Research Paper

Effect of pH and Fat Levels on the Physico-chemical and Sensory Properties of Beetroot and Carrot-based Bread Spreads

H.R.P. Fernando[1]*, B.M.D.S. Piyasena[2], U.M. Thenabadhu[2], C.D. Kodithuwakkuarachchi[1], T. Dharmathilake[1], U.L. Manage[1]

[1] *Food Research Unit, Department of Agriculture, Gannoruwa, Peradeniya, Sri Lanka*

[2] *Department of Agriculture and Food Science, Faculty of Industrial Technology, University of Vocational Technology, Rathmalana, Sri Lanka*

[*]*Corresponding author: hrpf100@gmail.com*

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Abstract

This study explores the utilization of carrot and beetroot in developing a low-fat, low-sugar bread spread, aiming to extend their shelf life as value added products. Four recipes were formulated, altering the percentages of virgin coconut oil and citric acid (T_1, T_2, T_3, and T_4 were 3.2 and 0.12, 3.5 and 0.15, 3.2 and 0.15, and 3.5 and 0.12 of virgin coconut oil and citric acid, respectively) using Taguchi method. Sensory evaluation by a panel of 30 semi-trained members revealed the recipe with carrot/beetroot (92.56%), virgin coconut oil (3.5%), as main ingredients as the optimal choice. The developed bread spreads maintained quality for four months when stored in glass bottles under ambient conditions. Analysis showed pH, Total Soluble Solids (TSS), moisture, titratable acidity, fat, protein, and microbial counts at regular intervals. The final products contained 5.2-5.0 pH, 11.3-13.3% TSS, 0.41-0.46% acidity, 79.43-84.35% moisture, and 8.29-9.69% ash. Microbial analysis indicated that total plate count and yeast/mold counts below 10 CFU g^{-1}, with zero *Escherichia coli* counts over four months. The cost for 250 g was Rs. 350.37. These findings highlighted the potential for preserving carrot and beetroot spreads for up to four months under ambient conditions. Overall, both beetroot and carrot-based bread spreads offer a variety of nutrients, with carrot spread generally containing higher levels of protein and carbohydrates compared to beetroot spread. These spreads can contribute to a balanced-diet when consumed in moderation and as part of a varied meal plan.

Keywords: Beetroot, Bread spread, Carrot, Low fat, Microbial analysis, Quality and sensory properties

Introduction

Childhood malnutrition is a critical issue in Sri Lanka, exacerbated by the consumption of low nutrient foods and high carbohydrate intake (Abeywickrama *et al.*, 2018). Many children start their day with inadequate breakfasts, predominantly carbohydrate based, due to busy life styles. Unfortunately, these choices lack essential nutrients and may contain unhealthy fats, such as those found in margarine products (Tiina *et al*., 2012). Addressing this concern requires the urgently, and introducing of highly nutritious breakfast alternatives that offer essential nutrients.

In recent years, there has been a notable shift towards healthier dietary choices and sustainable food practices globally (Guerrero *et al*., 2009). As a part of this trend, traditional food items have been reinvented to provide more nutritious alternatives, and vegetable based bread spreads have emerged as a popular choice among health-conscious consumers. These spreads offer a flavourful and wholesome alternative to conventional spreads, tapping into the natural goodness of vegetables. Moreover, concerns regarding the nutritional content and health impact of traditional spreads have highlighted the potential of vegetable based alternatives to address health and sustainability challenges (Augustin *et al*., 2020).

Carrot (*Daucus carota* L.) and beetroot (*Beta vulgaris* L.) are highly prized vegetables in Sri Lanka. They are a favourite among children, who are drawn to their appealing taste and colours. Notably, these vegetables boast a wealth of nutrients, including vitamins, minerals, phytochemicals, and dietary fiber, while being low in fat (Shyamala & Jamuna, 2010). However, post-harvest losses of these vegetables are often substantial due to their perishable nature and susceptibility to diseases and microbial decay. Additionally, abnormal roots are typically unsuitable for market sale and are often sold at reduced prices or discarded (Dharmathilake *et al*., 2020). To address these issues, carrot and beetroot can be utilized to prepare nutritious bread spreads (Raees-ul & Prasad, 2015; Mudgal *et al*., 2022), which can be popularized among children and the young community to combat malnutrition and reduce post-harvest losses. Currently, there's a scarcity of vegetable based nutritious bread spreads in the local market, and research on such products in Sri Lanka is limited. Thus, this study aimed to formulate nutritious, low-fat, vegetable-based bread spreads and promote them as breakfast options to help address children's malnutrition. Physico-chemical properties, shelf life, and sensory attributes were also evaluated to identify the best formulations and production processes.

Materials and Methods

Preliminary studies were conducted to develop recipes for bread spreads using beetroot and carrot. In the initial phase, various formulations were tested to determine the ingredients and their respective quantities. After conducting

nine trials, one formulation was selected as the best one (Table 1). To make this formulation, several factors were assessed after one month of storage under ambient conditions, including pH, °Brix value, colour change, appearance, odor, oil separation, lactic acid bacteria fermentation, and microbial growth (data is not shown). The recipe selected below was used as the basis of experiment aimed at optimizing pH and oil content through several treatments.

Table 1. Selected best formulation for beetroot and carrot bread spread

Ingredients	Quantity (%)
Carrot /Beetroot	92.56
Virgin coconut oil	3.20
Garlic powder	1.67
Mustard powder	1.21
Salt	0.81
Citric acid	0.12
Pepper	0.13
Lecithin	0.1
Corn flour	0.67
Sodium metabisulfite	0.1

Treatments

In the second preliminary study, three levels of virgin coconut oil and citric acid content were tested as lower, middle, and upper concentrations to select the favorable concentrations. The middle values were selected for formulation as 3.20% for virgin coconut oil and 0.12% for citric acid. Lower values were 2.9% for virgin coconut oil and 0.09% for citric acid, while upper values were 3.5% for virgin coconut oil and 0.15% for citric acid. The lower levels (2.9% virgin coconut oil and 0.09% citric acid) were observed as unsuccessful in preparing beetroot and carrot based bread spreads. Consequently, only middle and upper values (Table 2) were used in the final experiment to study physico-chemical properties, taste, shelf life, and spreadability, while reducing microbial growth, fat percentage, and oil separation of the final products.

Table 2. Selected treatments for the final experiment

Variables	T_1	T_2	T_3	T_4
Virgin coconut oil (%)	3.2	3.5	3,2	3.5
Citric acid (%)	0.12	0.15	0.15	0.12

Note: T_1, T_2, T_3 and T_4= different combinations of virgin coconut oil and citric acids as different treatments.

Based on the above combinations, four treatments were conducted for the experiment as detailed below:

T_1 = 3.2% Virgin coconut oil and 0.12% citric acid

T_2 = 3.5% Virgin coconut oil and 0.15% citric acid

T_3 = 3.2% Virgin coconut oil and 0.15% citric acid

T_4 =3.5% Virgin coconut oil and 0.12% citric acid

Material preparation for the formulations

Beetroot and carrot (commercial varieties, Tera Cota and Crimson Globe respectively) were obtained from Green Fresh Organic Farm in Nuwara Eliya, Sri Lanka, ensuring quality and uniformity. After careful selection, the vegetables were cleaned, peeled, and baked at 190 °C for 1 hour. Ingredients including Virgin coconut oil, garlic powder, mustard powder, salt, citric acid, pepper, lecithin, corn flour, and sodium metabisulphite (SMS) were utilized as per the quantities listed in Table 1, except for Virgin coconut oil and citric acid, which varied as experimental variables. Garlic and mustard were used in dry powder form.

Preparation method of bread spreads

The baked carrots or beetroot were diced and blended in to a paste. Other ingredients, excluding virgin coconut oil and citric acid, were added and blended until homogenized. Half of the virgin coconut oil was then mixed thoroughly. The remaining oil was heated in a stainless-steel pan at 80±2 °C for four minutes, and the mixture was cooked. Citric acid and SMS were swiftly added, and after 2-3 minutes, the spread was hot-filled into sterilized glass bottles. These bottles underwent an exhausting process at 80 °C before final sterilization at temperature up to 100 °C .

Determining of physico-chemical parameters

Determination of pH: One gram of the bread spread was dissolved in 4 mLof distilled water, with each treatment receiving appropriately labeled samples. Beakers were stirred until a smooth suspension formed and then left for 30 minutes. The suspension was transferred to another beaker, and the pH was measured using a pre-calibrated pH meter (MI 150, Milwaukee, Rumania).

Total Soluble Solid (TSS): The refractometer was calibrated with distilled water to ensure a zero reading. Then, a small amount (1 or 2 drops) of the prepared bread spread solution was placed on the prism of the digital refractometer, and reading was taken. Three readings were taken sequentially from each sample.

Colour: Each sample was taken individually, and a small amount of bread spread was placed in a petri dish. The spread was then smoothed out, and the petri dishes were positioned on a white background. Comparisons were made between the chart (Royal Horticulture Society) and the food sample, and the colour was noted.

Moisture content: Moisture content of carrot and beetroot bread spreads was measured using AOAC official method 934.01 (AOAC, 1990). The moisture content was calculated using the Formula 1.

$$\text{Moisture content (\%)} = \frac{\text{Weight of dried sample (g)}}{\text{Weight of initial sample (g)}} \times 100 \qquad (1)$$

Total ash content: The ash content of carrot and beetroot bread spread samples was determined following AOAC official

method 942.05 (AOAC, 1990). The total ash content was calculated accordingly to the Formula 2.

Titratable Acidity (TA): Titratable Acidity (TA) of carrot and beetroot bread spreads was determined following the AOAC official method 942.15 (AOAC, 1990). The TA values were calculated accordingly Formula 3.

V = volume of NaOH solution used (mL), N = normality of NaOH solution, W = weight of the spread sample (g), and EW = Equivalent weight of citric acid (g)

Free fat: The free fat percentage was determined using AOAC official method 960.39 (AOAC, 1990) according to the Soxhlet extraction. The free fat percentage was calculated using the Formula 4.

M_1 = weight of the empty round bottom flask and M_2 = weight of the empty round bottom flask + fat

Protein content: The crude protein content of the samples was determined following AOAC method 960.52 (AOAC, 1990) with minor adjustments. Each homogenized bread spread sample (50 mg) was placed into separate 50 mL Kjeldhal titration flasks. A Kjeldhal tablet (5 g, Supelco®, Germany) and 2 mL of concentrated sulfuric acid were added to each flask. After digestion, the samples were cooled, dissolved in ammonia free distilled water, and transferred to a semi micro Kjeldhal distillation apparatus. In the distillation flask, a mixture of 8 mL Sodium hydroxide-sodium thiosulphate (0.1 mol L^{-1}), 5 mL 4% boric acid solution, and 3 drops of indicator mixture (methyl red and methylene blue) was added. Ammonia liberated during distillation was captured in a titration flask containing boric acid and indicator mixture. The distillate was titrated with standard hydrochloric acid solution until a pink colour endpoint was reached. Protein content was calculated using the Equation 5.

Ws = weight (g) or volume (mL) of sample, a = volume (mL) of 0.1N H_2SO_4 used in blank titration, b = volume (mL) of 0. 1N H_2SO_4 used in sample titration, 14.00 = atomic weight of nitrogen, 1000 = the conversion of mg N/100 g to g N/100 g sample, and 6.25 = the protein-nitrogen conversion factor

$$\text{Ash content (\%)} = \frac{\text{Weight of final ash sample (g)}}{\text{Weight of initial sample (g)}} \times 100 \quad (2)$$

$$\text{TA (\%)} = \frac{\text{V} \times \text{N} \times \text{Volume made up} \times \text{EW}}{\text{Aliquot taken for the titration} \times \text{W} \times 1000} \times 100 \quad (3)$$

$$\text{Free fat (\%)} = \frac{M_2 - M_1}{\text{Weight of the sample}} \times 100 \quad (4)$$

$$\text{Nitrogen (\%)} = \frac{(b - a) \times 0.1 \times 14.00 \times 6.25}{Ws \times 1000} \times 100 \quad (5)$$

Carbohydrate concentration: The carbohydrate content of bread spread samples was determined using the method previously suggested by Chronakis and Kasapis (1995). This involves subtracting the sum of total crude fiber, total protein, ash, and moisture from 100. The calculation was done by using Equation 6.

Microbiology analysis

Determination of yeast and mold growth: Yeast and mold count of the samples were conducted according to the standard method SLS 516 part 2-1991. Potato dextrose agar (PDA) media was prepared by dissolving 39 g of PDA powder in 1 liter of distilled water and autoclaving at 121 °C and 15 PSI for 20 minutes. All glassware was sterilized at 160 °C for 2 hours, and test tubes filled with 9 mL of distilled water were sterilized at 121 °C and 15 PSI for 20 minutes. All bread spread samples were diluted to 10^{-1}, 10^{-2}, and 10^{-3} dilutions. Prepared PDA was poured into petri plates, and inoculated samples were plated and sealed with Para film. Plates were incubated at 30–35 °C for 48 hours, and colonies were counted. Petri dishes containing 30-300 colonies were considered accurate. The calculation was performed using Equation 7.

N – Number of yeasts and molds per ml, Σ C – sum of colonies counted on all plates, n_1 – number of plates retained in the first dilution, n_2 – number of plates retained in the second dilution, and d – dilution factor corresponding to the first dilution

Determination of total plate count: The total plate count of the samples was assessed using the standard method SLS 516 part 1 -1991. Nutrient agar powder (17.5 g) was dissolved in 1 L of distilled water in a conical flask and sterilized in an autoclave at 121 °C for 20 minutes at 15 PSI. All glassware was sterilized at 160 °C for 2 hours, and test tubes filled with 9 mL of distilled water were sterilized at 121 °C and 15 PSI for 20 minutes. All samples were diluted to 10^{-1}, 10^{-2}, and 10^{-3} dilutions. Agar was poured into plates, inoculated, and sealed with para film. Plates were then incubated at 30–37 °C for 24 hours, and colonies were counted. Petri dishes containing 30-300 colonies were considered accurate, with calculations performed as per the Equation 8.

N – Number of total colonies per ml or per g, Σ C – sum of colonies counted on all plates, n_1 – number of plates retained in the first dilution, n_2 – number of plates retained in the second dilution, and d – dilution factor corresponding to the first dilution

$$\text{Carbohydrate (\%)} = 100 - (\text{\% protein} + \text{\% fat} + \text{\% ash} + \text{\% moisture} + \text{\% fiber}) \quad (6)$$

$$N = \frac{\Sigma C}{(n_1 + 0.1n_2)^d} \quad (7)$$

$$N = \frac{\Sigma C}{(n_1 + 0.1n_2)^d} \quad (8)$$

Determination of *Escherichia coli*: The determination of *E. coli* in the samples followed standard method SLS 516 part 3 – 1982. *E. coli* broth (ECB) (17 g) was diluted in 1000 mL of distilled water. Then, 9 mL of the prepared ECB was dispensed into test tubes, with Durham tubes carefully placed into each without introducing air bubbles. All tubes were sterilized in an autoclave at 121 °C for 20 minutes at 15 PSI. Subsequently, 1 mL of the samples was transferred into the test tubes using a sterilized pipette. The labeled test tubes were incubated at 37 °C for 48 hours. The presence of *E. coli* was indicated by the formation of air bubbles inside the Durham tube. Gas production and pink colouration in the upper part of the solution indicated the presence of *E. coli*.

Sensory attributes

The study employed Taguchi's design methodology, utilizing a factorial design (2-2) with the addition of one center point. Four bread spreads, labeled as T_1, T_2, T_3, and T_4 as mentioned above included in the complete design. A sensory evaluation involved thirty panelists from the Food Research Unit, Gannoruwa, assessing attributes like appearance, aroma, colour, taste, texture, spreadability, and overall acceptability using a seven-point Hedonic scale. Samples labeled with three digit numbers were provided to each panelist for evaluation.

Statistical analysis procedure

The experimental design for the current study employed a Completely Randomized Design (CRD). Two-way analysis of variance was conducted using Minitab 20® for Windows (Minitab, LLC) to analyze all physicochemical and micro-biological parameter datasets. The significance of differences between means was assessed using Tukey's multiple range test at a significance level of $p<0.05$. Friedman's non parametric analysis and Minitab's two-way ANOVA were utilized for data analysis in the sensory evaluation to ascertain any significant differences in mean liking scores among the selected samples. The sum of ranks was calculated to derive a test statistic.

Results and Discussion

Physico-chemical parameters

pH change: The pH values of all samples exhibited a progressive trend during storage, with beetroot and carrot-based bread spreads (T_1-T_4) showing an average pH range of 5.0 to 5.2 after two months (Table 3). Despite fluctuations between 4.4 and 5.2, the quality of these spreads remained consistently high over the two-month period, with pH values ranging from 5.0 to 5.2 (Table 3). Microbiology counts confirmed product safety, with a microbial count of <10 CFU g^{-1} after two months. Treatment T4 beetroot- based bread spread

displayed superior stability, with pH values of 4.6 after one month and 5.2 after two months. The final carrot-based bread spread under treatments T_1 and T_3 showed higher pH values (5.2) after two months. However, no significant differences were noted among treatments and days based on pH results (p>0.05). Generally, a decrease in pH values indicates microbial growth in products, which affects product quality and shelf life. Conversely, higher pH levels in products promote microbial growth. Therefore, maintaining a moderate pH range (4.1 to 5.4) is advisable for preserving both product quality and shelf life (Holmer *et al*., 2009). Mendiratta *et al*.(2015) reported that maintaining a moderate pH, such as 4.8 to 5.1, enhances the quality and shelf life of chicken sandwich spread. Furthermore, Andrés-Bello *et al*. (2013) explained that pH significantly influences the quality and shelf life of processed foods. Additionally, a moderate pH range ensures product quality by slowing down microbial growth and enzyme deterioration.

Colour change: Both beetroot and carrot-based bread spreads exhibited colour changes during storage. Heating processes notably affected the colour of the beetroot spread due to the instability of red pigments like Betacaine under heat (Hobbs *et al*., 2014). Similar findings were observed in this study. Initially, beetroot based samples (T_1-T_4) displayed gray orange 165 (T_1), gray-orange 174 (T_2 and T_3), and red 53 (T_4) colours post-heating, transitioning to gray brown 199 (T_1, T_2, and T_3) and gray orange 164 (T_4) after four months. Carrot based spreads (T_1-T_4) initially had a yellow orange 17 colour, shifting to yellow orange 21 except for T_4, which maintained its original colour code (yellow-orange 17) after four months. Both spreads experienced colour reduction during storage, with T_4 of each spread showing the least change over the four month.

Table 3. pH fluctuations in beetroot and carrot-based bread spreads at two-week intervals over a two-month storage period at ambient temperature

Sample	Time	T_1	T_2	T_3	T_4
Beetroot-based bread spread	0W	4.7±0.01^{abc}	4.6±0.02^{bcd}	4.5±0.01^{bcd}	4.7±0.01^{abcd}
	2W	4.5±0.11^{bcd}	4.5±0.09^{cd}	4.4±0.03^{d}	4.6±0.04^{bcd}
	4W	4.9±0.26^{a}	4.5±0.09^{bcd}	4.5±0.08^{bcd}	4.6±0.08^{bcd}
	6W	4.8±0.09^{ab}	4.6±0.08^{bcd}	4.6±0.05^{bcd}	4.5±0.17^{bcd}
	8W	5.1±0.14^{a}	5.0±0.03^{abc}	5.0±0.04^{ab}	5.2±0.00^{a}
Carrot-based bread spread	0W	5.2±0.28^{a}	4.7±0.03^{bc}	4.8±0.03^{abc}	4.9±0.01^{ab}
	2W	4.5±0.07^{bcd}	4.3±0.06^{d}	4.4±0.09^{cd}	4.5±0.08^{bcd}
	4W	4.9±0.39^{ab}	4.5±0.17^{bcd}	4.7±0.07^{bcd}	4.7±0.10^{bcd}
	6W	4.6±0.05^{bcd}	4.6±0.05^{bcd}	4.8±0.09^{bc}	4.6±0.03^{bcd}
	8W	5.2±0.01^{ab}	5.1±0.04^{abc}	5.2±0.02^{a}	5.0a±0.03^{bcd}

Note: The mean values are presented based on three replicates, with ± standard deviation. Mean values with the same superscript letters within the same column do not differ significantly at p<0.05 significant level. W=week

Total soluble solids: The initial TSS values of beetroot and carrot-based spreads (T_1-T_4) ranged from 14-15% and from 10.7- 12.5%, respectively (Figure 1). After one month, beetroot spreads showed values between 13% and 15%, while carrot spreads ranged from 10.4-13.3%. Unlike sugar-added spreads, this study's sugar-free spreads exhibited lower TSS values. After two months, beetroot spreads decreased to 11-12%, while carrot spreads increased to 13-15%. While no significant differences were found among treatments for beetroot spreads (p>0.05), significant variations were observed over time (p<0.05). Carrot spreads showed significant differences among treatments, with the foutrh treatment showing the least fluctuation and maintaining higher TSS levels throughout storage.

Treatable Acidity: Over a span of two months, notable differences in acidity were observed among the bread spreads stored in glass bottles at room temperature. However, there were no significant variations among the treatments and days (p>0.05), as indicated by the comprehensive results. Within a one month period, both products maintained excellent pH levels (Table 3). Consequently, throughout this timeframe (1st and 2nd months), acidity levels remained satisfactory, as evidenced by the data in Table 4. Notably, the sensory evaluation of T_4 sample remained excellent even after 2 months, corroborated with the data in Table 4.

Moisture content:

Both carrot and beetroot bread spread samples experienced a decrease in moisture content during storage. Significant differences (p<0.05) in moisture content were observed among all treatments except for samples stored in glass bottles at ambient temperature (Figure 2). Regarding beetroot bread spread, there was a significant difference between days, whereas for carrot bread spread, there were no significant differences (p>0.05) among the days.

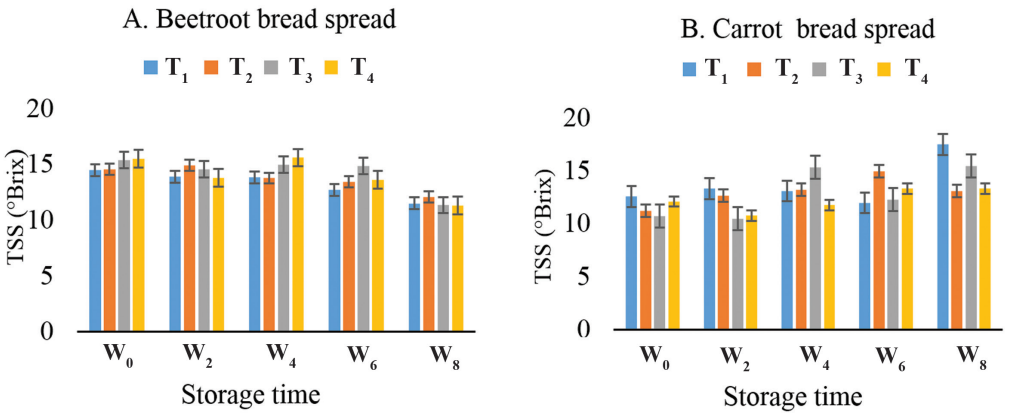

Figure 1. Variation of total soluble solid content of the beetroot (A) and carrot-based (B) bread spreads within two month storage at ambient conditions.

W= week. Vertical bars indicate standard error of the mean for three replicates.

Table 4. Change of titratable acidity (TA) of beetroot and carrot-based bread spreads for two-month storage at ambient conditions.

Sample	Storage time	T_1	T_2	T_3	T_4
Beetroot-based bread spread	W_2	0.73 ± 0.03^{abcd}	0.77 ± 0.00^{abc}	0.79 ± 0.07^{ab}	0.85 ± 0.18^{a}
	W_4	0.55 ± 0.05^{bcde}	0.58 ± 0.00^{abcde}	0.64 ± 0.00^{abcde}	0.64 ± 0.00^{abcde}
	W_6	0.49 ± 0.07^{cde}	0.53 ± 0.15^{bcde}	0.47 ± 0.03^{de}	0.45 ± 0.06^{de}
	W_8	0.40 ± 0.03^{e}	0.64 ± 0.28^{abcde}	0.49 ± 0.04^{cde}	0.46 ± 0.03^{de}
Carrot-based bread spread	W_2	0.53 ± 0.04^{cd}	0.70 ± 0.22^{bc}	0.49 ± 0.04^{cd}	0.45 ± 0.11^{d}
	W_4	0.91 ± 0.07^{ab}	0.98 ± 0.04^{a}	0.87 ± 0.03^{ab}	0.85 ± 0.07^{ab}
	W_6	0.38 ± 0.00^{d}	0.46 ± 0.03^{cd}	0.36 ± 0.07^{d}	0.46 ± 0.03^{cd}
	W_8	0.49 ± 0.09^{cd}	0.38 ± 0.06^{d}	0.36 ± 0.03^{d}	0.41 ± 0.03^{d}

Note: The mean values are presented based on three replicates, with ± standard deviation. Mean values with the same superscript letters within the same column do not differ significantly at $p<0.05$ significant level. W=week

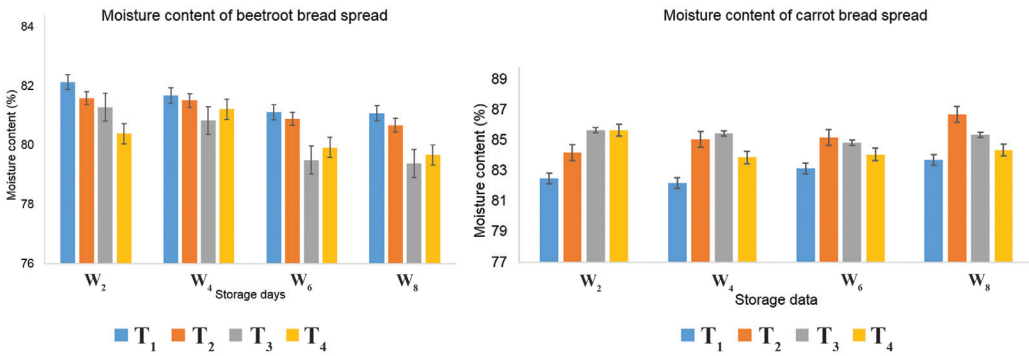

Figure 2. Variation of moisture content of the beetroot and carrot-based bread spreads within two month storage at ambient conditions.

Vertical bars indicate standard error of the mean for three replicates. W= week.

After the first month, moisture content was measured at 80.38% for beetroot spread and 85.65% for carrot spread. After 2 months' period, the moisture content decreased to 79.43% for beetroot spread and 84.35% for carrot spread. Figure 2 illustrates the variation in moisture content over the 2-month storage period for both products. However, the reduction of moisture content in the bread spreads helped to control physicochemical characters and microbial growth, thereby improving the quality and shelf life (Lee *et al*., 2004).

Sensory evaluation: After conducting the micro biology tests, the sensory evaluation was conducted on the product after one month later. Treatment 4, comprising 3.5% virgin coconut oil and 0.12% citric acid, was identified as the optimal recipe for both beetroot and carrot bread spreads based on sensory evaluation (Figure 3).

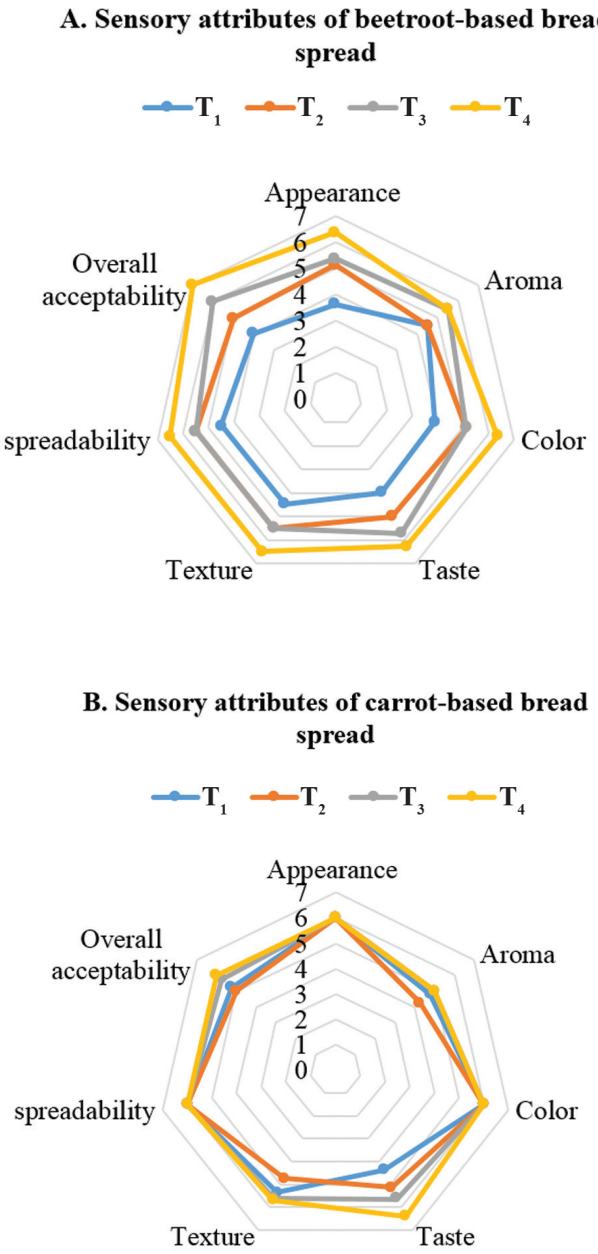

Figure 3. Descriptive rating chart of beetroot (A) and carrot (B) based bread spread sensory evaluation.

The higher fat content and lower citric acid levels were favored by panelists, enhancing attributes like appearance, aroma, taste, texture, and spreadability. These findings are supported by Limbad *et al*. (2020), who noted that increased fat content improves mouthfeel and spreadability. Additionally, Patel *et al*. (2019) reported that higher pH levels enhance sensory attributes in processed foods. While all carrot-based treatments yielded similar sensory results, variations were observed among beetroot-based treatments, with treatment 4 performing best across attributes like aroma, taste, texture, mouthfeel, and overall acceptability. Thus, the ingredient combination of the fourth tratment offers optimal formulations for both carrot and beetroot based spreads.

Microbial growth: Microbial growth tests, including total plate count, yeast and mold growth, and *E. coli* testing, were conducted after a two-month storage period at room temperature. The microbial count should adhere to the standard limits specified by the Sri Lanka Standards Institution, such as total plate count at 1.0 x 10^4 CFU g^{-1}, yeast and mold at 1 x 10^2 CFU g^{-1}, *E. coli* at 1 x 10^2 CFU g^{-1}, and the absence of salmonella (SLS/MOH 1980). Following the two-month storage period at ambient conditions, microbial growth tests yielded negative results for all TPC, yeast and mold, and *E. coli* tests. Therefore, both products are deemed safe after a two-month storage period under ambient conditions.

Based on the overall performance indicated by measured parameters (physicochemical, nutritional, sensory, and microbial analyses), T_4 demonstrated superior quality in both beetroot and carrot-based bread spreads, when compared to other treatments (T_1, T_2, and T_3). Consequently, the beetroot and carrot based bread spreads produced under the T_4 recipe displayed the highest quality, extended shelf life, and tested negative for all microbial assessments.

Microbial assessment: After four months, both products maintained microbial counts below the standard limits set by the Sri Lanka Standards Institution (1.0 × 10^4 CFU g^{-1}), ensuring their safety throughout the storage period. *E. coli* test results also indicated levels within acceptable limits during the four-month period (Table 5). The final product's microbial quality was notably high, attributed to proper ingredient levels, pre-processing conditions, addition of preservatives (SMS), pH control, quality raw materials, handling practices, aseptic procedures, and storage conditions. Both products remained microbiologically stable under ambient temperature storage for the entire four-month duration (Table 5).

Table 5. The microbial assessment of the final products (T_4) after 4-months storage at ambient conditions

Product	TPC (CFU g^{-1})	Yeast and mold (CFU g^{-1})	E. coli (CFU g^{-1})
Beetroot-based bread spread	$<10^4$	$<10^2$	Negative
Carrot-based bread spread	$<10^4$	$<10^2$	Negative

Notes: The measurements are presented based on three replicates. TPC= total plate count, CFU = Colony forming units, g= grams.

Fat concentration: In this study, low-fat bread spreads were developed with reduced fat levels compared to regular spreads, targeting individuals mindful of their fat intake for a healthier diet. Lower fat content also reduces overall calorie count, beneficial for managing caloric intake. Additionally, it may offer health advantages by limiting saturated and trans-fat intake, associated with increased heart disease risk. Per the CODEX Alimentarius standard, blended fat spreads should contain <80% crude fat, while margarine >80% crude fat (WHO, 2021). Previous research on pumpkin-based spread showed a fat content of 25.10% (Samarathunga, 2010). Food standards stipulate fat products should contain 10-90% fat (Gunarathne *et al*., 2021). Carrot (17.32%) and beetroot (14.55%) based bread spreads in this study meet these standards, making them safe, low-fat alternatives (Table 6).

Nutritional profile: Beetroot bread spread has approximately 69.92% moisture, slightly higher than the 65.73% found in carrot spread (Table 7). Moisture content influences texture and shelf life. The total fat content is 12.60% in beetroot spread and slightly higher at 13.41% in carrot spread.

Table 6. The crude fat concentration of the final selected products (T_4) after 4-months storage at ambient conditions.

Sample	Treatment	Crude fat (%)
Beetroot-based bread spread	$<10^2$	Negative
Carrot-based bread spread	$<10^2$	Negative

The measurements are presented based on three replicates. TPC= total plate count, CFU = Colony forming units, g= grams.

Table 7. Nutritional profile of the developed beetroot and carrot-based bread spreads

Nutrient	Percentage on wet basis (%)	
	Beetroot spread	Carrot spread
Moisture	69.92	65.73
Total fat	12.60	13.41
Crude fat	6.83	10.32
Ash	8.53	6.55
Protein	2.11	3.98
Carbohydrates	6.84	10.33

Fat provides energy and essential fatty acids. Beetroot spread has 6.83% crude fat, while carrot spread has 10.32%. Ash content is 8.53% in beetroot and lower at 6.55% in carrot spread, representing mineral content. Protein content is 2.11% in beetroot and higher at 3.98% in carrot spread. Carbohydrate content is 6.84% in beetroot and 10.33% in carrot spread, serving as the body's main energy source.

Cost estimation: In the Sri Lankan market, popular fat-based bread spreads are priced at 250 g for Rs. 450, Rs. 495, and Rs. 850. The price of the products currently of this project can be managed to remain within a lower or similar range compared to that of commercial products. Additionally, these commercial spreads often contain high percentages of fat. Therefore, carrot and beetroot based bread spreads can be recommended as healthy alternatives at reasonable prices for consumers. These spreads are sugar free and low fat, offering a nutritious option for consumers seeking healthier choices. Table 8 illustrates the cost of beetroot and carrot based bread spreads for 250 g quantities.

Table 8. Cost estimation to produce 250 g of beetroot and carrot based bread spreads

Items	Amounts for 250 g (g)	Cost for 250 g (Rs)
Carrot /Beetroot	231	92.40
Virgin coconut oil	8.75	15.00
Garlic	4.175	6.97
Mustard powder	3.025	5.00
Salt	2.025	2.90
Citric acid	0.3	2.60
Pepper	0.325	7.50
Lecithin	0.25	3.50
Corn flour	1.675	2.50
SMS	0.25	3.50
Other cost (Labour, electricity, water, machinery)	-	213.50
Total		**350.37**

Conclusions

In summary, the beetroot and carrot-based bread spreads maintained quality for four months when stored in glass bottles at ambient conditions. Microbiological analysis over this period revealed total plate counts and yeast and mold counts below 10 CFU g^{-1}, with *E. coli* counts testing negative. These results were within acceptable limits, indicating that the products remained microbiologically stable and safe during the four-month storage period. Both carrot and beetroot were successfully utilized in the development of bread spreads. These spreads can be recommended as low-fat and low-sugar options, offering satisfactory fat levels compared to market alternatives. Overall, both beetroot and carrot spreads offer a variety of nutrients, with carrot spread generally containing higher levels of protein and carbohydrates compared to beetroot spread. These spreads can contribute to a balanced diet when consumed in moderation and as part of a varied meal plan.

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