Potential use of Cashew Almond Aqueous Extract for Yoghurt Production

Abstract

Kernels of cashew (*Anacardium occidentale* L.) possess an enormous nutritional and technological potential. However, the almond is less consumed and processed by food industries in Côte d'Ivoire. The present study aims to bring added value to cashew kernel. The effect of this cashew almond extract was tested on fermentative activity of two lactic bacteria (*Lactobacillus delbrueckii subsp bulgaricus* and *Streptococcus salivarius subsp thermophilus*) specific for yoghurt production.

The study consisted of making seven formulations composed of milk and aqueous extract of cashew almond. Different formulations namely F0, F1, F2, F3, F4, F5 and F6 contained 0%, 5%, 10%, 15%, 20%, 25% and 30% respectively of aqueous extract of cashew almond. Lactic ferment containing the above lactic bacteria was used for fermentation of each formulation.

Effect of aqueous extract from cashew almond on fermentative activity of the two bacteria was carried out by pH, titratable acidity with duration of fermentation to obtain 80 Dornic degrees.

The results showed that the fermentative activity of lactic bacteria tested was improved with formulations with 15% (F3) and 20% (F4). With these two formulations, the acidity of the yogurt reaches 80° D compared to the standard (F0%). The extract therefore seems to stimulate the activity of bacteria at these two rates. Beyond 20% of cashew almond extract, the extract seems to inhibit the activity of lactic acid bacteria. The aqueous extract of cashew almond may play a role in yogurt-like fermented milk technology.

Keywords: *yogurt, lactic acid bacteria, extract, cashew almond, acidity 80 ° D.*

1. INTRODUCTION

The health of humans is largely dependent on the food they eat. Diseases such as diabetes, stroke, obesity, atherosclerosis, etc. are eating habits [1]. According to [2], the consumption of food of plant origin would decrease the risk of diseases in humans. Milk, given its nutritional richness, occupies an important place in human food intake. It is the staple food of all mammals. But since man could not consume breast milk forever, he turned to milk from another animal species. The various milk for consumption found in trade come mainly from cows. Cow's milk can be consumed raw or can undergo processing before consumption. But many consumers are turning to plant-based dairy products either for health reasons or as a lifestyle choice [3]. The consumption of plant-based milk substitutes has spread rapidly around the world due to its numerous positive health effects on the human body [4]. One of the main health reasons dictating this choice is the prevalence of lactose intolerance, which is due to the presence of lactose in cow's milk and other milk of animal origin [5]. Plant-based milk is known for its anti-hypertensive, anti-diabetic, anti-tumor and antioxidant properties effects [6]. Today the biggest challenge is to integrate ingredients of plant origin (vegetable milk) in the manufacturing process of food products in general and dairy products in particular in order to optimize the quality and quantity of finished products. Given the positive influence of plants on our health, it is therefore necessary to do extensive research on the possible intervention of plant extracts, which can permanently replace the chemical and animal ingredients used, in milk technology. In order to add value to the cashew kernel, we tried to integrate it into yogurt technology. Thus we studied the influence of the aqueous extract of the cashew kernel on the activity of lactic acid bacteria specific to yogurt.

2. MATERIALS AND METHODS

2.1. Chemicals and Biological material

The biological materials used for this study is consists of cashew nuts, powdered milk and lactic ferment. The cashew nuts samples are collected in different farms located in Dikodougou at 50 km of Korhogo (northern Côte d'Ivoire). The powdered milk (LP®, Irland) is purchased from Korhogo market and lactic ferment (Yalacta®, France) provided by "Pharmacie de la rue du commerce" (Abidjan, Côte d'Ivoire).
Reagents [(glucose, vitamin C, phenolphthalein, sodium hydroxide, 3.5-Dinitrosalicylic acid (DNS), metaphosphoric acid, acetic acid, dichlorophenol-indophenol (DCPIP)] were purchased from Sigma-Aldrich (Germany). All chemicals used in this study were of analytical grade

2.2. Production of aqueous extract from cashew almonds

The cashews were boiled in autoclave at 100°C for 20 minutes and then cooled to room temperature. The boiled cashews were split in half to separate almonds from shells using pliers for shilling cashew nuts and small knife. Almonds obtained were dried in oven (Memmert, Germany) at 80°C for 2 hours before pruning them with small knife. The pruned almonds were put in plastic box for storage. For production of aqueous extract, cashew almonds were ground using blinder (Nasco, South Korea). The paste obtained was mixed in 1 liter of distilled water at 20 % using blinder. The mixture was then filtered over a sieve and filtrate was pasteurized at 85°C for 5 minutes to give the aqueous extract of cashew almonds. After cooling, this extract was put in a sterile glass bottle before storage in a refrigerator at 4°C until used for experimentation.

2.3. Physicochemical analysis of aqueous extract of cashew almond

2.3.1.Moisture and dry matter content

Moisture and dry matter contents of aqueous extract of cashew almond were determined according to method of the [7]. Five (05) grams of almond extract sample were put in crucible previously weight (m0) and the whole (m1) were dried with oven at 105°C. Successive weighing of the whole (crucible and dried sample) until obtaining after 4 hours constant weight (m2). The percentages of moisture and dry matter are calculated with relations

$$\text{Moisture (\%)} = \frac{m1 - m2}{m1 - m0} \times 100 \qquad \text{Dry Matter (\%)} = 100 - \text{Moisture \%}$$

2.3.2.Protein content

The protein content of aqueous extract of cashew almond was determined according the official Kjeldahl method. This method consists to determine total nitrogen content of almond extract and calculated it protein content by allocated a factor of 6.25. Kjeldahl method based on three (03) stages: mineralization, distillation and titration. A sample of 5 mL of aqueous extract of almond and 15 mL of sulfuric acid were respectively put into Matras flask. The mixture contained in the flask was heated at 400°C for two (02) hours in digester. The mineralization of extract was carried out when mixture becomes clear bluish or very light greenish during the heating. After cooling at room temperature, the mineralized extract was transferred into 100 mL volumetric flask. Distilled water was added to adjust the volume of flask up to the gauge line. Ten (10) mL of sodium hydroxide at 40 % (w/v) were added to 10 mL of mineralized extract of almond into distillation flask. The condensed vapors will be collected in a beaker containing 20 mL of boric acid and two indicators (methyl red + bromocresol green). The distillation was carried out for 10 min until obtaining a purple distillate. The distillate obtained was titrated with a sulfuric acid solution (0.1 N) until it turns green. **A control samples was prepared using distilled water.** The total protein content was expressed by those formulas

$$\text{Total nitrogen (\%)} = \frac{(V1 - V0) \times N \times 0.014}{me} \times 100 \qquad \text{Protein content (\%)} = 6.25 \times \text{Total nitrogen (\%)}$$

With V0: volume (mL) of sulfuric acid solution (0.1 N) for blank titration; V1: volume (mL) of sulfuric acid solution (0.1 N) for sample titration; N: normality of sulfuric acid solution; me: weight of sample (g); 14: nitrogen atomic mass and 6.25: conversion coefficient of nitrogen proportion to protein.

2.3.3.Lipid content

The lipid content of aqueous extract of cashew almond was determined according to [7] method with Soxhlet extractor. Three (3) grams of almond extract (P0) were put into extraction cartridge before introducing cotton to cover the contents. The cartridge loaded with almond extract was then put in extractor. Furthermore, 300 mL of extraction solvent (hexane) were introduced into the flask of extractor previously weighed (P1). After assembling flask containing hexane and refrigerant of extractor, the flask was heated at 60°C with a heater. The extraction of fat by solvent (hexane) was carried out by the flux and reflux system of extractor at 60°C for 8 hours. After extraction, mixture of fat and solvent contained in flask was treated in evaporator at 60°C to separate solvent. Therefore, the flask containing fat was weighed (P2). The lipid content of aqueous extract of cashew almond was expressed as a percentage by formula:

$$\text{Lipid content (\%)} = \frac{P2 - P1}{P0} \times 100$$

2.3.4.Ash content

Ash content of aqueous extract of cashew almond was determined with method described by [7]. Five (5) grams (m0) of almond extract were put into crucible and the whole (extract + crucible) is weighed (m1) before incineration in a muffle furnace at 550°C for 24 hours. After incineration, the crucible containing ash was cooled in desiccator and the whole (ash + crucible) was weighed (m2). The ash content was determined according to formula:

$$\text{Ash (\%)} = \frac{(m2 - m0)}{(m1 - m0)} \times 100$$

2.3.5.Carbohydrate content

The total carbohydrate content of aqueous extract of almond was gave in percentages of fresh sample mass according to the relation:

Total carbohydrate (%) = 100 – [Protein (%) + Moisture (%) + Lipid (%) + Ash (%)]

2.4. Production of yogurt

2.4.1.Different formulations of yogurt

For each formulation of yogurt, a part (volume) of milk was substituted with aqueous extract of cashew almond at different percentages (Table 1). These formulations was prepared by incorporating to volume of milk, the aqueous extract of cashew almond at 5%, 10%, 15%, 20%, 25% and 30%. The control was only formulation without incorporation of aqueous extract of cashew almond (F0%). For fermentation, to each yogurt formulation was added ferment at 4% of total volume consisting of milk and aqueous extract of cashew almond.

Table 1. Proportion of ingredients for different formulations of yogurt

	Formulations						
Ingredients	F0%	F5%	F10%	F15%	F20%	F25%	F30%
Milk (%)	100	95	90	85	80	75	70
Aqueous extract of cashew almond (%)	0	5	10	15	20	25	30
Ferment (%)	4	4	4	4	4	4	4

2.4.2. Analysis of the yogurt produced

Effect of aqueous extract of almond on fermentative activity of the two bacteria was evaluated by pH, titratable acidity with duration of fermentation to obtain 80 Dornic degrees. Mixture (milk and aqueous extract of almond) of each formulation was pasteurized at 85°C for 5 min. After cooling to 45°C, each formulation was inoculated at 4% with lactic ferment containing two lactic bacteria (*L. delbrueckii subsp bulgaricus* and *S. salivarius subsp thermophilus*). Effect of aqueous extract of cashew almond on fermentative activity of lactic bacteria was carried out during incubation at 45°C by measuring titratable acidity (TA) and pH of mixture inoculated with lactic ferment over time. Titratable acidity was recorded by titrated 10 ml of each mixture inoculated with sodium hydroxide solution (0.111 mol/L) in presence of phenolphthalein [7]. The quantity of soda in mL poured multiplied by 10 corresponds to the Dornic degree.The titratable acidity is obtained by the following relationship:

$$TA = C_{NaOH} \times V_{NaOH} \times M_{Lactic\ acid}$$

$$\text{Dornic degree (°D)} = 10 \times V_{NaOH}$$

With TA: Titratable acidity, C_{NaOH}: Concentration of sodium hydroxide solution (mol/L), V_{NaOH}: Volume of sodium hydroxide solution (L), $M_{Lactic\ acid}$: Molecular mass of lactic acid (90g/mol).

2.5. Statistical analysis

The statistical analyses were performed with Graph Pad Prism software version 8.0.2 (263). The variance analysis (ANOVA) was performed to determine differences between the averages according to method of Turkey at the 5% threshold ($p<0.05$ was considered significant). The results were expressed as averages with standard error on mean (mean ± SEM).

3. RESULTS AND DISCUSSION

3.1 Yields of products from cashew kernel

The yields of different products calculated from cashew kernel weight are shown in Table 2. Cashew kernel shelling produced 29.11 ± 0.01% of fresh almonds and 70.89 ± 0.01% of shell. The fresh almonds drying gives 25.81 ± 0.01% of dried almonds. The yield of pruned almond is 20.99 ± 0.03% and that of the dandruff is 4.82 ± 0.02%. The low level of pruned almonds is due to tool used for husking. This is because hulling tool splits the almond in half. This is the instrument used for quality control in the cashew sector. The fact that the almonds were not whole made pruning difficult. The almonds easily broke into small pieces. The high level of shell is due to poor cooking of nuts. Indeed, cooking should remove a large part of fat that is in hulls..

Table 2. Rate of products derived from cashew kernel.

Operations	Products	Yield (%)
Cashew kernel shelling	Shell	70.89 ± 0.01
	Fresh almond	29.11 ± 0.01
Fresh almond drying	Dried almond	25.81 ± 0.01
Almond pruning	Pruned almond	20.99 ± 0.03
	Dandruff	4.82 ± 0.02

3.2. Chemical and Physicochemical properties of cashew almond and its aqueous extract

Table 3 presents the results of physico-chemical analysis of the cashew kernel and its aqueous extract. These results showed that the moisture content and dry matter (respectively 3.77 ± 0.57 and 96.33 ± 0.57) of cashew kernel are inversely proportional to those of its extract (respectively 85.66 ± 0.57 and 14.33 ± 0.57). On the other hand, the other parameters such as carbohydrates, lipids, ash and proteins have a higher content in almonds than in the aqueous extract. The results showed that the almond extract is slightly acidic with pH and titratable acidities of 6.81 ± 0.12% and 0.21 ± 0.05%, respectively.

The high ash content (2.69 ± 0.04) could be explained by concentration of nutrients due to water loss during roasting [8]. This ash content is in agreement with that of [9] which are 2.6 ± 0.0 but low compared to that reported by [10] which are 3.32%. Ash is the inorganic substances which contains minerals. Minerals are essential for diet because they act as cofactors in many physiological processes. Cashew kernels are rich in minerals, mainly selenium, which acts as a powerful antioxidant [11].

Almonds dry matter content in our study is high (96.33 ± 0.57). This high rate is due to low water content of almonds. The moisture rate (3.77 ± 0.57) was similar to that obtain by [12] (3.04). According to [13], moisture content of almonds should not exceed 5%. The water content of our almonds was between 2% and 4% so it meets standards.

On the other hand, the protein content (17.09 ± 0.36) in the present study was similar to that reported by [14] in raw kernels (17.2%). The results support that cashew nuts are an abundant source of protein and amino acids. Rich in these compounds, such cashew kernels are useful for the food industry in providing value-added products [15].

The lipid content found (37.09 ± 0.36%) was lower to those found by [16] in dried cashew almond (47.2 %). Cashew almond are rich in oleic and linoleic acids, fatty acids with therapeutic properties against cardiovascular diseases, besides controlling glycemia [17].

Table 3: Chemical and physicochemical properties of the cashew almond and its aqueous extract

	Values (%)	
Parameters	**cashew almond**	**aqueous extract**
Moisture (%)	3.77 ± 0.57	85.66 ± 0.57
Dry matter (%)	96.33 ± 0.57	14.34 ± 0.57
Total carbohydrates (%)	38.57 ± 0.61	5.73 ± 0.61
Lipids (%)	37.09 ± 0.36	5.51 ± 0.36
Protein (%)	17.09 ± 0.36	2.5 ± 0.36
Ash (%)	2.69 ± 0.04	0.4 ± 0.04
pH	-	6.81 ± 0.12
Titratable acidity (%)	-	0.21 ± 0.05

3.3. Evaluation effect of aqueous extract of cashew almond on fermentation activity of lactic bacteria

In the present study, the effects of cashew almond extract on fermentative activity of the bacteria Lactobacillus *bulgaricus* and *Streptococcus thermophilus* were investigated. The acidity and pH in yoghurt were evaluated at 30 min intervals till reaching 80°Dornic degree for yoghurt. The duration of fermentation to obtain 80°Dornic degrees was also evaluated.

3.3.1. pH and titratable acidity variation of different formulations

Table 4 and 5 shows the evolution of the acidity and pH. The pH ranged from 5.20±0.45 to 6.94±0.02 while the acidity (Dornic degree) ranged from 23.33 ±1.02 to 97±1.09. It was observed in this study that pH values of yoghurt decreased with increased acidity. Increase in the Dornic degree was in agreement with the results of other workers [18] who reported the same for yoghurt produced from blend of cashew nut and cow-milk and [19] with the used of "baobab" and "néré" flours as food additives in the production of yoghurt. This trend could be attributed to the fermentation process. This is because, during fermentation, microorganism uses sugar such as lactose and glucose for their metabolic activity and in the process secret acids as by-products [20].

Table 4. pH values of different formulations during fermentation

Durations (min)	Formulations						
	F0%	**F5%**	**F10%**	**F15%**	**F20%**	**F25%**	**F30%**
T0	6.94±0.02	6.91±0.02	6.90±0.06	6.88±0.12	6.87±0.23	6.86±0.12	6.84±0.03
T90	6.68±0.05	6.73±0.15	6.68±0.21	6.60±0.30	6.61±0.15	6.52±0.21	6.60±0.16
T120	6.26±0.14	6.23±0.38	6.10±0.04	6.11±0.04	6.10±0.29	6.02±0.05	6.01±0.09
T150	5.81±0.29	5.50±0.09	5.46±0.22	5.61±0.21	5.61±0.19	5.52±0.22	5.57±0.20
T180	5.37±0.18	5.32±0.10	5.27±0.15	5.23±0.60	5.20±0.45	5.34±0.33	5.35±0.25
T210	5.21±0.33	5.16±0.28	5.13±0.20	5.10±0.31	5.00±0.23	5.20±0.33	5.24±0.36

Tableau 5. Variation of Dornic degree of different formulations during fermentation

Durations (min)	Formulation						
	F0%	F5%	F10%	F15%	F20%	F25%	F30%
T0	23,33±1.02	24,33±0.43	24,66±0.72	28,33±0.60	31±0.02	32±0.40	32,66±0.62
T90	31,66±1.2	33,33±0.52	33,66±0.42	37±0.24	39±0.32	33,66±0.28	35±0.27
T120	42±0.90	42,33±0.67	47±0.30	49±0.17	52±0.19	52,33±0.30	52±0.42
T150	56,33±0.7	59±0.23	62±0.20	65±0.52	67,33±0.50	67±0.70	61±0.81
T180	65±0.32	67±0.80	67±0.72	80±0.63	80,66±0.81	78±0.71	74,33±0.56
T210	80,66±0.56	81±1.08	81,33±1.22	85±1.32	97±1.09	86,66±0.62	76±0.82

3.3.2. Fermentation time to obtain 80°Dornic degrees

Table 6 shows the fermentation time to obtain 80°Dornic degree for each yogurt formulated. Among the different formulations, only the 15 and 20% formulations gave better results. The incorporation of 15 and 20% cashew almond extract reduced the fermentation time. For these two formulations, 80° D is reached at 180 min against 210 min for the control. Below 15 and 20% incorporation, 80° D is reached at 210 min of fermentation. On the other hand, from 30% incorporation, 80° D is not yet reached at 210 min. This reduction in the production time of F15% and F20% yoghurts could be due to the contribution of nutrients contained in the aqueous extract of the cashew almond such as proteins (23%), carbohydrates (29.30%), essential fatty acids (44%) and unsaturated fats (82%) [21] [22], vitamins and minerals [23]. These nutrients can stimulate the growth of probiotics, which resulted in improved fermentation of yogurts with aqueous cashew almond extract compared to the control [24]. This may indicate that cashew milk can be used as a rich growth medium to enhance the growth and metabolic activity of *Lactobacillus*. According to [25], *Streptococcus thermophilus* grows faster and produces formic acid and CO_2. which stimulate growth of *Lactobacillus bulgaricus*. They therefore act in symbiosis. Similar result was reported by [26] who found a very significant increase ($P < 0.01$) in the of Dornic degree which on average was 70.29°D at 2h to reach 90.50°D after 4h with the production of yogurt added with pectin. However, all increases in the percentage of incorporation of cashew almond extract above 25% significantly increase it fermentation time compared to the control. This could be explained by the low pH and high acidity with the presence of many organic acids preventing any activity of bacteria [27], [28]. According to [29], any high acidity would significantly inhibit the development of this species of lactic bacteria. Indeed, for lactic acid production.

Table 6: The variation in time to reach 80° dornic degree of the different formulations

Formulation	Fermentation time (min)	Dornic degree
F0%	210	80,66±0.56
F5%	210	81±1.08
F10%	210	81,33±1.22
F15%	180	80±0.63
F20%	180	80,66±0.81
F25%	210	86,66±0.62
F30%	210	76±0.82

4. CONCLUSION

In general, aqueous extract acts on kinetics of acidity and pH of yogurt. The acidity of the formulations containing the aqueous extract of cashew almond all reached 80 ° D one step ahead of the standard. In addition to these positive effects on our health, cashew kernel may play a role in the technology of fermented yogurt-type milk. At levels less than 30% based on the amount of milk, aqueous cashew kernel extract does not appear to affect the viability of lactic acid bacteria. Above this level, the aqueous extract of cashew almond appears to have an inhibitory effect against lactic acid bacteria. It therefore contains elements having inhibitory effects vis-à-vis lactic acid bacteria. It is therefore clear that at a reasonable incorporation rate, the aqueous extract can be used as an ingredient in yogurt technology. In addition to its technological role, the aqueous extract of cashew almond provides nutrients to yogurt. Thus with this extract we can compensate for the loss of nutrients due to different heat treatments. It would therefore be very interesting to identify compounds likely to have antagonistic effects vis-à-vis lactic acid bacteria specific to yogurt. These compounds could have antimicrobial effects.

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