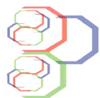

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Utilization of *Lactobacillus fermentum* and *Saccharomyces cerevisiae* as starter cultures in the production of '*dolo*'

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ABSTRACT

Objective: The present study was carried out to evaluate the potential of representative strains of predominant lactic acid bacteria and yeast as starter cultures in the production of *dolo* (a type of sorghum beer) of comparable consistency and organoleptic quality to the commercial product in Burkina Faso.

Methodology and results: Two strains each of *Lactobacillus fermentum* and *Saccharomyces cerevisiae* from previous studies (Sawadogo- Lingani *et al.,* 2007; Glover *et al*., 2005) were used as starter cultures in producing *dolo* both under laboratory conditions, and in the field (pilot plant). pH, lactic acid bacteria and yeast growth were determined at the beginning and end of fermentation. Products were subjected to sensory evaluation for taste, aroma and mouth feel and results analyzed using the Students (t) test. *Dolo* produced from starter combinations of one strain of *L. fermentum* and both *S. cerevisiae* strains had taste and aroma that did not differ significantly from the commercial product, while single isolate combinations gave better mouth feel.

Conclusions and applications of findings: Single-strain and double-strain combinations of lactic acid bacteria (LAB) and yeast starter cultures could be used successfully to produce *dolo* of different quality indices. Combinations of either LAB strain with one yeast strain (AC17) produced *dolo* that was more comparable to the commercial product than combinations with the other yeast strain (TK25). LAB strain ZN4.1 in combination with yeast strain AC17 gave better *dolo* in all attributes evaluated. On the whole, *dolo* produced with double-strain combinations of LAB and yeast under laboratory conditions possessed consistent organoleptic quality and stability comparable to the commercial product. Varied combinations of these starters should be investigated in order to determine a suitable combination for producing *dolo* possessing all the desired organoleptic qualities and consistency.

Key words: *Lactobacillus fermentum*, *Saccharomyces cerevisiae,* starter cultures, *dolo*

INTRODUCTION

Dolo, a type of sorghum beer, is a traditional alcoholic beverage produced in Burkina Faso (Konlani *et al*., 1996). Sorghum beer is also produced in several sub-Saharan African

countries, e.g. Nigeria, Ghana, Benin and Togo where it is referred to variously as *pito, burukutu, sulom, dam, akadamu, merissa and sekete* (Demuyakor & Ohta, 1991; Sanni, 1993; Owuama, 1997; Sanni *et al*., 1999). Like many other indigenous fermented foods and beverages, sorghum beer contributes significantly to the diet of the people in the regions where it is produced (Sanni, 1993; van der Aa Kühle *et al*., 2001). *Dolo* is golden-yellow to dark-brown in color with taste varying from slightly sweet to sour and contains lactic acid, unfermented sugars, amino acids and 2-3% alcohol (v/v), as well as vitamins and proteins (Ekundayo, 1969; Bansah, 1990).

Since it is closely related to *kaffir* beer on which extensive nutritional studies have been conducted (Aucamp *et al*., 1961; Novellie, 1963; Sanni, 1993), it would be expected that *dolo* is a good source of B vitamins, including thiamine, folic acid, riboflavin, and nicotinic acid (Platt, 1964; Ekundayo, 1977). The processing of *dolo* basically involves malting, drying, milling, souring (lactic fermentation), boiling, mashing and alcoholic fermentation (Haggblade & Holzapfel, 1989) in which variations may occur depending on local practices. The alcoholic fermentation is achieved by inoculating the wort with either a portion of a previous brew or dried yeast harvested from a previous brew (Sefa-Dedeh *et al*., 1999).

The production of *dolo*, like *pito* includes a 12- 36 h spontaneous mixed fermentation involving lactic acid bacteria and yeasts (Sefa-Dedeh, 1991; Sefa-Dedeh *et al*., 1999). Bacteria of the genera *Lactobacillus* and *Leuconostoc* spp. are the major contributors to the acidity of *pito* during the initial souring stage (Sefa-Dedeh, 1991). Most of the acid produced is lactic acid with only traces of acetic and formic acid being present (Sefa-Dedeh, 1991). *Lactobacillus fermentum* (264 strains) was found to be the dominant lactic acid bacteria species during the entire process of *dolo* production, particularly the acidification (souring) stage together with some *Lactobacillus delbrueckii* subsp. *delbrueckii* (9 strains), *Pediococcus acidilactici* (7 strains), *Lactococcus lactis* (1 strain) and *Leuconostoc lactis* (1 strain) (Sawadogo-Lingani *et al*., 2007). Demuyakor and Ohta (1991) reported yeasts associated with alcoholic fermentation of Konkomba and Nandom *pito* in the northern regions of Ghana as *Saccharomyces cerevisiae* (33%), *Kluyveromyces* spp. (23%), *Candida* spp. (17%) and members of six other genera. Sefa-Dedeh *et al*. (1999) also reported the isolation and characterization of 21 yeast strains belonging to 7 genera from Dagarti *pito* produced in the Greater Accra Region of Ghana. These comprised eight *S. cerevisiae*, four *Candida tropicalis*, three *Torulaspora delbrueckii*, two *Kloeckera apiculata*, two *Hansenula anomala*, one *Schizosaccharomyces pombe* and one *Kluyveromyces africanus*. These results, while agreeing on the dominance of *S. cerevisiae*, seem to suggest some locality-dependent diversity in yeast populations involved in alcoholic fermentation of *pito* in Ghana.

Sanni (1993) and Sanni and Lonner (1993) attributed the diversity of the associated yeast micro flora of traditional alcoholic beverages including *pito,* in sub-Saharan Africa, to the spontaneous nature of the fermentation, sources, and types of ingredients used. This assertion was countered by the findings of a **(**van der Aa Kühle *et al*., 2001), which highlighted the almost exclusive occurrence of *Saccharomyces cerevisiae* strains in yeast associated with production of Dagarti *pito* and *dolo* from northern Ghana and neighboring Burkina Faso, respectively. Another study by Glover *et al.* (2005) also reported that *Saccharomyces cerevisiae* predominate the alcoholic fermentation of sorghum wort during production of *pito* within eight geographical regions of Ghana and *dolo* at four production sites in Burkina Faso. That study also investigated technological properties of representative strains, including sensory attributes of *pito*, to evaluate their potential for starter culture development common to Ghana.

Even though lactic acid bacteria and yeast associated with *dolo* production in Burkina Faso have been variously characterized, no link seems to be established between the predominant species and *dolo* quality. The present study evaluated the potential of representative strains of predominant lactic acid bacteria and yeast as

starters in the production of *dolo* of comparable consistency and organoleptic quality to the traditional product in Burkina Faso.

MATERIALS AND METHODS

Microbial isolates: Two *Lactobacillus fermentum* strains (ZN4.1 and SF6.1a) previously isolated from dolo in Burkina Faso and two strains of *Saccharomyces cerevisiae* (AC17 and TK25) isolated from two pito production sites in Ghana were used.

Preparation of inocula: The lactic acid bacteria (LAB) stock cultures were each subcultured at 37ºC for 48 hours on de Man-Rogosa-Sharpe (MRS) agar (MERCK, Darmstadt, Germany) followed by two successive rounds of subculturing in MRS broth (MERCK) with incubation at 37ºC for 24 hours and 16-18 hours, respectively. Cells were then harvested by centrifuging at 3900 x g for 20 min and pellets reconstituted in sterile distilled water and held at room temperature (25ºC) until use. The yeast stock cultures were grown in Malt extract-Yeast extract-Glucose-Peptone (MYGP) broth (OXOID, Hampshire, England) incubated at 30ºC for 48 hours with agitation at 120-160 revolutions per minute (rpm). Cells were subsequently harvested by centrifuging at 3000 x g for 5 minutes, the pellets reconstituted in sterile distilled water and held at room temperature until use.

Fermentation trials

Laboratory trials: *Dolo* mash was collected hot and aseptically from a commercial *dolo* producer at Samandin, a suburb of Ouagadougou, Burkina Faso. The mash was reheated to boil and left to cool. Acidification and souring were achieved by inoculating 1000ml lots of cooled mash with 10 ml of each LAB strain and incubating at room temperature overnight (9-10 hours). The clear supernatant (wort) was subsequently decanted and boiled for 30-60 minutes to arrest the acidification process as well as concentrate the wort. At the beginning (t_0) and end (t_{10}) of acidification, pH was determined using the pH meter (JENWAY 3310, Jenway Ltd., Essex, Cambridge, UK) while LAB cell growth was checked in total counts by microscopy (OLYMPUS CH30, Olympus Opt. Co. Ltd, Tokyo, Japan) and a counting chamber (Bright-Line Haemacytometer, REICHERT, Buffalo, NY, USA). Alcoholic fermentation was achieved by adding 50ml of each yeast strain to a 500ml lot of cooled wort and incubating at room temperature overnight (11-12 hours). pH and yeast cell growth were again checked at the beginning (t_0) and end (t_{12}) of the alcoholic fermentation process, using the same methods as for the LAB.

Field (pilot plant) trials: Hot mash from a commercial *dolo* producer at Samandin was dished out with clean calabashes into clean, sterile, covered pots and allowed to cool. For acidification, 160ml of each LAB inoculum was added to a 16L of cooled mash and incubated at 25ºC for 9-10 hours. The clear supernatant (wort) was subsequently decanted into clean, covered pots, boiled for 3-4 hours to arrest acidification and ensure concentration, and then cooled. pH and LAB cell growth were checked at the beginning (t_0) and end (t_{10}) of acidification (as in laboratory trials). Five hundred milliliters of the cell suspension of each yeast strain was inoculated into a 5L lot of cooled wort and incubated at 25ºC overnight (11-12 hours) for alcoholic fermentation to take place. pH and yeast cell growth were again checked at the beginning (t_0) and end (t_{12}) of the alcoholic fermentation process (as in laboratory trials). Multiple yeast strain inoculum pitching was also done involving the use of both yeast strains at the same time.

All trials were carried out in duplicate and repeated once. Results of all analysis therefore represent means of four replicate trial fermentations with duplicate measurements.

Sensory evaluation of *dolo*: Samples of *dolo* produced under both laboratory and field conditions were evaluated by a four-member panel of non-professional assessors (but conversant with *dolo*) at the Sensory Evaluation Unit of the Département de Technologie Alimentaire (DTA), Ouagadougou, Burkina Faso. Panelists were asked to rate samples for taste, aroma and mouth feel compared to traditional *dolo*, using a ten-point Hedonic scale ranging from (0--not different and 10--extremely different from commercial *dolo*).Categorization as different meant worse than the commercial product. The order of presentation of samples was randomized.

Statistical analysis: The evaluation was repeated once for each type of trial and results were subjected to the Student Test (t) analysis.

RESULTS

pH and cell growth: The pH of *dolo* wort reduced from 5.0 to 4.9 immediately upon addition of the LAB strains, stabilizing at between 3.6 and 3.4 at the end of acidification in 10 hours for the two strains (Table 1). A final pH of 3.4 was achieved at the end of the alcoholic fermentation with each yeast strain, this being close to the value of 3.5 recorded for traditional *dolo*. LAB numbers increased significantly from 10^6 to 10^9cfu ml^{-1} during the acidification process while yeast cell numbers only increased slightly from 10^6 to 10^7cfu ml^{-1} during alcoholic fermentation. The yeast cell numbers however compare favourably to the numbers found in traditional *dolo*.

Table 1: pH of growth medium and cell numbers of starters during fermentation of *dolo* in laboratory trials.

Parameter	Fermentation Time (t)	*Dolo* mash	LAB fermentations		Fermentation Time (t)	Alcoholic (Yeast) fermentations		Commercial *Dolo*
			ZN4.1	SF6.1a		AC17	TK25	
PH	t_0	5.0±0.07	4.9±0.05	4.9±0.05	t_0	3.5±0.09	3.4±0.02	-
	t_{10}	--	3.6±0.29	3.4±0.19	t_{12}	3.5±0.05	3.4±0.06	3.5±0.1
Cell density (cfu ml^{-1})	t_0	0.0	0.9±0.38 (x 10^6)	1.2±1.15 (x 10^6)	t_0	4.0±1.13 (x 10^6)	0.9±0.19 (x 10^6)	-
	t_{12}	--	1.5±0.00 (x 10^9)	1.4±0.00 (x 10^9)	t_{12}	6.0±1.36 (x 10^7)	2.7±2.07 (x 10^7)	3.0±0.00 (x 10^7)

LAB = lactic acid bacteria (*Lactobacillus fermentum* strains); Yeast = (*Saccharomyces cerevisiae* strains) t_o =fermentation time at 0hour; t_{10} = fermentation time at 10hours; t_{12} = fermentation time at 12hours; values shown indicate mean of four independent readings ± SD.

As in the laboratory trials, pH of mash in the field trials decreased from an initial 5.0 to 3.5 with both LAB strains while cell number increased sharply from 10^6 to 10^9cfu ml^{-1} during acidification. A similar situation was observed for the alcoholic fermentation where pH of the final product stabilized at 3.5 and yeast cell number reached 10^7cfu ml^{-1} for single strains and also with a combination of the two. For the two stages of fermentation (i.e. LAB and Alcoholic (Yeast), the pH and cell number values compared favourably with those of commercial *dolo*.

Sensory evaluation of *dolo* produced with starter cultures: Yeast strain AC17 in combination with either LAB strain produced *dolo* with one or more attributes much more comparable to commercial *dolo* than yeast strain TK25 (Table 3).

Table 2: pH of growth medium and cell numbers of starters during fermentation of *dolo* in field trials.

Parameter	*Time (t)	*Dolo* wort	LAB fermentations		*Time (t)	Alcoholic (Yeast) fermentations			Commercial *Dolo*
			ZN4.1	**SF6.1a**		**AC17**	**TK25**	**AC17 + TK25**	
PH	t_0	5.9±0.07	5.2±0.10	5.1±0.02	t_0	3.6±0.13	3.6±0.10	3.6±0.11	-
	t_{10}	--	3.5±0.17	3.5±0.13	t_{12}	3.5±0.05	3.5±0.06	3.5±0.10	3.2±0.1
Cell density (cfu ml^{-1})	t_0	0.0	0.1±0.04 (x 10^6)	0.2±0.05 (x 10^6)	t_0	1.6±0.17 (x 10^6)	0.9±0.50 (x 10^6)	1.4±0.68 (x 10^6)	-
	t_{12}	--	0.7±0.31 (x 10^9)	1.5±0.35 (x 10^9)	t_{12}	2.6±1.07 (x 10^7)	2.1±1.39 (x 10^7)	5.7±4.30 (x 10^7)	2.8±0.00 (x 10^7)

LAB= lactic acid bacteria (*Lactobacillus fermentum* strains); Yeast = (*Saccharomyces cerevisiae* strains); * time = fermentation time; t_o =fermentation time at 0hour; t_{10} = fermentation time at 10hours; t_{12} = fermentation time at 12hours; values shown indicate mean of four independent readings ± SD.

Table 3: Sensory evaluation of *dolo* (average ratings of panelists in laboratory and field trials).

Starter combination	Characteristic		
	Taste	Aroma	Mouth feel
ZN4.1 + TK 25	5.93 ± 1.08	4.42 ± 0.01	4.03 ± 0.17
ZN4.1 + AC 17	4.13 ±1.04	4.01 ± 0.70	3.63 ± 1.37
SF6.1a + TK 25	5.06 ± 0.98	6.54 ± 1.56	5.92 ± 0.95
SF6.1a + AC17	4.70 ± 1.30	5.43 ± 0.57	4.09 ± 0.49
ZN4.1 + AC 17 + TK 25	3.50 ± 0.00	5.50 ± 0.00	3.16 ± 0.00
SF6.1a + AC 17 + TK 25	2.58 ± 0.42	3.96 ± 0.30	5.50 ± 1.00

Legend: Evaluation scale used varies from 0—not different from commercial *dolo*; to10—extremely different from commercial *dolo;* ZN4.1 & SF6.1a = *Lactobacillus fermentum* strains from Burkina Faso; AC17 & TK 25 = *Saccharomyces cerevisiae* strains from Ghana

According to the results yeast strain AC17 in combination with LAB strain ZN4.1 produced *dolo* with average ratings for mouth feel, aroma and taste being 3.6, 4.0 and 4.1, respectively, while a combination of yeast strain TK25 and LAB strain ZN4.1 gave *dolo* with average ratings of 4.0, 4.4 and 5.93 for the three parameters, respectively. A similar pattern was observed for combinations of either yeast strain with LAB strain SF6.1a though ratings were much lower. Double-strain combination of both yeast strains with LAB strain SF6.1a produced *dolo* of more acceptable taste and aroma than with LAB strain ZN4.1 which gave *dolo* of better mouth feel as observed with the single-strain combinations of each of the yeast strains with LAB strain ZN4.1. *Dolo* produced with double yeast starter combinations with each LAB strain (i.e. SF6.1a + AC 17 + TK 25 and ZN4.1 + AC 17 + TK 25) had minimum mean ratings of 2.58 and 3.16 for taste and mouth feel respectively, and was not significantly different from commercial *dolo* ($t_{1-\alpha/2} = t_{0.95} = 3.17$) at 5% confidence level ($p < 0.05$)

DISCUSSION

The observed drop in pH of sorghum mash during acidification (lactic fermentation) from 4.9 to 3.4 (laboratory trials) and 5.2 to 3.5 (field trials) is necessary for yeast growth and elimination of spoilage bacteria and moulds. The observed lower initial pH of sorghum mash of 4.9 during acidification in the laboratory trials as compared to the 5.2 of the field trials could have resulted from the reheating and concentration of the mash after it had been taken from the producer for the laboratory trials. This action ostensibly could have facilitated the production of more lactic acid by the LAB from the concentrated carbohydrate. Statistically, however, this difference was insignificant. During alcoholic (yeast) fermentation pH dropped from 3.5 to 3.4 (laboratory trials) and 3.6 to 3.5 (field trials). This final pH is normal for *dolo* which like *pito* is known to have a pH value ranging between 3.4 and 3.6 (Sefa-Dedeh, 1991). Glover *et al*. (2005) also reported an initial pH of *pito* wort in the acidic range of 3.58, this experiencing very little change over the 12h alcoholic fermentation stage with values ranging between 3.39 and 3.55. Again, the minimal drop in pH during alcoholic fermentation was similar to findings by Damiani *et al*. (1996) who found in wheat sourdough that the pH did not drop below 5.4 in yeast started dough compared to values between 3.72 and 4.35 for dough started with lactic acid bacteria or with associations of lactic acid bacteria and yeasts. This pH, it is believed, ensures preservation of the final product from spoilage microorganisms.

The observed significant increase in cell numbers of LAB within 10 hours (10^6 –10^9 cfu ml^{-1}) under both laboratory and field trial conditions is advantageous in providing enough enzymes to hydrolyze the polysaccharides, proteins and lipids to give the taste, aroma and mouth feel that is pleasant and attractive to *dolo* consumers (Glover *et al.,* 2005). Gassem (2002) reported lactic acid bacteria counts ranging from 4.01 to 8.19 log cfu per ml in *sobia*, a traditional fermented beverage from wheat and malt flours in the Western province of Saudi Arabia.

The moderate increase in yeast cell numbers (10^6 –10^7 cfu ml^{-1}) within 12 hours under both test conditions was suitable for producing the desirable alcohol level of 2-3% (v/v) as well as moderate quantities of amino acids, vitamins and proteins (Ekundayo, 1969; Bansah, 1990).

Wood and Hodges (1985) and Martinez-Anaya *et al*., (1990) have suggested that interactions between lactic acid bacteria and yeasts during the production of fermented foods involve a "symbiotic" association due to mutual growth stimulation based on their amino acids and carbohydrate metabolisms. A rapid growth of lactic acid bacteria lowers the pH favouring yeast growth during the subsequent alcoholic (yeast) fermentation stage (Yong & Wood, 1976; Jespersen *et al*., 1994). According to Gobbetti *et al*. (1994), growth of *S. cerevisiae* 141 was enhanced from 10^7 to 10^8 cfu g^{-1} during wheat sourdough fermentation due to the ability of the yeast to sequentially utilize free amino acids produced by lactic acid bacteria. They further maintained that this stable co-metabolism between the LAB and yeasts is common to many African indigenous fermented foods, enabling the utilization of substances that are otherwise non-fermentable (e.g. starch) and thus increasing the adaptability of these microbes to complex food systems.

The ability of combinations of either LAB strain with yeast strain AC17 to produce *dolo* that is more comparable to the traditional product than combinations with yeast strain TK 25 is rather surprising as TK25 is known to have a broader carbohydrate assimilation spectrum (9 sugars) than AC17 (4 sugars)(Glover *et al.,* 2005). Perhaps, as suggested by Sefa-Dedeh *et al.* (1999), the yeast strains were involved in the assimilation of nutrients for cell growth to the detriment of fermentation activities to enhance flavour and general acceptability of the final product. Thus LAB strain ZN4.1 in combination with yeast strain AC17 gave good *dolo* in all attributes evaluated. Teniola and Odunfa (2001) reported the use of mixed cultures of *S. cerevisiae* and *Lactobacillus brevis* for fermentation of Nigerian *ogi*, resulting in a product much more improved in terms of acceptability and increased concentrations of lysine and methionine.

Double-strain starter culture combinations of lactic acid bacteria and yeasts have been reported in several studies on sourdough to produce more aroma compounds and in many cases to improve flavour than when used individually (Martinez-Anaya *et al*., 1990; Hansen & Hansen, 1994; Meignen *et al*., 2001).

According to Annan *et al.* (2003), one of the major aims of isolating starter cultures for use in the production of fermented foods has is to ensure consistency and to preserve the unique flavour, aroma and texture attributes of these products. The fact that isolates of *S. cerevisiae* from African fermented products have properties different from those of well recognized starter cultures (Hayford & Jespersen, 1999; van der Aa Kühle *et al*., 2001; Glover *et al*., 2005) demonstrates that starter cultures for indigenous fermented foods and beverages should be isolated from the products they are supposed to be used for, and selected according to the technological properties required for the actual type of product (Jespersen, 2003). Our study, which involved the use of strains of lactic acid bacteria and yeast isolated from *dolo* from Burkina Faso and *pito* from Ghana, is yet another example of isolating strains of microorganisms from African fermented foods and beverages and thereafter successfully using them as starter cultures.

The present study has generated information on the isolation of dominant microorganisms (lactic acid bacteria and yeast) from commercially –produced *dolo* and using them as starter cultures under both laboratory and pilot-scale conditions to get products of organoleptic quality quite similar to that of the commercial product. Single and double-strain combinations of the starter cultures have been used successfully to produce *dolo* of different quality indices. Single –strain combinations of LAB strain ZN4.1 and yeast strain AC 17 produced *dolo* that was most comparable to the traditional product while double-strain combination SF6.1a + AC 17 + TK 25 produced *dolo* of more comparable acceptability than combination ZN4.1 + AC 17 + TK 25

Varied combinations of the starters should be investigated in order to discover a suitable combination for producing *dolo* possessing all the desired organoleptic qualities and consistency.

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