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Antimicrobial activity of crude biosurfactants extracted from a locally fermented milk (*pendidam*) on yellow *Achu* soup produced in Ngaoundere, Cameroon

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ABSTRACT

The consumption of yellow *Achu* soup in Ngaoundere presents a potential risk as alteration and pathogenic microorganisms are present in it. In order to ameliorate the microbiological characteristics of this soup, the extraction of crude biosurfactants, a biopreservative-like substance from *pendidam* and the study of its *in vitro* and *in situ* antibacterial effects against some groups of bacteria isolated from yellow *Achu* soup in Ngaoundere; constituted the objective of this study. Analyses were carried out on seven samples of the soup from some restaurants in Ngaoundere town. The soup samples presented a total aerobic and facultative anaerobic count ranging between 15.43±1.23 × 10^3cfu/mL and 38.30 ± 1.08 × 10^3 cfu/mL. Most of the samples contained both pathogenic and/or spoilage bacteria such as *Salmonella* spp., faecal streptococci and faecal coliforms. The antibacterial activity of crude biosurfactants against some strains isolated from yellow *Achu* soup was studied by determining their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Results obtained show that crude biosurfactants had bactericidal effect on all the three strains of bacteria tested. Faecal coliform strain had an MIC of 25 mg/mL and an MBC of 50 mg/mL while faecal *Streptococcus* sp. and *Salmonella* sp. had the same value of MIC (50 mg/mL) with MBCs of 50 and 100 mg/mL for faecal *Streptococcus* sp. and *Salmonella* sp., respectively. The crude biosurfactants extracted from *pendidam* had an MBC/MIC ratio value of less than 4 for all the three bacteria strains tested with an MBC/MIC ratio value of 2 for faecal coliform strain and *Salmonella* sp.; and 1 for the faecal *Streptococcus* sp. The application of crude biosurfactants in yellow *Achu* soup to study its *in situ* antimicrobial activity has significantly reduced the microbial load of this soup. These results indicate that crude biosurfactants could be used as a potential food biopreservative, considering its *in vitro* and *in situ* antibacterial effects on micro-organisms found in yellow *Achu* soup.

INTRODUCTION

At a time when there is a global cry for food security and with the prevailing circumstances of postharvest loss at the increase, there is a dire need to improve quality and quantity in agricultural production so as to alleviate this situation of insufficiency which has become a call for concern. Food that contains any trace of pathogenic microorganisms or their toxin is not wholesome thereby posing a threat to the health and wellbeing of its potential

consumers and the community as a whole. As consumers now have much concern about what they eat, safe foods cannot be neglected. A key concerns in food industry is the contamination by pathogens which are frequent cause of food borne diseases (Parada et al., 2007).

Although numerous strategies have been established and are currently in used to control pathogenic and spoilage microorganisms in foods, the research for novel, natural and effective antimicrobial agents still continues. Recently, the use of biosurfactants as alternatives to control microorganisms in foods has been explored. Biosurfactants have been shown to exhibit antimicrobial activity and control the proliferation of pathogenic microorganisms in food. Biosurfactants are structurally diverse groups of surface-active molecules synthesized by microorganisms. They represent a promising alternative in controlling the proliferation of pathogenic microorganisms in foods. These molecules have been proven to have some emulsifying properties (Rodrigues et al., 2006; Gudiña et al., 2010; Mbawala et al., 2015a; Tchougang, 2016), antibacterial effect (Mbawala et al., 2013a, b), antifungal and antiviral effect (Ilse, 2009; Gudiña et al., 2010; Inès and Dhouha, 2015), biodegradability and non-toxic effect on man and the environment (Wang et al., 2006; Perfumo et al., 2009).

Biosurfactants as biopreservatives have been exploited by several researchers to ameliorate the quality of some foods such as meat and meat products (Mbawala et al., 2013a; Tchougang, 2016), fermented milk product "*pendidam"* (Pahane, 2013) and yellow *Achu* soup (Mbawala et al., 2015a), etc. However, most foods are pasteurised and even sterilized in some instances after mixing all the ingredients except emulsion based foods among which we have yellow *Achu* soup. This soup also called '*Nah poh*' is a palm oil/water emulsion partially emulsified (stabilised) by *kanwa* a local earthy material or *nikih* (liquid extract from the ash of plantain or banana peelings) (Ekosse, 2010). The over 19 spices used in the preparation of this soup confers important chemical, biochemical, therapeutic functions to this prestigious soup (Tchiégang and Mbougueng, 2005). What then becomes the health risk attributed to such a food which is consumed without pasteurisation? Within limit of our knowledge, no study on the antimicrobial activity of crude biosurfactants extracted from the locally fermented milk (*pendidam*) on yellow *Achu* soup has been carried out so far.

The objective of this research work is to determine the activity of crude biosurfactants extracted from locally fermented milk (*pendidam*) against some groups of microorganisms present in yellow *Achu* soup sold in Ngaoundere.

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MATERIALS AND METHODS

Yellow *Achu* soup

Seven samples of yellow *Achu* soup of 100 mL each were collected in sterile plastics containers in some restaurants in the town of Ngaoundere and transported to the Laboratory in a sterile hermetically sealed container for analysis.

Pendidam

Pendidam samples were bought from Dang market a neighboring locality in Ngaoundere (Cameroon), stored in sterile glass bottled containers and transported in an ice containing flask to the Laboratory.

Palm oil

Palm oil was obtained from Limbe main market (Head quarter of Fako division in the Southwest region of Cameroon) and preserved in a hermetically sealed container before transporting to Ngaoundere.

Spices

Nineteen of the commonly used yellow *Achu* soup spices as reported by (Abdou, 2009) was bought from market B of Bafoussam (capital of the West region of Cameroon) and dried in an oven at 45°C to a constant weight followed by milling to a granulometry of less than 0.5 mm using a blender and stored in a sterile glass bottled containers.

Kanwa

Kanwa as one of the non-biological material was obtained from Dang market in Ngaoundere and transported to the Laboratory in a sterile hermetically sealed container.

Microbiological analysis of yellow *Achu* soups

The aim of this analysis was to evaluate the health risk attributed to such a food which is consumed without pasteurisation. As such, total aerobic count, faecal and total coliforms, faecal streptococci, *Salmonella* spp., yeasts and moulds were search using the classical

techniques of inoculation, serial dilution and plate counting.

To this end, 25 mL of each yellow *Achu* soup sample was transferred into flask containing 225 mL of sterile physiological water (NaCl 8.5‰, m/v) containing tween 80 (2‰, v/v) under sterile and aseptic conditions. The mixture was then vortexed and allowed to stand for 30 min so as to revive the microorganisms present. After revivification, serial dilution ranging from 10^{-1} to 10^{-8} were made.

Total aerobic and facultative anaerobic count

One millilitre (mL) of the respective sample dilutions was transferred into their respective Petri dishes followed by pouring about 20 mL of sterile plate count agar (PCA) (Scharlau) culture media after which it was homogenised. It was then allowed to stay at room temperature until it became solidified. The Petri dishes were then inverted and incubated at 30°C for 48 h. All the colonies were then counted after incubation and recorded.

Faecal and total coliforms

Twenty mL of sterile ENDO culture media (Scharlau) were poured into their respective Petri dishes and allowed to solidify at room temperature followed by surface inoculation with 0.1 mL of the respective sample dilutions. The culture media were then incubated at 37°C for total coliforms and 44°C for faecal coliforms for 24 to 48 h.

Faecal streptococci

Twenty mL of sterile Slanetz and Bartley culture media (Scharlau) were poured into their respective Petri dishes and allowed to solidify at room temperature followed by inoculation with 0.1 mL of their respective dilutions. The inoculated culture media was incubated at 37°C for 24 to 48 h. Red violet or brown colonies were then counted and recorded after incubation.

Yeasts and moulds

Sabouraud agar medium (Scharlau) together with 0.1 mg/mL of chloramphenicol was used to make the medium more selective for yeasts and moulds. The inoculation was made by spreading 0.1 mL of each respective sample dilution on the surface of the culture media present in the Petri dish. The Petri dishes were then incubated at 25°C for 3 to 5 days. The colonies present after incubation were then counted and recorded.

***Salmonella* spp.**

The isolation of *Salmonella* spp. was carried out on Salmonella-Shigella (SS) agar (Scharlau) after pre-enrichment of 25 mL of the yellow *Achu* soup samples in 225 mL of peptone water which were incubated at 37°C for 16 h. This step was followed with enrichment by transferring 1 mL of pre-enriched solution in a tube containing 10 mL of sterile selenite-cystine broth. The mixture was then vortexed and incubated at 37°C for 24 h followed by a surface inoculation with 0.1 mL of the mixture in Petri dishes containing 20 mL of SS agar medium and incubation at 37°C for 24 h. The presence of colonies with black spot or colourless colonies was then observed after the incubation period.

The microbiological qualities of the various yellow *Achu* soup samples were then determined based on the presence or absence of pathogenic and alteration microorganisms isolated using their respective culture media as described above.

Extraction of crude biosurfactants

Crude biosurfactants were extracted from *pendidam* using the method described by Mbawala et al. (2015b). Thus, the *pendidam* was centrifuged (6500 g, 20 min, 4°C), using a refrigerated Biofuge centrifuge (Primo R *Heraeus*). The supernatant was filtered (Whatman No. 1) followed by acidification with hydrochloric acid 6 N to a pH of 2. It was then incubated for 24 h at 4°C. The isoelectric point (lipoprotein nature) of the crude biosurfactants is attained at this pH resulting in a drop in solubility leading to precipitation. The crude biosurfactants were then extracted from the broth using a mixture of ethyl acetate and methanol (4:1v/v), respectively. The organic fraction was then evaporated to dryness under vacuum condition with a rotary evaporator (RV BASIC plus VWR by IKA) until film of biosurfactants remains; acetone was added to recover the crude biosurfactants. The acetone was evaporated and the crude biosurfactants collected was then weighed and stored at room temperature using aluminium foil paper.

The surface tension activity and the emulsification activity of the extracted compounds were assessed in order to confirm its biosurfactants properties using the method described by Abouseoud et al. (2008).

Antibacterial activities of crude extract of biosurfactants

To evaluate the effect of crude extract of biosurfactants against the microorganisms isolated from yellow *Achu* soup, we had to choose some strains of bacteria based on their most occurring capacity in almost all the yellow

Achu soup samples and on their degree of pathogenicity. Thus, the bacteria used for this test were some strains of faecal coliforms, faecal streptococci and *Salmonella* sp. In this study, the MIC and MBC of the crude biosurfactants against the above bacterial strains were evaluated.

Determination of the MIC

The inoculum was first prepared using the method of Clinical and Laboratory Standards Institute (CLSI, 2008). To this effect, pure strains of the bacteria to be used were streaked on their specific culture media and incubated for 16 h at their optimal growth temperature. Then, 4 to 5 pure colonies were collected using platinum wire and introduced in tubes containing 5 mL of sterile physiological water. The mixture was then homogenized using vortex apparatus for few seconds so as to obtain turbidity around the neighbourhood of standard of Mac Farland 0.5. In this case, the standard of Mac Farland 0.5 absorbance with a wavelength of 620 nm is supposed to be between 0.08 and 0.1. This absorbance corresponds to a microbial load of 10^7 to 10^8 cfu/mL (Hellal, 2011).

For antimicrobial tests, the minimum inhibitory concentrations were achieved for the crude biosurfactants concentrations of 12.5, 25, 50 and 100 mg/mL. Thus 0.5 mL of each inoculum was introduced in a tube containing 4 mL of tryptic soy broth (TSB), and then 0.5 mL of solutions containing crude biosurfactants at different concentrations was added. After homogenization, the tubes were incubated for 24 h at the respective growth temperatures of each strain. After incubation, the tube in which there was no visible growth of the respective microorganism was considered as the tubes with MIC.

Determination of the MBC

These tubes representing the MIC were then stored for the subsequent evaluation of MBC. In order to evaluate the MBC, 0.1 mL of liquid culture media in which there was no visual microbial growth when evaluating the MIC was used in inoculating specific culture media of each strain previously poured in Petri dishes. The inoculum were spread at the surface of culture medium followed by incubation for 24 h at the respective growth temperatures of each strain. The Petri dishes corresponding to the lowest concentration of biosurfactants, into which no visible colony appears, were considered as MBC.

In situ antibacterial activity of biosurfactants

To evaluate the antibacterial activity of crude extract of biosurfactants on yellow *Achu* soup, the soup was prepared while using the crude biosurfactants as emulsifier based on its emulsification activity. To this end, 200 g of the 19 spices was boiled in 300 mL of distilled water for 5 min, followed by filtration using Whatman No. 1 to obtain a solution of the spices which was then preserved in a sterile glass container for subsequent analysis (Mbawala et al., 2015a). Biosurfactants solutions were then prepared at a concentration of 40 mg/mL using sterile distilled water. The solutions were then vortexed for 2 min and preserved in sterile glass containers for the different analysis.

Preparation of yellow *Achu* soup was carried out using the method described by Mbawala et al. (2015a). As control, a yellow *Achu* soup prepared while using *kanwa* solution of 40 mg/mL in sterile distilled water as found in restaurant in the town of Ngaoundere, was used.

Thereafter, the microbiological analysis of the prepared soup was carried out using the same method previously described for samples collected in the town of Ngaoundere.

Statistical analysis

In this study, each test was carried out in triplicate and statistical analysis were done using Statgraphics centurion XVI.I software. Data were assessed by the analysis of variance (ANOVA). Duncan's multiple range tests were used to separate the means. Significance was accepted at ($p<0.05$).

RESULTS AND DISCUSSION

Microbiological quality of the soup samples

Total aerobic and facultative anaerobic count

Table 1 shows that the total aerobic and facultative anaerobic count of all samples analysed varies significantly ($p<0.05$) from one sample to the other with a microbial load greater than the expected norms (<10 cfu/mL) as stipulated by the Health Protection Agency (2009).

Among the samples collected and analysed in the town of Ngaoundere, samples C, D, E and F showed higher levels of contamination compared to samples A, B and G. This difference could probably be due to the variability of culinary techniques from one producer to another. In the same way the non-respect of good hygienic practices and good manufacturing practices equally account for this variation.

Total and faecal coliforms count

Total coliforms isolated in all the seven yellow *Achu* soup

Table 1. Microbial load of yellow *Achu* soups samples collected from some restaurants in the town of Ngaoundere (CFU/mL).

Microorganisms	A	B	C	D	E	F	G	Norms
Total aerobic and facultative anaerobic count	$17.30\pm1.36\times10^{3a}$	$15.43\pm1.23\times10^{3a}$	$36.00\pm2.81\times10^{3b}$	$33.30\pm5.77\times10^{3b}$	$38.30\pm1.08\times10^{3b}$	$34.00\pm5.20\times10^{3b}$	$17.73\pm1.02\times10^{3a}$	<10
Yeasts	$8.23\pm2.44\times10^{3b}$	$3.20\pm1.73\times10^{3a}$	$3.77\pm1.04\times10^{3a}$	$3.53\pm1.16\times10^{3a}$	$3.73\pm0.52\times10^{3a}$	$4.63\pm1.69\times10^{3a}$	$4.60\pm1.00\times10^{3a}$	$<10^2$
Moulds	-	-	-	-	-	-	-	<10
***Salmonella* spp.**	+	+	+	+	+	+	-	absent/25g
Faecal streptococci	$3.70\pm0.65\times10^{3a}$	$3.17\pm0.52\times10^{3a}$	$4.93\pm1.62\times10^{3b}$	$5.20\pm1.00\times10^{3b}$	$5.57\pm1.03\times10^{3b}$	$7.03\pm2.00\times10^{3b}$	$6.97\pm2.51\times10^{3b}$	<10
Total coliforms	$2.02\pm0.09\times10^{3a}$	$3.53\pm0.62\times10^{3a}$	$15.20\pm2.17\times10^{3b}$	$13.60\pm4.73\times10^{3b}$	$12.30\pm2.43\times10^{3b}$	$12.00\pm2.50\times10^{3b}$	$3.23\pm1.21\times10^{3a}$	$10^2\leq10^4$
Faecal coliforms	$1.32\pm0.14\times10^{3a}$	$1.20\pm0.26\times10^{3a}$	$3.40\pm0.58\times10^{3b}$	$3.33\pm1.08\times10^{3b}$	$3.30\pm0.65\times10^{3b}$	$3.27\pm1.06\times10^{3b}$	$1.23\pm0.29\times10^{3a}$	$20\leq10^2$

Values of mean ± standard deviation bearing the same letters at the exponent on the same row show that they have no statistical significant difference at $p<0.05$. **A, B, C, D, E, F and G** are yellow *Achu* soup samples collected from some restaurants in the town of Ngaoundere; **+,** presence of the microorganisms; **-,** absence of the microorganisms as stipulated by Health Protection Agency (2009). The experiments were done in triplicate.

samples revealed a heavy and significant contamination from one sample to another far above the norms ($10^2 \leq 10^4$ cfu/mL) as recommended by the Health Protection Agency (2009).

These coliforms bacteria can be a reasonable indication of the presence of other pathogenic bacteria thus a pointer to the sanitary condition of the yellow *Achu* soup.

The results of faecal coliforms isolated in all the seven yellow *Achu* soup samples also revealed a heavy and significant contamination from one sample to the other far above the norms ($20\leq10^2$ cfu/mL) of Health Protection Agency (2009). This substantial increase in contamination in this food suggests a general lack of cleanliness, improper storage and poor hygiene practices from one producer to the other as stipulated by Aboubakar et al. (2008).

Yeasts and moulds count

Yeast was present in all samples collected in the town of Ngaoundere with a load ranging from $3.20 \pm 1.73 \times 10^3$ to $8.23 \pm 2.44 \times 10^3$ cfu/mL. The contamination of the various yellow *Achu* soups with yeast cells varies from one sample to another. This variation of yeast load might be due to the spices used during the preparation.

However, the yeast load of all these samples obtained in this study was high than that recommended by the norms ($<10^2$) of Health Protection Agency (2009).

Concerning moulds, none of the sample was positive for the development of moulds. This could probably be due to the antifungal activity of spices used in the preparation of yellow *Achu* soup.

***Salmonella* spp.**

Salmonella spp. are pathogenic bacteria responsible for the diseases called salmonellosis which happen to be one of the most frequently reported food-borne diseases worldwide. The microbial analysis of the various yellow *Achu* soup above reveals that only one out of the seven samples was negative (sample G) for the presence of *Salmonella*. *Salmonella* spp. being pathogenic microorganisms must not be present in food as stipulated by Health Protection

Table 2. Minimum inhibitory concentration of crude extract of biosurfactants present in some selected strains isolated from yellow *Achu* soups obtained from restaurants in Ngaoundere town.

Microorganisms	Concentration of crude extract of biosurfactants (mg/mL)			
	100	50	25	12.5
Faecal coliform strain	-	-	-	+
Faecal *Streptococcus* sp.	-	-	+	+
Salmonella sp.	-	-	+	+

+, Presence of microbial growth; **-,** Absence of microbial growth.

Agency (2009).

The presence of *Salmonella* spp. in the remaining seven samples reveals a health risk attributed to the consumption of this food. Poor quality of raw materials or food components, undercooking, cross-contamination, poor temperature and poor cleaning may likely be the reason behind this contamination. The absence of heat treatment (pasteurisation) after preparing the yellow *Achu* soup is also a contribution to their presence in this food.

Faecal streptococci

Faecal streptococci represent with faecal coliforms a large group of biochemically and genetically related bacteria which are used to assess the general hygienic status of a food product. Their presence in food indicates a recent contact of this food with faecal matter of animals or humans being. In this study, all the yellow *Achu* soup samples collected in the town of Ngaoundere were positive and heavily contaminated with these bacteria far above the norms (<10) of Health Protection Agency (2009). Their presence in all samples may be due to the poor quality of raw materials or food components, undercooking, cross-contamination, poor temperature and poor cleaning, inadequate cooking or post-processing contamination as observed during sampling.

At the end of the microbiological analyses of the yellow *Achu* soup samples collected, the results obtained show that all the samples had a poor microbiological quality as pathogenic and spoilage microorganisms were present. In order to solve this problem, the antibacterial activity of crude biosurfactants against these microorganisms was carried out.

Antibacterial activities of crude extract of biosurfactants

Minimum inhibitory concentration of crude extract of biosurfactants

The MIC of the crude extract of biosurfactants was determined on three strains of microorganisms belonging to three different genera. The choice of such strains was based on the frequency of occurrence in most of the yellow *Achu* soup sample, danger attributed to their consumption, pathogenicity etc. Table 2 present the different MIC of crude biosurfactants on the test strains. The results show that the MIC values obtained for all the test strains varies from 25 to 50 mg/mL. The faecal coliform strain was the most sensitive to the activity of the crude biosurfactants (25 mg/mL) while the other strains which presented the same MIC were more resistant (50 mg/mL). The antimicrobial activity of the crude biosurfactants could be explained by the fact that, the fatty acid moieties of biosurfactants inserting into the cell membrane instigating a proliferation of membrane size and ultra-structural changes (Gomaa, 2013). In the same way, the ability of biosurfactants to form pores and disrupt the plasma membrane (Inès and Dhouha, 2015) could also be an explanation to this effect.

The MIC value registered for the faecal coliform strain is in agreement with that obtained by Mbawala et al. (2015b) who found out in their study that crude biosurfactants extracted from *pendidam* collected at Wakwa in Adamawa Region of Cameroon, have an MIC of 50 mg/mL against *E. coli.*

Minimum bactericidal concentration of crude extracts of biosurfactants

The MBC of crude biosurfactants against the test strains was equally evaluated and the results presented in Table 3. The results presented in Table 3 show that faecal coliform strain and the faecal *Streptococcus* sp. had the same value of MBC (50 mg/mL), meanwhile strain of *Salmonella* sp. had a higher value of MBC (100 mg/mL). The MBC value obtained in this study on the strain of faecal coliform is higher than those obtained by Mbawala et al. (2015b). Thus in their research works, they had an MBC of 100 mg/mL on faecal coliform strain (*E. coli*). This difference could be explained by the fact that the variation in the resistance of a microorganism varies with the specie and the strain used.

Table 3. MIC, MBC and MBC/MIC ratio of crude biosurfactants on some selected strains of bacteria isolated from yellow *Achu* soup collected from some restaurants in Ngaoundere town.

Microorganisms	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
Faecal coliform strain	25	50	2
Faecal *Streptococcus* sp.	50	50	1
Salmonella sp.	50	100	2

Table 4. Microbial load of yellow *Achu* soup samples prepared with and without crude extract of biosurfactants (CFU/mL).

Microorganisms	H	I	Norms
Total aerobic and facultative anaerobic count	$36.00\pm4.36\times10^{3a}$	$3.40\pm1.53\times10^{3b}$	<10
Yeasts	$3.03\pm1.52\times10^{3a}$	$0.31\pm0.05\times10^{3b}$	$<10^2$
Moulds	-	-	<10
Salmonella spp.	+	-	Absent/25 g
Faecal streptococci	$2.20\pm1.14\times10^3$	-	<10
Total coliforms	$11.50\pm3.21\times10^{3a}$	$6.03\pm0.04\times10^{2b}$	$10^2 \leq 10^4$
Faecal coliforms	$3.13\pm1.03\times10^{3a}$	$0.42\pm0.06\times10^{2b}$	$20 \leq 10^2$

Values of mean ± standard deviation bearing the same letters at the exponent on the same row show that they have no statistical significant difference at $p<0.05$. **H**, sample without biosurfactants; **I**, sample with biosurfactants, **+**, presence of the microorganisms, **-**, absence of the microorganisms as stipulated by Health Protection Agency (2009). The experiments were done in triplicate.

The results obtained show that the crude biosurfactants molecules extracted has bacteriostatic properties since the MIC was less than MBC at a limited dose and bactericidal at a very high dose. The results also reveal that the degree of bactericidal properties of the crude biosurfactants depends on the groups of microorganisms tested. Studies carried out by Oussou et al. (2008) revealed that when the ratio of MBC/MIC of an antibacterial substance is less than or equal to 4, then such a substance is considered to be bactericidal.

The ratio of MBC/MIC stands at 2 for faecal coliform strain and *Salmonella* sp. strains while that of faecal streptococci is 1. These results thus confirm the great potentials of crude biosurfactants as an antibacterial molecule that can be greatly exploited in yellow *Achu* soup preservation and other food products in agro-food industry.

In situ activities of biosurfactants

After evaluating the *in vitro* activity of crude extract of biosurfactants on some strains of bacteria, their emulsifying and antibacterial properties were exploited in the preparation of yellow *Achu* soup. A yellow *Achu* soup sample without crude biosurfactants was used as control. The results obtained is presented in Table 4.

Total aerobic and facultative anaerobic count

The results presented in Table 4 shows that the total count of yellow *Achu* soup without crude biosurfactants is very high ($36.00 \pm 4.36 \times 10^3$ cfu/mL) than those with crude biosurfactants ($3.40 \pm 1.53 \times 10^3$ cfu/mL) as recommended by the Health Protection Agency (2009). The significant drop in the sample with crude biosurfactants could probably be due to the antibacterial effect of the crude biosurfactants molecules.

However, the fact that a complete inhibition of microorganism in yellow *Achu* soup was not realise could be due to the poor microbiological quality of the palm oil as well as the spices used. In the same way, the interaction of crude biosurfactants with some compound present in yellow *Achu* soup such as palm oil fatty acid or phenolic compound of spices has probably reduced the activity of crude biosurfactants.

Total and faecal coliforms

Crude biosurfactants based yellow *Achu* soup sample presented the least contamination load of faecal and total coliforms compared to sample without crude biosurfactants. In the same way, the microbial load of this yellow *Achu* soup, was within the norms of Health Protection

Agency (2009). The decrease in faecal and total coliforms load observed in the sample made with crude biosurfactants may be attributed to the antibacterial effect of the crude biosurfactants molecules on this yellow *Achu* soup. Similar results have been reported by Mbawala et al. (2015b) who reported that the introduction of crude biosurfactants in *pendidam* contributed to a significant reduction of it faecal coliforms load.

Salmonella spp. *and faecal streptococci*

A complete inhibition of *Salmonella* spp. and faecal streptococci was observed in the sample made with crude biosurfactants. This may be due to the antibacterial activity of biosurfactants which has completely inhibited these microorganisms.

Yeast and moulds

No moulds growth was observed in any of the *Achu* soup sample unlike yeast that had $3.03 \pm 1.52 \times 10^3$ cfu/mL greater than the norm ($<10^2$) in the sample made without crude biosurfactants while $0.31 \pm 0.05 \times 10^3$ cfu/mL was observed in the sample with crude extract of biosurfactants. The reduction of yeast load in the crude biosurfactants sample may be due to the antifungal effect of the crude biosurfactants. This results are in accordance with that reported by Mbawala et al. (2015b) who found out that the application of crude biosurfactants into *pendidam* contributed to a significant reduction of *Candida* spp.

Conclusion

This research work has revealed that yellow *Achu* soup sold in the town of Ngaoundere are contaminated by pathogenic and spoilage microorganisms such as faecal streptococci, *Salmonella* spp., total and faecal coliforms and yeasts. The level of contamination of these microorganisms in all the yellow *Achu* soup samples analysed was higher than those recommended by the standards. The crude biosurfactants extracted from *pendidam* presented antibacterial activity against strains of faecal coliforms, faecal streptococci and *Salmonella* isolated from yellow *Achu* soup samples. The use of crude biosurfactants as emulsifier in the preparation of yellow *Achu* soup contributed significantly in reducing its microbial load thereby improving on the microbiological quality of yellow *Achu* soup. Based on this study, crude extract of biosurfactants is efficient against some harmful microorganisms and at the same time, a good alternative to chemical emulsifier. This dual functional properties exhibited by these bio-molecules can thus be exploited by food industries in food formulation.

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Competing interests

Authors have declared that no competing interests exist.

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