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Physicochemical properties of probiotic *Lactobacillus acidophilus* fermented soy yoghurt supplemented with enzymes hydrolyzed African breadfruit

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Abstract
Physicochemical properties of *L. acidophillus* fermented soy yoghurt supplemented with enzymes hydrolysed African breadfruit (HABF) and inulin was evaluated. Three sets of yoghurt coded SMY, SINY and SBFY respectively for soymilk, 96% soymilk with 4% HABF and 96% of soymilk with 4% of inulin were produced and evaluated. African breadfruit (ABF) flour was hydrolysed with 0.1% glucanase enzymes and supplemented into soymilk. The milk was pasteurized at 720C for 30 min in a water bath. *L. acidophillus* was inoculated and the samples incubated at 420C for 6 - 8 h to make the yoghurts. The yoghurts were stored for 30 days at refrigeration temperature (4±2oC) and were analysed using standard method after 6 days' interval for pH, titratable acidity (TTA) and total soluble solids (TSS). There was significant ($p<0.05$) decrease in the pH from 5.13 - 3.14, 5.13 - 3.55 and 5.32 - 3.18 and TSS of the samples 15.00 - 6.05, 15.00 - 7.05 and 15.00 - 7.50 Brix respectively for SBFY, SINY and SMY. While TTA increased significantly ($p<0.05$) from 0.14 - 1.36, 0.84 -1.77 and 0.23 -1.77 % lactic acid. The result confirmed utilization of sugar and production of lactic acid by the fermenting organism *L. acidophilus.* This work suggests the effective production of yoghurt from HABF and inulin which can serve as a probiotic and prebiotic yoghurt.

Keywords: hydrolyzed African breadfruit, inulin, lactobacillus acidophilus, soymilk, yoghurt

Introduction
Yoghurt is one of the oldest fermented milk products known in human history. It is produced by inoculating concentrated milk with a yoghurt starter culture consisting of a mixture of homofermentative lactic acid bacteria, *Strephococcus thermophilus* and *Lactobacillus bulgaricus* at 45°C until the pH of about 4.0 is attained and cooled rapidly to about 4°C. Yoghurt is consumed for its refreshing and appealing flavour. The need for value addition to underutilized raw agricultural produce have necessitated the investigation of raw materials like African breadfruit in yoghurt production.
Soybean soymilk and other soy products have gained wide spread acceptability by consumers though with some limitations due to the intrinsic beany flavour associated with soybean. Soybean, (*Glycine max* Merr) is economically the most important bean in the world, providing vegetable protein for millions of people and ingredients for hundreds of chemical products and a potential source of bioactive peptides (Ani, *et al.*, 2010) [1]. On average, soybean contains more than 37% (Ogbemudia *et al.*, 2017) [7]. It also contains carbohydrates, phytochemicals, saponins, phytic acid and fiber. All this have health benefits.
African breadfruit (AFB) (*Treculia africana*) seeds contain 35 - 60 % carbohydrate with a considerable percentage being oligosaccharides which makes it a possible source of prebiotics (Ifediba and Ozoh, 2018) [10]. *Treculia africana* is an underutilized legume with high dietetic value and medicinal properties. It is very rich in micronutrients which contribute to its wound healing and antioxidant properties. Furthermore, showed that The fructose oligosaccharide content (prebiotic) of ABF are hydrolysable by glucanase a are cell wall degrading enzymes and such hydrolysis enhanced the growth of *L. acidophilus* a probiotic (Barber *et al.*, 2020) [4].
The combination of soybean and African bread fruit in the production of yoghurt will provide a probiotic and prebiotic refreshing drink. This study was therefore aimed at evaluation of the physico-chemical properties (pH, total titratable acidity and total soluble solid) of probiotic soy yoghurt supplemented with glucoamalyse hydrolyzed African breadfruit.

Materials and Methods
Materials
Soybean and African breadfruit
Soya beans (*Glycine max Merr*) was obtained from mile 3 market in Rivers State, Nigeria. African breadfruit ((*Treculia africana*) seeds were purchased from an African breadfruit tree owner in Kabangha, Khana Local Government Area of Rivers State, Nigeria.

Enzyme and Fermenting microorganisms
Glucoamylase was obtained from Novozymes (Novoenzymes A/S, Bagsvaered, Denmark). The fermenting microorganism used was probiotic species of *Lactobacillus acidophilus* (Nature source, UK).

Microbial media and analytical reagents
The microbial media used for isolation and enumeration of *L. acidophilus* was De Man Rogosa Sharpe (MRS) agar and broth (Oxoid, UK). Buffered Peptone water was used as diluents for serial dilutions. Analytical grade reagents that was used includes hydrochloric acid (HCL), calcium hydroxide (Ca (OH)2), sodium hydroxide (NaOH) and phenolphthalein This was obtained from the Department of Food Science and Technology, Rivers State University, Port Harcourt.

Methods

Production of African Breadfruit (Treculia africana) Flour

ABF seed flour was produced as described by Barber *et al.*, (2020) [4] Fresh ABF seeds were parboiled in boiling water for 5 min. The seeds were drained, manually dehulled and dried at 50°C for 18 h in a hot air oven (Gallenkamp UK). The dried seeds were milled and sieved through a 150 µm sieve to obtain the ABF seed flour. This was packaged in airtight plastic bottles and stored in a deep freezer and was gradually used for analyses.

Hydrolysis of ABF Flour

A slurry (1:3.5 w/v ABF seed flour: water) was made with distilled water with the pH adjusted to 6.0 - 6.5 with Ca (OH)2 solution that was prepared at pH 11.00. 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 slurry was held at 50 °C in a water bath and the Beta-glucanase [endo-1,3(4)-] (0.01 ml/100g flour) was added to the it with regular stirring for 2 h to partially hydrolyse the ABF. Thereafter mixture was boiled to inactivate the enzyme. The ABF hydrolysates was labeled as HABF (Barber *et al*,2020) [4].

Production of soy milk

The method of Champagne *et al.*, (2012) [5] 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 as soymilk in a refrigerator at 4°C. The milk was used within 3 h for the production of soy yoghurt.

Production of yoghurt samples

Three sets of yoghurt were produced following the method of Barber *et al*, (2020) [4]. One set was from soymilk, the second set was made with 4% HABF and 96% of soymilk and the third set was made with 96% of soymilk and 4% of inulin. The samples were coded SMY, SBFY, SINY for soymilk, soy/HABF and soy/inulin yoghurts respectively as shown in Table 1. Sample with only soymilk served as control while sample with inulin served as standard. The three samples were fermented with probiotic *L. acidophilus* mono-culture. All the formulations were pasteurized at 72 °C for 30 min in a water bath, cooled to 42°C and then inoculated with 4% activated culture (i.e. 20 ml of the starter containing 6.71 and 6.43 Log10 CFU/ml) for the mono-cultures *L. acidophillus* respectively, according to (Barber *et al.*, 2020) [4]. 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.

Table 1: Formulation of the probiotic *L. acidophilus* fermented soy yoghurt supplemented with HABF and Inulin

Yoghurt Samples	Composition (%)			Starter
	Soymilk	HABF	Inulin	
SMY	100	0	0	*L. acidophilus*
SBFY	96	4	0	*L. acidophilus*
SINY	96	0	4	*L. acidophilus*

Determination of the Physicochemical properties of probiotic L. acidophilus fermented soy yoghurt supplemented with HABF and Inulin

Determination of pH

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 was measured using a digital pH meter. The electrode was completely submerged in the sample and the pH read from the digital LCD read-out. This method was according to the standard AOAC method (AOAC 2012) [2].

Determination of the Total Titratable Acidity (TTA) as %Lactic Acid

Titratable acidity (TTA) of the yoghurt samples was determined according to the method described in AOAC (2012) [2]. Ten milliliters (10 ml) of each of yoghurt sample was each pipetted into Erlenmeyer flasks (100 ml). Phenolphthalein indicator (0.5 ml) was added and mixed thoroughly. The yoghurt samples were titrated against 0.1 M NaOH solution until the first tinge of pink will appear persisted for 30secs. TTA of the samples as percentage of lactic acid was calculated by the formula: (Titre X Normality of base X 0.09 X100)/Volume of sample.

Determination of Total soluble solid

The TSS as percentage brix was determined using the refractometer as described by AOAC (2012) [2]. Briefly, a drop of sample was introduced into the refractometer using a pasture pipette and the TSS was read off at the point of separation of the white and blue bars.

Statistical Analysis

All experiments and analysis were carried out in duplicates and the data obtained were subjected to analysis of variance (ANOVA) using Minitab (Release 18.0) under the general linear model and turkey pairwise comparison at 95% confidence level.

Results and Discussion

pH of probiotic *L. acidophilus* fermented soy yoghurt supplemented with HABF and inulin during refrigeration storage

pH of the yoghurt samples is presented in Table 2. The initial pH of the samples ranged from 5.13 – 5.30 for the SMY and SINY respectively. There was significant ($p<0.05$) decrease in pH with the storage. The values decreased from 5.13 – 3.14, 5.13 – 3.55 and 5.32 – 3.11 for SMY, SINY and SBFY respectively, after 30 days of storage at refrigeration temperature. The decrease was significantly ($p<0.05$) higher in SMY which is the control and lower in SBFY which is the test sample. This indicates that there was continuous metabolic activity of the probiotic *L. acidophilus* and such activities was higher in the control sample. Diary milk is generally considered to be slightly acidic, with a pH of 6.5-6.7 (Hajirostamloo, 2009) [9]. Studies on plant-based milk have shown that the pH of samples like soybeans is usually in the range of 6.0 - 6.4 (Ogbonna, *et al.*, 2013) [8]. The decrease in pH of the samples in this study could be attributed to the more sufficient utilization of the substrate by the probiotic *L. acidophilus* in medium and storage time in refrigerator. This is in trend with the report of Barber *et al.*, (2020) [4]

Table 2: pH of probiotic *L. acidophilus* fermented soy yoghurt supplemented with HABF and inulin during refrigeration storage

Time (Days)	Yoghurt Samples		
	SMY	SINY	SBFY
Initial	5.13g1±0.20	5.13e1±0.01	5.32f2±0.01
1	4.61f1±0.49	4.60d1±0.13	4.73e2±0.02
7	4.05e1±0.40	4.44d2±0.03	4.55d3±0.03
13	3.78d1±0.06	4.23c2±0.03	4.44c3±0.03
19	3.53c1±0.03	4.19c2±0.01	4.29b2±0.00
24	3.23b1±0.01	4.01b2±0.00	4.20b3±0.02
30	3.14a1±.0.01	3.55a2±.0.74	3.18a1±.0.03

Means with the same alphabetic superscript along each column are not significantly (P>0.05) different. Means with the same numeric superscript along each row are not significantly (p>0.05) different. Values are mean ± standard deviation of duplicate samples.
SYM =Soymilk yoghurt, SINY = Soymilk with 4% inulin yoghurt, SBFY = Soymilk with 4% hydrolyzed African bread fruit yoghurt

Table 3: TTA (%Lactic acid) of probiotic *L. acidophilus* fermented soy yoghurt supplemented with HABF and inulin during refrigeration storage

Time (Days)	Yoghurt Samples		
	SMY	SINY	SBFY
Initial	0.14e3±0.00	0.39f1±0.23	0.24f2±0.01
1	0.46d2±0.01	0.36f3±0.00	0.66e1±0.00
7	0.75c2±0.01	0.66e3±0.00	0.91d1±0.01
13	0.77 c3±0.00	0.91d2±0.00	1.13c1±0.00
19	1.45b1±0.02	1.33c2±0.02	1.27b3±0. 00
24	1.36b2±0.01	1.43b2±0.01	1.76a1±0.50
30	1.97a1±0.00	1.77a2±0.01	1.77a2±0.00

Means with the same alphabetic superscript along each column are not significantly (P>0.05) different. Means with the same numeric superscript along each row are not significantly (P>0.05) different. Values are mean ± standard deviation of duplicate samples
SYM =Soymilk yoghurt, SINY = Soymilk with 4% inulin yoghurt, SBFY = Soymilk with 4% hydrolyzed African bread fruit yoghurt

Titratable acidity (TTA) of probiotic *L. acidophilus* fermented soy yoghurt supplemented with HABF and inulin during refrigeration storage

The TTA of the yoghurt samples are shown in Table 3. The initial TTA of the samples varied significantly (p<0.05). The soymilk yoghurt SMY had the least initial TTA of 0.14% lactic acid and the soy-inulin yoghurt (SIINY) had the highest value of 0.39% lactic acid. There was significant (p<0.05) increase in TTA with storage. The values ranged from 0.14 – 1.97, 0.39 – 1.77 and 0.24 – 1.77 Brix for SMY, SINY and SBFY respectively. These values are more acidic than the values reported by (Barber, *et al.,* 2020) [4], which could attribute to storage time. The increase in TTA amongst the samples was significantly (p<0.05) higher in the soy milk yoghurt (SMY). Faster increase in TTA of soy milk during fermentation has been attributed to the low buffering capacity of soy milk (Zhang, *et al.*,2005, 2017) [12, 13]. Generally, the increased in TTA is a confirmation of the metabolic activity of the probiotic *L acidophilus* a homo-fermenter characterised with the production of two molecules of lactic acid from a molecule of sugar (Komesu *et al* 2017) [11].

Table 4: Total soluble solid of probiotic *L. acidophilus* fermented soy yoghurt supplemented with HABF and inulin during refrigeration storage

Time (Days)	Yoghurt samples		
	SMY	SINY	SBFY
Initial	15.05a1±0.05	15.05a1±0.05	15.25a1±0.05
1	7.05b2±0.05	9.10b1±0.10	9.05b1±0.05
7	7.05 b1±0.05	8.05c1±0.05	8.05c1±0.05
13	6.60 c2±0.10	8.10c1±0.10	8.00c1±0.00
19	6.55c2±0.05	7.70d1±0.20	7.55d1±0. 50
24	6.50c2±0.00	7.50d1±0.05	7.55d1±0.50
30	6.05d3±0.05	7.05e2±0.05	7.50d1±0.00

Means with the same alphabetic superscript along each column are not significantly (P>0.05) different. Means with the same numeric superscript along each row are not significantly (P>0.05) different. Values are mean ± standard deviation of duplicate samples
SYM =Soymilk yoghurt, SINY = Soymilk with 4% inulin yoghurt, SBFY = Soymilk with 4% hydrolyzed African bread fruit yoghurt

Total Soluble Solid (TSS) of probiotic *L. acidophilus* fermented soy yoghurt supplemented with HABF and inulin during refrigeration storage

Table 4 presents the total soluble solid (TSS) content of probiotic *L. acidophilus* fermented soy yoghurt supplemented with HABF and inulin during refrigeration storage. There was no significant (p>0.05) difference in the initial TSS of the yoghurt samples, the value ranged from 15.05 – 15.25 for SMY and SBFY. TSS decreased significantly (p<0.05) with storage time. The value ranged from 15.05 - 6.05, 15.05 - 7.05 and 15.25 - 7.50 for SMY, SINY and SBFY respectively. The decrease was significantly (p<0.05) higher in SMY. The values obtained in this study were similar to the report by Nnam, (2003) [6]. The decrease in sugar content is suitable for those that need less sugar. Aydar, *et al.*, (2020) [3] reported that soy milk had lower sugar content than other tubers and nuts, making them suitable for good health.

Conclusion

The pH, TTA and TSS of The yoghurt samples made from soymilk, soymilk supplemented with 4%HABF and soymilk supplemented with 4% inulin showed significant (p<0.05) decrease in pH, increase in TTA and decrease in TSS with storage time. Amongst the samples, soymilk yoghurt which is the control showed significant (p<0.05) difference from the test sample and the standard. However, the test sample and the standard provided utilizable nutrient for fermentation and production of yoghurt. The presence of probiotic fermenter L. acidophilus and a prebiotic inulin suggests that a refreshing pro and prebiotic yoghurt can be achieved with hydrolysed African Bread Fruit (HABF). Further studies into the phytochemical and microbiological quality of the yoghurt are recommended.

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