

Storage Stability Study of Sesame (*Sesamum Indicum*) Seed-Based Salad Cream

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Submitted: July 27th, 2024; Revised: January 19th, 2025; Accepted: January 31st, 2025; Published: May 29th, 2025

ABSTRACT: The quality and storage stability of sesame seed-based salad cream containing varied quantities of acetic acid were compared with a renowned commercial salad cream. Variations in pH, peroxide value, total titratable acidity, free fatty acid, thiobarbituric acid, viscosity, and microbial parameters of samples stored in an airtight container for 6 months at 20 °C were determined using standard methods. Storage stability parameters (pH, peroxide oxide, free fatty acid, total titratable acidity, thiobarbituric acid, viscosity, and microbial analysis) had significant differences ($P < 0.05$) during six months of storage. The samples were chemically stable, but the viscosity of the samples decreased below the Standards Organization of Nigeria specification at week 24. Sample 462 and sample 795 were not microbiologically stable. The decrease in total plate count and the inhibitory effect of acetic acid on microorganisms were observed to be proportional to the concentration of acetic acid.

Keywords: salad cream, sesame seeds, storage

INTRODUCTION

Salad cream is a viscous product of oil in water emulsion, containing fat not less than 30% as stated by Thai Industrial Standard Institute (TISI) No. 1402-2540, produced from ingredients such as vinegar, vegetable oil, water, sugar, mustard paste, salt, pasteurized egg yolk, starch, and riboflavin for coloring as stated by Standards Organization of Nigeria (SON) No. 591. Salad cream makes raw vegetables palatable. Therefore, it is used for the consumption of vegetable salad (Adeleke *et al.*, 2020). Mustard seed, one of the ingredients of imported salad cream, is a spice uncommon in tropical regions such as Nigeria. Therefore, there is a need for a locally available crop for the production of salad cream.

Sesame seed (beniseed), a member of the family *Pedaliaceae*, is an oil seed crop widely grown in the northern and central parts of Nigeria (Toungos, 2020). In Africa, Sudan is the largest producer of sesame (Imoloame & Abubakar, 2023), while Nigeria is the second-largest producer of sesame and the third-largest in the world, with an estimated production of 580,000 tonnes in 2017 (Toungos, 2020). Sesame seed is one of the neglected and underutilized food crops (Nkwonta *et al.*, 2023) that are necessary for providing solutions to malnutrition and food insecurity (Nkwonta *et al.*, 2023). The production of sesame seed salad cream will introduce diversification in the use of sesame seeds, thereby

increasing the cultivation of the seed, reducing its postharvest loss (by using it in value-added products like salad cream), and providing salad cream with health benefits that guarantee food security and provide solutions to nutrient deficiency. Sesame seeds are affected by a wide variety of pathogens. According to Ara *et al.* (2017), annual losses of about 7 million tonnes of sesame seeds have been recorded as a result of diseases caused by these pathogens. This loss could be a result of its underutilization, as stated by Nkwonta *et al.* (2023). To combat this loss, storage measures free of harmful chemicals are necessary. Previous studies on the storage of salad cream constitute the use of mustard seed powder and vinegar using different cassava starch varieties, coconut vinegar and carboxymethyl cellulose, and extra virgin olive oil and apple vinegar. However, a study on six months of storage for salad cream containing sesame seeds with varied concentrations of white vinegar and sugar (sucrose) is yet to be carried out. This study provides information (especially for those who consume little or no vinegar and sugar due to health issues) on how long it can remain safe for consumption when produced at the household level. The choice of vinegar and sugar emanates from consumers' demand for food containing little or no chemical preservatives. Also, research conducted by Park *et al.* (2014) proved the effect of sugar and vinegar on poultry meat that has 4.5 - 5.0 log CFU/g of *Campylobacter jejuni* and *Salmonella typhimurium* during storage. Vinegar contains acetic acid, which is

used to increase the acidity of food in order to reduce the deterioration of food. De Leonardis *et al.* (2022) reported that the peroxide value of salad dressing containing olive leaf vinegar remained low (4.5 meqO₂/kg) after 12 months of storage. Sugar acts by lowering the water activity in food products. This creates an environment that limits microbial growth by facilitating the production of alcohol or acid that acts as a preservative in some food products (Goldfein & Slavin, 2015), as seen in the research conducted by Ashaver *et al.* (2023) who reported that tiger nut moringa seed, sugar, and citric acid drink significantly lowered microbial growth as compared to plain tiger nut moringa seed drink.

MATERIALS AND METHODS

Materials

Sugar (sucrose), table salt (sodium chloride), egg, white vinegar, corn, Kings® vegetable oil, and sesame seeds were purchased from the *Wurukum* market, Makurdi, Benue State, Nigeria, while the chemicals used were of analytical grade.

Sample preparation

Clean and dried sesame seeds were milled into a paste according to the method described by Akusu *et al.* (2020). Corn starch was prepared according to the method described by Dongmo *et al.* (2020). Eggs were pasteurized according to the method of Froning *et al.* (2019).

Production of sesame seed-based salad cream

Sesame seed-based salad cream was produced according to the method described by Nwosu & Eke-Ejiofor (2021). It was produced by mixing measured quantities of 45 g corn starch, 5 g, 10 g, 15 g sugar, 2.5 g salt, 60 mL, 120 mL white vinegar, and 70 mL distilled water. The mixture was heated at 121 °C for 5 min with continuous stirring to obtain a slurry. It was allowed to cool before adding 36 g pasteurized egg yolk, 90 mL vegetable oil (Kings®), 15 g sesame seed paste, and 0.01 g riboflavin. Then, it was homogenized using an electric blender until a consistent paste was formed.

The study includes initial production, obtained from a 3 × 4 randomized experimental design comprising 3 levels of acetic acid (0%, 2%, 4%) and 4 levels of sugar (0%, 2%, 4%, 6%) to give twelve samples: sample 367 (0% acetic acid + 0% sugar); sample 931 (0% acetic acid + 2% sugar); sample 183 (0% acetic acid + 4% sugar); sample 462 (0% acetic acid + 6% sugar); sample 273 (2% acetic acid + 0% sugar); sample 564 (2% acetic acid + 2%

sugar); sample 815 (2% acetic acid + 4% sugar); sample 795 (2% acetic acid + 6% sugar); sample 648 (4% acetic acid + 0% sugar); sample 926 (4% acetic acid + 2% sugar); sample 852 (4% acetic acid + 4% sugar) and sample 319 (4% acetic acid + 6% sugar). Sensory evaluation was used to select the three best samples for chemical and microbial analyses in comparison to the commercial salad cream.

Storage stability test

The formulations were filled into clean bottles, covered with a lid, labeled, and stored at 20 °C for six months. The samples were labeled thus: sample 462 (contains 6% sugar without vinegar), sample 795 (contains 2% acetic acid and 6% sugar), sample 852 (contains 4% acetic acid and 4% sugar), and sample 734 (a commercial sample). Chemical analyses such as pH (Adeleke *et al.*, 2020), peroxide value (Say *et al.*, 2024), total titratable acidity (Almasi *et al.*, 2021), free fatty acid (Pardeshi, 2020), thiobarbituric acid (Zeb & Ullah, 2016) and viscosity (Adeleke *et al.*, 2020) were carried out on each sample during the storage period. Microbiological examination of the samples was carried out using the American Public Health Association's official methods as described by Kurtzman *et al.* (1971).

Data analysis

Analysis was conducted in triplicates. The results were expressed as mean ± standard deviation. Using the statistical package SPSS version 20 software, the difference in mean was determined using one-way analysis of variance (ANOVA), while differences between means were determined by Duncan's multiple range test. Values are considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

The results for changes in the storage properties of salad cream samples during storage are presented in Table 1. There were significant differences ($P < 0.05$) that were observed during the six-month storage period. The pH of sample 462, containing 6% sugar and no vinegar content, was close to neutral but became slightly acidic at the end of the storage due to the conversion of starch and sugar to organic acids by bacteria. Justifiable amounts of vinegar were added to samples 795 and 852, just as it is present in the commercial salad cream. This was done to lower the pH of the salad cream, thereby limiting the growth of microorganisms. The addition of acidulants to foods must

Table 1. Changes in storage properties of salad cream samples during storage

Sample	Storage time (weeks)	pH	Peroxide value (meq/kg)	Titratable acidity (%)	Free fatty acid (%)	Thiobarbituric acid (mg/kg)	Viscosity (cP)
462	0	6.36±0.05 ^a	3.05±0.05 ^h	0.10±0.01 ^g	0.10±0.01 ^d	0.12±0.01 ^h	2440.37±0.68 ^a
	2	6.16±0.10 ^b	4.55±0.05 ^g	0.11±0.01 ^f	0.11±0.01 ^{cd}	0.14±0.01 ^g	2400.13±0.01 ^b
	4	5.80±0.10 ^c	4.63±1.00 ^f	0.13±0.03 ^e	0.11±0.01 ^{cd}	0.15±0.01 ^f	2113.11±0.23 ^c
	8	5.70±0.10 ^d	4.76±0.03 ^e	0.14±0.01 ^d	0.12±0.01 ^{bc}	0.23±0.01 ^e	2107.00±0.03 ^d
	12	5.66±0.10 ^e	5.17±0.01 ^d	0.15±0.01 ^c	0.12±0.01 ^{bc}	0.27±0.01 ^d	1901.98±0.15 ^e
	16	5.07±0.06 ^f	5.60±0.29 ^c	0.16±0.01 ^c	0.13±0.01 ^{ab}	0.31±0.01 ^c	1732.45±0.78 ^f
	20	4.87±0.05 ^g	6.13±0.01 ^b	0.22±0.03 ^b	0.14±0.01 ^a	0.32±0.01 ^b	1587.62±0.01 ^g
	24	4.70±0.10 ^h	7.12±0.23 ^a	0.41±0.05 ^a	0.14±0.01 ^a	0.34±0.01 ^a	1223.20±0.34 ^h
795	0	4.30±0.10 ^a	3.64±0.54 ^h	1.34±0.01 ^h	0.10 ^c ±0.01	0.10±0.01 ^h	2599.10±0.06 ^a
	2	4.27±0.05 ^b	4.03±0.01 ^g	1.35±0.01 ^g	0.10 ^c ±0.01	0.13±0.01 ^g	2564.52±0.77 ^b
	4	4.16±0.10 ^c	4.58±0.29 ^f	1.36±0.01 ^f	0.11 ^c ±0.01	0.14±0.01 ^f	2442.00±0.12 ^c
	8	4.06±0.16 ^d	4.90±0.01 ^e	1.38±0.01 ^e	0.12 ^b ±0.01	0.19±0.01 ^e	2256.97±0.54 ^d
	12	3.96±0.10 ^e	5.15±0.14 ^d	1.39±0.01 ^d	0.14 ^a ±0.01	0.20±0.01 ^d	2100.52±0.01 ^e
	16	3.80±0.10 ^f	5.78±0.02 ^c	1.41±0.01 ^c	0.14 ^a ±0.01	0.25±0.01 ^c	1886.36±0.04 ^f
	20	3.77±0.10 ^g	6.44±0.01 ^b	1.43±0.01 ^b	0.15±0.01 ^a	0.26±0.01 ^b	1698.00±0.25 ^g
	24	3.67±0.10 ^h	7.09±0.56 ^a	1.44±0.01 ^a	0.15±0.01 ^a	0.37±0.01 ^a	1253.21±0.12 ^h
852	0	4.06±0.10 ^a	4.01±0.19 ^h	1.50±0.01 ^h	0.11±0.01 ^d	0.23±0.01 ^h	2600.10±0.20 ^a
	2	3.96±0.10 ^b	4.99±0.97 ^g	1.51±0.01 ^g	0.12±0.01 ^{cd}	0.25±0.01 ^g	2502.12±0.01 ^b
	4	3.87±0.10 ^c	5.11±0.23 ^f	1.52±0.01 ^f	0.12±0.01 ^{cd}	0.28±0.01 ^f	2590.45±0.07 ^c
	8	3.77±0.05 ^d	5.23±0.11 ^e	1.55±0.02 ^e	0.13±0.01 ^{bc}	0.33±0.01 ^e	2221.00±0.03 ^d
	12	3.67±0.05 ^d	6.11±0.29 ^d	1.56±0.01 ^d	0.14±0.01 ^{ab}	0.34±0.01 ^d	2151.11±0.06 ^e
	16	3.66±0.05 ^d	6.35±0.12 ^c	1.57±0.11 ^c	0.14±0.01 ^{ab}	0.35±0.01 ^c	2076.98±0.21 ^f
	20	3.60±0.05 ^d	6.73±0.01 ^b	1.58±0.13 ^b	0.14±0.01 ^{ab}	0.39±0.01 ^b	1955.19±0.55 ^g
	24	3.47±0.10 ^e	7.11±0.17 ^a	1.64±0.01 ^a	0.15±0.01 ^a	0.44±0.01 ^a	1299.03±0.73 ^h
734	0	3.77±0.05 ^a	2.08±0.01 ^h	1.45±0.01 ^h	0.10±0.01 ^d	0.21±0.01 ^h	2504.10±0.06 ^a
	2	3.76±0.05 ^a	2.61±0.01 ^g	1.47±0.03 ^g	0.11±0.01 ^{cd}	0.22±0.01 ^g	2443.76±0.01 ^b
	4	3.67±0.05 ^a	3.00±0.01 ^f	1.52±0.01 ^f	0.11±0.01 ^{cd}	0.24±0.01 ^f	2445.99±0.01 ^c

Values are mean±standard deviation. Mean values of each sample within a column with the same letters are not significantly different ($P > 0.05$).

Key:

462 = 0% acetic acid + 6% sugar

795 = 2% acetic acid + 6% sugar

852 = 4% acetic acid + 4% sugar

734 = commercial salad cream

be done with caution, taking note of the quantity to avoid toxicity. Lhotta *et al.* (1998) reported that the consumption of 250 mL/day of vinegar caused hypokalemia, hyperreninemia, and osteoporosis in a patient. The Nigerian Industrial Standard (NIS) for salad creams approved by the Standards Organization of Nigeria (SON) governing council stated that the pH of salad creams must be within the range of 3.1 - 4.2. Sample 852 and the commercial salad cream sample in the initial stages of storage met this standard. There was a decrease in pH for all the samples, which may be due to organic matter decomposition and microbial activities (Ahmad *et al.*, 2019). The presence of sugar in the samples must have facilitated the production of acid by the microbes. As pH values decreased, total titratable acidity increased with time (Table 1). The Nigerian Industrial Standard (NIS) for salad creams approved by the Standards Organization of Nigeria (SON) governing council stated that the acidity of salad creams must be within the range of 1.5%-1.6%, inferring that only sample 852 and the commercial sample meet the SON standard. Adeleke *et al.* (2020) observed a decrease in the pH values for a three-month storage study of salad cream made from different cassava starch varieties. Likewise, Rathnayake *et al.* (2019) observed a decrease in pH in their research. However, an increase in pH for six weeks of storage study of salad cream was observed in the findings of Eke-Ejiofor & Beleya (2015). The increase in total titratable acidity with time observed in this study agrees with the findings of Eke-Ejiofor & Beleya (2015) and Rathnayake *et al.* (2019), who worked on 6 weeks and 7 weeks of salad cream storage, respectively. To combat this, the use of active packaging to store salad creams would extend its shelf-life (Settier-Ramirez *et al.*, 2022).

Edible oils could be oxidized over a long period, especially in the presence of high temperature, light, and oxygen (Karami *et al.*, 2020); when the product is not packaged appropriately. Free fatty acid, peroxide value, and thiobarbituric acid were measured to determine the extent of lipid oxidation. According to David (2002), peroxide values above 10 - 20 meq/kg bring about rancidity in edible oils or fatty foods. It was observed that though there was an increase in the peroxide values, it did not exceed 10 meq/kg. The values obtained for sample 462, containing 6% sugar and no vinegar content, were perhaps as a result of the antioxidant, sesamin, and sesamolin, present in sesame seeds, as reported by Kato *et al.* (1998) and Dravie *et al.* (2020). The findings also agree with Izadi *et al.* (2022), who reported that after one

month, salad dressing samples formulated with sesame seed oil and rice bran oil had the lowest peroxide values compared to samples containing olive oil and soybean oil. Besides the antioxidant properties of riboflavin (Jacobsen, 2016) and sesame seeds (Kato *et al.*, 1998; Dravie *et al.*, 2020), vinegar also possesses antioxidant and antimicrobial properties (Antoniewicz *et al.*, 2021; Kara *et al.*, 2022). Therefore, the addition of vinegar to samples 795, 852, and 734 may explain the decrease in the rate of oxidation (Table 1). The Nigerian Industrial Standard (NIS) for salad creams approved by the Standards Organization of Nigeria (SON) governing council stated that free fatty acid value should be 0.1% and peroxide value should not exceed 15 meq/kg, inferring that the salad creams meet the SON standard. Peroxide value measures the amount of hydroperoxide formed during the primary oxidation of oil, while thiobarbituric acid measures secondary oxidation in lipids. Thiobarbituric acid values that were recorded in all the samples were below the Food and Agriculture Organization acceptable limit of less than 0.5 mg/kg, indicating that the salad creams may not have undergone extensive oxidation. The increase in peroxide value of the samples with time observed in this study agrees with the findings of Eke-Ejiofor & Beleya (2015) and Adeleke *et al.* (2020), who worked on 6 weeks and 3 months of salad cream storage, respectively.

The viscosities of all the samples decreased significantly ($P < 0.05$) during the period of storage. The Nigerian Industrial Standard (NIS) for salad creams approved by the Standards Organization of Nigeria (SON) governing council gave the specification of viscosity (centipoises) of salad cream to be 1300 – 2600 cP, inferring that the salad creams meet SON standards. However, at week 24, only the commercial salad cream (1425.32 cP) maintained the standard, while the viscosity of samples 462, 795, and 852 was below the stipulated standard. The decrease in viscosity with time may be due to the velocity of agitation that was used for mixing during production (Salehi, 2020). The homogenization process reduces particle size in fluid, and this prevents the conglomeration of particles during storage (Delmas & Cleary, 2023). According to Salehi (2020), the application of high-pressure homogenization to fruit and vegetable juices can enhance the viscosity and stability of suspended solids and retard color change. Paredes *et al.* (1989) reported that a decrease in velocity may be a result of the coalescence of oil droplets in the samples, which is a function of storage time. It could also be a result of the interaction between ingredients

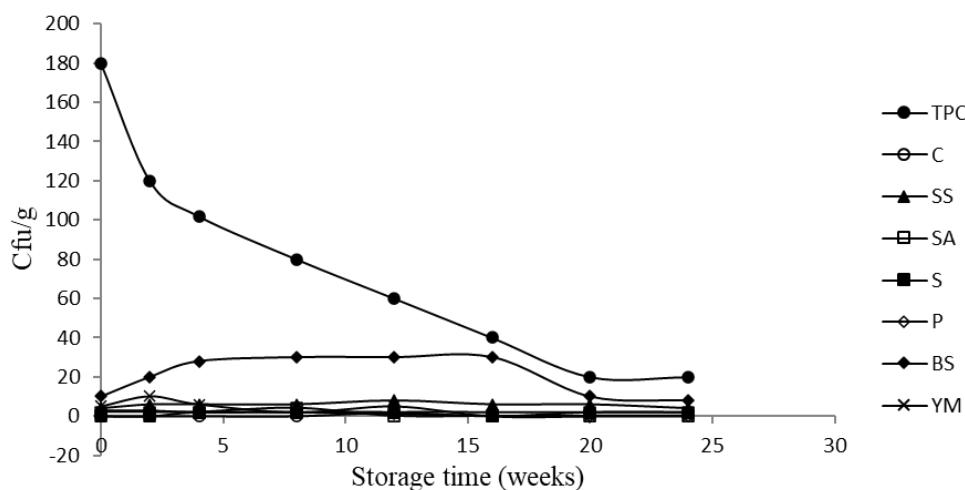


Figure 1. Changes in microbial parameters of sesame seed-based salad cream containing 0% acetic acid and 6% sugar during storage

Key: TPC = total plate count, C = coliforms, SS = *Streptococcus* spp., SA = *Staphylococcus aureus*, S = *Salmonella* spp., P = *Pseudomonas* spp., BS = *Bacillus* spp., YM = yeast and mould

(Schirmer *et al.*, 1986) or the settling of particles when left standing for a long time (Kikuchi *et al.*, 2017). The decrease in viscosity observed in this study agrees with the observation of Adeleke *et al.* (2020), who worked on 3 months of salad cream storage.

Changes in microbial parameters for sample 462, containing 6% sugar and no vinegar content, are presented in Figure 1. The sample had the highest total plate count (180 CFU/g) at week 0. A significant decrease ($P < 0.05$) was observed in the total plate count of the sample. This may be due to several factors, such as nutrient depletion (the growth of microbes decreases as the available nutrient depletes) (Dai *et al.*, 2022) or competition (competition for available nutrients may eliminate certain species) (Ghoul & Miti, 2016) or as a result of the production of byproducts that accumulate over a long period, which may not be conducive for microbial growth (Price & Sowers, 2004). The Nigerian Industrial Standard (NIS) for salad creams approved by the Standards Organization of Nigeria (SON) governing council stated that the total viable count in salad creams should not exceed 1000 CFU/g, inferring that the salad cream meets the SON standard.

The presence of coliforms was observed in week 12 and was found to remain at 2 CFU/g all through the period of storage. The quantity (2 CFU/g) of coliforms in this sample was significantly different ($P < 0.05$) from samples 795, 852, and the commercial salad cream. It is also below the permissible level (< 10 CFU/g).

Vatanasuchart & and Stonsaovapak (2000) also observed the presence of coliform below 10 CFU/g in their oatrim-5 salad cream formulation.

The quantity of *Streptococcus* spp. ranged from 4 CFU/g – 8 CFU/g, which was significantly different ($P < 0.05$) from the commercial salad cream and above the Food and Agriculture Organization permissible limit (1.0 CFU/g), inferring that the salad cream is not fit for consumption.

Staphylococcus aureus was found present in the sample within the range of 2 – 4 CFU/g, which is below the permissible level of $< 10^3$ CFU/g allowed in ready-to-eat foods (Tsehayneh *et al.*, 2021) but it was not detected from week 12 through week 24.

The quantity of *Salmonella* spp. in the sample was recorded to be 2 CFU/g, which is significantly different from sample 852 and the commercial salad cream, and its presence is against the Nigerian Industrial Standard (NIS) for salad creams approved by the Standards Organization of Nigeria (SON) governing council. The specification for the quantity of *Salmonella* spp. is nil, inferring that the salad cream is not fit for consumption.

Pseudomonas spp. was found present in the sample within the range of 0 – 3 CFU/g, which was significantly different ($P > 0.05$) from samples 795, 852, and the commercial salad cream but below the Food and Agriculture Organization permissible limit ($< 10^4$ CFU/g).

Bacillus spp. was found to be present in the sample within the range of 8 - 30 CFU/g, which was significantly different ($P < 0.05$) from the commercial salad cream and

The quantity of *Streptococcus* spp. ranged from 2 CFU/g – 3 CFU/g which was significantly different ($P < 0.05$) from the commercial salad cream. The inhibitory effect of

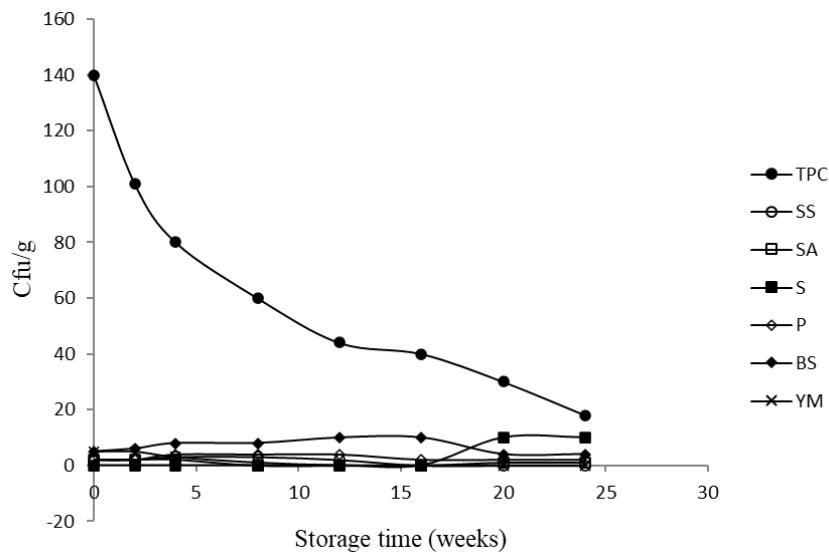


Figure 2. Changes in microbial parameters of sesame seed-based salad cream containing 2% acetic acid and 6% sugar during storage

Key: TPC = total plate count, SS = *Streptococcus* spp., SA = *Staphylococcus aureus*, S = *Salmonella* spp., P = *Pseudomonas* spp., BS = *Bacillus* spp., YM = yeast and mold

below the Food and Agriculture Organization permissible limit ($< 10^3$ CFU/g).

The Nigerian Industrial Standard (NIS) for salad creams approved by the Standards Organization of Nigeria (SON) governing council stated that the quantity of yeast and mold in salad creams should not exceed 100 CFU/g, Yeast and mold was found to be present in the sample within the range of 2 - 10 CFU/g which is below the permissible limit and also significantly different ($P < 0.05$) from samples 795 (2% acetic acid and 6% sugar), 852 (4% acetic acid and 4% sugar) and the commercial salad cream.

Changes in microbial parameters for sample 795, containing 2% acetic acid and 6% sugar, are presented in Figure 2. The salad cream had a total plate count of 140 CFU/g at week 0. A significant decrease ($P < 0.05$) was observed in the total plate count of the sample during storage. This may be due to the antimicrobial effect of acetic acid (Park *et al.*, 2021).

Coliforms and *E. coli* were not found all through the period of storage, indicating the inhibitory effect of acetic acid in the salad cream when compared with sample 462, which had no vinegar content.

acetic acid was observed from week 16 through week 24.

Staphylococcus aureus was found present in the sample within the range of 1 – 2 CFU/g, which was not significantly different ($P > 0.05$) from the commercial salad cream, and the quantity present is below the permissible level of $<10^3$ CFU/g allowed in ready-to-eat foods (Tsehayneh *et al.*, 2021) but it was not detected in week 8, week 12, and week 16. At week 20 (1.00 CFU/g) and week 24 (1.00 CFU/g), it was detected.

The quantity of *Salmonella* spp. in the sample was recorded to be 10 CFU/g at weeks 20 and 24, and it is significantly different ($P < 0.05$) from the commercial salad cream. The specification for the quantity of *Salmonella* spp. is nil, inferring that the salad cream is not safe for consumption at week 20.

Pseudomonas spp. was found present in the sample within the range of 2 – 4 CFU/g, which is not significantly different ($P > 0.05$) from sample 852 (4% acetic acid and 4% sugar) and the commercial salad cream but below the Food and Agriculture Organization permissible limit ($<10^4$ CFU/g). This observation agrees with the findings of Ahmed *et al.* (2022), who reported that *Pseudomonas aeruginosa* can survive at low pH (4.0) and high sugar concentrations. Dejsirilert *et al.* (1991) also reported the adaptability of *P. pseudomallei* to acidic medium (pH 4.5).

Bacillus spp. was found to be present in the sample within the range of 4 – 10 CFU/g, which was not significantly different ($P > 0.05$) from the commercial salad cream and sample 852 (4% acetic acid and 4% sugar) and below the

Coliforms, *E. coli*, and *Salmonella* spp. were not found all through the period of storage, indicating the inhibitory effect of acetic acid in the salad cream when compared with sample 462, which had no vinegar content.

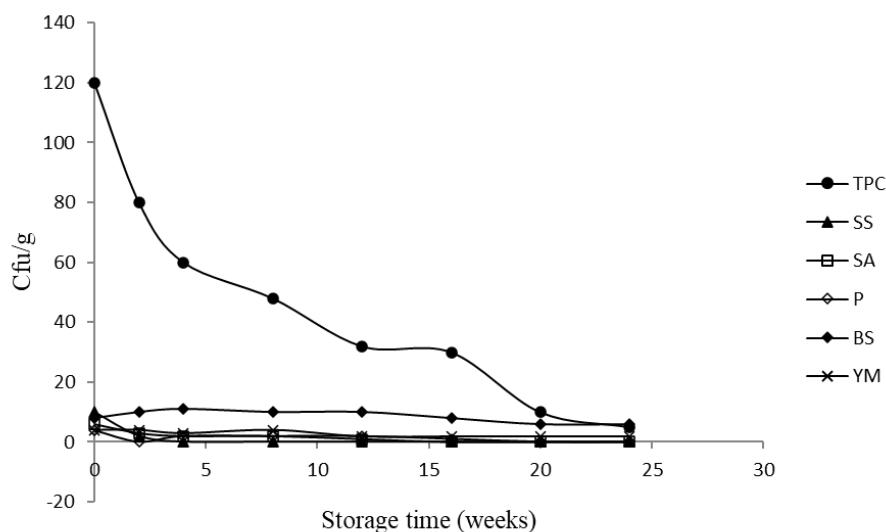


Figure 3. Changes in microbial parameters of sesame seed-based salad cream containing 4% acetic acid and 4% sugar during storage

Key: TPC = total plate count, SS = *Streptococcus* spp., SA = *Staphylococcus aureus*, P = *Pseudomonas* spp., BS = *Bacillus* spp., YM = yeast and mold

Food and Agriculture Organization permissible limit ($<10^3$ CFU/g). The observation agrees with the findings of Chirakkara & Abraham (2022), who reported that *Bacillus cereus* and *Bacillus velezensis* exhibited 50% survival at pH 2.5.

Yeast and mold were found to be present in the sample within the range of 1 – 5 CFU/g, which was below the permissible limit and was not significantly different ($P > 0.05$) from the commercial salad cream. This observation agrees with the findings of Pitt & Hocking, (2022), who reported that yeast and mold can survive at lower pH.

Changes in microbial parameters for sample 852 containing 4% acetic acid and 4% sugar are presented in Figure 3. The salad cream had a total plate count of 120 CFU/g at week 0. A significant decrease ($P < 0.05$) was observed in the total plate count of the sample during storage. This may be due to the antimicrobial effect of acetic acid (Park *et al.*, 2021). The decrease in total plate count was observed to be proportional to the concentration of acetic acid when compared with sample 462 (0% acetic acid and 6% sugar) and sample 795 (2% acetic acid and 6% sugar). Manios *et al.* (2014) also reported that salad cream with higher acetic acid content showed higher microbial stability.

Streptococcus spp. was only present at the initial stage after preparation (10 CFU/g), and in week 2 (2 CFU/g), and this was significantly different ($P < 0.05$) from the commercial salad cream. The inhibitory effect of acetic acid was observed from week 4 through week 24.

Staphylococcus aureus was found present in the sample within the range of 1 – 6 CFU/g, which was not significantly different ($P > 0.05$) from the commercial salad cream, and the quantity present is below the permissible level of $<10^3$ CFU/g allowed in ready-to-eat foods (Tsehayneh *et al.*, 2021) but it was not detected in week 16, week 20, and week 24.

Pseudomonas spp. was found present in the sample within the range of 1 – 4 CFU/g. However, it is below the Food and Agriculture Organization permissible limit ($<10^4$ CFU/g) and is not significantly different ($P > 0.05$) from sample 795 (2% acetic acid and 6% sugar) and the commercial salad cream. It was not detected in week 20 and 24. This observation is similar to the findings of Ahmed *et al.* (2022), which reported that *Pseudomonas aeruginosa* can survive at low pH (4.0) and high sugar concentration. Dejsirilert *et al.* (1991) also reported the adaptability of *P. pseudomallei* to acidic medium (pH 4.5).

Bacillus spp. was found to be present in the sample within the range of 6 – 11 CFU/g, which was not significantly different ($P > 0.05$) from the commercial salad cream and sample 795 (2% acetic acid and 6% sugar) and below the Food and Agriculture Organization permissible limit ($<10^3$ CFU/g). The observation agrees with the findings of Chirakkara & Abraham (2022), who reported that *Bacillus cereus* and *Bacillus velezensis* exhibited 50% survival at pH 2.5.

CFU/g, which was significantly different ($P < 0.05$) from samples 852, 795, and 462. The quantity present (2 - 4 CFU/g), is below the permissible level of $<10^3$ CFU/g allowed in ready-to-eat foods (Tsehayneh *et al.*, 2021).

Pseudomonas spp. was found present in the sample within the range of 2 – 6 CFU/g, which was below the Food and Agriculture Organization permissible limit ($<10^4$ CFU/g) and is significantly different ($P < 0.05$) from sample 795 (2% acetic acid and 6% sugar), 852 (4% acetic acid and

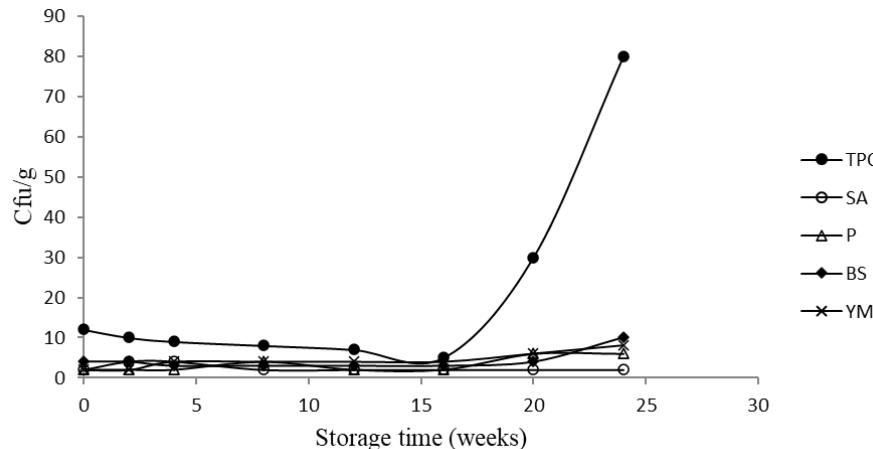


Figure 4. Changes in microbial parameters of a commercial salad cream during storage

Key: TPC = total plate count, *Staphylococcus aureus*, P = *Pseudomonas* spp, BS = *Basillus* spp, YM = yeast and mould

Yeast and mold were found to be present in the sample within the range of 2 – 4 CFU/g, which was below the permissible limit and was not significantly different ($P > 0.05$) from the commercial salad cream. This observation agrees with the findings of Pitt & Hocking (2022), who reported that yeast and mold can survive at lower pH.

Changes in microbial parameters of a commercial salad cream are presented in Figure 4. The salad cream had the lowest total plate count of 12 CFU/g at week 0. A significant increase ($P < 0.05$) was observed in the total plate count of the sample at week 20 (30 CFU/g) and week 24 (80 CFU/g). However, the total plate count is still below the permissible limit. This may be due to the sample reaching close to its shelf life or exposure of the salad cream to microorganisms from the environment once the seal is broken.

Coliforms, *E. coli*, *Streptococcus* spp., and *Salmonella* spp. were not found all through the period of storage, indicating the inhibitory effect of acetic acid in the salad cream.

Staphylococcus aureus was found present all through the period of storage in the sample within the range of 2 – 4

4% sugar) and sample 462 (0% acetic acid and 6% sugar). This observation agrees with the findings of Ahmed *et al.* (2022), who reported that *Pseudomonas aeruginosa* can survive at low pH (4.0) and high sugar concentration. Dejsirilert *et al.* (1991) also reported the adaptability of *P. pseudomallei* to acidic medium (pH 4.5).

Bacillus spp. was found to be present in the sample within the range of 3 – 10 CFU/g which was significantly different ($P < 0.05$) from sample 852 (4% acetic acid and 4% sugar) and sample 795 (2% acetic acid and 6% sugar) and below Food and Agriculture Organization permissible limit ($<10^3$ CFU/g). The observation agrees with the findings of Chirakkara & Abraham (2022), who reported that *Bacillus cereus* and *Bacillus velezensis* exhibited 50% survival at pH 2.5.

Yeast and mold were found to be present in the sample within the range of 2 – 8 CFU/g, which was below the permissible limit and was significantly different ($P < 0.05$) from sample 462 (0% acetic acid and 6% sugar), 852 (4% acetic acid and 4% sugar) and sample 795 (2% acetic acid and 6% sugar). This observation agrees with the findings of Pitt & Hocking (2022), who reported that yeast and mold can survive at lower pH (2.0).

Vinegar acts as an antimicrobial by lowering the pH of food samples to a state that is unfavorable for microbial growth. (Antoniewicz *et al.* 2021; Kara *et al.* 2022). The build-up of acid as a result of organic decomposition, which is facilitated by the presence of sugar, made the growth of microorganisms unfavorable and this led to the decrease of microorganisms. Therefore, the combined effect of sugar and acetic acid (vinegar) brought about reduction in the total plate count; slowed down the oxidation of oil present in the salad cream, and increased acidity, as seen in the results for microbial analysis (Figure 1-4), pH, titrable acidity, peroxide value, free fatty acid and thiobarbituric acid (Table 1).

Overall, the presence of *Pseudomonas* spp. and *Salmonella* spp. in the salad cream samples explains the change in the color (from pale yellow to brown) of the salad creams. The deduction is based on the findings of Muller (1985). He observed the production of brownish pigment by bacteria of the morganella-proteus-providencia group on agar containing L-forms of aromatic amino acids (that is, histidine, phenylalanine, tryptophan, and tyrosine) in the presence of iron ions under aerobic conditions. *Morganella morganii* belongs to the *Proteaceae* of the Enterobacteriaceae family. Enterobacteriaceae is a large family of Gram-negative bacteria such as *Salmonella*, *E. coli*, *Pseudomonas*, and so on. To combat this challenge, the use of vacuum packaging (Maqsood *et al.*, 2016) or modified atmosphere packaging will retard the growth of these microorganisms and preserve the color of the salad cream (Koyuncu & Batur, 2023).

CONCLUSION

All the samples were chemically stable; as such, extensive oxidation was not observed. Samples 462 (containing no vinegar and 6% sugar) and 795 (containing 2% acetic acid and 6% sugar) were not microbiologically stable during the six months of storage. The decrease in total plate count and the inhibitory effect of acetic acid on some microorganisms were observed to be proportional to the concentration of acetic acid present in the samples.

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