Journal of Advances in Biology & Biotechnology

24(5): 26-35, 2021; Article no.JABB.69617
ISSN: 2394-1081

Changes in the Population of *Schizosaccharomyces japonicus* in *Carissa edulis* Vahl (Simple-spined Carissa) Juice Treated with Extracts of *Citrus aurantifolia* Christm. (lime) and *Citrus limon* Burm F. (lemon) Peels as Natural Preservatives during Storage

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Authors' contributions

This work was carried out in collaboration among all authors. Author UEI designed the study, performed the statistical analysis, and wrote the protocol and the first draft of the manuscript. Author NMA supervised the work. Author OAI managed the analysis and literature reviews of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2021/v24i530215
Editor(s):
(1) Dr. Joana Chiang, China Medical University, Taiwan.
Reviewers:
(1) Dina Ezzat Kodary Ahmed El-Ghwas, National Research Center, Egypt.
(2) Luis Cesar Maffei Sartini Paulillo, Centro Universitário de Tecnologia e Ciências (UNIFTC), Brazil.
(3) Ruta Lavinia, University of Bucharest, Romania.
Complete Peer review History: http://www.sdiarticle4.com/review-history/69617

Original Research Article

Received 15 April 2021
Accepted 21 June 2021
Published 26 June 2021

ABSTRACT

Aim: This study was aimed at evaluating the changes in the population of *Schizosaccharomyces japonicus* in locally produced simple-spine Carissa juice and the effects of lemon peel, lime peel and combination of lime and lemon peels extract as preservatives on the juice samples.
Methodology: Fruit juice produced was treated with different concentrations of citrus peels extracts (lemon, lime and lime+lemon) and their shelf lives were determined at room temperature for 30 days. The effects of the different preservatives on the pH of the juices were assessed and the Yeast count of the juice samples treated with the various concentrations of the citrus peels and the untreated samples were assessed using standard microbiological methods. The sugar fermentation

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and assimilation test of the isolate was also determined.
Results: Yeast population increased in the juice samples that were without treatments (control) from $5.6\times10^{3}\pm200$ to $1.52\times10^{4}\pm200$ cfuml^{-1} indicating significant increase ($p\geq0.05$). Treated juice samples showed significant decrease ($p\leq0.05$) in yeast population in the order of 300>200>100 mg/ml of the natural preservatives. Juice samples treated with highest concentration (300 mg/ml) of combined lime+lemon peels recorded the least colony forming units of $2.0\times10^{3}\pm100$ ml^{-1} during the storage period. This showed that the highest concentration (300 mg/ml) of combined lime+lemon peels had more effects on yeast load reduction in the Carissa juice. The results of the preservative properties of the citrus peels revealed the combined preservative (lime+lemon) as the best among the individual preservatives of lime and lemon. The sugar fermentation and assimilation tests showed that *Schizosaccharomyces japonicus* is a good fermenter of most sugars and can also assimilate sugars for its growth. The pH (3.40±0.01 and 4.09±0.01) of the treated fruit juices were within the acidic ranges that support the growth of yeast cells in culture.
Conclusion: From the findings, organic extracts of citrus peels can be used to extend the shelf-life of Carissa juice for up to three weeks.

Keywords: Carissa juice; citrus peels, yeast; preservative; Schizosaccharomyces japonicus.

1. INTRODUCTION

Fruit juices are known as important sources of nutrients [1] and possess pleasant tastes and aroma. They contain several important therapeutic properties that may reduce the risk of various diseases. They also contain large amounts of antioxidants including vitamins C and E [2]. Juices produced from tropical fruits have increasingly gained global importance and attention due to their health effects.

Preservatives are natural or synthetic substances that are added to the pharmaceuticals, cosmetics, and food products [3] to maintain and enhance their quality and to increase their shelf-life [4]. Preservation of fruits in the form of juices has turn out to be the business activity of great significance. Countries with rich fruit resources but with short harvesting season are emphasizing more for established storage to keep up quality of fruits, enhances shelf-life and preserve fruit juices for availability in off season [5]. There are different types of tropical fruits such as pineapple, watermelon, orange, mango and banana readily available for the production of fruit juices. The juice may be produced from single fruit or combination of fruits and sold by the street vendors. Meanwhile, there are other underutilized tropical fruits such as Simple-spined Carissa (*Carissa edulis* Vahl.) that have not been used for the processing of fruit juices.

Simple-spined Carissa (*Carissa edulis* Vahl.) is a spiny, much branched, small tree up to 5 meters in height which belongs to *Apocynaceae* family. The fruits are ovoid to almost spherical, red-black ripening to purple black. Its common names are; English (Simple-Spined Carissa, Simple Spined Num-Num), Arabic (emir) Northern Nigeria Hausa (Leemun tsun tsuu). *Carissa* fruits are mostly eaten raw or fresh in the Northern part of Nigeria. However, this fruit can become a potential source of raw material for fruit juice production. Freshly processed Carissa juice can be consumed immediately or stored in the refrigerator or treated with preservatives. The juice can be extracted and packaged for human consumption.

Lime and lemon peels are accepted as food ingredients mainly for flavoring intentions as well as to add on the acidity [6]. Freshly processed juices are subject to rapid microbial, enzymatic, chemical and physical deterioration [1]. Therefore, any possible way of processing that will minimize theses undesirable reactions and enhance inherent quality of the starting fruit should be encouraged.

The present study therefore was designed to produce juice from the fruit of *Carissa edulis* and investigate the possible ways to increase its shelf-life using natural preservatives as alternative to synthetic preservatives which possess harmful health effects on humans. It will also help to reduce the large economic losses reoccurring as a result of fruits deterioration by microorganisms.

2. MATERIALS AND METHODS

2.1 Source of Samples

The experimental samples including seventy (70 kg) of fresh and ripe simple spine-Carissa

(*Carissa edulis*) fruits, *Citrus limon* Burm F. and *Citrus aurantifolia* Christm were obtained from Faringada Market, Jos North Local Government Area, Plateau State, Nigeria. These experimental fruits were put in clean, well labeled polyethene bags and were then conveyed to the Applied Microbiology Laboratory in Department of Plant Science and Biotechnology, University of Jos for processing and analysis.

2.2 Fruit Juice Extraction

The extraction of the fruit juice of simple-spine Carissa was done in accordance to the method reported by Emelike and Ebere [7]. The simple-spine Carissa fruits were surface sterilized with 70 % ethanol and were washed in three successive sterile distilled water. A volume of 1.5 liters of Carissa juice was aseptically extracted using Moulinex blender (Type 278 China) and Philip multifunction juicer (Q/GDL001-2013, China). The extracted juice was filtered into some clean sterile bowls using sterile muslin cloth. A 100ml portion from the juice was dispensed into each of 150 sterile bottle containers using a sterile measuring cylinder.

2.3 Preparation of Preservatives

The natural preservatives were prepared from lime and lemon peels using cold maceration method described by Akinyemi and Oladapo [8]. With the aid of a knife, the peels were removed from lime and lemon and cut into smaller pieces before oven drying at low temperature of 45^{o}C to constant weight. Using Laboratory blender Moulinex (Type 278, China), the peels were finely pulverized. The pulverized peel materials were stored in airtight containers before used.

2.3.1 Preparation of different concentrations of the peel extracts

A weight of 3g of the pulverized peels of each of the lime and lemon were macerated in 10 ml of distilled water for 48 hours to obtain a concentration of 300 mg/ml. The mixtures were filtered into sterilized beakers using Whatman No.1 filter paper. The 300 mg/ml concentrations of lime and lemon were serially diluted respectively to obtain 200 mg/ml and 100 mg/ml concentrations respectively. The extracts were collected in airtight bottles and stored in a refrigerator before used.

2.3.2 Treatment of juice samples

A total of 150 bottles of juice samples were used for the experiment. With the aid of different sterile syringes, 1ml each of the various concentrations of the preservatives; lime, lemon and combination of lime+lemon was added separately into each batch of 15 bottles of 100 ml of the simple-spine Carissa juice and shaken thoroughly. All the treatments were in triplicate. Also, a total number of 100 ml 15 bottles of juice samples served as control (juice samples without treatment). All the 150 bottles of juice samples were stored at room temperature for the period of 30 days. The treated fruit juice samples and the control were further used to assess the total yeast count, yeasts isolation and identification and determination of pH.

2.4 Determination of Total Yeast count

For the determination of total yeast count, pour plate method of Vulindlu et al. [9] was used. One milliliter of the juice samples was serially diluted to nine fold sterile peptone water. A volume of 0.1 ml was taken from each dilution (10^{-4}) and poured on the sterile Petri dishes and were each covered with 20 ml of molten Sabouraud Dextrose Agar medium in triplicates. The Petri plates were allowed to solidify and then incubated at 37^{o}C for 24-48 hours. At the end of the incubation period, total colonies on the surface of the Petri plates ranging from 10-200 were counted and the results expressed as $\log_{10}$ colony forming units per milliliter ($\log_{10}$cfu/ml) of the fruit juices. The average of colonies formed on the triplicate plates for each treatment was recorded. The total yeast count was calculated using the formulae below:

Colony forming units (CFU/ml) = (Average number of colonies in the replicates X reciprocal of dilution factor) / Weight of inoculum

Source: Lutchmedia et al. [10].

2.5 Isolation and Identification of Yeast Species from Simple-spine Carissa Juice Samples

For the isolation of the yeast species, pour plate method of Vulindlu et al. [9] was employed. Sabouraud Dextrose Agar (SDA) was prepared based on the manufacturers' instruction and sterilized in an autoclave at 121^{o}C at 15psi for 15minutes. Serial dilution of juice samples were carried out and 0.1ml of appropriate aliquot (10^{-2}) diluent was then added unto the agar plate. About 20 ml of the molten agar medium was poured on the Petri dishes containing the juice samples and were allowed to solidify. Different

sterile 1ml pipette for individual treatments was employed. The inoculated plates were incubated at 37^{o}C for 24-48 hours. Pure cultures of isolates were obtained by repeated sub-culture on Sabouraud Dextrose Agar. Each experiment was carried out in triplicates. The growth characteristics were determined by their appearance on culture plates while the morphological features were determined microscopically with reference to existing Atlas for Microbial Identification Barnett et al. [11].

2.6 Determination of pH Values for the Juice Samples

The pH of the various juice samples containing different concentrations of the citrus peels natural preservatives and their controls were determined using a digital pH meter (Jenway pH meter Model 3310) according to standard methods of Association of Official Analytical Chemist AOAC,[12]. Fifty (50) ml of the various juice samples were weighed into different 100ml conical flasks. The pH meter was calibrated with buffer solution of pH 7.0 before inserted into the juices. The average of three readings was taken and recorded. This was repeated thrice for the various samples respectively at day (0) and one (1) week intervals throughout the period of storage.

2.7 Sugar Fermentation and Assimilation Tests

Sugar fermentation and assimilation tests were determined by multiple tube fermentation technique. Liquid medium containing peptone (1%), sodium chloride (0.5%) which was added Andrade´s indicator was prepared in different test tubes. Filter sterilized sugar solutions were also prepared at the concentration of (2%). One milliliter (1 ml) of the different sugar solutions were then poured into different test tubes containing inverted Durham tubes to show formation of gas. The test tubes containing the sugar solutions were then sterilized by autoclaving. The sugars used were glucose, fructose, maltose, galactose, lactose, sucrose and raffinose. A set of these seven sugars was used for identification of the Yeast isolates. Each tube was inoculated with 0.1 ml of 10^{-5} *Schizosaccharomyces japonicus* inoculum. The tubes were incubated at 37^{o}C for a period of 24 hours and were examined. The formation of gas in the Durham tubes (which was equivalent to the amount of water displaced from the tube) and production of acid indicated by pink colour was considered as 'fermentation positive' while only acid production with no formation of gas in the Durham tubes was considered positive for 'sugar assimilation' Meseret et al.[13].

2.8 Statistical Analysis

Using a Graph pad Prism version 8.2, the data were statistically analyzed with the help of a one-way ANOVA. Results were presented as mean} SEM. At $P<0.05$, the results were considered statistically significant.

3. RESULTS

The effects of different treatments on the yeast population of Carissa juice throughout the period of storage is shown in Table 1. The results varied significantly ($p\leq0.05$) and also revealed that, the Carissa juice treated with mixed preservative of (lime+lemon peel extracts) exhibits the least Colony Forming Unit (CFUml^{-1}) of $1.16x10^{3}\pm100^{d}$, followed by those treated with lime, $1.22x\ 10^{4}\pm200^{c}$ and lemon $1.44x10^{4}\pm100^{b}$ respectively on day 28. Carissa juice without treatment (Control) has the highest Colony Forming Unit (CFUml^{-1}) of $1.52x10^{4}\pm200^{a}$ on day 28. The results also revealed the effectiveness of the concentration of the various preservatives in this order; (300mgml^{-1}>200mgml^{-1}>100mgml^{-1}) respectively.

The effect of the different citrus peels treatments on the pH of Carissa juice is shown in Table 2. The pH of the treated juice samples decreased significantly ($p\leq0.05$) from 4.03 to 3.95. There was a significant increase ($p\leq0.05$) in pH for juice samples treated with lemon extracts from 5.49 to 6.23. Juice samples treated with lime increased in pH from 4.89 to 4.98 while juice treated with (lime + lemon) extract had a slight increase in pH from 3.32 to 4.27.

The results of the sugar fermentation and assimilation tests of *Schizosaccharomyces japonicus* isolated from Carissa juice during the storage period is presented in Table 3. The results revealed that the isolate was able to ferment and assimilate most of the sugar used. The yeast species was found to ferment and assimilate glucose, fructose, sucrose, galactose, maltose and raffinose. Lactose was neither fermented nor assimilated by the yeast.

The cultural characteristics and the structure of *Schizosaccharomyces japonicus* are shown in Plates 1a and 1b respectively.

4. DISCUSSION

The microbial quality of locally produced Carissa juice and the effects of lemon, lime and the combination of lemon and lime peel extracts as preservatives were studied with a view to prolong the shelf life of the juice. The peels of *Citrus aurantifolia* (lime) and *Citrus limon* (lemon) were used as sources of naturally occurring phenolic compounds. Shelf life is a major consideration in developing, producing and marketing of fruit juices and refers to the time during which a fruit juice sample remains acceptable to the consumer in terms of sensory properties. Factors that influence shelf life of fruit juice include moisture loss, enzymatic changes, deterioration due to microbial growth and oxidation. The addition of preservatives (synthetic or natural) in fruit juices helps to prevent decomposition, acidification and fermentation Adegoke et al. [14]. Preservatives from natural sources are acceptable to consumers, considering their being non-toxic, economical, availability, having both antimicrobial and anti oxidant potentials.

The Carissa juice produced was treated accordingly with various concentrations of the different citrus peels preservatives. The results showed that there is preservative potential of the citrus peels used on the fruit juice samples. Also the results showed that there are differences among lemon, lime and the combination of (lime+lemon) as inhibitors for yeast growth. Treated juice samples showed a significant decrease ($p \leq 0.05$) in yeast population. This result affirms the report by Nwachukwu et al. [1] which recorded a significant decrease in microbial population of fruit juices treated with lime and lemon juice.

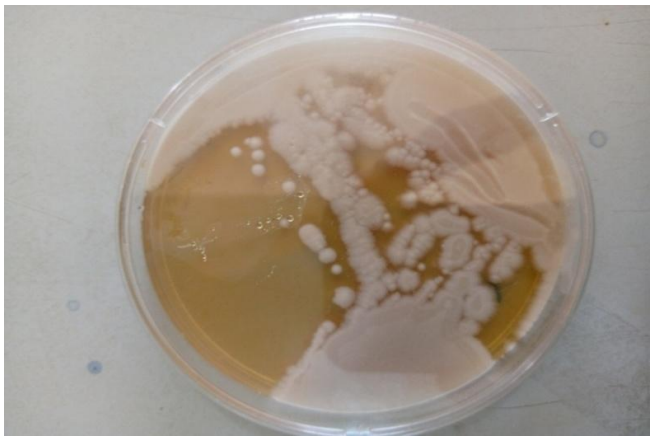

Plate 1a. *Schizosaccharomyces japonicas* on Sabouraud Dextrose Agar

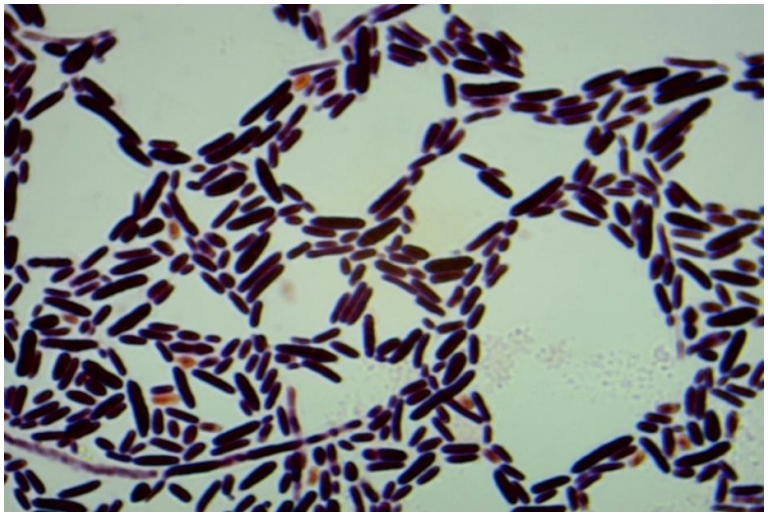

Plate 1b. *Schizosaccharomyces japonicas* with true hyphae (×1000)

Table 1. Effects of Varying Concentrations of Preservatives on the Population of *Schizosaccharomyces japonicus* in Carissa Juice during the Period of Storage

		Days				
Concentration mg/ml	**Treatment**	**0**	**7**	**14**	**21**	**28**
100	Lime	$3.8 \times 10^3 \pm 100^b$	$6.7 \times 10^3 \pm 100^b$	$8.9 \times 10^3 \pm 100^c$	$1.06 \times 10^4 \pm 100^a$	$1.22 \times 10^4 \pm 200^c$
	Lemon	$3.4 \times 10^3 \pm 200^b$	$6.6 \times 10^3 \pm 200^b$	$9.8 \times 10^3 \pm 100^b$	$1.29 \times 10^4 \pm 100^a$	$1.44 \times 10^4 \pm 100^b$
	Lime+ Lemon	$2.8 \times 10^3 \pm 200^c$	$5.3 \times 10^3 \pm 100^c$	$7.4 \times 10^3 \pm 200^d$	$9.0 \times 10^3 \pm 100^b$	$1.16 \times 10^3 \pm 100^d$
200	Lime	$3.1 \times 10^3 \pm 100^b$	$6.1 \times 10^3 \pm 100^b$	$8.3 \times 10^3 \pm 100^c$	$1.01 \times 10^4 \pm 200^a$	$1.18 \times 10^4 \pm 100^d$
	Lemon	$3.2 \times 10^3 \pm 200^b$	$6.3 \times 10^3 \pm 200^b$	$9.1 \times 10^3 \pm 100^b$	$1.21 \times 10^4 \pm 200^a$	$1.36 \times 10^4 \pm 200^b$
	Lime+ Lemon	$2.4 \times 10^3 \pm 200^c$	$5.2 \times 10^3 \pm 100^c$	$7.0 \times 10^3 \pm 100^d$	$8.8 \times 10^3 \pm 200^c$	$1.11 \times 10^3 \pm 100^d$
300	Lime	$2.0 \times 10^3 \pm 100^c$	$5.0 \times 10^3 \pm 200^c$	$7.5 \times 10^3 \pm 100^d$	$9.8 \times 10^3 \pm 100^b$	$1.12 \times 10^4 \pm 100^d$
	Lemon	$2.9 \times 10^3 \pm 100^c$	$5.9 \times 10^3 \pm 100^c$	$8.8 \times 10^3 \pm 100^c$	$1.16 \times 10^4 \pm 200^a$	$1.31 \times 10^4 \pm 100^b$
	Lime+ Lemon	$2.0 \times 10^3 \pm 200^c$	$4.7 \times 10^3 \pm 100^d$	$6.8 \times 10^3 \pm 200^c$	$7.7 \times 10^3 \pm 100^d$	$1.02 \times 10^4 \pm 200^a$
	Control	$5.6 \times 10^3 \pm 200^a$	$7.3 \times 10^3 \pm 200^a$	$1.03 \times 10^4 \pm 100^a$	$1.33 \times 10^4 \pm 200^a$	$1.52 \times 10^4 \pm 200^a$
	L.S.D	252				
	L.S	***				

Values are mean ±standard deviation of triplicate determinations
Means with different superscripts along a row are significantly different (p≤0.05)

Table 2. Effects of Varying Concentrations of Preservatives on the pH of Carissa Juice during the Period of Storage

		Days				
Concentration mg/ml	Treatment	0	7	14	21	28
100	Lime	3.75±0.01^{b}	3.93±0.01^{a}	3.89±0.01^{b}	3.86±0.01^{a}	3.99±0.01^{b}
	Lemon	3.60±0.01^{c}	3.95±0.01^{a}	3.87±0.01^{b}	3.90±0.01^{a}	3.96±0.01^{b}
	Lime+ Lemon	3.73±0.01^{b}	3.93±0.01^{a}	3.80±0.01^{b}	3.88±0.01^{a}	3.92±0.02^{b}
200	Lime	3.70±0.10^{b}	3.78±0.01^{b}	3.97±0.01^{a}	3.90±0.01^{a}	3.56±0.01^{e}
	Lemon	3.71±0.01^{b}	3.88±0.01^{b}	3.88±0.01^{b}	3.92±0.01^{a}	3.90±0.01^{b}
	Lime+ Lemon	3.71±0.01^{b}	3.91±0.01^{a}	3.91±0.01^{a}	3.92±0.01^{a}	3.91±0.01^{b}
300	Lime	3.65±0.01^{c}	3.93±0.0l^{a}	3.82±0.01^{b}	3.88±0.01^{a}	4.09±0.01^{a}
	Lemon	3.70±0.01^{b}	3.90±0.01^{a}	3.87±0.01^{a}	3.95±0.01^{a}	3.40±0.01^{f}
	Lime+ Lemon	3.74±0.01^{b}	3.88±0.01^{b}	3.57±0.28^{b}	3.93±0.01^{a}	3.81±0.01^{c}
	Control	3.18±0.01^{a}	3.88±0.01^{b}	3.83±0.03^{b}	3.83±0.01^{a}	3.71±0.01^{d}
	L.S.D	0.06				
	L.S	***				

Values are mean ±standard deviation of triplicate determinations.
Means with different superscripts along a row are significantly different (p≤0.05)

Table 3. Sugar Fermentation and Sugar Assimilation Tests of the *Schizosaccharomyces japonicus* isolated from the Carissa Juice

Organism	**Sugar Fermentation**							**Sugar Assimilation**							**Gram reaction**
	Gl	**Fr**	**Su**	**Gal**	**La**	**Mal**	**Ra**	**Gl**	**Fr**	**Su**	**Gal**	**La**	**Mal**	**Ra**	
Yeast															
Schizosaccharomyces japonicus	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+

Gl = Glucose, Fr = Fructose, Gal = Galactose, Su = Sucrose, Mal = Maltose, La = Lactose, Ra = Raffinose+ = Present, - = Absent

The present finding also revealed the combination of lemon and lime peel extracts as the best among the preservatives; this was observed as the storage period proceeded to over 14 days and demonstrated the ability to reduce the viable count of the yeast species as compared to the controls. Reena and Priyanka [15] reported the biological activity of fruit peel and juice extracts from *C. limon*, *C. sinensis* and *C. aurantifolia* for possible use as antimicrobial agents. Piccinelli et al. [16] studied the presence of secondary metabolites in different parts of Citrus plants.

On the other hand, juice samples treated with lime extracts was more effective on reduction of viable count of the yeast species than those treated with lemon extracts as seen from the results (Table 1). The result agreed with the report of Fapohunda et al. [17] who recorded a decrease in microbial population (CFU/ml) of Kunu Zaki treated with lime and lemon juice in a quest to prolong its storage period.

The results varied significantly ($p \leq 0.05$) and also revealed that, the Carissa juice treated with mixed preservative of lime+lemon extracts exhibited the least Colony Forming Unit ($CFUml^{-1}$), followed by those treated with lime and then lemon respectively across all the concentrations of the preservatives. The results also revealed the effectiveness of the concentration of the various preservatives in this order; ($300mgml^{-1} > 200mgml^{-1} > 100mgml^{-1}$) respectively. The juice samples treated with highest concentration (300mg/ml) recorded more effect on yeast load reduction with the least colony forming units (CFU/ml) of ($2.0 \times 10^3 \pm 100$) during the storage period. The results of this investigation also revealed that untreated (control) juice samples recorded a significant increase ($p \leq 0.05$) in yeast population which ranged from ($5.6 \times 10^3 \pm 100$) to ($1.52 \times 10^4 \pm 200$) CFU/ml throughout the period of storage. This could probably be due to activities of the yeast favored by the absence of refrigeration and preservatives. Biodeterioration of fruits and its products are influenced by factors such as pH, temperature, chemical composition microbial population Lutchmedial et al. [10].

The assessment of yeast flora of the juice samples revealed the presence of *Schizosaccharomyces japonicus* in the Carissa juice. This agrees with similar observation reported by Nzabuheraheza et al. [18] who isolated *Saccharomyces spp* from locally produced passion and mango fruit juices respectively. Various researchers have reported isolation of yeast species from different fruit juices [19,20,21,22].

The excellent keeping quality of fruit juices and soft drinks are influenced by low pH Bates et al. [23].The pH values of the juice sample also varied significantly ($p \leq 0.05$) across the various concentration of different preservatives at each evaluation intervals. Also the results of the investigation showed that the pH of the fruit juice samples was generally acidic irrespective of the various treatments (Table 2). The high acidity of the fruit juice samples could be due to the presence of organic acids and the citric acid components of the experimental fruit (*Carissa edulis*) samples and the various citrus peels used as preservatives whose composition varies depending on the nature of the treatment. The pH level of Carissa juice samples at the first evaluation day (before fermentation) recorded low values with the untreated juice (Control) having the lowest value (3.18 ± 0.01). The results of this research work agree with that of Oranusi et al. [24] in their work on Microbiological and chemical quality assessment of some commercially packed fruit juices sold in Nigeria.

There was a little increase in pH level of Carissa juice treated with lime 300 mg/ml concentration as the storage prolongs with the highest value (4.09 ± 0.01) recorded on day (28) as shown in Table 2. The increase in the pH level though still within acidic range for Carissa juice samples could probably be as a result of the metabolic activities of the yeast species leading to fermentation and production of organic acids such as acetic and citric acids. An increase in acidity as shown could also be as a result of the accumulation of microbial metabolites over time. At low pH, microbial growth decreases and shelf life of fruit juice increases. The reduction of yeast population in the experimental fruit juices following the addition of lemon and lime peel extracts could be related to their high acidic components which help in acidification of the juice samples hence reducing their pH level which in turn inhibit the growth of most pathogenic microorganisms. The high acidity of the juices could account for the low numbers and few types of microorganisms associated with its deterioration Willey et al. [25].

Schizosaccharomyces japonicus was found to ferment and assimilate most of the sugars including glucose, fructose, sucrose, galactose, maltose and raffinose. Lactose was not

fermented and assimilated. Carbon fermentation and assimilation are important criteria in the identification of yeasts. Yeasts generally depend on organic carbon sources for their energy source for growth and development. Galactose is a non-conventional nutrient for yeasts and can be used for as a sole carbon source when glucose is absent from the medium. The ability of yeast strain of *Schizosaccharomyces japonicus* isolated in this study to assimilate galactose indicated that the strains have the GAL genes responsible for galactose fermentation as reported in a similar work by Yun et al. [26].

5. CONCLUSION

The findings of this study generally revealed that citrus peels are excellent natural source of preservatives for simple-spine Carissa juice. However, the combined preservative of lime+lemon peel extracts proved the best for preservation of Carissa juice for up to four weeks without refrigeration. Also, the study demonstrated that the higher the concentration of the preservatives the higher the effect on *Schizosaccharomyces japonicus* inhibition.

6. RECOMMENDATION

Since this method is simple, inexpensive and convenient, it can be adopted for industrial use in the processing and preservation of Carissa juice. The utilization of preserved juice should be encouraged as health/therapeutic drink.

Above all, preservation of Carissa juice is important because of its season ability which makes it abundantly available during its season and scarce during off season. Therefore, low cost preservation of Carissa juice using combination of lemom+lime peel extracts is recommended for extending the shelf-life of the juice stored at ambient temperature.

ACKNOWLEDGEMENTS

The authors wish to appreciate and acknowledge the technical staff of Department of Plant Science and Biotechnology of University of Jos, for their support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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