Preservation of *Oenococcus oeni* by different methods in a new culture medium

Martínez Chamás JJ[1,2]; Rubio MC[2]; Aredes Fernández PA[1,2]

[1]CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), [2]Instituto de Biotecnología, Facultad de Bioquímica, Química y Farmacia- UNT

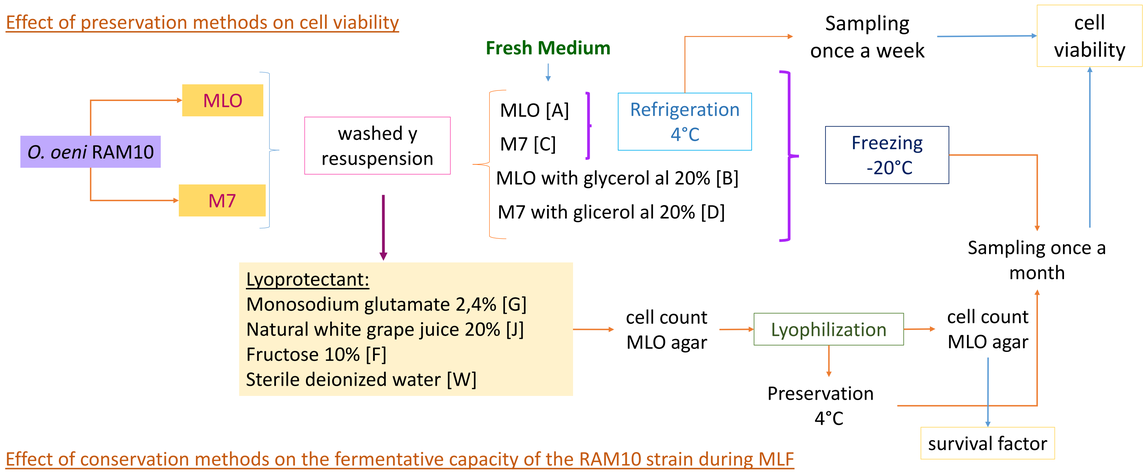
E-mail:pedroaredes@conicet.gov.ar

Introduction

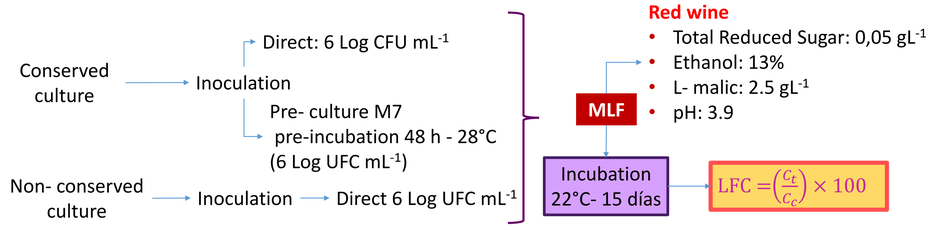
The preservation of starter cultures for malolactic fermentation (MLF) of wines is necessary for their following use. The conservation is regularly carried out at low temperatures, in a suitable medium. In refrigeration conservation, the cultures are kept at 4°C, for which the culture is required to be at high cellular concentrations in a suitable medium. Freezing is usually carried out at –20°C, however, it is necessary to use cryoprotectants such as glycerol to avoid the formation of ice crystals and thus avoid cell rupture. Lyophilization is a preservation method by which it is possible to maintain a high number of viable microorganisms. The product obtained is a culture in powder form, which allows the inoculation of the wine in a simple and homogeneous way. However, this preservation process can cause cell damage due to the freezing that the culture must undergo to be lyophilized since the cells are subjected to low temperatures. Also the conservation of a starter culture and its fermentation activity depend on the environment where the conservation is carried out. *Oenococcus oeni* RAM10 is a strain corresponding to the group of lactic acid bacteria, it was isolated from red wines from Tucuman in Argentina, which exhibited a significant potential to carry out MLF. It also showed excellent resistance properties to wine stress conditions (high alcohol content, low pH, presence of sodium metabisulfite and low temperatures). On the other hand, it was possible to design a culture medium, named M7, capable of favoring the production of high concentration of bacterial biomass as well as its adaptation to hostile winemaking conditions. It was constituted in gL^{-1} or mLL^{-1} by 5.0 yeast extract; DL-Malic Acid 6.0; Tween 80 0.5; dealcoholized wine 400.0; tomato juice 23.0; grape juice 357.7 and ethanol 40.0 at pH 4.8. These components are easy to get and cheap. The aim of this work was to evaluate the effect of the medium on the cell viability and the loss of fermentative capacity (LFC) after culture conservation by refrigeration, freezing with glycerol and lyophilization with glutamate, fructose and grape juice as protective agents.

Materials and Methods

Effect of preservation methods on cell viability



Effect of conservation methods on the fermentative capacity of the RAM10 strain during MLF



Results

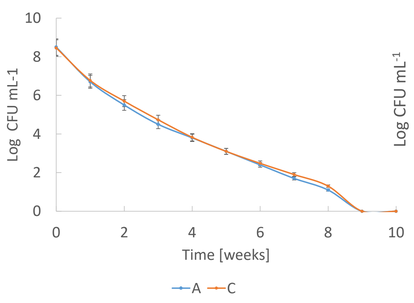


Figure 1. Effect of refrigeration on the cell concentration of the *O. oeni* RAM10 strain during 10 weeks of storage in MLO (A) and M7 medium (C)

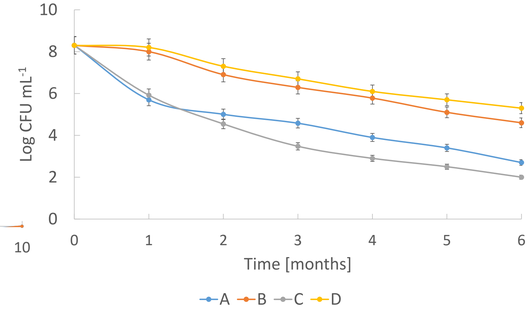


Figure 2. Effect of freezing on the cell viability of *O. oeni* RAM10 during 6 months of storage in MLO medium (A); MLO added with 20% glycerol (B); MOP7 (C) and MOP7 added with 20% glycerol (D).

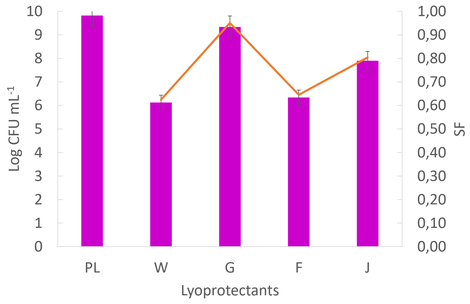


Figure 3. Survival factors associated with the different lyoprotectants (line) and bacterial concentration immediately after the lyophilization process (bars). PL: culture prior to lyophilization; W: sterile deionized water, G: glutamic acid, F: fructose, J: grape juice

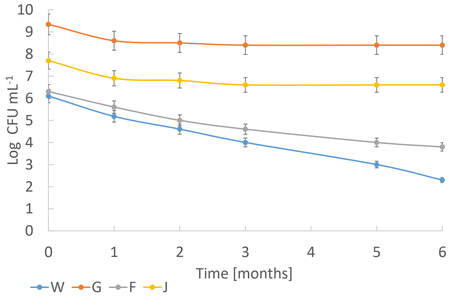


Figure 4. Variation of the cell concentration of cultures of *O. oeni* RAM10 stored after lyophilization with different lyoprotective agents for 6 months at 4°C. W: sterile deionized water, G: glutamic acid, F: fructose, J: grape juice.

Table 1. Loss of percentage fermentative capacity (LFC) during MLF of cultures preserved by different methods at different storage times

Conservation Methods	Culture Medium	Inoculation	Time in weeks 1	2	3	4	5	6
Refrigeration	A	d	15,2±0,1	NE	-	-	-	-
		pc	14,8±0,3	24,3±0,1	NE	-	-	-
	C	d	15,0±0,2	NE	-	-	-	-
		pc	14,9±0,1	23,8±0,2	NE	-	-	-
			Time in months 1	2	3	4	5	6
Freezing	A	d	NE	-	-	-	-	-
		pc	23,8±0,2	NE	-	-	-	-
	B	d	5,2±0,4	11,3±0,1	24,2±0,2	NE	-	-
		pc	4,9±0,3	11,0±0,2	24,1±0,2	30,2±0,3	NE	-
	C	d	NE	-	-	-	-	-
		pc	15,2±0,2	NE	-	-	-	-
	D	d	4,8±0,1	10,8±0,3	17,1±0,4	NE	-	-
		pc	4,8±0,1	11,1±0,4	16,9±0,4	22,2±0,3	NE	-
Lyophilization	C + W	d	NE	-	-	-	-	-
		pc	19,8±0,2	NE	-	-	-	-
	C + G	d	7,6±0,4	8,1±0,3	10,3±0,1	15,4±0,3	17,3±0,5	20,1±0,2
		pc	7,5±0,3	7,9±0,2	11,2±0,1	15,2±0,3	18,2±0,5	20,4±0,3
	C + J	d	7,2±0,3	8,4±0,2	12,3±0,3	17,1±0,2	20,4±0,3	24,8±0,2
		pc	7,7±0,4	8,1±0,2	12,7±0,2	16,5±0,3	19,5±0,3	25,2±0,2
	C + F	d	NE	-	-	-	-	-
		pc	15,3±0,2	23,2±0,3	NE	-	-	-

d: direct inoculation
pc: inoculation by preculture
NE: not tested due to lack of critical bacterial biomass to initiate MLF, in subsequent times it was not possible to perform the LFC test (-).

Conclusions

Refrigeration
- Greater loss of viability over time in MLO and M7
- Recommended for storage periods of up to two weeks in M7 medium

Freezing
- Glycerol favors the conservation of cultures
- Allows direct inoculation of cultures up to 3 months without appreciable loss of viability
- It is convenient to keep in M7 with glycerol since it allows a MLF with a lower LFC

Lyophilization
- Lyiophilization of *O. oeni* RAM10 in desionized water showed an intrinsic resistance to this conservation method
- Fructose has less lyoprotective effect
- Glutamic acid and grape juice maintain a high viability of the culture which allows direct inoculation of the wine up to 6 months with a low LFC