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UTILIZATION OF MORINGA, MINT AND THYME EXTRACTS AS NATURAL PRESERVATIVES TO AMELIORATE SHELF STABILITY OF CARROT JUICE

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Abstract

Carrot juice is a popular and an economical beverage consumed worldwide and commonly accepted as a vital source of its valuable nutrients. The juice is normally spoiled within few hours of extraction and difficult to preserve for a long time thus cannot be utilized properly. The present research was conducted to explore the potential of moringa, mint and thyme leaves ethanolic extracts as natural preservatives at different level (0, 1, 2 and 3%) for preservation of carrot juice during 20 days of storage period at 4 ± 2°C temperature. The effect of extracts incorporation and storage time was studied on physico-chemical parameters (TSS, PH, Titratable acidity %), antioxidant potential (DPPH, FRAP), bioactive compounds (TPC, TFC), enzymatic activity, microbial load and overall acceptability. The 2% thyme extract (T_8) significantly ($p<0.05$) maintain the pH (6.00±0.04-5.80±0.01) during 20 days of storage as compared to control (6.20±0.02-5.60±0.01). The total phenolic contents was also observed to be retained in 2% thyme extract (32.26±1.16-31.70±1.14 mg GAE/100 ml) as compared to control (27.75±1.11- 25.80±1.29 mg GAE/100 ml) at the end of storage period. Regarding polyphenol oxidase (PPO), per oxidase (POD) and pectin methyl esterase (PME), the highest value was recorded in control pasteurized juice (32.95±0.07%, 26.30±0.08, 14.27±0.03% respectively). However, the corresponding enzyme activity (PPO, POD and PME) decreased as the concentration of extracts increased and thyme incorporation at 2% level showed minimum changes in PPO (28.94±0.05 to 29.40±0.06%), POD (22.80±0.09 to 23.30±0.07%)and PME (10.66±0.02 to 11.18±0.02%) during storage. Other physicochemical and phytochemical analysis including total soluble solids, titratable acidity%, flavonoid content, 2, 2-Diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) showed that carrot juice incorporated with thyme extract was significantly ($p<0.05$) stable during storage. However, the inclusion of moringa and mint extracts were also effective in preserving the juice as compared to control. The microbial safety and shelf stability of juices was attained by (T_8) 2% and (T_9) 3% thyme extracts as compared to control. Sensory scores of juice samples decreased with the progression of storage time. Regarding the overall acceptability of treatments T_8 (7.53±0.70) containing 2% thyme extract

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attained the maximum score throughout the storage period. The addition of natural ingredients into carrot juice such as thyme extract may add other health promoting elements in addition to make product more stable with acceptable quality.

Index Terms: Carrot, Juice. Moringa, Mint, Thyme, Natural Preservatives, Shelf Stability.

1. INTRODUCTION

Consumer's preference has ominously increased for using health promoting food products. This practice has extensively highlighted the innovative research for the development of healthier foods and juices for maintaining the healthy lifestyle of community. In the context of global market, people have sought more alternative to juices for more shift in their eating habits. However, this interest can be increased if fruits and vegetables with higher health benefits are processed into juices as expected by consumers [1]. Drinking juice is an effective method to combat lower consumption of fruits and vegetables in a quick and convenient manner. Moreover, Fruits and vegetable juices are gaining more popularity as an alternative to the caffeinated, non-alcoholic beverages such as coffee, black and green tea as well as carbonated soft drinks. Hence, biological active compounds (BACs) in these commodities are easier to digest and assimilate when consumed as juices rather than whole plant tissues [2].

Carrot (*Daucus carota* L.), a member of Apiaceae family is one of the important biennial root vegetable. It exhibits enormous health benefits and produced all around the world. Globally, annual carrot and turnip production was approximately 41 million tonnes, half of which produced by china [3]. Commonly, carrots are eaten as fresh vegetable but spoiled rapidly due to its perishable nature. It is necessary to process it into a variety of products like juices, canned carrots, candies, drinks and intermediate moisture products. Carrot juice has gained vast popularity being a rich source of dietary fibre and phytonutrients such as carotenoids, minerals and vitamins [4].

However, juices are highly susceptible to spoilage by microorganisms that thrive in juices during storage [5]. The endogenous enzymes including polyphenol oxidase (PPO), peroxidase (POD), and pectin methyl esterase (PME) are also considered conducive for juice deterioration. The inactivation or inhibition of the growth of microorganisms and enzymes is precarious for extending the shelf-life of carrot juice [6, 7] Chemical preservatives such as sodium benzoate and potassium sorbate are extensively employed for enhancing storage life of juices. Most of these chemical preservatives are costly and cause harmful effects on health in the form of allergy, headache, asthma and digestive and respiratory problems [8]. Moreover, consumers have shifted their trend towards natural, nontoxic, and environment friendly food preservatives having less cost, highly nutritive and that are available easily [9, 10].

The natural preservatives are thus being preferred by consumers and valued in food manufacturing [11]. Plants possess phenolic compounds that are of considerable importance due to their bioactive functions and have received growing attention in recent years. Plant based active compounds like flavonoids and polyphenols in their respective essential oils and extracts has been used as natural preservatives with strong capability

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to retard oxidation and microbial proliferation in both raw and processed foods [12]. Selected plant extracts like (moringa, mint and thyme) can be used as natural preservatives to extend shelf life of juices.

Moringa (*Moringa oleifera)*, member of Moringaceae family is well famous as drumstick tree. The leaves of this plant are rich in phytochemical constituents that make them suitable to be used in food products as natural preservative and as well as alternative to chemical preservative. Antioxidant and antimicrobial triggering properties of moringa leaves are associated with important bioactive moieties like glucosinolates, zeatin, quercetin, caffeic acid, and kaempferol [13].

Mint *(Mentha piperita* L.) belongs to Lamiaceae family and another well-known plant based preservative. Leaves of this plant contain high phytonutrients mainly polyphenol content, vitamin C, phosphorus, potassium, fiber, iron, chlorophyll and calcium [14] and are mostly used in extract form that linked with prominent amount of phenolics and flavonoids. These phenolic compounds are responsible for antimicrobial and antiviral activities of [15]. *Thymus* genus, a part of the Lamiaceae family is one of the most important and taxonomically complex genera containing 250–350 taxa [16].

The members of this family exhibit antimicrobial and antioxidant activities that might enhance the shelf life of food [17]. With this objective, the research was performed to study the effect of different levels of moringa, mint and thyme leaves extracts on physicochemical parameters, bioactive compounds, antioxidant potential, residual enzyme activity, microbial analysis and sensory attributes of carrot juice and also evaluate the shelf stability of incorporated juices during storage.

2. MATERIALS AND METHODS

Raw material: Fresh carrots (T-29 variety), moringa and mint leaves were obtained from Ayub Agricultural Research Institute, Faisalabad Pakistan while thyme leaves were procured from the National Agriculture Research Centre (NARC) Islamabad, Pakistan. The chemicals required (analytical and HPLC grade) including standards were obtained from local suppliers of Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan).

2.1 Preparation of extracts: Leaves were destalked and washed with tap water to eliminate dirt and other extraneous matter. Clean leaves were dipped in potassium metabisulphite (KMS) (0.1%) for ten minutes. After treatment, leaves were removed from KMS solution and dehydrated in cabinet dryer at 50±5°C. Dried leaves were ground to obtain fine powders. 1.5 g of each powder material was extracted with 20 ml of aqueous ethanol 80% (v/v) for 4 hours at 38℃ in thermo shaker at 280 rpm. The method with slight modification was adopted as described by [18]. Centrifugation of samples was done at 3500 rpm for 20 minutes and filter paper (Whatman No.1) was used to obtain clear filtered extract. The solvent was removed by rotary evaporator (VP30, LabTech, UK) at a temperature of 40°C near to dryness of material. The extracts were kept into glass bottles for further study.

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2.2 Preparation of carrot juice: Carrot juice samples were prepared following the procedure of [19] with slight modifications. Fresh carrots were washed thoroughly with water to get rid of dirt and other undesired stuffs, peeled and cut with stainless steel knife followed by juice extraction from locally available juice extractor. The juice was sieved with eight layers of muslin cloth to make it clear from coarse particles. Clear carrot juice was homogenized in homogenizer to reduce the particle size. Carboxy methyl cellulose at 0.1% was added in juice samples. Pasteurization of carrot juice was done at 85℃ for 5 minutes to enhance the shelf life by inactivating microbes. Moringa, mint and thyme leaves ethanolic extracts were incorporated @ 0, 1%, 2% and 3% in pasteurized carrot juice. Carrot juice was hot filled in polyethylene terephthalate (PET) bottles and lids were sealed properly. Finally, juice was kept at 4±2℃ in refrigerator for 20 days for further quality evaluation.

2.3 Physicochemical analysis of carrot juice: Physico-chemical analysis including total soluble solids (TSS), pH and titratable acidity% was conducted by using the methods followed by [20] during 20 days storage at an interval of four days.

2.4. Bioactive compounds

2.4.1. Total phenolic contents (TPC): Total phenolic contents (TPC) of juice samples was assayed following Folin-Ciocalteau method as mentioned by [21] after diluting the extract supplemented carrot juice samples with distilled water (1:20 v/v).The absorbance was noted at 760 nm against blank using spectrophotometer.

2.4.2. Total flavonoids contents (TFC): Total flavonoid content (TFC) of Juice samples was analyzed by following procedure as reported by [22]. Quercetin at different concentrations was used to construct curve and (80% ethanol (v/v) was used for preparing standard solutions. 415 nm was set to note the reading of the reaction mixture and expressed as Quercetin Equivalent (mg QE/100ml).

2.5. Antioxidant analysis

2.5.1. Free radical scavenging activity (DPPH): The DPPH assay of juice samples was determined according to the procedure of [23]. For this purpose, DPPH (0.1mM) in one 1 mL of methanolic solution was incorporated to each juice sample (4 ml) and kept for 30 min. The mixture was then incubated at room temperature. The spectrophotometer (Cecil CE7200) was used to note absorbance at 520 nm. The findings are expressed in percent inhibition

2.5.2. Ferric reducing antioxidant power (FRAP): Juice samples were analysed for FRAP by adopting the protocol of [24] with minor changes. Briefly, ferrous tripyridyltriazine, a color product was formed and absorbance was noted using spectrophotometer at 593 nm. Standard reading was noted by using ferrous sulfate ($FeSO_4$) at different concentrations (25-1000 µM) and FRAP values were expressed on basis of dry matter as (µmol Fe^{2+}/100ml).

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2.6. Enzymatic activity

2.6.1. Polyphenol oxidase (PPO) activity: PPO activity in control and treated carrot juices was determined according to protocol given by [25]. Centrifugation of juice samples was done at 10 000g at 4℃ for 10 min. The reaction mixture consisted of 1.5 ml sample, 0.5 ml catechol and 3 ml of potassium phosphate buffer (pH 6.8) with total volume of 5 ml. Absorbance was taken at 410 nm for 10 min from zero second starting to every 60 seconds. Storage study for PPO activity was repeated after every 4 days for 20 days. Following equation was used to measure residual enzymatic activity

$$\text{Enzymatic activity (\%)} = \frac{\text{enzyme – specific activity after treatment enzyme} \times 100}{\text{Specific activity in control}}$$

2.6.2. Peroxidase (POD) activity: Juices were evaluated for POD using pyrogallol as substrate according to [26]. Centrifugation of juice samples was done at 10 000 g for 10 min at 4℃. The reaction mixture contained 2.2 ml sample, potassium phosphate buffer (pH 6.0) (0.32 ml) and pyrogallol (0.32 ml) and H_2O_2 (0.16 m). H_2O_2 was used to initiate the process and absorbance was measured by using spectrophotometer at 420 nm for 3 min. The samples were subjected to evaluate POD activity at 4 days interval for 20 days. The above said equation was applied for determination of POD.

2.6.3. Pectin methyl esterase (PME) activity: Pectin Methyl Esterase activity was determined by evaluating the free carboxyl groups formed as a reaction of enzymatic action on pectin as described by [27]. Sample centrifugation was done at 10 000g at 4℃ for 10 min. 40 ml of pectin solution in NaCl and 10 ml of the juice sample was included in reaction mixture. The pH (7.7) was maintained by adding 100 µl NaOH. Storage study for PME activity was repeated after every 4 days for 20 days. The % PME activity was taken by equation as given above.

2.7. Microbiological analysis: The microbial testing of each juice sample was investigated for the growth of microorganisms as described by [28] with minor modification. The aerobic mesophiles were carried out by pour plate method. Sequential dilutions were made with distilled water and then poured into sterilized petri dishes. Each petri dish was embedded with melted agar (15 ml) and 30 min stay time was given to set the media at 25℃.

The petri plates were placed in incubator with upside turned down for 48 hrs at 37℃. Likewise yeast and mold count was carried out on agar plates embedded with potato dextrose after incubating at 25±1℃ for 120 hrs. Developed colonies in juice samples were calculated by multiplying with reciprocal. The data obtained were showed as log colony forming units (CFU/ml) of sample.

2.8. Sensory evaluation: Sensory evaluation was performed using 9-point hedonic scale system following [29] during storage at an interval of four days up to twenty days. Hedonic scale (9=like extremely; 1=dislike extremely) was followed. In this context, parameters for sensory response like overall acceptability were noted by judges.

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The sensory testing was performed in sensory evaluation laboratory of NIFSAT, University of Agriculture Faisalabad. Fifteen judges were selected as panelist for sensory evaluation and they were provided with fluorescent light in separate booths and juice samples in clear glasses randomly in coded form. Judges were supplied with water and unsalted crackers to counteract their taste for precarious testing to accurately find out the best treatments.

2.9. Statistical analysis: The whole experiment was laid out in two factor factorial under CRD, having treatment and storage intervals as two factors. All readings were taken in triplicate and findings were expressed as average value along with standard deviation. The obtained data for each parameter was then statistically analyzed using statistix 8.10 software. Furthermore, variation between different means was carried out using post ANOVA HSD test as multiple mean comparison to check the level of significance [30].

3. RESULTS AND DISCUSSION

3.1 Total Soluble Solids: The results regarding total soluble solids (TSS) of untreated juice and natural extracts (moringa, mint and thyme) incorporated juices are shown in figure 1. Treatments and storage period have significant effect ($P<0.05$) on °Brix values. The maximum TSS was noted in T_9 (8.28±0.04 °B) i.e. carrot juice with incorporation of 3% thyme extract followed by T_6 (8.22± 0.05ºB) with 3% mint extract.

This extract addition exhibited increase in TSS thereby meaning direct relationship of extracts with comparatively higher percentages. All The leaf extract supplemented samples showed higher value of TSS as compared to control i.e., T_0 (7.92±0.05°B). Storage study delineated lowest TSS (7.91±0.07°B) at day zero that progressively increased to (8.28±0.03°B) on 20th day exhibiting linear relation with storage time.

This increase in total soluble solids might be due to enzymes responsible for the breakdown of poly and disaccharides into simple sugars while high temperature may be another reason for the inversion of sucrose resulting in higher TSS during storage. TSS% increased due to dissolved solids in form of sugars, vitamins and minerals present in juices. Moreover, during processing operations of juices, the upsurge in TSS% might be due to the alteration of insoluble polysaccharides to simple form as well as insoluble pectin to simple pectic substances.

The findings from present research are in close collaboration with [31] who reported an increase in TSS with increasing the percentage of extract increased than control juice ranged from 6.50-8.50 °Brix in carrot juice supplemented with *Aframomun danielli* spice aqueous extract at 5, 10 and 15% level as preservative. Arif *et al.* [32] also found that TSS among treatments ranged from 1.11 and 1.47 in ready to serve (RTS) strawberry juice supplemented with moringa juice at 5, 10 and 15% during the entire storage period.

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Quality Attributes	Treatments	Storage Days					
		0	4	8	12	16	20
TSS (°Brix)	T_0	7.63±0.02^{s}	7.70±0.03^{rs}	7.87±0.01^{n-r}	8.00±0.05^{i-p}	8.09±0.04^{f-m}	8.22±0.02^{a-h}
	T_1	7.72±0.05^{rs}	7.76±0.02^{qrs}	7.83±0.05^{p-s}	7.95±0.01^{k-q}	8.07±0.03^{g-n}	8.20±0.05^{a-j}
	T_2	7.86±0.04^{o-r}	7.90±0.05^{m-r}	7.97±0.04^{j-p}	8.05±0.02^{g-o}	8.12±0.01^{d-l}	8.25±0.03^{a-g}
	T_3	8.00±0.06^{i-p}	8.05±0.04^{g-o}	8.12±0.02^{d-l}	8.17±0.16^{b-j}	8.23±0.05^{a-h}	8.30±0.01^{a-e}
	T_4	7.83±0.07^{p-s}	7.90±0.03^{m-r}	7.97±0.08^{j-p}	8.10±0.05^{e-m}	8.14±0.04^{d-k}	8.20±0.02^{a-j}
	T_5	8.00±0.08^{i-p}	8.05±0.01^{g-o}	8.12±0.03^{d-l}	8.21±0.03^{a-h}	8.30±0.02^{a-e}	8.36±0.04^{abc}
	T_6	8.09±0.16^{f-m}	8.12±0.02^{d-l}	8.19±0.05^{b-j}	8.25±0.01^{a-g}	8.30±0.03^{a-e}	8.37±0.05^{ab}
	T_7	7.85±0.08^{o-r}	7.90±0.05^{m-r}	8.05±0.04^{g-o}	8.13±0.02^{d-l}	8.22±0.03^{a-h}	8.30±0.03^{a-e}
	T_8	7.93±0.08^{l-q}	7.97±0.04^{j-p}	8.03±0.24^{h-p}	8.08±0.03^{f-m}	8.12±0.05^{d-l}	8.15±0.01^{d-k}
	T_9	8.16±0.07^{c-j}	8.20±0.02^{a-j}	8.27±0.03^{a-f}	8.31±0.01^{a-d}	8.36±0.03^{abc}	8.40±0.05^{a}
	Storage	**					
	Treatment	**					
	Storage ×treatment	**					

Figure 1: Effect of treatments and storage on TSS (°Brix) of naturally preserved carrot juice

T_0= control pasteurized carrot juice, **T_1=** Carrot juice with 1% moringa extract, **T_2=** Carrot juice with 2% moringa extract, **T_3=** Carrot juice with 3% moringa extract, **T_4=** Carrot juice with 1% mint extract, **T_5=** Carrot juice with 2% mint extract, **T_6=** Carrot juice with 3% mint extract, **T_7=** Carrot juice with 1% thyme extract, **T_8=** Carrot juice with 2% thyme extract, **T_9=** Carrot juice with 3% thyme extract.

3.2 pH and Titratable Acidity%: The results pertaining to pH and titratable acidity% of naturally preserved carrot juice with different levels of moringa, mint and thyme leaves

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extracts and untreated juice are expressed in [figure 2]. Treatments and storage had a significant effect ($p < 0.05$) on pH and titratable acidity% of incorporated juices.

The lowest observed value for pH was noticed in T_6 (5.79±0.02) with 3% mint extract supplemented carrot juice followed by T_9 i.e. carrot juice enriched with 3% thyme extract (5.80±0.03) and T_3 (5.84±0.02) juice with 3% moringa extract. The results depicted that pH of moringa, mint and thyme containing juices was significantly ($p < 0.05$) lower than untreated juice samples.

The maximum changes were observed in juice samples that without incorporation (T_0) from 6.20±0.02 to 5.60±0.01 while minimum rise in pH was observed in T_8 (6.00±0.04 to 5.80±0.01) i.e., in juice supplemented with 2% thyme extract during entire period of storage. The decline in pH could be due to formation of acidic compounds and degradation of reducing sugars.

Within treatments, the maximum titratable acidity% was noticed in T_3 (0.66±0.04%) containing 3% moringa extract followed by T_7 (0.63±0.04%) with 1% thyme extract and T_9 (0.62±0.03%) with 3% thyme extract whereas the lowest mean value was observed in T_8 (0.56±0.03%) carrot juice without 2% thyme extract addition followed by T_6 (0.57±0.05%) supplemented with 3% mint extract. The average values for storage days ranged from 0.44±0.04% to 0.77±0.04% which showed increasing pattern of acidity in all juice samples with the passage of time irrespective of treatments applied.

This rise in juices titratable acidity% might be due to acids formation by hydrolysis of polysaccharides, oxidation of reducing sugars and breakdown of pectic constituents. The outcomes are closely associated with the results of Mokhtar and Ibrahim [33] who incorporated pomegranate and guava leaf extract in guava nectar and showed decrease in pH and increase in acidity%.

Storage days has momentous effect on guava nectar titratable acidity% which is in agreement with findings from current research. Similarly according to El-sadanoy [5] pH significantly decreased from 4.4 to 3.6 in treated samples during six month storage period in cucumber juice. Natural additives incorporation lessen the changes in pH as compared to chemical treated and control samples which is in conformation to present research results.

The reduction in pH leads to concurrent rise in acidity due to fermentation of sugars present in juices during storage. Amanyunose *et al*. [31] illustrated that total titratable acidity of untreated and *Aframomum danielli* extract treated carrot juices ranged from 0.63-0.70 % which is in line with current results.

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Quality Attributes	Treatments	Storage Days					
pH	T_0	6.20±0.02a	6.10±0.02bc	5.87±0.01cde	5.80±0.03g-j	5.72±0.03 r-v	5.60±0.01w
	T_1	6.09±0.0ab	6.10±0.02bc	6.00±0.04bcd	5.92±0.01d-g	5.84±0.02f-i	5.77±0.03 k-q
	T_2	6.00±0.03def	5.97±0.04d-h	5.92±0.03f-i	5.86±0.01h-l	5.80±0.01k-q	5.76±0.02q-v
	T_3	5.93±0.03g-j	5.90±0.04h-m	5.87±0.01i-n	5.84±0.02k-q	5.78±0.03q-v	5.70±0.01r-v
	T_4	6.15±0.03bc	6.10±0.02def	6.03±0.01d-h	5.94±0.04g-j	5.86±0.03i-n	5.78±0.01r-v
	T_5	5.98±0.01d-h	6.00±0.01g-j	5.92±0.02k-q	5.81±0.02n-t	5.76±0.01p-u	5.70±0.03tuv
	T_6	5.88±0.01h-m	5.90±0.01i-o	5.83±0.03j-q	5.77±0.01n-t	5.7±0.02q-v	5.68±0.03tuv
	T_7	6.10±0.02cde	6.00±0.04d-h	5.92±0.03h-l	5.85±0.01i-n	5.8±0.01n-t	5.72±0.02l-r
	T_8	6.00±0.04e-h	5.95±0.03h-k	5.89±0.01j-p	5.85±0.02n-t	5.83±0.03s-v	5.80±0.01uv
	T_9	5.90±0.05i-n	5.86±0.01k-q	5.84±0.04m-s	5.80±0.01o-u	5.73±0.02tuv	5.68±0.03vw
	Storage	**					
	Treatments	**					
	Storage ×treatment	**					
Acidity%	T_0	$0.35±0.04^{v}$	$0.40±0.08^{tuv}$	$0.52±0.03^{k-u}$	$0.60±0.06^{f-p}$	$0.72±0.04^{b-g}$	$0.90±0.06^{a}$
	T_1	$0.41±0.08^{s-v}$	$0.44±0.01^{r-v}$	$0.53±0.03^{k-u}$	$0.59±0.04^{g-p}$	$0.69±0.03^{b-i}$	$0.76±0.04^{a-d}$
	T_2	$0.46±0.04^{p-v}$	$0.49±0.01^{n-v}$	$0.55±0.11^{i-s}$	$0.62±0.03^{d-n}$	$0.66±0.02^{c-i}$	$0.72±0.04^{b-g}$
	T_3	$0.53±0.05^{k-u}$	$0.58±0.05^{g-q}$	$0.64±0.03^{c-m}$	$0.70±0.04^{b-h}$	$0.75±0.04^{b-e}$	$0.78±0.04^{abc}$
	T_4	$0.42±0.02^{r-v}$	$0.48±0.02^{n-v}$	$0.60±0.03^{f-p}$	$0.66±0.03^{b-l}$	$0.78±0.04^{abc}$	$0.80±0.04^{ab}$
	T_5	$0.47±0.04^{o-v}$	$0.50±0.03^{m-u}$	$0.56±0.03^{h-r}$	$0.62±0.03^{d-n}$	$0.67±0.03^{b-k}$	$0.75±0.04^{b-e}$
	T_6	$0.39±0.02^{uv}$	$0.45±0.05^{q-v}$	$0.53±0.03^{k-u}$	$0.60±0.03^{f-p}$	$0.70±0.07^{b-h}$	$0.75±0.07^{b-e}$
	T_7	$0.44±0.02^{q-v}$	$0.50±0.03^{m-u}$	$0.56±0.06^{h-r}$	$0.72±0.04^{b-g}$	$0.76±0.04^{a-d}$	$0.80±0.04^{ab}$
	T_8	$0.47±0.02^{o-v}$	$0.50±0.03^{m-u}$	$0.54±0.03^{j-t}$	$0.58±0.03^{g-q}$	$0.60±0.03^{f-p}$	$0.65±0.03^{c-l}$
	T_9	$0.50±0.03^{m-u}$	$0.55±0.03^{i-s}$	$0.61±0.03^{e-o}$	$0.65±0.03^{c-l}$	$0.68±0.03^{b-j}$	$0.74±0.04^{b-f}$
	Storage	**					
	Treatment	**					
	Storage ×treatment	**					

Figure 2: Effect of treatments and storage on pH and titratable acidity% of naturally preserved carrot juice

T_0= control pasteurized carrot juice, **T_1=** Carrot juice with 1% moringa extract, **T_2=** Carrot juice with 2% moringa extract, **T_3=** Carrot juice with 3% moringa extract, **T_4=** Carrot juice with 1% mint extract, **T_5=** Carrot juice with 2% mint extract, **T_6=** Carrot juice with 3% mint extract, **T_7=** Carrot juice with 1% thyme extract, **T_8=** Carrot juice with 2% thyme extract, **T_9=** Carrot juice with 3% thyme extract.

3.3 Total phenolic and flavonoid content: The results pertaining to total phenolic content and flavonoid content of naturally preserved juices have been demonstrated in figure 3. It is exhibited that the total phenolic content and flavonoid content of carrot juice followed an incremental trend with rise in level of extract addition irrespective of extract source. The maximum mean value for polyphenols and flavonoid was noticed in T_9 (32.87±1.19 mg GAE/100ml, 12.79±0.21 mg QE/100ml) containing 3% thyme extract followed by T_8 (31.99±1.19 mg GAE/100ml, 12.51±0.20 mg QE/ 100ml) having 2% thyme extract and T_3 (31.54±1.26 mg GAE/100 ml) supplemented with 3% moringa extract. The reduction level in bioactive compounds in juices with 2% thyme leaves extracts was comparatively lower than untreated carrot juice and other treatments with extract addition. However, at the end of shelf life the significant reduction in Total phenolic content

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(30.78±1.19 mg GAE/100 ml to 29.47±1.21 mg GAE/100 ml) and flavonoid content (11.60±0.19 to 9.93±0.17 mg QE/100ml) was noticed. The reduction in TPC might me due to the oxidation and degradation in polyphenolic components and their polymerization with protein during storage [34]. Recently, Khan *et al*. [35] documented the declining trend in total phenolic content and flavonoid content of sugarcane juice with moringa and amla extracts incorporation during storage. Similarly, Mena *et al*. [36] reported a declining trend of total phenolics in investigation done on the combined effect of thermal treatment and blending on the quality of pomegranate juice. Muzaffar *et al*. [37] reported that the diminution in total phenolic content could be due to the degradation of polyphenols into brown pigments quinones. In agreement with the present work, total phenolic content of chemical and natural additives supplemented cucumber juice was respectively 9% to 23% during storage of six month [38].

Quality parameters	Treatments	Storage days					
		0	4	8	12	16	20
TPC (mgGAE/100ml)	T_0	$27.75\pm1.11^{h\,n}$	$27.46\pm1.10^{j\,n}$	$27.15\pm1.09^{k\,n}$	26.68 ± 1.20^{lmn}	26.16 ± 1.05^{mn}	$25.80\pm1.29n$
	T_1	$29.74\pm1.19^{a\,n}$	$29.60\pm1.24^{a\,n}$	$29.14\pm1.31^{c\,n}$	$28.86\pm1.36^{c\,n}$	$28.56\pm1.20^{c\,n}$	$28.24\pm1.10^{f\,n}$
	T_2	$30.36\pm1.21^{a\,j}$	$30.25\pm1.21^{a\,m}$	$29.70\pm1.31^{a\,n}$	$29.46\pm1.06^{b\,n}$	$29.27\pm1.17^{b\,n}$	$28.85\pm1.44^{c\,n}$
	T_3	$31.96\pm1.28^{a\,g}$	$31.90\pm1.24^{a\,g}$	$31.72\pm1.3^{a\,j}$	$31.46\pm1.23^{a\,j}$	$31.24\pm1.31^{a\,k}$	$30.94\pm1.18^{a\,k}$
	T_4	$29.30\pm1.05^{a\,n}$	$29.15\pm1.17^{b\,n}$	$28.74\pm1.15^{d\,n}$	$28.37\pm1.28^{f\,n}$	$28.09\pm1.32^{g\,n}$	$27.65\pm1.30^{j\,n}$
	T_5	$30.75\pm1.26^{a\,j}$	$30.62\pm1.22^{a\,j}$	$30.39\pm1.25^{a\,j}$	$30.13\pm1.18^{a\,m}$	$29.75\pm1.13^{a\,n}$	$29.47\pm1.33^{a\,n}$
	T_6	$31.50\pm1.20^{a\,j}$	$31.40\pm1.19^{a\,j}$	$31.23\pm1.34^{a\,k}$	$30.96\pm1.11^{a\,k}$	$30.75\pm1.08^{a\,j}$	$30.51\pm1.10^{a\,j}$
	T_7	$30.82\pm1.23^{a\,j}$	$30.62\pm1.13^{a\,j}$	$30.24\pm1.03^{a\,m}$	$29.89\pm1.26^{a\,n}$	$29.50\pm1.09^{a\,n}$	$29.16\pm1.02^{b\,n}$
	T_8	$32.26\pm1.16^{a\,f}$	$32.20\pm1.26^{a\,f}$	32.06 ± 1.28^{abc}	$31.90\pm1.18^{a\,g}$	$31.79\pm1.11^{a\,h}$	$31.70\pm1.14^{a\,j}$
	T_9	33.40 ± 1.17^{a}	33.25 ± 1.13^{ab}	32.95 ± 1.25^{abc}	$32.70\pm1.18^{a\,d}$	$32.56\pm1.24^{a\,e}$	$32.34\pm1.16^{a\,f}$
	Storage	**					
	Treatment	**					
	Storage ×treatment	NS					
Flavonoid content (mg QE/100ml)	T_0	9.45 ± 0.19^{uv}	$8.93\pm0.13^{v\,y}$	8.32 ± 0.16^{yzab}	8.15 ± 0.08^{zab}	7.77 ± 0.11^{B}	7.00 ± 0.12^{C}
	T_1	$10.80\pm0.13^{o\,r}$	10.64 ± 0.15^{pqr}	10.37 ± 0.21^{rst}	9.94 ± 0.10^{stu}	9.34 ± 0.16^{uvw}	8.19 ± 0.18^{zab}
	T_2	$11.70\pm0.22^{h\,j}$	$11.60\pm0.16^{i\,m}$	$11.39\pm0.28^{j\,o}$	$11.16\pm0.19^{j\,p}$	$10.87\pm0.20^{o\,r}$	9.86 ± 0.23^{tu}
	T_3	$12.25\pm0.12^{d\,h}$	$12.20\pm0.15^{d\,i}$	$11.96\pm0.20^{f\,j}$	$11.79\pm0.26^{g\,k}$	$11.64\pm0.21^{h\,l}$	$11.41\pm0.18^{j\,o}$
	T_4	$10.45\pm0.17^{q\,t}$	10.32 ± 0.09^{rst}	9.15 ± 0.16^{vwx}	$8.75\pm0.15^{w\,z}$	8.41 ± 0.19^{yza}	8.10 ± 0.08^{AB}
	T_5	$11.52\pm0.25^{i\,n}$	$11.40\pm0.16^{j\,o}$	$11.17\pm0.16^{k\,p}$	$10.92\pm0.20^{n\,r}$	10.76 ± 0.17^{pqr}	10.50 ± 0.23^{qrs}
	T_6	$12.80\pm0.22^{a\,d}$	12.68 ± 0.11^{bcd}	$12.42\pm0.21^{c\,f}$	$12.25\pm0.27^{d\,h}$	$11.98\pm0.19^{e\,j}$	$11.00\pm0.21^{m\,q}$
	T_7	$10.90\pm0.13^{n\,r}$	$10.84\pm0.15^{o\,r}$	10.50 ± 0.18^{qrs}	9.14 ± 0.15^{vwx}	$8.92\pm0.15^{v\,y}$	$8.64\pm0.09^{x\,a}$
	T_8	$12.75\pm0.18^{a\,d}$	12.70 ± 0.11^{bcd}	$12.58\pm0.23^{c\,f}$	$12.50\pm0.20^{c\,f}$	$12.34\pm0.27^{c\,g}$	$12.20\pm0.23^{d\,i}$
	T_9	13.34 ± 0.29^{a}	13.22 ± 0.19^{ab}	12.95 ± 0.28^{abc}	12.59 ± 0.20^{cde}	$12.37\pm0.17^{c\,g}$	$12.25\pm0.12^{d\,h}$
	Storage	**					
	Treatment	**					
	Storage ×treatment	**					

Figure 3: Effect of treatments and storage on Total phenolic content and total flavonoid content of naturally preserved carrot juice

T_0= control pasteurized carrot juice, **T_1=** Carrot juice with 1% moringa extract, **T_2=** Carrot juice with 2% moringa extract, **T_3=** Carrot juice with 3% moringa extract, **T_4=** Carrot juice with 1% mint extract, **T_5=** Carrot juice with 2% mint extract, **T_6=** Carrot juice with 3% mint extract, **T_7=** Carrot juice with 1% thyme extract, **T_8=** Carrot juice with 2% thyme extract, **T_9=** Carrot juice with 3% thyme extract.

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3.4 Antioxidant Activity: The antioxidant activity (DPPH and FRAP) observed during storage period in carrot juice incorporated with moringa, mint and thyme extracts showed statistically significant changes ($p < 0.05$) as shown in figure 4. The highest percentage0 of DPPH was observed in 3% mint extract incorporated carrot juice (37.45±1.69%) and juice with 3% moringa extract (36.50±1.57%) at zero day while the lowest value was observed in T_0 (29.95±1.35%) containing 100% carrot juice.

The level of reduction was significantly increased ($P<0.05$) after 20 days of storage in control and 1% of moringa, mint and thyme extract addition. Mean values for DPPH radical scavenging potential explicated a decreasing trend from (33.90±1.38 to 32.05±1.43%) at 20th day of storage. Ferric reducing power (FRAP) mean value of control juice and treated juices ranged from (14.85±0.22 µmol Fe^{2+}/100ml) to (20.54±0.297µmol Fe^{2+}/ml**)** at zero day that decreased to (8.50±0.17 µmol Fe^{2+}/100ml to 19.42±0.10 µmol Fe^{2+}/100ml) at 20th day of storage among moringa, mint and thyme extract added juices respectively.

It was observed that 3% mint and 2% thyme incorporated juices remained stable in antioxidant potential throughout storage period. However, carrot juice supplementation with natural plants extracts (moringa, mint and thyme) showed better antioxidant potential elaborating that each extract source owing linear relation in DPPH % and FRAP with extract quantity.

The reason for reduction in antioxidant potential could be owing to loss of polyphenolic components and enzyme reactions i.e., PPO and POD that cause juices this antioxidant triggering activity. The results regarding decline in DPPH% of all juice samples with the progression of storage is accordance with the outcomes of [39] who stated a reduction in DPPH in fortified guava whey juice with moringa aqueous extract at 1.5% during two months of storage.

Findings of [40] reported carrot juice supplementation with winter savory herbal extract found the DPPH% of carrot juice with savory extract at initial 172.66 (mg Trolox/mL) that decreased to 68.42 (mg Trolox/mL) at fifteen days of storage, they are in collaboration with our findings.

This decrease in reducing power of all treatments during storage time is in accordance with the outcomes of [35] who stated a decrease in Ferric Reducing Power in sugarcane juice preserved with anola and moringa extracts as in 5% aonla extract (86.23-79.23%) and in 5% moringa extract (88.78%-79.67%). The highest decrease was observed in control juice with 77.12% to 56.12% during storage of 21 days.

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Quality Attributes	Treatments	Storage days					
		0	4	8	12	16	20
DPPH%	T_0	$29.95\pm1.35^{k\text{-}p}$	$29.32\pm1.41^{m\text{-}p}$	$28.67\pm1.26^{n\text{-}q}$	27.00 ± 1.30^{pqq}	25.50 ± 1.28^{qq}	24.32 ± 1.31^{q}
	T_1	$32.78\pm1.21^{a\text{-}n}$	$32.60\pm1.34^{a\text{-}n}$	$32.27\pm1.48^{b\text{-}n}$	$31.56\pm1.55^{c\text{-}o}$	$30.95\pm1.21^{l\text{-}o}$	$30.50\pm1.25^{l\text{-}o}$
	T_2	$35.05\pm1.65^{a\text{-}j}$	$34.90\pm1.36^{a\text{-}k}$	$34.36\pm1.58^{a\text{-}l}$	$33.95\pm1.53^{a\text{-}m}$	$33.12\pm1.24^{a\text{-}n}$	$33.50\pm1.41^{a\text{-}n}$
	T_3	$36.50\pm1.57^{a\text{-}e}$	$36.40\pm1.46^{a\text{-}f}$	$36.29\pm1.52^{a\text{-}f}$	$36.14\pm1.63^{f\text{-}o}$	$35.98\pm1.62^{a\text{-}h}$	$35.82\pm1.68^{a\text{-}j}$
	T_4	$32.50\pm1.40^{a\text{-}n}$	$32.34\pm1.29^{c\text{-}o}$	$31.89\pm1.66^{g\text{-}o}$	$31.45\pm1.54^{g\text{-}h}$	$31.18\pm1.40^{h\text{-}o}$	$30.84\pm1.45^{k\text{-}l}$
	T_5	$35.17\pm1.65^{a\text{-}j}$	$35.05\pm1.37^{a\text{-}j}$	$34.78\pm1.60^{a\text{-}k}$	$34.42\pm1.55^{a\text{-}l}$	$33.63\pm1.26^{a\text{-}n}$	$33.86\pm1.52^{h\text{-}o}$
	T_6	37.45 ± 1.69^{a}	37.40 ± 1.57^{a}	37.22 ± 1.49^{ab}	36.87 ± 1.62^{abc}	$36.71\pm1.58^{a\text{-}d}$	$36.60\pm1.68^{a\text{-}d}$
	T_7	$31.95\pm1.50^{c\text{-}o}$	$31.86\pm1.31^{d\text{-}o}$	$31.37\pm1.44^{g\text{-}o}$	$30.69\pm1.50^{l\text{-}o}$	$30.42\pm1.19^{l\text{-}p}$	$29.52\pm1.21^{l\text{-}p}$
	T_8	$34.70\pm1.56^{a\text{-}k}$	$34.60\pm1.31^{a\text{-}k}$	$33.40\pm1.47^{a\text{-}n}$	$33.15\pm1.59^{a\text{-}n}$	$32.81\pm1.43^{a\text{-}n}$	$32.34\pm1.42^{a\text{-}n}$
	T_9	$34.65\pm1.28^{a\text{-}k}$	$34.50\pm1.41^{a\text{-}j}$	$34.24\pm1.58^{a\text{-}m}$	$33.94\pm1.66^{a\text{-}m}$	$33.50\pm1.31^{a\text{-}n}$	$33.24\pm1.36^{a\text{-}n}$
	Storage	**					
	Treatment	**					
	Storage xtreatment	NS					
FRAP (µmol Fe^{2+}/100ml)	T_0	14.85 ± 0.22^{yza}	14.50 ± 0.15^{zab}	12.40 ± 0.19^{e}	11.00 ± 0.20^{f}	9.74 ± 0.10^{g}	8.50 ± 0.17^{h}
	T_1	17.56 ± 0.16^{nop}	17.45 ± 0.14^{nop}	$17.22\pm0.17^{n\text{-}r}$	16.50 ± 0.15^{tuv}	15.90 ± 0.16^{uvw}	14.80 ± 0.13^{yza}
	T_2	$19.75\pm0.18^{e\text{-}j}$	$19.86\pm0.20^{e\text{-}j}$	19.18 ± 0.35^{ijk}	18.75 ± 0.19^{kl}	18.34 ± 0.28^{lm}	17.90 ± 0.18^{mn}
	T_3	22.34 ± 0.11^{a}	22.25 ± 0.22^{a}	21.94 ± 0.20^{ab}	21.50 ± 0.28^{bc}	21.27 ± 0.21^{bc}	20.90 ± 0.29^{cd}
	T_4	15.40 ± 0.14^{wxy}	15.20 ± 0.27^{xy}	14.45 ± 0.19^{ab}	13.90 ± 0.22^{bc}	13.50 ± 0.12^{cd}	13.19 ± 0.12^{d}
	T_5	16.45 ± 0.28^{tu}	16.32 ± 0.16^{tu}	15.86 ± 0.14^{ux}	15.57 ± 0.25^{vwx}	15.18 ± 0.12^{wyz}	14.72 ± 0.19^{yza}
	T_6	17.84 ± 0.21^{mn}	17.70 ± 0.14^{mno}	$17.36\pm0.21^{n\text{-}q}$	$17.14\pm0.17^{o\text{-}s}$	$16.90\pm0.17^{p\text{-}t}$	$16.69\pm0.15^{q\text{-}t}$
	T_7	17.54 ± 0.11^{nop}	17.40 ± 0.16^{nop}	16.62 ± 0.33^{rst}	16.24 ± 0.11^{tuv}	15.62 ± 0.14^{vwx}	15.24 ± 0.18^{wxy}
	T_8	$19.90\pm0.18^{e\text{-}h}$	$19.82\pm0.34^{f\text{-}j}$	$19.58\pm0.27^{g\text{-}j}$	$19.42\pm0.17^{h\text{-}k}$	$19.27\pm0.17^{h\text{-}k}$	19.07 ± 0.19^{jk}
	T_9	20.54 ± 0.27^{de}	20.41 ± 0.10^{def}	20.19 ± 0.30^{efg}	$19.94\pm0.26^{e\text{-}h}$	$19.65\pm0.16^{g\text{-}j}$	$19.42\pm0.10^{h\text{-}k}$
	Storage	**					
	Treatment	**					
	Storage xtreatment	*					

Figure 4: Effect of treatments and storage on DPPH% and FRAP of naturally preserved carrot juice

T_0= control pasteurized carrot juice, **T_1=** Carrot juice with 1% moringa extract, **T_2=** Carrot juice with 2% moringa extract, **T_3=** Carrot juice with 3% moringa extract, **T_4=** Carrot juice with 1% mint extract, **T_5=** Carrot juice with 2% mint extract, **T_6=** Carrot juice with 3% mint extract, **T_7=** Carrot juice with 1% thyme extract, **T_8=** Carrot juice with 2% thyme extract, **T_9=** Carrot juice with 3% thyme extract.

3.5 Enzyme activity: The results regarding Polyphenol oxidase (PPO), Per oxidase (POD) and Pectin methyl esterase (PME) of moringa, mint and thyme leaves extract incorporated juices have been expressed in figure 5 [a, b, c]. It was noted that different concentrations of extracts significantly ($P<0.05$) affect the action of PPO, POD and PME in carrot juice. Highest PPO, POD and PME activity was shown by control juice T_0 (31.56±0.06%, 25.54±0.06% and 13.56±0.03%) at zero day that was significantly decreased as the level of extracts increased within treatments and increased (34.89±0.07%, 27.30±0.11% and 15.16±0.03%) during the storage period of 20 days. Minimum increase in PPO (28.94±0.05% to 29.33±0.03%), POD (22.80±0.09% to 23.26±0.05%) and PME (10.66±0.02% to 11.10±0.02%) was observed within the juice samples incorporated with 2% thyme leaves extract followed by T_9 containing 3% thyme

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extract PPO (27.50±0.05% to 28.15±0.03%), POD (20.84±0.02% to 23.38±0.06%) and PME (10.16±0.02% to 10.70±0.03%) as compared to mint and moringa incorporated extracts throughout the storage period. This upsurge in enzyme activity triggered the browning of carrot juice during entire storage days. The reason could be due to phenolic compounds oxidation, the reaction of organic acid with sugars resulting in development of insoluble dark pigments such as melanin and degradation of chlorophyll [36]. Khan *et al*. [35] investigated the effect of moringa and aonla extract incorporation on POD and PPO and found that these extract have the ability to inactivate enzymes at higher concentrations and upsurge in POD and PPO activity during storage of sugarcane juice for 21 days. The outcomes are supported by [41] who found a rise in enzymatic activity of kinnow juice prepared by blending 3% ginger juice and 5% of aonla juice for storage time of six months. Similarly, [42] also reported that enzymatic activity increased in aonla juice preserved in water during storage period of 30 days.

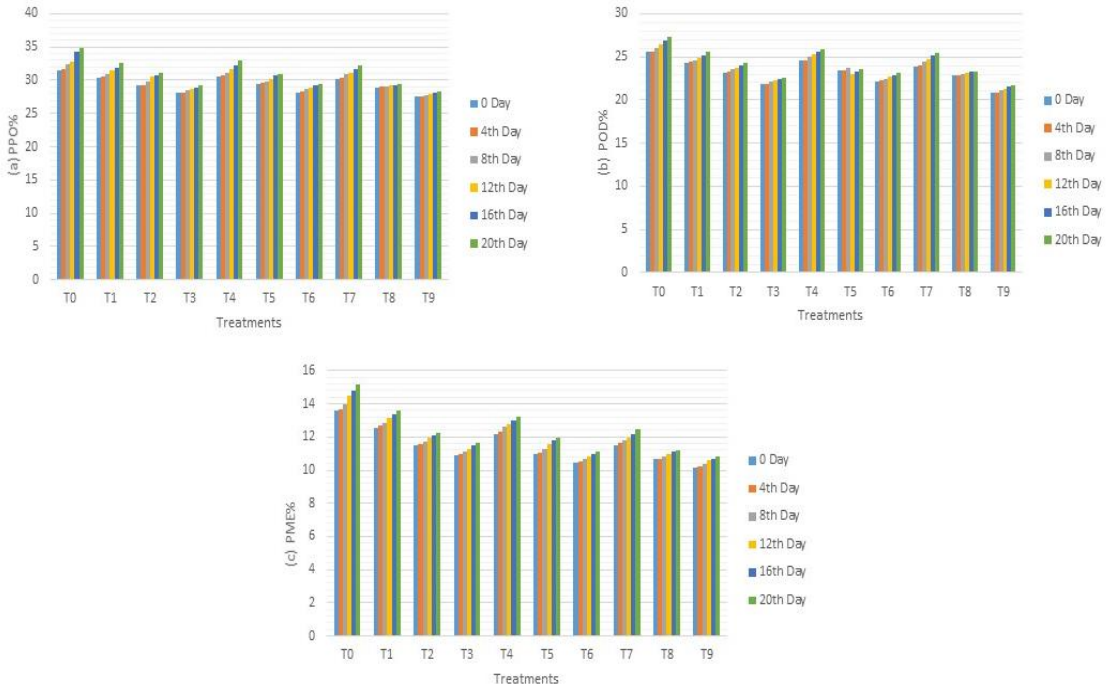

Figure 5: (a, b, c) Effect of treatment and storage on enzymatic activity of naturally preserved carrot juice

T_0= control pasteurized carrot juice, **T_1=** Carrot juice with 1% moringa extract, **T_2=** Carrot juice with 2% moringa extract, **T_3=** Carrot juice with 3% moringa extract, **T_4=** Carrot juice with 1% mint extract, **T_5=** Carrot juice with 2% mint extract, **T_6=** Carrot juice with 3% mint extract, **T_7=** Carrot juice with 1% thyme extract, **T_8=** Carrot juice with 2% thyme extract, **T_9=** Carrot juice with 3% thyme extract.

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3.6 Microbiological analysis: The changes in microbial load of carrot juice that are naturally preserved with moringa, mint and thyme extracts and control juice are presented in figure 6 [a, b]. The maximum mean value for microbial analysis was noticed in T_0 (2.24±0.07 log CFU/ml) i.e. control carrot juice followed by T_4 (1.85±0.05 log CFU/ml) juice containing 1% mint extract and T_7 (1.75±0.04 CFU/ml) juice with 1% thyme extract whereas minimum microbial load was showed by T_8 (1.33±0.07 CFU/ml) containing 2% thyme extract and T_9 (1.52±0.06 log CFU/ml) with 3% thyme extract respectively. The higher levels of extract incorporation from all sources in carrot juices showed the minimum growth of aerobic microbes during storage. However, 2% thyme extract retained the quality of juices with respect to microbial growth and showed comparatively lower microbial growth on time dependent manner. Mean microbial population among intervals of storage revealed that bacterial count increases with increasing the time of storage from (1.19±0.05 log CFU/ml) at 0 day to higher count (2.29±0.06 log CFU/ml) after 20 day of storage. With respect to yeast and mold, the highest count was observed in control carrot juice (0.62±0.02 log CFU/ml) followed by T_4 (0.52±0.05 log CFU/ml) juice with 1% mint extract and T_7 (0.50±0.05 log CFU/ml) while the minimum population was found in 3% extract supplementation from each source. Significantly ($p<0.05$) increasing trend in yeast and mold count was observed (0.24±0.01-0.64±0.02 log CFU/ml) during 20 days of storage. Inclusion of peppermint, basil, lavender and lemongrass herb extracts in cucumber juice reduced the bacterial count (3.1-5.0 CFU/mL) than control juice (4.0-7.5 CFU/mL) and was found most effective in controlling the bacterial count up to 40% during 60 days of storage resulting in extending shelf life [38]. Similar results were observed in guava whey juice preservation supplemented with 1.5% and 2% of moringa leaves extract [39]. Addition of *Aframomum danelli* spice in carrot juice also resulted in reduction of microbial load that control and lower level of spice [31].

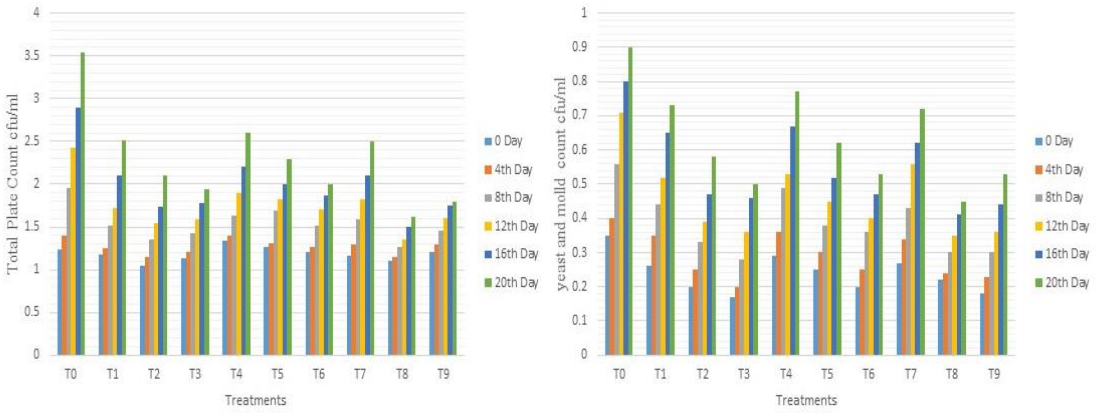

Figure 6: [a, b]. Effect of treatments and storage on microbial activity of naturally preserved carrot juice

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T_0= control pasteurized carrot juice, **T_1=** Carrot juice with 1% moringa extract, **T_2=** Carrot juice with 2% moringa extract, **T_3=** Carrot juice with 3% moringa extract, **T_4=** Carrot juice with 1% mint extract, **T_5=** Carrot juice with 2% mint extract, **T_6=** Carrot juice with 3% mint extract, **T_7=** Carrot juice with 1% thyme extract, **T_8=** Carrot juice with 2% thyme extract, **T_9=** Carrot juice with 3% thyme extract.

3.7 Sensory evaluation: Sensory scores for overall acceptability of control and treated juices showed significant variation ($p<0.05$). Figure 7 showed that the maximum mean score was attained by T_8 (7.53±0.70) juice with 2% thyme leaves extract and T_5 (7.07±0.65) juice with 2% mint incorporation. At zero day pasteurized carrot juice and extract supplemented juices were rated higher (7.76±0.66) which was decreased significantly at the 20th day of storage to (5.71±0.55) at refrigerator temperature showing decreasing trend in overall acceptability of extract incorporated carrot juices.

The reason could be the highest amount of extract addition leads towards the less acceptability from the consumers due to strong flavor notes from moringa, mint and thyme extracts. The decline in scores could be due to development of brown pigments function of non-enzymatic browning reactions, the forfeiture of volatile compounds, the collapse of polysaccharides and the degradation of protein and complex formation with pectic substances and phenols could be other factors for decline in sensory scores during storage.

The overall acceptability reported by [43] in mint-ginger drink significantly changed during sixty days of storage. Decrease in overall acceptability from 5.60 to 4.50 with 0.5mg GAE of extract and 5.60 to 3.50 in 4 mg GAE extract was reported during storage upto 20 days in pasteurized carrot juice with orange peel extract [21].

Treated samples with naturally derived preservatives including aonla and moringa gained high scores for sensory attributes during storage as compared to untreated sugarcane juice [35]. Value added drinks prepared from melon byproducts (melon peel powder 5% and 10% and seed powder 5% and 10%) and extract (1% and 3% of melon peel and seed) exhibited a significant decrease in sensory scores by adding high percentages of powder and extract from zero day upto 90 days of storage.

The reason behind decrease in color score could be millard reactions that leads to enzymatic and non-enzymatic reactions during storage. Mokhtar and Ibrahim [33] also found decline in sensory attributes of pomegranate peel and guava leaf extracts incorporated pasteurized guava nectar.

This decreasing trend of sensory scores is ascribed to oxidation of ascorbic acid that convert it into dehydro ascorbic acid and oxidation of tannin into gallic acid that result in sour taste and increased acidity of product [45].

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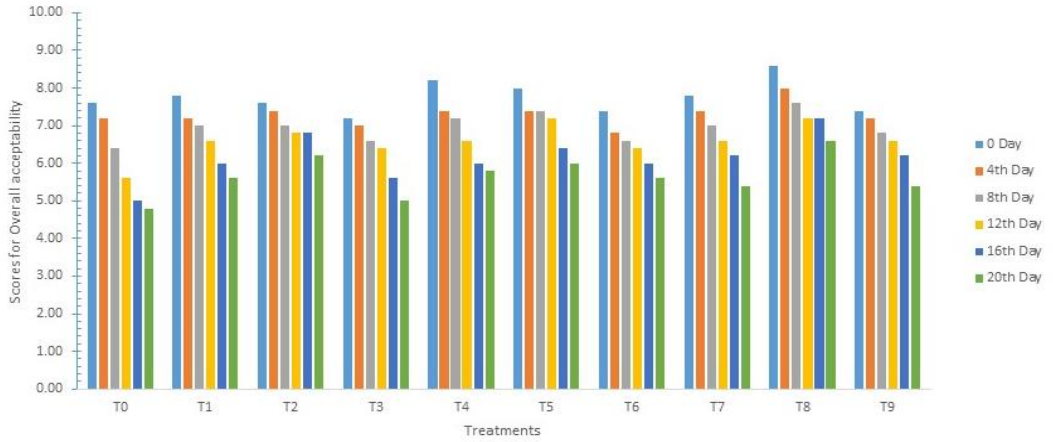

Figure 7: Effect of treatments and storage on overall acceptability of carrot juice

T_0= control pasteurized carrot juice, **T_1=** Carrot juice with 1% moringa extract, **T_2=** Carrot juice with 2% moringa extract, **T_3=** Carrot juice with 3% moringa extract, **T_4=** Carrot juice with 1% mint extract, **T_5=** Carrot juice with 2% mint extract, **T_6=** Carrot juice with 3% mint extract, **T_7=** Carrot juice with 1% thyme extract, **T_8=** Carrot juice with 2% thyme extract, **T_9=** Carrot juice with 3% thyme extract.

4. CONCLUSION

The present research reveals that the utilization of moringa, mint and thyme leaves extracts as an alternative to chemical preservatives enhance the quality and shelf stability of carrot juice. All juice samples were studied for physicochemical, bioactive compounds, enzyme activity, microbial analysis and overall acceptability for 20 days at the interval of 4 days. Carrot juices incorporated with 2% thyme extract remained stable in pH, titratable acidity, phenolic contents and flavonoid contents as well as in inhibition of residual enzyme activity poly phenol oxidase (PPO), per oxidase (POD) and pectin methyl esterase (PME). However, mint and moringa extracts were also effective in preserving the carrot juices as compared to control. The extract incorporation in juices reduce microbial load and enzyme activity whereas also improve nutritional status. This can help to extend shelf life of perishable carrot juice up to 20 days at refrigerated storage as compared to freshly squeezed juice. Moreover, this may potentially enhance the economic value by increasing its availability at market level.

Conflict of Interest

All authors have declared no conflict of research

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