in un-pasteurized juice results in loss of cloudiness (**Wicker *et al*., 2003).** Herbs and spices contain volatile chemicals that are used in the production of preservatives via distillation and enzymatic action. These natural herbs are used in the form of powder, extracts or essential oils to check the microbial growth. The preservative activity of these natural herbs / spices depends on the type of test organism, nature and concentration of herb/spice. Herbs have been used as flavoring agents and

*** Corresponding author:** Tel.: +201066384282
E-mail address: leilamagdy831@yahoo.com

preservatives due to their antimicrobial activity against certain pathogens, and antioxidant property **(Meena and Sethi, 1997; Archana *et al.*, 2009)**. Green tea extract contains several polyphenolic components with antioxidant properties, but the predominant active components are the flavanol monomers known as catechins, where epigallocatechin-3-gallate and epicatechin- 3-gallate are the most effective antioxidant compounds. Additional active components of green tea extract include the other catechins such as epicatechin and epigallocatechin. Among these, epigallocatechin-3-gallate is the most bioactive and the most scrutinized one **(Namal Senanayake, 2013**). Chemical constituent as preservative of green tea: Polyphenols predominantly flavonoids, Preservative action: It shows antimicrobial activity against *Staphylococcus* spp., *Steptococcus* spp., *E. coli* and *Helicobacter pylori*. It has strong antioxidant activity **(Chou *et al.*, 1999; Simonetti *et al*., 2004).**

So our objective in this study were to extend the shelf life of fresh orange juice through addition of green tea extracts, packaging and study effects of these treatments on the microbial and physicochemical quality characteristics of fresh, stored orange juices.

MATERIALS AND METHODS

The present investigation was carried out in Horticultural Products. The food Technology Research Institute, Agricultural Research Center, El Giza, Egypt.

Materials

Raw materials

Orange fruits, (*Citrus sinensis*) Egyptian Baladi Orange fruits at the full ripe stage of maturity were brought from local markets, Giza, Egypt.

Green tea leaves (*Camellia sinensis*) was purchased from a local retail spice market, Giza, Egypt.

Chemicals and reagents

All chemicals (analytical grade) were purchased from El-gomhouria pharmaceuticals Co., Cairo, Egypt.

(2.2-diphenyl-1-1picryl-hydrazyl (DPPH)), Folin-Ciocalteu reagents, gallic acid, were obtained from Sigma-Aldrich Chime, Steinheim, Germany.

Media

Plate count agar, potato dextrose agar and nutrient broth were obtained from Oxoid, Hampshire, England.

Methods

Preparation of orange juice

Orange juice preparation

- Orange fruits were washed and left to drain.
- Cut into halves (stainless knives).
- Then, squeezed using a juicer (Moulinex Brand).
- The juice filtered by using stainless drainer.

Green tea Extracts

Water Extract

One hundred grams of dried green tea leaves were suspended in 1000 ml of distilled hot water in a water bath at 80°C ±1 for 30 min. The extracts were cooled, filtered and the supernatant was collected (**El-Shemy *et al*., 2007**).

Ethanol extract

One hundred grams of dried green tea leaves were mixed with 80% (v/v) ethanol and stirred mechanically (IKA RW 20 Digital Homogenizer) for 2 hrs.at room temperature, then allowed to stay in the refrigerator for 24 hrs. at 4˚C±1. The extracts were filtered and the supernatant was collected (**Khalafalla *et al*., 2009).** Then dried in a rotary evaporator (Stuart Rotary Evaporator Model RE300) at 40°C

± 1 and completely dried in freeze drying (Snijders Scientific type 2040). The lyophilized extracts were stored at 4°C±1 for further uses.

Filtered Fresh orange juice was blended and mixed with green tea extracts as follows:

- The control sample, orange juice without any addition of extracts.
- Sodium benzoate (0.1)% plus orange juice.
- A_1: green tea water extract 0.1% (GTWE) Plus orange juice
- A_2: green tea water extract 0.2% (GTWE) Plus orange juice
- A_3: green tea water extract 0.3% (GTWE) Plus orange juice
- B_1: green tea alcoholic extract 0.1% (GTWE) Plus orange juice
- B_2: green tea alcoholic extract 0.2% (GTWE) Plus orange juice
- B_3: green tea alcoholic extract 0.3% (GTWE) Plus orange juice

These mixtures were mixed well for two minutes using the blender. Then pasteurized at 82ºC for 2 minutes packed in sterilized packaging materials (100 ml glass bottles), and subjected to heat up to 90ºC for another two minutes. Finally, the orange juice cooled down by using tap water and stored at 4ºC± 1^{o}C until analyses**.**

Analytical methods

Gross chemical composition

Moisture content, ash, total soluble solids (TSS) and total acidity were determined according to the **AOAC (2007).** The pH values were measured at 25°C using a pH meter (Jenway, 3510, UK).

Determination of Vitamin C

Ascorbic acid was quantitatively determined according to2,6- dichlorophenol -indophenol dye method **Ranganna (1977**).

Determination of total phenolic compounds

Total phenolic compounds content was determined using the Folin-Ciocalteau reagent according to the method described by **Maurya and Singh (2010).**

Fractionation and identification of phenolic compounds

Phenolic compounds were fractionated and identified by HPLC according to the method described by **Goupy *et al*. (1999).**

Determination of total flavonoids

Total flavonoids content was determined according to the method described by **Jia *et al*. (1999).**

Fractionation and identification of flavonoid compounds

Flavonoids compounds were fractionated and identified by HPLC according to the method of **Loon *et al*. (2005).**

Determination of antioxidant activity by 2, 2'-diphenyl 1- picrylhydrazyl (DPPH)

Antioxidant activities of the samples were analyzed by investigating their ability to scavenge the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals using the method of **Baraca *et al*. (2001).**

Sensory evaluation test procedures

Orange juice was sensory evaluated by 10 panelists, 5 were staff and 5 were random consumers. The quality attributes were measured according to the method described by **Norman (1986).** The weight of parameters of sensory evaluation was as following, color 10%, odor 10%, taste 10% and palatability 10 % **Ahmed (2000).**

Microbiological analyses

Aerobic plate (AP) count was determined using serial dilutions on plate count agar (PCA) with pour plate method. The duplicate plates were incubated at 30ºC for 48 h. the enumeration of total yeasts and molds (YM) count with the same dilutions was also carried out on potato Dexterous

agar (PDA) at 25ºC for 5 days using the pour plate method. Coliform group (CG) count with same dilutions was also carried out on Mackoncy agar (MA) at 37ºC for 24 h. Results were expressed as "cfu (colony-forming units)/ml" **APHA (1992)**.

Statistical analysis

The statistical analysis was carried out using one-way analysis of variance (ANOVA) under significant level of 0.05 for the whole results using the statistical program Costat (Ver. 6.400**)** and data were treated as complete randomization design according to **Steel *et al.* (1997).** To ascertain the significant among means of different samples, LSD test was applied.

RESULTS AND DISSECTION

The Chemical Composition of Green Tea Dried Leaves

Data presented from Table 1 show that the dried green tea leaves moisture contains 7.06%, ash 5.67%, protein contains 0.57%, fat contain 2.43%, crude fiber contain 13.67%, total carbohydrates contain 12.14, total sugars contain 3.70%, reducing sugar contain 0.47 and non-reducing sugar contain 2.29%, these result were in agreement with **Adnan *et al.* (2013)** who found that moisture content in dried green tea was 6.46% and **Ahmad *et al.* (2014)** who found that moisture content in dried green tea was 4.88% and **Rahman *et al.* (2014)** who found that fiber content in dried green tea was 12.05%.

Total Phenolic Compounds, Flavonoids Compounds and Antioxidant Activity of Green Tea and Fresh Orange Juice

Table 2 showed the total phenolic compounds of green tea and fresh orange juice. The content of total phenolic compounds was 74.44, 202.55 and 299.61 (mg/g) for green tea powder, green tea water extract and green tea alcoholic extract, respectively. While, orange juice presented 0.65 mg/g. This result nearly in agreement with **Erol *et al.* (2009)** who found that total phenolic compounds in green tea ethanolic extract was 276.5 (mg/g) and **Chanson-Rolle *et al.* (2016)** who found that total phenolic compounds in orange juice was 62.9 ± 5.94 mg/100 ml. The content of total flavonoid compounds was 25.14, 39.21, 59.73 and 0.16 (mg/g) for green tea powder, green tea water extract and green tea alcoholic extract and orange juice, respectively. This result nearly in agreement with **Dharti and Dhvanika (2014**). While, the content of antioxidant activity was 87.70, 86.39, 89 and 36.33% for green tea powder, green tea water extract, green tea alcoholic extract and orange juice, respectively. This result nearly in agreement with **El-Kady *et al.* (2015)** who found that antioxidant activity in orange juice was 31.10% and **Glevitzky *et al.* (2008)** who found that antioxidant activity in green tea ethanol extract was 95.68%.

Fractionation of Green Tea Total Flavonoids Compounds by HPLC Analysis

Flavonoids compounds presented in Table 3 showed that Hesperidin, Rutin and Naringin were predominate flavonoid compounds in green tea powder, water and ethanol extracts. The highest values of flavonoid compounds in green tea powder were Hesperidin, Rutin, Naringin and Quercetin with 98.07% of total flavonoids compounds. While the result also showed that the highest values of flavonoid compounds in green tea water extract were Hesperidin, Rutin, Naringin and Quercetin with 98.80%. Whereas the highest values of flavonoid compound in green tea ethanol extract were Hesperidin, Rutin, Naringin and Hespirtin with 98.17%.

Pyrogallol and P-OH-Benzoic from Table 4 were found to be predominate phenolic compounds in green tea powder, water and ethanol extracts. Catachein contains 52.41 mg/100g in green tea powder and this results in agreement with result found by **Pekal (2012)**.

Table (1): Chemical composition of dried green tea leaves.

Chemical composition (%)	Dried green tea (%)
Moisture	7.06
Ash	5.6
Protein	0.57
Fat	2.43
Crude fibers	13.67
Total carbohydrates	12.14
Reducing sugar	0.47
Non reducing sugar	2.29
Total sugars	3.70

Table (2): Total phenolic, flavonoid compounds and antioxidant activity of green tea and fresh orange juice.

Sample	Green tea powder	Green tea water extract	Green tea ethanol extract	Fresh orange juice
Total phenolic compounds (mg/g) (as gallic acid)	74.44	202.55	299.61	0.65
Total flavonoids (mg/g) (as gallic acid)	25.14	39.21	59.73	0.16
Antioxidant (%)as DPPH	87.70	86.39	89.07	36.33

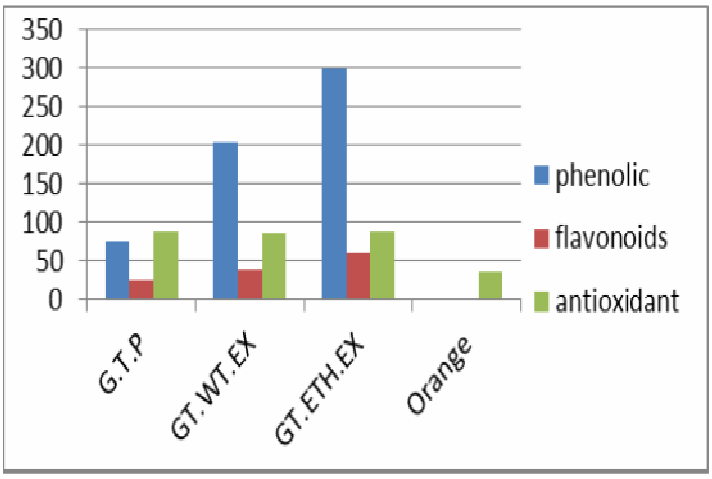

Fig. (1): Total phenolic, flavonoid compounds and antioxidant activity of green tea and fresh orange juice.

Table (3): Fractionation of total flavonoid compounds in green tea by HPLC analysis.

Compound	Green tea powder (mg/100g)	Green tea water extract (mg/100g)	Green tea alcoholic extract (mg/100g)
Naringin	62.57	188.96	360.84
Rutin	93.69	1025.17	511.20
Hesperidin	4587.60	2301.92	19741.80
Quercetrin	46.72	82.07	266.78
Quercetin	39.69	14.68	87.30
Naringenin	16.53	6.63	17.16
Hespirtin	30.81	18.01	306.72
Apigenin	3.20	2.79	7.71
Kampferol	3.75	1.29	10.04
Total	4884.38	3640.82	21309.55

Fractionation of Green Tea Total Phenolic Compounds by HPLC Analysis

Pyrogallol and P-OH-Benzoic from Table 4 were found to be predominate phenolic compounds in green tea powder, water and ethanol extracts. Catachein contains 52.41 mg/100g in green tea powder and this results in agreement with result found by **Pekal *et al.* (2012).**

The highest values of phenolic compounds in green tea powder were Caffeine, Vanillic, Pyrogallol, P-OH-benzoic, Chlorogenic and Rosmarinic with 83.96% from total phenolic compounds. While the results showed that the highest values of total phenolic compounds in green tea water extract were each of Catachein, Catechol, Pyrogallol, P-OH-benzoic, Benzoic and Rosmarinc with 83.26% from total phenolic compounds the result showed that the highest values of phenolic compounds in green tea ethanol extract were Caffeine, Pyrogallol, Vanillic , P-OH-benzoic, Gallic, Catechol with 82.88% from total phenolic compounds.

Effect of the Storage on the Chemical Properties of Orange Juice with Green Tea

Antioxidant activity

The green tea water and alcoholic extracts showed a higher scavenging activity in the fortified juices with (0.1, 0.2 and 0.3%) and sodium benzoate 0.1% during storage period up to 6 months. It was 82.4, 83.5, 90.8, 78.79, 81.04 and 90.81% in A1, A2, A3, B1, B2 and B3 and 35.5%, 84.01% in control and benzoate 0.1% after 6 months of storage Table 5. This may be due to the higher amounts of polyphenols in green tea extracts Consequently orange juice fortified with green tea extracts could be considered as a good source of antioxidant activity as a free radical scavenger and also prolonging the shelf-life of the product antioxidant activity were decreased during storage period up to 6 months This decrease in antioxidant activity during storage period is in agreement with **El-Kady *et al.* (2015)**.

Table (4): Fractionation of total phenolic compounds in green tea by HPLC analysis.

Compound	Green tea powder (mg/100g)	Green tea water extract (mg/100g)	Green tea alcoholic extract (mg/100g)
Gallic	2.58	6.49	764.12
Pyrogallol	292.94	292.89	3098.64
4-Amino-benzoic	1.94	2.76	4.37
Protocatchuic	29.83	70.80	79.35
Catechein	52.41	889.89	451.36
Chlorogenic	250.19	59.68	99.22
Catechol	85.78	507.43	582.41
Caffeine	508.85	12.96	4210.77
P-OH- benzoic	283.01	201.48	972.04
Caffeic	26.13	20.58	110.01
Vanillic	335.96	67.40	1061.46
P-Coumaric	13.22	15.69	59.48
Ferulic	17.59	10.65	147.71
Iso-Ferulic	3.75	23.75	20.66
Rosmarinic	156.77	132.35	273.91
Ellagic	18.79	123.21	83.04
Benzoic	63.58	164.04	494.07
Alpha-Coumaric	3.85	3.40	39.15
3,4,5-methoxy-cinnamic	5.81	4.11	37.93
Coumarin	6.60	2.94	63.70
Salycilic	15.01	14.52	231.12
Cinnamic	2.11	0.70	11.56
Total	2176.7	2627.72	12896.08

Table (5): Effect of the storage period on the chemical properties of orange juice with green tea water and alcoholic extracts.

Treatment	Storage period (month)		
	Zero	3	6
	Antioxidant activity(DPPH)%		
Control	35.59^{g}	19.08^{h}	15.59^{h}
Benzoate	84.01^{cd}	21.09^{g}	17.93^{g}
A1	82.84^{c}	50.47^{f}	41.91^{f}
A2	83.55^{d}	70.01^{d}	51.23^{d}
A3	90.81^{b}	77.00^{c}	65.55^{c}
B1	78.79^{f}	54.17^{e}	45.19^{e}
B2	81.04^{e}	70.01^{d}	69.52^{b}
B3	90.81^{b}	86.08^{a}	76.01^{a}
LSD 0.05%	1.214	1.701	1.697
	Total phenolic compounds(mg/g)		
Control	0.61^{g}	0.50^{h}	0.34^{f}
Benzoate	0.73^{f}	0.53^{g}	0.34^{f}
A1	0.80^{e}	0.71^{f}	0.56^{e}
A2	1.038^{c}	1.02^{d}	0.69^{c}
A3	1.24^{ab}	1.11^{c}	0.78^{a}
B1	0.87^{d}	0.78^{e}	0.61^{d}
B2	1.19^{b}	1.17^{b}	0.72^{b}
B3	1.25^{a}	1.11^{c}	0.77^{a}
LSD 0.05%	0.053	0.020	0.0135
	Total flavonoid compounds(mg/g)		
Control	0.15^{f}	0.13^{f}	0.12^{e}
Benzoate	0.15^{f}	0.14^{f}	0.13^{e}
A1	0.25^{e}	0.23^{e}	0.20^{d}
A2	0.32^{d}	0.30^{d}	0.29^{c}
A3	0.45^{b}	0.43^{b}	0.36^{b}
B1	0.26^{e}	0.24^{e}	0.14^{e}
B2	0.37^{c}	0.37^{c}	0.31^{c}
B3	0.57^{a}	0.56^{a}	0.40^{a}
LSD 0.05%	0.0105	0.0239	0.033

- The control sample, orange juice without any addition of extracts.
- Sodium benzoate (0.1)% plus orange juice
- A1,2,3 water extract, orange juice + green tea (0.1,0.2,0.3% respectively)
- B1,2,3 alcoholic extract, orange juice + green tea (0.1,0.2,0.3% respectively)

Total phenolic compounds (mg/g)

Data in the Table 5 showed that total phenolic compounds in treatments A1, A2, A3, B1, B2 and B3 for orange juice with green tea water and alcoholic extracts were 0.80, 1.03, 1.24, 0.87, 1.19 and 1.25 mg/g, respectively. While, it was 0.61and 0.73 mg/g in control and benzoate respectively. Total phenolic compounds were decreased during storage period up to 6 months. This decrease in total phenolic compounds during storage is in agreement with **Klimczak *et al*. (2007)**

Total flavonoid compounds (mg/g)

Results in Table 5 ascertained that the total flavonoid compounds in treatments A1, A2, A3, B1, B2 and B3 for orange juice with green tea water and alcoholic extracts were 0.25, 0.32, 0.45, 0.26, 0.37 and 0.57 mg/g respectively, and 0.15 and 0.15 mg/g in control and benzoate, respectively .Total flavonoids compounds were decreased during storage period up to 6 months .This decrease of total flavonoid compounds during storage This decrease in total phenolic compounds during storage is in agreement with **Klimczak *et al*. (2007)**

Acidity

Table 6 show the acidity value of orange juice with green tea water and alcoholic extracts. It was clear that the acidity values of all treatments slightly decreased during the storage period up to 6 months. These decrements in total acidity could be attributed mainly to the breakdown of ascorbic acid during storage.

pH value

Table 6 show PH value in treatments A1, A2, A3, B1, B2, B3, benzoate and control which were 3.30, 3.35, 3.40, 3.35, 3.37, 3.39, 3.49 and 3.41 respectively. At zero time of storage PH values increased to 3.47, 3.53, 3.56, 3.5, 3.52, 3.53, 3.61 and 3.53, respectively. pH values slightly increased during storage period up to 6 months on contrary to the acidity values which gradually decreased and this result is in agreement with **Hussain *et al.* (2017)**.

TSS

Table 6 ascertained that the total soluble solid in treatments A1, A2, A3, B1, B2 and B3 for orange juice with green tea water and alcoholic extracts were 11, 11, 11, 11, 11, 11 and 11 respectively, and 11 and 11.5 in control and benzoate , respectively .T.S.S were increased during storage period up to 6 months and this result is in agreement with **Hussain *et al.* (2017)**.

Ascorbic acid (mg/100g)

Data in Table 6 clear that the percentage of ascorbic acid content was decreased during the time of storage. The initial percentage of ascorbic acid content in treatments A_1, A_2, A_3, B_1, B_2, B_3, benzoate and control were 18.2, 16.9, 14.43, 20.07, 17.01, 14.63, 22.42 and 23.47 mg/100 g, respectively. Storage period led ascorbic acid values to be decreased to 3.14, 2.78, 2.45, 3.41, 2.91, 2.48, 3.74 and 3.51, respectively, these results agree with those reported by **El Kady *et al.* (2015) and Klimczak (2007)**.

From the same Table 5 it could be seen that treatments B_3, A_3, B_2 were significantly higher than other treatments on antioxidant activity total phenolic and flavonoids.

Effect of Storage Period on Total Visible Counts of Orange Juice with Green Tea

In this study, we focused initially on microbiological evaluation (total bacterial count and yeast and mold count). All

Table (6): Effect of the storage period on the chemical properties of orange juice with green tea water and alcoholic extracts.

Treatment	Storage period (months) Zero	3	6
		pH value	
Control	3.41[b]	3.44[e]	3.53[c]
Benzoate	3.49[a]	3.50[bc]	3.61[a]
A1	3.30[f]	3.45[e]	3.47[f]
A2	3.35[e]	3.51[b]	3.53[c]
A3	3.40[bc]	3.53[a]	3.56[b]
B1	3.35[e]	3.48[d]	3.50[e]
B2	3.37[d]	3.49[cd]	3.52[d]
B3	3.39[c]	3.49[c]	3.53[c]
LSD 0.05%	0.0105	0.0145	0.0079
		Acidity%	
Control	1.00[c]	0.97[c]	0.90[d]
Benzoate	1.01[c]	1.00[bc]	1.01[ab]
A1	1.07[b]	1.00[b]	0.968[bc]
A2	1.09[b]	1.03[b]	1.03[a]
A3	1.09[b]	1.09[a]	1.03[a]
B1	1.06[b]	1.02[b]	0.961[c]
B2	1.09[b]	1.08[a]	1.02[a]
B3	1.14[a]	1.09[a]	1.03[a]
LSD 0.05%	0.043	0.030	0.047
		TSS%	
Control	11.0[b]	11.5[b]	12.3[a]
Benzoate	11.5[a]	12.0[a]	11.6[b]
A1	11.0[b]	11.0[c]	11.6[b]
A2	11.0[b]	11.0[c]	11.6[b]
A3	11.0[b]	11.0[c]	11.6[b]
B1	11.0[b]	11.0[c]	11.6[b]
B2	11.0[b]	11.0[c]	11.6[b]
B3	11.0[b]	11.0[c]	11.6[b]
LSD 0.05%	0.223	0.681	0.857
		Ascorbic acid (mg/100g)	
Control	23.47[a]	5.79[a]	3.74[b]
Benzoate	22.42[b]	5.61[b]	3.51[a]
A1	18.26[d]	4.55[d]	3.14[c]
A2	16.90[e]	4.27[e]	2.78[e]
A3	14.43[f]	3.59[f]	2.45[f]
B1	20.07[c]	5.02[c]	3.41[b]
B2	17.01[e]	4.35[e]	2.91[d]
B3	14.63[f]	3.35[f]	2.48[f]
LSD 0.05%	0.778	0.132	0.162

- The control sample, orange juice without any addition of extracts.
- Sodium benzoate (0.1)% plus orange juice .
- A1,2,3 water extract, orange juice + green tea (0.1,0.2,0.3%, respectively).
- B1,2,3 alcoholic extract, orange juice + green tea (0.1,0.2,0.3%, respectively).

evaluation tests were done 3 times (at zero time, after 60 days, and at the end of the storage period 90 days. The effect of adding green tea water and alcohol extracts, at the concentration of 0.1%, 0.2% and 0.3% and sodium benzoate 0.1% on total bacterial counts and Yeast and Mold during storage refrigerator temperatures were investigated in Table 8 and the results show that no detectable any microbial spoilage in all treatments at zero time. This may be due that thermal pasteurization was the most intense treatment reaching counts < 1cfu/ml in citrus juice **Rivas *et al.* (2006)**. Total bacterial count was not detected in all treatments after three months. Mold and yeast were absent at zero time, but after 3 months they appeared in control only with 2×10^2. After 6 months of storage total bacterial count was not detected in all treatments except (control, A_1 and A_2) with (3×10^2, 2×10^2 and 1×10^2), respectively. Mold and yeast were not detected in all treatments except (control, A_1 and A_2) with (3×10^2, 2×10^2 and 1×10^2) respectively. The results proved that green tea extracts can used to enhance the shelf-life of various food products **(Namal Senanayake, 2013).** Green tea contains catechin and polyphenols which are highly sensitive to the oxidation process. The catechin and polyphenols have been found to possess antibacterial and antiviral action **(Archana and Abraham, 2011**).

Gulf Standard (2000), put some important microbiology standard guidelines for any fruit juice as in Table 7.

Sensory evaluation

Organoleptic evaluation could be considered as one of the most important aspects in juice blend technique since it reflects the consumer/preference. Data concerning sensory evaluation of orange juice fortified with or without green tea water and ethanol extracts by percentages (0.1, 0.2, 0.3%), backed in glass bottles and stored up to 6 months, are shown in Table 9 after preparation of the juices, no major changes occurred in odor of all juices. These samples were sensory evaluated for color, taste, odor and palatability. It could be clearly that nearly all samples products were almost palatable among different panelists. Orange juice with sodium benzoate 0.1% and orange juice with green tea alcoholic extracts (0.1, 02, 0.3%) recorded a slight decrease in sensory parameters compared to control, at zero time of storage. On the other hand, high scores of sensory attributes plus acceptability were given in samples of orange juice with green tea water extracts (0.1, 02, and 0.3%) after storage in glass bottles up to 6 months.

Conclusion

From the obtained results it could be conclude that 0.1%, 0.2% and 0.3% of green tea water and alcoholic extracts addition to orange juice can expand the storage period for a long time due to its antioxidant and antimicrobial properties. Thus, green tea can be considered a promising natural source of extracts that are rich in antioxidant and antimicrobial compound in order to replace synthetic antioxidants in the food industry, due to its low cost and high availability.

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Table (7): The recommended microbiological standards for any fruit juice consumed.

Parameter	Total viable count	Yeast and Mold
Maximum count	5.0×10^3	100
Maximum count permitted	1.0×10^4	1.0×10^3

Table (8): Effect of the storage period on total visible counts of orange juice with green tea water extracts (CFU/g).

Blends	Total bacterial count				Yeast and mold			
	Storage period (month)			Maximum count permitted	Storage period (month)			Maximum count permitted
	0	3	6		0	3	6	
Control	ND	ND	3×10^2	1.0×10^4	ND	2×10^2	3×10^2	0.1×10^3
Benzoate	ND	ND	ND		ND	ND	ND	
A1	ND	ND	2×10^2		ND	ND	2×10^2	
A2	ND	ND	1×10^2		ND	ND	1×10^2	
A3	ND	ND	ND		ND	ND	ND	
B1	ND	ND	ND		ND	ND	ND	
B2	ND	ND	ND		ND	ND	ND	
B3	ND	ND	ND		ND	ND	ND	

*** ND Not detected**

- The control sample , orange juice without any addition of extracts.
- sodium benzoate(0.1)% plus orange juice
- A1,2,3 water extract, orange juice + green tea (0.1,0.2,0.3% respectively)
- B1,2,3 alcoholic extract, orange juice + green tea (0.1,0.2,0.3% respectively)

Table (9): Changes in Sensory evaluation of orange juice with green tea water extract during the storage period.

Treatment	Storage period (month)		
	Zero	3	6
		Taste	
Control	8.05^{a}	7.55^{a}	7.05^{a}
Benzoate	7.40^{abc}	6.09^{abc}	6.40^{abc}
A1	7.55^{abc}	7.05^{abc}	6.55^{abc}
A2	7.85^{ab}	7.25^{ab}	6.75^{ab}
A3	7.80^{ab}	7.30^{ab}	6.75^{ab}
B1	7.25^{bc}	6.75^{bc}	6.25^{bc}
B2	6.90^{c}	6.40^{c}	5.90^{c}
B3	5.65^{d}	5.40^{d}	5.15^{d}
LSD 0.05%	0.754	0.724	0.705
		Odor	
Control	8.55^{a}	8.10^{a}	7.65^{a}
Benzoate	7.15^{b}	6.70^{bc}	6.20^{cd}
A1	8.10^{a}	7.70^{a}	7.20^{ab}
A2	8.00^{a}	7.60^{a}	7.25^{ab}
A3	7.90^{a}	7.40^{ab}	6.90^{bc}
B1	7.00^{bc}	6.55^{c}	6.05^{c}
B2	6.40^{c}	6.15^{c}	6.85^{c}
B3	6.60^{bc}	6.10^{c}	6.65^{c}
LSD 0.05%	0.745	0.720	0.735
		Color	
Control	8.80^{a}	8.30^{a}	7.80^{a}
Benzoate	7.55^{b}	7.05^{c}	6.55^{c}
A1	8.00^{b}	7.50^{bc}	6.90^{bc}
A2	8.25^{ab}	7.80^{ab}	7.15^{ab}
A3	8.05^{ab}	7.70^{abc}	7.30^{abc}
B1	7.50^{b}	7.00^{c}	6.50^{c}
B2	6.60^{c}	6.10^{d}	5.60^{d}
B3	5.60^{d}	5.40^{d}	5.10^{d}
LSD 0.05%	0.775	0.737	0.736
		Palatability	
Control	8.35^{a}	7.85^{a}	7.35^{a}
Benzoate	7.45^{bc}	7.00^{bc}	6.70^{b}
A1	7.50^{bc}	7.20^{abc}	7.55^{ab}
A2	8.20^{ab}	7.70^{ab}	7.35^{a}
A3	7.75^{abc}	7.35^{abc}	6.87^{ab}
B1	7.35^{c}	6.85^{c}	6.40^{b}
B2	5.90^{d}	5.60^{d}	5.30^{c}
B3	5.25^{d}	5.10^{d}	4.90^{c}
LSD 0.05%	0.772	0.772	0.803

- The control sample, orange juice without any addition of extracts.
- sodium benzoate(0.1)% plus orange juice
- A1,2,3 water extract, orange juice + green tea (0.1,0.2,0.3% respectively)
- B1,2,3 alcoholic extract, orange juice + green tea (0.1,0.2,0.3% respectively)

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إطالة فترة صلاحية عصير البرتقال باستخدام الشاي الأخضر

ليلى مجدي احمد تغيان[١]، سامح حسن مصيلحى[٢]، محمد عبدالشافي عبدالسميع[٢]، لبنى عبدالفتاح محمود هريدي[١]

١-قسم بحوث تكنولوجيا الحاصلات البستانية، معهد بحوث تكنولوجيا الأغذية، مركز البحوث الزراعية، الجيزة، مصر.

٢-قسم علوم وتكنولوجيا الأغذية والألبان، كلية العلوم الزراعية البيئية، جامعة العريش، مصر.

تهدف الدراسة إلى استخدام المستخلص الكحولي والمائي للشاي الأخضر كمادة حافظة طبيعية بدلاً من المواد الحافظة الكيماوية (بنزوات الصوديوم) وذلك باستخدام التركيزات المختلفة من الشاي الأخضر (٠,١، ٠,٢، ٠,٣%) لكلاً من المستخلص المائي والكحولي للشاي الأخضر، وأوضحت نتائج هذا البحث التأثير الإيجابي لاستخدام المستخلص المائي والكحولي للشاي الأخضر علي عصير البرتقال أثناء فترة التخزين (٦ شهور)، وارتفاع محتوى العصير المدعم بمستخلص الشاي الأخضر من مضادات الأكسدة والفينولات والفلافونويد بالمقارنة بعينة الكنترول، وظهور تأثير مستخلص الشاي الأخضر بصورة ملحوظة على العدد الكلى للبكتريا حيث نقصت فى العينات المدعمة بالشاي الأخضر عن عينة المقارنة حتى الشهر السادس من التخزين، وبصورة عامة فإن هذا البحث يوصي باستخدام مستخلص الشاي الأخضر المائي والكحولي لحفظ عصير البرتقال و لرفع قيمته الغذائية والصحية.

الكلمات الإسترشادية: الشاي الأخضر، المستخلص المائي، المستخلص الكحولي، عصير البرتقال، المركبات النشطة بيولوجيا، مضادات الأكسدة، مضادات الميكروبات، تحليل HPLC.