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Full Length Research Paper

Impact of partial milk substitution with cocoa powder on the properties of milk fermented by *Lactobacillus bulgaricus and Streptococcus thermophilus*

Jean J. Essia Ngang[1]*, Christiant P. Kouebou[2], Sylvain L. Sado Kamdem[1] and Darman R. Djoulde[3]

[1]Département de Microbiologie, Faculté des Sciences, Université de Yaoundé I B.P. 812 Yaoundé, Cameroun.
[2]Institut de Recherche agricole pour le développement (IRAD) , Station polyvalente de Garoua, B.P. 415 Garoua, Cameroun.
[3]Higher Institute of Sahel, University of Maroua, P. O. Box 46 Maroua, Cameroon.

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Milk powder was substituted by 0, 10, 20, 30 and 40% (w/w) cocoa powder, and used for yoghurt production. Microbiological and physico-chemical analyses were carried out during fermentation and sensory profile of fermented products evaluated in comparison to plane commercial yoghurt. Adding cocoa powder induced an increase of the initial pH ($p<0.05$). A reduction ($p<0.05$) of the fermented products viscosity was observed with an increase in the amount of cocoa powder. Coagulation times increased with substitution level up to 20%. Under 20% substitutions, the products were accepted by the panellist with no significant difference with respect to the control.

Key words: Milk, cocoa, fermentation, physico-chemicals properties, sensorial evaluation, lactic acid bacteria.

INTRODUCTION

In sub-Saharan regions, high temperature and the lack of refrigeration facilities have led to the transformation of fresh raw milk to many fermented products such as “Alaska, pendidam, kindirmou, dackéré”, and yoghurt which is the most popular and the most consumed (Libouga et al., 2005). Yoghurt, whose nutritional properties are derived from the milk used, has more nutritional advantages than milk. In fact, during yoghurt production, the milk proteins are partially hydrolysed, increasing their digestibility. In the same way, acidification that induces casein demineralisation increases soluble calcium and phosphorus, thereby improving mineral uptake into the body (Adolfsson et al., 2004). Moreover, the conversion of lactose into lactic acid allows lactose intolerant people who can not consume milk to be protected from diarrhoeal episodes and to benefit from the nutritional value of milk protein which is well preserved during fermentation. The low folic acid content in the milk can also be increased by lactic acid bacteria, improving on the vitamin content in human diet, while the presence of probiotic bacteria in yoghurt strengthens children’s resistance to enteric infections (Kailasapathy and Chin, 2000). Moreover, although milk is considered as one of the most complete food products, this quality can still be enhanced to meet other nutritional and nutraceutical requirements now claimed by the consumers and the market.

In spite of those beneficial effects, an important shortage of milk product demand is observed in this part of the world because of the seasonal production of milk (Libouga et al., 2001). An annual importation of milk powder worth about 15 billions CFA is thus resorted to in

*Corresponding author. E-mail: essia_ngang@yahoo.fr.

Table 1. Experimental plan and physico-chemical properties of reconstituted milk products before fermentation.

Run	Cocoa Powder (%)	Milk Powder (%)	Fermentation time (hour)	Protein (% w/w)	Lipid (% w/w)	Ash (% w/w)	pH
1	0	100	1	26.28±0.2[a*]	25.66±0.4[a]	5.51±0.3[a]	6.0±0.1[a]
2	10	90	1	25.75±0.2[b]	25.71±0.4[a]	5.29±0.2[ab]	6.20±0.1[b]
3	20	80	1	25.12±0.1[c]	25.96±0.2[a]	5.04±0.1[b]	6.40±0.1[c]
4	30	70	1	24.64±0.3[d]	25.59±0.9[a]	4.82±0.2[bc]	6.50±0.1[c]
5	40	60	1	23.97±0.1[e]	26.20±0.3[a]	4.45±0.2[c]	6.70±0.1[d]
6	0	100	2				
7	10	90	2				
8	20	80	2				
9	30	70	2				
10	40	60	2				
11	0	100	3				
12	10	90	3				
13	20	80	3				
14	30	70	3				
15	40	60	3				
16	0	100	4				
17	10	90	4				
18	20	80	4				
19	30	70	4				
20	40	60	4				

*Different superscripts letters on the same row (within each block of five runs) means values are significantly different ($p<0.05$)

order to cover up about 60% of milk product requirements (Dieye et al., 2002).

Considering the nutritional importance of milk products as well as the increasing demand attributed to population growth, there is a need to develop new products from locally available food material as a way of reducing milk importation. Among the available agricultural resources, cocoa (*Theobroma cacao*), with more than 50% of world production coming from Africa is worth valorising as it has a good nutritional value. Cocoa is an excellent source of minerals: iron, phosphorus, potassium, vitamins (vitamin B12, B1 and B2,) and fibre. Cocoa is also rich in poly-phenols such as catechins and procyanidins, which are well known for their antioxidant and anti-inflammatory effects (Wollgast and Anklam, 2000). Buijsse et al. (2006) in a study on 470 elderly men during 15 years observed that cocoa intake is inversely associated with blood pressure, cardiovascular and all cause mortality. In an attempt to make consumers benefit from the nutraceutical properties of cocoa powder, Polagruto et al. (2006) observed that the consumption of phytosterol-enriched snacks for 6 weeks effectively reduced total plasma and LDL cholesterol levels in a population with hypercho-lesterolemia.

In this perspective, and in order to reduce cost of production by exploiting our local resources, the influence of different percentages of milk substitution by cocoa powder on the fermentation parameters, macro element composition and consumers' acceptance of the fermented product was assessed.

MATERIALS AND METHODS

Materials

Whole milk powder (Flanders, BBDP, SA, Holland), cocoa powder (manufactured by Chococam, Cameroon) and sugar (Sosucam, Cameroon) were purchased from a local market in Ngaoundéré. Strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were obtained from the Microbiology Laboratory, University of Yaoundé I (Cameroon).

Inoculum and fermented products preparation

Lactic starter cultures of *L. bulgaricus* and *S. thermophilus* maintained on De Man Rogosa and Sharpe (MRS) agar at 4°C were grown overnight in MRS broth at 40°C and subcultured twice at the same temperature in UHT hold milk before used for the production. Production was done in 6 steps as described by Acollas et al. (1980). Milk powder substituted is shown in Table 1 and solubilised to 14% (w/v). The mixtures were then diluted in distilled water at the concentration of 14% (w/v). Inoculation was done at 2.50% (v/v) of a mixed culture of 1:1 ratio of *L. bulgaricus* and *S. thermophilus*, giving about 6.20 Log cfu/ml final concentration. The

inoculated milk were incubated at 45°C in an incubator (Edmund Bühler, Germany) for 4 h during which fermentation was monitored every hour.

Physico-chemical analysis

Protein, fat, and ash content of the milk mixtures were determined using AOAC methods (AOAC, 1990). pH was determined using pH meter (Eutech, Cybernetics, Cyberscan1000, Singapore), total titratable acidity was evaluated according to AFNOR methods (AFNOR 1980). pH and total acidity were determined at 1 h interval during fermentation. Viscosity was determined at the end of fermentation using a ball viscosimeter (Haake viscosimeter, Germany).

Lactic flora enumeration

Lactic acid bacteria were enumerated at 1 h interval during fermentation using poured plated method on MRS agar. Plates were incubated anaerobically at 37°C for 24 to 36 h.

Sensory analysis

Sensory evaluation was carried out by 20 panellist selected between students and lecturers of the University based on their familiarity with fermented milk product and their experience on sensory evaluation. Refrigerated samples of milk product were assessed for colour, flavour, texture, odour and overall acceptability. Samples were evaluated using 6-point hedonic scale ranging from 6 (like extremely) to 1 (dislike extremely).

Statistical analysis

Experiments were performed in triplicate and results subjected to analysis of variance. Duncan's multiple range test (Steel and Torrie, 1980) and multivariate analyses using Excel of Microsoft 2003 and statistica 7, (StatSoft).

RESULTS AND DISCUSSION

In this work, milk powder was substituted with different percentages of cocoa powder before reconstitution with water and fermentation, in order to increase the nutriational and functional properties of the product. Physico-chemical parameters of the powder mixtures are shown in Table 1. Increasing substitution of milk by cocoa powder decreased protein and ash contents. These results were expected since the protein content in cocoa powder is less than in milk powder. Fat content of the mixture were not significantly affected by this substitution while the pH of reconstituted milk mixtures increased with increasing amounts of cocoa powder. The nature of this increase in pH can be explained by the fact that cocoa powder production generally includes an alkalization process which increases the pH of the natural product from 5.2-5.6 to values between 6.8 and 8 depending on the conditions of alkalization (Li et al., 2012).

Data recorded for the three repetitions of the experimental plan indicated in Table 1 were merged together and a multiple non linear regression was performed to assess the effect of cocoa powder substitution and fermentation time on the lactic acid bacteria (LAB) count, pH and titratable acidity. The models equations and qualifying parameters obtained are indicated as follows:

$$\text{LAB count (Log(cfu/mL))} = 6.65 + 0.004*\text{Time}*\text{Subt} - 0.00022*\text{Subt}^2 + 0.1044*t^2 \quad (R^2=0.83;\ F=120.2;\ P=0)$$

$$\text{Titratable acidity (°D)} = 24.86 + 0.382*\text{Subt} + 3.28*t^2 \quad (R^2=0.96;\ F=928.6;\ P=0)$$

$$\text{pH} = 6.049 + 0.00045*\text{Subt}^2 - 0.12*t^2 \quad (R^2=0.94;\ F=586.2;\ P=0)$$

The results indicate that the substitution of milk powder (Subt) had a high influence only on the titratable acidity (Figure 1). The influence of fermentation time on the LAB count, titratable acidity and pH is not questionable as it is an expected result (see equations). In fact, the same kind of influence was observed in the model, also indicating that these three measurements are correlated. Decrease of pH correlated with acid evolution whose production accounted for the drop in the pH as was also observed by Mortada and Omer (2013).

Increasing cocoa powder percentages led more to an increase in titratable acidity than the growth of LAB, both at the beginning and at the end of fermentation (Figure 1). Moreover, our initial concern about the possible growth limiting effect of the cocoa powder was not observed with the LAB used for fermentation.

The coagulation times observed were 3 h, 3 h 10 min, 3 h 30 min for the 0, 10 and 20% substitution respectively, while it was longer than 4 hours for the higher percentages of substitution. These results were in agreement with those of O'connell et al. (1998) who noted an increase of skimmed milk coagulation time in the presence of 0.40 - 4.80 g/100 ml cocoa. Moreover, a decrease of the product viscosity following an increase in percentage cocoa substitution was observed (Figure 2). The low viscosity of samples containing high proportions of cocoa powder could be due to the combined effect of the reduced casein content and the preservation of casein stability by cocoa polyphenol compounds reported by o'connell et al. (1998).

The products obtained were then submitted for consumer appreciation. From the results reported in Figure 3, it can be observed that for substitution up to 20%, products were well accepted and scored the same as the control sample. No significant differences were observed ($p>0.05$) on the texture, flavour and odour as compared to the control.

Samples with more than 20% substitution had low texture score as no coagulation was observed after 4 h of fermentation. The fermentation process may be the cause of the non-acceptance of the 30 and 40% substituted products compared to chocolate milks that are not or less fermented. Non-jellified products obtained

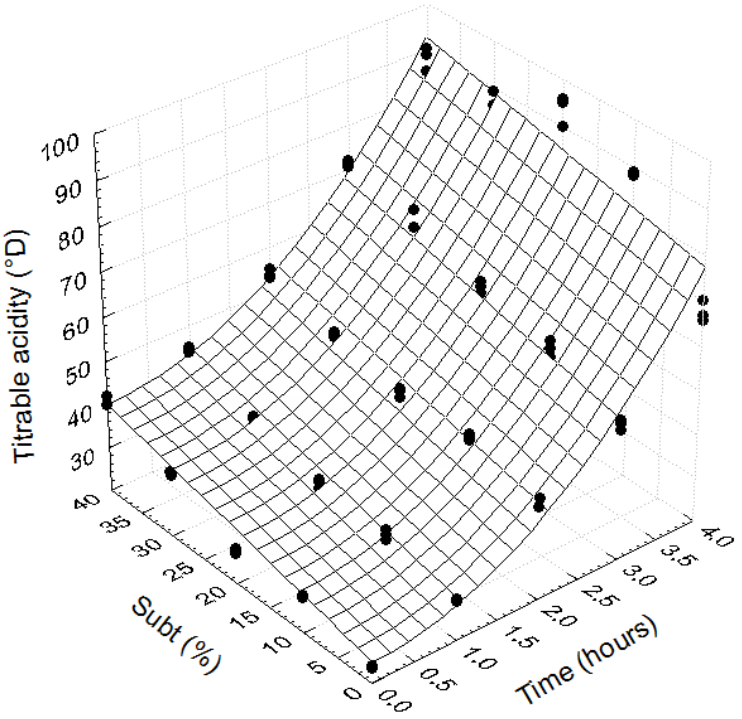


Figure 1. Influence of time of fermentation (time) and percentage of milk substituted with cocoa (subt), on the titrable acidity.

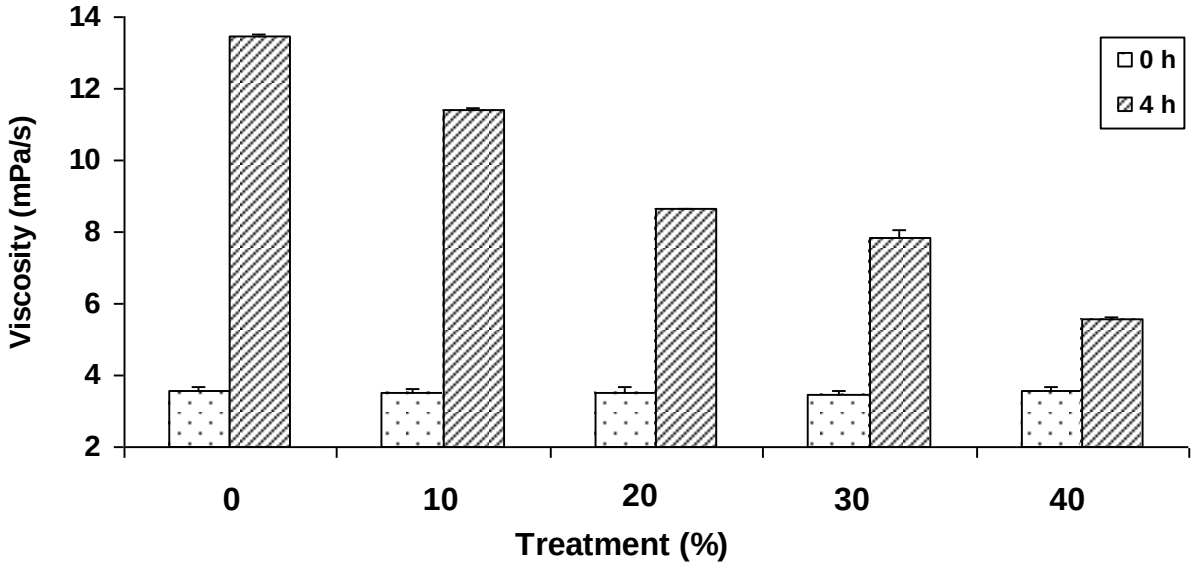
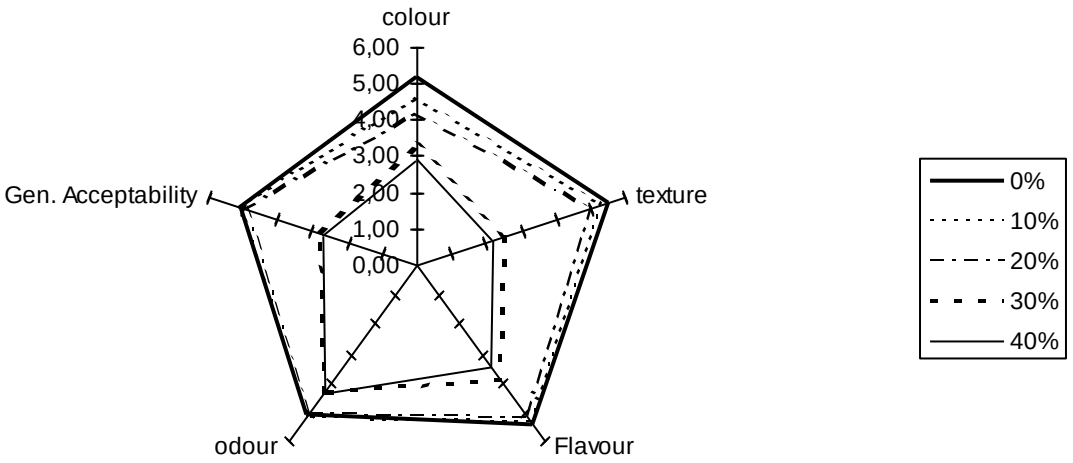


Figure 2. Viscosity variation at the end of fermentation over the level of milk powder substitution (treatment).

during this experiment are proposed to be tested as drinkable fermented milk as such products are highly appreciated in European countries but not yet in this part of the world. This will go a long way in increasing the market share of fermented cocoa milk products.

Conclusion

Results of the present study show that a percentage of powder milk can be substituted by cocoa powder in order to enhance the products nutritional properties without



Parameters	Cocoa percentage in milk				
	0%	10%	20%	30%	40%
Colour	5,13 ± 0,1[a*]	4,56 ± 0,3[b]	4,16 ± 0,3[b]	3,2 ± 0,3[c]	2,91 ± 0,1[c]
Texture	5,51 ± 0,3[a]	5,26 ± 0,3[a]	4,9 ± 0,1[a]	2,53 ± 0,5[b]	2,16 ± 0,3[b]
Flavour	5,41 ± 0,1[a]	5,26 ± 0,3[a]	5,13 ± 0,1[a]	3,91 ± 0,1[b]	3,43 ± 0,4[c]
Odour	5,06 ± 0,1[a]	5,13 ± 0,1[a]	5,03 ± 0,1[a]	4,3 ± 0,3[b]	4,33 ± 0,1[b]
Overall Acceptability	5,06 ± 0,1[a]	5,1 ± 0,1[a]	4,83 ± 0,1[a]	2,75 ± 0,2[b]	2,66 ± 0,3[b]

Figure 3. Influence of the percentage of milk substituted by cocoa on the sensory analysis parameters (raw data table is introduced in the figure).

causing any change in the LAB growth capacity. Moreover, less than 20% of cocoa powder substitution does not cause significant changes in the product quality as assessed by consumers. This approach is thus promising as it can help increase the use of local agricultural resources while reducing the cost of production.

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