ANTIOXIDANT, MINERAL AND HYDROPHOBICITY PROPERTIES OF VALUE ADDED YOGHURT MADE FROM TROPICAL FRUITS

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ABSTRACT

In recent times, interests have been generated globally in the use of plant derived natural enhancers in the food industries due to their acceptability, palatability and minimal side effects on consumers' health. This study examined the antioxidant, mineral and hydrophobicity qualities of yoghurts made from raw cow's milk enhanced with tropical fruits and synthetic pineapple flavourants at 1, 7 and 14 days of storage. The antioxidant capacity showed that DPPH was highest (26.95 ± 4.56 %) in orange flavoured yoghurt, while the FRAP value was superior (13.72 mg/ml) in grape yoghurt. All samples showed low (0.01 mmol/g) scavenging activity with ABTS. Storage effect indicated that antioxidants capacity assay was highest at day 1 with values 0.02 mmol/g ABTS, 38.53 ± 1.00 % DDPH and 15 mg/ml FRAP. Interaction effect revealed that grape yoghurt at day 1 had a stronger FRAP activity of 18.08 ± 0.04 mg/ml, while the DPPH activity was superior (43.51 ± 0.29 %) in pawpaw yoghurt at 14 day storage. Lemon yoghurt recorded the highest potassium and zinc concentrations (1164.14 ± 46.54, 50.08 ± 3.88 mg/L respectively). Calcium concentration was highest in orange yoghurt (1629.83 ± 7.94 mg/L). Pawpaw flavoured yoghurt showed superiority in sodium and iron contents (32.96 ± 2.01 mg/L and 12.25 ± 0.23 mg/L respectively), while synthetic pineapple yoghurt had the highest phosphorus (439.40 \pm 8.93 mg/L) concentration. L. delbrueckii subsp. bulgaricus had the strongest hydrophobicity. Conclusively, indigenous fruits could enhance the nutritional value of yoghurt and also replace synthetic flavourants in yoghurt production.

Keywords: Fermented milk, Tropical fruits, Value addition, Antioxidant, Hydrophobicity, Mineral

INTRODUCTION

Yoghurt is a fermented semi-fluid milk product prepared from fresh whole or skimmed milk, which is usually carried out by the addition of bacterial starter culture (Akpan *et al.*, 2007). *Streptococcus salivarus* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are the major predominant cultures associated with the milk fermentation into yoghurt (Gonçalves *et al.*, 2009). They are rich sources of bioactive peptides with antioxidant activity that are produced during fermentation. The healthy food

bulgaricus are associated with are (Gonçalves of bioactive vity that are healthy food cholester of bioactive associated with are healthy food cholester of bioactive associated with are healthy food cholester blood cholester of bioactive cancer, lower blood cholester of, improved immune response and help the body assimilate protein, calcium and iron (Van de Water, 2008). Usually, yoghurt is flavoured by adding natural ingredients or synthetic flavour compounds (Coisson *et al.*, 2005). Yoghurt is mainly pigmented and flavoured by adding fruit juices

yoghurt

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image of yoghurt is due to the probiotic effect of yoghurt bacteria. The health promoting

properties of live lactic acid bacteria (LAB) in

gastrointestinal upsets, enhanced digestion of

lactose by maldigesters, decreased risk of

protection

against

include

or pulp from berry fruits such as strawberries, blueberries and raspberries, which provide natural colour and flavour as well as bioactive compounds. Fruit juices, powders and extracts have potential as functional ingredients in the food industry, including the dairy sector (Wallace and Giusti, 2008). The effects of different types of plant materials added as functional ingredients on the quality and antioxidant properties of yoghurt have been studied (Kim et al., 2009; Oh and Kang, 2015). Antioxidant compounds in foods play a significant role as a health-protecting factor. They are capable of deactivating free radicals which can cause cells and tissue damage. These damages cause malfunctioning of cells or cell death (Senadeera et al., 2018). Consumption of probiotic bacteria via food products such as voghurt is an ideal way to improve the intestinal microflora balance. Toxic effects have been associated with synthetic antioxidants and consequently, limitations have been imposed on their usage. This has necessitated researchers focus on natural antioxidants inherent in plants. These natural antioxidants usually come from a diet rich in fruits and vegetables or are carried in creams or topically applied. Fruits are excellent considered as an source of antioxidants and prebiotic fibres and polyphenols (Fernandez and Marette, 2017). Fruit juices add colour, flavour, essential vitamins and minerals, in addition to providing phytochemicals which impact positive health benefits of consumers. Fruits (strawberry, apple, watermelon, pawpaw, mango, orange, banana and grape) and vegetables are rich sources of vitamins, mineral, fibers and anti-oxidants and can be used in producing value-added yoghurt (Erdogan and Zekai, 2003; Vahedi et al., 2008; Ibhaze et al., 2020). This study is therefore aimed at evaluating the antioxidant, mineral and hydrophobicity properties of yoghurt enriched with tropical fruits.

MATERIALS AND METHODS

Milk Collection Site: Raw cattle milk from White Fulani cows was obtained at the cattle breeders' settlement at Ita-Ogbolu, Ondo State and transported in a cold chain immediately to the nutrition laboratory of the Department of Animal Production and Health for refrigeration.

Yoghurt Production Site: Yoghurt production was carried out in the Nutrition Laboratory of the Department of Animal Production and Health, Federal University of Technology, Akure (FUTA), Ondo State, Nigeria, located on longitude 4.944055° E and 5.82864° E, and latitude 7.491780° N with annual rainfall ranging between 1300 and 1650 mm and maximum and minimum daily temperature of 38° C and 27° C respectively (Daniel, 2015).

Procurement of Experimental Materials: Starter culture, pineapple flavourant and sucrose were purchased from a reputable store in Lagos, while the fruits (ripe orange, grape, pawpaw and lemon) were sourced from fruit shops in Akure.

Preparation of Flavoured Fruit Juices: The commercial (synthetic) pineapple flavour was reconstituted with distilled water at a ratio of 1:2 v/v, the pH value was determined using a pH meter and kept in a labeled container. The fruits (orange, pawpaw, lemon and grape) were washed properly with water. The oranges, lemon and grape were cut, squeezed to obtain the juice. The juice was filtered to obtain a pure filtrate, while the pawpaw fruit was peeled and the seeds removed and the edible parts was blended using the electric blender (Philip Model) and the juice was extracted using the cheese cloth. All fruits extracts were placed in labeled containers. The pH value of the juices and reconstituted synthetic pineapple flavourant were determined using pocket-sized digital pH meter. The juice obtained from each fruit was pasteurized at 80°C for 3 minutes and cooled to room temperature.

Preparation of Flavoured Yoghurt: The yoghurt was prepared according to the method described by Ibhaze *et al.* (2020). The raw cow milk (18 L) was clarified, homogenized and pasteurized at 80°C for 3 minutes. Sucrose (5%) was then added as sweetener. Thereafter, the milk was cooled to a temperature of 42°C for inoculation. Commercial freeze-dried starter

culture was added to 18 litres of the milk at 5 g/litre. The inoculated milk was divided into six portions representing the treatments as; Reconstituted synthetic pineapple flavour, plain, orange, lemon, grape and pawpaw juices. Each treatment was replicated thrice. The flavourants were added at 200 mL into 1 litre each of the inoculated milk excluding the plain milk. The samples were incubated at 43°C for 14 hours using an incubator. The flavoured yoghurts produced were stored in a refrigerator at 4°C for analyses at storage periods of 1, 7 and 14 days.

Determination of Antioxidant Properties

Antioxidant capacity of flavoured yoghurts at different storage periods was determined by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Ferric reducing antioxidant's property (FRAP) and 1,1-diphenyl-2picrylhydrazyl (DPPH) methods.

ABTS scavenging ability: ABTS radical scavenging activity was determined according to the method described by Re et al. (1999) with some modifications. The ABTS was generated by reacting ABTS (7mM) aqueous solution with $K_2S_2O_8$ (2.45 mM/L, final concentration) in the dark for 16 hours and adjusting the absorbance at 734 nm to 0.700 with ethanol 0.2 of the appropriate dilution of the extract was then added to 2.0 mL of ABTS solution and the absorbance was read at 732 nm after 15 minutes. The TROLOX equivalent antioxidant capacity was subsequently calculated: ABTS (mg/g) = Absorbance of Sample / Slope of Standard Curve.

Determination of ferric reducing properties: The reducing property of the yoghurt samples was determined using the method described by Pulido *et al.* (2000). 0.25 ml of the yoghurt sample was mixed with 0.25 mL of 200 mM of sodium phosphate buffer pH 6.6 and 0.25 mL of 1 % potassium ferrocyanate (KFC). The mixture was incubated at 50°C for 20 minutes, thereafter 0.25 mL of tricarboxylic acid (TCA) was also added and centrifuged at 2000 rpm for 10 minutes, 1 mL of the supernatant was mixed with 1 mL of distilled water and 0.1% of FeCL₃ and the absorbance was measured at 700 nm.

4299

Determination of (DPPH) free radical scavenging ability: The free radical scavenging ability of the yoghurt against DPPH (1, 1- diphenyl-2-picryhydrazyl) was determined using the method described by Mensor et al. (2001). 1 mL of the yoghurt sample was mixed with 1 mL of the 0.4 mM methanolic solution of the DPPH. The mixture was left in the dark for 30 minutes before measuring the absorbance at 516 nm. The scavenging activity percentage was determined thus: DPPH Scavenged (%) = AControl – A test / A Control × 100.

Evaluation of Hydrophobicity: Bacterial adhesion is a method to measure relative hydrophobicity to different bacteria strain. The LAB isolate was first grown on De Man, Rogosa and Sharpe (MRS) agar at 37°C for 24 hours. They were centrifuged at 5000 rpm for 15 minutes, pellets were washed twice with phosphate buffer saline (PBS) having pH 7.0, and the optical density measured at 600 nm. Then, one millilitre of the bacterial suspension was added to 1 mL of different hydrocarbons (chloroform and xylene) and vortexed for 30 seconds. After 30 minutes of phase separation, the optical density of aqueous separation was measured again at 600 nm and compared with initial value. Hydrophobicity was calculated using the equation: % hydrophobicity = (A_{600nm}) initial value – A_{600nm} aqueous solution/ A_{600nm}) × 100 (Rosenberg et al., 1980).

Mineral Content Determination: To measure the minerals content, 2 g of each yoghurt sample was ashed and digested with 10 ml of 10 % hydrochloric acid (HCL) to almost dryness. Then 50 ml of sterile deionized water was added, filtered and the filtrate was made up to 100 ml. Jenway Flame Photometer (Model PFP7) was used to assay potassium, calcium, magnesium and sodium while the UV-Visible Spectrophotometer (Pec Medical, Model 721) was used to measure phosphorus, zinc and iron according to the methods of AOAC (1990).

Data Analysis: Data were subjected to analysis of variance (ANOVA) and treatment means were separated using New Duncan's Multiple Range Test (NDMRT). The data analysis was performed with SPSS 25.0 software (SPSS, 2017).

RESULTS

Antioxidant Properties of Yoghurt Made from Tropical Fruits and **Synthetic** Flavourants: Presented in Table 1 are the antioxidant capacities of flavoured yoghurts at 1, 7 and 14-day storage periods. Storage effect showed that significant differences (p<0.05)existed in DPPH, FRAP and ABTS. ABTS value was 0.02 ± 0.00 mmol/g in day 1, while day 7 and 14 remained 0.00 ± 0.00 mmol/g. The DPPH and FRAP activities were at their peak at day 1 (38.53 ± 1.00 % and 15.97 ± 0.30 mg/ml respectively) and depressed at day 14 (10.68 \pm 1.15 % and 5.43 \pm 0.30 mg/ml respectively). Treatment effect showed significant differences (p<0.05) in DPPH and FRAP only. Orange yoghurt recorded the highest DPPH value of 26.95 ± 4.56 %, while the least (22.90 ± 6.07 %) was observed in plain yoghurt. The interaction effect revealed significant differences (p<0.05) only in DPPH and FRAP activities, where DPPH activity was highest (43.51 ± 0.29) %) in pawpaw flavoured yoghurt at day 14, while the least $(3.04 \pm 0.04 \%)$ was observed in plain yoghurt at day 7. FRAP activity was strongest in grape flavoured yoghurt (18.08 ± 0.04 mg/ml) and least in plain yoghurts $(3.70 \pm$ 0.00 mg/ml) at day 14.

Hydrophobicity Analysis of the Cell Surface of Lactic Acid Bacteria (LAB): The results on voghurt hydrophobicity of the cell surface of LAB using two solvents (n-hexane and xylene) are presented in Table 2. The results showed that L. delbrueckii subsp. bulgaricus had the highest value of 72.89 ± 6.57 %, followed by 21.51 ± 0.01 % and 11.65 ± 0.44 % for *Lactobacillus* spp. and *casei* in n-hexane solvent Lactobacillus respectively. In xylene solvent, L. delbrueckii subsp. *bulgaricus* had the highest value of 73.47 \pm 5.08 %, followed by 22.31 \pm 0.02 % and

14.78 \pm 0.33 % for *Lactobacillus* spp. and *L. casei* respectively.

Mineral Composition of Flavoured Yoghurt at Different Storage Periods: The mineral compositions of yoghurt made from different flavours at 1, 7 and 14 days of storage are presented in Table 3. At the treatment levels and interaction between the treatment and storage period, significant differences (p<0.05) existed in all mineral contents except for iron.

Lemon yoghurt recorded the highest potassium (1164.14 \pm 46.54 mg/L) and zinc (50.08 \pm 3.88 mg/L) concentrations. Lemon yoghurt was least in calcium (1568.40 \pm 22.17 mg/L) and sodium (32.08 \pm 2.12 mg/L) concentrations. Calcium concentration was highest in orange yoghurt (1629.83 \pm 7.94 mg/L).

Pawpaw flavoured yoghurt was superior in sodium and iron contents $(32.96 \pm 2.01 \text{ mg/L})$ and 12.25 ± 0.23 mg/L respectively). Synthetic pineapple flavoured yoghurt had the highest phosphorus (439.40 ± 8.93 mg/L) concentration. Storage effect was significant (p<0.05) as it revealed that all the minerals investigated were at their peak at day 1 and least at day 14 of storage. The interaction between storage periods and treatments revealed significant effect (p<0.05) on all minerals except iron. The interaction effect showed that lemon yoghurt highest potassium and had the zinc concentrations (1344.83 \pm 0.41 and 61.76 \pm 0.34 mg/L respectively) at day 1, grape yoghurt was superior in calcium (1683.55 \pm 0.26 mg/L) at day 1, while orange yoghurt had the peak sodium and magnesium concentrations of 43.40 \pm 0.35 and 1098.37 \pm 0.32 mg/L respectively at day 1. Synthetic pineapple flavoured yoghurt had the highest phosphorus (473.18 ± 0.43) mg/L) concentration at day 1.

DISCUSSION

Antioxidant compounds in foods play a significant role as a health-protecting factor. They are capable of deactivating free radicals which can cause cells and tissue damage. These damages cause malfunctioning of cells or cell death (Senadeera *et al.*, 2018).

1, 7 and 14 days	APTS (mmol/g)							
Treatment Grape	ABTS (mmol/g) 0.01 ± 0.00	DPPH (%) 23.07 ± 3.51 ^c	FRAP (mg/ml) 13.72 ± 1.66 ^a					
-								
Lemon	0.01 ± 0.00	$23.72 \pm 3.66^{\circ}$	$11.31 \pm 1.55^{\circ}$					
Orange	0.01 ± 0.00	26.95 ± 4.56^{a}	10.53 ± 1.38^{d}					
Pawpaw	0.01 ± 0.00	$23.14 \pm 6.11^{\circ}$	$11.39 \pm 1.50^{\circ}$					
Synthetic pineapple	0.01 ± 0.00	26.53 ± 3.52^{a}	11.67 ± 1.66 ^b					
Plain	0.01 ± 0.00	22.90 ± 6.07^{b}	10.11 ± 1.65^{d}					
Storage period (day)								
1	0.02 ± 0.00^{a}	38.53 ± 1.00^{a}	15.97 ± 0.30^{a}					
7	0.00 ± 0.00^{b}	23.94 ± 0.80^{b}	$15.43 \pm 0.30^{\circ}$					
14	0.00 ± 0.00 ^b	10.68 ± 1.15^{c}	11.83 ± 0.29^{b}					
Treatment and storage period (day)								
Grape (1)	0.02 ± 0.00	33.47 ± 0.29^{d}	18.08 ± 0.04^{a}					
Lemon (1)	0.00 ± 0.00	24.00 ± 0.00^{h}	$6.75 \pm 0.05^{\circ}$					
Orange (1)	0.00 ± 0.00	12.05 ± 0.05^{m}	14.00 ± 0.00^{f}					
Pawpaw (1)	0.02 ± 0.00	32.50 ± 0.29^{e}	14.61 ± 0.31^{e}					
Synthetic pineapple (1)	0.00 ± 0.00	26.15 ± 0.15^{g}	4.40 ± 0.00^{p}					
Plain (1)	0.00 ± 0.00	10.65 ± 0.03^{n}	12.58 ± 0.30^{9}					
Grape (7)	0.02 ± 0.00	41.60 ± 0.30^{b}	$15.27 \pm 0.13^{\circ}$					
Lemon (7)	0.00 ± 0.00	24.50 ± 0.50^{h}	5.74 ± 0.00^{n}					
Orange (7)	0.00 ± 0.00	13.93 ± 0.07^{I}	10.58 ± 0.01^{k}					
Pawpaw (7)	0.02 ± 0.00	$39.66 \pm 0.33^{\circ}$	16.88 ± 0.06^{b}					
Synthetic pineapple (7)	0.00 ± 0.00	28.50 ± 0.50^{f}	6.58 ± 0.01^{m}					
Plain (7)	0.00 ± 0.00	3.04 ± 0.04^{p}	10.70 ± 0.02^{j}					
Grape (14)	0.02 ± 0.00	$40.41 \pm 0.21^{\circ}$	16.05 ± 0.03^{d}					
Lemon (14)	0.00 ± 0.00	21.55 ± 0.29^{i}	$4.70 \pm 0.00^{\circ}$					
Orange (14)	0.00 ± 0.00	17.64 ± 0.32^{k}	11.92 ± 0.06^{h}					
Pawpaw (14)	0.02 ± 0.00	43.51 ± 0.29^{a}	14.89 ± 0.11^{e}					
Synthetic pineapple (14)	0.00 ± 0.00	20.15 ± 0.15^{j}	3.70 ± 0.00^{q}					
Plain (14)	0.00 ± 0.00	$6.79 \pm 0.01^{\circ}$	11.20 ± 0.10^{i}					

Table 1: Antioxidant capacity of yoghurts made from tropical fruits at storage periods of
1, 7 and 14 days

^{*a-m*} Means along the same column with different superscripts are significantly (p<0.05) different

Table 2: Cell surface hydrophobicity (CSH) of yoghurts made from tropical fruits at 600nm wavelength

Bacterial strains	Cell surface hydrophobicity (%)		
	n-Hexane	Xylene	
Lactobacillus casei	$11.65 \pm 0.44^{\circ}$	$14.78 \pm 0.33^{\circ}$	
Lactobacillus delbrueckii subsp. bulgaricus	72.89 ± 6.57^{a}	73.47 ± 5.08^{a}	
Lactobacillus spp.	21.51 ± 0.01^{b}	22.31 ± 0.02^{b}	

^{a-c} Means along the same row with different superscripts are significantly (p<0.05) different

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Treatment	K	Ca	Na	Mg	Р	Zn	Fe
Grape	1137.01 ± 39.33^{f}	$1588.11 \pm 28.49^{\circ}$	32.44 ± 1.61^{ab}	$1005.85 \pm 22.34^{\circ}$	$434.30 \pm 9.47^{\circ}$	$48.27 \pm 3.52^{\circ}$	12.00 ± 0.2
Lemon	1164.14 ± 46.54^{a}	1568.40 ± 22.17 ^f	32.08 ± 2.12^{ab}	1005.50 ± 20.05^{cd}	433.88 ± 6.50 ^c	50.08 ± 3.88^{a}	12.04 ± 0.19
Orange	1147.77 ± 27.54 ^c	1629.83 ± 7.94^{a}	32.61 ± 2.85^{a}	1010.41 ± 22.53^{b}	433.16 ± 8.53^{d}	46.78 ± 2.50^{d}	12.17 ± 0.15
Pawpaw	1144.78 ± 36.65 ^d	1578.88 ± 20.72 ^e	32.96 ± 2.01 ^a	1000.93 ± 22.11 ^e	435.11 ± 7.21 ^b	$48.53 \pm 3.18^{\circ}$	12.25 ± 0.23
Synthetic pineapple	1143.39 ± 28.08 ^e	1591.06 ± 20.11^{b}	32.46 ± 2.42^{ab}	1005.09 ± 21.66^{d}	439.40 ± 8.93^{a}	49.02 ± 2.83 ^b	12.13 ± 0.15
Plain	158.41 ± 40.87 ^b	1586.22 ± 12.18^{d}	32.68 ± 1.10^{b}	1011.87 ± 20.90^{a}	433.44 ± 8.55 ^d	49.30 ± 3.13 ^b	12.21 ± 0.23
Storage period (day)							
1	1290.36 ± 8.01^{a}	1641.58 ± 6.00^{a}	40.14 ± 0.42^{a}	1091.19 ± 1.32^{a}	463.10 ± 1.49^{a}	58.80 ± 0.45^{a}	12.71 ± 0.09^{a}
7	1110.04 ± 1.64^{b}	1609.75 ± 3.71^{b}	32.06 ± 0.40^{b}	977.59 ± 1.71^{b}	434.82 ± 0.73^{b}	50.02 ± 0.58^{b}	12.04 ± 0.01^{b}
14	1047.36 ± 4.72 ^c	1519.91 ± 9.95 ^c	25.42 ± 0.36 ^c	951.05 ± 2.31 ^c	406.73 ± 0.97 ^c	$37.16 \pm 0.44^{\circ}$	11.65 ± 0.08^{c}
Treatment and storage period (day)							
Grape (1)	$1287.19 \pm 0.09^{\circ}$	1683.55 ± 0.26^{a}	38.74 ± 0.38^{d}	$1093.11 \pm 0.16^{\circ}$	468.58 ± 0.21^{b}	58.06 ± 0.06^{d}	12.35 ± 0.17
Grape (7)	1102.48 ± 0.29^{k}	1594.35 ± 0.33^{k}	30.38 ± 0.19^{h}	978.85 ± 0.45^{i}	431.13 ± 0.13 ^j	52.12 ± 0.12^{g}	12.03 ± 0.02
Grape (14)	1021.38 ± 0.24^{q}	1486.43 ± 0.30^{p}	28.21 ± 0.11^{i}	945.59 ± 0.30 ^p	403.19 ± 0.31°	34.62 ± 0.32 ^p	11.61 ± 0.06
Lemon (1)	1344.83 ± 0.41^{a}	1634.19 ± 0.16^{d}	$39.22 \pm 0.33^{\circ}$	1082.34 ± 0.12^{f}	455.26 ± 0.03^{f}	61.76 ± 0.34^{a}	12.55 ± 0.29
Lemon (7)	1112.58 ± 0.82^{g}	1586.99 ± 0.57 ^I	32.44 ± 0.29 ^f	986.99 ± 0.05^{9}	436.01 ± 0.24^{h}	53.10 ± 0.10^{f}	12.07 ± 0.04
Lemon (14)	1035.01 ± 0.01^{p}	1484.03 ± 0.03^{q}	24.57 ± 0.30 ¹	$947.17 \pm 0.09^{\circ}$	$410.37 \pm 0.32^{\circ}$	$35.38 \pm 0.19^{\circ}$	11.51 ± 0.29
Orange (1)	1257.26 ± 0.13^{d}	1657.15 ± 0.58^{b}	43.40 ± 0.35^{a}	1098.37 ± 0.32^{a}	463.17 ± 0.09 ^c	55.78 ± 0.40 ^e	12.65 ± 0.32
Orange (7)	1103.35 ± 0.33 ^j	1630.18 ± 0.13^{e}	30.38 ± 0.19 ^h	983.35 ± 0.33^{h}	432.17 ± 0.44^{i}	46.01 ± 0.30^{k}	12.00 ± 0.06
Orange (14)	1082.69 ± 0.31^{1}	1602.14 ± 0.14^{j}	24.06 ± 0.03 ¹	949.52 ± 0.29^{n}	404.13 ± 0.13^{n}	38.55 ± 0.2^{m}	11.87 ± 0.03
Pawpaw (1)	$1285.39 \pm 0.31^{\circ}$	1615.82 ± 0.59^{9}	38.59 ± 0.30^{d}	1086.24 ± 0.03^{e}	458.18 ± 0.09^{e}	58.10 ± 0.06^{d}	13.04 ± 0.09
Pawpaw (7)	1110.40 ± 0.35^{h}	1624.65 ± 0.33^{f}	35.11 ± 0.06 ^e	978.44 ± 0.23 ⁱ	438.52 ± 0.29 ⁹	50.99 ± 0.05^{h}	12.06 ± 0.03
Pawpaw (14)	$1038.54 \pm 0.29^{\circ}$	$1496.16 \pm 0.09^{\circ}$	25.17 ± 0.16^{k}	938.11 ± 0.22^{q}	408.62 ± 0.3^{m}	36.49 ± 0.28^{n}	11.64 ± 0.32
Synthetic pineapple (1)	1249.35 ± 0.32 ^e	$1647.43 \pm 0.43^{\circ}$	40.85 ± 0.45^{b}	1091.64 ± 0.32^{d}	473.18 ± 0.43^{a}	$59.11 \pm 0.06^{\circ}$	12.67 ± 0.09
Synthetic pineapple (7)	1122.72 ± 0.36^{f}	1612.54 ± 0.29^{h}	32.40 ± 0.30^{f}	965.56 ± 0.28^{10}	432.62 ± 0.32^{i}	48.37 ± 0.19^{j}	12.01 ± 0.01
Synthetic pineapple (14)	1058.10 ± 0.06^{m}	1513.19 ± 0.19^{n}	24.13 ± 0.06 ¹	958.09 ± 0.14^{m}	412.41 ± 0.21^{k}	39.57 ± 0.30 ¹	11.71 ± 0.05
Plain (1)	1318.12 ± 0.06^{b}	1611.35 ± 0.33^{h}	40.02 ± 0.58^{b}	1095.44 ± 0.29^{b}	460.23 ± 0.12^{d}	60.01 ± 0.07^{b}	13.01 ± 0.12
Plain (7)	1108.72 ± 0.37^{i}	1609.80 ± 0.16^{i}	31.65 ± 0.33 ^g	972.35 ± 0.22 ^j	438.45 ± 0.24 ⁹	49.52 ± 0.29 ⁱ	12.08 ± 0.04
Plain (14)	1048.40 ± 0.30^{n}	1537.51 ± 0.26^{m}	26.35 ± 0.18 ^j	967.82 ± 0.43^{k}	401.65 ± 0.18^{p}	38.35 ± 0.1^{m}	11.54 ± 0.27
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Means along the same column with different superscripts are significantly (p<0.05) different, K= Potassium, Ca=Calcium, Na=Sodium, Mg= Magnesium, P=Phosphorus, Zn= Zinc, Fe=Iron

Most synthetic additives used as flavourants in the food industry contain antioxidants such as butylated hydroxyl anisole (BHA), ethoxyguin, metabisulfite and butylated hydroxyl toluene (BHT). These synthetic antioxidants have been reported to be toxic and harmful to human being, therefore their use are being restricted and substituted by natural antioxidants (Imaida et al., 1983). The principle of ABTS involves the scavenging activity of extracts against free radicals, but ABTS salt must be generated by enzymatic or chemical reaction (Arnao, 2000). DPPH is a stable free radical with characteristic absorption at 517 nm. Antioxidants react with DPPH and convert it to 2.2-diphenyl-1-picrylhydrazine (Von Gadow et al., 1997). The FRAP assay is the only assay that directly measures antioxidants (or reductants) in a sample compared to other assays measuring inhibition of free radicals (Halvorsen et al., 2002).

In this study, all the fruits enriched yoghurts showed stronger DPPH antioxidant potential than the plain yoghurt. The higher the value, the higher its potency to inhibit free radical induced oxidative damage. It was shown that all fruit-enriched yoghurts showed an increment in percentage of inhibition compared with plain yoghurt. Grape, orange and pawpaw flavoured yoghurt had increased antioxidant capacity with increase in storage period, while lemon, synthetic pineapple and plain yoghurt had reduced antioxidant capacity at day 14 of the storage period. The antioxidant activity of these fruit flavoured yoghurt could be attributed to the phytochemical compounds present in the fruits. However, grape flavoured yoghurt showed stronger FRAP antioxidant potential than other samples which was in accordance with the report of Raikos et al. (2019). The storage period also had significant effect on the antioxidant capability of the yoghurt samples as this decreased in all the samples at day 14 of storage which corroborates the reports of Raikos et al. (2019) that beyond four weeks of storage, the FRAP antioxidant potential of yoghurt containing salal berry (SB) reduced by around 18 %. The decrease in antioxidant activity of yoghurts fortified with fruit extract could be attributed to the loss of anthocyanin

activity (Lawin and Kongbangkerd, 2010; Kumar and Kumar, 2016). Decrease antioxidant activity of samples during storage may be related to milk polyphenol interactions which may lead to decreased antioxidant capacity (Arts et al., 2002). The radical scavenging abilities of the voghurt could be beneficial in the management of type 2 diabetes as free radicals are involved in the development and complications of diabetes in a number of ways; the white blood cell production of reactive oxygen species mediates the autoimmune destruction of the beta cells in the islets of Langerhans in the pancreas, abnormalities in transition metal metabolism are postulated to result in the establishment of diabetes and diabetes associated hyperglycaemia causes intracellular oxidative stress, which contributes to vascular dysfunction (Ademosun and Oboh, 2015).

Cell surface hydrophobicity (CSH) is an important property for probiotic bacteria. It is a prerequisite for probiotics to adhere to the intestinal epithelium in order to colonise the gastrointestinal tract by exerting beneficial effect of probiotics, such as exclusion of enteropathogenic bacteria (Sharma et al., 2016). Microbial hydrophobicity plays an important role in processes such as food production, spoilage, etc. due to interactions between microorganisms and food components such as lipids and proteins. For example, species of LAB such as Lactococcus lactis subsp. lactis biovr diacetylactis, which have a key role in the production of yoghurts, cheese or sausages, could influence and change the stability of food emulsions (Krasowska and Sigler, 2014). According to Falah et al. (2019), hydrophobicity examination can be considered a pre-test of the adhesion capacity of probiotic bacteria to epithelial cells. They also consider hydrophobicity as one of the important properties improving the first contact between bacteria and host cells. De Souza et al. (2019) reported bacteria with high hydrophobicity have better ability to bind to epithelial cells. The ability to adhere to the mucus produced by intestinal epithelium is one of the main criteria for selecting probiotics. Having this ability may increase their chances of survival in the gastrointestinal tract and thus allow bacteria to

exert their positive health effects (García-Cayuela et al., 2014; Okochi et al., 2017). Their attachment to intestinal epithelium can have a protective role against harmful bacteria via competition for host cell binding sites (Monteagudo-Mera et al., 2019). The degree of hydrophobic interaction between bacteria and surfaces revealed that the L. delbrueckii subsp. *bulgaricus* had the strongest CSH in the yoghurt samples. The results obtained in this study are in agreement with those of Haitham et al. (2017) and Dlamini et al. (2019). However, varied result was obtained in the research of Lv and Zhao (2011) who reported strong CSH for Lactobacillus acidophilus than Lactobacillus *bulgaricus* and *Streptococcus* thermophiles; stating time, temperature, pH, concentration, Ca²⁺ and protease as influencing factors. Although, some substances which mediates CSH of L. delbrueckii subsp. bulgaricus may be proteins. Mineral ions play vital roles in metabolism as transport co-factor and stabilize enzymes (Mishra et al., 2012). They are essential nutrients that are involved in all animal metabolism and body functions, like providing strength to skeletal structure, maintenance of acid-base equilibrium in body, sodium is an important mineral for the control of water balance in the body and also helps with normal impulse regulation and muscle contraction, calcium is needed for bone formation and neurological function of the body (Cashman et al., 2002). Magnesium is essential to good health because it helps to maintain normal muscle and nerve function, keeps heart rhythm steady, supports a healthy immune system and keeps bones strong. Lack of magnesium is associated with abnormal irritability of muscle and convulsions and excess magnesium associated with depression of the central (Igbabul al., nervous system et 2014). Magnesium functions as a co-factor for many enzymes involved in energy metabolism, protein synthesis and maintenance of the electrical potential of nervous tissues and cell membranes (Onimawo and Akubor, 2012). The decrease in mineral content as storage time increased observed in this study could be attributed to fermentation process during storage. This result is in concord with the report of Akubor (2016)

who reported decrease in mineral composition of yoghurt and pineapple juice as storage period increased from day 1 to 7. The differences in the mineral constituents of the flavours (grape, lemon, orange, pawpaw and synthetic pineapple) could be implicated. The study showed that all the flavoured yoghurts are good sources of sodium, while the orange flavoured yoghurt is a good source of calcium indicating their idealness for individuals that need supplementary intake of these minerals.

Conclusion: Fruit juices could be used as alternative flavourants in yoghurt production as they compared favourably with the commonly used synthetic flavourants. This could help in preventing health conditions associated with the use of synthetic flavourants. However, due to the decrease in the mineral concentration and antioxidant capacity with increased storage, value-added yoghurt made from tropical fruits should be consumed within seven days of production for optimal health benefits.

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REFERENCES

- ADEMOSUN, A. O. and OBOH, G. (2015). Inhibition of carbohydrate hydrolyzing enzyme associated with type 2 diabetes and anti-oxidative properties of some edible seeds in vitro. *International Journal of Diabetes in Developing Countries*, 35(3): 516 – 521.
- AKPAN, U. G., MOHAMMED, A. D. and AMINU, I. (2007). Effect of preservative on the shelf life of yoghurt produced from soya beans milk. *Leonardo Electronic Journal* of Practices and Technologies, 11: 131 – 142.
- AKUBOR, P. I. (2016). Physicochemical, microbiological and sensory properties of yoghurt supplemented with carrot

juice. *Nigerian Journal of Nutritional Sciences*, 32(1): 15 – 20.

- AOAC (1990). *Official Methods of Analysis*. 15th Edition, Association of Official Analytical Chemist, Washington, DC, USA.
- ARNAO, M. B. (2000). Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practical case. *Trends in Food Science and Technology*, 11(11): 419 – 421.
- ARTS, M. J. J. J., HAENEN, G. R. M. M., WILMS, L. C., BEETSTRA, S. A. J. N., HEIJNEN, C. G. N., VOSS, H. and BAST, A. (2002). Interactions between flavonoids and proteins effect on the total antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 50(5) 1184 – 1187.
- CASHMAN, K. D. ROGINSKI, H. and FUQUAY, J.
 W. (2002). Macroelements nutritional significance. Pages 2051 2058. In: ROGINSKI, H., FUQUAY, J. W. and FOX, P. F. (Eds.). *Encyclopedia of Dairy Sciences*. Number 3, Academic Press, London.
- COISSON, J. D., TRAVAGLIA, F., PIANA, G., CAPASSO, M. and ARLORIO, M. (2005). *Euterpe oleracea* juice as a functional pigment for yogurt. *Food Research International*, 38(8-9): 893 – 897.
- DANIEL, O. A. (2015). Urban extreme weather: a challenge for a healthy Living environment in Akure, Ondo State, Nigeria. *Climate*, 3(4): 775 – 791.
- DE SOUZA, B. M. S., BORGONOVI, T. F., CASAROTTI, S. N., TODOROV, S. D. and PENNA, Α. L. B. (2019). Lactobacillus casei and Lactobacillus fermentum strains isolated from mozzarella cheese: probiotic potential, safety, acidifying kinetic parameters and viability under gastrointestinal tract conditions. Probiotics and Antimicrobial Proteins, 11(2): 382 - 396.
- DLAMINI, Z. C., LANGA, R. L., AIYEGORO, O. A. and OKOH, A. I. (2019). Safety evaluation and colonisation abilities of four lactic acid bacteria as future probiotics. *Probiotics and Antimicrobial Proteins*, 11(2): 397 – 402.

- ERDOGAN, K. and ZEKAI, T. (2003). Influence of different fruit additives on some properties of stirred yoghurt during storage. Yüzüncü Yıl Üniversitesi, Ziraat Fakültesi, Tarım Bilimleri Dergisi, Journal of Agricultural Science, 13(2): 97 – 101.
- FALAH, F., VASIEE, A., BEHBAHANI, B. A., YAZDI, F. T., MORADI, S., MORTAZAVI, S. A. and ROSHANAK, S. (2019).
 Evaluation of adherence and antiinfective properties of probiotic *Lactobacillus fermentum* strain 4-17 against *Escherichia coli* causing urinary tract infection in humans. *Microbial Pathogenesis*, 131: 246 – 253.
- FERNANDEZ, M. A. and MARETTE, A. (2017). Potential health benefits of combining yogurt and fruits based on their probiotic and prebiotic properties. *Advances in Nutrition*, 8(1): 1555 – 164S.
- GARCÍA-CAYUELA, T., KORANY, A. M., BUSTOS, I., DE CADIÑANOS, L. P. G., REQUENA, T., PELÁEZ, C. and MARTÍNEZ-CUESTA, M. C. (2014). Adhesion abilities of dairy *Lactobacillus plantarum* strains showing an aggregation phenotype. *Food Research International*, 57: 44 – 50.
- GONÇALVES, M. M., FREITAS, R., NERO, L. A. and CARVALHO, A. F. (2009). Enumeration of starter cultures during yogurt production using Petrifilm[™] AC plates associated with acidified MRS and M17 broths. *Journal of Dairy Research*, 76(2): 229 – 233.
- HAITHAM, A. R., ZAITON, H., NORRAKIAH, A.
 S. and HUDA-FAUJAN, N. (2017).
 Assessment of potential probiotic properties lactic acid bacteria from shrimp paste of belacan. *International Journal of Advances in Science Engineering and Technology*, 5(1): 90 98.
- HALVORSEN, B. L., HOLTE, K., MYHRSTAD, M. C., BARIKMO, I., HVATTUM, E., REMBERG, S.
 F., WOLD, A. B., HAFFNER, K., BAUGERØD, H., ANDERSEN, L. F. and MOSKAUG, Ø. (2002). A systematic screening of total

antioxidants in dietary plants. *Journal of Nutrition*, 132(3): 461 – 471.

- IBHAZE, G. A., AMANZE, U. R. and ONIBI, G. E. (2020). Mineral, vitamin concentrations and sensory properties of artificial and naturally flavoured yoghurts. *Nigerian Journal of Animal Science* 22(2): 318 – 323.
- IGBABUL, B., SHEMBER, J. and AMOVE, J. (2014). Physicochemical, microbiological and sensory evaluation of yoghurt sold in Makurdi metropolis. *African Journal of Food Science and Technology*, 5(6): 129 – 135.
- IMAIDA, K., FUKUSHIMA, S., SHIRAI, T., OHTANI, M., NAKANISHI, K. and ITO, N. (1983). Promoting activities of butylated hydroxyanisole and butylated hydroxytoluene on 2-stage urinary bladder carcinogenesis and inhibition of γ-glutamyl transpeptidase-positive foci development in the liver of rats. *Carcinogenesis*, 4(7): 895 – 899.
- KIM, K. H., HWANG, H. R., JO, J. E., LEE, S. Y., KIM, N. Y. and YOOK, H. S. (2009). Quality characteristics of yogurt prepared with flowering cherry (*Prunus serrulata* L. var. *spontanea* Max. wils.) fruit powder during storage. *Journal of the Korean Society of Food Science and Nutrition*, 38(9): 1229 – 1236.
- KRASOWSKA, A. and SIGLER, K. (2014). How microorganisms use hydrophobicity and what does this mean for human needs?. *Frontiers in Cellular and Infection Microbiology*, 4: 112. https:// doi.org/10.3389/fcimb.2014.00112
- KUMAR, A. and KUMAR, D. (2016). Development of antioxidant rich fruit supplemented probiotic yogurts using free and microencapsulated Lactobacillus rhamnosus culture. Journal of Food Science and Technology, 53(1): 667 – 675.
- LAWIN, P. and KONGBANGKERD, T. (2010). Development of probiotic yoghurt mixed with roselle syrup. *Asia-Pacific Journal of Science and Technology*, 15(9): 803 – 808.

- LV, J. L. and ZHAO, W. (2011). Analysis of the cell surface hydrophobicity of yoghurt fermentation bacteria. *Advanced Materials Research*, 361-363: 1274 – 1278.
- MENSOR, L. L., MENEZES, F. S., LEITÃO, G. G., REIS, A. S., SANTOS, T. C. D., COUBE, C. S. and LEITÃO, S. G. (2001).
 Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, 15(2): 127 – 130.
- MISHRA, B. K, RASTOGI, A. and SHUKLA, S. (2012). Regulatory role of mineral elements in the metabolism of medicinal plants. *Medicinal and Aromatic Plant Science and Biotechnology*, 6(1): 1 23.
- MONTEAGUDO-MERA, A., RASTALL, R. A., GIBSON, G. R., CHARALAMPOPOULOS, D. and CHATZIFRAGKOU, A. (2019). Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. *Applied Microbiology and Biotechnology*, 103(16): 6463 – 6472.
- OH, H. and KANG, S. (2015). Quality characteristics and antioxidant activity of yogurt added with acanthopanax powder. *Korean Journal of Food Science and Technology*, 47(6): 765 771.
- OKOCHI, M., SUGITA, T., ASAI, Y., TANAKA, M. and HONDA, H. (2017). Screening of peptides associated with adhesion and aggregation of *Lactobacillus rhamnosus* GG in vitro. *Biochemical Engineering Journal*, 128: 178 – 185.
- ONIMAWO, I. A. and AKUBOR, P. I. (2012). *Food Chemistry Integrated (Approach with Biochemical Background)*. 2nd Edition, Joytal Printing Press, Ibadan, Nigeria.
- PULIDO, R., BRAVO, L. and SAURA-CALIXTO, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, 48(8): 3396 – 3402.
- RAIKOS, V., NI, H., HAYES, H. and RANAWANA, V. (2019). Antioxidant properties of a

yogurt beverage enriched with salal (*Gaultheria shallon*) berries and blackcurrant (*Ribes nigrum*) pomace during cold storage. *Beverages*, 5(1): 2. http://dx.doi.org/10.3390/beverages501 0002

- RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M. and RICE-EVANS, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9-10): 1231 – 1237.
- ROSENBERG, M., GUTNICK, D. and ROSENBERG, E. (1980). Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiology Letters*, 9(1): 29 – 33.
- SENADEERA, S. S., PRASANNA, P. H. P., JAYAWARDANA, N. W. I. A., GUNASEKARA, C. S., SENADEERA, D. Ρ. and CHANDRASEKARA, A. (2018). Antioxidant, physicochemical, microbiological, and sensory properties of probiotic yoghurt with various incorporated Annona species pulp. *Heliyon*, 4(11): e00955. https://doi.org/10.1016/j.heliyon.2018.e 00955
- SHARMA, K., SHARMA, N. and SHARMA, R. (2016). Identification and evaluation of in vitro probiotic attributes of novel and potential strains of lactic acid bacteria isolated from traditional dairy products of North-West Himalayas. *Journal of*



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Clinical Microbiology and Biochemical Technology, 2(1): 018 – 025.

- SPSS (2017). *Statistical Package for Social Sciences Version 25.* SPSS Incorporated, Chicago, Illinois, USA.
- VAHEDI, N., TEHRANI, M. M. and SHAHIDI, F. (2008). Optimizing of fruit yoghurt formulation and evaluating its quality during storage. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 3(6): 922 – 927.
- VAN DE WATER, J. (2008). Yogurt and immunity: The health benefits of fermented milk products that contain lactic acid bacteria. Pages 113 – 144. *In:* FARNWORTH, E. R. T. (Ed.). *Handbook of Fermented Functional Foods.* Second Edition, CRC Press, Boca Raton, Florida, United State.
- VON GADOW, A., JOUBERT, E. and HANSMANN,
 C. F. (1997). Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), a-tocopherol, BHT, and BHA. *Journal of Agricultural and Food Chemistry*, 45(3): 632 638.
- WALLACE, T. C. and GIUSTI, M. M. (2008). Determination of color, pigment, and phenolic stability in yogurt systems colored with nonacylated anthocyanins from *Berberis boliviana* L. as compared to other natural/synthetic colorants. *Journal of Food Science*, 73(4): C241 – C248.