Effect of Lactic Acid Bacteria Starter Cultures on Vitamin and Oligosaccharide Composition of Milk Extracted from Three Common Bean (*Phaselous Vulgaris* L) Varieties

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Abstract

Fermented foods have in recent times attracted consumer interest mainly due to perceived health benefits of probiotic microorganisms. This study characterized changes in the concentrations of selected B-complex vitamins and oligosaccharides of common bean milk during fermentation by a common dairy starter culture, YF L-903 (*Streptococcus thermophilus* + *Lactobacillus Bulgaricus subs Debulgaricus*), and three probiotic cultures namely ABT (*Lactobacillus acidophilus La-5* + *Bifidobacterium animalis Bb-12* + *Streptococcus thermophilus*), Yoba (*Lactobacillus rhamnosus yoba* + *Streptococcus thermophilus*), and Yoba Fiti (*Lactobacillus rhamnosus yoba* + *Streptococcus thermophilus*), and Yoba Fiti (*Lactobacillus rhamnosus yoba* + *Streptococcus thermophilus*), fermentation with each of the mixed cultures caused significant increase in the vitamin B complex. Significant reductions (p<0.05) in the oligosaccharides sugars of 77.8% was found in milk from pinto bean variety fermented with ABT culture. These findings suggest that LAB probiotic cultures have a potential for improving biosynthesis of vitamins and removal of the verbascose, stachyose and raffinose oligosaccharides, thus making the product more digestible and the nutrients more bioavailable.

Keywords: common bean, bean milk, fermentation, vitamin biosynthesis

1. Introduction

In developing nations dietary deficiencies, especially in vitamins is reported to cause various health disorders (UNICEF, 2011). However, in developed nations consumers are concerned with their recommended dietary intake and usually use vitamins as supplements to promote health and prevent chronic diseases (Burgess, Smid, & Sinderen, 2009; Fortmann et al., 2013). Although the major role of food in the body is to provide adequate nutrients to meet daily metabolic requirements, recent findings suggest that food may regulate several functions beyond the predictable nutritional benefit (Stanton et al., 2001). Therefore, fermented foods, particularly those fermented with probiotic cultures have in recent times attracted the interest of the consumers, due to their perceived health benefits including bioavailability of nutrients such as vitamins (LeBlanc et al., 2010) and reduction or improved oligosaccharides digestion (Difo et al., 2015).

Vitamins are generally classified into two groups, the fat soluble vitamins (A, D, E and K) and the water soluble vitamins which include a series of B-vitamins, vitamin C and biotin (Burgess et al., 2009). The B-complex or B group vitamins is comprised of thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), biotin (H or B7), folate (M, B9 or B11) and cobalamin (B12) (Capozzi et al., 2012). Owing to their water solubility the B group vitamins play an important role in cellular metabolism of fats and protein (pyridoxine and riboflavin) and carbohydrates (thiamine), where they act as coenzymes, principally as carriers of a specific chemical group (Baku & Dickerson, 1996). These molecules are normally present in a number of foods, but can easily be destroyed or removed during food processing which potentially explains why their deficiency is rather common in a large population (Capozzi et al., 2012). Thus, food industries have been subjected by laws in the country of their operations to fortify certain foods with specific B-complex vitamins (LeBlanc et al., 2010).

Prokaryotes, including some lactic acid bacteria (LAB) utilize B-vitamins to meet their nutritional requirements (Snell, 1993). However, production of these vitamins by LAB has also been established (LeBlanc et al., 2011). This natural ability for vitamin B-complex biosynthesis by LAB has the potential to be utilized, either to harness the natural biosynthetic pathway of these microorganisms in fortification of fermented foods or to replace costly chemical synthesis of such foods (Burgess et al., 2009).

Oligosaccharides in common beans are about 31 to 76% of the total sugars and are known to cause flatulence and discomfort in the stomach (Campos et al., 2009; Campos et al., 2013). Traditional processing methods such as soaking and de-hulling followed with thermal treatment can eliminate most of these oligosaccharides in common bean but require a lot of energy, which is costly, particularly in developing countries (Nakitto, Muyonga, & Nakimbugwe, 2015). Little research has been done on the role of combined processing methods such as soaking, de-hulling, fermentation and steaming on nutritional quality and production of nutritious fast cooking common bean product (Nakitto et al., 2015), yet fermentation could sequestrate oligosaccharides (Kort et al., 2015). Therefore, this study was carried out to develop a milk product from beans and to characterize changes in the concentrations of the B-complex molecules and oligosaccharides sugars of common bean milk.

2. Materials and Methods

2.1 Bean Collection and Storage

Local varieties of dry pinto beans, red haricot beans and yellow kidney beans were procured from a trader in Nairobi County, Kenya. One packet containing 2 Kg of each of the bean variety was bought, wrapped in a kraft paper and transported to the food technology workshop in the School of Food and Nutritional Sciences of the Jomo Kenyatta University of Agriculture and Technology (JKUAT). The dry beans were stored in sealed plastic containers at room temperature (20 to 25 $^{\circ}$ C) until use.

2.2 Bean Milk Preparation and Fermentation

Bean milk was prepared using methods of Anino et al. (2019). Briefly, 100 g of common beans was rinsed and soaked in a 1 L of deionized water for 16 h at room temperature (23 °C). Water was drained off and the seeds dehulled by hand and ground in a blender (MBLR4314/WH, Mika, Dubai, UAE) for 3 min at 550W with 1L boiling water. The resulting slurry was filtered through 2 layers of muslin cloth to allow only water soluble common bean milk to pass through. The strained milk was heated in a heavy bottom pan to 100 °C for 20 min, stirring frequently to prevent sticking. The heat treated bean milk was placed at room temperature (20 to 25 °C) and left to cool for 2 hours and thereafter stored at 4 °C.

With regards to fermentation method used by Mani, Palou, & López (2014) was adopted with slight modifications. Briefly, LAB strains contained in a common dairy starter culture, YF L-903 (*Streptococcus thermophilus* + *Lactobacillus Bulgaricus subs Debulgaricus*), and three probiotic cultures namely ABT (*Lactobacillus acidophilus La-5* + *Bifidobacterium animalis Bb-12* + *Streptococcus thermophilus*), Yoba (*Lactobacillus rhamnosus yoba* + *Streptococcus thermophilus*), and Yoba Fiti (*Lactobacillus rhamnosus* GR1 + *Streptococcus thermophilus*) were used to ferment the bean milks. Each of the four starter cultures was prepared to yield equal amount of fermented milk for the three different bean milk varieties as follows. A 0.5 g of each of the starter culture was inoculated in 500 ml of raw milk. The inoculated bean milk was incubated at 45 °C in a Heratherm microbilogical incubator until a pH \leq 4.3 was attained. The fermented bean milk was placed at room temperature (20 to 25 °C) and left to cool for 2 hours and thereafter stored at 4 °C. Eppendorf tubes of 5 ml fermented milk were taken in triplicates to determine the concentration of B-vitamins (thiamine, riboflavin, niacin, pyridoxine and folic acid) and eppendorf tubes of 10 ml oligosaccharides (raffinose, stachyose and verbascose).

2.3 Determination of Thiamine, Riboflavin, Pyridoxine and Folic Acid

Extraction of thiamine, riboflavin, pyridoxine, niacin and folic acid was based on the modified methods of Chase et al. (1993); Ekinci & Kadakal (2005) and Kamman, Labuza, & Warthesen (1980). The extractions were carried out in triplicates by adding 20 ml of deionized water to 5 ml of bean milk (dilution factor, $F_{r} = 5$ ml). The mixture was homogenized using a homogenizer at medium speed for 1 min. The homogenized mixture was centrifuged for 15 min at 1500 rpm and Sep-Pak C₁₈ (500 mg) cartridges method of Cho et al. (Cho, Ko, & Cheong, 2000) was used to extract the water-soluble vitamins. The extracts were filtered through a 0.45 µm micropore membrane FP 30/45 CA-S filters (Schleicher and Schuell, Darmstadt, Germany). A 0.45 µm of the filtrate was injected with a syringe into HPLC column (20A Series, Shimadzu Co-operation, Kyoto, Japan) C₁₈ 150 mm x 4.6 mm with a flow rate of 0.1 mol L⁻¹ and KH₂PO₄ (PH 7.0)-methanol, 90:10, as mobile phase (0.7 mL min⁻¹) in isocratic mode. The vitamins were identified by comparing their retention times and UV-visible

spectra with those of standards stored in a data bank at 266 nm for riboflavin, 282 nm for folic acid, 234 nm for thiamine, 324 nm for pyridoxine and 261 nm for niacin.

2.4 Determination of Oligosaccharides

The methods of Brenes et al. (2003) and Campos et al. (2009) were modified to determine three forms of oligosaccharides; raffinose, stachyose and verbascose in triplicates. A10 ml sample of common bean milk was homogenized in aqueous ethanol (100 ml, 80%, v/v) and placed in a Soxhlet at 80°C for 60 min. The ethanol extracts was recovered, concentrated under vacuum, and the water phase frozen and lyophilized. A 7 mg sample of the extracted oligosaccharides was re-dissolved in 1 ml of deionized water, filtered and subjected to HPLC analysis. Standard curves were determined by injecting 20 μ l of raffinose, stachyose and verbascose standards into HPLC column (20A Series, Shimadzu Cooperation, Kyoto, Japan) connected to a refractive index detector fitted with a Zorbax NH₂ pre-column (4.6 x 12.6 mm, 5 μ m) and Zorbax column (250 x 4.6 mm). A 20 μ l of the extracted oligosaccharides was also injected into HPLC column to obtain peak areas. Water/acetonitrile (65:35) was used as mobile phase at 1 ml/min. Column and detector temperatures were maintained at 25 °C.

2.5 Statistical Analysis

All data were subjected to two way full factorial ANOVA using STATA/SE 12.0 software for windows to identify significant treatment effects. Comparison among means for different groups was made using Bonferroni least significant difference (LSD) test at $p \le 0.05$.

3. Results and Discussion

3.1 Vitamin Concentration of Fermented Bean Milk

Table 1. Vitamin concentration in milk extracted from red haricot (RH), yellow kidney (YK) and pinto (P) common bean varieties fermented with LAB probiotic starter cultures; ABT (*Lactobacillus acidophilus La-5* + *Bifidobacterium animalis Bb-12* + *Streptococcus thermophilus*), YF L-903 (*Streptococcus thermophilus* + *Lactobacillus Bulgaricus subs Debulgaricus*), Yoba (*Lactobacillus rhamnosus yoba* + *Streptococcus thermophilus*) and Yoba Fiti (*Lactobacillus rhamnosus GR1* + *Streptococcus thermophilus*)

	Thiamine (mg/100g)			Riboflavin (µg/100g)			Niacin (mg/100g)			Pyridoxine (mg/100g)			Folic acid (mg/100g)		
	RH	YK	Р	RH	YK	Р	RH	YK	Р	RH	YK	Р	RH	YK	Р
NF	0.2±0.0 ^a	0.2±0.0 ^a	0.2±0.0 ^a	$88.1{\pm}1.6^{\rm c}$	52.2±3.4 ^b	40.0±4.7 ^{ab}	0.5±0.0 ^a	0.4±0.0 ^a	0.5±0.1 ^a	0.1±0.0 ^a	0.1±0.0 ^a	0.1 ±0.0 ^a	0.3±0.0 ^a	$0.4\pm\!0.0^{b}$	0.4±0.0 ^{bc}
Yoba	ND	ND	ND	27.8±0.5 ^a	57.7±3.0 ^b	49.9±9.1 ^b	1.2±0.1 ^b	2.3±0.0°	1.3±0.0 ^b	0.3±0.0°	0.2 ± 0.0^{b}	0.5±0.0 ^e	0.4 ± 0.0^{b}	0.6±0.1 ^{cd}	0.6±0.1 ^{cd}
YF	ND	ND	ND	50.1 ± 0.2^{b}	60.1 ± 10.6^{b}	33.9±4.7 ^{ab}	3.7 ± 0.4^{d}	2.8±0.2 ^{cd}	3.0±0.4 ^{cd}	$0.2\pm\!0.0^{b}$	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	$0.5 \pm 0.0^{\circ}$	0.9±0.1 ^e	0.6±0.1 ^{cd}
L-903															
ABT	ND	ND	ND	433.0±63.7 ^d	$1121.9 \pm 15.9^{\rm f}$	670.9±26.1°	2.8±0.2 ^{cd}	2.2±0.1°	2.8±0.1 ^{cd}	$1.0\pm0.1^{\rm f}$	0.5 ± 0.1^{de}	$1.0\pm\!0.0^{\rm f}$	0.6 ± 0.0^{d}	0.9±0.1 ^e	0.7±0.1 ^{de}
Yoba	ND	ND	ND	23.8±0.6ª	27.8±1.1ª	21.2±0.1ª	3.6±0.3 ^d	2.4±0.4 ^c	3.0±0.8 ^{cd}	0.4 ± 0.0^d	0.3±0.0°	0.4 ± 0.1^{cd}	$0.5 \pm 0.0^{\circ}$	0.8±0.0 ^e	$0.5 \pm 0.0^{\circ}$
Fiti															
SE	0.01			8.8			0.26			0.01			0.01		
Р	< 0.01			< 0.01			< 0.01			< 0.01			< 0.01		

NB: Results are means \pm standard deviation (SD). Different superscript letters within the same column and row indicate statistical significance (Bonferroni, p<0.05); NF, non-fermented; ND, not detectable.

The effects of fermentation on vitamin concentration of common bean milk extracted from the three bean varieties are shown in Table 1. There were no significant inter-varietal differences in the contents of thiamine, niacin and pyridoxine in raw bean milk, while the differences in riboflavin and folic acid were significant (p<0.05). Thiamine was not quantifiable in bean milk fermented with any of the cultures. This is consistent with the results of (Granito et al., 2002) who observed notable losses in thiamine after natural fermentation of lentils and red beans.

The starter cultures had different effects on the riboflavin content of beans (Table 1) which is consistent with previous reports that LAB strains can produce or utilize individual vitamin B molecules, depending with the genome of the fermenting microorganism (Burgess et al., 2009). Fermentation with Yoba Fiti culture significantly reduced (p<0.05) riboflavin in milk extracted from the three bean varieties by 46.7% to 73%. An earlier study by Elmadfa et al. (2001) showed that most probiotic strains of lactobacilli consume riboflavin thereby decreasing its bioavailability. Additionally, riboflavin biosynthesis has been shown to occur when the four genes; ribG, ribB, ribA and ribH are present in the microbes genome (Bacher et al., 2015). However, absence of the ribG is previously reported for *L. rhamnosus* GR1, *L. rhamnosus yoba, L. bulgaricus* and *S. thermophilus* (Thakur et al., 2015; Valle et al., 2014). On the hand, the bean variety from which milk was extracted had great influence on the riboflavin concentration of the fermented milk. For example, fermentation

with Yoba significantly reduced riboflavin content in milk extracted from red haricot but not in milk extracted from the other two varieties, while fermentation with ABT caused great increases of this vitamin in milk extracted from all the varieties, especially in milk extracted from yellow kidney beans (>2000% increase). *Bifidobacterium animalis Bb-12* and *L. acidophilus La-5* which are the fermenting bacteria in the mixed probiotic ABT culture contain the four gene operons needed to catalyze biosynthesis of riboflavin (Thakur et al., 2015). Thus, appropriate selection of species and/or strains is essential in increasing riboflavin of fermented bean milk.

Fermentation caused significant increase (p<0.01) in niacin concentration of the milk extracted from the three bean varieties. The niacin values in milk extracted from red haricot beans fermented with Yoba and L-903 cultures increased from 0.5 ± 0.0 mg/100g to 1.2 ± 0.1 mg/100g and 3.7 ± 0.4 mg/100g respectively (Table 1). The highest increase in niacin concentration in milk extracted from the three bean varieties, an increase of 640% was found in milk extracted from red haricot beans fermented with YF-L903 culture. Increase in niacin values of cheese and yoghurt fermented with lactic acid producing bacteria was earlier reported (Gu & Li, 2016). These strains may be useful in enriching niacin composition of bean milk and could be exploited for other legumes.

With regards to pyridoxine the highest concentration was quantified in milk fermented with ABT culture (Table 1). Similarly, the highest increase of 900% was found in milk extracted from red haricot and pinto beans fermented with ABT cultures. A previous study by Vajaranant & Fields (1989) reported increase (p<0.05) in pyridoxine values of corn meal (0.52 ± 0.0 to 0.72 ± 0.1 mg/100g) fermented with different strains of *Bacilus licheniformis*. Similarly, fermentation of soy with different species and strains of *Streptococcus thermophilus, Lactobacilluis helveticus and Bifidobacterium longum* was previously reported to cause significant increases in pyridoxine concentration (Champagne et al., 2010). The biosynthesis of pyridoxine was previously reported to depend on the microbial ecological niche (Qaidi et al., 2013). This could imply that *L. acidophilus* and *B. animals* have got specific metabolic properties that make them more efficient in the biosynthesis of folic acid than other LAB (Table 1).

Milk extracted from yellow kidney beans and pinto beans had statistically higher (p<0.05) folic acid of 0.4 ± 0.0 mg/100g than those extracted from red haricot beans which contained 0.3 ± 0.0 mg/100g. Folic acid was significantly higher (p<0.05) in corresponding fermented bean milk than the non-fermented milk (Table 1). This agrees with earlier studies which reported increase in folic acid in milk fermented with *L. rhamnosus* (Hugenschmidt et al., 2010), *S. thermophilus* (Lai~no, LeBlanc, & Savoy, 2012), *L. acidophilus* (Lai~no et al., 2014), and *B. animalis* (Pompei et al., 2007). Milk extracted from yellow kidney beans exhibited relatively higher increase in folate than the other two bean varieties. Additionally, the highest increase in folic acid in each milk category was found in those fermented with ABT culture, an increase of (75 to 125%). *L. acidophilus* (DHPP) to folic acid biosynthesis precursor parabenzoic amino acid (pABA) (Gu & Li, 2016). However, *L. rhamnosus* and *S. thermophilus* use an alternative biosynthesis pathway (KEGG, 2014) which could be less efficient in the biosynthesis of folic acid (Table 1).

3.2 Oligosaccharides Concentration of Fermented Bean Milk

Table 2. Oligosaccharides concentration (mg/100g) in milk extracted from red haricot (RH), yellow kidney (YK) and pinto (P) common bean varieties fermented with LAB probiotic starter cultures; ABT (Lactobacillus acidophilus La-5 + Bifidobacterium animalis Bb-12 + Streptococcus thermophilus), YF L-903 (Streptococcus thermophilus + Lactobacillus Bulgaricus subs Debulgaricus), Yoba (Lactobacillus rhamnosus yoba + Streptococcus thermophilus) and Yoba Fiti (Lactobacillus rhamnosus GR1 + Streptococcus thermophilus)

	Raff	inose (mg/10	0g)	Verba	ascose (mg/1	.00g)	Stachyose (mg/100g)			
	RH	YK	Р	RH	YK	Р	RH	YK	Р	
NF	0.4 ± 0.0^{d}	0.4 ± 0.0^{d}	0.4 ± 0.0^{d}	0.2 ± 0.0^{b}	0.4 ± 0.0^{d}	$0.3 \pm 0.0^{\circ}$	3.4±0.1 ^g	4.2 ± 0.2^{h}	3.6±0.4 ^{gh}	
Yoba	0.2 ± 0.1^{abc}	0.2 ± 0.0^{b}	$0.3 \pm 0.0^{\circ}$	0.1 ± 0.0^{a}	$0.1\pm\!\!0.0^{a}$	0.1 ± 0.0^{a}	1.0 ± 0.0^{b}	1.3 ± 0.1^{bcd}	2.3 ± 0.4^{ef}	
YF L-903	0.2 ± 0.0^{ab}	0.2 ± 0.1^{abc}	0.2 ± 0.0^{b}	0.2 ± 0.1^{abc}	$0.1\pm\!\!0.1^{ab}$	0.1 ± 0.0^{a}	2.6 ± 0.4^{f}	1.6±0.1 ^{de}	1.1±0.2 ^{acd}	
ABT	0.1 ± 0.1^{ab}	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	$0.1\pm\!0.0^{a}$	$0.1\pm\!\!0.0^{a}$	$0.1\pm\!0.0^{a}$	0.9 ± 0.2^{ab}	1.0±0.1 ^{ab}	$0.8\pm\!0.0^{a}$	
Yoba Fiti	0.2 ± 0.0^{ab}	0.2 ± 0.1^{abc}	0.2 ± 0.0^{b}	$0.1\pm\!0.0^{a}$	0.1±0.1 ^a	$0.1\pm\!0.0^{a}$	1.6 ± 0.2^{bcde}	1.1±0.1 ^{abc}	1.0±0.1 ^{ab}	
SE		0.02			0.02			0.12		
Р		< 0.01			< 0.01			< 0.01		

NB: Results are means \pm SD. Different superscript letters within the same column and row indicate statistical significance (Bonferroni, p<0.05); NF, non-fermented.

There are various types of oligosaccharide sugars, including raffinose, verbascose and stachyose. These sugars

are known to cause flatulence and are partly the reason for low consumption of common beans and its associated products (Paredes & Harry, 1989). Table 2 shows the effects of fermentation on oligosaccharides concentration of common bean milk extracted from three bean varieties. A narrow range of values have been reported for raffinose in legumes. For instance, Difo et al. (2015) found that *racemosa* seeds contained 1.9±0.0 mg/100g of raffinose sugars while Akinyele & Akinlosotu (1991) reported concentration of 2.0±0.0 mg/100g in cowpeas (*Vigna unguiculata*). Thus, the concentration of 0.4 ± 0.0 mg/100g contained in non-fermented bean milk was far much lower than the previously reported values for most legumes. Similar to the results of Da et al., (2006) stachyose (3.4 ± 0.1 to 4.2 ± 0.2 mg/100g) was the most abundant oligosaccharide sugar in non-fermented bean milk (Table 2). The highest reduction in raffinose concentration (75%) was recorded in pinto bean milk fermented with ABT culture. A previous study by Granito & Alvarez (2006) reported similar results when black beans varieties were fermented with lactic acid bacteria. The reduction in raffinose could be attributed to the utilization of the oligosaccharides for energy by the microorganisms. The current finding is of great interest as it suggests that fermentation could be used to reduce flatulence causing raffinose.

Fermentation with Yoba, ABT and Yoba Fiti cultures caused significant decreases (p<0.05) in verbascose concentration of the milk extracted from red haricot beans (p>0.05) (Table 2). Fermented milk extracted from yellow kidney and pinto beans were also found to contain statistically lower (p<0.05) verbascose values on average (0.1±0.0 mg/100g) than non-fermented milk (0.4±0.0 mg/100g) and (0.3±0.0 mg/100g) respectively, a 75% reduction in verbascose concentration in milk extracted from yellow kidney beans. This agrees with earlier reports which had shown reduction in verbascose values of common beans upon fermentation (Starzynska, Bozena, & Mickowska, 2014). LAB contains α galactosidase enzyme which potentially enables them to utilize verbascose sugars. However, there was variation in the utilization rate of verbascose (Table 2) among the fermenting LAB which could be due to differences in the expression of the α -galactosidase enzyme.

Fermentation triggered significant reduction (p<0.05) in stachyose values for the three bean milk, with the highest reduction of 77.8% observed in milk extracted from pinto bean variety fermented with ABT culture. Stachyose could have been hydrolyzed by α -galactosidase into sucrose and galactose, and the latter metabolized through the galactose-utilization system (Da et al., 2006). Additionally, significantly higher (p<0.05) stachyose value was found in milk extracted from red haricot beans fermented with YF L903. This could be an indication that the ability of fermenting microorganisms to hydrolyze bonds in oligosaccharide moieties is dependent on enzymatic properties of the bacterial strain and the efficiencies of the α -galactosidase activity of that particular strain. Thus, appropriate selection of the fermenting culture is a necessity in reducing stachyose in fermented bean milk.

4. Conclusion

Fermentation with each of the four cultures increased pyridoxine, niacin and folic acid concentrations of the three bean milks. However, thiamine was non-quantifiable in fermented milks while riboflavin values were lowered for all the fermenting cultures, except ABT culture. This implies that combination of probiotic strains of *Lactobacillus acidophilus La-5* + *Bifidobacterium animalis Bb-12* + *Streptococcus thermophilus* could be exploited for natural fortification of riboflavin in bean milk. It was also observed that fermentation significantly lowered the oligosaccharide compositions of stachyose, raffinose and verbascose. Thus, fermentation of bean milk with any of the four cultures could be utilized for removal of the flatulence causing oligosaccharides.

Authors' contribution

CA and AO participated in bean milk development and experimentations. SI and JM participated in conceptualization of the research design and performing the experiments. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Availability of data and materials

All the data and material supporting the conclusion of this work are included within the manuscript. Additional information may be provided on request by the corresponding author.

Consent for publication

All authors have agreed to submit the manuscript in its current form for publication in the Journal of Food Research.

Ethical approval and consent to participate

Not applicable. No tests, measurements or experiments were performed on humans as part of this work.

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