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Prolonging the Shelf life of Orange Juice Via Lemongrass Extracts

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Abstract

Freshly squeezed, unpasteurized orange juice is very desirable for the consumer because of its fresh aroma and flavor, but the shelf life is less than 6 days at 4 °C. Microbiological quality is the bottleneck for safety and shelf life. So,this study aimed to extend the shelf life of orange juice by using lemongrass extracts as natural preservatives. The effect of adding lemongrass extracts of 0.1, 0.2, and 0.3% to orange juice on the physicochemical, microbial, and sensory properties during storage up to 6 months were studied. The most important findings showed that the highest content of antioxidants, phenols, and flavonoids were found in orange juice plus (0.3%) lemongrass aqueous and ethanolic extracts for treatments A3 and B3, compared to all treatments. As a result, orange juice fortified with lemongrass extracts could be considered a good source of antioxidant activity as a free radical scavenger and extending the product's shelf life. Furthermore, lemongrass extracts had the best way to minimize the total number of bacteria in juice samples compared with controls during the storage up to 6 months. Fractionation of phenolic and flavonoid compounds of dried and extracted lemongrass by HPLC method showed that pyrogallol was the predominant phenolic compound of lemongrass powder, aqueous, and ethanolic extracts, while rutin was the predominant flavonoid compound. Results indicated that orange juice fortified with lemongrass aqueous extracts was more favorable than that fortified with ethanolic extracts or with sodium benzoates. Generally, it could be concluded that using lemongrass aqueous and ethanolic extracts extended the shelf life and raised the nutritional and health value of orange juice**.**

Key words: Natural preservatives,Lemongrass, Aqueous and Ethanolic extract, orange juice, Bioactive compounds, Antioxidant activity, Antimicrobial, HPLC analysis.

Introduction

Orange Baladi (**Citrus Sinensis**) is one of the most important citrus cultivar groups grown worldwide, accounting for roughly 70% of total annual Citrus species production ***(Navarro et al., 2011)***. 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 fiber ***(Stella et al., 2011; Stincoet al., 2012).*** Orange juice consumption has been associated with a reduced risk of chronic diseases, largely because of the presence of bioactive compounds such as ascorbic acid, carotenoids, and flavonoids ***(Aptekmann and Cesar, 2013; Ghanim*et al*., 2010; and Morand*et al*., 2011).*** It also contributes to vascular health and helps reduce a thermogenesis. Ascorbic acid is considered the main antioxidant compound in orange juice ***(Ness et al., 1996; Simon, 1992).*** Flavonoids, particularly hesperidin and naringin, also exhibit antioxidant activity ***(Tripoli et al., 2007),*** anti-inflammatory properties ***(Milenkovicet al., 2011),*** lipid-lowering properties ***(Monforte*et al*., 1995),*** and anti-carcinogenic properties ***(Birtet al., 2001; Yang et al., 2001).*** Carotenoids exhibit provitamin A activity and also reduce the risk of developing macular degeneration ***(Krinsky and Johnson, 2005).*** The bioactive compounds present in orange juice, particularly polyphenols, may also be associated with the metabolism of gut microbiota ***(Laparra and Sanz, 2010; Pereira-Caro*et al*., 2015a, and Pereira-Caro et al., 2015b).***

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 microbial growth. The natural herbs and spices' preservative activity is determined by the type of organism tested as well as the nature and concentration of the herb or spice.Herbs have been used as flavouring agents and preservatives due to their antimicrobial activity against certain pathogens and antioxidant properties ***(Meena and Sethi, 1997; Archanaet al., 2009).***

Lemongrass (Cymbopogoncitratus) is a rich source of bioactive compounds (flavonoids and vitamin C). The natural flavonoids are also attracting more and more attention, not only due to their antioxidant properties but also as anti-carcinogenic and anti-inflammatory agents because of their lipid anti-peroxidation effects ***(Martin* et al*., 2002).*** Several benefits of using lemongrass as a food preservative have been identified, including extended shelf life, hypoallergeniccity, the improvement of aroma and taste, and possible health benefits to consumers due to its antioxidant and anticancer effects ***(Hernández* et al*., 2016). Leite*et al*., (1986)*** reported that Cymbopogoncitratus has no toxic effect in humans. So, our objective in this study was to extend the shelf life of fresh orange juice through the addition of lemongrass extracts as natural preservatives, to study the effects of these treatments on the microbial and physicochemical quality characteristics of fresh, stored orange juice.

Materials and Methods

Fruits: Orange fruits (*Citrus sinensis),* Egyptian Baladi Orange fruits at full maturity were brought from local markets in Giza, Egypt.

Plant material :Lemongrass (*Cympopogoncitratus)* was obtained from Medicinal and Aromatic Plants Research, Horticulture Research Institute, Agricultural Research Center, El Giza, Egypt, by some specialist researchers in botany classification and identification. The fresh leaves of lemon grass were

collected, washed in running tap water to remove impurities, drained of excess water, and shade dried for 4 days. The dried leaves were ground into a fine powder *(**Thorat** et al**., 2017)***

Chemicals and reagents: All chemicals (analytical grade) were purchased from El-Gomhouria Pharmaceuticals Co., Cairo, Egypt. (2.2-diphenyl-1-1 picrylhydrazyl (DPPH)), Folin-Ciocalteu reagents, and gallic acid were obtained from Sigma-Aldrich Chemical, Steinheim, Germany.

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

Methods

Fresh juice preparation:

Orange fruits were sorted, washed, and left to drain.
Using stainless knives, cut into halves.
The juice was then extracted using a juicer (Moulinex brand).
The juice was filtered using a stainless drainer.

Lemongrass Extracts

Aqueous extract: According to ***Thorat et al., (2017),*** one hundred grams of dried lemongrass leaves were suspended in 1000 ml of distilled hot water in a water bath (w/v) at 80 °C for 30 minutes. The extracts were cooled, filtered, and the supernatant collected and stored in an airtight bottle for further use ***(El-Shemy et al., 2007).***

Ethanolic extract: One hundred grams of dried lemongrass leaves were mixed with 80% (v/v) ethanol and mechanically stirred for 2 hours at room temperature (IKA RW 20 Digital Homogenizer), then refrigerated for 24 hours at 4 °C±1.The extracts were filtered, and the supernatant was collected (**Khalafalla *et al*., 2009).** The product was then dried in a Stuart Rotary Evaporator Model RE300 at 40 °C1 and completely dried in a Snijders Scientific Type 2040 freeze dryer**.** Lyophilized extracts were kept at 4 ±1 °C for future use.

Dried lemongrass extracts were added to filtered, freshly prepared orange juice at different percentages as follows:

Orange juice with no extracts added (Control)
Sodium benzoate (0.1%) plus orange juice (benzoate)
Lemongrass ethanolic extract (L.G.E.E.) 0.1% + 99.9 ml orange juice
A2 lemongrass ethanolic extract (L.G.E.E.) 0.2% + 99.8 ml orange juice
A3 lemongrass ethanolic extract (L.G.E.E.) 0.3% + 99.7% orange juice
Lemongrass aqueous extract (L.G.W.E.) 0.1% + 99.9 ml orange juice
B2 lemongrass aqueous extract (L.G.W.E.) 2% + 99.8 ml orange juice
Lemongrass aqueous extract (L.G.W.E.): 0.3% + 99.7% orange juice

These mixtures were mixed well for two minutes using the blender. The juices' temperature was raised to 82 ^{o}C in a water bath for 2 minutes (ORTO ALRSA). The juice was packed in sterilized packaging materials (100-ml glass bottles) and subjected to heat up to 90 ºC for another two minutes ***(Mousa, 2012).*** Finally, the orange juice was cooled with tap water before being stored at 4°C±1 until analysis.

Analytical methods

Gross chemical composition

Moisture content, ash, total soluble solids (TSS%), and total acidity were determined according to the ***A.O.A.C (2012).*** The pH values were measured at 25 °C using a pH meter(Jenway, 3510, UK).Ascorbic acid was quantitatively determined according to the 2,6-dichlorophenol—indophenol dye method of ***Ranganna (1977).***Total phenolic compound was determined using the Folin-Ciocalteau reagent according to the method describedby ***Singleton and Rossi (1965)*** Phenolic compounds were fractionated and identified by HPLC according to the method described by***Goupyet al., (1999*)**.** Total flavonoid content was determined according to the method of ***Jiaet al., (1999).***Flavonoid compounds were fractionated and identified by HPLC according to the method recommended by ***Loon et al., (2005).*** Antioxidant activity: The ability of the samples to scavenge the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was investigated using the method developed by ***Baracaet al.,(2001).***

Microbiological analyses:

The aerobic plate (AP) count was determined using serial dilutions on plate count agar (PCA) with the pour plate method. The duplicate plates were incubated at 30 °C for 48 h. The total yeasts and moulds (YM) count was also determined using the pour plate method and the same dilutions on potato Dexterous Agar (PDA) for 5 days at 25 °C. A coliform group (CG) count with the same dilutions was also carried out on Mackoncy agar (MA) at 37 °C for 24 h. Results were expressed as "cfu (colony-forming units)/mL" ***(APHA, 1992).***

Antimicrobial activities of plant extracts

Dimethyl sulfoxide (DMSO) (3% w/v) was used to prepare the stock solution of aqueous and ethanolic extract solutions to evaluate their activities against the standard pathogenic bacteria that were used in this study for the preparation of the stock solution, having evaluated its activity at 100, 200, and 400 mg/ml concentrations of dried aqueous and ethanolic extracts, according to the method described by ***Fadeyi et al.,(2015).*** The antibacterial activity of aqueous and ethanolic extracts was determined using the agar well diffusion method according to the method described by ***Patel et al., (2014).*** Nutrient broth cultures of *Escherichia coli*, *Staphylococcus aureus, Bacillus cereus,* and *Salmonella sp*. were grown at 35ºC for 22 hours. Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells of 6 mm were punched in the agar and filled with plant extracts. On the same plate, control wells containing neat solvents (negative controls) were also run in parallel. After 24 hours of incubation at 37 °C 1, the antimicrobial activity was measured by measuring the diameter of the inhibition zone.

Sensory evaluation test procedures:

Orange juice was sensory evaluated by 10 panelists, of whom 5 were staff and 5 were random consumers. The quality attributes were measured according to the method described by ***Potter (1986).*** The weights of the parameters of sensory evaluation were as follows: colour 10%, odour 10%, taste 10 % and palatability 10 % ***Ahmed (2000).***

Statistical analysis:

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

Results and Discussion

The Chemical Composition of Fresh and Dried Materials:

The data in Tables 1 and 2 revealed a significant difference (**P ≤ 0.05**) in the means of the various samples. Dried lemongrass contained 8.95, 8.78, 23.59,and 13.83**%** moisture, ash, fiber, andtotal carbohydrates, respectively. These results were in agreement with ***Pereira et al., (2015c), Joshua et al., (2012), Nambiar and Matela (2012), and Asaoluet al., (2009)***. On the other hand, the moisture and ash contents of fresh orange juice (F.O.J.) were 90.77% and 0.36%, respectively. The total carbohydrate was found to be 3.42%. This results in almost complete agreement with ***Begum et al., (2018).***

Table (1):

Chemical composition of dried lemongrass leaves

Chemical composition (%)	Dried lemongrass
Moisture	8.95±0.01
Ash	8.78±0.19
Crude Protein	0.64±0.01
Total lipids	3.64±0.09
Crude fibers	23.59±0.04
Total carbohydrates	13.83±0.22
Total sugars	2.54±0.28
Reducing sugars	0.39±0.01
Non reducing sugars	8.95±0.01

Values are means ± SD of three measurements.

Data in Table 2showed also that total soluble solids (TSS) and total solids (TS) in F.O.J were 11% and 9.23%, respectively; This result is nearly in agreement with those obtained by ***Leahu et al., (2013) and Begum et al.,(2018).*** The ascorbic acid content of F.O.J. was found to be 28.69 mg/100ml. This result was almost in agreement with those found by ***Leahu et al., (2013) and Begum et al., (2018)*** who stated that, ascorbic acid content in (F.O.J) was 30.8 and 30.0 mg/100ml, respectively. The different percentages of chemical properties of fruit juice may be due to affected by the variety stage of maturity and growing conditions ***(Tressler and Joslyn, 1961).***

Table (2):
Physicochemical analysis of fresh orange juice

Chemical composition (%)	Fresh orange juice(F.O.J)
Moisture	90.77±0.02.
Ash	0.36±0.02
Total carbohydrates	3.42±0.10
Total sugars	9.26±0.19
Reducing sugars	2.62±0.15
Non reducing sugars	6.63±0.08
T.S.S.	11.00±0.02
T.S.	9.23±0.00
Acidity	0.63±0.02
V.C mg/100ml	28.69±0.04
pH value	4.00±0.00

Values are means ± SD of three measurements.

Total Phenolic, Flavonoid, and Antioxidant Activity of Lemongrass and Fresh Orange Juice:
The data in Table 3 showed the total phenolic compounds of lemongrass and fresh orange juice. The contents of total phenolic compounds were 7.69, 28.28, and 48.19 mg/g for lemongrass powder, aqueous, and ethanolic extracts, respectively. while orange juice presented 0.65 mg/g. This result is nearly in agreement with ***Soliman et al., (2017),*** who found that the total phenolic compounds in lemongrass leaves were 7.55 mg/g,and ***Chanson-Rolle et al.,(2016),*** who found that the total phenolic compounds in orange juice were 0.63 ± 0.04 mg/mL. The content of total flavonoid compounds was 9.41, 12.36, and 30.49 mg/g for lemongrass powder, aqueous extracts, and ethanolic extracts, respectively, followed by 0.16 mg/g for fresh orange juice. while the content of antioxidant activity was 81.94%, 83.33 % and 87.12% for lemongrass powder, aqueous extracts, and ethanolic extracts, respectively, and 36.33% for fresh orange juice. This result is nearly in agreement with ***Dharti and Dhvanika (2014),*** who mentioned that the antioxidant activity in orange juice was 36.6% **.**and ***Boeira et al., (2018)*** found that the antioxidant activity in lemongrass alcoholic extract was 89.47%. The decreased value of polyphenolic compounds in aqueous extract may be associated with the prevention of microbial growth. Further, the increase in antioxidant activity of juices may be due to the reactions between oxidized polyphenols and the formation of new antioxidant compounds during juice storage ***(Castro-Lópezet al., 2016).***

Table (3):

Total phenolic, flavonoid compounds and antioxidant activity of lemongrass and fresh orange juice

Sample	Lemongrass powder	Lemongrass ethanolic extract	Lemongrass aqueous extract	Fresh orange juice
Total phenolic compounds (mg/g) (as gallic acid)	7.69^{c} ±0.13	48.19^{a}± 0.11	28.28^{b} ±0.17	0.65^{d}±0.01
Total flavonoids (mg/g) (Asrutin)	9.41^{c}±0.25	30.49^{a}± 0.17	12.36^{b}± 0.10	0.16^{d}±0.01
Antioxidant (%) as DPPH	81.94^{c}±0.26	87.12^{a}± 0.20	83.33^{b}±0.60	36.33^{d}±0.01

Values are means ± SD of three measurements.

Means in the same row with different letters are significantly different (p< 0.05).

Fractionation and identification of phenolic compounds (mg/100 g) of powder and extracts of lemongrass by HPLC analysis.

Table 4 shows the HPLC phenolic compound fractions content of powder and extracts of lemongrass.The obtained data clearly indicated that the highest phenolic compound fraction content was found in lemongrass ethanolic extract, followed by aqueous extract, while the lowest phenolic compound fraction content was found in lemongrass powder.The phenolic compound fraction content was found in the following descending order for the ethanolic extract:

Pyrogallol,Ellagic,Rosmarinic,Catechein,Chlorogenic,Caffeic,Cinnamic, and Gallic.

Their values were 241.32, 168.88, 119.96, 110.17, 109.22, 66.63, 38.44, and 29.70 mg/100 g, respectively. It is also observed that, their values in lemongrass powder and aqueous extract followed the same trend but with lower concentrations and fewer differences among the fractions, as shown in Table.

Table (4):

Phenolic compounds fractions content as identified by HPLC analysis of lemongrass leaves powder; and its ethanolic and aqueous extracts (mg/100g dry weight).

Compounds	Lemongrass powder	Lemongrass aqueous extract	Lemongrass Ethanolic extract
Gallic	0.48	19.53	29.70
Pyrogallol	32.19	115.49	241.32
Catechein	10.58	41.41	110.17
Chlorogenic	5.47	16.17	109.22
Caffeic	0.82	2.22	66.63
Rosmarinic	10.86	22.68	119.96
Ellagic	5.37	17.27	168.88
Cinnamic	0 .69	10.32	38.44

Fractionation and identification of flavonoid compounds (mg/100 g) of powder and extracts of lemongrass by HPLC analysis

The data in Table 5 showed that rutin was the major flavonoid in all investigated lemongrass samples (powder, aqueous, and ethanolic extracts) among the three detected flavonoids. It is also found that its content was in the following decreasing order: Lemongrass ethanolic extract > Lemongrass aqueous extract > Lemongrass powder, with values of 511.20, 362.47, and 134.48

mg/100 g, respectively. The obtained data also revealed that the detected three flavonoids were found in the following decreasing concentration order in both aqueous and ethanolic extracts: rutin>quercetin>apigenin.

Table 5 :

Content of flavonoid compound fractions in lemongrass powder, aqueous and ethanolic extracts (mg/100 g dry weight).

Compounds	Lemongrass powder	Lemongrass aqueous extract	Lemongrass Ethanolic extract
Rutin	134.48	362.47	511.20
Quercetin	2.47	8.29	87.30
Apigenin	4.85	4.88	7.71

While this order was reversed in lemongrass powder, apigenin fraction content was higher than quercetin fraction content, which could be attributed to their polarity and solubility in polar solvents such as aqueous and ethanol because they are primarily glycosides.It could be concluded that lemongrass contained pyrogallol, catechin, rosmarinic, and ellagic phenolic compounds, as well as rutin, quercetin, and apigenin flavonoids, which were the predominant compounds. Furthermore, lemongrass was the strongest extraction medium that was able to dissolve most of the phenolic and flavonoid compounds from the samples. Methanol can extract semi-polar phenolic acid, whereas aqueous solutions prefer polar phenolic acid.

The HPLC analysis (after hydrolysis) is the most suitable process for fractionation of phenolic compounds (unconjugated with sugars) **(Khoddami *et al*., 2013),** which revealed that phenolic compounds may bind with other sample elements such as proteins and carbohydrates and therefore need to break down these bindings through the addition of some enzymes to release phenolic compounds.

Antibacterial activity of aqueous and ethanolic extracts of dried lemongrass on pathogenic bacteria

The zones of inhibition of microorganism growth are a function of the relative antibacterial activity of the extracts ***(Oloyede, 2009).*** Lemongrass leaf ethanolic and aqueous extracts have antibacterial activities against four foodborne pathogenic bacteria, E. coli and Salmonella Sp.Table (6) shows Staph. aureus and Bacillus cereus as gram-negative bacteria, as well as Staph. aureus and Bacillus cereus as gram-positive bacteria.

The aqueous extracts of lemongrass have no inhibitory effect on the growth of tested bacteria at all concentrations (100, 200, and 400 mg/ml). Meanwhile, a relatively high inhibition zone (5.0 mm) was observed against *E. coli* at 400 mg/ml of lemongrass ethanolic extracts, and the inhibition zone diameter increased with increasing the extract concentration from 100, 200, and 400 mg/ml for*Salmonella. Sp. (2.5, 5, and 9 mm), Staph. aureus (5, 8.5, and 12 mm), and Bacillus cereus (3, 5, and 9 mm, respectively).* These results are in agreement with ***Asaolu et al., (2009) and Fagbemi et al., (2009).***This result may be due to the polyphenolics and flavonoids extracted from lemongrass, which have attracted considerable interest as natural plant components with antioxidant and antibacterial activity. The data of HPLC phenolic and flavonoid compound fractions content of

lemongrass extracts also revealed that the lemongrass ethanolic extract had the highest phenolic and flavonoid compound fractions content, followed by the lemongrass aqueous extract.

Table 6.

Antibacterial activity of aqueous and ethanolic extracts of dried lemongrass on pathogenic bacteria

Test organism / extract concentration		Gram-Negative		Gram-Positive	
		E. coli	Salmonella. Sp	Staph. Aureus	Bacillus cereus
		Diameter of inhibition zone (mm)			
Aqueous ext.(mg/ml)	100	ND	ND	ND	ND
	200	ND	ND	ND	ND
	400	ND	ND	ND	ND
Ethanolic ext. (mg/ml)	100	ND	2.5	5	3
	200	ND	5	8.5	5
	400	5	9	12	9

ND = Not Detected

The Effect of Storage on Orange Juice and Lemongrass Antioxidant Activity, Phenolic and Flavonoid Compounds Antioxidant activity

The ethanolic and aqueous extracts of lemongrass demonstrated higher scavenging activity in fortified orange juice with (0.1, 0.2, and 0.3%) and sodium benzoate (0.1%) and increased antioxidant activity with increasing the level of plant extracts from 0.1 to 0.3% over a 6-month storage period.It was 66.9, 81.7, and 84.7, respectively, for orange juice with lemongrass ethanolic extracts (A1, A2, and A3) and 68.8, 73.5, and 83.2%, respectively, for orange juice plus lemongrass aqueous extracts (B1, B2, and B3); 35.5% and 84.01% in control; and benzoate 0.1% after 6 months of storage (Table 7). This may be due to the higher amounts of polyphenols in lemongrass extracts. Consequently, orange juice fortified with lemongrass extracts could be considered a good source of antioxidant activity as a free radical scavenger and also prolong the shelf-life of the product ***(Porter et al., 2006)***. Antioxidant activity was decreased during the storage period of up to 6 months. This decrease in antioxidant activity during storage was in agreement with those reported by ***Klimczaket al., (2007),*** who studied the effect of storage on the content of polyphenols, vitamin C, and the antioxidant activity of orange juice. They found that the reduction in the content of polyphenols and vitamin C during storage was reflected by the decrease in the antioxidant capacity of orange juice.

Table (7):

Effect of the storage period on Antioxidant activity (DPPH)%of orange juice with lemongrass ethanolic and aqueous extracts.

Storage period (month)			
Treatment	Zero	3	6
Antioxidant activity (DPPH)%			
Control	35.59^{e}±0.24	19.08^{f}±0.37	15.59^{f}±0.23
Benzoate	84.01^{a}±0.50	21.09^{e}±0.16	17.93^{d}±0.56
A1	66.98^{e}±0.15	25.03^{c}±0.28	17.86^{d}±0.51
A2	81.77^{b}±0.87	27.70^{b}±0.99	23.58^{b}±0.97
A3	84.72^{a}±0.13	31.98^{a}±0.82	27.56^{a}±0.92
B1	68.87^{d}±1.82	22.50^{d}±0.26	16.31^{ef}±0.17
B2	73.53^{c}±0.15	24.37^{c}±0.47	17.47^{de}±0.24
B3	83.20^{ab}±0.2	28.26^{b}±0.26	20.17^{c}±0.12
LSD 0.05%	1.589	1.1241	1.208

Total phenolic compounds (mg/g)

Data in Table 8 showed also that total phenolic compounds in treatments (A1, A2, A3, B1, B2, and B3) for orange juice with lemongrass ethanolic and aqueous extracts were 0.69, 0.83, 0.93, 0.62, 0.77, and 0.84 mg/g, respectively, while they were 0.61 and 0.73 mg/g in control and benzoate, respectively. Total phenolic compounds decreased over a 6-month storage period.

Table (8):

Effect of the storage period on Total phenolic (as gallic acid) of orange juice with lemongrass ethanolic and aqueous extracts

Storage period (month)			
Treatment	Zero	3	6
Total phenolic compounds(mg/g)			
Control	0.61^{f}±0.02	0.50^{f}±0.01	0.34^{g}±0.01
Benzoate	0.73^{d}±0.00	0.53^{e}±0.01	0.34^{g}±0.01
A1	0.69^{e}±0.00	0.62^{d}±0.00	0.46^{d}±0.01
A2	0.83^{b}±0.02	0.75^{b}±0.02	0.58^{b}±0.01
A3	0.93^{a}±0.01	0.87^{a}±0.01	0.64^{a}±0.01
B1	0.62^{f}±0.01	0.53^{e}±0.01	0.36^{f}±0.01
B2	0.77^{c}±0.01	0.61^{d}±0.01	0.44^{e}±0.01
B3	0.84^{b}±0.00	0.72^{c}±0.00	0.56^{c}±0.01
LSD 0.05%	0.20	0.0137	0.0136

This decrease in total phenolic compounds during storage was in agreement with those reported by ***Klimczak et al., (2007)*** and ***Assous et al., (2012),*** who produced products having high nutritional value from lemon grass and Valencia orange and found that total polyphenols decreased during the storage period.

Total flavonoid compounds (mg/g)

Results in Table 9 ascertained that the total flavonoid compounds in treatments A1, A2, A3, B1, B2, and B3 for orange juice with lemongrass ethanolic and aqueous extracts were 0.23, 0.28, 0.43, 0.19, 0.23, and 0.40 mg/g, respectively, and 0.152 and 0.158 mg/g in control and benzoate, respectively. Total flavonoids compounds decreased over a 6-month storage period.This decrease of total flavonoid compounds during storage is in agreement with those reported by ***Klimczak et al., (2007)*** and ***Oszmianski and Wojdylo (2009),*** who found that flavonols in apple juice decreased from 37.28 to 50.50% after 6 months of storage at 30 °C**.**

Table (9):

Effect of the storage period on Total flavonoid compounds (mg/g) of orange juice with lemongrass ethanolic and aqueous extracts

Storage period (month)			
Treatment	Zero	3	6
Total flavonoid compounds(mg/g)			
Control	0.15^{f}±0.00	0.13^{e}±0.01	0.12^{e}±0.01
Benzoate	0.15^{f}±0.00	0.14^{e}±0.01	0.13^{e}±0.01
A1	0.23^{d}±0.00	0.22^{c}±0.01	0.17^{c}±0.01
A2	0.28^{c}±0.01	0.27^{b}±0.01	0.24^{b}±0.01
A3	0.43^{a}±0.04	0.34^{a}±0.01	0.31^{a}±0.01
B1	0.19^{e}±0.00	0.19^{d}±0.01	0.14^{de}±0.01
B2	0.23^{d}±0.00	0.22^{c}±0.01	0.16^{cd}±0.01
B3	0.40^{b}±0.00	0.26^{b}±0.01	0.21^{b}±0.01
LSD 0.05%	0.028	0.017	0.033

The control sample, orange juice without any addition of extracts.

Sodium benzoate (0.1) % plus orange juice

A1,2,3 ethanolic extract, orange juice + lemongrass (0.1,0.2,0.3% respectively)

B1,2,3 aqueousextract, orange juice + lemongrass (0.1,0.2,0.3% respectively)

Effect of the storage period on the other chemical properties of orange juice with lemongrass ethanolic and aqueous extracts

Titratable acidity

Results in Table 10 revealed that the acidity value of orange juice with lemongrass ethanolic and aqueous extracts. It was clear that the acidity values of all treatments slightly decreased during the storage period, which lasted up to 6 months. These decreases in total acidity could be attributed mainly to the breakdown of ascorbic acid during storage or to the reaction between acid and sugar. This observation is in agreement with the studies of ***Molinari and Silva (1996); Assous et al., (2012).***

Table (10):

Effect of the storage period on the chemical properties of orange juice with lemon grass ethanolic and aqueous extracts

Storage period (months)			
Treatment	Zero	3	6
pH value			
Control	3.41^{e}±0.01	3.44^{d}±0.01	3.53^{c}±0.01
Benzoate	3.49^{d}±0.01	3.50^{c}±0.01	3.61^{a}±0.01
A1	3.32^{h}±0.01	3.33^{g}±0.01	3.41^{f}±0.01
A2	3.34^{g}±0.01	3.35^{f}±0.01	3.45^{c}±0.01
A3	3.36^{f}±0.01	3.38^{e}±0.01	3.47^{b}±0.01
B1	3.54^{c}±0.01	3.55^{b}±0.01	3.67^{e}±0.01
B2	3.56^{b}±0.01	3.57^{b}±0.01	3.71^{d}±0.01
B3	3.58^{a}±0.01	3.59^{a}±0.01	3.75^{c}±0.01
LSD 0.05%	0.0122	0.0173	0.0093
Titratable acidity%			
Control	1.006^{b}±0.01	0.97^{c}±0.01	0.90^{b}±0.03
Benzoate	1.014^{b}±0.03	1.00^{bc}±0.01	1.01^{a}±0.05
A1	1.016^{ab}±0.01	1.01^{b}±0.01	1.01^{a}±0.00
A2	1.025^{ab}±0.01	1.01^{b}±0.01	1.04^{a}±0.03
A3	1.044^{a}±0.01	1.09^{a}±0.01	1.06^{a}±0.03
B1	0.888^{c}±0.01	0.78^{d}±0.01	0.80^{c}±0.03
B2	0.888^{c}±0.01	0.81^{d}±0.01	0.81^{c}±0.02
B3	0.889^{c}±0.01	0.87^{c}±0.01	0.83^{c}±0.00
LSD 0.05%	0.025	0.044	0.0608
T.S.S%			
Control	11^{b}±0.01	11.5^{b}±0.01	11.6^{c}±0.02
Benzoate	11.5^{a}±0.01	12^{a}±0.01	12.3^{a}±0.02
A1	11.5^{a}±0.01	12^{a}±0.01	12.1^{b}±0.02
A2	11.5^{a}±0.01	12^{a}±0.01	12.1^{b}±0.01
A3	11.5^{a}±0.01	12^{a}±0.01	12.1^{b}±0.01
B1	11.5^{a}±0.01	12^{a}±0.01	12.1^{b}±0.01
B2	11.5^{a}±0.01	12^{a}±0.01	12.1^{b}±0.01
B3	11.5^{a}±0.01	12^{a}±0.01	12.1^{b}±0.03
LSD 0.05%	0.2498	1.852	0.395
Ascorbic acid(mg/100ml)			
Control	23.47^{a}±0.56	5.79^{a}±0.01	3.74^{a}±0.01
Benzoate	22.42^{b}±0.33	5.61^{a}±0.01	3.51^{b}±0.01
A1	19.99^{c}±0.47	4.88^{b}±0.01	3.39^{c}±0.01
A2	18.40^{d}±0.16	4.20^{c}±0.01	3.14^{c}±0.01
A3	16.72^{e}±0.17	4.19^{d}±0.01	2.87^{d}±0.01
B1	19.85^{c}±0.24	4.81^{bc}±0.02	3.52^{b}±0.01
B2	16.84^{e}±0.34	4.20^{d}±0.01	2.88^{d}±0.02
B3	15.20^{f}±0.02	3.73^{e}±0.01	2.56^{e}±0.01
LSD 0.05%	0.905	0.225	0.182

The control sample, orange juice without any addition of extracts.
Sodium benzoate (0.1)% plus orange juice .
A1,2,3ethanolic extract, orange juice + lemongrass (0.1,0.2,0.3%, respectively).
B1,2,3aqueousextract, orange juice + lemongrass (0.1,0.2,0.3%, respectively).

pH value

Data in Table 10 show that pH values in treatments A1, A2, A3, B1, B2, B3, benzoate, and control ranged from 3.32, 3.34, 3.36, 3.54, 3.56, 3.58, 3.49, and 3.41, respectively, at zero time of storage and increased to 3.41, 3.45, 3.47, 3.67, 3.71, 3.75, 3.61, and 3.53, respectively, during the storage period up to 6 months, in contrast to acidity values, which gradually decreased and this result were in agreement with those reported by ***Hussain et al., (2017)***

TSS %

Data in Table 10 ascertained that the total soluble solids in treatments A1, A2, A3, B1, B2, and B3 for orange juice with lemongrass ethanolic and aqueous extracts were 11.5, 11.5, 11.5, and 11.5%, respectively, and 11 and 11.5 in control and benzoate, respectively. TSS decreased during the storage period, which lasted up to 6 months. This observation is in agreement with the study of ***Hussain et al., (2017).***

Ascorbic acid(mg/100 g)

Table 10 shows that the percentage of ascorbic acid content decreased during storage. The initial percentage of ascorbic acid content in treatments A1, A2, A3, B1, B2, B3, benzoate, and control decreased from 19.90, 18.40, 16.70, 19.80, 16.80, 15.20, 22.42, and 23.47 mg/100 g, respectively. Ascorbic acid values decreased during storage to 3.39, 3.14, 2.87, 3.52, 2.88, 2.56, 3.74, and 3.51, respectively; these results agree with those reported by ***Klimczak (2007).***

The Influence of Storage Period on Orange Juice with Lemongrass Total Visible Counts

The effect of storage for 0, 3, and 6 months at 4.0±1.0 ºC for processed orange juice fortified with lemongrass extracts on total bacterial counts and yeast and mould were investigated, and the results are presented in **Table 11**..

Table (11):

Effect of storage period on total viable counts of orange juice with lemongrasss ethanolic and aqueous 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	1×10^2	2×10^2	3×10^2	1.0×10^4	1×10^2	2×10^2	3×10^2	0.1×10^3
Benzoate	ND	ND	ND		ND	ND	ND	
A1	ND	ND	ND		ND	ND	ND	
A2	ND	ND	ND		ND	ND	ND	
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**

**** Gulf Standard (2000)**

The control sample, orange juice without any addition of extracts.

sodium benzoate (0.1) % plus orange juice

A1,2,3 ethanolic extract, orange juice + lemongrass (0.1,0.2,0.3% respectively)

B1,2,3 aqueousextract, orange juice + lemongrass (0.1,0.2,0.3% respectively)

As shown in Table 11, total bacterial counts were not detected in all treatments at 0, 3, and 6 months. Meanwhile, total bacterial count, yeast, and mould were detected in unfortified control (A) at (1×10^2, 2×10^2 and 3×10^2) (C.F.U/g) at zero time, 3 and 6 months of storage, respectively. This could be because thermal pasteurisation was the most intense treatment, achieving counts of 1 cfu/ml in citrus juice. ***Rivas et al., (2006).***

Furthermore, from the result presented in Table 11, it is clear that mould and yeast were not detected for all treatments except control with 1×10^2, 2×10^2 and 3×10^2 (C.F.U/g)at zero time, 3 and 6 months of storage, respectively.

Sensory evaluation

Sensory attributes are the most significant quality parameters for determining consumer acceptance ***(Lawless and Classen, 1993)***.Results in Table 12 revealed that orange juice fortified with lemongrass aqueous extracts was more favourable than those of ethanolic extracts and orange juice fortified with sodium benzoates.Moreover, the control sample (pure orange juice) had the highest score in all parameters of sensory evaluation; this may be due to the natural taste of orange juice to panelists.Data showed that all treatments' taste, color, odor, and palatability scores decreased slightly after 3 and 6 months of storage.

Table (12):

Changes in Sensory evaluation of orange juice with lemongrass ethanolic and aqueous extract during the storage period

Storage period (months)			
Treatment	Zero	3	6
Taste			
Control	8.05^{ab} ± 0.79	7.55^{ab} ± 0.79	7.05^{ab}± 0.79
Benzoate	7.40^{bc} ±0.94	6.09^{bc}± 0.49	6.40^{bcd}± 0.49
A1	7.25^{de}±0.80	6.05^{de}± 0.68	5.95^{d}± 0.68
A2	6.80^{cd} ±0.71	6.30^{cd}± 0.71	6.10^{cd} ± 0.80
A3	5.77^{e} ±0.84	5.50^{e}± 0.64	5.27^{e}± 0.45
B1	8.05^{ab}±0.85	7.55^{ab}± 0.85	6.70^{abc}± 0.90
B2	8.10^{ab}±0.80	7.60^{a}± 0.80	7.10^{a}± 0.80
B3	8.15^{a}± 0.90	7.55^{ab}± 0.85	7.05^{ab}± 0.85
LSD 0.05%	0.73	0.69	0.68
Odor			
Control	8.55^{a}±0.65	8.10^{a}± 0.70	7.65^{a}± 0.70
Benzoate	7.15^{b}±0.71	6.70^{b}± 0.78	6.20^{bc}± 0.78
A1	7.45^{b}±0.91	7.05^{b}± 0.91	6.55^{b}± 0.91
A2	7.45^{b}±0.96	7.15^{b}± 0.84	6.70^{b}± 0.84
A3	5.95^{c}±0.86	5.68^{c} ± 0.75	5.50^{c}± 0.56
B1	8.75^{a}±0.93	8.35^{a}± 1.00	7.90^{a}± 0.94
B2	8.75^{a}±0.81	8.35^{a}± 0.81	7.95^{a}± 0.82
B3	8.65^{a}±0.95	8.25^{a}± 0.93	7.75^{a}± 0.93
LSD 0.05%	0.80	0.79	0.76
Color			
Control	8.08^{a}±0.71	8.30^{a}± 0.71	7.8^{a}± 0.71
Benzoate	7.55^{b} ± 0.85	7.05^{b}± 0.85	6.55^{b}± 0.85
A1	6.30^{c}±0.90	6.05^{c}± 0.86	5.55^{c}± 0.49
A2	6.15^{c}±0.87	5.85^{c}± 0.67	5.50^{c}± 0.50
A3	5.68^{c}±0.96	5.45^{c}± 0.50	5.18^{c}± 0.32
B1	8.45^{a}±0.47	8.10^{a}± 0.54	7.65^{a}± 0.63
B2	8.55^{a}±0.65	8.30^{a}± 0.45	7.70^{a}± 0.45
B3	8.60^{a}±0.49	8.15^{a}± 0.55	7.70^{a}± 0.60
LSD 0.05%	0.71	0.64	0.58
Palatability			
Control	8.35^{a}±0.67	7.85^{a}± 0.67	7.35^{ab}± 0.67
Benzoate	7.45^{b}±0.79	7.00^{b}± 0.84	6.7^{bc}± 0.91
A1	6.15^{c}±0.95	5.86^{c}± 0.78	5.60^{cd}± 0.66
A2	6.41^{c}±0.70	6.00^{c}± 0.67	5.65^{cd}± 0.71
A3	5.86^{c}±0.91	5.63^{c}± 0.71	5.36^{d}± 0.48
B1	8.51^{a}±0.81	8.00^{a}± 0.81	7.90^{a}± 0.83
B2	8.62^{a}±0.80	8.40^{a}± 0.58	7.25^{ab}± 2.32
B3	8.71^{a}±0.93	8.50^{a}± 0.87	7.80^{a}± 0.90
LSD 0.05%	0.77	0.70	1.01

The control sample, orange juice without any addition of extracts.

sodium benzoate (0.1) % plus orange juice

A1,2,3 ethanolic extract, orange juice + lemongrass (0.1,0.2,0.3% respectively)

B1,2,3 aqueousextract, orange juice + lemongrass (0.1,0.2,0.3% respectively)

Conclusion

From the obtained results, we can conclude that 0.3% of lemongrass aqueous and ethanolic extracts plus orange juice can raise nutritional value and expand the storage period for a long time due to its antioxidant and antimicrobial properties. Thus, lemongrass can be considered a promising natural source of extracts that are rich in antioxidant and antimicrobial compounds in order to replace synthetic antioxidants in the food industry, due to its low cost and high availability.

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إطالة فترة صلاحية عصير البرتقال باستخدام مستخلص حشيشة الليمون

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تهدف الدراسة إلى استخدام المستخلص الكحولي والمائي لحشيشة الليمون كمادة حافظة طبيعية بدلاً من المواد الحافظة الكيماوية (بنزوات الصوديوم) وذلك بإستخدام التركيزات المختلفة من حشيشة الليمون (0.1، 0.2 ،0.3%) لكل من المستخلص المائي والكحولي ، وأوضحت نتائج هذا البحث التأثير الإيجابى لإستخدام المستخلص المائي والكحولي لحشيشة الليمون علي عصير البرتقال أثناء فترة التخزين (6 شهور)، وارتفاع محتوى مضادات الأكسدة والفينولات والفلافونويد للعصير المدعم بمستخلص حشيشة الليمون بنسبة 0.3%بالمقارنة بعينة الكنترول، واظهرت النتائج تأثير مستخلص حشيشة الليمون على العدد الكلى للبكتريا حيث انخفض بصورة ملحوظة فى العينات المدعمة بالمقارنة بالعينة الضابطة وذلك خلال مراحل التخزين حتى ستة اشهر. ،واوضحت النتائج لتفريد محتوى الفينولات والفلافونات لحشيشة الليمون المجففة والمستخلصة بكلا من الماء والكحول ارتفاع محتوى البيروجالول والروتين كمركبات فعالة على التوالى. وبصورة عامة فإن هذا البحث يوصي بإستخدام مستخلص حشيشة الليمون المائي لاطالة صلاحية عصير البرتقال و لرفع قيمته الغذائية والصحية.

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