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Quality Characteristics of Probiotic Soy Yoghurts with Enzyme Hydrolyzed African Breadfruit and Rice Additives

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Abstract Effect of enzyme hydrolyzed African breadfruit (HABF) and culture type on the physicochemical and nutrient composition of soy yoghurt sweetened with rice syrup was evaluated. The effect of the HABF on the final counts of the cultures; *Bifidobacterium bifidum* (ATCC 11883) and *Lactobacillus acidophilus* in mono- and co-cultured soy yoghurt was also determined. African breadfruit (ABF) flour was hydrolysed with a mixture of cell wall degrading enzymes: Xylanase [endo-1,4-] and [endo-1,3(4)-] Beta-glucanase (Ultraflowmax^{R}). HABF was added at concentrations of 0 - 5 % into soymilk containing 25 % hydrolysed rice syrup and then pasteurized at 80 °C for 30 min in a water bath. Probiotic *B. bifidum and L. acidophillus* as mono- and co-cultures were separately inoculated into the soy-HABF milk and fermented at 42°C for 6 - 8 h. Samples were analyzed using standard methods. The pH (4.46 - 4.30) and syneresis index (32.35 - 25.00) decreased significantly ($P \leq 0.05$) with increase in HABF concentration for the cultures, while TTA (0.62 - 0.93 % lactic acid) and viscosity (1.20 - 1.84 Pa.s^{-1}) increased significantly ($P \leq 0.05$). No significant ($P \leq 0.05$) effect on moisture (85.79 - 89.16 %), crude protein (3.44 - 3.75 %) and crude fat (1.24 - 1.58 %) content of the soy yoghurt was observed. The ash (0.17 - 0.50 %), crude fibre (0.24 - 0.47 %) and carbohydrate (7.10 - 8.53 %) varied significantly ($P \leq 0.05$) amongst the cultures with HABF concentrations. *B. bifidum* and *L.acidophillus* monoculture counts ranged from 7.36 - 7.69 and 7.16 - 8.49 Log_{10} CFU/ml respectively, and 6.52 - 7.66 and 7.79 - 8.92 Log_{10} CFU/ml respectively in co-culture fermentation. Viable cells were > 10^6 recommended for probiotic products. This work therefore reveals the possibility of the production of probiotic yoghurt with soymilk and at least 4% HABF inclusion and rice syrup as sweetner.

Keywords: *enzyme hydrolysis, African breadfruit, soy yoghurt, probiotics, bifidobacterium bifidum, lactobacillus acidophillus*

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1. Introduction

Yoghurt is described as milk that has been fermented and acidified with viable and well-defined bacteria, creating a thickened, often flavored, product with an extended shelf life [1]. Yoghurt is originally produced from cow's milk and has also been produced from the milk of other ruminants such as goats, sheep, buffaloes and camels [2,3]. The production of acceptable yoghurt or yoghurt-like products from cereals, and vegetable milks such as soy, maize, oats etc. have also been reported [4]. Yoghurt starters are mainly lactic acid bacteria which include *Lactobacillus bulgaricus*, *Lactococcus lactis*, *Streptococcus thermophillus*, *Bifidobacterium sp*. and *Leuconostoc sp*.

Bifidobacterium spp possess *α*-galactosidase activity which enables them to metabolize lactose. Hence, during fermentation, it has the ability to metabolize milk components with the production of various components that enhances flavours [5]. *Lactobacillus acidophilus* on the other hand, utilizes sucrose more efficiently than lactose as an energy source, and this has been ascribed to the fact that *ß*-galactosidase may be an inducible enzyme [6]. *Lactobacillus* spp. and *Bifidobacterium* spp. amongst others are regarded as probiotic bacteria and have been used in the production of probiotic fermented soymilk and yoghurt [7]. Probiotic bacteria are live and beneficial organisms which have positive influence on human health by maintaining or restoring microbiological equilibrium in the digestive tract [8].

Soybean (*Glycine max)* is an excellent source of good quality proteins and is widely consumed by large

populations especially in Asia [9]. It serves as a rich source of bioactive compounds such as isoflavones, antioxidants and bioactive peptides [10,11]. However, its utilization and acceptance is still limited by its beany flavor and flatulence producing properties. Fermentation of soybeans has been suggested as a means of removing the objectionable beany flavor of soy milk and also as a means of increasing variety of soy products. Several authors have reported on various aspects of fermented soy milk to produce yoghurt [12,13]. Acceptable probiotic soy yoghurt containing various prebiotics has also been developed [14]. African breadfruit (*Treculia africana*) seeds contain 35-60 g carbohydrate/100 g with a considerable percentage being oligosaccharides which makes it a possible source of prebiotics. The seeds are traditionally eaten by boiling or roasting. African breadfruit seed flour has been used in production of breakfast cereals and snacks [15]. Ifediba and Ozoh [16] reported the successful production of yoghurt -like product from aqueous extracts of African breadfruit and corn. However, there are no reports of the use of partially hydrolyzed African breadfruit in the production of soy yoghurt. Partial hydrolysis with glucanases is expected to breakdown African breadfruit seed cell wall and release of prebiotic materials from the cells which may enhance probiotic growth. Rice, (*Oryza sativa*) is one of the most important cereal crops and staple in developing countries. Rice is processed into various food products by dehusking and polishing the grains with or without parboiling to produce cooking rice, puffing to produce breakfast cereals and processed into different beverages Kunze [17]. Rice flour is produced by milling the kernel and starch can be extracted from the flour. Rice starch can be hydrolyzed to produce glucose and high fructose syrups used as substitutes for sucrose in the production of beverages.

Demand for healthy beverages based on increased consumer awareness can be met by developing alternative beverages with healthier ingredients. The use of rice syrup and enzyme hydrolyzed African breadfruit additives could improve the survival (growth and metabolism) of probiotic cultures in soymilk to produce soy yoghurt. Hence, this study was aimed at evaluation of the effect of enzyme hydrolyzed African breadfruit on physicochemical and microbiological composition of *Bifidobacterium bifidum* and *Lactobacillus acidophillus* fermented soy yoghurt with rice syrup.

2. Materials and Methods

2.1. Soya Beans and African Breadfruit

Soya beans (Samsoy1variety) was obtained from National Root Crop Research Institute (NRCRI) Umudike, Nigeria. African Breadfruit (ABF) seeds were purchased from processors in Oyigbo Local Government Area of Rivers State, Nigeria. Improved rice variety (NERICA FARO L19) was obtained from Africa Rice Center, IITA Ibadan, Oyo State, Nigeria.

2.2. Enzymes

Bacterial and fungal alpha amylases, glucoamylase, Ultraflow max^{TM} {mixture of xylanase [endo-1,4-] and beta glucanase [endo-1,3(4)-]}, invertase (β-fructofuranosidase E.C.3.2.1.26) and proteases were obtained from Novozymes Company.

2.3. Microbial Cultures and Media

Probiotic species used were *Bifidobacterium bifidum* (ATCC 11883*)* and *Lactobacillus acidophilus (Nature source UK).* De Man Rogosa Sharpe (MRS) agar and broth (Oxoid) were used for isolation and enumeration of *Lactobacillus acidophillus*. MRS agar supplemented with 0.05% L-cysteine known as modified (mMRS) agar was used for isolation and enumeration of *Bifidobacterium bifidum*. Buffered Peptone water was used as diluent for serial dilution.

2.4. Reagents

Analytical grade reagents used included hydrochloric acid (HCL), calcium hydroxide ($Ca(OH)_2$), and sodium hydroxide (NaOH).

2.5. Production of African Breadfruit (ABF) Flour

ABF seed flour was produced by parboiling fresh ABF seeds in boiling water for 5 min. The seeds were drained, manually dehulled and dried at 50 °C for 18 h in an air oven (Gallenkamp UK). The dried seeds were milled and sieved through a 150µm sieve to obtain the ABF seed flour. This was packaged in airtight plastic bottles and stored in a deep freezer until required for further analyses.

2.6. Hydrolysis of ABF Flour

A slurry (1:3.5 w/v ABF seed flour: water) was made with distilled water (the pH was adjusted to pH 11.00 with $Ca(OH)_2$ solution). The mixture was stirred and its pH checked with a digital pH meter (Thomas Scientific Germany) to ensure that it was between 6.0 - 6.5. The temperature of the slurry was held at 50 $^{\circ}$C in a water bath. The Xylanase [endo-1,4-] Beta-glucanase [endo-1,3(4)-] (ultraflowmax^{TM}) (0.01 ml/100g flour) was added to the mixture with regular stirring for 2 h to partially hydrolyse the ABF. The mixture was brought to the boil to inactivate the enzyme. The ABF hydrolysates were labeled as HABF.

2.7. Preparation of Rice Syrup

Rice syrup was produced by the method of Osuji and Nwosu, [18]. The sugar content of the rice syrup for use in the yoghurt production was maintained at 30 B^0. This was confirmed with a hand held refractometer. The syrup was stored in sterile glass bottles in a deep freezer and used within 24 h.

2.8. Production of soy milk

The method of Champagne *et al.*, [19] was used to produce the soymilk. Briefly, 300 g of soybeans was sorted and soaked in 900 ml distilled water (1:3 w/v) for 16 h. The beans were manually dehulled and blended with

1.5 L hot distilled water at high speed for 3 min. The slurry was sieved through a double folded muslin cloth and the resulting filtrate simmered for 10 min, cooled and stored as soymilk in a refrigerator at 4°C. The milk was used within 3h for the production of soy yoghurt .

2.9. Formulation and Production of Probiotic Soy-HABF Yoghurt

The probiotic soy-HABF yoghurt was formulated as shown in Table 1. A total of fifty-four (54) yoghurt sample were prepared using the *B. bifidum* and *L. acidophillus* mono- and co-culture. For each probiotic starter six (6) sets of soy yoghurt were produced in triplicate. The rice syrup sweetened soymilk was supplemented with 0, 1, 2, 3, 4 and 5% HABF to give the 6 sets. In each case, to produce 400 ml of yoghurt, 100 ml of rice syrup was added to 300 ml of the rice syrup sweetened soy milk and an appropriate quantity of HABF to give 1, 2, 3, 4 and 5% HABF in the soymilk was used. To obtain a 1% HABF concentration in the rice syrup sweetened soymilk, 4 g of HABF was added to 396 ml of the soymilk. All the formulations were pasteurized at 80 °C for 30 min in a water bath, cooled to 42°C and then inoculated with 5% activated culture i.e. 20 ml of the starter containing 6.71 and 6.43 Log_{10} CFU/ml for the mono-cultures of *B.bifidum* and *l.acidophillus* respectively, while the co-culture was with 10 ml of each of the 2 starters with the same cell densities as in the mono cultures. The inoculated samples were incubated at 42°C for 6 - 8 h. At the end of the incubation period the samples were used for the various analyses. The tests were carried out in uniform conditions and the samples without HABF served as control.

Table 1. Composition of Soy-HABF yoghurt with rice syrup

Ingredients	Quantity					
Soymilk (ml)	300	296	292	288	284	280
Rice syrup (ml)	100	100	100	100	100	100
HABF Conc (g)	0	4	8	12	16	20
*Starter culture (ml)	20	20	20	20	20	20

*Three starter cultures: *B. bifidum*, *L. acidophillus* as mono-culture and *B. bifidum* (10 ml) and *L. acidophillus* (10 ml) together as co-culture. Control for each of the organism had no HABF.

2.10. Physicochemical Evaluation of Soy- HABF Yoghurt with Rice Syrup

2.10.1. Determination of pH of Probiotic Soy - HABF Yoghurts with Rice Syrup

Prior to pH determinations, the pH meter (Thomas Scientific, Germany) was calibrated using buffers of pH 4.00, 7.00 and 9.00. The pH of 20 ml of the yoghurt samples were measured using a digital pH meter. The electrode was completely submerged in the sample and the pH read from the digital LCD read-out

2.10.2. Determination of the Total Titratable Acidity (TTA) of Probiotic Soy - HABF Yoghurts with Rice Syrup

Titratable acidity (TTA) of the yoghurt samples were determined according to the method described in AOAC [20]. Ten milliliters (10 ml) of each of yoghurt sample were each pipetted into Erlenmeyer flasks (100 ml). Phenolphthalein indicator (0.5 ml) was added and mixed thoroughly. The yoghurt samples were then titrated against 0.1 M NaOH solution until the first tinge of pink that appeared persisted for 30secs. TTA of the samples as percentage of lactic acid was calculated by the formular:

$$\%TTA = \frac{\text{vol NaOH} \times \text{NBase} \times 0.09}{\text{vol of sample}} \times 100.$$

2.10.3. Determination of Viscosity of Probiotic Soy - HABF Yoghurts with Rice Syrup

The method of Unal and Akalin, [21] was used to determine the viscosity of the samples. Each of the yoghurt sample (200 ml) was homogenized separately in a homogenizer (FJ 300-S China) at medium speed for 3min. The viscosity of the thoroughly homogenized samples was measured using a digital display viscometer (NDJ-85, China) with No. 4 spindle at 120 rpm. Viscosity was expressed as $Pa.s^{-1}$.

2.10.4. Determination of Syneresis Index of probiotic Soy - HABF Yoghurts with Rice Syrup

The method of Unal and Akalin, [21] was used to measure this parameter. Twenty grams (20 ml) of each of the yoghurt formulations (20 ml) was centrifuged (L-600 China centrifuge) at 5000 g for 10 min. The extracted whey was weighed and syneresis index (SI) in percentage was calculated as:

$$SI = \frac{\text{weight of whey}}{\text{weight of sample}} \times 100.$$

2.11. Determination of the Proximate Composition of Probiotic Soy - HABF Yoghurts with Rice Syrup

The proximate composition was determined using the standard methods of AOAC [20]. Moisture was determined by an automated method using a moisture analyzer. The displayed values were noted as the percentage moisture content. Crude protein was determined by macro-Kjeldahl method. Protein was obtained by multiplying the percentage nitrogen obtained by a conversion factor (6.25). Rose Gotlieb method was used to determine the percentage fat content of the samples. The crude ash content was determined by difference after incineration of charred samples to a white ash by heat in a muffle furnace at 500°C for about 3 h until. Acid hydrolysis method was used in the determination of crude fibre. Total carbohydrate was determined by difference: [100 - (moisture + crude protein + crude fat + ash + crude fibre)].

2.12. *B. bifidum* and *L. acidophilus* Counts in Probiotic Soy - HABF Yoghurt with Rice Syrup

Starter bacteria in soy yoghurt supplemented with 0 - 5% HABF were enumerated using the spread plate method at the start and end of the fermentation. For each fermentation period, 10 - fold serial dilutions from stock of 10 ml of sample in 90 ml of sterile diluent were made up to $10^{9.}$ Aliquots of 0.1 ml from 10^{7}, 10^{8} and 10^{9} dilutions were plated in duplicate by spread plating

technique onto MRS agar and incubated anaerobically at 37°C for 48 h and 42°C for 24 h respectively, for *B. bifidum* and *L. acidophilus*. At the end of the incubation period, plates showing between 30 - 300 colonies were counted on an electronic counter. The average number of organisms was obtained and expressed as colony forming units per ml (CFU/ml) using the formula:

Cell counts (CFU/ml) = (Average No. of colonies × Dilution Factor)/ Volume plated. Colony counts were converted to Log_{10} CFU/ml.

2.13. Experimental Design and Statistical Analysis

A completely randomized 3 x 6 full factorial experimental design was applied. All data were expressed as means of three independent trials with standard deviation. SPSS statistic 20 was used to assess differences between treatments and the data subjected to analysis of variance (ANOVA). Means were compared and Duncan's multiple range test used to separate means where differences existed.

3. Results and Discussion

3.1. Effect of HABF concentration and culture type on pH of probiotic soy yoghurt with rice syrup.

Effect of enzyme hydrolyzed African breadfruit and culture type on pH of single and co-cultures of *B. bifidum* and *L. acidophilus* soy yoghurt with rice syrup are shown in Figure 1. There was no significant ($P \geq 0.05$) difference in pH among the fermenting microorganisms at HABF concentration of 0 - 3%, but pH decreased significantly ($P \leq 0.05$) with increase in concentration of HABF for the fermenting organisms. The pH at HABF concentrations of 0 - 5 % varied from 4.46 ± 0.03 - 4.32 ± 0.02, 4.44 ± 0.03 - 4.30 ± 0.01 and 4.45 ± 0.00 - 4.35 ± 0.01 respectively for *B. bifidum*, *L. acidophillus* and co-culture of *B. bifiduum* and *L.acidophillus*. *B. bifidum* had the least pH of 4.32 at HABF concentration of 5 % while sample with *L. acidophilus* had their least pH at concentration of 4 %. This may be attributed to more efficient utilization of the substrates in the medium by *B.bifidum.*

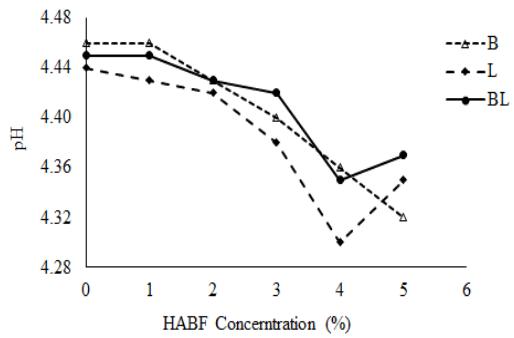

Figure 1. Effect of enzyme hydrolyzed African breadfruit and culture type on pH of probiotic soy yoghurt with rice syrup, HABF = Hydrolyzed African breadfruit, B = Soy-HABF yoghurt produced with *B. bifidum*, L = Soy-HABF yoghurt produced with *L. acidophilus*, BL = Soy-HABF yoghurt produced with co-culture of *B. bifidum* and *L. acidophilus*

There was significant ($P \leq 0.05$) interaction between HABF concentration and culture type on pH of soy yoghurts. The concentration of HABF had significant ($P \leq 0.05$) effect on the final pH of the samples. There was a significant ($P \leq 0.05$) decrease in pH with increase in HABF concentration. The decrease in pH of the samples with increase in HABF concentration up to 4% could be as a result of increased concentration of metabolizable carbohydrates provided by addition of HABF as well as enhanced microbial activity arising from increased fermentable solutes. HABF inclusion up to 4% may have provided additional fermentable substrates in form of oligosaccharides which enhanced the ability of the cultures to ferment the products. Fermentation with the single and co-culture of the probiotic *B. bifidum* and *L. acidophillus* resulted in significant ($P \leq 0.05$) decrease in the pH of the samples. The highest significant ($P \leq 0.05$) pH drop of 0.14 units occurred in samples fermented with *L. acidophilus* at HABF concentration of 4%. The final pH of all samples fermented with the probiotics was < 4.50 indicating complete fermentation of the substrate. The *L. acidophillus* and *B. bifidum* singly and in combination with each other produced enough acid to form coagulum of the soy milk. This could be attributed to the beta galactosidase activities of the probiotic species in hydrolyzing FOS present in HABF. Similar decrease in pH of soy milk yoghurt formulated with saccharified rice solution and fermented with probiotic bacteria was reported by Park [22]. The pH values obtained for all the soy HABF samples were lower than the reported 4.70, 4.78 and 4.73 for *L. plantarum*, *L. brevis* and *L. reuteri* respectively by Niyibituronsa *et al.*, [23] for soy milk. Garro *et al.*, [24] reported pH of 5 for soy milk fermented with mixed cultures.

3.2. Effect of HABF Concentration and Culture Type on the Total Titratable Acidity (TTA) (% Lactic Acid) of Probiotic Soy Yoghurts with Rice Syrup

Shown in Figure 2 is the effect of enzyme hydrolyzed African breadfruit and culture type on TTA as % Lactic acid of single and co-cultures of *B. bifidum* and *L. acidophilus* soy yoghurt with rice syrup.

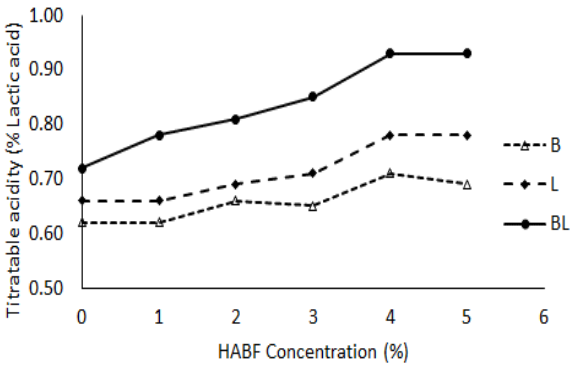

Figure 2. Effect of enzyme hydrolyzed African breadfruit and culture type on total titratable acidty (% Lactic acid) of probiotic soy yoghurt with rice syrup, HABF = Hydrolyzed African breadfruit, B = Soy-HABF yoghurt produced with *B. bifidum*, L = Soy-HABF yoghurt produced with *L. acidophilus*, BL = Soy-HABF yoghurt produced with co-culture of *B. bifidum* and *L. acidophilus*

The TTA varied significantly ($P \leq 0.05$) for the yoghurt samples from different fermenting organisms and for the different concentrations of HABF. Amongst the fermenting organisms, significant ($P \leq 0.05$) increase was observed at concentration of 4 and 5 % for *B.bifidum*, while *L. acidophillus* and co-culture of *B. bifidum* and *L. acidophillus* had significant ($P \leq 0.05$) increase from the concentration of 3 %. The TTA of the co-culture fermentation was the highest while the single culture of *B. bifidum* had the least TTA. The TTA ranged from 0.62 ± 0.02 - 0.71 ± 0.04, 0.66 ± 0.00 - 0.78 ± 0.01 and 0.72 ± 0.02 - 0.93 ± 0.00 respectively, for *B. bifidum*, *L. acidophillus* and co-culture of *B. bifidum* and *L. acidophillus*. *L acidophilus* is a homofermenter and this may account for the increased TTA values as this is calculated as lactic acid. The control samples without HABF had significantly ($P \leq 0.05$) the least TTA values of 0.62, 0.66 and 0.72 for *B.bifidum*, *L.acidophillus* and their co-culture respectively. The increase in acidity observed with the increase in HABF concentration can be attributed to the fact that the HABF offered the probiotics more suitable nutrients particularly metabolizable carbohydrates that were metabolized to short chain fatty acids during fermentation. Lactic acid bacteria utilize the Embden -Meyerhof -Parnas (EMP) pathway to metabolise carbohydrates leading to increase in cell mass and concomitant increase in lactic acid as an end product. For all the samples, HABF concentration beyond 4% did not produce any significant ($P \geq 0.05$) increase in TTA. Obadina *et al.,* [25] did not report significant ($P \geq 0.05$) differences in TTA of soy yoghurt produced with different lactic acid bacteria. The final TTA at the inclusion of 1 % for the co-culture of *B. bifidum* and *L. acidophillus* was lower than the report by Niamah *et al.,* [26] for fermented milk with 1.0% gum Arabica, but the TTA at 4 - 5 % HABF was higher. The increased acidity could be as a result of synergism in lactic acid production between the cultures. Faster increase in TTA of soy milk during fermentation has also been attributed to the low buffering capacity of soy milk [27]. The significantly ($P \leq 0.05$)) lower TTA observed in sample B produced with single culture of *B. bifidum* is comparable with the report by Chou and Hou [28]. They reported that *Bifidobacterium* produced low amounts of acid in soy milk although not lower than that of *L. casei.*

3.3. Effect of HABF Concentration and Culture Type on the Viscosity of Probiotic Soy Yoghurts with Rice Syrup

Effect of HABF and culture type on viscosity of single and co-cultures of *B. bifidum* and *L. acidophilus* soy yoghurt with rice syrup are shown in Table 2. The viscosity ranged from 1.25 - 1.55, 1.20 - 1.84 and 1.26 - 1.42 $Pa.s^{-1}$ respectively, for *B. bifidum*, *L. acidophillus* and co-culture of *B. bifidum* and *L.acidophillus*. There were significant ($P \leq 0.05$) differences in the viscosity of the samples. Among the probiotic bacteria, the viscosity of sample L with 0 and 2 % HABF were the least while at HABF concentration of 4 and 5 % it had the highest viscosity. Generally, there was significant increase ($P \leq 0.05$) in viscosity with increase in HABF concentration. The concentration of 5% HABF had the highest viscosity for the single cultures while the highest viscosity for the mixed culture was at a concentration of 2 - 4 %. Increase in viscosity of soy yoghurt with addition of HABF may be attributed to the enhanced fermentation and reduction in pH of the medium which causes isoelectric point precipitation of proteins as well as interaction between these substances and soy proteins [29]. It has been established that probiotic bacteria produce exopolysaccharides which increases viscosity, water retention and other components of soy milk resulting in increased viscosity [30]. The viscosity obtained for the soy-HABF yoghurt are lower than those reported for soy yoghurt containing cherry fruits [31] and high amylose corn starch powder and inulin [7]. Gel formation of soy milk proteins is a key process step in the manufacture of non-diary fermented products like soy/ HABF yoghurt.

Table 2. Effect of HABF and culture type on viscosity ($Pa.s^{-1}$) of probiotic soy yoghurt with rice syrup

Samples	HABF Concentration (%)					
	0	1	2	3	4	5
B	$1.25^{1c} \pm 0.11$	$1.31^{1c} \pm 0.07$	$1.36^{12cb} \pm 0.04$	$1.43^{1ab} \pm 0.08$	$1.50^{23ab} \pm 0.06$	$1.55^{2a} \pm 0.02$
L	$1.20^{2c} \pm 0.05$	$1.31^{1cb} \pm 0.13$	$1.31^{2cb} \pm 0.08$	$1.34^{1bc} \pm 0.08$	$1.76^{1c} \pm 0.06$	$1.84^{1a} \pm 0.06$
BL	$1.26^{1c} \pm 0.02$	$1.35^{1b} \pm 0.03$	$1.40^{1a} \pm 0.01$	$1.41^{1a} \pm 0.02$	$1.42^{3a} \pm 0.02$	$1.33^{3b} \pm 0.01$

Values are means of triplicate determinations ± SD. Means with the same superscript numbers in the same column are not significantly ($P \geq 0.05$) different. Means with the same alphabets in the same row are not significantly ($P \geq 0.05$) different. HABF = Hydrolyzed African breadfruit, B = Soy-HABF yoghurt produced with *B. bifidum*, L = Soy-HABF yoghurt produced with *L. acidophilus*, BL = Soy-HABF yoghurt produced with co-culture of *B.bifidum* and *L. acidophilus.*

Table 3. Effect of HABF and culture type on Syneresis Index of probiotic soy yoghurt with rice syrup

Sample	HABF Concentration (%)					
	0	1	2	3	4	5
B	$31.10^{3a} \pm 2.60$	$29.30^{3b} \pm 0.00$	$26.52^{3e} \pm 2.7$	$28.57^{3c} \pm 3.26$	$28.13^{2d} \pm 0.00$	$26.34^{2e} \pm 1.73$
L	$34.27^{1a} \pm 1.80$	$32.36^{12b} \pm 0.00$	$27.41^{2f} \pm 1.85$	$29.48^{2c} \pm 4.60$	$28.70^{1d} \pm 2.60$	$28.00^{1e} \pm 1.40$
BL	$32.35^{2a} \pm 2.36$	$32.00^{2a} \pm 1.60$	$31.41^{1b} \pm 1.51$	$31.00^{1c} \pm 4.30$	$26.93^{3d} \pm 1.54$	$25.00^{3e} \pm 1.32$

Values are means of triplicate determinations ± SD. Columns with the same superscript (number) are not significantly ($P \geq 0.05$) different. Rows with the same alphabets are not significantly ($P \geq 0.05$) different. HABF = Hydrolyzed African breadfruit, B = Soy-HABF yoghurt produced with *B. bifidum*, L = Soy-HABF yoghurt produced with *L. acidophilus*, BL = Soy-HABF yoghurt produced with co-culture of *B. bifidum* and *L. acidophilus.*

3.4. Effect of HABF and Culture Type on Syneresis Index of Probiotic Soy Yoghurt with Rice Syrup

Table 3, showed the effect of HABF on syneresis jndex of single and co-cultures of *B. bifidun* and *L. acidophilus* soy yoghurt with rice syrup. There was significant ($P \leq 0.05$) difference in the syneresis index of the samples. The syneresis index for *B. bifidum*, *L. acidophillus* and co-culture of *B. bifidum* and *L.acidophillus*. ranged from 26.34 - 31.10, 27.41 - 34.27 and 25.00 - 32.35 respectively. The range of values for the concentration were 31.10 - 34.27, 29.30 - 32.36, 26.52 - 31.41, 28.57 - 31.00, 26.93 - 28.70 and 25.00 - 28.00 for HABF concentrations of 0, 1, 2, 3, 4 and 5 % respectively. Samples fermented with *B. bifidum* had least syneresis index up to 3 % HABF inclusion. The mixed culture fermentation had the least syneresis index at HABF inclusion of 4 and 5 %.

The culture type and HABF concentration had significant ($P \leq 0.05$) effect on syneresis index of the samples. There was significant ($P \leq 0.05$) decrease in the syneresis index of the soy-HABF yoghurt samples with increase in HABF concentration. Increased levels of HABF increased the amount of polysaccharides and total solids in the samples thus serving as a stabilizer in the samples which may account for the low syneresis index. The values obtained in this study at 4-5% HABF inclusion are comparable to those (24 to 26%) reported by Vareltzis *et al.*, [32] for yoghurt stabilized with albumin and protein concentrate and for cow yoghurt containing Date pulp [33]. The culture type also affected the syneresis index of the samples. The co-culture of *B. bifidum* and *L, acidophilus* had significantly ($P \leq 0.05$) the lowest syneresis index at 5% HABF inclusion. Lactic acid bacteria produce exopolysaccharides which increase water holding capacity thus decreasing syneresis by acting as natural texturizers in many dairy products [34].

3.5. Effect of HABF and Culture Type on Proximate Composition of Probiotic Soy yoghurt with Rice Syrup

The effect of HABF and culture type on proximate composition of single and mixed cultures of *B. bifidum* and *L. acidophilus* soy yoghurt with rice syrup are shown in Table 4. The culture type had no significant ($P \geq 0.05$) difference in the moisture content of the samples but there was a significant ($P \geq 0.05$) decrease with HABF concentration. The moisture content was between 85.79 - 89.16, 86.00 - 88.27 and 86.93 - 88.68 % respectively for *B. bifidum*, *L. acidophillus* and co-culture of *B. bifidum* and *L. acidophillus*. The moisture content of the samples was comparable to the report by Ifediba and Nwafor [35] for ABF corn yoghurt but was higher than the 81.32% reported by Junior *et al.*, [36] and lower than 91.63% for soy yoghurt [37]. The low moisture content of the soy-HABF yoghurt could be attributed to the inclusion of HABF which may have also increased the solid contents of the samples.

The protein and fat content of the probiotic soy yoghurt did not differ significantly ($P \geq 0.05$) among the cultures and with the different concentration of HABF. The protein content for B, L, and BL samples was between 3.52 - 3.72, 3.44 - 3.75 and 3.50 - 3.67 % respectively. Fat content was between 1.24 - 1.36, 1.55 - 1.54 and 1.34 - 1.58 % for B, L and BL respectively. The protein content of the samples at 5% HABF ranged from 3.66 - 3.78%. African bread fruit is classified as a high protein food and also has lower fat content than typical oil seeds Akubor and Badifu [38]. However, the inclusion of even 5 % HABF did not contribute enough protein and fat to make a significant ($P \leq 0.05$) change in the protein and fat contents of the samples. The results obtained in this study are lower than those reported by Ifediba and Nwafor [35], but higher than the report by Amanze and Amanze [37] and Ndife *et al.*, [39].

The ash content varied significantly ($P \leq 0.05$) from 0.21 - 0.50 and 0.17 - 0.39 % for *B. bifidum* and co-culture of *B. bifidum* and *L. acidophillus* respectively, while it was constant at 0.29 % for *L. acidophillus*. There were significant ($P \leq 0.05$) differences in the ash content of the soy yoghurt. Among the microorganisms, there was no significant ($P \geq 0.05$) difference at HABF concentration of 2 %. Ash increased significantly ($P \leq 0.05$) with increase in HABF concentration but for *L. acidophillus* samples there was no significant ($P \leq 0.05$) difference. The ash content of the sample was similar to the report for soymilk yoghurt and soy-corn yoghurt reported by Amanze and Amanze [37] and Makunjuola [4] but lower than those reported for other plant based yoghurts [40]. The ash content of a product is an indication of its mineral content. Although the mineral content of the samples was not analyzed, addition of HABF which increased the ash content may contribute to increased mineral content of these yoghurt samples.

The crude fibre content of the samples varied significantly ($P \leq 0.05$) amongst microorganism and with the different levels of HABF. The values ranged from 0.24 - 0.35, 0.31 - 0.47 and 0.24 - 0.39 % respectively, for B, L and BL. Sample fermented with L had significantly ($P \leq 0.05$) the highest crude fibre content. Crude fibre increased significantly ($P \leq 0.05$) with increase in HABF concentration for B and L while BL had significantly ($P \leq 0.05$) higher values at concentration of 3 and 4 %. Increasing the amount of significantly ($P \leq 0.05$) increased the crude fibre content of the samples. This increase could be attributed to the HABF because the crude fibre content of the soymilk is much less than that of ABF. Crude fibre content in all the samples in the study, are higher than that reported by Amanze and Amanze [37] for soy yoghurt. Fibre in food materials are indigestible and are selectively utilized as prebiotics by probiotics. The increased fibre content could also account for the increase in probiotic cell counts observed in this study for the soy yoghurt samples.

The carbohydrate content of the yoghurt varied significantly ($P \leq 0.05$) among the microorganisms and with the concentrations of HABF. The values were 7.10 - 8.53, 7.05 - 7.56 and 7.36 - 8.50 for *B. bifidum*, *L. acidophillus* and their co-culture respectively. Amongst the microorganisms, the co-culture had significantly ($P \leq 0.05$) the highest content of carbohydrate and *L. acidophillus* the least. There was increase in carbohydrate content with increase in HABF concentration attributable to the carbohydrate content of ABF. African breadfruit contains 73% carbohydrate [41]. The inclusion of 5% HABF produced a significant ($P \leq 0.05$) change in the carbohydrate content

of the samples. The carbohydrate content obtained in this study were within the range reported by Olubamiwa and Kolakpo [42] but lower than the report of Ifediba and Nwafor [35] for ABF corn yoghurt.

There was significant ($P \leq 0.05$) variation in the total solid content of the samples between culture and HABF concentrations. The total solid content increased as moisture content decreased. The mean for *B. bifidum*, *L. acidophillus* and their co-culture samples varied respectively, from 10.84 - 14.21, 11.73 - 14.00 and 11.32 -11.32 %. The control sample without the probiotic bacteria had the least total solid content. These results are comparable with the report for fruit-soy yoghurt by Osundahunsi *et al.*, [43]. Total solid content is an indication of dry matter content (macro nutrients) in yoghurt and a function of the moisture content of the samples. It is also related to quality characteristics of soy yoghurt such as viscosity and syneresis. According to Estevez *et al.*, [11], in soy yoghurt, syneresis is significantly reduced at solid content higher than 8%. Samples produced with the probiotic *L. acidophillus* and *B. bifidum* cultures had significantly ($P \leq 0.05$) higher solid contents than control for all HABF concentrations. This increase in the total solid content of the culture fermented soy yoghurt could be attributed to the inclusion of HABF.

3.6. Effect of HABF on Final Counts of *B bifidum* and *L.acidophillus* in Single and Co-culture Probiotic Soy Yoghurt with Rice Syrup

The final counts of *B. bifidum* and *L. acidophillus* in the mono- and co-culture probiotic soy yoghurt containing 0 - 5 % HABF are shown in Figure 3. The viable counts of *B. bifidum* and *L. acidophillus* increased significantly ($P \leq 0.05$) with increase in HABF concentration. The final viable probiotic counts in the monoculture fermentation ranged from 7.36 - 7.69 and 7.16 - 8.49 Log_{10} CFU/ml respectively, for *B. bifidum* and *L. acidophillus* counts, while in the co-culture fermentation *B. bifidum* and *L. acidophillus* increased from 6.52 - 7.66 and 7.79 - 8.92 Log_{10} CFU/ml respectively.

In the monoculture, *B. bifidum* showed a final increase of 1 log cycle at 0 - 1 % HABF inclusion and an increase > 1.5 log cycle beyond 2 % HABF concentration. For *L. acidophilus*, at HABF concentration of 0 - 2 % there was an increase of 1 log cycle while at 3 - 5 % HABF inclusion a 2 log cycle increase was observed. The increase in cell counts with increasing HABF concentration could mean increase in metabolizable carbohydrate for microbial growth and may also account for the decrease in pH and increase in acidity of the soy-HABF yoghurt (Figure 1 and Figure 2). Niamah and Al-Manhel [44], reported lower final counts of probiotic *L. acidophillus* in milk supplemented with 4 % and 6 % gum Arabic and *B. bifidum* in 6 % gum Arabica. The final counts of *L. acidophillus* as mono culture in soymilk with more than 2 % HABF were higher than the 10^6 viable cells recommended for probiotic products [7]. For *Bifidobacterium sp.* to provide therapeutic benefits in a product, the cell count must be > 10^6 CFU/g [45]. Martensson *et al.*, [46] reported viable counts of log 7 - 8 for *L.acidophillus* in oat based on dairy products, and a count of log 8 - 9 for *B. bifidum* in the same product. HABF contains FOS and FOS which have been shown to contribute to the growth of the probiotics in the soy yoghurt. FOS are known to selectively stimulate the growth of probiotics [7].

Table 4. Effect of HABF and culture type on proximate composition of probiotic soy yoghurt with rice syrup

HABF Conc. (%)	Culture	Moisture	Protein	Fat	Ash	Crude Fibre	CHO	Total Solid
0	B	$89.16^{1a} \pm 0.28$	$3.52^{1a} \pm 0.18$	$1.24^{1a} \pm 0.06$	$0.21^{2d} \pm 0.02$	$0.24^{2b} \pm 0.02$	$7.10^{1c} \pm 0.80$	$10.84^{2e} \pm 0.28$
	L	$86.35^{2b} \pm 0.17$	$3.44^{1a} \pm 0.26$	$1.55^{1a} \pm 0.20$	$0.29^{1a} \pm 0.01$	$0.34^{1c} \pm 0.03$	$7.05^{1b} \pm 0.06$	$13.65^{1c} \pm 0.17$
	BL	$88.68^{1a} \pm 0.85$	$3.50^{1a} \pm 0.35$	$1.34^{1a} \pm 0.11$	$0.17^{3d} \pm 0.04$	$0.24^{2c} \pm 0.01$	$7.36^{1b} \pm 0.09$	$11.32^{2c} \pm 0.85$
1	B	$88.90^{1a} \pm 0.70$	$3.53^{1a} \pm 0.06$	$1.29^{1a} \pm 0.01$	$0.26^{2c} \pm 0.04$	$0.25^{2b} \pm 0.02$	$7.40^{1bc} \pm 0.11$	$11.97^{2e} \pm 0.70$
	L	$86.22^{2b} \pm 0.14$	$3.49^{1a} \pm 0.22$	$1.57^{1a} \pm 0.11$	$0.29^{1a} \pm 0.00$	$0.34^{1c} \pm 0.04$	$7.15^{1b} \pm 1.82$	$13.75^{1b} \pm 0.14$
	BL	$87.50^{1ab} \pm 0.50$	$3.50^{1a} \pm 0.00$	$1.36^{1a} \pm 0.06$	$0.26^{2c} \pm 0.04$	$0.27^{2c} \pm 0.01$	$7.57^{1b} \pm 1.63$	$12.50^{2b} \pm 0.50$
2	B	$85.79^{1c} \pm 0.25$	$3.57^{1a} \pm 0.32$	$1.34^{1a} \pm 0.09$	$0.29^{1c} \pm 0.07$	$0.28^{2b} \pm 0.01$	$7.70^{1b} \pm 0.15$	$14.21^{1a} \pm 0.25$
	L	$88.27^{1a} \pm 0.16$	$3.53^{1a} \pm 0.16$	$1.60^{1a} \pm 0.00$	$0.29^{1a} \pm 0.03$	$0.31^{2c} \pm 0.03$	$7.18^{1b} \pm 1.20$	$11.73^{3d} \pm 0.16$
	BL	$87.42^{1ab} \pm 0.04$	$3.55^{1a} \pm 0.31$	$1.36^{1a} \pm 0.05$	$0.31^{1bc} \pm 0.03$	$0.35^{1b} \pm 0.02$	$8.32^{1a} \pm 1.77$	$12.58^{2b} \pm 0.04$
3	B	$87.56^{1b} \pm 0.10$	$3\ 64^{1a} \pm 0.21$	$1.34^{12a} \pm 0.08$	$0.35^{1b} \pm 0.05$	$0.33^{2a} \pm 0.01$	$8.14^{1a} \pm 0.13$	$12.44^{3d} \pm 0.10$
	L	$86.09^{1b} \pm 0.08$	$3.57^{1a} \pm 0.26$	$1.45^{12a} \pm 0.11$	$0.29^{2a} \pm 0.00$	$0.38^{1b} \pm 0.01$	$7.31^{1a} \pm 1.44$	$13.91^{1b} \pm 0.08$
	BL	$87.13^{1bc} \pm 0.13$	$3.61^{1a} \pm 0.22$	$1.58^{1a} \pm 0.13$	$0.34^{1ab} \pm 0.00$	$0.39^{1a} \pm 0.05$	$8.58^{1a} \pm 1.47$	$12.87^{2ab} \pm 0.13$
4	B	$87.00^{1b} \pm 0.50$	$3.68^{1a} \pm 0.34$	1.341a ± 0.05	$0.47^{1a} \pm 0.04$	$0.35^{3a} \pm 0.01$	$8.47^{1a} \pm 0.18$	$13.00^{2c} \pm 0.50$
	L	$86.00^{1b} \pm 0.05$	$3.74^{1a} \pm 0.43$	1.541a ± 0.17	$0.29^{3a} \pm 0.01$	$0.42^{1a} \pm 0.03$	$7.35^{1a} \pm 1.09$	$14.00^{1a} \pm 0.05$
	BL	$86.93^{1bc} \pm 0.23$	$3.64^{1a} \pm 0.00$	1.581a ± 0.08	$0.34^{2ab} \pm 0.05$	$0.39^{2a} \pm 0.05$	$8.58^{1a} \pm 1.27$	$13.07^{2a} \pm 0.23$
5	B	$86.54^{1a} \pm 0.04$	$3.72^{1a} \pm 0.04$	$1.36^{1a} \pm 0.07$	$0.50^{1a} \pm 0.00$	$0.35^{2a} \pm 0.02$	$8.53^{1a} \pm 1.74$	$13.46^{1b} \pm 0.04$
	L	$86.28^{1b} \pm 0.14$	$3.75^{1a} \pm 0.48$	$1.54^{1a} \pm 0.28$	$0.29^{3a} \pm 0.06$	$0.47^{1a} \pm 0.08$	$7.56^{1a} \pm 1.50$	$13.72^{1b} \pm 0.14$
	BL	$86.93^{1b} \pm 0.05$	$3.67^{1a} \pm 0.22$	$1.58^{1a} \pm 0.13$	$0.39^{2a} \pm 0.07$	$0.32^{ba} \pm 0.02$	$8.50^{1a} \pm 1.50$	$13.03^{2a} \pm 0.05$

Values are means of triplicate determinations ± SD. Means with the same superscript (number) among the microorganisms for each concentration are not significantly ($P \geq 0.05$) different. Means with the same superscript (alphabets) among the concentration for each microorganism are not significantly ($P \geq 0.05$) different. HABF = Hydrolyzed African breadfruit, B = Soy-HABF yoghurt produced with *B. bifidum*, L = Soy-HABF yoghurt produced with *L. acidophilus*, BL = Soy-HABF yoghurt produced with co-culture of *B.bifidum* and *L. acidophilus*

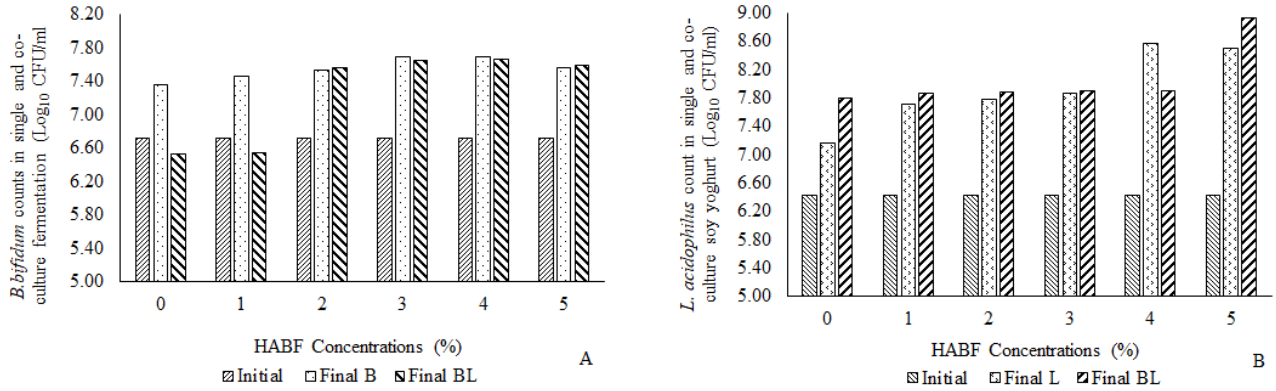

Figure 3. Effect of enzyme hydrolyzed African breadfruit on final counts of (A) *B bifidum* and (B) *L.acidophillus* in single and co-culture probiotic soy - HABF yoghurt with rice syrup. HABF = Hydrolyzed African breadfruit, Final B = Final count of *B. bifidum* in single culture probiotic Soy-HABF yoghurt, Final L = Final count of *L. acidophilus* in single culture probiotic Soy-HABF yoghurt, Final BL = Final count of *B. bifidum* and *L. acidophilus* in co-culture probiotic Soy-HABF yoghurt.

In co-culture with each other, the ratio of *B. bifidum* and *L. acidophillus*, was approximately 1:1 in samples containing 0 - 5 % HABF. However, *L. acidophillus* counts were higher than those of *B. bifidium*. Akalin *et al*, [47] reported *Bifidobacterium* count of log 7 CFU/ml in yoghurt containing 2 % FOS which is similar to the counts in the soy-HAFB yoghurt in this study with counts of Log 7 CFU/ml at 1 - 5 % HABF concentrations. In co-culture microbial growth maybe symbiotic or antagonistic. The growth of *L. acidophilus* and *B. bifidum* in HABF supplemented soy yoghurt is probably symbiotic and the enzyme hydrolysis of the ABF and the rice syrup at 25 % provided sufficient metabolizable sugars for the growth and production of soy milk probiotic yoghurt.

4. Conclusion

The concentration of HABF and the type of culture had significant ($P \leq 0.05$) effect on pH, titratable acidity (TTA), viscosity and syneresis index of probiotic soy yoghurt. pH and syneresis index decreased significantly ($P \leq 0.05$) with increase in concentration of HABF in all the, while TTA and viscosity increased significantly ($P \leq 0.05$). Neither type of culture nor the concentration of HABF had any significant ($P \leq 0.05$) effect on moisture, crude protein and crude fat content of the soy yoghurt. The ash, crude fibre and carbohydrate content of the soy yoghurt varied significantly ($P \leq 0.05$) amongst microorganism and at the different levels of HABF. HABF concentration increased significantly ($P \leq 0.05$) the final counts of *L.acidophillus* and *B. bifidum* in mono and co-culture in soymilk yoghurt. The values were greater than the 10^6 viable cells recommended for probiotic products, hence it could be concluded that the soymilk yoghurt produced with added enzyme hydrolyzed African breadfruit and sweetened with rice syrup could be considered a probiotic beverage with potential health benefits.

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