PRODUCTION AND QUALITY EVALUATION OF YOGHURT ENRICHED WITH TWO ACCESSIONS OF PASSION FRUITS (Passiflora edulis flavicarpa)

Abstract
Aims: The broad objective of the research is to produce acceptable yoghurt flavoured with two accessions of passion fruit (pulp and skin) and evaluate its quality (physicochemical, phytochemical, microbiological, selected mineral and vitamin content).

Study Design: The experimental design that was used is Completely Randomized Design.

Place and Duration of Study: The study took place at the Department of Food Science and Technology, University of Nigeria, Nsukka between August 2016 and September 2017.

Methodology: Yoghurt is a dairy product obtained from lactic acid fermentation of milk. Yoghurt and two accessions of passion fruit juices (Passiflora edulis flavicarpa) were processed to formulate enriched yoghurt in the following ratios 90:10, 80:20, 70:30, 60:40 and 50:50. Yoghurt without the passion fruit juice (100:0) served as the control. Based on sensory evaluation, the best samples were subjected to physicochemical, phytochemical, microbiological, selected mineral and vitamin content evaluation using standard methods. The best enriched yoghurt samples were those in the ratio 90:10 and 80:20. Proximate composition of the enriched yoghurt samples significantly (p<0.05) differed with the controls.

Results: Enriched yoghurt showed an increase in the protein content (3.70, 3.52, 3.86, 3.93, 3.94, 3.52%) than control (2.81%). The control had higher fat content (3.43%) than enriched yoghurt (2.93, 3.12, 3.14, 2.78, 3.03 and 2.90%). The ash content ranges from 0.59-0.82% while addition of passion fruit juice caused pH to drop from 4.24 in the plain yoghurt to 4.18, 4.20 and 4.23 in the enriched yoghurt. The titratable acidity of yoghurt samples increased from 0.18 in the control to 0.72, 0.54, 0.52, 0.50 and 0.45 in enriched yoghurt. No direct relationship was observed between the pH and the titratable acidity. The total solid content ranges from 4.57-30.03. The phytochemicals were in trace amount. There was no significant (p>0.05) difference in the tannin content. The titratable acidity of yoghurt samples increased from 0.013 µg in the control to 0.015, 0.027, 0.028, 0.016, 0.020 and 0.024 in enriched yoghurt. Significant (p<0.05) difference in the phenolic content value was observed among all the samples. The phenolic content in the yoghurt samples ranges from 0.06 to 0.10 mg/g. The mineral content and vitamin content of the flavoured yoghurt samples significantly (p<0.05) differed with that of the control. An increase in the sodium content was observed in the samples from the control (168.24mg/100g) to the enriched sample (209.31, 202.66, 169.48 and 192.82 mg/100g). Similar increases were observed for potassium, calcium, magnesium and phosphorus content of the samples. Enriched yoghurt showed an increase in the vitamin C content (6.40, 7.53, 7.29, 7.15 and 7.13 mg/g) than in the control (5.10 mg/g). Vitamin A content ranged from 14.96 µg/100g - 20.67 µg/100g. The total viable count was higher in the control (5.67 × 10⁵ cfu/ml) than in enriched yoghurt. The mould count of the yoghurt samples varied from 0.33 × 10¹ cfu/ml - 1.58 × 10³ cfu/ml. All the sensory attributes tested in the flavoured yoghurt samples significantly (p<0.05) differed. Yoghurt enriched with local specie passion fruit juice from pulp, was the most accepted.

Keywords: Fermentation, Yoghurt, Passion fruit,
functions in addition to the traditional nutritional effects [3]. Yoghurt as a functional food is
grouped as probiotics, prebiotics and symbiotics. Probiotics are live bacteria and yeasts that are
good for the health, especially in the digestive system. Probiotics are often called "good" or
"helpful" bacteria because they help keep the gut healthy. Probiotics are found in supplements and
some foods, like yogurt. Prebiotics as “non-digestible food ingredient that beneficially affects the
host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in
the colon” [4]. Prebiotics are non-digestible carbohydrates that act as food for probiotics.
Symbiotic is a combination of probiotics and prebiotics that “beneficially affects the host by
improving the survival and the implantation of live microbial dietary supplements in the gastro-
intestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or
a limited number of health promoting bacteria” [5]. The two main types of yoghurt are set and
stirred yoghurt. Yoghurts are also available in many varieties including plain, flavoured, mixed
with fruit purees and whole or sliced fruit.

With increasing sensitization on consumption of healthy foods, and increasing campaign against
artificial flavours in beverages and drinks (which are associated with various carcinogens believed
to be cancer causing), there is a need to explore the use of natural fruit flavours in yoghurt
production which not only acts as a flavouring base but also significantly contributes valuable
nutrients [6]. A wide assortment of flavours, typically fruit flavours such as strawberry, pineapple
and mango among others could be used. In the processing of flavoured yoghurt, natural fruits
could be used. Nigerian Industrial Standard [7] defined flavored yoghurt as yoghurt to which has
been added flavoring food or other flavouring agents (like fruits). There are some tropical and
local underutilized fruits that can be utilized as flavors such as the passion fruits.

Passion fruit is native to tropical America and widely grown in Brazil [8]. In India it is found
to be growing wild in many parts of Western Ghat such as Nilgiris, Wynad, Kodaikanal,
Shevroys, Coorg and Malabar as well as Himachal Pradesh and North Eastern States like
Manipur, Nagaland and Mizoram [9]. Again, Kenya is one of the leading producers of passion
fruit in Africa. Other large producers world-wide include Hawaii, Brazil, Australia, Columbia,
Zimbabwe and South Africa [10, 11]. The yellow passion fruit is well suited to the ecology of
southern Nigeria, it is relatively unknown by farmers and hardly grown [12]. Passion fruit is not
available in Nigeria but it was recently introduced.
Passion fruit, like any other fruit, are susceptible to damage due to poor storage condition,
handling, pest attack, disease and deterioration. Also, they do not stay for a long period of time
hence the need to utilize them when they are in season.

The fruit is valued for its pronounced flavor and aroma which helps not only in producing
a high quality squash but also in flavouring several other products [13]. The most economical
importance of passion fruit is in the form of concentrated juice [14]. The juice of passion fruit
with an excellent flavour is quite delicious, nutritious and liked for its blending quality [15]. The
juice is extensively used in confectionery and preparation of cakes, pies and ice cream [13]. It can
also be used in jam and jelly production. It is a rich source of Vitamin A and contains fair
amounts of sodium, magnesium, sulphur and chlorides [15]. It is also rich in vitamin C, calcium
and phosphorus [16].

As an edible fruit, it contains several components such as acids and sugars and nutrients
that make passion fruit a tasteful and healthy addition to the diet [17]. Passion fruit is known for
its natural attractive colouring, unique flavor properties and medicinal purposes [13]. It is a very
nutritious fruits and should be grown in Nigeria as this would widen the food base of fruit used as
natural flavourant. Furthermore, commercial experience has also shown that flavouring of yoghurt
is an important additional sales prospect due to introduction of a wide variety of flavours and also adds on therapeutic properties of the product [6]. The demand for fruity yoghurt with different flavors is increasing [6]. Adding fruit juice to yoghurt decreases viscosity [18] (and increases some minerals such as magnesium, zinc, iron and copper [19].

The determination of the quality of this product would widen the food base of fruit, increase utilization of passion fruit and also increase the value addition. There are so many natural fruits used in flavouring yoghurt. Passion fruit has its unique properties. Yellow passion fruit consumed mainly as juice in many parts of the world, is a new crop in Nigeria [20]. It is exploited for its economic importance due to the presence of volatile compounds and a comparatively high acid content, which are responsible for its characteristic exotic flavor and aroma [21, 22, 23]. Information on the safety of passion fruit will further increase its acceptability. Again, the risk of post-harvest losses have given rise to alternative means of processing the fruit into valued products such as flavoring for yoghurt.

This study could also enhance the cultivation of passion fruit in Nigeria and provide employment. Passion fruit cropping offers a revenue earning opportunity for developing countries like Nigeria with an emerging economy [20]. Therefore, the research was aimed to produce acceptable yoghurt flavoured with two accessions of passion fruit (pulp and skin) and evaluate its quality.

2. MATERIALS AND METHODS

2.1 Raw materials
Yellow passion fruits (Passiflora edulis flavicarpa), the Kenyan and local specie were obtained from a garden in Department of Crop Science, University of Nigeria, Nsukka. The local specie was originally obtained from University of Agriculture, Abeokuta. Other ingredients for the yoghurt production were procured from Ogige main market, Nsukka, Enugu State, Nigeria.

2.2 Sample preparation

2.2.1 Processing of passion fruit juice
Passion fruit pulp and skin was processed using the method [24] and modified by the method [6]. The passion fruit was sorted. The fruits were rinsed in warm water, peeled and deseeded. The resulting pulp (400 g) was blended with 300 ml of sterile water and skin (200 g) was blended with 1000 ml of sterile water inside a blender (Kenwood, FP730, UK). The homogenized pulp and skin was filtered with a muslin cloth. The flow chart of passion fruit pulp and skin juice production in Figure 2

2.2.2 Production of yoghurt

Yoghurt was processed in accordance with the procedure [25]. The milk mix (400g of powdered milk to 1l of water) was pasteurized at 85 °C for 20 minutes to inactivate the pathogens in a water bath (Gallenkamp, model BKS - 350) and homogenized at pasteurization temperature. Subsequently, the milk was cooled to inoculation temperature of 43 ± 2 °C and then inoculated with 10 % yoghurt starter culture (yoghurmet) consisting of Lactobacillus bulgaricus, Streptococcus thermophilus and Lactobacillus acidophilus. The yoghurt was fermented for 12 hours at room temperature after which it was homogenized, smoothened and flavoured. The formulated flavoured yoghurt was chilled in a refrigerator, stored and presented for analysis. The flow diagram for the processing of flavoured yoghurt is as given in Figure 3.

2.2.3 Formulation of enriched yoghurt from passion fruit pulp and skin blends of the Kenyan and local specie.
Table 2 shows the proportions of the Kenyan and local species of the passion fruit (Passiflora edulis Flavicarpa) used in the formulation of flavoured yoghurt.
Passion Fruit

- Sorting/Washing
- Peeling
- Removal of seed
- Cutting of the skin (with knife)
- Addition of sterile water
- Blending (Kenwood, FP730, UK)
- Filtration
- Pasteurisation (71.5°C at 15 sec)
- Passion fruit juice (pulp)

**Figure 2:** Production of passion fruit juice (from pulp and skin) [24, 6]

Milk mix (powdered milk and water)
- Pasteurization (85°C at 20 minutes)
- Cooling
- Inoculation of starter culture (L. bulgaricus, S. thermophilus and L. acidophilus)
- Fermentation
- Homogenisation
- Flavouring
- Flavoured yoghurt

**Figure 3:** Modified Production of Flavoured Yoghurt [25]

**Table 2:** Formulation of enriched yoghurt from passion fruit pulp and skin blends for the Kenyan and local specie.

<table>
<thead>
<tr>
<th>Sample codes for Kenyan specie</th>
<th>Proportions (ml)</th>
<th>Sample codes for local specie</th>
<th>Proportions (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>kp1</td>
<td>90:10</td>
<td>lp1</td>
<td>90:10</td>
</tr>
</tbody>
</table>
kp2  80:20  lp2  80:20
kp3  70:30  lp3  70:30
kp4  60:40  lp4  60:40
kp5  50:50  lp5  50:50
ks1  90:10  ls1  90:10
ks2  80:20  ls2  80:20
ks3  70:30  ls3  70:30
ks4  60:40  ls4  60:40
ks5  50:50  ls5  50:50
NY (control)  100:0
ks= Kenyan Passion fruit skin; kp= Kenyan Passion fruit pulp; lp= Local passion fruit pulp; ls= Local passion fruit skin; NY = Unflavoured yoghurt

2.3 Analysis
Analysis was carried out on the flavoured yoghurt blends, the passion fruit pulp and skin (for Kenyan and local specie), unflavoured yoghurt and market yoghurt was used as control.

2.3.1 Analysis of the physico-chemical composition of flavoured yoghurt using passion fruit

2.3.1.1 pH determination
The pH was carried out using the method [26]. The pH meter was standardized using a buffer solution pH 4.0 and 7.0. Ten minutes was allowed for stabilization before the readings was taken. Ten percent (10 %) w/v suspension of the sample was prepared using distilled water. The mixture was mixed vigorously by shaking manually, their pH was measured with a functional pH meter (Extech instruments, model DO700, China).

2.3.1.2 Determination of total titrable acidity (TTA)
The total titrable acidity was determined by the method [27]. Ten milliliters of the sample was measured into a conical flask and about 3 drops of phenolphthalein indicator was added to the sample and titrated with 0.1 N sodium hydroxide (NaOH) until colour change was observed. The end point was taken and the TTA expressed as % lactic acid was calculated using the relationship:

\[
\% \text{ TTA as lactic acid} = \frac{n (\text{NaOH}) \times N(\text{NaOH}) \times 0.09 \times 100}{\text{Volume of sample}}
\]

Where; \( n = \text{volume of titre} \), \( N = \text{number of moles} \)

2.3.1.3 Determination of total solids
The total solid content of the samples was determined by drying 5ml of the sample to constant weight in a hot air oven (Gallenkamp) at 130 °C. The total solid content was obtained as percentage (%) total solids [27].

\[
\% \text{ Total solids} = \frac{\text{Weight of dried sample} \times 100}{\text{Weight of sample}}
\]

2.3.2. Proximate composition of passion fruit flavoured yoghurt

2.3.2.1 Moisture content
The moisture content of the samples was determined according to the standard method of Association of Official Analytical Chemists [27]. The crucible was washed and dried in the oven at 100 °C for 1 hour (\( W_1 \)). The hot dried crucible was cooled in the desiccators. The weight was taken when cooled. Two milliliters of the sample was weighed into the crucible (\( W_2 \)) and then placed inside the oven (zitalo Z0502P, Nigeria) at 100 °C for 4 hours. The crucible and contents
were removed, cooled in desiccators and weighed \((W_3)\). The drying continued until a constant weight is obtained. The percentage moisture content was calculated from weight loss of the sample. Thus:

\[
\% \text{ Moisture content} = \frac{W_2 - W_3 \times 100}{W_2 - W_1}
\]

Where; \(W_1\) = initial weight of empty crucible, \(W_2\) = weight of crucible + weight of sample before drying, \(W_3\) = weight of dish + weight of sample after drying.

### 2.3.2.2 Ash content

The ash content of the sample was determined according to the standard methods of Association of Official Analytical Chemists [27]. Two milliliter (2 ml) of the sample was weighed into a preheated cooled crucible \((W_2)\). The sample was charred on a bunsen flame inside a fume cupboard. The sample was transferred into a preheated muffle furnace at 550 ⁰C for 2 hours until a white or light grey ash was obtained \((W_3)\). It was cooled in a desiccator and weighed. The ash content was calculated mathematically as follows:

\[
\% \text{ Ash content} = \frac{W_3 - W_1 \times 100}{W_2 - W_1}
\]

Where: \(w_1\) = weight of empty crucible; \(w_2\) = weight of crucible + weight of sample before ashing; \(w_3\) = weight of crucible + weight of sample after ashing

### 2.3.2.3. Crude protein

The protein content of the flavoured yoghurt was determined according to the standard Kjeldahl method [27]. The sample (2 ml) was weighed into Kjeldahl flask. Anhydrous sodium sulphate (5 g or 4 tablet of Kjeldahl catalyst) was added to the flask. Twenty five milliliters (25 ml) of concentrated tetraoxosulphate (VI) acid \((H_2SO_4)\) was added with few boiling chips. The flask with the content was heated in the fume chamber until the solution become clear, cooled to room temperature, transferred into a 250 ml volumetric flask and made up to the level with distilled water. A 100 ml conical flask (receiving flask) containing 5 ml of 2 % boric acid solution with few drops of methyl red indicator was placed under the condenser. Then, 5 ml of the sample digest was pipetted into the apparatus through the small funnel and washed down with distilled water. Five milliliters of 60 % \(NaOH\) (sodium hydroxide) solution was added to the digest and heated until 100 ml of distillate \((ammonium sulphate)\) was collected in the receiving flask. The solution in receiving flask was titrated with 0.049 M \(H_2SO_4\) to a pink coloured end point. A blank with filter paper was subjected to the same procedure.

Calculation: \%

\[
\text{Nitrogen of sample} \ (\%N) = \frac{V_s - V_B \times N \text{ acid}}{W} \times 0.01401 \times 100
\]

Where: \(V_s\) = volume (ml) of acid required to titrate the sample; \(V_B\) = volume (ml) of acid required to titrate the blank; \(N\text { acid}\) = Normality of acid \((0.1N)\); \(W\) = weight of sample in gram

\%

\[
\text{crude protein} = \% N \times 6.25 \ (conversion \ factor).
\]

### 2.3.2.4 Fat

The fat content of the sample was determined using the standard method [26]. A Soxhlet extractor with a reflux condenser and a 500 ml round bottom flask was fixed. The extraction thimble was sealed with cotton wool. The Soxhlet apparatus after assembling was allowed to reflux for about 6 hour. The thimble was removed with care and petroleum ether (boiling point of
40-60 °C) collected in the top and drained into a container for reuse. When the flask was free of ether, it was removed and dried at 105 °C for 1 hour in an oven. It was cooled in a desiccator and then weighed.

Calculation

\[
\% \text{ Fat} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100
\]

2.3.2.5 Crude fibre

The crude fibre content of the sample was determined using the standard method [27]. Petroleum ether (boiling point of 40-60 °C) was used to defat 2 ml of sample. This was put in boiled 200 ml of 1.25 % H₂SO₄ and boiled for 30 minutes. The solution was filtered through linen or muslin cloth on a fluted funnel. It was washed with boiling water until it is free from acid. The residue was returned into 200 ml boiling NaOH and allowed for 30 minutes. It was further washed with 1 % HCl, boiling water, to free it of acid. The final residue was drained and transferred to silica ash crucible (porcelain crucible), dried in oven at 100 °C for 2 hours and cooled, until a constant weight is obtained. The cooled sample was incinerated or washed in a muffle furnace at 600 °C for 5 hours, cooled in a desiccator and weighed.

Calculation;

\[
\% \text{ crude fiber} = \frac{\text{Loss of weight after ignition}}{\text{Weight of original sample}} \times 100
\]

2.3.2.6 Carbohydrate

Using the standard methods [27], carbohydrate content of the samples was determined by difference as follows:

\[
\% \text{ Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ protein} + \% \text{ fat} + \% \text{ crude fibre} + \% \text{ ash})
\]

2.3.3. Determination of phytochemical content of flavoured yoghurt using passion fruit

2.3.3.1 Tannin

The Folin-Denis spectrophotometric method was used [28]. A measured weight of each sample (1 ml) was dispersed in 10 ml distilled water and agitated. This was left to stand for 30 minutes at room temperature, being shaken every 5 minutes. At the end of 30 minutes, it was centrifuged and the extract gotten. 2.5 ml of the supernatant was dispersed into a 50 ml volumetric flask. Similarly, 2.5 ml of standard tannic acid solution was dispersed into a separate 50 ml flask. Then, 1 ml Folin-Denis reagent was measured into each flask, followed by 2.5 ml of saturated Na₂CO₃ solution. The mixture was diluted to mark in the flask (50 ml), and incubated for 90 minutes at room temperature. The absorbance was measured at 250 nm. Readings were taken with the reagent blank at zero. The tannin content was given as follows:

\[
\% \text{ tannin} = \frac{\text{An}}{\text{As}} \times \frac{\text{C}}{\text{W}} \times \frac{100}{\text{Vf/Va}}
\]

Where, An = Absorbance of test sample; As = Absorbance of standard solution; C = Concentration of standard solution; W = Weight of sample used, Vf = Total volume of extract; Va = Volume of extract analyzed.
2.3.3.2 Determination of total phenolic content

Total phenolic content (TPC) was measured spectrophotometrically based on a method [29]. Firstly, 1 ml of sample extract was mixed with 4 ml of Folin-Ciocalteu reagent (previously diluted 10 times) and the mixture was allowed to stand for 3 minutes at room temperature. Then, 5 ml of 7.5% sodium carbonate solution was added to the mixture, vortexed vigorously and kept at room temperature in dark for 30 minutes. The absorbance was measured at 765 nm using a PRIM Light spectrophotometer (Secomam, Cedex, France) against a blank (distilled water). The standard curve of gallic acid was $y = 0.0165x + 0.0003$ ($R^2 = 0.9972$). Total phenolic content was expressed as $\mu g$ gallic acid equivalent (GAE) per gram sample (fresh weight).

2.3.4. Determination of micronutrients of formulated flavoured yoghurt

2.3.4.1 Determination of calcium, sodium, potassium and magnesium

Micronutrients (calcium, sodium, potassium and magnesium) were determined using method [30]. Two millilitres (2 ml) of the sample was weighed and subjected to dry ashing for five (5) hours in well-cleaned porcelain crucibles at 550 °C. The resultant ash was dissolved in 5 ml of HNO$_3$/HCl/H$_2$O (1:2:3) and heated gently on a hot plate until brown fumes disappeared, remaining the material in each crucible. Five (5 ml) of deionized H$_2$O was added and heated until a colourless solution was obtained. The solution on each crucible was filtered into 100 ml volumetric flask and the volume made up to 100 ml with deionised water. The solution was then used to analyse for calcium, sodium, potassium and magnesium using an atomic absorption spectrophotometer. The results were expressed as mg / 100 ml.

2.3.4.2 Determination of phosphorus content

Preparation of standard solution

Phosphorus was determined using method [31] with slight modification [26]. Then, 1.1224 g of K$_2$HPO$_4$ (potassium phosphate) was dissolved in 500 ml of water and transferred to one litre volumetric flask. 8 ml of concentrated HCl is added and diluted to one litre with water.

For working standard solution: Stock standard (25 ml) was diluted to 100 ml with 10% trichloroacetic acid and 0.005 mg/ml, 0.10 mg/ml, 0.15 mg/ml and 0.20 mg/ml are prepared from this working solution.

Sample preparation

To a 16 X125 mm test-tube of the mineral digest and 9.5 ml of 10% trichloroacetic acid was added. The mixture was agitated to mix, centrifuged for 5 minutes and then filtered through 7 cm filter paper. Five millilitres of the filtrate was measured into 19 mm cuvet. Five millilitres of the filtered trichloroacetic acid and five millilitres of the working standard was measured into two cuvets as a blank and standard respectively. These were treated the same way as the sample filtrate. To each tube, 0.5 ml of molybdate reagent was added and mixed. Sulphuric acid reagent (0.2 ml) was added. The contents was stoppered, mixed and allowed to stand for 10 minutes. The absorbance of the test and standard was read in a spectrophotometry at 660 nm with the blank set at zero.

$$\text{Absorbance of test} \times \text{concentration of standard (5mg/ml)} = P \text{ (mg/ml)}$$

Absorbance of standard

2.3.4.3 Determination of Vitamin C content

The 2,6 dichlorophenol titrimetric method) was adopted [27]. Two millilitres (2 ml) of the sample was extracted by homogenizing sample in acetic acid solution.

Procedure
The standard solution was prepared by dissolving 50 mg standard ascorbic acid tablet in 100 ml in a volumetric flask with water. The solution was filtered to get clear solution. A 10 ml of the filtrate was added into a flask in which 2.5 ml acetone has been added. This was titrated with indophensol solution (dye 2, 6, dichlorophenol indophenols) to a faint pink colour which persists for 115 seconds. The standard was treated identically.

**Calculation**

\[ \text{mg ascorbic acid } 1 \text{ g} = C \times V \times \frac{DF}{WT} \]

Where; \( C = \text{mg ascorbic acid } 1 \text{ ml dye} \); \( V = \text{Volume of dye used for titrate of diluted sample} \); \( DF = \text{Dilution factor} \); \( WT = \text{Weight of sample in ml} \)

### 2.3.4.4 Determination of pro-vitamin A

Pro-vitamin A was determined using standard method [27]. Five milliliters (5 ml) of the sample was pipetted in duplicate into a glass stoppered test tube and equal volume of ethanol was added drop wise with mixing to give 50 % solution (v/v). At this concentration, the protein precipitated and free from retinol and retinyl esters was extracted by addition of 3 ml hexane. The tube was stoppered and the content mixed rigorously on the vortex for 2 minutes to ensure complete extraction of carotene for 5 – 10 minutes at 600 – 1000 g to obtain a clean separation of phases. Then, 2 mg/ml of the upper hexane extract was pipetted. Absorbance due to carotenoids at 450 nm was used against a hexane blank (\( A_{450} \)). A standard curve was plotted from the \( A_{620} \) values on ordinary rectangular coordinate paper, where the ordinate was at the \( A_{620} \) values and the abscissa was the \( \mu g \) vitamin A/tube and a factor (\( FA_{620} \)) calculated as below.

\[ FA_{620} = \frac{\mu g \text{ vitamin A/tube}}{A_{620}} \]

Pro-vitamin A was calculated using the formula: Total carotenoid (as lycopene/dl) = \( A_{620} \times Fc_{450} \times 150 \)

Where, \( Fc_{450} = \text{constant determined on the laboratory} \), 150 = dilution factor

Likewise, pro-vitamin A (as \( \mu g \) retinol/dl) was calculated:

\[ (\text{as } \mu g \text{ retinol/dl}) \left[ A_{620} - \frac{2 \times A_{450} \times FC_{450}}{FC_{620}} \right] \times FA_{620} \times 75 \]

### 2.3.5 Microbial analysis of formulated enriched yoghurt

This analysis was carried out on the sample using the pour plate method [32].

#### 2.3.5.1 Determination of total viable count

The fermenting slurry (1 ml) was dissolved into 9 ml of Ringer’s solution in a test tube and mixed thoroughly by shaking. This was a 10\(^{-1}\) dilution; one millilitre (1 ml) of the mixture was pipetted into another 9 ml of Ringer’s solution to give 10\(^{-2}\), 10\(^{-3}\), 10\(^{-4}\), 10\(^{-5}\) and 10\(^{-6}\) dilution. 1 ml aliquot from different dilutions (10\(^{-3}\) and 10\(^{-4}\)) was used to check the total viable count per ml on nutrient agar media. The Petri dishes were made in triplicate for each sample and in each plate, 15 ml of sterile nutrient agar medium was added and 1 ml of each sample dilution was pipette into each medium containing plate respectively. This was followed by shaking and rocking in a circular movement for about 10 seconds to uniform homogenisation. The plates were allowed to set and
were incubated (inverted) for 24 - 48 hours at 37 °C. The colonies formed were counted and recorded as colony forming units (cfu).

\[ \text{No of colonies (cfu/ml)} = \text{average count} \times \text{dilution factor (Df)} \]

### 2.3.5.2 Determination of mould count

This was determined using the method described using potato dextrose agar (PDA) as the nutrient medium [33]. Ringer’s solution was prepared by dissolving a tablet of quarter strength Ringer’s tablet in 500 ml of distilled water and autoclaved at 121 °C for 15 minutes at 15 psi. Then, 2 ml of the sample was ground and put into serial dilution bottles which had been previously autoclaved and shaken for 2 minutes. Following this, 1 ml of the appropriate diluent was pipetted into the sterilised Petri dish and potato dextrose agar was used for plating and the set up left in an incubator for 72 hours. The count was determined and expressed as colony forming units per gram (cfu/ml) of the sample.

### 2.3.5.3 Determination of lactic acid bacteria using deManRogosa Sharpe (MRS) agar

The lactic acid bacteria (LAB) in the formulated yoghurt were determined using deMan Rogosa Sharpe (MRS) Agar (CM 361) as described by Oxoid Manual [34]. Samples were serially diluted in triplicate and inoculated 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 and represented as colony forming unit per milliliter (cfu/ml).

\[ \text{Cfu/ml} = \text{average count} \times \text{dilution factor (D.F)} \]

### 2.3.6 Sensory evaluation of the formulated enriched yoghurt blended with passion fruit blends

Sensory properties of the samples were evaluated by 20 semi-trained panelists consisting of students of University of Nigeria, Nsukka for various sensory attributes (colour, taste, flavour, mouthfeel, consistency, aftertaste and overall acceptability). The extent of differences between the yoghurt samples for each sensory quality was measured on a nine- point Hedonic scale, (where “9” represents extremely like and “1” represents extremely dislike [35].

### 2.3.7 Data analysis and experimental design of the formulated flavored yoghurt

The data generated was subjected to a one-way analysis of variance (ANOVA) under split-plot in completely randomized design using Statistical product for service solution (SPSS) version 20.0 computer programme. Mean separation was by the Duncan’s new multiple range test. Significant difference was accepted at \( p < 0.05 \) [36].

## 3. RESULTS AND DISCUSSION

### 3.1 Passion fruit enriched yoghurts
Plate 9 shows the passion fruit juices (from skin and pulp), plain yoghurt and formulated yoghurt enriched with passion fruit (pulp and skin).
<table>
<thead>
<tr>
<th>Samples</th>
<th>Colour</th>
<th>Flavour</th>
<th>Taste</th>
<th>Aftertaste</th>
<th>Mouthfeel</th>
<th>Overall Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>kp1</td>
<td>8.15&lt;sup&gt;ghi&lt;/sup&gt; ± 0.88</td>
<td>6.05&lt;sup&gt;abcd&lt;/sup&gt; ± 1.67</td>
<td>5.95&lt;sup&gt;bcde&lt;/sup&gt; ± 1.54</td>
<td>5.75&lt;sup&gt;bcde&lt;/sup&gt; ± 1.92</td>
<td>6.40&lt;sup&gt;fghi&lt;/sup&gt; ± 1.73</td>
<td>6.55&lt;sup&gt;def&lt;/sup&gt; ± 1.40</td>
</tr>
<tr>
<td>kp2</td>
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<td>7.05&lt;sup&gt;fgi&lt;/sup&gt; ± 1.15</td>
<td>6.60&lt;sup&gt;defg&lt;/sup&gt; ± 1.35</td>
<td>6.60&lt;sup&gt;efghi&lt;/sup&gt; ± 1.35</td>
<td>7.30&lt;sup&gt;ijkl&lt;/sup&gt; ± 1.22</td>
<td>7.50&lt;sup&gt;fgijh&lt;/sup&gt; ± 1.15</td>
</tr>
<tr>
<td>kp3</td>
<td>7.85&lt;sup&gt;fgi&lt;/sup&gt; ± 0.99</td>
<td>7.00&lt;sup&gt;fgi&lt;/sup&gt; ± 1.12</td>
<td>6.70&lt;sup&gt;efghi&lt;/sup&gt; ± 1.33</td>
<td>6.65&lt;sup&gt;efghi&lt;/sup&gt; ± 1.27</td>
<td>6.95&lt;sup&gt;ghij&lt;/sup&gt; ± 1.32</td>
<td>6.90&lt;sup&gt;defg&lt;/sup&gt; ± 1.21</td>
</tr>
<tr>
<td>kp4</td>
<td>7.90&lt;sup&gt;fgi&lt;/sup&gt; ± 1.12</td>
<td>6.40&lt;sup&gt;cdefg&lt;/sup&gt; ± 1.31</td>
<td>6.00&lt;sup&gt;bcd&lt;/sup&gt; ± 1.56</td>
<td>5.65&lt;sup&gt;bcde&lt;/sup&gt; ± 1.84</td>
<td>6.15&lt;sup&gt;cdefg&lt;/sup&gt; ± 0.93</td>
<td>6.55&lt;sup&gt;def&lt;/sup&gt; ± 1.10</td>
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<td>kp5</td>
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<td>5.30&lt;sup&gt;ab&lt;/sup&gt; ± 1.84</td>
<td>4.90&lt;sup&gt;ab&lt;/sup&gt; ± 2.15</td>
<td>4.90&lt;sup&gt;ab&lt;/sup&gt; ± 1.80</td>
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<td>5.35&lt;sup&gt;abc&lt;/sup&gt; ± 1.84</td>
</tr>
<tr>
<td>lp1</td>
<td>8.40&lt;sup&gt;hi&lt;/sup&gt; ± 0.82</td>
<td>8.05&lt;sup&gt;jk&lt;/sup&gt; ± 1.28</td>
<td>7.60&lt;sup&gt;ij&lt;/sup&gt; ± 1.65</td>
<td>7.65&lt;sup&gt;ij&lt;/sup&gt; ± 1.81</td>
<td>7.80&lt;sup&gt;ijkl&lt;/sup&gt; ± 1.67</td>
<td>8.00&lt;sup&gt;ijkl&lt;/sup&gt; ± 1.38</td>
</tr>
<tr>
<td>lp2</td>
<td>7.30&lt;sup&gt;defg&lt;/sup&gt; ± 1.59</td>
<td>7.00&lt;sup&gt;fgi&lt;/sup&gt; ± 1.49</td>
<td>7.25&lt;sup&gt;ghij&lt;/sup&gt; ± 1.62</td>
<td>6.95&lt;sup&gt;fgijh&lt;/sup&gt; ± 1.57</td>
<td>7.20&lt;sup&gt;ijkl&lt;/sup&gt; ± 1.54</td>
<td>7.20&lt;sup&gt;efghi&lt;/sup&gt; ± 1.43</td>
</tr>
<tr>
<td>lp3</td>
<td>6.45&lt;sup&gt;bcd&lt;/sup&gt; ± 1.67</td>
<td>6.10&lt;sup&gt;bcd&lt;/sup&gt; ± 1.55</td>
<td>5.95&lt;sup&gt;bcd&lt;/sup&gt; ± 1.57</td>
<td>5.25&lt;sup&gt;bcd&lt;/sup&gt; ± 1.48</td>
<td>5.95&lt;sup&gt;bcddefg&lt;/sup&gt; ± 1.57</td>
<td>6.45&lt;sup&gt;de&lt;/sup&gt; ± 1.39</td>
</tr>
<tr>
<td>lp4</td>
<td>6.00&lt;sup&gt;b&lt;/sup&gt; ± 1.94</td>
<td>5.65&lt;sup&gt;bcd&lt;/sup&gt; ± 1.42</td>
<td>5.60&lt;sup&gt;bcd&lt;/sup&gt; ± 1.42</td>
<td>5.10&lt;sup&gt;b&lt;/sup&gt; ± 1.37</td>
<td>5.35&lt;sup&gt;bcd&lt;/sup&gt; ± 1.14</td>
<td>5.30&lt;sup&gt;b&lt;/sup&gt; ± 1.13</td>
</tr>
<tr>
<td>lp5</td>
<td>5.10&lt;sup&gt;a&lt;/sup&gt; ± 2.13</td>
<td>5.65&lt;sup&gt;bcd&lt;/sup&gt; ± 1.35</td>
<td>5.10&lt;sup&gt;b&lt;/sup&gt; ± 1.65</td>
<td>5.00&lt;sup&gt;b&lt;/sup&gt; ± 1.65</td>
<td>4.90&lt;sup&gt;ab&lt;/sup&gt; ± 1.86</td>
<td>5.10&lt;sup&gt;ab&lt;/sup&gt; ± 1.62</td>
</tr>
<tr>
<td>ks1</td>
<td>8.40&lt;sup&gt;ghi&lt;/sup&gt; ± 0.68</td>
<td>7.20&lt;sup&gt;ghi&lt;/sup&gt; ± 0.83</td>
<td>6.90&lt;sup&gt;cdefg&lt;/sup&gt; ± 1.68</td>
<td>6.75&lt;sup&gt;cdefg&lt;/sup&gt; ± 1.02</td>
<td>6.95&lt;sup&gt;ghij&lt;/sup&gt; ± 1.23</td>
<td>7.10&lt;sup&gt;efghi&lt;/sup&gt; ± 1.07</td>
</tr>
<tr>
<td>ks2</td>
<td>7.00&lt;sup&gt;cdef&lt;/sup&gt; ± 1.07</td>
<td>6.40&lt;sup&gt;cdefg&lt;/sup&gt; ± 1.10</td>
<td>5.75&lt;sup&gt;bcd&lt;/sup&gt; ± 1.40</td>
<td>5.80&lt;sup&gt;bcd&lt;/sup&gt; ± 1.36</td>
<td>6.05&lt;sup&gt;cdefg&lt;/sup&gt; ± 1.47</td>
<td>6.30&lt;sup&gt;cde&lt;/sup&gt; ± 1.26</td>
</tr>
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<td>ks3</td>
<td>6.70&lt;sup&gt;bcd&lt;/sup&gt; ± 0.98</td>
<td>5.00&lt;sup&gt;a&lt;/sup&gt; ± 1.72</td>
<td>4.45&lt;sup&gt;a&lt;/sup&gt; ± 1.88</td>
<td>4.65&lt;sup&gt;a&lt;/sup&gt; ± 1.79</td>
<td>4.50&lt;sup&gt;a&lt;/sup&gt; ± 1.99</td>
<td>4.65&lt;sup&gt;a&lt;/sup&gt; ± 1.79</td>
</tr>
<tr>
<td>ks4</td>
<td>6.70&lt;sup&gt;bcd&lt;/sup&gt; ± 1.53</td>
<td>5.47&lt;sup&gt;abc&lt;/sup&gt; ± 1.84</td>
<td>5.41&lt;sup&gt;abc&lt;/sup&gt; ± 2.06</td>
<td>5.11&lt;sup&gt;abc&lt;/sup&gt; ± 1.69</td>
<td>5.29&lt;sup&gt;bcd&lt;/sup&gt; ± 1.72</td>
<td>5.47&lt;sup&gt;abc&lt;/sup&gt; ± 1.70</td>
</tr>
<tr>
<td>ks5</td>
<td>7.30&lt;sup&gt;cdefg&lt;/sup&gt; ± 1.84</td>
<td>6.50&lt;sup&gt;cdefg&lt;/sup&gt; ± 1.56</td>
<td>6.34&lt;sup&gt;cdefg&lt;/sup&gt; ± 1.77</td>
<td>6.34&lt;sup&gt;cdefg&lt;/sup&gt; ± 1.77</td>
<td>6.52&lt;sup&gt;fgijh&lt;/sup&gt; ± 1.65</td>
<td>6.69&lt;sup&gt;cdefg&lt;/sup&gt; ± 1.06</td>
</tr>
<tr>
<td>ls1</td>
<td>8.75&lt;sup&gt;hi&lt;/sup&gt; ± 0.55</td>
<td>8.20&lt;sup&gt;k&lt;/sup&gt; ± 0.89</td>
<td>8.30&lt;sup&gt;j&lt;/sup&gt; ± 0.86</td>
<td>8.00&lt;sup&gt;j&lt;/sup&gt; ± 0.79</td>
<td>8.15&lt;sup&gt;j&lt;/sup&gt; ± 0.81</td>
<td>8.45&lt;sup&gt;j&lt;/sup&gt; ± 0.76</td>
</tr>
<tr>
<td>ls2</td>
<td>8.35&lt;sup&gt;hi&lt;/sup&gt; ± 0.88</td>
<td>7.50&lt;sup&gt;hi&lt;/sup&gt; ± 1.05</td>
<td>7.55&lt;sup&gt;ghi&lt;/sup&gt; ± 1.19</td>
<td>7.35&lt;sup&gt;hi&lt;/sup&gt; ± 1.31</td>
<td>7.55&lt;sup&gt;ijkl&lt;/sup&gt; ± 1.10</td>
<td>7.75&lt;sup&gt;ghi&lt;/sup&gt; ± 1.21</td>
</tr>
<tr>
<td>ls3</td>
<td>7.70&lt;sup&gt;fgi&lt;/sup&gt; ± 1.45</td>
<td>6.75&lt;sup&gt;cdefg&lt;/sup&gt; ± 1.71</td>
<td>6.40&lt;sup&gt;cdefg&lt;/sup&gt; ± 1.60</td>
<td>6.25&lt;sup&gt;cdefg&lt;/sup&gt; ± 1.45</td>
<td>6.60&lt;sup&gt;ghi&lt;/sup&gt; ± 1.31</td>
<td>6.65&lt;sup&gt;cdef&lt;/sup&gt; ± 1.35</td>
</tr>
<tr>
<td>ls4</td>
<td>6.65&lt;sup&gt;bcd&lt;/sup&gt; ± 1.42</td>
<td>5.80&lt;sup&gt;bcd&lt;/sup&gt; ± 1.54</td>
<td>5.70&lt;sup&gt;bcd&lt;/sup&gt; ± 1.75</td>
<td>6.00&lt;sup&gt;bcd&lt;/sup&gt; ± 1.62</td>
<td>5.45&lt;sup&gt;bcd&lt;/sup&gt; ± 1.50</td>
<td>5.95&lt;sup&gt;bcd&lt;/sup&gt; ± 1.50</td>
</tr>
<tr>
<td>ls5</td>
<td>6.25&lt;sup&gt;bc&lt;/sup&gt; ± 1.33</td>
<td>5.10&lt;sup&gt;ab&lt;/sup&gt; ± 1.89</td>
<td>4.95&lt;sup&gt;ab&lt;/sup&gt; ± 1.47</td>
<td>5.35&lt;sup&gt;bcd&lt;/sup&gt; ± 1.79</td>
<td>4.75&lt;sup&gt;a&lt;/sup&gt; ± 1.59</td>
<td>5.20&lt;sup&gt;ab&lt;/sup&gt; ± 1.61</td>
</tr>
<tr>
<td>NY</td>
<td>8.00&lt;sup&gt;ghi&lt;/sup&gt; ± 0.97</td>
<td>7.65&lt;sup&gt;ijk&lt;/sup&gt; ± 1.04</td>
<td>7.60&lt;sup&gt;ghi&lt;/sup&gt; ± 1.27</td>
<td>7.10&lt;sup&gt;ghi&lt;/sup&gt; ± 2.15</td>
<td>7.60&lt;sup&gt;ijkl&lt;/sup&gt; ± 2.32</td>
<td>7.70&lt;sup&gt;ghi&lt;/sup&gt; ± 1.84</td>
</tr>
</tbody>
</table>

Means ± standard deviation (n = 22); Means within a column with the same superscript are not significantly (p > 0.05) different.

Samples were evaluated on a 9-point Hedonic scale (1 = dislike extremely and 9 = like extremely).

**Key:**
- kp1 = Pulp Kenya 90; kp2 = Pulp Kenya 80; kp3 = Pulp Kenya 70; kp4 = Pulp Kenya 60; kp5 = Pulp Kenya 50; lp1 = Pulp Local 90; lp2 = Pulp Local 80; lp3 = Pulp Local 70; lp4 = Pulp Local 60; lp5 = Pulp Local 50; ks1 = Skin Kenya 90; ks2 = Skin Kenya 80; ks3 = Skin Kenya 70; ks4 = Skin Kenya 60; ks5 = Skin Kenya 50; ls1 = Skin Local 90; ls2 = Skin Local 80; ls3 = Skin Local 70; ls4 = Skin Local 60; ls5 = Skin Local 50; NY = Unflavoured yoghurt

**Table 3:** Sensory scores of the formulated yoghurt enriched with passion fruit juices (pulp and skin)
3.2 Sensory scores of formulated yoghurt enriched with passion fruit.

The sensory scores for the formulated yoghurt enriched with passion fruit juices (skin and pulp) are shown in Table 3.

Table 3 shows the mean sensory scores of the enriched yoghurt and the controls for colour, flavour, taste, aftertaste, mouthfeel and overall acceptability. The samples containing 10 – 20 % passion fruit had more acceptable colour and there was a decrease in the level of acceptance as the percentage of passion fruit juice increased. There was no significant (p > 0.05) difference in the colour of samples NY (unflavoured yoghurt = 8.00), kp1 (8.15), kp2 (8.25), lp1 (8.4), ks1 (8.40), ls1 (8.75) and ls2 (8.35). The samples mentioned were enriched with 10- 20 % passion fruit and had higher sensory score. This agreed with result obtained by other researchers [37, 38].

The flavor of the yoghurt followed the same trend as the colour. There was a reduction in the acceptance of the enriched yoghurt as the percentage of passion fruit juice added increased. Samples lp1 (8.05), ks1 (7.20), ls1 (8.20) and ls2 (7.50) compares well with the control (NY = 7.65) and there is no significant (p > 0.05) difference between them.

There was a reduction in the acceptance of the taste and aftertaste of the flavor yoghurt as the percentage of passion fruit added increased. Samples lp1 (8.00 and 7.65), lp2 (7.25 and 6.95), ls1 (8.30 and 8.00) and ls2 (7.55 and 7.35) compared favourably with the control (NY) and they are the most acceptable samples for taste and aftertaste.

The mouthfeel of samples kp2 (7.30), lp1 (7.80), lp2 (7.20), ls1 (8.15) and ls2 (7.55) compared favourably with the control (NY = 7.60). Just as in other attributes, there was a decrease in the acceptability of the mouthfeel as the percentage of passion fruit juice added increased. This agreed with result obtained by other researchers [37].

The overall acceptability of samples kp2 (7.50), lp1 (8.00), ls1 (8.45) and ls2 (7.75) compares well with the control (NY = 7.70). Samples enriched with 10 – 20 % of passion fruit juice were most accepted. This was the basis for the selection of enriched yoghurt that underwent further analysis. Meanwhile, Sample ls1 (%) (colour = 8.75, flavor = 8.20, taste = 8.30, aftertaste = 8.00, mouthfeel = 8.15 and overall acceptability = 8.45) and Sample lp1 (colour = 8.40, flavor = 8.05, taste = 8.00, aftertaste = 7.65, mouthfeel = 7.80 and overall acceptability = 8.00) had the highest scores in all the attributes. Generally, the mean sensory scores for the whole samples compared favourably with the control (NY) in taste, colour, flavor, aftertaste, mouthfeel and overall acceptability and there were significant (p< 0.05) differences in the evaluated attributes.

From Table 3, colour, flavour, taste, aftertaste mouthfeel and general acceptability decreased with increase in the proportion of passion fruit juice. This trend was also observed in the work on yoghurt flavoured with beetroot (Beta vulgaris l.) where the sample that had 90 ml yoghurt and 10 ml beetroot had the highest score (colour = 7.65, flavour = 6.50, taste = 7.15, aftertaste = 5.65, mouthfeel = 6.75, general acceptability = 7.60) and the sample that contained 50 ml yoghurt and 50 ml beetroot had the least score (colour = 5.25, flavour = 5.15, Taste = 4.65, mouthfeel = 5.30, overall acceptability = 6.16) [37]. Also, in the work on yoghurt flavoured with solar-dried bush mango (Irvingia gabonensis) pulp where the sample that was flavoured with 0.80% dried bush mango had the highest score (colour = 6.90, flavour = 7.30, aftertaste = 6.75, mouthfeel = 6.45 and overall acceptability = 6.75) and sample flavoured with 4.80% dried bush mango had the highest score (colour = 4.20, flavour = 4.30, aftertaste = 4.25, mouthfeel = 3.70 and overall acceptability = 4.00) [40]. However, there was no significant (p > 0.05) difference in colour of plain yoghurt (NY = 8.00) and samples (both the Kenyan and local, skin and pulp) containing 10 % passion fruit juice (kp1 = 8.15, lp1 = 8.40, ks1 = 8.40, ls1 = 8.75). The result obtained on yoghurt flavoured with fresh and dried cashew (Anacardium occidentale) apple pulp
observed the same trend in the colour of the flavoured (8.20) and unflavoured (6.95) yoghurt [41].

### 3.3 Nutritional Composition of passion fruit juices (From skin and pulp)

Table 4 shows the nutritional composition of local and Kenyan specie of passion fruit passion fruit juices (From skin and pulp). There was significant (p<0.05) difference in the pH of the fruit juice samples. The result obtained corresponds with the assertion (pH = 3.2) [10]. It also corresponds with the result (3.11, 3.09) obtained in work done on passion fruit pulp [20]. Low pH is observed in passion fruit thereby making it a high acid food. Passion fruit is a high acid food due to the predominance of two acids, citric acid (93 – 96 % of total) and malic acid (3- 6 % of total) [10]. No significant (p<0.05) difference was observed in the titratable acidity among the passion fruit samples.

Other researchers reported 0.63 – 0.81 as titratable acidity values for passion fruit which is higher than the value obtained in this work [16]. The variation in the results could be as a result of the dilution of passion fruit juice samples used in this work. Again, the passion fruits may have been on different ripening stage and this may have affected the titratable acidity. Total soluble solids content for passion fruit (Table 4) are samples lp (79.18 %) and kp (80.73 %) for the passion fruit juice (pulp) and ls (96.80 %) and ks (95.48 %) for passion fruit juice (skin). The value for the passion pulp agreed with the reports [10].

There was significant (p<0.05) difference in the protein content of the fruit juice samples. Other researchers reported protein value of 0.6 – 2.8 for passion fruit juice and these values corresponds with the values in this work [42]. The protein content of passion fruit could be as a result of the manure applied [20]. Reports [43, 44] showed potassium concentration in manure activates biochemical processes in plant particularly its ability to make protein. The passion fruit pulp juice had carbohydrate content of 14.01 and 15.98 % (samples lp and kp respectively) while the passion fruit skin juice had values of 9.01 and 7.05 % (samples ls and ks, respectively). The Carbohydrate content could be as a result of citric acid in passion fruit [45]. The authors further stated that citric acid has an important role in the metabolism of carbohydrate and higher acidity may therefore be a precursor for high sugar (carbohydrate) in the juice.

The concentration of tannin in the passion fruit juice samples as seen in table 4 was in trace amount. Samples (lp = 0.021, kp = 0.011, ls = 0.008, ks = 0.004) samples was negligible. This result is slightly lower than the result (0.070 mg/100g) obtained on passion fruit juice (pulp) [20]. The work on passion fruit (skin) had a higher amount of 0.17 of tannin which is a negligible amount [46]. The phenolic content in the passion fruit samples were lp = 0.02, kp = 0.03, ls = 0.01, ks = 0.01. The researchers obtained a phenolic content value of 4.20 which is higher than the result of Table 4 [46]. The lower value of phenolic content could probably be due to leaching and dilution effect of the phytochemical into the medium (water).

### 3.4 Physicochemical composition of formulated yoghurt enriched with Two Accessions of passion fruit

Table 5 shows the physicochemical composition of enriched yoghurt using passion fruit juices (skin and pulp). There was significant (p<0.05) difference in the pH value between the enriched samples and control. No significant (p>0.05) difference was observed between samples (lp1 = 4.23, lp2 = 4.18, kp2 = 4.20) enriched with passion fruit pulp (both for Kenyan and local specie). This trend was also observed between the samples (ls2 = 4.34, ks1 = 4.37) enriched with passion fruit skin (both for Kenyan and local specie). The control NY (4.24) had a higher pH value than yoghurt enriched with passion fruit pulp (lp1 = 4.23, lp2 = 4.18, kp2 = 4.20) and lower pH value.
Table 4: Nutritional Composition of passion fruit juices (skin and pulp)

<table>
<thead>
<tr>
<th>Nutritional Composition</th>
<th>LP ( ±0.06)</th>
<th>KP ( ±0.06)</th>
<th>LS ( ±0.06)</th>
<th>KS ( ±0.06)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.11 ±0.05</td>
<td>3.22 ±0.06</td>
<td>4.46 ±0.46</td>
<td>4.17 ±0.06</td>
</tr>
<tr>
<td>Acidity</td>
<td>0.05 ±0.01</td>
<td>0.06 ±0.01</td>
<td>0.31 ±0.02</td>
<td>0.93 ±0.02</td>
</tr>
<tr>
<td>Total Solids</td>
<td>79.18 ±0.46</td>
<td>80.73 ±0.41</td>
<td>96.80 ±0.81</td>
<td>95.48 ±0.46</td>
</tr>
<tr>
<td>Protein</td>
<td>0.91 ±0.03</td>
<td>1.19 ±0.02</td>
<td>0.61 ±0.03</td>
<td>0.37 ±0.02</td>
</tr>
<tr>
<td>Fat</td>
<td>0.58 ±0.02</td>
<td>0.77 ±0.02</td>
<td>0.32 ±0.02</td>
<td>0.25 ±0.02</td>
</tr>
<tr>
<td>Fibre</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ash</td>
<td>0.47 ±0.02</td>
<td>0.53 ±0.02</td>
<td>0.40 ±0.01</td>
<td>0.28 ±0.03</td>
</tr>
<tr>
<td>Moisture content</td>
<td>84.26 ±0.56</td>
<td>81.46 ±0.09</td>
<td>89.68 ±0.02</td>
<td>91.99 ±0.07</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>14.01 ±0.49</td>
<td>15.98 ±0.09</td>
<td>9.01 ±0.05</td>
<td>7.05 ±0.03</td>
</tr>
<tr>
<td>Tannin (µg/g)</td>
<td>0.021 ±0.00254</td>
<td>0.011 ±0.00006</td>
<td>0.008 ±0.00000</td>
<td>0.004 ±0.00006</td>
</tr>
<tr>
<td>Phenolic content (mg/g)</td>
<td>0.02 ±0.00000</td>
<td>0.03 ±0.00100</td>
<td>0.01 ±0.00058</td>
<td>0.01 ±0.00000</td>
</tr>
<tr>
<td>TVC (cfu/ml)</td>
<td>1.35 × 10^5</td>
<td>1.50 × 10^5</td>
<td>1.49 × 10^5</td>
<td>4.78 × 10^4</td>
</tr>
<tr>
<td>LAB (cfu/ml)</td>
<td>1.71 ±10^2</td>
<td>2.24 ±10^2</td>
<td>2.28 ±10^2</td>
<td>2.68 ±10^4</td>
</tr>
<tr>
<td>Mould (cfu/ml)</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>1.16 ±10^3</td>
</tr>
<tr>
<td>Sodium (mg/100g)</td>
<td>46.18 ±10.30</td>
<td>38.92 ±11.27</td>
<td>37.23 ±10.62</td>
<td>29.20 ±1.11</td>
</tr>
<tr>
<td>Potassium (mg/100g)</td>
<td>47.60 ±14.99</td>
<td>40.79 ±19.94</td>
<td>37.40 ±21.72</td>
<td>30.02 ±12.29</td>
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<td>Calcium (mg/100g)</td>
<td>317.85 ±7.24</td>
<td>197.16 ±19.06</td>
<td>281.87 ±6.92</td>
<td>256.57 ±7.96</td>
</tr>
<tr>
<td>Magnesium (mg/100g)</td>
<td>34.97 ±6.84</td>
<td>29.27 ±2.55</td>
<td>27.79 ±2.19</td>
<td>23.24 ±6.89</td>
</tr>
<tr>
<td>Phosphorus (mg/100g)</td>
<td>3.20 ±1.17</td>
<td>2.25 ±0.40</td>
<td>1.92 ±0.31</td>
<td>2.20 ±1.10</td>
</tr>
<tr>
<td>Vitamin A (µg/100g)</td>
<td>1.58 ±0.03</td>
<td>1.91 ±0.04</td>
<td>1.34 ±0.06</td>
<td>1.19 ±0.02</td>
</tr>
<tr>
<td>Vitamin C (mg/g)</td>
<td>19.94 ±0.09</td>
<td>23.42 ±0.06</td>
<td>18.56 ±0.21</td>
<td>16.44 ±0.48</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of triplicate determinations. Means with different superscripts in the same column are significantly (p < 0.05) different.

LP = Local Specie of passion fruit juice from pulp; KP = Kenyan Specie of passion fruit juice from pulp; LS = Local Specie of passion fruit juice from skin; KS = Kenyan Specie of passion fruit juice from skin.
than yoghurt enriched with passion fruit skin (ls = 4.26, ls2 = 4.34, ks1 = 4.37). Addition of passion fruit as flavor caused pH in the formulated yoghurt to drop from 4.24 (NY) to 4.23 (sample lp1), 4.18 (sample lp2) and 4.20 (sample kp2). This could be attributed to the appreciable quantity of ascorbic acid. The result obtained in this study is comparable to earlier researchers on flavoured yoghurt using carrot, pineapple and spiced yoghurt [1] and on yoghurt flavoured with solar dried bush mango [40].

The values observed in this study are comparable with researchers [47, 48, 49]. All the same pH results are in accordance with FDA specifications for the pH of yoghurt (4.6 or lower). Also, it is the range of Standards Organisation of Nigeria [50] specification for pH of yoghurt (3.7 - 4.5).

Generally, there was significant (p<0.05) difference in the titratable acidity value between the enriched samples and control. However, No significant (p>0.05) difference was observed between the enriched samples (ls1 = 0.45, ls2 = 0.50, lp1 = 0.52, lp2 = 0.54, kp2 = 0.72) except for sample ks1 which is not significantly (p>0.05) different with the control (NY = 0.47). The titratable acidity of yoghurt and enriched yoghurt are shown in Table 5. Samples kp2 (0.72) had the highest titratable acidity while sample ks1 (0.14) had the lowest titratable acidity. The values obtained for titratable acidity are generally below the standard which is 0.7% [51] except for sample kp2 (0.72). No direct relationship was observed between pH values and titratable acidity as has been previously reported [47, 49].

There was significant (p<0.05) difference in the total acid value between the enriched samples and control. The enriched yoghurts contained more total solids than the plain yoghurt. Other researchers reported values for fruit and natural yoghurts ranging from 15.0 - 22.8 % and 13.6 – 18.8 %, respectively [47]. The total solid of yoghurts enriched with passion fruit pulp were within the range.

Table 5: Physicochemical composition of plain yoghurt and yoghurt enriched with passion fruit juice (pulp and skin)

<table>
<thead>
<tr>
<th>Samples</th>
<th>pH</th>
<th>Acidity</th>
<th>Total solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>ls 1</td>
<td>4.26c</td>
<td>0.45abc</td>
<td>4.68g</td>
</tr>
<tr>
<td></td>
<td>±0.04</td>
<td>±0.31</td>
<td>±0.73</td>
</tr>
<tr>
<td>ls 2</td>
<td>4.34b</td>
<td>0.50ab</td>
<td>30.03d</td>
</tr>
<tr>
<td></td>
<td>±0.00</td>
<td>±0.35</td>
<td>±0.07</td>
</tr>
<tr>
<td>Ks1</td>
<td>4.37b</td>
<td>0.14cd</td>
<td>4.57g</td>
</tr>
<tr>
<td></td>
<td>±0.01</td>
<td>±0.02</td>
<td>±1.38</td>
</tr>
<tr>
<td>lp 1</td>
<td>4.23cd</td>
<td>0.52ab</td>
<td>26.51e</td>
</tr>
<tr>
<td></td>
<td>±0.01</td>
<td>±0.35</td>
<td>±0.03</td>
</tr>
<tr>
<td>lp 2</td>
<td>4.18de</td>
<td>0.54ab</td>
<td>11.85f</td>
</tr>
<tr>
<td></td>
<td>±0.01</td>
<td>±0.05</td>
<td>±0.16</td>
</tr>
<tr>
<td>Kp 2</td>
<td>4.20cde</td>
<td>0.72a</td>
<td>12.26f</td>
</tr>
<tr>
<td></td>
<td>±0.01</td>
<td>±0.11</td>
<td>±0.82</td>
</tr>
<tr>
<td>NY (control)</td>
<td>4.24cd</td>
<td>0.18cd</td>
<td>3.35g</td>
</tr>
<tr>
<td></td>
<td>±0.04</td>
<td>±0.04</td>
<td>±0.66</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of triplicate determinations. Means with different superscripts in the same column are significantly (p < 0.05) different. NY= unflavoured yoghurt; ls 1= Yoghurt + passion fruit skin local (90:10); YPFls 2= Yoghurt + passion fruit skin local (80:20); ks1 = Yoghurt + passion fruit skin Kenya (90:10); lp 1 = Yoghurt + passion fruit pulp local (90:10); lp 2= Yoghurt + passion fruit pulp local (80:20); kp2 = Yoghurt + passion fruit pulp kenya (80:20).
Table 6 shows the proximate composition (%) of enriched yoghurt. There was significant (p<0.05) difference in the moisture content value between the enriched samples and control. No significant (p>0.05) difference was observed between samples (lp1 = 68.59 %, lp2 = 68.29 %) enriched with passion fruit pulp (local specie). This trend was also observed between the samples (ls1 = 80.46 %, ls2 = 79.72 %) enriched with passion fruit skin (local specie). The high moisture content of the product could be as a result of dilution (reconstitution) of milk prior to fermentation.

For the fat content, there was significant (p<0.05) difference in the value between the enriched samples and control. No significant (p>0.05) difference was observed between samples (ls2 = 3.12 %, ks1 = 3.14 %) flavoured with passion fruit skin (both local and Kenyan specie). The fat content of yoghurt could be attributed to the oil content of milk which was the major substrate of the yoghurt produced. This corresponds with work that the fat level of yoghurt depends on oil content of milk whether skimmed or full cream milk [52]. He stated categorically that yoghurt manufactured from skimmed milk would likely have very low fat content (within range of 1 - 2 %) while that produced from full cream milk would have fat content in the region of 4 %. Since full cream milk was used in the yoghurt production, fat content of the yoghurt sample (Table 6) was within the acceptable range (4%) for high fat yoghurt hence it corresponds with the work of other researchers [52].

The fiber content was in trace amount and ash content was low. No significant (p>0.05) difference was observed between samples (lp2 = 0.67 %, kp =0.67 %) enriched with passion fruit pulp (both local and Kenyan specie) and the control (NY = 0.71 %). The result agreed with the observation of researchers who stated that generally yoghurts have poor fiber level because they are milk and water based products [53]. Even with addition of passion fruit there was a slight increase in ash content. The amount of passion fruit juice added to the yoghurts (10 - 20 %) maybe too little to cause a remarkable increase in the ash content. Besides addition of water, the pulp and skin were sieved after grinding.

Table 6 shows that there were significant (p<0.05) differences in the protein value between the flavoured samples and control. No significant (p>0.05) difference was observed between samples (ks1 = 3.86 %, lp1 = 3.93 %, lp2 = 3.94 %). The protein contents of flavoured yoghurt were between 3.52 (ls2) and 3.94 % (lp2). The sample NY (control) contains 2.81 % protein. This result compared favourably with other results which reported protein content for yoghurt as 3.5% [4]. The result did not compare favourably with the result (9.97 %) [1] but corresponds with work done on effect of different concentration of fruit additives on some physicochemical properties of yoghurt during storage [54]. The work reported protein content for plain yoghurt as 3.41 % and flavoured yoghurt as 4.01. Other researchers obtained the value (4.30) slightly higher [55] than formulated yoghurt enriched with passion fruit juice in this work. There was significant (p<0.05) difference in the moisture content value between the enriched samples and control. No significant (p>0.05) difference was observed between samples lp1 and lp2 (24.03 and 23.97 % respectively) enriched with passion fruit pulp (local specie). Carbohydrate is the major constituent of milk that is converted to lactic acid during yoghurt production. The conversion of lactose to lactic acid accounts for low carbohydrate content of yoghurt. Yoghurt enriched with passion fruit juice from pulp had higher carbohydrate content.
than yoghurt enriched with passion fruit juice from skin. This could probably be due to the higher carbohydrate content in the pulp compared to the skin.

Table 6: Proximate composition (%) of plain yoghurt and enriched yoghurt using passion fruit juices (pulp and skin)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Protein</th>
<th>Fat</th>
<th>Fibre</th>
<th>Ash</th>
<th>Moisture content</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ls 1</td>
<td>3.70 ±0.04</td>
<td>2.93 ±0.04</td>
<td>-</td>
<td>0.63 ±0.03</td>
<td>80.46 ±0.51</td>
<td>12.42 ±0.58</td>
</tr>
<tr>
<td>ls 2</td>
<td>3.52 ±0.05</td>
<td>3.12 ±0.03</td>
<td>-</td>
<td>0.59 ±0.02</td>
<td>79.72 ±0.67</td>
<td>13.28 ±0.63</td>
</tr>
<tr>
<td>ks 1</td>
<td>3.86 ±0.04</td>
<td>3.14 ±0.03</td>
<td>-</td>
<td>0.82 ±0.02</td>
<td>81.80 ±0.24</td>
<td>10.31 ±0.19</td>
</tr>
<tr>
<td>lp 1</td>
<td>3.93 ±2.02</td>
<td>2.78 ±0.05</td>
<td>-</td>
<td>0.60 ±0.02</td>
<td>68.59 ±0.16</td>
<td>24.03 ±0.14</td>
</tr>
<tr>
<td>lp 2</td>
<td>3.94 ±0.10</td>
<td>3.03 ±0.03</td>
<td>-</td>
<td>0.67 ±0.01</td>
<td>68.29 ±0.41</td>
<td>23.97 ±0.45</td>
</tr>
<tr>
<td>Kp 2</td>
<td>3.52 ±0.11</td>
<td>2.90 ±0.04</td>
<td>-</td>
<td>0.67 ±0.05</td>
<td>78.76 ±1.08</td>
<td>14.63 ±1.22</td>
</tr>
<tr>
<td>NY (control)</td>
<td>2.81 ±0.14</td>
<td>3.43 ±0.05</td>
<td>-</td>
<td>0.71 ±0.02</td>
<td>77.56 ±0.03</td>
<td>15.48 ±0.12</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of triplicate determinations. Means with different superscripts in the same column are significantly (p < 0.05) different. NY = unflavoured yoghurt; ls 1 = Yoghurt + passion fruit skin local (90:10); YPFls 2 = Yoghurt + passion fruit skin local (80:20); ks 1 = Yoghurt + passion fruit skin Kenya (90:10); lp 1 = Yoghurt + passion fruit pulp local (90:10); lp 2 = Yoghurt + passion fruit pulp local (80:20); kp 2 = Yoghurt + passion fruit pulp Kenya (80:20).

3.6 Phytochemical composition of plain yoghurt and formulated yoghurt enriched with passion fruit (pulp and skin)

Table 7 shows the phytochemical composition of flavoured yoghurt using passion fruit juice (skin and pulp). There was no significant (p > 0.05) difference in the tannin content value among all the samples. The control (NY) had the least tannin content. Addition of passion fruit juice as flavour caused a slight increase in the tannin content of the enriched yoghurt. Significant (p < 0.05) difference in the phenolic content value was observed among all samples. The phenolic content in the yoghurt samples ranged from 0.06 to 0.10 mg/g. Results given in Table 7 shows the total phenolic content in plain and enriched yoghurt. With the addition of the passion fruit juice, there was a slight increase in phenolic content of enriched yoghurt. The control (NY) had the least phenolic content. The increase in phenolic content of enriched yoghurt could be due to addition of passion fruit juice as flavor.

3.7 Microbiological count of plain yoghurt and formulated yoghurt enriched with passion fruit juice (pulp and skin)

Table 8 shows the microbial load of formulated yoghurt enriched with passion fruit using passion fruit (pulp and skin). The total viable count of the microbiological analysis of the yoghurt and enriched yoghurt samples ranges from $7.72 \times 10^2$ cfu/ml to $5.67 \times 10^5$ cfu/ml. High bacteria count was expected because of the presence of starter cultures, mainly lactic acid bacteria [56].
The standard count is $10^6 - 10^7$ cfu/ml [57, 58]. Very high count however is used as an indication of post-pasteurization contamination [59]. The plain yoghurt, sample NY (control) had higher viable count ($5.67 \times 10^5$) than enriched yoghurts. Microorganisms used as starter culture may have contributed to the total viable count of the yoghurt samples. Passion fruit is typically an unexplored tropical fruit that has anti-bacterial activity [60]. Studies also show that passion fruit are natural antioxidant. The fruit skin or peel has higher antioxidant activity as compared to the pulp [61, 62, 63]. According to other researchers in their research on antimicrobial activity of pineapple and passion fruit juice reported that bacteria were relatively resistant to antibiotic but sensitive to fruit juices [60]. The antibacterial activity of the passion fruit may have led to the decrease of viable count in enriched yoghurt.

Mould count ranged from $0.33 \times 10^1$ to $2.47 \times 10^3$ cfu/ml. The control (NY = $1.20 \times 10^3$ cfu/ml) and enriched yoghurts (sample ls1 = $8.27 \times 10^2$ cfu/ml and sample ls2 = $1.58 \times 10^3$ cfu/ml). These values are above the limits stipulated [57, 59]). Yoghurt enriched with passion fruit pulp lp1 ($0.33 \times 10^1$ cfu/ml), lp2 (not detected), kp2 (not detected) conformed to the standard [57]. According to Codex Alimentarius [57], yoghurt should contain no greater than 1 yeast cell per gram (10 cfu/ml). High counts of yeast and mould have also been reported in yoghurts [64, 65, 66, 67]. Fruit purees added to yoghurt are usually the main source of moulds and yeast due to the dry ingredients (sugar) and fruits [68]. Also, it was stated that Talaromyces spp might be present in fruit flavoured yoghurt [69]. Sample PY (control) had the highest mould count $2.47 \times 10^3$ cfu/ml. This might be due to insufficient hygiene practices during processing by the producers. Other researchers also added that the fungal contamination might occur during transformation processes and/or packaging, storage, transport and sale [70].

The lactic acid bacteria of the yoghurt were least in sample ls2 ($0.33 \times 10^1$ cfu/ml). Passion fruit have anti-bacterial properties and could have rendered some lactic acid bacteria in the yoghurt non-viable. The mould count in sample ls2 was relatively high and could have also suppressed some of the lactic acid bacteria in yoghurt.

### Table 7: Phytochemical composition of plain and formulated yoghurt enriched with passion fruit juice (skin and pulp)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Tannin (µg/g)</th>
<th>Phenolic content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ls 1</td>
<td>0.024abc ±0.00012</td>
<td>0.09e ±0.00058</td>
</tr>
<tr>
<td>ls 2</td>
<td>0.020abcd ±0.00012</td>
<td>0.06f ±0.00153</td>
</tr>
<tr>
<td>Ks 1</td>
<td>0.016abcde ±0.00006</td>
<td>0.08e ±0.00153</td>
</tr>
<tr>
<td>lp 1</td>
<td>0.028ab ±0.00012</td>
<td>0.10a ±0.00058</td>
</tr>
<tr>
<td>lp 2</td>
<td>0.027a ±0.00012</td>
<td>0.10b ±0.00153</td>
</tr>
<tr>
<td>Kp 2</td>
<td>0.015abcde ±0.00012</td>
<td>0.06f ±0.00200</td>
</tr>
<tr>
<td>NY (control)</td>
<td>0.013bcde ±0.00006</td>
<td>0.06f ±0.00115</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of triplicate determinations. Means with different superscripts in the same column are significantly (p < 0.05) different. NY= unflavoured yoghurt (negative control); ls 1= Yoghurt + passion fruit skin local (90:10); YPFls 2= Yoghurt + passion fruit skin local (80:20); ks 1= Yoghurt + passion fruit skin Kenya (90:10); lp 1= Yoghurt + passion fruit pulp local (90:10); lp 2= Yoghurt + passion fruit pulp local (80:20); kp 2= Yoghurt + passion fruit pulp kenya (80:20).
3.8 Micronutrient content of formulated yoghurt enriched with passion fruit juice (pulp and skin)

Table 9 shows the micronutrient content of plain and enriched yoghurt. Yoghurt contains high amount of minerals. The result justifies the assertion that yoghurt is a very good source of essential mineral needed for human metabolism or functionality of cells [71]. Addition of passion fruit caused an increase in the mineral content. The mineral contents of the enriched yoghurts revealed that samples enriched with pulp juice (local specie) had highest mineral content (Table 9). That is samples lp2 and lp1 had the highest mineral content. Sodium, potassium and phosphorus content were the highest (209.31 mg/100g, 209.81 mg/100g and 38.10 mg/100g respectively) in sample lp2 while calcium and magnesium content were the highest (2395.65 mg/100g and 135.94 mg/100g respectively) in sample lp1. There was a slight difference in sample kp2 for all mineral content analysed. Sample kp2 contains passion fruit pulp but its value is lower than the yoghurt sample containing passion fruit skin. This could be as a result of the microbial load as it contains a very high microbial load.

The microorganisms might have utilized the nutrients thereby reducing the value. Samples ls2 and kp which had the highest microbial load of all the enriched yoghurt samples (1.82 × 10^4 and 1.05 × 10^4 cfu/ml) and they both had the least mineral contents.

There were significant (p<0.05) difference in the sodium value between the enriched samples and control. No significant (p>0.05) difference was observed between samples (lp1 = 202.66 mg/100g and lp2 = 209.31 mg/100g) enriched with passion fruit pulp (for local specie). No significant (p>0.05) difference was also observed between some enriched samples (ls2 = 161.21 mg/100g, ls1 = 169.48 mg/100g, kp2 = 166.88 mg/100g) and the control (NY = 168.24 mg/100g). The sodium content of the yoghurt sample ranged from ls2 = 161.21 to lp2 = 209.31 mg/100g. Samples lp2 and lp1 had the highest sodium content (209.31 mg/100g and 202.66 mg/100g), respectively. Also, sodium content of the passion fruit ranged from ks1 = 29.20 to lp = 46.18 mg/100g.

The passion fruit juice from pulp had higher sodium content than passion fruit skin (refer to table 4) and as such the yoghurt enriched with passion fruit pulp contains higher sodium content (lp1 = 202.66 mg/100g, lp2 = 209.31 mg/100g and kp2 = 166.88 mg/100g, respectively) than the samples enriched with passion fruit skin (ls1 = 192.82 mg/100g, ls2 = 161.21 mg/100g and ks1 = 169.48 mg/100g) respectively. The result obtained in this work is higher than the result (41.02 mg/100g) obtained by other researchers [38].

There was significant (p<0.05) difference in the potassium value between the enriched samples and control. No significant (p>0.05) difference was observed between samples (lp1 = 204.46 mg/100g and lp2 = 209.81 mg/100g) enriched with passion fruit pulp (for local specie). Similarly, no significant (p>0.05) difference was observed between samples (ls1 = 177.42 mg/100g, ls2 = 171.42 mg/100g) and the control (NY = 178.03 mg/100g). The potassium content of the yoghurt samples ranged from ls2 = 166.64 mg/100g to lp2 = 209.81 mg/100g. Samples lp2 and lp1 had the highest potassium content (209.81 mg/100g, 204.46 mg/100g), respectively. Also, potassium content for passion fruit samples ranged from ks = 30.03 mg/100g to kp = 476.02 mg/100g.

The passion fruit juice from pulp had higher potassium content than passion fruit skin from skin (refer to table 4) and as such the yoghurt flavoured with passion fruit pulp contained higher potassium content (lp1 = 204.46 mg/100g, lp2 = 209.81 mg/100g and kp2 = 165.15 mg/100g,
respectively) than the samples containing passion fruit skin (ls1 = 177.42 mg/100g, ls2 = 166.64 mg/100g and ks = 171.42 mg/100g, respectively). The potassium content obtained in the control (plain yoghurt sample) NY = 178.03 mg/100ml were slightly lower than the optimum figures (280 mg/100g) [73]. The result obtained in this work is lower than the result (561.42 mg/100g) [72] and higher than the results of other researchers (109.55 mg/100g) [38].

Table 8: Microbiological count of plain yoghurt and yoghurt enriched with passion fruit using passion fruit (pulp and skin)

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>TVC (cfu/ml)</th>
<th>LAB (cfu/ml)</th>
<th>Mould (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ls 1</td>
<td>4.54 × 10^3</td>
<td>2.37 × 10^3</td>
<td>8.27 × 10^2</td>
</tr>
<tr>
<td>ls 2</td>
<td>1.82 × 10^4</td>
<td>0.33 × 10^3</td>
<td>1.58 × 10^3</td>
</tr>
<tr>
<td>Ks 1</td>
<td>7.72 × 10^2</td>
<td>2.90 × 10^3</td>
<td>Not detected</td>
</tr>
<tr>
<td>lp 1</td>
<td>2.64 × 10^3</td>
<td>1.53 × 10^3</td>
<td>0.33 × 10^1</td>
</tr>
<tr>
<td>lp 2</td>
<td>5.62 × 10^2</td>
<td>4.98 × 10^2</td>
<td>Not detected</td>
</tr>
<tr>
<td>Kp 2</td>
<td>1.05 × 10^4</td>
<td>7.85 × 10^3</td>
<td>Not detected</td>
</tr>
<tr>
<td>NY (control)</td>
<td>5.67 × 10^5</td>
<td>1.50 × 10^3</td>
<td>1.20 × 10^3</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of triplicate determinations. Means with different superscripts in the same column are significantly (p < 0.05) different. NY= unflavoured yoghurt (negative control); ls 1 = Yoghurt + passion fruit skin local (90:10); YPFls 2 = Yoghurt + passion fruit skin local (80:20); ks1 = Yoghurt + passion fruit skin Kenya (90:10); lp 1 = Yoghurt + passion fruit pulp local (90:10); lp 2 = Yoghurt + passion fruit pulp local (80:20); kp2 = Yoghurt + passion fruit pulp kenya (80:20).

There was significant (p<0.05) difference in the calcium value between the enriched samples and control. The calcium content of the yoghurt samples ranged from YPFls2 = 433.04 mg/100g to YPFFlp1 = 2395.65 mg/100g. Samples YPFFlp2 and YPFFlp1 had the highest calcium content of 1448.10 mg/100g and 2395.65 mg/100g respectively. The passion fruit juice from pulp lp (local specie) had higher calcium content than passion fruit juice from skin but passion fruit juice from pulp kp (Kenya specie) had lower calcium content than passion fruit juice from skin (refer to table 4). Yoghurt enriched with passion fruit juice from pulp contained higher calcium content (samples lp1 = 2395.65 mg/100g, lp2 = 1448.10 mg/100g and kp2 = 396.25 mg/100g respectively) than the samples containing passion fruit juice from skin (ls1 = 924.62 mg/100g, ls2 = 433.04 mg/100g and ks1 = 779.26 mg/100g, respectively). The calcium content of passion fruit did not follow a similar trend like potassium and sodium. The calcium content of the passion fruit juice from skin was higher than passion fruit juice from pulp (Kenya specie). The calcium content of the passion fruit juice from skin was lower than passion fruit juice from pulp (local specie) (of table 4). The calcium content obtained in the control NY = 178.03 did not compare favourably with result (200 mg/100g) reported by Dairy council (2013). The result obtained in this work was higher than the result (281.43 mg/100g) [72] and the result (111.69 mg/100g) [38]. Again, the work of other researchers (16.99 – 62.14 mg /100g) was lower than the result obtained in this work [39].

There was significant (p<0.05) difference in the magnesium value between the enriched samples and control. The magnesium content of the yoghurt samples ranged from Is2 = 58.84
mg/100g to \( \text{ls1} = 135.94 \text{ mg/100g} \). Samples \( \text{lp2} \) and \( \text{lp1} \) had the highest magnesium content (115.38 mg/100g and 135.94 mg/100g respectively). The passion fruit juice from pulp had higher magnesium content than passion fruit juice from skin (refer to Table 4). The yoghurt enriched with passion fruit juice from pulp contains higher magnesium content (samples \( \text{lp1} = 135.94 \text{ mg/100g} \), \( \text{lp2} = 115.38 \text{ mg/100g} \) and \( \text{kp2} = 53.35 \text{ mg/100g} \)) than the samples containing passion fruit juice from skin (samples \( \text{ls1} = 75.75 \text{ mg/100g} \), \( \text{YPFls2} = 58.84 \text{ mg/100g} \) and \( \text{YPFks} = 70.36 \text{ mg/100g} \)). The magnesium content obtained in this work did not compare favourably with the result (0.17 to 4.20 mg/100g) [37]. The result obtained in this work is higher than the result (23.52 mg/100g) [38].

There was significant (p<0.05) difference in the phosphorus value between the enriched samples and control. The phosphorus content of the yoghurt samples ranged from \( \text{ls2} = 2.98 \) to \( \text{lp} = 38.10 \text{ mg/100g} \). Samples \( \text{lp2} \) and \( \text{lp1} \) had the highest phosphorus content (38.10 mg/100g and 30.53 mg/100g, respectively). Enriched passion fruit juice from pulp contained higher phosphorus content than the samples containing passion fruit juice from skin. This could be as a result of higher phosphorus content in passion fruit juice from pulp than passion fruit juice from skin (Table 4). The phosphorus content obtained in the unflavoured yoghurt (\( \text{NY} = 11.02 \text{ mg/100g} \)) did not compare favourably with the figures (170 mg/100g) [73].

The phosphorus content of yoghurt without passion fruit flavour (control, \( \text{NY} = 11.02 \text{ mg/100g} \)) was higher than that containing passion fruit flavour with the exception of samples \( \text{lp1} (30.53 \text{ mg/100g}) \) and \( \text{lp2} (38.10 \text{ mg/100g}) \). This indicates that the phosphorus content reduced with the addition of the passion fruit flavour. This trend was observed in the work done [40] on yoghurt flavoured with solar-dried bush mango (\textit{Irvingia gabonensis}) pulp, where the phosphorus content of the unflavoured yoghurt (7.91 mg/100g) was higher than the yoghurt containing the bush mango flavour (1.90 mg/100g, 1.20 mg/100g and 1.364 mg/100g at 3.20 %, 0.80 % and 1.60 % respectively). The reduction in phosphorus content of the enriched yoghurt could be as a result of presence of phytochemicals or anti-nutrients which may have interfered with the bio-availability of phosphorus. The result obtained in this work for phosphorus content is lower than the result (202.25 mg/100g) obtained [72] and the result (114.08 mg/100g) obtained by [38]. The recommended (81 mg/100g) [73] for drinkable yoghurt is higher than the result obtained in this project. The phosphorus content obtained in the control \( \text{NY} = 31.24 \) did not compare favourably with result (170 mg/100g) [73]. Again, the work (32.44 – 73.59 mg/100g) done by other researchers [39] is slightly higher than the result obtained in this work.

A similar trend observed in mineral content was also found in vitamin C content of the samples. Addition of passion fruit caused an increase in vitamin C. Similar trends were recorded by other researchers [52, 40, 1]. Addition of passion fruit flavour caused increase in yoghurt enriched with passion fruit juice. There was no significant difference between the yoghurt enriched with passion fruit juice from skin (\( \text{ls1} = 18.30 \) and \( \text{ks} = 18.29 \)) and control (\( \text{NY} = 18.86 \)). However, it was also observed that samples YPFls2 (Vitamin A = 14.96 \( \mu \text{g/100g} \), Vitamin C = 5.58 mg/g) had slightly lower values when compared to other yoghurt samples enriched with passion fruit skin and sample kp2 (Vitamin A = 18.84 \( \mu \text{g/100g} \), Vitamin C = 6.40 mg/g) had slightly lower values when compared to other yoghurt enriched with passion fruit juice from pulp. This could be as a result of the microbial load as it contains a very high microbial load. The microorganisms might have utilized the nutrients, thereby reducing the vitamin content. Samples YPFls2 and YPFkp contained the highest
Table 9: Micronutrient content of plain yoghurt and yoghurt enriched with passion fruit juices (pulp and skin).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Sodium (mg/100g)</th>
<th>Potassium (mg/100g)</th>
<th>Calcium (mg/100g)</th>
<th>Magnesium (mg/100g)</th>
<th>Phosphorus (mg/100g)</th>
<th>Vitamin A (µg/100g)</th>
<th>Vitamin C (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ls 1</td>
<td>192.82&lt;sup&gt;b&lt;/sup&gt;±1.27</td>
<td>177.47&lt;sup&gt;b&lt;/sup&gt;±94.66</td>
<td>924.62&lt;sup&gt;c&lt;/sup&gt;±10.72</td>
<td>75.75&lt;sup&gt;c&lt;/sup&gt;±17.42</td>
<td>20.52&lt;sup&gt;d&lt;/sup&gt;±2.35</td>
<td>18.30&lt;sup&gt;c&lt;/sup&gt;±0.63</td>
<td>7.13&lt;sup&gt;f&lt;/sup&gt;±0.14</td>
</tr>
<tr>
<td>ls 2</td>
<td>161.21&lt;sup&gt;c&lt;/sup&gt;±115.90</td>
<td>166.64&lt;sup&gt;c&lt;/sup&gt;±45.00</td>
<td>433.04&lt;sup&gt;g&lt;/sup&gt;±9.00</td>
<td>58.84&lt;sup&gt;f&lt;/sup&gt;±8.49</td>
<td>2.98&lt;sup&gt;g&lt;/sup&gt;±0.28</td>
<td>14.96&lt;sup&gt;d&lt;/sup&gt;±0.35</td>
<td>5.58&lt;sup&gt;i&lt;/sup&gt;±0.25</td>
</tr>
<tr>
<td>Ks 1</td>
<td>169.48&lt;sup&gt;c&lt;/sup&gt;±34.88</td>
<td>171.42&lt;sup&gt;bc&lt;/sup&gt;±13.20</td>
<td>779.26&lt;sup&gt;c&lt;/sup&gt;±30.88</td>
<td>70.36&lt;sup&gt;d&lt;/sup&gt;±14.95</td>
<td>9.80&lt;sup&gt;f&lt;/sup&gt;±0.50</td>
<td>18.29&lt;sup&gt;c&lt;/sup&gt;±0.29</td>
<td>7.15&lt;sup&gt;f&lt;/sup&gt;±0.04</td>
</tr>
<tr>
<td>lp 1</td>
<td>202.66&lt;sup&gt;a&lt;/sup&gt;±10.74</td>
<td>204.46&lt;sup&gt;a&lt;/sup&gt;±94.14</td>
<td>2395.65&lt;sup&gt;a&lt;/sup&gt;±83.72</td>
<td>135.94&lt;sup&gt;a&lt;/sup&gt;±23.95</td>
<td>30.53&lt;sup&gt;c&lt;/sup&gt;±4.18</td>
<td>20.67&lt;sup&gt;b&lt;/sup&gt;±0.69</td>
<td>7.29&lt;sup&gt;ef&lt;/sup&gt;±0.10</td>
</tr>
<tr>
<td>lp 2</td>
<td>209.31&lt;sup&gt;a&lt;/sup&gt;±122.43</td>
<td>209.81&lt;sup&gt;a&lt;/sup&gt;±91.75</td>
<td>1448.10&lt;sup&gt;b&lt;/sup&gt;±49.61</td>
<td>115.38&lt;sup&gt;b&lt;/sup&gt;±36.02</td>
<td>38.10&lt;sup&gt;a&lt;/sup&gt;±7.61</td>
<td>21.16&lt;sup&gt;b&lt;/sup&gt;±0.59</td>
<td>7.53&lt;sup&gt;c&lt;/sup&gt;±0.06</td>
</tr>
<tr>
<td>Kp 2</td>
<td>166.88&lt;sup&gt;c&lt;/sup&gt;±33.64</td>
<td>165.15&lt;sup&gt;c&lt;/sup&gt;±24.42</td>
<td>396.25&lt;sup&gt;h&lt;/sup&gt;±11.43</td>
<td>53.35&lt;sup&gt;g&lt;/sup&gt;±18.94</td>
<td>9.54&lt;sup&gt;c&lt;/sup&gt;±1.06</td>
<td>18.84&lt;sup&gt;c&lt;/sup&gt;±0.90</td>
<td>6.40&lt;sup&gt;g&lt;/sup&gt;±0.21</td>
</tr>
<tr>
<td>NY</td>
<td>168.24&lt;sup&gt;c&lt;/sup&gt;±2.92</td>
<td>178.03&lt;sup&gt;b&lt;/sup&gt;±64.52</td>
<td>516.33&lt;sup&gt;f&lt;/sup&gt;±8.89</td>
<td>63.55&lt;sup&gt;e&lt;/sup&gt;±11.64</td>
<td>11.02&lt;sup&gt;e&lt;/sup&gt;±0.76</td>
<td>18.86&lt;sup&gt;c&lt;/sup&gt;±0.19</td>
<td>5.10&lt;sup&gt;h&lt;/sup&gt;±0.04</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of triplicate determinations. Means with different superscripts in the same column are significantly (p < 0.05) different. NY= unflavoured yoghurt (negative control); ls 1= Yoghurt + passion fruit skin local (90:10); YPFls 2= Yoghurt + passion fruit skin local (80:20); ks1 = Yoghurt + passion fruit skin Kenya (90:10); lp 1 = Yoghurt + passion fruit pulp local (90:10); lp 2= Yoghurt + passion fruit pulp local (80:20); kp2 = Yoghurt + passion fruit pulp kenya (80:20).
microbial load of all the flavoured yoghurt samples (1.82 × 10^4 and 1.05 × 10^4 cfu/ml respectively) and had minimal vitamin C content.

There was a significant (p<0.05) difference in the vitamin A value between the flavoured samples and control. No significant (p>0.05) difference was observed between samples (YPFlp1 = 20.67 µg/100g, YPFlp2 = 21.16 µg/100g) flavoured with passion fruit pulp (for local specie).

No significant (p>0.05) difference was also observed between some flavoured samples (YPFls1 = 18.30 µg/100g, YPFks = 18.29 µg/100g, YPFkp = 18.84 µg/100g) and the controls (NY = 18.86 µg/100g). The vitamin A content of the yoghurt samples ranges from YPFls2 = 14.96 µg/100g to PY = 24.78 µg/100g. The positive control (PY) had the highest vitamin A content (24.78 µg/100g). Also, the vitamin A content of passion fruit samples ranged from PFks = 1.19 to PFkp = 1.91 µg/100g. The passion fruit pulp had higher vitamin A content (PFPlp1 = 1.58 µg/100g, PFPlp2 = 1.91 µg/100g) than passion fruit skin (samples PFls = 1.34 µg/100g, PFks = 1.19 µg/100g) and as such the yoghurt flavoured with passion fruit pulp contains higher vitamin A content (samples YPFlp1 = 20.67, YPFlp2 = 21.16 and YPFkp = 18.84 µg/100g) than the samples containing passion fruit skin (samples YPFls1 = 18.30, YPFls2 = 14.96 and YPFks = 18.29 µg/100g). The result of the vitamin A content of the yoghurt samples obtained was lower than that reported (70.04 RE) [40] which had 70.04 RE for unflavoured yoghurt and 175.11, 44.20 and 70.04 RE at 3.20, 0.80 and 1.60 % respectively for yoghurt flavoured with bush mango but it is within range (59.68 IU converted to 17.90 µg/100g) with that reported [72].

There was significant (p<0.05) difference in vitamin C value between the flavoured samples and control. The vitamin C content of the yoghurt samples ranges from 5.58 mg/g to 7.53 mg/g. Yoghurt flavoured with passion fruit pulp juice had higher vitamin C content (lp1 = 7.29 mg/g, lp2 = 7.53 mg/g, kp2 = 6.40 mg/g) than yoghurt flavoured with skin juice (ls1 = 7.13, ls2 = 5.58 and ks = 7.15 mg/g). Sample lp1 and lp2 had the highest Vitamin C content (7.29 and 7.53 mg/g). The result obtained [1] for vitamin C were 3.90 mg (plain yoghurt), 4.01 mg (yoghurt spiced with pepper fruit), 3.91 mg (yoghurt spiced with ginger), 4.25 mg (yoghurt flavoured with carrot), 4.48 mg (yoghurt flavoured with pineapple).

4. CONCLUSION AND RECOMMENDATION

The result of this study shows that addition of passion fruit juice to yoghurt as flavouring agent improved the physicochemical and sensory properties of yoghurt, especially when flavoured in the range of 10 – 20%. The addition of passion fruit juice in yoghurt improved the colour, flavour, taste, aftertaste, mouthfeel and overall acceptability as seen in the sensory scores obtained with the highest scores being in the flavoured yoghurt that contained 10 - 20 % passion fruit juices.

The utilization of passion fruit as a natural flavouring agent improved the nutritional properties of the product. The enriched yoghurt contained higher protein content than the unflavoured yoghurt. The fat and carbohydrate contents were lower in enriched yoghurt and higher in unflavoured yoghurt making it an ideal drink for obese or weight conscious individuals. The samples enriched with passion fruit pulp had more minerals and vitamins than those flavoured with passion fruit skin. The high nutrient content of the enriched yoghurt makes it a very nutritious and healthy drink. The phytochemicals (tannins and phenolic content) in the product were in trace amount and hence makes it an ideal drink for all classes of people in the world: children, aged, sick, pregnant women and among others. Yoghurt enriched with 10 – 20% passion fruit pulp conformed to the standard stated in Codex alimentarius for yoghurt, thereby establishing the fact that it is safe and healthy for human consumption.
Based on the study, the research on passion fruit flavoured yoghurt (especially those flavoured with pulp juice) at commercial level is highly recommended. Passion fruit should be included in the wide range of fruit used to flavor yoghurt as the result obtained in this piece of work had shown it to add to the nutritional content of yoghurt. It is also recommended that information on the production of yoghurt be disseminated to domestic and commercial manufacturers of yoghurts. It is very necessary that further work should be done where passion fruit maybe incorporated in the yoghurt formulation before fermentation. There is need for further studies on other minerals (iron and zinc) and vitamins B (B₁, B₂, B₃, B₆ and B₁₂). The storage stability of the formulated product should be investigated.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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