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PRODUCTION AND QUALITY EVALUATION OF YOGHURT FLAVOURED WITH BLACK VELVET TAMARIND (*Dailium guineense*)

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ABSTRACT
Yoghurt was produced and flavoured by blending with 0.2, 0.4, 0.6, 0.8, and 1.0 g black velvet tamarind powder (Dialium guineense) in ten different samples, five flavoured before fermentation (VTB1, VTB2, VTB3, VTB4 and VTB5) and five flavoured after fermentation (VTA1, VTA2,VTA3,VTA4 and VTA5) while VTO was coded as control. The black velvet tamarind flavoured yoghurt was subjected to proximate, micronutrients, microbial and sensory evaluation using standard procedures. Data obtained was subjected to statistical analysis. Results showed that the moisture content of the samples decreased with increased level of the black velvet tamarind powder both before and after fermentation. The ash content ranged from 0.04 to 1.72% in the samples flavoured after fermentation while the highest value for samples flavoured before fermentation was 1.04% and the concentration increased with increase in concentration both before and after fermentation. The fat content increased with increase in concentration. Protein content ranged from 6.48 to 9.31%, and there was a significant ($p<0.05$) different between the control sample and the formulated samples. The carbohydrate content increased as the concentration of the black velvet tamarind powder increased before and after fermentation. The vitamin C content increased as the concentration increased which differed significantly ($p<0.05$) between the control and the formulated samples. The vitamin A content ranged from 11.09 to 74.17 IU and fermentation favoured the increased concentration of the vitamin A. The samples flavoured before fermentation increased calcium and phosphorus. The total viable count ranged from 1.4×105 (control) to 2.9×105 cfu/ml (VTA1). The lactic acid bacteria ranged from 1.1×105 (VTA5) to 2.6×105 cfu/ml (VTA1). The most acceptable flavoured yoghurt by the panellists contained 0.2 g black velvet tamarind in samples before and after fermentation.

1. Introduction

Dairy foods like yoghurt have been consumed since ages as almost complete foods to meet body energy requirements (Santosh *et al.,* 2018). Yoghurt is one of the essential cultured milk products which have been in use long before the recognition of its nutritious and health value. With time, yoghurt has been continually modified to obtain a more desirable product. The modification, however, has been impacting on the flavour and aroma component of the food. The popularity of yoghurt as a food component depends mainly on its sensory characteristics, of which aroma and flavour are most important (Weerathilake *et al*., 2014). Sensory appeal also is one of the essential associated with market success of fermented product like yoghurt (Getenesh *et al*., 2017). The odour and taste of soured milk products are characterized by numerous volatile bacterial metabolites, some of which are by-products of lactic acid fermentation or are produced by other reaction mechanisms. Lactic acid itself is suggested to be one of the significant compounds significantly contributing to yoghurt flavour. More than 90 flavour compounds have been identified so far (Winny & Mishra, 2011). Kaminarides *et al*. (2007) reported that the aroma and taste of yoghurt are mainly because of the presence of nonvolatile or volatile acids and carbonyl compounds, and especially the group of carbonyl compounds is believed to have a significant influence on the final yoghurt aroma due to their relatively higher concentrations. The most critical aromatic components are acetaldehyde, acetone, acetoin, and diacetyl in addition to acetic, formic, butanoic, and propanoic acids. The typical aroma of yoghurt is characterised chiefly by acetaldehyde, so it is suggested as a significant flavour compound.

The flavour is a critical factor for food stuff acceptability by consumers. Organoleptic evaluations have shown a marked preference for the fruity yoghurt. Addition of different fruit in yoghurt manufacture has been attempted increasingly. Use of fruit in yoghurt makes it more delicious. Fruit yoghurt has more taste and pleasant flavour (Mbaeyi & Anyanwu, 2010). The pectin and sugars from the fruit are mixed with the yoghurt, causing an increase in its consistency and viscosity and therefore, mouth-feel is improved. Pectins added to acidified dairy products to avoid syneresis. It adsorb on casein reversibly, inducing an increase in the steric repulsion and thus decreased their aggregation (Nongonierma *et al*., 2007).

The introduction of various fruit-flavoured yoghurts has significantly contributed to the consumption of yoghurt from all ages. Fruits may be added to yoghurt formulae as single or blends in the form of refrigerated, frozen, canned fruit, juice or syrup. Most common exotic fruits used in yoghurt formulae are peach, cherry, orange, lemons, purple plum, boysen-berry, spiced apple, apricot, pineapple, strawberry, raspberry and blueberry. The incorporation of fruits endorses the good image of yoghurts (Amal *et al.,* 2016). Thereby increasing dependency on exotic flavours, which are expensive leading to a subsequent increase in the cost of yoghurt production.

On that note, black velvet tamarind fruit pulp which is reported to have a unique sour taste due to the natural occurrence of sugars and plant acids together possesses characteristic flavour similar to that of yoghurt and should probably enhance yoghurt flavour (Obasi *et al.,* 2013). Black velvet tamarind (*Dialium guineense*) is a woody plant that grows better in the rain forest region of West Africa. It grows up to the height of 30m with dark blue glossy leaves each measuring 5cm to 8cm long and 2.5cm wide. It produces fruit seasonally, normally between January and May but the peak of harvest is March and April. The pulp is called Icheku or Nchinchi in south-eastern part of Nigeria and Awin in south-western part (Obasi *et al.,* 2013). There are three species of this plant which includes: *Dialium dinklagei, Dailium pachyphyllum* and *Dailium guineense* (Onwuka *et al*., 2010). *Dailium guineense*

belong to the leguminosae family and has small typically grape sized edible fruits with brown hard inedible shells (Okudu *et al*., 2017). Apart from its natural taste, it also contains some essential minerals and vitamins especially rich in vitamin C, which are also the basic body requirements (Obasi *et al.*, 2013) despite its nutritive quality, it is underutilized in Nigeria. It also contains some essential minerals such as magnesium and calcium and other health benefits, including antioxidant effect (Nguyen, 2015).

Plate 1: A maturing black velvet tamarind pulp. Plate 2: A matured velvet tamarind pulp.
Source: Asoiro *et al.* (2017).

The mean length, width and thickness of unshelled, shelled and kernel of the fruit are 0.0174, 0.016 and 0.0081 m; 0.015, 0.01 and 0.0052 m; and 0.0077, 0.0072 and 0.0036 m respectively. The fruit kernel has the highest sphericity and aspect ratio values of 0.7594 and 0.94 respectively, when compared with the unshelled which have values of 0.7520 and 0.926 m respectively; and the shelled which had values of 0.6132 and 0.674 m respectively. The porosity of the unshelled (12.471%), shelled (4.049%) and kernel (18.066%) is generally low. Natural convection is not advisable during aeration or drying, based on this observation. Individual unshelled fruits are denser than water, consequently would quickly sink in water. This makes separation of the unshelled fruit from the shelled, kernel and other contaminants less dense than water possible, during separation. The mean surface area of unshelled *Dialium guineense* fruit is about two times higher than that of the shelled fruit and five folds higher than the kernel (Asoiro *et al.*, 2017). Abiodun *et al*. (2012) reported the total percentage (%) acidity as in tartaric acid of the mature fruit pulp ranges between 16.8 and 36.2% while the crude protein, crude fat, ash as well as total crude carbohydrate of mature tamarind fruit pulp ranged from 3.5 to 7.4, 3.5 % to 7.4, 3.0 to 6.9 % and 52.0 to 62.7 %, respectively. The ascorbic acid, colour and soluble solid vary between 3.7 and 11.3 %, 0.30 and 1.42 % and 5.2 and 6.4 %, respectively. The mature tamarind fruits are high in tartaric acid (pH 2.3-3.3) but low in total carotenoids, antinutrients and micronutrients. Calcium and sodium are the most abundant macro-and micro-nutrients. The variation in compositions would be attributed to the nature of the land where the plant grows. Gnansounou *et al*. (2014) also reported vitamin C, Iodine, magnesium, calcium and potassium contents of tamarind pulp in mg/100g dry weight are: 0.45, 0.43, 14.75, 30.84 and 366 mg, respectively. Okudu *et al*. (2017) observed the abundant presence of sugars in the pulp of *Dialium*

guineense makes it a right supplier of this nutrient quickly usable by cells. Its high contents in major minerals (calcium, sodium, magnesium, potassium) and minor minerals (iodine, iron) open the way for use in order to palliate minerals deficiency problems. The sticky pulp of velvet tamarind is a rich source of non-starch polysaccharides (NSP) or dietary fiber such as gums, hemicelluloses, mucilage, pectin, and tannins. The authors reported that one hundred grams of fruit pulp provided 5.1 or over 13% of dietary fiber. NSP or dietary fiber in food increased its bulk thereby, augmenting bowel movements and preventing constipation. This fiber binds toxins in the food and help to protect the colon mucosa from cancer-causing chemicals. Also, these dietary fibers in the pulp bind to bile salts (produced from cholesterol) and decrease their reabsorption in the colon as seen in the expulsion of "bad" or low-density lipoprotein (LDL) cholesterol levels from the body.

Nguyen (2015) reported to find various applications, apart from its consumption as snacks and lactating meal for mothers, it can also be utilized as a major component of beverage production It is also utilized in the food industry for the production of candy (Obasi et al., 2013). The pulp has been used in many traditional medicines as a laxative, digestive, and as a remedy for biliousness and bile disorders. It can be used as a spicy condiment and as an emulsifying agent in syrups, decoctions and different pharmaceutical products. From previous studies, it was observed that black velvet tamarind is a common ingredient in curries, "rasam," chutneys, vegetable and lentil recipes all over India and South-East Asia. Also, the pulp is used in marinades, "hot and sour" soups. The juice made of tamarind pulp combined with dates, sugar, honey, cardamom, cloves, and coriander seeds produced a refreshing drink marketed in different parts of the world. Its pulp is also employed in confectionaries as a solidifying agent. The pulp can also be used for seasoning, in prepared foods, to flavour confections, curries, and sauces, and as a major ingredient in juices and other beverages (Caluwe *et al.*, 2009). The tree is reported to be highly valued for its pulp. Apart from that, its fresh leaves have some medicinal application in a traditional setting (Nguyen, 2015).

Its utilization as a yoghurt supplement is a way of preserving its nutritional requirement in a product even beyond its season. It is also a demonstration of the suitability of the use of traditionally neglected plant product in an industrial process (Obasi *et al.,* 2013). Furthermore, black velvet tamarind pulp being naturally acidic should be able to facilitate the desired environment in the yoghurt medium. This is a way of preventing the annual harvest loss of the flesh and as well promotion of local fruit utility which is left to grow wild in most of the place it is found in Nigeria. Among the fruits used in yoghurt production from literature, none has been reported to contain more than one prominent vitamin, and that could give velvet tamarind pulp an average edge over others. So, its use in supplementing in the yoghurt production would not only increase the nutritional value but also impacts health benefits and reduces post-harvest lost. Also, its minerals and vitamins content should promote the nutritive value of the product thereby leading to the encouragement of its utility in yoghurt industry. Therefore, the broad thrust of this study was to produce and evaluate the qualities (proximate, micronutrient, sensory and microbial properties) of flavoured yoghurt using black velvet tamarind pulp.

2. Materials and Methods

Sample procurement: Skimmed milk, yoghurment (yoghurt culture) and black velvet tamarind fruit was purchased from Ogige Market Nsukka, Enugu State.

Processing of black velvet tamarind pulp (*Dialium guineense*)

The fresh fruits of the velvet tamarind were processed according to the method described by Obasi *et al*. (2013). The fresh fruits were first sorted to remove any extraneous materials and rottened fruit, it was then cleaned with water and then dried under the sun, and the shells were removed manually. The

seeds were removed and the pulp subjected to size reduction using mortar to obtain black velvet tamarind pulp powder. Black velvet tamarind pulp powder production is summarized in the Figure 1.

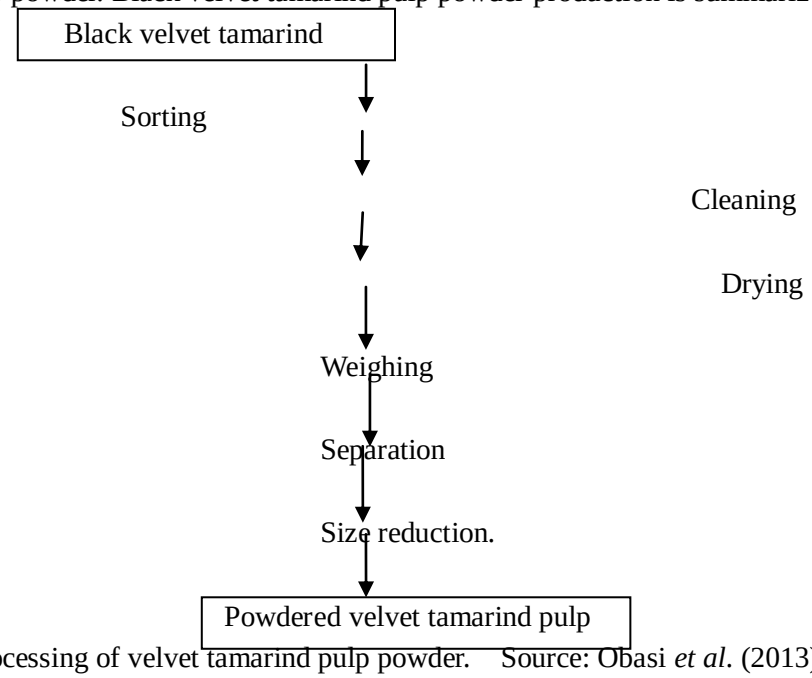

Figure 2: Processing of velvet tamarind pulp powder. Source: Obasi *et al*. (2013).

Processing of yoghurt mixes

The velvet tamarind flavoured yoghurt was prepared using the method described by Ihekoronye (1999). The powdered milk (skimmed milk) was dissolved in water equivalent of one or more litres and was used as the control. Another ten samples were prepared using the same method but with the addition of different concentrations of the powdered velvet tamarind pulp. The yoghurt mixes were then homogenized to obtain a more uniform product. It was then pasteurized at 82-85 ^{0}C for 30 minutes to destroy any unwanted microorganisms (pathogenic and spoilage microorganisms). Cooling of the product was followed to a temperature of 42±2 ^{0}C (the ideal temperature for starter culture growth). The starter culture was added to the ten formulated portions. Five samples out of the ten contained different concentrations of the black velvet tamarind powder (added before fermentation) while the other five was fermented plain but the black velvet tamarind powder was added at varying concentration after fermentation. The different formulations of the samples' concentrations are summarized in Table 1 and the samples of the yoghurt produced are shown in Plate 1.

Sample analysis

Proximate analysis: The following proximate analysis was carried out on the black velvet tamarind powder, formulated samples of the black velvet tamarind flavoured yoghurt and the control sample.

Determination of the crude protein content: The crude protein determination was achieved using the standard method (Kjeldahl method) described by AOAC (2010). This involves sample digestion, 2 ml of the sample was measured into Kjeldahl digestion flask, anhydrous barium sulphate would be added and then copper sulphate served as catalyst. Twenty five millimeters (25 ml) of concentrated tetraoxosulphate (VI) acid (H_2SO_4) (denoted by V_S) was added with few boiling chips. The flask with its content was heated in the fume chamber until a clear solution obtained. The resultant solution was cooled to room temperature after which it would be transferred into a 250 ml volumetric flask and made up to the level with distilled water. The solution was then measured into a 100 ml receiving flask (conical flask) containing boric acid. The flask was placed under condenser after adding an

indicator (methyl orange). The volume of the digest that was added to the set up was 5 ml. This was followed by addition of the 60% NaOH solution. The digestion flask was heated until a quantifiable amount of distillate would be collected (100 ml) into the receiving flask.

Titration

The solution in the receiving flask was titrated with about 0.04 M HCl to get a pink colour, and the titre value was obtained.

The crude protein would then be calculated as follows:

Crude protein of yoghurt sample = Percentage (%) nitrogen content =

$$\frac{V_S - V_B \times N_{acid} \times 0.0001410 \times 100}{\text{Weight of sample}} \quad \frac{\quad}{1}$$

Percentage (%) crude protein = % N × 6.25 (conversion factor)

Where V_S = Volume (ml) of acid required to titrate the sample; V_B = Volume (ml) of acid required to titrate the blank; N_{acid} = Normality of acid (0.1N) and 0. 01410 = Millilitre equivalent weight of nitrogen.

Table 1: Blending ratios of black velvet tamarind pulp powder for formulated flavoured yoghurt (before and after fermentation)

Samples	Black velvet tamarind flour(g)
VTO	0
VTA1	0.2
VTA2	0.4
VTA3	0.6
VTA4	0.8
VTA5	1.0
VTB1	0.2
VTB2	0.4
VTB3	0.6
VTB4	0.8
VTB5	1.0

Key: VTO = plain yoghurt, VTA = flavoured yoghurt after Fermentation,
VTB= before fermentation.

Determination of the crude fibre content: The crude fibre was determined using the method described by AOAC (2010). Five millilitres of the sample (W_3) was digested with 200 ml of 0.22 NH_2SO4. It was filtered and washed severally and transferred into another conical flask. The mixture was then dissolved in a 200 ml of 1.25 % NaOH solution, boiled for 30 minutes, cold filtered and washed with boiling water. The residue was dried at 105 ºC for 2 hours, cooled in a desiccator and weighed (W_1). It was incinerated at 550 ºC for 2 hours in a muffle furnace, cooled again in a desiccator and weighed (W_2).

The percentage of crude fibre was calculated as;

$$\% \text{ crude fibre} = \frac{W_2 - W_1}{W_3} \times \frac{100}{1}$$

Where: W_1 = weight of the sample before incineration, W_2 = weight of the sample after incineration, W_3 = weight of the original sample.

Determination of crude fat content: The fat content of the samples was determined using the standard AOAC (2010) method. A Soxhlet extractor with a reflux condenser and a 250 ml round bottom flask was fixed. Three grams of sample was placed in a thimble and petroleum ether (200 ml) was filled into the round bottom flask. The extraction thimble was sealed with cotton wool. The Soxlet apparatus after assembling would be allowed to reflux for 3 hours. The thimble was removed with care, and the petroleum ether collected on the top and drained into a container for reuse. When the flask would be free of ether, it was removed and dried at 70^{o}C for 1 hour in an oven. It was cooled in a desiccator and then weighed.

$$\text{Percentage (\%) fat content} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times \frac{100}{1}$$

Determination of moisture content: The moisture content of the samples was determined according to the hot air oven method described by the Association of Official Analytical Chemist (AOAC, 2010). The crucibles were cleaned thoroughly and afterwards dried in the oven at 110^{o}C for 1 hour. The hot dried crucibles were cooled in a desiccator and the weight was designated as W_1. Three grams (3 g) of the sample was weighed into the crucible, and the importance of the crucible with the sample was noted as W_2. The sample was then dried at 103 ^{o}C until a constant weight (W_3) is obtained.

$$\text{Percentage moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where; W_1 = initial weight of empty crucible; W_2 = weight of crucible + weight of sample before drying and W_3 = weight of crucible + weight of sample after drying.

Determination of ash content: The ash content of the samples was determined according to the standards of AOAC (2010). A preheated and cooled crucible was weighed (W_1) and 2 grams of each of the samples were weighed into two preheated cooled crucibles (W_2). The samples were charred on a Bunsen flame inside a fume cupboard. The charred sample in the crucible was then transferred into a preheated muffle furnace at 550 ^{o}C for 2 hours until a white or light grey ash was obtained (W_3). It was then cooled in a desiccator, weighed and documented.

$$\text{Ash content (\%)} = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Determination of calcium content: Calcium content was determined by titration method according to Egan *et al.* (1981). Ten millilitres of the sample was pipetted into 250 ml conical flask. Twenty-five millilitres (25 ml) of potassium hydroxide (KOH) and a pinch of calcine indicator was added and titrated against ethylene diamine tetra-acetate (EDTA) solution to the end point. The volume of EDTA is the equivalent volume of calcium in the sample.

$$\text{Percent calcium} = \frac{\text{Volume of EDTA} \times \text{atomic weight of calcium} \times 100 \times DF}{1000 \times \text{weight of sample used} \times 10}$$

Where: DF = dilution factor.

Determination of phosphorus content: Phosphorus in the sample was determined according to

Onwuka (2018) by molybdate method using hydroquinone as a reducing agent. A mixture of 1.0 ml ammonium molybdate, 1.0 ml sodium sulphate, 1.0 ml hydroquinone and 0.5 ml of the mineral digest was agitated and allowed to stand for 30 minutes. The blue colour developed was quantified using a colorimeter at 660 nm against a standard.

$$\text{Phosphorus} = \frac{\text{Absorbance of test} \times \text{dilution factor}}{W \times 5}$$

Where: W = Weight of the sample.

Determination of Vitamin A content: Vitamin A content was determined according to Prentice & Langridge (1992) procedure. Ten ml of the sample was first saponified using an alcoholic solution of potassium hydroxide in the presence of pyrogallol. The unsaponified matter containing vitamin A was extracted using a mixture of diethyl ether and petroleum spirit. The extract was evaporated under nitrogen and the residue was dissolved in methanol. The extract was chromatographed using a reverse phase octa deccyl silane (ODS) column with the mobile phase consisting of 95% acetonitrile with 5% water. The separated retinol would quantified using a UV absorbance detector at 328nm.

Determination of vitamin C content: The vitamin C content was determined using the methods described by Osborne & Voogt (1978). Two millimetres (2 ml) of each of the samples were weighed out and 100 ml of distilled water would be added to it. It was then filtered to get a clear solution. A 10 ml of the distilled volume of the solution was pipetted into a small flask containing 2.5 ml acetone. The solution was titrated with the indo-phenol solution (2, 6-dichloro indophenols) to a faint pink colour which persists for 15 seconds. The vitamin C content was calculated as follows:

Vitamin C (mg/100 ml of sample) = $20 \times V \times C$

Where V= indophenols solution in titration (ml); C = Mg (milligram) vitamin C / ml indophenols; 20 = Dilution factor.

Determination of total viable count: The total viable count test was carried out using the method described by Prescott *et al*. (2005). One millilitre (1 ml) of the sample and 9 ml of ringer solution would be used to make serial dilutions up to 10^{-3}. The diluted sample was pipetted into a marked Petri dish, 15 ml of prepared nutrient agar solution was added; the solution was swirled to mix and incubated at the temperature of about 37^{o}C for 24 hours. After incubation, the number of colonies was counted and represented as colony forming unit per gram (cfu/ml).

Determination of the lactic acid bacteria (LAB): The lactic acid bacteria (LAB) in the formulated yoghurt was determined using de gMan Rogosa Sharpe (MRS) Agar (CM 361) described by Oxoid manual (Oxoid, 1982). One millilitre of the Samples were serially diluted in duplicates using the surface pour plate method. The plates were incubated under anaerobic conditions at 37 °C for 48 hours. After incubation, the number of colonies was counted and represented as colony forming unit per milliliter (cfu/ ml).

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

Sensory evaluation of the tamarind pulp flavoured yoghurt: Twenty semi-trained panellists randomly selected from the Department of Food Science and Technology, University of Nigeria, Nsukka were used to evaluate the tamarind pulp flavoured yoghurt samples. The samples were evaluated for taste, colour, aroma, flavour, aftertaste and overall acceptability. The extent of differences among samples for each sensory quality was measured using a 9-point Hedonic scale where nine represents extremely like and 1 represents extremely dislike, according to Ihekoronye & Ngoddy (1985).

Determination of pH: A standard pH meter (model 20 pH conductivity meter, Denver Instrument,

United Nations Inventory Database), beakers and buffer solution would be used for the determination. The pH meter would be standardized using buffer solutions of pH 4.0 and 9.0. The pH electrode was dipped into a solution of the sample and after five minutes of equilibration, the pH of the sample was taken.

Experimental design and data analysis: This was based on completely randomized design in one way analysis of variance (ANOVA). Means separation was done using Duncan's multiple range tests with a statistical package for service solution (SPSS) version 20. Significance would be accepted at $p < 0.05$ according to Steel & Torrie (1980).

3. Results and Discussion

Proximate composition and Vitamin C content of Formulated black velvet tamarind powder

The moisture content of the black velvet tamarind after processing to powder was 7.01 %. There was an increase in moisture content compared to a previous report (6.71 %) by Obasi *et al* (2013). This can be attributed to the effect of processing the material was subjected to as reported by Obasi, *et al*. (2013). The ash content of the black velvet tamarind powder was 1.59 %. This was in agreement with the value (1.6 %) as reported by Nguyen. (2015). Although there was a little decrease in value compared with the report of Obasi, *et al*. (2013) which was 1.49 %, but that could be attributed to different in soil composition and other environmental factors. The fibre content of the raw material (black velvet tamarind powder) was 1.72%. This was in agreement with 1.74 % as was reported by Nicholas *et al*. (2014). The crude fat content of the raw material was 3.04 %. There was a reduction compared with the 5.1 % as report by USDA. (2009). The protein content of the powder was 5.3 %. There was a slight increase in percentage compared with the 4.2 % as reported by Nicholas *et al*. (2014). This could be as a result of geographical difference and land composition. The carbohydrate content of the velvet tamarind powder was 81.34%. There was a relative increase in the carbohydrate content compare to reports in the literature specifically the 82.64 % reported by Obasi *et al*. (2013). This could be attributed to difference in land composition and subjection of the black velvet tamarind to drying during processing could have caused the carbohydrate content to be more concentrated Obasi *et al*. (2013).

The micronutrient content of the black velvet tamarind concurred with the previous reports except for the vitamin C that has a little increase in value. The samples were a bit brownish in colour slightly deviated from the normal creamy colour of yoghurt. The panelists appreciated the fact that it turned out chocolate-like in appearance.

Proximate composition of yoghurt samples flavoured with different concentration of black velvet tamarind

Table 2 shows the proximate composition (%) of black velvet tamarind flavoured yoghurt. The moisture content of the flavoured yoghurt ranges from 72.27% for VTB5 to 84.35% for VTO. There was a significant ($p<0.05$) different between the flavoured yoghurt samples and sample VTO, which was plain and serves as a control sample. The difference in moisture decreases as the concentration increases the effect was higher on the samples that were flavoured before fermentation. The decrease in moisture could be attributed to the high water absorption capacity of black velvet tamarind as reported by Obasi *et al*. (2013).

The ash content of the samples ranges from 0.04 % for sample VTO (control sample) to 1.72 % for sample VTB5 which has the highest concentration in the category that was flavoured before fermentation. There was a significant ($p<0.05$) different between the flavoured samples and the

control sample. The progression in the difference increases as the concentration increases with significant increase being more eminent at the category that was flavoured before fermentation. This implies that fermentation may have contributed to the increase in solid residues in the samples. The ash content was similar to ash content of *Tamarind indica* pulp as reported by Obasi *et al*. (2013).

The fat content ranges from 2.39 % for sample VTA1 to 4.25 % for sample VTA4. There was a significant ($p<0.05$) different between sample VTO and sample VTA1 and VTA4, but there was no significant ($p<0.05$) different between sample VTO and the rest of the sample. This could be attributed to the low fat content of black velvet tamarind as reported by Adetuyi and Ibrahim (2014).

The protein content of the formulated samples of the flavoured yoghurt showed a variation in concentration. The progression indicated that there was a little influence of the flavouring on the protein composition of the samples. The sample with the highest protein content was sample VTB2 (9.31 %) and the lowest was sample VTA2 (6.48 %) which is not significantly ($p>0.05$) different from the control sample (VTO). Between sample VTO and the rest of the samples (apart from VTA2) there was a significant ($p<0.05$) different. The variation in concentration was in line with the variation of the total viable count; this could be that the microbial concentration may have had influence on the protein concentration since they are proteineous in nature. This agreed with the report of Ammara & Imran. (2010).

The carbohydrate content of the samples ranges from 6.65 % of VTB1 to 14.74 % of sample VTB5. There was a significant ($p<0.05$) different between the samples and the increase in value was simultaneous with increase in concentration. This could be because black velvet tamarind is rich in glucose and fructose (Obasi *et al.*, 2013).

Selected micronutrients of black velvet tamarind flavoured yoghurt

Table 3 shows some selected micronutrient composition of black velvet tamarind flavoured yoghurt. The vitamin C content of the yoghurt samples ranges from 10.01 mg/100 g in the control sample to 16.71 mg/100 g in sample VTB5. There was a significant ($p<0.05$) difference between the control sample (VTO) and the rest of the samples. The increase in vitamin C content follows a simultaneous increase in the concentration of the black velvet tamarind flour.

Table 2: Proximate composition of the yoghurt flavoured with black velvet tamarind

Yoghurt samples	Moisture	Ash	Fat	Protein	Carbohydrates
VTA1	$81.27^{b}\pm2.02$	$0.15^{de}\pm0.03$	$2.39^{c}\pm0.06$	$8.65^{ab}\pm0.52$	$7.51^{b}\pm2.50$
VTA2	$78.98^{bcd}\pm1.12$	$0.47^{cd}\pm0.48$	$3.81^{ab}\pm0.15$	$6.48^{e}\pm0.13$	$10.64^{ab}\pm1.05$
VTA3	$77.41^{de}\pm0.61$	$0.86^{bc}\pm0.01$	$3.51^{b}\pm0.59$	$7.68^{cd}\pm0.02$	$10.55^{ab}\pm1.17$
VTA4	$75.65^{ef}\pm0.37$	$0.73^{bc}\pm0.01$	$4.25^{a}\pm0.00$	$9.26^{a}\pm0.44$	$10.11^{ab}\pm0.82$
VTA5	$73.61^{fg}\pm0.88$	$1.71^{a}\pm0.01$	$3.45^{b}\pm0.14$	$8.60^{abc}\pm0.23$	$12.64^{ab}\pm0.95$
VTB1	$80.29^{b}\pm0.59$	$0.74^{bc}\pm0.05$	$3.77^{ab}\pm0.62$	$8.57^{abc}\pm0.26$	$6.65^{b}\pm0.18$
VTB2	$79.87^{bc}\pm1.19$	$1.04^{b}\pm0.06$	$3.31^{b}\pm0.13$	$9.31^{a}\pm0.11$	$6.98^{b}\pm0.66$
VTB3	$79.47^{bcd}\pm0.88$	$0.84^{bc}\pm0.54$	$3.95^{ab}\pm0.10$	$9.04^{ab}\pm0.00$	$6.71^{b}\pm0.74$
VTB4	$77.76^{cde}\pm0.04$	$0.85^{bc}\pm0.00$	$3.73^{ab}\pm0.28$	$6.98^{de}\pm0.04$	$10.69^{ab}\pm0.35$
VTB5	$72.27^{g}\pm0.76$	$0.72^{a}\pm0.16$	$3.38^{b}\pm0.00$	$8.26^{bc}\pm0.71$	$14.74^{ab}\pm0.74$
VTO	$84.35^{a}\pm0.53$	$0.04^{e}\pm0.02$	$3.27^{b}\pm0.26$	$6.70^{e}\pm0.80$	$10.58^{ab}\pm7.68$

Values are means ± standard deviation of duplicate determinations. Values in the same column with the same superscript are not significantly ($p>0.05$) different.

Key: VTO = plain yogurt; VTA = yoghurt flavoured after fermentation; VTB = yoghurt flavoured before fermentation.

Plate 3: Samples of black velvet tamarind flavoured yoghurt

confirms the high vitamin C content of black velvet tamarind as reported by Gnansounou, *et al*. (2014). There was a progressive increase in the category of the samples that were flavoured before fermentation compare to the ones that were flavoured after fermentation. This could mean that fermentation brings about an increase in the concentration of vitamin C in yoghurt. The increase could be attributed to the microbial synthesis of vitamin C which shows a relative increase during 24 hours period of fermentation and decreases as the fermentation time increase due to the activity of an enzyme (ascorbate oxidase) as reported by Adetuyi & Ibrahim (2014).

The vitamin A content of the formulated yoghurt samples ranges from 11.09±0.83 mg/100g of sample VTA1 to 74.70±1.71 mg/100g in sample VTB5. The result indicated that addition of black velvet tamarind after fermentation masks the presence of vitamin A in food sample because of the decrease in the sample category that were flavoured after fermentation was even below the vitamin A in the plain sample (VTO). The sample categories that were flavoured before fermentation experienced a progressive increase in vitamin A content. This confirms that fermentation brings about a higher concentration of micronutrients in yoghurt sample (Reade, 2012).

The calcium content of the formulated yoghurt ranges from 40.02±0.13 mg/100g in sample VTB4 to 51.23±0.48 mg/100 g in sample VTA1. There is no significant ($p<0.05$) different within the group that were flavoured after fermentation and as well no significant ($p<0.05$) different between the group that were flavoured before fermentation. But there is significant ($p<0.05$) different across the group. The result shows that the addition of black velvet tamarind after fermentation would lead to an increase in calcium content. The values of the calcium content obtained in the samples concurred with the report of Ogungbenle (2015).

The phosphorus content of the formulated yoghurt samples ranges from 136.35±5.78 mg/100g in sample VTB4 to 173.09±5.61 mg/100g in sample VTA1. It is significantly ($p<0.05$) different between the samples. The increase in concentration of the black velvet tamarind leads to decrease in the phosphorus content between the samples. The values obtained agreed with the report of Ogungbenle (2015).

Table 3: Selected micronutrient composition of black velvet tamarind flavoured yoghurt

Yoghurt samples	VitaminC (mg/100g)	Vitamin A (IU)	Calcium (mg/100g)	Phosphorus (mg/100g)
VTA1	11.05^{g}±0.01	11.09^{a}±0.83	51.23^{b}±0.48	173.09^{d}±5.61
VTA2	12.36^{f}±0.42	69.01^{abcd}±3.34	50.70^{b}±0.47	160.23^{bcd}±0.48
VTA3	14.39^{c}±0.30	66.69^{b}±0.07	49.72^{b}±1.18	161.79^{bcd}±1.44
VTA4	13.00^{e}±0.10	68.43^{bc}±2.66	48.31^{b}±0.83	164.12^{bd}±5.79
VTA5	14.59^{c}±0.06	70.27^{bcde}±0.21	48.06^{b}±4.32	161.73^{bcd}±5.60
VTB1	13.40^{d}±0.05	74.08^{de}±5.45	41.90^{a}±1.38	143.66^{ab}±3.16
VTB2	16.20^{b}±0.09	73.20^{cde}±0.27	43.50^{a}±2.14	140.88^{a}±14.14
VTB3	16.25^{b}±0.07	73.02^{cde}±0.28	40.85^{a}±1.46	143.62^{ab}±2.89
VTB4	16.27^{b}±0.09	73.64^{cde}±0.48	40.02^{a}±0.13	136.35^{a}±5.78
VTB5	16.71^{a}±0.03	74.70^{e}±1.71	40.58^{a}±2.39	140.34^{a}±14.91
VTO	10.01^{h}±0.02	70.17^{bcde}±0.07	40.75^{a}±0.25	151.46^{abc}±7.87

Values are mean ± standard deviation of duplicate readings. Means on the same column with different superscript are significantly ($p<0.05$) different.

Key: VTO = plain yoghurt, VTA = yoghurt flavoured after fermentation, VTB = yoghurt flavoured before fermentation

Microbial count (cfu/ml) of yoghurt flavoured with black velvet tamarind (before and after fermentation).

Table 4 showed the microbial population of the formulated yoghurt. The samples show a total viable count range of 1.4×10^5 in VTO and VTA5 to 2.9×10^5 in sample VTA1. There was a decrease in TVC as the concentration of the flavouring substance increases within the samples that were flavoured after fermentation while there was simultaneous increase as the concentration increases within the samples that were flavoured before fermentation. This could be as a result of the antimicrobial effect of the phytochemicals such as tannins, alkaloids, phenols, among others reported to be contained in the black velvet tamarind. Although, fermentation causes a decrease in the concentration of the phytochemicals hence, an increase in the samples that were flavoured before fermentation (Sani *et al.* 2013)

The lactic acid bacteria in the samples range from 1.1×10^5 in sample VTO and VTB1 to 2.6×10^5 in sample VTA1. There was a decrease in LAB as the concentration of the black velvet tamarind increases across the samples that were flavoured after fermentation but a little increase was shown in the sample categories that were flavoured before fermentation. This could be because of the release of concentrated nutrient and in vitro antibacterial efficacy of flavonoids in the black velvet tamarind (Eze, *et al*., 2018).

Table 4: Total viable count (TVC), lactic acid bacteria (LAB) and mould count of the formulated yoghurt

Samples	TVC (Cfu/ml)		LAB (Cfu/ml)	Mould (Cfu/ml)
VTA1	2.9×10^5	2.6×10^5	ND	
VTA2	1.8×10^5	2.1×10^5	1.0	
VTA3	2.0×10^5	1.5×10^5	ND	
VTA4	2.8×10^5	2.2×10^5	2.0	
VTA5	1.4×10^5	1.2×10^5	3.0	
VTB1	2.6×10^5	1.1×10^5	ND	
VTB2	1.8×10^5	2.0×10^5	ND	
VTB3	1.6×10^5	2.1×10^5	ND	
VTB4	2.7×10^5	2.0×10^5	ND	
VTB5	2.8×10^5	2.0×10^5	1.0	
VTO	1.4×10^5	1.1×10^5	ND	

Key: VTO= plain yoghurt; VTA= yoghurt flavoured after fermentation; VTB= yoghurt flavoured before fermentation; TVC= total viable count, LAB= lactic acid bacteria; ND= not detected; Cfu/ml = coliform forming unit per milliliter.

The mould count was detected in sample VTA2, VTA4, VTA5 and VTB5. It has been reported that black velvet tamarind can easily support mould growth by Obasi *et al.* (2013). So the mould may have arisen from the black velvet tamarind powder that was added.

Sensory scores of yoghurt flavoured with black velvet tamarind powder

Table 5 showed the sensory scores of yoghurt flavoured with black velvet tamarind powder. The mean score of colour ranged from 4.15 ±1.50 for sample VTA5 to 7.95 ± 0.89 for sample VTO. The plain yoghurt (sample VTO) has the highest score for colour. There was significant ($p<0.05$) different in the colour of the samples across the group. There is no significant different ($p<0.05$) between sample

VTA3, VTA4, VTB3 and VTB4. The more preferred one apart from plain yoghurt are sample VTA1 and VTB1 that has 0.2 g concentration of black velvet tamarind pulp powder. This corresponds with the report of Mbaeyi & Anyanwu (2010).

The value for flavour ranged from 4.45 ± 1.15 for sample VTA5 to 7.90 ± 0.97 for sample VTO. The highest score goes to sample VTO, followed by VTA1 and VTB1. There was significant ($p<0.05$) different between sample VTO, VTA1 and VTB1 and the rest of the samples. This could be attributed to the concentration of the black velvet tamarind powder. The panellists tend to appreciate the sample with lesser concentration (0.2 g).

The mouthfeel score ranged from 4.30 ±0.93 for sample VTA5 to 8.00 ±0.73for sample VTO. There was no significant ($p<0.05$) different between sample VTA1 and VTB1, but there was significant ($p<0.05$) different between the two stated samples and the rest of the sample. The whole sample that had a concentration from 0.4 g to 1.0 g has no significant ($p<0.05$) different across the treatments.

The consistency ranged from 4.30 ± 1.22 for sample VTB4 to 7.75 ± 0.64 for sample VTO. There was significantly different between sample VTO and the rest of the sample across the treatments. Sample VTA1 and VTB1 have no significant ($p<0.05$) different.

The score for taste ranged from4.45 ±1.64 for sample VTA5 to 8.10 ± 0.97 for sample VTO. There was significant ($p<0.05$) different between sample VTO and the rest of the samples and samples with concentrations of 0.4 to 1.0 g are not significantly ($p<0.05$) different. Sample VTO has the highest score for taste.

The score for aftertaste ranged from 4.45 ± 1.36 for sample VTA5 to 8.10 ± 1.02 for example VTO. Sample VTO has the highest rating for the aftertaste. There was a significant ($p<0.05$) different between sample VTO and the rest of the samples. There was no significant ($p<0.05$) different between samples with 0.4 to 1.0 g concentration of the black velvet tamarind powder.

The overall acceptability ranged from 4.70 ± 1.78 for sample VTB5 to 8.45 ± 1.02 for sample VTO. The plain yoghurt (sample VTO) had the highest score in the overall acceptability followed by sample VTB1 and then VTA1 which are significantly ($p<0.05$) different from each other and from the rest of the samples which had no significant ($p<0.05$) different.

The result of the sensory score denoted that at 0.2 g concentration, flavoured yoghurt can be comfortably produced and accepted by the consumers. Following the result of the sensory, it can be deduced that fermentation process had no significant ($p<0.05$) different in the acceptability in terms of flavour of the yoghurt samples.

pH of the formulated samples of yoghurt flavoured with black velvet tamarind

Table 11 contained the pH of the formulated samples of yoghurt flavoured with black velvet tamarind. The pH was taken at the first day of production. The mean score of the samples flavoured before fermentation ranges from 4.30±0.14 for sample VTA5 while the sample range for the category that were flavoured after fermentation is from 4.30 for sample VTO to 5.46 for sample VTA2.

Table 5: Sensory scores for the formulated yoghurt flavoured with black velvet tamarind pulp powder and the plain yoghurt

Samples	Colour	Flavour	Mouthfeel	Consistency	Taste	Aftertaste	Overall acceptability
VTA1	$7.55^{a}\pm1.19$	$7.15^{a}\pm1.35$	$6.85^{b}\pm1.46$	$6.70^{b}\pm1.34$	$7.10^{b}\pm1.41$	$6.95^{b}\pm1.47$	$7.45^{b}\pm1.32$
VTA2	$5.45^{b}\pm0.94$	$5.30^{bc}\pm1.22$	$5.05^{c}\pm1.61$	$5.05^{cd}\pm1.47$	$5.20^{cd}\pm1.20$	$4.75^{c}\pm1.33$	$4.95^{c}\pm1.43$
VTA3	$4.95^{bcd}\pm1.39$	$4.65^{bc}\pm1.31$	$4.80^{c}\pm1.47$	$4.80^{cd}\pm1.54$	$4.85^{cd}\pm0.81$	$4.45^{c}\pm1.47$	$4.80^{c}\pm1.32$
VTA4	$4.55^{bcd}\pm1.23$	$5.20^{bc}\pm1.20$	$4.95^{c}\pm1.23$	$4.75^{cd}\pm1.25$	$4.90^{cd}\pm1.21$	$4.85^{c}\pm1.53$	$4.80^{c}\pm1.40$
VTA5	$4.15^{d}\pm1.50$	$4.45^{c}\pm1.15$	$4.30^{c}\pm0.98$	$4.30^{d}\pm1.17$	$4.45^{d}\pm1.64$	$4.45^{c}\pm1.36$	$4.65^{c}\pm1.35$
VTB1	$7.65^{a}\pm1.04$	$7.35^{a}\pm1.42$	$7.10^{b}\pm1.48$	$7.30^{ab}\pm1.17$	$7.30^{ab}\pm1.59$	$7.45^{ab}\pm1.23$	$7.75^{ab}\pm1.25$
VTB2	$5.30^{bc}\pm1.69$	$5.45^{bc}\pm1.43$	$5.05^{c}\pm1.28$	$5.25^{c}\pm1.37$	$5.55^{c}\pm1.36$	$5.30^{c}\pm1.26$	$5.35^{c}\pm1.27$
VTB3	$5.00^{bcd}\pm1.52$	$4.80^{bc}\pm1.15$	$4.80^{c}\pm1.54$	$4.70^{cd}\pm1.34$	$5.25^{cd}\pm1.02$	$5.20^{c}\pm1.44$	$5.30^{c}\pm1.26$
VTB4	$4.90^{bcd}\pm1.17$	$4.90^{bc}\pm0.85$	$4.85^{c}\pm1.60$	$4.30^{d}\pm1.22$	$5.05^{cd}\pm1.10$	$4.60^{c}\pm1.43$	$4.90^{c}\pm0.97$
VTB5	$4.45^{cd}\pm1.36$	$5.05^{bc}\pm1.64$	$4.65^{c}\pm1.79$	$4.40^{cd}\pm1.43$	$5.05^{cd}\pm1.85$	$4.50^{c}\pm2.16$	$4.70^{c}\pm1.78$
VTO	$7.95^{a}\pm0.89$	$7.90^{a}\pm0.97$	$8.00^{a}\pm0.73$	$7.75^{a}\pm0.64$	$8.10^{a}\pm0.97$	$8.10^{a}\pm1.02$	$8.45^{a}\pm0.69$

Values are means ± standard deviation of 20 panelists. Values with the same superscript in a row are not significantly ($p<0.05$) different.
Key: VTO = plain yoghurt; VTA = yoghurt flavoured after fermentation; VTNB = yoghurt flavoured before fermentation

Table 9: pH of yoghurt samples flavoured with black velvet tamarind.

Sample VTB	pH	Sample VTA	pH
VTB1	4.30±0.67	VTA1	5.00±0.28
VTB2	4.47±0.77	VTA2	5.46±0.66
VTB3	4.80±0.28	VTA3	5.16±0.18
VTB4	4.30±0.59	VTA4	4.39±0.45
VTB5	4.30±0.14	VTA5	4.77±1.10
VTO	4.36±0.63	VTO	4.30±0.28

Values are mean ± standard deviation of duplicate readings.

Key: VTA = Yoghurt samples flavoured after fermentation, VTB= Yoghurt samples flavoured before fermentation, VTO = Plain yoghurt.

The reduction in pH for the samples that were flavoured before fermentation confirms the report of Ammara & Imran. (2015) which stated that some of the naturally occurring fungal isolates from the natural fermentation of the fruit pulp of *D. guineense* are capable of utilizing the substrate for the production of citric acid and could be employed for the production of citric acid on large scale. Which implies that the present of the test raw material in a fermenting medium promotes the acidic concentration of the medium.

4. Conclusion

From the result of this work, it can be deduced that the addition of black velvet tamarind in plain yoghurt could improve the nutritional quality of yoghurt especially in terms of vitamins analyzed. The therapeutic potency of yoghurt could also be improved in that vitamin C has been regarded as anti-scurvy vitamin. Also, flavouring before fermentation was advantageous in that the concentrations of the targeted micronutrient were more in the samples that were flavoured before fermentation. There was also a positive impact in the proximate composition of the formulated products especially in terms of protein and carbohydrates.

The sensory attributes (colour, flavour, consistency, mouth-feel, taste, after taste and overall acceptability) evaluated showed an appreciable degree of acceptability by the panellists which could add to the number of yoghurt variety in the market. The results also showed that black velvet tamarind with a concentration of 0.2 g in both treatments was most preferred among the formulated yoghurt samples with an overall acceptability of 7.45 and 7.75. Others were not appreciated as such apart from the 0.2 g concentration. This justifies the economic importance of using black velvet tamarind pulp.

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Conflict of Interests

The authors declare no conflict of interest.

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