FACULTY OF SCIENCE
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Title: Microbiological quality of “Kosam daanidam” an indigenous fermented milk from Burkina-Faso

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Abstract

“Kosam daanidam” is a traditional spontaneous unpasteurized fermented milk product from Burkina Faso. The microbiota of this indigenous food product was studied throughout the processing. Samples were harvested from three local markets and one neighbouring village of Bobo-Dioulasso in the south-west of Burkina Faso. The processing was determined through interviews and following up of sets of fermentation. The microbiota was assessed on a total of 21 samples consisting of raw, intermediary and end products. Determinations of total mesophilic bacteria, *Enterobacteriaceae*, yeast and lactic acid bacteria were performed by plate count. From samples throughout the processing 186 yeasts, 170 *Lactococci* and 183 lactic acid bacteria were isolated and identified to species level by $(GTG)_5$ based rep-PCR in addition to 16S rRNA and 26S rRNA sequencing.

The fermented milk end product had a pH range of 3.49 to 4.57. The *Enterobacteriaceae* enumeration was 2.15 ± 2.82 10^7CFU/g whereas for lactic acid bacteria and *Lactococci* counts were 1.62 ± 1.53 10^9 and 1.69 ± 1.61 10^9 CFU/g, respectively. The level of total aerobic microorganisms was 1.44 ± 1.11 10^9 CFU/g. The preliminary morphological observations, catalase and gram tests confirmed that the fermentative flora was dominated by lactic acid bacteria.

The data revealed the need for improvement of the standard quality of “Kosam daanidam” sour milk. Future work will focus on screening microorganisms with potential technological properties for development of multifunctional starter cultures, specially targeted “Kosam daanidam”.

Figure 1. Picture of a ”fulani” milking his cow.

Materials and methods

Sampling

The study site was Bobo-Dioulasso at the south-west of Burkina-Faso. The chosen sampling sites were one border village and three local markets.

The fermentation was monitored for 3 days by sampling every 7 hours. The fermentation room temperature was also monitored. The samples were collected aseptically and transported in ice box to the laboratory for analyses.

The markets samples consisted of end products and were also collected aseptically and transported to the laboratory for analyses. A total 10 samples from different steps of fermentation and 11 samples of end products from the market were collected for microbiological and some physio-chemical analyses.

Flow process determination

The processing chart was determined through interviews and following up of sets of fermentation. The interviews included 4 village-based producers at the periphery of the town and three local markets.

Determination of pH and Acidity

The pH was determined using a pH-meter and acidity was determined by titration with NaOH 0.1N.

Microbial count, isolation and purification

Enumeration of total mesophilic flora, *Enterobacteriaceae* and yeast were determined respectively on Plate Count Agar (PCA), Violet Red Bile Glucose Agar (VRBG) and Sabouraud Agar with cloramphenicol. Lactic Acid Bacteria (LAB) count was performed on the Agar medium developed by Man, Rogosa & Sharpe (MRS) and *Lactococci* count were determined on M17. From samples throughout the processing 186 yeasts, 170 *Lactococci* and 183 lactic acid bacteria were isolated. Isolates colonies and cells were characterized visually and microscopically.

Genotypic identification of lactic bacteria and yeasts

The isolates were grouped by $(GTG)_5$ based rep-PCR fingerprints. (Adimpong et al. 2012; Akabanda et al., 2013). Briefly, DNA extraction was performed using the InstaGene (Bio-Rad Laboratories, Hercules, CA, USA) following the instructions of the manufacturer. After electrophoresis, gels were stained with ethidium bromide, photographed under UV illumination and documented using an AlphaImager gel imaging system (Alpha Innotech, San Francisco, CA, USA). Cluster analysis was performed using Bionumerics 7.1 (Applied Maths, Sint-Martens-Latem, Belgium). The dendrograms were calculated on the basis of the Dice’s Coefficient of similarity using the unweighted pair group method with arithmetic averages clustering algorithm (UPGMA).

Results

Process chart

The processing is similar for most of the producers (figure 3). The milking is manual (figure 1) and starts with stimulation by the calf. Then follows hand milking and collection of the milk in a container. The Filtration (figure 2a; pore size of the filter about 1mm) can be done on the site or after transportation to the familly agglomeration which is the fermentation site. The milk destined to produce sour milk is left in a covered container until the characteristics of the fermented product are reached (figure 2b; in cold weather at least about 36h ; in warm weather :overnight).

Microbial count

The end product ”Kossam daanidam” both at production site and market level expressed a high level of *Enterobacteriaceae* of 2.15 ± 2.82 107CFU/g, although the product was quite sour with an average pH of 4.15 ± 0.12 (figure 4). The dominant microorganisms were lactic acid bacteria and *Lactococci* whose counts were 1.62 ± 1.53 10^9 and 1.69 ± 1.61 10^9 CFU/g, respectively. The level of total aerobic microorganisms was 1.44 ± 1.11 10^9 CFU/g, whereas yeast count was 3.01 10^7 CFU/g. The preliminary morphological observations, catalase and gram tests confirmed that the fermentative flora was dominated by lactic bacteria.

During fermentation at 25.7± 2.2 C° the stationary phase for LAB seemed to be reached between 28h and 35h.

Identification of yeast

Yeast colonies and cells were described then grouped by rep-PCR fingerprint using $GTG)_5$ as primer. The cluster analysis showed 15 groups with one group as dominant (figure 5c) .

Identification of LAB and *Lactococci*

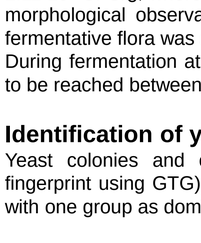
Colonies isolated from MRS and M17 were presumptively considered as lactic acid bacteria and *Lactococci*. Following initial characterization, the isolates were genotypically grouped by $(GTG)_5$-based rep-PCR fingerprinting. For the isolates from MRS plates, cluster analysis showed 35 groups of LAB with 1 group as the dominant and for Lactococci cluster analysis resulted in 41 groups with 2 dominant groups (Figure 5a and b).

Conclusion

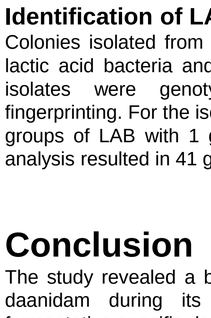
The study revealed a big diversity of microorganisms species in Kosam daanidam during its processing and suggested the existence of fermentative specific dominant strains .The study also showed the need for improvement of the hygienic quality of “Kosam daanidam” sour milk and a need of standardisation of the product. This can be achieved through trainings of producers and development of starters cultures.

Future perspectives

The representative isolates from each group will be identified by sequencing by 16S rRNA and 26S rRNA gene. Futhermore the technological properties will also be determined in order to select some strains as potential starter cultures. Implementation of starter cultures for fermented food products in West Africa would up-grade the food sector and facilitate up-grading from house-hold production to semi-industrial scale.



a



b

Figure 2. Pictures of fresh milk in filtration (a) and of Kosam daanidam (b).

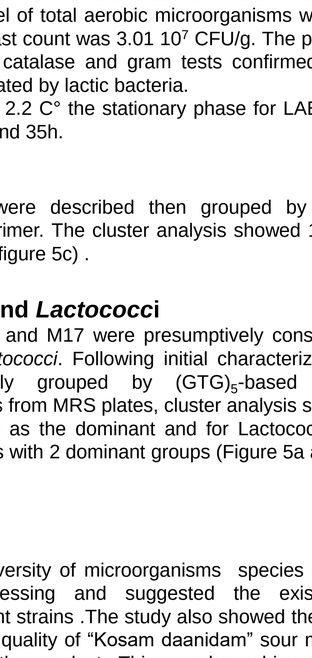


Figure 3. Flow chart of Kosam daanidam processing.

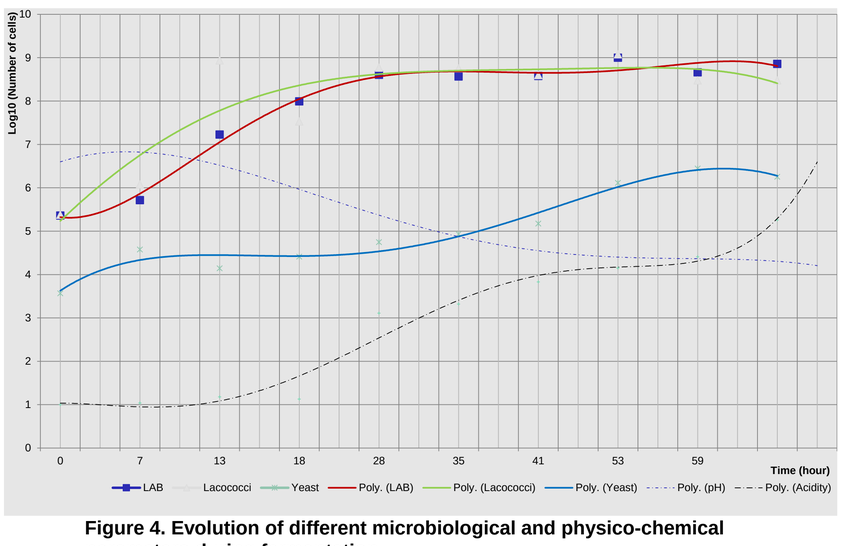
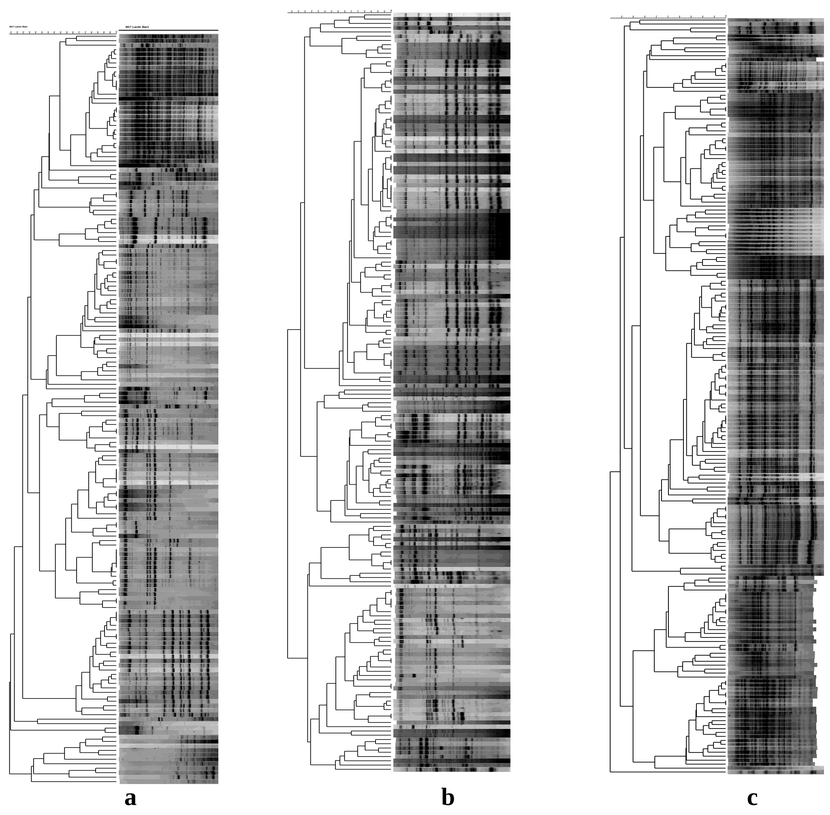


Figure 4. Evolution of different microbiological and physico-chemical parameters during fermentation.



a b c

Figure 5. Dendrograms obtained by cluster analysis of (GTG)5-based rep-PCR fingerprints of *Lactococci* (a), lactic acid bacteria (b) and yeast (c) isolated during Kosam daanidam processing in Burkina Faso. Dice (Tol 1.0% -1.0 %) (H>0.0% S>0.0 %) [0.0 %-100.0 %].

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