

The Effect of Moringa Oleifera Leaves Extract on Extending the Shelf Life and Quality of Freshly Sweet Orange Juice

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Abstract

Background: Moringa has been the most spread and extensive species in the world, which makes this tree important in the economic field because of having nutritional and medical properties. Various kinds of fresh fruit juice, especially orange (*Citrus sinensis*) juice has been very desirable for the consumers because of its delicious flavor, however, its shelf life is less than 3 days at $4\pm 1^\circ\text{C}$. **Objective:** to evaluate the sensorial, physicochemical and microbiological properties of the Moringa oleifera leaves extract (M.O.L.E) as a natural preservative of fresh orange juice prolonging its shelf life. **Methods:** This study treated sweet orange juice by different percentages of M.O.L.E, Ginger extract, Hibiscus extract and Beetroot juice, respectively. Sensorial, physicochemical and Microbiological analysis was conducted on the juice during the storage up to one month at $4\pm 1^\circ\text{C}$. **Results:** This study showed that the treatment B (Orange juice 70%+Hibiscus extract 20%+Ginger extract 10%) and D (Orange juice 70%+M.O.L.E.10%+Beetroot juice 10%+Ginger extract 10%) were highly accepted in overall acceptability throughout the storage period up to one month. Proximate analysis ascertained that treatment D had the highest contents of total soluble solids (17.4%), pH value (3.66), ascorbic acid (83.34 mg/100ml), total phenolic contents (71.44 mg GAE/ml) and antioxidant activity (75.63%), respectively during the storage up to one month compared to the control A (Orange juice 70%+water 20%+Ginger extract 10%). Microbiological analysis indicated that adding M.O.L.E by 20% (treatment C) preserved the juice up to one month of storage compared to the control A. **Conclusion:** from all the aforementioned obtained results, M.O.L.E. had antimicrobial effects and also functional ingredient with reasonable safety margins to inhibit the bacterial growth in the pharmaceutical and food applications. Thus, Moringa crop has been applicable and accessible to be utilized in producing many edible and preferable manufactured products.

Keywords: Moringa Oleifera Leaves, Fruit juices, Moringa leaves extract, Bioactive components, Shelf Life.

Introduction

Food quality is an important aspect for assuring public health and safety. Assuring and monitoring of food quality are important steps in food manufacturing, storage and distribution (Rajni Kant,

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et al. 2018). Moringa Oleifera Lam (*Pterygosperma Gaertner*), as a medical tree has numerous economic applications and utilizations in human consumption (Kasolo, J.N. et al., 2010; El- Massry, et al., 2013). Moringa Oleifera leaves (M.O.L) can be utilized in the food industry as a natural preservative, that can be used as an alternative to synthetic preservatives in the future, it can also be utilized to market healthier products without synthetic additives (Bukar et al., 2010; El- Sohaimy et al., 2015; Mangundayao and Yasurin, 2017). M.O.L is a rich source of nutrients and phytochemical components which allows its leaves to be utilized for producing important products (El- Massry et al., 2013). Furthermore, M.O.L is a perfect source of ascorbic acid, vitamin A, vitamin B and minerals (specifically iron and calcium), plus sulphur- amino acids (methionine and cysteine) (Gopalakrishnan et al., 2016). The extract of the leaves has a significant antimicrobial activity, and it is effective in preventing growth of fungi (Farooq et al., 2012). Thus, M.O. extract might be considered as a microbial inhibitor, and it can increase the preservative activity of fortified juices. Through the phenolic, hydrocarbon and alcoholic contents of Moringa extract, these compounds also increase the shelf-life of juices (Ali et al., 2015).

The importance of studying M.O. trees which have been planted locally in Saudi Arabia is critical and more research-on the way of its use in industrial applications in Saudi Arabia is required. Thus, raising the awareness on the effective uses of this underutilized crop and encouraging the consumption of it are needed (Badejo et al., 2014). In recent years, consumers prefer natural food with fresh taste with fewer preservatives. Consumers are trying partial or complete replacement of the synthesized preservatives due to their negative health effects. This matter has led to an increasing interest in improving natural, harmless and eco-friendly alternatives to food preservatives in order to extend the shelf life and safety of foods. Besides, substitute processing technologies has emerged in order to produce foods with little nutritional, physicochemical organoleptic changes in the food industry (El- Kady et al., 2015). Furthermore, it was found that the dried leaf powder has more nutrients than fresh leaves in the following quantities: 9 times more protein than yogurt, 7 times more ascorbic acid than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 15 times more potassium than bananas and 25 times more iron than spinach (Gopalakrishnan et al., 2016; Mishra et al., 2012). (El- Massry et al., 2013) evaluated the

chemical composition of the M.O. plant parts. They found that fresh and dried (at 45°C in air oven) M.O.L. contained high amounts of crude protein (20.94 and 26.79 %), and carbohydrates (37.35 and 35.90 %), and the moisture content was (74.32 and 5.48%), fiber content was (7.68 and 18.67 %), ash content was (3.64 and 7.92 %) and caloric values were (2869.1 and 2955.9 cal/g). It was observed that M.O.L. both fresh and dried have been good sources of protein, ash and fiber when compared to legumes and have had high quality of protein (amino acids). All essential amino acids content was higher in the dried leaves [tryptophan 8.45, leucine 39.41, lysine 26.21, methionine 8.26, valine 25.19, phenylalanine 24.84 (mg/100g)]. Moreover, minerals such as [K 494.12, P 1498.75, Ca 2078.98, Mg 403.56, and Na 72.50 Fe 27.76, Zn 5.43 Cu 0.76 and Mn 32.45 (mg / 100 g)], were higher in dried leaves than in other plant parts. The potential of D.M.O.L. as a functional food showed that the leaves can be a powerful vitamin factory in practical concentrations for individual needs. Since the M.O.L. has been found to immense nutritional values such as [phytochemicals, vitamins, minerals, proteins, vitamins and amino acids], they might be used to combat malnutrition, especially among infants and nursing mothers (El- Sohaimy et al., 2015). F.M.O.L. and D.M.O.L. have been rich in many nutrients required for human health. (Otu et al., 2013) investigated the nutritional composition and assessed the shelf life of composite juice that had a ratio of 50:38:12 for F.M.O.L.: Pineapple: Carrot juice, respectively (the juice heat treatment at 62°C for 30 minutes). The study was conducted under 3 variance storage conditions of temperature (refrigerated 5°C, room 26°C, sunlight above 37°C) for 8 weeks. The shelf life analysis during the storage showed a reduction in the rate of ascorbic acid as 2.5, 4 and 6 (mg/week) in all conditions. But ascorbic acid was saved better in a refrigerator than other conditions. (El- Kady et al., 2015) Studied how to extend the shelf life of fresh Orange juice (O.J.) using the treatment by ozone (4, 7 ppm), and added 5% green tea extract (*Camellia Sinensis*) and 15% carrot (*Daucus Carota*) juice. The evaluation was done before and after 15 days at 4°C. Then, it was packaged in glass bottles, multilayer polyethylene terephthalate (PET) bottle, and three-layer Polyester/Aluminum foil/Polyethylene (PAP). It was observed that the addition of carrot juice decreased the total soluble solid (10.6%) in comparison with 12% of the control (only orange juice). Also, there was a small decrease during the storage period in all juices. This was due to the occurrence of microorganism which caused deterioration of juices as a result of sugar fermentation. (Otu et al., 2013) conducted microbial analysis (total Plate Count, Yeast /Mold and, total Coliform, *Staphylococcus* areas) of F. M.O. L. beverage (50% moringa juice, 38% pineapple juice and 12% carrot juice). The results showed no microbial growth in all beverages for an 8 weeks period of storage. Consequently, the aim of this study was to extend the shelf life and improve the quality of fresh fruit juices fortified with *Moringa oleifera* leaves extract, and produce the untraditional healthy products.

Materials and Methods

Materials:

Fresh *Moringa oleifera* lamarck leaves (*Pterygosperma Gaertner*) were purchased from Durat Al-Ezdihar Farm in jizan, the Kingdom of Saudi Arabia (KSA). The fresh fruits and vegetables varieties namely, sweet orange (*Citrus sinensis*), beetroot (*Beta vulgaris* L.) were purchased from the local market in Jeddah. The hibiscus (*Sabdariffa linn*) and ginger (*Zingiber officinale*) powders were purchased from local herbs market in Jeddah. All samples were purchased during the years of (2016 - 2017). The muslin cloth was purchased from a local shop for fabrics (Al-Dahab street, Al-balad), Jeddah, Saudia Arabia.

Chemicals for physicochemical analysis:

Ascorbic acid, meta phosphoric acid and 2,6 di-chloro phenol indophenol and Folin-Ciocalteu's phenol reagent were purchased from Scientific Supply House, Jeddah. Sodium carbonate was obtained from Al-Shafei establishment. The chemicals used in crude protein analysis were obtained from Halwani factory, jedahh. Standard Gallic acid (GAE) and deionized water were obtained from King Fahd for Medical Research Center. Sodium hydroxide (NaOH) and Methanol were obtained from the laboratory of food and nutrition department. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich company, Jeddah.

Packaging materials:

The glass bottles and air-tight glass jars were purchased from Bin Shihoun Commercial Center, Al-balad, Jeddah.

Methods

Materials preparation

1. *Moringa Oleifera* Leaves (M.O.L): The fresh M.O.L (20 kg) was harvested, separated from stalks, sorted to remove the yellow and brown leaves, which would have altered the color of the final product. The sorted leaves were washed to remove the dusts, blanched at 45°C for 30 sec and left to drain, then dried in air circulated oven at 50°C for 5h to avoid the loss of active compounds. The dried leaves were ground to fine powder using a hand milling machine (Badejo et al., 2014). The powdered M.O.L. extract (M.O.L.E.) was stored in an air-tight glass jars and kept at room temperature (Mishra et al., 2012) until added to the orange juice.

2. Ginger, hibiscus, and *Moringa oleifera* extracts (M.O.L.E.) were prepared by adding 2 g of powdered ginger, hibiscus and M.O.L.E. to three clean bottles containing 100 mL water (at 25°C). Each extract was vigorously shaken for 30 s, allowed to stand for 30 s, and then filtered through a sieve with the sterilized muslin cloth (Badejo et al., 2014).

3. Beetroots were washed, the outer skin was peeled off, cut into big pieces (Vanajakshi et al., 2015), with some modifications made by grinding in juice extractor (SJ-BJ12, SANYO) to get beetroot juice. All above filtrated solutions material were kept

individually in sterilized glass bottles in the refrigerator at $4\pm 1^\circ\text{C}$ until used.

4. Sweet orange fruits were washed, and left to drain, cut into halves (stainless Knives), then squeezed using a juicer (citrus press/JE 280, Kenwood) to get the orange juice (El- Kady et al., 2015), to mix immediately by different percentage of M.O.L.E., ginger, hibiscus and beetroots.

Processing technology:

Moringa Oleifera Leaves Extract (M.O.L.E.) were added to freshly prepared juices immediately by different percentages as follows:

- A. Orange juice 70% + Water 20% + Ginger extract 10% (control sample).
- B. Orange juice 70% + Hibiscus extract 20% + Ginger extract 10%
- C. Orange juice 70% + M.O.L.E. 20% + Ginger extract 10%
- D. Orange juice 70% + M.O. L.E. 10% + Beetroot juice 10% + Ginger extract 10%.

The blends were packed in sterilized glass bottles, the packaged materials were stored in the refrigerator at $4.0 \pm 1^\circ\text{C}$. The samples of different treatments were conducted for different tests: Physico-chemical, microbiological, sensorial analysis at zero time and during the storage period up to one month.

Analytical Methods:

Physico-chemical analysis:

Moisture content, crude protein, pH value and total soluble solids (T.S.S %) were determined according to the method described by (AOAC, 2007). The total titratable acidity and ascorbic acid content were detected by the methods of (Ranganna, 1977). Color hunter (L^* , a^* , b^* values) was determined by using Chroma Meter CR-400, Konica Minolta, SN: 888212539.

Antioxidant activity (DPPH radical scavenging assay) was detected by the methods of (Su and Silva, 2006) using spectrophotometer, Model: PD-303UV, APEL. 0.5 g of sample with 15 ml of acid methanol was extracted by 1% HCL at room temperature for 1 hour. Then, the extract was centrifuged on 2500 RPM for 15 minutes, the up layer was collected in a beaker. The pH of collected extract was adjusted to reach (3). Then, it was added to 50 ml methanol, and 0.05 or 1ml (fresh sample) was mixed with 5 ml of DPPH solution (0.025 g/L). The mixture and blank (1ml methanol mixed with 5 ml DPPH solution) were kept in the dark for 30 min at 23°C , after that the absorbance was read at 715 nm. Then, the juice sample was diluted (1ml of the juice to 10 ml of distilled water). And finally, 1 ml of diluted juice was mixed with 5 ml DPPH solution.

Total phenolic compounds (TPC) were detected by the methods of (Saeed et al., 2012) using spectrophotometer, Model: PD-303UV, APEL. "In brief, 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin- Ciocalteu's phenol reagent. Ten ml of

Na_2CO_3 (7%) solution was added to the mixture after 5 min. Then, 13 ml of deionized water was added and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C , after which the absorbance was read at 750 nm. The TPC was determined from the extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of Gallic acid equivalents (GAE) per g of dried sample", or per ml of the juice sample.

Microbiological analysis:

The total bacterial count was determined using serial dilutions (10^{-3}) on plate count agar (PCA). The duplicate plates were incubated at 30°C for 48 h. The enumeration of total yeasts and molds (YM) count with the same dilutions was also carried out on Rose Bengal Chloramphenicol agar (RBC) at 25°C for 5 days. The results were expressed as " colony-forming units (CFU) /ml ". The analysis was conducted using the methods of (Gulf standards and Saudi standards, metrology and quality organization, 1994, No. 261 and 514; No.409 and 757; No.842 and 1152).

Sensory evaluation:

The sensory evaluation was conducted in a laboratory of food and nutrition department, girls' section, King Abdul Al-Aziz University, Jeddah. 69 students were chosen randomly, the first stage included 37 students, and the second stage included 32 students. They seated in booths with proper illumination, and were asked to judge each juice on five attributes: taste, color, odor, texture and overall acceptability. The form of sensory evaluation test contained five-point hedonic scale, 5-like extremely, 4-like moderately, 3- neither like nor dislike, 2- dislike moderately, 1- dislike extremely (Spangler and Mook, 1978).

Statistical analysis:

The obtained data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (SPSS Inc., Chicago, IL, USA). The collected data were presented as mean \pm standard deviation (SD). The analysis of Variance (ANOVA) test was used for determining the significance among different groups. All differences were considered significant for P -values ≤ 0.05 .

Results

The present investigation was designed to study the effect of the Moringa Oleifera leaves extract on extending the shelf life of fresh fruit juices.

Proximate composition:

The data in **Table (1)** shows that there was a significant difference at ($P \leq 0.05$) of the means values for the different

studied samples. Fresh M.O. leaves (F.M.O.L) contained a high percentage of moisture, antioxidant activity and ascorbic acid by 69.85 %, 68.65%, and 139.46 mg/100g; respectively. Meanwhile, it also contained the total phenolic and crude protein by 13.68 mg GAE/g and 7.77 %; respectively. Dried Moringa Oleifera Leaves (powder) (D.M.O.L) had a high content of ascorbic acid (147.45 mg/100g), crude protein, total phenolic and antioxidant activity by 31.07 %, 29.29 mg GAE/g and 54.23 %; respectively. It could be concluded that D.M.O.L had the highest contents of crude protein, ascorbic acid, total phenolic and antioxidant activity than fresh one. Also, it was established that all fresh and dried M.O.L had a good source of bioactive components and nutrients for human around the world especially for infants and nursing mothers.

Table 1: The chemical analysis of fresh and dried Moringa Oleifera leaves (M.O.L)

Chemical analysis (%)	M.O. Samples	
	F.M.O. L	*D.M.O. L
Moisture	69.85 ± 0.24 ^a	7.91 ± 0.13 ^b
Crude protein	7.77 ± 0.10 ^b	31.07 ± 0.47 ^a
Ascorbic acid mg/100g	139.46 ± 0.64 ^c	147.45 ± 0.64 ^b
Total phenolic mg GAE/g	13.68 ± 0.04 ^c	29.29 ± 0.4 ^b
Antioxidant activity	68.65 ± 0.32 ^b	54.23 ± 0.21 ^c

Means ± standard deviation (N= 3±SD): For each sample within a column, means not sharing the same alphabetical letter are significantly different ($p \leq 0.05$) *calculated on dry weight basis.

Physicochemical properties of different processed fruit juices:

Physicochemical analyses were done at zero time and at the end of storage period (30 days at $4.0 \pm 1^\circ\text{C}$). The evaluation treatments of different juices indicated the following results:

Total Soluble Solids (T.S.S%):

Results in Table (2) and Figure (1) illustrated that the significant percentage of total soluble solids (T.S.S %) in treatment D

(Orange juice 70% + M.O. L.E. 10% + Beetroot juice 10% + Ginger extract 10%) was 17.40%, compared with the control A (Orange juice 70% + water20% + Ginger extract 10%) (15%).

Total titratable acidity (T.T.A%) and pH value:

Total titratable acidity (T.T.A%) of the treatment D recorded low percent (0.038%) compared with the control (0.070%). All treatments showed a significant lower content of T.T.A% than those of control A, especially the treatment (D). There were no significant differences between the treatments B, C and D of pH value of 3.59, 3.60 and 3.66. Whereas, the results also showed that the treatment D recorded a significant difference of pH value content. It was the highest in pH value (lowest content of T.T.A%) compared with the control one (A).

Ascorbic acid content:

Ascorbic acid contents of treatment D was significantly higher than the content by 83.34 mg/100ml juice followed by C (49.17 mg/100ml juice) than those of the control treatment A (32.29 mg/100ml juice).

Total phenolic compounds:

A significant difference in total phenolic contents showed that the treatments D and C were 74.44 and 65.70 mg GAE/ml juice; respectively, compared with the control A (55.23 mg GAE/ml juice).

Antioxidant activity:

A significant difference in the antioxidant activity showed that the percentage of antioxidant activity in treatment D and C was 75.63 and 72.68 %; respectively, compared with the control A (66.83%).

It could be concluded that after the preparation of juices, the treatment D had the highest contents of all the physicochemical parameters.

Table 2: The physicochemical analysis of fresh orange juice mixed with or without M.O.L E at zero time of storage

Contents%	After processing (at zero time)			
	Treatments			
	A	B	C	D
T.S.S	15.00 ± 0.00 ^c	17.20 ± 0.00 ^a	16.00 ± 0.00 ^b	17.40 ± 0.00 ^a
Titratable acidity	0.070 ± 0.00 ^a	0.051 ± 0.00 ^b	0.045 ± 0.00 ^c	0.038 ± 0.00 ^d
pH value	3.53 ± 0.07 ^b	3.59 ± 0.07 ^{ab}	3.60 ± 0.04 ^{ab}	3.66 ± 0.6 ^a
Ascorbic acid (mg/100ml)	32.29 ± 0.00 ^d	39.58 ± 0.00 ^c	49.17 ± 0.00 ^b	83.34 ± 0.00 ^a
Total phenolic (mg GAE/ml)	61.66 ± 1.55 ^b	60.10 ± 1.84 ^{bc}	65.70 ± 1.91 ^{ab}	74.44 ± 1.69 ^a
Antioxidant activity	66.83 ± 0.57 ^{bc}	68.96 ± 0.28 ^b	72.68 ± 0.00 ^{ab}	75.63 ± 0.00 ^a

Means ± standard deviation (N= 3±SD): or each treatment within a column, means not sharing the same alphabetical letter are significantly different ($p \leq 0.05$)

- Orange juice 70% + Water 20% + Ginger extract 10% (control).
- Orange juice70% +Hibiscus extract 20% + Ginger extract 10%.
- Orange juice 70% + M.O.L.E. 20% + Ginger extract 10%.
- Orange juice 70% + M.O. L.E. 10% + Beetroot juice 10% + Ginger extract 10%

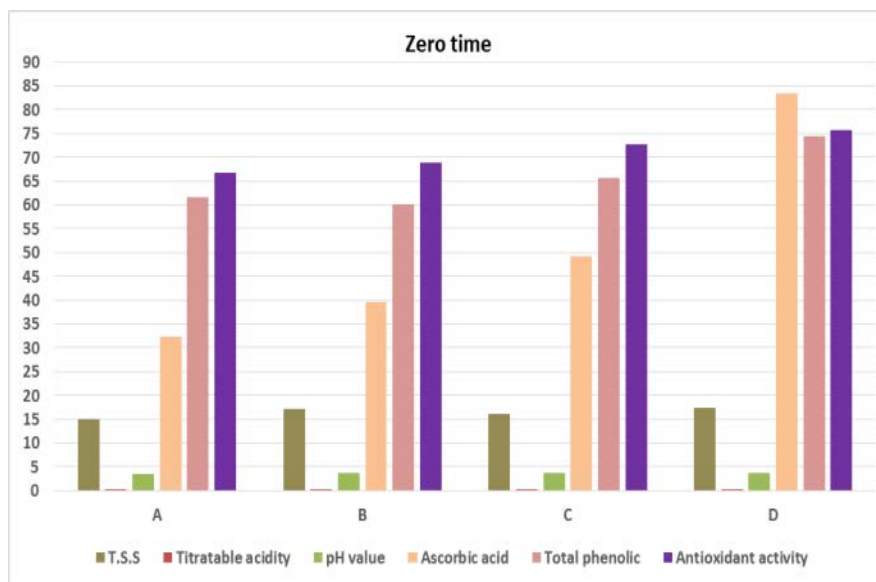


Fig. 1: The physicochemical analysis of fresh orange juice mixed with or without M.O.L.E. at zero time of storage

The statistical analysis data for the physicochemical properties of different orange juices mixed with or without M.O.L.E. during the storage at 4°C±1 up to one month have been presented in **Table (3) and Figure (2, 3, 4, 5)** Total titratable acidity (T.T.A%) of all the treatments was slightly and significantly changed at ($p \leq 0.05$). Meanwhile, the total soluble solids (T.S.S%), pH value, ascorbic acid, phenolic compounds and antioxidants activity of all treatment were significantly decreased at ($p \leq 0.05$) by storage up to one month compared to the treatments at zero time of storage. The data in the same table illustrated that, after one month of storage, the percentage of T.S.S of treatment D (Orange juice 70% + M.O. L.E. 10% + Beetroot juice 10% + Ginger extract 10%) was 17 % compared with the control A (Orange juice 70% + water20% + Ginger extract 10%) (14%). The treatment D had the highest content of the pH value by 3.66% and the lowest content of T.T.A by

0.038%, compared to the control A (3.53% and 0.070%); respectively. Meanwhile, the ascorbic acid contents of the treatment D and C were 81.51 and 40.17 mg/100ml juice; respectively after one month compared with the control A (30.21 mg/100ml juice). The results of the treatment D and C had 61.29 and 59.92 mg GAE/ml juice; respectively during the storage up to one month compared with the control A (55.93 mg GAE /100ml juice). Also, the treatment D and C had 72.35 and 69.8% of antioxidant activity, during the storage up to one month compared with the control A (63.95%).

It could be concluded that the treatment D had the highest contents of all the physicochemical parameters and the good lowest content of total acidity at zero time and up to one month of storage.

Table 3 The physicochemical properties of fresh orange juice mixed with or without M.O.L.E during storage at 4±1°C up to one month

Contents%	Storage period (months)							
	Zero time				After one month			
	A	B	C	D	A	B	C	D
T.S.S	15.00 ± 0.00 ^c	17.20 ± 0.00 ^a	16.00 ± 0.00 ^b	17.40 ± 0.00 ^a	14.0 ± 0.00 ^c	16.0 ± 0.00 ^{ab}	15.5 ± 0.00 ^b	17.0 ± 0.00 ^a
Titratable acidity	0.070 ± 0.00 ^a	0.051 ± 0.00 ^b	0.045 ± 0.00 ^c	0.038 ± 0.00 ^d	0.19 ± 0.00 ^a	0.16 ± 0.00 ^b	0.13 ± 0.00 ^c	0.09 ± 0.00 ^d
pH value	3.53 ± 0.07 ^b	3.59 ± 0.07 ^{ab}	3.60 ± 0.04 ^{ab}	3.66 ± 0.6 ^a	3.11 ± 0.01 ^b	3.17 ± 0.01 ^a	3.17 ± 0.01 ^a	3.18 ± 0.01 ^a
Ascorbic acid mg/100ml	32.29 ± 0.00 ^d	39.58 ± 0.00 ^c	49.17 ± 0.00 ^b	83.34 ± 0.00 ^a	30.21 ± 0.0 ^d	36.46 ± 0.0 ^c	40.17 ± 0.0 ^b	81.51 ± 0.0 ^a
Total phenolic mg GAE/ml	61.66 ± 1.55 ^b	60.10 ± 1.84 ^{bc}	65.70 ± 1.91 ^{ab}	74.44 ± 1.69 ^a	55.93 ± 1.62 ^b	51.39 ± 1.94 ^c	59.92 ± 1.74 ^a	61.29 ± 1.58 ^a
Antioxidants activity	66.83 ± 0.57 ^{bc}	68.96 ± 0.28 ^b	72.68 ± 0.00 ^{ab}	75.63 ± 0.00 ^a	63.95 ± 0.0 ^d	65.10 ± 0.52 ^c	69.8 ± 0.52 ^b	72.35 ± 0.57 ^a

Means ± standard deviation (N= 3±SD): or each treatment within a column, means not sharing the same alphabetical letter are significantly different ($p \leq 0.05$).

- A. Orange juice 70% + Water 20% + Ginger extract 10% (control).
- B. Orange juice70% +Hibiscus extract 20% + Ginger extract 10%.
- C. Orange juice 70% + M.O.L.E. 20% + Ginger extract 10%.
- D. Orange juice 70% + M.O. L.E. 10% + Beetroot juice 10% + Ginger extract 10%

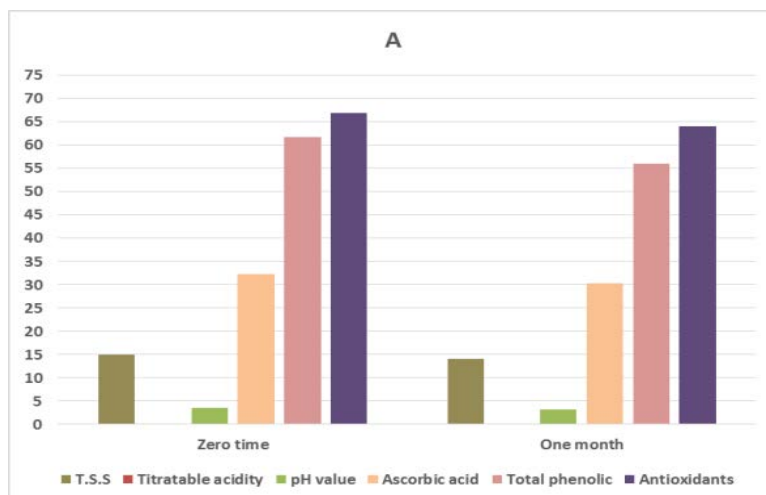


Fig. 2: The physicochemical properties of Control treatment A mixed with or without M.O.L.E during the storage at $4\pm 1^\circ\text{C}$ up to one month

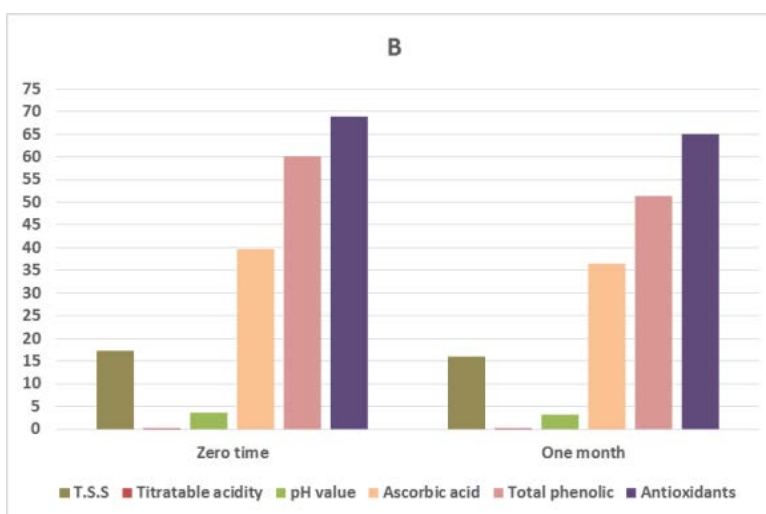


Fig. 3: The physicochemical properties of treatment B mixed with or without M.O.L.E during the storage at $4\pm 1^\circ\text{C}$ up to one month

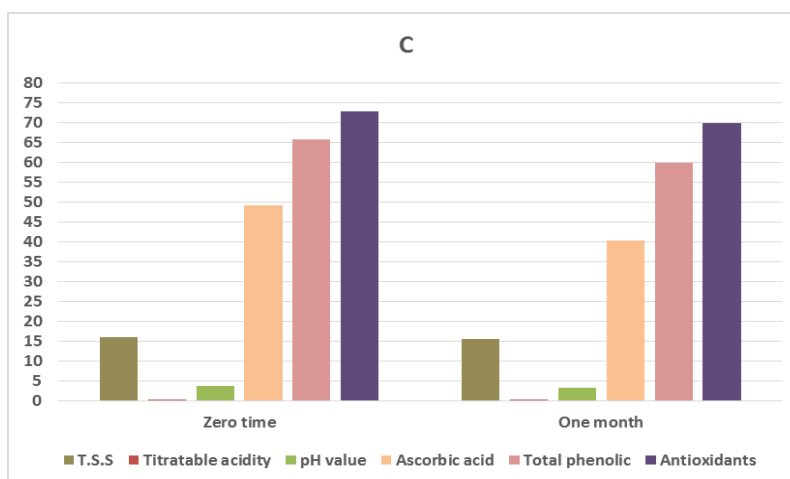


Fig. 4: The physicochemical properties of treatment C mixed with or without M.O.L.E during the storage at $4\pm 1^\circ\text{C}$ up to one month

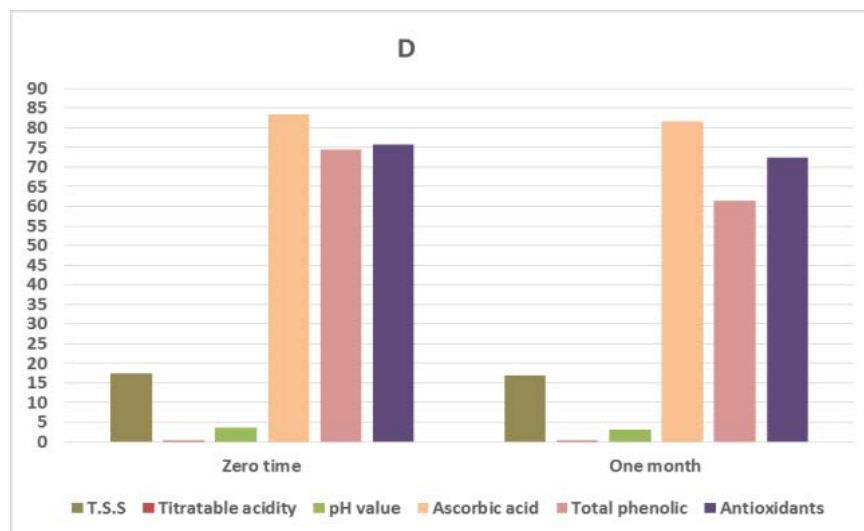


Fig. 5: The physicochemical properties of treatment D mixed with or without M.O.L.E during the storage at 4±1 °C up to one month

Statistical analysis data for the color values of L*, a* and b* of different processed fruit juices mixed with or without M.O.L.E. at zero time have been presented in Table 4. The L* parameter indicated a lightness which ranged from 0 (black) to 100 (white). The L* values for the juices were significantly (p ≤ 0.05) affected by different treatments from A to D. The data showed that there was a significant difference of color (L*) value between the treatments at zero time of storage. The color lightness (L*) value of treatment C (Orange juice 70% + M.O. L.E. 20% + Ginger extract 10%) was higher (32.28) followed by the control A (Orange juice 70% + water 20% + Ginger extract 10%) (31.70) than those of the other treatments.

The a* parameter indicated red color at positive value (+a*) or green at negative value (-a*). The a* values for the juices were significantly (p ≤ 0.05) affected by different treatments from A to D. The data showed that there was a significant difference of color (a*) value between the treatments at zero time of storage. The color redness (+a*) positive value of treatment D (Orange juice 70% + M.O. L.E. 10% + Beetroot juice 10% + Ginger extract 10%) was higher (11.92) than those of the other treatments.

The b* parameter indicated a degree of yellow at a positive value (+b*) or blue at a negative value (-b*). The b* values for the juices were significantly (p ≤ 0.05) affected by different treatments from A to D. The data showed that there was a significant difference of color (b*) value between the treatments at zero time of storage. The color yellowness (+b*) value of the treatment C had a higher content (11.61), followed by the control A (10.37) than those of the other treatments.

It could be concluded that the experiment A, the treatments C, followed by control A were lighter (L* value) than those of the other treatments. Meanwhile, both treatments tended to yellowness +b* value more than greenness -a* value, and the

treatment D was darker (L* value) than the other treatments, and tended to redness +a* value.

The L* values for the juice were significantly (p ≤ 0.05) affected by the storage period up to one-month (Table (5) ; and Figure (6, 7, 8)). There was a significant difference in color lightness (L*) value between the treatments after one month of storage. The color lightness (L*) value of the experiment A, the control A (Orange juice 70% + water + Ginger extract 10%) (30.45) and the treatment C (Orange juice 70% + M.O.L.E. 20% + Ginger extract 10%) (30.03) had a higher content than those of the other treatments. The color lightness (L*) value was slightly decreased in the treatment A and C during the storage up to one month compared to zero time.

Table 4: The color values of L*, a*, and b* of fresh orange juice mixed with or without M.O.L.E

Color values	After processing (at zero time)			
	Treatments			
	A	B	C	D
L*	31.70 ± ^a 0.76	28.35 ± ^b 1.23	32.28 ± ^a 0.01	17.35 ± ^c 0.21
a*	-1.38 ± ^c 0.24	3.46 ± ^b 0.02	-1.22 ± ^c 0.05	11.92 ± ^a 0.24
b*	10.37 ± ^b 0.46	7.39 ± ^c 1.03	11.61 ± ^a 0.05	-0.05 ± ^d 0.07

Means ± standard deviation (N= 3±SD): or each treatment within a column, means not sharing the same alphabetical letter being significantly different at (p ≤ 0.05)

- A. Orange juice 70% + Water 20% + Ginger extract 10% (control).
- B. Orange juice 70% + Hibiscus extract 20% + Ginger extract 10%.
- C. Orange juice 70% + M.O.L.E. 20% + Ginger extract 10%.
- D. Orange juice 70% + M.O. L.E. 10% + Beetroot juice 10% + Ginger extract 10%

The a* values for the juice were significantly (p ≤ 0.05) affected by the storage period up to one month. There was a significant

difference in color redness/greenness (a^*) values between all the treatments up to one month of storage. The color redness ($+a^*$) value of experiment A, treatment D (Orange juice 70% + M.O. L.E. 10% + Beetroot juice 10% + Ginger extract 10%) was higher (10.23) than those of the other treatments, and there was a slight decrease during the storage up to one month compared to zero time.

The b^* values for the juice were significantly ($p \leq 0.05$) affected by the storage period up to one-month. There was a significant difference in yellowness (b^*) values between all the treatments up

to one month of storage. The color yellowness ($+b^*$) value of experiment A, the control A and C were higher (being 10.07 and 9.77; respectively) than those of the other treatments, and a slight decrease was shown during the storage up to one month compared at zero time.

It could be concluded that in the experiment A, the treatments A, C and D (L^* value), ($+b^*$ value) and ($+a^*$ value) were slightly decreased during the storage up to one month compared at zero time.

Table 5. The color values of L^* , a^* , and b^* of fresh orange juice mixed with or without M.O.L.E during the storage at $4 \pm 1^\circ\text{C}$ up to one month

Color values	Storage period (months)							
	Zero time				After one month			
	A	B	C	D	A	B	C	D
L^*	31.70 ± 0.76^a	28.35 ± 1.23^b	32.28 ± 0.01^a	17.35 ± 0.21^c	30.45 ± 0.07^a	25.14 ± 0.13^c	30.03 ± 0.18^a	16.15 ± 0.03^d
a^*	-1.38 ± 0.24^c	3.46 ± 0.02^b	-1.22 ± 0.05^c	11.92 ± 0.24^a	0.20 ± 0.03^c	6.26 ± 0.07^b	-0.35 ± 0.02^d	10.23 ± 0.11^a
b^*	10.37 ± 0.46^b	7.39 ± 1.03^c	11.61 ± 0.05^a	-0.05 ± 0.07^d	10.07 ± 0.02^a	5.83 ± 0.05^c	9.77 ± 0.15^b	-0.54 ± 0.04^d

Means \pm standard deviation ($N = 3 \pm \text{SD}$); or each treatment within a column, means not sharing the same alphabetical letter being significantly different ($p \leq 0.05$).

- A. Orange juice 70% + Water 20% + Ginger extract 10% (control).
- B. Orange juice 70% + Hibiscus extract 20% + Ginger extract 10%.
- C. Orange juice 70% + M.O.L.E. 20% + Ginger extract 10%.
- D. Orange juice 70% + M.O. L.E. 10% + Beetroot juice 10% + Ginger extract 10%

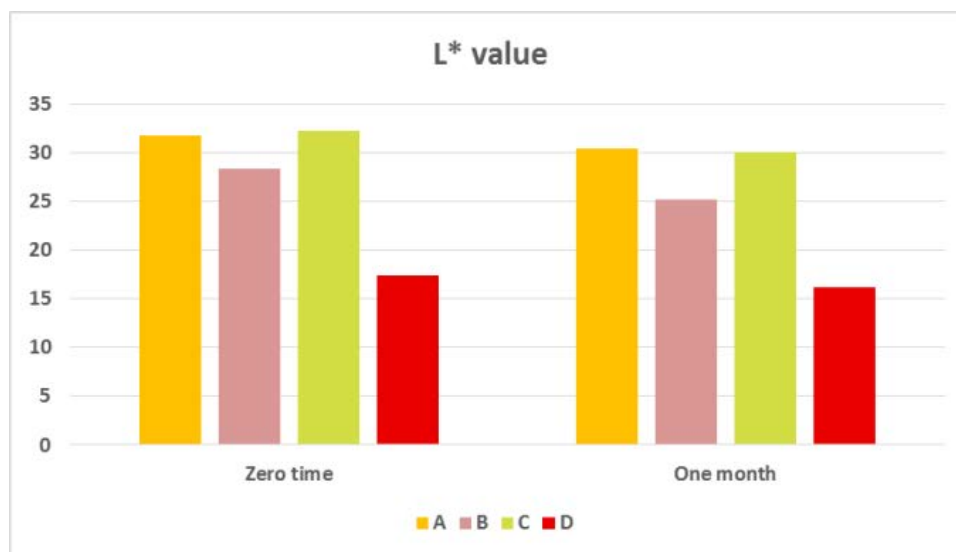


Fig. 6: The color values of L^* value of fresh orange juice mixed with or without M.O.L.E during the storage at $4 \pm 1^\circ\text{C}$ up to one month

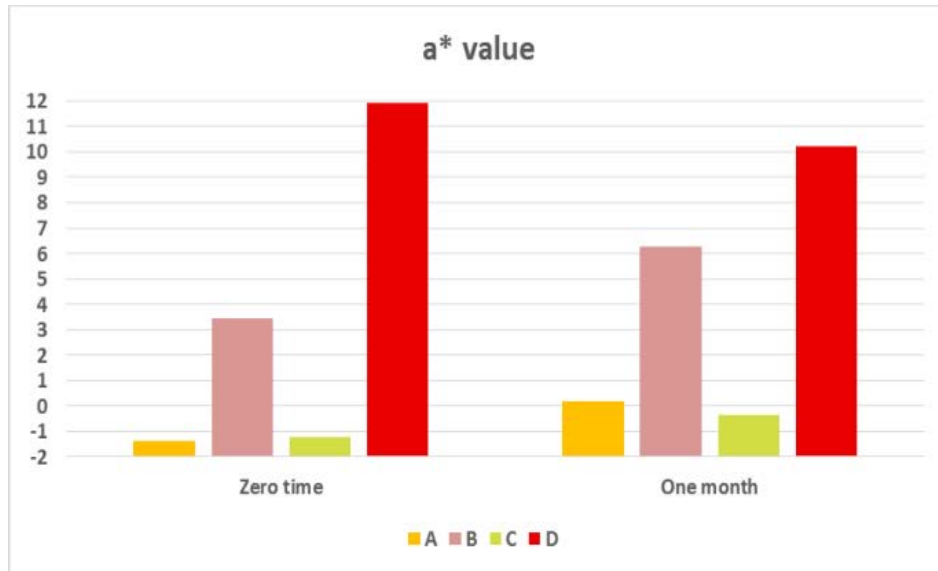


Fig. 7: The color values of a* value of fresh orange juice mixed with or without M.O.L.E during the storage at 4±1°C up to one month

Microbiological quality analysis of juices:

In this study, it was initially focused on the microbiological evaluation (total bacterial count and yeast & mold count). All the evaluation tests were done 3 times (at zero time, after 15 days and after 30 days of storage). The microbiological analysis of orange juices mixed with or without M.O.L.E. during the storage at 4°C±1 up to one month in glass bottles have been shown in **Table (6)**. The results illustrated that the control A (Orange juice 70% + Water 20% + Ginger extract 10%) exceeded the limits of Gulf standards. It was reached to (21x10³) for the total bacterial counts and (32x10³cfu/ml) for yeasts counts during the storage

up to one month compared with the control A at zero time of storage. Data in the same table also showed that in the treatment D (Orange juice 70% + M.O. L.E. 10% + Beetroot juice 10% + Ginger extract 10%), the total bacterial count and yeasts counts also exceeded the limits of the gulf standards (13x10³cfu/ml) compared to the other treatments namely, B (Orange juice70% +Hibiscus extract 20% + Ginger extract 10%) and C (Orange juice70% +Hibiscus extract 20% + Ginger extract 10%).

Based on the microbiological analysis of juices, it could be concluded that all juices in the experiment (A) were not analyzed for the sensory quality attributes after one month of storage.

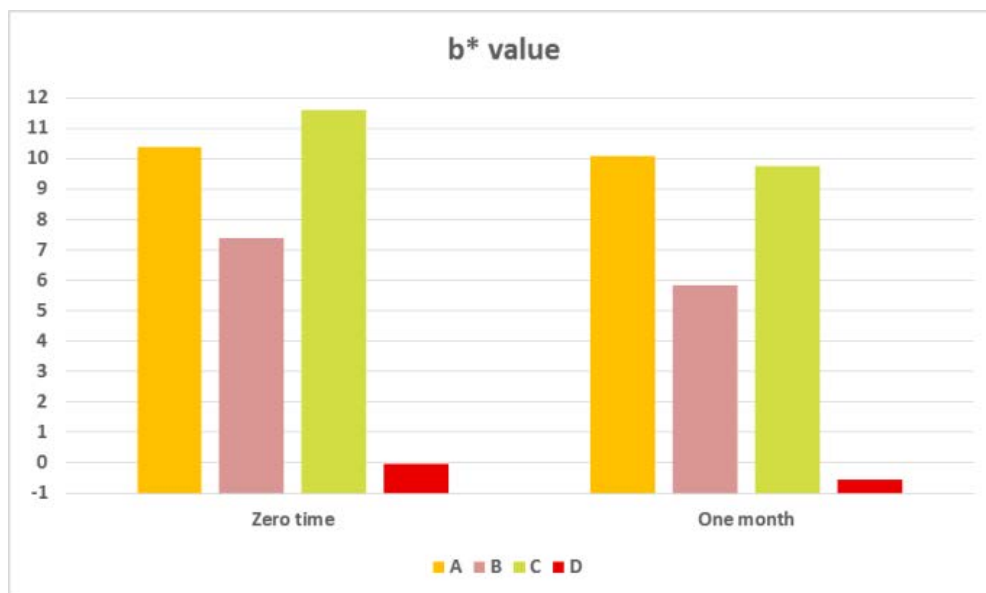


Fig. 8: The color values of b* value of fresh orange juice mixed with or without M.O.L.E during the storage at 4±1°C up to one month

Table 6: The microbiological analysis of fresh orange juice mixed with or without M.O.L extract during the storage at 4±1 °C up to one month

Microorganism	Storage period (months)							
	Zero time				After one month			
	A	B	C	D	A	B	C	D
Total Bacterial	5x10 ³	4x10 ³	1x10 ³	ND	21x10 ³	7x10 ³	4x10 ³	13x10 ³
Molds	ND	ND	ND	ND	ND	ND	ND	ND
Yeasts	ND	ND	ND	ND	32x10 ³	7x10 ³	2x10 ³	13x10 ³

- A. Orange juice 70% + Water 20% + Ginger extract 10% (control).
 B. Orange juice 70% + Hibiscus extract 20% + Ginger extract 10%.
 C. Orange juice 70% + M.O.L.E. 20% + Ginger extract 10%.
 D. Orange juice 70% + M.O. L.E. 10% + Beetroot juice 10% + Ginger extract 10%

Sensory evaluation analysis of juices:

Organoleptic evaluation could be considered as one of the most important aspects in juice blend technique since it reflects the consumer/preference. The statistical analysis data for the sensory evaluation of different orange juices mixed with or without M.O.L.E. have been presented in **Table (7)**. The data showed that there was a significant difference ($p \leq 0.05$) in taste, odor and overall acceptability between the control A, the treatments B, C and D. The observed taste, odor, color, texture and overall acceptability of the orange juice (70%) that have been mixed with water (20%) and ginger extract (10%) (control A) had the highest scores followed by the treatment B (Orange juice 70% mixed with hibiscus 20% and ginger extract 10%) compared with the other treatments. On the other hand, the sensory results (taste, odor, color and texture) of the treatment C (Orange juice 70% mixed with M.O.L.E 20% and ginger extract 10%) and D (Orange juice 70% mixed with M.O.L.E 10%, beetroot juice 10% and ginger extract 10%) had non-significant differences between all the parameters at zero time of storage in glass bottles.

Orange juices mixed with or without M.O.L.E. was sensory evaluated after the storage at 4°C±1 up to one month. The data in the same **Table (7)** showed that there were non-significant differences observed for all the treatments in taste, odor, color and texture after the storage up to one month. On the other hand, it could be noticed that there were significant differences in the overall acceptability between the treatment B and other treatments. The overall acceptability scores of control A and treatment B significantly decreased during the storage up to one month. Meanwhile, the overall acceptability scores of treatment C and D significantly increased after one month of storage in glass bottles. Generally, those results showed that all the treatments had high acceptability especially the treatments B and D.

Thus, all the results from the sensory evaluation concluded that, it was acceptable to obtain orange juice mixed with M.O.L.E. and ginger, hibiscus extract and beetroot especially by the treatment B and D throughout the storage period after one month.

Table 7: The sensory evaluation of fresh orange juice mixed with or without M.O. L.E during the storage at 4±1 °C up to one month

Treatments code	Storage period (months)									
	Zero time					After one month				
	Taste	Odor	Color	Texture	Overall acceptability	Taste	Odor	Color	Texture	Overall acceptability
A	4.68 ± 0.53 ^a	4.50 ± 0.84 ^a	4.68 ± 0.67 ^a	4.43 ± 0.84 ^a	4.65 ± 0.54 ^a	3.88 ± 1.16 ^b	3.81 ± 1.03 ^b	4.53 ^a ± 0.88	4.31 ^a ± 0.86	3.88 ^b ± 1.16
B	4.27 ± 0.84 ^{ab}	4.19 ± 0.97 ^b	4.43 ± 0.80 ^a	4.30 ± 0.85 ^a	4.35 ± 0.75 ^a	3.97 ± 0.97 ^b	3.81 ± 1.06 ^b	4.34 ^a ± 1.83	4.41 ^a ± 0.92	4.22 ± 0.79 ^{ab}
C	3.54 ± 1.30 ^b	3.62 ± 1.36 ^b	4.41 ± 0.73 ^a	4.49 ± 0.61 ^a	3.81 ± 1.08 ^b	3.53 ± 1.11 ^b	3.53 ± 1.05 ^b	4.31 ^a ± 0.69	4.50 ^a ± 0.80	3.88 ^b ± 1.87
D	3.65 ± 1.25 ^b	3.54 ± 1.33 ^b	4.95 ± 0.23 ^a	4.62 ± 0.49 ^a	3.86 ± 1.11 ^b	3.59 ± 1.29 ^b	3.47 ± 1.05 ^{bc}	4.44 ^a ± 0.88	4.34 ^a ± 0.94	4.09 ^b ± 1.06

Means ± standard deviation: or each treatment within a column, means not sharing the same alphabetical letter are significantly different ($p \leq 0.05$)

- A. Orange juice 70% + Water 20% + Ginger extract 10% (control).
 B. Orange juice 70% + Hibiscus extract 20% + Ginger extract 10%.
 C. Orange juice 70% + M.O.L.E. 20% + Ginger extract 10%.
 D. Orange juice 70% + M.O. L.E. 10% + Beetroot juice 10% + Ginger extract 10%

Discussion:

In this study, the application of Moringa Oleifera Leaves Extract (M.O.A.E) was used for extending the shelf life of fresh fruit juices as a natural preservative and adding value to the functional drinks (untraditional healthy product). Recently, consumers prefer natural food with fresh taste with fewer preservatives. This matter has led to an increasing interest in improving natural, harmless and eco-friendly alternatives to food preservatives in order to extend the shelf life and safety of food (El- Kady et al., 2015; Salem et al., 2015). Processing fresh fruit juices fortified with plant extract such as moringa oleifera has been important to produce high quality fruit juice that meets consumers' interest. This is because of that moringa leaves are a rich source of nutrients and phytochemical components which allow these leaves to be utilized for producing important products such as Moringa tea and juice and dairy products (El-Massry et al., 2013).

Moisture content in F.M.O.L was decreased by 88.68% after the dehydration of (D.M.O.L). Crude protein in D.M.O.L showed higher content than the fresh one. This result was approximate to the finding by (Emelike and Ebere, 2016). The result of protein value in D.M.O.L was the highest when compared to the dry weight of quinoa seed (13.72%) and wheat (13.90%) (Villa et al., 2014).

The differences in ascorbic acid content of F.M.O.L varieties induced the damage occurred during the enzymatic processes which continued for long periods of collection and marketing of leaves (Adeyemi et al., 2014). The recent investigation by (Amaglo et al., 2010) revealed that M.O. extract contains a high antioxidant activity due to the high concentrations of polyphenolics. Therefore M. oleifera tissues could be an important dietary source of antioxidants and polyphenols.

Highly significant increase in all the sensory scores (taste, odor, color, texture and overall acceptability) of control A (orange juice 70% and ginger extract 10% with 20% water) was followed by the treatment B (with hibiscus extract 20%) at zero time compared to the treatment C (with 20% M.O.L.E) and D (with 10% M.O.L.E +10% beetroot juice). This increase might be due to the high amounts of orange juice, which had a sweet taste. Plus, mixed orange juice with hibiscus gave a special flavor and hibiscus flavor was common between panelists. Meanwhile, the scores of taste, odor and overall acceptability was a little decreased when adding M.O.L.E. (treatment C and D), but it was still acceptable between the panelists at zero time of storage. Meanwhile, all treatments had high acceptability especially treatment B and D throughout the storage period after one month. This was due to the strong herbal flavor of M.O.L.E. (Out et al., 2013). Plus, most of the panelists didn't know how the M.O.L taste was.

Physicochemical analysis of juices at zero time of storage showed that the total soluble solids (T.S.S%) were significantly increased by the percentage of 16% in the treatment D (Orange juice 70% +

M.O. L.E. 10% + Beetroot juice 10% + Ginger extract 10%) compared with the control A (Orange juice 70% + water20% + Ginger extract 10%) at zero time of storage. This increase might be due to adding M.O.L.E. plus beetroot juice and ginger extract. (Ameh et al., 2015) reported that the increase in T.S.S% of juice was due to the high concentration of solids, typically sugars, vitamins, minerals etc, which were dissolved in the juice. Also, during the processing treatment of juices, the increase of T.S.S% might be due to converting insoluble polysaccharides to soluble polysaccharides and insoluble pectin to soluble pectin.

Total titratable acidity (T.T. A%) was a measure of the acid-taste of the juices (Badejo et al., 2014). The experiment A and D had a significantly low percentage of (T.T.A) (0.038%) compared with the control A (0.070%) (orange juice with ginger only). This was due to adding M.O.L.E. to beetroot juice, orange juice and ginger extract. The results ascertained that the treatments D (experiment A) had the highest content of pH value by the percentage of 3.7and 4.70 ,5.38%, and the lowest content of T.T.A% by 45.7and 9.1,9.1%; respectively compared with the control (A). This might be due to the fact that fortified fruit juices with plant extract (M.O.L.E.) had the ability to decrease fruit titratable acidity based on its acid-binding properties, this result was in agreement with those found by (Adeogun et al., 2017). Also, the decrease in T.T.A% might be due to the interaction between the organic acids and sugars, which produced forming monoesters.

Also, the results proved that the treatment D followed by C showed a high contents of ascorbic acid, total phenolic compounds and antioxidant activity by the percentage of 158, 20.7 and 13.17; respectively for the treatment D, and by the percentage of (52, 6.6 and 8.75) for the treatment C compared with the control A. This might be due to the addition of M.O.L.E. by 10% and 20%; respectively plus beetroot juice by 10%. Several authors reported that beetroot juice had high ascorbic acid contents of 362 mg/100g, the total antioxidant capacity 110 mg TEAC /100g, and the total phenolic contents of 2.25 mg GAE/g (Guldiken et al., 2016; Vasconcellos et al., 2016). The highest in radical scavenging activity of DPPH (antioxidant activity) might be due to a synergistic effect of the antioxidant capacity of orange ginger juice and M.O.L.E. with or without beetroot juice. These results were in agreement with those found by (Badejo et al., 2014).

It could be concluded that orange juices blended with M.O.L.E. (treatment D) plus ginger extract and beetroot juice were a good source of antioxidant activity against free radicals, which can be consumed by human as a healthy functional drink.

Physicochemical analysis of juices during the storage up to one month also showed that T.T.A % of all the treatments were slightly but significantly increased. Meanwhile, T.S.S%, pH value, ascorbic acid, phenolic compounds and antioxidants activity of all the treatments were significantly decreased by the storage up to one month compared to the treatments at zero time of storage. The T.S.S of the treatment D had a higher content by 17% than the control A (14%). This increase in T.S.S. % might

be due to the addition of M.O.L.E. plus beetroot juice to orange juice compared with the control one. These results were in agreement with those found by (Ameh et al., 2015). The total soluble solids (T.S.S%) in treatment D were significantly and slightly decreased by the percentage of 2.3 % after one month, compared to the control A (6.7%). These results were in agreement with those found by (El- Kady et al., 2015).

Treatment D had a higher content of the pH value by 3.66%, and the lowest content of T.T.A by 0.038% than the control A (3.53% and 0.070%); respectively. This might be due to the addition of M.O.L.E. plus beetroot juice to orange juice compared with the control one. The total titratable acidity (T.T.A%) in treatment D was increased by the percentage of 136.8 %, by decreasing the pH value with the percentage of 13.1 % after one month of storage, compared to the control A by 171.4 and 11.9%; respectively. Decreasing pH value during the storage was in agreement with (Ramachandran et al., 2017) that found that pH value of sugarcane juice fortified with moringa seed extract was decreased during the storage, this was due to the fermentation of sugars by the bacteria.

Meanwhile, the ascorbic acid contents of treatment D had a higher content by 81.51 mg/100ml juice than the control A (30.21 mg/100ml juice). This might be due to the addition of M.O.L.E. plus beetroot juice. Whereas, the ascorbic acid contents of treatment D and control A were slightly decreased by the percentage of (2.2 and 6.4%, respectively) after one month of storage, compared to zero time of storage. The loss in ascorbic acid content was increased with increasing the storage period. This decrease in ascorbic acid during the storage was due to the oxidation of ascorbic acid. This result was in agreement with those found by (El- kady et al., 2015; Out et al., 2013).

The total phenolic contents of treatment D had a higher content of 61.29 mg GAE/ml juice than the control A (63.95 mg GAE/ml juice). This might be due to the addition of M.O.L.E. plus beetroot juice. Total phenolic contents of treatment D were decreased during the storage up to one month by the percentage of (17.7%), compared with the control A (9.3%). (Castro-López et al., 2016) mentioned that the decrease of polyphenolic compounds might be associated to the prevention of microbial growth. Some others revealed that the difference in total phenolic contents during the storage might be due to several factors such as, type of extraction conditions used and the same as the yield and bioactivities of the samples (Castro-López et al., 2016). Several authors reported the increment of polyphenolic compounds associated to the microbial growth (Castro-López et al., 2016; Kallithraka et al., 2009; Martínez-Flores et al., 2014).

Besides, the treatment D had a higher percentage by 72.35 % of antioxidant activity than the control A (63.95 %). This increase might be due to the addition of M.O.L.E. plus beetroot juice. On the other hand, the antioxidant activity of the same treatment was slightly decreased by the percentage of (4.3%) after one month of storage, compared to zero time of the storage. This decrease might be related to the decrease in phenolic compounds and ascorbic acid contents which were observed in all the treatments

during the storage up one month (Klimczak et al., 2007; Owczarek et al., 2004).

The results of the color analysis of L*, a* and b* values in the experiment A of juices at zero time of the storage indicated that the treatments C and the control A were significantly lighter (L* value) than those of the other treatments. This might be due to the adding of M.O.L.E by 20% to orange juice that did not affect its lightness and greenness (treatment C). Meanwhile, treatment D was darker than the other treatments and tended to redness +a* value, this might be due to the adding beetroot juice by 10%. The data also indicated that the treatments A, C and D (L* value) , (+b*value) and (+a* value) were slightly decreased during the storage up to one month compared with zero time. (Naderi et al., 2015) reported the decreased L* and b* values of Cherry juice as a result of the breakdown of carotenoids and chlorophyll as well as the formation of brown pigments. Also, oxidative and non-oxidative reactions of polyphenols and browning reactions in food included: Maillard reaction, ascorbic acid degradation and caramelization (Koca et al., 2003). These changes in color properties (L*, a* and b* value) of juices might be due to the precipitation caused by the instability of the juice during the storage (Adiamo et al., 2017).

The results of the microbiological analysis indicated that the shelf life of all juices ended after one months of the storage. Meanwhile, adding M.O.L.E. by 20% to orange juice mixed with ginger (treatment C) preserved the juice up to one month of storage compared with the control A (orange with ginger only). This might be due to M.O.L.E. which had antimicrobial activity, as mentioned by (Gopalakrishnan et al., 2016; Farooq et al., 2012).

Conclusion

- Fresh and dried M.O.L had a good source of bioactive components.
- M.O.L.E preserved the orange juices mixed with ginger extract (treatment C) up to one month compared to the control A.
- The treatments D has the highest contents of all physicochemical parameters.

Recommendations

- More researches on fortified fresh fruit juices with Moringa Oleifera extract or Moringa Oleifera leaves Aqueous Extract are needed in food industry.
- The agricultural and food industrial sectors should be encouraged to invest on Moringa Oleifera in Saudi Arabia. And, the consumption of the underutilized crops such as Moringa Oleifera should be enhanced in food industry to achieve food safety.
- Because moringa oleifera leaves have worthy nutritional value, they are recommended to be added in juices offered especially to children.

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