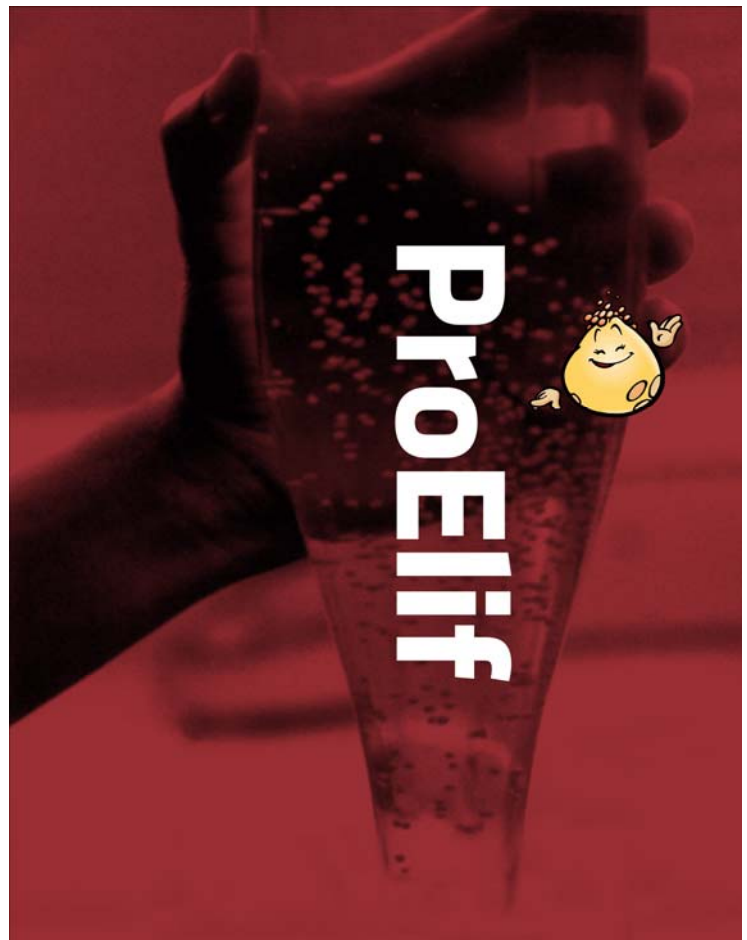


ProElif[®]

USER'S GUIDE

*Encapsulated Yeasts
for Sparkling Wine
Production*



Index



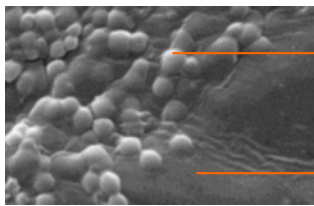
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1. ProElif® Description

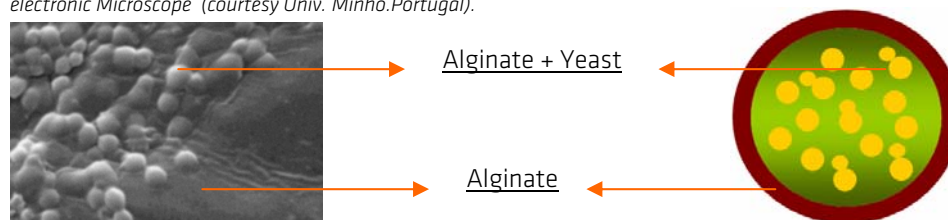
ProElif® is a dehydrated product in the form of 2mm diameter beads, composed of *Saccharomyces cerevisiae* yeasts enclosed in an alginate capsule. The capsule's external layer of alginate prevents the release of the yeast cells into the wine (picture 1 and 2).

The alginate is a polysaccharide extracted from the algae *Phaeophytaeae* (sea weed), and is used in winemaking as riddling adjuvants.

Picture 1 – Photo of a bead's interior, taken with an electronic Microscope (courtesy Univ. Minho, Portugal).



Picture 2 – Drawing of an encapsulated yeast



2. Why ProElif®

In sparkling wine production using the *Méthode Traditionnelle*, a second alcoholic fermentation occurs in the bottle after the inoculation of a yeast suspension. At the end of alcoholic fermentation, the sediment and the yeast's elimination require long and expensive procedures – Riddling. The yeast encapsulation procedure allows the yeast (after the beads introduction into the bottle together with the base wine) to perform its fermentative activity inside the capsule via the entrance of substrates and the release of metabolites. This occurs without releasing the yeast cells into the wine (Picture 2). **ProElif®** beads drop quickly when inverting the bottle, making them easier to be removed by *disgorging*, without requiring riddling.



ProElif® Main advantages

- **Reduction of production costs and labour time**, thanks to the **suppression of the riddling step**. **ProElif®** helps avoiding cloudiness in the wine. The bottle's inversion is sufficient to allow the beads to sediment in the bottleneck within a few seconds;
- **Fast response to the market needs and efficient planning** of deliveries to satisfy urgent orders, as the wines fermented with **ProElif®** are easily ready for disgorging;
- **Direct inoculation**, the encapsulated yeast cells can be directly introduced into the bottle. There is no need to build up a culture;
- **Better space management in the cellar**, as most riddling equipment becomes unnecessary;
- **Similar organoleptic characteristics** as in fermentation using *free* yeasts.

Therefore, using **ProElif®** helps significantly reduce the production cost, while bringing similar organoleptic properties as in non-encapsulated yeast fermentation.

3. Product development

In partnership with a Bioprocess researcher's team from the Chemical Engineering Laboratory - *Centre National Recherche Scientifique* - Toulouse, France and a researcher's team from several Portuguese Institutions of Science and Technology, Proenol developed an industrial production process, unique worldwide, for the encapsulation and dehydration of microorganisms. Currently, Proenol has a production facility for encapsulation/drying yeasts and bacteria, that allows it not only to respond easily to the market needs (foreign and domestic), but also to assist customers through technical support geared to optimize their individual needs.



4. Requirements prior to ProElif® use

4.1. Base wine characteristics

- Ideal condition for the use of ProElif®:**
- Free SO₂ < 15 mg/L
 - pH ≥ 3.0
 - Alcohol ≤ 11.5% (v/v)
 - Assimilable nitrogen ≥ 100mg/L
 - Protein and tartaric stability
 - Fermentation temperature ≥ 12°C
 - Saturation temperature:
 - White wines < 10°C
 - Red wines < 16°C.

Alcohol, pH, free SO₂ and temperature act in synergy. If the values of these parameters are too close to the limit, the fermentative kinetics will be slower. It is important to verify the sugar content of the base wine, in order to calculate the volume of *liqueur de tirage* to be added on the tirage day.

Wine corrections

If Assimilable Nitrogen is <100mg/L it is necessary to correct this value with DAP (100 mg/L of ammonium phosphate is equivalent to 27 mg/L of Assimilable Nitrogen).

Just like for regular active dry yeast fermentation, we recommend the Thiamine addition. Add 0,3mg/L of Thiamine to the base wine. Don't add antimicrobial agents such as velcorin or sorbate.

Tartaric stability

The base wine to be used on the sparkling wines production must have a good tartaric stability. Our trials show that the beads tend to agglomerate in wines that do not have a proper tartaric stability, which does not allow an efficient disgorging.

Therefore, we recommend the following saturation temperature values (Stabisat equipment):

- **White** wines: Saturation temperature <10°C;
- **Red** wines: Saturation temperature <16 C.

Protein stability

The base wine must be protein stabilised, in order to avoid insolubilizing of the unstable proteins during the second fermentation.

4.2. Wine preparation for tirage

The following procedures must be carried out on the **TIRAGE DAY**:

1. **Addition of "liqueur de tirage"**: It must be added at the beginning of the tirage day. The riddling step is no longer required, therefore it is not necessary to add riddling adjuvants. Thiamine, DAP as well as tannins or proteins (to enhance volume and mouthfeel) should be added before the final filtration;
2. **Filtration**: The wine must be filtered using a 0.45µm absolute pore membrane filter. It is essential that the filtration procedures are followed properly. Testing the membrane integrity before and after each bottling cycle and respecting the differential pressure recommended, by the cartridge supplier are particularly important. Filtration must be performed on the bottling day and directly into the bottle, in order to avoid any post filtration contamination.

4.3. Equipment Hygiene

When using **ProElif®** strict attention to hygiene of all material and equipment used during tirage (appendix I) is crucial. This procedure is very important to prevent the growth of microorganisms that may cause bottle cloudiness. If possible, proceed settling with a hygiene control using classic microbiology or quick methods (i.e. bioluminescence). The control of hygiene chemical product residues is also important since even in small quantities, sanitizing agents have a microbicide action which may interfere with **ProElif®**. After using the production line for the tirage with *free* yeasts it is recommended to disassemble the bottling machine and proceed to a rigorous cleaning, before using **ProElif®**.

The Most critical points of hygiene are the following:

DAY BEFORE TIRAGE

Tubes and connections:

1. Check machine seals. If the joints are used, it is convenient to replace them before the tirage;
2. Rinse;
3. Make a cleaning cycle with a detergent solution rinse and then proceed with a sanitizing cycle with a disinfectant solution;
4. Respect the concentrations and contact time recommended by the product supplier. If possible, use a detergent solution at high temperatures (max.60°C);
5. On the joints and connections of difficult access use a foam solution for a better hygiene;
6. Rinse with clean water and make sure that there are no hygiene solution residues left.

Walls, floor and tirage area:

1. Rinse;
2. Apply a cleaner/sanitizing foam solution;
3. Respect the concentration and contact time recommended by the product supplier;
4. Rinse with clean water;
5. Proceed with a visual monitoring of all surfaces.

- Bottles washing machine (If there is any):**
1. Rinse;
 2. Apply a cleaner/sanitizing foam solution;
 3. Rinse with clean water at a low pressure, assuring total product's elimination.

- Bottle filler:**
1. Prepare a hygiene plan that must consist of a cleaner phase with an alkaline detergent solution and a sanitizing phase with a disinfectant solution;
 2. Be sure that all parts of the machine are fully covered by the action of detergent/sanitizing solutions. (i.e. reservoir's cover, valves interior, etc);
 3. It also important to apply cleaning foam on the exterior of the filler (i.e. valves).

- ProElif® Doser:**
1. Set up the doser;
 2. Check the bottle's filling process;
 3. Hand work cleaning and sanitizing of the doser with alcohol.
- Note:** The use of compressed air (4.5 bar) is essential in order to make the doser work.

TIRAGE DAY – BEFORE FILLING

- Tubes, filter plates, filter membranes and bottle filler:**
1. Set up all the equipment on the circuit;
 2. Rinse all the circuit;
 3. Prepare a sanitizing solution, using a higher concentration than usual, preferably based on peracetic acid;
 4. Disinfect all the circuit according to the recommended contact time. It is important that the filler tubes are completely immersed in the solution. It is also important that the deposit cover (interior part) is in contact with this solution;
 5. Rinse abundantly with clean water and be sure that no cleaning residues are left.
- Note:** During breaks in bottling, filler valves must be sprayed with alcohol.

TIRAGE DAY – AFTER FILLING

- Tubes, plates and filter membranes, bottle filler:**
1. Rinse all the circuit;
 2. Prepare an alkaline detergent solution, using a higher concentration than usual;
 3. Wash all the circuit according to the recommended contact time. If possible use a solution at high temperatures (max. 60°C);
 4. Rinse abundantly with clean water and make sure that there are no cleaning residues left;
 5. Prepare a sanitizing solution using a higher concentration than normal, preferably based on peracetic acid;
 6. Sanitize all the circuit with the solution. Be sure that the filler tubes are submerged in this solution, also the interior of the reservoir cover must be in contact with this solution;
 7. If there is no information otherwise, left the solutions operate during the night.
 8. Rinse abundantly with clean water and be sure that no cleaning residues are left.

5. ProElif® dosage

Beads are directly introduced into the bottle during the bottling process. The **ProElif®** doser must be placed before the bottle filler (Appendix 1). If there is a lack of space, the doser can be placed after the filling process. However in this case, it is important to monitor the bottle neck after the dosage to be sure that the beads do not stick on it.

The beads dosage must be 1.2 to 1.5 g/bottle (depending on the wine analyses profile), meaning 4.8 to 6 millions of viable cellules/mL of wine.



6. ProElif® application check list

To be sure that all requirements prior to bottling are fully complied, we set up a Check List (appendix 2).

7. Fermentative kinetics control

We suggest the examination of the following fermentation parameters:

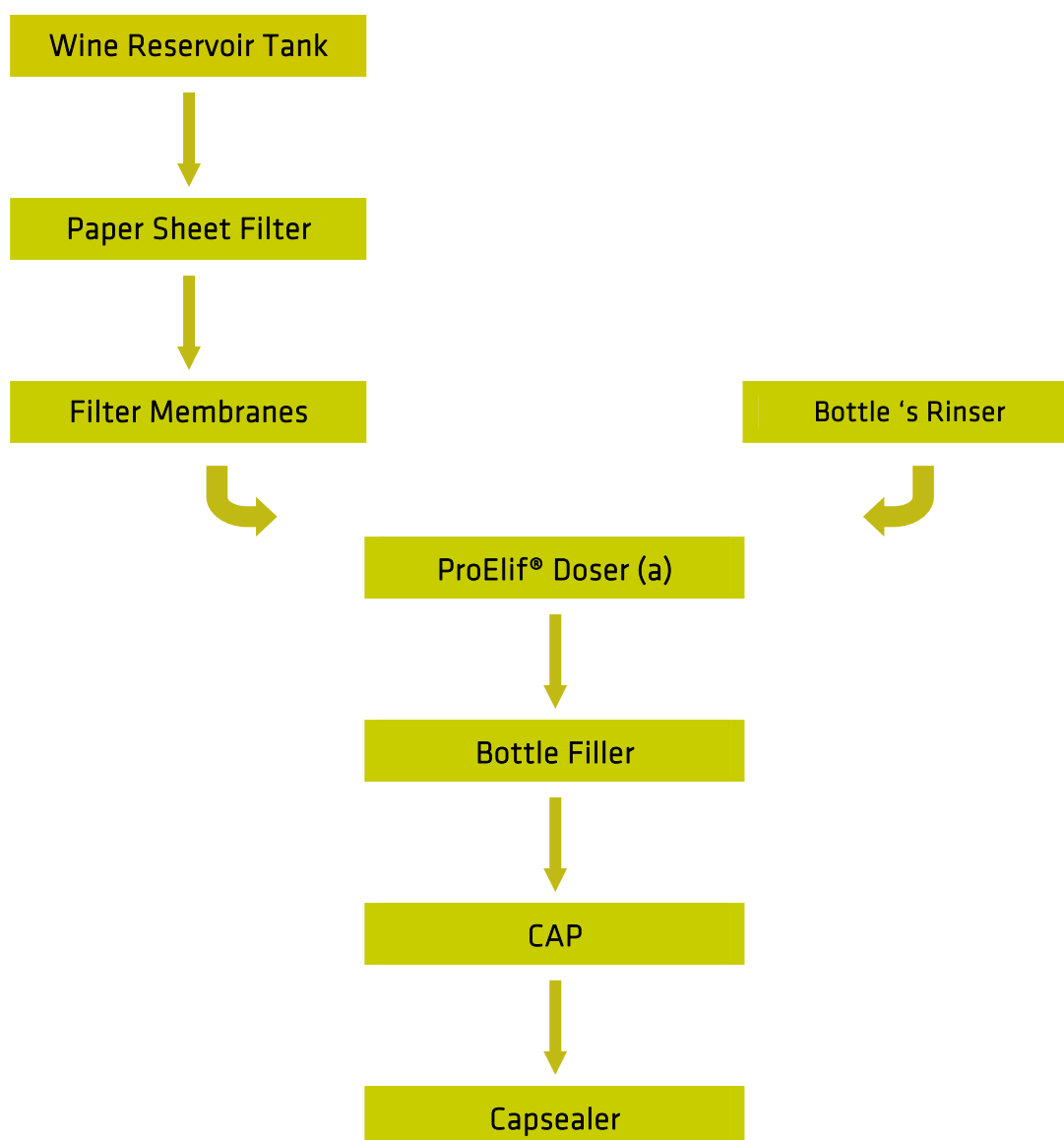
1. Pressure;
2. Temperature;
3. Sugar;
4. Bottle visual monitoring



We recommend keeping control bottles, meaning bottles with and without **ProElif®** (Witnesses), which must be stored at the same temperature as the other bottles. This procedure will be very useful to identify the origin of contamination, in case of cloudiness in bottles.

Appendix I

DIAGRAM



(a) If there is a lack of space, the doser can be placed after the filling process. However in that case, it is important to monitor the bottle neck after the dosage to be sure that the beads do not stick on it.

Appendix II

ProElif® Application Check List

Company's name:

Date:

Wine:

Grape Variety:

Signature:

1. Chemical analysis of the base wine:

	Result	Limit
Free SO ₂ (mg/L)		< 15
pH		≥ 3
Alcohol (%v/v)		≤ 11,5
Assimilable nitrogen - NFA (mg/L)		≥100
Protein stability		Stable
Tartaric stability		Stable
- Saturation temperature in White wines (°C)		< 10
- Saturation temperature in Red wines (°C)		< 16

2. Addition of nutrients and *liqueur de tirage* to the wine:

DAP (if NFA<100mg/L) _____g/hL	Yes	No
Thiamine 0.3mg/L	<input type="checkbox"/>	<input type="checkbox"/>
Nutrients and liqueur de tirage added to the wine before filtration and only on the tirage day	<input type="checkbox"/>	<input type="checkbox"/>
Wine free of antimicrobial agents (velcorin, sorbates...)	<input type="checkbox"/>	<input type="checkbox"/>

3. Filtration

Final filtration filter with 0.45 microns absolute pore size	Yes	No
Filter integrity test	<input type="checkbox"/>	<input type="checkbox"/>
Filtration made on the tirage day	<input type="checkbox"/>	<input type="checkbox"/>
Filtration processed in line and wine sent directly to the bottle	<input type="checkbox"/>	<input type="checkbox"/>
Filtration differential pressure recommended by the cartridge supplier	<input type="checkbox"/>	<input type="checkbox"/>

4. Equipment Hygienization:

Day before tirage:	Yes	No	Tirage day – prior to filling:	Yes	No
Tubes and connections	<input type="checkbox"/>	<input type="checkbox"/>	Tubes, filters and filler	<input type="checkbox"/>	<input type="checkbox"/>
Wall, floor and tirage area	<input type="checkbox"/>	<input type="checkbox"/>			
Bottle Rinsers	<input type="checkbox"/>	<input type="checkbox"/>			
Filler	<input type="checkbox"/>	<input type="checkbox"/>			

Hygiene control results:

5. ProElif® dosage ≥ 1.2g/bottle	Yes	No
	<input type="checkbox"/>	<input type="checkbox"/>

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6. Fermentation temperature $\geq 12^{\circ}\text{C}$
7. Control bottles (with and without ProElif®)
8. Notes