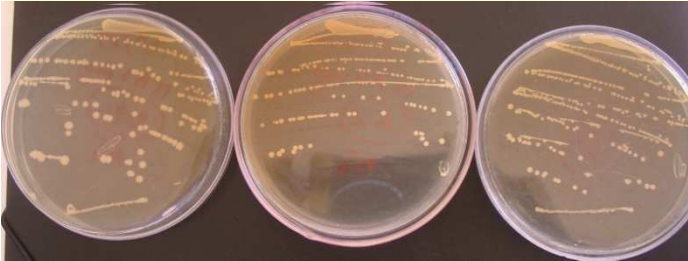


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The use of selected strains of *Lact. fermentum* and yeast isolate for controlled fermentation of *dolo*, a traditional sorghum based beverage

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Outline

- **Introduction**
- **Objective of the study**
- **Experimental methods**
- **Results**
- **Conclusion & perspectives**

INTRODUCTION (1)

- Dolo: traditional beer from Burkina Faso, fermented beverage made from sorghum,**
- Like the other fermented beverages and foods in West Africa, dolo is produced by traditional processing with natural and uncontrolled fermentation.**
- In such fermentation processes, microorganisms involved were provided by raw material, utensils and environment**

INTRODUCTION(2)

- ❑ Disadvantages of uncontrolled fermentation: low yield, greater variability of quality and stability of the final product with risks of contamination by undesirable microorganisms and metabolites (Motarjemi, 2002)

- ❑ Traditional brewing of dolo from sorghum malt comprises 2 types of fermentation:
 - Natural lactic acid fermentation lasts for 12-16 h; *Lact. fermentum* is found to be the dominant bacteria involved (Sawadogo-Lingani *et al.*, 2007).

 - Alcoholic fermentation of the acidified wort lasts for 9 -12 h. *Saccharomyces cerevisiae* is found to be the dominant species of yeast (van der Aa Kühle *et al.*, 2001; Glover *et al.*, 2005)

INTRODUCTION (3)

- Our Previous study on technological properties of *Lact. fermentum* isolates involved in the natural fermentation of the wort concluded that rapid acidifying isolates with antimicrobial activity and producing EPSs have the potential to be used as starter cultures for a controlled lactic fermentation of the wort (Sawadogo-Lingani *et al.*, 2008).
- Formation of EPSs by LAB may function in foods as:
 - viscosifying, stabilizers, emulsifiers, gelling agents or water-binding agents (van den Berg *et al.*, 1995; De Vuyst *et al.*, 2001),
 - and play important role in the rheological behavior and texture of the products (de Vuyst and Degeest, 1999).

Objectives of this study

- ❑ To experiment the use of pure cultures (*Lact. fermentum* and yeast isolates) in dolo production
- ❑ To constitute data base on starter cultures use for traditional fermented beverages
- ❑ To promote the development of new technologies for semi-industrial scale processing of fermented beverages

EXPERIMENTAL METHODS

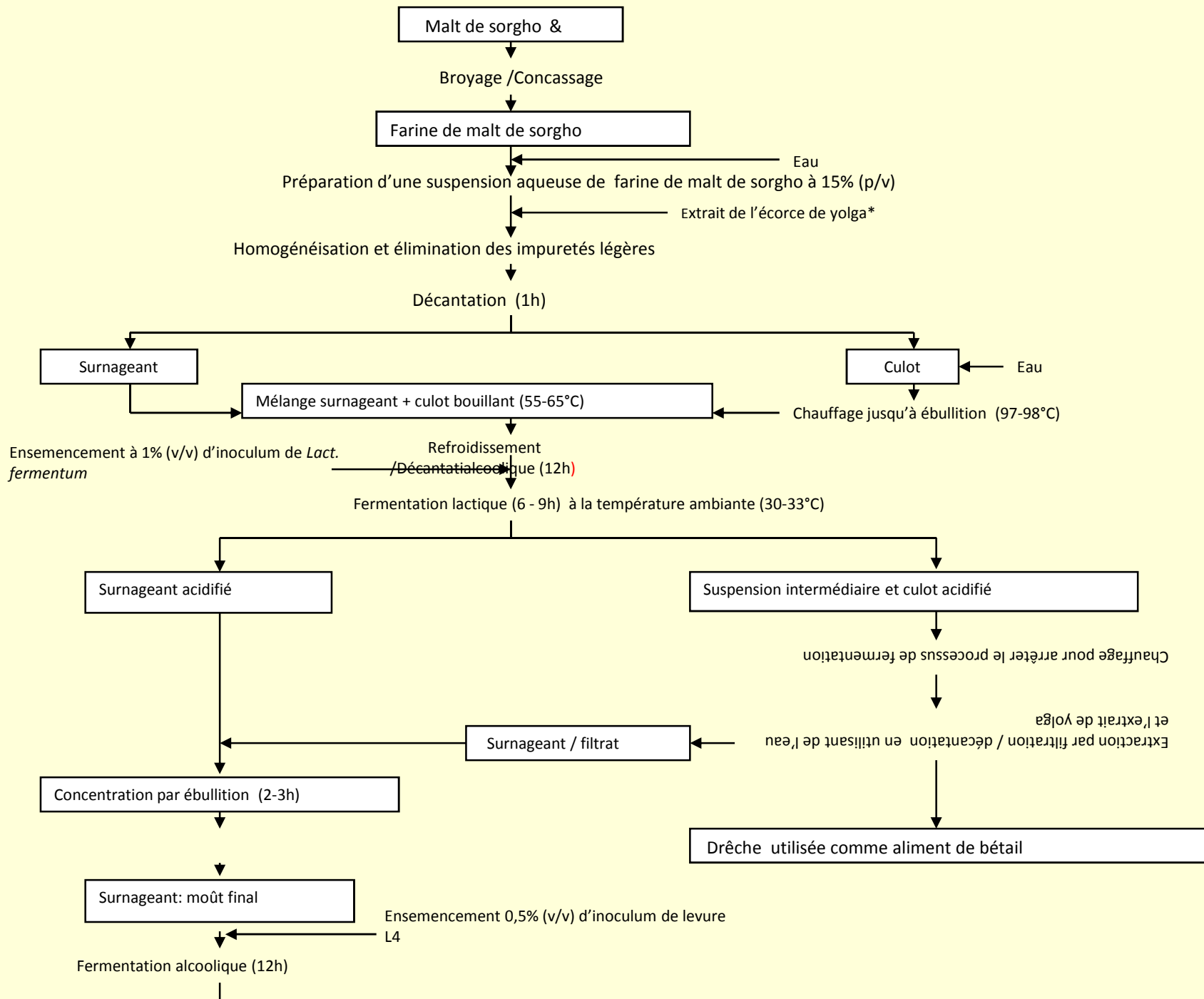
- Characteristics of LAB isolates used

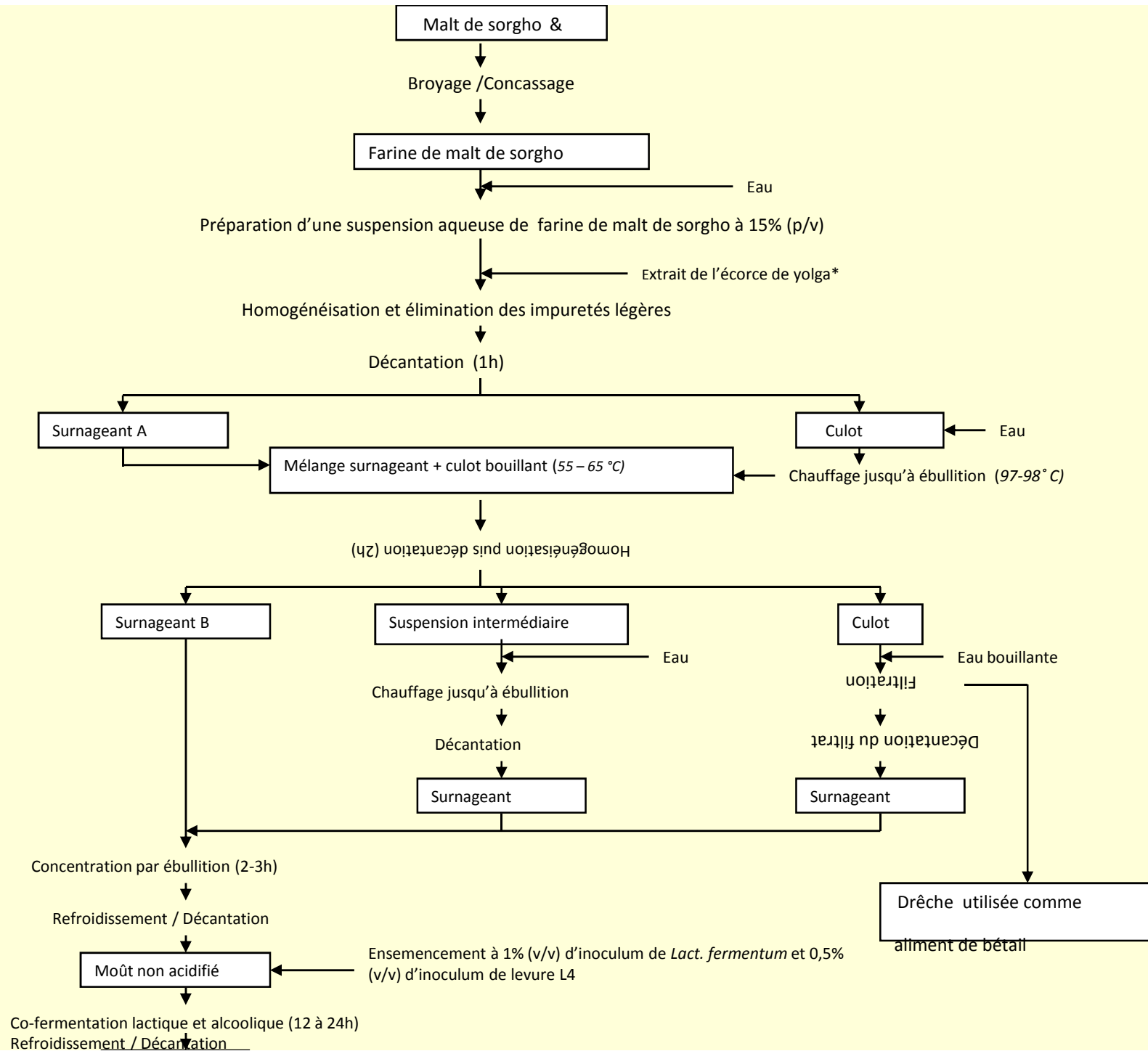
References of the isolates	Characteristics
ZN4.1	<ul style="list-style-type: none">-Rate of acidification (in ΔpH) of 1.12 ± 0.15 pH unit in 9h of fermentation of sorghum malt broth: G2 group of isolates;-Capable of inhibition the growth of <i>Staph. aureus</i>, <i>E. coli</i> and <i>L. innocua</i>- Not capable of producing EPSs
SF6.1a	<ul style="list-style-type: none">-Rate of acidification: G2 group of isolates;-Capable of inhibition the growth of <i>Staph. aureus</i>, <i>E. coli</i> and <i>L. innocua</i>;-Capable of producing EPSs
SF8.2	<ul style="list-style-type: none">-Rate of acidification: G2 group of isolates;-Capable of inhibition the growth of <i>Staph. aureus</i> and <i>E. Coli</i>- Not capable of producing EPSs
SD1.8	<ul style="list-style-type: none">-Rate of acidification of 1.15 ± 0.15 pH unit in 6h of fermentation of sorghum malt broth: G1 group of isolates-Capable of inhibition the growth of <i>Staph. aureus</i>- Capable of producing EPSs
Yeast L4	Isolated and purified from dolo sample (Samandin)

EXPERIMENTAL METHODS

Trials conducted in the laboratory

Trials	Based on the traditional brewing method				Based on lactic and alcoholic co-fermentation (mix LAB and yeast isolates)			
Conditions	<p>Lactic fermentation of sterilized mash by isolates of <i>Lact. fermentum</i> used in single culture. Incubated at 37°C for 24h; Inoculum: overnight culture of each <i>Lact. fermentum</i> used at 1% (v/v)</p>				<p>Concentrated and clear wort was prepared on the base of the traditional method with modifications; Inoculation of sterilized wort with the isolates of <i>Lact. fermentum</i> (in single culture at 1%) and yeast L4 (0.5 %); incubated at 37°C for 24h</p>			
	ZN 4.1	SF6.1a	SF8.2	SD1.8				
	<p>Alcoholic fermentation: each acidified wort (clear supernatant) is concentrated by boiling, sterilized cooled down and fermented by yeast L4; incubated at 30°C for 24h; Inoculum: overnight culture of yeast L4 used at 0.5% (v/v)</p>							
Dolo samples	ZN4.1/L4	SF6.1a/L4	SF8.2/L4	SD1.8/L4	ZN 4.1& L4	SF6.1a & L4	SF8.2 & L4	SD1.8 & L4
<p>Sampling every 3h for pH, viable cell counts; dolo were evaluated by panel of 5 persons for the sensory analysis at 12h and 24h of fermentation</p>								





□ Experimental conditions at the production site:

➤ Traditional method with the use of :

- *Lact. fermentum* isolates (4) in single culture for a controlled lactic fermentation of the wort
- Yeast L4 for controlled alcoholic fermentation of each acidified wort
- Traditional dolo was produced and used as control: spontaneous lactic fermentation & local yeast used for alcoholic
- Sampling: beginning and end of each fermentation
- Sensory analysis of dolo samples : panel of 21 persons

RESULTS

Laboratory

- A rapid decrease in pH was observed as expected.
- Isolates ZN4.1, SF6.1a, and SF 8.2 confirmed their acidifying characteristic : 9 h to record pH necessary for dolo wort (3.5-3.8)
- Isolate SD1.8 was lightly below its acidifying characteristic

- A good growth of *Lact. fermentum* and yeast was observed during lactic & alcoholic co-fermentation and during the separated lactic acid and alcoholic fermentations.

Laboratory

Times (h)	Traditional Brewing Method : separated lactic et alcoholic fermentations			Co-fermentation		
	Lactic fermentation		Alcoholic fermentation			
	<i>Lact ferm</i> (4 strains (log cfu/ml))	pH	Yeast L4 (log cfu/ml)	<i>Lact ferm</i> (4) (log cfu/ml)	Yeast L4 (log cfu/ml)	pH
0h	5.94- 6.41	4.86	5.17- 5.28	5.76 - 6.08	4.92 - 5.04	4.83
6h	7.34- 8.30	3.81- 4.33	6.18 - 6.30	7.63 – 8.78	5.90 - 6.15	4.08 - 4.54
9h	9.28 - 9.46	3.62 - 3.94	6.94-7.08	8.76 – 9.11	6.25 - 6.75	3.74- 4.05
12h	9.28 - 9.46	3.52 - 3.67	7.67 - 7.79	9.23 - 9.59	6.98 - 7.34	3.68 - 3.75
24h	9.34 - 9.82	3.38 - 3.51	7.82 - 8.0	9.36 - 9.76	7.11 - 7.50	3.43 - 3.55

Trials based on Lactic & alcoholic co-fermentation

❖ LAB growth/ lactic & alcoholic co-fermentation

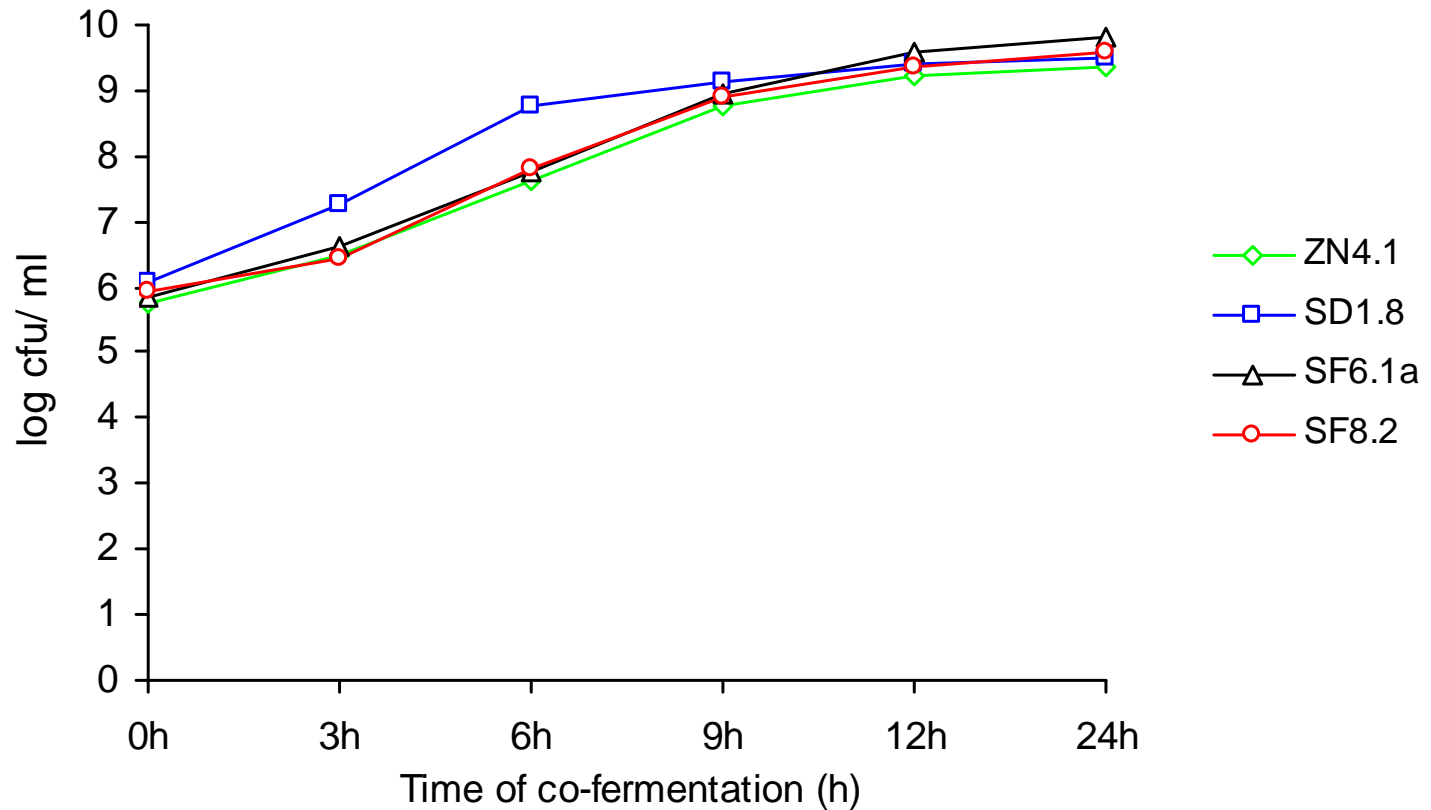


Fig 5.3: Growth of *Lact. fermentum* ZN4.1, SD1.8, SF6.1a and SF8.2 during the lactic and alcoholic co-fermentation of the wort

❖ Yeast L4 growth / lactic & alcoholic co-fermentation

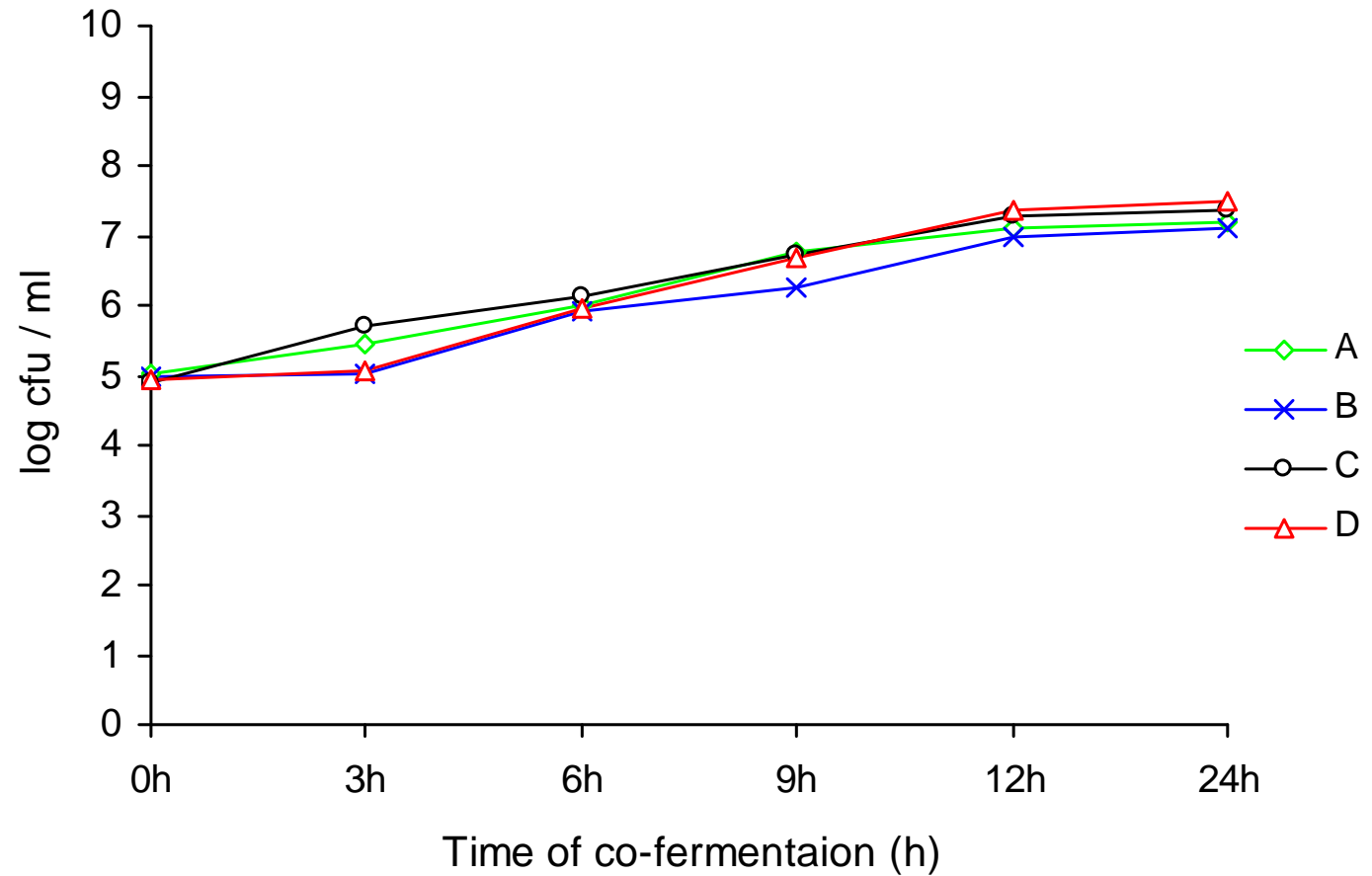


Fig. 5.4: Growth of yeast L4 during the lactic and alcoholic co-fermentation of the wort. A: ZN4.1/L4; B: SD1.8/L4; C: SF6.1a/L4 et D:SF8.2/L4

❖ pH evolution / lactic & alcoholic co-fermentation

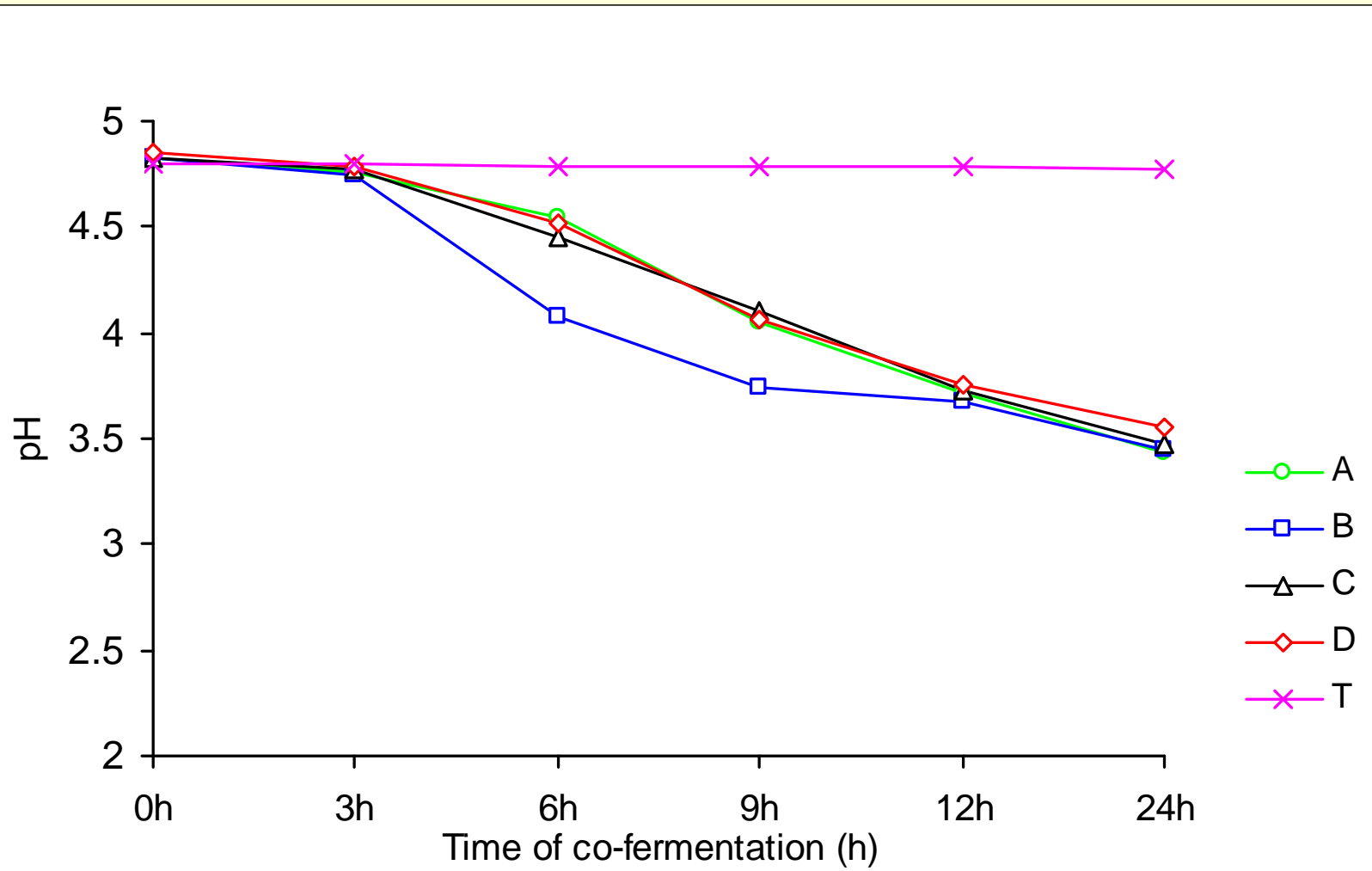
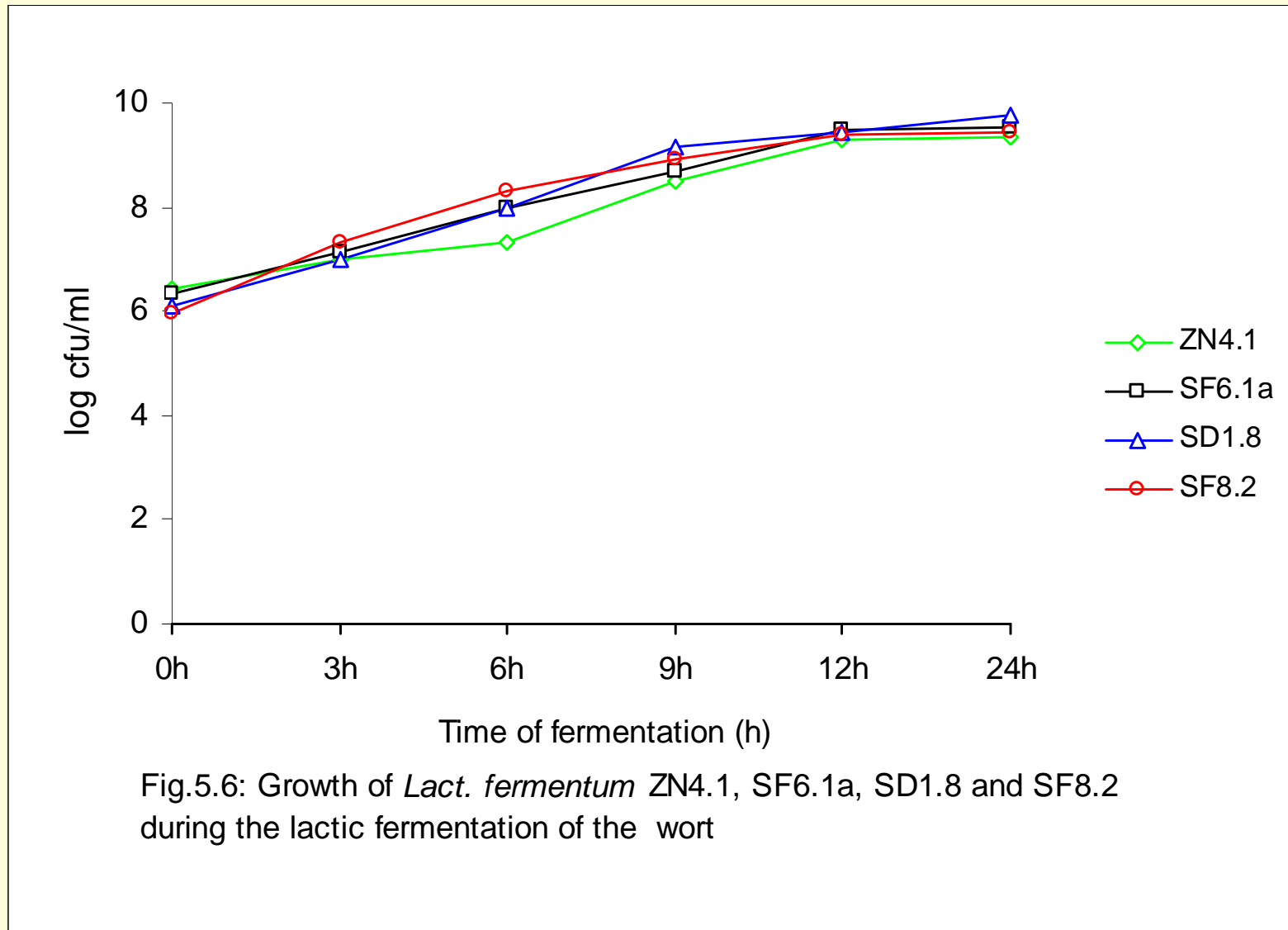


Fig. 5.5: Evolution of pH during the lactic and alcoholic co-fermentation of the wort. A: ZN4.1/L4; B: SD1.8 /L4; C: SF6.1a/L4 and D: SF8.2 /L4; T: negative control

Trials based on traditional brewing method: separated lactic and alcoholic fermentations

❖ LAB growth/lactic fermentation



❖ Evolution of pH/lactic fermentation

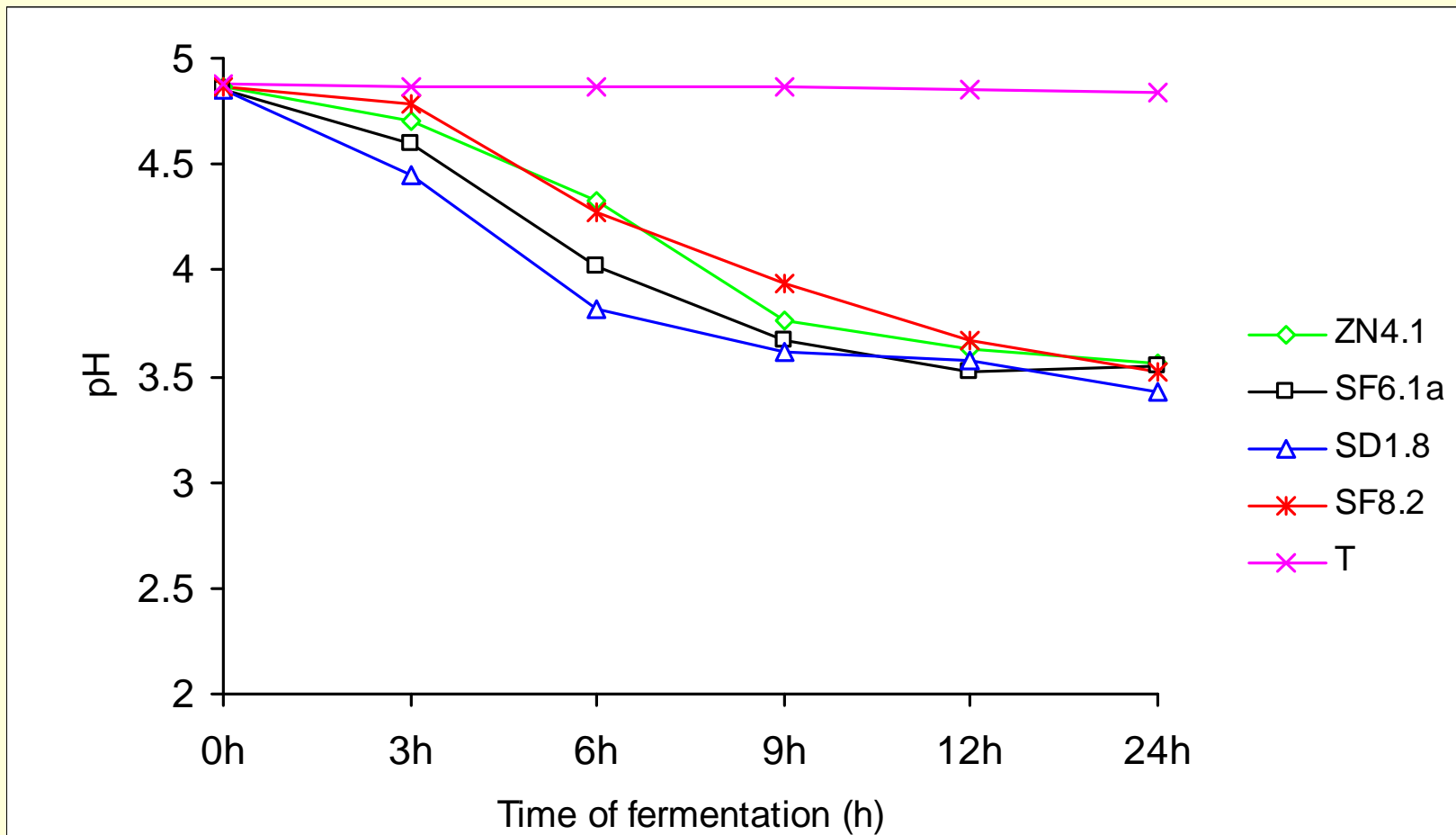


Fig.5.7: Evolution of pH during the lactic fermentation of the worts by *Lact. fermentum* ZN4.1, SF6.1a, SD1.D et SF6.2; T: negative control ensemencé

❖ Yeast growth/Alcoholic fermentation

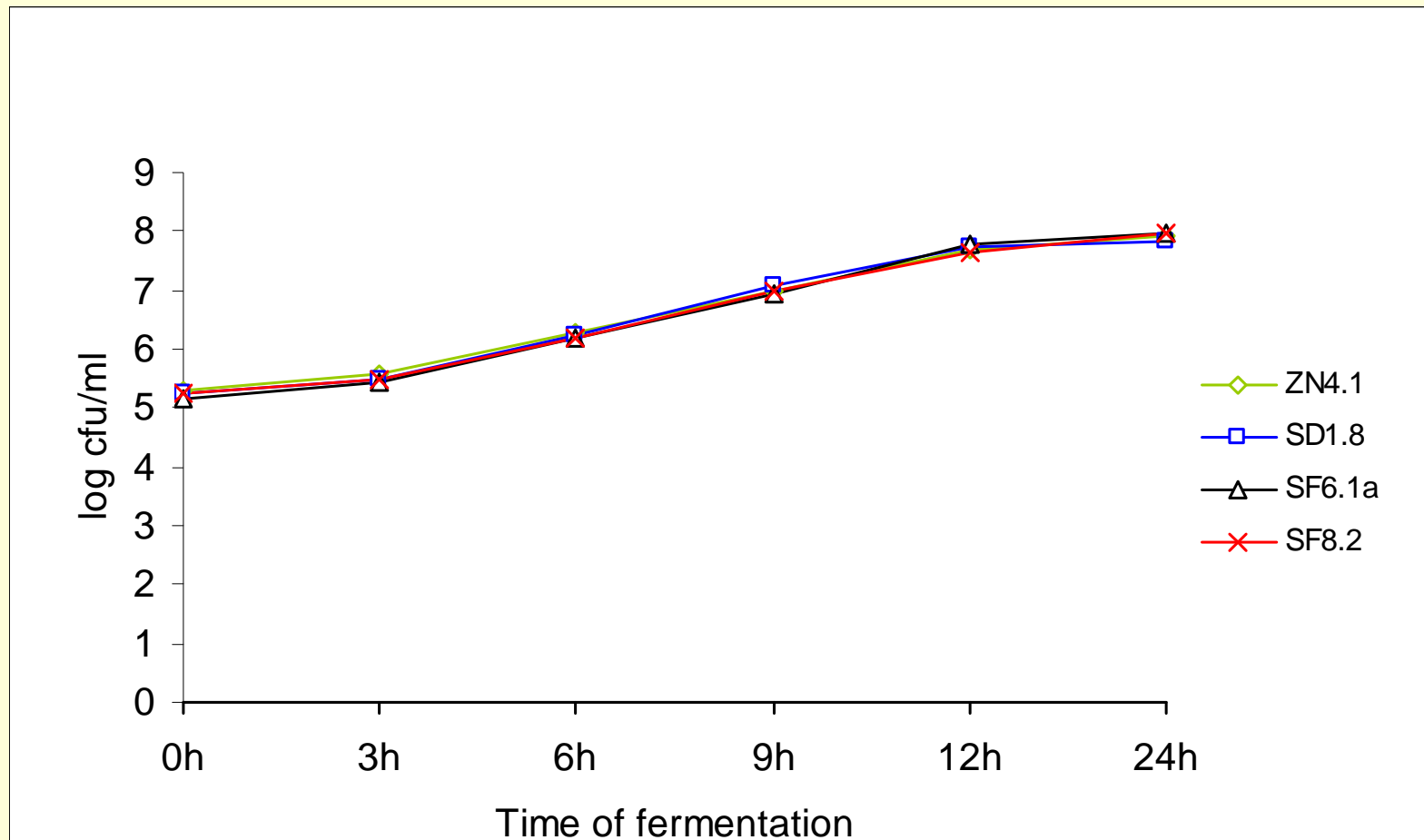


Fig.5.8: Growth of yeast L4 during the alcoholic fermentation of worts previously fermented by *Lact. fermentum* ZN4.1, SD1.8, SF6.1a and SF8.2

RESULTS

- ❑ When comparing the 2 methods, lactic & alcoholic co-fermentation and those based on the traditional processing (separated lactic and alcoholic fermentations):
 - Similar levels of yeast L4 growth, and *Lact. fermentum* isolates growth were observed
 - But a delay of around 2-3h on the decrease of pH was observed with the co-fermentation compared to the separated lactic fermentation

RESULTS

- The reduction of the duration of lactic fermentation by the use of selected isolates has been observed as expected. That has also been observed for african fermented products: Halm *et al.*, (1996) for Kenkey, Viéra-Dalodé *et al.* (2008) for gowè.
- The simultaneous growth of LAB (*Lact. fermentum*) and yeast (L4) noticed during the co-fermentation of the wort has also been reported in previous results (Orji *et al.*, 2003; Muyanja *et al.*, 2003).

Production site results

- LAB counts, Yeast counts and pH recorded during the trials conducted at the production site were comparable to those of the laboratory:
 - pH of 3.61-3.76 and LAB counts of 9.32 - 9.57 log cfu/ml in 9h of controlled lactic fermentation;
 - yeast L4 counts of 7.67-7.80 log cfu/ml in 12h of alcoholic fermentation of the final wort.

Sensory analysis results

- Dolo produced in the Laboratory : the panelists (5) found that the four samples of *dolo* (ZN4.1/L4, SF6.1a/L4, SD1.8/L4 and SD8.2/L4) had *dolo* organoleptic characteristics for taste, flavour and general aspect; but found that the samples obtained by co-fermentation were too cloudy
- Dolo produced at the production site : the panelists (21) found that the *dolo* samples had sensory characteristics comparable to the traditional *dolo* and were acceptable. The general tendency of their classification in order of preference has been: traditional *dolo*, *dolo* SF6.1a/L4, *dolo* SD1.8/L4, *dolo* SD8.2/L4 and *dolo* ZN4.1/L4.

CONCLUSION & PERSPECTIVES

- Selected strains of *Lact. fermentum* allowed to ferment the wort at the production site in 9h instead to 12-16h by the spontaneous fermentation (traditional brewing)
- Used in mix culture, *Lact. fermentum* and yeast L4 allowed to produce a kind of *dolo* in 12h of co-fermentation instead to 21 - 48h (combined duration of the both fermentations in the traditional processing), contributing to reduce the duration of the entire process.
- General flow diagrams of the 2 methods with the use of starter cultures have been established
- Scale-up of the production of *dolo* using starter cultures can be experimented at a pilot plan with the implementation of Good Hygienic and Good Manufacturing Practices to master the processes

Acknowledgements

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- **THANK YOU FOR KIND ATTENTION!**