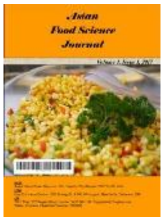

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Assessment of Nutrient and Storage Stabilizing Potential of Ginger and Garlic on Composite Fruit Smoothies

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Authors' contributions

This work was carried out in collaboration between both authors. Author OSJ designed the study, supervised the project, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OAA managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The study aimed at demonstrating the influence of natural spices (ginger and garlic) and storage conditions on nutritional, microbial and organoleptic characteristics of composite fruit smoothies.
Study Design: Completely Randomized General Factorial Design and Multivariate Pattern Recognition Technique were used to achieve the study.
Place and Duration of Study: The study took place at the Department of Food Science and Technology, Federal University of Technology, Akure between January and May 2018.
Methodology: Smoothies were produced from the blends of pineapple, watermelon and banana, treated with garlic and ginger, bottled, pasteurized and stored for 21 days to obtain a total of 12 samples and 2 control (prior to storage). Chemical parameters such vitamin C, total phenol, total sugar, moisture and crude fibre contents, titratable acidity, pH, minerals (calcium, sodium, potassium and magnesium), microbiological and sensory qualities were monitored weekly throughout the storage periods.

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Results: Univariate data analysis results showed type spices as the most significant factor (p = 0.05) with respect to variables considered. There was a decline in vitamin C, titratable acidity and pH as storage time advanced. Total and reducing sugars and ^{o}Brix increased with storage while other chemical parameters did not change significantly. Garlic and ginger extracts enhanced microbial stability and contributed to better sensory acceptability of the samples. Principal component analysis (PCA) multivariate model further showed the distinct trends between the impacts of garlic and ginger on the score and loading plots. Smoothies enriched with garlic had comparatively higher TPC, crude fibre, pH, vitamin C, sodium and its ratios with potassium and magnesium, within the first week, total and reducing sugars on the third week. Conversely, ginger enriched smoothies were high in acidity, sensorial scores, magnesium and potassium in the first week of storage and ^{o}Brix afterward.

Conclusion: Garlic and ginger are potent natural preservatives with additional nutritional benefits that could replace synthetic additives.

Keywords: Smoothie; natural preservatives; storage stability; principal component analysis.

1. INTRODUCTION

The nutritional and health benefits of continuous consumption of adequate amount of fruits and vegetables are generally well recognized. Due to seasonal gluts and postharvest losses, fruits are yet to become readily available throughout the year especially in developing countries. Countries with less attention to effective postharvest handling and storage facilities can record as high as 65% loss of fruits produced [1]. However, processing of fruit into fruit juice classified as those without pulp (“clarified” or “not clarified”) and those with pulp (“pulps”, “purees”, and “nectars”) and dry fruits have become prominent as a way of extending freshness and availability of fruit especially during off-season [2]. The comparative evaluation of chemical properties of fresh fruit and its secondary products did not show any significant different. Nevertheless, the desires for freshness, health consciousness and diversity in consumer taste have recently led to the emergence of “fruit smoothie” [3]. This is a kind of fruit puree relatively thick in consistency; obtained from blending the same or different kind of fruits together with pulp with or without other adjuncts and normally consumed fresh or preserved under refrigerator within a short time [4]. Smoothie is one of the means of extending the storage stability of highly perishable fruits and vegetables with improved aesthetic properties as an added advantage.

Composite smoothie synergistically combines the sensory and nutritional properties of two or more fruits and other ingredients added. Therefore, the degree of acceptability of the resultant products may be higher than that of individual fruits. In addition, smoothie is recently recognized as an emerging functional food with maximum flexibility for nutritional adjustment [5]. Fruits high in acidity content and astringency have a limited scope for table consumption even when rich in functional ingredients. Combination of two or more fruits creates novel flavor and taste and consequently leading to expansion and diversification in fruit and vegetable industry and household consumption [6]. The consumption of fruit smoothies had also been seen as one means of achieving the target of five servings of fruits and vegetables per day by consumers [7]. The storage stability of fruit smoothie remains a major challenge due to its susceptibility to spoilage [8]. In view of this, non-thermal and mild thermal pasteurization treatments were found effective in the preservation of fruit smoothie [9,10,11]. Satisfactory extended shelf life was obtained from the application of these sophisticated techniques. However, application of these methods is constrained by economic capacity of producers and willingness of consumers to pay slightly more for better quality.

An alternative and possibly more nutritionally beneficial way of ensuring sustainable prolonged storage life of smoothie is application of natural plant materials such as garlic and ginger that are rich in bioactive compounds. Nutritional and preservative benefits of these natural ingredients have been adequately studied. Vwioko et al. [12] observed a significant reduction in microbial counts and improved overall storage quality of soursop juice treated with a mixture of ginger and garlic. Similarly, Fijelu, Yanshun, Qixing & Wenshui, [13] investigated the storage stability effect of both spices on fish period, with satisfactory outcome. Hence, inclusion of ginger (*Zingiber officinalae*) and garlic (*Allium sativum*) which are both popular for their distinct sharp and hot flavor due to an oily organic substance known as *gingerol* in the case of ginger and *allicin* in the case of garlic could enhance both sensory and storage qualities of fruit smoothies. Among the bioactive compounds found in ginger and garlic, flavonoids and flavanols are specifically bactericidal [14,15]. Therefore, this research aims at investigating the impact of natural preservatives (ginger and garlic) and storage conditions (cold and ambient) on the physicochemical, quality characteristics and storage stability of fruit smoothie prepared from a combination of banana (*Musa spp*.), pineapple (*Ananas comosus*), and watermelon (*Citrullus lanatus*). The combined effects of the factors were further elaborated using Principal Component Analysis – an unsupervised pattern recognition multivariate data analytical technique.

2. MATERIALS AND METHODS

2.1 Sources of Raw Materials

Wholesome banana (*Musa sapientum*), watermelon (*Citrullus vulgaris*), pineapple (*Ananas comosus*), were obtained at their optimum maturity and ripening stage, from NIHORT (National Horticultural Research Institute) Ibadan, Oyo State Nigeria. The natural preservatives: Ginger (*Zingiber officinale*) and garlic (*Allium sativum*) were purchased from shoprite in Akure, Ondo State Nigeria. Fruits were processed within two days of arrival in the laboratory

2.2 Preparation of Smoothie and Selection of Preferred Blends

Fruits were thoroughly washed before peeling with a cleaned sharp knife. The fruits were chopped into small bits and chunks to facilitate blending. The sliced fruits were blended separately using a domestic electrical blender (Sharp EM-125L, China) with a volume of 1.5 Liters. The blended fruits were mixed based on different proportions (banana 20-30%; pineapple 20-40%; and watermelon 30-50%). Preliminary preference test was conducted on the smoothie samples to select the most preferred blends based on appearance, flavor, taste, mouth feel and overall acceptability as described by Wang et al. [16]. The blend containing 40%, 30% and 30% watermelon, banana and pineapple, respectively was found to be comparatively more preferred to other blends in virtually all the sensory parameters considered.

2.3 Preparation and Incorporation of Natural Preservatives

Ginger and garlic were peeled and 50 g each of both the ginger and garlic were blended separately using an electrical blender (Sharp EM-125L (W), China) with 100 ml distilled water. The mixture was then filtered to remove the fibrous content of the mixture. The clear solution was then added to the most preferred smoothie blend in the ratio of 1:100. The enriched smoothies were bottled and pasteurized at 72°C for 15 minutes batch-wisely using an electrical water bath (Memmert, China). The samples were cooled after pasteurization and stored under refrigeration (4°C) and ambient (25°C) conditions for a period of three (3) weeks. The two samples (garlic-enriched and ginger-enriched smoothies) prior to storage constitute the reference samples (at week 0). A total of 14 samples were obtained at the end of 3 weeks storage time using a 2^2-general factorial design with two replicates in each week. Effects of preservative and storage conditions on chemical and quality characteristics of the smoothie samples were determined.

2.4 Chemical Properties Analysis

2.4.1 Moisture, crude fibre and ascorbic acid contents

AOAC Official Methods of Analysis [17] was used in the determination of moisture content and crude fibre of smoothie samples. Ascorbic acid content was determined using a standard solution of indophenols (dye solution) according to the procedures described in AOAC [18] and reported in mg/100g as shown in the equation below:

Ascorbic acid (mg/100 g) = (Titre value x dye factor x volume (100 ml)/ aliquot of extract x volume of sample) x 100

2.4.2 Total and reducing sugar contents

Total and reducing sugars were estimated by Lane and Eynon's method as reported by Ranganna [19].

2.4.3 Total phenol content

Total phenol content (mgGAE/kg) was determined by measuring the color developed by Folin-Ciocalteu assay at 765 nm using modified method of Divya et al. [20].

2.4.4 Mineral analysis

The procedure described by AOAC Official Methods of Analysis AOAC [17] was adapted to determine the following minerals: Sodium (Na), calcium (Ca), potassium (K) and magnesium (Mg).

2.5 Physicochemical Properties Analysis

2.5.1 pH, Titratable acidity (TA) and total soluble solid (°Brix) determination

The degree of acidity and alkalinity of the prepared smoothies were determined with a Digital Handheld Thermal Orion 868 pH meter (Thermo Fisher Scientific, Inc., Massachusetts, USA) at room temperature. The meter was calibrated with buffers at pH 4.0 and 7.0 prior to usage [21]. Titratable acidity was determined by titrating diluted samples against 0.1 N sodium hydroxide using phenolphthalein as indicator and the result was expressed as percentage equivalent of malic acid, while total soluble solid was measured using a hand refractometer (Erma, Japan).

2.6 Microbial Analysis

Preservative potentials of the enrichments (garlic and ginger) on the samples were estimated by enumerating the total viable growth for bacteria and fungi using standard plate counts procedure. The growth was reported as colony forming unit per milliliter according to Swanson et al. [22].

2.7 Sensory Evaluation

Variations in the sensorial characteristics of the samples were evaluated weekly. A total of 30 panelists (14 male and 16 female) who were affiliates or members of the Food Science and Technology Department with prior knowledge of food sensory assessment, were selected. Differences in appearance, flavor, taste and overall acceptability of the samples were reported.

2.8 Statistical Data Analysis

The significance of factors (preservatives and storage conditions) and their interactions were determined by Analysis of Variance (ANOVA) at 95% confidence levels using Minitab Statistical Package (Minitab 16.0, Minitab Inc., State College, USA). In order to further explore some underlying trends and patterns of relationship between factors and variables, a PCA model was built on the data. It is the most commonly applied multivariate technique with the ability to transform data matrix linearly into a reduced dimension space (principal components - PCs) for better visualization of data clustering. The reduction in the data dimensions does not lead to any loss of information as maximum amount of variance in the original data set is preserved [23]. PCA generates score and loading plots for data interpretation. The score shows the position of the observations (smoothies) as described by the measured variables (loading plot). The loading shows the extent to which each variable contributes to the principal components [24]. Data matrix **X** of size 14 x 21 consisting of 14 smoothie samples (**n** observations) and 21 measured variables (**k** variables) was analyzed. The variables include: total phenol content (TPC), moisture, crude fibre, pH, titratable acidity, total sugar, reducing sugar, °Brix, sodium, potassium, calcium, magnesium , four mineral ratios (Ca/K, Na/K, Na/Mg and Ca/Mg) and four sensory parameters. SIMCA software (ver. 13.0, Umerics Umea, Sweden) was used for the PCA modelling and the results were reported as number of PCs, total explained variance and position of observations and variables on one dimensional hyperplane (score and loading plots).

3. RESULTS AND DISCUSSION

3.1 Influence of Preservatives and Storage Techniques on Chemical Properties

Effects of preservatives and storage conditions on the chemical characteristics of the samples were monitored on weekly basis over a storage period of three weeks. There was no significant difference in the smoothies' moisture contents with respect to the main effects ($P > 0.05$) as shown in Fig. 1A. However, garlic-enriched smoothies showed a slight decline in moisture within the first week. This decline was slightly more under ambient than cold condition. The relatively constant moisture content during storage may be due to the slight increase in soluble content. Similar inverse relationship has been reported on fruit blends during storage [25]. Quantitatively, garlic-enriched smoothies stored under cold temperature had higher moisture content. The slight temperature gradient between the sample and storage environment may be responsible for moisture loss during ambient condition. The significance of preservative on moisture content of smoothies was not pronounced throughout the storage period as the moisture contents were within the expected current 87.22% USDA standard for smoothies [26]. Similarly, crude fibre did not show any significant difference with respect to preservatives and storage conditions. Garlic-enriched smoothies had fibre ranged between 0.83 – 0.86% and 0.82 – 0.92% for ambient and cold storage, respectively, whereas ginger-enriched samples were between 0.76 – 0.86% and 0.82 – 0.87%. The contributions of preservatives in raising the crude fibre contents of the smoothie were apparent when compared the results to literature [27]. Considering 5 serving per day - WHO recommendation for fruits and vegetables which is equivalent to 400 g/day; the sample could approximately account for 15% of the recommended daily nutrient intakes (RNIs) for fibre (25 g/day). According to The Code of Federal Regulations [28], food products capable of supplying up to 20% or more of the recommended daily intakes for fibre are considered as “excellent sources of fibre’’.

There was a notable decline in vitamin C content with respect to storage time and type of preservative ($p < 0.05$). Vitamin C showed more variation with storage time, than virtually all other parameters (Fig. 1C). However, it seemed that vitamin C was comparatively more preserved under cold temperature than ambient conditions. There was about 60% loss in vitamin C by the end of 3rd week in ambient stored smoothies as compared to about 40% for those under low temperature. A similar magnitude of vitamin C depletion during storage was reported [29] on fruits blends. Ascorbic acid is highly susceptible to oxidation during long time storage. Being a potent antioxidant, factors such as oxidative enzyme, molecular oxygen, alkaline pH, metal ions, and temperature influence the fluctuation of vitamin C in fruits and vegetables [30]. The vitamin C contents of fresh smoothie samples from both categories (garlic and ginger-enriched) could provide approximately more than 120% of the recommended nutrient intake requirement for adults and about 70% that of lactating women. These two populations constitute the highest vitamin C RNIs according to FAO & World Health Organization, [31]. These percentages reduced significantly as the storage weeks advanced. This is in agreement with the common perception that fruits and vegetables are best utilized for maximum vitamin C content, at fresh or minimally processed conditions [32]. However, smoothies treated with garlic had higher initial vitamin C than those with ginger. This could possibly be as a result of the protective effect of bioactive compounds in garlic against thermal degradation of vitamin C during pasteurization.

Total and reducing sugar contents of the samples were found significant with respect to the main effects (preservative and storage conditions). Generally, there was close to a linear increase in total and reducing sugar contents with storage time, such that at the end of 3rd week, smoothies had close to three times the initial total sugar content (Fig. 2A-B). However, there was no significant difference between cold and ambient stored smoothies with respect to sugar. Earlier study of Karav et al. [33] indicated a slight increase in total sugar after two months of cold storage of pomegranate fruit juice. In the same vein, Akusu et al. [34] observed a significant increase in reducing sugar of apple juice during long time storage. Prior to storage, total phenol contents of garlic-enriched smoothies were significantly higher than ginger-enriched (Fig. 2). The reason may be due to higher folin-reducing capacity of garlic extract than that of ginger. Cold and ambient-stored smoothies exhibited the same pattern with slight variation especially with garlic-enriched (Fig. 2C). This pattern of change may be as a result of oxidative protection of the samples by phenols leading to the depletion of total phenols and other related compounds. The disproportionate reduction in ascorbic acid with storage time as compared to that of total phenol content may be as a result of higher antioxidant potency of vitamin C in fruits smoothie than phenolic compounds. In order to maximize total phenol content of enriched-smoothie, extended storage time is not encouraged (Fig. 2). However, cold-stored smoothies had slightly better retention of total phenol content than the ambient. This was in agreement with a recent report [35] on different fruit juices and smoothies.

The results of mineral composition of the samples were presented with respect to magnesium, calcium, sodium and potassium. Smoothies enriched with different preservatives were not significantly different in magnesium at the initial stage prior to storage (Table 1). There was no remarkable change in this mineral even after storage at cold and ambient temperature. Garlic-enriched smoothies had slightly higher calcium content (9.48 mg/100 g) than that of ginger-enriched samples (7.69 mg/100 g).

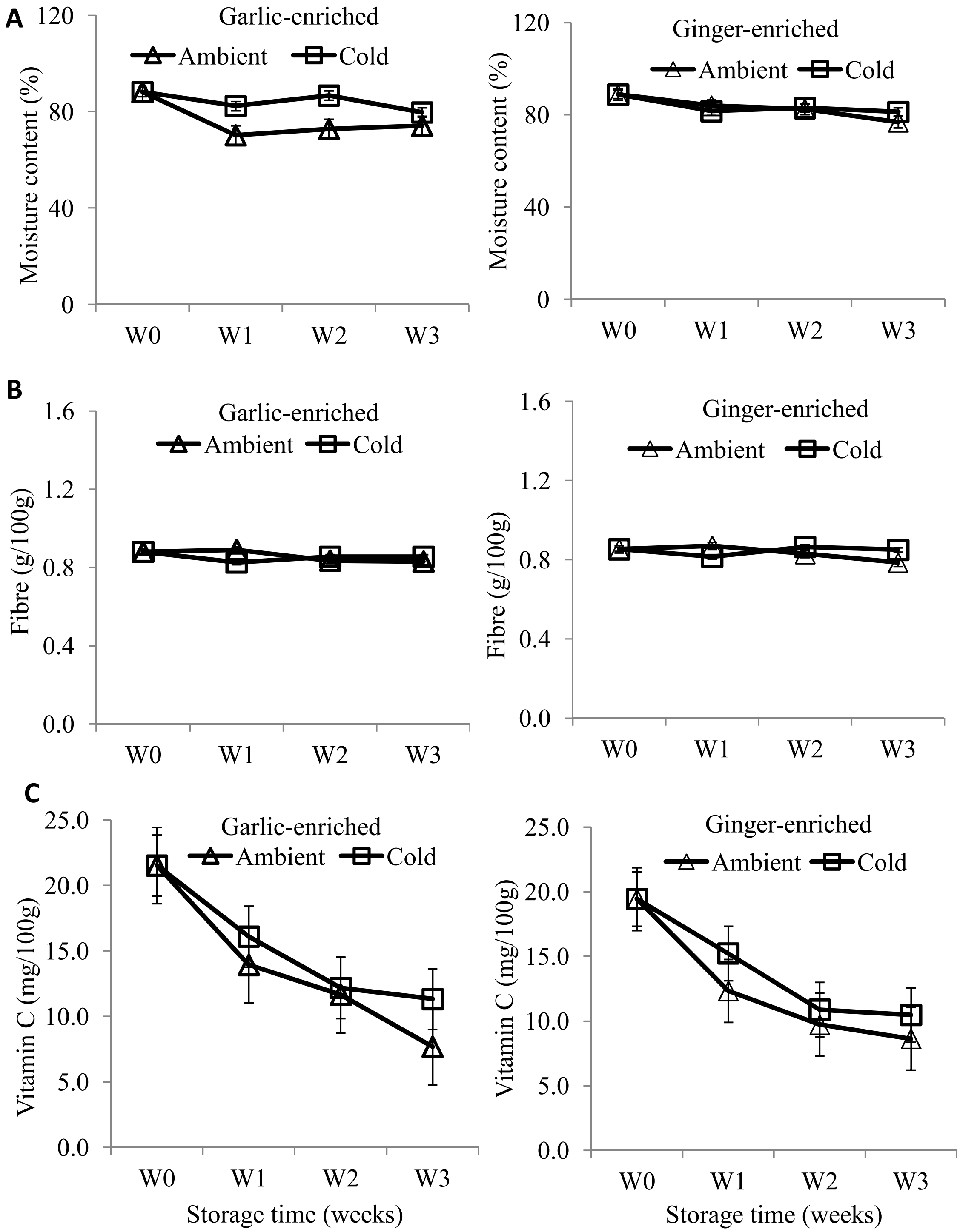

Fig. 1. Dynamic changes in (A) Moisture content, (B) Crude fibre and (C) Vitamin C contents of composite smoothies stored for 3 weeks at cold and ambient temperatures. Data are shown as mean ± standard deviation (n = 3)

The reason may be due to higher calcium contribution from garlic than ginger [36]. There was no difference in sodium among the smoothie samples with respect to preservatives and storage conditions. A wider difference was observed in potassium between garlic and ginger-enriched smoothie samples before storage. However, the values of these minerals at the end of storage period were within the acceptable range reported in USDA [26].

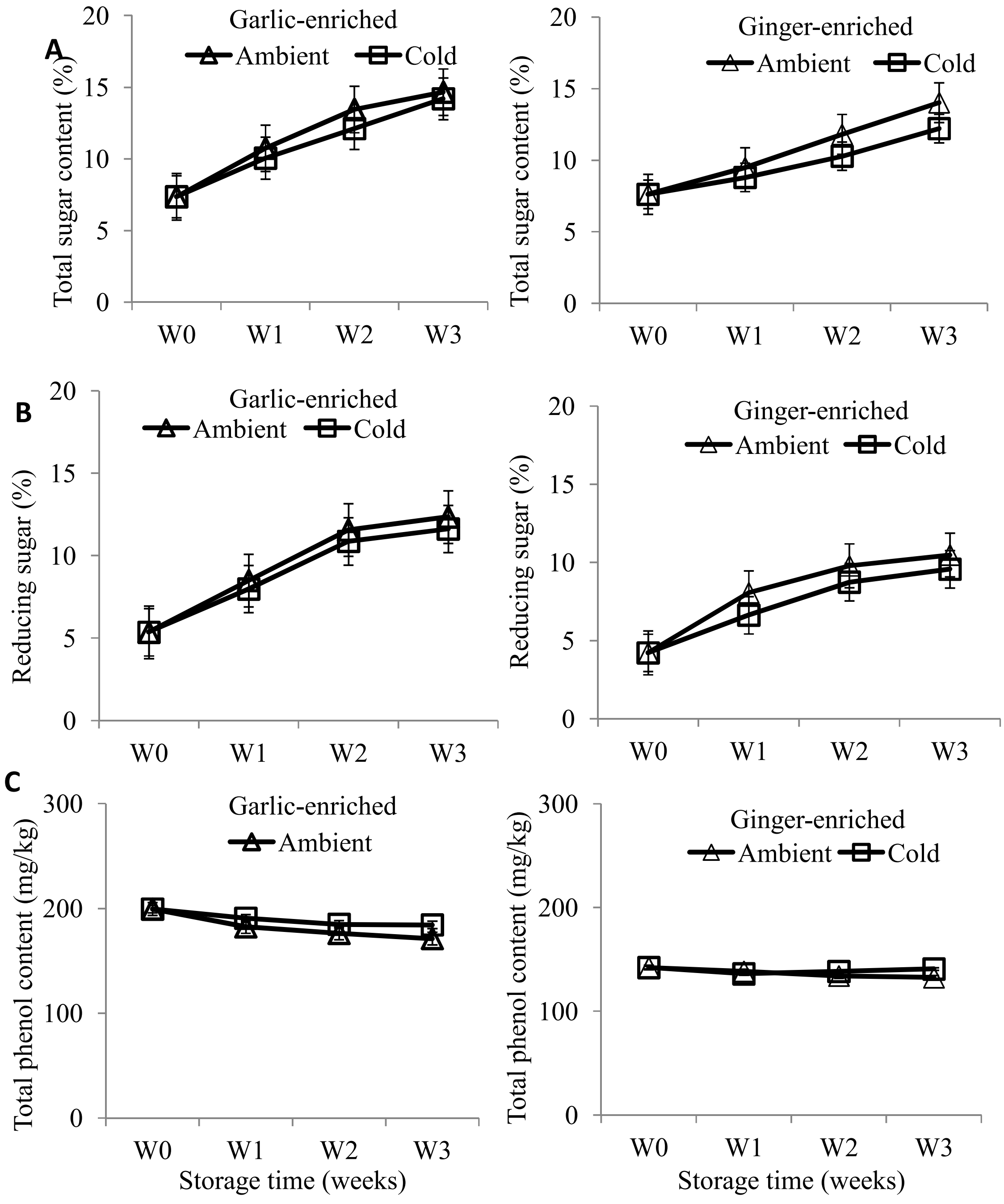

Fig. 2. Dynamic changes in (A) total sugar (B) reducing sugar and (C) total phenol contents of composite smoothies stored for 3 weeks at cold and ambient temperatures. Data are shown as mean ± standard deviation (n = 3)

Table 1. Mineral composition of composite smoothies stored at ambient (25°C) and cold (4°C) temperature

Mineral	Garlic		Ginger	
Composition	**Ambient**	**Cold**	**Ambient**	**Cold**
Magnesium (mg/100 g)				
Week 0	12.82±0.30		13.25±0.45	
Week 1	13.17±0.69	13.37±0.96	13.36±0.17	12.60±0.11
Week 2	10.36±0.16	12.11±0.62	10.80±1.01	10.11±0.89
Week 3	12.65±1.17	12.81±1.22	13.45±0.13	12.65±0.51
Calcium (mg/100 g)				
Week 0	9.84±0.47		7.69±0.35	
Week 1	9.43±0.96	8.65±1.41	9.25±0.86	9.87±1.07
Week 2	8.53±0.64	8.64±0.58	7.99±0.18	9.36±0.31
Week 3	8.27±0.57	8.56±0.64	7.98±0.15	9.11±0.18
Sodium (mg/100 g)				
Week 0	1.86±0.11		1.94±0.17	
Week 1	2.19±0.05	1.91±0.11	2.01±0.08	1.88±0.01
Week 2	2.03±0.12	1.94±0.11	1.99±0.18	1.90±0.16
Week 3	2.17±0.22	2.07±0.48	1.83±0.19	1.94±0.06
Potassium (mg/100 g)				
Week 0	149.64±4.39		189.60±2.79	
Week 1	140.82±1.61	145.91±4.74	191.72±7.30	194.24±7.88
Week 2	140.56±10.85	145.61±8.24	185.82±11.36	191.76±7.44
Week 3	136.95±10.47	147.41±8.55	197.12±5.98	192.81±4.87

3.2 Influence of Preservatives and Storage Techniques on Physicochemical Properties

pH, titratable acidity and total soluble solid (^{o}Brix) of fruit and vegetable products are primarily used to assess some intrinsic quality attributes, which may determine the degree of acceptability of the products to consumers. In the first two weeks of storage, there was a significant difference in the pH of the samples based on preservatives, with garlic-enriched smoothies having higher pH (4.17 – 5.72) than ginger-enriched ones (3.78 – 4.68) (Fig. 3A). However, garlic-enriched smoothies showed a slightly different pH pattern during storage, especially in cold stored samples. pH of the samples decreased slightly with storage time. Microbial activities were suggested to be responsible for rapid decline in pH of fruit blends by some authors [37,38]. Similarly, titratable acidity of the smoothies decreased with storage; which is consistent with other study [39]. Acid hydrolysis of polysaccharides has been suggested to be responsible for the decline in acidity of fruit blends during storage [40]. Influence of preservatives and storage conditions were not significant on the pattern of change in acidity of the samples. Higher reduction was observed between 0 and 2nd week compare to that of 2nd and 3rd week (Fig. 3B). In the case of total soluble solid content (^{o}Brix), a gradual increase was observed in the enriched-smoothies stored at both temperatures (Fig. 3C). Total soluble solid of the fruit blends increased slightly as storage progressed. A minimum increase in °Brix has been previously linked to a more desirable quality fruit blend [4].

3.3 Influence of Preservatives and Storage Techniques on Microbiological Qualities

The microbial inhibitory potential of garlic and ginger extracts on smoothie during storage, were demonstrated and the results were presented in Table 2. The microbial inhibitory potentials of garlic and ginger extracts varied and their effectiveness was more evident after 1st week of storage. Smoothies enriched with garlic and ginger had lower bacteria growth compared to negative control, even after 3rd weeks of ambient storage. The bactericidal potential of natural herbs and spices in various foods had been earlier reported [41,42]. Generally, this minimal microbial growth at the end of storage time can also be attributed to the combined effect of pasteurization and sanitary preparatory conditions. Synthetic additives (potassium sorbate and sodium benzoate) in conjunction with pasteurization had similar preservative effects on apple juice [43]. All the samples were also below the maximum United States Department of Agriculture (USDA) standard limits for commercial fruit juices with respect to bacteria, fungi and coliform (<5x10^4, 1x10^3 and 100 cfu/ml, respectively) [44]. Both preservatives were highly effective against fungi compared to the control sample. There was no observable coliform growth in all the samples. This eliminates the possibility of faecal contamination, which is a pointer to hygienic preparation [45]. The results were similar to the observation of Francis et al. [46] who reported a delay in microbial deterioration and extended storage life of fruit juice on addition of natural preservatives.

3.4 Sensorial Changes in Enriched Smoothies with Storage Time and Conditions

The results of sensory assessment of the smoothies by panelist on appearance, flavor, taste and overall acceptability were presented in Table 3. Prior to storage, there were no significant differences between garlic and ginger-enriched smoothies with respect to all the sensory parameters considered. However, sensorial ranking of smoothies began to decline with storage time. There were slight changes in appearance of both garlic and ginger-enriched smoothies even after three weeks of cold and ambient storage. Although taste is considered to be the determinant of food choice [47], appearance is the primary factor that motivates a consumer to try a product in the first place [48]. Smoothies stored at ambient temperature declined in flavor and taste with storage. These changes can be attributed to certain chemical reactions; the extents to which depend on storage time and temperature [49]. Therefore, samples stored under cold temperature were sensorially preferred to those at room temperature. The same inference was true for overall acceptability. Generally, the performance of the samples organoleptically was considerably fair enough as none of the sensory parameters had a mean score lower than 5 even after one week of cold and ambient storage.

3.5 PCA Multivariate Analysis of All Measured Variables

PCA statistical model built with all measured variables was used to highlight the differences and similarities among the smoothie samples with respect to the significant factors. The results of the PCA model with 2PCs and 67% explained variance as shown in Fig. 4 indicated clearly that the factors influencing the separation of observations (smoothies) were preservatives and storage time rather than storage conditions. The most recognizable pattern in this unsupervised model is the distinctive difference between smoothies enriched with garlic and ginger, with the upper part of the control ellipse predominantly occupied by ginger-enriched smoothies and the lower part by garlic-enriched ones. As shown in the loading plot (Fig. 4B), garlic-enriched smoothies were best described by their comparatively higher TPC, fibre, total

Table 2. Microbial quality (standard plate count and coliform) of enriched and control smoothie samples

Microbial quality	Garlic		Ginger		Control	
	Ambient	Cold	Ambient	Cold	Ambient	Cold
TVC bacteria (cfu/mL)						
Week 0	1.2x10^1		1.0x10^1		2.1x10^1	
Week 1	2.1x10^1	1.1x10^1	2.2x10^1	2.3x10^1	4.3x10^1	3.8x10^1
Week 2	2.0x10^1	1.6x10^1	1.5x10^1	1.8x10^1	3.6x10^1	3.1x10^1
Week 3	3.3x10^1	2.0x10^1	2.6x10^1	1.5x10^1	4.8x10^1	4.1x10^1
TVC fungi (cfu/mL)						
Week 0	Nil		Nil		Nil	
Week 1	1.0x10^1	Nil	1.2x10^1	1.0x10^1	3.0x10^1	2.5x10^1
Week 2	1.3x10^1	Nil	2.1x10^1	Nil	1.8x10^1	2.4x10^1
Week 3	1.5x10^2	2.2x10^1	3.5x10^1	2.8x10^1	3.2x10^2	5.6x10^1
Coliform (cfu/mL)						
Week 0	Nil		Nil		Nil	
Week 1	Nil	Nil	Nil	Nil	Nil	Nil
Week 2	Nil	Nil	Nil	Nil	Nil	Nil
Week 3	Nil	Nil	Nil	Nil	Nil	Nil

TVC: Total viable count, CFU: Colony forming unit

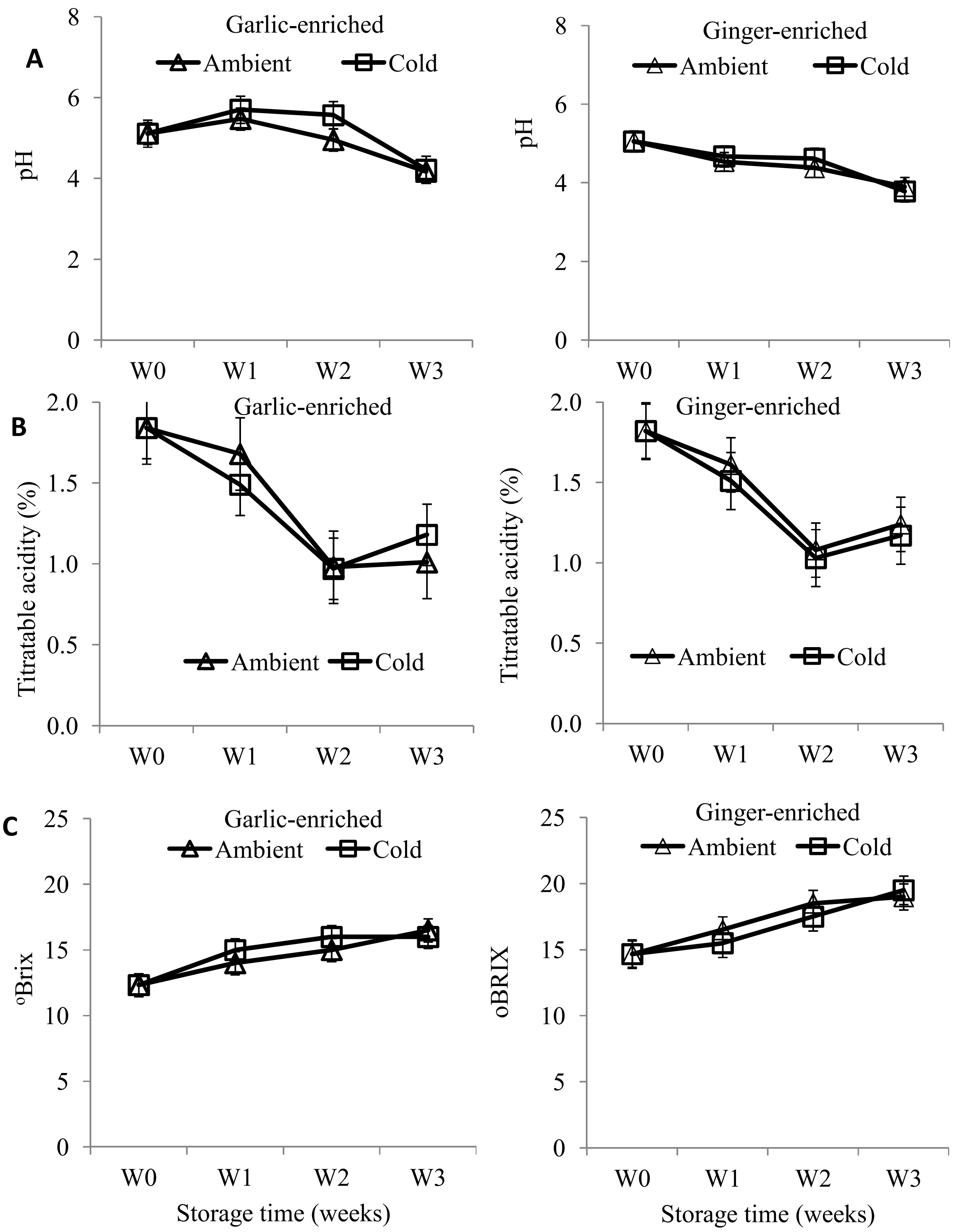

Fig. 3. Dynamic changes in (A) pH (B) titratable acidity and (C) °Brix of composite smoothies stored for 3 weeks at cold and ambient temperatures. Data are shown as mean ± standard deviation (n = 3)

and reducing sugar contents, pH, sodium, mineral ratios and to an extent vitamin C. Na/K molar ratio of less than 1.0 has been linked with some health benefits such as reduced hypertension and cardiovascular disease [50]. Garlic-enriched smoothies had comparatively higher Na/K, Ca/Mg, Ca/K and Na/Mg molar ratios than ginger-enriched. On the other hands, variables most responsible for the separation of ginger-enriched smoothies were titratable acidity, magnesium, potassium and °Brix and all sensory parameters determined. Another observable trend in the samples' projection is the movements of the samples from the positive axis of the PC 1 to the negative axis as storage weeks advanced (Fig. 4A). This indicated a gradual decrease in quality parameters such as TPC, vitamin C, fibre, minerals, mineral ratios and corresponding increase in total and reducing sugar and °Brix. This further buttressed the earlier suggestion of Castillejo et al. [8] that, mildly thermal treated smoothie is at its nutritionally optimum condition when consumed fresh or within few days of production. PCA score plot further showed the effect of garlic and ginger; as fresh (GIR and GAR) and one week stored samples were located on the same quadrant. The difference in the quality characteristics became pronounced as the storage time increased.

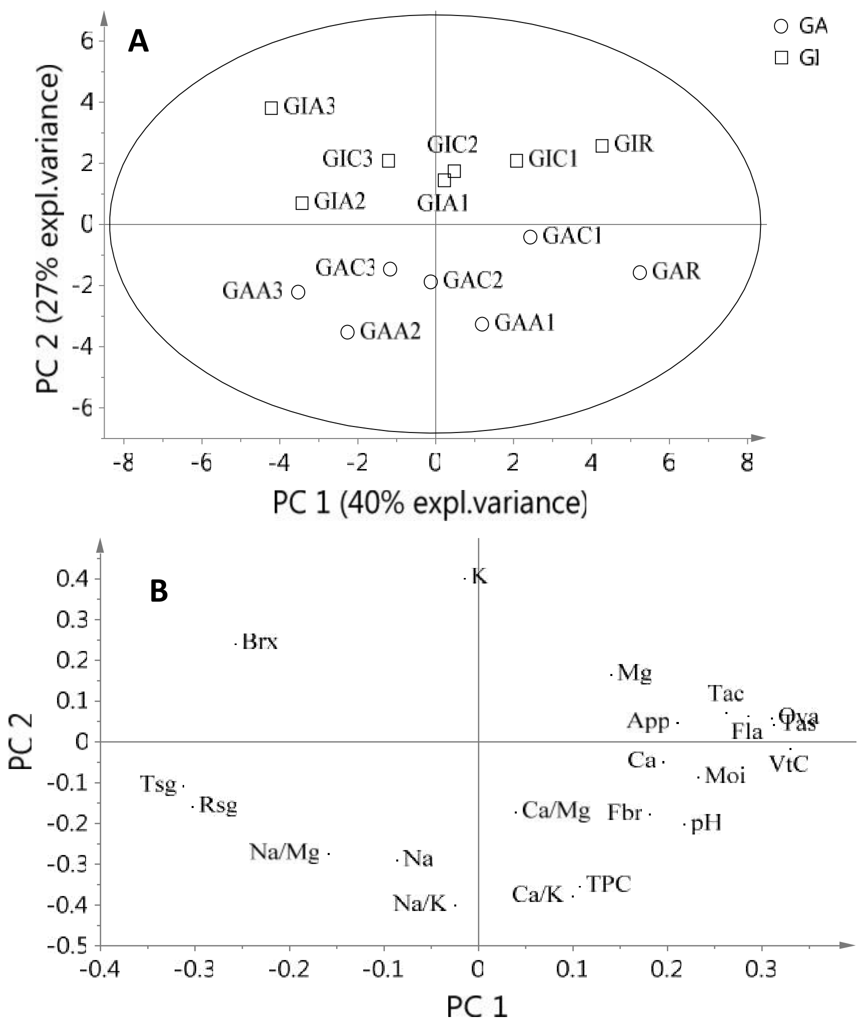

Fig 4. PCA model results: (A) score and (B) loading plots of garlic (GA) and ginger-enriched (GI) composite smoothies stored under ambient (GAA) and cold (GAC) conditions for four weeks (1, 2, 3 and 4) and reference/fresh smoothies before storage (GAR and GIR)

Keys: Moi: Moisture content, TPC: Total phenol content, Fbr: Crude fibre, VtC: Vitamin C, Tsg: Total sugar, Rsg: Reducing sugar, Brx: °Brix, Tac: Titratable acidity, Na: Sodium, Mg: Magnesium, K: Potassium, Fla: Flavor, Tas: taste, App: Appearance, Ova: overall acceptability

Table 3. Changes on sensory quality of enriched smoothies with storage conditions and time

Sensory quality	Garlic		Ginger	
	Ambient	Cold	Ambient	Cold
Appearance				
Week 0	6.68±0.38[a]		7.13±0.22[a]	
Week 1	5.86±0.05	6.62±0.18	6.01±0.11	6.88±0.10
Week 2	6.03±1.09	5.94±0.28	5.06±1.03	6.80±0.33
Week 3	6.82±0.22	6.07±0.47	5.85±0.63	5.93±0.88
Flavor				
Week 0	7.05±0.35[a]		7.75±0.95[a]	
Week 1	5.75±0.65	7.15±0.65	5.65±1.05	7.52±0.47
Week 2	5.45±0.25	6.52±0.15	5.05±0.82	6.85±0.61
Week 3	4.81±0.44	6.62±0.39	4.32±1.04	6.94±0.87
Taste				
Week 0	7.23±0.53[a]		7.58±0.26[a]	
Week 1	6.25±0.33	6.84±0.14	4.45±1.23	6.17±0.58
Week 2	3.45±0.25	5.51±0.25	4.17±0.92	6.33±0.40
Week 3	3.08±0.66	5.64±0.19	3.11±0.76	5.92±0.73
Overall acceptability				
Week 0	6.36±0.81[a]		7.22±0.25[a]	
Week 1	5.25±0.89	6.44±0.37	5.04±0.26	6.16±0.96
Week 2	4.64±0.55	5.35±0.46	4.84±0.86	5.77±0.42
Week 3	4.11±0.67	5.76±0.09	3.83±0.42	5.46±0.51

**Means that do not share a letter (superscript) are significantly different (P<0.05)*

4. CONCLUSION

The study evaluated the effects of natural preservatives and storage conditions on nutrients and quality stability of fruit smoothies formulated from the blend of 40% watermelon, 30% banana and 30% pineapple and kept under cold and ambient conditions for three weeks. Types of preservative and storage time were found significant in determining nutrient and quality stability of smoothies. Storage conditions affected vitamin C, total and reducing sugar and TPC between 0 and 3rd week of storage with cold stored smoothies having higher amounts of these parameters. Ginger and garlic in conjunction with cold storage conferred positive influence on microbiological quality of the samples during storage. PCA multivariate model, showed distinctive nutritional and quality characteristics of smoothies based on preservatives. Garlic-enriched smoothies were best defined by higher TPC, sugar contents, fibre, sodium, mineral ratios, and vitamin C as compared to ginger-enriched samples which were high in total soluble solids, titratable acidity, magnesium and potassium. There was no remarkable change in the desirable properties such as: TPC, vitamin C, mineral compositions, fibre contents, and sensory properties of smoothies within the first week of storage especially under cold conditions. However, reduction in the sensory attributes of the enriched smoothies became significant as storage time progressed. Garlic and ginger are potent natural preservatives with additional nutritional benefits that may replace synthetic additives such as citric acid, sodium benzoate, propionate and metabisulphite, potassium sorbate, methyl paraben etc that are commonly used in fruits juices, nectars and smoothies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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