

2022-03-25

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Enviro Research Publishers.

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<http://dx.doi.org/10.12944/CRNFSJ.10.1.20>

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## Development of Plant-Based Yoghurt Rich in Bioavailable Essential Nutrients and Bioactive Compounds from Ingredients Available in East Africa

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### Abstract

The main aim of the present study was to formulate a convenient plant-based yoghurt (PBY) with essential nutrients and bioactive compounds comparable to that of cow's milk using locally available ingredients in East Africa. Linear programming (LP) was applied for ratios optimization. The fermentation technique was deployed using commercial yoghurt culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) to develop a palatable and functional yoghurt. Laboratory analysis was conducted to validate the nutritional and functional values calculated by LP, and their relative difference was also calculated. PBY was analyzed for lactic acid bacteria (LAB) viability and storage stability for the 1<sup>st</sup>, 7<sup>th</sup>, and 14<sup>th</sup> day of refrigeration storage (4°C). Results showed that LP-optimized PBY could be formulated at a low cost of USD 0.9/kg, which is 60% cheaper than Alpro natural PBY. The formulation contained 37.87% and 18.88% of total Monounsaturated Fatty acids (MUFAs) and total Polyunsaturated Fatty acids (PUFAs), respectively. PBY rich in essential nutrients and functional properties enough to meet the Recommended Daily Intake (RDI) for 2-10-year old children. Formulated PBY were microbiologically stable for 14 days of storage and were found within acceptable standards specified by the Food Standard Australia New Zealand (FSANZ) and the East African Standard for yoghurt (EAS33:2006). Plant-based yoghurt rich in nutrient bioavailability and bioactive compounds can be formulated using locally available ingredients and considered as an alternative to yoghurt.



### Article History

Received: 21 December 2021

Accepted: 12 March 2022


### Keywords

Essential Nutrients;  
Lactic Acid Bacteria;  
Linear Programming;  
Locally Available Ingredients;  
Plant-Based Yoghurt.

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Doi: <http://dx.doi.org/10.12944/CRNFSJ.10.1.20>

## Introduction

Humans have consumed dairy products for centuries, and today, Eastern African countries still consume substantial amounts of dairy products.<sup>1</sup> They are considered as a good source of calcium, fats, carbohydrate, and proteins essential to human nutrition, and comparable balance is hard to find in others. This advantage is connected to a shortage of milk availability compared to the global demand, especially in low-income countries.<sup>2</sup> Despite the benefits of milk, its consumption may significantly elevate the amounts of saturated fats in diets, increasing the risk of heart diseases and prostate and breast cancers.<sup>3-1</sup> Moreover, health concerns and risks such as cow milk allergy, lactose intolerance, veganism, and cholesterol concerns have compelled some consumers to switch to dairy-free foods and beverages as alternatives to cow milk.<sup>4</sup> Reports show that about 75% of the global population are lactose intolerant,<sup>5</sup> whereas 85% of African people are intolerant to cow's milk.<sup>2</sup> In Tanzania, 10% of individuals in Morogoro and 20% in Njombe testified to have had lactose intolerance after consuming goat and cow milk, respectively.<sup>6</sup> Additionally, allergies to animal milk are increasingly becoming an emerging disease condition in Africa, where 18% of children under the age of five years in Kenya are allergic to milk products and 20 % of asthmatic children had reactivity to milk allergens and chronic constipation in South Africa.<sup>7</sup>

For these reasons, plant-based beverages and foods are rapidly expanding segments of the functional food market as dairy-free alternatives. Their demand is rising, and their future global market is anticipated to hit USD 2.89 billion by 2026, which can aid as an inexpensive alternative in areas with a shortfall to animal milk accessibility.<sup>8</sup> Plant materials such as coconut, rice, and sesame seeds are naturally free from lactose. They can be used as essential ingredients in plant-based beverages for consumers who are intolerant to lactose or allergic to cow's milk.<sup>9</sup> Plant-based yoghurt (PBY) is obtained by fermenting water-based extracts (plant-based milk), suspensions from plant source materials like cereals, nuts, legumes, or fruits pulp.<sup>8-10</sup> Numerous trials to obtain milk and yoghurt with similar consumers acceptability to dairy-derived products have been performed by academics and industries in recent years.<sup>10</sup> Despite that effort, among the

existing alternatives to dairy products are highly-priced and face processing or preservation problems associated with poor texture or flavour.<sup>9-10</sup> Besides, the majority of these alternatives contain health-benefiting bioactive composites, but others are unbalanced in nutritional values relative to animal-derived products.<sup>8-10</sup>

Today, yoghurt is consumable as a source of probiotic live microorganisms (*Bifidobacterium* and/or *Lactobacillus* genera) which are widely employed in the fermentation of commercial products. They have shown to present some health-promoting benefits, including producing particular organic acids to stimulate the immunological responses of the host, improving digestion and enhance the gut microbiota, producing bacterial metabolites to inhibit the growth of pathogenic bacteria and successfully competing in terms of foods and space.<sup>11</sup>

The proteins in yoghurt are more digestible than those in milk. They can be a staple diet for children who cannot tolerate milk and consumers with allergies to milk protein or intolerant to milk lactose.<sup>12</sup> The poor sensory attributes (particularly texture and flavour) in PBY arise from the absence of cow's milk's lactose and fat contents. On the other hand, the enjoyable creamy texture of dairy yoghurt emanates partly from the fat content of milk and partly from the lactic acid produced during fermentation which interacts with the casein and whey proteins present in cow's milk.<sup>13</sup> Due to the absence of these proteins in plant-based milk, creating the desired "creamy" texture in PBY is a daunting task. As such, plant-based beverages are scanty in the Eastern African region. Even if they are accessible, most consumers cannot afford them due to their poor economic profiles. The known plant-based beverage in the region is soybean milk, a common food allergen to individuals who are allergic to cow's milk, especially young children.<sup>9</sup> All these factors make it difficult for lactose-intolerant people and those allergic to animal milk proteins to access palatable dairy alternatives in the East African market.

Nowadays, consumers are pursuing products with minimal or no sugar additives and those made from simple and natural ingredients so that the products have a so-called clean label.<sup>8</sup> The present research

aimed to formulate a palatable plant-based yoghurt rich in the bioavailability of essential nutrients and biologically active compounds from locally available ingredients apart from dairy sources with the aid of LP. Furthermore, the optimal developed PBY can expand a choice space for consumers with lactose intolerance, allergies to milk proteins and all consumers in general in the East African Community (EAC).

## Materials and Methods

### Materials

The food samples such as whole coconut, broken rice, sesame seeds (LINDI 02 variety); oyster mushroom (*Pleurotus ostreatus*) powder and date palm fruits (Medjool variety) were conveniently collected from farmers' local markets in Arusha, Tanzania. The commercial plain yoghurt was purchased from local supermarket at Tengeru, Tanzania. The guar gum (Bob's Red Mill Natural Foods Inc, USA) and freeze-dried lactic culture for Direct Vat Set (DVS), which are thermophilic yoghurt culture YF-L811 (50: 50 *Streptococcus thermophilus* and *Lactobacillus bulgaricus*), were obtained from suppliers of food additives in Mbezi, Dar es Salaam. For proto type formulation, all ingredients were transported to the Nelson

Mandela African Institution of Science and Technology (NM-AIST) food kitchen. The Research Ethical Clearance Committee approved the present study protocol at NM-AIST, Tanzania (KNCHREC00034, 2020).

### Formulation and Testing the Prototype

Three important steps were involved in the overall process of formulating and testing the model: The first stage involved determining the potential ingredients. All possible raw materials that could be cultivated in Tanzania and the EAC were shortlisted in the created checklist (Table 1). Local and global food composition databases and available published reports were surveyed to find data on nutritional composition. Hence, based on the local availability, nutrient composition, ingredients prices, and cultural acceptability, the selection of ingredients was made and completed. The second stage created definite LP model key elements: the decision variables (DV), objective function (OF), and constraints. With Microsoft Excel Office 2010 (version 14.0.7268.5000) and the Solver add-in, these parameters were used to set up and solve the LP model as described by.<sup>14</sup> The last stage included the formulation (preparation) of the prototype.

**Table 1: Checklist of raw materials screenings**

Brand	Names	Energy (kcal)	Protein (g)	Fats (g)	Carbs (g)	Fiber (g)	Ca (mg)	B <sub>9</sub> (μg)	Fe (mg)
FDC ID: 1100522	Coconut	354	3.33	33.49	15.23	9	14	26	2.43
FDC ID: 1100608	Sesame	631	20.45	61.21	11.73	11.6	60	11.5	6.36
FDC ID: 1578329	Quinoa	357	14.29	7.14	64.44	6.7	44	ns	4
FDC ID: 170162	Cashew	553	18.22	43.85	30.19	3.3	37	6.68	25
FDC ID: 170556	Pumpkin seeds	559	30.23	49.05	10.71	6	46	8.82	58
FDC ID: 1097552	Rice	47	0.28	0.97	9.17	0.3	118	0.2	2
FDC ID: 1581178	Pea	48	3.2	0.4	8	2.4	16	ns	1.15
FDC ID: 169702	Millet	378	11.02	4.22	72.85	8.5	8	85	3.01
FDC ID: 170178	Macadamia	718	7.91	75.77	13.82	8.6	85	11	3.69

FDC ID: 1517737	Sunflower seeds	283	11.67	25	10	5	67	ns	3
FDC ID: 175034	Mushroom powder	33	2.9	0.2	6.9	3.48	2.5	63	0.7
E 017	Dates fruits	310	2.38	0.35	72.67	9.10	ns	ns	ns

Data values were determined from USDA<sup>15</sup>, SELF Nutrition Data<sup>16</sup>, and are expressed per serving 100g, ns: not specific.

### Preparation of Coconut Milk, Rice Milk and Sesame Milk

Coconut milk was prepared following the method described by<sup>17</sup> with slight modification. Coconut milk was prepared by shelling the nut, and by use of a dull knife, the meat was separated. The brown skin of the meat was removed by a sharp knife and followed by washing with clean water. Later coconut meat was chopped into small pieces. In a bowl of warm water (65-75°C), the chopped meat pieces were soaked for 30 min to allow the extracted oil and aromatic compounds. The coconut meat was homogenized with water in a blender and filtered through cheesecloth. The obtained milk (supernatant) was stood where fat and water separated to form a float coconut cream.

The method described by<sup>18</sup> was used to prepare rice milk. Broken rice was manually sorted and washed with clean potable water. The rice was cooked with 1:3 parts of water at maintained temperature of 80°C for 15 min, and  $\alpha$ -amylase (0.22%) was added to faster the cooking rate. The soupy gelatinized filtered through cheesecloth and extracted milk was obtained. Sesame milk was prepared following the method reviewed by.<sup>19</sup> Sesame seeds were roasted in an oven (145°C for 20 min) and soaked overnight for 16 h at room temperature. These processes occurred to reduce chalkiness and bitterness by improving the flavour and acceptability of milk. The seeds drained, rinsed in tap water and blanched for 15 min in boiling water (65°C). After draining, the blanched sesame seeds were wetly milled in the blender with water (5:1) for 20 min. The resulting slurry remained at room temperature (25°C) for about 1 hour and was later filtered through a double-layered cotton cloth to get sesame milk.

### Preparation of Date Syrup

A natural sweetener extracted from dried dates fruits soaked in hot water at fixed temperature of 80°C

for 3 hours using water bath, mashed and filtrated to get the water extract. The extract was boiled by stirring until a thick consistency like honey was obtained.<sup>20</sup>

### Culture Preparation

The 100 mg of thermophilic yoghurt culture YF-L811 (YoFlex®, Denmark) packed with *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* (50:50) were inoculated to 100 mL of De Man, Rogosa, and Sharpe (MRS) broth (HiMedia, M369-500G, India) that had been sterilized at 121°C for 15 minutes. The incubation was placed overnight at 37°C. The 20% of glycerol was added to the stock culture and fractioned into 10 mL aliquots. For future usage, the stock culture aliquots were kept at -20°C.

### Developing a Linear Programming Model

Linear programming is an appropriate mathematical model for formulating novel optimized food products. It aids in using the possible cheapest food ingredients of a region to set and meet the nutritional necessities while respecting the multiple linear constraints.<sup>20</sup> In the present study, LP was used to lessen the objective function Z, which is the cost of the formulation. The decision variables are values of ingredients weights that can be changed to reduce the cost of formulation Z. The LP model is expressed in Equation 1.

$$Z = A_1X_1 + A_2X_2 + \dots + A_nX_n \quad \dots(1)$$

Where Z is the total cost for ingredients;  $A_1, A_2, \dots, A_n$  are objective function coefficients which are constant equivalent to cost per unit weight of food ingredients, and  $X_1, X_2, \dots, X_n$  is the values of DV in formulation Z. The set of linear constraints is the optimization process limitations. The main purpose was to reduce the cost of formulation Z while meeting various constraints. These limitations or constraints, such as greater than,

equality, or less than, are imposed on one or several DV to ensure that the product's nutritional composition meets the designed requirements and does not surpass the upper thresholds. The solution is feasible upon solving the LP when all of its constraints are achievable. Due to the lack of plant-based yoghurt standards for East Africa, the Food Standard Australia New Zealand (FSANZ)<sup>22</sup> for plant-based substitutes and the East African Standards (EAS33:2006) set the constraints. Also, the peer review journals of related similar products to design optimal formula were surveyed. The constraints for the LP model were as follows: nutrients concentration and energy, texture, palatability, anti-nutrients, total food ingredients, and ratios to fats, carbohydrates and proteins to energy. In the prototype development, a LP was used to avoid the traditional trial-and-error method and minimize the production cost.

Nutrient concentration and energy constraints: LP constraints were set to ensure that the optimized formula met the FSANZ specifications for energy proportion and energy ratios to fats, carbohydrates and protein. Care was taken to obtain the energy quantity between 67-272kJ/100g and caloric distribution to be 20-33% from fat, 5 to 6% from protein as per the FSANZ specifications.<sup>14</sup> and<sup>23</sup> explained the use of LP in designing a consistent, palatable prototype. In computing the values to be used in the formulation of the food prototype using LP, the palatability constraint was introduced to obtain the acceptable taste, and dried date (7-10 g/100g) was included to enhance the sweetness of the formulation. Therefore, based on 14 study, the low sugar content of 15-25% was constrained in LP. The existence of texture of food in prototype formulation is paramount, as is the specific consistency in the food mix that determines the uniformity in composition and stability. The texture-related constraint, the solid contents of yoghurt expected to be 8.25% and fat content with a range from 0.8-6.8% to provide a better body and texture that is smooth and firm enough to be spooned.<sup>24</sup> Since the fat composition makes the texture of the product softer, squeezable, and swallowable more easily by consumers, fat compositions and yoghurt total solid content was constrained since they can affect the texture and consistency of the

prototype. Anti-nutrients factors were inserted in the LP model to ensure that the optimized formula met the FSANZ<sup>22</sup> specifications for anti-nutrient factors in food. The phytic acid content less or equal to 22.8 mg per 100 g were delimited and constrained in LP.

The overall weight of food ingredients was limited to give space for including vitamins and minerals during the final product premixing. In this exercise, the inclusion of equality constraint was considered to weigh the food ingredients at 97 g. This setting was based on a preceding calculation which established that up to 3% would be required for premixes of the final product weight. For obtaining the optimized values using LP, mathematical computation and software involved five steps as previously applied by<sup>25</sup> and<sup>26</sup>

- i. Creating the data layout in a Microsoft Excel spreadsheet,
- ii. Excel installation standard allows for the activation of the add-in Solver Function,
- iii. Assignment of the objective function (OF), Decisions variables (DV) and Constraints
- iv. Resolution of the objective function by running LP, and
- v. Sensitivity analysis.

### Plant-Based Yoghurt Production

Based on the above evaluation, the optimized ingredients' ratios were calculated in LP<sup>23</sup> and were used to formulate four prototypes different on the added concentration of date's syrups. The milk blends (Table 2) was triple sieved with the muslin cloth. The final four formulations were prepared and blended until a homogenized smooth solution was achieved. The milk samples were pasteurized in 30 minutes at 85°-87 °C, filtered with gauze filters and cooled at 40°C (Figure 1). The milk blends of 100 mL were poured in four sterile containers with different portions of date syrup (0.0, 6.0, 8.0 and 10.0% v/v), 0.05% (w/v) of guar gum, and 1% (w/v) of oyster mushroom powder. By stirring, 0.1% (w/v) of culture was added. The milk blends were incubated at 43°C for 8-12 hours up to the dropped pH of 4.5. Then four types of yoghurt were cooled rapidly and stored at + 4°C during 14 days for further analysis. Thereafter, experienced panelists carried out the sensory evaluation. The best ranked optimized formulation was selected and evaluated

for proximate analysis, fatty acids profile, minerals bio-availability, healthy bioactive compounds, and

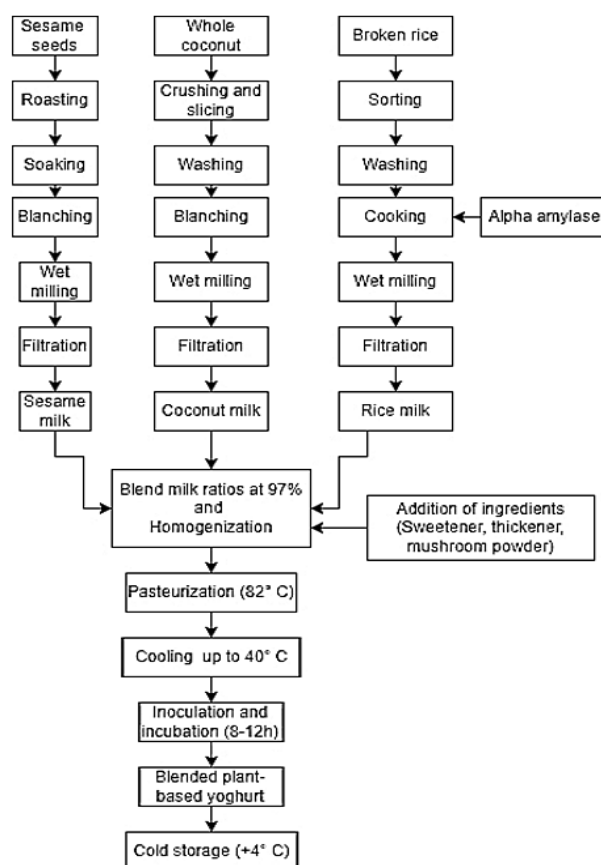
microbiological stability compared to the commercial plain yoghurt (control).

**Table 2: Blending of coconut milk, rice milk and sesame milk at different ratios for PBY formulation**

Ingredients	Plain Yoghurt	PBY -0%	PBY - 6%	PBY -8%	PBY -10%
Cow's milk yoghurt	100	-	-	-	-
Coconut milk	-	-	-	8540.7	38.5
Rice milk	-	-	92	539.3	38.5
Sesame milk	-	-	4	-8	9
Mushroom powder	-	1	1	1	1
Guar gum	-	0.05	0.05	0.05	0.05
Date palm syrup	-	0	6	8	10
<b>Total (%)</b>	<b>100</b>	<b>97.00</b>	<b>97.00</b>	<b>97.00</b>	<b>97.00</b>

PBY-0%: Plant-based yoghurt without date palm syrup; PBY-6%: Plant-based yoghurt with 6% date palm syrup; PBY-8%: Plant-based yoghurt with 8%

of date palm syrup; and PBY-10%: Plant-based yoghurt with 10% of date palm syrup.



**Figure 1: Flowchart for the production of the blended plant-based yoghurt.**<sup>8,10</sup>



### The Sensory Evaluation

The sensory testing was accomplished to assess consumers' satisfactoriness level of the formulated non-dairy yoghurt relative to cow's milk yoghurt. Twenty panelists from NM-AIST, including students and staff members, were involved in the sensory test. The panelists were chosen based on their socioeconomic statuses, such as education, willingness, capacity, and experience in conducting the sensory evaluation. The sensory attributes acceptability of produced yoghurt was determined based on a 9-hedonic scale ranging from like extremely (9) to dislike strongly (1) concerning taste, texture, odour, colour, and overall acceptability between optimized PBV prototypes and cow's milk yoghurt (control).<sup>4</sup>

### Laboratory Analysis of the Optimized Prototype Proximate Analysis

Proximate analyses (moisture, fiber, fat, protein, ash, and carbohydrate content) of the yoghurt were determined according to the Association of Official Analytical Chemists (AOAC).<sup>27</sup> The fat content was analyzed using the Gerber fat method. The Kjeldahl method was used to quantify the crude protein content in the PBV. The carbohydrate value was expressed as the difference of the protein, fat, crude fiber, total ash, and moisture content of the sample from 100%.

### Determination of Phytoconstituents

The phytate content was determined as described by.<sup>28</sup> The phytic acid concentration was determined using waste reagents of 0.03%  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  and 0.3% sulfosalicylic acid. A standard phytic acid curve was constructed under the same conditions, and results were expressed as phytic acid mg/100 g of fresh weight of the sample. The total phenolic contents (TPC) and the Total Flavonoid Content (TFC) were determined using the method described by.<sup>29</sup> For TPC, 10% of Folin-Ciocalteu's reagent (FCR) and 7%  $\text{Na}_2\text{CO}_3$  were used. Gallic acid solutions in methanol (5-500 mg/L) were prepared for the standard curve, and TPC was calculated as mg Gallic acid equivalents per gram of fresh weight of the sample (GAE/g). TFC of the extract was investigated using the aluminium chloride colourimetric. The standard calibration curve was prepared for Quercetin; the TFC was expressed

as milligram quercetin equivalent per gram of extracted sample based on a standard curve of Quercetin (mg QCE/g sample).

### Vitamins, Minerals and Fatty Acid Profile Determination

The sample's fat-soluble vitamins (vitamin A, E, and  $\beta$ -Carotene) and water-soluble vitamins (B1, B9 and vitamin C) were quantified by the methods described by.<sup>30</sup> Analysis of minerals was performed as described by.<sup>31</sup> A Flame Atomic Absorption Spectrophotometer (FAAS) was used to determine the zinc (Zn), iron (Fe), and magnesium (Mg) contents. A flame photometer was used to determine calcium (Ca), potassium (K), and sodium (Na) according to,<sup>27</sup> while a DR 2700 spectrophotometer was used to quantify phosphorus (P) content. The quantification of fatty acids profile was assessed using a Gas Chromatography Hewlett-Packard 5890:5971A system (Hewlett-Packard, Walbronn, German) with an SP 2331 column (0.25 mm of diameter, 60 m of length, and 0.25  $\mu\text{m}$  of film thickness) following a previously published method by.<sup>32</sup>

### Microbiological Analysis

The enumeration of viable lactic acid bacteria (LAB) colonies was tested following the method applied previously by.<sup>33</sup> The sample was subjected to a 10-fold serial dilution. Aliquots portions (0.1 mL) were picked and transferred by spread-plating on MRS agar (HiMedia, M641-500G, India) plates per dilution factor. The incubation occurred at 37°C for 24-48 hours. The same quantity of aliquot was inoculated on Potato dextrose agar (PDA) (HiMedia, M096-500G, India) to enumerate yeasts and moulds. Plates were incubated at 25°C for 3-5 days. Coliform bacteria were determined on MacConkey agar (HiMedia, MM081-500G, India), incubated at 37°C for 24 hours.<sup>34</sup> For *salmonella* counting, dilution was spread plated on *Salmonella Shigella* agar (SSA) and incubated for 24-48h at 37°C.<sup>35</sup> For all enumerations, the plates with between 30 and 300 bacteria colonies were counted. The total microbial counts were expressed as log Colony Forming Units per mL of yoghurt (CFU/mL). The microbiological properties were evaluated at the 1<sup>st</sup>, 7<sup>th</sup>, and 14<sup>th</sup> days of refrigerated storage.



### The Relative Difference Between the Results Generated by the Lp and the Values Analyzed in the Lab

The relative difference between the LP computed and the lab analyzed values were calculated as described by.<sup>14</sup> An absolute difference (AD) for each nutrient was calculated by subtracting the LP values (C) from the lab analyzed results (E). The relative difference (RD) was computed by dividing the absolute difference values (AD) over the calculated LP values (C), as shown in Equation 2.

$$RD = (B) \times 100 / C \quad \dots(2)$$

Where C is the calculated values from LP, E is the analyzed value from the lab, and B is the absolute value (AD or E-C)

### Statistical Analysis

Means of the LP-calculated nutritional values were compared to the lab-analyzed values using a paired

t-test. The mean differences were compared using one way ANOVA (analysis of variance). The main goal was to check if there were significant differences between the LP-designed product and those from the lab at  $P=0.05$ . Data were processed and analyzed in SPSS 23 (23 IBM@SPSS@Statistics, USA) and R (library version 3.6.1) software.

## Results and Discussions

### Sensory Evaluation of the Optimized Prototypes

The absence of significant statistical differences between the sensory attributes of the formulated PBV was shown by the Kruskal-Wallis H test. The likely mean scale score for taste, odour, colour, texture, and overall acceptability is presented in Table 3. The yoghurt derived from cow's milk was more acceptable than the formulated PBV-10% date syrup (liked very much toward moderately) in terms of overall acceptability.

**Table 3: Average results of sensory evaluation**

Sample	Odour	Texture	Colour	Taste	Overall acceptability
PBY-0%	5.0±0.14 <sup>b</sup>	5.2±0.26 <sup>e</sup>	6.1±0.21 <sup>f</sup>	7.9±0.19 <sup>a</sup>	6.3±0.25 <sup>c</sup>
PBY-6%	6.2±0.12 <sup>a</sup>	6.0±0.15 <sup>b</sup>	6.9±0.18 <sup>a</sup>	8.0±0.15 <sup>e</sup>	7.5±0.27 <sup>a</sup>
PBY-8%	7.7±0.11 <sup>d</sup>	7.0±0.18 <sup>f</sup>	7.7±0.14 <sup>c</sup>	8.2±0.12 <sup>e</sup>	7.7±0.15 <sup>a</sup>
PBY-10%	8.5±0.19 <sup>c</sup>	7.9±0.25 <sup>d</sup>	7.8±0.28 <sup>c</sup>	8.45±0.52 <sup>b</sup>	8.0±0.48 <sup>d</sup>
Plain Yoghurt	8.85±0.10 <sup>e</sup>	8.88±0.13 <sup>a</sup>	9.0±0.10 <sup>d</sup>	8.9±0.09 <sup>f</sup>	8.93±0.13 <sup>b</sup>

Values expressed in form of mean±sd (standard deviation, n=3). Means which differ on superscripts within columns are significantly different from each other ( $p<0.05$ ). PBY-0%: Plant-based yoghurt without date palm syrup; PBY-6%: Plant-based yoghurt with 6% date palm syrup; PBY-8%: Plant-based yoghurt with 8% of date palm syrup; and PBY-10%: Plant-based yoghurt with 10% of date palm syrup.

The formulated prototype (PBY-10%) satisfactorily met consumers' sensory preferences regarding odour, taste, and overall acceptability (liked very much), whereas the texture and colour were moderately liked. The odour acceptability was increased with increases of roasted sesame milk concentration in formulation. The pleasant taste obtained was enhanced by the increases of date syrup concentration and fermentation result of natural sugar present in blended milk by use of mixed culture microbial species: *S. thermophilus* and *L. bulgaricus*, which have been shown to

improve the sensory attributes, organoleptic quality, bioavailability of mineral and vitamins, and the shelf life of the fermented plant-based products.<sup>36</sup> The control (Plain yoghurt) was liked extremely in all parameters compared to the PBY formulated. This acceptance was due to the creaminess, thickness, and pleasant aroma of cow's milk yoghurt linked to milk proteins (casein and whey).

### Attributes of the final optimized product

The mathematical LP method was used to create the functional PBY. The prototype was created using

locally grown commodities by smallholder farmers in Tanzania and East Africa like coconut, broken rice, roasted sesame seeds, date palm syrup, and mushroom powder. The prices per kilogram of the main ingredients were about 0.4 USD for broken rice and coconut, USD 0.6 for sesame, USD 0.7 for mushroom powder, and USD 0.5 for dried date fruit. In this pilot study, the LP tool was valuable to certify that the cost was minimized while the nutritional value requirements and product palatability were met. The LP analysis result of the formulated LP model given from Equation 1 indicated a low cost of 0.9 USD/kg (2078.10 TZS/kg), which is 60% cheaper than Alpro (Soya-coconut blend), cost 2.50 Euro/kg and have a comparable price to the commercial plain yoghurt available in Tanzania. The analyses of the present study showed that LP could be used to develop a nutritious PBV rich in health-promoting bioactive compounds from locally available ingredients (other than animal milk) in East Africa. In general, the LP study showed that it is technically possible to design suitable culturally acceptable formulas rich in nutrient bioavailability. Essential nutrients and bioactive compounds (Tables 4, 5, and 6) were found in significant amounts in 100g of blended PBV.

The ingredients were rationed to improve acceptability while meeting the essential nutritional goal of the formulation based on raw materials

combination. The micronutrients and macronutrients of the proposed optimized formula yoghurt matched A500 and A1104 FSANZ for plant-based alternatives and EAS (33:2006) standards. According to FSANZ,<sup>22</sup> at least 20–33% of the total energy must be provided from fats and the rest from proteins and carbohydrates, and a total energy density of 67-272 kJ/100 g is suggested. The total energy density content of 265.23 kJ/100g was obtained with a protein-to-energy ratio (0.198), fat-to-energy ratio (0.404) and carbohydrate-to-energy ratio (0.408). Most of the nutrient contents in the proposed PBV were in the range of the dairy alternatives standards (Table 4). The formulated PBV contained fats content above FSANZ standards (2.9%) because of the high amount of fat present in coconut and sesame raw materials (Table 1). Despite the scarcity of scientific literature on blending plant-based milk,<sup>9</sup> established that blending two or more plant-based milk varieties to produce a product with a high nutritional value comparable to cow's milk is crucial in food production. The blending of ingredients as art to improve nutritional balance and sensory acceptability of PBV has also been studied by.<sup>37</sup> The present study discourages the use of highly-priced imported raw materials to formulate ready-to-use therapeutic foods as a means of addressing the cost constraints in developing countries, according to UNICEF.<sup>38</sup>

**Table 4: Proximate composition, phytoconstituents and minerals ratios of formulated PBV and plain (cow's milk) yoghurt**

Analyzed nutrients	Optimized PBV (100 mL)	Plain yoghurt (100g)	FSANZ (100 mL)
Moisture, %	85±0.66 <sup>a</sup>	84.90±0.46 <sup>a</sup>	50-100
Proteins, %	3.1± 0.25 <sup>a</sup>	3.95±0.53 <sup>b</sup>	not less than 3%
Fibers, %	0.88±0.06 <sup>a</sup>	0.00±0 <sup>b</sup>	0-1.9
Ash,%	1.93±0.02 <sup>a</sup>	0.59±0.18 <sup>b</sup>	NS
Carbohydrate, %	6.38±0.41 <sup>b</sup>	7.60±0.40 <sup>b</sup>	NS
Fats,%	2.90±0.22 <sup>a</sup>	3.00±0.30 <sup>a</sup>	no more than 2.5%
TPC, (mg GAE/100g)	120.10±0.61 <sup>a</sup>	0.00±0 <sup>b</sup>	NS
TFC, (mg QCE/100g)	69.01±1.06 <sup>a</sup>	0.00±0 <sup>b</sup>	NS
Phytic acid, mg	0.23±0.01 <sup>a</sup>	0.00±0 <sup>b</sup>	<22.8
Molar ratio :			
Phytic acid: Fe	0.0020	NS	<2.5
Phytic acid: Zn	0.0145	NS	<15
Phytic acid: Ca	0.0001	NS	<0.24

Ascorbic acid: Fe	0.1162	NS	<3.8
Energy, kJ	265.23±14.54a	305.87±6.04b	67-272

Values expressed in form of mean±sd (standard deviation, n=3). Means which differ on superscripts within rows are significantly different from each other (p<0.05).PBY: Plant-based Yoghurt; FSANZ: Food Standards Australia and New Zealand; NS: Not specified, TPC: Total Phenolic Content, GAE: Gallic Acid Equivalent, TFC: Total Flavonoids Content, and QCE: Quercetin Equivalent.

The optimized formulation met the standard ratios of minerals (Ca, Fe, and Zn) to phytic acid (Table 4) are within the ranges that favour micronutrients bioavailability which are the determinants of minerals absorption in the body. This range shows no impairment of nutrient absorption due to interactions between minerals and phytate. The low amount of anti-nutrients phytic acids in the formulation was caused by the processing techniques of ingredients. For instance, roasting and soaking raw sesame seeds have been established to minimize the level of phytic acids and tannins while increasing the extraction yield of milk.<sup>8</sup> The fermentation process can also have beneficial effects on minerals absorption. According to,<sup>39</sup> *Lactobacillus* genus boost calcium bioavailability in some fermented foods by enhancing metabolism and absorption of available calcium. They create phytase and short-chain fatty acids, which help to release calcium that has been stored in the body and increase its solubility. The study demonstrates that the formulated product has a potential TPC profile and TFC, absent in dairy products (control) (Table 4). A similar range of TPC (49.60-74.75mgGAE/100g)

was obtained by.<sup>40</sup> According to the approved requirements for ready-to-use foods and beverages, the current formulation does not contain artificial antioxidants or flavourings

According to FAO and the World Health Organisation,<sup>41</sup> at a single-serving (100g), the optimized formula meets the RDI requirements for women of reproductive age, which are about 70% (Fe) and 30% (Vitamin A)<sup>41</sup> (Table 5). Likewise, one serving (100g) of formulated yoghurt can contribute to the RDI of 15% for Mg, Na, vitamin E, B1 and B9; 30% for Ca, 60-70% for Zn and P, and ≥ 100% RDI for K, Fe, and vitamin C for the infants under three years.<sup>41</sup> Additionally, the formulated PBY met 80-100% of FSANZ for vitamin A, B1, B9, Mg, K, P and Ca (Table 5). These attributes make the optimized formula be consumers' better source of limiting micronutrients such as vitamin A, vitamin B<sub>9</sub>, Fe, Zn and Mg, which are cofactors of α-linolenic acid (ALA) conversion to docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and docosapentaenoic acid (DPA) in the human body.<sup>26</sup>

**Table 5: Minerals and vitamins composition of formulated PBY and plain yoghurt**

Components	Optimized PBY (100g)	Plain yoghurt	FSANZ (100 mL)	<sup>1</sup> RDI (100mL)
Calcium, mg	117±2.08 <sup>b</sup>	120±0.0 <sup>c</sup>	120	300-500
Zinc, mg	1.59±0.02 <sup>e</sup>	0.90±0.05 <sup>b</sup>	0.8	2.4-3.6
Potassium, mg	362±1.53 <sup>f</sup>	231.00±2.53 <sup>d</sup>	200	400-600
Phosphorus, mg	95±1.14 <sup>e</sup>	112±6.66 <sup>b</sup>	100	270-460
Iron, mg	9.6±0.38 <sup>b</sup>	0.07±0.02 <sup>c</sup>	5-7	0.27-1.5
Sodium, mg	14.00±1.52 <sup>a</sup>	50.00±2.52 <sup>e</sup>	NS	44.3
Magnesium	10.81±0.2 <sup>c</sup>	16.86±0.07 <sup>f</sup>	11	54-60
Vitamin A, <sup>μg</sup> RE	54.00±0.61 <sup>a</sup>	27.50±1.04 <sup>b</sup>	55-62.5	200-400
Vitamin B9, <sup>μg</sup>	8.08±0.12 <sup>d</sup>	10.90±0.15 <sup>a</sup>	6	150
Vitamin C, mg	35±0.01 <sup>f</sup>	0.009±0.05 <sup>d</sup>	NS	8-35
β- carotene, <sup>μg</sup>	86.50±1.11 <sup>a</sup>	21.00±0.06 <sup>f</sup>	NS	2000-6000

Vitamin E, µg	29.01±1.06 <sup>b</sup>	50.00±0.23 <sup>e</sup>	NS	>320
Thiamine, µg	50.00±1.52 <sup>f</sup>	59.01±0.57 <sup>c</sup>	50	500

Values expressed in form of mean±sd, (standard deviation, n=3). Means which differ on superscripts within rows are significantly different from each other (p<0.05). PBV: Plant-based Yoghurt; FSANZ: Food Standards Australia and New Zealand; <sup>1</sup>RDI: Recommended Daily intake data values were reported from FAO/WHO<sup>41</sup>

In the present study, the plant-based milk and yoghurt encompassed enough Polyunsaturated Fatty Acids (PUFAs) of 15.52 -18.88% (Table 6), which increased after fermentation. Formulae contained natural cofactors required for long-chain fatty acids metabolism (carbon atom of 20-22) in the body and contained a balanced omega-6 to the omega-3 fatty acid ratio (3:1), which are rare in other common dairy free-alternatives. Additionally, PBV is also sugar-free, relying on dried fruits as a natural sweetener. A combination of local ingredients acted as sources of monounsaturated and polyunsaturated functional fatty acids such as palmitoleic acids, linoleic acid (LA), and oleic, which augmented after fermentation (Table 6) and their quality was maintained because of the high content of vitamin E and C antioxidants in the formulation (Table 5). Additionally, coconut is a source of lauric acid and monolaurin-functional compounds against harmful pathogens, such as bacteria, viruses, and fungi.<sup>42</sup> Consumers should get access to omega-6 fatty acids, represented by linoleic acid (LA, 18:2) and omega-3 fatty acids like alpha-linolenic acid (ALA, 18:3) in a ratio that does not compromise the bioavailability of omega-3 fatty acids upon

consumption. Both are essential fatty acids that act as cofactors to be metabolized into long-chain fatty acids in the body. During metabolism, LA converts into arachnoid acid (C20:4), whereas ALA converts into eicosapentaenoic acid (EPA, C20: 5) and docosahexaenoic acid (DHA, 20:6). At a single-serving (100g), the analysis of this study showed that PBV could provide the recommended amount of linoleic acid (10g) and alpha-linolenic acid (0.9 g) for children between 4-8 years, which are negligible in many dairy alternatives.<sup>43</sup> There is no current dietary omega-6: omega-3 guideline ratio, but the recommended intake of omega-6 and omega-3 can be used to access the amount of dietary intake a consumer would have if they followed them.<sup>43</sup> reported that omega-6 to omega-3 lower ratios (below 10:1) were linked to a healthy diet and adequate intake of various other nutrients. Moreover, the presence of sesame in the formulation can enable consumers to access sesame proteins that contain adequate essential amino acids to meet 100% RDI for methionine, tryptophan, and cysteine, which are the most limiting micronutrients among children < 3 years old in developing countries.<sup>26</sup>

**Table 6: Fats acids profile of optimized formula before and after fermentation**

Fatty acids	Non-fermented blended milk (% TFA)	PBV (% TFA)
Butyric acid, C4:0	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Caprylic acid, C8:0	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Capric acid, C10:0	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Lauric acid, C12:0	2.25±0.19 <sup>a</sup>	3.01±0.26 <sup>b</sup>
Tridecanoic acid, C13:0	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Myristic acid, C14:0	5.65±0.46 <sup>c</sup>	4.00±0.30 <sup>b</sup>
Myristoleic acid, C14:1	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Pentadecanoic acid, C15:0	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Palmitic acid, C16:0	38.86±3.90 <sup>c</sup>	34.44±2.49 <sup>d</sup>
Palmitoleic acid, C16:1	1.44±0.12 <sup>c</sup>	2.07±0.20 <sup>c</sup>
Heptadecanoic acid, C17:0	0.12±0.01 <sup>d</sup>	0.00±0.00 <sup>a</sup>

Stearic acid, C18:0	3.23±0.30 <sup>e</sup>	1.11±0.12 <sup>f</sup>
Oleic acid, C18:1	31.18±2.81 <sup>f</sup>	34.85±2.49 <sup>e</sup>
Elaidic, C18:1 (Trans)	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Linoleic acid, C18:2	15.03±1.05 <sup>e</sup>	18.05±1.70 <sup>b</sup>
Linoelaidic, C18:2 (Trans)	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
α-Linolenic acid, C18:3	0.49±0.038 <sup>c</sup>	0.83±0.07 <sup>d</sup>
Arachidic, C:20:0	1.17±0.1 <sup>d</sup>	0.69±0.05 <sup>b</sup>
Eicosenoic acid, C20:1	0.58±0.04 <sup>b</sup>	0.95±0.08 <sup>b</sup>
<b>Total saturated</b>	<b>51.28±4.90<sup>e</sup></b>	<b>43.25±4.25<sup>d</sup></b>
<b>Fatty acid</b>		
<b>Total monounsaturated</b>	<b>33.2±2.78<sup>c</sup></b>	<b>37.87±2.98<sup>a</sup></b>
<b>Fatty acid</b>		
<b>Total polyunsaturated</b>	<b>15.52±1.43<sup>f</sup></b>	<b>18.88±1.78<sup>b</sup></b>
<b>Fatty acid</b>		

PBY: Plant-based Yoghurt; TFA: Total Fatty Acids, % values expressed as means ± SD (Standard deviation, n=3), Means which differ on superscripts within rows are significantly different from each other (p<0.05).

Microbiological quality of developed plant-based was assessed to expel doubts of the product microbiological deterioration during its anticipated shelf-life and ensure consumer protection against exposure to any health hazard. Yoghurt and alternative yoghurt must contain at least 10<sup>6</sup> CFU/mL (g) LAB Colonies during consumption time to provide a therapeutic advantage to the host.<sup>11</sup> In present study, the number of LAB identified is within the accepted quantitative standard of a minimum of 10<sup>6</sup> to 10<sup>7</sup> CFU/mL which is corresponding to 6-7 log CFU/mL.<sup>44</sup> The LAB count was almost constant during 14 days of refrigerated storage (+4°C), with minor decreases especially for the control which had a slight drop in LAB towards the end of storage due to the type of strain used (Table 7). Similarly, the slight constant of LAB during storage time of 15 days agrees with the results obtained by<sup>11</sup> and.<sup>33</sup> Yeast and mould (YMC) concentrations of no more than 2 log cfu/mL are allowed in yoghurt as because yoghurts with YMC more than 2 log cfu/mL deteriorate quickly even before being refrigerated.<sup>34</sup> In current study, Yeast and moulds were not detected at 10<sup>-1</sup> using spread plate, thus less than 100 CFU/mL was reported at the end of storage (Table 7). This can be attributed to the presence of LAB, which prevents the proliferation of fungus in yoghurt during storage. A previous study by<sup>45</sup> also obtained least amount of yeasts and moulds during storage time of 14 days (+4° C). *Salmonella* spp, and coliforms were not detected in PBY during storage times. This absence indicates Good

Manufacturing Practices (GMP), such as effective cleaning and pasteurization employed during the production. For instance, blanching occurred for coconut inactivated natural enzymes linked to odour loss, texture, lipid oxidation, and decreased microbial load. Moreover, organic acids and sensory metabolites such as bacteriocins produced by starter culture acted against pathogenic and spoilage bacteria during fermentation. Therefore, the lack of Enterobacteria indicates the safety level of optimized PBY.

#### Relative Difference of Nutritional Values Between Lab Analyzed Values and Lp Calculated Values

The drawback of the LP analysis is the discrepancies among ingredients' nutritional values from various nutrient data sources. The local Food Composition Data like Tanzanian Food Composition Tables does not have all nutritional information for the selected ingredients, thus other publicly available sources such as SELF Nutrition Data,<sup>16</sup> USDA nutritional databases,<sup>15</sup> and peer-review papers were used to obtain the nutritional composition data. The ingredients' nutritional composition may differ according to the geographical locations across the world and these deviations can affect the final product composition.<sup>26</sup> Hence, the optimized PBY was analysed for nutritional values in the laboratory to validate the quality of the LP formulated prototype. Therefore, the calculated RD (Table 8) confirmed that the LP-computed nutritional values of developed PBY were in line with the laboratory analysed values.

**Table 7: Microbial count (log CFU/mL) of PBY during storage (4°C)**

Storage days	Parameters	PBY <sup>1</sup>	Plain Yoghurt <sup>2</sup>	EAS and Codex Alimentarius specifications (log CFU/mL or g)
Day 1	LAB	8.24 ± 0.28 <sup>a</sup>	8.50±0.36 <sup>e</sup>	Minimum of 6-7 log cfu/mL
	YMC	ND	ND	2 log cfu/mL
	Coliforms	ND	ND	Absent
	<i>Salmonella</i>	ND	ND	Negative in 25mL
Day 7	LAB	8.19± 0.33 <sup>a</sup>	8.46± 0.26 <sup>e</sup>	Minimum of 6-7 log cfu/mL
	YMC	ND	ND	2 log cfu/mL
	Coliforms	ND	ND	Absent
	<i>Salmonella</i>	ND	ND	Negative in 25mL
Day 14	LAB	8.14 ± 0.19 <sup>a</sup>	7.85±0.36 <sup>e</sup>	Minimum of 6-7 log cfu/mL
	YMC	2.00±0.0 <sup>b</sup>	ND	2 log cfu/mL
	Coliforms	ND	ND	Absent
	<i>Salmonella</i>	ND	ND	Negative in 25mL

<sup>1,2</sup>formulated plant-based yoghurt with 10% of date palm syrup and cow's milk yoghurt, respectively. LAB (Lactic Acid Bacteria), YMC (Yeast and Mould Count), ND (Not Detected). Means which differ on superscripts within columns are significantly different from each other (p<0.05).

**Table 8: Relative difference between the LP calculated values and lab analyzed values**

Component	C (LP)	E (Lab)	<sup>1</sup> A.D E-C=B	<sup>2</sup> R.D (B×100)/C
Moisture, %	84.7	85	0.3	0.34
Proteins, %	3.06	3.1	0.04	1.30
Fiber, %	0.90	0.88	0.02	2.2
Ash, %	1.87	1.93	0.06	3.20
Carbohydrate, %	6.55	6.38	-0.17	-2.59
Fats, %	2.92	2.90	-0.002	-0.68
Energy, KJ	271.41	265.23	-6.18	-2.27
Protein energy/ Total Energy, KJ	52.02	52.7	0.68	1.30
Fat energy/Total energy, KJ	108.04	107.3	-0.74	-0.68
Calcium, mg	115	117	2	1.73
Zinc, mg	1.55	1.59	0.04	2.58
Potassium, mg	362	362	0.00	0.00
Phosphorus, mg	94	95	1	1.06
Iron, mg	9.4	9.6	0.2	2.17
Sodium, mg	13.8	14	0.2	1.44
Magnesium	10.5	10.81	0.31	2.95
Vitamin A, µg	53	54	1	1.88
Vitamin B9, µg	8	8.08	0.08	1



Vitamin C, mg	34	35	1	2.94
β-carotene, µg	85.8	86	0.2	0.23
Vitamin E, µg	29	29.01	0.01	0.03
Thiamine, µg	51.5	50	-1.5	-2.91
TPC, mg GAE/g	119	120.10	1.1	0.92
TFC, mg QCE/g	70	69.01	-0.04	-0.57
Phytic acid, mg				
	0.22	0.23	0.01	4.54
Molar ratio:				
Phytic acid: Fe	0.0020	0.0020	0.00	0.00
Phytic acid: Zn	0.0145	0.0145	0.00	0.00
Phytic acid: Ca	0.0001	0.0001	0.00	0.00
Ascorbic acid:Fe	1.162	1.162	0.00	0.00

<sup>1</sup>AD: Absolute Difference, 2RD: Relative Difference, TPC: Total Phenolic Content, GAE: Gallic Acid Equivalent, TFC: Total Flavonoids Content, QCE: Quercetin Equivalent

## Conclusion

The presented results and discussions show that affordable lactose-free yoghurt rich in essential nutrients and functional biological compounds can be processed from locally known ingredients other than costly animal sources in East Africa. With the aid of LP, the present study showed that the use of primary ingredients (broken rice, coconuts, and sesame oilseeds) is one of the possible cost-effective ways to reduce the high cost of importing raw materials from foreign countries, which is always incurred by food industries that manufacture nutritious and complementary foods and beverages. Therefore, the study recommends that researchers and food industries switch to the use of locally available commodities for food product development. Despite this achievement, clinical trials are needed to validate the efficacy of the developed ready-to-serve

PBY among individuals with lactose intolerance or who have milk allergies.

## Acknowledgements

The authors acknowledge the Inter-University Council for East Africa (IUCEA) for sponsoring the research at NM-AIST and Arusha Technical College (ATC) for collaboration and contribution while conducting this study.

## Funding

The authors have received no financial support for this article's research, authorship, and publication.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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