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RESEARCH ARTICLE

DEVELOPMENT AND SHELF STABILITY OF FUNCTIONAL DRINK PREPARED FROM *CITRULLUS LANATUS* AND *CUCUMIS SATIVUS*.

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ABSTRACT

Background: *Cucumis sativus* commonly known as cucumber has countless benefits related to skin, body, and general health conditions. Watermelon (*Citrullus lanatus)* juice is highly nutritious and perishable. Recent studies focus on using it with a combination of potential functional ingredients. Watermelon-Cucumber drink is a healthy combination of beverages that can provide many nutrients and health benefits. **Objectives:** In this study, cucumber juice was used to improve watermelon juice's nutritional profile and sensory attributes by utilizing honey as a sweetener. This study aimed to evaluate the functional drink's proximate parameters, oxidative stability, microbial load, and organoleptic evaluation. **Methodology:** Statistics was applied using Statistix 8.1, and data was analyzed using two-way ANOVA and Tukey HSD Post Hoc analysis. The functional drink was prepared using altering concentrations of cucumber juice and watermelon juice (80:10, 70:20, 60:30, 50:40) and stored at 4°C for 28 days. Results revealed that adding cucumber juice significantly improved antioxidant potential ($p<0.05$) up to 1.6%. The current study revealed that a non-significant effect was observed on ash contents (0.06-0.04%), total solids (0.36-0.32%), and degree Brix (8.7°-9.7°) of functional drink. Increasing concentration of cucumber juice led to an increase in titratable acidity (0.57%) while pH was decreased (3.6). **Conclusions:** Using watermelon and cucumber juice in combinations improved the antioxidant potential of the functional drink. The organoleptic evaluation revealed that treatment with 80% watermelon juice and 10% cucumber juice was observed best in sensorial attributes.

Keywords: Watermelon, Cucumber, Functional Drink, Beverage, Shelf-life.

INTRODUCTION

Beverages are necessary for growth, development, and various physiological processes significant for healthy living. Beverage consumption maintains water content in the body and also prevents dehydration. They are used for the body's healing, regeneration, and detoxification (1). Water also helps to remove the toxic substances present in the body (2). It also regulates body temperature through the sweating process. The antioxidant profile of pure fruit and vegetable juices is much more complex and includes carotenoids, tocopherols, ascorbic acid, and polyphenols (3).

Fruits are necessary foods because of their nutritional content and health advantages. They are excellent providers of nutrients that the body needs to operate correctly, including vitamins, fatty acids, amino acids, minerals, phytonutrients, dietary fibres, and others (4). Fruits are incredibly perishable; therefore, proper storage is essential to keeping them readily available (5). Fruits may be stored as drinks, such as fruit juice. The health advantages of consuming fruit juice are linked to essential vitamins and polyphenol substances, such as flavonoids, carotenoids, and tannins (6). These phytochemicals are potent antioxidants against degenerative illnesses and have also demonstrated promising outcomes in treating different infections (7).

Red, scented, and sweet watermelon (*Citrullus lanatus*) are refreshing and smoothly digested (8). It is rich in vitamins A and ascorbic acid and contains beta-carotene, vitamin B6 (9), potassium, and thiamine. Watermelon is included in the genus *Citrullus,* native to Africa (10). It is a better choice of functional food because of its nutraceutical potential, as demonstrated by many researchers. Many ailments like cardiovascular, aging, ulcers, obesity, diabetes, and many types of cancer have been treated by watermelon. Important phytochemicals i.e., citrulline, lycopene, and polyphenolic compounds in watermelon, enhance its medicinal properties. By inhibiting DNA mutation and reacting counter to tumor matastasis, lycopene, an active compound in watermelon, could be involved in the modification of cancer development (11).

Secondary metabolites present in watermelon are involved in reducing the spread of cancer cells. Moreover, its supplementation increases the antioxidant ability in obese adults and lowers triglyceride levels and low-density lipoprotein cholesterol (12). Lycopene, vitamin A and C content, and antioxidant potential in watermelon made it the best functional food (13). Vascular reactivity is increased in animal models by l-arginine (14–16) and hyper cholesterol patients (14). Watermelon can significantly reduce appetite and be involved in managing weight compared to refined carbohydrate snacks (11). Different watermelon tissues, e.g., seeds, leaves, sprouts, fruits, and rinds, have secondary metabolites with nutraceutical potential on several potential drug targets in different diseases like cancer, inflammation, obesity, and diabetes. As a good potassium source (>100mg/g), cucumber-based beverages take part in electrolyte levels balance in case of dehydration (17).

About 95% of water present in cucumber offers superior hydration. The fiber in cucumber boosts to stay regular and avoid constipation. For many skin benefits, soothing properties for digestion, and therapeutic uses, cucumber have been used for decades. They augment cellular water and deal with common conditions such as bone health, connective tissue disorders, aging, cardiovascular and cancerous diseases, and skin discoloration. The hydrating properties of cucumber and the skin's cooling effect also benefit the eyes and surrounding tissues, which helps reduce dehydration. High-level vitamin K in cucumber help to reduce dark circles, and the lignans in it reduces inflammation (18). By inhibiting tyrosinase, cucumbers have been used as skin brighteners and moisturizers and for treating sunburns and wrinkles (19). The speed of muscle wasting, which occurs with aging, can be reduced by an alkaline diet (20). An antioxidant such as beta carotene in cucumbers fights against free radicals in the body, unpaired electrons that damage cells. It contains 19.9 milligrams of trusted source of calcium. According to sex and age, adults must trust 1,000–1,200 mg of calcium daily. Cucumbers may also have anti-inflammatory benefits. Honey plays a significant role in functional beverages as a sweetening agent. Honey contains 76-80% glucose, fructose, 17-20% water, wax, pollen, and mineral salts (21). Pinocembrin (Dihydrochrysin, Galangin flavanone, 5,7-Dihydroxyflavanone) is only present in honey as an antioxidant (22). Keeping in view the nutritional importance and functional potential of watermelon and cucmber, the present study was designed with the aim:

- To develop a functional drink with improve nutritional profile and sensory attribute
- To analyze the physicochemical, oxidative potential and organoleptic characteristics of the drink
- To evaluate the storage stability of the prepared functional beverage

MATERIALS AND METHODS

Procurement of raw material

The research was carried out at the University of the Punjab's Food Safety Laboratory, Department of Food Sciences, Faculty of Agricultural Sciences, Lahore. Fresh watermelon and cucumbers were obtained from the local market (Sabzi Mandi Multan Chungi, Kamran Block Allama Iqbal Town, Punjab 54000, Pakistan).

Preparation of functional drink

Functional beverage was perepared by the method described by (21) with some modifications. Fresh watermelon and cucumbers were washed, peeled, blended, and strained separately. Four different treatments were made using varied ratios of cucumber and watermelon juices. The treatment plan followed for treatment preparation is provided in Table 1. by keeping the quantity of honey constant. They were stored at refrigeration temperature (4°C), and different analyses were performed.

Table 1. Detail of Treatments

Serial no	Ingredient	T1 (%)	T2(%)	T3(%)	T4(%)
1.	Watermelon Juice	80	70	60	50
2.	Cucumber Juice	10	20	30	40
3.	Honey	9	9	9	9
4.	Sod. Benzoate	0.2	0.2	0.2	0.2
5.	Gum Arabic (Stabilizer)	0.8	0.8	0.8	0.8
	Total	**100**	**100**	**100**	**100**

Analysis of the product

Proximate parameters like ash content, moisture, pH, total acidity, total solids, viscosity, and antioxidant potential were determined by following the methods (22). Analysis was performed on storage intervals of 7 days. The first analysis was performed for fresh sample and then on days 7, 14, 21, and 28.

Determination of pH

pH meter Inolab pH 720, WTW 82362 was used to analyze the pH of the final product. Determination of the pH of samples was done (23). The pH meter was calibrated with pH 4.0 and 7.0 before analysis. On days 0, 7, 14, and 21, all sample analyses were carried out in triplicate.

Determination of total acidity

The total acidity of the sample was determined by the titration method.10mL sample was pipette out in a 250mL beaker. As soon as a light pink colour appeared, the sample was tested against 0.1N NaOH while using 2–3 drops of phenolphthalein indicator.Titratable acidity was calculated by using the equation (24).

$$\text{Total acidity (\%)} = \frac{0.1 \times \text{equivalent weight of acid} \times \text{normaltity of NaOH} \times \text{Titer}}{10}$$

Determination of total solids

Total solids were determined by following AOAC (Association of Official Agricultural Chemists) standard method. The 15mL of each treatment was filtered separately using Whatman filter paper. Each filter paper was weighed before being used. After filtration, filter papers were placed in Petri plates and were oven dried (DOF-230E, Bievopeak, Japan) at 100^{o}C for ten minutes. The weight of filter papers was measured by weighing balance, and total solids were obtained by subtracting the final weight from the initial weight.

Determination of Brix

Brix was determined using a refractometer. It was first cleaned with tissue paper gently, and a drop of drink was placed on it with the help of a pipette. Digital results were indicated on the screen.

Determination of ash content

Ash content was determined by ashing the sample in a muffle furnace. 20mL sample was dried using a hot plate and then placed in a muffle furnace at 550^{o}C for 24 hours, and readings were noted. Then the mineral content was calculated using the formulae given by (25).

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Oxidative Stability

Determination of antioxidant potential

The antioxidant potential of the sample was measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay by the method (26) using methanol extracts. 1-3ml sample was extracted in 10ml of 80% methanol, and 2 hours of stay time was given. DPPH reagent was prepared by dissolving 4mg of DPPH in 100ml methanol.50µl extract was added to 2ml of DPPH solution. At the experiment's conclusion, absorbance was measured using a spectrophotometer at 515 nm compared to a control. Values for the sample and the control both were noted.

$$\text{Antioxidant activity (\%)} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100$$

Organoleptic evaluation

The organoleptic evaluation was performed according to the method described (27) with some modifications. Sensory analysis was performed using a 9-point hedonic scale with scores from 1 (dislike very) to 9 (like very) in the form of a questionnaire to evaluate the functional drink samples. A group of 5 people was presented with samples of all treatments at described time intervals. Parameters for result recording were color, aroma, taste, mouthfeel, consistency, and overall acceptability. Results were recorded and analyzed.

Statistical analysis

Statistical analysis was performed following the method described by (28). Descriptive statistics (mean, standard deviation, etc.) and inferential statistics (t-test, ANOVA etc.) were used to examine the study's primary goal. Software Statistix 8.1 was used to analyze and compare data statistically. For multiple comparisons, Tukey HSD Post Hoc analyses were performed. Significance level was kept at p value 0.05.

RESULTS AND DISCUSSION

The samples were stored at $4^{o}C$ for a storage interval of 28 days, and analysis was performed at 0, 7, 14, 21, and 28 days. Proximate, oxidative, microbial, and sensory analyses were performed for freshly prepared and stored products. The results of the above parameters are reported.

Proximate parameters

Proximate parameters observed for Functional drinks in a combination of watermelon and cucumber juice are displayed in Table 2.

pH

The statistical results regarding the pH of functional drink are presented in table 1. These results indicate that the pH of functional drink decreas0es significantly ($p<0.05$) with storage time. The maximum pH was observed in T3 treatment with 70% watermelon and 30% cucumber juice at 0 Day, which is 4.91, and the lowest pH was observed in treatment T2 with 80% watermelon and 20% cucumber juice at day 21, which is 3.33. The change in the concentration of the cucumber in watermelon has a non-significant ($p>0.05$) effect on pH. The increased concentration of cucumber juice in functional drink significantly ($P<0.05$) decreased the pH value.

Titratable acidity

The results of titratable acidity of functional drink are presented in Table 2. These results show that the total acidity of the functional drink increases significantly ($p<0.05$) with storage time and non-significant ($p>0.05$) with different concentrations of cucumber and watermelon juice. The maximum acidity was observed in T4 at 28-day storage, and the lowest acidity was observed in T2 at 0-day storage. The maximum acidity was observed in treatment with 60% watermelon and 40% cucumber juice at 28 days storage, and the lowest acidity was observed in treatment with 80% watermelon and 20% cucumber juice at 0 Days. It shows the acidic nature of functional drink with storage. The study (25) also revealed that the relation between pH and total acidity is inverse, i.e., decreasing pH increases the total acidity.

Total solids

The results of the total solids of functional drink are presented in Table 2. These results indicate the non-significant ($p>0.05$) change in total drink solids with storage time and with change in concentration of cucumber juice in the functional drink. (29) reported similar results related to change in total solids of functional drink prepared by using orange, lemon, honey, and ginger.

Brix

The results indicated the non-significant ($p>0.05$) change in Brix of drink with storage time. Changing the cucumber juice concentration in drink indicates a significant decrease in Brix. The maximum Brix was observed in the T1 treatment at 28-day storage, and the minimum Brix was observed in the T4 treatment at 7-day storage. The studies performed (30), also proved that storage has very little or no effect on the sweetness level of functional drink.

Ash content

These results indicate the non-significant ($p>0.05$) change in ash content of drink with storage time, and a significant change was observed by changing the concentration of cucumber in the functional drink. The maximum value was observed in T1 treatment at 0 day, and the minimum value was observed in T4 at 0 day storage. Storage did not affect the inorganic material present in the functional drink. A similar study was also performed (27), which reported that ash content stays constant over time and was found same with 0, 10, 20, 30, and 40 days of product storage.

Table 2. Proximate composition of Functional Drink

Proximate Composition		T1	T2	T3	T4
pH	0 Day	4.913[a] ± 0.08	4.800[a] ± 0.10	4.933[a] ± 0.05	4.833[a] ± 0.05
	7 Days	4.266[bc] ± 0.05	4.466[b] ± 0.15	4.466[b] ± 0.15	4.233[bc] ± 0.15
	14 Days	4.116[cd] ± 0.02	3.333[g] ± 0.11	4.200[bc] ± 0.10	3.800[e] ± 0.10
	21 Days	3.820[de] ± 0.07	3.700[ef] ±0.1	3.833[de] ± 0.05	3.766[e] ± 0.11
	28 Days	3.640[ef] ± 0.05	3.466[fg] ± 0.05	3.7000[ef] ± 0.10	3.600[efg] ± 0.10
Total acidity (%)	0 Day	0.233[h] ± 0.01	0.143[i] ± 0.05	0.220[h] ± 0.01	0.273[h] ± 0.04
	7 Days	0.450[ef] ± 0.01	0.363[g] ± 0.01	0.436[f] ± 0.02	0.523[abcd] ± 0.04
	14 Days	0.476[cdef] ± 0.01	0.450[ef] ± 0.02	0.453[def] ± 0.01	0.546[abc] ± 0.02
	21 Days	0.496[bcdef] ± 0.01	0.453[def] ± 0.01	0.483[cdef] ± 0.03	0.560[ab] ± 0.01
	28 Days	0.513[abcde] ± 0.05	0.463[def] ± 0.01	0.540[abc] ± 0.04	0.576[a] ± 0.01
Total Solids (%)	0 Day	0.360[ab] ± 0.07	0.363[ab] ± 0.01	0.373[ab] ± 0.02	0.323[b] ± 0.07
	7 Days	0.363[ab] ± 0.02	0.380[ab] ± 0.04	0.370[ab] ± 0.07	0.320[b] ± 0.05
	14 Days	0.356[ab] ± 0.03	0.396[a] ± 0.02	0.370[ab] ± 0.01	0.313[b] ± 0.03
	21 Days	0.346[ab] ± 0.14	0.366[ab] ± 0.14	0.343[ab] ± 0.21	0.310[b] ± 0.01
	28 Days	0.343[ab] ± 0.01	0.363[ab] ± 0.01	0.320[b] ± 0.35	0.313[b] ± 0.07
Brix°	0 Day	9.700[a] ± 0.30	9.686[a] ± 0.08	8.666[b] ± 0.15	8.700[b] ± 0.10
	7 Days	9.666[a] ± 0.28	9.666[a] ± 0.05	8.800[b] ± 0.10	8.633[b] ± 0.05
	14 Days	9.766[a] ± 0.28	9.800[a] ± 0.10	8.700[b] ± 0.1	8.733[b] ± 0.115
	21 Days	9.866[a] ± 0.28	9.666[a] ± 0.15	8.966[b] ± 0.05	8.800[b] ± 0.10
	28 Days	9.933[a] ± 0.23	9.733[a] ± 0.11	8.666[b] ± 0.15	8.733[b] ± 0.15
Ash (%)	0 Day	0.061[a] ± 0.01	0.033[c] ± 0.01	0.007[d] ± 0.002	0.047[b] ± 0.01
	7 Days	0.063[a] ± 0.02	0.034[c] ± 0.002	0.009[d] ± 0.02	0.048[b] ± 0.001
	14 Days	0.063[a] ± 0.01	0.035[c] ± 0.002	0.008[d] ± 0.01	0.047[b] ± 0.001
	21 Days	0.064[a] ± 0.01	0.033[c] ± 0.001	0.008[d] ± 0.01	0.047[b] ± 0.02
	28 Days	0.063[a] ± 0.01	0.032[c] ± 0.000	0.006[d] ± 0.02	0.047[b] ± 0.02

Antioxidant potential

The results of antioxidants in a functional drink are presented in Table 3. These results indicate the non-significant ($p>0.05$) change in antioxidants in a drink with storage time. In contrast, a highly significant ($p<0.05$) result was observed with an increased concentration of cucumber juice in a functional drink. The minimum value was observed in the T1 treatment with 90% watermelon and 10% cucumber juice at 0 Days. The maximum value was observed in T3 treatment with 70% watermelon and 30% cucumber juice at 14 Day storage. (28) also observed similar results and reported the same storage effect on antioxidants.

Table 3. Antioxidant Potential of Functional Drink

Storage Days	Antioxidant potential (%)			
	T1	T2	T3	T4
0 Day	$1.390^{e} \pm 0.01$	$1.597^{cd} \pm 0.02$	$1.603^{b} \pm 0.03$	$1.604^{ab} \pm 0.01$
7 Days	$1.890^{e} \pm 0.02$	$1.598^{cd} \pm 0.05$	$1.604^{ab} \pm 0.04$	$1.604^{ab} \pm 0.01$
14 Days	$1.390^{e} \pm 0.04$	$1.599^{c} \pm 0.01$	$1.604^{ab} \pm 0.02$	$1.605^{ab} \pm 0.03$
21 Days	$1.391^{e} \pm 0.01$	$1.596^{d} \pm 0.02$	$1.603^{ab} \pm 0.03$	$1.605^{a} \pm 0.01$
28 Days	$1.391^{e} \pm 0.02$	$1.598^{cd} \pm 0.03$	$1.604^{ab} \pm 0.01$	$1.604^{ab} \pm 0.04$

Microbial analysis

Microbial analysis results of functional drink are shown in Table 4. These results indicate that the microbial load of the functional drink increases significantly ($p<0.05$) with storage time. The highest microbial load was seen in T1 at 28-day storage, and the lowest microbial load was seen in T4 at 0 days. The maximum level of bacterial load was observed in treatment with 90% watermelon and 10% cucumber juice at 28 days of storage. The minimum level of microbes was observed in treatment with 60% watermelon and 40% cucumber juice at 0 Days. This change in microbial content may be due to acidic conditions in a drink with storage. The relevant study was also provided by (31), who reported that no significant effect of storage time was observed on the microbial load of kunu drink solids.

Table 4. Microbial Analysis of Functional drink

Storage Days	Total Plate Count (CFU x 10^{1}/ml)			
	T1	T2	T3	T4
0 Day	$0.740^{l} \pm 0.05$	$0.823^{l} \pm 0.04$	$0.833^{l} \pm 0.05$	$0.633^{l} \pm 0.15$

7 Days	$2.543^{j} \pm 0.13$	$2.846^{i} \pm 0.05$	$2.203^{k} \pm 0.07$	$2.440^{j} \pm 0.04$
14 Days	$4.433^{h} \pm 0.04$	$4.360^{h} \pm 0.05$	$4.813^{g} \pm 0.03$	$4.353^{h} \pm 0.03$
21 Days	$6.413^{d} \pm 0.03$	$4.813^{g} \pm 0.09$	$5.393^{ef} \pm 0.03$	$5.213^{f} \pm 0.01$
28 Days	$8.336^{a} \pm 0.05$	$5.576^{e} \pm 0.04$	$6.643^{c} \pm 0.05$	$6.846^{b} \pm 0.05$

Organoleptic evaluation

Sensory scores of treatments over the storage time of 28 days are provided in Fig 1. The juice or drink is initially accepted based on its colour. Red is a functional drink colour that is also highly appealing. It was found that, to some extent, the hue changes and gets lighter with storage. Treatment T1, with a high percentage of watermelon, exhibits minimal colour change after storage. However, Treatment 4 with a high percentage of cucumber exhibits a pronounced colour shift due to the natural colour of the drink occasionally fading. Utilizing natural hues can stop it. The findings were comparable to those of a prior study on other beverages. Since artificial color was introduced during processing, they showed that storage time had no discernible impact on the samples' colour scores (29). When doing a sensory review, the product's scent is crucial. The scent influences our brain's decision to accept or reject a thing. The functional drink's components have a strong scent that imparts freshness to the drinker. Cucumber concentration increases and the results are significant ($p<0.05$). The storage duration of the functional drink produced non-significant ($p>0.05$) findings. Contradictory findings from earlier studies with fruit juices added with vegetable extracts and spices showed that the product's scent was not significantly impacted (32). The third aspect of the product's impression is taste. During the product's development, sweeteners are added to the product to improve its flavour profile since natural flavours can occasionally become so sour and bitter that they overpower the flavour of the entire product. The flavour of the functional drink changed significantly ($p<0.05$) when the concentration of cucumber changed. However, there were no statistically significant changes in taste with storage time ($p>0.05$). The results were conflicting, showing that the product's flavour was not considerably impacted (33). The aftertaste perceptual quality of the mouthfeel itself is constant in persons to the groups referred to as tingling and others. The results show that varying concentrations of cucumber juice and watermelon juice result in a significant ($p>0.05$) change in the mouthfeel or aftertaste of the product and a non-significant ($p>0.05$) change in the mouthfeel. Whereas the mouth feel of the product was not significantly impacted, contradicting findings were displayed (25). Consistency is the perceptual feature of texture that is constant throughout

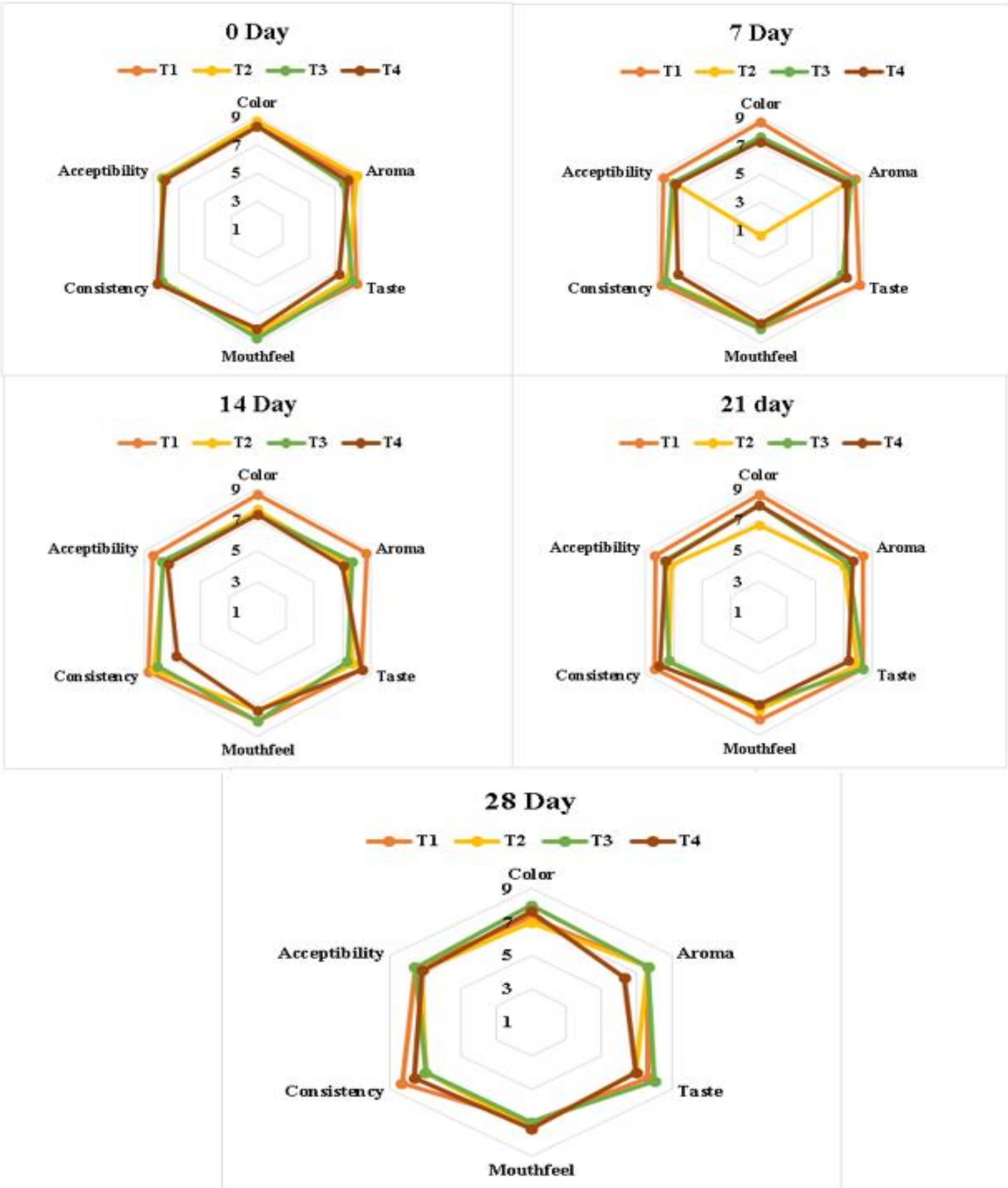

Fig 1. Sensory Scores of treatments over 28 days of storage of Functional Drink

various textural categories such as thick, thin, viscous, and others. Our brain reacts to flow rate and bases its analysis of product consistency on it. The functional drink showed a significant change ($p>0.05$) with storage time and a non-significant change ($p>0.05$) with various amounts of cucumber and watermelon juice. Overall acceptability was assessed based on all factors, including colour, taste, scent, consistency, and mouthfeel. ANOVA (analysis of variance) also revealed that the interaction between storage time and treatments had a significant impact on the outcome ($p<0.05$). When inconsistent findings were displayed, the product's acceptance was not significantly impacted (33). A key predictor of possible consumer preference is sensory evaluation of food, and the primary criterion used to assess the quality of beverages is hue and colour (34).

CONCLUSION

A combnination of watermelon and cucmber juice was developed to observe the nutritionally and organoleptically best treatment in the current study. This study revealed that adding cucumber juice significantly improved antioxidant activity and was observed maximum in T4 with a 40% concentration of cucumber juice. Ash contents, degree Brix and total solids were non significantly affected by the addition of cucumber juice. Antioxidant activity of functional drink was significantly affected by the addition of cucmber and was observed to improve with increasing concentration of cucumber. Microbilogical Evaluation depicted that bacterial load of samples was significantly reduced by the rising concentration of cucmber content. An organoleptic evaluation revealed that the treatment observed with the highest sensory scores was T1, with watermelon juice at 80% and cucumber juice at 10%.

Authors' Contributions

ZB Identified the research area, prepared the project, and performed all the research analytically. MRT interpreted the findings and assisted in the project's design. SWA set up a research facility and assisted with the writing. ZU Aided in the purchase and procurement of raw materials and data collection. MTA assisted in writing the article and final proofreading. MK supported in formulating the project and assisted in data collection. All the authors contributed equally and approved the final manuscript.

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DECLARATIONS

Ethics approval

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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