

# 1 PRODUCTION AND QUALITY EVALUATION OF YOGHURT ENRICHED WITH 2 TWO ACCESSIONS OF PASSION FRUITS (*Passiflora edulis flavicarpa*)

## 3 4 Abstract

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

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

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

11 **Methodology:** Yoghurt is a dairy product obtained from lactic acid fermentation of milk. Yoghurt  
12 and two accessions of passion fruit juices (*Passiflora edulis flavicarpa*) were processed to  
13 formulate enriched yoghurt in the following ratios 90:10, 80:20, 70:30, 60:40 and 50:50. Yoghurt  
14 without the passion fruit juice (100: 0) served as the control. Based on sensory evaluation, the best  
15 samples were subjected to physicochemical, phytochemical, microbiological, selected mineral and  
16 vitamin content evaluation using standard methods. The best enriched yoghurt samples were those  
17 in the ratio 90:10 and 80:20. Proximate composition of the enriched yoghurt samples significantly  
18 ( $p < 0.05$ ) differed with the controls. **Results:** Enriched yoghurt showed an increase in the protein  
19 content (3.70, 3.52, 3.86, 3.93, 3.94, 3.52%) than control (2.81%). The control had higher fat  
20 content (3.43%) than enriched yoghurt (2.93, 3.12, 3.14, 2.78, 3.03 and 2.90%). The ash content  
21 ranges from 0.59- 0.82% while addition of passion fruit juice caused pH to drop from 4.24 in the  
22 plain yoghurt to 4.18, 4.20 and 4.23 in the enriched yoghurt. The titratable acidity of yoghurt  
23 samples increased from 0.18 in the control to 0.72, 0.54, 0.52, 0.50 and 0.45 in enriched yoghurt.  
24 No direct relationship was observed between the pH and the titratable acidity. The total solid  
25 content ranges from 4.57-30.03. The phytochemicals were in trace amount. There was no  
26 significant ( $p > 0.05$ ) difference in the tannin content. The titratable acidity of yoghurt samples  
27 increased from 0.013  $\mu\text{g}$  in the control to 0.015, 0.027, 0.028, 0.016, 0.020 and 0.024 in enriched  
28 yoghurt. Significant ( $p < 0.05$ ) difference in the phenolic content value was observed among all the  
29 samples. The phenolic content in the yoghurt samples ranges from 0.06 to 0.10 mg/g. The mineral  
30 content and vitamin content of the flavoured yoghurt samples significantly ( $p < 0.05$ ) differed with  
31 that of the control. An increase in the sodium content was observed in the samples from the  
32 control (168.24mg/100g) to the enriched sample (209.31, 202.66, 169.48 and 192.82 mg/100g).  
33 Similar increases were observed for potassium, calcium, magnesium and phosphorus content of  
34 the samples. Enriched yoghurt showed an increase in the vitamin C content (6.40, 7.53, 7.29, 7.15  
35 and 7.13 mg/g) than in the control (5.10 mg/g). Vitamin A content ranged from 14.96  $\mu\text{g}/100\text{g}$  -  
36 20.67  $\mu\text{g}/100\text{g}$ . The total viable count was higher in the control ( $5.67 \times 10^5$  cfu/ml) than in  
37 enriched yoghurt. The mould count of the yoghurt samples varied from  $0.33 \times 10^1$  cfu/ml -  $1.58 \times$   
38  $10^3$  cfu/ml. All the sensory attributes tested in the flavoured yoghurt samples significantly  
39 ( $p < 0.05$ ) differed. Yoghurt enriched with local specie passion fruit juice from pulp, was the most  
40 accepted.

41 **Keywords:** Fermentation, Yoghurt, Passion fruit,

## 42 1. Introduction

43 Yoghurt is a fermented dairy product obtained from lactic acid fermentation of milk [1]. It is one  
44 of the most popular fermented milk products in the world [2]. Nowadays, healthy foods mean  
45 “functional foods”. Food is labelled functional if it exerts beneficial effects or more specific body

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

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

68 Passion fruit is native to tropical America and widely grown in Brazil [8]. In India it is found  
69 to be growing wild in many parts of Western Ghat such as Nilgiris, Wynad, Kodaikanal,  
70 Shevroys, Coorg and Malabar as well as Himachal Pradesh and North Eastern States like  
71 Manipur, Nagaland and Mizoram [9]. Again, Kenya is one of the leading producers of passion  
72 fruit in Africa. Other large producers world-wide include Hawaii, Brazil, Australia, Columbia,  
73 Zimbabwe and South Africa [10, 11]. The yellow passion fruit is well suited to the ecology of  
74 southern Nigeria, it is relatively unknown by farmers and hardly grown [12]. Passion fruit is not  
75 available in Nigeria but it was recently introduced.

76 Passion fruit, like any other fruit, are susceptible to damage due to poor storage condition,  
77 handling, pest attack, disease and deterioration. Also, they do not stay for a long period of time  
78 hence the need to utilize them when they are in season.

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

87 As an edible fruit, it contains several components such as acids and sugars and nutrients  
88 that make passion fruit a tasteful and healthy addition to the diet [17]. Passion fruit is known for  
89 its natural attractive colouring, unique flavor properties and medicinal purposes [13]. It is a very  
90 nutritious fruits and should be grown in Nigeria as this would widen the food base of fruit used as  
91 natural flavourant. Furthermore, commercial experience has also shown that flavouring of yoghurt

92 is an important additional sales prospect due to introduction of a wide variety of flavours and also  
93 adds on therapeutic properties of the product [6]. The demand for fruity yoghurt with different  
94 flavors is increasing [6]. Adding fruit juice to yoghurt decreases viscosity [18] (and increases  
95 some minerals such as magnesium, zinc, iron and copper [19]).

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

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

## 109 **2. MATERIALS AND METHODS**

### 110 **2.1 Raw materials**

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

### 115 **2.2 Sample preparation**

#### 116 **2.2.1 Processing of passion fruit juice**

117 Passion fruit pulp and skin was processed using the method [24] and modified by the method [6].  
118 The passion fruit was sorted. The fruits were rinsed in warm water, peeled and deseeded. The  
119 resulting pulp (400 g) was blended with 300 ml of sterile water and skin (200 g) was blended with  
120 1000 ml of sterile water inside a blender (Kenwood, FP730, UK). The homogenized pulp and skin  
121 was filtered with a muslin cloth. The flow chart of passion fruit pulp and skin juice production in  
122 Figure 2

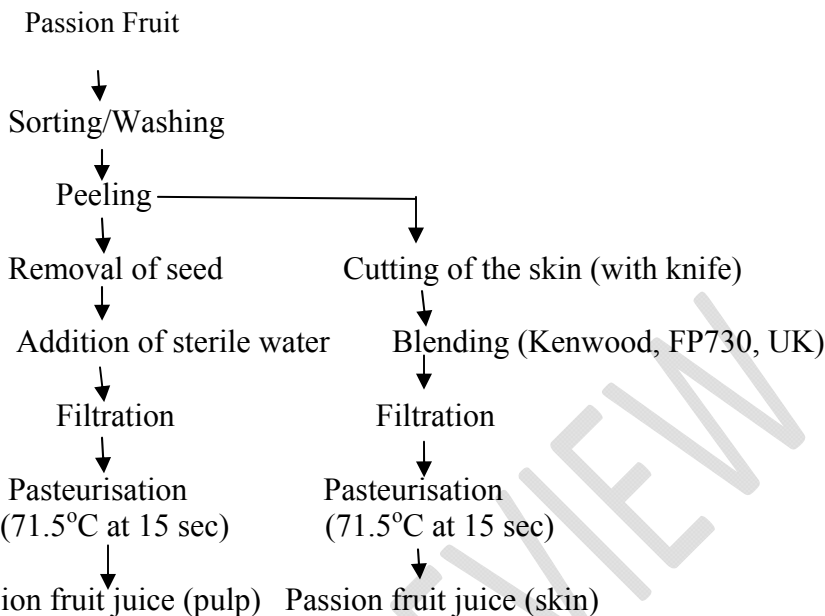
#### 123 **2.2.2 Production of yoghurt**

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

#### 133 **2.2.3 Formulation of enriched yoghurt from passion fruit pulp and skin blends of the Kenyan and local specie.**

134 Table 2 shows the proportions of the Kenyan and local species of the passion fruit (*Passiflora*  
135 *edulis Flavicarpa*) used in the formulation of flavoured yoghurt.  
136  
137

138

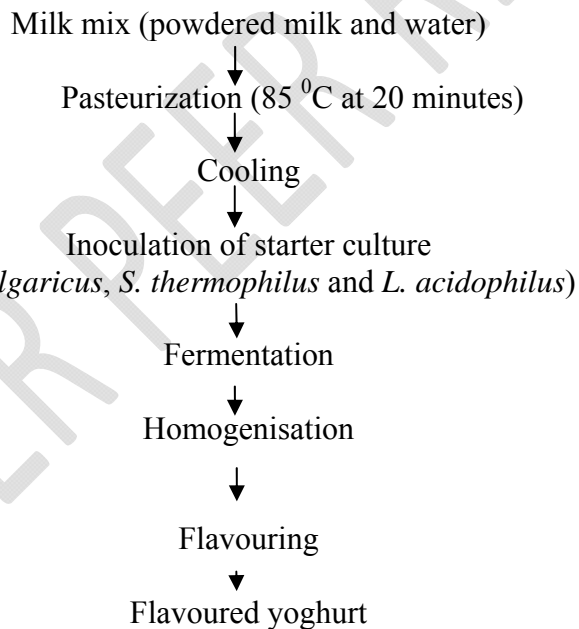


154 **Figure 2:** Production of passion fruit juice (from pulp and skin) [24, 6]

155

156

157



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

176

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

179 Sample codes	Proportions ( ml )	Sample codes	Proportions ( ml )
180 for Kenyan specie		for local specie	
181 kp1	90:10	lp1	90:10

182

183	kp2	80:20	lp2	80:20
184	kp3	70:30	lp3	70:30
185	kp4	60:40	lp4	60:40
186	kp5	50:50	lp5	50:50
187	ks1	90:10	ls1	90:10
188	ks2	80:20	ls2	80:20
189	ks3	70:30	ls3	70:30
190	ks4	60:40	ls4	60:40
191	ks5	50:50	ls5	50:50
192	NY (control)	100:0		

193 ks= Kenyan Passion fruit skin; kp= Kenyan Passion fruit pulp; lp= Local passion fruit pulp; ls=  
 194 Local passion fruit skin; NY = Unflavoured yoghurt

195

## 196 2.3 Analysis

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

### 199 2.3.1 Analysis of the physico-chemical composition of flavoured yoghurt using passion fruit

#### 200 2.3.1.1 pH determination

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

#### 206 2.3.1.2 Determination of total titrable acidity (TTA)

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

$$211 \text{ \% TTA as lactic acid} = \frac{n(\text{NaOH}) \times N(\text{NaOH}) \times 0.09 \times 100}{\text{Volume of sample} \times 1}$$

212

213 Where; n = volume of titre, N= number of moles

214

#### 215 2.3.1.3 Determination of total solids

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

$$219 \text{ \% Total solids} = \frac{\text{Weight of dried sample} \times 100}{\text{Weight of sample} \times 1}$$

220

### 221 2.3.2. Proximate composition of passion fruit flavoured yoghurt

#### 222 2.3. 2.1 Moisture content

223 The moisture content of the samples was determined according to the standard method of  
 224 Association of Official Analytical Chemists [27]. The crucible was washed and dried in the oven  
 225 at 100 °C for 1 hour (W<sub>1</sub>). The hot dried crucible was cooled in the desiccators. The weight was  
 226 taken when cooled. Two milliliters of the sample was weighed into the crucible (W<sub>2</sub>) and then  
 227 placed inside the oven (zitalo Z0502P, Nigeria) at 100 °C for 4 hours. The crucible and contents

228 were removed, cooled in desiccators and weighed ( $W_3$ ). The drying continued until a constant  
229 weight is obtained. The percentage moisture content was calculated from weight loss of the  
230 sample. Thus:

$$231 \text{ \% Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

232  
233 Where;  $W_1$  = initial weight of empty crucible,  $W_2$  = weight of crucible + weight of sample before  
234 drying,  $W_3$  = weight of dish + weight of sample after drying.

### 235 2.3.2.2 Ash content

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

$$242 \text{ \% Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

243  
244 Where:  $w_1$  = weight of empty crucible;  $w_2$  = weight of crucible + weight of sample before ashing;  
245  $w_3$  = weight of crucible + weight of sample after ashing

### 246 2.3.2.3. Crude protein

247 The protein content of the flavoured yoghurt was determined according to the standard Kjeldahl  
248 method [27]. The sample (2 ml) was weighed into Kjeldahl flask. Anhydrous sodium sulphate (5 g  
249 or 4 tablet of Kjeldahl catalyst) was added to the flask. Twenty five milliliters (25 ml) of  
250 concentrated tetraoxosulphate (VI) acid ( $\text{H}_2\text{SO}_4$ ) was added with few boiling chips. The flask with  
251 the content was heated in the fume chamber until the solution become clear, cooled to room  
252 temperature, transferred into a 250 ml volumetric flask and made up to the level with distilled  
253 water. A 100 ml conical flask (receiving flask) containing 5 ml of 2 % boric acid solution with  
254 few drops of methyl red indicator was placed under the condenser. Then, 5 ml of the sample  
255 digest was pipetted into the apparatus through the small funnel and washed down with distilled  
256 water. Five milliliters of 60 % NaOH (sodium hydroxide) solution was added to the digest and  
257 heated until 100 ml of distillate (ammonium sulphate) was collected in the receiving flask. The  
258 solution in receiving flask was titrated with 0.049 M  $\text{H}_2\text{SO}_4$  to a pink coloured end point. A blank  
259 with filter paper was subjected to the same procedure.

$$260 \text{ Calculation: \% Nitrogen of sample (\%N)} = \frac{V_s - V_B \times N \text{ acid} \times 0.01401}{W} \times 100$$

261  
262 Where:  $V_s$  = volume (ml) of acid required to titrate the sample;  $V_B$  = volume (ml) of acid required  
263 to titrate the blank; N acid = Normality of acid (0.1N); W = weight of sample in gram  
264 % crude protein = % N X 6.25 (conversion factor).

### 266 2.3.2.4 Fat

267 The fat content of the sample was determined using the standard method [26]. A Soxhlet  
268 extractor with a reflux condenser and a 500 ml round bottom flask was fixed. The extraction  
269 thimble was sealed with cotton wool. The Soxhlet apparatus after assembling was allowed to  
270 reflux for about 6 hour. The thimble was removed with care and petroleum ether (boiling point of

271 40-60 °C) collected in the top and drained into a container for reuse. When the flask was free of  
272 ether, it was removed and dried at 105 °C for 1 hour in an oven. It was cooled in a desiccator and  
273 then weighed.

274 Calculation

$$275 \quad \% \text{ Fat} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times \frac{100}{1}$$

277

### 278 2.3.2.5 Crude fibre

279 The crude fibre content of the sample was determined using the standard method [27].  
280 Petroleum ether (boiling point of 40-60 °C) was used to defat 2 ml of sample. This was put in  
281 boiled 200 ml of 1.25 % H<sub>2</sub>SO<sub>4</sub> and boiled for 30 minutes. The solution was filtered through linen  
282 or muslin cloth on a fluted funnel. It was washed with boiling water until it is free from acid. The  
283 residue was returned into 200 ml boiling NaOH and allowed for 30 minutes. It was further washed  
284 with 1 % HCl, boiling water, to free it of acid. The final residue was drained and transferred to  
285 silica ash crucible (porcelain crucible), dried in oven at 100 °C for 2 hours and cooled, until a  
286 constant weight is obtained. The cooled sample was incinerated or washed in a muffle furnace at  
287 600 °C for 5 hours, cooled in a desiccator and weighed.

288 Calculation;

$$289 \quad \% \text{ crude fiber} = \frac{\text{Loss of weight after ignition}}{\text{Weight of original sample}} \times \frac{100}{1}$$

291

### 292 2.3.2.6 Carbohydrate

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

$$295 \quad \% \text{ Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ protein} + \% \text{ fat} + \% \text{ crude fibre} + \% \text{ ash})$$

### 296 2.3.3. Determination of phytochemical content of flavoured yoghurt using passion fruit

#### 297 2.3.3.1 Tannin

298 The Folin-Denis spectrophotometric method was used [28]. A measured weight of each sample (1  
299 ml) was dispersed in 10ml distilled water and agitated. This was left to stand for 30 minutes at  
300 room temperature, being shaken every 5 minutes. At the end of 30 minutes, it was centrifuged and  
301 the extract gotten, 2.5 ml of the supernatant was dispersed into a 50 ml volumetric flask.  
302 Similarly, 2.5 ml of standard tannic acid solution was dispersed into a separate 50 ml flask. Then,  
303 1 ml Folin-Denis reagent was measured into each flask, followed by 2.5 ml of saturated Na<sub>2</sub>CO<sub>3</sub>  
304 solution. The mixture was diluted to mark in the flask (50 ml), and incubated for 90 minutes at  
305 room temperature. The absorbance was measured at 250 nm. Readings were taken with the  
306 reagent blank at zero. The tannin content was given as follows:

$$307 \quad \% \text{ tannin} = \frac{A_n}{A_s} \times C \times 100/W \times V_f/V_a$$

308 Where, A<sub>n</sub> = Absorbance of test sample; A<sub>s</sub> = Absorbance of standard solution; C =  
309 Concentration of standard solution; W = Weight of sample used, V<sub>f</sub> = Total volume of extract; V<sub>a</sub>  
310 = Volume of extract analyzed.

311

### 312 **2.3.3.2 Determination of total phenolic content**

313 Total phenolic content (TPC) was measured spectrophotometrically based on a method [29].  
314 Firstly, 1 ml of sample extract was mixed with 4 ml of Folin-Ciocalteu reagent (previously diluted  
315 10 times) and the mixture was allowed to stand for 3 minute at room temperature. Then, 5 ml of  
316 7.5 % sodium carbonate solution was added to the mixture, vortexed vigorously and kept at room  
317 temperature in dark for 30 minutes. The absorbance was measured at 765 nm using a PRIM Light  
318 spectrophotometer (Secomam, Cedex, France) against a blank (distilled water). The standard  
319 curve of gallic acid was  $y = 0.0165x + 0.0003$  ( $R^2 = 0.9972$ ). Total phenolic content was  
320 expressed as  $\mu\text{g}$  gallic acid equivalent (GAE) per gram sample (fresh weight).

### 321 **2.3.4. Determination of micronutrients of formulated flavoured yoghurt**

#### 322 **2.3.4.1 Determination of calcium, sodium, potassium and magnesium**

323 Micronutrients (calcium, sodium, potassium and magnesium) were determined using method  
324 [30]. Two millilitres (2 ml) of the sample was weighed and subjected to dry ashing for five (5)  
325 hours in well-cleaned porcelain crucibles at 550 °C. The resultant ash was dissolved in 5 ml of  
326  $\text{HNO}_3/\text{HCl}/\text{H}_2\text{O}$  (1:2:3) and heated gently on a hot plate until brown fumes disappeared,  
327 remaining the material in each crucible. Five (5 ml) of deionized  $\text{H}_2\text{O}$  was added and heated until  
328 a colourless solution was obtained. The solution on each crucible was filtered into 100 ml  
329 volumetric flask and the volume made up to 100 ml with deionised water. The solution was then  
330 used to analyse for calcium, sodium, potassium and magnesium using an atomic absorption  
331 spectrophotometer. The results were expressed as mg / 100 ml.

#### 332 **2.3.4.2 Determination of phosphorus content**

##### 333 **Preparation of standard solution**

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

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

##### 340 **Sample preparation**

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

$$350 \quad \frac{\text{Absorbance of test} \times \text{concentration of standard (5mg/ml)}}{\text{Absorbance of standard}} = P \text{ (mg/ml)}$$

#### 352 **2.3.4.3 Determination of Vitamin C content**

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

### 355 **Procedure**

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

#### 361 **Calculation**

$$362 \text{ mg ascorbic acid } 1 \text{ g} = C \times V \times \frac{DF}{WT}$$

363 Where; C=mg ascorbic acid 1ml dye; V=Volume of dye used for titrate of diluted sample;  
364 DF=Dilution factor; WT=Weight of sample in ml

365

#### 366 **2.3.4.4 Determination of pro-vitamin A**

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

$$377 FA_{620} = \frac{\mu\text{g vitamin A/tube}}{A_{620}}$$

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

380 Where,  $Fc_{450}$  = constant determined on the laboratory, 150 = dilution factor

381 Likewise, pro-vitamin A (as  $\mu\text{g}$  retinol/dl) was calculated:

$$382 (\text{as } \mu\text{g retinol/dl}) [A_{620} - \frac{2 \times A_{450} \times Fc_{450}}{Fc_{620}}] \times FA_{620} \times 75$$

#### 383 **2.3.5 Microbial analysis of formulated enriched yoghurt**

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

##### 385 **2.3.5.1 Determination of total viable count**

386 The fermenting slurry (1 ml) was dissolved into 9 ml of Ringer's solution in a test tube and mixed  
387 thoroughly by shaking. This was a  $10^{-1}$  dilution; one millilitre (1 ml) of the mixture was pipetted  
388 into another 9 ml of Ringer's solution to give  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  dilution. 1ml aliquot  
389 from different dilutions ( $10^{-3}$  and  $10^{-4}$ ) was used to check the total viable count per ml on nutrient  
390 agar media. The Petri dishes were made in triplicate for each sample and in each plate, 15 ml of  
391 sterile nutrient agar medium was added and 1 ml of each sample dilution was pipette into each  
392 medium containing plate respectively. This was followed by shaking and rocking in a circular  
393 movement for about 10 seconds to uniform homogenisation. The plates were allowed to set and

394 were incubated (inverted) for 24 - 48 hours at 37 °C. The colonies formed were counted and  
395 recorded as colony forming units (cfu).

396 No of colonies (cfu/ml) = average count X dilution factor (Df)

### 397 **2.3.5.2 Determination of mould count**

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

### 406 **2.3.5.3 Determination of lactic acid bacteria using deManRogosa Sharpe (MRS) agar**

407 The lactic acid bacteria (LAB) in the formulated yoghurt were determined using deMan Rogosa  
408 Sharpe (MRS) Agar (CM 361) as described by Oxoid Manual [34]. Samples were serially diluted  
409 in triplicate and inoculated using the surface pour plate method. The plates were incubated under  
410 anaerobic conditions at 37 °C for 48 hours. After incubation, the number of colonies were counted  
411 and represented as colony forming unit per milliliter (cfu/ml).

412 Cfu/ml = average count × dilution factor (D.F)

### 413 **2.3.6 Sensory evaluation of the formulated enriched yoghurt blended with passion fruit 414 blends**

415 Sensory properties of the samples were evaluated by 20 semi-trained panelists consisting of  
416 students of University of Nigeria, Nsukka for various sensory attributes (colour, taste, flavour,  
417 mouthfeel, consistency, aftertaste and overall acceptability). The extent of differences between the  
418 yoghurt samples for each sensory quality was measured on a nine- point Hedonic scale, (where  
419 "9" represents extremely like and "1" represents extremely dislike [35].

### 420 **2.3.7 Data analysis and experimental design of the formulated flavored yoghurt**

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

425

## 426 **3. RESULTS AND DISCUSSION**

### 427 **3.1 Passion fruit enriched yoghurts**

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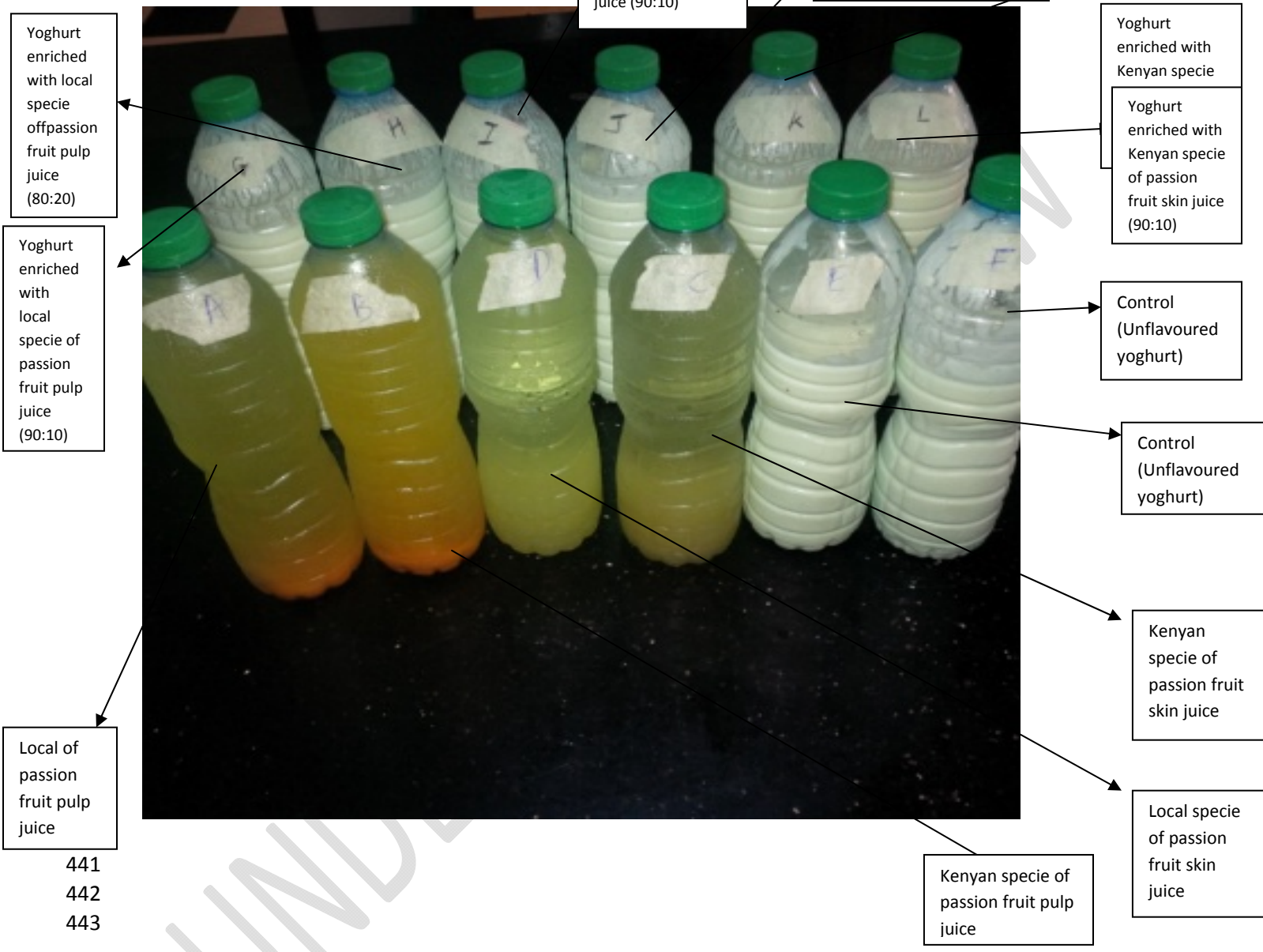
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436 Plate 9 shows the passion fruit juices (from skin and pulp), plain yoghurt and formulated yoghurt  
437 enriched with passion fruit (pulp and skin)

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**Plate 9: Passion fruit juices, plain yoghurt and enriched yoghurt using passion fruit (pulp and skin)**

Samples	Colour	Flavour	Taste	Aftertaste	Mouthfeel	Overall Acceptability
kp1	8.15 <sup>ghi</sup> ± 0.88	6.05 <sup>abcdef</sup> ± 1.67	5.95 <sup>bcde</sup> ± 1.54	5.75 <sup>abcde</sup> ± 1.92	6.40 <sup>efghi</sup> ± 1.73	6.55 <sup>def</sup> ± 1.40
kp2	8.25 <sup>ghi</sup> ± 0.96	7.05 <sup>fghi</sup> ± 1.15	6.60 <sup>defgh</sup> ± 1.35	6.60 <sup>efghi</sup> ± 1.35	7.30 <sup>ijkl</sup> ± 1.22	7.50 <sup>fghij</sup> ± 1.15
kp3	7.85 <sup>fghi</sup> ± 0.99	7.00 <sup>fgh</sup> ± 1.12	6.70 <sup>defgh</sup> ± 1.30	6.65 <sup>efghi</sup> ± 1.27	6.95 <sup>ghijk</sup> ± 1.32	6.90 <sup>defgh</sup> ± 1.21
kp4	7.90 <sup>fghi</sup> ± 1.12	6.40 <sup>cdefg</sup> ± 1.31	6.00 <sup>bcde</sup> ± 1.56	5.65 <sup>abcde</sup> ± 1.84	6.15 <sup>defg</sup> ± 0.93	6.55 <sup>def</sup> ± 1.10
kp5	6.55 <sup>bcd</sup> ± 1.82	5.30 <sup>ab</sup> ± 1.84	4.90 <sup>ab</sup> ± 2.15	4.90 <sup>ab</sup> ± 1.80	5.05 <sup>abc</sup> ± 1.54	5.35 <sup>abc</sup> ± 1.84
lp1	8.40 <sup>hi</sup> ± 0.82	8.05 <sup>jk</sup> ± 1.28	8.00 <sup>ij</sup> ± 1.65	7.65 <sup>ij</sup> ± 1.81	7.80 <sup>kl</sup> ± 1.67	8.00 <sup>ij</sup> ± 1.38
lp2	7.30 <sup>defg</sup> ± 1.59	7.00 <sup>fgh</sup> ± 1.49	7.25 <sup>fghij</sup> ± 1.62	6.95 <sup>fghij</sup> ± 1.57	7.20 <sup>hijkl</sup> ± 1.54	7.20 <sup>efghi</sup> ± 1.43
lp3	6.45 <sup>bcd</sup> ± 1.67	6.10 <sup>bcdef</sup> ± 1.55	5.95 <sup>bcde</sup> ± 1.57	5.25 <sup>abcd</sup> ± 1.48	5.95 <sup>bcdefg</sup> ± 1.57	6.45 <sup>de</sup> ± 1.39
lp4	6.00 <sup>b</sup> ± 1.94	5.65 <sup>abcd</sup> ± 1.42	5.60 <sup>bcd</sup> ± 1.42	5.10 <sup>abc</sup> ± 1.37	5.35 <sup>abcde</sup> ± 1.14	5.30 <sup>ab</sup> ± 1.13
lp5	5.10 <sup>a</sup> ± 2.13	5.65 <sup>abcd</sup> ± 1.35	5.10 <sup>ab</sup> ± 1.65	5.00 <sup>ab</sup> ± 1.65	4.90 <sup>ab</sup> ± 1.86	5.10 <sup>ab</sup> ± 1.62
ks1	8.40 <sup>hi</sup> ± 0.68	7.20 <sup>ghijk</sup> ± 0.83	6.90 <sup>efghi</sup> ± 1.68	6.75 <sup>efghi</sup> ± 1.02	6.95 <sup>ghijk</sup> ± 1.23	7.10 <sup>efghi</sup> ± 1.07
ks2	7.00 <sup>cdef</sup> ± 1.07	6.40 <sup>cdefg</sup> ± 1.10	5.75 <sup>bcde</sup> ± 1.40	5.80 <sup>abcdef</sup> ± 1.36	6.05 <sup>cdefg</sup> ± 1.47	6.30 <sup>cde</sup> ± 1.26
ks3	6.70 <sup>bcde</sup> ± 0.98	5.00 <sup>a</sup> ± 1.72	4.45 <sup>a</sup> ± 1.88	4.65 <sup>a</sup> ± 1.79	4.50 <sup>a</sup> ± 1.99	4.65 <sup>a</sup> ± 1.79
ks4	6.70 <sup>bcde</sup> ± 1.53	5.47 <sup>abc</sup> ± 1.84	5.41 <sup>abc</sup> ± 2.06	5.11 <sup>abc</sup> ± 1.69	5.29 <sup>abcd</sup> ± 1.72	5.47 <sup>abc</sup> ± 1.70
ks5	7.30 <sup>defg</sup> ± 1.84	6.56 <sup>defg</sup> ± 1.56	6.34 <sup>cdef</sup> ± 1.77	6.34 <sup>defgh</sup> ± 1.77	6.52 <sup>fghij</sup> ± 1.65	6.69 <sup>defg</sup> ± 1.06
ls1	8.75 <sup>hi</sup> ± 0.55	8.20 <sup>k</sup> ± 0.89	8.30 <sup>j</sup> ± 0.86	8.00 <sup>j</sup> ± 0.79	8.15 <sup>l</sup> ± 0.81	8.45 <sup>j</sup> ± 0.76
ls2	8.35 <sup>hi</sup> ± 0.88	7.50 <sup>hijk</sup> ± 1.05	7.55 <sup>ghij</sup> ± 1.19	7.35 <sup>hij</sup> ± 1.31	7.55 <sup>ijkl</sup> ± 1.10	7.75 <sup>hij</sup> ± 1.21
ls3	7.70 <sup>fgh</sup> ± 1.45	6.75 <sup>efgh</sup> ± 1.71	6.40 <sup>cdef</sup> ± 1.60	6.25 <sup>cdefgh</sup> ± 1.45	6.60 <sup>ghij</sup> ± 1.31	6.65 <sup>def</sup> ± 1.35
ls4	6.65 <sup>bcde</sup> ± 1.42	5.80 <sup>abcde</sup> ± 1.54	5.70 <sup>bcd</sup> ± 1.75	6.00 <sup>bcdefg</sup> ± 1.62	5.45 <sup>abcdef</sup> ± 1.50	5.95 <sup>bcd</sup> ± 1.50
ls5	6.25 <sup>bc</sup> ± 1.33	5.10 <sup>ab</sup> ± 1.89	4.95 <sup>ab</sup> ± 1.47	5.35 <sup>abcd</sup> ± 1.79	4.75 <sup>a</sup> ± 1.59	5.20 <sup>ab</sup> ± 1.61
NY (control)	8.00 <sup>ghi</sup> ± 0.97	7.65 <sup>ijk</sup> ± 1.04	7.60 <sup>hij</sup> ± 1.27	7.10 <sup>ghij</sup> ± 2.15	7.60 <sup>ijkl</sup> ± 2.32	7.70 <sup>ghij</sup> ± 1.84

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

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

448

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

453

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

### 455 3.2 Sensory scores of formulated yoghurt enriched with passion fruit.

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

458 Table 3 shows the mean sensory scores of the enriched yoghurt and the controls for colour,  
459 flavour, taste, aftertaste, mouthfeel and overall acceptability. The samples containing 10 – 20 %  
460 passion fruit had more acceptable colour and there was a decrease in the level of acceptance as  
461 the percentage of passion fruit juice increased. There was no significant ( $p > 0.05$ ) difference in  
462 the colour of samples NY (unflavoured yoghurt = 8.00), kp1 (8.15), kp2 (8.25), lp1 (8.4), ks1  
463 (8.40), ls1 (8.75) and ls2 (8.35). The samples mentioned were enriched with 10- 20 % passion  
464 fruit and had higher sensory score. This agreed with result obtained by other researchers [37, 38]

465 The flavor of the yoghurt followed the same trend as the colour. There was a reduction in  
466 the acceptance of the enriched yoghurt as the percentage of passion fruit juice added increased.  
467 Samples lp1 (8.05), ks1 (7.20), ls1 (8.20) and ls2 (7.50) compares well with the control (NY =  
468 7.65) and there is no significant ( $p > 0.05$ ) difference between them.

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

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

477 The overall acceptability of samples kp2 (7.50), lp1 (8.00), ls1 (8.45) and ls2 (7.75)  
478 compares well with the control (NY = 7.70). Samples enriched with 10 – 20 % of passion fruit  
479 juice were most accepted. This was the basis for the selection of enriched yoghurt that underwent  
480 further analysis. Meanwhile, Sample ls1 (%) (colour = 8.75, flavor = 8.20, taste = 8.30, aftertaste  
481 = 8.00, mouthfeel = 8.15 and overall acceptability = 8.45) and Sample lp1 (colour = 8.40, flavor  
482 = 8.05, taste = 8.00, aftertaste = 7.65, mouthfeel = 7.80 and overall acceptability = 8.00) had the  
483 highest scores in all the attributes. Generally, the mean sensory scores for the whole samples  
484 compared favourably with the control (NY) in taste, colour, flavor, aftertaste, mouthfeel and  
485 overall acceptability and there were significant ( $p < 0.05$ ) differences in the evaluated attributes.

486 From Table 3, colour, flavour, taste, aftertaste mouthfeel and general acceptability decreased  
487 with increase in the proportion of passion fruit juice. This trend was also observed in the work on  
488 yoghurt flavoured with beetroot (*Beta vulgaris* l.) where the sample that had 90 ml yoghurt and  
489 10 ml beetroot had the highest score (colour = 7.65, flavour = 6.50, taste = 7.15, aftertaste =  
490 5.65, mouthfeel = 6.75, general acceptability = 7.60) and the sample that contained 50 ml  
491 yoghurt and 50 ml beetroot had the least score (colour = 5.25, flavour = 5.15, Taste = 4.65,  
492 mouthfeel = 5.30, overall acceptability = 6.16) [37]. Also, in the work on yoghurt flavoured with  
493 solar-dried bush mango (*Irvingia gabonensis*) pulp where the sample that was flavoured with  
494 0.80% dried bush mango had the highest score (colour = 6.90, flavour = 7.30, aftertaste = 6.75,  
495 mouthfeel = 6.45 and overall acceptability = 6.75) and sample flavoured with 4.80% dried bush  
496 mango had the highest score (colour = 4.20, flavour = 4.30, aftertaste = 4.25, mouthfeel = 3.70  
497 and overall acceptability = 4.00) [40]. However, there was no significant ( $p > 0.05$ ) difference in  
498 colour of plain yoghurt (NY = 8.00) and samples (both the Kenyan and local, skin and pulp)  
499 containing 10 % passion fruit juice (kp1 = 8.15, lp1 = 8.40, ks1 = 8.40, ls1 = 8.75). The result  
500 obtained on yoghurt flavoured with fresh and dried cashew (*Anacardium occidentale*) apple pulp

501 observed the same trend in the colour of the flavoured (8.20) and unflavoured (6.95) yoghurt  
502 [41].

### 503 **3.3 Nutritional Composition of passion fruit juices (From skin and pulp)**

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

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

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

529 The concentration of tannin in the passion fruit juice samples as seen in table 4 was in  
530 trace amount. Samples (lp = 0.021, kp = 0.011, ls = 0.008, ks = 0.004) samples was negligible.  
531 This result is slightly lower than the result (0.070 mg/100g) obtained on passion fruit juice (pulp)  
532 [20]. The work on passion fruit (skin) had a higher amount of 0.17 of tannin which is a negligible  
533 amount [46]. The phenolic content in the passion fruit samples were lp = 0.02, kp = 0.03, ls =  
534 0.01, ks = 0.01. The researchers obtained a phenolic content value of 4.20 which is higher than  
535 the result of Table 4 [46]. The lower value of phenolic content could probably be due to leaching  
536 and dilution effect of the phytochemical into the medium (water).

### 537 **3.4 Physicochemical composition of formulated yoghurt enriched with Two Accessions of** 538 **passion fruit**

539 Table 5 shows the physicochemical composition of enriched yoghurt using passion fruit juices  
540 (skin and pulp). There was significant ( $p < 0.05$ ) difference in the pH value between the enriched  
541 samples and control. No significant ( $p > 0.05$ ) difference was observed between samples (lp1 =  
542 4.23, lp2 = 4.18, kp2 = 4.20) enriched with passion fruit pulp (both for Kenyan and local specie).  
543 This trend was also observed between the samples (ls2 = 4.34, ks1 = 4.37) enriched with passion  
544 fruit skin (both for Kenyan and local specie). The control NY (4.24) had a higher pH value than  
545 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)**

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

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

548 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=

549 Kenyan Specie of passion fruit juice from skin

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

556 The values observed in this study are comparable with researchers [47, 48, 49]. All the  
 557 same pH results are in accordance with FDA specifications for the pH of yoghurt (4.6 or lower).  
 558 Also, it is the range of Standards Organisation of Nigeria [50] specification for pH of yoghurt  
 559 (3.7 - 4.5).

560 Generally, there was significant ( $p < 0.05$ ) difference in the titratable acidity value  
 561 between the enriched samples and control. However, No significant ( $p > 0.05$ ) difference was  
 562 observed between the enriched samples (ls1 = 0.45, ls2 = 0.50, lp1 = 0.52, lp2 = 0.54, kp2 =  
 563 0.72) except for sample ks1 which is not significantly ( $p > 0.05$ ) different with the control (NY =  
 564 0.47). The titratable acidity of yoghurt and enriched yoghurt are shown in Table 5. Samples kp2  
 565 (0.72) had the highest titratable acidity while sample ks1 (0.14) had the lowest titratable acidity.  
 566 The values obtained for titratable acidity are generally below the standard which is 0.7% [51]  
 567 except for sample kp2 (0.72). No direct relationship was observed between pH values and  
 568 titratable acidity as has been previously reported [47, 49].

569 There was significant ( $p < 0.05$ ) difference in the total acid value between the enriched  
 570 samples and control. The enriched yoghurts contained more total solids than the plain yoghurt.  
 571 Other researchers reported values for fruit and natural yoghurts ranging from 15.0 - 22.8 % and  
 572 13.6 – 18.8 %, respectively [47]. The total solid of yoghurts enriched with passion fruit pulp  
 573 were within the range.

574  
 575 **Table 5: Physicochemical composition of plain yoghurt and yoghurt enriched with passion**  
 576 **fruit juice (pulp and skin)**

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

577 Values are means ± standard deviation of triplicate determinations. Means with different  
 578 superscripts in the same column are significantly ( $p < 0.05$ ) different. NY= unflavoured yoghurt;  
 579 ls 1= Yoghurt + passion fruit skin local (90:10); YPF1s 2= Yoghurt + passion fruit skin local  
 580 (80:20); ks1 = Yoghurt + passion fruit skin Kenya (90:10); lp 1 = Yoghurt + passion fruit pulp  
 581 local (90:10); lp 2= Yoghurt + passion fruit pulp local (80:20); kp2 = Yoghurt + passion fruit  
 582 pulp kenya (80:20).

584 **3.5 Proximate composition (%) of plain yoghurt and formulated yoghurt enriched with**  
585 **passion fruit**

586 Table 6 shows the proximate composition (%) of enriched yoghurt. There was significant  
587 ( $p < 0.05$ ) difference in the moisture content value between the enriched samples and control. No  
588 significant ( $p > 0.05$ ) difference was observed between samples ( $lp1 = 68.59\%$ ,  $lp2 = 68.29\%$ )  
589 enriched with passion fruit pulp (local specie). This trend was also observed between the samples  
590 ( $ls1 = 80.46\%$ ,  $ls2 = 79.72\%$ ) enriched with passion fruit skin (local specie). The high moisture  
591 content of the product could be as a result of dilution (reconstitution) of milk prior to  
592 fermentation.

593 For the fat content, There was significant ( $p < 0.05$ ) difference in the value between the enriched  
594 samples and control. No significant ( $p > 0.05$ ) difference was observed between samples ( $ks2 =$   
595  $3.12\%$ ,  $ks1 = 3.14\%$ ) flavoured with passion fruit skin (both local and Kenyan specie). The fat  
596 content of yoghurt could be attributed to the oil content of milk which was the major substrate of  
597 the yoghurt produced. This corresponds with work that the fat level of yoghurt depends on oil  
598 content of milk whether skimmed or full cream milk [52]. He stated categorically that yoghurt  
599 manufactured from skimmed milk would likely have very low fat content (within range of 1 - 2  
600 %) while that produced from full cream milk would have fat content in the region of 4 %. Since  
601 full cream milk was used in the yoghurt production, fat content of the yoghurt sample (Table 6)  
602 was within the acceptable range (4%) for high fat yoghurt hence it corresponds with the work of  
603 other researchers [52].

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

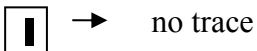
612 Table 6 shows that there were significant ( $p < 0.05$ ) differences in the protein value  
613 between the flavoured samples and control. No significant ( $p > 0.05$ ) difference was observed  
614 between samples ( $ks1 = 3.86\%$ ,  $lp1 = 3.93\%$ ,  $lp2 = 3.94\%$ ). The protein contents of flavoured  
615 yoghurt were between 3.52 ( $ls2$ ) and 3.94 % ( $lp2$ ). The sample NY (control) contains 2.81 %  
616 protein. This result compared favourably with other results which reported protein content for  
617 yoghurt as 3.5% [4]. The result did not compare favourably with the result (9.97 %) [1] but  
618 corresponds with work done on effect of different concentration of fruit additives on some  
619 physicochemical properties of yoghurt during storage [54]. The work reported protein content for  
620 plain yoghurt as 3.41 % and flavoured yoghurt as 4.01. Other researchers obtained the value  
621 (4.30) slightly higher [55] than formulated yoghurt enriched with passion fruit juice in this work.  
622 There was significant ( $p < 0.05$ ) difference in the moisture content value between the enriched  
623 samples and control. No significant ( $p > 0.05$ ) difference was observed between samples  $lp1$  and  
624  $lp2$  (24.03 and 23.97 % respectively) enriched with passion fruit pulp (local specie).  
625 Carbohydrate is the major constituent of milk that is converted to lactic acid during yoghurt  
626 production. The conversion of lactose to lactic acid accounts for low carbohydrate content of  
627 yoghurt. Yoghurt enriched with passion fruit juice from pulp had higher carbohydrate content

628 than yoghurt enriched with passion fruit juice from skin. This could probably be due to the  
629 higher carbohydrate content in the pulp compared to the skin.

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

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

632 Values are means ± standard deviation of triplicate determinations. Means with different  
633 superscripts in the same column are significantly ( $p < 0.05$ ) different. NY= unflavoured yoghurt;  
634 ls 1= Yoghurt + passion fruit skin local (90:10); YPFIs 2= Yoghurt + passion fruit skin local  
635 (80:20); ks1 = Yoghurt + passion fruit skin Kenya (90:10); lp 1 = Yoghurt + passion fruit pulp  
636 local (90:10); lp 2= Yoghurt + passion fruit pulp local (80:20); kp2 = Yoghurt + passion fruit  
637 pulp kenya (80:20).

638  → no trace

639

### 640 **3.6 Phytochemical composition of plain yoghurt and formulated yoghurt enriched with** 641 **passion fruit (pulp and skin)**

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

652

### 653 **3.7 Microbiological count of plain yoghurt and formulated yoghurt enriched with passion** 654 **fruit juice (pulp and skin)**

655 Table 8 shows the microbial load of formulated yoghurt enriched with passion fruit using passion  
656 fruit (pulp and skin). The total viable count of the microbiological analysis of the yoghurt and  
657 enriched yoghurt samples ranges from  $7.72 \times 10^2$  cfu/ml to  $5.67 \times 10^5$  cfu/ml. High bacteria  
658 count was expected because of the presence of starter cultures, mainly lactic acid bacteria [56].

659 The standard count is  $10^6 - 10^7$  cfu/ml [57, 58]. Very high count however is used as an indication  
 660 of post-pasteurization contamination [59]. The plain yoghurt, sample NY (control) had higher  
 661 viable count ( $5.67 \times 10^5$ ) than enriched yoghurts. Microorganisms used as starter culture may  
 662 have contributed to the total viable count of the yoghurt samples. Passion fruit is typically an  
 663 unexplored tropical fruit that has anti-bacterial activity [60]. Studies also show that passion fruit  
 664 are natural antioxidant. The fruit skin or peel has higher antioxidant activity as compared to the  
 665 pulp [61, 62, 63]. According to other researchers in their research on antimicrobial activity of  
 666 pineapple and passion fruit juice reported that bacteria were relatively resistant to antibiotic but  
 667 sensitive to fruit juices [60]. The antibacterial activity of the passion fruit may have led to the  
 668 decrease of viable count in enriched yoghurt.  
 669 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)  
 670 and enriched yoghurts (sample ls1 =  $8.27 \times 10^2$  cfu/ml and sample ls2 =  $1.58 \times 10^3$  cfu/ml).  
 671 These values are above the limits stipulated [57, 59]). Yoghurt enriched with passion fruit pulp  
 672 lp1 ( $0.33 \times 10^1$  cfu/ml), lp2 (not detected), kp2 (not detected) conformed to the standard [57].  
 673 According to Codex Alimentarius [57], yoghurt should contain no greater than 1 yeast cell per  
 674 gram (10 cfu/ml). High counts of yeast and mould have also been reported in yoghurts [64, 65,  
 675 66, 67]. Fruit purees added to yoghurt are usually the main source of moulds and yeast due to the  
 676 dry ingredients (sugar) and fruits [68]. Also, it was stated that *Talaromyces* spp might be present  
 677 in fruit flavoured yoghurt [69]. Sample PY (control) had the highest mould count  $2.47 \times 10^3$   
 678 cfu/ml. This might be due to insufficient hygiene practices during processing by the produces.  
 679 Other researchers also added that the fungal contamination might occur during transformation  
 680 processes and/or packaging, storage, transport and sale [70].  
 681 The lactic acid bacteria of the yoghurt were least in sample ls2 ( $0.33 \times 10^1$  cfu/ml). Passion fruit  
 682 have anti-bacterial properties and could have rendered some lactic acid bacteria in the yoghurt  
 683 non-viable. The mould count in sample ls2 was relatively high and could have also suppressed  
 684 some of the lactic acid bacteria in yoghurt.

685  
 686 **Table 7: Phytochemical composition of plain and formulated yoghurt enriched with passion**  
 687 **fruit juice (skin and pulp)**

Samples	Tannin ( $\mu\text{g/g}$ )	Phenolic content (mg/g)
ls 1	$0.024^{abc} \pm 0.00012$	$0.09^c \pm 0.00058$
ls 2	$0.020^{abcd} \pm 0.00012$	$0.06^g \pm 0.00153$
Ks 1	$0.016^{abcde} \pm 0.00006$	$0.08^e \pm 0.00153$
lp 1	$0.028^{ab} \pm 0.00012$	$0.10^a \pm 0.00058$
lp 2	$0.027^a \pm 0.00012$	$0.10^b \pm 0.00153$
Kp 2	$0.015^{abcde} \pm 0.00012$	$0.06^f \pm 0.00200$
NY (control)	$0.013^{bcde} \pm 0.00006$	$0.06^f \pm 0.00115$

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

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### 3.8 Micronutrient content of formulated yoghurt enriched with passion fruit juice (pulp and skin)

698 Table 9 shows the micronutrient content of plain and enriched yoghurt. Yoghurt contains high  
699 amount of minerals. The result justifies the assertion that yoghurt is a very good source of  
700 essential mineral needed for human metabolism or functionality of cells [71]. Addition of  
701 passion fruit caused an increase in the mineral content. The mineral contents of the enriched  
702 yoghurts revealed that samples enriched with pulp juice (local specie) had highest mineral  
703 content (Table 9). That is samples lp2 and lp1 had the highest mineral content. Sodium,  
704 potassium and phosphorus content were the highest (209.31 mg/100g, 209.81 mg/100g and 38.10  
705 mg/100g respectively) in sample lp2 while calcium and magnesium content were the highest  
706 (2395.65 mg/100g and 135.94 mg/100g respectively) in sample lp1. There was a slight  
707 difference in sample kp2 for all mineral content analysed. Sample kp2 contains passion fruit pulp  
708 but its value is lower than the yoghurt sample containing passion fruit skin. This could be as a  
709 result of the microbial load as it contains a very high microbial load.

710 The microorganisms might have utilized the nutrients thereby reducing the value. Samples ls2  
711 and kp which had the highest microbial load of all the enriched yoghurt samples ( $1.82 \times 10^4$  and  
712  $1.05 \times 10^4$  cfu/ml) and they both had the least mineral contents.

713 There were significant ( $p < 0.05$ ) difference in the sodium value between the enriched samples  
714 and control. No significant ( $p > 0.05$ ) difference was observed between samples (lp1 = 202.66  
715 mg/100g and lp2 = 209.31 mg/100g) enriched with passion fruit pulp (for local specie). No  
716 significant ( $p > 0.05$ ) difference was also observed between some enriched samples (ls2 = 161.21  
717 mg/100g, ks1 = 169.48 mg/100g, kp2 = 166.88 mg/100g) and the control (NY = 168.24  
718 mg/100g). The sodium content of the yoghurt sample ranged from ls2 = 161.21 to lp2 = 209.31  
719 mg/100g. Samples lp2 and lp1 had the highest sodium content (209.31 mg/100g and 202.66  
720 mg/100g), respectively. Also, sodium content of the passion fruit ranged from ks1 = 29.20 to lp  
721 = 46.18 mg/100g.

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

728 There was significant ( $p < 0.05$ ) difference in the potassium value between the enriched samples  
729 and control. No significant ( $p > 0.05$ ) difference was observed between samples (lp1 = 204.46  
730 mg/100g and lp2 = 209.81 mg/100g) enriched with passion fruit pulp (for local specie).  
731 Similarly, no significant ( $p > 0.05$ ) difference was observed between samples (ls1 = 177.42  
732 mg/100g, ks1 = 171.42 mg/100g) and the control (NY = 178.03 mg/100g). The potassium  
733 content of the yoghurt samples ranged from ls2 = 166.64 mg/100g to lp2 = 209.81 mg/100g.  
734 Samples lp2 and lp1 had the highest potassium content (209.81 mg/100g, 204.46 mg/100g),  
735 respectively. Also, potassium content for passion fruit samples ranged from ks = 30.03 mg/100g  
736 to lp = 476.02 mg/100g.

737 The passion fruit juice from pulp had higher potassium content than passion fruit juice from skin  
738 (refer to table 4) and as such the yoghurt flavoured with passion fruit pulp contained higher  
739 potassium content (lp1 = 204.46 mg/100g, lp2 = 209.81 mg/100g and kp2 = 165.15 mg/100g,

740 respectively) than the samples containing passion fruit skin (ls1 = 177.42 mg/100g, ls2 = 166.64  
741 mg/100g and ks = 171.42 mg/100g, respectively).

742 The potassium content obtained in the control (plain yoghurt sample) NY = 178.03 mg/100ml  
743 were slightly lower than the optimum figures (280 mg/100g) [73]. The result obtained in this  
744 work is lower than the result (561.42 mg/100g) [72] and higher than the results of other  
745 researchers (109.55 mg/100g) [38].

746  
747 **Table 8: Microbiological count of plain yoghurt and yoghurt enriched with passion fruit**  
748 **using passion fruit (pulp and skin)**

SAMPLES	TVC (cfu/ml)	LAB (cfu/ml)	Mould (cfu/ml)
ls 1	$4.54 \times 10^3$	$2.37 \times 10^3$	$8.27 \times 10^2$
ls 2	$1.82 \times 10^4$	$0.33 \times 10^1$	$1.58 \times 10^3$
Ks 1	$7.72 \times 10^2$	$2.90 \times 10^2$	Not detected
lp 1	$2.64 \times 10^3$	$1.53 \times 10^3$	$0.33 \times 10^1$
lp 2	$5.62 \times 10^2$	$4.98 \times 10^2$	Not detected
Kp 2	$1.05 \times 10^4$	$7.85 \times 10^3$	Not detected
NY (control)	$5.67 \times 10^5$	$1.50 \times 10^3$	$1.20 \times 10^3$

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

755  
756 There was significant ( $p < 0.05$ ) difference in the calcium value between the enriched samples and  
757 control. The calcium content of the yoghurt samples ranged from YPFIs2 = 433.04 mg/100g to  
758 YPFIs1 = 2395.65 mg/100g. Samples YPFIs2 and YPFIs1 had the highest calcium content of  
759 1448.10 mg/100g and 2395.65 mg/100g respectively. The passion fruit juice from pulp lp (local  
760 specie) had higher calcium content than passion fruit juice from skin but passion fruit juice from  
761 pulp kp (Kenya specie) had lower calcium content than passion fruit juice from skin (refer to  
762 table 4). Yoghurt enriched with passion fruit juice from pulp contained higher calcium content  
763 (samples lp1 = 2395.65 mg/100g, lp2 = 1448.10 mg/100g and kp2 = 396.25 mg/100g  
764 respectively) than the samples containing passion fruit juice from skin (ls1 = 924.62 mg/100g,  
765 ls2 = 433.04 mg/100g and ks1 = 779.26 mg/100g, respectively). The calcium content of passion  
766 fruit did not follow a similar trend like potassium and sodium. The calcium content of the  
767 passion fruit juice from skin was higher than passion fruit juice from pulp (Kenya specie). The  
768 calcium content of the passion fruit juice from skin was lower than passion fruit juice from pulp  
769 (local specie) (of table 4). The calcium content obtained in the control NY = 178.03 did not  
770 compare favourably with result (200 mg/100g) reported by Dairy council (2013). The result  
771 obtained in this work was higher than the result (281.43 mg/100g) [72] and the result (111.69  
772 mg/100g) [38]. Again, the work of other researchers (16.99 – 62.14 mg/100g) was lower than  
773 the result obtained in this work [39].

774 There was significant ( $p < 0.05$ ) difference in the magnesium value between the enriched  
775 samples and control. The magnesium content of the yoghurt samples ranged from ls2 = 58.84

776 mg/100g to ls1 = 135.94 mg/100g. Samples lp2 and lp1 had the highest magnesium content  
777 (115.38 mg/100g and 135.94 mg/100g respectively). The passion fruit juice from pulp had higher  
778 magnesium content than passion fruit juice from skin (refer to table 4). The yoghurt enriched  
779 with passion fruit juice from pulp contains higher magnesium content (samples lp1 = 135.94  
780 mg/100g, lp2 = 115.38 mg/100g and kp2 = 53.35 mg/100g) than the samples containing passion  
781 fruit juice from skin (samples ls1 = 75.75 mg/100g, YPFIs2 = 58.84 mg/100g and YPFks = 70.36  
782 mg/100g). The magnesium content obtained in this work did not compare favourably with the  
783 result (0.17 to 4.20 mg/100g) [37]. The result obtained in this work is higher than the result  
784 (23.52 mg/100g) [38].

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

793 The phosphorus content of yoghurt without passion fruit flavour (control, NY = 11.02 mg/100g)  
794 was higher than that containing passion fruit flavour with the exception of samples lp1 (30.53  
795 mg/100g) and lp2 (38.10 mg/100g). This indicates that the phosphorus content reduced with the  
796 addition of the passion fruit flavour. This trend was observed in the work done [40] on yoghurt  
797 flavoured with solar-dried bush mango (*Irvingia gabonensis*) pulp, where the phosphorus content  
798 of the unflavoured yoghurt (7.91 mg/100g) was higher than the yoghurt containing the bush  
799 mango flavour (1.90 mg/100g, 1.20 mg/100g and 1.364 mg/100g at 3.20 %, 0.80 % and 1.60 %  
800 respectively). The reduction in phosphorus content of the enriched yoghurt could be as a result of  
801 presence of phytochemicals or anti-nutrients which may have interfered with the bio-availability  
802 of phosphorus. The result obtained in this work for phosphorus content is lower than the result  
803 (202.25 mg/100g) obtained [72] and the result (114.08 mg/100g) obtained by [38]. The  
804 recommended (81 mg/100g) [73] for drinkable yoghurt is higher than the result obtained in this  
805 project. The phosphorus content obtained in the control NY = 31.24 did not compare favourably  
806 with result (170 mg/100g) [73]. Again, the work (32.44 – 73.59 mg/100g) done by other  
807 researchers [39] is slightly higher than the result obtained in this work

808 A similar trend observed in mineral content was also found in vitamin C content of the  
809 samples. Addition of passion fruit caused an increase in vitamin C. Similar trends were recorded  
810 by other researchers (52, 40, 1]. Addition of passion fruit flavour caused increase in yoghurt  
811 enriched with passion fruit juice. There was no significant difference between the yoghurt  
812 enriched with passion fruit juice from skin (ls1 = 18.30 and ks = 18.29) and control (NY = 18.86).  
813 However, It was also observed that samples YPFIs2 (Vitamin A = 14.96  $\mu\text{g}/100\text{g}$ , Vitamin C =  
814 5.58 mg/g) had slightly lower values when compared to other yoghurt samples enriched with  
815 passion fruit skin and sample kp2 (Vitamin A = 18.84  $\mu\text{g}/100\text{g}$ , Vitamin C = 6.40 mg/g) had  
816 slightly lower values when compared to other yoghurt enriched with passion fruit juice from  
817 pulp. This could be as a result of the microbial load as it contains a very high microbial load. The  
818 microorganisms might have utilized the nutrients, thereby reducing the vitamin content. Samples  
819 YPFIs2 and YPFkp contained the highest

820

821 **Table 9: Micronutrient content of plain yoghurt and yoghurt enriched with passion fruit juices (pulp and skin).**

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

822 Values are means ± standard deviation of triplicate determinations. Means with different superscripts in the same column are  
 823 significantly ( $p < 0.05$ ) different. NY= unflavoured yoghurt (negative control); ls 1= Yoghurt + passion fruit skin local (90:10); YPFIs  
 824 2= Yoghurt + passion fruit skin local (80:20); ks1 = Yoghurt + passion fruit skin Kenya (90:10); lp 1 = Yoghurt + passion fruit pulp  
 825 local (90:10); lp 2= Yoghurt + passion fruit pulp local (80:20); kp2 = Yoghurt + passion fruit pulp kenya (80:20).

826

827 microbial load of all the flavoured yoghurt samples ( $1.82 \times 10^4$  and  $1.05 \times 10^4$  cfu/ml  
828 respectively) and had minimal vitamin C content.

829 There was a significant ( $p < 0.05$ ) difference in the vitamin A value between the flavoured  
830 samples and control. No significant ( $p > 0.05$ ) difference was observed between samples (YPFfp1  
831 = 20.67  $\mu\text{g}/100\text{g}$ , YPFfp2 = 21.16  $\mu\text{g}/100\text{g}$ ) flavoured with passion fruit pulp (for local specie).  
832 No significant ( $p > 0.05$ ) difference was also observed between some flavoured samples (YPFfs1  
833 = 18.30  $\mu\text{g}/100\text{g}$ , YPFks = 18.29  $\mu\text{g}/100\text{g}$ , YPFkp = 18.84  $\mu\text{g}/100\text{g}$ ) and the controls (NY =  
834 18.86  $\mu\text{g}/100\text{g}$ ). The vitamin A content of the yoghurt samples ranges from YPFfs2 = 14.96  
835  $\mu\text{g}/100\text{g}$  to PY = 24.78  $\mu\text{g}/100\text{g}$ . The positive control (PY) had the highest vitamin A content  
836 (24.78  $\mu\text{g}/100\text{g}$ ). Also, the vitamin A content of passion fruit samples ranged from PFks = 1.19  
837 to PFkp = 1.91  $\mu\text{g}/100\text{g}$ . The passion fruit pulp had higher vitamin A content (PFfp = 1.58  
838  $\mu\text{g}/100\text{g}$ , PFkp = 1.91  $\mu\text{g}/100\text{g}$ ) than passion fruit skin (samples PFfs = 1.34  $\mu\text{g}/100\text{g}$ , PFks =  
839 1.19  $\mu\text{g}/100\text{g}$ ) and as such the yoghurt flavoured with passion fruit pulp contains higher vitamin  
840 A content (samples YPFfp1 = 20.67, YPFfp2 = 21.16 and YPFkp = 18.84  $\mu\text{g}/100\text{g}$ ) than the  
841 samples containing passion fruit skin (samples YPFfs1 = 18.30, YPFfs2 = 14.96 and YPFks =  
842 18.29  $\mu\text{g}/100\text{g}$ ). The result of the vitamin A content of the yoghurt samples obtained was lower  
843 than that reported (70.04 RE) [40] which had 70.04 RE for unflavoured yoghurt and 175.11,  
844 44.20 and 70.04 RE at 3.20, 0.80 and 1.60 % respectively for yoghurt flavoured with bush  
845 mango but it is within range (59.68 IU converted to 17.90  $\mu\text{g}/100\text{g}$ ) with that reported [72].

846 There was significant ( $p < 0.05$ ) difference in vitamin C value between the flavoured  
847 samples and control. The vitamin C content of the yoghurt samples ranges from 5.58 mg/g to  
848 7.53 mg/g. Yoghurt flavoured with passion fruit pulp juice had higher vitamin C content (lp1 =  
849 7.29 mg/g, lp2 = 7.53 mg/g, kp2 = 6.40 mg/g) than yoghurt flavoured with skin juice (ls1 = 7.13,  
850 ls2 = 5.58 and ks = 7.15 mg/g). Sample lp1 and lp2 had the highest Vitamin C content (7.29 and  
851 7.53 mg/g). The result obtained [1] for vitamin C were 3.90 mg (plain yoghurt), 4.01 mg  
852 (yoghurt spiced with pepper fruit), 3.91 mg (yoghurt spiced with ginger), 4.25 mg (yoghurt  
853 flavoured with carrot), 4.48 mg (yoghurt flavoured with pineapple).

#### 854 4. CONCLUSION AND RECOMMENDATION

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

861 The utilization of passion fruit as a natural flavouring agent improved the nutritional properties  
862 of the product. The enriched yoghurt contained higher protein content than the unflavoured  
863 yoghurt. The fat and carbohydrate contents were lower in enriched yoghurt and higher in  
864 unflavoured yoghurt making it an ideal drink for obese or weight conscious individuals. The  
865 samples enriched with passion fruit pulp had more minerals and vitamins than those flavoured  
866 with passion fruit skin. The high nutrient content of the enriched yoghurt makes it a very  
867 nutritious and healthy drink. The phytochemicals (tannins and phenolic content) in the product  
868 were in trace amount and hence makes it an ideal drink for all classes of people in the world:  
869 children, aged, sick, pregnant women and among others. Yoghurt enriched with 10 – 20%  
870 passion fruit pulp conformed to the standard stated in *Codex alimentarius* for yoghurt, thereby  
871 establishing the fact that it is safe and healthy for human consumption.

872 Based on the study, the research on passion fruit flavoured yoghurt (especially those flavoured  
873 with pulp juice) at commercial level is highly recommended. Passion fruit should be included in  
874 the wide range of fruit used to flavor yoghurt as the result obtained in this piece of work had  
875 shown it to add to the nutritional content of yoghurt. It is also recommended that information on  
876 the production of yoghurt be disseminated to domestic and commercial manufacturers of  
877 yoghurts. It is very necessary that further work should be done where passion fruit maybe  
878 incorporated in the yoghurt formulation before fermentation. There is need for further studies on  
879 other minerals (iron and zinc) and vitamins B (B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>6</sub> and B<sub>12</sub>). The storage stability of  
880 the formulated product should be investigated.

881

## 882 **COMPETING INTERESTS**

883 Authors have declared that no competing interests exist.

884

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