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Original Research Paper

Effects of ethanolic extracts of garlic, ginger and rosemary on the shelf-life of orange juice

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The effects of ethanolic extracts of garlic (*Allium sativum*), ginger *(Zingiber officinale*) and rosemary *(Rosemarinus officinales)* on the extension of the shelf life of orange juice were studied. Two and half milliliters of various extracts were introduced into the orange juice at concentrations of 125, 250, 500 and 1000mg L^{-1}. Positive control samples contained benzoic acid 2.5ml , while negative control sample consisted of orange juice only. It was observed that higher concentrations of 500mgL^{-1} and 1000mgL^{-1} of garlic and ginger extracts extended the shelf life of orange juice from Day 0 to Day 2 of storage while rosemary extract at similar concentrations extended the shelf life until Day 3 of storage. Indices for measurement of shelf life included significant loss in ascorbic acid and sugar, drop in pH, changes in colour (from pale yellow to brown) and odour (from good to foul) as well as changes in total fungal and bacterial counts. Rosemary extract showed the highest antibacterial and antifungal activities at all tested concentrations and the garlic extract displayed the least efficiency. Also, all the extracts displayed a higher suppressive action against fungal isolates than the bacterial isolates as well as against the Gram positive bacterial isolates than Gram negative bacterial isolates.

Key words: *Allium sativum, Zingiber officinale Rosemarinus officinale* , shelf-life, orange juice.

INTRODUCTION

Most of the fruits and vegetables are perishable especially in tropical and sub-opical regions (Surekha et al., 2010). The magnitude of post harvest losses – of fresh fruits and vegetables is estimated to be 25.80 % (Thirupathi et al., 2006). Most of the product is lost after harvest because of inadequate preservation method. The people in developing countries like Nigeria often cannot afford the use of cold storage either because of frequent power failure or lack of economic resource to acquire the necessary facilities.

Traditionally, the shelf- life stability of juices has been achieved by thermal processing (low temperature long time or high temperature short time treatments). However, thermal pasteurization tends to reduce the product quality and freshness. Therefore some non-thermal pasteurization methods have been proposed. These include: high hydrostatic pressure, high pressure homogenization, pulsed electric field and ultrasound. These emerging techniques seem to have the potential to provide fresh, safe juices with prolonged shelf-life but are quite expensive. Also, chemical preservatives such as sodium benzoate and potassium sorbate are widely used for the extension of the shelf-life of fruit juices and beverages. However, consumers' demand for natural origin, safe and environmental friendly food preservatives has been increasing over the last decade. Natural antimicrobials such as bacteriocins, organic acids, essential oils and phenolic compounds have shown considerable promise for use in some food products.

Several plant extracts or plant products have broad spectrum antimicrobial properties. They can be recognized as bio-preservatives, having no harmful effect on human health. Thus, herbal extracts have promise for use with fruits to enhance the shelf-life. They are safe and non- toxic, their application is simple and do not lose their efficacy at normal storage temperature. Hence this work aimed at the

determination of the efficacies of extracts obtained from three spices (garlic, ginger and rosemary) in extending the shelf-life of orange juice.

MATERIALS AND METHODS

Source of samples

Fresh healthy and ripe oranges, healthy garlic bulbs and ginger rhizomes were purchased from the main market at Eku in Delta State. Also, pre-packed rosemary spice was bought from the same market and particular notice of its expiration date was made.

Preparation of extracts of various spices

The garlic bulbs and ginger rhizomes were washed with sterile deionized water, aseptically peeled using a pre-sterilized knife and sun dried to constant weight, after which they were blended separately to powder and the weight of each powder was taken 550g . The weight of the rosemary 550g spice was also taken. Further, the ethanolic extract of each was obtained with 95 % ethanol using a soxhlet apparatus. Extracts obtained were evaporated to dryness using a rotary evaporator.

Preparation of orange juice sample

All the oranges purchased were washed with sterile deionized water. The rinds were cleaned with cotton wool soaked in methylated spirit and then rinsed in sterile deionized water. The orange fruits were carefully and aseptically peeled using a pre-sterilized knife. A juice extractor was then used to extract the juice from the orange fruits . Juice obtained was passed through a clean filter paper (Whatman no 1) to remove all roughages or fibers present and the filtrate was dispensed into sterile 250 ml Erlenmeyer flask in 100 ml volume.

Effect of various spice extracts on quality of fruit juice

One gram of each extract was re-constituted in 1000 ml of sterile distilled water and properly shaken to obtain a concentration of 1000 mgL^{-1}. Subsequent double fold serial dilutions were performed to obtain concentrations of 500 mgL^{-1}, 250 mgL^{-1} and 125 mgL^{-1}. The various extract concentrations were then introduced in 2.5 ml amounts into freshly prepared orange juice contained in 250 ml Erlenmeyer flask. These were left to stand for a period of 7 days. A flask of orange juice to which 2.5ml benzoic acid was added served as the positive control while another to which no preservative was added served as the negative control. At intervals of Day 0, 1, 2, 3 and 7, samples were aseptically withdrawn for analysis of pH, proximate composition, and total viable bacterial and fungal counts. Also, the colour and odour were noted on each day of analysis.

Proximate composition analysis

The proximate composition of the content of each flask was determined using methods as described by AOAC (2000). The parameters monitored include pH, titratable acidity, vitamin C (ascorbic acid) and sugar.

Determination of total viable bacterial and fungal counts

One milliliter of the content of each flask was withdrawn aseptically and ten-fold serial dilution was performed after which 0.1 ml of the dilution that produced 30-300 colonies was inoculated onto the surface of freshly prepared nutrient agar and Sabauraud Dextrose Agar plates for the enumeration and isolation of bacteria and fungi, respectively. Plates for bacterial counts were incubated at 37°C for 18-48 h. However, plates for fungal counts were incubated at ambient temperature for 4 – 7 days.

Identification of bacterial and fungal isolates

At the end of enumeration, individual well separated colonies were isolated and sub-cultured to obtain pure cultures of organisms associated with the juice. Bacterial identification was based on colonial morphology, Gram reaction and biochemical characterization following the method of Cruikshank et al*, (*1975). Fungal isolates were identified by the method of Barnet and Hunter (1994).
Statistical analysis of the observed parameters are needed data obtained were analyzed using the Student t-test and Analysis of Variance (ANOVA) at 0.05 probability level.
Economic analysis of using plant extracts compared to the existing positive control i.e using benzoic acid is needed. No economic analysis was done. Our focus was on encouraging a drift from the use of chemical preservatives to biological preservatives.

RESULTS

The effects of garlic, ginger and rosemary extracts on the proximate composition of orange juice samples are presented in Tables 1, 2 and 3, respectively. Results obtained revealed a gradual decrease in pH, ascorbic acid and sugar concentration of the samples as storage period increased. The percent sugar utilized at the end of the 7 day storage duration in samples that received 125, 250, 500 and 1000 mgL^{-1} of garlic extract were 79.85 , 59.69 , 42.32 and 34.74 %, respectively. Similarly, the amounts of sugar utilized in samples that contained 125, 250, 500

Table 1. Effect of garlic extract on changes in proximate composition of orange juice sample

Storage Duration	Extract Concentration (mg/L)	pH	Titratable acid	Ascorbic acid(g/100ml)	Sugar (g/100ml)	Colour/Odour
0	125	3.8	1.85	0.25	1.042	Pale yellow/Good
	250	3.81	1.86	0.25	1.042	Pale yellow/Good
	500	3.46	1.80	0.25	1.042	Pale yellow/Good
	1000	3.42	1.804	0.25	1.042	Pale yellow/Good
	C+	3.2	1.6	0.25	1.042	Pale yellow/Good
	C-	4.5	0.85	0.25	1.042	Pale yellow/Good
1	125	3.72	1.801	0.088	1.010	Pale yellow/Good
	250	3.8	1.86	0.087	0.911	Pale yellow/Good
	500	3.46	1.84	0.185	1.000	Pale yellow/Good
	1000	3.4	1.845	0.18	1.020	Pale yellow/Good
	C+	3.21	1.9	0.248	1.000	Pale yellow/Good
	C-	4.3	0.84	0.207	0.028	Brownish/Foul
2	125	3.71	1.8	0.085	0.600	Slightly brown/Foul
	250	3.8	1.86	0.080	0.804	Pale yellow/Good
	500	3.46	1.84	0.177	0.930	Pale yellow/Good
	1000	3.4	1.844	0.18	1.010	Pale yellow/Good
	C+	3.2	1.98	0.202	1.000	Pale yellow/Good
	C-	4.14	0.09	0.2	0.020	Brownish/Foul
3	125	3.7	1.8	0.084	0.380	Slightly brown/Foul
	250	3.8	1.85	0.08	0.509	Slightly brown/Good
	500	3.47	1.84	0.162	0.910	Pale yellow/Good
	1000	3.45	1.842	0.143	0.900	Pale yellow/Good
	C+	3.21	1.98	0.202	1.000	Pale yellow/Good
	C-	3.96	0.05	0.12	0.020	Brownish/Foul
7	125	3.61	1.8	0.081	0.210	Brownish/Foul
	250	3.77	1.85	0.08	0.420	Brownish/Foul
	500	3.43	1.81	0.162	0.601	Brownish/Foul
	1000	3.40	1.84	0.14	0.680	Brownish/Foul
	C+	3.00	1.99	0.2	1.000	Pale yellow/Fair
	C-	3.96	0.06	0.1	0.02	Brownish/Foul

Key: C^+: Positive Control ; C^-:Negative Control

and 1000mgL^{-1} of ginger extracts were 55.57, 51.92, 42.42 and 35.50 %, respectively. Also, orange juice samples that contained 125, 250, 500 and 1000mgL^{-1} of rosemary recorded sugar utilization of 5.37, 13.53, 4.99 and 4.03 %. In positive control that received benzoic acid, amount of sugar utilized was 4.03% while 98.08% was utilized in the negative control (without preservative). There was a significant difference ($P \leq 0.05$ in residual sugar concentrations among samples containing garlic, ginger and rosemary extracts.

Residual ascorbic acid were 0.081, 0.08, 0.162 and 0.14 in 100 gmL^{-1} in samples that received 125, 250, 500 and 1000 mgl^{-1} garlic extract. Corresponding values recorded in samples that contained ginger were of 0.14, 0.14, 0.2 and 0.24 in100 gml^{-1}, respectively. At the 7th day of storage, samples that received 125, 250, 500 and 1000 mgL^{-1} of rosemary extract, contained 0.204, 0.208, 0.221 and 0.236100gml^{-1} ascorbic acid. Positive and negative controls contained 0.2 and 0.19 mgml^{-1} , respectively.

It was observed that the activity of the microbial consortium in samples that received 125, 250, 500 and 1000 mgL^{-1} garlic resulted in a pH drop from 3.8 to 3.61, 3.81 to 3.77, 3.46 to 3.43 and 3.42 to 3.00. A reduction in pH from 3.68 to 3.07, 3.62 to 3.10, 3.61 to 3.42 and 3.6 to 3.17 was observed in orange juice samples that were treated with 125, 250, 500 and 1000mgL^{-1} of ginger extract. The values of pH in the juice samples treated with rosemary extract reduced from 4.40 to 4.10 in 125 mgL^{-1} , 4.40 to 4.22 in 250 mgL^{-1} , 4.61 to 4.20 in 500 mgL^{-1} and 4.60 to 4.20 in 1000 mgL^{-1} . The positive control showed a reduction from 3.2 to 3.00 while the pH of the negative control reduced from 4.5 to 3.96. Also, the colour of orange juice samples changed from pale yellow to brown and the taste became foul at day 7 of storage irrespective of the concentration of the various extracts introduced. Although, there was a difference in the intensity of the brown colouration depending on the extract present in the order: garlic extract ≥ ginger extract > rosemary extract. However, samples that received 500 and 1000 mgL^{-1} of garlic and ginger retained the initial colour and taste till Day 2 of storage while 125 to 1000 mgL^{-1} of rosemary extract maintained the colour and odour of various samples

Table 2. Effect of ginger extract on changes in proximate composition of orange juice sample

Storage Duration	Extract Concentration (mg/L)	pH	Titratable acid	Ascorbic acid(g/100ml)	Sugar (g/100ml)	Colour/Odour
0	125	3.68	0.84	0.25	1.042	Pale yellow/Good
	250	3.62	0.87	0.25	1.042	Pale yellow/Good
	500	3.61	0.82	0.25	1.042	Pale yellow/Good
	1000	3.6	0.8	0.25	1.042	Pale yellow/Good
	C+	3.2	1.6	0.25	1.042	Pale yellow/Good
	C-	4.5	0.85	0.25	1.042	Pale yellow/Good
1	125	3.66	0.88	0.186	1.030	Pale yellow/Good
	250	3.62	0.821	0.188	1.014	Pale yellow/Good
	500	3.61	0.82	0.214	1.020	Pale yellow/Good
	1000	3.6	0.8	0.231	1.026	Pale yellow/Good
	C+	3.2	1.9	0.248	1.000	Pale yellow/Good
	C-	4.3	0.84	0.207	0.028	Brownish/Foul
2	125	3.6	0.93	0.186	0.942	Slightly brown/Fair
	250	3.6	0.84	0.184	0.910	Slightly brown/Fair
	500	3.6	0.81	0.208	1.010	Pale yellow/Good
	1000	3.58	0.8	0.240	1.000	Pale yellow/Good
	C+	3.2	1.98	0.202	1.000	Pale yellow/Good
	C-	4.14	0.09	0.2	0.020	Brownish/Foul
3	125	3.1	1.06	0.140	0.482	Brownish/Foul
	250	3.25	1.064	0.147	0.566	Brownish/Foul
	500	3.5	0.84	0.200	0.946	Slightly brown/fair
	1000	3.55	0.81	0.246	0.982	Slightly brown/Fair
	C+	3.21	1.98	0.202	1.000	Pale yellow/Good
	C-	3.96	0.05	0.12	0.020	Brownish/Foul
7	125	3.07	1.09	0.14	0.463	Brownish/Foul
	250	3.1	1.101	0.14	0.501	Brownish/Foul
	500	3.42	0.972	0.2	0.600	Brownish/Foul
	1000	3.17	1.02	0.24	0.672	Brownish/Foul
	C+	3.00	1.99	0.2	1.000	Pale yellow/Fair
	C-	3.96	0.06	0.1	0.020	Brownish/Foul

C^+: Positive Control C^-:Negative Control

till Day 3 of storage. The colour and odour of the positive control remained pale yellow and good respectively throughout the storage period while negative control sample became brownish and foul smelling by Day 1 of storage.

Total viable fungal counts and total viable bacterial counts of samples that were treated with various concentrations of garlic, ginger and rosemary extracts are presented in Table 4 and 5 respectively. Total fungal counts and bacterial counts of samples containing 125 to 500mgL^{-1} of garlic and ginger increased from Day 0 to 3 of storage. Reductions in both fungal and bacterial counts were observed at Day 7. Slight reductions in total viable counts (fungal and bacterial) were observed in samples containing 1000mgL^{-1} of garlic, ginger as well as all concentrations of rosemary extracts from day 1 to 2 of storage and thereafter, gradual increase were noticed. In positive control sample, fungi were completely inhibited by Day 1 of storage while bacterial count reduced gradually from Day 1 to 7 of storage. Total fungal and bacterial counts in negative control sample increased significantly from Day 1 to 3 of storage. Slight reductions were observed at Day 7. There were significant differences at $P \leq 0.05$ among the total fungal count and total bacterial count of samples containing the various concentrations of garlic, ginger and rosemary extract. Rosemary extract showed the highest antibacterial and anti-fungal activities at all tested concentrations and the garlic extract displayed the least efficiency. Also, the extracts displayed a higher suppressive action against fungal isolates than the bacterial isolates as well as against the Gram positive isolates than gram negative isolates. Fungal isolates obtained in the study period include *Aspergillus sp*, *Rhizopus sp*, *Penicillium sp* and *Alternaria*. The prevalence of each of these isolates throughout the storage duration are as shown in Table 6. With garlic extract, persistence of fungal isolates decreased in the order: *Aspergillus* sp > *Penicillium* sp > *Rhizopus* sp > *Alternaria* sp. The trends at all concentrations of ginger and rosemary were: *Alternaria* sp > *Penicillium* sp > *Rhizopus* sp ≥ *Aspergillus* sp and *Penicillium* sp > *Alternaria* sp > *Rhizopus* sp ≥ *Aspergillus* sp respectively.

The prevalence of the bacterial isolates in orange juice

Table 3. Effect of rosemary extract on changes in proximate composition of orange juice sample

Storage Duration	Extract Concentration (mg/L)	pH	Titratable acid	Ascorbic acid(g/100ml)	Sugar (g/100ml)	Colour/Odour
0	125	4.40	0.800	0.250	1.042	Pale yellow/Good
	250	4.40	0.860	0.250	1.042	Pale yellow/Good
	500	4.61	0.840	0.250	1.042	Pale yellow/Good
	1000	4.60	0.846	0.250	1.042	Pale yellow/Good
	C+	3.20	1.600	0.250	1.042	Pale yellow/Good
	C-	4.50	0.850	0.250	1.042	Pale yellow/Good
1	125	4.37	0.800	0.246	1.040	Pale yellow/Good
	250	4.40	0.860	0.249	1.039	Pale yellow/Good
	500	4.40	0.840	0.250	1.042	Pale yellow/Good
	1000	4.39	0.845	0.250	1.040	Pale yellow/Good
	C+	3.21	1.900	0.248	1.000	Pale yellow/Good
	C-	4.30	0.840	0.207	0.028	Brownish/Foul
2	125	4.37	0.800	0.245	1.023	Slightly brown/Fair
	250	4.31	0.860	0.249	1.012	Slightly brown/Fair
	500	4.28	0.840	0.246	1.000	Pale yellow/Good
	1000	4.30	0.844	0.244	1.021	Pale yellow/Good
	C+	3.20	1.980	0.202	1.000	Pale yellow/Good
	C-	4.14	0.090	0.200	0.020	Brownish/Foul
3	125	4.12	0.800	0.208	1.020	Pale yellow/Good
	250	4.26	0.859	0.219	1.010	Pale yellow/Good
	500	4.20	0.840	0.226	1.000	Pale yellow/Good
	1000	4.20	0.842	0.240	1.020	Pale yellow/Good
	C+	3.21	1.980	0.202	1.000	Pale yellow/Good
	C-	3.96	0.050	0.120	0.020	Brownish/Foul
7	125	4.10	0.800	0.204	0.986	Slightly brown/Fair
	250	4.22	0.850	0.208	0.901	Slightly brown/Good
	500	4.20	0.810	0.221	0.990	Slightly brown/Good
	1000	4.20	0.840	0.236	1.000	Pale yellow/Good
	C+	3.00	1.990	0.200	1.000	Pale yellow/Good
	C-	3.96	0.060	0.100	0.020	Brownish/Foul

C^+: Positive Control C^-:Negative Control

Table 4. Changes in total viable fungal count in orange juice samples containing various extracts

Extract	Sample Concentration (mg/l)	Total Fungi Count ($cfuml^{-1}$) x 10^{-5}				
		Day 0	Day 1	Day 2	Day 3	Day 7
Garlic	125	1.0	1.4	2.2	2.4	1.6
	250	1.0	1.6	2.0	2.1	1.4
	500	1.0	0.4	0.4	0.2	0.2
	1000	1.0	0.4	0.4	0.6	0.3
	C+	1.0	0.0	0.0	0.0	0.0
	C-	1.0	1.3	2.8	1.9	0.9
Ginger	125	1.0	1.8	3.0	1.6	0.8
	250	1.0	1.4	2.4	1.7	1.0
	500	1.0	1.8	1.7	1.3	1.5
	1000	1.0	1.1	0.9	1.4	1.1
	C+	1.0	0.0	0.0	0.0	0.0
	C-	1.0	1.3	2.8	1.9	0.9
Rosemary	125	1.0	0.6	0.4	0.8	0.4
	250	1.0	0.6	0.2	0.1	0.0
	500	1.0	0.3	0.1	0.3	0.2
	1000	1.0	0.2	0.1	0.2	0.3
	C+	1.0	0.0	0.0	0.0	0.0
	C-	1.0	1.3	2.8	1.9	0.9

C^+: Positive Control C^-: Negative Control

Table 5. Changes in total viable bacterial count in orange juice samples containing various extracts

Extract	Sample Concentration(mg/l)	Total Fungal Count (cfuml^{-1}) x 10^{-3}				
		Day 0	Day 1	Day 2	Day 3	Day 7
Garlic	125	5.2	4.7	8.0	10.1	9.0
	250	5.2	4.0	5.3	7.4	7.1
	500	5.2	3.5	3.1	3.8	3.0
	1000	5.2	3.0	2.9	3.1	2.5
	C+	5.2	2.1	1.6	1.1	0.0
	C-	5.2	7.6	10.4	11.6	4.8
Ginger	125	5.2	6.7	8.8	10.0	6.1
	250	5.2	7.4	9.6	10.8	5.6
	500	5.2	5.0	5.8	7.3	6.4
	1000	5.2	5.6	5.1	6.9	6.0
	C+	5.2	2.1	1.6	1.1	0.0
	C-	5.2	7.6	10.4	11.6	4.8
Rosemary	125	5.2	5.0	4.5	6.2	4.8
	250	5.2	5.4	5.8	4.7	4.0
	500	5.2	4.8	4.1	4.9	4.2
	1000	5.2	5.0	4.5	5.3	4.8
	C+	5.2	2.1	1.6	1.1	0.0
	C-	5.2	7.6	10.4	11.6	4.8

C^{+}: Positive Control C^{-}:Negative Control

Table 6 . Prevalence of fungal isolates in orange juice samples treated with garlic, ginger and rosemary extracts

Extract	Sample Conc (mg/l)	Isolate / Storage period(Days)	*Aspergillus sp*	*Rhizopus sp*	*Penicillium sp*	*Alternaria sp*
			0 1 2 3 7	0 1 2 3 7	0 1 2 3 7	0 1 2 3 7
Garlic	125		+ + + + +	+ - + - +	+ + + + -	+ - - - -
	250		+ + + - -	+ - - + +	+ + + - -	- + + - -
	500		+ + - - -	+ - + - -	+ + - - -	- + - - -
	1000		+ - - - -	+ - - - -	+ - + - -	- - - - -
	C+		+ - - - - -	+ - - - -	+ - - - -	- - - - -
	C-		+ + + + +	+ + + + +	- + + + -	- - + + +
Ginger	125		+ + + + +	+ + + - -	+ + - - -	- + + + -
	250		+ + - - -	+ + - - -	+ + - - -	- + + + -
	500		+ - - - -	+ - - - -	+ + - - -	+ + + - -
	1000		+ - - - -	+ - - - -	+ + + - -	- + + - -
	C+		+ - - - - -	+ - - - -	+ - - - -	- - - - -
	C-		+ + + + +	+ + + + +	- + + + -	- - + + +
Rosemary	125		+ - - - -	+ + + - -	+ + + + +	+ + + + +
	250		+ + - - -	+ - + - -	- - + + -	+ + - - -
	500		+ - - - -	+ - - - -	+ + + + +	- - - + +
	1000		+ - - - +	+ + - - -	- + + + +	- - - + -
	C+		+ - - - - -	+ - - - -	+ - - - -	- - - - -
	C-		+ + + + +	+ + + + +	- + + + -	- - + + +

Key: + = present, - = absent C^{+}: Positive Control C^{-}: Negative Control

samples treated with various concentrations of the extracts of garlic, ginger and rosemary is shown in Table 7. The bacterial isolates obtained included *Pseudomonas* sp, *Streptococcus* sp, *Staphylococcus* sp, *Bacillus* sp, *Micrococcus* sp and *Esherichia* sp. Trends in the capability of garlic, ginger and rosemary extracts in suppressing the growth of various bacterial species include: *Pseudomonas* sp > *Esherichia* sp > *Bacillus* sp > *Streptococcus* sp ≥ *Staphylococcus* sp > *Micrococcus* sp, *Pseudomonas* sp > *Esherichia* sp > *Bacillus* sp > *Micrococcus* sp > *Streptococcus* sp ≥ *Staphylococcus* sp and *Pseudomonas* sp > *Esherichia* sp > *Bacillus* sp > *Micrococcus* sp ≥

Table 7. Prevalence of bacterial isolates in orange juice samples treated with garlic, ginger and rosemary extracts

Extract	Sample Conc. (mg/L)	Isolate / Storage period(Days)	IsP	IsS_1	IsS_2	IsB	IsM	IsE
			0 1 2 3 7	0 1 2 3 7	0 1 2 3 7	0 1 2 3 7	0 1 2 3 7	0 1 2 3 7
Garlic	125		+ + + + +	+ + + + +	+ + + + +	+ + + + +	+ + + + +	+ + + - -
	250		+ + + - +	+ - + - +	+ + + + +	+ + + + +	- + + + -	- + + - -
	500		+ + + - -	+ + - - -	+ + - - -	+ + + - -	+ + - + -	- - + + -
	1000		+ + + - -	+ - - - -	+ + - - -	+ + + - -	- - - - -	+ + - - -
	C+		+ + - - -	+ - - - -	+ + - - -	+ + - - -	+ - - - -	- - - - -
	C-		+ + + + +	+ + + + +	+ + + - -	+ + + + +	+ + + + +	+ - + + -
Ginger	125		+ + + + +	+ + + + +	+ + + + +	+ + + + +	+ + + + +	- - + - -
	250		+ + + - -	+ - + - -	+ + + + +	+ + + + +	+ + + - -	+ + + - -
	500		+ - + - -	+ - - - -	+ - - - -	+ - - - -	+ + + - -	+ + + - +
	1000		+ + + - -	+ - - - -	+ - - - -	+ + - - -	+ - - - -	+ + - - -
	C+		+ + - - -	+ - - - -	+ + - - -	+ + - - -	+ - - - -	- - - - -
	C-		+ + + + +	+ + + + +	+ + + - -	+ + + + +	+ + + + +	+ - + + -
Rosemary	125		+ + + + +	+ + + -	+ + - - -	- + - + +	+ + - - -	- - + - -
	250		+ + + - -	+ - - - -	+ - - - -	+ + - + +	+ - - - -	+ + - - -
	500		+ - - - -	+ - - - -	+ - - - -	+ - - - -	+ + + - -	+ + + - +
	1000		+ + + - -	+ - - - -	+ - - - -	+ + - - -	+ - - - -	+ + - - -
	C+		+ + - - -	+ - - - -	+ + - - -	+ + - - -	+ - - - -	- - - - -
	C-		+ + + + +	+ + + + +	+ + + - -	+ + + + +	+ + + + +	+ - + + -

Key: + = present, - = absent, IsP = *Pseudomonas* sp, IsS_1 = *Streptococcus* sp, IsS_2 = *Staphylococcus* sp, IsB = *Bacillus* sp, IsM = *Micrococcus* sp, IsE = *Esherichia* sp.
C^+: Positive Control C^-: Negative Control

Streptococcus sp ≥ *Staphylococcus*, respectively.

DISCUSSION

Two hundred and seventeen (217) bacterial isolates belonging to six genera and 124 fungal isolates belonging to four genera were isolated from the orange samples employed in the course of this study. These are likely to be part of the normal commensals of orange fruit. The activities of these organisms in the various juice samples might be responsible for the decline in the concentrations of sugar and ascorbic acid recorded. It is well known that the utilization of sugar (fermentation) results in the release of acidic by-products. This in turn may be accountable for the decrease in the pH levels noticed in all samples.

Also, the reductions in the pH values might explain the reason why the bacterial load of various samples reduced as storage period increased, but, more importantly, it was observed that the total fungal population that ought to thrive at low pH, also reduced with increasing storage duration, hence the reduction in pH is no longer a sufficient reason for the decrease in microbial load. The drop recorded in both the total viable bacterial and fungal counts is therefore most likely attributable to the antimicrobial properties of the various extracts used. Garlic had been shown to contain allicin, thiosulphuric acid and dially disulphate (Avato et al., 2000). The phytochemical components of ginger are zingiberol, zingiberine, gingerol, paradole and bisabolene(Michael, 1999) while rosemary extracts contain carnosic acid and carnosol (Del et al., 2000) . These components have been reported to have both anti-bacterial and anti-fungal properties (Joe et al., 2009). In this study, rosemary extract showed the highest antibacterial and antifungal activities at all tested concentrations. This probably is due to the peculiar phenolic antioxidant present in rosemary. All the extracts displayed a higher suppressive action against fungal than the bacterial isolates and also, against the Gram positive isolates than Gram negative isolates. The findings agree with those of other authors including Onyeagba et al., 2004, Joe et al., 2009, Kaushik and Goyal, 2011 and Hassan et al., 2012. The findings of Hassan et al., 2012 suggest that the antifungal effects may be a result of monoterpene which disrupt fungal membrane integrity. The comparative resistance of the Gram negative organisms may be due to the structural nature of their cell walls. The Gram negative bacterial cell wall contains outer membrane which probably has assisted in blocking the penetration of the extracts into the cell. Similar reports have been made by Mukhtar and Ghori, 2012 and Lawson, 1996. According to Odhav et al., 2002, the mechanism of antimicrobial action of spices involves the hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, membrane disruption and destruction of electron transport systems as well as cell disruption.

The results presented in Tables 6 and 7 indicate that different isolates respond differently to different concentrations of the various extracts. This may be responsible for the inability of the respective extracts applied singly, to extend the shelf life of the orange juice beyond day 2 or 3days of storage as there was no complete inhibition of all microorganisms present in the juice at any given point. Subsequently, it is important to assay the effects of these extracts when applied in combinations. Again, the concentrations of the various extracts may have only exhibited microbiostatic rather than microbiocidal effects and hence it may be necessary to step-up concentrations of these extracts in future studies. The microbiostatic effects of the spices could account for the loss in sensory quality and nutritional values (ascorbic acid and sugar) of the orange juice observed beyond Day 2 of storage.

The results of this study are quite encouraging as the three spices provided a significant increase in shelf life of orange juice in comparison with both positive and negative controls used. We therefore suggest that with the trend of increasing use of natural, biological and health friendly preservatives in food products, natural antimicrobial agents from garlic, ginger and rosemary may offer an innovative and interesting measure if better extraction and refining methods are employed.

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