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Modelling the Color and Microbial Properties of Canned Ngu by Response Surface Method

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HIGHLIGHTS:

- Increase in sterilization time increased the a* value and decreased the b* value of canned Ngu.
- Change of stabilizer from Carboxyl Methyl Cellulose to Akparata and then to Ofo resulted in increase in L* value, of canned Ngu.
- Total Fungal Count and Total Viable Count value of all the canned Ngu samples were within acceptable limits

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Abbreviations
CFU=Colony Forming Units
CIE=Comission Internationale de l'Eclairage
CMC=Carboxyl Methyl Cellulose
TFC=Total Fungal Count
TVC=Total Viable Count

ABSTRACT

Background: Ngu is an African salad dressing used to improve the stability and shelf life of African salad.

The aim of this study was to produce canned Ngu using three stabilizers at various concentrations, constant sterilization temperature, and varied sterilization times.

Methods: Ngu (50 L) was prepared from potash using three stabilizers at various concentrations. A three-level factorial response surface design was applied to generate the experimental runs for the production of the canned Ngu. The Ngu emulsion was filled inside 250 ml bottle jars, sterilized at 121 ^{o}C at various times, and canned. The color of the canned Ngu was evaluated using the Comission Internationale de l'Eclairage color scale, and its microbiological attributes, Total Viable Count (TVC) and Total Fungal Count was determined with standard procedures. The effect of the stabilizers (Akparata, Ofo, and Carboxyl Methyl Cellulose), stabilizer concentration, and sterilization time on the color and microbial properties of the canned Ngu was assessed. The Statistical Software Design Expert version 8.0.7 was utilized for response surface analysis and derivation of model equation.

Results: Increase in sterilization time increased the a* value and decreased the b* value of the canned Ngu. The quadratic effect of stabilizer, stabilizer concentration, and sterilization time indicated that the alteration of stabilizer from Carboxyl Methyl Cellulose to Akparata and then to Ofo increased the L* value, of the canned Ngu. An increase in the quadratic effect of stabilizer concentration increased the a* value but decreased the L* and b* values of the canned Ngu. However, the TVC and b* value of the canned Ngu reduced as the quadratic effects of the sterilization time increased.

Conclusion: Total Fungal Count and TVC value of all the canned Ngu samples were within acceptable limits, ensuring the samples safe for human consumption.

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Introduction

Ngu is an African salad dressing, an emulsifier–water in oil emulsion. Emulsifiers are included in food products to increase the stability and prolong the shelf life (Amadi and Nwankwo, 2021; Okafor-Elenwo and Imade, 2020). In general "emulsion" is described as a structure created through the dispersion of one of two immiscible liquids within the other one in the form of small droplets (generally oil and water) (Jia-hui et al., 2020; Thakur et al., 2023; Wan et al., 2023). Ngu emulsion is utilized in the preparation and consumption of African salad (Abacha Ncha), bitter yam, and processed oil bean seed ("Ugba") (Uzodinma et al., 2014). Ngu is produced by a manual mixture of palm oil and water filtrate of plant ash (potash). It is a convenient food, readily accessible, cost-effective food that has the potential to enhance food security and nutritional status. However, it could get contaminated principally due to improper handling and lack of hygiene in the processing/preparation environment, causing it a veritable source of food-borne diseases (Amadi and Nwankwo, 2021). Moreover, Ngu has a very short shelf life as prepared and left in utensils, (Okafor-Elenwo and Imade, 2020). Maintaining food for an extended period of time at a temperature range that promotes microbial proliferation (Time-Temperature abuse) has been reported as a measure which leads to food-borne infections and intoxication by *Staphylococcus aureus and Bacillus cereus* (Ndraha et al., 2018, 2020). Additionally, Ngu is served cold which makes it a major culprit of food poisoning (Kumar, 2020). Proper preservation is crucial for storing Ngu for an extended period without spoilage and microbial contamination. Canning is a traditional method of food preservation and the process involves cooking the food, and thereafter sealing it in sterilized jars or cans, and boiling the containers for sterilization (Kumar, 2019). In these circumstances, all microbes are killed. . Stabilizers are additives widely used in the food industry (Shao et al., 2020). They can be added to Ngu to enhance the viscosity, influence texture, creaminess, and mouth feel (Kamsiati and Herawati, 2021).

The superficial appearance and color of food are the primary parameters of quality evaluated by consumers, and are thus critical factors for acceptance of the food item by the consumer (Dey and Nagababu, 2022). Despite the existence of various color spaces, the most commonly utilized for measuring color in food is the Comission Internationale de l'Eclairage (CIE) L*, a*, b* color space due to the uniform distribution of colors, and since it is very close to human perception of color (Lozhkin and Kuzmenko, 2021; Sobol et al., 2020). Furthermore, the CIE-L* a* b* system is the most convenient, as it represents a versatile color space in Cartesian coordinates (Blattner, 2020). Response Surface Methodology (RSM) can be modified as a technique that involves complex calculation for optimization process (Weremfo et al., 2023). This approach develops an appropriate experimental design that integrates all independent variables and utilizes the data input from the experiment to ultimately generate a set of equations for theoretical value of an output (Athanasaki et al., 2024). The outputs are obtained from a well-designed regression analysis which is based on the controlled values of independent variables. Thereafter, the dependent variable can be predicted according to the new values of independent variables (Weremfo et al., 2023). The experimental runs generated from central composite response surface designs are reduced enormously compared to the number of runs determined using full factorial design and the obtained results are statistically acceptable (Breig and Luti, 2021).

This research aimed to produce Ngu using three stabilizers- Akparata (*Alfzelia africana*), Ofo (*Detarium microcarpum*), and Carboxyl Methyl Cellulose (CMC) at various concentrations, constant sterilization temperature and varied sterilization times. The Ngu was subsequently stabilized, homogenized, and canned. The color of the canned Ngu was determined using the L*, a*, b* color space and its microbial attributes were also analyzed. RSM was employed to derive a model equation which can later be applied for response prediction and the determination of optimal conditions. This study will enhance wider production and canning of Ngu for broader distribution and applications, ultimately making it readily accessible to consumers and Nigerians in diaspora.

Materials and methods

Materials

Ncha ighu (10 kg) was procured from a local professional ncha producer at Eke market, Amuda Isuochi, Umunneochi local government area, Abia State, Nigeria. The stabilizers Akparata and Ofo (10 kg each) were procured from a professional local food processor at Amuda Isuochi, Umunneochi local government area, Abia State, Nigeria. The CMC and the canning jars were purchased from Ariaria market in Aba, Aba South local government area, Abia State, Nigeria. All utilized chemicals/reagents originated from Sigma-Aldrich Co., Ltd. (Steinheim, Germany).

Experimental design

A Central Composite response surface Design (CCD) for K=3 as described by Athanasaki et al. (2024) was used in the equation below:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum\sum \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

Where, Y=dependent variable

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X_i and X_j=independent variables
K=number of independent variables
β_0 =intercept (constant and regression coefficient of the model)
ε=random error terms

Thirty-two experimental runs were generated based on CCD to study the interactions between factors in addition to identifying significant factors. The three factors considered in this experiment were: type of stabilizer, concentration of stabilizer, and sterilization time. The center point (0, 0, 0) with Akparatta as the stabilizer, 0.63 stabilizer concentration and 15 min sterilization time was replicated five times (Kiwu-Lawrence et al., 2021). The independent variables and levels for the production of canned Ngu are illustrated in Table 1 and the experimental design variable settings for the production of canned Ngu are demonstrated in Table 2.

Table 1: Independent variables and levels for production of canned Ngu

Independent variables	Variable levels		
	-1	0	1
Stabilizer X_1	CMC	Akparata	Ofo
Stabilizer Concentration (%) X_2	0.00	0.63	1.25
Sterilization time (min) X_3	10	15	20

CMC=Carboxyl Methyl Cellulose

Table 2: Experimental design for the production of canned Ngu

Run	Stabilizer	Stabilizer concentration (%)	Sterilization time (min)
1	CMC	0	10
2	Akparata	0	10
3	Ofo	0	10
4	CMC	0.63	10
5	Akparata	0.63	10
6	Ofo	0.63	10
7	CMC	1.25	10
8	Akparata	1.25	10
9	Ofo	1.25	10
10	CMC	0	15
11	Akparata	0	15
12	Ofo	0	15
13	CMC	0.63	15
14	Akparata	0.63	15
15	Ofo	0.63	15
16	CMC	1.25	15
17	Akparata	1.25	15
18	Ofo	1.25	15
19	CMC	0	20
20	Akparata	0	20
21	Ofo	0	20
22	CMC	0.63	20
23	Akparata	0.63	20
24	Ofo	0.63	20
25	CMC	1.25	20
26	Akparata	1.25	20
27	Ofo	1.25	20
28	Akparata	0.63	15
29	Akparata	0.63	15
30	Akparata	0.63	15
31	Akparata	0.63	15
32	Akparata	0.63	15

CMC=Carboxyl Methyl Cellulose

Production of homogenized and stabilized canned Ngu

Homogenized and stabilized Ngu was prepared in accordance with the procedure of Alakali et al. (2008) and Adheeb-Usaid et al. (2014) with several modifications. Ncha filtrate (10% w/v) was obtained by dissolving 5,000 g ncha ighu in 50 L distilled water and further filtered. The filtrate was mixed with 8,000 g of melted palm oil to acquire the Ngu emulsion. Distinct levels (0.0, 0.63 and 1.25%) of stabilizers (Akparata, Ofo, and CMC), were mixed into the Ngu emulsion according to experimental design. Subsequently, the dissolved stabilizer at each of the concentration was homogenized using a warring blender (Tm-800, China) for 5 min. Each of the concentration was filled into 250 ml bottle jar, maintaining the airspace and

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sterilized at 121 °C at various times (10, 15, and 20 min). The used Retort was New Life Model NL–50LD Vertical Pressure Steam Sterilizer Series No 18L–1217, made in China. The completed products were allowed to cool, and stored at room temperature for analysis.

Color determination

Color determination was performed using a Konica Minolta Chroma Meter CR-300 (Konica Minolta Sensing Inc., Milton Keynes, UK) following standardization with a white calibration plate. CIE L*, a*, b* system was deployed with the following values: L* -defined as the lightness of the sample ranging from 0 (black) to 100 (white), a* and b* represents two perpendicular color axes, with the values ranging from -60 to +60 in dimensionless values (Lozhkin and Kuzmenko, 2021). Parameter a* as (-) depicts greenness, when (+) represents redness. Whereas b* represents blueness when (-), and yellowness when (+) (Sobol et al., 2020).

Total Viable Count (TVC)

TVC test was conducted to estimate the total number of microorganisms, including bacteria, yeast or mould, in the Ngu samples (Feng, 2022; Vodyakova et al., 2023). Pour plate method as outlined by Onwuka (2018), was utilized. One g of the sample was macerated into 9 ml of ringer's solution and thoroughly mixed through shaking. This was further diluted to achieve concentrations of 10^{-2} and 10^{-3}. The 0.1 ml dilution (Onwuka, 2018) was transferred from each dilution bottle into the corresponding plate and 15 ml of sterile NutriSelect Plus (70, 148) nutrient agar medium was poured and mixed thoroughly with the inoculums by rocking the plates. The plates were incubated at 38 °C for 24 h after which the colonies were counted and reported as Colony Forming Units (CFU)/g (Feng, 2022; Hasan et al., 2023).

Total Fungal Count (TFC)

A total fungal test was conducted to identify the presence, total count excluding species of fungi in Ngu samples (Krnjaja et al., 2021). The pour plate method as described by Onwuka (2018) was employed. The sample dilution weighing 0.1 ml (Onwuka, 2018) was transferred from each dilution into corresponding plates and 15 ml of sterile NutriSelect Plus (70, 139) Potato Dextrose Agar (PDA) medium was poured and mixed thoroughly with the inoculums by rocking the plates. The plates were incubated at ambient temperature for 72 h, after which the number of colonies formed was counted and expressed in terms of CFU/g.

Statistical analysis

The statistical software Design Expert version 8.0.7 (Stat ease, Inc., Minneapolis, USA) was employed to generate the experimental design matrix and analyze the experimental data. The model was significant at $p \leq 0.05$. The terms statistically detected as non-significant were excluded from the model ($p \geq 0.05$).

Results

The results of the color of the canned Ngu evaluated using CIE L*, a*, b* which are demonstrated in Table 3. With regard to the obtained result, color of the Ngu emulsions was of average brightness (not too dark and not too bright).

The results of the microbiological analysis of the canned Ngu samples are revealed in Table 4. The five replicated runs in Tables 3 and 4 were explained in the experimental design and illustrated in Table 2.

Table 3: Color properties of canned Ngu

Run	Stabilizer	Stabilizer Concentration (%)	Sterilization Time (min)	Color		
				L*	a*	b*
1	CMC	0	10	40.03	14.16	20.15
2	Akparata	0	10	40.03	14.16	20.15
3	Ofo	0	10	40.03	14.16	20.15
4	CMC	0.63	10	41.61	12.15	21.18
5	Akparata	0.63	10	41.32	11.83	22.76
6	Ofo	0.63	10	41.32	12.35	22.69
7	CMC	1.25	10	41.94	12.12	21.27
8	Akparata	1.25	10	41.92	12.21	21.38
9	Ofo	1.25	10	41.81	11.93	21.43
10	CMC	0	15	40.92	14.03	20.05
11	Akparata	0	15	40.92	14.03	20.05
12	Ofo	0	15	40.92	14.03	20.05
13	CMC	0.63	15	41.47	12.65	22.62
14	Akparata	0.63	15	41.12	12.03	22.83
15	Ofo	0.63	15	41.22	12.32	22.52
16	CMC	1.25	15	41.76	13.04	22.66
17	Akparata	1.25	15	40.03	14.16	20.15
18	Ofo	1.25	15	41.64	12.18	22.48
19	CMC	0	20	40.92	14.03	20.05
20	Akparata	0	20	40.92	14.03	20.05

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Run	Stabilizer	Stabilizer Concentration (%)	Sterilization Time (min)	Color L*	a*	b*
21	Ofo	0	20	40.92	14.03	20.05
22	CMC	0.63	20	41.32	12.35	22.69
23	Akparata	0.63	20	40.86	14.12	20.12
24	Ofo	0.63	20	41.32	11.83	22.76
25	CMC	1.25	20	41.81	11.93	21.43
26	Akparata	1.25	20	40.92	14.03	20.05
27	Ofo	1.25	20	41.92	12.21	21.38
28	Akparata	0.63	15	41.12	12.03	22.83
29	Akparata	0.63	15	41.12	12.03	22.83
30	Akparata	0.63	15	41.12	12.03	22.83
31	Akparata	0.63	15	41.12	12.03	22.83
32	Akparata	0.63	15	41.12	12.03	22.83

L* represents clarity (L=0 black, and L*=100 colorless)
a* (green/red color component (a*>0 red, a*<0 green)
b* (blue/yellow (b*> 0 yellow, b*<0 blue)
CMC=Carboxyl Methyl Cellulose

Table 4: Microbial properties of canned Ngu

Run	Stabilizer	Stabilizer concentration (%)	Sterilization time (min)	TVC (CFU/g)	TFC (CFU/g)
1	CMC	0	10	6.75	2.00
2	Akparata	0	10	6.75	2.00
3	Ofo	0	10	6.75	2.00
4	CMC	0.63	10	9.05	6.00
5	Akparata	0.63	10	7.00	4.50
6	Ofo	0.63	10	12.9	4.00
7	CMC	1.25	10	5.95	1.50
8	Akparata	1.25	10	4.60	3.00
9	Ofo	1.25	10	14.85	13.00
10	CMC	0	15	9.55	6.00
11	Akparata	0	15	9.55	6.00
12	Ofo	0	15	9.55	6.00
13	CMC	0.63	15	7.00	5.00
14	Akparata	0.63	15	21.60	6.00
15	Ofo	0.63	15	8.40	8.00
16	CMC	1.25	15	21.60	4.00
17	Akparata	1.25	15	2.95	25.50
18	Ofo	1.25	15	26.40	7.00
19	CMC	0	20	6.85	10.00
20	Akparata	0	20	6.85	10.00
21	Ofo	0	20	6.85	10.00
22	CMC	0.63	20	11.00	2.00
23	Akparata	0.63	20	12.00	5.50
24	Ofo	0.63	20	9.25	6.50
25	CMC	1.25	20	24.00	3.00
26	Akparata	1.25	20	7.70	2.00
27	Ofo	1.25	20	5.75	-
28	Akparata	0.63	15	21.60	4.00
29	Akparata	0.63	15	21.60	4.00
30	Akparata	0.63	15	21.60	4.00
31	Akparata	0.63	15	21.60	4.00
32	Akparata	0.63	15	21.60	4.00

CFU=Colony Forming Unit; CMC=Carboxyl Methyl Cellulose; TFC=Total Fungal Count; TVC=Total Viable Count

Analysis of variance results have been added accordingly (Tables 5 to 9). The canned Ngu samples had TFC (2.00 to 25.50 CFU/g) and TVC (2.95 to 26.40 CFU/g) values below the acceptable limits (less than 104 CFU/g) as exhibited in Table 4, hence, they are microbiologically fit for human consumption. Results of the analysis of variance based on TVC and TFC of canned Ngu are observed in Tables 5 and 6, respectively. ANOVA results for the CIE of canned Ngu for L*, a*, and b* are uncovered in Tables 7, 8, and 9, respectively. Optimization was not performed since it is not within the scope of this study. Fresh and frozen fish were excluded from the study.

Following the elimination of insignificant (p>0.05) model terms, the model equation representing the impact of stabilizer (X_1), stabilizer concentration (X_2), and sterilization time (X_3) on the TVC of the canned Ngu was:

$$TVC=16.26-6.77\ X_3^2 \quad (2)$$

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The quadratic effect of sterilization time ($-X_3^2$) was the exclusively significant (p<0.05) model term. Figure 1 indicated the surface plot for the effect of stabilizer (X_1), and sterilization time (X_3) on the TVC of the canned Ngu.

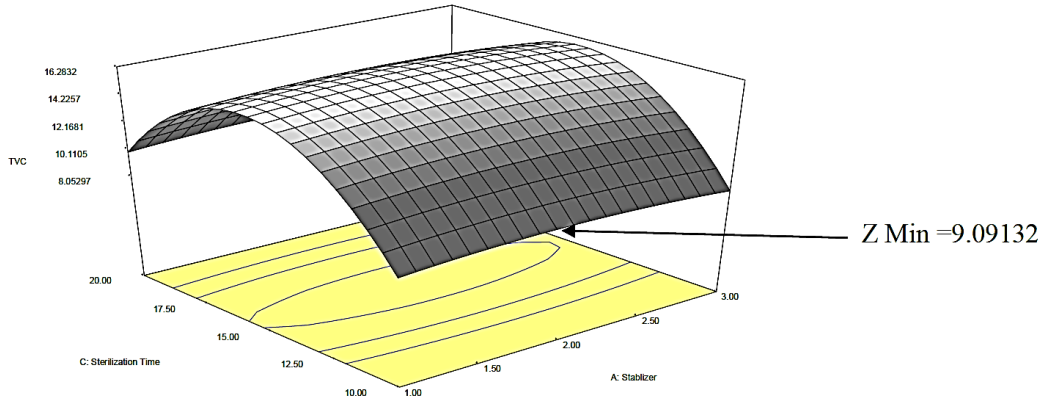

Figure 1: Response surface plot for the effect of sterilization time and stabilizer on Total Viable Count (TVC) of canned Ngu

The model was not significant (p>0.05) in analyzing the effect of stabilizer (X_1), stabilizer concentration (X_2), and sterilization time (X_3) on the TFC of the canned Ngu.

Figures 2 (a, b, c) declared the surface plot for the effect of stabilizer (X_1), stabilizer concentration (X_2), and sterilization time (X_3) on the L***** value of the canned Ngu. Following the elimination of insignificant terms, the model equation was:

$$L^* = 41.07 + 0.35X_1^2 - 0.24X_2^2 - 0.31X_2X_3 + 0.43X_1^2X_2 + 0.51X_2X_3^2 \quad (3)$$

Figures 3 (a, b, c) presented that the increase in sterilization time and stabilizer concentration resulted in the quadratic increase in a* value of the canned Ngu. After the elimination of non-significant (p>0.05) model terms, the model equation was:

$$a^* = 12.40 + 0.66X_3 + 1.16X_2^2 - 0.61X_1^2X_2 - 0.70X_1^2X_3 \quad (4)$$

The linear effect of the sterilization time (X_3), quadratic effect of stabilizer concentration (X_2^2), the sterilization time (X_3^2), interaction between the quadratic effect of stabilizer and the linear effect of stabilizer concentration ($X_1^2X_2$) and between the stabilizer and sterilization time ($X_1^2X_3^2$) were the significant (p<0.05) model terms. Furthermore, it was observed that these significant model terms uncovered a synergistic effect on Ngu.

The model equation following the removal of non-significant model terms was:

(5)

$$b^* = 22.52 - 0.68X_3 - 1.69X_2^2 - 0.57X_3^2 + 0.62X_1^2X_2 + 0.80X_1^2X_3$$

Figures 4 (a, b, c) betrayed the surface plots for the effect of stabilizer (X_1), stabilizer concentration (X_2), and sterilization time (X_3) on the b* value of the canned Ngu. From the plots (Figures 4a, b, c), it was observed that alteration of stabilizer state (from CMC to Ofo) led to quadratic increase in the b* value of the canned Ngu. On the account of sterilization time and stabilizer concentration, it was observed that as the magnitude enhanced, the value of b* increased as well, further increase in their magnitude resulted in a decrease in the b* value.

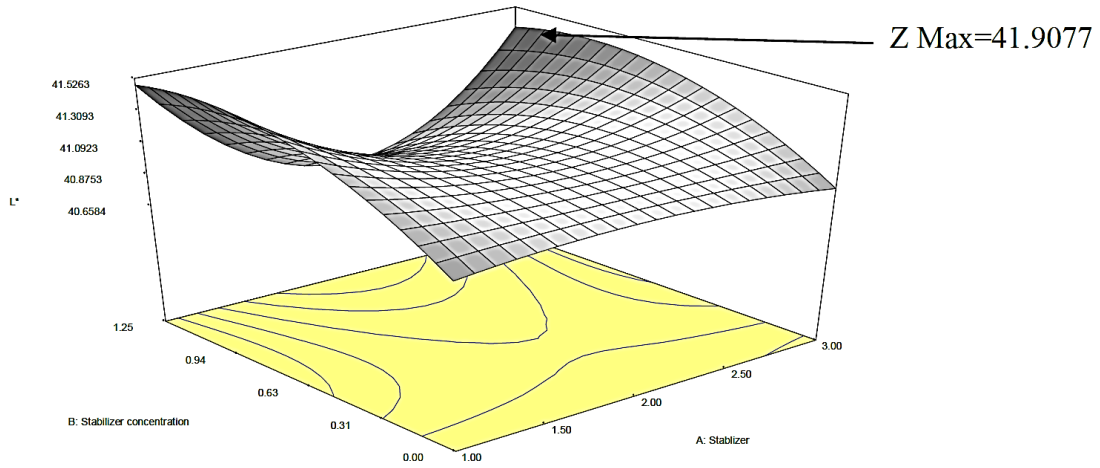

Figure 2a: Response surface plot for the effect of stabilizer concentration and stabilizer on the L* value of canned Ngu

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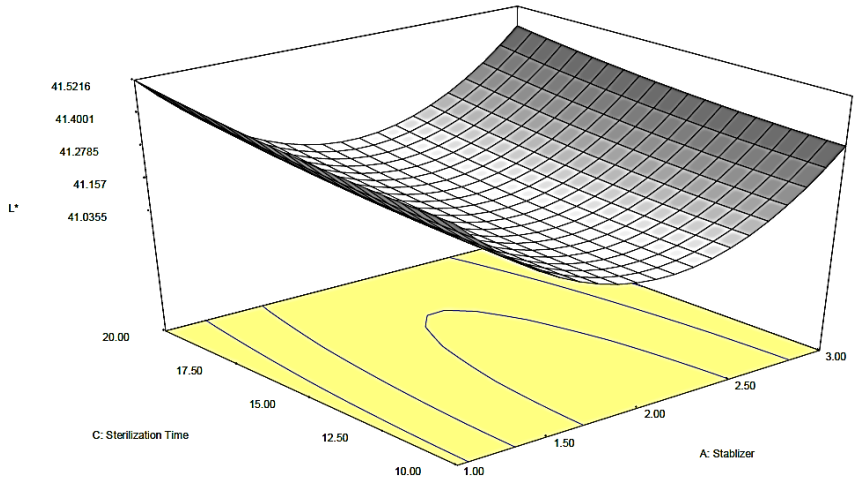

Figure 2b: Response surface plot for the effect of stabilizer and sterilization time on the L* value of canned Ngu

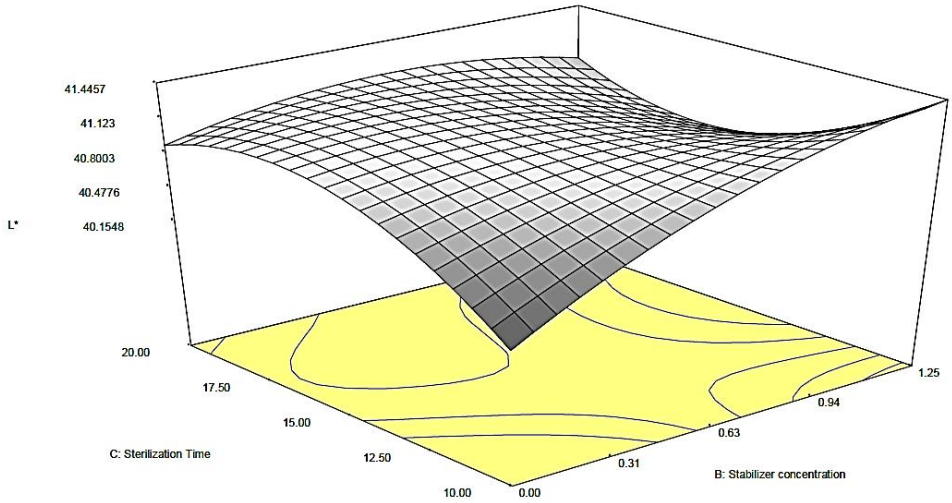

Figure 2c: Response surface plot for the effect of stabilizer concentration and sterilization time on the L* value of canned Ngu

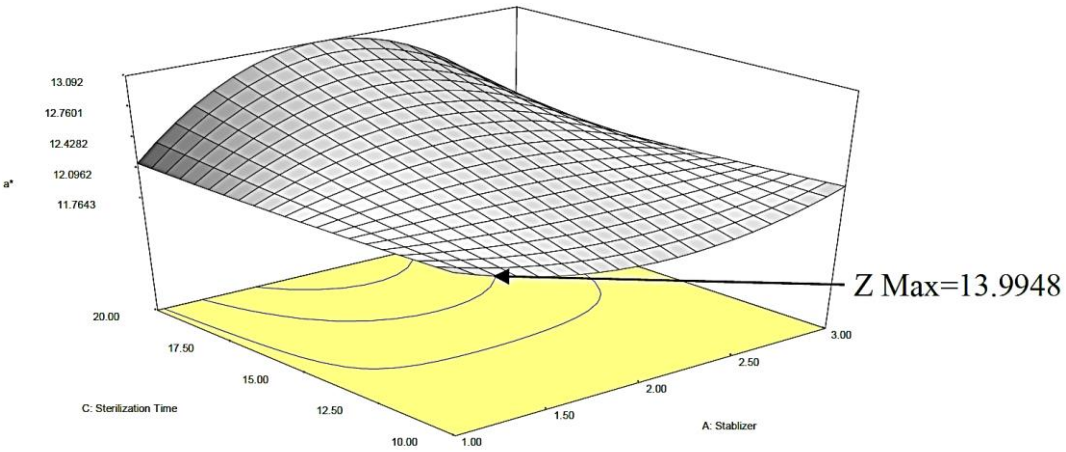

Figure 3a: Response surface plot for the effect of stabilizer concentration and stabilizer on the a* value of canned Ngu

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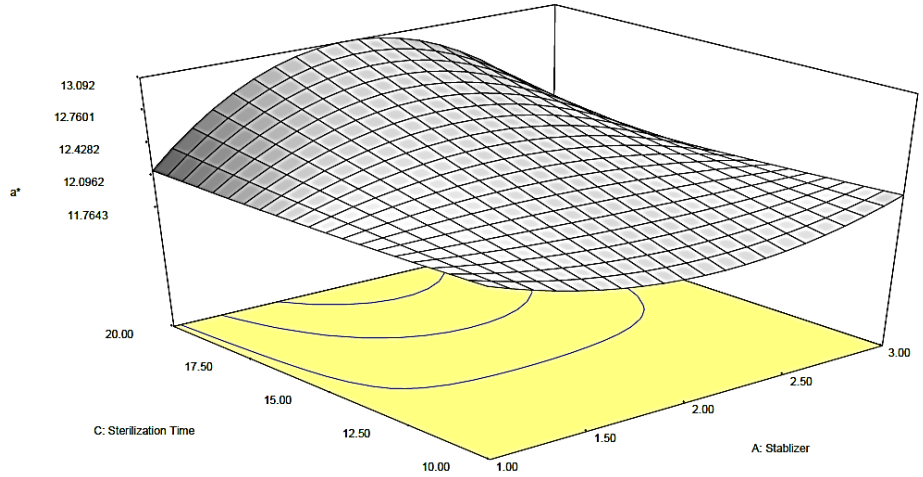

Figure 3b: Response surface plot for the effect of stabilizer and sterilization time on the a* value of canned Ngu

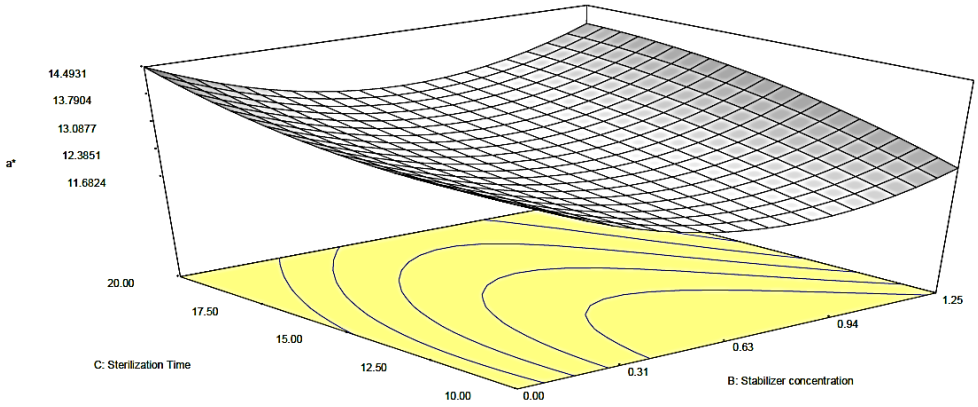

Figure 3c: Response surface plot for the effect of stabilizer concentration and sterilization time on the a* value of canned Ngu

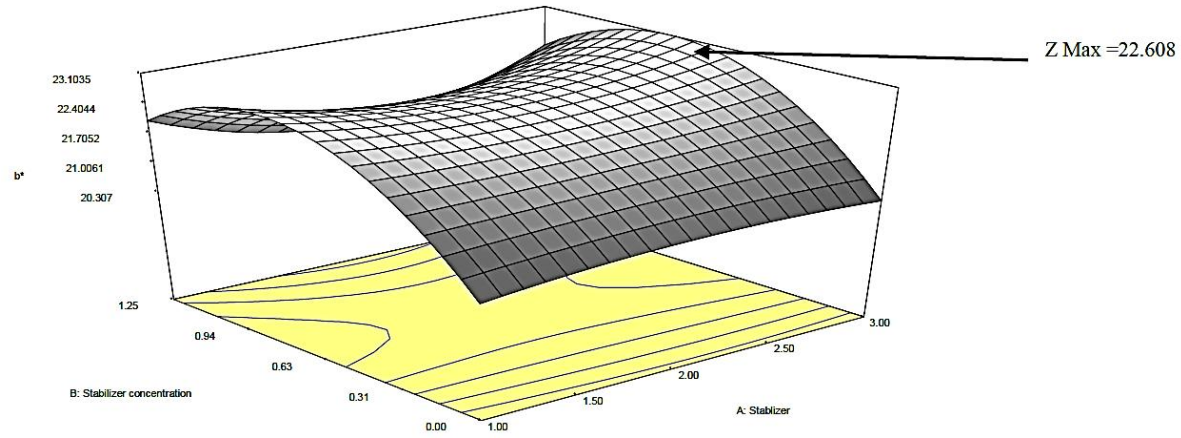

Figure 4a: Response surface plot for the effect of stabilizer concentration and stabilizer on the b* value of canned Ngu

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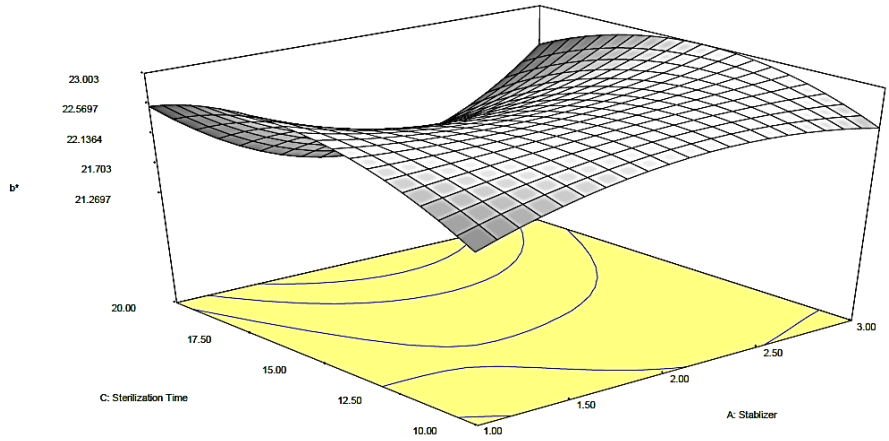

Figure 4b: Response surface plot for the effect of stabilizer and sterilization time on the b* value of canned Ngu

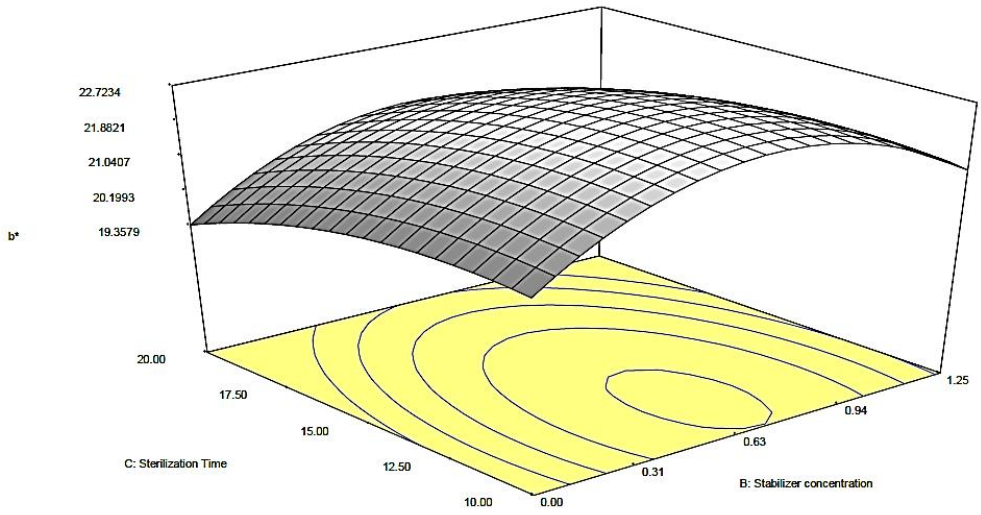

Figure 4c: Response surface plot for the effect of stabilizer concentration and sterilization time on the b* value of canned Ngu

Table 5: Analysis of variance for the total viable count of canned Ngu

Source	Sum of squares	Degree of freedom	Mean	F value	Probability>F
Model	627.02	7	89.57	2.47	0.0461
X_1	0.059	1	0.059	1.634E-003	0.9681
X_2	9.74	1	9.74	0.27	0.609
X_3	13.61	1	13.61	0.38	0.546
X_1^2	1.98	1	1.98	0.055	0.818
X_3^2	340.24	1	340.24	9.40	0.005
X_1X_2	1.70	1	1.70	0.047	0.830
$X_1^2X_2$	126.40	1	126.40	3.49	0.074
Residual	868.77	24	36.20		
Lack of fit	868.77	24	36.20		
Pure error	0.000	5	0.000		

Table 6: Analysis of variance for the total fungal count of canned Ngu

Source	Sum of squares	Degree of freedom	Mean	F value	Probability>F
Model	102.13	3	34.04	1.74	0.183
X_2	4.57	1	4.57	0.23	0.633
X_3	12.67	1	12.67	0.65	0.429
X_2X_3	77.31	1	77.31	3.94	0.057
Residual	529.15	27	19.60		
Lack of fit	525.81	22	23.90	35.85	0.0004
Pure error	3.33	5	0.67		

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Table 7: Analysis of variance for L* value of canned Ngu

Source	Sum of squares	Degree of freedom	Mean	F value	Probability>F
Model	7.77	10	0.78	11.40	<0.0001
X_1	0.026	1	0.026	0.38	0.546
X_2	0.11	1	0.11	1.58	0.222
X_3	0.046	1	0.046	0.68	0.418
X_1^2	0.86	1	0.86	12.60	0.002
X_2^2	0.40	1	0.40	5.82	0.025
X_3^2	2.735E-003	1	2.735E-003	0.040	0.843
X_1X_2	1.692E-003	1	1.692E-003	0.025	0.876
X_2X_3	1.14	1	1.14	16.73	0.0005
$X_1^2X_2$	0.73	1	0.73	10.74	0.004
$X_2X_3^2$	1.04	1	1.04	15.29	0.0008
Residual	1.43	21	0.068		
Lack of fit	1.43	16	0.089		
Pure error	0.000	5	0.000		

Table 8: Analysis of variance for a* value of canned Ngu

Source	Sum of squares	Degree of freedom	Mean	F value	Probability>F
Model	24.71	12	2.06	8.81	<0.0001
X_1	0.11	1	0.11	0.48	0.498
X_2	7.136E-003	1	7.136	7.136E-003	0.863
X_3	2.64	1	2.64	11.28	0.003
X_1^2	0.78	1	0.78	3.32	0.084
X_2^2	9.60	1	9.60	41.07	<0.0001
X_3^2	4.441E-003	1	4.441E-003	0.019	0.892
X_1X_2	0.050	1	0.050	0.21	0.650
X_1X_3	5.208E-003	1	5.208E-003	0.022	0.883
X_2X_3	0.44	1	0.44	1.90	0.184
$X_1^2X_2$	1.51	1	1.51	6.45	0.020
$X_1^2X_3$	1.98	1	1.98	8.49	0.009
$X_1X_3^2$	0.61	1	0.61	2.61	0.123
Residual	4.44	19	0.23		
Lack of fit	4.44	14	0.32		
Pure error	0.000	5	0.000		

Table 9: Analysis of variance for b* value of canned Ngu

Source	Sum of squares	Degree of freedom	Mean	F value	Probability>F
Model	38.71	10	3.87	12.31	<0.0001
X_1	0.11	1	0.11	0.35	0.560
X_2	0.30	1	0.30	0.95	0.341
X_3	2.76	1	2.76	8.78	0.007
X_1^2	1.20	1	1.20	3.81	0.064
X_2^2	20.40	1	20.40	64.90	<0.0001
X_3^2	2.30	1	2.30	7.32	0.013
X_1X_2	3.195E-004	1	3.195E-004	1.016E-003	0.975
X_1X_3	0.23	1	0.23	0.72	0.405
$X_1^2X_2$	1.55	1	1.55	4.93	0.038
$X_1^2X_3$	2.58	1	2.58	8.19	0.009
Residual	6.60	21	0.31		
Lack of fit	6.60	16	0.41		
Pure error	0.000	5	0.000		

Discussion

Color in the food industry is considered as an identifier which is applied by producers and processing engineers as well as consumers (Sobol et al., 2020). It is a meaningful feature as it is one of the initial characteristics to be evaluated by consumers and closely associated with the food quality (Pathare et al., 2013; Wu and Sun, 2013). Color is a frequently measured quality trait of products in postharvest handling and in the food processing research and industry (Pathare et al., 2013). The CIE L*, a*, b* model is utilized for assessing the quality of fruits, vegetables, dairy products, and potatoes (Zielińska and Markowski, 2012).

The impact of sterilization time on L* failed to manifest a consistent pattern except for the zero concentration of stabilizer which intensified as the sterilization time increased. However, as the stabilizer concentration was increased at constant sterilization time, the L* also increased except for Akparata which revealed reduction as the concentration of Akparata was increased from 0.63 to 1.25% at sterilization time of 15 min. In all cases, the L* values ranged from 40.03 to 41.94, suggesting that the emulsions were of average brightness (neither too dark, nor too bright). Nonetheless, Kamsiati and Herawati (2021)

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reported that the inclusion of stabilizers led to a decrease in the lightness of black pepper sauce.

It was noted that as the sterilization time increased, the L*value of the canned Ngu increased as well. Studies have indicated that the sterilization time can greatly affect the lightness of food. Sevenich et al. (2015) discovered that High Pressure Thermal Sterilization (HPTS) can result in improved food quality and decreased thermal load, potentially influencing the lightness of the food. In addition, Lazárková et al. (2011) detected that various heat sterilization regimes can cause alterations in the sensory properties of processed cheese, which could include modifications in lightness. These studies collectively suggest that the lightness of food can be affected by the sterilization time, method, and conditions.

Furthermore, the L* value rose as the stabilizer concentration increased. The concentration of stabilizers in food products can have a significant impact on their attributes, including lightness (Perez-Santaescolastica et al., 2020). On the account of the stabilizer, a decrease was noticed from CMC to Akparata, further movement from Akparata to Ofo resulted in an increase in the L* value of the canned Ngu. The model is highly significant (p<0.05) in analyzing the effect of stabilizer (X_1), stabilizer concentration (X_2), and sterilization time (X_3) on the L* value of the canned Ngu. The quadratic effect of stabilizer (X_1^2), stabilizer concentration (-X_2^2), interaction between the stabilizer concentration, and sterilization time (-X_2X_3), the interaction between the quadratic effect of stabilizer and the linear impact of stabilizer concentration ($X_1^2X_2$), and the interraction between the stabilizer concentration and quadratic effect of the sterilization time ($X_2X_3^{2)}$ are the significant (p<0.05) model terms. It was further observed that X_2^2 and X_2X_3 model terms exhibited an antagonistic effect on the L* value of the canned Ngu whereas the remaining significant model terms proved a synergistic influence on the canned Ngu.

Based on the effect of sterilization time on the a* of the canned Ngu, as the sterilization time increased the a* followed a parabolic path for all the stabilizers and their concentrations except for 0.63% Akparata and 1.25% Ofo stabilizers that displayed an increase in the a* value with a boost in the sterilization time. Maintaining the sterilization time constant and varying the stabilizer concentration, revealed that as the stabilizer concentration rose, the a* value followed a curved path except for Ofo (10 and 15 min sterilization time) and CMC (10 min sterilization time) which unveiled a reduction in the a* value as the stabilizer concentration increased. This aligns with the findings of Kamsiati and Herawati (2021) which disclosed the inclusion of stabilizers reduced the level of redness in black pepper sauce. In terms of color hue, the emulsions had positive a* values from 11.83 to 14.16, which indicate red in color (Sobol et al., 2020). The linear effect of the sterilization time (X_3), quadratic effect of stabilizer concentration (X_2^2), and the interaction between the quadratic effect of stabilizer and the linear impact of stabilizer concentration (-X_1^2X$_2$) and between the quadratic impact of both stabilizer and sterilization time ($X_1^2X_3^2$) are the significant (p<0.05) model terms. Additionally, these significant model terms indicated a synergistic effect on the Ngu, whereas (-X_1^2X$_2$) and ($X_1^2X_3^2$), proved antagonistic effect. Identically, an alteration of stabilizer from CMC to Ofo resulted in increase in a* value of canned Ngu. This is in agreement with the work of Kamsiati and Herawati (2021) who reported that the type and concentration of the stabilizer had significant effect on the color of black pepper sauce.

The impact of stabilizer concentration on b* content of the canned Ngu followed a curved path for all the stabilizer except for Akparata sterilization time of 20 min where the b* value slightly decreased with higher Akparata concentration although in a negligible manner. In the case of sterilization time effect on the b* concentration of the canned Ngu, a curved shape is also observed for all the samples except for 1.25% Akparata and 0.63% CMC which experienced a decrease and an increase respectively for b* with increase in sterilization time. The b* values were all positive ranging from 20.05 to 22.83, indicating that the emulsions had a yellow hue. In simpler terms, the emulsions all displayed yellow – red hue tending towards yellow due to the b* values being relatively higher than a* values (Lozhkin and Kuzmenko, 2021).

The TVC value of the canned Ngu (Table 4) failed to follow any particular trend as observed for all the stabilizer concentrations with increasing sterilization time except for 1.25% CMC concentrations and sterilization time of 20 min which uncovered an increase with boosting sterilization time and stabilizer concentration. Even though, Akparata, had a significant reduction in TVC (from 7.0 at 0.63% 10 min, to 2.95 at 1.25% 15 min). The depressing rate of TVC with increase in concentration of the local stabilizers (Akparata and Ofo), could suggest that the local stabilizers have anti-microbial effect (Mbaeyi-Nwaoha et al., 2017). However, the canned Ngu is microbiologically fit for human consumption since the plate count is less than the stipulated 1.0×104 CFU/g for plant products (Uneanya et al., 2019). 1.0×104 CFU/g is the microbial limit for plant products whether canned or not.

It was observed that the model term demonstrated an antagonistic effect on the TVC of the canned Ngu. This result contradicts the research conducted by Olurunnisomo et al. (2015) which detected an increase in microbial load of yoghurt with the addition of stabilizers. This could be as a result of the heat treatment subjected to the canned Ngu.

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It was evident that only sterilization time had a quadratic effect on the TVC. There was a reduction in TVC as the sterilization time increased. Reduced TVC of canned Ngu as compared to that of Ngu sold commercially (195 CFU/ml), as documented by Uzodinma et al. (2014) highlights the reduction of health hazards in using this innovative product as a salad dressing in Africa salad preparations.

The TFC value of the canned Ngu intensified as the sterilization time increased at various concentrations of CMC except for 0.63% CMC and 1.25% Ofo which decreased as the sterilization time increased. Shelf life studies are beyond the scope of our study. For 0.63% Ofo and Akparata, the TFC value followed a parabolic direction with increasing sterilization time. Keeping the sterilization time constant, the value of TFC decreased with increasing stabilizer concentration except for CMC at 10 and 20 min sterilization time, Ofo at 15 min sterilization time, and Akparata at 10 min sterilization time which demonstrated a parabolic shape with increasing stabilizer concentration. Although Ofo at 10 min sterilization time and Akparata at 15 min sterilization time displayed an increase in TFC as stabilizer concentration increased. Exposure time, temperature, the presence of organic matter, food pH, and the nature/concentration of stabilizers are key factors influencing the efficacy of sterilization of canned foods for significant reduction of fungal counts. Hu et al. (2020) concluded that a sterilization time of 0.5-1 h was effective in eliminating arbuscular mycorrhizal fungal colonization. Raits et al. (2021) emphasized the role of food pH in determining the survival of thermophilic bacteria during heat treatment. Sasaki and Yamanaka (2020) proposed a novel method combining a food preservative and low-temperature steaming for the treatment of lignocellulosic biomass, which could potentially be adapted for use in canned food sterilization. The canned Ngu had TFC values below the acceptable limits (<104 CFU/g), for canned and other foods, therefore, it is microbiologically safe for human consumption (Samuel, 2012).

Conclusion

This investigation has proved that Ngu can be transformed into a convenient form (canned) for the benefit of African salad consumers. The CIE L*, a*, b* method is effective in assessing the color of canned Ngu. Based on the microbial studies TFC and TVCs of the samples were within acceptable limits. The local stabilizers had the ability to bind water, thus preventing microbial growth. Moreover, as oil in water emulsion, the samples with the stabilizer failed to separate even after about 4 weeks of production, the products were still stable at room temperature. The results of this project promote the use of natural stabilizer particularly Akparata as a raw material for the development of new food products –canned Ngu (African salad dressing).

Author contributions

C.O. performed the experiments and prepared the manuscript; M.O.I. supervised the work and edited the manuscript; P.C.A. and S.U. conducted the statistical analysis; A.N.A. reviewed and edited the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

All the authors declared that there is no conflict of interest in the study.

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Ethical consideration

Not applicable. No human or animal was used for the study.

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