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Storage Stability of Probiotic Soy Yoghurts with Enzyme Hydrolyzed African Breadfruit and Rice Additives

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Authors' contributions

This work was carried out in collaboration among all authors. Author CMO designed the study. Author LIB managed the analysis, performed the statistical analysis, performed the literature search and wrote the manuscript. Authors NCO, CMO and CCO supervised the analysis and were part of the initial draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study investigated the effect of culture type and storage time on pH, titratable acidity (%Lactic acid), viscosity and syneresis, probiotic viability and sensory properties of probiotic soy yoghurts with enzyme hydrolyzed African bread fruit (HABF) and rice syrup.
Methodology: Three sets of Soy-HABF Yoghurt were formulated by supplementing soymilk with 4% HABF and the addition of enzyme hydrolyzed rice syrup. The formulations were inoculated separately with *Bifidiobacterium bifidum* and *Lactobacillus acidophillus* as mono- and co-cultures and fermented at 42 °C for 8 h. Using standard methods, the samples were analyzed after fermentation representing day 1 and on the 5^{th}, 10^{th}, 15^{th} and 20^{th} days of storage at 4^{o} C.
Results: Culture type and the storage time had significant ($P \leq 0.05$) effect on physicochemical, probiotic count and sensory properties of the probiotic soy-HABF yoghurt. pH decreased

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significantly (P ≤ 0.05) with storage time from 4.36 - 4.06, 4.30 – 3.97 and 4.35 - 4.26 for B, L and BL (*B. bifidum*, *L. acidophilus* and the co-culture of *B. bifidum*, and *L. acidophilus* fermented samples). *L. acidophilus* had highest pH decrease. Titratable acidity, viscosity and syneresis index increased significantly (P≤0.05) with increase in storage time. Probiotic counts varied from 7.72-6.54 and 8.56 – 7.70 Log CFU/ml for *B. bifidum* and *L. acidophilus* respectively in the mono-culture samples B and L and from 7.66 – 5.65 and 7.90 – 6.57 Log CFU/ml for *B.bifidum and L. acidophilus* in sample BL. Assessors' degree of likeness for the aroma, appearance, taste and texture of the probiotic soy-HABF yoghurt varied from neither like nor dislike to moderate likeness. The overall acceptability indicated that the *L. acidophilus* fermented product (sample L) was more acceptable to the assessors within the first 5 days of storage.

Conclusion: This study have shown *that B.bifidum* and *L.acidophillus* retained a viability of > log 6 within 15 days of storage. The physicochemical and sensory characteristics of soy/HABF yoghurt were also at optimum within the first 5 days at 4^{o} C.

Keywords: Probiotic viability; soy yoghurt; African breadfruit; storage stability; L acidophillus; B. bifidum.

1. INTRODUCTION

Functional foods have been described as products which confer health benefits in addition to basic nutrients. This term was first used by Hasler [1] in Japan in the mid 1980's and refers to processed foods which contain ingredients that aid specific bodily functions in addition to nutrition. These ingredients include, legumes, polysaccharides, vitamins, isoflavones, prebiotics, polyphenols or flavonoids amongst others. They promote welfare and health as well as reduce risk of metabolic and degenerative syndromes [2]. Probiotic products have been defined as foods which contain live organisms such as *Bifidobacterium* and *Lactobacillus* which beneficially affect the gut microbiota. Probiotic organisms are expected to withstand the harsh conditions in the stomach and transit to the colon. Fermented dairy products are the main vehicles to carry probiotics into the body. In recent times however, there is increased demand by consumers for non-dairy probiotic alternatives as a result of increasing health consciousness of consumers. Fermented soy products such as soy yoghurt is one such alternative to dairy yoghurt. Other workers have also developed, oat based, pea, lupin and quinoa based probiotic yoghurt- like products with success. Other non- dairy yoghurts include native black rice yoghurt [3], and probiotic soy yoghurt [4]. To be classified as a probiotic product, the probiotic species must be present in adequate numbers >than 10^{6} (log 6) CFU/g or mL at the point of consumption [5]. This implies that probiotics must be able to survive and maintain viability under conditions in which the products are stored. Survival of probiotics are enhanced by mainly indigestible carbohydrates called prebiotics. Gibson et al., [6], defined prebiotics as 'a selectively fermented ingredient that allows specific changes, both in the composition and/or activity of the gastrointestinal microflora that confer benefits upon host well-being and health'. Prebiotics include fructooligosaccharides, galactooligosaccharides, soy oligosaccharides, resistant starch etc. Food sources of prebiotics include unripe banana, tomatoes, onions, garlic, chicory roots, cereals, lentils, faba beans and other plants [7]. Zare et al. [8] demonstrated that lentil, chick peas and pea flours supported the growth of probiotics in milk. Inclusion of 4% green lentil flour increased the growth of *L. acidophilus* and *B. lactis* in yoghurt during 28 days storage [9]. Pea flour supplementation at 3% also enhanced growth and acid production of *L. rhamnosus* in yoghurt [8]. Faba bean supplementation at 4% stimulated bifidogenic microbial growth by more than 1 log cycle from log 7.0 to log 8.0 [10]. In yoghurt formulated with quinoa flour, the viability of the LAB during the 20 days-storage remained constant and always higher than 8.5 Log CFU/mL [11]. Agil et al. [9] reported that inclusion of green lentil flour in yoghurt decreased the pH with concomitant increase in titratable acidity. Inclusion of enzyme hydrolyzed African breadfruit flour has been reported to enhance the physical and microbiological characteristics of soy yoghurt sweetened with rice syrup [12].However there are no reports of the effect of enzyme hydrolyzed African breadfruit on the physical, sensory quality and viability of *L.acidophillus*, *B.bifidum* or their combination during refrigerated storage .The purpose of this study was to evaluate the effect of enzyme hydrolyzed African breadfruit supplementation on soy yoghurt sweetened with rice syrup during a 20 day refrigerated storage.

2. MATERIALS AND METHODS

2.1 Soya Beans and African Bread Fruit and Rice

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 (Switzerland AG) .

2.3 Microbial Cultures and Media

Probiotic species used were *Bifidobacterium bifidum* (ATCC 11883*)* and *Lactobacillus acidophilus (Nature source UK).* De Mann 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 for serial dilution.

2.4 Reagents

Analytical grade reagents used included HCL, CaOH, NaOH and H_2SO_4

2.5 Production of African Breadfruit (ABF) FLOUR

ABF seed flour was produced as reported by Barber et al. [12]. Briefly the seeds were dehulled by parboiling in water for 5 min, 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 150 µm sieve, packaged and stored in a freezer as ABF.

2.6 Hydrolysis of ABF Flour

African breadfruit flour (ABF) was hydrolysed using the method reported by Barber et al. [12] .Briefly, a slurry (1:3.5 w/v ABF seed flour: water) was made with distilled water adjusted to pH 11.00 with CaOH 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 ^{0}C in a water bath. The enzyme a mixture of β-glucanase and arabinoxylanase (Ultraflo^R Max) (0.01mL) 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 hydrolysate were labeled as HABF.

2.7 Preparation of Rice Syrup

Rice syrup was produced by the method of Osuji and Nwosu, [13]. The sugar content of the rice syrup for use in the yoghurt production was maintained at 58 ^{0}B. 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. [14] 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 1.5L 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. The milk was then used for the production of soy yoghurt.

2.9 Formulation, Fermentation and Storage of Soymilk-HABF Yoghurt with Rice Syrup

Yoghurt samples were formulated with soymilk supplemented with 4.0 %(w/v) HABF based on previous studies by Barber et al. [12]. Sugar was added in the form of rice syrup obtained by enzyme hydrolysis of rice. The volume of rice syrup added was calculated based on the total soluble solids (TSS) content of the soymlk to give a final TSS of 15 ^{0}B (8 % v/v). A 400 mL sample of each formulation was obtained as shown in Table 1, separately pasteurized at 80°C for 30 min in a water bath, cooled to 42°C and then inoculated with 20 mL (5 % v/v) of starter culture. The starter culture used were *B. bifidum and L. acidophillus* as mono- and co-cultures to give samples B, L and BL The inoculated samples were incubated at 42°C for 6 – 8 h to a final pH of 4.60.

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

Ingredients	Quantity
Soymilk	300 ml
Rice syrup	100 ml
(HABF)	4 % w/v
Probiotic starter cultures	5 % w/v

The yoghurt was dispensed in a total of 10 polyethylene tetraphthalate bottles per sample and after fermentation, were stored in the refrigerator at 4° C for 20 days. At 5-day intervals, 2 bottles per sample were withdrawn for physicochemical, microbiological and sensory analyses.

2.10 Determination of the Effect of Culture Type and Storage time on Physicochemical Characteristics of Probiotic Soy Yoghurt

The pH, TTA and viscosity were determined 2 h after fermentation to represent day 1 while the syneresis index was determined 24 h after fermentation to indicate day 1. Samples were subsequently analyzed on days 5, 10, 15 and 20.

2.10.1 Determination of pH and total titratable acidity (TTA) of probiotic soy - HABF yoghurts with rice syrup

The pH of 10 ml of the yoghurt samples were measured using a digital pH meter (Thomas Scientific, Germany). The electrode was completely submerged in the sample and the pH read from the digital LCD read-out. Prior to the determinations, the pH meter was calibrated using buffers of pH 4.00, 7.00 and 9.00. Thereafter , the titratable acidity (TTA) of the samples were determined by titrating against 0.1 M NaOH solution until the first tinge of pink that appeared persisted for30s. TTA of the samples as percentage of lactic acid was calculated as:

$$\frac{Vol\ of\ NaOH \times \text{Normality of base} \times 0.09}{\text{Volume of sample} \times 100}$$

2.10.2 Determination of viscosity of probiotic soy - HABF yoghurts with rice syrup

The method of Unal and Akalin, [15] 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 $Pa.s^{-1}$.

2.10.3 Determination of syneresis index of probiotic soy - HABF yoghurts with rice syrup

The method of Unal and Akalin, [13] 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:

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

2.11 Determination of the Effect of Culture and Storage Time on the Viability of Probiotic Bacteria in Soy Yoghurt

The starter bacteria in soy yoghurt supplemented with 4% HABF were enumerated using the spread plate method at each storage time. For each period, 10 - fold serial dilutions from stock of 10 ml of sample in 90 ml of sterile diluent were made up to 10^6. Aliquots of 0.1 ml from 10^4, 10^5 and 10^6 dilutions were plated in duplicate onto MRS agar and incubated anaerobically at 37 ^{0}C for 48 h and 42 ^{0}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) =

$$\frac{\text{Average No.of colonies} \times \text{Dilution Factor}}{\text{Volume plated}}.$$

Colony counts were converted to Log_{10} CFU/ml.

2.12 Evaluation of the Effect of Culture and Storage Time on Sensory Properties of Probiotic *soy* - HABF Yoghurts with Rice Syrup

The method of Iwe [16] was used in the determination of the sensory properties of the soy-HABF yoghurt. A 20-member untrained panel consisting of staff and students from Rivers State University who are familiar with the organoleptic characteristics of yoghurt

participated in the sensory evaluation of the products. Each sample (40 mL) was identified by a three-digit random number and was served cold (4 – 6^0C) in clear plastic tubs in a well-ventilated and naturally lit room. The panelists were required to evaluate each sample and rate their preference based on, appearance, aroma, taste, mouthfeel and overall acceptability. Panelists were requested to evaluate color first. The rating was on a nine -point hedonic scale where: 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely. Portable water was provided to rinse the palate and a covered cup was provided for expectoration for those who did not wish to swallow the samples

2.13 Experimental Design and Statistical Analysis

A completely randomized 3 × 5 full factorial experimental design was applied (culture/time of storage). 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 Culture Type and Storage Time on Physicochemical- Characteri stics of Probiotic Soy Yoghurt

3.1.1 Effect of culture type and storage time on pH of probiotic soy yoghurt

Fig. 1 showed the effect of culture type and storage time on pH of probiotic soy-HABF yoghurt with rice syrup. There was no significant interaction between culture and time. There was a significant (P<0.05) decrease in pH with increase in storage time. The pH of the soy-HABF decreased respectively from 4.36 - 4.06, 4.30 - 3.97 and 4.35 - 4.26 for B, L and BL *(B. bifidum, L. acidophilus* and the co-culture of *B. bifidum*, and *L. acidophilus* fermented samples). The least pH for *L. acidophilus* and the co-culture fermentation was observed on day 20 while *B. bifidum* sample had the least pH on day 15 with an increase to 4.20 on day 20 (Fig.1). Amongst the fermenting microorganisms, *L. acidophilus* produced significantly (P<0.05) the highest decrease in pH with time. Decreases in pH of soy-HABF yoghurt during storage can be attributed to continued metabolic activities of the probiotic cultures at low temperatures, as there was maintained viability of the fermenting microorganisms throughout the storage period. Progressive pH reduction in *Bifidobacterium* probiotic soy yoghurt has also been reported by Chou and Hou [17]. The change in pH reported in this study are within the range reported by Donkor [18] for probiotic yoghurt produced with *L. acidophilus*. The small but steady decline in pH of probiotic soy yoghurt has also been attributed to the low proteolytic activity of the starter cultures [19].

3.1.2 Effect of culture type and storage time on Titratble acidity (%Lactic acid) of probiotic soy yoghurt.

The effect of culture type and storage time on titratable acidity (%Lactic acid) of probiotic soy-HABF yoghurt with rice syrup is shown in Fig. 2. The fermenting microorganisms and the storage time had significant (P ≤0.05) effect on the TTA of the yoghurt. Sample L fermented by *L. acidophilus* and co-culture of *B. bifidum* and *L. acidophilus* (BL) had significant (P ≤ 0.05) increase in TTA with the storage time and the values varied from 0.78 -1.10 and 0.93 - 1.35 % lactic acid respectively. Sample B, fermented by *B. bifidum* alone after day 10 had significant (P ≤ 0.05) decrease. The values ranged from 0.67 - 0.94 for day 20 and day 10 respectively. The TTA at the start of storage were higher than those reported by Estevez et al. [20]. The final TTA was also higher than those reported by Wang et al. [21]. The continued increase in TTA is related to the decrease in pH of the samples during storage and is an indication that metabolic enzymes of the probiotics continued the production of acid even at refrigeration temperature. Increase in TTA of the soy yoghurt sample may also be as a result of lowered buffering capacity of soymilk proteins [21,22].

3.1.3 Effect of culture type and storage time on viscosity and syneresis of probiotic soy yoghurt

The viscosity and syneresis of the soy-AHBF soy yoghurt are shown in Table 3. The syneresis for the *B. bifidum*, *L. acidophilus* and the co-cultured yoghurts varied respectively, from 28.31 - 38.33, 28.09 - 34.00 and 26.93 - 33.49 for day 1 and day 20. Both the culture type and time of storage

had significant influence on the syneresis of soy-HABF yoghurt under refrigerated storage. The syneresis of the soy-HABF yoghurt samples increased significantly (P≤0.05) with time of storage and the highest syneresis was recorded on day 20. Amongst the cultures, sample BL fermented by the co-culture had significantly the least syneresis while sample B fermented by mono culture of *B. bifidium* fermented samples had the highest. Athar et al. [23] reported increase in syneresis in yoghurt. Syneresis is an important quality parameter which influences the appearance and consistency of products.

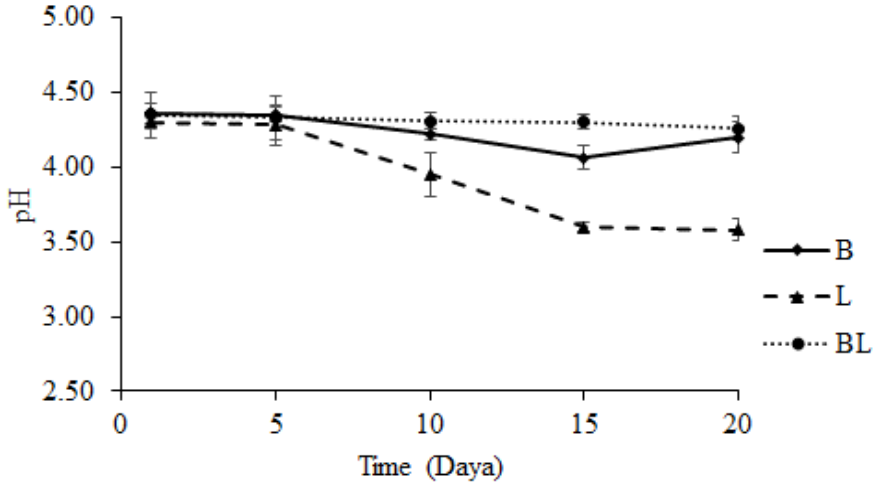

Fig. 1. Effect of culture type and storage time on pH of probiotic soy-HABF yoghurt with rice syrup

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

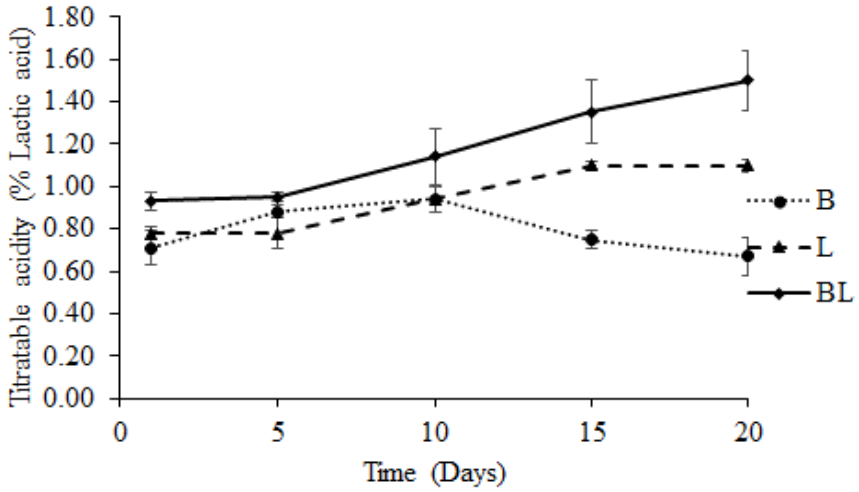

Fig. 2. Effect of culture type and storage time on Titratble acidity (%Lactic acid) of probiotic soy-HABF yoghurt with rice syrup

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

Table 3. Effect of culture type and storage time on viscosity (Pa.s^{-1}) and syneresis of probiotic soy-HABF yoghurt with rice syrup

Parameter	Sample	Time (Days)				
		1	5	10	15	20
Syneresis	B	28.31^{a}±4.93	30.21^{a}±2.80	30.90^{b}±3.90	35.63^{a}±2.73	38.33^{a}±3.05
	L	28.09^{a}±3.01	29.00^{c}±3.00	32.53^{a}±2.50	33.60^{b}±3.70	34.00^{b}±3.00
	BL	26.93^{c}±2.96	29.43^{b}±2.63	30.50^{c}±2.50	30.83^{c}±1.38	33.49^{c}±3.17
Viscosity	B	1.40^{2y}±0.12	1.75^{x}±0.27	2.00^{x}±0.00	2.00^{y}±0.07	2.10^{y}±0.10
	L	1.76^{1y}±0.04	1.78^{y}±0.12	2.00^{x}±0.01	2.31^{x}±0.09	2.44^{x}±0.25
	BL	1.42^{2y}±0.01	1.49^{y}±0.04	1.86^{y}±0.02	2.07^{y}±0.07	2.18^{y}±0.03

Values are means of triplicate determinations ± SD. Means with the same superscript in the same column are not significantly (P ≥ 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

The viscosity of the Soy-HABF yoghurt varied significantly (P≤0.05) as shown in Table 3. The viscosity on day 1 and day 20 for the *B. bifidum*, *L. acidophilus* and the co-culture yoghurts varied respectively, from 1.40 – 2.10 Pa.s $^{-1}$, 1.76 – 2.44 Pa.s $^{-1}$ and 1.42 – 2.18 Pa.s $^{-1}$. Both the culture type and time of storage had significant influence on the viscosity of soy-HABF yoghurt under refrigerated storage. For *L. acidophilus* mono-culture (L), the increase in viscosity up to the 10th day was not significant (P≥0.05). The increase in viscosity from the 15th to the 20th for this sample was significant (P<0.05). The viscosity of sample B fermented with *B. bifidum in* mono-culture increased significantly within the first five days of refrigerated storage from 1.40 to 1.75 Pa.s $^{-1}$ .The viscosity of the sample by the 20th day increased to 2.10 Pa.s $^{-1.}$. The highest viscosity of probiotic yoghurt in this study was however, lower than that reported for soymilk yoghurt by Estevez et al. [20].Viscosity is also influenced by solid content of samples. Increase in viscosity is also mediated by proteolysis of proteins producing short peptide chains resulting in more compact network of coagulated protein. The viscosity of yoghurt is an important quality parameter to evaluate texture and mouth feel and it also determines energy required to pump fluid products. The samples with the highest final viscosity also exhibited the lowest syneresis.

3.2 Effect of Culture and Storage Time on the Viability (Log CFU/ml) of Probiotic Bacteria in Mono and Co-culture Soy-HABF Yoghurt

The effect of culture type and storage time on the viability of the probiotic bacteria in mono- and co-culture of soy-HABF yoghurts with rice syrup are shown in Table 4.

The increase of *B. bifidum* count in the yoghurt samples fermented by the mono-culture (B) of *B. bifidum* ranged from 7.69 - 7.71 Log CFU/ml on day 1 and day 10 was not significant (P≥0.05), however there was a significant (P≤0.05) decrease to 6.54 Log CFU/ml on day 20. In the co-culture fermentation (BL), there was significant decrease in *B. bifidum* count with storage time from 7.66 – 5.65 Log CFU/ml for day 1 and day 20. For a product to have a probiotic claim, the viability of probiotics at the point of consumption must be a minimum of log 6.0 [16]. In this study *B. bifidum* retained viability of up to 6 Log CFU/ml at the end of the storage period in samples B while for sample BL log 6 was retained up to the 15th day thus, conferring on soy/HABF yoghurt one of the recommended criteria for a probiotic product. The counts obtained in this study are higher than those reported for *B. bifidum* grown in non -diary oat beverage by Martensson et al. [24]. They reported viability of 5.0 Log CFU/ml by the 20th day of storage. The high survival rate reported in this study could be attributed to the fact that the pH changes was not lower than 4.00. *B. bifidum* has a poor resistance to pH values less than 4.00. Boudjou et al. [10] suggested that the presence of Faba bean enables probiotic *B. lactis* to thrive in single or co -culture during storage of Kefir. *B. bifidum* has also been shown to possess alpha- galactosidase which hydrolyze raffinose family oligossacharides (RFOs) present in soymilk and HABF [25]. *B. bifidum* in this study served as an adequate starter for the fermentation of soymilk supplemented with 4% HABF. The reduced viability after day 10 could be attributed to increased acidity in the samples. It is probable that beyond the 10th day of storage the deleterious effect of increased acidity had started manifesting as reduced viability of *B. bifiduim*. By day 15, the acidity of the sample increased by 28%.

Table 4. Effect of culture and storage time on the viability (Log CFU/ml) of probiotic bacteria in mono and co-culture soy-HABF yoghurt

Days	*B. bifidum*	*B.bifidum*	*L. acidophilus*	*L.acidophillus*
	Mono-culture (B)	Co-culture (BL)	Mono-culture (L)	Co-culture
1	7.69^{2a}±0.02	7.66^{a1}±0.04	8.56^{a1}±0.07	7.90^{a2}±0.02
5	7.72^{1a}±0.19	7.54^{b2}±0.04	8.33^{b1}±0.30	7.79^{a2}±0.02
10	7.71^{12a}±0.27	6.81^{c2}±0.08	7.83^{c1}±0.05	6.67^{b2}±0.03
15	6.94^{3c}±0.04	6.75^{c2}±0.05	7.82^{c1}±0.03	6.63^{c2}±0.03
20	6.54^{4c}±0.03	5.65^{d2}±0.01	7.70^{d1}±0.02	6.57^{c2}±0.02

Values are means of triplicate determinations ± SD. Means with the same number superscript in the same column are not significantly (P ≤ 0.05) different. Means with the same superscript letter along the same row for each probiotic are not significantly (P ≤0.05) different. HABF = Hydrolyzed African breadfruit

There was significant (P≤0.05) decrease in *L. acidophilus* count both in the mono- and the co-culture fermentation (L and BL), though the decrease in the co-culture was significantly (P≤0.05) greater than in the mono-culture fermentation. The mono-culture fermentation had *L. acidophilus* counts decrease from 8.56 - 7.70 Log CFU/ml while the co-culture counts decreased from 7.20 - 6.23 Log CFU/ml. The viability of *Lactobacillus plantarum* was reported to decrease slightly over a period of 20 days in quinoa beverage [11]. In studies by Martinez-Villaluenga et al. [26], *L. acidophillus* counts were significantly reduced in yoghurt fortified with raffinose family oligossacharides. Although there was reduction in cell viability, the final counts of *L. acidophillus* after the storage period was within log 6.0 CFU/ml recommended as one of the criteria specified for a product to be considered as a probiotic Reduction in viability of *L. acidophillus* during storage could be as a result of reduced enzyme synthesis resulting in decreased metabolism and reduced growth. The increased pH and acidity of soy-HABF yoghurt during storage could also contribute to reduced viability of *L. acidophillus.*

3.3 Effect of Culture and Storage Duration on Sensory Properties of Probiotic Soy- HABF Yoghurts with Rice Syrup

The effect of culture type and storage time on the sensory properties: aroma, appearance, taste, texture (mouth feel) and overall acceptability of the probiotic soy-HABF yoghurts with rice syrup are shown in Fig. 3. There was no significant interaction between culture and duration of storage on appearance of the samples F= (8,30)=1.41,P=0.23. The storage duration had significant (P≤0.05) effect on the appearance of the soy-HABF yoghurt. There was significant (P≤0.05) decrease in the assessor's degree of likeness of the appearance with increase in storage time. The degree of likeness for appearance of samples B and BL were rated 'like slightly' (6.00 to 6.55) for the first ten days by assessors. Subsequently, sample B was rated neither like nor dislike (5.00-5.55) from the 15th to 20th day .Sample L was rated 'like slightly (6.08 – 6.55) up to the 5th day of storage and neither like nor dislike (5.73-5.50) from the 10th to the 20th day. Appearance of yoghurt samples is judged principally by the homogeneity of the samples (i.e. no signs of whey separation, absence of clumps and consistency). The decrease in the degree of likeness of the appearance of the samples can be related to the fact that syneresis increased significantly (P≤0.05) with time.

There was significant interaction between culture and time F= (8, 30) =2.23, P=0.05 on the aroma of the samples. Assessors rated the aroma of sample L 'neither like nor dislike' throughout the 20day storage period. Sample B was rated neither like nor dislike up to the 10th day and dislike slightly on 15th and 20th days. The co-cultured sample BL was rated 'neither like nor dislike' up to the 5th day of storage and subsequently rated 'dislike slightly' from day 10 to day 20. The storage time and type of culture had significant (P0≤ 0.05) on aroma of soy HABF yoghurt. Aroma is the result of production of volatile compounds during carbohydrate metabolism by microorganisms. The aroma scores could indicate that the probiotics could not produce enough volatile compounds from the substrates used in the yoghurt fermentation. Although, ratings obtained in sensory evaluations may be affected by many other factors apart from the characteristics of the samples. According to Donkor et al. [20] the characteristic of the assessors, the test situation, attitudes and assessors' expectations can influence the results obtained in sensory studies. Aroma compounds in soy yoghurt include aldehydes such as acetaldehyde and phenylacetaldehyde. Other compounds include alcohol and furans.

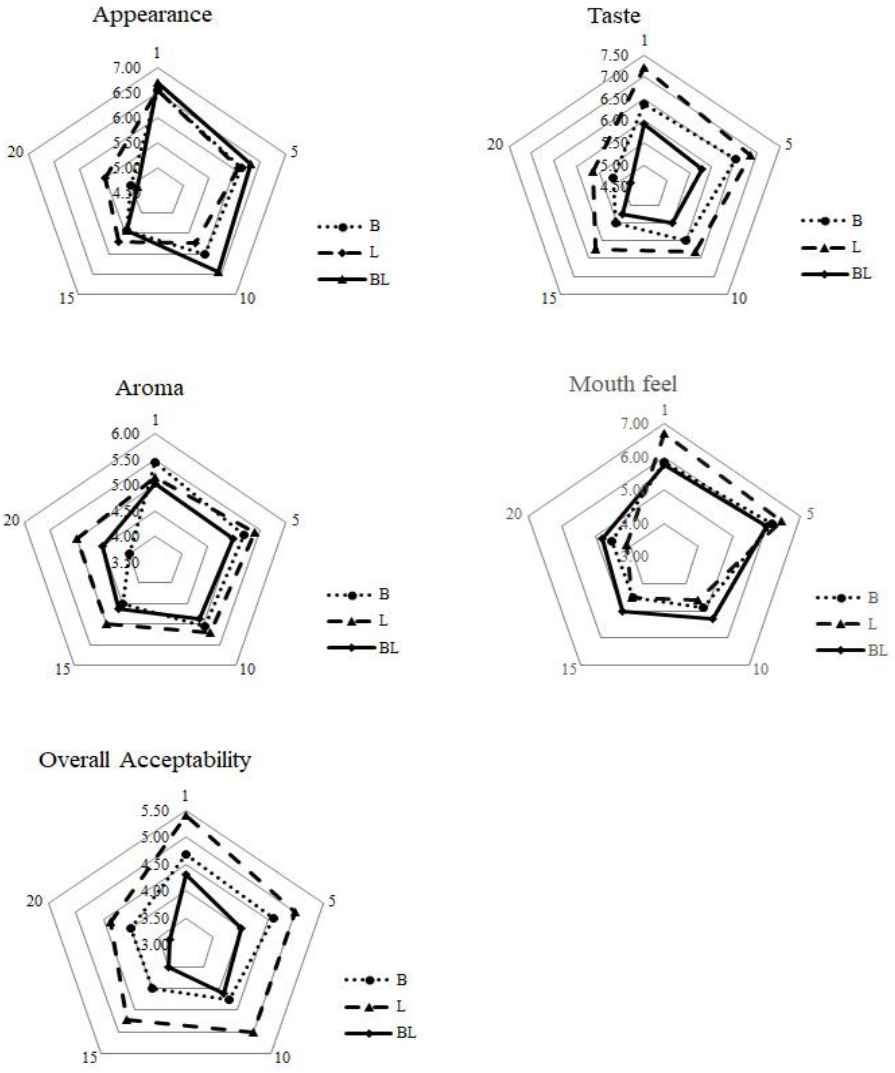

Fig. 3. Effect of culture and storage time on Sensory properties of probiotic soy - HABF yoghurts

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 culture type and the storage duration had significant (P≤0.05) effect on the taste of the soy-HABF yoghurt. There was significant (P≤0.05) decrease in the degree of likeness of the taste of the samples with increase in storage time. For *B. bifidum*, *L. acidophilus* and the co-culture (*B. bifidum* and *L. acidophilus*) samples B, L and BL respectively, the scores for taste decreased from 6.38 - 5.18, 7.20 - 5.63 and 5.94 - 4.81 respectively. The taste scores of the mono-cultured samples (B and L) was significantly (P≤0.05) higher than that of the co-culture sample BL. Assessors' degree of likeness for the taste of Sample L ranged from 'liked moderately' on the 1st day to 'neither liked nor disliked' by the 20th day of storage. For sample B, the taste was rated 'like slightly' for the first ten days of storage and 'neither liked nor disliked' between days 15 and 20.The taste of sample BL was rated 'neither liked nor disliked' between days 1 to 15 and 'disliked slightly' on day 20. The degree of likeness for taste and character of yoghurt samples which decreased with increasing time of storage could be as a

result of increased acidity observed with storage in the samples. BL which scored significantly (P≤0.05) the least for taste had significantly, the highest acidity value of 1.35 % lactic acid. Pinthong et al. [27] reported that acidity of more than 1.80% often imparted unpleasant taste to yoghurt and the optimum acidity is about 1.15%.This could also account for the higher score (5.63)recorded for the taste of sample L at the 20th day of storage.

The culture type and the storage duration had significant (P≤0.05) effect on the texture of the soy-HABF yoghurt. There was significant (P≤0.05) decrease in texture from the 10th day to the 20th day of storage. There was also, significant interaction between culture and duration of storage on the texture of the samples F= (8, 30) = 3.60, P= 0.005. For samples B, L and BL (*B. bifidum*, *L. acidophilus* and the co-culture *B. bifidum* and *L. acidophilus*) the texture decreased from 5.83 - 4.56, 6.72 - 4.11 and 5.76 - 4.82 respectively. This implied neither like nor dislike for samples B and BL while sample L was liked slightly to disliked slightly. There was an increase in the likeness of the co-culture sample on day 20 from dislike slightly to neither like nor dislike. Texture was evaluated as thickness and lack of smoothness before swallowing and chalkiness or mouth coating after swallowing. Chalkiness is related to total solids which increased significantly (P≤0.05) with time. Cliff et al. [28] suggested that solid content contributed to increased chalkiness and mouth coating in yoghurt formulated with higher milk solids.

The overall acceptability of the soy-HABF yoghurt was significantly (p≤ 0.05) affected by culture type and the storage duration. The values decreased from 4.68 - 4.00, 5.40 - 4.37 and 4.32 - 3. 30 for samples B, L and BL respectively. The overall acceptability of sample L was neither liked nor dislike from day 1-10 and was disliked slightly on day 15 and 20. The co-culture samples was significantly the least acceptable to the assessors. They were disliked slightly on day 1 -10 and disliked moderately on day 15 and 20. The B samples were disliked slightly throughout the storage period.

4. CONCLUSION

Soy-HABF yoghurt produced with mono –culture of *L.acidophillus* (sample L) maintained a viable count of ≥ log 6 throughout the 20day storage period. Physicochemical characteristics of this sample also indicated optimum decrease in pH to 4.1 on the 15th day and a TTA of 1.1% an adequate production of acidity in the sample. This sample also had the lowest percentage change in syneresis .In terms of sensorial assessment sample L was rated liked slightly for appearance, taste, texture on day 5 .It was also the only sample rated neither like nor dislike in terms of overall acceptability. *L.acidophillus* fermented soy /HABF yoghurt maintained better characteristics for 5 days under refrigerated storage than *B.bifidum* and co-cultured samples of *B.bifidum* and *L acidophilus.*

There is however, need for further studies to improve the physicochemical properties and acceptability of this product.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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