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Improvement of the physicochemical and microbiological properties of two traditional beers, Katata and Katubi, from the Haut-Katanga Province in the DRC

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Abstract
This article presents some physicochemical and microbiological properties of two traditional drinks from the province of Haut-Katanga, Katata and Katubi, in the DRC. After centrifugation and filtration on Kieselguhr, the analyzes showed that the two beers had an acid pH (respectively 3.65 and 3.85), the same alcohol content of 4.3%v, a transparent yellow colour, a pleasant appearance to view. Saccharomyces cerevisiae were highlighted there and no other bacteria.

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1. Introduction

In the past, several local fermented drinks were regularly brewed and widely consumed in rural areas of the province of Katanga, an area split into four new autonomous provinces in 2015 and also currently called Grand-Katanga, in the Democratic Republic of Congo (DRC). Today, mainly due to the rural exodus and the introduction of drinks of foreign origin in many of our rural areas, their number and the frequency of their preparation have significantly decreased. If, of all these local drinks, Munkoyo is the most documented [1-21], for most of them, on the other hand, even their names are only known to few people, generally those of the third age. One of the consequences of this state of affairs is the great drop in sorghum and finger millet production in the geographical area thus targeted.

Motivated by the virtual absence in the literature of data relating to traditional Katata and Katubi beers from the province of Haut-Katanga, we decided to carry out investigations on these two brewing specificities of Grand-Katanga which are respectively prepared from sorghum and germinated finger millet to which is added, in both cases, ungerminated corn flour [21].

As in an old study that had been carried out in our laboratory on another beer from Haut-Katanga, also very little known, Kikwekele, where the author aimed at improving the alcoholic degree [11], the present work would like to prompt the traditional fermented drinks from the DRC. To achieve this, it is necessary to improve the conditions for obtaining and storing them. Little by little, the artisanal aspect should give way to a more modern dimension, as it was the case with the industrialization of South African drinks, Mahewu, and from Zimbabwe, Chibuku [22].

2 Material and Methods

2.1 Laboratories

Our work was partially carried out in three laboratories in the city of Lubumbashi: the Biology and Chemistry laboratories of the Institut Supérieur Pédagogique de Lubumbashi (ISP de Lubumbashi) and the laboratory of Simba brewery (Brasimba).

2.2 Starch Material
Maize, sorghum and finger millet flour used as a source of starch and amylases were purchased in two markets in the city of Lubumbashi, the Mzee market in the commune of Lubumbashi and Zambia market in the commune of Ruashi.

2.3 Preparation Principle
The principle of the preparation of Katata and Katubi drinks can be summed up in the following three steps: cooking a porridge of ungerminated maize flour, adding a porridge of finger millet or sorghum malt flour, fermentation.

2.4 Physicochemical Analysis
Acidity was determined using the pH meter. While the measurements of density and alcohol content were made by injecting a previously filtered sample into the Alcoholysis Beer Anton DMA 4500M densimeter. The measurement of the color or clarity of the samples of the beers studied was carried out using a Hach Lange DR 6000 spectrophotometer at 430 nm.

2.5 Microbiological Analyses
These analyses were carried out on four solid culture media. Then, a PCR (Polymerase Chain Reaction) analysis using the Genedisc technology developed by Pall Corporation was requested for the characterization of the germs.
The four culture media that were used are: Wallerstein Laboratories Nutrient (WLN) for determining the presence of total germs, yeasts and molds; WLN-differential with added cycloheximide (WLD) for bacteria only; Yeast and Mold Agar (YM Agar/Géloset Levure et Mushrooms) for the isolation and enumeration of wild yeasts and moulds; finally, De Man Rogosa and Sharp (MRS), with a supplement of cycloheximide (Actidione) for the isolation and enumeration of lactic acid bacteria.

3. Results and Discussion
3.1 Results
3.1.1 Physicochemical analyses
The physicochemical analyses concerned the color or clarity, acidity, alcohol and sugar content as well as the density of two beers subjected to our investigations.

a. Katubi beer
Before being analysed, the beer had undergone two types of filtration. In one case, simple filtration on filter paper was carried out. In the other, the beer was previously centrifuged before being filtered through an activated earth, Kieselguhr.

Table 1: Determination of colour, pH, density, alcohol content and sugar content in Katubi beer filtered through filter paper

N°	Settings	Measures			Mean	Detour
		M1	M2	M3		
01	pH	3,85	3,85	3,85	3,85	0,00
02	Density	1,0065	1,0065	1,0065	1,0065	0,00
03	Alcohol (%v)	4,23	4,26	4,24	4,24	0,02
	Alcohol (%m)	3,32	3,35	3,33	3,33	0,02
04	Coloring (°EBC)	4,91	4,92	4,94	4,92	0,01
05	Sugar (°P)					
	a) primitive extract	9,75	9,81	9,78	9,78	0,03
	b) Apparent extract	1,66	1,67	1,68	1,67	0,01
06	Apparent attenuation (%)	82,94	82,98	82,81	82,91	0,09

Table 2: Determination of color, pH, density, alcohol content and sugar content in Katubi beer centrifuged and filtered through Kieselguhr

N°	Settings	Measures			Mean	Detour
		M1	M2	M3		
01	pH	3,85	3,85	3,85	3,85	0,00
02	Density	1,0065	1,0065	1,0065	1,0065	0,0000
03	Alcohol (%v)	4, 37	4,35	4,28	4,33	0,05
	Alcohol (%m)	3,43	3,42	3,38	3,41	0,03
04	Colouring (°EBC)	4,91	4,92	4,94	4,92	0,01
05	Sugar (°P)					
	a) primitive extract	10,02	10,00	9,90	9,97	0,06
	b) Apparent extract	1,70	1,71	1,74	1,72	0,02
06	Apparent attenuation (%)	83,07	82,91	82,38	82,79	0,36

b. Katata beer
Since the results recorded with the two treatments, simple filtration on filter paper and centrifugation followed by filtration, were practically identical, only the second treatment was applied to Katata beer.

Table 3: Determination of color, pH, density, alcohol content and sugar content in Katata beer centrifuged and filtered through Kieselguhr

N°	Settings	Measures			Mean	Detour
		M1	M2	M3		
01	pH	3,65	3,65	3,65	3,65	0,0
02	Density	1,0063	1,0063	1,0063	1,0063	0,0
03	Alcohol (%v)	4, 37	4,35	4,28	4,33	0,05
	Alcohol (%m)	3,43	3,42	3,38	3,41	0,03
04	Colouring (°EBC)	10,16	10,15	10,13	10,14	0,01
05	Sugar (°P)					
	a) primitive extract	9,72	9,79	9,78	9,76	0,04
	b) Apparent extract	1,01	1,01	1,01	1,01	0,00
06	Apparent attenuation (%)	89,66	89,66	89,66	89,66	0,00

Apart from the values of the results of the coloring and the apparent extract, those of the other parameters of the beer filtered on Kieselguhr and centrifuged are practically identical in the two drinks: density (Katata= 1.0063±0.00 and Katubi= 1 0.0065±0.00; pH Katata= 3.65±0.00 and Katubi= 3.85±0.00; Katata primitive extract= 9.56±0.04°P and Katubi= 9.97±0, 06°P). Regarding colouring or clarity, the value of Katata beer represented twice (10.14±0.01°EBC) than that of Katubi beer (4.92±0.01°EBC). Similarly, the sugar conversion rate expressed by apparent attenuation in Katata beer (89.7±0.00%) was slightly higher than that of Katubi drink (82.8±0.06%). On the other hand, the opposite situation was observed in the final sugar expressed by the apparent extract which was 1.01±0.02°P for Katata and 1.70±0.00°P for Katubi.

3.1.2 Microbiological analyses

In a first phase, the two samples of beer were inoculated on the four culture media selected, namely: WLN, WLD, YM and MRS to search for total germs, bacteria, wild yeasts and lactic acid bacteria respectively. In a second phase, the microorganisms sought were analyzed by the polymerase chain reaction (PCR).

Table 5: Search for colonies of microorganisms in Katata and Katubi beers inoculated on four different culture media

Beer	Cultural Environment			
	WLN Total germs	WLD bacteria	YM Yeasts Savages	MRS Lactobacillus
Katubi	38 Colonies (Yeasts and Molds)	Absence	14 Colonies	Absence
Katata	42 Colonies (Yeasts and Molds)	Absence	22 Colonies	Absence

It emerges from reading Table 5 that several colonies of molds and yeasts were demonstrated in the two traditional beers studied. Moreover, this double presence was observed in a particular way using trinocular optical microscopy (Magnification 40X / 0.65 Ph 2 plan-Achromat).

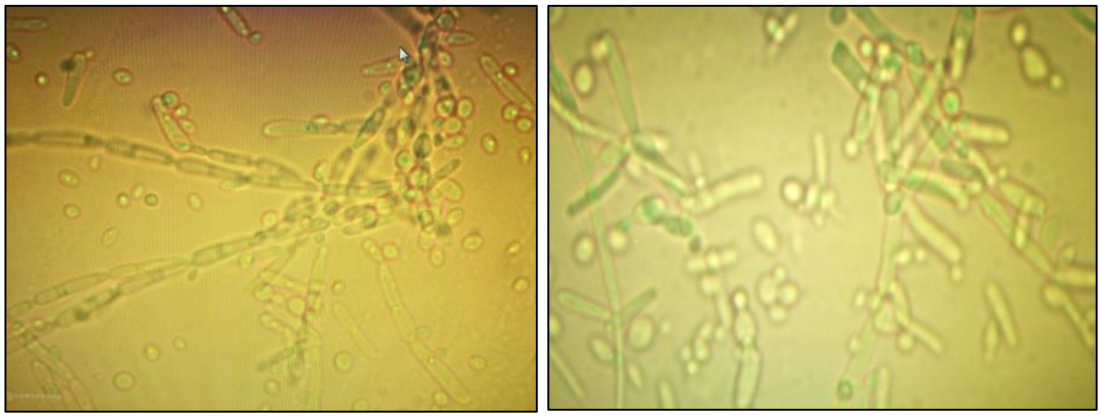

Fig 1: From left to right, observation of Katubi and Katata colony smears on a Trinocular Optical Microscope: Magnification 40X/0.65 Ph2 plan-Achromat

Indeed, from the morphology, oval-shaped wild yeasts and more elongated and segmented molds are easily detectable in the figure above.

Table 6: Type of yeast strains sought in Katata and Katubi beer samples

N°	Yeast analysed	Katubi		Katata	
		Ct	Results	Ct	Results
1	*B. bruxellensis*	0	Not detected	0	Not detected
2	*Brettanomyces spp.*	0	Not detected	0	Not detected
3	*Candida pichia spp.*	31	Presence	0	Not detected
4	*Saccharomyces cerevisiae diastaticus*	0	Not detected	0	Not detected
5	*Saccharomyces cerevisiae*	28,6	Presence	28,5	Presence
6	*Saccharomyces pastorianus*	0	Not detected	0	Not detected
7	*Saccharomyces spp.*	27,7	Presence	27,2	Presence
8	*Saccharomycodes spp.*	0	Not detected	0	Not detected
9	*Schizosaccharomyces spp.*	0	Not detected	0	Not detected
10	*Zygosaccharomyces bailii*	0	Not detected	0	Not detected
11	*Zygosaccharomyces spp.*	0	Not detected	0	Not detected

Ct: Cycle Threshold

The results of the last table show that it was detected in the two beers, Katata and Katubi, the presence of two strains of Saccharomyces cervisiae and spp. In addition, a second presence was noted in Katubi beer only, that of Candida pichia spp.

3.2 Discussion

3.2.1 Physicochemical analyses

If we consider that the usual pH of commercial beers oscillates between 3.8 and 4.423, we can conclude that the acidity of Katata beers (pH = 3.65) and Katubi (pH = 3.85)

hardly deviates from that of other beers. Likewise, the alcohol content of 4.3% recorded is not very far from that of the industrial beers Simba, Castel, 33 Export and Savana in which the alcohol content is 5%. Moreover, it is reported in the literature that during the first 15 hours of the preparation of the Munkoyo drink, the pH goes from 6.4-6.2 to 4.1-4.0. Between 42 and 90 hours, the value of this parameter stabilizes at 3.4-3.6 and the acidification of the drink is mainly attributed to lactic fermentation [24].

As we know, in the brewing industry, at the end of fermentation, the alcoholic degree can even reach 10%, because here the hydrolysis of the starch is more efficient since the α and β amylases are used separately by exploiting the optimum temperature for the enzymatic activity of each of them, 70-74°C for α amylases at an optimum pH of 5.6-5.8 and 62°C (5.8-65°C) for β amylases at optimum pH of 5.4-5.525. To remain consistent with the types of beers produced (Light, Blond, Brown), we obviously operate dilutions. In addition, when in the traditional manufacture of beers, fermentation takes place thanks to the action of wild mushrooms, in industry, the yeasts come from a pure strain which is multiplied.

Given that the EBC degree of Katata beer was practically twice (10.14°EBC) that of Katubi beer (4.92° EBC), we deduced, in accordance with the European Brewery Convention that the colouring was greater in the first beer than in the second one. We attributed this situation in particular to the nature of the raw materials.

3.2.2 Microbiological analyses

The wild yeasts (Saccharomyces cerevisiae, Saccharomyces spp, Candida pichia spp) detected in the two beers under study are generally carried in the air and cling to the walls of the calabashes in which the fermentation took place. Said calabashes are not smooth inside and almost always remain damp, so they also favor the production of molds and are favorite sites for the attachment of fungi.

The presence of Saccharomyces cerevisiae in the two drinks that constitute the object of the present study is in agreement with the data of previous studies according to which S. cerevisiae is the predominant species of fermentation [24, 26-28].

Generally, before consumption, traditional beers undergo a simple sieving and are transferred into other old calabashes previously rinsed with water only. With such treatment, yeasts and molds persist abundantly in the vases. However, under our operating conditions, the use of filtration has certainly made it possible to obtain drinks with very few substances in suspension and which are pleasant to the eye, but, for obvious sanitary and hygienic reasons, the total elimination mushrooms is necessary.

4 Conclusion

Our study focused on the valuation of two traditional fermented cereal-based drinks, Katata and Katubi beers from the province of Haut-Katanga, in the Democratic Republic of Congo (DRC). The results recorded concerned the physicochemical and microbiological aspects.

The physicochemical analyses showed that the two drinks under study were characterized by an acid pH (3.65 for the Katata and 3.85 for the Katubi) and an alcoholic degree of 4.3% alcohol by volume in both cases. The acidity and alcohol content thus obtained are very closed to those of commercial blond industrial beers. With regard to colouring or clarity, the value of Katata beer (10.14°EBC) having been practically double that of Katubi beer (4.92°EBC), we deduced that the colouring was greater in the first drink than in the second. For their part, the microbiological analyzes revealed the absence of bacteria and the presence of two types of wild yeasts, Saccharomyces cerevisiae in the two drinks studied and Candida pichia spp in Katubi beer only.

Given the real interest of the results recorded, the investigations initiated deserve to be deepened so that the step of industrialization of certain traditional drinks of the DRC comes to crown the desired valuation of the latter.

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