NISEB Journal Vol. 15, No. 1, March, 2015 1595-6938/2015
Printed in Nigeria (2015) Nigerian Society for Experimental Biology

Investigation of Unripe Plantain (*Musa Paradisiaca*) Powder as Possible Enrichment Ingredient for Yoghurt.

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Abstract
The investigation of the effect of supplementing yoghurt with unripe plantain powder has been carried out. Plantain is one of the healthiest foods in the world in terms of its nutrient content. The study was conducted by adding unripe plantain powder at different concentrations (5, 10, and 15% weight/weight) into reconstituted powdered milk solution, pasteurized, inoculated with lactobacillus delbrueckii spp. Bulgaricus and streptococcus thermophilus and then incubated at 45^{O}C to pH 4.30. A control without unripe plantain powder was also prepared. The resulting products were stored at 5^{O}C and evaluated for various physical and chemical properties. The lactic acid concentration profile and pH was monitored during incubation to ascertain the effect of the supplement on the viability of lactobacillus delbrueckii spp. Bulgaricus and streptococcus thermophilus. At all concentrations studied, unripe plantain addition neither significantly affected the growth of the microorganism nor caused any modification of nutritional parameters. In conclusion, these results suggests powdered unripe plantain as a valuable ingredient to enrich yoghurt

Keywords: Plantain, Fermentation, Microorganism, Yoghurt

Introduction
The increasing rise in health concern has led to growing interest in food substances that are beneficial to the health of consumers, especially with regard to foods that can be considered of greater nutritional value, low in calorie, low in fat and rich in bioactive compounds scientifically proven to be negatively correlated to disease risk [1, 2]. Yoghurt is one of such food. It is a coagulated milk product that results from the fermentation of lactose to lactic acid in milk by lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*).
Yoghurt, is one of the best known food that contain probiotics which upon ingestion in sufficient amount exert beneficial effect on the normal microbial population in the gastrointestinal track. [3].
Yoghurt is a popular food.[4]. Increased yogurt production and consumption are attributed to yogurt's perceived health benefits and wide consumer appeal [5, 6]. Approximately, 30% of the world's population buys probiotic dairy food on a regular basis, and in 2008, the global probiotic food market was over $15.7 billion, which was 18% the global functional food market [7]. The global yoghurt market is characterized by intense competition prompting leading players to differentiate themselves by focusing on health benefits, branding and incorporation of ingredients such as fruits and some health important plant products.
Believe it or not, plantain is one of the healthiest foods in the world in terms of its nutrient content. The nutritional value of plantain makes it stand out as a very important addition to any healthy living diet plan. Extensive investigation regarding anti-ulcerogenic and ulcer healing activity of plantain have been studied in the laboratory department of pharmacology institute of medical science Banaras Hindu University, Indian. [8], have reported that anti ulcerogenic activity of dried powder of unripe plantain against ulcer induced by Histamine in guinea pigs and phenylbutazone, refrain stress and predinsolone in rat [8,9]. In a comprehensive study done with dried unripe plantain and its various extracts against various experimental gastro-duodenal ulcer and ideologically proved case of peptic ulcer, dried unripe plantain showed its usefulness. [10].
Plantain is a staple food in the tropical regions of the world. Some countries with plantain crops includes, Nigeria, United States, Taiwan, Indonesia, Australia, etc.
The aim of this study is to prepare yoghurt product with additional health benefits, by fortifying yoghurt samples with unripe plantain powder and to evaluate how much influence it has on the yoghurt bacterial by studying the rate of production of lactic acid from lactose. And also to study how much effect the unripe plantain powder has on the nutritional and the physicochemical properties of the yoghurt products

Materials and methods.
The materials used for this research and their sources are, Non–fat milk powder (Dano milk), Yoghurt starter culture (*Lactobacillus bulgaricus, Streptococcus thermophilus*), Sodium hydroxide pellet, Phenolphthalein indicator,

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Sulphuric acid, Selenium tablet, Perchloric acid, Glucose powder, are all from BDH Chemicals Ltd, Poole England and are analytical grade, Peptone water was from Oxoid ltd. Basingstoke, UK. Electric weighing balance (Model: Scout Pro Spu 202. Max 200g). Incubator, Stomacher 400, was from PBI International, Milan, Italy. Autoclave, Microscope (Karl Zeis AG, Gottingen, Germany) pH meter (HaNNA-pH 210. $\pm$ 0.1 pH accuracy), Oven (Platinum; Model No: PL – 36L), Spectrophotometer (Model: Gallenkamp, Spec 120), Centrifuge (Model: Gallenkamp, Spec 210), Thermometer (Galleenkamp Griffin Nitrogen filled). Green Plantain was obtained from University of Benin Agric farm.

Preparation of plantain powder: Green plantain fingers were washed, sliced into small pieces and oven dried at 60^{O}C to constant weight. It was then ground into powder and filtered using a 160µm sieve to obtain fine powder.

Preparation of yoghurt mother culture: A mother culture was prepared by mixing 5g of low fat non-fat dry milk to 100ml of de-ionized, distilled water in a 250ml volumetric flask using a magnetic stirrer at room temperature, 28^{O}C for 5minutes. The reconstituted milk was covered with cheesecloth and aluminium foil, autoclaved at 115^{O}C at a pressure of 15psi for 15minutes and cooled to room temperature. 2%w/w of yoghurt starter culture was aseptically transferred to the milk and incubated for 24hours at 37^{O}C until pH 4.2 was achieved. The prepared mother culture which was used in subsequent studies was stored and refrigerated at 5^{O}C.

Yoghurt/ Plantain mix processing: 20g of the powdered milk was accurately weighed into four different flasks. 5%, 10% , and 15% of plantain powder weight by weight of milk was reconstituted with the weighed milk into de-ionized, distilled water in the four flask to make tests sample, A, B, C, and D. Sample A has no plantain powder and served as control. The test samples were stirred to achieve dispersion and pasteurized at 90^{O}C for 10minutes and cooled to about 43^{O}C. All test samples (A, B, C and D) were inoculated with 5g of the mother culture, mixed properly and incubated at 42^{O}C until a pH of 4.3 was attained and the time was noted. When the pH of 4.3 was attained, the samples were cooled in a water-bath, homogenized and then stored in a refrigerator at 5^{O}C for further analysis.

pH measurement: Equal quantities of yoghurt and distilled water were mixed vigorously in a beaker. The sample was allowed to settle for one minute and then the electrode of a digital potable pH meter calibrated at pH 4, 7 and 8 using buffer solution was inserted, allowed few seconds for the reading to stabilize and the results were taken. The pH of the samples were measured and recorded at intervals of 1 hour during fermentation.

Titratable acidity: Titrable acidity was measured as described by [11]. 10g of yoghurt was placed in a beaker and titrated to end point with 0.1 M sodium hydroxide solution using phenolphthalein as an indicator. The titratable acidity of each of the yoghurt samples was determined at intervals of 1 hour during fermentation. The results were given as percentage lactic acid (% lactic acid).

%TA = 9 X 0.1 X ml of NaOH/yoghurt weight.

Crude protein determination: Crude protein was estimated by determination of the total nitrogen in each sample. 1mL of each sample was digested in 2mL concentrated Sulphuric acid in the presence of Selenium catalyst, until a clear digest was obtained [12]. The nitrogen content of the digest was determined colorimetrically at 630nm. The conversion factor, 6.38 used was that recommended for milk products according to Davis and Mclachlan [13].

Moisture content: 5g of the sample was accurately weighed and transferred into a porcelain dish which has been dried and weighed. The weight of the sample with porcelain dish was recorded and the sample was dried in an oven at 105^{O}C for 6 hours and cooled in a desiccator for 10minuntes. The weight of the sample was taken at intervals of 1 hour until a constant weight was obtained. The percentage weight loss was recorded as the moisture content. [12]

$$\% \text{ moisture} = \frac{\text{weight of sample} - \text{weight of dry sample} \times 100}{\text{Weight of sample}}$$

Total solids: To determine the total solids in each yoghurt sample, the procedure for determination of moisture content was followed. The weight of residue obtained was expressed as percentage total solid. The total solid is given as:

% Total solid = 100 – moisture content

Ash Content : The method employed is as described by Association of Official Analytical Chemist. [12]. 5g of each sample was accurately weighed into a porcelain dish which had been washed, dried in an oven for about 2hours and cooled to room temperature in a dessicator. The sample was ashed at 550^{O}C in an oven for 24hours. At the end of the ashing period, the samples (in the porcelain dish) were placed in a dessicator to cool to room temperature and weighed. The results were given as percentage ash.

$$\% \text{ Ash} = \frac{\text{Weight of ash} \times 100}{\text{Weight of original sample}}$$

Carbohydrate determination: 13mL of 52% perchloric acid was used to hydrolyze 1mL of each yoghurt sample in 10mL of distilled water. The mixture was stirred with a glass rod for 20minutes in order to achieve complete hydrolysis. It was filtered into 25mL volumetric flask and made up to the mark with distilled water. 10mL of filtrate was taken and diluted to 100mL with distilled water. 1mL of the diluted solution, 0.1% glucose solution and distilled water were pipetted into a test tube. The test tube was placed in a hot water bath for about 12mins for development. Then the tube was removed from the water bath cooled to room temperature and the absorbance was read at 630nm against blank in a spectrophotometer. The absorbance was multiplied by the factor of 10 and the result was given as percentage carbohydrate.

Crude fibre determination: Determination of crude fibre was adopted from AOAC method 7.067 [12] with modifications. In the determination of crude fibre, 2mL of the sample was measured into a graduated beaker, and 100mL of boiling water was added. 25mL of 2.04M H_2SO_4 was also added and the content was made up to 200mL with water, the sample was boiled for 30mintes on a hot plate. After boiling, the sample was filtered using a filter paper and washed thoroughly with boiling water, then it was dried in an oven at 105^OC for 3 hours, after which the sample was cooled in a dessicator for 10 minutes and weighed. The weight gotten was expressed as percentage fibre content.

Fat determination: The method employed is as described by AOAC [12].

10mL of Sulphuric acid (H_2SO_4) was poured into a test tube containing 5mL of the yoghurt sample and thoroughly mixed, until no white particle was seen. The test tube containing the mixture was placed in a centrifuge and centrifuged at 1100 revolution per minute. And transferred to water bath at 65^OC for 3minutes and the columns of the fat was recorded immediately. The result was expressed as percentage fat.

Specific gravity: Specific gravity of yoghurt samples were evaluated using specific gravity bottles. The empty bottle was weighed, weighed with 1mL water, dried and weighed with 1g of the yoghurt sample. The specific gravity was calculated as the ratio of sample weight to weight of water

Sensory Evaluation: The sensory evaluation of the various yoghurt samples was done on 5-point hedonic scale (1- unacceptable; 5- excellent) by a 22 member panelist selected from final year students from various departments in university of Benin, Ugbowo campus.

The quality properties evaluated were: colour, taste, consistency, flavour and general acceptability.

Colour – Intensive white, ***Taste*** – Sour / sweet, ***Consistency*** – Uniform and compact, creamy, not lumpy, ***Flavour*** – Characteristic yoghurt-like (sharp, tangy flavor)

Storage study: The pH, titratable acidity and microbial count of streptococos and bacillus, of the yoghurt samples were measured on days 1, 7, 14, 21 and 28 at 10^Oc as a way of monitoring the stability of the products.

Statistical analysis: Results obtained were then subjected to statistical treatment using the one way analysis of variance (ANOVA).

Results and Discussions

The results of the influence of unripe plantain powder added to yoghurt preparation on the viability of *Lactobacillus delbrueckii spp. bulgaricus and Streptococus thermophilus* and on pH, titrable acidity and other proximate composition of yoghurt produced are shown below.

Table 1. Physicochemical quality of yoghurt samples obtained at the end of fermentation

Parameter s	**A**	**B**	**C**	**D**
pH (At the end of incubation)	4.300	4.300	4.300	4.300
% Titratable acidity	1.235	1.266	1.444	1.476
% Moisture Content	84.800	83.500	81.800	79.500
% Total Solid	15.200	16.500	18.200	20.500
% Ash	0.770	0.790	0.851	0.882
Specific gravity	1.043	1.043	1.055	1.058
% Crude fibre	1.800	2.200	2.400	2.800
% Crude protein	7.884	7.882	8.221	9.662
% Carbohydrate	3.361	5.123	6.690	7.166
% Fat	0.210	0.211	0.213	0.220

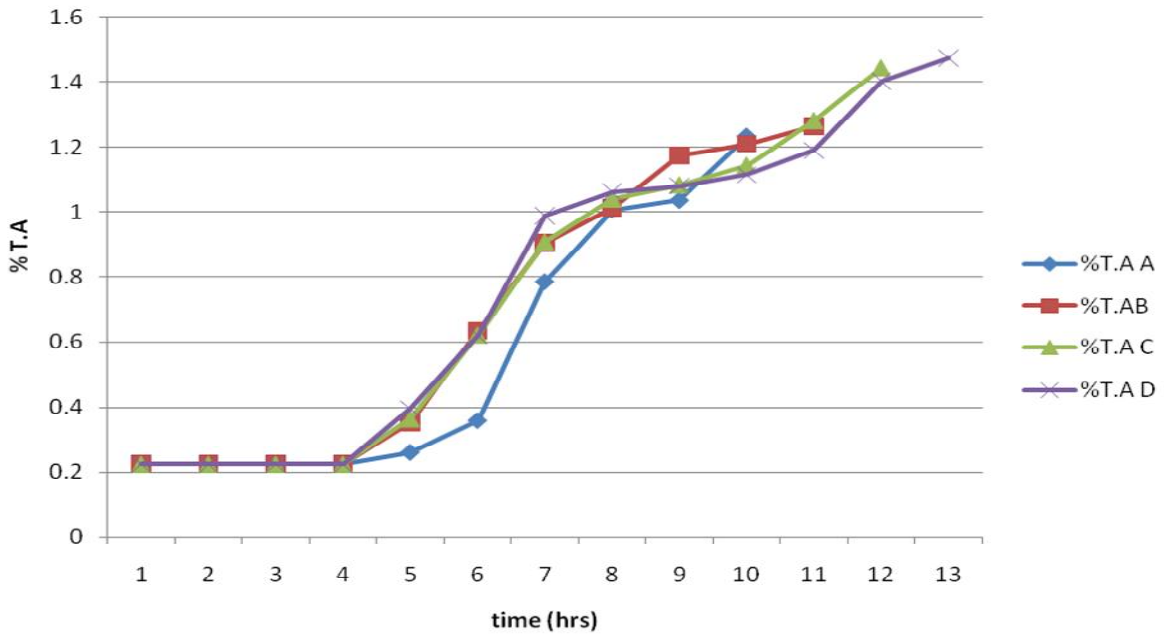

Fig.1. Titratable acidity versus time of fermentation

Table 1, shows the physicochemical quality of yoghurt obtained at pH 4.30. This pH is comparable to other works. [13]. Fig.1 shows the plot of titrable acidity versus time for fermentation. It shows that titratable acidity increases as fermentation progresses. The percentage titratable acidity increases with increasing supplementation whereas moisture content decreases with supplementation. The moisture content of the fortified yoghurt samples decreases with increase fortification as compared to the unfortified sample, which may be due to the absorption of water by the dried plantain powder. Moisture helps to control the viscosity and texture and promote softness of the yoghurt sample. The total solid increased from sample A through to D. This is in agreement the work of Davis and Mclachan [13]. The specific gravity and ash content increases with increasing amount of plantain powder in the mix. This suggests that plantain supplementation increased the mineral content of yoghurt. The same goes for crude fibre, protein and carbohydrate content of the yoghurt sample. There is no discernable amount of fat added to the yoghurt sample.

Figure 2**,** shows the time taken for milk/ plantain mix to get fermented till pH 4.30 was obtained. The result reveals that with more plantain powder in milk, the longer time it will take for fermentation to go to completion. Plantain powder absorbs water thereby reducing the amount of water that is available for microbial activity, act as thickening or bulking agent and hence slows down the rate of yoghurt production. Plantain powder therefore, affects the yoghurt culture and increases the lag phase of yoghurt preparation process where the microorganisms adapt themselves to growth condition.

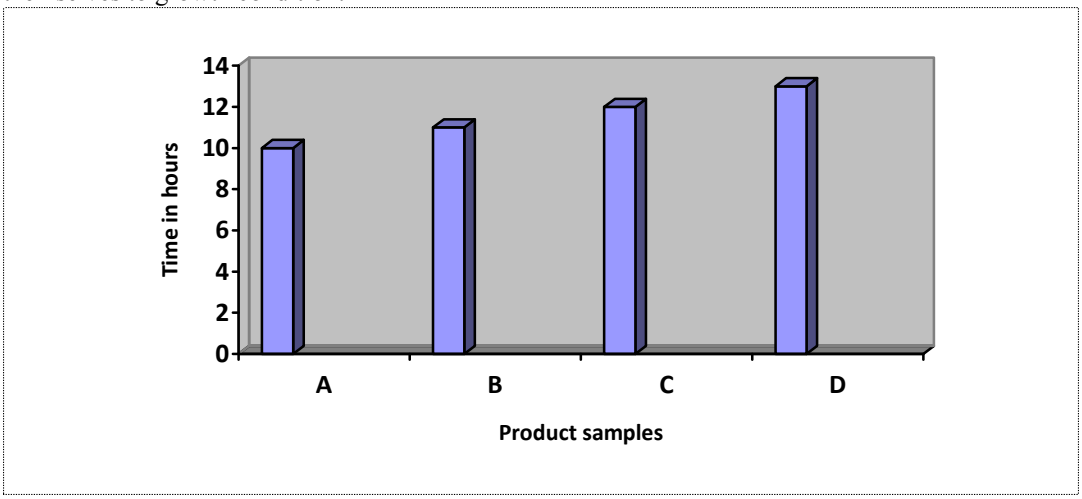

Figure 2: Time taken for product pH to get to 4.30

Storage study

pH values and titratable acidity of yoghurt samples monitored throughout storage are shown in Table 2 and Table 3 respectively. pH values after 28 days of storage at 10^{O}c , ranges from 3.700 to 3.900 for the control to the most supplemented sample respectively. No significant differences were observed between yoghurt with or without unripe plantain powder. All yoghurt samples also show similar titratable acidity on storage.

Table 2. Stability studies of yoghurt products; pH

Time (Days)	A	B	C	D
1	4.300	4.300	4.300	4.300
7	4.100	4.100	4.300	4.300
14	4.000	4.000	4.200	4.100
21	3.900	3.900	4.000	4.000
28	3.700	3.700	3.800	3.900

Table 3. Stability studies of yoghurt products; %TA

Time (Days)	A	B	C	D
1	1.235	1.266	1.447	1.476
7	1.234	1.266	1.447	1.475
14	1.236	1.267	1.449	1.477
21	1.236	1.267	1.448	1.477
28	1.237	1.267	1.449	1.478

Maintaining the characteristics of any food in food fortification is a major concern to any nutritionist. The above result shows relatively that the chemical, microbiological and nutritional qualities of yoghurt had no significant change at the end of experimental window of 28 days study.

Food acceptance and preference are functions of product quality. Table 3 shows the result of organoleptic tests carried out on yoghurt enriched with unripe plantain powder. The higher supplementation of yoghurt with plantain powder lowers the consistency of yoghurt sample whereas the taste appeal increases with increase in unripe plantain content of yoghurt. Often color is the first sensory characteristic perceived by the consumer and color tends to modify other perceptions such as flavor and aroma. The white colour of milk decreased alongside with the flavour with increase in the concentration of unripe plantain in yoghurt. Whiteness in fluid milk is a result of the larger particles, such as milk fat globules (2 to 10 μm) and casein micelles (~100 nm) which scatter light in the visible spectrum. Consumers show the highest appeal for fluid milks with visual properties characteristic of whole milk; thus, milk whiteness has a positive influence on increasing consumer appeal. [14, 15].

Table 3: Sensory evaluation of yoghurts samples.

Sensory property	A	B	C	D
Consistency	4.0	3.0	3.0	2.7
Taste	2.7	3.0	3.3	4.6
Colour	4.4	4.0	3.7	3.7
Flavour	4.0	3.3	3.0	3.0
General acceptability	3.3	3.6	3.0	3.3

There is no particular trend in the general acceptability of yoghurt with or without unripe plantain powder supplementation. Except that there is more acceptance for yoghurt mildly supplemented with unripe plantain than the other samples. However, results obtained from organoleptic analyses for consistency, taste, colour, flavour and overall acceptability, subjected to further statistical treatment using a one way analysis of variance (ANOVA) shows that there was no significant difference ($p > 0.05$) in consistency, taste, colour, flavour, and overall acceptability of the supplemented yoghurt.

Conclusion: In conclusion, the results obtained in this study suggest that powdered unripe plantain is a valuable ingredient for the enrichment of yoghurt preparation due to the additional nutritional quality of the products with good appeal, good taste, and cheap price because of the cheaper cost of plantain than milk.

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