African Journal of Biotechnology Vol. 11(30), pp. 7725-7730, 12 April, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3231
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Stabilization and preservation of probiotic properties of the traditional starter of African opaque sorghum beers

A. P. Polycarpe Kayodé[1], Deloris C. Deh[1], Lamine Baba-Moussa[2], Simeon O. Kotchoni[3] and Joseph D. Hounhouigan[1]

[1]Département de Nutrition et Sciences Alimentaires, Faculté des Sciences Agronomiques, Université d'Abomey-Calavi, 01 BP 526 Cotonou, Bénin.
[2]Laboratoire de Biologie et de Typage Moléculaire en Microbiologie; Faculté des Sciences et Techniques, Université d'Abomey-Calavi, 05 BP 1604 Cotonou, Bénin.
[3]Department of biology and Center for Computational and Integrative Biology, Rutgers University, 315 Penn St., Camden, NJ 08102, USA.

Accepted 28 February, 2012

This present study assessed the impact of drying process parameters, that is temperature and drying duration, on the dry matter content, pH, titratable acidity, yeasts and lactic acid bacteria content of granule starter of African opaque sorghum beer. Probiotic properties of the dry starter were tested. The aim was to establish levels of temperature and duration of drying that lead to a longer shelf life and optimum activity of the starter. Results show that the drying temperature has significant effects on the titratable acidity, yeasts and lactic acid bacteria contents of the granule starter while the level of dry matter was significantly affected by both temperature and duration of drying. The optimal drying conditions providing a stable granule starter with optimum viability of lactic acid bacteria and yeasts were established to 43°C and 24 h. Both wet and dried starters showed inhibitory effect on the meticillin resistant *Staphylococcus aureus*.

Key words: sorghum, opaque beer, starter, yeats, probiotic, Response Surface Methodology.

INTRODUCTION

Opaque sorghum beers are popular alcoholic beverages in Africa. They are known as *tchoukoutou* in Benin, *dolo* in Burkina-Faso*, pito* in Ghana*,* and *burukutu* or *otika* in Nigeria (Odunfa, 1985; Kayodé et al., 2005). The beers have a sour taste, a relatively high dry matter content and low alcohol content, which make them suitable beverages for adults (Agu and Palmer, 1998; Briggs et al., 2004). The nutritional attributes of eight commercial sorghum beers were reported by Novellie and De Schaepdrijver (1986) as follows: protein 5.4 g L^{-1}, ash 1.13 g L^{-1}, carbohydrate 47.6 g L^{-1}, iron (Fe) 1.4 g L^{-1} and zinc (Zn) 1.4 g L^{-1}. This suggests that such beer can be a significant source of dietary nutrients, considering the rather large quantity that is consumed daily in certain locations (Briggs et al., 2004; Kayode et al., 2005). The sorghum beers are largely consumed by the poorest people and therefore contribute to their dietary needs.

Tchoukoutou, the Benin opaque sorghum beer*,* is produced by women using various processes. In general, as in the conventional lager beer process, the manufacturing process consists of three phases: malting, mashing and fermentation. The grain is soaked in water overnight (9 to 12 h), germinated (72 to 85 h), sun dried (7 to 15 h), ground in a disc mill, mixed with water, decanted and divided into slurry and supernatant. The slurry is mashed under gradual heating until the boiling point is reached after 2 h, mixed with supernatant and allowed to ferment overnight, then filtered, boiled (6 to 9 h), cooled, and inoculated with a starter called *kpete-kpete*, which is generally harvested from the bottom of a previous fermenting beer (resulting from 13 to 14 h overnight fermentation). The fermentation step is a critical step in the process, and its success depends on the

*Corresponding author: E-mail: polykap@yahoo.fr. Tel +229 97870734.

accurate knowledge of the processor in terms of the starter handling. Several researches reported that the microorganisms contained in the traditional starters of African opaque beers mainly consist of yeasts and lactic acid bacteria (Van der Aa Kühle et al*.,* 2001; Demuyakor and Ohta, 1991; Sefa-Deheh et al., 1999; Sanni and Lönner, 1993). The microorganisms are kept alive by replacing the supernatant on a daily basis. The preservation of such starter is a tedious and a risky business since it is common that the starter to loose its fermenting properties and therefore fail to make the beer effervescent, because the involved microorganisms would have died. Preliminary data demonstrated that rural and urban women's groups in the processing chain of opaque sorghum beers derive a direct benefit from increased marketing opportunities (Kayodé et al., 2007). Thus, innovations in the traditional brewing technology and the product quality could significantly improve income and livelihood of rural households involved in this activity.

This present study aims at improving the shelf life of the traditional starter used to ferment opaque African beers. More specifically, the objective was to evaluate the effect of temperature and duration of drying, on several quality determining factors such as the dry matter content; titratable acidity, as well as yeast and lactic acid bacteria content of granule starter. It is quite likely that these factors are interdependent. However, interactions between factors cannot be detected using the one-factor-at-a-time approach (Giovani, 1983). Therefore, we decided to use a design methodology that is able to detect such interactions. The response surface methodology (RSM) was used applying a central composite design. Central composite designs are the basis for RSM and are used to estimate parameters of a full second-degree model. Such a quadratic model is usually sufficient for accuracy in product and process design (Giovani, 1983). In addition, the probiotic properties of the dry granules were evaluated by testing the anti-microbial effect of the starter extracts on methicillin resistant *Staphylococcus aureus* grown on agar plates.

MATERIALS AND METHODS

Starter sampling

Traditional starter, locally known as *kpete-kpete,* was harvested from one processing site in Abomey-Calavi. This consists of wet slurry from an actively fermenting sorghum beer. The samples were collected in sterile bottles, packed in ice cold box and transported to laboratory for microbiological and physicochemical analysis. To check for the variability in the starter, four samples were collected from the same processor on different days and analysed for pH, titratable acidity, total mesophilic aerobic bacteria, lactic acid bacteria, yeasts and enterobacteriaceae counts. The coefficients of variation for the measured parameters were consistently below 7% in the different samples. One batch of starter was sampled from this processor and use for the stabilization study.

Experimental design

Response surface methodology is a statistical method that uses quantitative data derived from an appropriate experimental design with quantitative factors to estimate the relationship between a response and the factors in order to optimize processes or products (Giovani, 1983). In this study, an orthogonal rotatable central composite design (Montgomery, 2001) for K = 2 factors was used to estimate the simultaneous effect of two process variables on titratable acidity, lactic acid bacteria, yeasts, and total mesophilic aerobic bacteria in a quadratic function. The variables (factors) were the drying temperature (35 to 50°C), and duration of drying (5 to 24 h). The responses were titratable acidity, dry mater, yeasts, lactic acid bacteria, and total mesophilic aerobic bacteria counts. The design generated 14 observations which are distributed as follows: four kernel points, four star points and six replications at the central point. The design matrix and variable combinations are presented in Table 1.

Experimental processing

4 kg of cleaned sorghum grains were dehulled using a mini-PRL dehuller (Thiès, Sénégal) and then ground. The flour obtained is mixed with distilled water (45% w/w), inoculated with 10% (w/w) of *kpete-kpete*, kneaded into dough and allowed to ferment in a plastic bucket with lid for 24 h. Fermented dough samples were oven dried for an indicated time and temperature as specified in the next experimental design (Table 1). Samples were withdrawn when the predefined time and temperature were reached and immediately analyzed for microbiological characteristics.

Counts of viable microorganisms

Total counts of mesophilic aerobic bacteria, lactic acid bacteria (LAB), yeasts, moulds and Enterobacteriaceae were enumerated according to the method described by Nout et al. (1987). Duplicate samples of stabilized starter (10 g) were diluted in 90 ml sterile peptone physiological saline solution (5 g peptone, 8.5 g NaCl, and 1000 ml distilled water, pH = 7.0) and homogenised with a Stomacher lab-blender (type 400, London, UK). Decimal dilutions were plated. Total mesophilic aerobic bacteria counts were determined on plate count agar (PCA, oxoid, CM 325, Hampshire, England) after incubation at 30°C for 72 h. Viable counts of lactic acid bacteria were determined on de Man, Rogosa and Sharpe Agar (MRSA, CM 361, Oxoid, Hampshire, England) containing 0.1% (w/v) natamycin (Delvocid, DSM, The Netherlands) with incubation in anaerobic jar (Anaerocult A, Merck KGaA, Germany). Viable yeasts were determined on oxytetracyclin glucose yeast extract agar (OGYA, Oxoid CM 0545, Basingstoke, Hampshire, England) containing oxytetracycline. Enterobacteriaceae were enumerated on violet red bile agar (VRBA) after incubation at 37°C for 24 h.

Physico-chemical analysis

Dry matter was determined according to the American Association of Cereal Chemists (AACC) approved methods (AACC, 1984). Titratable acidity and pH were determined as described by Nout et al. (1989).

Evaluation of probiotic properties

The probiotic properties of the dry granules were evaluated by testing the anti-microbial effect of the starter extracts on methicillin

Table 1. Design matrix and variable combinations.

Treatment code	Level code		Variable level	
	Temperature	Time	Temperature (°C)	Time (h)
1	0	0	42.5	14.5
2	0	0	42.5	14.5
3	0	0	42.5	14.5
4	0	0	42.5	14.5
5	0	0	42.5	14.5
6	0	0	42.5	14.5
7	+1	+1	47.0	20.2
8	-1	+1	38.0	20.2
9	-1	-1	38.0	8.79
10	+1	-1	47.0	8.79
11	+α	0	50.0	14.5
12	0	+α	42.5	24.0
13	-α	0	35.0	14.5
14	0	-α	42.5	5.0

resistant *S. aureus* grown on agar plates (Baba-Moussa et al., 2008). In order to confirm the preservation of the functional properties of the starter, we tested the starter before and after the drying under the optimum condition (43°C, 24 h). The dry starter was resuspended in water before use. The starters were centrifuged (13,000 rpm, at room temperature, for 5 min) and respective supernatants were used as probiotic extracts for the anti-microbial test. For the test, a drop (30 µl) of the supernatant was allowed to diffuse from a paper disk deposited at the center of *S. aureus* grown agar plate. The plate was incubated overnight at 37°C and the inhibition zone induced by the extract on the bacterial plate was recorded. A bacterial grown plate containing a paper disk with a drop (30 µl) of water was used as control.

Statistical analysis

Data were analyzed using the Minitab 14 statistical program. A second order polynomial model was proposed to establish the relationship between the responses (*Y*) and the variables (*X*) as follows:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_1^2 + b_4x_2^2 + b_5x_1x_2$$

In which b_0 is a constant, b_1 and b_2 are linear effect coefficients, b_3 and b_4 are quadratic effect coefficients, b_5 is an interaction effect coefficient. The fitted polynomial equations were expressed in a 3D response surface in which the response is presented on the vertical axis and two factors at the two horizontal perpendicular axes.

RESULTS AND DISCUSSION

Effect of drying parameters on dry matter

The response values for the different treatments are presented in Table 2. The polynomial equation was fitted to the experimental data using the Minitab program, and the linear regression coefficients estimates are presented in Table 3. The water content of a food product is a good indicator of its storage ability. After the drying treatments applied, the water content of the granules ranged between 6.19 and 49.36% (Table 2). The analysis of variance showed that the drying temperature (X_1) as well as the drying duration (X_2) significantly affected ($P \leq 0.05$) the dry mater content of the granules. Particularly, the linear and the quadratic effects of these factors are significant on the product dry matter content (Table 3). Figure 1 shows the trends in dry matter content of the granules as function of temperature, the drying duration, and their mutual interaction. At drying duration < 12 h, the dry matter content of the product is quite stable for temperatures values between 35°C and 44°C. Between 12 h and 24 h of drying duration, there is a significant increase in the granule dry matter content which reaches 80% (Figure 1a). Such increase in the product dry matter content, due to water loss, is desirable since it could improve shelf life of the product. Previous research demonstrated that cereal products preserve well with water content < 12% (Cecil, 1992). From our experiment it is apparent from the response surface plot that such level of water content could be achieved at drying temperature between 42 and 44°C.

Effect of drying parameters on viable yeast and lactic acid bacteria

The number of viable microorganisms varied among treatments and the major variation in the microorganisms counts were explained by the model. The coefficient of variation (R^2) was 0.75, 0.74, and 0.75 for total count of viable lactic acid bacteria, yeasts, and total mesophilic aerobic bacteria respectively. The analysis of variance showed that only the temperature exerted a significant effect on the viability of microorganisms contained in the granule starter. The number of viable microorganisms is

Table 2. Response for pH, titratable acidity, dry matter, lactic acid bacteria, yeasts, and total count.

Code	pH	Titratable acidity (% lactic acid)	Dry matter (%)	Lactic acid bacteria (log cfu/g)	Yeasts (log cfu/g)	Total mesophilic aerobic bacteria count (log cfu/g)
1	3.92	0.21	60.09	7.86	7.82	7.91
2	3.90	0.34	58.83	7.84	7.92	8.13
3	4.24	0.22	59.29	8.02	7.90	7.90
4	4.54	0.2	59.17	7.82	7.89	7.95
5	4.62	0.16	61.78	7.89	7.86	7.83
6	4.30	0.22	60.18	7.9	7.84	7.92
7	3.64	0.18	82.28	7.19	7.30	7.37
8	4.00	0.26	55.04	7.46	7.46	7.74
9	3.85	0.34	51.72	7.52	7.52	7.82
10	3.65	0.19	60.10	7.50	7.39	7.62
11	3.96	0.17	93.81	6.90	0.00	0.00
12	4.20	0.16	85.43	7,82	7.51	7.90
13	4.31	0.41	50.64	7.76	8.08	8.41
14	3.61	0.24	55.01	8.60	8.71	8.83

Table 3. Coefficients of the variables in the model and their corresponding R^2.

Coefficient	pH	Titratable acidity	Dry matter	Lactic acid bacteria	Yeast	Total mesophilic aerobic bacteria count
b_0	-4.031	0.225	398.549	-13.2092	-90.737	-86.63
b_1	0.329	-0.109[a]	-15.844[a]	1.0371[a]	5.044[a]	4.873[a]
b_2	0.232	-0.038	-9.099[a]	0.0119	-0.193	-0.195
b_3	-0.004	0.066	0.184[a]	-0.0123[b]	-0.063[a]	-0.061[a]
b_4	-0.005	-0.023	0.093[a]	0.0021	0.0058	0.008
b_5	-0.002	0.048	0.184	-0.0024	-0.0003	-0.001
$R^{2\,c}$	0.46	0.75	0.94	0.75	0.74	0.75

$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_1^2 + b_4x_2^2 + b_5x_1x_2$ where, X_1 = temperature (°C), X_2 = time (h); a, significatif at 5 %; b, significatif at 1 %; c; coefficient of determination R^2.

relatively constant at drying temperature between 36 and 44°C. At these temperatures, the level of viable yeasts, lactic acid bacteria and total mesophilic aerobic bacteria is in the range between 8.1 and 9.0 log cfu/g. These values are comparable to values reported for these microorganisms in the traditional starter of the African opaque sorghum beer (Hounhouigan, 2007). The effect of temperature on viability of the microorganisms is more pronounced between 44 and 50°C. At this temperature interval, viable yeasts decrease from 7.3 to 2.0 log cfu/g and the total mesophilic aerobic bacteria decreased from 7.19 to 6.6 log cfu/g. Similar to findings by Fields et al. (1981), our results confirm the relative susceptibility of yeasts to temperature compared to lactic acid bacteria which are able to survive at temperatures as high as 45°C (Giudici et al., 1998; Frazier, 1958). No significant effect of the drying duration was observed on the pH, while the titratable acidity of the granules was only affected by the temperature.

Optimization of the drying conditions

In order to identify the drying conditions that lead to the optimum viability and functionality of the microorganisms of the granule starter, we used the desirability function to optimize the drying duration and temperature. The target characteristics of the granule starter, except the dry matter content, were selected on the basis of the properties of the traditional starter and were as follows: viable yeasts 7.6 to 8.64 log cfu/g, viable lactic acid bacteria 7.19 to 8.26 log cfu/g and pH 3.20 to 4.62. The level of the dry matter was set to 80 to 89%. The optimum drying conditions for the granule starter were found to be: drying temperature: 42.84°C and drying duration: 24 h with a desirability of 0.78. To check for the adequacy of the predicted model, we conducted additional independent experiments at the suggested optimal drying conditions. The predicted and the experiment values for viable yeast and lactic acid bacteria, pH

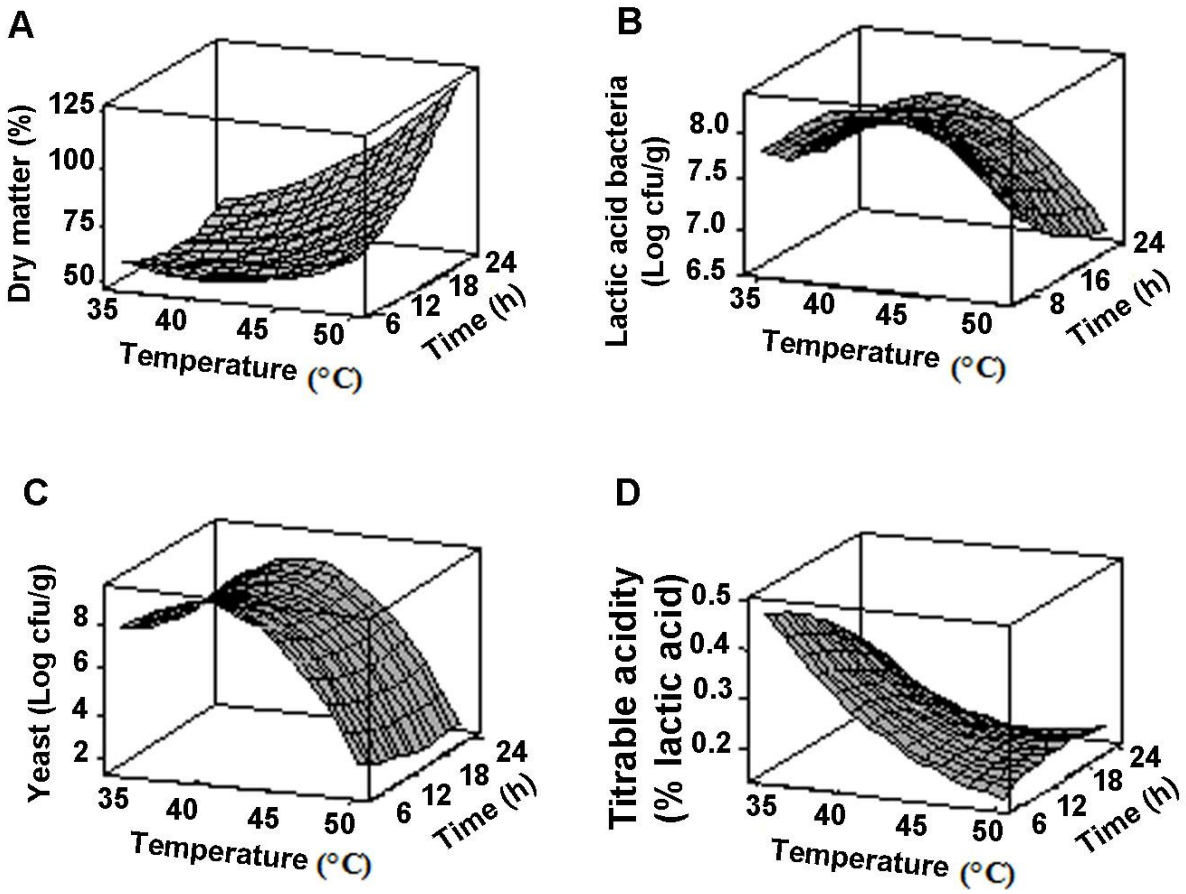

Figure 1. Response surfaces showing the effects of temperature and drying duration on (A) dry matter, (B) lactic acid bacteria, (C) yeast viability, and (D) titratable acidity of starter of opaque beer.

Table 4. Predicted and experimental value for pH, dry matter, lactic acid bacteria and yeasts.

Variable	Limit	Desirability (%)	Predicted value	Experimental value
pH	3.20- 4.62	0.88	3.94	4.15 ± 0.09
Dry matter (%)	80.0 – 89.0	0.95	82.00	84.39 ± 0.99
Lactic acid bacteria **(**Log cfu/g**)**	7.19 – 8.26	0.65	7.72	7.95 ± 0.76
Yeasts **(**Log cfu/g**)**	7.62 – 8.64	0.69	8.00	7.6 ± 0,10

and dry matter are presented in Table 4. The experimental and the predicted values are in close agreement with a desirability ranging between 0.65 and 0.95. A Chi-square test indicated that the observed values were statistically the same as the predicted values. Consequently the generated model adequately predicted the viability of the microorganisms as well as the pH and dry matter content of the granule starter.

Probiotic properties of the dry granule

The probiotic properties of the traditional starter have been here demonstrated (Figure 2). We hypothesized that the starter represents a biologically stable probiotic matter that can inhibit opportunist and disease inducing microorganisms. We tested our hypothesis by assessing the inhibitory effect of the undried and the dried starters on disease inducing *S. aureus* (Baba-Moussa et al., 2008). Our results show that even when dried, the starter was able to inhibit the meticillin resistant *S. aureus* (Figures 2A, B and C) as expected. The diameter of the inhibition zone can easily be seen on the agar plates (Figures 2B and C) compared to the control plate (Figure 2A) indicating the potential benefits of the starter as a powerful health promoting matter. Our results demonstrate the stability and preservation of the probiotic properties of the traditional starter under the optimum

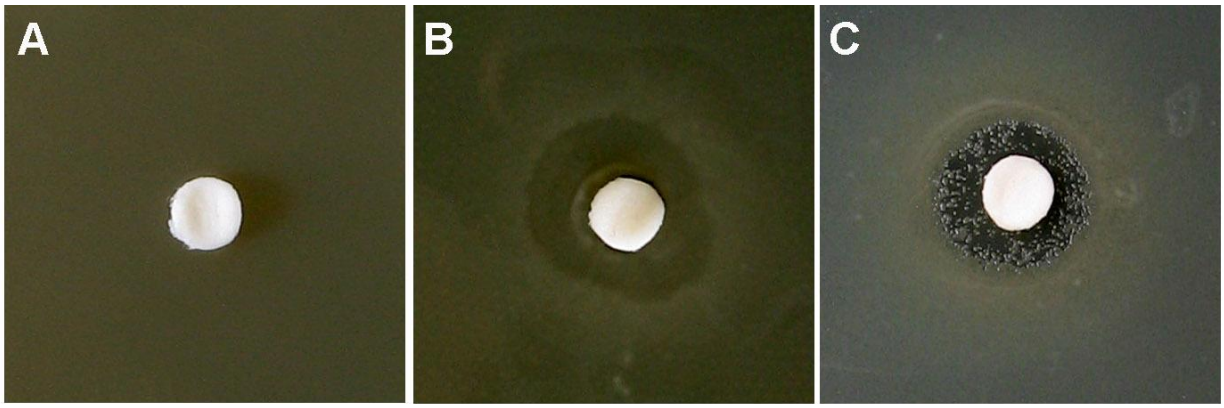

Figure 2. Inhibitory effects of probiotic on methicillin resistant *S. aureus*. The undried starter (B) and dried starter (43°C, 24 h) (C) inhibits the bacterial growth as revealed by significant inhibitory zone (B, C) compared to control plate (A) where we observed no inhibitory zone but a uniform growth of the bacteria.

drying conditions established.

Conclusion

This present study shows that the temperature of drying has significant effects on tritratable acidity; yeasts and lactic acid contents of the starter granules while the level of dry matter was significantly affected by both temperature and duration of drying. The optimal drying conditions ensuring a stable granules starter with optimum viability of lactic acid bacteria and yeasts were established to 43°C and 24 h. These drying conditions have no effect on the probiotic properties of the starter. The response surface methodology could be used to establish prediction model that adequately describe the changes in viability of microorganisms, pH and dry matter content of the granule starter for the fermentation of African opaque beers.

AKNOWLEDGEMENTS

The authors thank the *Projet Aires Sud Grant N° 7190, IRD, France,* for financial support.

REFERENCES

AACC (1984). Approved methods of the American Association of cereal chemists, 8th Edition, St. Paul, MN, USA.

Agu RC, Palmer GH (1998). A reassessment of sorghum for lager-beer brewing. Bioresour. Technol. 66: 253-261.

Baba-Moussa L, Anani L, Scheftel JM, Couturier M, Riegel P, Haıkou N, Hounsou F, Monteil H, Sanni A, Prevost G (2008). Virulence factors produced by strains of *Staphylococcus aureus* isolated from urinary tract infections. J. Hosp. Infect. 68: 32-38.

Briggs DE, Boulton CA, Brookes PA, Stevens R (2004). Native African beers. In *Brewing: Science and practice* Woodhead publishing Ltd, Cambridge, UK. pp. 589-605.

Demuyakor B, Ohta Y (1991). Characteristics of pito yeasts from Ghana. Food Microbiol. 8: 183-193.

Fields ML, Ahmed M, Hamad K, Duane KG (1981). Natural lactic acid fermentation of corn meal. J. Food Sci. 46: 900-902.

Giovanni M (1983). Response Surface Methodology and product optimization. Food Technol. 11: 41-45.

Giudici P, Caggia C, Pulvirenti A, Rainieri S (1998). Karyotyping of *Saccharomyces* strains with different temperature profiles. J. Appl. Microbiol. 84: 811-819.

Hounhouigan H (2007). Evaluation et amélioration de la technologie traditionnelle de production de *kpètè-kpètè*, un ferment utilisé pour la fermentation du *tchoukoutou*. Thèse d'Ingénieur Agronome, FSA / UAC.

Kayodé APP, Hounhouigan DJ, Nout MJR, Niehof A (2007). Household production of sorghum beer in Benin: technological and socio-economical aspects. Int. J. Cons. Stud. 3: 258-264.

Montogomery DC (2001). Design and analysis of experiments, 5 ed.; John Wiley and Sons: New York.

Nout MJR, Beernink G, Bonants-Van Laarhoven TMG (1987). Growth of Bacillus cereus in soyabean tempeh. Int. J. Food Microbiol. 4: 293-301.

Nout MJR, Rombouts FM, Havelaar A (1989). Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic micro-organisms. Int. J. Food Microbiol. 8: 351-361.

Novellie L, De Schaepdrijver P (1986). Modern developments in traditional African beers. Progr. Ind. Microbiol. 23: 74-157.

Odunfa SA (1985). African fermented foods. In Wood BJB (ed.) Microbiology of Fermented Foods Elsevier Applied Science, London, UK. pp. 167-195.

Sanni AI, Lönner C (1993). Identification of yeast isolated from Nigerian traditional alcoholic beverages. Food Microbiol. 10: 517-523.

Sefa-Dedeh S, Sanni AI, Tetteh G, Sakyi-Dawson E (1999). Yeasts in the traditional brewing of pito in Ghana. World J. Microbiol. Biotechnol. 15: 593-597.

Van der Aa Kuhle A, Jesperen L, Glover RLK, Diawara B, Jakobsen M (2001). Identification and characterization of *Saccharomyces cerevisiae* strains isolated from West African Sorghum beer. Yeast, 18: 1069-1079.