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Primary Characterization of a Novel Soymilk-Cashew Fermented with an Improving of Its Antioxidant and Anti-Inflammatory Contents

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Abstract

Cashew nut (*Anacardium occidental*) milk was included as a functional ingredient in soybean (*Glycine max* L.) milk at 20/80% and was fermented comparatively to commercial yogurt starters by two strains TC6 and A4 (recently isolated from two Ivorian highly flavored neglected traditional staple foods (beer and garlic)) identified as *Weissella paramesenteroides* and *Enterococcus faecalis*, after the screening of their probiotic, antioxydant and anti-inflammatory potentialities, respectively. The primary characterization of these plant-milks showed a strong improvement in their sensory, antioxidant and anti-inflammatory abilities. While TC6 from traditional beer Tchapalo increases anti-inflammatory activity by almost eighteen times, A4 isolated from garlic increases antioxidant activity by two times. Due to their aromatic and organosensory potentialities certainly, milks L138, L180 and 130 fermented respectively by the combination of TC6 and A4, TC4 alone and A4 alone were the most appreciated than the plant-milk fermented by the two strains of commercial yogurt. These probiotics, anti-oxidant and anti-inflammatory plant-milks should be further characterized in terms of their biological activities and tested in animal models to confirm their nutrition-health claims.

Keywords

Probiotic, Staple Food, Soy-Milk, Anti-Oxydant, Anti-Inflammatory

1. Introduction

Overeating due to the nutritional transition in sweet, salty, fatty and high-calorie foods, including drinks, causes in Côte d'Ivoire a high prevalence of metabolic diseases. In addition, in a fragile COVID sanitary context, the world market for "healthy" plant foods, including plant milks (fermented or not) and/or enriched with different natural plant-based ingredients is constantly increasing around the world [1]. Also, following the recommendations in favor of the consumption of plants (source of prebiotics favorable to the intestinal flora) of the FAO/WHO and in favor of the reduction of the consumption of meat and animal products and in the face of the controversy over milk of animal origin, functional soy milks enriched with cashew nut, fermented by potential probiotic lactic acid bacteria with health potential (antioxidant and/or anti-inflammatory) would be a promoter nutritional solution for a sustainable diet. Soy (*Glycine max*) is rich in high-quality protein (40% - 50%), carbohydrates (25% - 30%), lipids (20% - 30%), dietary fiber (15%) and micronutrients (vitamins, minerals) [2]. Soy milk is the most studied and popularized plant-milk in the world. Qualified as a functional food, it has high potential in the development of new beverages, due to its chemical and nutritional characteristics. Of a total of 36 plant-based milks (almond, coconut, hemp, oat, rice, spelt …), soy milk is the only plant-milk that would be comparable nutritionally to cow's milk [3]. Although, soymilk has a lower fat content and contains no cholesterol comparatively to cow milk, only the soy-based beverages reached similar protein amounts but had lower protein quality than cow milk. [4]. However, plant-drinks cannot be considered nutritionally equivalent to cow's milk in the long term without adjustments (enrichment, supplementation, etc.) to fully meet nutritional needs, taking into account digestibility, absorption and bioavailability of bioactive compounds (minerals, vitamins, etc.) [3]. Pure and expensive functional ingredients such as inulin and plants (fruits, vegetables, algae, spices, spices, seaweed, etc.) are therefore added to plant milks to improve their nutritional value [3]. The cashew nut (*Anacardium occidental*) has the most interesting vitamins and minerals and active ingredients giving it health benefits. Along with pistachios, they are the nuts with the lowest lipid content with almost 80% unsaturation, maintaining healthy cholesterol levels. They are also rich in tocopherols and phytosterols. Cashew nut milk, is low in calories and sugar, and has tryptophan (essential amino acid) precursor of well-being hormones. In view of its high protein content (18%) and quality, cashew nuts could be an ideal supplement to soy milk. As fermentation makes it possible to improve the nutritional value (including the digestibility, absorption and bioavailability of bioactive compounds) of foods, it is also used to improve nutritional quality of plant-milks. In this sense, probiotics are of great interest to the point of giving soy milk the ability to replace cow and buffalo milks as therapeutic foods in the future [5]. Probiotics give to soy-milk symbiotic aptitude because it has both prebiotic and probiotic activities and would therefore be functionally more interesting than unfermented soy milk [5]. Probiotics have indeed an increasingly growing health interest to the point that they are associated in synergy with existing conventional treatments in the search for new medical avenues against metabolic diseases due to their ability to restore the affected intestinal microbiota. The probiotic abilities of a microorganism are linked, among other things, to its ability to grow and survive in the hostile conditions of the gastrointestinal tract and to adapt by colonizing it (e.g., resistance to acid pH and bile salts). Beneficial characteristics are attributed to probiotic strains among which their anti-inflammatory and anti-oxidant effects. Currently, there is a great interest in the isolation of new probiotic LAB strains from unconventional sources, such as fermented staple foods [6] [7] [8]. Knowing that fermented garlic has important virtues against metabolic diseases [9], it could be an interesting source of isolation of anti-inflammatory and/or antioxidant lactic acid bacteria. Tchapalo or traditional African sorghum beer is rich in lactic bacteria probiotics [10] [11]. The use of new lactic bacteria from staple foods as starters would contribute to new innovations in the agri-food sector. Thus, this work leads to formulate a functional fermented plant-milk of high nutritional quality of soy milk enriched in cashew.

2. Materials and Methods

2.1. Microbial Strains Used in This Study

Two LAB of our Laboratory microbial collection, TC6 (isolated from the traditional sorghum fermented beer "tchapalo") and A4 (isolated from fermented gallic) and later identified as *Weissella paramesenteroides and Enterococcus faecalis*, respectively by their ARN 16S sequencing (article accepted for publication) were selected as starters comparatively to two LAB starters of a yogurt marketed in Côte d'Ivoire.

2.2. Screening of Technological Properties of TC6 and A4

For probioctic properties, the acidity resistance (for 3 hours in MRS broth adjusted to pH 2 using hydrochloric acid according to Hyronimus *et al.* (2000) [12], bile salt resistances (in MRS broth supplemented with 0.3% of a mixture of bile salts (Oxgall Powder B-3883 Sigma)) and Cell surface hydrophobicity aptitude) of the strains were screened [13]. Diluted pellet $OD_{600nm} = 0.5$ (after centrifugation at 8000 rpm for 15 min and washed twice with PBS (pH 7.4)) of 18 hours cultures (in MRS broth of each strain) were used. After acidity and bile salt resistance tests, successive dilutions of tested microbial suspension were done to inoculate MRS agar which was then incubated at 37˚C for 48 and the survival rate was evaluated comparatively to their controls. Adhesion of each strain to xylene (as hydrophobic substance) was used to screen their hydrophobicity aptitude ([1]). One milliliter of each pellet ($OD_{600nm} = 0.5$) was resuspended in PBS and the OD_{600nm} value (A_0) was determined. An egual proportion of xylene was again added and the mixture was then vortexed for 5 minute, incubated at 37˚C for 1 hour. The OD_{600nm} of organic matter (A_1) was read. The Bacterial Adhesion to Solvents (BATS) was calculated as follows: BATH% = $[(A_0 - A_1)/A_0] \times 100$.

Exopolysaccharides (EPS) synthesis by each strain was screened on MRS agar containing 40 g/l sucrose addition [14]. The 18 h bacterial pellet was inoculated on the Petri dishes by streaking and incubated at 37˚C for 48 h. EPS producers strains showed a slimy appearance with large, sticky colonies [14]. Antimicrobial activity of each strain against pathogens (*Staphylococcus*, *Pseudomonas*, *Escherichia coli*, *Salmonella*, *Aspergillus*) was aseptically done by the well (6 mm wells done by a Pasteur pipette) diffusion method [15] on MRS agar for 18 h at 37˚C. After solidification of the MRS agar containing the pathogens, each well was filled with 10 µl of every tested strains (OD_{600nm} = 0.5) and was refrigerated at 4˚C for 2 hours to allow a good diffusion of the substance. Inhibition zones diameters were measured at 37˚C after 24 h of incubation. Anti-inflammatory aptitude of each strain was done by its culture supernatant against bovine serum albumin (BSA) as protein denaturation inhibition method [16]. The reaction mixture (0.5 mL) consisted of 0.45 mL of BSA (5% aqueous solution) and 0.05 mL of each strain culture supernatant. The pH was adjusted to 6.3 using hydrochloric acid (1N) and samples were incubated at 37˚C for 20 min, then heated to 57˚C for 3 min. After cooling, 2.5 mL of phosphate buffer solution (pH = 6.3) was added. Absorbance was measured at 614 nm. For the control, distilled water replaced the microbial supernatant. Inhibition of protein denaturation (%) was calculated as follows:

$$\text{Inhibition (\%)} = 100 - [(\text{OD Sample} - \text{OD Control}/\text{OD Control})] \times 100$$

Antioxidant activity of every strain was obtained by the DPPH (2,2-diphenyl-1-picryl hydrazyl (DPPH•) scavenging test. One (1) ml of 48 h bacterial culture supernatants was added in 2 ml of ethanol (70%) and centrifuged (6000 rpm for 10 min) and the supernatant collected. 50 µL was added to 1950 µL of DPPH (0.6 mg/mL). The negative control was prepared by mixing 50 µL of 70% ethanol with 1950 µL of DPPH at the same concentration. After incubation in the dark for 30 min, OD_{517nm} was read and the percentage of DPPH decolorization was determined by the formula below: I (%) = ((OD control − OD sample)/OD control) × 100.

For the fermentative type of every strain, the presence or absence of gas in the Durham bell after 48 h of incubation at 37˚C in MRS broth indicates the heterofermentative or homofermentative metabolism. Sugars fermentation profile of each strain was studied by replaced dextrose by the tested sugar in MRS agar supplemented with bromocresol purple at 0.04% (pH indicator) at 37˚C for 48 h. Change from purple to yellow of the pH indicator due to the acidification of the medium reflects the fermentation of the tested sugar.

The enzymatic profile of every strain was studied on Modified MRS agars containing 2% of a sole carbon source (soluble starch, cellulose, pectin, tannic and phytic acids) which were sterilized, cooled at 50˚C and poured into Petri dishes. After solidification, they were inoculated per well by the microbial colonies, then incubated at 30˚C for 48 h. According to the tested substrate, enzymatic synthesis was revealed by the appearance of a clear halo around the wells.

2.3. Production of Fermented Plant Milk of Soya/Cashew

After buying at Gouro Market (Abidjan), soybeans and cashew nuts were separately used to extraction of their milks according to Marazza *et al.* (2012) [17] and Adedokun *et al.* (2013) [18], respectively. For soy (*Glycine max* L.) milk production, whole soybeans were washed and soaked in distilled water for 48 h, then washed in potable water to remove foreign matter that could influence milk taste. The swollen soybeans were dehulled manually and ground with water (1 kg per 5 L of water, *i.e.* a 1:5 w/v ratio). Then, the suspension was pressed using a muslin cloth to extract the milk. Cashew milk was chosen to more easily optimize the solubilization of compounds extracted from cashew nuts in soy milk. For cashew milk obtention, 450 g of cashew nuts were cleaned to remove undesirable and soaked in 250 mL of drinking water for 6 hours. After, the water was drained and the soaked nuts were crushed (using a blender) by mixing with tap water at a ratio of 1:6. The slurry was pressed with a muslin cloth to extract the milk. The soya and cashew milks were then mixed at a ratio of 80/20, homogenized to obtain the composite milk in sterile jars for pasteurization (82˚C for 10 min) before the controlled fermentation at room temperature by inoculation with 10^7 CFU/g of viable colonies of the starters.

2.4. Physico-Chemical Characterization of the Milks

pH determination was done with 10 mL of every milk [19]. For titratable acidity, 10 ml of each sample were titrated using a sodium hydroxide solution (NaOH, 0.1 N) with two drops of phenolphthalein. Dry matter and ashes content were evaluated by AOAC (1990) [20] method. The fat content was determined with 10 ml of every sample by the Soxhlet extraction method with 300 ml of hexane according to AFNOR (1982) [21] for 7 hours. After, the solvent was recovered using a HEIDOLPH brand rotary evaporator. The flask (initially tared) containing the fat was weighed to determine the mass of the extracted fat. The reducing sugars content of the milks was obtained with the DNS Bernfeld method (1955) [22] using a dextrose (1 mg/mL) standard curve at 540 nm. Proteins content was determined by the colorimetric assay of Bradford (1976) [23] at 595 nm with a standard curve from bovine serum albumin solution. Antioxidant (on 1 ml sample) and anti-inflammatory activities (on 0.05 mf sample) of the milks were obtained by the DPPH (2,2-diphenyl-1-picryhydrazyl) and the bovine serum albumin protein denaturation inhibition method according to Huang *et al.* (2005) [24] and Anoop *et al.* (2015) [16], respectively.

2.5. Microbiological Analysis of the Plant-Milks

Stock solutions (10^{-1} dilution) were aseptically prepared by 10 g of milk which were homogenized with 90 mL of buffered peptone water. Then, after 10^{-2} dilution (one (1) mL of each stock sterilely mixed with 9 mL Tryptone-salt (Bio-Rad)à, several successive decimal dilutions were done to 10^{-10} dilution. Enumeration of Total Mesophilic Aerobic Flora Count was performed by ISO 4833-1 (2013)

[25] method on Plate Count and, total coliforms count was obtained on lactose bile agar with crystal violet and neutral red (VRBL) according to ISO 4832 (2006) [26]. Lactic acid bacteria were enumerated on MRS agar (de Man, Rogosa, Sharpe) according to ISO 15214 (1998) [27]. *Bacillus* sp. counts were done on nutrient agar. The inoculation consisted of spreading 0.1 mL of the stock solution and its decimal dilutions on the nutrient agar pre-poured in the Petri dishes after solidification. After 24 h incubation at 30˚C, typical colonies (large, smooth or rough) were counted as *Bacillus* species. Yeasts enumeration was carried out on Sabouraud chloramphenicol agar according to ISO 21527-1 (2008) [28].

2.6. Sensory Analysis of Milks

Hedonic test based on the "pleasure" dimension and the personal feelings of the panelists was carried out according to the ISO 8587 (2006) [29] standard. A tasting, followed by an evaluation of the milk were done by 50 untrained or naive panelists. These panelists were selected to correspond to the product's target and were placed in close conditions. Evaluation of the milk samples was done by assigning them marks on a structured rating scale of 5 levels (from very unpleasant = 1 to very pleasant = 5), expressing the general impression of their preference. Finally, the samples were ranked according to their preference (from the most appreciated to the least appreciated). Descriptive analysis of milks were done by ISO 13299 (2016) [30] standard with 10 panelists which were trained on the basis of the descriptors (sour taste, sweet taste, smell, aroma, shine and color of milk) in order to familiarize themselves with the use of a rating scale, to assess the intensities of these sensory attributes. The intensities of the attributes, on the structured intensity scale of 1 to 9 points, were evaluated by the panel.

2.7. Statistical Analyzes

The tests were carried out in triplicate. The results were expressed as mean ± standard deviation. A one-factor analysis of variance (ANOVA) of the means obtained was carried out using the XLSTAT 2014 software and Duncan's test at the probability threshold ($\alpha = 0.05$) was used.

3. Results and Discussion

It has shown that the traditional sorghum beer Tchapalo contained probiotic lactic bacteria of the different species which would be good candidates as starter cultures [10]. Also, the probiotic, anti-oxidant and anti-inflammatory properties of microorganisms of interest from fermented garlic have been documented [9]. In these studies, the authors concluded that these lactic acid bacteria could be validly used as starters of fermented plant-based foods. Thus, two *Lactobacillus plantarum* strains TC6 and A4 isolated from these two Ivorian staple foods of our laboratory microbial collection were screened for their probiotic, anti-inflammatory and anti-oxidant properties comparatively to two lactic bacteria starters of an international brand of yoghurt marketed in Côte d'Ivoire. The two strains TC6 and A4 exhibited excellent resistance to pH 2 and bile salt as well as excellent hydrophobicity greater than 50%, respectively (**Table 1**). These criteria constitute the first bases for a probiotic microorganism to ensure its survival through the stomach and intestine, respectively. The resistance to this drastic living condition with high survival rates (more than 50%) suggests that TC6 and A4 were able to cross stomach acidity and reach the intestinal environment to multiply there. The denaturation of tissue proteins is a well-documented cause of inflammation which may be at the origin of the production of autoantigens in certain diseases (arthritic, rheumatoid …). Moreover, the ability to trap the free radical DPPH reflects the presence of antioxidant activity. So, the two strains significantly presented high anti-inflammatory (31.34% ± 2.22% for TC6 1 and 44.25% ± 2.25% for A4) and anti-oxydant activities (19.38% for TC6 and 23.11% for A4), respectively. Probiotic *Limosilactobacillus reuteri FEEL 6901* with the abilty to synthetyse anti-inflammatory substances was considered by its intact cells to have an excellent antioxidant activity with 21.6% DPPH inhibition rate (higher than that of WCFS1 (10.6%) or LGG (17.1%)) [7]. Also, the two strains presented exopolysaccharides (new source of cancer treatment) synthesis ability by their sticky and shiny colonies forms on MRS sucrose (40 g/l) agar. Compared to YB and YC with low antioxidant and anti-inflammatory activities on MRS, these characteristics led to use TC6 and A4 as probiotic starters for plant-milk fermentation, an emerging food whose market is constantly increasing due to the high demand for innovative health products including the addition of pure but expensive functional ingredients such inulin. Also, the antibacterial activity of a probiotic is essential against intestinal pathogenic bacteria. TC6 and A4 inhibited most of the indicator pathogen strains with good inhibition against *Salmonella* (27.00 ± 1.15 - 24.00 ± 2.32 mm inhibition diameter) while *Staphylococcus* exhibited the weakest inhibition (12.00 ± 0.45 - 12.00 ± 1.66 mm) diameter) (**Table 2**). These inhibitions could have several origins among which the production of antimicrobial compounds such as organic acids (lactic acid or acetic acid). In addition, the fermentation of soy milk by probiotic lactic acid bacteria gives it antimicrobial activities which were absent in unfermented milk [5]. The two strains TC6 and A4 fermented also several sugars including raffinose (an indigestible sugar) into lactic acid to prevent indigestion and stomach bloating. Indeed, in humans, these sugars are metabolized by microorganisms in

Table 1. Morphological and healthier activities of the starters used in this work.

Strains	Survival (%) at pH 2	Survival (%) at 0.3% Bile salts	Hydropho bicity (%)	Exopolysaccahari-desynthesis	Anti-oxydant activity (%)	Anti-inflammatory activity (%)
TC6	66.19 ± 0.41b	71.10 ± 0.50a	76.42 ± 0.15a	+	19.38b	31.34 ± 2.22a
A4	65.33 ± 0.39b	66.22 ± 0.44a	71.11 ± 0.02a	+	23.11b	44.25 ± 2.25b
YB	7.57 ± 0.15a	76.34 ± 0.11a	66.56 ± 0.01a	+	9.62a	0
YC	5.52 ± 0.51a	68.43 ± 0.17a	71.63 ± 0.07a	+	7.31a	0

Table 2. Characteristics of the two starters to environmental conditions.

Environmental conditions	TC6	A4
Pathogens (Inhibition diameter)		
Staphyloccocus	12.00 ± 0.45a	12.00 ± 1.66a
Pseudomona	17.00 ± 0.63a	14.00 ± 0.77a
E. coli	15.00 ± 0.31a	14.00 ± 2.10a
Samonella	27.00 ± 1.15b	24.00 ± 2.32b
Sugar (20 g/l) fermentation		
Tréhalose	+	+
Erythritol	–	–
Galactose	+	+
Saccaharose	+	+
Raffinose	+	+
Glucose	+	+
Maltose	+	+
Fructose	+	+
Sorbitol	–	–
Lactose	+	+
Soluble starch	–	–
Arabitol	–	–
Xylitol	–	–
Synthetysed enzymes		
Tannase	°	–
Amylase	+	+
Phytase	–	+
Cellulase	°	+
Produced titratable acidity on MRS		
	1.77 ± 0.18a	1.68 ± 0.42a

the large intestine, liberating huge amounts of gas, which can then cause gastrointestinal disorders. Also, their acidification ability after 48 hours would lead to decrease their environment pH in order to extend the lag phase of sensitive organisms including foodborne pathogens. In addition to these characteristics, they also synthesize interesting hydrolytic enzymes which are necessary for fermentation. Cellulase and amylase contribute to the softening of foods while tannase and phytase synthesis is useful to eliminate anti-nutritional factors during the fermentation process. In regarding to such interesting technological properties, these two isolates were used in further steps to ferment the plant milk of soybean/cashew nuts. Comparatively to the two strains of an international commercial yogourt which are certainly *Lactobacillus bulgaricus* (YB) and *Strepto-*

coccus thermophilus (YC), five composite plant milks of soya enriched by cashew have been developed. Soybeans contribute in Côte d'Ivoire modestly to the income of traders who engage in its marketing, while cashew is essentially exported without any form of processing. For this, the juice extracted from soybeans was supplemented with the juice extracted from cashew nuts in a ratio of 80/20. Table 3 presents the physico-chemical characteristics of the different plant milks.

Physico-chemical characterization of the five milks showed a slight drop in pH of the fermented milks between 4.8 ± 0.20 (naturally fermented milk control) to 3.3 ± 0.20 (milk L110 fermented with the two strains of commercial yogurt) and an increase in the titratable acidity from 0.59% ± 0.02% (naturally fermented control) to 0.99% ± 0.12% (milk L110) (Table 3). These results confirm the adhesional aptitude of TC6 and A4 in these matrices (pH ≤ 4) and so to tolerate and adapt with ability to survive. If the co-culture of the two commercial yogurt starters induced the lowest pH values and highest titratable acidities, the pH and titratable acidity values obtained with TC6 and A4 and their variations are however lower compared to those of soy milk-camel milk yogurt (4.05 ± 0.06) as well as plain soy milk yogurt (4.35 ± 0.02) and Soy-corn-yoghurt (4.50 - 4.80) obtained by Amal B. Shori (2013) [31] and Ibrahim *et al.* (2019) [32], respectively. The increase in titratable acidity could be due to the synthesis of organic acids, mainly lactic and acetic acids. These low pH values constitute foods safety factors against pathogens [33]. Except ashes, the fat, reducing sugar and protein contents of the five plant-milks are higher in the naturally fermented sample (control) than in the samples obtained by controlled fermentation. Also, while the naturally fermented sample (control milk) has very low anti-inflammatory and antioxidant activities (2.60% ± 0.00% and 4.03% ± 0.02%), the others four milks (L110, L130, L180 and L138) obtained by controlled fermentation, varied from a milk to another depending on the starter used. While A4 provides in L180 antioxidant activity (8.31% ± 0.37%) similar to that of the two yogurt strains in L110 (9.37% ± 0.29%), TC6 provides in L130 the highest content of anti-inflammatory substances (74.91% ± 2.53%) followed by A4 in L180 (34.80%

Table 3. Physico-chemical parameters of the plant-milks.

Fermented plant-milks	pH	TA (%)	Ashes (%)	Fat (%)	Reducing sugars (mg/ml)	Protein (mg/ml)	Anti-oxidant activity (%)	Anti-inflammatory activity (%)
Control	4.80 ± 0.20^a	0.59 ± 0.02^b	2.00 ± 0.00^a	39.75 ± 0.35^a	4.26 ± 0.00^a	12.81 ± 0.02^a	4.03 ± 0.02^a	2.60 ± 0.00^a
L110	3.3 ± 0.20^b	0.99 ± 0.12^a	2.25 ± 0.25^a	35.50 ± 3.75^a	1.15 ± 0.01^a	11.12 ± 0.02^a	9.37 ± 0.29^a	5.09 ± 0.00^a
L130	3.7 ± 0.10^b	0.70 ± 0.06^b	2.25 ± 0.35^a	36.75 ± 1.76^a	1.47 ± 0.01^a	11.33 ± 0.00^a	4.22 ± 0.01^a	74.91 ± 2.53^a
L180	3.9 ± 0.20^{bc}	0.66 ± 0.03^b	2.25 ± 0.00^a	38.25 ± 3.18^a	3.05 ± 0.34^a	12.17 ± 0.04^a	8.31 ± 0.37^a	34.80 ± 0.28^a
L138	3.83 ± 0.15^c	0.81 ± 0.10^a	2.50 ± 0.00^a	34.50 ± 0.70^a	3.18 ± 0.01^a	6.43 ± 0.04^a	4.72 ± 0.24^a	22.53 ± 0.25^a

Control: naturally fermented milk, L110: milk fermented with yoghurt strains; L130: milk fermented with tchapalo strain TC6; L180: milk fermented with garlic strain A4; L138: milk fermented with mixed strains (TC6 + A4); TA: titratable acidity; NB: In a column, the average values followed by an alphabetical letter differ are statistically different with a threshold $\alpha = 0.05$.

± 0.28%) and the combination of TC6 and A4 in L138 (22.53% ± 0.25%). In a recent study, anti-oxidant activities (% DPPH inhibition) of soy milk after fermentation by probiotic lactic bacteria was 80.0% ± 0.10% against 1.00% ± 0.0% in the non-fermented sample [5]. In addition to increased anti-oxidant activities, controlled fermentation also gives soy milk anti-inflammatory activities to make it one of the natural food promoters with nutrition-health claims [34]. *Streptococcus salivarius subsp. thermophilus* CCRC 14805 and/or *B. infantis CCRC 14603*-fermented soymilk exhibited anti-inflammatory activity *in vitro* [35]. *L. plantarum* fermented soymilk exhibited an anti-inflammatory activity in LPS-induced macrophages [36].

Microbiological analysis showed absence of Total aerobic mesophilic flora (TAMF), total coliforms, *Bacillus* and yeast in these milks and confirmed that these milks were hygienic and suitable for human consumption without risks (**Table 4**). To assure the health benefits of fermented plant-based milk products, probiotics should meet the minimum level requirement for probiotic bacteria between 10^6 and 10^7 cfu/ml until the expiry date [37]. This finding was reconfirmed in the present study. Viability reached more than 10^6 cfu/ml. The genus *Enterococcus* whose species *faecalis* are constantly used as a starter for the fermentation of soy milk [38] [39]. Moreover, recent studies show that *Weissela* strains can be safely used for the fermentation of dairy products [40].

Sensory evaluation is decisive in the quality control and marketing of food products; thus, it is essential for assessing the development of novel food products [41]. The characteristics (texture, taste, flavor, and overall acceptability) of the the four-promoter fermented plant-milks were evaluated. These plant-milks were affected by fermentation but they do not show too many significant differences with respect to their sensory profiles. The radar presented by the sensory profile reveals the characteristics of the milks according to the different descriptors. On a scale of 1 to 9, the more one tends towards 9, the milk presents the characteristic given by the descriptor. Let's take the example of L138 which was the best appreciated, according to the radar it is beige in color with a slight smell of milk compared to other milks, less astringent, sweeter, less bitter, but with a good aroma compared to milk L110 less popular (**Figure 1**). In terms of sensory profiles, the milk obtained with the mixed starter L138 (A4 + TC6) presented the best characteristics according to the descriptors followed by the L130 milk (inoculated with the TC6 starter of tchapalo). The fermentation profile of these strains showed that they were both capable of degrading the same sugars, so the combination of these two isolates would be complementary with regard to their respective technological properties. Thus, overall, this milk obtained by the combination of the two strains of TC6 and A4 (milk L 138) which also has both antioxidant (4.72 ± 0.24) and anti-inflammatory (22.53% ± 0.25%) quite interesting could be popularized in the fight against diseases and metabolic disorders related to oxidative stress. However, milk L180 (fermented with the A4 strain of garlic which has the best antioxidant (8.31% ± 0.37%) and anti-inflammatory

Table 4. Microbiological characteristics of the plant milks.

Plant-milks	Lactic bacteria (UFC/g)	*Bacillus* (UFC/g)	Yeasts (UFC/g)	TAMF (UFC/g)	Total coliforms (UFC/g)
Témoin	0	0	0	$3.61.10^4 \pm 1.33$b	0
L110	$2.94.10^7 \pm 1.35$a	0	0	$1.8.10^5 \pm 1.20$a	0
L130	$3.70.10^7 \pm 2.44$a	0	0	$7.10^4 \pm 1.30$c	0
L180	$2.13.10^7 \pm 1.80$a	0	0	$7.10^4 \pm 0.80$c	0
L138	$3.49.10^8 \pm 2.63$a	0	0	$1.05.10^4 \pm 1.20$a	0

Table 5. Averages and ranks of plant-milks.

Plant-milks	Average scores	Ranks
L110	2.60 ± 0.20b	4^{th}
L180	2.8 ± 0.10b	3^{th}
L130	3.10 ± 0.10a	2^{nd}
L138	3.30 ± 0.20a	1^{st}

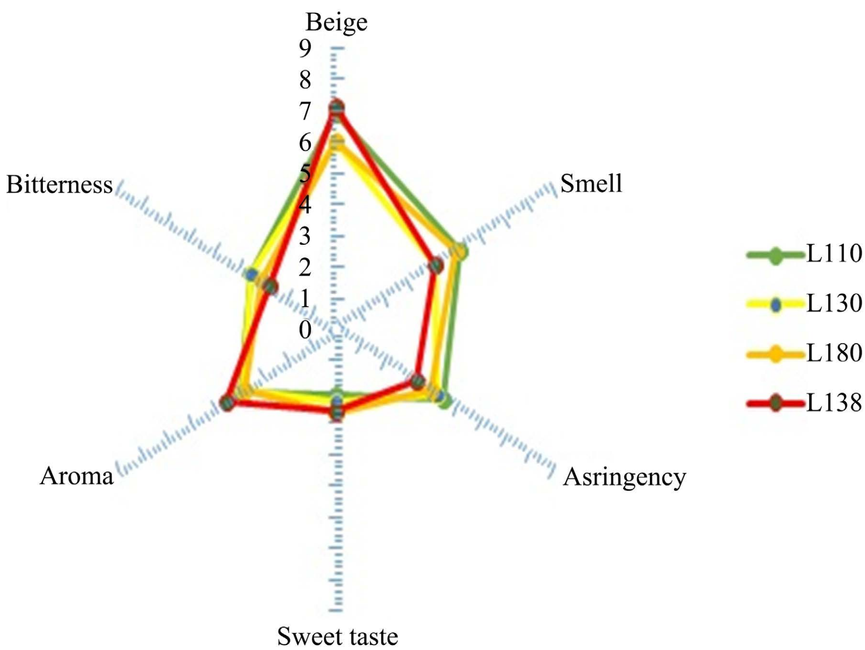

Figure 1. Sensorial profiles of the plant milks.

(34.80 ± 0.28) activities despite the fact that it was not appreciated (third) by the panelists, is not to be ruled out (Table 5).

4. Conclusion

This study aims to accompany the national policy of integrated agricultural economy aimed at reducing, from the cultivation of soybeans, poverty in the north of Côte d'Ivoire where cashew is also a main culture. It proposes a fermentative valorization of these two cultures in the new context of sustainable food against metabolic diseases. This work has also made it possible to diversify the potential for agro-food use of two microbial strains isolated from neglected traditional foods. As a result of the aromas and other distinctive floral notes observed during sensory analyzes of L180, L130 and 138 milks which are highly appreciated by consumers, it would be desirable in the future to study their aromatic profiles. Furthermore, the two strains TC6 and A4 can be excellent candidates for industrial use and in the fight against diseases issued from oxidative stress. Their GRAS "Generally Recognized as Safe" status could not be in doubt since their isolation matrices are usually consumed without any danger. Additional data are necessary about these plant-milks such as their viability in probiotic strains TC6 and A4 during one-month storage and the chemical nature of their antioxidant and anti-inflammatory substances.

Authors' Contributions

This work was carried out in collaboration among all authors. Authors read and approved the manuscript.

Conflicts of Interest

All the authors declare that they have no conflict of interest.

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