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Physicochemical and Microbiological Characteristics from "*Wagashi*" Production Whey in Abomey-Calavi (Benin)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OD, SBT, HS, LBM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AHS, SBT, AA and FBM managed the analyses of the study. Authors OD, AHS, HS and LBM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The production of "wagashi", induce the production of whey which is often directly drop in the environment. The aim of this study was to evaluate the microbiological and physicochemical qualities of wagashi's whey samples collected in Abomey-Calavi (Benin).
Methodology: Whey samples were collected from Abomey-Calavi (Parana and Akassato) in Southern Benin. The physicochemical analyses targeted the pH, titratable acidity, dry matter

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contents, protein and lactose. The microbiological analyzes carried out consisted in enumeration of total mesophilic flora (TMF), fecal coliforms, lactic acid bacteria, staphylococci, yeasts and molds, *Pseudomonas*, *Escherichia coli* and salmonella by cultures on specific synthetic nutrient media.
Results: This study revealed characteristics such as dry matter (5.30-5.66 g / l), pH (3.91-5.21), titratable acidity, protein (5.94-0.128 g / l), lactose; and microbial quality of the whey. Parana's whey was more acidic (pH = 3.91 ± 0.014) than Akassato's whey analyzed with higher titratable acidity (pH = 0.92 ± 0.01). The presence of total mesophilic aerobic flora, *Escherichia coli*, staphylococci, lactic acid bacteria, yeasts and molds reveals fecal, human and environmental contamination during cheese production or during whey storage. Thus, Parana's whey was more contaminated with TMF ($3.45.10^8$ CFU/ml), lactic acid bacteria ($4.82.10^8$ CFU/ml) and fecal coliforms (> 3.10^8 CFU/ml) while Akassato's whey was more contaminated by staphylococci ($4.70.10^8$ CFU/ml) and *Escherichia coli* (> 3.10^8 CFU/ml). Salmonella was not identified.
Conclusion: It thus important raising the awareness of cow's milk processors and / or whey producers in Benin. We therefore project to use this whey as a substrate for bioproduction.

Keywords: Whey; cow's milk; microbiological quality; physicochemical; Benin.

1. INTRODUCTION

The current challenge for our society is ago-food transformations waste recycling into new products in order to minimize the damage caused to the environment. The recovery of agro-industrial residues or by-products is back on the agenda and the concept of waste has evolved to be replaced by the term by-product [1]. Among these by-products is whey, which is the main by-product from the dairy industry [2]. It is undoubtedly a noble and rich material. Indeed, it has become an interesting source of active compounds and specific nutrients, exhibiting incomparable properties, both nutritionally and techno-functionally [3]. Whey is reported to be rich in many components such as lactose, soluble proteins, water-soluble vitamins, fats and mineral elements [4]. In recent years, whey has aroused the interest of industries. To this end, several products with high added value have emerged, some in a concentrated form and others in a fractional form of this whey [2]. Among these products it is the production of whey powder (intended for human and animal consumption) and lactose which predominate. However, the current trend is to obtain new products such as carboxylic acids or succinic acid. The management of whey can generate both economic and ecological interest. The quality of any product depends in part on the conditions of its production and storage. The conditions for producing milk cheese are those which lead to the production of whey. In Benin, the production of "wagashi", a traditional milk cheese is mainly done in precarious conditions. The objective of this study was to evaluate the microbiological and physicochemical qualities of whey samples collected in Abomey-Calavi (Benin).

2. MATERIALS AND METHODS

2.1 Samples Collections

Whey samples were collected from Parana and Akassato, two localities of Abomey-Calavi (between 6.45 latitudes and 2.35 longitudes, at 6 ° 27 ′ 0 ″ in the north and 2 ° 21 ′ 0 ″ in the east) in Southern Benin. Samples were taken in 1.5L plastic bottles with closed caps. A total of 7 samples were collected in each locality at the rate of one sample every 2 days for 2 weeks where each daily sample consisted of 3 different samples. Once collected, samples were carried out at the Laboratory of Biology and Molecular Typing in Microbiology (LBTMM) of the University of Abomey-Calavi (UAC) in Benin for physicochemical and microbiological analyzes.

2.2 Physicochemical Analyzes of Collected whey

These analyses targeted the pH, titratable acidity, dry matter contents, protein and lactose, as described below.

2.2.1 PH determination

The pH was determined using a digital pH-meter calibrated with buffers (pH 4 and 7). To 10 ml of whey was added 20 ml of distilled water. The whole was homogenized before the pH reading using a pH meter.

2.2.2 Determination of titratable acidity

The titratable acidity was determined according titrimetric method as described by AOAC (2000).

2.2.3 Determination of dry matter content

The water and dry matter contents were determined by the AACC method from 5g of product, by desiccation (105 ±2°C for 72h) and by differential weighing [5]. This dry matter content was determined by the formula: MS (%) = [(M_1-M_2) / M] X 100.

Where MS is the dry matter, M_1 is the mass (g) of the capsule and the test portion before drying, M_2 is the mass (g) of the capsule and the test portion after drying, and M is the mass (g) of the empty capsule.

2.2.4 Protein assay

The determination of proteins in the whey samples was carried out according to the Kjeldahl method [5]. This method consists of three phases: mineralization, distillation and titration. The following equation helps determine the protein content of whey.

Protein Content (%) = [((V-Vt) x N x1.041 x 6.25) / M] X 100

Where V is the volume of HCL used for the titration in ml, Vt is the volume in ml of HCL used for the titration of the control, N is the titer of acid used for the titration, M is the mass in grams of the sample, 6.25 is the conversion factor of nitrogen to protein, and 1.041 is a constant.

2.2.5 Lactose determination

The lactose detection assay was performed by a DNS colorimetric method [6]. In order to determine the amount of lactose present in the unknown solutions, a standard curve with a 0.5 g/l lactose solution was carried out with known solutions of lactose. Fig. 1 below illustrates the standard curve for lactose assay by the DNS colorimetric method.

2.3 Microbiological Analyzes of Collected Whey

The microbiological analyzes carried out consisted in enumeration of total mesophilic flora (TMF), fecal coliforms (FC), lactic acid bacteria, staphylococci, yeasts and molds, *Pseudomonas*, *Escherichia coli* and salmonella by cultures on synthetic nutrient media specific to each germ. The enumeration was done by counting the colonies [7]. In accordance with the AFNOR NF V 08-051 [8] standard, stock solutions were prepared and diluted.

2.3.1 Enumeration of the total Mesophilic flora

The count was carried out with reference to the ISO 4833 standard [9]. To do this, 1 ml of the initial suspension and / or of each of the dilutions retained was taken and inoculated into sterile Petri dishes. A volume (15 to 20 ml) of Plate Count Agar (PCA-OXOID CM 0463) previously melted and supercooled were poured onto the inoculum. After homogenization of the inoculum on the agar by gentle rotary movements and solidification, a second layer of 5 ml of PCA was poured for the realization of the double layer. The petri dishes were then incubated at 30°C. for 72 h ±3 h before reading.

2.3.2 Enumeration of fecal coliforms

The enumeration of fecal coliforms (CF) was carried out according to standard NF V 08-050 and NFV 08-060 [10]. A quantity (1 ml) of the initial suspension and / or of each of the dilutions retained was taken and inoculated into sterile petri dishes; 15 to 20 ml of solid crystal violet and neutral red lactose bile agar (VRBL), previously melted and brought at 45°C, were poured onto the inoculum. After homogenization of the inoculum on the agar by gentle rotary movements and solidification, a second layer of 5 ml of VRBL was poured for the realization of the double layer. These dishes were incubated at a temperature of 44°C for 24 h.

2.3.3 Enumeration of lactic acid bacteria

Lactic flora was counted on the Man Rogosa Sharp (MRS) culture medium [11]. A quantity (1 ml) of the initial suspension and / or of each of the dilutions retained was taken and inoculated into sterile petri dishes. A volume (15 to 20 ml) of solid MRS previously melted and supercooled was poured onto the inoculum. After homogenization of the inoculum on the agar by gentle rotary movements and solidification, a second layer of 5 ml of SRM was poured for the realization of the double layer. Petri dishes were incubated at a temperature of 37°C for 24-48 h before reading.

2.3.4 Enumeration of staphylococci

The enumeration of staphylococci was carried out on Baird Parker agar according to standard [12]. The seeding technique used here was that of surface seeding. Staphylococci were counted by addition and plating using a sterile 0.1 ml

spreader of the initial suspension or dilutions considered on BP agar added with egg yolk and potassium tellurite. Incubation was done at a temperature of 37°C for 48 h.

2.3.5 Enumeration of fungal flora: yeasts and molds

The enumeration of the fungal flora was carried out with reference to standard [13]. The medium used for the search for fungal flora was Dichloran Rose Bengal Chloramphenicol (DRBC) agar. The presence of chloramphenicol, a thermostable antibiotic and of chlortetracycline had made it possible to reinforce the selectivity of the medium with respect to most contaminating bacteria. Thanks to tergitol, the proliferation of *Mucoraceae* was limited. Previously prepared and sterilized, 10 to 15 ml of DRBC were pre-poured into the sterile petri dishes. Using a sterile pipette, 0.1 ml of the stock suspension or selected dilutions was transferred to the surface of the pre-cast dishes. The inoculum was spread until completely absorbed by the medium. The whole was incubated at 25±1°C for 5 days.

2.3.6 Enumeration of *Escherichia coli*

The enumeration of Escherichia coli was demonstrated by the Tryptone Bile X-Glucuronide (TBX) culture medium according to the ISO standard [14]. A quantity (0.1 ml) of the stock suspension or of the dilutions considered was added and spread over 15 to 20 ml of the TBX culture medium previously prepared in sterile petri dishes. The thus seeded petri dishes were incubated at a temperature of 44°C for 21 to 24 h.

2.3.7 Enumeration of *Pseudomonas* ssp

The medium used for the detection of *Pseudomonas* was cetrimide Agar with the addition of glycerol during the preparation of the culture medium [15]. Cetrimide was the selective agent and inhibited most bacteria by acting as a detergent. Previously prepared and sterilized, 10 to 15 ml of cetrimide Agar was pre-poured into the sterile petri dishes. Using a sterile pipette, 0.1 ml of the stock suspension or selected dilutions was transferred to the surface of the pre-cast dishes. The inoculum were spread using a sterile Pasteur pipette until completely absorbed by the medium. The whole was incubated at a temperature of 37°C for 24 h.

2.3.8 Enumeration of Salmonella

The detection of salmonella was carried out in four stages according to ISO 6579-1 [16]: pre-enrichment, enrichment, selective isolation and identification.

2.3.9 Enumeration in solid medium (calculated by CFU)

The calculation was made according to the ISO 7218 [17] standard which stipulates that:

- when the count in the first box is $1 \leq n1 < 4$, we have $N < 4 / d1$;
- when the count in the first dilution is less than 300, we have $N = (n1 + n2) / ((V \times 1.1) \times d1)$;
- when the count in the first dilution is $4 \leq n1 \leq 10$, we have $N = n1 / d1$;
- when the dish of the first dilution contains more than 334 colonies and the second dilution $n2 \geq 8$, we have $N = n2 / d2$;
- when each of the two dishes contains more than 300 colonies, we have $N > 300 / d2$; and
- when there are no colonies in the first dish, we have $N < 1 / d1$,

Where n1 is the number of colonies on the first dish, n2 is the number of colonies on the second dish, d1 is the first dilution, d2 is the second dilution, V is the volume of the inoculum, and N is the number d 'Colony Forming Units per gram (CFU / g).

2.4 Data Proceeding and Statistical Analysis

The means and standard deviations were calculated from the experimental results using an Excel 2013 spreadsheet. The Graph Pad Prism 8 software was used to draw the graphs of the microbiological analyzes and to determine the significant differences at the 5% threshold between the means. calculated from physicochemical and microbiological tests.

3. RESULTS AND DISCUSSION

3.1 Result

3.1.1 Physicochemical parameters of whey

Table 1 shows the physicochemical parameters of the different whey samples. The pH values of the whey analyzed varied between 3.91 ± 0.014 and 5.21 ± 0.014 (Table 1). Parana's whey was more acidic (pH = 3.91 ± 0.014) than Akassato's whey analyzed with higher titratable acidity (pH = 0.92 ± 0.01). There is a highly significant difference (p <0.0001) between the pH of the two-whey analyzed as well as their titratable

acidity (p <0.0001). Considering the lactose content, Akassato's whey (43.2 g / l) was slightly higher than that of Parana (41.6 g / l). In addition, the whey analyzed had a dry matter content that varied between 5.30 ± 0.019 and 5.66 ± 0.004. There is a highly significant difference between the dry matter content of the whey tested (p <0.0001). Akassato's whey has a higher dry matter content (5.66 ± 0.004). The results obtained show that the whey analyzed have a protein level which varied between 5.94 ± 0.091 and 6.23 ± 0.128 (Table 1). However, Parana whey has a higher protein content (6.23 ± 0.128). There is a highly significant difference between the dry matter content of the whey tested (p <0.0001).

3.1.2 Microbiological parameters of whey

The Fig. 2 shows the microbial load of the whey collected from Parana and Akassato (Abomey-Calavi). Analysis of variance showed that there is no significant difference between the presence of microorganisms in whey (p> 0.05). However, difference recorded was significant between the microorganism load of the whey (p<0.0477). It can be seen from this figure that regardless of the type of microorganism involved, the contamination rates in the whey samples were high. Parana's whey was more contaminated with TMF ($3.45.10^8$ CFU/ml), lactic acid bacteria ($4.82.10^8$ CFU/ml) and fecal coliforms (> 3.10^8 CFU/ml) while Akassato's whey was more contaminated by staphylococci ($4.70.10^8$ CFU/ml) and *Escherichia coli* (> 3.10^8 CFU/ml). Salmonella was not identified (Fig. 2).

3.2 Discussion

3.2.1 Physicochemical parameters of whey

The physicochemical characteristics of the whey sampled at Parana and Akassato indicate that these two-whey tested are acidic. The measured pH values for Parana's whey (3.91 ± 0.014) and Akassato's (5.21 ± 0.014) are comparable with those found by some authors [18]. The acidic pH obtained in Parana whey can be explained by the presence of certain lactic acid bacteria such as lactic acid bacteria which can produce lactic acid during sampling and storage. In addition, the mean titratable acidity of whey is 0.635 ± 0.285. It should be noted that the acid whey must have an acidity greater than 18D° [19]. These results confirm that the whey analyzed is obtained following lactic fermentation.

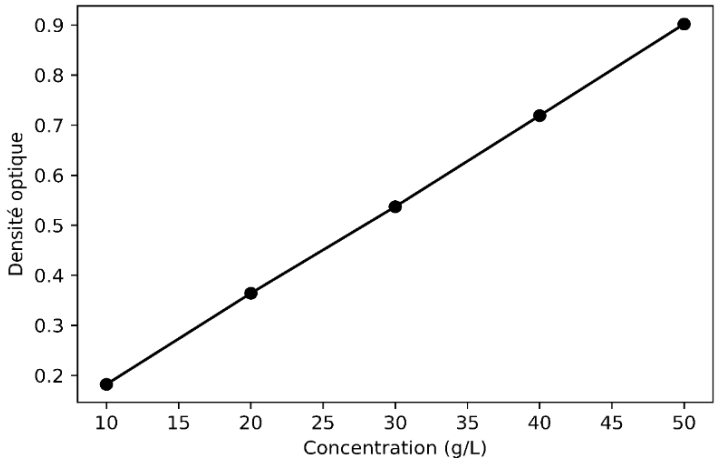

Fig. 1. Standard curve for lactose assay by the DNS method

Table 1. Physicochemical parameters of whey

Samples	Dry matter (g / 100 g)	pH	Titratable acidity	Proteins (g / 100 g b.s.)	Lactose (g / l)
Akassato whey	5.66 ± 0.004	5.21 ± 0.014	0.35 ± 0.01	5.94 ± 0.091	43.2±0.071
Whey Parana	5.30 ± 0.019	3.91 ± 0.014	0.92 ± 0.01	6.23 ± 0.128	41.6±0.102
Average	5.48 ± 0.18	4.56 ± 0.65	0.635±0.285	6.085±0.145	42.4 ± 0.8

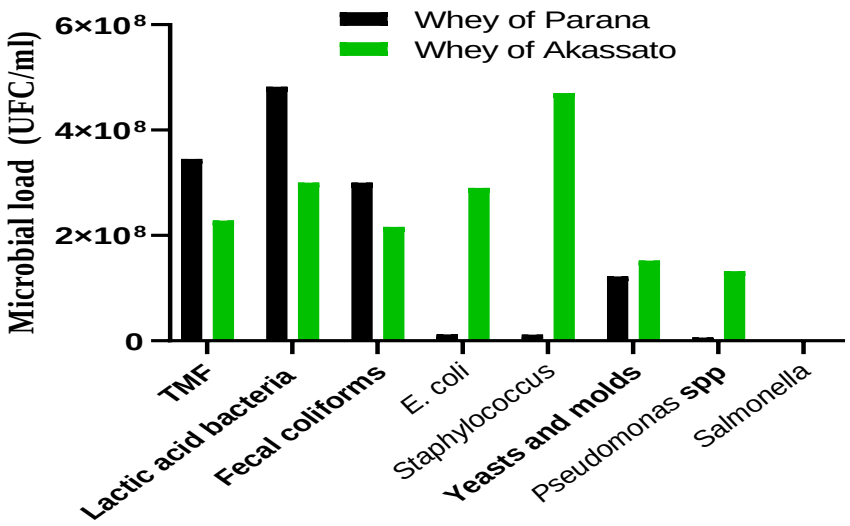

Fig. 2. Comparison of the microbial load between two zones before pasteurization

The average of the values found during the determination of whey proteins from is 6.085 ± 0.145 in g / 100g b.s. This result is lower than the 12g/l found by Woo (2002) [20]. The protein content of the whey sampled is higher than that reported for sweet [21] or sour [3] whey. The low level of protein obtained is probably due to the protein-deficient raw material used in the manufacture of cheese. The average dry matter content value noted in the whey studied is 5.48 ± 0.18 in g/100g. The dry matter contents noted in each whey were lower than the 6.6% reported by Blaschek et al. [22].

Depending on the coagulation process and the initial composition of the milk (season, breed of animals, type of feed, etc.), the composition of whey can vary significantly (Schuck et al., 2004). Whether sweet or sour, whey contains over 90% water. It also contains a high content of lactose (75% of the dry matter), a low content of soluble milk protein (about 20% of total milk protein) and various minerals [23]. In our study, Parana whey is more acidic (pH 3.91 ± 0.014) with a higher protein content. According to previous work, sweet whey has a higher protein content than sour whey due to the acid precipitation of some proteins [24,25]. The major difference between these two categories of whey lies in the content of lactose and lactic acid. In addition, the protein content could depend on the quality of the milk used and the coagulant. These problems are in part due to the high mineralization, low lactose content, and variability in the composition of acidic whey [26].

Lactose is the most important constituent of whey, as well as the essential substrate of lactic ferments [20-27]. Its initial content in whey can induce the course of fermentation. Also, the reduction in the concentration of lactose during the fermentation process can lead to a drop in the pH of the medium. This will have effects on the texture and the aging process [28]. In our study, the average lactose value is estimated at 42.4 g/l. This low content could be linked to the high microbial activity during cheese manufacturing as explained by Sweeney et al. [29].

3.2.2 Microbiological parameters of whey

Concerning the microbiological quality of the whey, the results obtained indicate a significant difference between the microorganism load of the analyzed whey. Total mesophilic aerobic flora is significantly present in whey samples. This value shows a fairly high level of contamination. This may be due to the environment in which the cheese is produced. With the composition of whey, it would constitute a good broth for the development of aerobic mesophilic germs. This contamination has also been reported in raw milk samples in Mali [30], Benin [31] and Togo [32]. The presence of *Escherichia coli* strains ($4.38.10^8$ CFU/ml) and fecal coliforms (> 3.10^8 CFU/ml) in the collected whey samples indicate fecal contamination and the potential presence of pathogens similar from ecological point of view. In addition, *Escherichia* contaminate milk directly (by direct contact with the udder), or multiply

following poor cleaning of dairy utensils [33]. This indicator is a measure that calls for the adoption of good hygiene practices by women during the cheese-making process, for the prevention of the occurrence and spread of fecal contamination during production and storage of whey. Indeed, the *Escherichia coli*, being an environmental bacterium, the observation of a good hygiene practical during cheese production and the recovery of the whey could help to solve the problem [34].

Each collected whey was contaminated with staphylococci which indicate human and animal contamination during the production and storage of whey. This pathogenic germ constitutes a real risk for public health in processed products because it has the capacity to produce, under certain conditions, heat-stable enterotoxins which can withstand heat treatments [35]. This has been reported by several authors in African countries [36-37]. Indeed, as Staphylococci are bacteria present on the humans' skin [38], can be avoid by observing good manufacturing hygiene.

Microbiological analysis showed a complete absence of salmonella in the whey samples. This complies with the microbiological criterion applicable to raw cow's milk. These results are similar to those obtained by Mennane et al. [49]) in raw milk. The whey analyzed are apparently healthy with regard to salmonella. The absence of salmonella in the whey analyzed may be due to the heating process during cheese making. Indeed, salmonella is one of the most dangerous groups of microorganisms responsible for food poisoning [40].

Lactic bacteria were significantly present in all the samples analyzed with a higher contamination rate at the level of Parana's whey ($> 3.10^8$ CFU / ml). This rate is greater than 6.10^4 CFU/ml obtained by Seme et al. [32] in Togo in raw milk. Lactic acid bacteria, which are of significant technological interest, are producers of lactic acid and bacteriocins [41].

Yeasts and molds come from the environment and their presence in milk is due to poor hygienic conditions during cheese processing. They also reflect the fact that during handling, whey is very exposed to the ambient air. However, they allow the fermentation necessary for the production of dairy derivatives. The microbial load of yeasts and molds obtained in the samples analyzed was higher than those obtained in Benin [31] on milk from Borgou, Lagunaire and Girolando cows.

4. CONCLUSION

This study revealed characteristics such as dry matter, pH, titratable acidity, protein, lactose; and microbial quality of the whey. The presence of total mesophilic aerobic flora, *Escherichia coli*, staphylococci, lactic acid bacteria, yeasts and molds reveals fecal, human and environmental contamination during cheese production or during whey storage. Lactic acid bacteria are responsible for the production of lactic acid by fermentation in whey. By the presence of the latter in an acceptable quantity, this whey can be used as a substrate for the production of certain organic acids. It thus important raising the awareness of cow's milk processors and / or whey producers in Benin. In addition, quality control of whey intended for recovery, is an essential point to reduce contamination.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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