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Influence of chemical preservatives on quality attributes of orange juice

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A B S T R A C T

The aim of this study is to evaluate the chemical, microbiological and sensory characteristics of chemically preserved orange juice samples. Orange juice was produced from sweet orange fruit (*Citrus sinensis*). Four samples were developed by treating the orange juice with 0.03% preservative sodium benzoate (SB), sodium metabisulphite (SM), potassium sorbate (PS) and their combination (AP). The control juice (UNT) sample was used as a control. Chemically preserved and unpreserved orange juice was stored at refrigeration temperature (4 ± 2 °C) for 3 weeks. The pH, total titratable acidity, ascorbic acid, total solids, total soluble solids, microbiological qualities and sensory attributes of samples stored for 3 weeks were determined using standard methods. The pH of the juice ranged from 3.30 to 3.60, total titratable acidity (1.50 - 1.77 mL), total soluble solids (9.40 – 10 °Brix), total solids (23.97 - 19.17 mg/L) and vitamin C content (270.20 - 150.90 mg/100g). The microbiological analysis showed no growth of coliform. The yeast and total viable count ranged from 3×10^3 - 14×10^3 CFU/mL and 3×10^3 - 10×10^3 CFU/mL, respectively. At 0.03%, sodium benzoate, sodium metabisulphite and potassium sorbate could be effective preservative for orange juice preservation. The use of chemical preservatives in orange juice preservation does not influence negatively the consumer acceptability of the product. The combined usage of sodium benzoate, sodium metabisulphite and potassium sorbate influenced the quality attributes of orange juice.

Introduction

Fruits and vegetables are among the most important foods of mankind because of their nutritive and indispensable effect in the management of health (Dauda and Adegoke, 2014). Sweet orange (*Citrus sinensis* L. Osbeck) fruit is a cultivated crop in the tropical and sub-tropical regions of the world that is widely consumed because of its excellent source of vitamin C (Ismail and Zhang, 2004) and bioactive properties (Rampersaud and Valim, 2017). Orange fruit contains biologically active compounds (natural antioxidant) that could prevent the development of degenerative diseases such as arteriosclerosis, cancer, kidney stones, stomach ulcers and high blood pressure (Etebu and Nwauzoma, 2014). Sweet orange contains folacin, calcium, potassium, thiamine, niacin, magnesium and phytochemicals like liminoids, synephrine, hesperidin, flavonoid and polyphenols (Gorinstein et al., 2001; Hashem et al., 2014).

Juice is a liquid drink that is contained naturally in fruit and vegetable. Citrus juice is a widely consumed beverage in the world (Rampersaud and Valim, 2017). Fruit juice could help meet the daily requirement of fruits and vegetables in the diet (Jan and Masih, 2012). The preservation of fruits and vegetables can be achieved through processing and

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the use of preservatives. There are many pathways to the deterioration of fruit juices. However, a lot of effective preservation methods could fight spoilage (Olurankinse, 2014) and wastage. Food preservation aims at maintaining the quality and nutritional attributes of foods while preventing their spoilage. Shelf stability in fruit juices is the major concern of this investigation. Several efforts have been made in the preservation of fruit juices, this includes; the report of Mehmood et al. (2008) on the effect of pasteurization and preservatives on quality and shelf stability of apple juice. Also, Sarkar et al. (2014) reported the effect of storage and preservatives on the antioxidant content of orange, apple, black grapes, sweet lime, litchi, pomegranate and mango fruit juices. Hence, little or none has been reported on the effect of chemical preservatives on the physicochemical, sensory and storage stability of orange fruit juices. Therefore, this study evaluated the quality of chemically preserved orange juice.

Materials and methods

Materials

Ripe sweet orange (*Citrus sinensis*) fruit was purchased from a local market in Akure, Ondo State, Nigeria. Sodium benzoate, sodium metabisulphite and potassium sorbate were purchased from Pascal Scientific Limited, (Akure, Nigeria). Nutrient agar, McConkey agar was obtained from Merck, (Merck, South Africa) and Sabouraud medium (Biomark Laboratories, India) while other reagents used in the study were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Methodology

Production of orange juice

Orange juice was produced by the modified method of Bates et al. (2001) as shown in Fig. 1. Orange fruits were manually sorted to remove damaged or bruised fruit, washed with calcium hypochlorite solution and rinsed in potable water. Cleaned orange fruits were fed into the Zumex robot type orange squeezer machine (PODIUM Version, 418560, Spain) for extraction of juice. Orange juice was divided into portions. About 0.45 g of sodium benzoate, sodium metabisulphite and potassium sorbate were added singly to each juice portion of 1.5 L. All selected preservatives were randomly combined and 0.45 g of each was added to portions of the juice. The untreated portion was used as a control. Juice samples were stored in opaque glass

bottles and pasteurized at 77 °C for a minute. The chemically preserved and control juice samples were stored under refrigeration temperature (4 ± 2 °C) for three weeks. Stored juice samples were investigated for its chemical, sensory properties and microbial loads.

Chemical analyses

The pH, total titratable acidity (TTA), total soluble solids (TSS), sugar-acid ratio and total solids (TDS) of orange juice were determined for 3 weeks of storage following the standard methods of AOAC (2014).

Determination of pH

The pH of the samples was determined using a standard pH meter (Expandable Ion Analyzer EA 920). The pH meter was calibrated using a buffer solution of pH 4 and 7. About 10 mL of sample was measured into a beaker and the pH meter was dipped into the beaker to measure the pH of the sample. The pH values were then recorded.

Determination total titratable acidity (TTA)

The total titratable acidity was determined according to the method described by AOAC (2014). About 5 mL of the juice was made up with distilled water to 50 mL mark in a beaker. Five (5) mL aliquot of the sample solution was pipetted into a conical flask and two drops of phenolphthalein indicator was added. Titration was done with 0.1 M NaOH until the endpoint was reached. The used factor for calculating citric acid was 0.007005%. Total acidity was calculated as citric acid percentage (% citric acid) as shown in Eq. 1.

$$\% \text{ TTA} = V(0.1 \text{ M NaoH}) \times M(\text{NaOH}) \times 0.007005 \% \quad (1)$$

Determination of total soluble solids (TSS)/°Brix

The soluble sugar was determined by using a Fisher Scientific™ Abbe Bench-top Refractometer at 20 °C (AOAC, 2014). The refractometer was connected to water bath for temperature control, sample replaceable prism was inserted and the light source was turned on prior to determination. The refractometer was cleaned with distilled water before drops of samples were placed on the slide of the refractometer and the lid replaced. The reading was regarded as the total soluble solids of the samples in degree brix (°Brix).
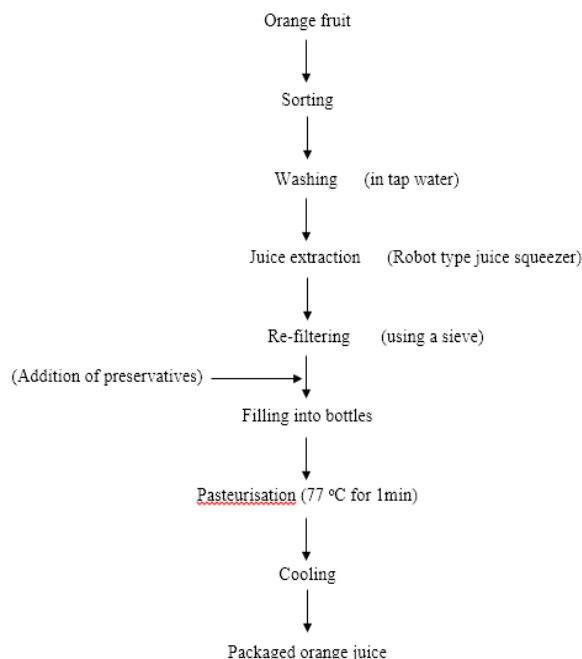
Fig. 1. Production of packaged orange juice

Determination of the sugar-acid ratio of the juice

The sugar-acid ratio of samples was determined by calculation as described below in Eq. 2.

$$Sugar - acid\ ratio = (TSS\ (°Brix))/(\%\ TTA) \qquad (2)$$

Determination of total solids (TS)

About 5 mL of the juice was weighed together with a dried and pre-weighed petri dish. The petri dish with its content was evaporated in a boiling water bath and dried to a constant weight in hot air oven at 70 °C. The total solids were calculated as the percentage of the insoluble solids in the sample (AOAC, 2014).

$$Total\ solids\ (\%) = \frac{W1 - W2}{W3} \times \frac{100}{1} \qquad (3)$$

Where; w_1 = weight of the sample + petri dish before drying; w_2 = weight of the sample + petri dish after drying; w_3 = weight of the sample; TS = Total solids (%).

Determination of vitamin C content

The vitamin C content of the juices was determined as described by the method of AOAC (2014). About 5 mL of the prepared standard solution (1 mg/mL of ascorbic acid) was measured into a beaker and made up to 50 mL with 4%

oxalic acid. Five (5) mL were pippeted from the above solution into a conical flask, 5 mL of 4% oxalic acid was added and titrated against indophenol dye to a pink colour end point. Each sample (10 g) was weighed into conical flasks and 20 mL of dissolved 4% oxalic acid was added. The solutions were filtered using a filter paper and about 5 mL of the filtrate was added to 5 mL of 4% oxalic acid and titrated against the indophenol dye to a pink end point. The titre values were recorded and the vitamin C content (mg/100g) was calculated as described in Eq. 4.

$$Vitamin\ C\ (mg/100)(C_A \times V_D \times 10000)/(V_A \times V_B \times W) \qquad (4)$$

Where; C_A = concentration of the Standard Vitamin C (mol/L); V_D = titre value of the dye for the sample (L); V_A = volume of the dye for Standard Vitamin C (L); V_B = volume of the sample used (L) and W = weight of the sample (mg).

Microbiological analysis

One (1) mL of each juice sample was serially diluted in 9 mL of sterile peptone water. One (1) mL aliquot of diluted samples was aseptically placed in petri dishes, after which sterile media were poured. The agar used includes; Nutrient agar (Merck) for the total bacteria

count, McConkey Agar (Merck) for coliform counts and Sabouraud medium (Biomark Laboratories, India) for fungal counts. The nutrient and McConkey agar plates were incubated at 37 °C for 24 h while Sabouraud medium plates were incubated for 7 days at 28 °C. The colony enumeration was done using a digital colony counter and values were expressed as colony forming units / one mL of sample.

Sensory evaluation

The consumers' acceptance of the juice was evaluated by 15 consumers (untrained panelists). The sensory panel comprised of staff and students of the Department of Food Science and Technology, Federal University of Technology, Akure, Ondo State, Nigeria. Samples were presented in white plastic cups, and the panelists were asked to evaluate the coded samples for colour, flavour, taste and general acceptability using a nine-point hedonic scale, varying from "dislike extremely" (score 1) to "like extremely" (score 9).

Statistical analysis

Data were obtained in triplicates and the level of significance was analyzed using one-way analysis of variance (ANOVA) on Statistical Package for Social Sciences (SPSS version 17.0). The means were separated using Duncan's New Multiple Range Test at $p < 0.05$.

Results and discussion

Chemical properties of chemically preserved orange juice

The chemical properties of chemically preserved orange juice are presented in Table 1. The pH of the examined orange juices ranged from 3.30 to 3.66. The pH decreased as the number of weeks of storage increased, indicating acidity in orange juices. The use of sodium benzoate, sodium metabisulphite and potassium sorbate as preservatives significantly influenced the pH of orange juice. The pH of samples was stable throughout the first and second week of storage. However, on the third week of storage, the pH of orange juice preserved using sodium metabisulphite and the control sample decreased and was significantly different to other samples ($p \geq 0.05$).

The pH of orange juice preserved with sodium benzoate (SB), potassium sorbate (PS) and combined selected preservatives (AP) were not significantly different from other samples. Thus implying, SB, PS and AP are effective in maintaining the pH of orange juice over three weeks of storage. Fruits are agricultural products that are rich in organic acids (Mgaya-Kilima et al., 2014). However, this acid/acidity reduces with ripening. The slight reduction in pH, observed in sample SM and UNT might be due to the biochemical degradation of sugars by colonizing microorganisms resulting in the production of acids.

Table 1. Chemical properties of chemically preserved orange juice

Samples	Storage weeks	pH	Total titratable acidity (mL)	Total solid (mg/mL)	Total soluble solids (°Brix)	Sugar-acid ratio (°Brix/mL)
UNT	Day 0	3.66±0.23	1.50±0.13	19.44±0.03	9.40±0.03	6.26±0.01
	Week 1					
AP		3.60±0.00[a]	1.56±0.03[a]	22.10±0.14[b]	9.60±0.05[b]	6.15±0.02[c]
SB		3.60±0.01[a]	1.54±0.00[a]	19.57±0.01[d]	9.80±0.00[a]	6.36±0.00[b]
SM		3.60±0.00[a]	1.55±0.05[a]	23.97±0.00[a]	9.80±0.01[a]	6.32±0.05[b]
PS		3.60±0.02[a]	1.50±0.15[a]	21.83±0.04[c]	9.50±0.15[bc]	6.42±0.03[a]
UNT		3.60±0.03[a]	1.54±0.00[a]	19.99±0.01[d]	9.40±0.00[c]	6.10±0.00[cd]
	Week 2					
AP		3.60±0.05[a]	1.70±0.28[c]	21.55±0.00[c]	9.60±0.01[a]	5.65±0.06[a]
SB		3.60±0.00[a]	1.72±0.00[bc]	19.20±0.01[d]	9.60±0.03[a]	5.58±0.00[c]
SM		3.60±0.03[a]	1.75±0.21[b]	24.03±0.00[a]	9.80±0.33[a]	5.60±0.01[b]
PS		3.60±0.02[a]	1.71±0.05[c]	18.32±0.12[e]	9.60±0.10[a]	5.61±0.03[b]
UNT		3.60±0.00[a]	1.77±0.00[a]	22.93±0.01[b]	9.35±0.06[b]	5.28±0.00[d]
	Week 3					
AP		3.55±0.05[a]	2.18±0.20[a]	23.80±0.30[a]	9.60±0.20[b]	4.40±0.02[b]
SB		3.55±0.01[a]	2.19±0.22[a]	21.45±0.01[c]	9.80±0.02[a]	4.48±0.05[a]
SM		3.45±0.02[b]	2.20±0.21[a]	21.29±0.01[c]	9.60±0.01[b]	4.36±0.01[bc]
PS		3.55±0.03[a]	2.16±0.28[b]	22.84±0.01[b]	9.60±0.22[b]	4.44±0.00[a]
UNT		3.30±0.02[c]	2.22±0.05[a]	19.17±0.00[d]	9.20±0.30[c]	4.14±0.03[c]

Values are mean of triplicate determination ± standard deviation. Means with different superscript along the same column are significantly different at 5% confidence level ($p > 0.05$). Keys: AP = orange juice + sodium benzoate + sodium metabisulphite + potassium sorbate; SB = orange juice + sodium benzoate; SM = orange juice + sodium metabisulphite; PS = orange juice + potassium sorbate ; UNT = control orange juice.

The pH of orange juice preserved with sodium benzoate (SB), potassium sorbate (PS) and combined selected preservatives (AP) were not significantly different from other samples. Thus implying, SB, PS and AP are effective in maintaining the pH of orange juice over three weeks of storage. Fruits are agricultural products that are rich in organic acids (Mgaya-Kilima et al., 2014). However, this acid/acidity reduces with ripening. The slight reduction in pH, observed in sample SM and UNT might be due to the biochemical degradation of sugars by colonizing microorganisms resulting in the production of acids. The work of Shahnawaz et al. (2013), Makanjuola et al. (2013) and Ajibola et al. (2009) support this thesis/finding. They reported a decrease in pH with increasing storage periods in tropical fruit juices. Reduced pH is indicative of high acidity which may provide longer shelf life on juices (Nzeagwu and Undugwu, 2009) although the consumers did not like it.

The total titratable acidity (TTA) of orange juice ranged from 1.50 – 2.22 mL. The TTA increased as number of weeks of storage increased and was the highest in the control sample and the lowest in potassium sorbate preserved sample. The increase in the TTA of the juices might be due to fermentation activities by colonizing microorganisms (Nwachukwu and Ezejiaku, 2014). In the first week of storage, the total solids of samples ranged from 18.32 – 24.03 mg/mL. The total solids increased with increasing week of storage. The total solids of samples increased during storage except for the juice treated with sodium benzoate (SM) and control (UNT). The total solids were the highest in SM in week two compared to the control and other samples. There is no significant difference (p≥0.05) between SB and SM in the third week of storage. This might be due to the high solubility index of sodium benzoate and sodium metabisulphite in water. This is in conformity with the report of Kaur and Aggarwal (2014) who evaluated the effect of different chemical preservatives on storage characteristics of bitter gourd.

The total soluble solids of chemically preserved orange juice ranged from 9.20 - 9.80 °Brix. The total soluble solids of samples were constant throughout the three weeks of storage except in SM and UNT. The Total soluble solids of samples SM and UNT reduced with storage, especially in week three of storage. Total soluble solids were the lowest in the control juice (9.20 °Brix) while the total soluble solids were the highest in SB (9.80 °Brix) in the third week of storage. This

finding showed that the combined use of the selected preservatives used in this study, i.e. sodium metabisulphite and potassium sorbate could inhibit the fermentation activities of colonizing microorganisms in fruit juice. As earlier reported by Leahu et al. (2013), the total soluble solids of juice samples remained almost constant in the initial weeks of storage. The stability in the total soluble solids of orange juice within the first two weeks of storage observed in this study confirms the findings of Leahu et al. (2013) that total solids of juice made from orange, kiwi and apple remained almost constant in the initial weeks of storage. Also, this finding supports the work of Rivas et al. (2006) who reported the decrease in total soluble solids of juice stored for seven weeks. However, the slight disparity between the values reported in this study and that of Rivas et al. (2006) might be due to the different method of juice extraction, type of fruit, degree of ripeness and geographical location of orange fruit used in the study.

The sugar-acid ratio of chemically preserved orange juice ranged from 4.14 - 6.12 °Brix/mL. The sugar-acid ratio decreased throughout the three weeks of storage. After three weeks of storage samples SB and PS had the highest sugar acid ratio. Higher sugar content of the samples SB and PS indicates the effectiveness of sodium benzoate and potassium sorbate as a preservative for orange juice preservation. Microorganisms could breakdown substrates such as sugar in the process of fermentation, producing metabolites which include acid and gas (Krishna, 2005). The sugar-acid ratio of juice samples was lowest in the untreated sample signifying the utilization of sugars in fermentation process thereby resulting in increased acidity in the juice. In the last week of storage, samples SB, PS and AP, SM, were not significantly different from each other. They were, however, different from the control except sample SM. Thus, sodium metabisulphite might not present a good preservative property on the stored orange juice as opposed to the findings of Davidson and Taylor (2007). The sulfite groups of chemical preservatives have been reported to have a high antimicrobial activity, especially in acidic food (Davidson and Taylor, 2007).

The ascorbic acid (vitamin C) contents of the samples are presented in Fig. 2. The vitamin C content of the chemically preserved orange juice ranged from 150.90 – 238.60 mg/100 g and decreased with increase in weeks of storage. This is in support of the report of Akinola et al. (2017) on a juice blend of orange, watermelon, carrot and

ginger. However, higher values of vitamin C were reported in this study. This higher value might be due to the source of the fruits used in the study. In the third week of storage, the vitamin C content was the highest in SM (164.90 mg/100 g), but not significantly different to AP (161.40 mg/100 g) at $p \geq 0.05$. The vitamin C content was the lowest in the control sample. The ascorbic acid is sensitive to oxygen, light and heat. The reduction observed in the vitamin C content of samples with storage might be due to the degradation ability of ascorbic acid to dehydroascorbic acid in orange juice when exposed to heat, light or oxygen. However, the vitamin C contents of the samples were within the recommended dietary allowance for vitamin C in juices (40 mg/100 g). This finding is in conformity with the report of Shahnawaz et al. (2013) on the use of sodium benzoate as a chemical preservative to improve the shelf life of orange juice. The analysis of variance indicated that the effect of various preservatives on Vitamin C in orange juice was not statistically significant ($p \geq 0.05$) especially in the second and the third week of storage.

Microbiological quality of chemically preserved orange juice

The total viable count of the chemically preserved orange juice stored at refrigeration temperature is presented in Table 2. The total bacteria count ranged from $2 \times 10^3 - 10 \times 10^3$ CFU/mL and total yeast count ($2 \times 10^3 - 14 \times 10^3$ CFU/mL). There was no growth of indicative organisms in the samples such as coliforms which are capable of causing health hazards when consumed. The coliform growth observed in SM (1×10^3 CFU/mL) in week two of the storage might be due to contamination during the storage. The total bacteria and yeast count increased as the weeks of the storage increased. No growth was observed in the first week of storage indicating good hygiene conditions during the sample preparation. The control juice sample had the highest total bacterial (10×10^3 CFU/mL) and fungal count (14×10^3 CFU/mL) while the orange juice sample treated with SB and AP had the lowest total bacterial and fungal count. The use of selected preservatives influenced the microbial quality of orange juice. Sodium benzoate may be a good preservative against colonizing microorganisms in orange juice. This finding is in agreement with the report of Adeola and Aworh (2013) in the work on the shelf life study on tamarind juice. The increase in population of microorganisms in the samples could be due to the inability of the used preservatives to inhibit the action of the colonizing microorganism in the third week of storage. However, the microbial population obtained in this study were within the acceptable limits (1×10^6 CFU/mL) in foods Kornacki (2017).
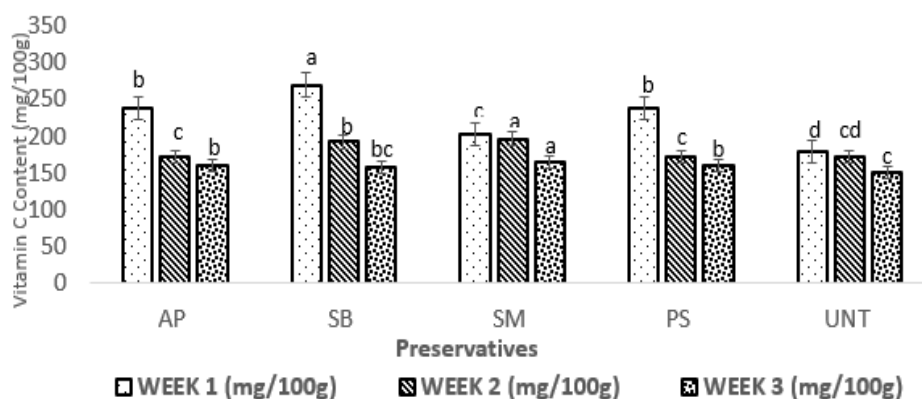
Fig. 2. Vitamin C content of chemically preserved orange juice (mg/100g). Values represent mean ± standard deviation. Means with different superscript are significantly different at 5% confidence level (p > 0.05). Keys: AP = orange juice + sodium benzoate + sodium metabisulphite + potassium sorbate; SB = orange juice + sodium benzoate; SM = orange juice + sodium metabisulphite; PS = orange juice + potassium sorbate ; UNT = control orange juice.

Table 2. Microbiological quality of chemically preserved orange juice (CFU/mL)

Samples	Storage weeks	Total Bacteria Count (CFU/mL)	Total Coliform Count (CFU/mL)	Total Yeast Count (CFU/mL)
UNT0	Day 0	NG	NG	NG
	Week 1			
AP		NG	NG	NG
SB		NG	NG	NG
SM		NG	NG	NG
PS		NG	NG	NG
UNT		NG	NG	NG
	Week 2			
AP		NG	NG	NG
SB		NG	NG	2×10^3
SM		2×10^3	1×10^3	6×10^3
PS		NG	NG	3×10^3
UNT		2×10^3	NG	7×10^3
	Week 3			
AP		3×10^3	NG	3×10^3
SB		5×10^3	NG	5×10^3
SM		7×10^3	NG	8×10^3
PS		7×10^3	NG	6×10^3
UNT		10×10^3	NG	14×10^3

Keys: AP = orange juice + sodium benzoate + sodium metabisulphite + potassium sorbate; SB = orange juice + sodium benzoate; SM = orange juice + sodium metabisulphite; PS = orange juice + potassium sorbate; UNT = control orange juice. TBC = total bacteria count, TCC = total coliform count, TYC = total yeast count, NG = no growth.

Table 3. Sensory evaluation of chemically preserved orange juice

Samples	Colour	Aroma	Taste	Overall Acceptability
AP	7.50 ± 1.08^a	7.00 ± 0.82^a	7.70 ± 1.06^a	7.50 ± 1.08^a
SB	7.30 ± 0.82^a	6.80 ± 1.03^a	7.30 ± 0.82^{ab}	6.70 ± 1.08^b
SM	7.20 ± 0.79^a	6.90 ± 1.20^a	6.80 ± 0.79^b	6.40 ± 1.07^b
PS	7.10 ± 1.20^a	6.60 ± 1.07^b	6.60 ± 1.65^b	6.50 ± 1.51^b
UNT	6.20 ± 0.92^b	6.20 ± 0.92^{bc}	6.70 ± 1.70^b	6.20 ± 1.0^c

Values represents mean ± standard deviation. Means with different superscript along the same column are significantly different at 5% confidence level ($p > 0.05$). Keys: AP = orange juice + sodium benzoate + sodium metabisulphite + potassium sorbate; SB = orange juice + sodium benzoate; SM = orange juice + sodium metabisulphite; PS = orange juice + potassium sorbate; UNT = control orange juice.

The sensory evaluation

The sensory evaluation of the chemically preserved orange juice stored under refrigeration temperatures for three weeks is presented in Table 3. The colour of the samples ranged from like slightly to like very much (6.20 – 7.50). The colour and taste (6.60 – 7.70) was adjudged *moderately like* and samples were not significantly different from each other except from the control. The aroma ranged from *like slightly* to *moderately like* and samples were adjudged not different from the control (6.20 – 7.00). The overall acceptability of the juice ranged from 6.20 – 7.50 and was the highest in the AP sample. The choice of preservative (sodium benzoate, sodium metabisulphite and potassium sorbate) and its combination does not influence the acceptability of orange juice. Hence, the orange juice preserved by using chemical preservatives (sodium benzoate, sodium metabisulphite, potassium sorbate and combination of preservatives) could be an acceptable orange juice to consumers.

Conclusion

The use of sodium benzoate, sodium metabisulphite, potassium sorbate as preservatives influences the chemical and microbiological quality of orange juice. The quality of chemically preserved orange juice reduces with storage. The use of chemical preservatives in orange juice preservation does not influence negatively the consumer acceptability of the product. An acceptable orange juice of both chemical and microbiological quality can be stored effectively for three weeks using 0.03% sodium benzoate, sodium metabisulphite, potassium sorbate or its combinations as preservatives.

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