Journal of Agricultural, Food Science & Biotechnology

JAFSB, 1(2), 128-139, 2023
https://doi.org/10.58985/jafsb.2023.v01i02.15
ISSN (Print): 2959-3417

*Research Article*

# Quality assessment of yoghurt formulated with aqueous extract of Roselle calyx, Moringa leaf and Lemon grass

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***Article Information***
*Received:* 08 January 2023
*Revised:* 22 March 2023
*Accepted:* 29 March 2023

***Academic Editor***
Gian Carlo Tenore

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***Keywords***
Moringa leaf, Roselle calyx, Lemon grass, aqueous extract, herbal yoghurt.

## Abstract

The Moringa leaf, Roselle calyx and Lemon grass leaf extracts were added separately at a proportion of 5, 10, 15% into the yoghurt and refrigerated at 5 ± 1 $^0$C for further analysis. The samples were coded as RCAE1, RCAE2, RCAE3 for Roselle calyx extract; MLAE1, MLAE2 for Moringa leaf extract and LGAE1, LGAE2, LGAE3 for Lemon grass leaf extract while YWNE was the control. The formulated yoghurts were subjected to proximate, micronutrients, phytochemicals, physicochemical, microbial and sensory evaluation. Data obtained were subjected to statistical analysis. The results showed that moisture and carbohydrate increased with an increase in concentration. The ash content decreased also. There showed no significant difference ($p>0.05$) between the control and formulated samples. The micronutrients increased except for Vitamin A of the Moringa leaf and lemon grass leaf (aqueous extract) samples decreased as the concentration increased. The phytochemical content of the samples increased; except tannin of Moringa leaf and Lemongrass which decreased on addition of aqueous extracts. pH of the formulated yoghurt samples decreased leading to an increase in titratable acidity. The total viable count for the yoghurt sample formulated with Roselle and Moringa leaf extract decreased with increase in the concentration of the extracts. The most acceptable herbal yoghurt by the panelists in terms of palatability is RCAE2 while the product best with respect to the nutritional quality is LGAE3.

## 1. Introduction

With the emergence of fortified foods, there is a worldwide increase in health awareness and interest in adding herbs as prized food additives in dairy and food products. Due to the increased consumer awareness and interest to follow healthy nutrition and dietary strategy in achieving health benefits from foods beyond their basic nutrition, the market for value added functional foods has expanded [1]. Herbs have multifarious role such as food flavorings, preservative and medicinal ingredients and various herbs are documented for their therapeutic properties such as, anti-oxidative, anti-hypertensive, anti-inflammatory, anti-diabetic and anti-microbial properties [2].

Some herbs that have been used in the past are Moringa plant (*Moringa oleifera*) is used to improve weight in malnourished children by adding Moringa powder to food. Drinking of Moringa tea relieves headache, heart burns and gastritis. Yoghurt is one of the most popular fermented milk products worldwide and has gained widespread consumer acceptance as a healthy food. It provides an array of nutrients in significant amounts, in relation to its energy and fat content, making it a nutrient-dense

food. In particular, yoghurt could provide the body with significant amounts of calcium in a bioavailable form. Furthermore, yoghurt has many health benefits beyond the basic nutrition it provides, such as improved lactose tolerance, a possible role in body weight and fat loss, and a variety of health attributes associated with probiotic bacteria [3]. Yoghurt is an excellent source of protein, calcium, iodine, phosphorus, riboflavin (vitamin $B_2$ ), thiamin (vitamin$B_1$) and vitamin$B_{12}$, and a valuable source of folate, niacin, magnesium and zinc. Yoghurt provides many of the nutrients needed for optimal bone health such as calcium, protein, magnesium, zinc and phosphorus.

*Hibiscus sabdariffa* commonly named as "red sorrel" or "Roselle" is a member of malvaceae family. Different extracts from Roselle play a crucial role in treating different medical problems including many cardiovascular disorders, helmenthic disease and cancer. The plant also acts as an anti-oxidant and used in obesity management [4]. *Moringa* leaves contain seven times the vitamin C of oranges, four times the vitamin A of carrots, four times the calcium of milk, three times the potassium of bananas and two times the protein in yoghurt.

*Cymbopogon citratus* commonly known as "Lemon grass" or "fever grass" is an aromatic perennial tall grass with rhizomes and densely tufted fibrous roots. Nutritionally, lemon grass is a good source of vitamins A and C, folic acid, magnesium, zinc, copper, iron, potassium, calcium and manganese. Lemon grass has been used as a food ingredient, in cosmetics and as folk medicines in several regions of the world. Yoghurt from cow's milk is lacking in certain phytochemicals such as carotenoids, flavonoids, anthocyanins, isothiocyanates, saponins, tannins, lutein and zeaxanthin, that play functional roles in the body. The lack of these phytochemicals has contributed to certain disease conditions such as cancer, heart disease, inflammation, ulcers and diabetes. The main aim of this study was to produce and evaluate yoghurt formulated with aqueous extracts of Roselle calyx (*Hibiscus sabdariffa*), Moringa leaves (*Moringa oleifera*) and Lemon grass leaves (*Cymbopogon citratus*). Therefore, the addition of herbs would add phytochemicals to the yoghurt to make a functional yoghurt. These phytochemicals would improve the nutritional status of the yoghurt and when taken into the body improves one's immune system. The use of indigenous herbs would reduce the cost of production as they are readily available.

## 2. Materials and methods

### *2.1 Raw materials*

The raw materials were whole milk powder, granulated sugar, stabilizer (CMC), yoghurt culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*), dried Roselle calyx, fresh Moringa leaves and fresh Lemon grass leaves.

#### *2.1.1 Sample procurement*

The whole milk powder, granulated sugar, stabilizer (CMC) and dried Roselle calyx were procured from Ogige market, Nsukka, Enugu State. The fresh Moringa leaves and fresh lemon grass leaves were obtained from farms. These leaves were authenticated by the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The yoghurt culture was purchased from the International Bakery and Confectionary market, Ogidi/Ogwunike Onitsha Metropolis, Anambra State.

### *2.2 Sample Preparation*

#### *2.2.1 Processing of aqueous Roselle leaf drink* (*Hibiscus sabdariffa*)

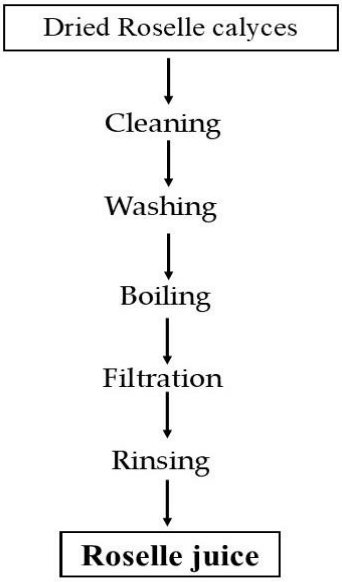
Sixty grams of dried Roselle calyces were cleaned by picking off dirt, foreign matters and washed with cold water. The calyces were boiled in 300mL of water for 30 minutes to ensure proper extraction of the juice from the calyces. The juice was filtered using a sieve to remove the Roselle calyces from the aqueous extract [5]. The production of the Roselle juice is summarized in Scheme 1.

#### *2.2.2 Processing of aqueous Moringa leaf* (*Moringa oleifera*)

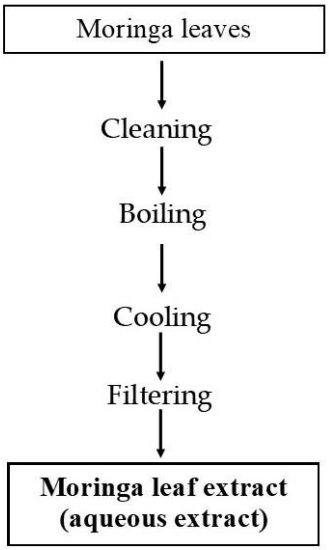
Sixty grams of fresh Moringa leaves was boiled with 300mL of water for 20 minutes at 80° C and cooled for 10 minutes. The extract was separated from the leaves using a sterile muslin cloth and filtered through a sterile Whatman filter paper [6]. The production of Moringa leaf extract is summarized in Scheme 2.

#### *2.2.3 Processing of Lemon grass (Cymbopogon citratus) aqueous extract*

Sixty grams of the leaves were washed with clean water, cut into pieces, boiled with 300mL of water for 30 minutes, cooled for 10 minutes and sieved using a




**Scheme 1:** Processing of Roselle calyx extract [5]

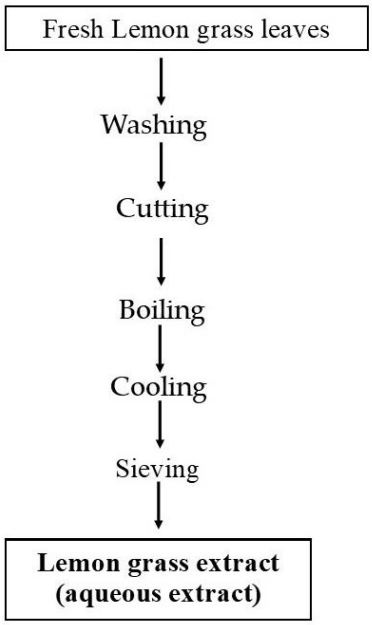



**Scheme 2.** Processing of Moringa leaf extract [6]

sterile muslin cloth to obtain the Lemon grass aqueous extract [7]. The production of Lemon grass aqueous extract is summarized in scheme 3.

*2.3 Production and formulation of yoghurt*

Seventy-five grams of milk was mixed with 0.6g of stabilizer, 6g of sugar and 215mL of water as an aqueous medium for mixing. Yoghurt mixes were homogenized to obtain a uniform product. It was pasteurized at 80 ℃ for 30 minutes to destroy unwanted microorganisms. The product was cooled to 43 ± 2 ℃ and starter culture was added and sample was incubated for 4 -6 hours. The leaf extracts were added separately at a proportion of 5, 10 and 15%. The product was kept refrigerated at 5 ± 1 ℃ for further analysis [8]. There were ten samples coded as RCAE1,




**Scheme 3.** Processing of lemon grass aqueous extract [8]

RCAE2, RCAE3, MLAE1, MLAE2, MLAE3, LGAE1, LGAE2, LCAE3, YWNE. Sample YWNE served as the control sample (Table 1). The pictorial representation of the formulated yoghurt samples (Plate 1-4) is shown in Fig 1.

**Table 1.** Formulation of herbal yoghurt using different concentrations of Roselle calyx, Moringa leaves and Lemongrass leaves (aqueous extract)

| Sample code | Roselle calyx (mL) | Moringa leaves (mL) | Lemongrass leaves (mL) |
|---|---|---|---|
| RCAE1 | 5 | - | - |
| RCAE2 | 10 | - | - |
| RCAE3 | 15 | - | - |
| MLAE1 | - | 5 | - |
| MLAE2 | - | 10 | - |
| MLAE3 | - | 15 | - |
| LGAE1 | - | - | 5 |
| LGAE2 | - | - | 10 |
| LGAE3 | - | - | 15 |
| YWNE (Control) | - | - | - |

Key:RCAE1=Herbal yoghurt with 5 mL Roselle calyx (aqueous extract); CAE2=Herbal yoghurt with 10 mL Roselle calyx (aqueous extract); RCAE3=Herbal yoghurt with 15 mL Roselle calyx (aqueous extract); MLAE1= Herbal yoghurt with 5 mL Moringa leaf (aqueous extract); MLAE2= Herbal yoghurt with 10 mL Moringa leaf (aqueous extract); MLAE3=Herbal yoghurt with 15 mL Moringa leaf (aqueous extract); LGAE1=Herbal yoghurt with 5 mL Lemongrass leaf (aqueous extract); LGAE2=Herbal yoghurt with 10 mL Lemongrass leaf (aqueous extract); LGAE3= Herbal yoghurt with 15 mL Lemongrass leaf (aqueous extract); YWNE=Herbal yoghurt with 0 mL of the herbs used as the control.


**Plate 1.** Yoghurt samples with Roselle calyx aqueous extract


**Plate 2.** Yoghurt samples with Moringa leaf aqueous extract


**Plate 3.** Yoghurt samples with Lemongrass aqueous extract

**Plate 4.** Yoghurt control sample

**Figure 1.** Pictorial representation of the formulated yoghurt samples (Plate 1-4)

*2.4 Proximate analysis of the herbal yoghurt samples*

The following proximate analysis was carried out on the formulated samples of the Roselle, Moringa and lemon grass flavoured yoghurt and the control.

*2.4.1 Determination of moisture content*

The moisture content of the samples was determined using the hot air oven method described by Association of Official Analytical Chemists [9].

*2.4.2 Determination of crude fat*

The fat content of the samples was determined using the standard method described by Association of official Analytical Chemists [9].

*2.4.3 Determination of crude protein*

The crude protein determination was achieved using the standard method (Kjeldahl method) described by Association of Official Analytical Chemist [9].

*2.4.4 Determination of ash content*

The ash content of each yoghurt sample was determined according to Association of Official Analytical Chemists [9].

*2.4.5 Determination of carbohydrate*

Total carbohydrate content was calculated by difference as follows:

% carbohydrate = 100 – (% moisture + % fat + % protein + % ash + % crude fibre).

*2.5 Determination of micronutrient composition*

*2.5.1 Determination of vitamin A content*

Vitamin A content was determined according to Association of Official Analytical Chemists method [9].

*2.5.2 Determination of vitamin C content*

Vitamin C content was determined using the Association of Official Analytical Chemists method [9].

*2.5.3 Determination of phosphorous content*

Phosphorous content in the sample was determined according to Onwuka by molybdate method using hydroquinone as a reducing agent [10].

*2.5.4 Determination of calcium content*

The calcium content was determined by the titration method according to Kirk and Sawyer [11].

*2.6 Determination of phytochemicals*

*2.6.1 Determination of flavonoids*

Total flavonoids content was determined by a colorimetric assay using the Kapoor's method [12].

*2.6.2 Determination of saponins*

Total saponins was determined using Kapoor method [12].

*2.6.3 Determination of tannins*

Total tannins were determined using Kapoor method [12].

*2.7 Microbial analysis*

*2.7.1 Determination of lactic acid bacteria (LAB)*

The lactic acid bacteria in the formulated yoghurt was determined using deMan Rogosa Sharpe (MRS) Agar (CM 361) as described by Oxoid manual. Samples were serially diluted in duplicates using the surface pour plate method. The plates were incubated under anaerobic conditions at 37° C for 48 hours. After incubation, the number of colonies were counted using the colony counter and represented as colony forming unit per millilitre (cfu/ml) [13].

No of colonies (cfu/ml) = average count × dilution factor (D.F)

*2.7.2 Determination of total viable count (TVC)*

The total viable count test was carried out using the method described by Prescott [14]. Using sample and sterilized quarter strength ringer solution as diluents, one millilitre (1 mL) of the water sample was pipetted into a sterile test tube and 9 ml ringer solution pipetted into it and other test tubes arranged for serial dilutions ($10^{-3}$). The diluted sample was pipetted into a marked petri dish and sterile nutrient agar of 20 mL was poured into the same petri dish and swirled to mix and incubated (under conditions that permit microbial reproduction so that colonies develop could be seen without the aid of a microscope at a temperature of 37 °C for 24 hours. After incubation, the number of colonies were counted and represented as colony forming unit per millilitre (cfu/ mL).

No of colonies (cfu/mL) = average count × dilution factor (D.F)

*2.7.3 Determination of mould count*

The mould count test was carried out using the method described by Prescott [14] and Sabouraud dextrose agar (SDA) was used as the media. Fifteen milliliters of the media was added to one gram of sample in the Petri dish and mixed. The media was allowed to settle before incubating at 37 $^{0}$C for 48 hours. After incubation, the number of colonies were counted and represented as colony forming unit per millilitre (cfu/mL).

No of colonies (cfu/mL) = average count × dilution factor (D.F)

*2.8 Titratable Acidity (TTA)*

The titratable acidity was determined according to Association of Official Analytical Chemist Method [9].

*2.9 pH*

The pH of the samples was measured in a 10% (w/v) dispersion of the samples in distilled water. Each suspension was mixed thoroughly and a standard pH meter (Hanna meter model H196107) was used for pH determination. The pH electrode was dipped into mixture and reading taken [9].

*2.10 Sensory evaluation*

The sensory properties of samples were evaluated by a semi-trained panel consisting of 20 panelists (including students and staff in the Department of Food Science and Technology, University of Nigeria, Nsukka). A 9-point Hedonic scale was used to assess the formulated herbal yoghurt, 0 = like extremely and 1 = dislike extremely. The samples were served in plastic cups and randomly presented. A questionnaire comprising of the five sensory attributes namely, color, taste, aftertaste, flavor, texture, consistency and overall acceptability was given to each panelist [15].

*2.11 Experimental design and data analysis*

The experiment was laid on Completely Randomized Design (CRD). Data obtained was subjected to statistical analysis. The analysis of variance (ANOVA) tests were carried out by using the general linear model procedure of the SPSS (Version 23.0). Means were separated by Duncan's new multiple range test (DNMRT). Significance was accepted at $p<0.05$ [26].

## 3. Results and discussion

*3.1 Proximate composition of the yoghurt samples formulated with Roselle calyx, Moringa leaf and Lemon grass (aqueous extract)*

*3.1.1 Moisture content*

The moisture content of the flavored yoghurt samples ranged from 70.80 % for MLAE1 to 76.92 % for LGAE3. There showed a significant difference ($p<0.05$) between the flavored yoghurt samples and the sample YWNE which was plain and served as control sample. The difference in moisture increased as the concentration of the extracts increased. The increase in moisture could be attributed to the increasing water content of the aqueous extracts [16]

*3.1.2 Ash content*

The ash content of the samples ranged from 0.20 % for LGAE3 to 0.85 % to RCAE1. There showed a significant difference ($p<0.05$) between the flavoured samples and the control sample. The ash content of the flavoured yoghurts decreased as the concentration of the extract increased. This implies that increase in the concentration of the extracts did not contribute to the increase in the solid residues in the yoghurt samples. The ash content was similar to the report by Salisu [17].

*3.1.3 Crude fat*

The fat content ranged from 12.97 % for RCAE3 to 15.83 % for MLAE2. There showed no significant difference ($p>0.05$) between the flavoured yoghurt samples and sample YWNE. This could be attributed

to the low fat content of Roselle calyx, Moringa and Lemon grass fresh leaves as with the report of Lakshmipriya [18].

*3.1.4 Protein content*

The protein content ranged from 5.11 % for YWNE to 5.59 % for MLAE2. There was no significant difference ($p>0.05$) between the flavoured yoghurt samples and sample YWNE. This could be attributed to the low protein content of Roselle calyx, Moringa and Lemon grass fresh leaves as to the report of Lakshmipriya [18].

*3.1.5 Carbohydrate content*

The carbohydrate content ranged from 3.84 % for LGAE2 to 7.51 % for MLAE1. There was no significant difference ($p>0.05$) between the flavoured yoghurt samples and sample YWNE. This could be due to the small amount (5, 10 and 15 ml) of Roselle calyx, Moringa and Lemon grass aqueous extracts added [16]. The proximate compositions are shown in Fig. 2.

*3.2 Selected micronutrient composition of the yoghurt samples formulated with Roselle calyx, Moringa leaf and Lemon grass (aqueous extract)*

*3.2.1 Vitamin C content*

The vitamin C content of the yoghurt samples ranged from 1.06 mg/100g for YWNE to 3.60 mg/100g for LGAE3. There was a significant difference ($p>0.05$) between the control sample (YWNE) and the flavoured yoghurt samples. The increase in vitamin C content follows a simultaneous increase in the concentration of the aqueous extracts. This is due to Roselle calyx, Moringa and Lemon grass leaves already containing a high vitamin C content as reported by Martha [19].

*3.2.2 Vitamin A content*

The vitamin A content ranged from 0.19 mg/100g for MLAE2 to 0.26 mg/100g for RCAE3. There was no significant difference ($p>0.05$) between the control sample (YWNE) and the flavoured samples except for sample RCAE3 which increased the vitamin A content of the yoghurt at Roselle calyx extract concentration of 15 ml. This could be due to increase in the extract at 15 ml contributing to the increase in the vitamin A content of the yoghurt as reported by Fasoyiro [20].

*3.2.3 Phosphorous content*

The phosphorous content of the yoghurt samples ranged from 174.59 mg/100g for YWNE to 588.41 mg/100g. There was a significant difference ($p<0.05$) between the control sample and the flavoured yoghurts. The increase in the concentration of the aqueous extracts led to the increase in the phosphorous content of the flavoured yoghurt samples and this is due to the high phosphorus content of Roselle calyx, Moringa and Lemon grass leaves [18, 20].

*3.2.4 Calcium content*

The calcium content of the yoghurt samples ranged from 81.32 mg/100g for LGAE1 to 104.26 mg/100g for RCAE3. There was a significant difference ($p<0.05$) between the control sample and the flavoured yoghurts. The result shows that addition of aqueous extract with increasing concentration led to increase in the calcium content of the yoghurt samples. The values of the calcium content obtained agreed with the report of Tajidin [21]. The micronutrient composition of the yoghurt samples is shown in Fig. 3.

*3.3 Physicochemical composition of the yoghurt samples formulated with Roselle calyx, Moringa leaf and Lemon grass (aqueous extract)*

*3.3.1 Titratable acidity*

The titratable acidity of the yoghurt samples ranged from 0.90 % for YWNE to 1.09 % for RCAE3. There was a significant difference ($p<0.05$) between the control sample and the flavoured yoghurt samples. The titratable acidity of the yoghurt samples was increasing with increase in the concentration of the aqueous extract [20].

*3.3.2 pH*

The pH of the yoghurt samples ranged from 3.75 for LGAE3 to 4.57 for YWNE. There was a significant difference ($p<0.05$) between the control sample and the flavoured yoghurt samples. The pH of the flavoured yoghurt samples decreased with increase in the concentration of the aqueous extract [19, 20]. The physicochemical composition of the yoghurt samples is shown in Fig. 4.

*3.4 Physiochemical composition of the Roselle calyx, Moringa leaf and Lemon grass (aqueous extract)*

*3.4.1 Titratable acidity*

The titratable acidity (Table 2) of Roselle calyx aqueous extract is 1.22 % showing the acidic level of the Roselle calyx, Moringa leaf is 0.64 % due to its alkaline nature and lemon grass is 0.41 % [17, 19, 20].

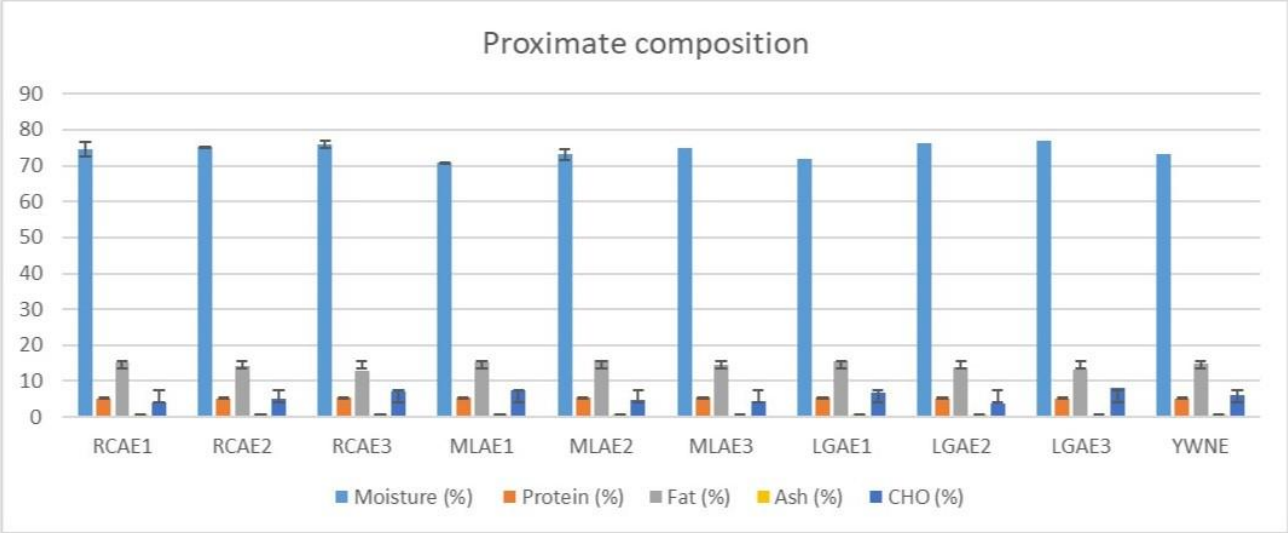



**Figure 2.** Graphical representation of the proximate composition of the formulated yoghurt samples. Values are means±standard deviation of triplicate determinations. Means with different superscripts in the same column are significantly different at $p< 0.05$).

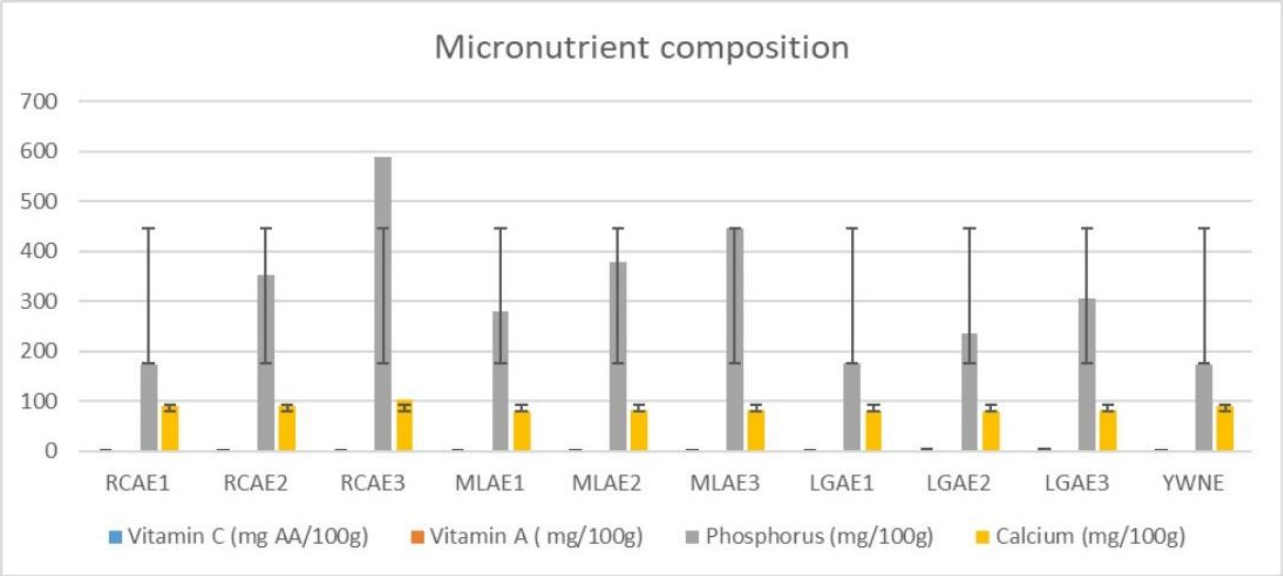



**Figure 3.** Graphical representation of the micronutrient of the formulated yoghurts. Values are means ± standard deviation of triplicate determinations. Means with different superscripts in the same column are significantly different at $p< 0.05$).

*3.4.2 pH*

The pH (Table 2) of Roselle calyx aqueous extract is 3.88 due to the acidic level of Roselle calyx [20], Moringa leaf is 6.89 due to its alkaline nature and lemon grass is 5.92 [17, 19].

*3.5 Phytochemical composition of the yoghurt samples formulated with Roselle calyx, Moringa leaf and Lemon grass (aqueous extract)*

*3.5.1 Flavonoid content*

The flavonoid content ranged from 0.17 mgQE/100g for YWNE to 8.76 mgQE/100g for RCAE3. There was no significant difference ($p<0.05$) between the control sample and flavoured yoghurt samples. This could be due to the amount (5, 10 and 15 ml) of Roselle calyx, Moringa and Lemon grass aqueous extracts added not been enough to cause a significant increase in the flavonoid content of the yoghurt samples [16].

*3.5.2 Tannin content*

The tannin content ranged from 0.02 mgTA/100g for YWNE to 0.97 mgTA/100g for RCAE3. There was a significant difference ($p<0.05$) between the control sample and the flavoured yoghurt samples. The tannin content of the yoghurt formulated with Roselle

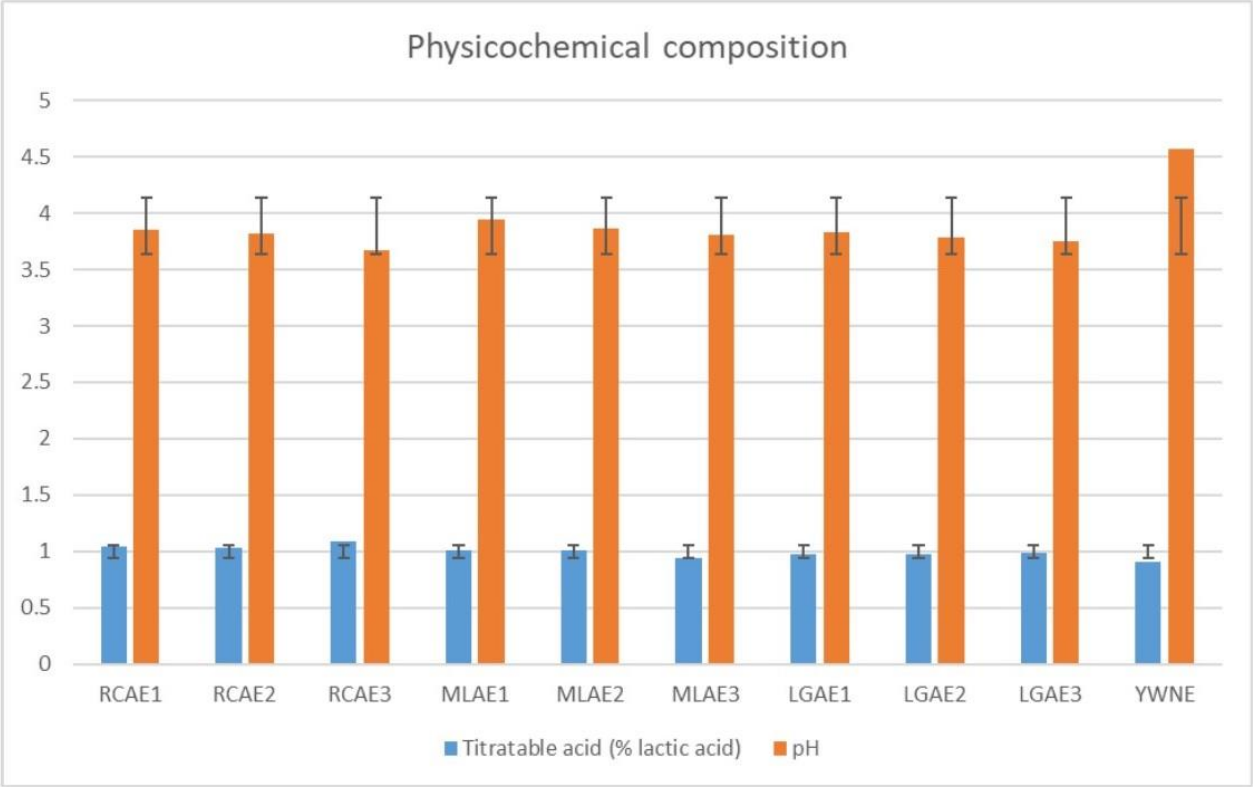



**Figure 4.** Graphical representation of the physicochemical composition of the formulated yoghurts. Values are means ± standard deviation of triplicate determinations. Means with different superscripts in the same column are significantly different at $p< 0.05$).

**Table 2.** Selected physiochemical composition of the Roselle calyx, Moringa leaf and Lemongrass leaf (aqueous extract).

| Sample | TTA (% lactic acid) | pH |
|---|---|---|
| RCAE | $1.22^{g} \pm 0.06$ | $3.88^{cd} \pm 0.01$ |
| MLAE | $0.64^{b} \pm 0.01$ | $6.89^{f} \pm 0.01$ |
| LGAE | $0.41^{a} \pm 0.00$ | $5.92^{e} \pm 0.01$ |

Values are means ± standard deviation of triplicate determinations. Means with different superscripts in the same column are significantly different at ($p< 0.05$). Values are means±standard deviation of duplicate determinations. Values in the same columns with the different superscript are significantly ($p<0.05$) different. KEY: RCAE=Yoghurt with Roselle calyx extract; MLAE=Yoghurt with Moringa leaf extract; LGAE=Yoghurt with Lemon grass leaf extract.

calyx extract increased with increase in concentration of extract and this could be related to Roselle calyx containing non-hydrolyzable tannin, namely catechin [17]. Also, the tannin content of the yoghurt formulated with Moringa leaf and Lemon grass leaf aqueous extract was decreasing with increase in the concentration of the extracts and this could be due to Moringa and Lemon grass leaf containing hydrolysable tannin, namely tannic acid, which gets easily hydrolysed in water [17].

*3.5.3 Saponin content*

The saponin content ranged from 0.05 mg/g for YWNE to 4.83 mg/g for MLAE3. There was a significant difference ($p<0.05$) between the control sample and the flavoured yoghurt samples. The saponin content of the yoghurt samples was increasing with increase in concentration of the extracts and this is due to the saponin content of Roselle calyx, Moringa leaf and Lemon grass leaf [18]. The phytochemical composition of the yoghurt samples is shown in Fig. 5.

*3.6 Phytochemical composition of the Roselle calyx, Moringa leaf and Lemon grass (aqueous extract)*

The aqueous extracts showed (Table 3) high values in flavonoid content than in tannin and saponin content. This could be attributed to the part of the plant and method used to produce the extract [18].

*3.7 Microbial count of the yoghurt formulated with Roselle calyx, Moringa leaf and Lemon grass (aqueous extract)*

The samples show (Table 4) a total viable count range of $2.77\times 10^{3}$ for RCAE3 to $7.33\times 10^{3}$ cfu/g for LGAE3. The total viable count for the yoghurt sample formulated with Roselle and Moringa leaf extract

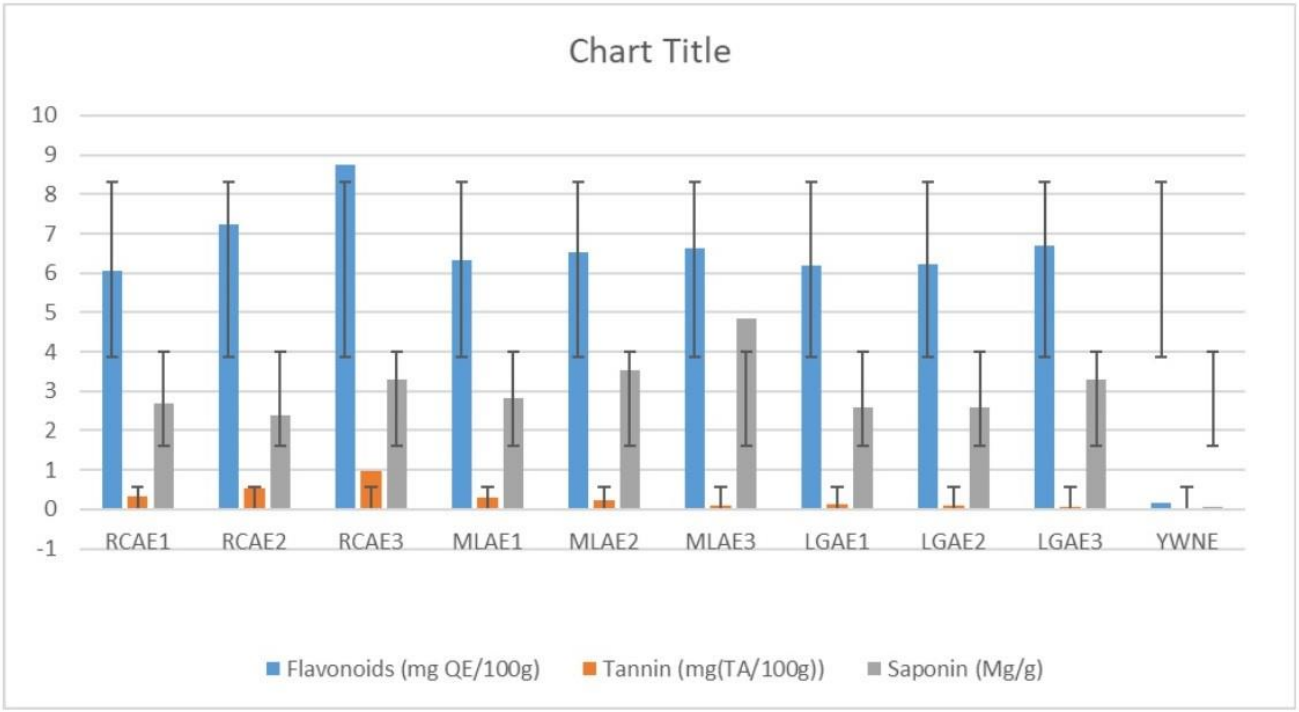



**Figure 5.** Graphical representation of the phytochemical composition of the formulated yoghurts. Values are means ± standard deviation of triplicate determinations. Means with different superscripts in the same column are significantly different at p< 0.05).

**Table 3.** Selected phytochemical composition of the Roselle calyx, Moringa leaf and Lemongrass leaf (aqueous extract)

| Sample | Flavonoids (mg QE/100g) | Tannin (mg TA/100g) | Saponin (Mg/g) |
|---|---|---|---|
| RCAE | $93.94^{c}$±20.29 | $1.67^{f}$±0.05 | $3.91^{de}$±1.03 |
| MLAE | $100.94^{c}$±31.92 | $0.43^{cd}$±0.00 | $4.05^{de}$ ± 0.13 |
| LGAE | $60.09^{b}$±1.58 | $0.25^{abc}$±0.07 | $2.80^{bc}$ ± 0.08 |

Values are means ± standard deviation of duplicate determinations. Values in the same columns with the different superscript are significantly ($p<0.05$) different. RCAE=Yoghurt with Roselle calyx extract; MGAE=Yoghurt with Moringa leaf extract; LGAE= Yoghurt with Lemon grass leaf extract.

decreased with increase in the concentration of the extracts. The total viable count of yoghurts was within acceptable standard <1x$10^6$ cfu/mL [24, 27, 28]. The standard count is $10^6$-$10^7$cfu/mL [29].

The lactic acid bacteria in the samples ranged from 1.02× $10^4$ for RCAE3 to 2.81× $10^4$ cfu/g for LGAE3. For yoghurt samples formulated with Roselle and Moringa leaf extract, the lactic acid bacteria decreased with an increase in the concentration of the extracts except for the yoghurt samples with Lemon grass aqueous extract which increased with the increasing concentration of the extracts. This could be attributed to the stimulating effect lemon grass has on lactic acid bacteria by enhancing their growth and acid production [17].

**Table 4.** Microbial count of the yoghurt flavoured with Roselle calyx, Moringa leaf and Lemon grass leaf (aqueous extract)2

| Samples | LAB (cfu/g) | Mould (cfu/g) | TVC (cfu/g) |
|---|---|---|---|
| RCAE1 | 1.90 x 104 | ND | 5.58 x 103 |
| RCAE2 | 1.12 x 104 | ND | 3.66 x 103 |
| RCAE3 | 1.02 x 104 | 5.55 x 102 | 2.77 x 103 |
| MLAE1 | 1.91 x 104 | 6.25 x 102 | 4.41 x 103 |
| MLAE2 | 1.41 x 104 | 2.94 x 102 | 4.16 x 103 |
| MLAE3 | 1.11 x 104 | 5..88 x 102 | 4.11 x 103 |
| LGAE1 | 1.08 x 104 | 8.33 x 102 | 3.05 x 103 |
| LGAE2 | 1.46 x 104 | ND | 6.36 x 103 |
| LGAE3 | 2.81 x 104 | 9.09 x 102 | 7.33 x 103 |
| YWNE | 2.07 x 104 | ND | 5.35 x 103 |

Values are means ± standard deviation of duplicate determinations. Values in the same columns with the different superscripts are significantly ($p<0.05$) different. YWNE=Plain yoghurt; RCAE=Yoghurt with Roselle calyx extract; MGAE=Yoghurt with Moringa leaf extract; LGAE=Yoghurt with Lemon grass leaf extract; LAB = lactic acid bacteria; TVC=total viable count; ND =Not detected.

Mould was detected in sample RCAE3, MLAE1, MLAE2, MLAE3, LGAE1 and LGAE3. Increased

acidity or a decrease in potential oxygen during the fermentation process are likely to have created favorable circumstances for the growth of yeasts and molds, which is why the number of molds has increased. Mold metabolite concentrations exceeding 10.0 cfu/g can result in food poisoning and human liver cancer by creating poisonous metabolites (mycotoxin, such as aflatoxin) [30, 31].

*3.8 Sensory scores of the yoghurt samples formulated with Roselle calyx, Moringa leaf and Lemon grass (aqueous extract)*

*Appearance:*

The mean score of appearance ranged from 6.30 for sample LGAE3 to 7.60 for sample RCAE2. There was a significant difference ($p<0.05$) between samples except for sample RCAE3, MLAE1, MLAE2, MLAE3, LGAE1 and LGAE2. Also, there was a color change in the yoghurt samples with Roselle calyx extracts due to the colour characteristics of the Roselle calyx.

*Aroma:*

The score for the aroma ranged from 6.80 for sample LGAE2 to 7.35 for sample RCAE2. There was no significant difference ($p>0.05$) between the control sample and formulated yoghurt samples.

*Taste:*

The score for taste ranged from 6.15 for sample LGAE3 to 7.40 for sample RCAE2. There was no significant ($p>0.05$) difference in taste for sample RCAE1, MLAE1, MLAE2, MLAE3, LGAE1, LGAE2 and YWNE.

*Aftertaste:*

The score for aftertaste ranged from 6.10 for sample LGAE2 to 7.15 for sample RCAE2. There was no significant difference ($p>0.05$) in taste for sample RCAE1, MLAE1, MLAE2, MLAE3, LGAE1, LGAE2 and YWNE.

*Mouthfeel:*

The score for mouthfeel ranged from 5.60 for sample LGAE3 to 7.25 for sample RCAE2. There was no significant difference ($p>0.05$) in mouthfeel for sample RCAE3, MLAE1, MLAE3, LGAE1, LGAE2, LGAE3 and YWNE.

*Consistency:*

The score for consistency ranged from 4.90 for sample LGAE3 to 7.05 for sample RCAE2. There was a significant difference ($p>0.05$) in consistency between the formulated samples due to the addition of the aqueous extracts.

*Overall acceptability:*

The score of the overall acceptability ranged from 6.05 for sample LGAE3 to 7.45 for sample RCAE2. There was a significant difference ($p>0.05$) in the overall acceptability of each sample. The yoghurt sample RCAE2 had the highest score in the overall acceptability followed by sample MLAE2 and then RCAE1 which are significantly different ($p>0.05$) from each other.

The result of the sensory score (Fig. 6) denoted that at 10 mL Roselle calyx extract, flavored yoghurt could be comfortably produced and accepted by the consumers. Following the result of the sensory scores, it could be deduced that the increased addition of the aqueous extract had no significant difference ($p>0.05$) in the acceptability in terms of flavor of the yoghurt samples.

## 4. Conclusions

From the result of this work, it could be deduced that addition of Roselle calyx, Moringa leaf and Lemon grass leaf aqueous extracts in plain yoghurt could improve the nutritional quality of yoghurt especially in terms of minerals and phytochemicals analyzed. The therapeutic potency of yoghurt could also be improved in vitamin A content which is essential for maintaining a good eyesight. There was also an impact on the proximate composition of the formulated products especially in terms of moisture and ash. The sensory attributes (appearance, aroma, taste, aftertaste, mouthfeel, consistency and overall acceptability) evaluated by 20 panelists showed an appreciable degree of acceptability which could add to the number of yoghurt varieties in the market. The results also showed that Roselle calyx yoghurt sample with the concentration of 10 mL was most preferred among the formulated yoghurt samples with an overall acceptability of 7.45. For the yoghurt with Moringa and Lemon grass extracts, sample MLAE2 and LGAE2 were also preferred with an overall acceptability of 7.00 and 6.45, respectively. Therefore, there should be adequate awareness creation on the importance of flavouring yoghurt with Roselle calyx to the general public and especially commercial producers. Quality evaluation should be carried out on sample RCAE2 with focus on water holding capacity, viscosity, syneresis rate and storage stability of the product and further research should be done on these herbal extracts to produce effervescent incorporation into yoghurt.

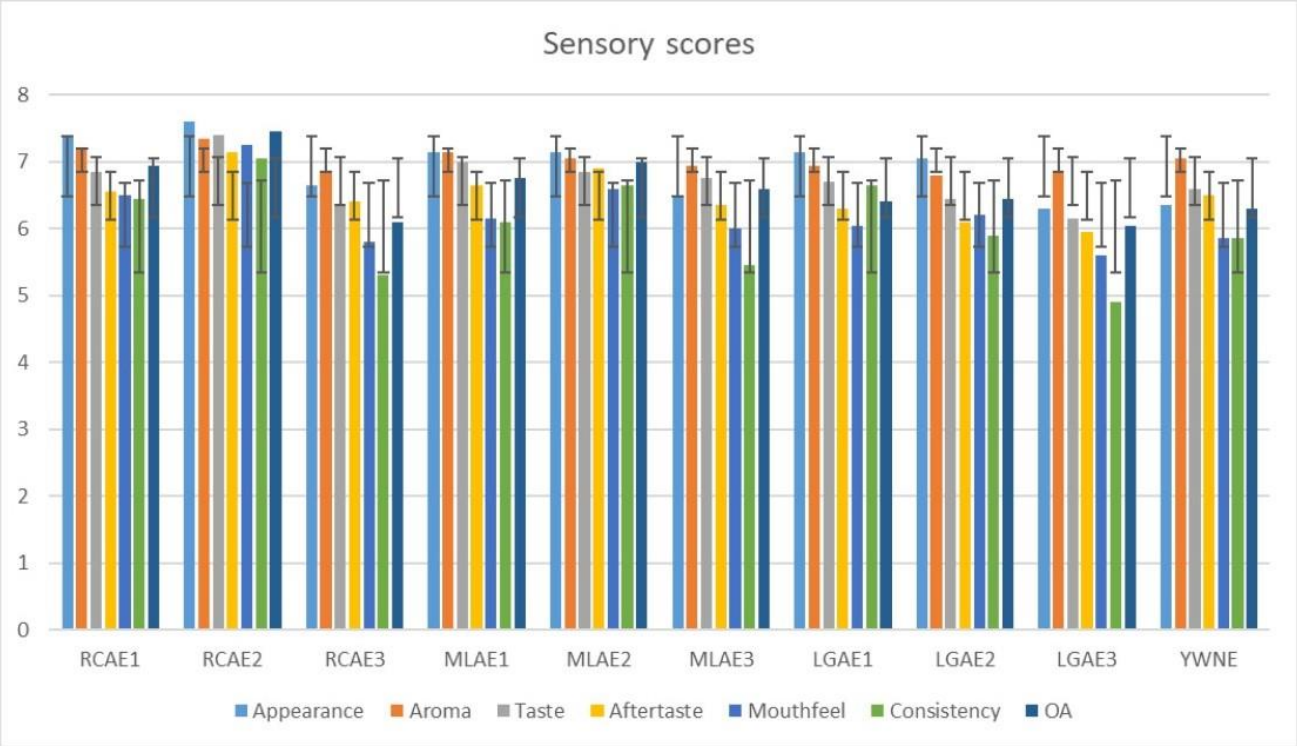



**Figure 6.** Graphical representation of the sensory scores of the formulated yoghurt samples.

## Authors' contributions

Conceptualization, M.I.E.; Methodology, M.I.E.; Software, M.I.E and N.J.C.; Validation, M.I.E.; Formal analysis, N.J.C.; Investigation, N.J.C.; Resources, M.I.E and N.J.C.; Data curation, N.J.C., Data preparation, M.I.E and N.J.C.; Writing original draft preparation, M.I.E and N.J.C.; Writing- review and editing, O.D.C.; Visualization, M.I.E and N.J.C.; Supervision, M.I.E.; Project administration, M.I.E and N.J.C.; Funding acquisition, N.J.C.

## Acknowledgements

We acknowledge the Department of Food Science and Technology, Faculty of Agriculture, University of Nigeria, Nsukka for their support in the actualization of this project.

## Funding

This research received no specific grant from any funding agency.

## Conflicts of interest

The authors declare no conflict of interest.

## Availability of data and materials

All data will be made available on request according to the journal policy.

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