Study of Physicochemical Parameters of Baobab and Néré Flours and their Influence on Fermentative Activity of *Lactobacillus delbrueckii subsp bulgaricus*

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Abstract: ***In this work, we are interested in studying the use of Baobab (Adansonia digitata) and Néré (Parkia biglobosa) flours in production of fermented milk (yoghurt type). This study aims to diversify dairy and specifically creates another type of yoghurt, which can meet the market requirement. Yoghurts were prepared by fermentation of milk added Baobab or néré flour using Lactobacillus delbrueckii subsp. bulgaricus. Biochemical and microbial properties of yoghurt prepared with Baobab and Néré were studied in order to determine the best preparation depending on the rate of the flour. The results obtained showed that the low rate of incorporation (5%, 10%, and 15%) of baobab and Néré flour improve considerably the process of fermentation time, the total and Dornic acidity, pH. Even if the in L. delbrueckii subsp. Bulgaricus decreases when the rate of incorporation increases, this load remains always higher than that necessary by the international standards. It is thus possible to make formulations of yoghurt by incorporating baobab and Néré flour in milk.***

Keywords: Yoghurt, fermentation, Baobab and Néré flour, Biochemical properties and microbial load.

1. Introduction

Yoghurt is a very popular product around the world. This is due to both its organoleptic and nutritional characteristics [1]. To meet the growing demands of consumers, manufacturers use food additives to improve organoleptic, technological and nutritional characteristics [2] of their products. But currently, the tendency is to reject synthetic additives that are considered dangerous for health [3] in favor of natural additives and safe for the consumer.

The food practices being different from one area to another took along the producers to diversify the ingredients to satisfy the requirements of the consumers. Thus, in Europe and North America there is a trend towards probiotic, low-acid, low-fat yoghurt [2, 1]. This explains the growing popularity of products such as high protein yogurts that are obtained by adding serum proteins or fruit yoghurts that are obtained by adding pieces of certain fruits or their puree [1, 4].

Yoghurt is the product of the fermentation of milk by *Staphyloccocus salivarius subsp thermophilus and Lactobacillus delbrueckii subsp bulgaricus* [5]. Today, several species of bacteria are used for the production of yoghurt. These bacteria are used to, in addition to fermentation, give organoleptic and nutritional characteristics to yoghurt. Some strains of *S. salivarius subsp thermophilus* and Lactobacillus acidophilus are used for their ability to produce less lactic acid and more exopolysascharides [6]. Yoghurt containing these bacteria are labeled "probiotic" provided that they are alive and in sufficient number during consumption because they have a positive impact on the intestinal flora [7]. *L. delbrueckii subsp. bulgaricus* may be replaced in this mixed culture by any other species of the genus Lactobacillus provided that it is clearly specified to the consumer [8].

In Ivory Coast, a significant share of the production of yoghourt is ensured by small producers. One of "yoghurts" more snuffed is the "dêguê" which is obtained by addition of semolina flour of millet. Simple formulations with new organoleptic characteristics could increase local consumption. One possibility is the incorporation of known substances and consumed by these populations such as Baobab (*Adansonia digitata*) and Néré (*Parkia biglobosa*) flours.

Flours from Baobab and Néré pulp are known and traditionally consumed by sub-Saharan African populations [9, 10]. They also have good nutritional values [11, 12]. The incorporation of these flours as a food additive in the formulation of yogurt would improve its nutritional and organoleptic qualities in order to valorize and popularize in the food field. This incorporation in the formulation of yoghurt would not influence the fermentative activity lactic bacteria?

Thus, the objective of this study is to evaluate the influence of the incorporation of Baobab and Néré pulp flours on the development of fermentative bacteria in milk during the production of yoghurt.

2. Material and Methods

2.1 Material

2.1.1 Biological material

Biological material consists of Baobab and Néré flours (purchased on the market of Korhogo, north of Ivory Coast), powdered milk (LP brand), ferment (YALACTA brand which is a mixture of *S. salivarius subsp thermophilus* and *L. delbrueckii subsp bulgaricus*).

2.1.2Chemicals

Reagents (glucose, phénolphtaléine, soude, DNS) were purchased from Sigma-Aldrich. MRS was purchased from Sharlau. All chemicals used in the study were of analytical grade.

2.2 Methods

2.2.1 Flour treatments

After their purchases, the flours were sieved (with a 10 µm mesh sieve) and oven-dried (Memmert, Germany) at 60°C for 72 hours [13]. The dried powdered samples obtained were stored in polythene bags at 4°C.

2.2.2 Physicochemical analysis

Proximate analysis was performed using official methods [14]. pH was determined as follow: 10 g of dried powdered sample was homogenized with 100 mL of distilled water and then filtered through Whatman No. 4 filter paper. The pH value was recorded after the electrode of pH-meter (Hanna, Spain) was immersed into the filtered solution. For acidity 10 mL of filtrate have been titrated by NaOH 0.1N. The moisture content was determined by the difference of weight before and after drying sample (10 g) in an oven (Memmert, Germany) at 105°C until constant weight. Ash fraction was determined by the incineration of dry matter sample (5 g) in a muffle furnace (Pyrolabo, France) at 550°C for 12 h. The percentage residue weight was expressed as ash content. Proteins were determined through the Kjeldhal method and the lipid content was determined by Soxhlet extraction using hexane as solvent. Carbohydrates were calculated using the following formula [15]:

Carbohydrates = 100 – (% moisture + % proteins + % lipids + % ash).

2.2.3 Different formulations

Flours were incorporated into the milk powder with percentage of 5, 10, 15, 20 and 25%. This formulation is summarized in the table below.

F_{B1}, F_{B2} F_{B3} F_{B4} F_{B5}: Baobab formulation
F_{N1}, F_{N2}, F_{N3}, F_{N4}, F_{N5} : Néré formulation

Table 1: Percent of flour incorporation

Formulation	Percent of flour incorporation (%)	Total quantity (g)	Flour (g)	Milk (g)
F_T	0	500	0	500
F_{B1} ou F_{N1}	5	500	25	475
F_{B2} ou F_{N2}	10	500	50	450
F_{B3} ou F_{N3}	15	500	75	425
F_{B4} ou F_{N4}	20	500	100	400
F_{B5} ou F_{N5}	25	500	125	375

2.2.4 Yoghurt production

2.2.4.1 Preparation of the inoculum

Preparation of the inoculum is carried out according to the instructions of the manufacturer. Preparation of the inoculum began with the reconstitution of milk at 14% dry matter from milk powder. This was between 25 and 30°C followed by sterilization at 85°C for 5 minutes. Sterilized milk is cooled to 45°C before being seeded with YALACTA powder at 1g/L. The ferment is ready when its Dornic degree reaches 80. It is preserved thereafter at 4°C.

2.2.4.2 Production

Production of yoghurt is carried out according to the method of [16].Milk was reconstituted at 14% dry matter between 50°C and 60°C from the different formulations (milk /farine and a control). Milk obtained is then pasteurized at 85°C for 5 minutes. After pasteurization, milk is cooled to 45°C before being seeded by adding the inoculum at a rate of 2% and baked at 45°C. The determination of the Dornic degree was carried out after 1 hour of fermentation and every 30min until a Dornic degree of 80. Fermentation is then stopped by fast refrigeration followed by conservation with 4°C.

2.2.5 Yoghurt analysis produced

2.2.5.1 Determination of Ph and Dornic degree

Ph determination consisted of dipping the electrode of a previously calibrated pH meter in 50 mL of yoghurt produced [14]. Dornic degree determination consisted of titrating 10 mL of yoghurt produced by NaOH (0.111 N) in the presence of phenolphthalein.

$$°D = CNaOH * Veq * 90$$

2.2.5.2 Enumeration of *Lactobacillus delbrueckii subsp bulgaricus*

Dilutions were performed with physiological water (9% NaCl). The first decimal dilution consisted in homogenizing 10ml of yoghurt produced in 90 mL of physiological solution. From this dilution, decimal dilutions were realized up to 10^{-7}. Seeding was anaerobic in the mass for 24 hours at 37°C. Seeding was carried out on the last three dilutions (10^{-5}, $10^{-6}$and 10^{-7}) by introducing 1 mL of each dilution in a Petri dish to which 15 mL of supercooled MRS were added. The whole was homogenized and after solidification, 5mL of MRS (Man - Rosa - Sharp) was added and the incubation was carried out at 37°C for 24 hours. The reading was done by counting the colonies formed on the Petri dish. To know the microbial load, the following formula made it possible of determined the microbial load:

$$N = \frac{\sum C}{d(n_1 + (0.1 \times n_2)V}$$

N: colony number (CFU / mL)
ΣC: number of colonies counted
d: dilution rate corresponding to the first boxes retained
n_1: number of boxes corresponding to the first dilution selected
n_2: number of boxes corresponding to the second dilution retained
V: volume of the inoculum

2.2.6 Statistical analysis

The statistical analyses were performed with the Graph Pad Prism software version 8.0.2 (263) of January 30, 2019. The correlations of Spearman were determined at the significance level of 5%. The variance analysis (ANOVA) was performed to determine the differences between the averages according to the method of Turkey at the 5% threshold (P <0.05 is considered significant). The results were expressed as averages with the standard error on the mean (mean ± SEM).

3. Results and Discussion

3.1 Powder physicochemical properties

Proximate composition of the flour is presented in Table 2. The physicochemical parameters generally differ significantly (p<0.05) from a flour to another. Acidity content of the investigated flours ranged from 14.1 ± 0.01 meq/100g (Milk) to 118.00 ± 3.12 meq/100g (Baobab). The high acidity content of baobab (118.00 ± 3.12 meq/100 g) is in agreement with the results of [17] on baobab (68 à 201 meq/100 g) in Senegal. Acidity could be with the presence of the organic acids like the citric acid, the tartaric acid, the acid mallic, the succinic acid, the pyruvic acid, the fumaric acid and the acid hydroxybutanoïc [18, 19, 20]. Flour of baobab could be used in infant flours in the fight against the diarrhoea.

All samples contained between 2.54 and 4.12 % moisture. The lower moisture content of flour is in variance with the results of [21] on Baobab (10.94%) and [22] on Néré (16.52%). According to [23], food substances, with high moisture content are subjected to the growth of microorganisms and moisture content higher than15% is said to promote enzymatic reactions leading to loss of vitamins [24]. In view to their ash contents (4.40±0.17-7.50±0.01%) the selected flour may be considered as good sources of minerals when compared to values (2-10%) obtained for cereals and tubers [25].Proteins content ranged from 9.43 0.09% in Néré to 26±0.02% in Milk. The proteins content of Baobab (9.57 ± 0.33 %) and Néré (9.43±0.09%) was more than that reported by [21] and [26] in the flour of Baobab (8.73%) and Néré (5.12%) respectively. But that of the Baobab is included in the value of [27] in Tanzanie (2.5 - 17%). Flours of Baobab and Néré studied could take part in the proteinic contribution of desirable vegetable origin. The lipids content of the flour was in the range of 1.75-26%. These lowest values of lipids in baobab and Néré corroborate the findings of many authors which showed that Baobab and Néré flour are poor sources of lipids [17, 28]. In addition, diet providing 1-2% of its caloric energy as fat is said to be sufficient to human beings, as excess fat consumption yields to cardiovascular disorders such as atherosclerosis, cancer and aging [29]. Therefore, the consumption of the studied flour could be advantageous for individuals suffering from obesity. Carbohydrate content was significantly higher for flours of Baobab (78.89 ± 0.55%) and Néré (80.65 ± 0.88%) compared to milk (36.95 ± 0.02%). Baobab and Néré flours are been good sources of carbohydrate. Carbohydrate profile should promote its consumption in order to reach the official recommendations (50 to 55% of daily calories must come from carbohydrates).Baobab and Néré flours could be used as a complement in infant flours.

Table 2: Physicochemical properties of Milk, Baobab and Néré

Parameters	Milk	Baobab	Néré
pH	6.30 ± 0.01^c	3.30±0.07^a	5.06±0.03^b
Acidity (meq/100g)	14.1 ± 0.01^c	118.00±3.12^a	23.66±1.66^b
Moisture %	3.55 ± 0.02^b	4.12±0.02^a	2.54±0.05^c
Ash %	7.50 ± 0.01^a	5.67±0.12^b	4, 40±0.17^c
Lipids %	26.00 ± 0.03^a	1.75±0.05^c	2.98±0.11^b
Proteins %	26.00 ± 0.02^a	9.57±0.33^b	9.43±0.09^b
Carbohydrates %	36.95 ± 0.02^c	78.89±0.55^b	80.65±0.88^a

Data are represented as Means±SD (n = 3). Means in the lines with no common superscript differ significantly (p<0.05)

3.2. Produced yoghurt

3.2.1 Effect of incorporated flours on yoghurt total Acidity

Figure 1 shows the flours acidity of the different formulations. The acidity of the flours increases with the percentage of incorporation. Flour of Baobab has the most acidity compared to Néré. The Néré acidity is similar to that of milk for the respectively formulations of 5; 10 and 15%. This high acidity of Baobab formulation could be due to the original acidity of baobab which is rich in citric acid, tartaric acid, mallic acid, succinic acid, pyruvic acid, fumaric acid, hydroxybutanoïc acid and vitamin C [18, 19, 20].

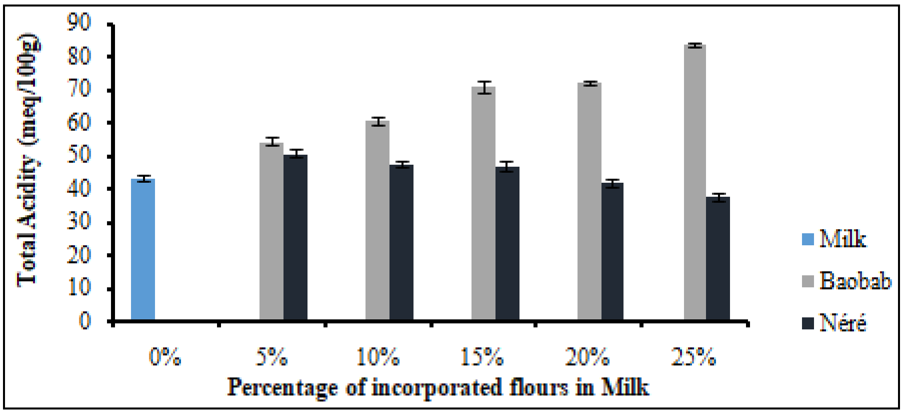

Figure 1: Total Acidity of formulated flours

3.2.2 Effect of incorporated flours on yoghurt pH and Dornic Acidity

The figure 2 and 3 shown the results of the pH and Dornic acidity evaluation of yogurt supplemented with baobab and Néré flour. The pH and Dornic acidity are important and convenient parameters for the development of the flavor and texture characteristics of yoghurt. The pH is dropped from 6.31 for control milk to 4.81 for the milk incorporated with 25% of Baobab flour and 6.17 for witch with incorporated 25% of Néré flour. According to [30], lactic strains have the ability to ferment lactose into lactic acid, with an increase of acidity and a decrease in pH of yoghurt. The Dornic acidity increase from 39 to 71°D for baobab incorporated milk and no significant increase for Néré flour incorpoted milk. This result is similar to those of [31] who observe the increasing Dornic acidity of yogurt added with pectin.

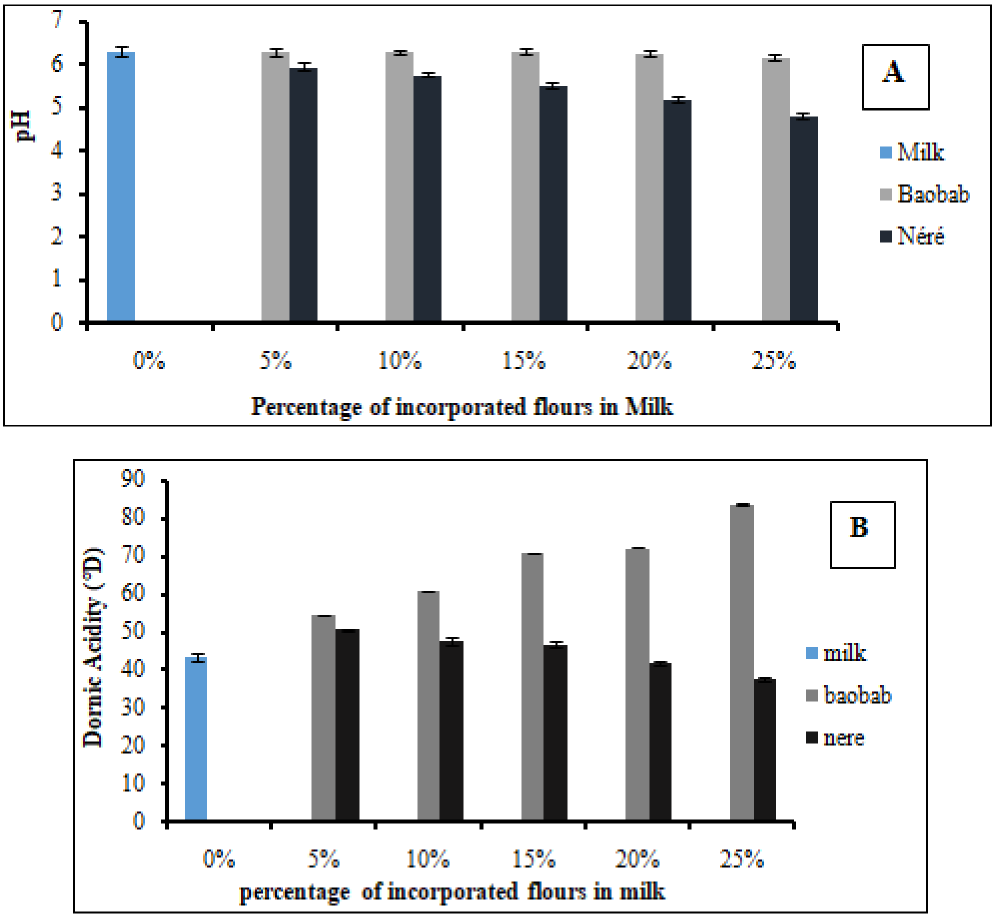

Figure 2: Evolution pH (A) and Dornic Acidity (B) of yogurt added with Baobab and Néré flours

3.2.3 The fermentation time and number of the Colony Forming Units of *Lactobacillus delbrueckii subsp bulgaricus*:

The result is presented in Table 3. The incorporation of 5 and 10% of flour entrained a significant reduction of fermentation time. This reduction is due to the reduction of the latency time. The latency time corresponds to the step of adaptation of bacteria to the medium. During this step, the bacteria produce compounds, such as amino vitamins and acids, which are necessary to their growth. These compounds which were to be synthesized by the bacteria are brought by the flours. Indeed, the incorporated flours contain vitamins and free acids amino as indicated by [12, 10].Baobab flour entrained high reduction of fermentation time than the néré flour. That is the fact of its wealth of certain growth promoters such as vitamin C concentration, according to [10], range from 125.5 to 312mg/100g.

Table 3: Evaluation of the fermentation time and the CFU of *Lactobacillus delbrueckii subsp bulgaricus* of yogurt added with Baobab or Néré flour.

Formulation	Rate of incorporation	*Lactobacillus bulgaricus* (CFU/g)	Fermentation time (minutes)
F_T	0%	$4, 75.10^8$	205
F_{B1}	5%	$9, 75.10^8$	115
F_{B2}	10%	$6, 70.10^8$	117
F_{B3}	15%	$5, 75.10^8$	123
F_{B4}	20%	$4, 50.10^8$	201
F_{B5}	25%	$3, 90.10^8$	210
F_{N1}	5%	$5, 05.10^8$	133
F_{N2}	10%	$4, 55.10^8$	136
F_{N3}	15%	$3, 22.10^8$	150
F_{N4}	20%	$1, 95.10^8$	165
F_{N5}	25%	$1, 32.10^8$	174

4. Conclusion

The biochemical and microbiological characteristics of Baobab and Néré flours incorporated in yoghurt was the subject of the study recorded in this report. The objective of this study was to evaluate the influence of the incorporation of Baobab and Néré flour on the fermentative activity of *L. delbrueckii subsp bulgaricus* during yoghurt production. The determination of the biochemical characteristics of the flours shows that baobab and Néré flours are significant sources of protein and carbohydrates that could be influenced by fermentative activity. The milks incorporated at percentages of 5, 10 and 15% have pHs close to the control. The rate of 5; 10 and 15% considerably reduces the fermentation time. The yogurt incorporated with 5; 10 and 15% contains a high concentration of *L. delbrueckii subsp bulgaricus*. All these proven potentials could be exploited to contribute to reduce the production time of yoghurt. Baobab and Néré flour has the potential of providing nutritional benefits and enhancing the quality of fermented dairy products on the basis of the biochemical and microbial properties. Baobab and Néré flour is a natural supplement that can further enhance health benefits of yogurt.

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