Portuguese traditional sourdough, manufactured from maize and rye: long term fermentation

João M. Rocha & F. Xavier Malcata

Escola Superior de Biotecnologia, Universidade Católica Portuguesa
Rua Dr. António Bernardino de Almeida, P-4200-072 Porto, Portugal

INTRODUCTION

BROA **is a traditional type of bread manufactured, in the absence of a starter culture, at the farm level in Northern Portugal, following ancient manufacture protocols. This food is obtained from maize and rye flour, and inoculation is via a given amount of old fermented dough.**

Previous microbiological studies, have shown that sourdough contains a diverse and rather complex wild microflora. Total viable counts for a large group of microorganisms were obtained after inoculation on 19 different selective culture media , incubated under a total of 22 distinct conditions: such microorganisms encompassed yeasts, molds, Gram$^-$ rods, Gram$^+$ rods (endospore-forming and nonsporing), Gram$^+$ cocci (catalase$^+$ and catalase$^-$), and general mesophilic and thermophilic viable forms (vegetatives and spores).

A similar approach was followed in attempts to study the microbiological profile throughout the fermentation process and storage: dough was prepared following the traditional process and kept during several weeks under controlled temperature and relative humidity.

EXPERIMENTAL METHODS

Samples of sourdough were taken along the fermentation and total viable counts were performed after inoculation and incubation. In order to check for the existence of a wide diversity of microorganisms, a large number of culture media and incubation conditions were selected according to the schematic procedure showed below:

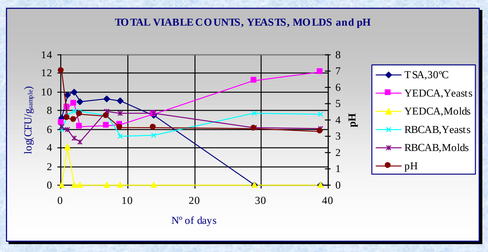
Group	Culture media	Conditions
Total Viable Counts	TSA	T=30ºC; 24-48h; aerobiose
Yeasts and Molds	YEDCA	T=30ºC; 48h; aerobiose
	RBCAB	Room Temperature; 64h; aerobiose; dark

Group	Culture media	Conditions
Gram$^-$ Rods		
Enterobacteriaceae	VRBDA	T=37ºC; 24h; anaerobiose
Pseudomonas	PAB	T=30ºC; 48h; aerobiose
Acinetobacter	*Herella* agar	T=30ºC; 24-48h; aerobiose
Alcaligenes	ANAYES	T=30ºC; 24-48h; aerobiose
Salmonella; Shigella; Yersinia; (and other facultative anaerobe coliforms)	MacConkey	T=37ºC; 24h; anaerobiose

Group	Culture media	Conditions
Gram$^+$ Rods		
Bacillus cereus	BCM	T=37ºC; 48h; aerobiose
Bacillus	TSYES	T=37ºC; 48h; aerobiose
Clostridium	RCM	T=30ºC; 64h; anaerobiose
Lactobacillus	MRS	T=30ºC; 64h; aerobiose

Group	Culture media	Conditions
Gram$^+$ Cocci		
Staphylococcus	BPM	T=37ºC; 48h; aerobiose
Micrococcus	FM	T=30ºC; 24-48h; aerobiose
Streptococcus (Lactococcus)	M17	T=30ºC; 48-64h; anaerobiose
Lactococcus	LSA	T=30ºC; 48-64h; anaerobiose
Streptococcus (Enterococcus)	KF	T=37ºC; 48-64h; anaerobiose
Enterococcus	KEAA	T=37ºC; 48-64h; anaerobiose
Leuconostoc	MSE	T=30ºC; 48-64h; aerobiose

RESULTS and DISCUSSION

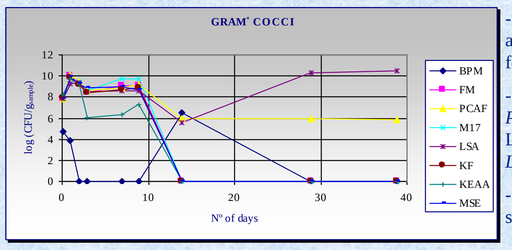


- pH reach values near 4 after 24 hours fermentation; from 9th day pH keep almost constant near 3.5; pH decreasing was especially due the metabolic activity of Lactic Acid Bacteria (LAB)

- Important differences were detected for yeasts and molds growing in the two culture media. Actually, YEDCA is more selective for yeasts and RBCAB for molds

- After 14 days fermentation, there is a general tendency for high viable numbers of yeasts and maintenance of molds with similar values than time 0

- Microflora growing on TSA disappeared completly: this show the decrease of the microflora diversity existent due the low pH values



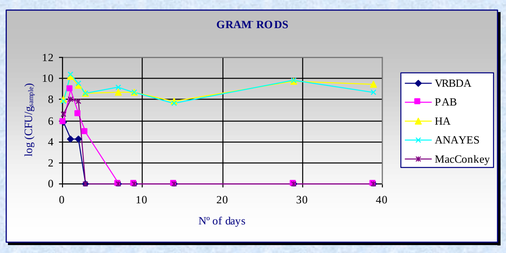
- It was observed the absence of *Staphylococcus* (Gram$^+$ cocci, catalase$^+$) after 2 days fermentation; unexpectedly, they appeared in the middle of fermentation

- Gram$^+$ cocci, catalase$^-$ from genus of *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Enterococcus* and some strains of *Streptococcus* below to the LAB; although they were all present in high values at the begin, *Lactobacillus* were the only LAB able to survive in all period studied

- These values show the importance of Gram$^+$ cocci, catalase$^+$ for the 1 day sourdough fermentation

- Only Gram$^-$ rod-shaped growing on HA and ANAYES have Colony-Forming Unit (CFU) values different from 0, after the 3th day; these media were inoculated on anaerobiose and the counts yielded final values similar to the yeasts

- There is an absence of anaerobic microflora (belonging to this group) after 3 days, with exception to *Pseudomonas* that disappeared after 7 days; this is a result of the dough acidification

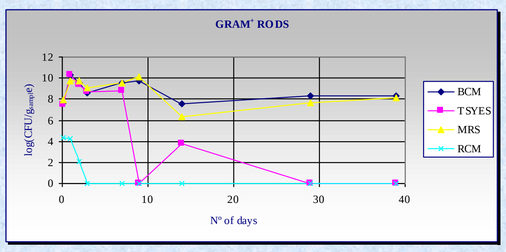


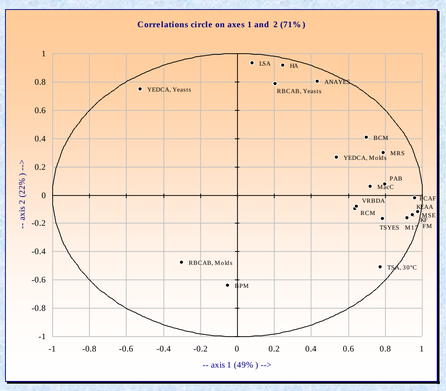
- *Bacillus* and *Lactobacillus* were the only Gram$^+$ rods present at the end of fermentation and with similar values

- In the 2nd day *Lactobacillus* were in higher numbers than yeasts, showing their importance for a short time fermentation

- *Clostridium* genus do not found good conditions to grow

- Important differences were detected between BCM and TSYES (both for the genus *Bacillus*)





- First Principal Component (PC1), explains 49% of the variance in the initial matrix and PC2 explains 22%

- PC1 and PC2 together describes 71% (cumulative percentage) of the total variance in the initial matrix

- Higher order PCs therefore influence than 29% of the model

- It is possible to define some clusters from the correlations circle plotted on axes 1 and 2 (or loading plot), for instance:

  = TSA, FM, MSE, M17, KF, PCAF, KEAA, TSYES (correlation between TSA and the other variables higher than 73%);

  = YEDCA,Yeasts and LSA, HA, RBCAB,Yeasts and ANAYES with correlations higher than 35%;

  = RBCAB,Molds and BPM with a correlation of 27%

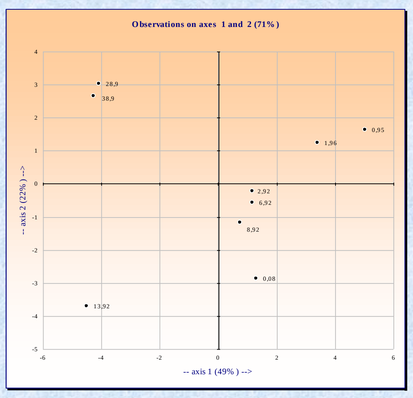
- Variables from each cluster (high correlation) or variables with high negative correlations such as TSA with YEDCA,Yeasts (-85%) or PAB with RBCAB,Molds (-64%) not allow a good distinction for the objects studied (log(cfu/g$_{sample}$)) with the days of fermentation

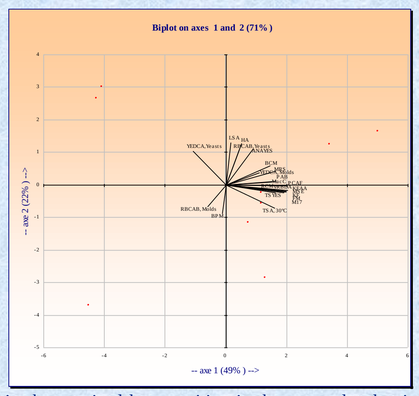
- VRBDA and RBCAB,Molds (7E-3) or VRBDA and LSA (6E-3) could be the axis to use to differentiate the objects, for instance

- Looking to the plot of observations on axes 1 and 2 (or score plot) there seems to be a time trend along PC1: from time 0 to 1 the PC1 score increases and then decreases until 14th day. PC2 has a similar trend. Following from the first to the last time of fermentation it is easy to see that the objects go from 4th to the 1st quadrant turns to the 3rd and finish in the 2nd

- Can be concluded that PC1, which is the dominant PC, has something to do with time of fermentation. In other words, PC1 lies along the maximum variance of the dependent variable "time of fermentation"

- This leads to the expected conclusion that typical microflora changes along the fermentation, as expected





- The corresponding score and loading plots is "complementary" and give valuable information about both the objects and the variables when studied together

- A great change in the typical microflora occurs from the 1st to the 2nd day of fermentation

- The last period studied (from 29th day) is characterized by a position in the score plot that is equivalent to the position of the variable YEDCA,Yeasts in the loading plot

- This means that those samples have high relative log(cfu/g$_{sample}$) values of YEDCA,Yeasts, (they have the most relative importance)

- The same reasoning can be done for the other observed points, making possible to describe the relevant microflora in each instant

GENERAL CONCLUSIONS

- The results produced indicates that yeasts, *Lactobacillus*, *Bacillus*, Acinetobacter and Alcaligenes are the predominant groups of microorganisms presented at the end of a long term fermentation; however, after 1 day fermentation there is a large diversity of microorganisms

- The pH decreasing (especially due the LAB activity) play an important rule on the control of the sourdough microflora and is determinant in the final taste and texture of *BROA*

- Is advisable to increase the fermentation time of *BROA* so as to take full advantage against undesirable microflora, and thus eventually increase its shelf-life, as happens with other sourdoughs

- An important step was also taken towards understanding the role of several microorganisms prevailing in dough, in addition to the very few yeasts and bacteria normally studied in sourdough breads

ACKNOWLEDGEMENTS

The authors are grateful to several members of the Regional Directorate of Agriculture of Entre Douro e Minho (DRAEDM) and several local farmers for cooperation within the experimental program described. Financial support for author J. M. R. was provided by a Ph.D. fellowship within the program for the Creation of National Infrastructures in Applied Science and Investigation (CIÊNCIA), administered by the National Board for Scientific and Technological Investigation (JNICT, Portugal) and by Calouste Gulbenkian Fundation. Partial financial support was received from PAMAF – IED (Ministry of Agriculture, Portugal) through a research grant entitled "Pão de milho: caracterização do processo tradicional de produção e melhoramento tecnológico".