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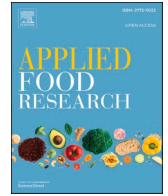


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Optimised combination of rosemary, ginger and garlic improves microbial shelf-life and sensory acceptability of vitamin A and iron-enriched cassava-based pancake (*Kabalagala*)

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ABSTRACT

In Uganda, the prevalence of vitamin A deficiency is 8.3 and 9.0 % in the urban and rural areas, respectively, while iron deficiency is at least 53 % in CUF. This necessitated the development of vitamin A and iron-enriched cassava-based pancakes (*Kabalagala*) with desirable sensory and physical properties. However, enrichment reduced microbial shelf life by 66.67 %, from 72 to 24 h. Individual application of spices (rosemary, ginger, and garlic) resolved the microbial shelf-life challenge but the products were sensorially unacceptable. A special cubic-constrained simplex lattice mixture design approach was used to generate different ternary combinations of the three spices. Ridge analysis was used to determine the optimal spices' proportions based on the pancakes' total plate count and sensory properties. Optimal values were 1.0 g rosemary, 0.5 g ginger, and 0.5 g garlic; and 0.5 g rosemary, 0.5 g ginger, and 1.0 g garlic, for products targeting children 2–3- and 4–5-years-old, respectively. Optimal spices combination improved microbial shelf-life by 44 and 34 h for products targeting children 2–3- and 4–5-years-old, respectively. The acceptability scores of the optimised products were largely better than the control products. It is therefore recommended that local processors adopt the new products and make them available to improve the intake of Vitamin A and iron among CUF in Uganda.

1. Introduction

Undernutrition in children under five (CUF) is an endemic developmental challenge in low-income countries typical of Uganda and is strongly associated with micronutrient deficiencies (UNICEF et al., 2023). The common micronutrients of public health importance include; Vitamin A, Iron, Folate, Zinc, and Iodine (Alba, 2021). The World Health Organization (WHO) (2020), estimated that 2 billion people globally suffer from micronutrient undernourishment of which 17 % were CUF. Vitamin A and iron deficiencies are serious nutritional constraints that negatively impact the health and well-being of CUF in Uganda (FAO et al., 2021; Development Initiative Poverty Research, 2020). Data on the global prevalence of vitamin A deficiency (VAD) in CUF is lacking. However, regional statistics show that Sub-Saharan Africa has the highest rates of VAD in 6–59-months-old children at 48 %, which is above the WHO cut-off of ≤ 40 % for micronutrient deficiencies in a healthy population (Gedfie et al., 2022; UNICEF et al., 2023). Conversely, the global prevalence of iron deficiency (ID) in CUF is 17.95

% (Gedfie et al., 2022). In Uganda, according to the Uganda Bureau of Statistics (UBOS) and Inner City Fund (ICF) (2018), the prevalence of VAD is 8.3 and 9.0 % in the urban and rural areas, respectively, while ID is at least 53 % in CUF. This implies that ID in Uganda is above the WHO cut-off by 13 %, whereas VAD largely puts children under five at risk (Adebisi et al., 2019). The high prevalence predisposes a significant number of CUF to increased incidences of anaemia, nyctalopia, impaired cognitive functions, and mental retardation (Regan et al., 2015; Keith, West & Ian, 2008). The long-term impact of these deficiencies is the reduced productive workforce among adults and increased rates of infections due to weakened immunity (FAO et al., 2021).

Composite food-based approaches can potentially address VAD and ID (Adoko et al., 2021; Mkambula et al., 2020; Nair et al., 2016). In Northern Uganda, cassava-based pancake, a common delicacy for most CUF, was nutritionally enriched with Vitamin A and iron by adding orange-fleshed sweet potato and iron-rich bean flour, respectively, targeting 2–3- and 4–5-years-old children (Adoko et al., 2021). The new products had better sensory acceptability, about 100 % vitamin A and

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iron content, and desirable physical properties (thickness, specific volume, and consistency in size). They were stable to oxidative rancidity during storage compared to the original pancake (Adoko et al., 2021). However, the enrichment of the pancakes resulted in microbial shelf-life reduction by 67 %, from 72 h to 24 h. Assocle et al. (2024) improved the microbial shelf-life of the pancakes developed by Adoko et al. (2021), using the individual application of selected spices (rosemary, ginger, and garlic) in the range of 0.5 to 1.0 % (w/w), to about 72 h. However, pancakes containing spices were largely unacceptable sensorially during storage depending on spice type and concentration due to low aroma and taste scores. Based on the significance of sensory acceptability and from a food security point of view, inferior sensory qualities of the products would therefore hinder the utilization of individual spice application in the production of the pancakes to address VAD and ID among CUF. This limitation necessitated the need for optimisation of the selected spices to target the simultaneous production of pancakes with good microbial shelf-life and acceptable sensory attributes.

In food science, “optimisation refers to the variation of certain production conditions and/or raw materials’ attributes to minimise or maximise pre-defined criteria such as product quality or profitability” (Banga et al., 2003). Data on the optimisation of rosemary, ginger, and garlic is lacking in research findings but their double or triple-randomised applications in previous research formed a strong basis for optimisation in this study. According to Carlsen, Blomhoff and Andersen (2011), spices are consumed in small quantities and are mainly used in a range of 0.5 to 1 % in the finished product of the food systems. Previous studies have shown superior improvement in microbial shelf-life and sensory acceptability of nutrient-dense foods when the spices are combined, and food is prepared over specific pH and temperature ranges (Olaniran et al., 2015). Zhang, Zhang, Ju, & Mujumdar, 2022 showed that combined essential oil mixtures of garlic, ginger, and mountain pepper, had better antibacterial activity thereby improving the microbial shelf-life of Chinese white cabbage by three days, and the product had better sensorial qualities (flavour and colour) than the control. Edward and Ohaegbu (2012), demonstrated that Kunun-zaki (a non-alcoholic beverage) treated with combined ginger and garlic, was more organoleptically tolerable due to the additional flavours by the combined spices and an increase in their concentration, and had a longer microbial shelf-life compared to the control. Olaniran et al. (2015) established that whereas adding combined ginger and garlic into tomato paste improved microbial shelf-life by at least eight weeks, key sensory attributes influencing consumer choice and preferences (aroma and taste) were rated low compared to the control. This indicates that the effect of spices on sensory quality depends on product type. Conclusively, from the aforementioned studies, the randomisation of spices does not guarantee the concurrent improvement of a microbial shelf-life and sensory qualities of a product. Thus, a need for optimisation of the selected spices.

Simplex lattice mixture design (SLMD) has been applied in food product development to improve or provide desirability qualities without compromising on the already existing ones. Deshmukh et al. (2024), optimised cabbage chips which resulted in improved nutritional composition. The nutritional qualities of millet-based ice cream cones were improved while revealing that moisture is a key control variable during storage (Mahulkar et al., 2024). In addition, Patil et al. (2023) developed a plant-based alternative (an optimised fish analogue) that was safe for consumption even after 30 days. Furthermore, the product had better antioxidant capacity and flavonoid content. Relatedly, SLMD was also successfully applied in the development of optimised sucrose-free milk chocolate in a ball mill without undesirable variations in rheological, quality, and sensory properties (Homayouni et al., 2019). From the aforementioned research shreds of evidence, simplex lattice mixture design serves as an appropriate tool for determining the optimal ternary combinations of the selected spices (rosemary, ginger, and garlic) for application in producing pancakes with improved microbial shelf-life and sensory acceptability. However, limited information is

available on the effects of optimal spices combinations on the microbial shelf-life and sensory acceptability of nutritionally enriched cassava-based pancake (Kabagalala) that is essential for improving intake of vitamin A and iron among CUF in Uganda. Therefore, this study investigated the effect of optimised spices’ combinations (rosemary, ginger and garlic) on the microbial shelf-life and sensory acceptability of vitamin A and iron-enriched cassava-based pancake (Kabagalala).

2. Materials and methods

2.1. Materials

The main raw materials were sweet bananas, cassava, orange-fleshed sweet potato (OFSP), and iron-rich beans (IRBs). The spices used were rosemary, ginger, and garlic. Sweet bananas, fresh cassava tubers, and spices were bought from Gulu’s main market, Gulu City in Northern Uganda. NAROBAN 4C variety of IRBs was procured from the National Agricultural Research Organization, Kampala, Uganda (Adoko et al., 2021). Fresh OFSP tubers were procured from the Koro-Bobi Sweet Potatoes Producers Association. Preparation and milling of dry raw materials and spices were executed according to Birungi (2022), Adoko et al. (2021), and Maziya-Dixon et al. (2017). The dried spices were processed into powder since spices in powder form easily dispersed into the flour and effectively penetrated the final food product (Balestra et al., 2011). The flours of cassava, OFSP, and IRBs were packed in separate sacks while milled spices were packed in separate low-density polythene bags (LDPB), and stored at room temperature (25–30 °C) till used.

2.2. Development of optimal spices combination

The pancakes were prepared according to the procedure described by Adoko et al. (2021) and Kayacier et al. (2014) (See schematic diagram in supplementary material 1). Table 1 shows the theoretically randomised spice combinations generated by Design Expert software for pancakes designed for 2–3- and 4- 5-year-olds targeting each age subgroup. The Special cubic-constrained Simplex Lattice Mixture of Design Expert software version 13.0.5.0 was used to generate theoretically randomised combinations of spices based on the lower limit (0.5 g) and higher limit (1.0 g) according to findings of Assocle et al. (2024). Spices’ proportions

Table 1
Spices formulations for a special cubic-constrained simplex-lattice mixture design for 2–3- and 4–5-year-old children.

Formulation	Spices proportions in 200 g of ingredient composition		
	Rosemary	Ginger	Garlic
1	0.67	0.83	0.50
2	0.67	0.67	0.66
3	0.67	0.50	0.83
4	0.50	0.50	1.00
5	0.83	0.67	0.50
6	0.50	1.00	0.50
7	0.50	0.67	0.83
8	0.58	0.84	0.58
9	0.50	0.83	0.67
10	0.50	0.50	1.00
11	0.50	1.00	0.50
12	0.83	0.50	0.67
13	1.00	0.50	0.50
14	0.58	0.58	0.84
15	1.00	0.50	0.50
16	0.84	0.58	0.58

The pancake formulation was adopted with modifications from Adoko et al. (2021). The pancake was made of 84 g cassava, 70 g OFSP, 26 g IRBs and 20 g sweet banana (for children 2–3-years old), and 66 g cassava, 86 g OFSP, 38 g IRBs and 10 g sweet banana (for children 4–5-years-old).

were expressed as fractions of the mixture with a sum ($X_1 + X_2 + X_3$) of two, where X_1 is rosemary, X_2 ginger, and X_3 garlic.

The design was constrained since it did not have any combination with no spices added. According to Adoko et al. (2021), the pancake formula that had the best consumer sensory acceptability score ratings and desirable physical properties for each age subgroup had the following combination of ingredients per 100 g: For children 2–3 years old; 42 % Cassava, 35 % OFSP, 13 % IRBs, and 10 % Banana. For children 4–5 years old; 33 % Cassava, 43 % OFSP, 19 % IRBs, and 5 % Banana. However, this composition was doubled in the reformulation of the pancake to satisfy the constrained aspect of the design. These pancake formulae were reformulated by incorporating the randomised spices combinations generated by the Design Expert software (Table 1), stored at ambient temperature, and subjected to microbial analysis (Total plate count) over 96 h (Section 2.2.1), and sensory analysis based on microbial shelf-life (Section 2.2.2) to generate data for optimisation.

2.2.1. Determination of total plate count (TPC)

TPC was determined according to procedures described by Assocle et al. (2024) and Adoko et al. (2021) with modification. This was done at 12-hour intervals for 96 h. In performing microbial analysis, three pancakes from each formulation were randomly picked from three different spots in the container and were cut into small pieces using a sterile scissor in the biosafety cabinet (CLEATECH, USA). The pieces were aseptically blended (HEAVY DUTY Blender, USA) for 3 min in the biosafety cabinet. Twenty-five grams were suspended in 225 ml of sterile buffered peptone water (Himedia, India) and aseptically homogenized for a minute at 8 strokes per second using a stomacher (Lab blenders Stomacher® 400 circulator- 0400/001/EU). An aliquot of 1 ml of the suspension was diluted in 0.1 % sterile buffered peptone water to a dilution factor of up to 10^{-7} , depending on the sample and interval. The diluted samples (1 ml) of varying dilution powers were pour-plated in duplicate using Plate Count Agar (PCA) (Condalab, Madrid, Spain) and incubated at 35 °C for 48 h for enumeration of TPC according to Da Silva et al. (2018); Yousef and Carlstrom (2003). The Log_{10} CFU/g derived from the nine data points for each formulation were averaged to get a value for use in optimisation.

2.2.2. Sensory evaluation

Sensory evaluation was carried out in the Unyama sub-county, Gulu district of Uganda using fifty untrained panellists who were mothers or female caregivers of children 2–5 years old. The sensory attributes investigated were appearance, aroma, texture, taste, and overall acceptability. The evaluation was done at 12-hour intervals until the microbiologically acceptable (safe) shelf-life point according to TPC for each formulation was attained. During the evaluation, panellists were provided with a product sample and a score sheet for those who knew how to write. Research assistants aided those with challenges in writing. Each sample was coded with 3-digit random numbers and was balanced ordered during testing to keep samples anonymous, and to avoid bias. Each formulation was tested in duplicate. Mineral water at ambient temperature was provided for the panellists to rinse their mouths in between samples. The five-point hedonic scale (1=dislike extremely, 2=dislike, 3=neither like nor dislike, 4= like, and 5= like extremely) was used for the assessment of the sensory attributes. Panellists were placed at least 2 m apart to avoid interference and interaction among them. Samples were served in pre-coded white disposable plates. The average values of Log_{10} CFU/g for TPC from the nine data points and mean sensory scores for each formulation were used in optimisation using response surface methodology. Data for TPC and sensory attributes for pancakes targeting 2–3- and 4–5-years-old children are presented in the supplementary material 2.

2.2.3. Statistical models, ANOVA, and validation of response factors (TPC and sensory attributes)

The following polynomial equation of function X_i was fitted for each

factor assessed at each experimental point. The polynomial special cubic model equation was:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (1)$$

where; Y was the estimated responses (TPC and sensory attributes); β was the constant coefficients for both linear and non-linear interaction (slope of the response variable); and X was the proportion of pseudo-components. Before the actual optimisation, a preliminary assessment of the data was conducted. Estimated ridge analysis was done to address multicollinearity challenges by determining the variance inflation factors (VIF). A VIF of 1 was considered ideal (orthogonal), but a VIF below 10 was generally accepted. To represent the fitted response values, cubic and quadratic models were used. The quality of the models for TPC and sensory attributes was assessed using the Analysis of Variance (ANOVA) by Fisher's test (F-value); the probability value ($p < 0.05$), and the significance of the lack of fit in determining whether the model correctly summarized the results of the design of experiment (Ashenafi et al., 2023; Coriandrum et al., 2023). A statistically insignificant lack of fit F-statistic ($p > 0.1$) relative to the pure error implied that the terms in the model accounted for all the assignable cause variations in the response variables. The predictive superiority of the models was checked using the coefficients of the multilinear regression (R^2) of ≥ 80 % or ≥ 0.80 , the predicted coefficient ($R^2_{\text{predicted}}$), and the adjusted coefficient (R^2_{adjusted}), and their difference of < 0.2 between a high predicted R^2 and a high adjusted R^2 was treated as being in reasonable agreement indicating that the model adequately represented the actual relationship between the randomized spices combination. A response with an Adequate precision of > 4 was treated as desirable. Response factors with negative predicted R^2 were not considered in the optimisation phase. The validity and accuracy of the models for each response were confirmed using model graphs and diagnostic plots. Numerical optimisation was applied using the desirability function (D) since modification of weight and importance of both the components of the mixture and the responses is possible. The importance of five pluses and a weight of 0.1 was applied to all the response factors of interest. The generated solutions were first confirmed for validation. The target was to minimise Log_{10} CFU/g TPC to between 1 and 4.9 and maximise sensory attributes to between 4 and 5 score units based on a five-point hedonic scale. Where two solutions were found, the one with the highest desirability index of ≥ 0.80 (≥ 80 %), was chosen as the optimal solution and used in the subsequent microbial, and sensorial analyses in comparison with the control products (Coriandrum et al., 2023).

2.3. Determination of the effect of optimal spice combinations

2.3.1. Preparation of pancakes

Pancakes were prepared in two sets (control and product with optimised spices) for each age subgroup as per the procedure provided in supplementary material number 1. For each age subgroup, the control or product with optimised spices was prepared in three replicates for microbial and sensory acceptability analyses. Details are provided in the subsequent Sections 2.3.2 and 2.3.3.

2.3.2. Determination of microbial shelf-life

Four microbial groups were analysed at 12-hour intervals over 96 h. The microbial groups enumerated were total plate count (TPC), *Enterobacteriaceae*, yeasts and moulds, and lactic acid bacteria (LAB). These were key in the determination of products' shelf-life (TPC), indicators of good hygiene and manufacturing practices (*Enterobacteriaceae*), and spoilage and fermentation indicators (LAB, and yeasts and moulds). The preparation of the pancakes involved the participation of women who usually produce the snacks for sale. Pancake samples were packed in food-grade plastic containers and stored at ambient temperature in the Gulu University Multifunctional Research Laboratories to

mimic the common practice among the processors.

Sample preparation for plating was done according to the procedure described under [Section 2.2.1](#) except that different media were used for each microbial group investigated. Briefly, an aliquot of 1 ml was pour-plated in; Plate Count Agar, Dicloran Rose Bengal Chloramphenicol Agar (DRBCA), Violet Red Bile Glucose Agar (VRBGA), and de Man Rogosa and Sharpe agar (MRS) (All media from Condalab, Madrid, Spain), for enumeration of TPC, yeasts and moulds, *Enterobacteriaceae* and LAB, respectively. The plates were incubated (Neo-Tech SA incubator, Germany) at; 30 °C for 72 h; 25 °C for 120 h; and 37 °C for 24 h, for PCA, DRBCA, and VRBGA, respectively. For LAB, an aliquot of 1 ml was pour-plated in a petri dish using a two-layered method in de Man Rogosa and Sharpe Agar (MRS; Condalab, Madrid, Spain). The plates were incubated under anaerobic conditions at 30 °C for 72 h (Thermo Scientific, CO₂ Incubator, Germany) (Cordoso et al., 2019). Following incubation, the counts of organisms in each plate were determined using a Colony counter (Interscience International, France) and the microbial load was reported as Log₁₀ CFU/g. The limit of detection (LOD) for the pour-plate method was taken as 1 Log₁₀ CFU/g (Caro et al., 2020). Microbial shelf-life was determined based on the duration (hours) taken by a given product formulation to reach the acceptable limit in ready-to-eat-foods for TPC, *Enterobacteriaceae*, yeasts and moulds, and LAB according to the International Commission for Microbiological Specifications of Foods (ICMSF) (2021) that stands at 5 Log₁₀ CFU/g, 4 Log₁₀ CFU/g, 3 Log₁₀ CFU/g, and 5 Log₁₀ CFU/g, respectively.

2.3.3. Determination of sensory acceptability

The evaluation exercise was conducted using untrained panellists who are frequent processors and/or consumers of the *Kabalagala*. The participants were residents of the Omoro district for at least six months (Adoko et al., 2021; Assocle et al., 2024). Panellists were volunteer women from Omoro district who were mothers or caregivers of children between 2 and 5 years old and willing to participate in the sensory evaluation. This study did not incorporate mothers or caregivers with children under two and over five years old. A multistage sampling method was used to select the untrained panellists. Two sub-counties were purposively selected from the Omoro district i.e., Koro and Bobi, followed by a random choice of two parishes from each of the selected sub-counties. Two villages were randomly selected from the two parishes followed by a purposive choice of participants from the families with children aged 2–5-years-old and those that process and/or are consumers of *Kabalagala*. The caretakers and processors/consumers of *Kabalagala* were reached through a formal process involving district and local leaders. This process led to the selection of sixty-six untrained panellists for sensory evaluation. According to Sharif et al. (2017) and Hough et al. (2006), in consumer acceptability tests, taking into account the mean standard error and difference between the sample means of 10 % of the sensory scale, at least 50–150 panellists are required. Untrained panellists were preferred since they were frequent consumers and/or processors of the *Kabalagala*. Sensory evaluation was carried out in three replicates for the control and optimised products targeting 2–3- and 4–5-years-old children. The product's storage time depended on the microbiologically acceptable shelf-life based on TPC (Adoko et al., 2021; Assocle et al., 2024). The evaluation exercise was conducted according to the procedure by Adoko et al. (2021) and Assocle et al. (2024) as per sub-Section 2.2.2.

2.4. Data analysis

Independent sample *t*-test was used to (i) Compare the overall microbial load and sensory attributes between control and optimised products prepared for each age subgroup over 96 h of storage time, (ii) compare the microbial load and sensory attributes between control and optimised products at each sampling point, and (iii) determine the time taken to attain the microbiologically safe shelf-life for each product. ANOVA and Tukey's test of SPSS version 26 were used to determine the

significant response factors during storage. Correlation of sensory attributes with overall acceptability was done using bivariate correlations. The level of significance was set at 5 %. Before running the *t*-test and ANOVA, data were subjected to normality tests using the box-plot approach, and it turned out that data were normally distributed, hence justifying the application of the two parametric tests.

3. Results and discussion

3.1. Development of optimal spice combinations

Tables 2 and 3 show results indicating the performance of each factor for the product designed for 2–3- and 4–5-years-old children, respectively. From ANOVA tables, the response factor with a coefficient of determination (R^2) and adjusted R^2 close to 1 (≥ 0.8) indicated that the analyzed factor model was statistically significant. The adequacy of precision of >4 indicated that the model equation had an adequate signal to predict TPC and sensory attributes. For 2–3-year-old children, only TPC, taste, and overall acceptability satisfied the conditions and criteria for a factor to be considered in optimisation. For 4–5-year-old children, only TPC and overall acceptability satisfied the conditions. The estimated 3-D response plots showing effects of rosemary, ginger, and garlic on (A) Total plate count, (B) Taste, and (C) Overall acceptability, for products designed for 2–3-years children and (D) Total plate count and (E) Overall acceptability for product targeting 4–5-years-old, respectively is provided in supplementary material 3. The current findings and representation are corroborated by Ashenafi et al. (2023), Coriandrum et al. (2023), and Hazarika et al. (2013).

Fig. 1 shows the contours of the estimated response surface illustrating the point where the optimum spices combination was obtained for a product targeting 2–3-years old children. One optimal spices combination with a desirability index (D) of 85.8 % was generated, i.e., 1.0 g rosemary, 0.5 g ginger, and 0.5 g garlic (Fig. 4). Based on these spices' combinations, the predicted values for TPC, taste, and overall acceptability were 4.815 Log₁₀ CFU/g, and 4.779 and 4.589 sensory score units, respectively. Fig. 2 shows the contours of the estimated response surface illustrating the points where the optimum spice combinations were obtained for a product targeting 4–5-years old children. Three optimal spice combinations were generated with a D of 89.9 %, 74.8 %, and 70.4 %, and the combination with the highest D was selected which corresponded with 0.5 g rosemary, 0.5 g ginger and 1.0 g garlic. The predicted response values for TPC and overall acceptability based on the combination of the selected spices were 3.856 Log₁₀ CFU/g and 4.447 sensory score units.

The predicted correlation of selected sensory attributes with overall acceptability was perfectly positive for both optimal spice combinations generated. The choice of the highest D is corroborated by a study done by Coriandrum et al. (2023). Application of the response surface methodology produced optimal spices combination that theoretically could result in products with acceptable microbial quality and sensory appeal. The validity of the generated optimal spice combinations was proven by the better performance of the spiced products in terms of microbial quality and consumer acceptability compared to the control. These findings are similar to those of Ashenafi et al. (2023) who developed field pea shiro flour of acceptable sensory quality through spices' optimisation. Moreover, Coriandrum et al. (2023), successfully optimised the extraction of phenolic antioxidants from three plants of the Apiaceae family.

3.2. Microbial shelf-life

Table 4 shows results based on pooled data for various microbial groups for products designed for 2–3- and 4–5-years-old children, for the entire storage time of 96 h.

For 2–3 years old; products formulated with optimised spices combination had significantly lower counts of TPC, *Enterobacteriaceae*, and

Table 2

ANOVA table and experimental data analysis for TPC and sensory attributes of pancake designed for 2–3-years-old children.

Model	TPC		APPEARANCE		AROMA		TEXTURE		TASTE		OVERALL ACCEPTABILITY	
	Reduced quadratic		Mean		Quadratic		Reduced cubic		Quadratic		Reduced quadratic	
Statistics	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Model	20.85	<0.0001*	N/A	N/A	2.08	0.1569**	4.44	0.0264*	26.61	<0.0001*	11.76	0.0006*
Linear mixture	24.83	<0.0001*	–	–	0.6567	0.5362**	1.69	0.2440**	8.76	0.0063*	16.85	0.0004*
AB	–	–	–	–	4.92	0.0467*	0.117	0.7411**	74.87	<0.0001*	–	–
AC	19.46	0.0010*	–	–	–	–	15.08	0.0046*	37.60	0.0001*	5.11	0.0451*
BC	15.37	0.0024*	–	–	–	–	0.0003	0.9866**	8.45	0.0156*	8.66	0.0134*
ABC	–	–	–	–	–	–	3.51	0.0977**	–	–	–	–
AC(A-C)	–	–	–	–	–	–	11.44	0.0096*	–	–	–	–
Lack of fit	2.71	0.2222**	3.52	0.1641**	4.43	0.1237**	8.88	0.0510**	1.02	0.5438**	4.19	0.1330**
R ²	0.88		0.00		0.34		0.80		0.93		0.81	
R ² adjusted	0.84		0.00		0.18		0.62		0.89		0.74	
R ² predicted	0.73		–0.14		–0.37		–0.83		0.84		0.55	
Adequate precision	14.26		N/A		4.42		8.23		15.29		10.26	

TPC means total plate count; Level of significance: *significant ($p < 0.05$), **Not significant ($p > 0.05$) and N/A means not applicable.**Table 3**

ANOVA table and experimental data analysis for TPC and sensory attributes of pancake designed for 4–5-years-old children.

Model	TPC		APPEARANCE		AROMA		TEXTURE		TASTE		OVERALL ACCEPTABILITY	
	Cubic		Mean		Special cubic		Special cubic		Special cubic		Special quartic	
Statistics	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Model	180.1	<0.0001*	–	–	1.47	0.2898**	1.60	0.2517*	2.14	0.1460**	16.74	0.0002*
Linear mixture	481.1	<0.0001*	–	–	1.15	0.3579**	0.76	0.4961**	1.03	0.3968**	13.61	0.0019*
AB	5.37	0.0597**	–	–	5.02	0.0518**	5.00	0.0521**	9.23	0.0141*	35.04	0.0002*
AC	283.62	<0.0001*	–	–	1.27	0.2883**	0.27	0.6154**	0.74	0.4116**	37.83	0.0002*
BC	97.07	<0.0001*	–	–	1.30	0.2836**	1.25	0.2932**	0.85	0.3812**	19.70	0.0016*
ABC	7.27	0.0358*	–	–	3.51	0.0936**	5.89	0.0382*	5.51	0.0435*	–	–
AB(A-B)	14.40	0.0090*	–	–	–	–	–	–	–	–	–	–
AC(A-C)	51.43	0.0004*	–	–	–	–	–	–	–	–	–	–
BC(B-C)	149.23	<0.0001	–	–	–	–	–	–	–	–	–	–
AB ² C	–	–	–	–	–	–	–	–	–	–	16.96	0.0026*
Lack of fit	0.2813	0.8373**	0.66	0.7404**	1.12	0.5045**	1.31	0.4467**	2.06	0.2956**	3.65	0.1575**
R ²	0.996		0.00		0.49		0.52		0.59		0.92	
R ² adjusted	0.99		0.00		0.16		0.194		0.31		0.86	
R ² predicted	0.98		–0.14		–0.83		–0.78		–0.54		0.72	
Adequate precision	41.02		N/A		3.92		3.93		4.79		12.59	

TPC means total plate count; Level of significance: *significant ($p < 0.05$), **Not significant ($p > 0.05$) and N/A means not applicable.

LAB compared to the control. Quantitatively, TPC, Enterobacteriaceae, and LAB counts were significantly lower in the product containing spices than in the control by 2.62, 1.95, and 1.99 Log₁₀ CFU/g. However, optimised spices application did not affect overall counts of yeasts and moulds. Similarly, for the product targeting 4–5-year-old children, overall counts of TPC, Enterobacteriaceae, and LAB were significantly lower in the optimised product than the control by 2.53, 1.79, and 1.75 Log₁₀ CFU/g, respectively. Notably, the optimised spices application did not affect the counts of yeasts and moulds. This implies that the concentration of the active ingredients in the optimised spice combination was not sufficient to confer a significant reduction in yeasts and moulds count. Therefore, future studies should consider yeasts and moulds in addition to the total plate count during optimisation. Data on the effect of the optimised spices combination on microbial growth during storage in food samples is largely lacking. However, combinations of ginger and garlic in equal proportions have been shown to reduce the growth of microorganisms during storage. For instance, [Edward and Ohaegbu \(2012\)](#) demonstrated that combining ginger and garlic significantly reduced microbial growth resulting in an improved shelf life of Kunun-zaki (a non-alcoholic beverage) by four days compared to when the spices were individually applied. [Zhang et al. \(2016\)](#) established the effects of different spice extracts in raw chicken meat during storage, where total viable counts in spice-treated samples were lower and acceptable for an entire storage time of fifteen days and the effectiveness

of which increased when the extracts were used in combination. In addition, [Zhang et al. \(2022\)](#) demonstrated that garlic, ginger, and mountain pepper (*Listea cubeba*) essential oil greatly reduced the growth of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* of ready-to-eat pakchoi (Chinese celery cabbage) by 56.76 %, 70.10 %, 73.95 % compared to non-treated samples when used in combination. According to [Chaichi et al. \(2021\)](#), a triple combination of essential oils from thyme, cinnamon, and clove spices resulted in a 6–8-fold decrease in minimum inhibitory concentration, compared to the double-combination and single-use of essential oils against *E. coli*, *Staphylococcus aureus* and *Pseudomonas fluorescens*.

[Figs. 3 and 4](#) show results for counts of various microbial groups as a function of storage time (96 h) for products designed for 2–3- and 4–5-years-old children, respectively. In all cases, there was an increase in the counts of microbial groups investigated as a function of time. However, counts of the organisms in optimised products were significantly below that of the control for the entire storage time. In the context of shelf-life, peculiarities were observed for products targeting 2–3-year-old children. Considering TPC, the control product reached and exceeded the satisfactory limit of 5Log₁₀ CFU/g at 43 h, whereas the optimised product reached the acceptable limit at 87 h, and had a longer shelf-life of 44 h. For *Enterobacteriaceae*, the control product exceeded the limit of acceptability of 4Log₁₀ CFU/g at 31 h of storage, while the optimised product reached this limit at 56 h of storage, thereby improving the

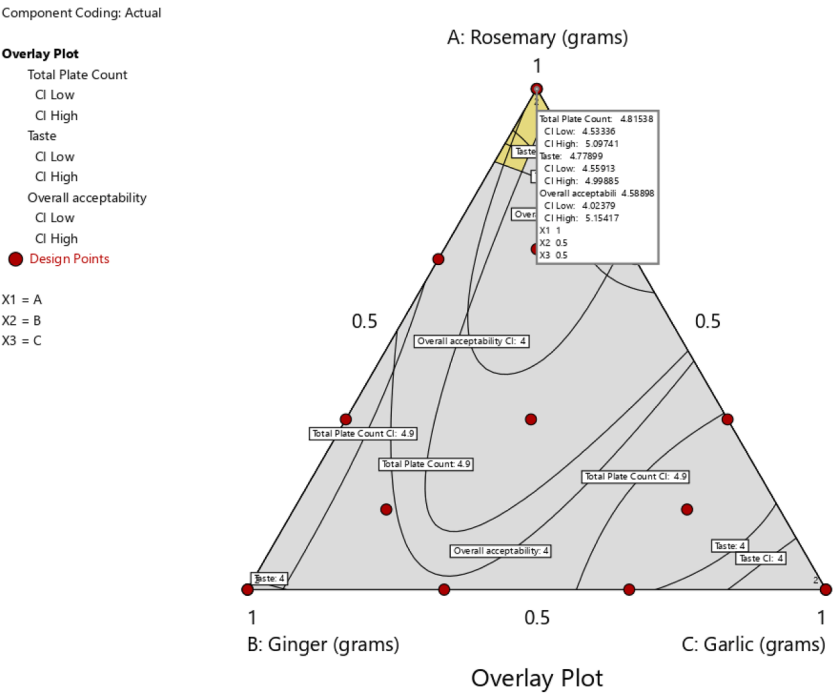


Fig. 1. Contours of estimated response surface illustrating the point where the optimum level was obtained. A=Rosemary; B=Ginger and C = Garlic targeting 2–3-years old children.

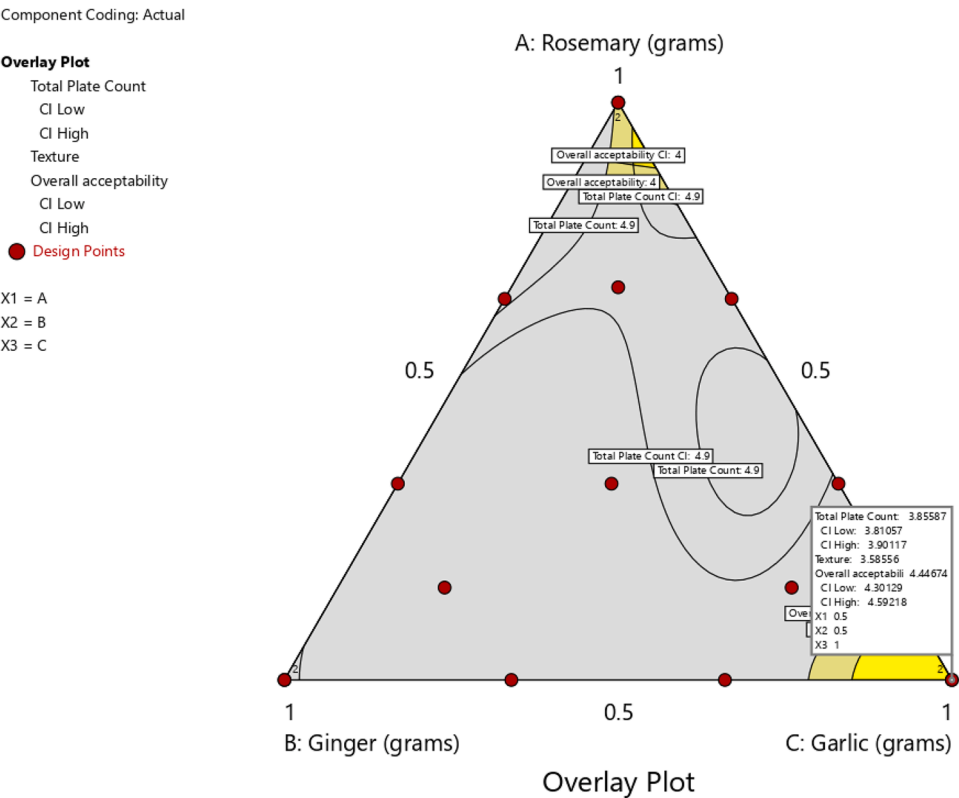


Fig. 2. Contours of estimated response surface illustrating the point where the optimum level was obtained. A=Rosemary; B=Ginger and C = Garlic targeting 4–5-years old children.

shelf-life (in the context of *Enterobacteriaceae*) by 25 h. Concerning yeasts and moulds, the control product reached the acceptable limit of 3Log₁₀ CFU/g at 58 h, while the optimised product remained acceptable past the storage time. For LAB, the control product reached the

acceptable limit of 5Log₁₀ CFU/g at 64 h, while that of the optimised product exceeded the storage time of 96 h.

For product targeting 4–5-years-old children, and in terms of TPC, the control product reached and surpassed the acceptable limit of 5

Table 4
Results based on pooled data on the effect of optimal spice combinations on various microbial groups for the entire store time of 96 h.

Target age (years)	Treatment	Total plate count	<i>Enterobacteriaceae</i>	Yeasts and molds	Lactic acid bacteria
2–3	Control	5.78 ± 2.50 ^a	5.37 ± 2.33 ^a	2.59 ± 1.50 ^a	4.81 ± 1.56 ^a
	Optimised	3.16 ± 1.72 ^b	3.42 ± 1.46 ^b	1.54 ± 1.22 ^a	2.82 ± 1.63 ^b
4–5	Control	6.38 ± 2.31 ^a	5.84 ± 1.67 ^a	2.28 ± 1.60 ^a	6.32 ± 2.03 ^a
	Optimised	3.85 ± 1.70 ^b	4.05 ± 1.28 ^b	1.75 ± 1.69 ^a	4.57 ± 1.23 ^b

Values indicate an average of means ± SD of three replicates for control and optimised products over 96 h of storage time (*n* = 9) in Log₁₀ CFU/g. Means with different superscripts in the same column for each parameter based on age are significantly different at 5 % (*p* ≤ 0.05). SD: standard deviation.

Log₁₀ CFU/g at 32 h, whereas the optimised product reached the acceptable limit at 66 h, and therefore exhibited better quality by 34 h. For *Enterobacteriaceae*, the control product exceeded the acceptable limit of 4 Log₁₀ CFU/g at 15 h, while the optimised product reached this limit at 66 h, thereby exhibiting better quality by 51 h. Concerning yeasts and moulds, the control product reached the acceptable limit of 3 Log₁₀ CFU/g at 54 h, while the optimised product reached the acceptable limit at 66 h implying the optimised product was better by 12 h.

For LAB, the control product attained the satisfactory limit of 5 Log₁₀

CFU/g at 26 h, while that of the optimised product attained the limit at 45 h, implying the optimised product was better by 19 h. The limit of *Enterobacteriaceae* of 4 Log₁₀ CFU/g for the products targeting 2–3 and 4–5-years-old was reached faster than for TPC.

The increase in TPC in both the control and optimised products could be attributed to the tolerance capacity of microorganisms to pH during storage. According to Mennah-Govela et al. (2019), the inclusion of iron-rich beans was shown to improve the buffering capacity of the pancake. The optimised product had lower TPC than the control due to the synergistic antimicrobial activities of the applied spices. Vwioko et al. (2013) demonstrated that combined garlic and ginger powder reduced microbial flora in homemade soursop fruit juices (*Annona muricata* L.) compared to their single usage during storage for thirty days. *Enterobacteriaceae* includes some toxigenic and pathogenic enteric bacteria mainly *E. coli*, *Salmonella spp.*, and *Shigella* which are used as indicators for poor post-handling practices, poor hygiene and sanitation, cross-contamination, and inadequate cooking (Britton et al., 2021). These microorganisms majorly inhabit the gut of humans and warm-blooded animals (Britton et al., 2021). Therefore, *Enterobacteriaceae* are usually considered by food manufacturers as hygiene indicators (Arvanitoyannis, 2009). The optimised spices improved microbial shelf life by 25 and 51 h for products targeting 2–3- and 4–5-years-old in the context of *Enterobacteriaceae*. However, there is a need to specifically determine the effect of the optimal spices on *E. coli*, *Salmonella spp.*, and *Shigella spp.* In this context, therefore, TPC was used to estimate the shelf-life of the control and optimised products similar to studies that were done by Adoko et al. (2021), Karam et al. (2018), and

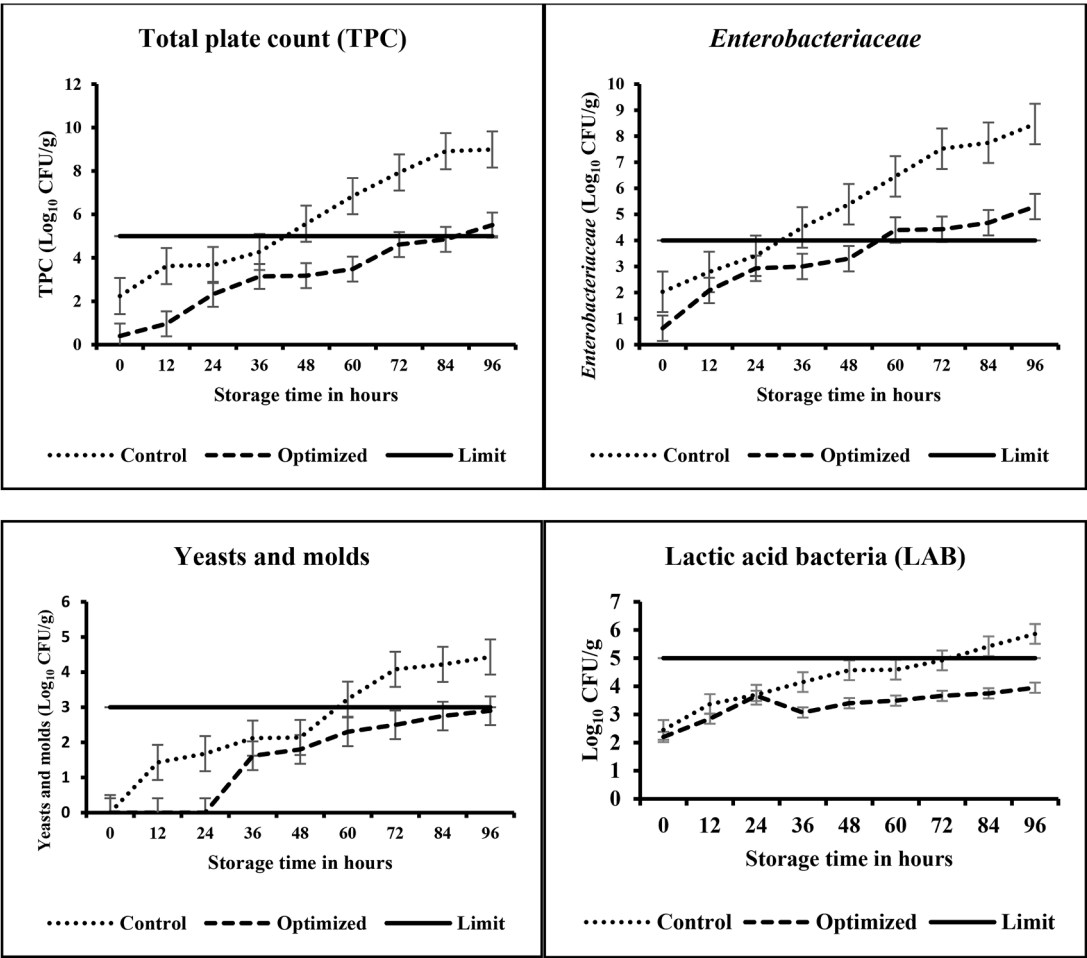


Fig. 3. Microbial load of TPC, *Enterobacteriaceae*, Yeasts and moulds, and LAB in cassava-based pancake enriched with vitamin A and iron for control and optimised product targeting 2–3-year-old. Data points represent each sample's mean of triplicate analysis, and error bars indicate the standard error.

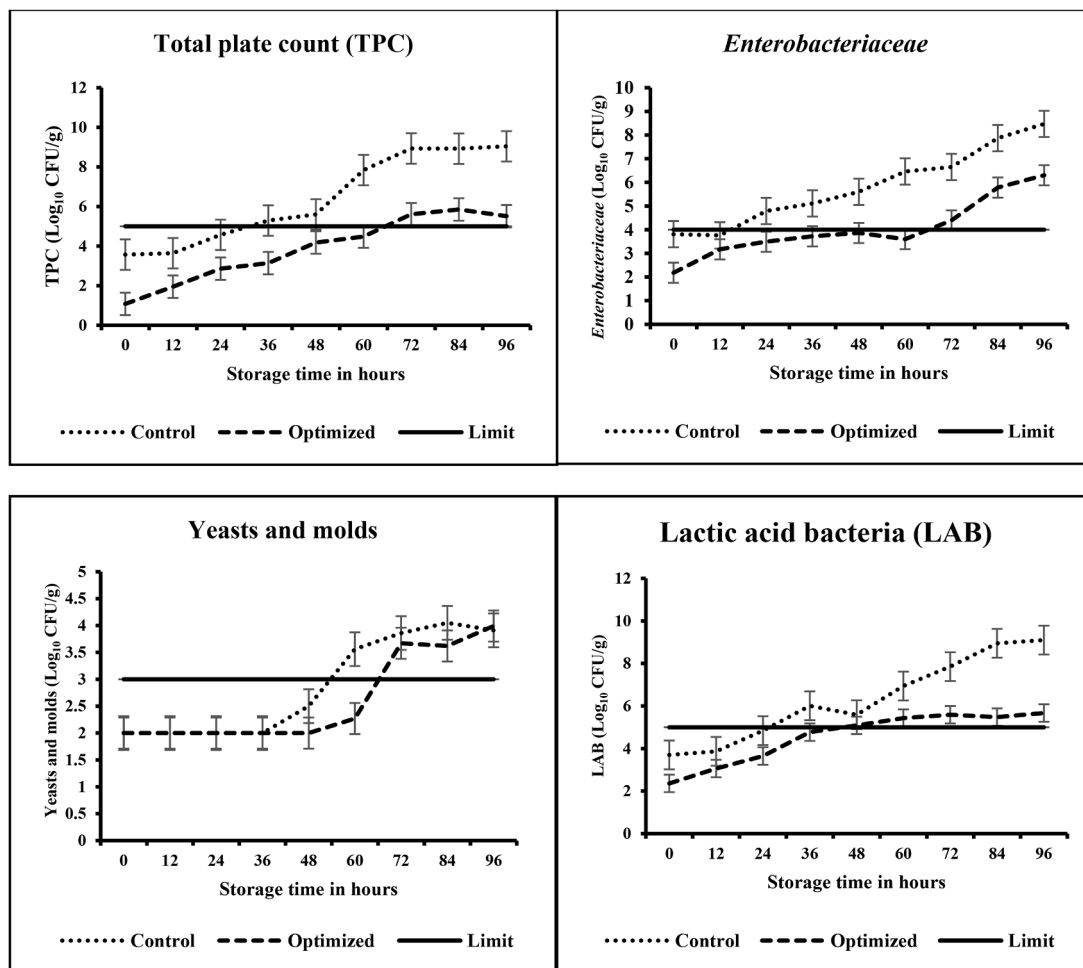


Fig. 4. Microbial load of TPC, Enterobacteriaceae, Yeasts and moulds, and LAB in cassava-based pancake (Kabalagala) enriched with vitamin A and iron for control and optimised product targeting 4–5 years old. Data points represent each sample's mean of triplicate analysis, and error bars indicate the standard error.

Assocle et al. (2024).

The growth of yeasts and moulds was similar between optimised products and their control contrary to a study done by Karam et al. (2018) where a combination of essential oil extracts (carvacrol and thymol) coupled with vacuum packaging on marinated chicken, effectively reduced yeasts and moulds. Additionally, a combination of garlic and ginger displayed better fungistatic characteristics in homemade soursop based on the findings of Olaniran et al. (2015). Optimised spices combination considerably reduced LAB proliferation. This finding is in agreement with a study done by Olaniran et al. (2015) who established the bio-preservative effects of a combination of 2 % garlic and 4 % ginger in tomato paste, where the lowest level of LAB was established after eight weeks of storage compared to their single usage.

The main components of ginger essential oil consist of zingiberene, β -sesquiphellandrene, sabinene, α -curcumin and β -bisabolene, which are known for their antimicrobial activity (Habibi et al., 2014). The rhizomes contain active chemical constituents (volatile oils) such as zingiberene, curcumin, borneol, geranial, geraniol, citronyl acetate, α -terpineol and linalool, and pungent compounds such as gingerols and shogaols (Habibi et al., 2014). The ginger phenolic compounds (eugenol, shogaols, zingerone, ginger diols, gingerols, etc.,) and their synergistic relationship with other compounds such as β -sesquiphellandrene, cis-caryophyllene, zingiberene, α -farnesene, α - and β -bisabolene, are mainly responsible for the antimicrobial activity found in ginger essential oil, extracts and oleoresins (Beristain-Bauza et al., 2019). On the other hand, the main antimicrobial effect of rosemary is attributed to three phenolic diterpenes: carnosic acid, carnosol, and

rosmarinic acid. In garlic, the main phytochemical with antimicrobial properties is allicin. The findings of the current study indicate that optimised spices combinations reduced the microbial load of different microbial groups due to the synergistic action of the three spices, and prolonged the shelf-life of cassava-based pancakes enriched with vitamin A and iron, by about 44 and 34 h, for products targeting 2–3- and 4–5-years-old children, respectively. This would contribute to the increased vitamin A and iron intake in CUF. However, there is a need to investigate the interaction of the spices' phytochemicals to understand how synergism occurs.

3.3. Sensory acceptability

Table 5 shows results based on pooled data for the entire sampling period based on microbial shelf-life, for products designed for 2–3- and 4–5-years-old children. For product targeting 2–3 years old; scores for all the sensory attributes were significantly higher for the optimised product compared to the control except for appearance for which scores were identical. Similar results were observed for products targeting 4–5-year-old children. Optimised products targeting both age subgroups largely had better sensory appeal than the control. Acceptability scores for the optimised product were better than the control product by about 9.55 % and 26.45 % for products targeting 2–3- and 4–5-years-old children, respectively. The optimised product targeting 2–3- and 4–5-years-old children, had better taste and aroma by about 42.06 % and 52.65 %; and 59.5 % and 52.33 %, respectively. Taste and aroma are key sensory attributes that majorly contribute to the overall acceptability of foods,

Table 5

Pooled data on the effect of optimised spices combination on sensory acceptability of vitamin A and iron-enriched pancakes based on microbial shelf-life.

Target age (years)	Treatment	Appearance	Aroma	Texture	Taste	Overall acceptability
2–3	Control	4.06 ± 0.22 ^a	2.64 ± 0.28 ^a	3.23 ± 0.47 ^a	2.15 ± 0.12 ^a	3.58 ± 0.46 ^a
	Optimised	3.97 ± 0.24 ^a	4.03 ± 0.20 ^b	3.62 ± 0.23 ^a	3.31 ± 0.69 ^b	3.90 ± 0.22 ^b
4–5	Control	4.13 ± 0.06 ^a	2.58 ± 0.15 ^a	3.24 ± 0.32 ^a	2.42 ± 0.30 ^a	3.56 ± 0.55 ^a
	Optimised	3.87 ± 0.30 ^a	3.93 ± 0.16 ^b	3.93 ± 0.39 ^b	3.86 ± 0.31 ^b	4.35 ± 0.29 ^b

Values indicate means ± SD ($n = 594$) sensory scores based on a five-point hedonic scale for control and optimised products taken within the microbiologically safe shelf life. Values with different superscripts in the same row for each age subgroup and column (parameter) are significantly different at 5 % ($p \leq 0.05$). SD: standard deviation.

and are highly limited to the ingredients used in food preparation and preservation (Cordero et al., 2015; Elsamani et al., 2014; Song et al., 2022).

Generally, the sensory preference of the optimised products improved during storage. This can be attributed to the fermentation process during storage that results in organic acid formation such as lactic acid, which improves organoleptic properties such as taste and aroma (Sharma et al., 2020). This is contrary to findings of a previous study by Olaniran et al. (2015) who reported a reduction in sensory qualities of tomato paste during storage when garlic and ginger were combined. However, a study by Edward and Ohaegbu (2012) is in tandem with these findings that a combination treatment of ginger and garlic on *Kunun-zaki* (a non-alcoholic beverage) was more acceptable by the panellists, with acceptability increasing with concentration during storage for four days of microbiologically safe shelf-life.

The appearance of control and optimised products targeting both age subgroups remained the same. Data about the effect of the triple or double combination of garlic, ginger and rosemary on the appearance of food during storage is limited. Existing data includes the individual application of the spices. For instance, Hamdy & Hafaz, 2018, demonstrated that the appearance scores of the Ricotta Cheese significantly improved during storage for 20 days when combined rosemary, thyme and basil with fresh garlic were used.

Figs. 5 and 6 show results for scores of sensory attributes as a function of time for product targeting 2–3- and 4–5-years-old children, respectively. Appearance scores were largely the same throughout the entire storage time for both products. For products targeting children

2–3 years old; appearance scores for both control and optimised products were identical throughout. Sensory scores for aroma and taste were significantly higher for the optimised product compared to the control throughout the storage time. However, sensory scores for texture and overall acceptability for the optimised product were significantly higher from 18 to 24 h, respectively, till the end of storage time.

For products designed for 4–5-year-olds; sensory scores for aroma, texture, taste, and overall acceptability were significantly higher for the optimised product compared to the control during storage. Generally, the sensory preference of the optimised products targeting 2–3- and 4–5-years-old children improved during storage. This can be attributed to the enzymatic and chemical degradation of the spices thereby reducing the undesirable sensation associated with spices (Sharma et al., 2020). A study by Edward and Ohaegbu (2012) is in tandem with these findings that a combination treatment of ginger and garlic on *Kunun-zaki* (a non-alcoholic beverage) was more acceptable by the panellists, with acceptability increasing with concentration during storage for four days of microbiologically safe shelf-life. However, Olaniran et al. (2015) reported a reduction in the sensory qualities of tomato paste during storage when garlic and ginger were combined.

All sensory attributes (appearance, aroma, texture and taste) had a perfectly positive linear correlation of +1 with overall acceptability for all the optimised products. This is in tandem with the predicted correlation values as derived from the output of optimisation. This implies that sensory attributes influence the overall acceptability of the food. The findings are in agreement with a study done by Maina (2018) which concluded that the overall acceptability of a food hinges on the

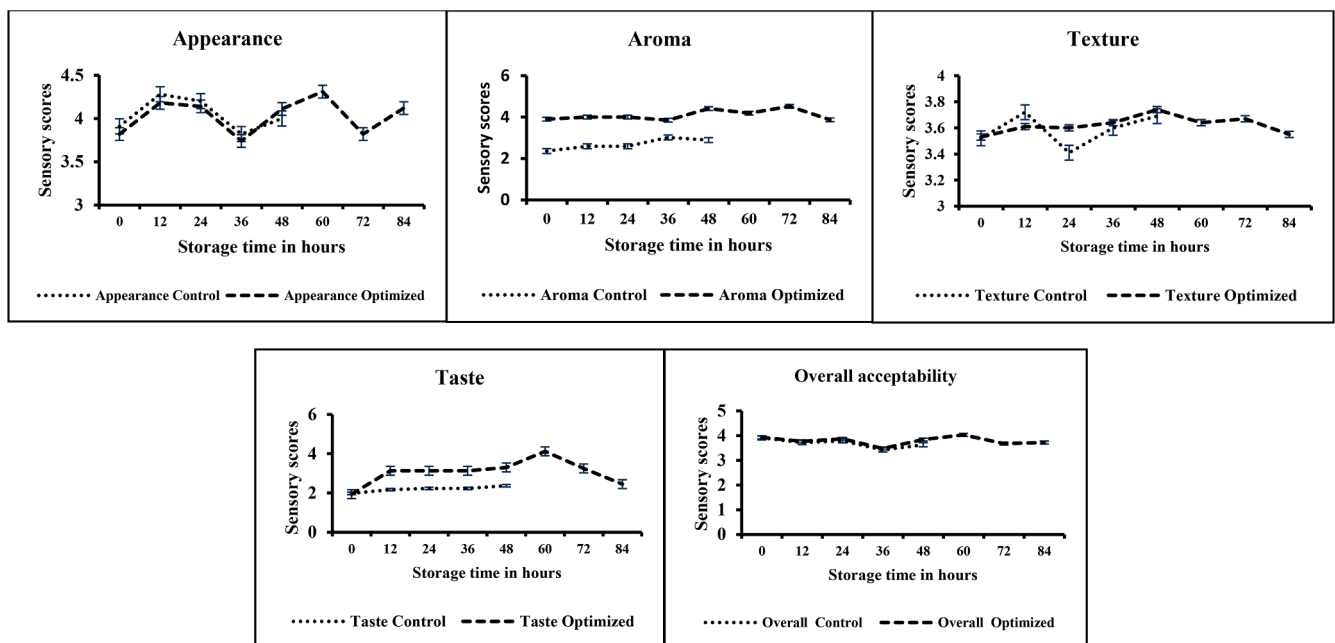


Fig. 5. Variation in sensory attributes as a function of time of control and optimised products targeting 2–3-year-old children. Data points represent each sample's mean of triplicate analysis, and error bars indicate the standard error.

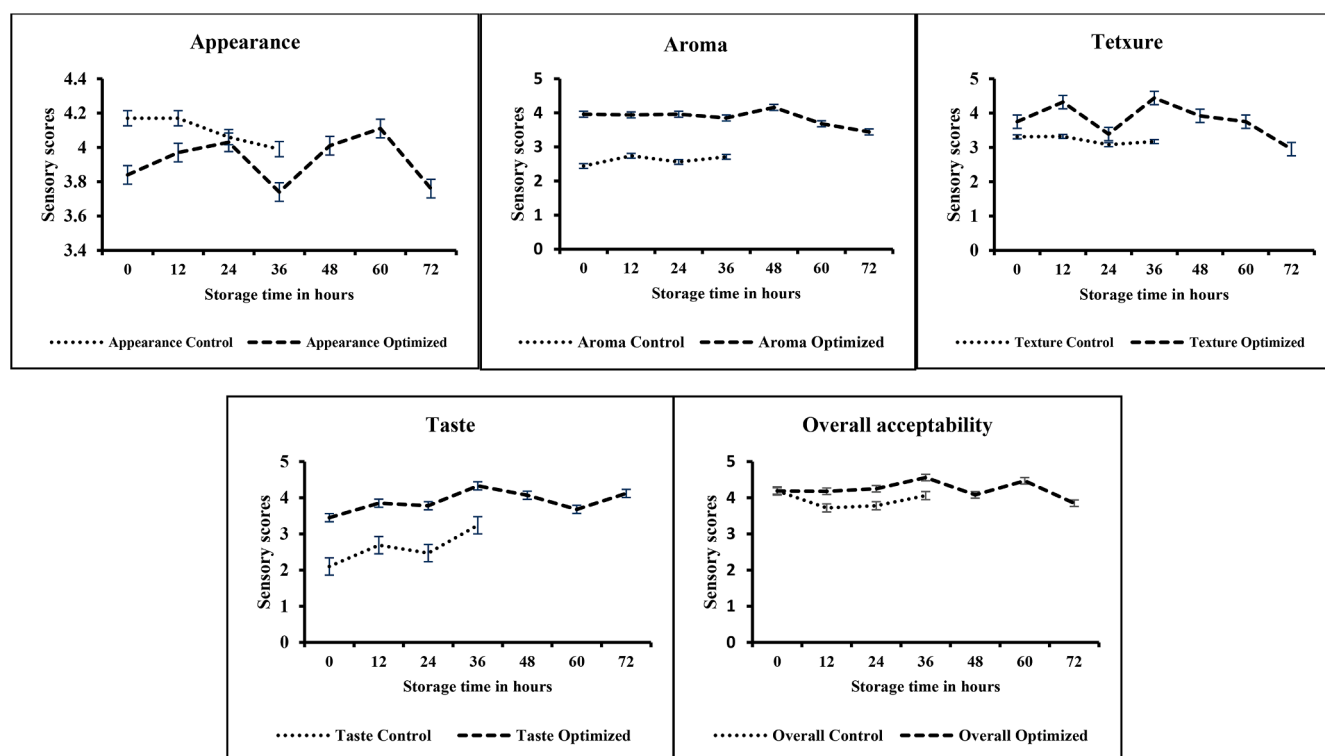


Fig. 6. Variation in sensory attributes as a function of time of control and optimised products targeting 4–5-years-old children. Data points represent each sample's mean of triplicate analysis, and error bars indicate the standard error.

preference of consumers for sensory attributes. Furthermore, research conducted by Favalli et al. (2013) revealed that the sensory attributes such as appearance, texture and taste of rye bread were strongly correlated with the overall acceptability.

4. Conclusion

Response surface methodology was successfully utilized to optimise the combinations of rosemary, ginger, and garlic, for use in the production of vitamin A and iron-enriched cassava-based pancakes (*Kabalagala*). The optimal spices combinations for *Kabalagala* designed for children 2–3 years were 1.0 g rosemary, 0.5 g ginger and 0.5 g garlic which improved the microbial shelf life by 44 h, making the product safe for up to 87 h. On the other hand, the optimal spices combination for *Kabalagala* designed for children 4–5 years was 0.5 g rosemary, 0.5 g ginger and 1.0 g garlic which improved microbial shelf life by 34 h, making the product safe for consumption for up to 66 h. This study has therefore demonstrated that the optimal spices' combinations result in pancakes with superior microbial shelf-life and sensory acceptability, hence should be promoted as a strategy for improving iron and vitamin A intake among children under five.

NGALA-Ethical statement

Ethical approval for the involvement of human subjects for sensory evaluation in this study was granted by the Gulu University Research Ethics Committee, Reference number (GUREC-2022–452), dated 6th April 2023. Participants gave informed consent via the statement "Participation in the research study is voluntary and I may join on my own free will. I have the right to withdraw from the study at any time without penalty. If I have any issues about my rights and participation in the study, I will contact the Chairperson, Gulu University Research Ethics Committee, Dr. Gerald Obai Tel: No., 0,772,305,621; email: lekobai@yahoo.com/lekobai@gmail.com; or the Uganda National Council for Science and Technology."

CRediT authorship contribution statement

John Ngoka Ngala: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Lawrence Okidi:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Duncan Ongeng:** Writing – original draft, Validation, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.afres.2024.100573](https://doi.org/10.1016/j.afres.2024.100573).

Data availability

Data will be made available on request.

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