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Optimization of Ingredients for the Production of Probiotic Yoghurt from Blends of Tigernut Milk, Coconut Milk and Flaxseed Powder Using Response Surface Methodology

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Abstract
Parameters influencing the quality of yoghurt were optimized using response surface methodology and analysis of variance statistical techniques to analyze the experimental results in order to reach an optimal formulation were used. Volume of tiger nut milk, coconut milk and flaxseed powder are the factors monitored while the pH, titratable acidity (TTA), total soluble solids (TSS), viscosity and syneresis are the expected responses. Milk extracted from these plants sources were pasteurized and inoculated with two strains of commercial starter cultures (1% w/v): Lactobacillus bulgaricus and Streptococcus thermophiles and incubated at 43 °C for 12 h. Commercial yoghurt made from cow milk was used as control. Effect of optimization on the physicochemical properties, proximate composition and sensory characteristics of the yoghurt produced were studied. Results showed that the pH decreased significant ($P<0.05$ % lactic acid) as total titratible acidity increased. All the factors were considered significant by ANOVA. The R^2 of all response variables was more than 0.70 indicating that a high proportion of variability was explained by the model. Optimum level of ingredients generated from the models was; Tigernut milk (62.9%), coconut milk (34.1%) and flaxseed (3.0%) at 65% desirability which yielded yoghurt of pH (3.96), viscosity (0.73 Pa.s), total titritable acidity (3.29%), total soluble solids (6.87%) and Syneresis (6.95%). The proximate analysis and sensory characteristics of yoghurt formulated were in line with standards for yoghurts. The model is appropriate to explain the results and the experimental values fit with the predicted ones and are within the norms. Tigernut milk, coconut milk and flaxseed powder can be blended and utilized as components in yoghurt production.

Keywords: *Optimization, Tigernut, coconut, flaxseed, probiotic Yoghurt*

1. Introduction

Yoghurt, is grouped among fermented dairy products obtained from fermentation of milk by lactic acid producing bacteria such as *Streptococcus thermophiles* and *Lactobacillus debrueckii subsp.*, *Lactobacillus bulgaricus* others include *Lactobacillus acidophilus*, *Bifidobacterium animalis* and *Enterococus faecium* (Crittenden *et al.*, 2003). During the fermentation, milk proteins hydrolysis occurs; the pH drops, latic acid production increases, total soluble solids decreases while viscosity increased and bacterial metabolites are produced causing a tart flavor that affects its organoleptic quality. The drop in pH of the yoghurt from lactic acid production limits the growth of food poisoning bacteria (Heydari et al. 2018). Barkallah et al. (2017) also stated that dairy products ensure an important role in human diet due to their valued nutrients comprising minerals, proteins, sugars and vitamins (water-soluble). The fermentation process makes these nutrients easier to absorb in the body (Dairy Council of California, 2015); studies has revealed many health benefits of eating yogurt, such as boosting immunity, reducing yeast infections, and lowering the risk of colon cancer, improve immune system, reduce cholesterol in the body, aid lactose intolerance and relief antibiotic side effects in consumer (Heydari et al. 2018).

According to Heydari et al. (2018), fermented milk, are good vehicle for probiotics delivery in the human body due to their good compatibility. Probiotics are live microorganisms which can exert health benefits on the host when taken in adequate level (García-Burgos et al. 2020). In order for probiotics to have a beneficial effect on health, they must remain alive in the passages of the gastrointestinal tract. The health-enhancing effects of probiotic-fermented foods like hypocholesterolemia, antihypertension, improvement of immunity, alleviation of lactose intolerance, reduction of ovarian cancer and cardiovascular disease risks have been well studied (Wagar, Champagne, Buckley, Raymond, Green-Johnson, 2009).

Cow milk and other dairy products is the commonly used ingredient in making yogurt and some studies had also used plant -based sources. Cow milk is highly valued because it contains more nutrients than any other single food including water, protein, fat, carbohydrates, cholesterol, minerals, vitamins and energy, Hence; it is regarded as a complete diet. Consumption of milk or milk products can have a positive impact on health and prevention of such diseases as osteoporosis, colon cancer, diabetes and help with weight management (Turler-Inderbitzin, 2012). However, consumption of cow milk products has the problem of lactose-intolerant individuals and strict vegetarians are restricted to consume the animal based yogurt and again milk is easily contaminated by microbes leading to spoilage as a result of its mild acidic nature. Therefore, it is of importance to seek for other non-dairy sources as alternative ingredient for yogurt production. In this study, tigernut milk, coconut milk and flaxseed powder have been chosen as plant based materials for the production of probiotic yoghurt.

Tigernut is extensively grow in Nigeria and are eaten as snack and as a drink. Tigernut is rich in mineral. According to the Adeyemi *et al.* (2022), iron and magnesium content of tigernut is higher than cow milk. The nut also contains very good source of fat, dietary fibre and carbohydrate. The protein content of tigernut is not as high as cow milk protein but can be increased when incorporated with other plant-based milk.

Coconut is a famous tropical fruits used for many types of food products. Coconut milk describes the liquid obtained from coconut meat of mature coconut (Cocos nucifera L) (Abdullah, Taip, Kamal and Rahman, 2020, Ajogun *et al* 2023). Coconut milk contains sucrose rather than lactose, this property can benefit an individual with lactose intolerance; therefore, coconut-based ingredients are highly desirable, at least from a "low sugar" and "lactose-free" standpoint (Walther, Guggisberg, Badertscher, et al., 2022). Coconut milk is an important ingredient to give additional flavor in curries, cakes, desserts, and others. It adds creamy taste, smooth, and aromatic flavor to the delicacies. Coconut milk contains moisture, fat, and solid non-fat (SNF) and also soluble and non-soluble fibres.

Flaxseed (*Linum usitatissimmum*) also known as linseed is obtained from blue flowering annual herb that belongs to the Linaceae family (Ganorkar, 2013). Flaxseed contains B vitamins, manganese, magnesium, phosphorus, calcium, iron, copper, zinc, potassium, antioxidant and selenium (Goyal, 2014). They contain no sodium. Flaxseed and flaxseed oil is considered to have potential health benefits due to presence of linolenic acid, linoleic acid, lignans, cyclic peptides, polysaccharides, alkaloids, cyanogenic glycosides, and cadmium (Shima, 2002). Flaxseed is the richest plant sources of ω-3 fatty acid i.e. α-linolenic acid (ALA), low in saturated fatty acids (9%), moderate in monounsaturated fatty acids (18%), and rich in polyunsaturated fatty acid (73%). Soluble to insoluble fiber varies between 20:80 and 40:60. Though, flaxseed is low in carbohydrates (sugars and starches).

Response surface methodology (RSM) is reported to be effective in optimizing various process parameters involving levels of ingredients and formulation. It is a useful model in studying factors that affect the responses by varying them simultaneously and can be used to study the relationships between one or more responses (dependant variables) and factors (independent variables) (Yolmeh and Jafari, 2017). RSM reduces the number of trials. Plant milks have been shown to be highly nutritious, beneficial to health and cheap. The development of yoghurt from plant base milk is useful for lactose-intolerant individuals since in the fermentation process, the milk sugar (lactose) is converted to lactic acid. This is a healthy advantage when compared with the conventional yogurts sold in the market. This study is aimed at optimizing tigernut milk, coconut milk and flaxseed powder to produce probiotic yoghurt.

2.0 Materials and Methods

2.1 Materials:

Coconut fruit, dry tigernut tubers, flaxseed used in this study were purchased from Oil mill market in Port Harcourt, Rivers State Nigeria. Starter culture used were composed of *Lactobacillus bulgaricus* and *Streptococcus thermophiles* manufactured by Lallemand Specialty Cultures 19 rue des Briquetiers, 31700 Blagnac, France. The microbial media and chemicals of analytical grade were obtained from the Department of Food Science and Technology, Rivers State University.

2.2 Extraction of coconut milk

The coconut milk was prepared according to method described by Ajogun *et al.* (2020) with slight modification. The coconut fruit was cracked manually and the meat removed with a dull knife. The brown skin of the meat was scraped off, then washed with clean water. The coconut meat was chopped in to small pieces to aid the blending process. 2 kg of grated coconut meat were blended with 1.5 mL of distilled water. It was then sieved with cheese cloth. The slurry obtained (the coconut milk) was stored in a bottle in a refrigerator for the processing of the plant base yoghurt.

Coconut fruits
↓
Unshelling
↓
Removing of brown particles
↓
Washing
↓
Wet blending
↓
Filtering (muslin cloth)
↓
Extracted coconut milk

Figure 1: Extraction of coconut milk

2.3 Extraction of Milk from Tiger Nut

The dry tiger nuts were properly picked to remove stones, infected nut and other debris. After which, 1 kg of the tigernut was washed and soaked in 4 L of clean water and kept in refrigerator for 24 h. The soaked nut was washed and blended with a blender (Panasonic mixer 105 MX, Japan). The milled tiger-nut was filtered with a clean muslin cloth to separate the milk from the insoluble chaff. The filtered tiger nut milk was then stored in a refrigerator until required for use in yogurt production.

Tiger nut tubers
↓
Sorting
↓
Soaking (24 h)
↓
Washing
↓
Wet blending
↓
Filtering (muslin cloth)
↓
Tiger nut milk

Figure 2 Extraction of Milk from Tiger Nut

2.4 Production of Probiotic Yoghurt

The production of plant-based yoghurt as shown in Figure 3 Was carried out according to the procedure of Lee *et al*., (2010). Optimized milk blend ratio 62.9 % (Tiger nut milk), 34.5%, (Coconut milk) and 3% (flaxseed) was used in the production. The milk was pasteurized at 72°C for 15 min, it was then allowed to cool to 42°C in water bath. The starter culture was prepared following the manufacturers instruction and inoculated (1% w/v) into the pasteurized milk samples followed by incubation at 42 °C for 12 h. The yogurt produced was stored in a refrigerator at 5 ℃ for 21 days for further analysis.

Pasteurization of milk (72 °C, 15 min)
↓
Cooling (42°C)
↓
Inoculation (1% w/v)
↓
Fermentation
↓
Stirring & bottling
↓
Cooling & storage (5 °C)
↓
Plant based yoghurt

Figure 3 Production of Probiotic Yoghurt

2.5 Experimental Design and Statistical Analysis

I-optimal design was used in this study for the design of experiments for the production of plant-based yoghurt. Response surface methodology (RSM) was applied to optimize the factors and study the influences of the volume of tiger nut milk, volume of coconut milk and the mass of flaxseed on the responses. The pH, viscosity, titratable acidity (TA), total soluble solids (TSS), Syneresis and Microbial count of the yogurt were evaluated as responses for the factors studied. The software was also used to develop the model equation, surface plot, and predict the optimum independent variable values for six response variables. Statistical differences between the samples and the controls were evaluated by one-way analysis of variance (ANOVA) in Minitab statistical software (Minitab 19.0, Minitab Incorporation, USA). Results were expressed as the mean of duplicate determinations standard deviation (SD). Mean value differences at $p < 0.05$ were considered statistically significant. Actual levels of the variables are indicated in Table 1.

Table 1: Experimental range and levels of independent variables

Component	Name	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Tiger nut	0.518	0.68	+0 ↔ 0.510	+0.9444↔0.68	0.599	0.0557
B	Coconut milk	0.28	0.444	+0 ↔ 0.28	+0.9449 ↔0.45	0.360	0.0569
C	Flaxseed	0.03	0.05	+0 ↔ 0.03	+0.1111 ↔0.05	0.040	0.0079
		Total =	1.0000	**L_Pseudo Coding**			

Table 2: Experimental results for the optimization of tiger nut milk, coconut milk and flaxseed response variables for different experimental runs

Run	pH	Viscosity (cst)	TA (%)	TSS (°Brix)	Syneresis (%)	Microbial count (cfu/mL)
1	4.03±0.01^{abc}	0.72±0.00^{a}	1.13±0.01^{a}	6.40±0.14^{c}	6.40±0.14^{a}	4.20±0.14^{a}
2	3.88±0.02^{cd}	0.73±0.05^{a}	0.95±0.07^{b}	6.86±0.01^{c}	5.65±0.21^{c}	4.04±0.05^{a}
3	4.04±0.02^{abc}	0.72±0.10^{a}	1.15±0.00^{a}	6.6±0.14^{c}	6.25±0.07^{ab}	4.30±0.14^{a}
4	3.88±0.02^{cd}	0.71±0.02^{a}	0.94±0.01^{b}	6.76±0.01^{c}	6.55±0.07^{a}	4.45±0.21^{a}
5	4.04±0.01^{abc}	0.71±0.00^{ab}	1.14±0.01^{a}	7.76±0.14^{b}	6.25±0.07^{ab}	4.80±0.14^{a}
6	4.02±0.01^{abc}	0.71±0.01^{ab}	1.12±0.01^{a}	7.45±0.07^{b}	6.40±0.07^{a}	4.60±014^{a}
7	3.75±0.01^{de}	0.72±0.01^{a}	0.66±0.14^{f}	6.75±0.07^{c}	6.30±0.14^{ab}	4.50±0.14^{a}
8	3.87±0.02^{cd}	0.69±0.01^{ab}	0.74±0.01^{d}	8.44±0.01^{a}	5.50±0.14^{c}	3.55±0.07^{c}
9	3.97±0.01^{bc}	0.73±0.01^{a}	0.66±0.01^{f}	6.45±0.07^{c}	5.75±0.07^{c}	4.45±0.07^{a}
10	4.13±0.01^{ab}	0.71±0.02^{a}	1.17±0.01^{a}	7.7±0.14^{b}	6.60±0.14^{a}	4.95±0.07^{a}
11	3.75±0.01^{de}	0.71±0.10^{a}	0.86±0.01^{c}	7.54±0.01^{b}	5.70±0.14^{b}	4.20±0.14^{a}
12	4.02±0.00^{abc}	0.74±0.11^{a}	1.12±0.01^{a}	6.55±0.07^{c}	6.20±0.14^{a}	4.45±0.07^{b}
13	4.20±0.14^{a}	0.65±0.06^{b}	1.20±0.01^{a}	8.44±0.01^{a}	5.30±0.14^{c}	3.60±0.14^{c}
14	4.20±0.14^{a}	0.69±0.07^{ab}	1.20±0.01^{a}	8.44±0.00^{a}	5.50±0.14^{c}	3.40±0.14^{c}
15	3.74±0.01^{de}	0.70±0.03^{ab}	0.76±0.02^{e}	7.50±0.01^{b}	5.90±0.14^{cb}	3.91±0.14^{ab}
16	3.57±0.00^{e}	0.74±0.05^{a}	0.66±0.01^{f}	7.55±0.00^{b}	6.30±0.14^{a}	4.21±0.14^{a}

Values are means ± standard deviation of duplicate determination. Means that do not share a letter are significantly different ($p<0.05$). TA = titratable acidity, TSS = total soluble solids

2.6 Optimization and verification

Optimal ingredients levels of tigernut milk, coconut milk and flaxseed were determined by superimposing the plots for all response variables. The optimum formulations were selected and used for calculating the predicted values of response variables using the prediction equations derived by RSM. Verification of the optimum formulation was performed. The yoghurt made using optimal ingredient level was experimentally analyzed and the results were statistically compared to the predicted values of the mathematical model.

Table 3: Analysis of variance (ANOVA) for the regression models of pH, Viscosity, Total titratible acidity, total soluble solids, Syneresis, Microbial count of plant yoghurt

Model Source	**Sum of Squares**	**DF**	**Mean Square**	**F-value**	**p-value**	R^2	Adjusted R^2	Predicted R^2	Adeq Precision	C.V. %
pH	0.3217	6	0.0536	12.04	0.0007	0.8892	0.8153	0.7289	10.9769	1.70
Viscosity (cSt)	0.0040	5	0.0008	27.66	< 0.0001	0.9326	0.8988	0.7268	13.0781	0.7510
TA (g lactic acid/100g)	1.86	8	0.2326	4.71	0.0277	0.8433	0.6642	0.6570	10.0406	7.02
TSS (°brix)	6.77	5	1.35	11.95	0.0006	0.8566	0.7849	0.6429	8.7188	4.59
Syneresis (%)	2.07	2	1.03	27.97	< 0.0001	0.8114	0.7824	0.7365	13.5256	3.19

*Significant at 0.05, TA = Titratable acidity, TSS = Total soluble solids, CV= coefficient of variability

The regression equations predicting the relationship between the factors are shown below Table 3.

The response surface fitted regression model equations in terms of coded factors are as follows:

pH = +4.04A+3.63B-58.86C+71.46AC+76.05BC-26. (Eq. 1)

Viscosity = +0.6901A+0.6797B-1.75C+0.1806AB+2.95AC+2.61BC (Eq. 2)

TA = +3.49A+1.57B-734.42C+834.71AC+810.30BC-622.48A2BC-319.60AB2C +4228.31ABC2 (Eq. 3)

TSS = +8.22A+9.03B+99.80C-7.23AB-102.70BC (Eq. 4)

Syneresis = +6.42A+5.32B+8.12C (Eq. 5)

Microbial count = +4.87A+3.50B+4.76C (Eq. 6)

2.7 Set Goals, Constraints and Desirability Function for Numerical Optimization of plant yogurt Variables and Responses

The objective of this study was to achieve a model for optimization of blend ratio for the production of probiotic plant yoghurt. The chosen optimization criteria and the achieved solutions are summarized in Table 4. For the independent variable; tiger nut milk and coconut milk were maximized, while flaxseed was minimized. In the case of dependent variables, no goal was set for pH; in yoghurt production the variables that most affect the response variable (pH) are the incubation time, incubation temperature and availability of carbohydrate. Even when the source of sugar is finished, microorganisms utilize proteins from the environment, leading to increase in pH; as reported by Shahbandari et al. (2016). Viscosity was maximized, Viscosity values of yogurt samples are affected by several factors: composition, starter cultures, heat treatment (Velez-Ruiz et al. 2012; Mohan et al. 2020). Research has shown that the viscosity of yoghurt is dependent on the lactic acid production. As the concentration of lactic acid increased, the proteins present in milk formed gel to give the end result as a viscous yogurt. Total titratible acidity was maximized to allow the decrease in pH of the yoghurt. Normally total titratable acidity content increase after fermentation owing to the LAB metabolic biosynthesis pathway (Yang et al., 2022). TTA improves the sensory characteristics of yoghurt during storage. Total soluble solids were put in range. At different values of TSS, the changes in basic physicochemical parameters such as TTA, reducing sugars and pH value were not statistically different Syneresis was also put in range, there was gradual decreased in syneresis in the yoghurt with increase in storage. The tendency for whey separation showed that the maximum whey was removed from the probiotic yoghurt at week 4 of storage. The desirability function was generated after limiting the preferred goal of plant yoghurt variables and responses. The desirability function represents the closeness of a response to its ideal value (Nwabueze 2010) it lays between 0 and 1.

Table 4: Optimization criteria and solution for the formulated plant based yoghurt

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:tiger nut milk	Maximize	0.510087	0.68	1	1	3
B:coconut milk	Maximize	0.28	0.45	1	1	3
C:flaxseed	Minimize	0.03	0.05	1	1	3
pH	None	3.58	4.12	1	1	3
Viscosity	Maximize	0.691	0.735	1	1	3
TTA (%)	Maximize	2.25	3.93	1	1	3
TSS (°brix)	is in range	6.5	8.43	1	1	3
Syneresis (%)	is in range	5.4	6.5	1	1	3

Table 5: Optimal formulation condition for the formulated plant based yoghurt

Number	Tiger nut milk	Coconut milk	flaxseed	pH	Viscosity cst	TA %	TSS °brix	Syneresis (%)	Desirability	
1	0.629	0.341	0.030	3.967	0.727	3.293	6.872	6.045	0.653	Selected
2	0.600	0.370	0.030	3.909	0.730	3.039	6.818	5.869	0.613	

2.8 Validation and Verification of the Predictive Model

To confirm the predicted value of response variables, the optimum blend ratio were revalidated and experimental values were compared to the predicted values. The predicted and experimental values are presented in Table 6. The result showed that there was no significant difference ($p > 0.05$) on the corresponding experimental values between the predicted and actual properties of probiotic yoghurt in pH and viscosity. However, significant difference ($p < 0.05$) in response was noticed in titatible acidityt (TA) 2.59 and 3.29 % for Predicted and Experimental values, respectively, total soluble solids (TSS) 6.52 and 6.87 °Brix, Syneresis (S) 5.99 and 6.05%, microbial count 4.22 and 4.40 cfu/mL. This result attests to the effectiveness of this design for optimum and effective production of probiotic yoghurt. This variation could be because the optimization has been carried out by software and the variable in range has been selected to obtain the optimum response (Edem and Elijah 2016).

Table 6: Validation and Verification of the Predictive Model

Response Variables	Maximum/Minimum Values	
	Predicted Mean	Experimental (n = 3)
pH	3.95	3.96±0.02
Viscosity	0.73	0.73±0.11
TA	2.59	3.29±0.07
TSS	6.52	6.87±0.04
Syneresis (%)	5.99	6.05±0.01

Values are expressed as mean ± SD of triplicate determination.

3.0 Physicochemical analysis of yoghurt

3.1 Determination of the pH

Direct measurement using a pH meter was employed to determine the pH of yoghurt samples according to AOAC, (2016) method. The yoghurt samples (200 mg) were placed in beakers and stirred with a magnetic stirrer; pH was measured in triplicates by pH electrode connected to an ion analyzer. Electrode calibration was done at the commencement of each assay by buffer solutions with pH 4.0, 7.0 and 9.0 as standards. Results were recorded as they appeared digitally.

3.2 Determination of Sugar Brix

Ten (10) mL aliquot of the sample was diluted with 200 mL water. A few drops of the diluted sample were dropped on the prism surface of the refractometer and the brix read. The value obtained was multiplied by the dilution factor because of the dilution made. The value obtained was expressed as percentage.

3.3 Determination of total solids

Total solids contents of the samples were determined as follows; a flat aluminium dish with cover was cleaned and dried at 105 ℃ for 15 min and cooled in a desiccator for 10 min and weighed (W1). Five grams of the sample was then weighed into the dish (W2) and the dish and the content dried at 150 ℃ for 4 h in an air oven until a constant weight was obtained. The dish and its content were then removed from the oven, cooled in a desiccator and weighed again (W3). The weight of the solid was then calculated using the formula:
% Total Solid = 100 - % Moisture content

% Moisture content = $\frac{W3-W2}{W2-W1}$ x $\frac{100}{1}$

3.4 Determination of viscosity

Viscosity of the samples were determined with the aid of a Rotary Digital Viscometer (NDJ - 8S). Using spindle number 2 at 6 rpm, 300 mL of the samples was transferred into a beaker. The content of the beaker was introduced onto the rotating spindle and value of viscosity displayed on the LCD screen in Pa.s was taken as the viscosity of the sample.

3.5 Determination of Total Titratable Acidity

The titratable acidity was determined using the AOAC, (2016) method. The titratable acidity was reported as the percentage lactic acid equivalent according to the expression: For titration acidity determination (TA), 10 g of yoghurt was diluted with distilled water then titrated with 0.1NNaOH using phenolphthalein as the indicator. TA was expressed as a percentage of lactic acid equivalents.

3.6 Determination of Syneresis

Forty (40 mL) milliliters of yoghurt sample was centrifuged at 5000 rpm and 40^{o}C for 20 min. At the end of the centrifugation, the separated whey was weighed. The value of syneresis S [%] was calculated according to this formula:
$S = A/B \times 100\%$,
Where:
A—mass of whey separated during centrifugation [g]
B—yogurt mass before centrifugation [g]

3.7 Proximate analysis of the yoghurt

3.7.1 Determination of Moisture Content

The moisture content of the samples was determined using AOAC, (2016) procedure. An aliquot of about two millilitres (2 mL) of the sample was weighed into a moisture can and kept in an air current oven at a temperature of 105 °C for 4 h. The can was then removed from the oven, cooled in a desiccator and weighed. This was repeated until a constant weight was obtained. The difference in weight was used to calculate the moisture content.
Moisture (%) = $\frac{weight\ of\ water\ loss}{weight\ of\ sample}$ x $\frac{100}{1}$

3.7.2 Determination of Ash Content

The ash content of the processed samples was determined according to the method described by AOAC (2016). Three milliliters [3 mL] of each of the milk/yoghurt samples were weighed into crucibles of known weights respectively. The samples were ignited at 550°C for 3 h in a muffle furnace (SXL). The crucibles were then transferred to desiccators to cool for 30 min before weighing. The percentage ash in the sample was calculated as follows:

Ash (%) = $\frac{weight\ of\ crucible\ ash - weight\ of\ crucible\ water\ loss}{sample\ weight}$ x $\frac{100}{1}$

3.7.3 Determination of Crude Fat Content

The crude fat was determined using the Rose-Gotlieb method according to AOAC, (2016). O.5 ml of the sample was weighed, wrapped in Whatman number 1 filter paper and placed in an extraction unit for 3 hours using petroleum ether for the extraction. At the end of the extraction process, the ether was evaporated and the weight of the extraction flask taken. The difference in weight in the extraction flask before and after extraction was recorded as the amount of fat or ether extract and was calculated as follows:

Crude Fat (%) = $\frac{\text{weight of fat}}{\text{weight of sample}}$ x $\frac{100}{1}$

3.7.4 Determination of Protein

Determination of crude protein content of the various blends followed the method of Association of Official Analytical Chemist (2016). A sample volume (0.5 mL) was weighed into a 100 mL kjedahl flask. One and a half tablet of kjedahl catalyst and 10 mL of Nitrogen free concentrated sulphuric acid were then added. The mixture was heated slowly for digestion in a fume cupboard with the flask placed at an angle of 40° for 30 min.; heating was then increased and continued until frothing ceased. The sample was allowed to cool and then transferred into a 100 mL volumetric flask and made up to volume with distilled water. A 10 mL of the digest was introduced into 100 mL Kjedahl distillation flask and 10 mL of 45% NaOH was added. The ammonia liberated was steam distilled into a 5 mL boric acid indicator in a conical flask until 50 mL of the distillate was obtained. This was back titrated against 0.05 N H_2SO_4 to give the nitrogen content of the sample. A blank determination was also carried out and subtracted from the sample reading and the %N calculated thus.

3.7.5 Carbohydrate (By Difference)

The carbohydrate content was determined by difference i.e. 100 – (% + % Ash + % Fat + % crude protein + % fibre)

3.8 Bacterial Count.

Yoghurt sample (1 g) was weighed and diluted 9 mL peptone water (Merck); then, serial dilutions were carried out. *S. thermophilus* and *L. Bulgaricus* were counted, reported as the log cfu/mL (Kundu, Dhankhar, Sharma, 2018), and incubated at 37°C for 48 h and 72 h, respectively, under anaerobic conditions. The incubation was done in 3 replications according to the following storage time 24 h, 7, 14, and 21 days at 5 ºC in the refrigerator.

3.8.1 Biochemical Identification of Bacterial Isolates

Conventional identification of isolates from samples was done using gram staining technique and biochemical test. For gram staining, smear fixation was carried out by spreading loopful of isolate on a glass slide and passing it over low flame 3 times. Smear was covered with 1 % crystal violet, Lugol's iodine solution and washed with 95 % ethanol and stained with 2 % safranin before being observed under light microscope. Biochemical test carried out were indole, citrate, catalase and methyl red test. For indole test, the LAB was inoculated in 5 mL of tryptone broth and incubated at 37 °C for 24 h. Five (5) drops of 0.5 % Kovac's reagent was added after incubation and mixed by gently shaking. For citrate test, LAB culture was inoculated on slants of Simmon's citrate agar then incubated at 37 °C for 24 h. For catalase test, a drop of 3 % hydrogen peroxide (H_2O_2) was added to a loopful of LAB culture. For methyl red test, LAB cultures were inoculated in 5 mL glucose phosphate peptone water and incubated at 37 °C for 24 h. Following incubation, drops of 0.02 % methyl red solution were added. The LAB isolates were identified using rapid identification method that involved the use of API 50 CH kit (Biome-rieux) was used to differentiate LAB isolates at strains level. Identified strains were then transferred onto fresh medium and subcultured every 2 weeks for proper storage.

4.0 Storage studies

The optimized plant-based yogurt was stored in a container at 5 ºC in the refrigerator for 21 days. The viable count, physicochemical, proximate analysis was determined at 24 h, 7, 14 and 21 days of storage in the refrigerator.

5.0 Sensory evaluation

Preference test for the produced plant yogurt was conducted among 30-untrained panelists. The yoghurt characteristics were evaluated using various characteristics such as taste, odor, color, appearance, and aroma. Sample of 25 mL was put and served in a plastic container and coded 926 and 431. The untrained panelists evaluated the samples by marking the scale of preference. Panelists were also served with a glass of water to wash their mouth before analyzing the next sample.

6.0 Statistical analysis

All data were expressed as means of three independent trials with standard deviation. Minitab (19.0 versions) statistical software was used to assess difference between treatments and data was subjected to analysis of variance (ANOVA). Means were compared and Duncan's multiple range tests was used to separate means where differences exist, 5% significance was accepted.

7.0 Results and Discussion

Experimental results for formulated yoghurt from tiger nut milk, coconut milk and flaxseed are shown in Table 2. The physicochemical properties of the yoghurt were evaluated as the responses for the factors considered. The employed variables and responses were fitted to the quadratic model by performing the analysis of variance (ANOVA). Results obtained from the ANOVA are shown in Table 3. R^2 values ranged from 80.0% to 93.26%. According to Lima et al. (2010), the goodness-of-fit of the model is recognized by the coefficient of determination (R^2) and it should be at least 80% and above. The p-values below 0.05 indicate that the quadratic model of the yoghurt formulation is statistically significant at a 95% confidence interval. Greater F-value and smaller p-value obtained represent better significant of the corresponding coefficient.

Effect of independent variables on pH

The significant effects of tiger nut milk, coconut milk and flaxseed on pH ($p < 0.05$) were observed for the linear term, where the ANOVA data demonstrates R^2 values for the developed model were 88%. The interaction effects are illustrated in Figure 1**.** The pH values showed a decrease when the activity of inoculum increased, which could be explained by higher microbial activity during fermentation. The microbes consume the sugars available and lowered the pH.

Effect of independent variables on viscosity

The interaction effects of tiger nut milk, coconut milk and flaxseed on the viscosity of plant yoghurt are illustrated in Figure 2. The protein content, whey protein denaturation, and exopolysaccharides concentration are key factors affecting the viscosity of yoghurts. Viscosity values of yogurt samples are affected by several factors: composition, starter cultures, heat treatment (Velez-Ruiz et al. 2012; Mohan et al. 2020). Likewise, as the total solids increase (e.g. fat content from coconut), the viscosity and firmness of the yogurt increased (Tarrega et al. 2016). Viscosity is related to mouthfeel and may influence the request of consumers. The curd stability of yogurt can be affected by numerous factors such as protein and total solids content, homogenization process, storage conditions, microbial activity, and acidity (Guven et al., 2005).

Effect of independent variables on Titratable acidity

The results concerning the effect of independent variables on the titratable acidity of plant yoghurt is presented in figure 3. The interaction effect was significant ($P < 0.05$). Fermentation time engendered a decrease in pH, which can be explained by the fact that higher fermentation promotes an increase in the metabolic activity of LAB bacteria and, as a result, an increase in the production of organic acids (Wu, Li, Li, Bhandari, Yang, Chen, Mao, 2009).

Effect of independent variables on total soluble solids

Figure 4 presents the effect of independent variables on the plant yoghurt from tiger nut milk, coconut milk and flaxseed. The interaction effect was significant ($P < 0.05$).

Effect of independent variables on syneresis

Interaction effects of tiger nut milk, coconut milk and flaxseed on the syneresis of plant yoghurt are illustrated in Figure 5. The interaction effect was significant ($P < 0.05$). Syneresis occurs when serum drains from the yoghurt gel resulting from the appearance of two phases, whey, and water and it is considered a defect in yogurt. Whey separation and pH could be inversely related, as low pH decreases the colloidal stability of casein micelles (Azari-Anpar et al., 2017). Among the main causes of this behavior are low milk quality, very high incubation temperature, low acidity, enzymes that clot the protein and low viscosity. Ibrahim and Khalifa (2015) reported that increasing total solids can result in higher water-holding capacity and reduced syneresis.
Flaxseeds contain high concentrations of total solids, such as mucilage, which is a substance that becomes a slick gel when mixed with water. Mucilage has various hexoses, pentoses, and methyl pentoses that are capable of trapping water and developing a gel. In addition, the starter culture proteolytic activity could break down the protein network, which is essential to hold the whey within the yogurt gel structure during storage. The increase in syneresis during storage could be due to the increase in cross-linking of peptides formed by microbial proteolytic activity, leading to smaller gel network and releasing whey (Hernández-Rodríguez et al., 2017).

Effect of storage on the physicochemical properties of tiger nut milk, coconut milk and flaxseed yoghurt

The value of pH, viscosity, titratable acidity (TA), total soluble solids (TSS) and syneresis of tiger nut milk, coconut milk and flaxseed yogurt were taken after 24 h from the time of storage at 4℃ and is illustrated in Table 7. Changes in physicochemical properties of yogurt were affected as storage progressed.

Effect of storage on pH

The pH of the tiger nut milk, coconut milk and flaxseed ranged from 3.57- 4.20. Run 16 produced the lowest value of pH of 3.57. Meanwhile, runs 13 and 14 showed the highest values. The analysis of variance (ANOVA) and fit statistics (table 7) for pH showed p-value less than 0.05 and the coefficient of determination R^2 indicated that the model accounts for 88.92% of the pH variability. The adjusted R-squared was 81.53% while Predicted R^2 gave a value of 72.89%. A pictorial illustration of pH relationship with independent variables is shown in Figure 1 while its mathematical relationship is expressed in Equation (1). There was a decrease in pH and increase in titratable acidity (TA) during storage for all the treatments. The decrease in pH and simultaneous increase in acidity observed are due to availability of carbohydrate sources from materials used to the metabolic activity of both yoghurt starter cultures resulting higher level of organic acids. According to Obi, Henshaw, Atanda (2010), the different values of pH might be due to the metabolic activities of the lactic acid bacteria in the yogurt culture. The pH is a measure of the hydrogen ion concentrations in any compound. Lower pH affected the casein (milk protein), causing it to coagulate and precipitate, thereby forming the solid or thick curd that made up the yoghurt (Falade et al., 2015). Guler-Akin and Akin (2007) have stated that the value of pH is inversely proportional to the lactic acid content in yoghurt.

Effect of storage on Viscosity

Values obtained for viscosity ranged from 0.691 to 0.735 cst (table 7). Statistical analysis of R^2 indicates that the model explains 93.26 % of the variability of the viscosity. Other statistical parameters were Adjusted R^2 (81.53%), Predicted R^2 (72.68%) and Adeq Precision (13.07%). The mathematical relationship is expressed in Equation (2). Lactic acid bacteria proliferate at 45˚C and metabolize sugars to acids. This increased acidity causes denaturation of the proteins and forms a gel resulting from the viscous nature characteristic of yoghurts (Ajibade, Olusegun, and James, 2015). A visual illustration of the relationship between the viscosity and independent variables is shown in Figure 2. Viscosity is affected by the strength and number of bonds between casein micelles in yoghurt, as well as their structure and spatial distribution (Izadi et al., 2014). Viscosity is an essential factor for yoghurt consistency, texture and flow, viscosity enhances yoghurt sensory properties, and inhibits syneresis. Viscosity of yogurt depends on the acidity level; this is because, when the acidity increased, the protein present in yoghurt milk forms gel resulting yogurt with high viscosity (Morales, Montes, de Gante, 2007). But in the view of Abdelmoneim and Sherif, (2016), the starch content of materials used in yoghurt production may increase viscosity. The materials may absorb water and swell resulting in an increased viscosity.

Effect of storage on Titratable acidity (TA)

Analysis of variance (ANOVA) for titratable acidity showed p-value below 0.05. Coefficient of determination R^2 indicates that the model accounts for 84.33% of Titratable acidity variability whereas Adjusted R^2 was 66.42% and Adeq Precision 10.04%. Values for titratable acidity (table 7) ranged from 1.25 to 1.63%. Pictorial image of the relationship between the titratable acidity and independent variables is shown in Figure 3 and the mathematical relationship is expressed in Equation (3). The TTA range after fermentation was higher than 0.91 – 0.95 % lactic acid reported by Makut et al. (2018). Lactic acid bacteria (LAB) activities causes increase in acidity by breaking lactose and other sugars into lactic acid. It has been reported that changes in yoghurt acidity depend on fermentation time, type of substrate and the starter culture used in the yoghurt production. Total acidity obtained in this study meets the standard yoghurt quality, which are > than 1.0 % lactic acid recommended by EAC, (2018).

Effect of storage on Total soluble solids (°Brix)

Total soluble solid of the yoghurt samples are shown in table 7. The coefficient of determination R^2, Adjusted R^2 and Adeq precision respectively, gave values of 85.66%, 78.49% and 64.29%. Analysis of variance (ANOVA) for total soluble solids showed p-value of 0.0277. A Pictorial plot of the relationship between the total soluble solids and independent variables is shown in Figure 4 while the mathematical relationship is expressed in Equation (4). TSS (°Brix) ranged from 6.03 - 7.50 and 6.53 -7.58 respectively, for the plant yoghurt and control sample. These values are in agreement with the values published by Ezeonu et al. (2016) for different plant based yoghurt. Low TSS implies faster utilization of the soluble sugars in the substrate by the fermenting microorganisms.

Effect of storage on Syneresis

The analysis of variance, estimated regression coefficient and fit statistics for syneresis of the plant yoghurt is presented in table 7 and equation 5. The model was significant with p-value of < 0.0001 ($p<0.05$). Coefficient of determination R^2 indicates that the model accounts for 81.14% of syneresis variability. Adjusted R^2 was 78.24% and Adeq Precision 13.52%. A visual illustration of syneresis relationship with independent variables is shown in Figure 5. The highest syneresis (8.53%) was observed in the control sample at week 1 whereas the least value was found in the plant yoghurt (6.08%) in week 4. reduction in pH of yoghurts during storage leads to contraction of the casein network resulting in higher level of syneresis as explained by Sah et al. (2026). Syneresis is a major visible issue in commercial yoghurt manufacturing which is the accumulation of whey on the surface of the gel, this can lead to reduced consumer acceptance of the product. Syneresis could also be due to thermodynamic incompatibility between polysaccharides of plant extracts and milk proteins.

Table 7: Effect of storage on the physicochemical properties of optimal plant-based yogurt

Parameter	Week 1		Week 2		Week 3		Week 4	
	Produced yoghurt	Control	Produced yoghurt	Control	Produced yoghurt	Control	Produced yoghurt	Control
pH	4.89 $\pm0.08^a$	4.24 $\pm0.04^a$	4.11 $\pm0.73^a$	3.81 $\pm0.01^a$	3.92 $\pm0.01^a$	3.26 $\pm0.02^a$	3.42 $\pm0.03^a$	3.18 $\pm0.05^a$
Total Titratable Acidity (TTA)%	0.52 $\pm0.03^b$	0.45 $\pm0.03^a$	0.76 $\pm0.26^a$	0.48 $\pm0.03^b$	0.92 $\pm0.06^a$	0.50 $\pm0.00^a$	1.26 $\pm0.06^a$	0.98 $\pm0.16^a$
Total Soluble Solid (TSS) %	6.03 $\pm0.04^b$	6.53 $\pm0.04^a$	5.53 $\pm0.04^b$	4.73 $\pm0.04^a$	4.03 $\pm0.04^b$	3.50 $\pm0.00^a$	2.08 $\pm0.04^b$	2.88 $\pm0.04^a$
Viscosity cst	0.70 $\pm0.00^a$	0.69 $\pm0.03^a$	0.71 $\pm0.01^b$	0.70 $\pm0.00^a$	0.72 $\pm0.03^a$	0.71 $\pm0.00^a$	0.73 $\pm0.01^a$	0.72 $\pm0.03^a$
Syneresis %	7.03 $\pm0.04^b$	7.53 $\pm0.04^a$	7.00 $\pm0.00^b$	8.35 $\pm0.07^a$	6.85 $\pm0.07^b$	8.50 $\pm0.00^a$	6.08 $\pm0.04^a$	8.63 $\pm0.04^a$

Values are expressed as mean ± SD of duplicate determination. Different alphabet superscripts within the same column are significantly different ($p<0.05$)

Effect of storage on the proximate composition of optimal plant-based yogurt

The proximate composition such as moisture, Ash, protein, fat and carbohydrates of tiger nut milk, coconut milk and flaxseed yoghurt sample are summarized in Table 8.

The result revealed moisture content of 82.05 % and 80.85 % (Table 8) for plant yoghurt and the control sample respectively, after 24 h fermentation. However, the moisture content decreased to 79.8 and 77.58 % after 12 days storage which is close to value (75-80%) reported from commercial yoghurt by Sagita et al. (2020). Low moisture could come from added ingredients resulting from lower value. Ash content increased with storage. Value increased respectively, from 0.75 and 0.55 % to 0.91 and 0.67 % in the plant yoghurt and control sample. The ash content of the products indicates the mineral content of the products. The ash content depends on many factors, including the milk composition and the type of plant based material used. Crude protein content of the yoghurt increased with storage. Obtained value showed an increase from 3.00 to 5.96 % and from 2.78 to 5.75 % correspondingly, in the plant yoghurt and the control. Increase may be due to the breakdown of protein by proteolytic organisms, producing a larger amount of free amino groups (Li et al., 2019). Also, protein increased may be due to the loss of moisture during storage. Similar observation was reported by Ammar et al. (2015). There was a decrease in the fat content with storage. Fat content reduced from 5.10 to 4.34 % in the plant yoghurt and from 4.55 to 3.44 % in the control sample. According to Smith et al. (2016), during fermentation bacteria decompose lactose into lactic acid and hydrolyse casein into peptides and free amino acids (proteolysis), and break down the milk fat into free fatty acids (lipolysis). Ndife et al. (2014) reported that fat in plant based yoghurt has better rheology and acts as an aroma solvent compared to skimmed and low-fat yoghurt. The carbohydrate content decreased from 9.10 to 8.99 % and from 11.27 to 10.82 %, the difference was significant ($p< 0.05$). This can be caused by the carbohydrate utilization for bacteria metabolism, as carbohydrate provides energy for LAB growth and metabolism. A similar result where the carbohydrate content decreased was reported by Obadina, (2013).

Table 8: Effect of storage on the proximate composition of optimal plant-based yogurt (%)

Yoghurt Sample	Moisture	Ash	Protein	Fat	Carbohydrate
After 24 h	82.05 ± 0.10^{a}	0.75 ± 0.00^{b}	3.00 ± 0.12^{c}	5.10 ± 0.22^{a}	9.10 ± 0.18^{c}
Control	80.85 ± 0.14^{b}	0.55 ± 0.11^{d}	2.78 ± 0.17^{d}	4.55 ± 0.04^{a}	11.27 ± 0.11^{a}
After 12 d storage	79.80 ± 0.07^{c}	0.91 ± 0.10^{a}	5.96 ± 0.13^{a}	4.34 ± 0.11^{b}	8.99 ± 0.10^{c}
Control	78.58 ± 0.04^{d}	0.67 ± 0.02^{c}	5.75 ± 0.11^{b}	3.54 ± 0.07^{c}	10.82 ± 0.14^{b}

Values are expressed as mean ± SD of triplicate determination. Different alphabet superscript within the same column are significantly different ($p<0.05$)

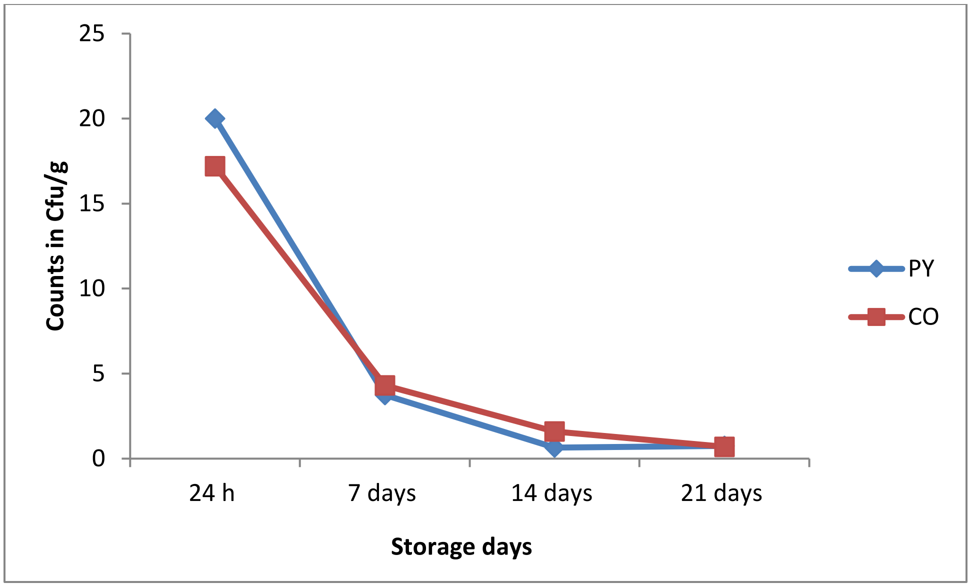
8.0 Biochemical Identification of Bacterial Isolates

Table 9 Biochemical Identification of Bacterial Isolates

Isolate codes	Gram Rxn	Cell Shape	Catalaqse	Citrate	Oxidase	Indole	Motilit	Lactose	Glucose	Mannitol	Sucrose	STH	MR	VP	Isolated Organism
1	+ve	Rods	-	+	+	-	+	A	A	A	A	+	-	-	*Lactobacillus sp*
1.2	+ve	Rods	+	+	+	-	-	A	AG	A	A	+	+	-	*Lactobacillus sp*
3.1	+ve	Rods	-	+	+	-	-	A	AG	AG	A	+	+	-	*Lactobacillus sp*
3.2	+ve	Rods	-	+	-	-	+	A	AG	AG	AG	-	+	-	*Bacillus sp*
6.1	+ve	Rods	-	+	+	-	+	A	AG	A	A	+	+	-	*Bacillus sp*
6.2	+ve	Cocci	+	+	-	-	-	A	A	A	A	+	-	-	*Staphylococcus sp*
7.1	+ve	Rods	-	+	+	-	-	A	A	A	A	+	-	-	*Lactobacillus sp*
10.1	+ve	Rods	-	+	-	-	-	A	A	A	A	+	-	-	*Lactobacillus sp*
10.2	+ve	Rods	-	+	-	-	-	A	A	A	A	+	+	-	*Lactobacillus sp*

8.1 Effect of storage on the microbial count of yoghurt

Figure 7 showed the effect of storage on bacterial count of the yoghurt samples stored at refrigeration temperature (4℃). The result obtained showed that there was a significant difference ($p < 0.05$) between the produced yoghurt and the control samples. For instance, the produced yoghurt sample had the highest count 20.10 cfu/mL after 24 h of storage while the control sample had 17.2 cfu/mL but declined to 0.75 and 0.7 cfu/mL respectively at the last day of storage. The high total viable count of the onset of storage could be as a result of presence endogenous microorganism from the plant milk. Meanwhile, the total viable bacteria count declined storage progressed. Similar observation was also made by Aderinola and Olanrewaju (2014), where decline was also experienced as the days of storage increased, which could be as a result of the low temperature storage. Palova Charvat Masopust, Klapkova and Kvapil (2007) have previously reported the probable cause of death of bacterial cells to be as a result of damage in cell membranes and DNA denaturation during low temperature storage. The result (Fig. 4) further showed that the yoghurt produced from tiger nut milk, coconut milk and flaxseed, would have a longer shelf life compared with the control yoghurt sample due to its least bacteria count after 21 days of refrigeration storage. During yoghurt storage, the decrease of lactic acid level correlates with the decreasing LAB activities in the yoghurt. Lactic acid is one of the major lactose products in milk degradation due to bacterial fermentation. The production depends on the involved microorganisms, in which milk fermentation proceeds through the glycolysis pathway and yields lactic acid.



Key: PY – Plant yoghurt: CO – Control sample
Figure 7 Effect of storage on the microbial count of yoghurt

5.0 Sensory evaluations of yoghurt

The preference test scores of plant yoghurt in terms of texture, taste, appearance and mouthfeel is displayed in the column chart below (figure 8). In the sensory evaluation chart panelist preferred the texture of control yogurt over the plant yoghurt. The control sample exhibited good custard-like body which is preferred by panelists over the produced yoghurt. One important characteristic for evaluating yoghurt is its thickness and water content. In sensory evaluation of yoghurt texture relate to mouthfeel, oral viscosity and consistency of yoghurts. Thickness can be caused by enzyme activity or acid (Sunario, (2010). Judging from the mean score displayed on the chart, both yoghurt samples were preferred. Weerathilake et al. (2014) pointed out that the preference for certain yoghurt taste is attributable to its sweetness. There was no significant difference (p>0.05) in the plant yoghurt and the control sample**.** The average preference point score for "aroma" were found to be same; aroma is considered as the most important and has very strong influence to consumer acceptability (Olugbuyiro and Oseh, 2011).

The panelists accepted the appearance of the control sample over the plant yoghurt. The appearance of plant yoghurt samples had the least preference score as it lacked velvety feeling and was not fully homogenous in content; this must have resulted from the inclusion of flaxseed justified by its texture which the panelists rated very low. The "appearance" was also influenced by the colour of the yoghurt and the panellists showed preference for a brighter off-white colour of control sample. The control sample produced a clean natural colour with smooth velvety appearance which agrees with acceptable standard described by Rita, (2009). Though, there are no observed differences in terms of mouthfeel, the panelists gave their preference rating based on the tartness they felt in their mouth after tasting the yoghurt. This revealed that both yoghurt samples were accepted.

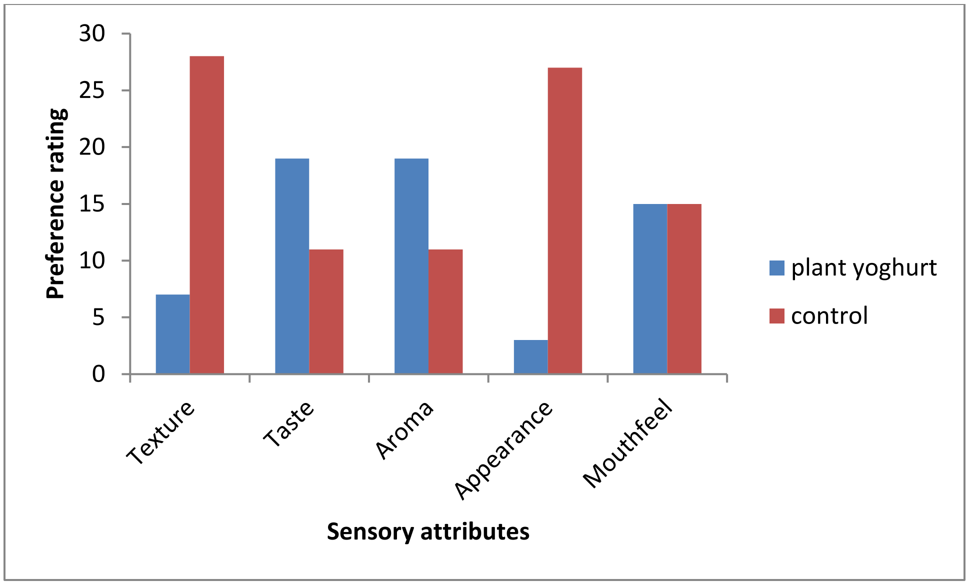


Figure 8 Effect of storage on sensory attributes of yoghurt

6.0 Conclusion

An optimal blend ratio of tiger nut milk (62.9%), coconut milk (34.1%) and flaxseed powder (3%) were obtained for the production of probiotic plant yoghurt. Physicochemical, proximate analysis of the plant yoghurt showed similar properties as the commercial yoghurt; however, the plant yoghurt was preferred in terms of taste and aroma. The optimized product was analyzed and *Lactobacillus sp* identified as the predominant probiotic bacteria. This study proved the validity of the selected regression models to sufficiently explain the factor-response relationship during plant yoghurt production with tiger nut milk, coconut milk and flaxseeds and that the predicted optimum blends are valid to generate probiotic metabolites

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Figures

The effect of independent variables on response variables

The interaction effects of tiger nut milk, coconut milk and flaxseed on all response variables is shown in surface plots Fig. 1-6. From the figures, when the independent variable level increased, a negative coefficient showed a decrease in the response variable, while a positive coefficient indicated an increase in the response variable (Marzlan, Muhialdin, Abedin, Mohammed, Abadl, Roby, Hussin, 2020).

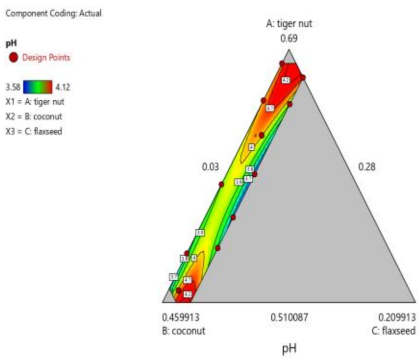


Figure 1

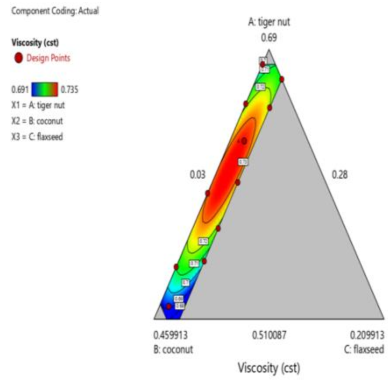
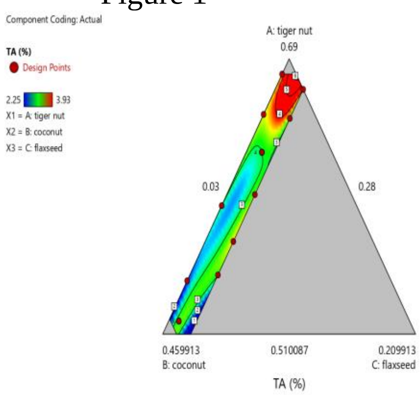


Figure 2



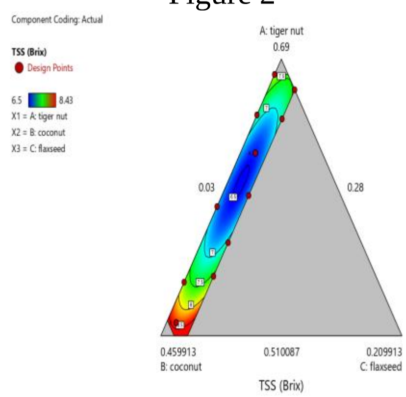


Figure 3

Figure 4

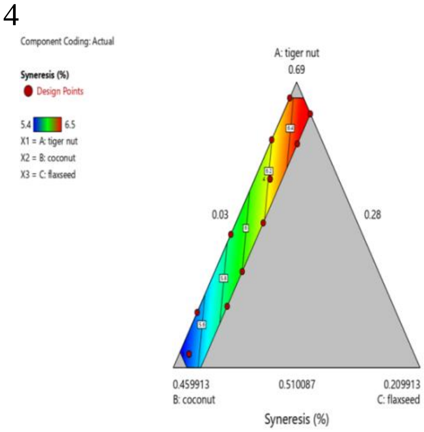


Figure 5

Figure 6: contour plots representing the effect of (1) pH (2) viscosity (3) titritable acidity (4) total soluble solids (5) syneresis of yoghurt