Microbiology Research Journal International

31(5): 21-26, 2021; Article no.MRJI.71226
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Evaluation of the Microbiological Quality of Soy Cheeses Sold at the Dantokpa Market in the Municipality of Cotonou in Benin

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Authors' contributions

This work was carried out in collaboration among all authors. Author CKCT designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors CTRK, RTMB, KCMS, AAMD, PSAS and JSBB managed the analyses of the study and performed the statistical analysis. Authors KTA, EA and IPBY managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2021/v31i530317
Editor(s):
(1) Xing Li, Mayo Clinic College of Medicine, USA.
Reviewers:
(1) Ahmed Alluwaimi, King Faisal University, Saudi Arabia.
(2) Ratmawati Malaka, Hasanuddin University, Indonesia.
Complete Peer review History: https://www.sdiarticle4.com/review-history/71226

Original Research Article

Received 15 May 2021
Accepted 21 July 2021
Published 29 July 2021

ABSTRACT

The objective of this study is to contribute to improving the health quality of soy cheese produced and sold in Benin, through the evaluation of the microbiological quality of samples taken at the Dantokpa market in southern Benin. Samples were taken from the women producers and sellers of this cheese in this market. A total of two hundred and forty (240) samples were collected from five producer-sellers. The cheese samples were sent under suitable conditions to the laboratory where

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various microbiological analyzes were performed. The results revealed that salmonella, sulfite-reducing anaerobic bacteria (SRA), *Escherichia coli* and *Staphylococcus aureus* were absent in all samples. In contrast, aerobic mesophilic bacteria and total coliforms were present at loads above the limit values established for food intended for human consumption. The microbiological quality of the products analyzed was generally unsatisfactory. Consequently, it is necessary to put in place a policy to raise awareness among producers-sellers of the markets on hygiene rules in order to guarantee the safety of consumers.

Keywords: Public health; microbiological quality; cheese; soya; Dantokpa market; Benin.

1. INTRODUCTION

In Benin, as in most African countries, thousands of plants are used daily for both nutritional and medicinal reasons. Among these plants are soybeans. Soybean, *Glycine max* (L.) Merrill, is an annual, hairy herbaceous plant belonging to the legume family. It has a vegetative habit and a reproductive system characteristic of the Fabaceae family [1] and bears pods of 3 to 5cm, each of which contains 2 to 4 seeds [2]. Soy is a legume with a high protein content [3]. It represents an excellent protein substitute for less accessible meat products and offers several processing possibilities for human food thus contributing to the food and nutritional balance of the population [4]. This plant has become a star food not only for its nutritional value, but also for its multiple culinary and medical potential. For several years, it has been used in various forms and food preparations in both human and animal nutrition. Among the usual products derived from soybeans intended for human consumption, we can cite: soybean oil, soya yogurt, infant flours. Because of to the ingenuity of women, soybeans entered their last years into the dietary habits of the Beninese population in the form of soy cheese and quickly spread to all segments of the population. In Benin, soy cheese, locally called *Amonsoja*, is one of the products from the processing of soy beans. The soybean cheese manufacturing method uses soybeans as the main raw materials. Magnesium sulfate and *Guissin* (Acidic liquid corn starch supernatant) are ingredients that are used as coagulants. Two stages are necessary for the production of cheese: the production of raw soy milk and then the manufacturing of cheese. Soy cheese for its nutritional quality is a valid substitute for meat and fish in many households in Benin [5]. However, it is common to see ignorance and lack in hygiene rules during its production and commercialization, which does not guarantee the hygienic quality of the product. Indeed, because of the artisanal nature of the soybean cheese manufacturing process, the producer-sellers work in unsanitary conditions. This could allow bad microbiological quality of the product and by extension affect the health of consumers. Several previous studies have shown that food poisoning in Benin is generally caused by the consumption of street foods that are full of pathogenic microorganisms that have negative impacts on consumer health [6,7]. As soy cheese is currently part of these street foods, it is therefore important to evaluate the microbiological quality of cheese in order to assess the risks to consumer safety. The other major interest of this study is that it addresses a public health issue. The available literature revealed a lack of data on the quality of this food sold in the city of Cotonou in Benin. This study will fill this gap. The objective of this study is to contribute to improving the health quality of soy cheese produced and sold in Benin through the evaluation of the microbiological quality of samples taken at the *Dantokpa* market.

2. MATERIAL AND METHODS

2.1 Vegetable Material

The plant material was soy cheese samples (Fig 1). This cheese is classified as hard cheese. This cheese been has processed, and sold at sides of streets or in family at home in Benin.

2.2 Sampling Method

Sampling was carried out randomly from five soybean cheese sellers on three different occasions at a regular seven-day interval in the Dantokpa international market located in the commune of Cotonou. For each sampling, sixteen samples were collected per vendor for the evaluation of microbiological parameters. On each occasion, the 80 coded samples were sent to the laboratory in batches of sixteen in five different sterile bags (Photo1) a cooler containing cold accumulators for microbiological analyzes. Thus, the sixteen taking (240 samples) were characterized. Cheese from the five sellers were respectively codified cheese N^{o} 1, N^{o} 2, N^{o} 3, N^{o} 4, N^{o} 5.

Fig. 1. Photos of the different cheese samples

2.3 Microbiological Analysis Methods of Samples

Microbiological analyzes focused not only on the search for pathogenic microorganisms but also on germs indicative of good hygiene practices in food products [8,9]. The culture media were prepared according to the manufacturer's instructions and maintained in supercooled until the time of inoculation except for Baird Parker Agar (BPA) enriched with egg yolk and potassium tellurite which was pre-poured into a Petri dish. To prepare the stock solution, 10 g of each sample was taken with a sterile spatula in a sterile stomacher bag to which 90 ml of tryptone salt broth was added. This mixture was homogenized in a sample mixer. Successive decimal dilutions were made from the stock solution. The inoculation was performed using mass inoculation technique except for BPA which was inoculated on the surface. Total bacteria was counted on the Plate Count Agar (PCA) after incubation at 30 °C for 72 h ± 2 h [10], the total coliforms on the Violet Red Bile Glucose (VRBG) after incubation at 30 °C for 24h ± 2h [11], staphylococci on BPA with egg yolk and potassium tellurite after incubation at 37 °C for 48h ± 2h [12]. The search for *Escherichia coli* β-glucuronidase was carried out using coliform dishes on Violet Red Bile Lactose Agar (VRBLA) by performing the Mac-Kenzie test (indole and oxidase) using Kovacs and oxidase reagents [13]. The enumeration of the anaerobic sulphito-reducing bacteria was carried out on Tryptone Sulfite Neomycin Agar (TSNA) after incubation at 46 °C for 20 h ± 2 h [14]. Finally, the search for salmonella was carried out on Salmonella Shigella Agar (SSA) according to standard NF EN ISO 6579 [15]. The microbiological analysis were carried out in three repetitions on each sample. The number of germs was expressed in Colony Forming Units per gram (CFU/g).

2.4 Statistical Analysis

The data collected were analyzed using SPSS version 16 software, which was used to perform analysis of variance (ANOVA) and Tukey's test for means comparison. The significance level retained was 5%. The averages of the microbial loads (CFU/g) found in the samples were compared with the limit values “m” and “M” set for cheese according to the microbiological criteria applicable to foodstuffs [16]; "M" represents the number of CFU/g below which the samples have a good bacteriological quality. If the number of CFU/g obtained is between "m" and "M", the samples are judged to be acceptable (poor), and the samples containing numbers of CFU/g greater than "M" are non-compliant.

3. RESULTS AND DISCUSSION

3.1 Results

Analysis of Table 1 shows that *Escherichia coli*, *Staphylococcus aureus*, anaerobic sulphito-reducing bacteria and salmonella were absent in soy cheeses sold in the Dantokpa market. However, these samples contained significant loads of total coliforms and aerobic mesophiles. These microbial loads obtained were greater than the limit values set by the AFNOR criteria in

2001 cited by Tchekessi [17]. With the exception of cheese samples N°1 and N°4 whose microbial load in total coliforms does not exceed the criteria. The same is true for cheese samples N° 1, N° 3, N° 4 and N° 5 with regard to the total bacteria.

3.2 Discussion

Analysis of Table 1 shows that the total coliform microbial loads of the samples of cheese N°2; cheese N°3 and cheese N°5, respectively $1.15x10^3 \pm 11$; $6.5x10^2 \pm 9.1$ and $7.0x10^2 \pm 9.9$ CFU /g exceeded the set standard (<100 CFU/g) for food intended for human consumption. The presence of these germs in the samples testifies to contamination from faecal and environmental origin as pointed out by Mhone [18] and Bonfoh [19] who indicated that the contamination of food by total coliforms can come from the environment, the action of factors such as wind, dust and also contamination of human origin through handling and biological secretions such as saliva, sweat, etc. Thus, the presence of these germs can be explained non-compliance with hygiene rules during the marketing of soy cheese. We noted that the microbial load of mesophilic aerobic germs ($1.31x10^5 \pm 15$ CFU/g) for cheese N° 2 exceeded the limit value ($<10^5$ CFU/g) set by the standard, unlike the cheese N° 1, N° 3, N° 4 and N° 5 samples, which microbial loads were respectively $2.26x10^3 \pm 2.6$ CFU/g, $4.3x10^4 \pm 2.9$ CFU /g, $8.3x10^4 \pm 10$ CFU/g and $1.97x10^4 \pm 2.4$ CFU / g. The high level of total bacteria recorded in the samples N° 2 would be linked to the exposure of these cheeses to the open air during marketing. The difference at the 5% threshold ($p<0.5$) was observed at the level of Aerobic Mesophilic germs on the one hand and total coliforms on the other hand of the samples of the different media (N° 1, N° 2, N° 3, N° 4 and N° 5). These results can also be explained by the fact that the producers and the salesmen do not respect the rules of hygiene during the marketing of the cheese of soya and even sometimes contaminate the samples of cheese after cooking on the places of sale. These analyzes agree with those of Tchekessi [17] who showed that the aerobic mesophilic germs encountered in foodstuffs after cooking germs could come from the environment. The results also corroborate the work of Bayoi *et al.* [20] who underlined that the high microbial load of total germs encountered in fruit juices comes from the unsanitary production and / or sales sites conditions. The results also showed the absence of Salmonella, sulfitor-reducing anaerobic bacteria, *Escherichia coli* and coagulase-positive staphylococci in all samples. These results are in accordance with the thresholds fixed for cheese by the international standards EN ISO 6579 (absent/25 g), NF EN ISO 15213 (absent/20 g), ISO 16649-2 (100 CFU/g), EN ISO 6888-1(100 CFU/g). This is contrary to the results from Djogbe *et al.* [21] who counted *Staphylococcus aureus* up to $146.05x10^2 \pm 0.045$ CFU/g in samples of cheese sold in the streets of Cotonou. In addition, the absence of salmonella, anaerobic sulphitor-reducing bacteria, *Escherichia coli* and

Table 1. Microbial loads of the germs sought in the samples of soybean cheese taken from the Dantokpa market

SAMPLES	GERMS (CFU / g)					
	Aerobic Mesophilic Germs	**Total coliforms**	***Staphylococcus aureus***	***Escherichia coli***	**Anaerobic Sulfitor-Reducing Bacteria (ASR)**	**Salmonella**
Cheese N° 1	$2.26.10^3 \pm 2.6^a$	0.00 ± 00^a	$<1^a$	$<1^a$	$<1^a$	$<1^a$
Cheese N° 2	$1.31.10^5 \pm 15^b$	$1.15.10^3 \pm 1.1^b$	$<1^a$	$<1^a$	$<1^a$	$<1^a$
Cheese N° 3	$4.3.10^4 \pm 2.9^c$	$6.5.10^2 \pm 9.1^c$	$<1^a$	$<1^a$	$<1^a$	$<1^a$
Cheese N° 4	$8.83.10^4 \pm 10^d$	$3.0.10^1 \pm 0.42^d$	$<1^a$	$<1^a$	$<1^a$	$<1^a$
Cheese N° 5	$1.97.10^4 \pm 2.4^e$	$7.0.10^2 \pm 9.9^c$	$<1^a$	$<1^a$	$<1^a$	$<1^a$
Criteria	$<10^5$	$<10^2$	$<10^2$	$<10^2$	Absent in 20g	Absent in 25

Average values with the same letter in the same column are not significantly different at the 5% level. Data represents in table is mean of three replications. ± Standard deviation

coagulase-positive staphylococci in the samples could be justified by their inhibition by the acidity of the medium resulting from the fermentation. Banon [22] and Tchekessi *et al.* [23] reported that the absence of these germs is a good indicator of the level of hygiene during the production and/or sale of food. These authors also indicated that the presence of coliforms in food indicates contamination after heat treatment and poor hygiene during sale. Thus, with the presence of coliforms in almost all of the samples (Cheese N°2, Cheese N°3, Cheese N°4, Cheese N°5), we can also assimilate the absence of salmonella, anaerobic sulfitor-reducing bacteria (ASR), *Escherichia coli* and coagulase positive staphylococci to the fact that these germs were not present in the immediate environment of the production or marketing of the cheese samples analyzed. In total, only the Cheese N°1 samples were satisfactory hygienic quality.

4. CONCLUSION

The results of this work have shown that the samples analyzed do not meet microbiological quality standards. The degree of contamination detected reflects a lack of hygiene, whether in terms of producers or of storage and sale conditions. The problem has shown that consumers of this product are exposed to the risks of food poisoning. To remedy this, all actors in the production chain should be encouraged to adopt good hygiene practices through awareness programs in order to allow the population to enjoy good health by consuming good quality cheese. From the microbiological analyzes performed on the samples of soybean cheese, it appears that these were unsatisfactory microbiological quality. The level of contamination detected reflects a relative lack of hygiene especially in the production environment and food handlers during sale.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/71226