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Comparative assessment of natural and synthetic preservatives on shelf stability of spinach smoothies

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Abstract

In the present work, a novel, nutritious food product, a spinach smoothie, was developed. Due to microbial spoilage and quality degradation, the smoothie's limited shelf life was the main issue. For this purpose, natural preservatives (garlic filtrate, ginger filtrate, and a mixture of garlic + ginger filtrate) in comparison to synthetic preservatives (sodium benzoate, citric acid, and a combination of sodium benzoate + citric acid) were added to enhance the shelf life. Further, physicochemical analyses such as pH, specific gravity, antioxidant potential, mineral content, microbial content, and organoleptic properties were evaluated during storage of 28 days. Maximum antioxidant activities for DPPH (41.22 - 32.27%), FRAP (33.21 - 29.35%), TPC (27.11 - 20.84 mg/mL GAE), and vitamin C (31.65 - 27.37 mg/100 mL) were observed with garlic + ginger filtrate smoothie from initial to final day of storage. The highest total bacterial count was observed in the control sample of 30×10^3 - 530×10^3 CFU/mL, and the lowest count was seen in garlic-ginger filtrate smoothie of 12×10^3 - 20×10^3 CFU/mL during the 28-day storage. The maximum score for sensorial acceptability was observed with garlic + ginger filtrate. Therefore, bio-preservatives such as garlic + ginger filtrate at 1% had the highest efficacy level, and were economically suitable. Thus, combining natural preservatives like garlic and ginger with additional nutritional benefits could replace synthetic preservatives.

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Introduction

Spinach (*Spinacia oleracea* L.) is a cool-season annual green leafy vegetable that is economically significant and consumed worldwide. It belongs to the family Chenopodiaceae, a family of nutritional powerhouses, and is extensively consumed across the globe on account of its unique nutritional composition (Fiorito *et al*., 2019). Spinach has many therapeutic uses in addition to its food value. It provides health benefits such as increasing blood glucose regulation in people with diabetes, decreasing cancer risk, and enhancing bone health (Abu Al-Qumboz and Abu-Naser, 2019). Due to the global market demand, drinks, concentrated juices, smoothies, and purées are essential food products (Kumar *et al*., 2020). The value of minimally processed foods with longer shelf life and improved nutritional characteristics is growing daily (Akinola *et al*., 2018). The shelf life of "natural" (untreated) beverages is limited owing to deterioration caused by microbial activity (Nieva *et al*., 2022). In the food industry, preservatives are widely used to extend product shelf life and characteristics (Bruna *et al*., 2018).

Preservatives are commonly used in foods to extend their shelf life, and keep them stable over time, in varying quantities and concentrations. Food preservatives are ingredients that, once added to a food, can delay or prevent changes produced by the action of microorganisms or enzymes. Natural and synthetic preservatives are available (Olaniran *et al*., 2019b). Natural plant components rich in bioactive compounds, such as garlic and ginger, offer small-scale producers an alternative and cost-effective approach. They provide an alternate and potentially more nutritionally beneficial approach to preserving a product's shelf life (Bouarab Chibane *et al*., 2019). Combining zingerone, shogaols, gingerols, and volatile oils gives ginger its distinctive flavour.

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Ginger extends the shelf life of tomato paste by eight weeks, and contains antibacterial and antifungal properties (Olaniran *et al*., 2020a). Ginger is frequently used as a flavouring ingredient in drinks. The fresh garlic bulb contains allicin, alliin, and volatile oil, which are sulphur-containing chemicals. Gram-positive and Gram-negative bacteria resist garlic's antibacterial effects (Tiencheu *et al*., 2021). Garlic and ginger extracts improved the product's microbiological stability and sensorial acceptance. Garlic and ginger are natural preservatives with significant health benefits that can be used to replace synthetic preservatives like benzoates, sorbates, propionates, citric acid, metabisulphite, methylparaben, and others found in fruit juices, nectars, and smoothies (Jolayemi and Adeyeye, 2018).

Synthetic preservatives are chemical substances added to food during production, and have a wide range of antibacterial and antifungal action. They are increasingly used to prevent food from rotting or adversely affecting unnecessary chemical changes related to microbial degradation and oxidation (Kalpana and Devi Rajeswari, 2019). The sodium salt of benzoic acid, sodium benzoate (SB), is a bacteriostatic and fungistatic food preservative that is stable and water-soluble. It is used in food products such as smoothies, juices, pickles, fruit-based fillings, jams, salad dressings, and carbonated drinks (Olaniran *et al*., 2019a). The Food and Drug Administration (FDA) considers sodium benzoate a safe preservative (Khoshnoud *et al*., 2018). Citric acid, a naturally occurring organic acid in fruits and vegetables, has exhibited antibacterial activity against various bacteria, including *Escherichia coli*, *Salmonella* Typhimurium, and *Listeria monocytogenes* (Nieva *et al*., 2022). According to the FDA, citric acid is "Generally Recognised as Safe" (GRAS) (Mahe *et al*., 2021). In smoked fish, ginger powder has been contrasted with synthetic antimicrobials such as citric acid and potassium sorbate (Mancini *et al*., 2019). There have been some safety concerns regarding the use of synthetic preservative like sodium benzoate and citric acid, alone or in combinations. Some studies showed that in the presence of a high temperature and light, a carcinogenic compound benzene is formed in the soft drinks containing sodium benzoate and citric acid. Additionally, traces of some metals like iron and copper in tinned packs can lead to chemical reactions between sodium benzoate and ascorbic acid preservatives. The maximum doses for sodium benzoate and citric acid permitted for soft drinks are 150 mg/L (Ilhan *et al*., 2023).

Spinach is a type of vegetable with high mineral and phytochemical contents. So, there is a need for the availability and preservation of fresh, safe, and healthy spinach smoothies. Therefore, the present work aimed to increase spinach smoothie's shelf-stability by demonstrating the impact of natural (garlic and ginger) and synthetic (sodium benzoate and citric acid) preservatives. It is interesting to substitute synthetic with natural preservatives (bio-preservatives) because they do not negatively affect the consumers.

Materials and methods

The present work was conducted in the Food Analytical, Food Technology, Food Processing and Preservation, and Hi-Tech Central Material Analysis Laboratory, Government College Women University, Faisalabad, Pakistan. To investigate the end-product features, the process was carefully monitored.

Procurement of raw materials

Spinach leaves and all other ingredients required for smoothie formulation were purchased from the local market in Faisalabad. Sigma-Aldrich (Tokyo, Japan) and Merck supplied the chemicals and reagents (KGaA Merck Darmstadt, Germany).

Sample preparation

Spinach leaves were separated from their stems, and washed adequately under running tap water to remove dirt and other extraneous matter before being used for further processing. Spinach leaves were then chopped with a sharp knife.

Preparation of garlic + ginger filtrate

From a nearby market, fresh ginger and garlic were purchased. The garlic and the ginger were peeled and then separately sliced into cubes after being cleaned under running tap water. The chopped cubes (60 g) were weighed individually, and combined in a grinder. The filtrates were then placed into labelled, clean bottles once the suspensions had been filtered according to the method of Olaniran *et al*. (2019b).

Smoothie formulation

The spinach smoothie was prepared as described by Olaniran *et al*. (2020a) with some changes. Fresh leaves were sorted and separated from their stems. Thorough washing was done to remove any dirt particles. Next, leaves were chopped and weighed in a weighing balance. To make a semi-viscous liquid (smoothie), all of the ingredients for the smoothie were blended in an electric blender (smoothies). The ingredients' compositions (by weight) were spinach, mint, green chili, salt, lemon juice, and water. Natural preservatives (garlic and ginger filtrate), synthetic preservatives (sodium benzoate and citric acid), and their combinations were added at this point, bottled, and stored under refrigeration temperature (4 ± 1°C, 28 days). Smoothie was formulated as shown in Table 1.

Table 1. Treatment plan for shelf stability of spinach smoothies.

Ingredient (%)	**Treatment**			
N	**N_0S_0**	**N_1**	**N_2**	**N_3**
Spinach smoothie	100	99	99	99
Garlic filtrate	-	1	-	0.5
Ginger filtrate	-	-	1	0.5
S	**-**	**S_1**	**S_2**	**S_3**
Spinach smoothie	-	99.9	99.5	99.7
Sodium benzoate	-	0.1	-	0.05
Citric acid	-	-	0.5	0.25

N = natural preservatives; N_0S_0 = spinach smoothie (100%; Control); N1 = spinach smoothie (99%) + garlic filtrate (1%); N2 = spinach smoothie (99%) + ginger filtrate (1%); N3 = spinach smoothie (99%) + garlic filtrate (0.5%) + ginger filtrate (0.5%); S1 = spinach smoothie (99.9%) + sodium benzoate (0.1%); S2 = spinach smoothie (99.5%) + citric acid (0.5%); and S3 = spinach smoothie (99.7%) + sodium benzoate (0.05%) + citric acid (0.25%).

pH

Smoothie samples were analysed for pH using a digital pH meter per the standard method of Association of Official Analytical Chemists (AOAC, 2007). The pH was standardised using a 4.0 - 7.0 pH standard buffer solution after the sample was placed into a beaker, and the probe of the pH meter was then inserted into it. The pH was then recorded after the reading stabilised for 1 min on the screen of the pH meter.

Total titratable acidity

The titratable acidity was measured following the method of AOAC (2007). In a beaker, the sample was taken and titrated against 0.1 N NaOH. A conical flask was filled with 95 mL of distilled water and 5 mL of smoothie sample. Using one to two drops of phenolphthalein as an indicator, 10 mL of 0.1 N NaOH was poured into a burette, and titrated against the sample in the flask. It was titrated until a pink colour appeared. The result was shown as the percent equivalent of citric acid. Eq. 1 was used to calculate the total titratable acidity:

$$\%Acidity = \frac{0.064(equivalent\ weight\ of\ citric\ acid) \times Normality\ of\ NaOH \times Titer\ value}{Weight\ of\ sample(g)} \times 100$$

(Eq. 1)

Specific gravity

The specific gravity of each smoothie sample was determined using a hydrometer (which gives a particular gravity reading directly) following the standard procedure (AOAC, 2007). Water was used for rinsing the hydrometer after each reading. The liquid to be analysed was poured into a graduated cylinder, and the hydrometer was slowly lowered till it floated freely. The point when the liquid surface touched the hydrometer stem was noted. The specific gravity can be read directly due to the scale inside the hydrometer stem.

Colour

A colorimeter was used to measure the colour intensity of smoothie samples. The colour was determined by direct reading of the reflectance at different wavelengths of colour parameters L* (lightness), a* (redness/greenness), and b* (yellowness/blueness), as described by El-Saadony *et al*. (2020).

Antioxidant assay

DPPH free radical scavenging assay

The DPPH free radical scavenging activity was measured according to Lele and Kadam (2016). First, 5 mL of methanol was used to extract 1 g of sample. The extract solution was centrifuged for 15 min at 1,000 rpm as 3.9 mL of methanolic DPPH (0.025 g/L) and 0.090 mL of distilled water were used to make aliquots of 0.01 mL of supernatant. The resultant mixture was shaken with a vortex shaker before being incubated for 30 min in the dark. A UV-Vis spectrophotometer measured the mixture's absorbance to a blank at 700 nm. The results were calculated as a percentage decrease in absorbance compared to the absorbance of a reference DPPH solution. The inhibition of free radicals by DPPH was then calculated using Eq. 2:

$$\%DPPH = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad \text{(Eq. 2)}$$

Ferric-reducing antioxidant power (FRAP)

Ferric-reducing antioxidant power (FRAP) was analysed according to Aderinola (2018a). The reducing property of the extract was determined by taking 250 µL of sample into test tubes (with distilled water as blank), and 250 µL of 0.02 M of phosphate buffer (pH 6.9) was added with 250 µL of 1% KFeCN (potassium ferricyanide). It was then incubated for 20 min at 50°C before being added to 250 µL of 10% TCA (trichloroacetic acid), 200 µL of 0.1% freshly prepared $FeCl_3$ (ferric chloride), and 1 mL of distilled water. At 700 nm, the absorbance was measured.

Total phenolic contents

The total phenolic content (TPC) was measured spectrophotometrically by Folin Ciocalteu reagent assay as a standard follow-up method (Wijayanti *et al*., 2017). Briefly, 1 g of sample was suspended in a 20 mL of methanol (99.5%, v/v) mixture. After that, it was centrifuged for 15 min at 2,000 rpm. Next, 200 µL of the extract was mixed with 2 mL of 7.5% sodium carbonate and 2.5 mL of 10% Folin Ciocalteu reagent. After that, the reaction mixture was incubated at 45°C for 40 min, and the absorbance was measured with a UV-spectrophotometer at 700 nm. As a standard phenol, gallic acid was used. The TPC was then calculated using Eq. 3:

$$\%TPC = \frac{A_{blank} - A_{test}}{A_{blank}} \times 100 \quad \text{(Eq. 3)}$$

Vitamin C content

The vitamin C content of the smoothie samples was analysed using 2, 6-chlorophenol indophenol dye following the method of AOAC (2007). Briefly, 2 g of sample was extracted before being mixed with an acetic acid solution. In a 100 mL volumetric flask, a 50 mg standard ascorbic acid pill was dissolved in distilled water to create a vitamin C standard solution. After filtering the mixture, 2.5 mL of acetone was put into a conical flask, and 10 mL of the pure filtrate was added. By using an indophenol dye solution (2, 6-dichlorophenol indophenol), this filtrate was titrated for 15 s. The standard was also subjected to the same procedure. The vitamin C content was then calculated using Eq. 4:

$$Ascorbic\ acid\ (mg/g) = \frac{C \times V \times DF}{WT} \quad \text{(Eq. 4)}$$

where, WT = weight of sample (g), DF = dilution factor, V = volume of dye used for titration, and C = mg vitamin C.

Mineral analysis

The mineral contents were analysed following the method of AOAC (2007) using an atomic absorption spectrophotometer (Ca, K) and flame photometer (Na, Fe). Briefly, 5 g of sample was weighed, put in a crucible, and subjected to ashing in a muffle furnace at 550 - 600°C. Wet ashing can also be done. The ash was dissolved in 50 mL of 10% nitric acid (HNO_3) solution. The solution was heated on a hot plate for 15 min, and immediately filtered with filter paper into a 100 mL volumetric flask. The filtrate was prepared up to the 100 mL mark with distilled water, and analysed using an atomic absorption spectrophotometer. A reagent blank was prepared by boiling 50 mL of 10% HNO_3 solution for 15 min, filtered, and made up to the 100 mL mark of the volumetric flask.

Microbial analysis

Microbial analysis was done weekly for 28 days, utilising nutrient agar (Oxoid Limited, UK) for identification of total bacterial count incubated at 37°C for 24 h by using the standard pour-plate method according to Salfinger and Tortorello (2015).

Sensory evaluation

The sensory evaluation of the samples of the smoothie was accomplished with the aid of sensory evaluators using a nine-point Hedonic scale with ratings ranging from like significantly (9) to dislike highly (1) to evaluate attributes such as appearance, smell, taste, phase separation, and overall acceptability according to Aderinola (2018b). To avoid a carry-over effect, water was offered to each panellist for mouth cleaning after each sample was tested.

Statistical analysis

Data were statistically analysed by 3-way factorial, and differences in mean values were assessed using the Tukey's test. A value of $p < 0.05$ was used to indicate statistical significance.

Results and discussion

Physicochemical analysis

Effect of preservatives on pH of spinach smoothies during storage

For the product shelf-life extension, pH is more important and closely associated with processed beverage products' acidity. The present work showed a highly significant trend ($p < 0.05$) among treatments, preservatives, and storage days on the pH of all smoothie treatments. The effect of preservatives on treatments during storage was mentioned in Figure 1B. A maximum pH decrease was observed in the smoothie treatment with no added preservatives. Smoothie treatment preserved with ginger + garlic filtrate (N_3) showed a minimum reduction from (4.49 to 3.28), while synthetically preserved smoothie treatment (S_3) had a pH range from (4.12 - 3.12) during storage of 28 days. This indicated that pH showed a decreasing trend during storage. These findings were similar to an earlier study (Jolayemi and Adeyeye, 2018). They found that pH decreased during the 3-week storage of blended fruit smoothie by adding ginger (4.68 - 3.78) and garlic (5.72 - 4.17) as a preservative. The pH values decreased from 4.16 - 3.72 in the watermelon and soursop fruit blend during the storage of 10 days, as stated by Olalekan *et al*. (2017). With the addition of chemical and natural additives to cucumber juice, a decreasing trend in pH with the storage period has also been reported (El-Saadony *et al*., 2020). Prisacaru *et al*. (2023) demonstrated the impact of garlic and ginger powder in fruit juices, and showed pH values decreasing over time during nine days of storage. Lowering of pH occurs due to an increase in acidity during storage.

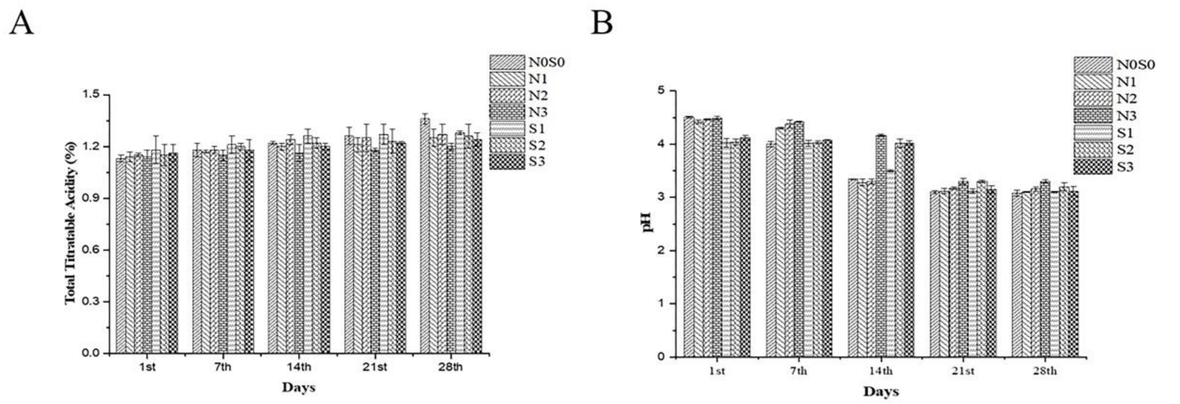

Figure 1. Total titratable acidity and pH. N_0S_0 = spinach smoothie (100%; Control); N1 = spinach smoothie (99%) + garlic filtrate (1%); N2 = spinach smoothie (99%) + ginger filtrate (1%); N3 = spinach smoothie (99%) + garlic filtrate (0.5%) + ginger filtrate (0.5%); S1 = spinach smoothie (99.9%) + sodium benzoate (0.1%); S2 = spinach smoothie (99.5%) + citric acid (0.5%); and S3 = spinach smoothie (99.7%) + sodium benzoate (0.05%) + citric acid (0.25%).

Effect of preservatives on titratable acidity (%) of spinach smoothies during storage

Titratable acidity indicates quality, as it is associated with a food's concentration of organic acids. Based on the titratable acidity, the storage stability of food is determined. A significant effect ($p < 0.05$) was noticed between days, treatments, and preservatives. Observed data of storage interval showed that total titratable acidity gradually increased with increased storage intervals. In Figure 1A, it is noted that the effect of preservatives concerning treatments and storage shows the highest titratable acidity (1.13 - 1.36%) in smoothie treatment that was stored without preservatives (N_0S_0) throughout storage. Smoothie treatment preserved with ginger + garlic filtrate (N_3) showed a minimum increase from 1.14 - 1.20%, while S_3 treatment showed a 1.16 - 1.24% increase during storage. The results observed during the storage of spinach smoothies were parallel to the concluded results of Kaur *et al*. (2018). They found that titratable acidity increased during the storage period. A similar increasing trend from 1.02 - 1.08% was also observed by Olaniran *et al*. (2020a) in chemically and synthetically preserved cashew apple juice. Haq *et al*.'s (2022) study also showed an increase in the titratable acidity in chemically preserved grape juice during 180 days of storage. Another study findings by Lele and Kadam (2016) showed an increase in titratable acidity of chemically preserved ash gourd carrot juice. The consistent increase in titratable acidity along storage period is vital in retaining the tartness of the product.

Effect of preservatives on specific gravity of spinach smoothies during storage

Specific gravity helps to assess the food value for consumers and processors. A significant difference ($p < 0.05$) was noticed regarding days, treatments, and preservatives. Observed data of storage interval showed that specific gravity gradually increased with storage intervals. The effect of preservatives concerning treatments and storage revealed that a maximum increase (1.25 - 1.71) was observed in N_0S_0 during the storage period. However, only slight increases of 1.27 - 1.47, 1.28 - 1.48, and 1.25 - 1.42 were observed in smoothie samples preserved with garlic, ginger, and a combination of garlic and ginger filtrate, respectively, as shown in Table 2. This could result from a synergistic effect of ginger and garlic. These results were similar to the earlier study of Olaniran *et al*. (2020b). They found that specific gravity increased during the storage of orange-watermelon juice. Another study (Odogwu, 2022) showed an increase in specific gravity up to 28 days of storage. Similar results were also reported by Olaniran *et al*. (2020b) in fruit juice blends using natural and chemical preservatives.

Table 2. Effect of preservatives, treatments, and storage days on specific gravity of spinach smoothies.

Preservative	Treatment	Storage Interval (day)				
		1st	7th	14th	21st	28th
N	N_0S_0	1.25 ± 0.01^{r}	1.42 ± 0.03^{h}	1.61 ± 0.05^{bc}	1.66 ± 0.02^{b}	1.71 ± 0.04^{a}
	N_1	1.27 ± 0.05^{op}	1.35 ± 0.01^{kl}	1.38 ± 0.02^{ij}	1.43 ± 0.04^{gh}	1.47 ± 0.03^{ef}
	N_2	1.28 ± 0.03^{o}	1.33 ± 0.02^{lm}	1.39 ± 0.01^{i}	1.44 ± 0.05^{g}	1.48 ± 0.06^{def}
	N_3	1.26 ± 0.02^{e}	1.30 ± 0.04^{mn}	1.36 ± 0.03^{j}	1.40 ± 0.01^{li}	1.42 ± 0.05^{h}
S	S_1	1.26 ± 0.04^{pq}	1.36 ± 0.06^{k}	1.46 ± 0.03^{ef}	1.48 ± 0.09^{def}	1.52 ± 0.02^{cd}
	S_2	1.27 ± 0.06^{op}	1.30 ± 0.02^{mn}	1.44 ± 0.01^{g}	1.49 ± 0.04^{de}	1.54 ± 0.03^{c}
	S_3	1.26 ± 0.07^{pq}	1.32 ± 0.05^{m}	1.41 ± 0.04^{hi}	1.46 ± 0.06^{ef}	1.50 ± 0.08^{d}

Values are mean ± standard deviation of triplicates. Means with different lowercase superscripts are significantly different. N = natural preservatives; N_0S_0 = spinach smoothie (100%; Control); N1 = spinach smoothie (99%) + garlic filtrate (1%); N2 = spinach smoothie (99%) + ginger filtrate (1%); N3 = spinach smoothie (99%) + garlic filtrate (0.5%) + ginger filtrate (0.5%); S1 = spinach smoothie (99.9%) + sodium benzoate (0.1%); S2 = spinach smoothie (99.5%) + citric acid (0.5%); and S3 = spinach smoothie (99.7%) + sodium benzoate (0.05%) + citric acid (0.25%).

Effect of preservatives on colour (L, a*, and b*) of spinach smoothies during storage*

The *L** value, an essential parameter in colour determination, represents the lightness of the sample where a low number (0 - 50) indicates darkness, and a high number (51 - 100) indicates lightness. A significant effect ($p < 0.05$) was noticed among days, treatments, and preservatives. The preservative effect between treatments and days showed that a minimum decrease (40.20 - 37.79) was noted in the naturally preserved smoothie sample (N_3), and a minimum value (30.04 - 25.21) was observed in S_1 (preserved synthetically) during storage. The value *a** represents red *vs*. green, where a positive number denotes redness, and a negative number indicates greenness. The ranges of *a** value of colour vary from ±60. The effect of preservatives concerning treatments and days showed the highest decrease (-2.50 - 7.87) in N_0S_0, and the lowest decrease (3.07 - 3.93) was observed in N_3 (garlic + ginger filtrate) during storage. The *b** value represents yellow *vs*. blue, with a positive value denoting yellowness and a negative value indicating blueness. The preservative effect among treatments and days showed that the highest decrease of 20.94 - 12.67 was seen in the control treatment, and the lowest value of 20.72 - 17.22 was noted in the smoothie sample preserved with garlic + ginger filtrate (N_3) followed by N_1 (20.07 - 16.10) preserved with garlic filtrate during the storage period. Results revealed that smoothie treatment preserved with ginger + garlic filtrate (N_3) showed a minimum decrease in colours (*L**, *a**, and *b**) during storage compared to other treatments. Similar findings were reported by Škegro *et al.* (2021), who observed colour degradation during storage. Moreover, Castillejo *et al.* (2016) also reported a decrease in colour in fresh red vegetable smoothies as anthocyanin contents showed degradation during storage, and a reduction in the colour of blueberry juice during storage of 10 days, as stated by Song *et al.* (2018). Moreover, Kiran (2023) demonstrated a similar observation in jamun juice.

Antioxidant assays

Effect of preservatives on DPPH free radical scavenging activity (%) of spinach smoothies during storage

DPPH is a free radical that accepts a hydrogen ion or electron for allocation in methanol or an aqueous solution. A stable free radical agrees with an electron or hydrogen radical to fit into an existing atom or molecule. A highly significant effect ($p <$ 0.05) was observed among days, preservatives, and treatments. Figure 2A shows the effect of interaction between preservatives, treatments, and days, with the highest free radical scavenging activity observed in N_3 (41.22 - 32.27%) preserved naturally, indicating that this blend had the highest efficiency and potential to inhibit the free radical formation, and lowest activity was found in N_0S_0 (39.24 - 17.18%) during 28 days of storage. The minimum decrease was seen in the natural and synthetically preserved smoothie sample compared to the non-preservative sample.

Further, the control sample was found to have lower DPPH activity and further reduced faster than treatment samples. The absence of phenolic compounds in the control sample leading to less inhibition of free radicals might be the primary reason for this, as stated by Sharma *et al.* (2020). This also corroborated Pandey *et al.* (2020), who showed that antioxidant activity decreased from 39.50 to 32.55% within 15 days of storage in carrot-based RTS beverages preserved with different preservatives. The antioxidant activity of orange and pomelo juice decreased from 300.23 to 160.14 µmol TE/100 g and 232.50 µmol to 101.76 TE/100 g, respectively, in the study of Chaudhry *et al.* (2023). Similar results were reported by Talasila *et al.* (2012) while studying the addition of chemical preservatives in prepared cashew apple juice. Lele and Kadam (2016) also reported a decrease in antioxidant activity by 14.43 to 6.43% during eight weeks of ash gourd carrot juice storage.

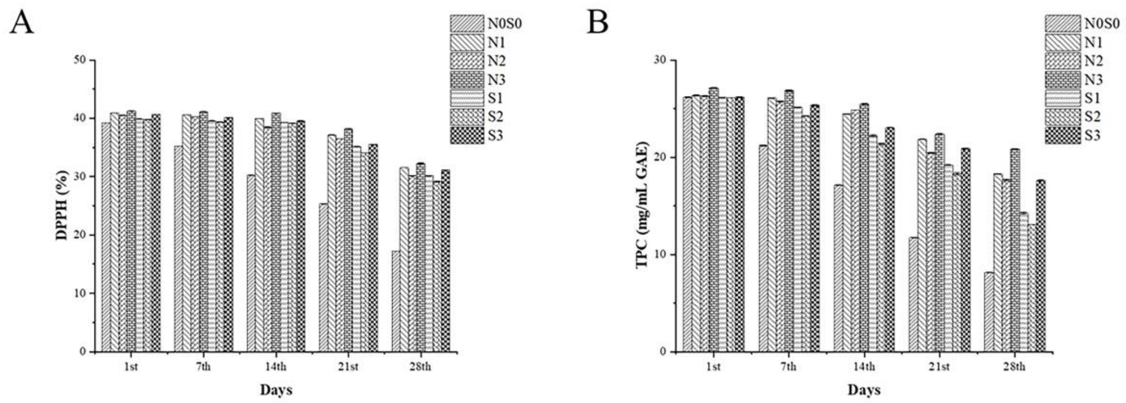

Figure 2. DPPH and TPC. N_0S_0 = spinach smoothie (100%; Control); N1 = spinach smoothie (99%) + garlic filtrate (1%); N2 = spinach smoothie (99%) + ginger filtrate (1%); N3 = spinach smoothie (99%) + garlic filtrate (0.5%) + ginger filtrate (0.5%); S1 = spinach smoothie (99.9%) + sodium benzoate (0.1%); S2 = spinach smoothie (99.5%) + citric acid (0.5%); and S3 = spinach smoothie (99.7%) + sodium benzoate (0.05%) + citric acid (0.25%).

Effect of preservatives on total phenolic contents of spinach smoothies during storage

Phenolic compounds are essential plant constituents with redox properties responsible for antioxidant activity. A highly significant result ($p <$ 0.05) was seen among days, preservatives, and treatments. The effect of preservatives in between treatments and days is exhibited in Figure 2B, in which the highest score (27.11 - 20.84 mg/mL GAE) is observed in N_3 (naturally preserved with ginger +

garlic filtrate) due to the presence of their essential oil, and the lowest score (26.17 - 8.13 mg/mL GAE) is observed in N_0S_0 during storage. A significant decrease was found in the TPC of control. Further, treatments demonstrated a slower rate of decrease than control, suggesting their oxidative stability for a longer duration. The order of TPC decrease in treatments throughout the storage period was ginger + garlic filtrate > garlic filtrate > ginger filtrate > sodium benzoate + citric acid > sodium benzoate > citric acid. Similar results were reported by Ahmed *et al.* (2021) while studying the addition of preservatives in blackberry juice. Moreover, degradation in total phenolic contents 38.515 - 29.727% of cucumber juice was seen by adding chemical and natural additives throughout six months of storage, as reported by El-Saadony *et al*. (2020). The decrease in antioxidant activity of fish finger by using natural preservatives was also reported by Abdel-Wahab *et al*. (2020).

Effect of preservatives on ferric-reducing antioxidant power of spinach smoothies during storage

The FRAP assay is a straightforward, repeatable, quick, and affordable technique that assesses the capacity of the total antioxidant system by measuring the reduction of Fe^{3+} to blue colour Fe^{2+}. A significant effect ($p < 0.05$) was noticed among days, preservatives, and treatments. The impact of preservatives concerning treatments and storage revealed that the highest activity, 33.21 - 29.35%, was noticed in smoothie sample preserved with natural preservatives (garlic + ginger filtrate), while S_3 treatments had a decreasing trend (32.22 - 25.45%), and minimum activity 31.02 - 11.02% observed in smoothie sample without preservatives throughout the storage period. The order of FRAP decrease in treatments throughout the storage period was ginger + garlic filtrate > garlic filtrate > ginger filtrate > sodium benzoate + citric acid > sodium benzoate > citric acid. This corroborated Tambunan *et al*. (2018), who showed that antioxidant activity decreased with time in tomato juice preserved with lemon juice. Similar results were reported by Ahmed *et al*. (2021) while studying the addition of preservatives in blackberry juice during the storage period of 60 days. Moreover, Deshaware *et al*. (2019) also reported a degradation in antioxidant activity of 60.4 - 52.08% during 90 days of storing bitter gourd juice.

Effect of preservatives on vitamin C of spinach smoothies during storage

In fruits and vegetables, vitamin C is naturally present in a significant amount. The presence of vitamin C is a sign of good nutritional qualities, and can act as an antioxidant. There was significant effect ($p < 0.05$) in the case of treatments, days, and preservatives. The effect of preservatives in between treatments and days showed the highest score of 31.65 - 27.37 mg/100 mL in sample preserved with a combination of ginger garlic filtrate, and the lowest score of 30.21 - 11.04 mg/100 mL in samples stored without any preservatives during storage period. Results showed that vitamin C contents decreased throughout the storage period. Similar results were reported by Ali and Muhammad (2021), who observed a decrease in vitamin C content, 43.62 to 13.50%, in mango loquat blended pulp during storage of 180 days due to its sensitivity.

A decrease in vitamin C content in tomato juice using chemical preservatives was also reported by Kabir *et al*. (2019), during the storage period of 30 days. Olalekan *et al*. (2017) also observed a decrease in vitamin C content during the storage of watermelon and soursop fruit blend by utilising preservatives during the storage period of 10 days.

Effect of preservatives on mineral contents of spinach smoothies

The mineral (Ca, Na, K, and Fe) composition of the spinach smoothies preserved with preservatives slightly decreased during storage.

Calcium and iron

Calcium is a mineral most frequently linked to strong bones and teeth. Calcium also significantly impacts blood clotting, muscular contraction, regular heartbeat, and nerve activity. The calcium content of spinach smoothies presented a highly significant trend ($p < 0.05$) among days, preservatives, and treatments. The effect of preservatives with treatments and storage showed that the maximum calcium content of 97.83 - 89.83 mg/mL was observed in N_3 (preserved naturally by using garlic and ginger filtrate combination), which might have been due to calcium contribution from garlic and ginger. The minimum content of 97.02 - 50.03 mg/mL was found in N_0S_0 (stored without any preservatives) during the storage period. Regarding synthetically preserved smoothies, S_3 yielded from

97.23 - 85.23 mg/mL during storage. It was observed that the calcium contents decreased throughout the storage period.

Iron is needed to make red blood cells. Iron is also a component of haemoglobin, the pigment in red blood cells, which binds to oxygen to facilitate its transportation from the lungs through the arteries to all of the body cells. A significant effect ($p < 0.05$) was noticed between days, treatments, and preservatives. The effect of preservatives with treatments and storage showed that the maximum iron content of 2.78 - 2.72 mg/mL was observed in N_3 (preserved naturally by using garlic and ginger filtrate combination), while S_3 exhibited 2.72 - 2.58 mg/mL, and the minimum content of 2.70 - 2.30 mg/mL was observed in N_0S_0 (stored without any preservatives) at the last day of storage period. It was found that a gradual increase in iron content was observed in those treatments preserved with natural preservatives as compared to synthetic preservatives. This agreed with Fagbohun and Ogundahunsi (2019), who showed that calcium content decreased in melon seeds during storage of 24 weeks. A similar decreasing trend was also observed by Jolayemi and Adeyeye (2018) in fruit smoothies preserved with preservatives.

Sodium and potassium

The human body needs a small amount of sodium to transmit nerve impulses, contract and relax muscles, and maintain an adequate balance of water and minerals. A significant effect ($p < 0.05$) was noticed between days, treatments, and preservatives. The interaction between preservatives, treatments, and days was observed with the highest sodium content found in N_3 (77.84 - 71.45 mg/100 mL) preserved naturally (using garlic + ginger filtrate), and the lowest content found in N_0S_0 (77.02 - 43.21 mg/100 mL) throughout the storage period.

Potassium can be obtained as a dietary supplement and naturally found in many foods. Its primary function in the body is maintaining proper fluid levels inside our cells. The present work showed a significant difference ($p < 0.05$) among treatments, preservatives, and storage days. The effect of preservatives between treatments and storage was obtained where the maximum content (557.27 - 545.85 mg/100 mL) was observed in N_3 (preserved naturally with garlic + ginger filtrate), while S_3 showed 557.24 - 540.27 mg/100 mL, and the minimum content was observed in N_0S_0 (557.02 - 460.04 mg/100 mL) during storage. It was observed that sodium and potassium contents decreased during the storage period. This agreed with Fagbohun and Ogundahunsi (2019), who showed that mineral contents decreased in melon seeds during storage of 24 weeks. Ajayi and Bankole (2020) also reported a decrease in mineral contents when using natural preservatives in tiger nut non-dairy milk during 28 days of storage.

Microbial analysis

Effect of preservatives on total bacterial count of spinach smoothies during storage

Microbial analysis is important in food safety. Results specified a significant difference ($p < 0.05$) among days, preservatives, and treatments. The preservative effect between treatments and days showed non-detectable total bacterial count in freshly prepared smoothie samples, with and without preservatives, on the first day of storage. The total bacterial count gradually increased from (30×10^3 - 530×10^5 CFU/mL) in N_0S_0 throughout the storage, and N_3 (garlic + ginger filtrate) showed non-detectable microbial count during the first three weeks of storage and minimum total bacterial count (12×10^3 - 20×10^3 CFU/mL) were seen during last two week of the storage period in comparisons to all other treatments. It was observed that the total bacterial count increased throughout the storage interval. The present work also found that using garlic and ginger as a preservative was the most effective since it significantly reduced the growth of microorganisms. These results were similar to an earlier study (Olaniran *et al*., 2019b), which reported that microbial content increased from 90×10^2 - 20×10^3 during the 5-week unpasteurised apple cashew juice storage. Another study (Akinola *et al*., 2018) showed an increase in total bacterial count in orange juice during 3-week storage using chemical preservatives. Similar results were reported by Pandhare *et al*. (2018) in pineapple juice stored with natural preservatives. Al Noman *et al*. (2023) reported the antibacterial efficacy of garlic and ginger crude extract against multidrug-resistant (MDR) poultry pathogens.

Sensory evaluation

Parameters of sensory evaluation are directly related to consumer acceptance of specific food products. Approval and rejection of the product depend on the sensory evaluation score. The results of the sensory assessment of the smoothies by panellists on appearance, taste, smell, phase separation, and overall acceptability are presented in Figure 3. Spinach smoothies stored with or without preservatives (natural and synthetic) were given a sensory score based on 9-point hedonic scale. Results indicated that reduction in the sensory score of treatments was preserved with or without preservatives. Control treatment deteriorated after third day of storage.

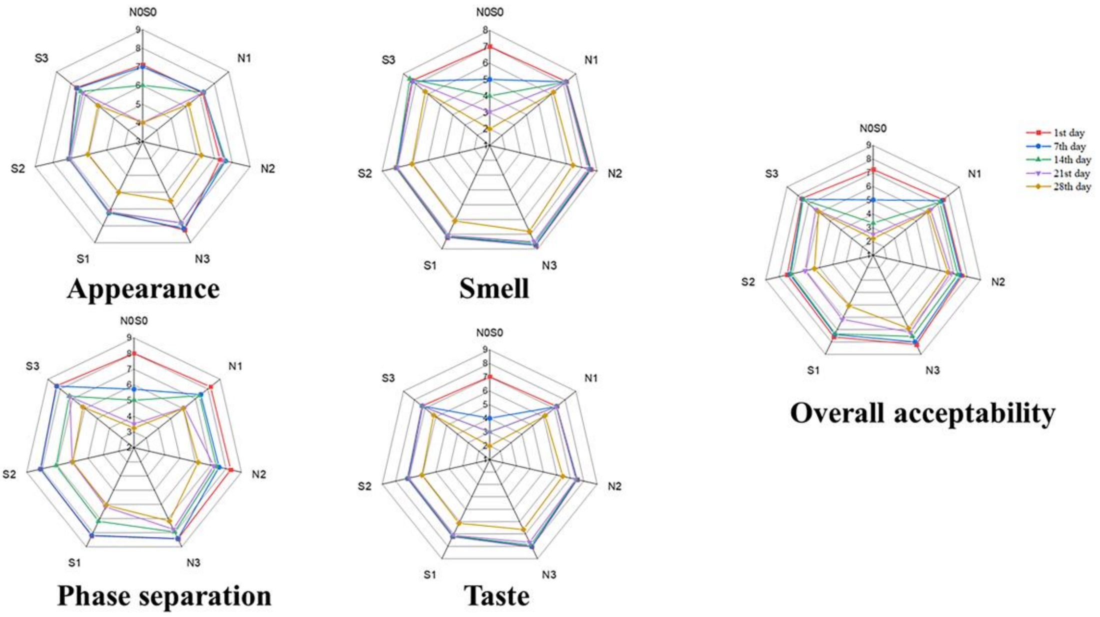

Figure 3. Appearance, smell, phase separation, taste, and overall acceptability. Values are means of 12 panellists ± standard deviation. N_0S_0 = spinach smoothie (100%; Control); N1 = spinach smoothie (99%) + garlic filtrate (1%); N2 = spinach smoothie (99%) + ginger filtrate (1%); N3 = spinach smoothie (99%) + garlic filtrate (0.5%) + ginger filtrate (0.5%); S1 = spinach smoothie (99.9%) + sodium benzoate (0.1%); S2 = spinach smoothie (99.5%) + citric acid (0.5%); and S3 = spinach smoothie (99.7%) + sodium benzoate (0.05%) + citric acid (0.25%).

Appearance is the primary factor that motivates a consumer to try a product in the first place. It has been evaluated from the results that there was a highly significant difference ($p < 0.01$) observed among days, preservatives, and treatments. There was a slight change in appearance in both naturally and synthetically preserved smoothie treatments. Effect of preservatives with respect to treatments and storage revealed that the sample preserved with 1% ginger-garlic had the highest score of 8.25 - 6.51 seen in N_3, and the lowest score (7.12 - 4.02) was observed in N_0S_0 (without preservatives) from first day of storage to final day of storage. In terms of appearance, the order of preference of the smoothie was garlic + ginger filtrate > ginger filtrate > garlic filtrate > citric acid + sodium benzoate > citric acid > sodium benzoate.

Taste is another determinant of food choice. The effect of preservatives concerning treatments and storage revealed that the highest score (8.05 - 6.65) was observed in N_3 (garlic + ginger filtrate), whereas the lowest score (7.05 - 2.01) was observed in N_0S_0 (without preservatives) throughout the storage period. Regarding synthetically preserved smoothie treatment, S_3 scored 7.28 - 6.20 throughout the storage period.

For smell, a maximum score (7.74 - 6.80) was found in N_3 (preserved naturally with garlic + ginger filtrate), while synthetically preserved smoothie (S_3) had 7.32 - 6.24 score, and a minimum score was observed in N_0S_0 (7.02 - 2.01) till last day of storage.

Phase separation is an important parameter that shows the division of solid and liquid particles. The preservative effect among treatments and days showed that the maximum score (8.44 - 7.18) was observed in N_3 (naturally preserved smoothie sample with ginger + garlic filtrate) and the minimum score (8.05 - 3.25) was found in N_0S_0 throughout storage.

Overall acceptability indicates the acceptance level of products in appearance, taste, smell, and phase separation. So, it is the compiled effect of all the crucial parameters of the product. Regarding overall acceptability, the best smoothie treatment was the one preserved with 1% ginger + garlic filtrate, showing a maximum score (8.21 - 6.90) throughout the storage period. This could have been due to ginger's pleasant fragrance to balance the garlic's strong flavour by mixing an equal volume of garlic and ginger filtrates. These changes can be attributed to specific chemical reactions, the extent to which they depend on storage time and temperature, as reported by Fernández-Vázquez *et al*. (2014). Mancini *et al*. (2019) also demonstrated a similar observation. However, Borode (2017) found that the preference for soymilk with preserved garlic was low. Kiran (2023) reported the impact of different preservative levels and packaging material in jamun juice, exhibiting that overall acceptability was decreased during storage.

Conclusion

The spinach smoothie was kept cold for 28 days while the present work assessed the effects of natural and synthetic preservatives on nutrients and quality stability. The preservative type and storage period significantly influenced the smoothie's nutrient and quality stability. With additional nutritional benefits, powerful natural preservatives like garlic and ginger can replace synthetic preservatives like citric acid and sodium benzoate. The present work also found that garlic-ginger filtrate at 1% had the highest efficacy of all the bio-preservatives, and was economically suitable.

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