Microbiological Profile in Sourdough: Evolution During Refrigerated Storage

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INTRODUCTION

Breadmaking is still much of an art, especially in those cases where no standard starter culture is deliberately added. One such type of bread, *BROA*, is widely manufactured at the farm level in Northern Portugal following ancient manufacturing procedures; this sourdough bread is obtained from maize and rye flours mixed with water and salt, and most chemical reactions that occur therein are brought about by adventitions microflora, which are passed from batch to batch. *BROA* is important from an economic point of view because of the large number of small farmers that depend considerably on this extra source of income for subsistence. It also helps fixating people in rural regions, thereby preventing extensive exodus towards urban areas.

Empiric know-how has been transmited between generations, and a few technological changes have become standard practices. One such alterations is the way the producers keep the sourdough for the next batch: today they keep more and more often the sourdough in a refrigerator. Note that, traditional *BROA* is not manufactured on a daily basis, but depends on the current needs.

One logical question is what happens to the wild microflora present in the sourdough as time elapses. Hence, samples of sourdough were taken throughout the preservation period at 4ºC, for up to 6 d, and total viable counting was duly performed.

This study has shown that the´general microbiological properties of sourdough remain essentially unchanged during refrigerated storage, so there are no important changes also during samples transportation from the producers until they reach control laboratory.

EXPERIMENTAL METHODS

Samples of sourdough were taken along its preservation period at 4ºC, and total viable counts were obtained 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 shown below:

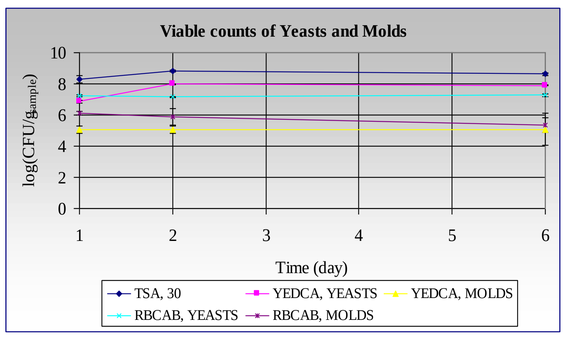
Group	Culture medium	Conditions
Total Viable Counts	TSA	T=30ºC; 24-48h; aerobiose
Yeasts and Molds	YEDCA	T=30ºC; 48h; aerobiose
	RBCAB	Room temperature; 64h; aerobiose; darkness

Group	Culture medium	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

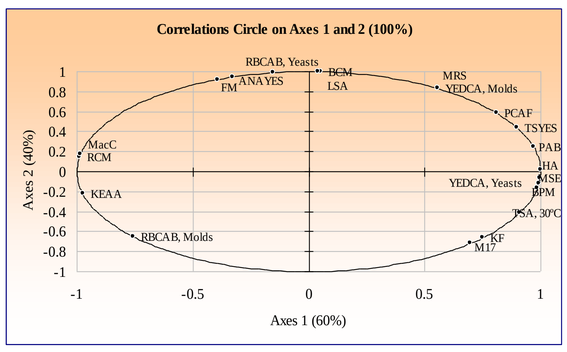
Group	Culture medium	Conditions
Gram⁺ Rods		
Bacillus cereus	BCM	T=37ºC; 48h; aerobiose
Bacillus	TSYES	T=37ºC; 48h; aerobiose
Clostridium	RCM	T=30ºC; 64h; anaerobiose
Lactobacillus	MRS	T=30ºC; 64h; aerobiose

Group	Culture medium	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



Mean log(CFU/g_{sample}):
TSA,30 - 8.6
YEDCA, Yeasts - 7.6
YEDCA, Molds - 5.1
RBCAB, Yeasts - 7.2
RBCAB, Molds - 5.8



- First Principal Component (PC1) explains 60% of the variance in the initial matrix, whereas the second Principal Component (PC2) explains the remaining 40%

- PC1 and PC2 together describe the total variance in the initial matrix

- It was possible to reduce the 22 initial variables to only 2, with essentially no loss of information

- Some clusters can be defined from the correlation circle plotted on axes 1 and 2 (or loading plot), *e. g.*:

= PAB with TSYES, HA, MSE, YEDCA,Yeasts, PCAF and BPM, with correlation coeficients (c.c.) higher than 91%, or either with MRS and YEDCA, Molds, with c.c. higher than 75%;

= TSA with BPM, YEDCA,Yeasts, KF, MSE, M17 and HA (c.c. >90%);

= BCM with LSA, RBCAB,Yeasts, Anayes and FM (c.c. >90%);

= MacConkey and RCM (c.c. = 100%); and

= KEAA with RBCAB,Molds (c.c. = 88%);

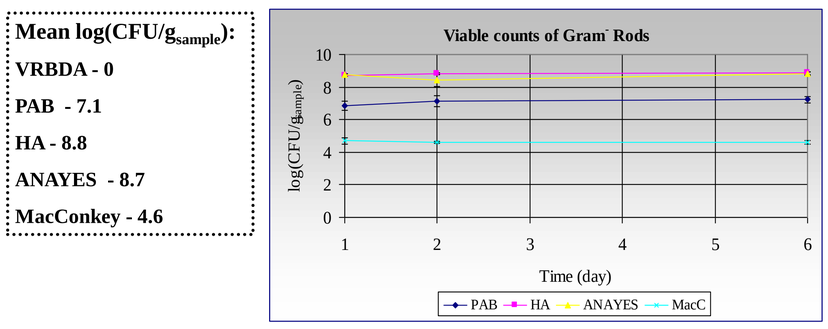
- Variables from each cluster (with high positive or negative correlations) do not allow a good distinction for the objects studied, in terms of log(CFU/g_{sample})

- Variables with low correlation could account for the axis that distinguishes the objects studied, *e. g.*: PAB and RBCAB,Yeasts (10%); PAB and ANAYES (-8%); YEDCA,Yeasts and LSA (-8%). However, the best two axis are obtained with the Principal Components (PC)

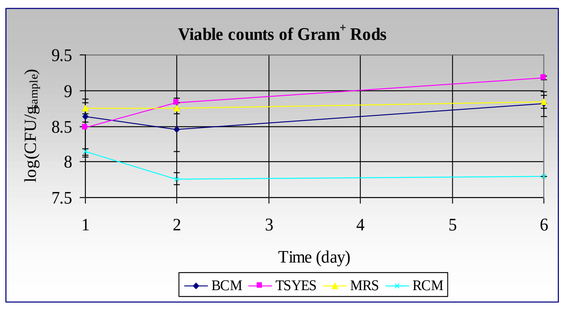
- The closer a variable is to the axis and the circle, the higher the correlation with that factor (Principal Component) is. As observed, all variables placed in the circle border, which justifies the 100% variance of the initial variables

- HA contributes fully to PC1; MSE, YEDCA,Yeasts, PAB and TSA contribute with values higher than 91%, and RCM and KEAA with values of -99% and -97%, respectively, to PC1

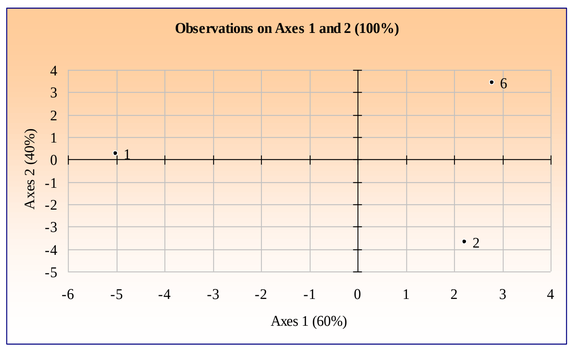
- LSA contributes fully to PC2; BCM, RBCAB,Yeasts and ANAYES contribute with values above 95% tp PC1



Mean log(CFU/g_{sample}):
VRBDA - 0
PAB - 7.1
HA - 8.8
ANAYES - 8.7
MacConkey - 4.6

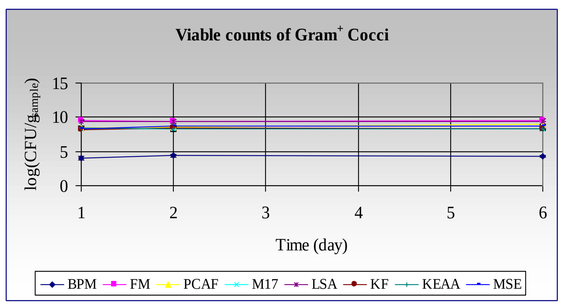


Mean log(CFU/g_{sample}):
BCM - 8.6
TSYES - 8.8
MRS - 8.8
RCM - 7.9



- The objects, in terms of number of storage days, are well dispersed, and can be distinguished from one another by the culture medium

- The general microbiological properties of sourdough remain almost unchanged during their preservation at 4ºC, with no important changes during samples transportation from the producers to the laboratory



Mean log(CFU/g_{sample}):

BPM - 4.3	**LSA - 9.4**
FM - 9.5	**KF - 8.3**
PCAF - 8.6	**KEAA - 8.3**
M17 - 8.3	**MSE - 8.6**

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