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Sulfur Dioxide as a Stabilizing Agent of Local Beers from Kara City in Togo

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Abstract: Beer is a drink consisting of an extremely complex mixture of over 3000 different compounds in an aqueous environment. In Togo, one of the most popular drinks is “Tchoukoutou”, a local beer made by fermenting sorghum. This sorghum beer, also popular in many African countries, is produced in a traditional way by women, often in unhygienic conditions. The production of this beer is not reproducible and also, lacks the necessary physicochemical, chemical and microbiological analyses to evaluate its quality. Moreover, because of its instability, it cannot currently be stored for more than 72 h. In this study, we used sulfur dioxide as a stabilizing agent for sorghum beer and studied its impact on certain physico-chemical and microbiological parameters. The parameters analyzed are pH by pH meter, density by areometry, volatile and total acidity by titrimetry, alcoholometry’s degree by densimetry, sugar content by enzymatic method, carbon dioxide rate by manometry and microbiological quality; the free and total SO_2 rate. The results show that sulfity (sulphity) samples are more acidic than those without SO_2. Thus, the pH values of the samples of «Tchoukoutou» are between 1.52±0.01 and 3.31±0.00; the density of the drinks varies between 1.0042±0.0002 and 1.0489±0.0001 g/cm. The total acidity of the Tchoukoutou studied varies between 1.54 and 4.30 g/L of H_2SO_4; the sugar content in the local beers analyzed varied between 26.1 and 93.2 mg/L. The fermentation process did not take place in the sulfity sample, confirmed by the alcohol content which is between 0 and 7.48% in the non sulfited; the CO_2 content varied between 359 and 653 mg/L. Microbiological analysis revealed that the sulfity samples were preserved from the microorganisms sought. We can retain from this study that the sulfity of Tchoukoutou beer can help prolong the quality of this local drink by inhibiting the growth of microorganisms causing deterioration of the organoleptic quality of Tchoukoutou.

Keywords: Tchoukoutou, Physico-Chemical Parameters, Bacteriological

Introduction

Sulfur Dioxide (SO_2) is a chemical product used as an additive for stabilizing alcoholic beverages such as wine or beer, combining antioxidant and antibacterial properties (Bennett and Hammond, 1992). When sulfur dioxide is added to beverages, a balance is established between the different forms of this compound (Ilet, 1995). At pH values of wine and beer (3-5), most free SO_2 is present as bisulfite (HSO_3^-), a combination of SO_2 and hydroxide ion OH^-. SO_2 that reacts with naturally occurring carbonyl compounds (carbon atom bonded to an oxygen atom) in wine or beer to produce carbonyl bisulfite is called "bound" SO_2 (Divol *et al*., 2012). As usual, several mmol/L of total SO_2 is required to obtain 0.20-0.50 mmol/L of active SO_2 (free), the necessary concentration to avoid further yeast fermentation and bacterial alteration. SO_2, in the form of H_2SO_3 (active SO_2), penetrates cells quickly (about 2 min), disrupts their development, growth and multiplication and ultimately causes cell death (Jackowetz and de Orduña, 2013). Sorghum beer is a craft beer recognized in many African countries under names like billi-billi, Dolo, or Tchakpalo, (Lango-Yaya *et al*., 2020; Aka *et al*., 2008; Coulibaly *et al*., 2014). This beer is also very popular in Togo and is called "Tchoukoutou". To produce Tchoukoutou, the sorghum is first malted, then the malt is brewed and finally, the resulting must is fermented (Kayodé *et al*., 2007). This traditional beer is of major economic and social importance. Firstly, it is a major source of income for the women who are its main producers (Dossou *et al*., 2015; Fokou *et al*., 2016). Also, unlike industrial beer which is expensive and reserved for the wealthy classes of African populations, sorghum beer is accessible to all economic strata of the population because it is cheap. Bars reserved exclusively for drinking Tchoukoutou are veritable institutions, as they are places for socializing and meeting friends or lovers. It is mainly the people of northern Togo, particularly the Kabiye, who produce the Tchoukoutou. In the Kara region, home to the Kabiye people, sorghum beer is a must at traditional ceremonies such as funerals and initiation rites for young boys, which are moments of celebration and reunion with family and friends (Coffi Aholou, 2010). Tchoukoutou is then produced and consumed in large quantities, causing health problems such as stomach aches and diarrhea, especially for people who don't drink it often. This is essentially due to the lack of respect for good hygiene practices during the production and storage of this beverage.

For example, yeasts from previous fermentations, whose microbiological composition is unknown, are added to the must to trigger fermentation. N'tcha *et al*. (2015); Dahouenon-Ahoussi *et al*. (2012) "Tchouk bars" are often surrounded by all kinds of waste and stagnant water, which attracts pests such as flies (Fokou *et al*., 2016). Tchoukoutou is an unstabilized and actively fermenting medium; therefore it must be consumed within 24-48 h of production, otherwise it will become too acidic and unpalatable (Osseyi *et al*., 2011).

Studies have been conducted to try to stabilize tchoukoutou with essential oils or plant extracts (Christian *et al*., 2012) but no studies have been conducted with sulfur dioxide, a recognized additive that is commonly used in beverages. Sulfur dioxide is a known and available stabilizing agent used in food and beverages, which does not alter organoleptic properties. Thus, in our study, we decided to use sulfur dioxide as a stabilizing agent for samples of sorghum beer produced in Kara in order to see its influence on certain previously determined physico-chemical parameters and on microbiological composition. We also wanted to have some indications of the chemical composition of this craft beer. Specifically, the actual study aims to extend the shelf life of tchoukoutou by inhibiting microbial activity with SO_2.

Materials and Methods

Framework Study

The experimental work including sampling and sulfiting bottling was carried out using appropriate equipment by the research team of the Laboratory of Organic Chemistry and Environmental Sciences (LaCOSE) of the University of Kara (Togo). The analyses were performed at the analytical laboratory of Laffort Oenologie (Bouliac, France), a benchmark laboratory for the analysis of wines and beverages, equipped with innovative analytical tools.

Sampling

In total, eight (08) samples of sorghum beers, called «Tchoukoutou», specifically six (06) fermented for about 8 h and two (02) unfermented, of the Kabyè-Missine type, were collected from three current production and sales sites: The Chaminade district, the south campus district and the village of Yadè. This choice was made because these sites were accessible and covered the south, center and north of the city of Kara. In addition, several regular consumers of Tchoukoutou recommended these locations as being more hygienic and offering better-quality, better-tasting drinks. Sterile 1.5 L plastic bottles were used to collect the samples. These bottles were carefully sealed with a screw cap, packed in a cooler and transported to the laboratory, where they were stored in a freezer at -8°C until analysis (Hounhouigan *et al*., 1994). In order to test the impact of SO_2 on the conservation and the quality of the microbiological and physico-chemical parameters of beverages, half of the samples to be tested were sulfated by aseptically incorporating a 10% sulfurous solution (0.1 mg/mL SO_2) followed by homogenization. The total SO_2 concentration did not exceed 200 mg/L in each sample. These samples were stored for analysis.

Physico-Chemical Characterization of Samples

pH, density, volatile acidity, total acidity, sugar concentration, alcohol percentage, CO_2 concentration, free SO_2 and total SO_2 concentration were determined in each sample:

- pH measurement: The pH was determined by direct reading using an INOLAB 1770 pH meter that was previously calibrated with commercially available pH = 4 and pH = 7 buffer solutions (Kayodé *et al*., 2007)
- Density measurement: The density of each sample was measured using a densitometer (OIV, 2009)
- Sugar content: The D-glucose and D-fructose content of each sample was determined by enzymatic assay (OIV, 2009)
- Alcohol content: After distillation, the densimetric method is used to determine the alcoholic strength of beverages (OIV, 2009)

- Volatile acidity: The volatile acids were separated from the local beers by steam stripping and then titrated (OIV, 2009)
- Total acidity: Total acidity was determined by titration in the presence of bromothymol blue as a colored indicator (OIV, 2009)
- Carbon dioxide concentration: Each sample's carbon dioxide content was determined using the manometric method (OIV, 2009)
- Free and total SO_2: Free and total sulfur dioxide levels were measured using the international official method (Frantz-Paul method) (OIV, 2009)

Microbiological Characterization of Samples

The fresh samples were observed under an optical microscope to assess the presence or absence of microorganisms. To identify these microorganisms, each sample was inoculated on different culture media: Total yeast medium, total bacteria medium (acetic bacteria and lactic acid bacteria; MIL-BT/BL ×10), acetic bacteria medium (MIL-BA ×10) according to the OIV-resolution OENO 206/2010. These bacteria are responsible for the deterioration of the taste and aroma of Tchoukoutou. So, genetic identification of yeast strains, using PCR, was attempted because the yeasts used by producers are uncharacterized wild yeasts. The results were expressed in terms of presence or absence.

Results and Discussion

Physico-Chemical Characteristics of the Thoukoutou Samples

The results presented in Fig. 1 and Table 1 show that the pH values of the "Tchoukoutou" samples are between 1.52±0.01 and 3.31±0.00. In general, the pH of the samples shows that the beers are acidic or even very acidic and the presence of sulfur dioxide further acidifies the environment. Indeed, apart from the KC-SO_2 sample, all other sulfite samples are more acidic than those without SO_2. The tchakpalo type beer is the most acidic (pH between 1.52 and 1.92) while the kabye-missine type beer is the least acidic (pH between 2.67 and 3.31). It should be noted that the lower the pH of beer, the more acidic its taste and the less appreciated by consumers. Togolese sorghum craft beers are more acidic than beers produced in Benin (Kayodé *et al*., 2007), Burkina-Faso (Sawadogo-Lingani *et al*., 2007), Ivory Coast (Aka *et al*., 2008), or the Central African Republic (Lango-Yaya *et al*., 2020). The variation of pH values between countries, types of beer and even sampling locations in the same country or city is due to the lack of control and standardization during the local beer production process, which depends on each individual brewer (Tcha-Sosso *et al*., 2020).

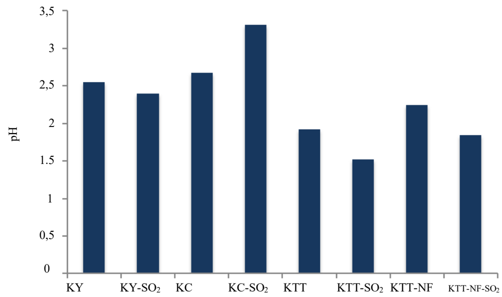

Fig. 1: pH of the Tchoukoutou beers analyzed

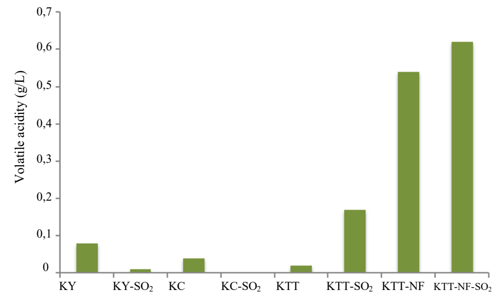

Fig. 2: Volatile acidity of analyzed samples

These African local «beers» are more acidic than industrial beers whose pH is between 4.2 and 4.4 (Declerck, 1957). This higher acidity can be explained by the production of organic acids by yeast and by the contamination of beverages by acetic bacteria that may be present in the environment. The sulfur dioxide dissolved in water is found in the form of sulfurous acid H_2SO_3 which is a bifunctional acid, which explains why the pH decreases further when SO_2 is added in the middle. Volatile acidity, which consists mainly of acetic acid, has a content below 0.90 g of H_2SO_4 per liter of beer (Fig. 2), which is the maximum allowed content in wines in France (Legifrance, 2013).

It is noted, however, that this volatile acidity is higher in sulfited beers than in non-sulfited beers, which is probably due to the acidity of sulfur dioxide in an aqueous medium. Note that if this acidity is too high in alcoholic beverages, they will taste pungent, sour and unpleasant: This is a sign of deterioration of the beverage (Seguin, 2011). The total acidity of the samples studied varied between 1.54 and 4.30 g/L of H_2SO_4, which is in the classical range of drinks (Fig. 3).

It is a parameter that makes it possible to appreciate the organoleptic qualities of a drink and to prevent possible contamination by non-acidophile microorganisms. The density of the beverages varies between 1.0042±0.0002 and 1.0489±0.0001 g/cm. Density provides information on the turbidity of the beer. These values are relatively similar to those obtained for Kapsiti white craft beer from Cameroon, which has a density of around 1.01 g/cm (Bayoï *et al*., 2016). The sugar content in the local beers analyzed varies between 26.1 and 93.2 mg/L of glucose + fructose (Fig. 4, Table 1).

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Table 1: Physico-chemical parameters of the local beers studied

	KY	KY-SO_2	KC	KC-SO_2	KTT	KTT-SO_2	KTT-NF	KTT-NF-SO_2
pH	2.5500±0,01	2.4000±0,01	2,67±0,02	3.31±0,02	1.92±0,01	1.52±0,01	2.24±0,00	1.84±0,00
Total acidity g/L	3.3100	3.6000	4.3000	3.9500	4.2500	3.7600	2.7500	1.54
Volatile acidity g/L	0.0800	0.0100	0.0400	0.0000	0.0200	0.1700	0.5400	0.62
Density	1.0188	1.0163	1.0063	1.0042	1.0179	1.0270	1.0248	1.0489
Alcohol content %	4.1500	5.6900	7.14000	6.9500	7.4800	5.8900	3.4500	0
Sugar content mg/L	64.5000	59.3000	34.7000	26.100	70.100	93.200	81.200	138.2
CO_2 mg/L	463.0000	449.0000	373.000	359.000	430.00	488.00	503.00	652
Free SO_2 mmol/L	0.0000	0.3900	000.000	0.6800	00.000	0.3100	00.000	0.84
Total SO_2 mmol/L	0.0000	1.7800	000.000	2.7200	00.000	1.5600	00.000	3.20

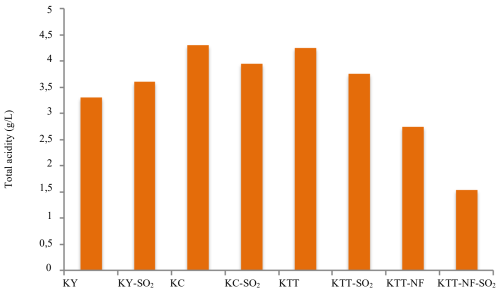

Fig. 3: Total acidity of analyzed samples

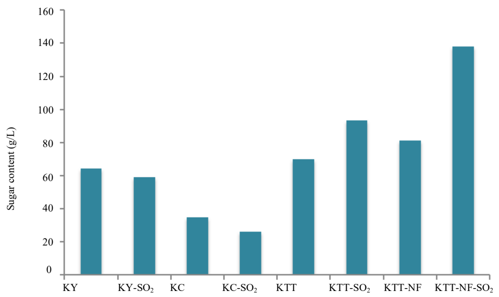

Fig. 4: Sugar level in mg/L of analyzed samples

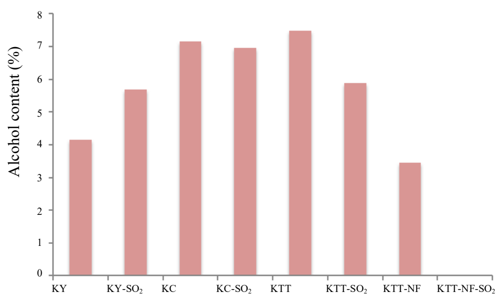

Fig. 5: Alcohol content (%)

It is the unfermented and sulfited sample (KTT-NF-SO_2) that contains the highest sugar content with a concentration of 138 g/L, due to the fact that the fermentation process did not take place. This is confirmed when we observe the alcohol level in this «unfermented» beer which is 0 (Fig. 5): There was no transformation of sugar into alcohol.

This observation is very important because, when we compare with the same unfermented and non-sulfited must, we find that the sugar level is 81.2 g/L and the alcohol level is 3.45. In the absence of SO_2 and although there was no yeast inoculation in the must, a spontaneous fermentation process occurs leading to the transformation of sugar into ethanol. This spontaneous fermentation is certainly due to the contamination of the environment by microorganisms, mainly yeasts and bacteria. Unknown yeasts and bacteria in the environment can produce a variety of aromatic compounds, including esters, phenols and acids, which can give beer undesirable characteristics. Sulfur dioxide, which has been used since the end of the 19th century for its antiseptic properties (fungicide and bactericide) (Millet and Lonvaud-Funel, 1999); Ribéreau-Gayon, 1976) therefore protects our Tchoukoutou samples from the uncontrolled and unwanted development of microorganisms and stops the fermentation process. For the other local beers, the alcohol level varies between 4.15% (KY sample) to 7.48% (KTT sample). Beers containing SO_2 have (except the KY-SO_2 sample) a lower alcohol content than the same non-sulfide sample, confirming that sulfur dioxide can block the fermentation process. This would allow local beers to be stored for a long time, as is the case for industrial beers. In addition, the level of sugar and alcohol has an impact on the organoleptic quality of the beer, particularly its taste. Overall, the alcohol content of these beers is of the same order of magnitude that "Tchoukoutou" beers analyzed in Lomé by Novidzro *et al.* (2018) and "tchakpalo" beers analyzed by Amane *et al.* (2005). The level of carbon dioxide in all these local beverages varies between 373 and 503 mg/L (Fig. 6).

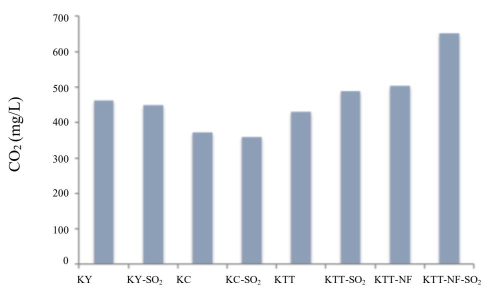

Fig. 6: CO_2 content (mg/L)

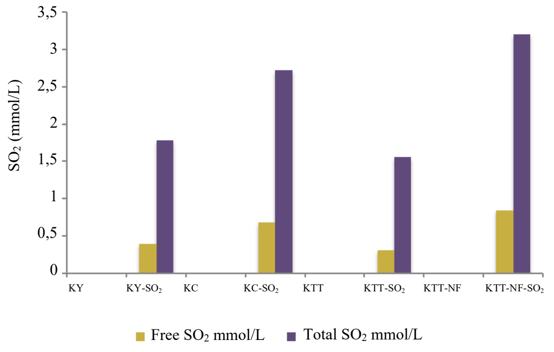

Fig. 7: Free and total SO_2 of beer samples studied (mmol/l)

This is consistent with the fact that, during the fermentation process, yeasts convert fermentable sugars into ethanol and carbon dioxide. No preliminary study on the CO_2 content in local beers was carried out to give a point of comparison. Non-sulfited beers do not contain sulfur dioxide. It is observed that for the sulphited samples, although they initially received the same amount of SO_2, the measured rates are different. This is explained by the chemical complexity of the medium constituting each of the beers studied. The proportion of active SO_2 or free SO_2 is greater than 0.3 mmol/L which is the dose necessary to ensure the preservation of a drink (Fig. 7).

The main properties of sulfur dioxide are attributed to its free form, the combined state being only weakly active (Ripper, 1892). The proportion of combined SO_2, which is the difference between total SO_2 and free SO_2, is relatively high and exceeds 1 mmol/L in all beers and is highest in the fermented must. Several dozen carbonyl compounds are known to combine SO_2 in musts and wines. These carbonyl compounds can be classified according to their chemical structure and are distinguished from neutral aldehydes and ketones, carbonyl acids, sugars and acids derived from sugars. The participation of each compound in the combination of SO_2 will depend on the dissociation constant Kd of each compound and its content in the wines. For compounds with a low Kd value (less than 0.01 mmol/L), the combination with SO_2 is practically total. This is the case for ethanal (Kd = 0,0024 mmol/L) and glyoxilic acid (Kd = 0.008 mmol/L). For compounds with a Kd value between 0.1 and 10 mmol/L, the combination with SO_2 takes place but it is never total. For example, pyruvic acid, 2-oxoglutaric acid, 5-oxofructose, glyoxal and glyceraldehyde. Sugars such as glucose or galacturonic acid, are probably present at high concentrations in beverages but have very high Kd values. They hardly participate in the combination of SO_2. We can therefore already assume the presence of these carbonyl compounds in Tchoukoutou beverages but, to have a precise knowledge of carbonyl compounds and their concentrations in beverages, it would be necessary to carry out the determination of each compound. Table 1 shows the details of the results obtained.

Microbiological Characteristics of the Tchoukoutou Samples Analyzed

There is no unequivocal difference between the samples of sulited and non-sulfited beers. Indeed, all samples of Tchoukoutou without SO_2, after analysis, contain both yeasts and lactic acid and acetic bacteria (Table 2). Craft beers are in the middle of fermentation after inoculation of ferment by the preparers which explains the presence of yeasts (Maoura *et al*., 2006). Unfermented beer that has not been inoculated by any ferment has undergone spontaneous fermentation caused by the surrounding microbiota. Almost all of the non-sulfited samples contain both *Saccharomyces* and wild yeasts whose genetic strains could not be identified. Lactic acid bacteria are, along with yeasts, the microorganisms generally found in products resulting from the fermentation of cereals (Oguntoyinbo *et al*., 2007; Oyewole, 2001). This explains the presence of yeasts (Maoura *et al*., 2006). Unfermented beer that has not been inoculated by any ferment has undergone spontaneous fermentation caused by the surrounding microbiota. Almost all of the non-sulfited samples contain both *Saccharomyces* and wild yeasts whose genetic strains could not be identified. Lactic acid bacteria are, along with yeasts, the microorganisms generally found in products resulting from the fermentation of cereals (Oguntoyinbo *et al*., 2007; Oyewole, 2001). It explains why they are also found in our non-sulfited samples. In samples containing SO_2, none contain microorganisms such as yeasts, lactic acid bacteria and acetic bacteria (Table 2). SO_2 is known to inhibit the development of microorganisms such as yeasts and lactic and acetic bacteria, which prevents the formation of yeast disorders, resealing and various bacterial alterations (Millet and Lonvaud-Funel, 1999). In addition, acetic bacteria convert alcohol into acetic acid, giving the beer a more acidic taste and an unpleasant odor that is not appreciated by consumers. The sulfitage of Tchoukoudou therefore seems to appear as an interesting possibility to stabilize and preserve this drink which, at present, must be consumed within 48-72 h of its manufacture under penalty of becoming undrinkable and therefore be thrown away. This is a source of waste, therefore causing economic loss for the manufacturers of Tchoukoutou and polluting the space surrounding the manufacture of this beer.

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Table 2: Total yeasts and bacteria (acetic and lactic) of the local beers studied

Sample Name/ Microorganisms	Wild yeast	Saccharomyces yeast	Acetic bacteria	Lactic bacteria
KTT NF	Presence	Presence	Presence	Presence
KTT NF-SO_2	Absence	Absence	Absence	Absence
KC	Presence	Presence	Presence	Presence
KC-SO_2	Absence	Absence	Absence	Absence
KY	Absence	Presence	Presence	Presence
KY-SO_2	Absence	Absence	Absence	Absence
KTT	Presence	Presence	Presence	Presence
KTT-SO_2	Absence	Absence	Absence	Absence

Conclusion

The stabilization of sorghum beer samples, commonly called Tchoukoutou and collected in the city of Kara, was achieved by adding a minimum dose of sulfur dioxide which is known for its antiseptic and antioxidant properties. The sulfitage of local beers has protected the samples from the uncontrolled development of microorganisms such as yeasts, lactic acid and acetic bacteria since all samples containing SO_2 are devoid of these microorganisms. This is positive because the physico-chemical and bacteriological tests carried out in several countries on sorghum craft beers have all highlighted the lack of hygiene during the production of this drink and the presence of microorganisms which can be a health problem such as stomach aches and diarrhea after consuming this drink since these drinks are highly prized and consumed due to their low cost. Stabilization and conservation is an interesting study in order to consider possibly the standardization and large-scale production of this local drink. The relatively high combined SO_2 levels in the sulfite samples show the presence of carbonyl derivatives, which are responsible for the bisulfite combination. This is important because, until now, no studies have been carried out to identify molecules contained in sorghum beers. It will remain to identify specifically the carbonyl derivatives and their contents by dosage. And by the way, will also improve this drink because the compounds have an influence on the organoleptic properties. A sensory analysis will be performed to see if the sulfitage of Tchoukoutou beers has an impact or not the organoleptic quality of beer

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Author's Contributions

Marie-France Bakaï: Conceptualized the research project, collected data, analyzed and interpreted the results and wrote and edited the manuscript.

Batcha Ouadja: Collected fieldwork samples, collected and analyzed data, conducted laboratory experiments and wrote and edited the manuscript.

Marie-Estelle Kipré-Naura: Designed the experimental methodology, collected and analyzed data and wrote and edited the manuscript.

Ethics

This article is original and contains unpublished material. It is confirmed that all of the authors have read and approved the manuscript and there are no ethical issues involved.

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