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Process for partial or total dealcoholization of wine using a post-fermentation microbiological technique

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Abstract. The dealcoholized wine sector is experiencing strong market growth, driven by increasing consumer demand. Current oenological practices allowed for wine dealcoholization by the OIV Code of Oenological Practices are based on physical processes. The Robert-Jean de Vogüé Research Center has developed an innovative, microbiology-driven post-fermentation method for wine dealcoholization. This process reduces or eliminates alcohol content while preserving the wine's essential organoleptic properties. Unlike existing techniques that can degrade quality, generate waste, or require additives, this method aims to produce a dealcoholized wine with a flavor profile closely resembling the original, achieved without exogenous water or external inputs. The process involves a combination of membrane separation and microbiological techniques. Initially, the wine is separated into two fractions using a membrane: a concentrated fraction (retentate) containing all the wine's essential components, and a permeate primarily composed of water and ethanol. The ethanol in the permeate is then metabolized by yeasts, specifically Saccharomyces cerevisiae, under aerobic conditions. These yeasts are previously cultured in a nutrient-rich medium, and controlled conditions are applied to initiate the diauxic shift. This shift involves the transition of yeast metabolism from fermentation to respiration once the primary sugar source is depleted, allowing the yeasts to consume ethanol as a secondary substrate. Subsequently, the yeasts are added to the permeate, where they metabolize the ethanol into carbon dioxide and water. Optionally, the retentate can undergo diafiltration with the dealcoholized permeate to further reduce its alcohol content. Finally, the retentate and dealcoholized permeate are recombined to produce a wine with significantly reduced alcohol content. This method ensures the preservation of the wine's aromatic and taste qualities, making it suitable for producing high-quality dealcoholized wines. The process is versatile, environmentally friendly, and compatible with various types of wines.

1. Introduction

For nearly a century, reducing or removing alcohol from alcoholic beverages, especially wine, has been driven by public health recommendations, evolving consumer preferences, and the impact of global warming on grape maturity and sugar content.

Traditional strategies for achieving lower alcohol content fall into two main categories: (1) limiting alcohol production during fermentation, and (2) acting on the alcoholic beverage post-fermentation. The first strategy involves interventions before and during the fermentation to reduce alcohol production. Several methods have been employed, including modification of raw materials such as partial reduction of sugar content in grape juices [1], use of genetically modified yeasts producing less alcohol during fermentation [2], stressed yeasts to reduce alcohol production capacity [3], reduced alcohol fermentation yield [4] and mixed yeast cultures with oxygen control [5]. However, a significant drawback of these methods is the potential alteration of the wine's gustatory properties, impacting the delicate balance of flavors and aromas, leading to a less desirable final product. Post-fermentation techniques act on the alcoholic beverage to remove or reduce the alcohol content. These techniques include separation methods that physically separate alcohol from the wine (using compounds with an affinity for ethanol, such as enzymes immobilized on a column [6] – however, this can lead to acetaldehyde accumulation, a compound detrimental to wine quality – or membrane separation

techniques like ultrafiltration, nanofiltration, reverse osmosis and membrane contactors; thermal methods that use heat such as vacuum distillation and spinning cone columns; microbiological methods using specific yeasts to metabolize ethanol into water and carbon dioxide under aerobic conditions; and combined methods [7]. These post-fermentation techniques, however, also have limitations. Membrane separation, especially diafiltration, often requires exogenous water, generally avoided in the wine industry. Reverse osmosis, while preserving wine taste due to its low-temperature operation [8] struggles to significantly reduce alcohol content (more than 2 vol.%) while maintaining organoleptic properties [9]. Microbiological methods can lead to oxidation of flavors, consumption of desirable compounds and production of undesirable compounds [10] and the Candida yeast method [11] can lead to loss of compounds of interest, oxidation, and glycerol consumption.

The primary challenge remains achieving a significant alcohol reduction without excessive quality loss, exogenous water or additives. Dealcoholized wines often exhibit undesirable flavors or require masking agents to compensate for taste defects, and existing methods can generate substantial waste. Therefore, there is a clear need for a sustainable dealcoholization process that does not require the use of exogenous water or other external inputs, reduces waste, and effectively preserves the organoleptic properties and the style of the original wine. aligning with the increasing demand for low-alcohol or alcohol-free options.

This synthesis describes the method developed and patented [12] by the RJDV center, highlighting its unique microbiology-driven approach.

2. Overall concept

This innovative, four-step method addresses the partial or total dealcoholization of wine. It aims to produce a dealcoholized product where initially present compounds of interest, especially those contributing to flavor, are conserved and preserved, avoiding denaturation from oxidation or excessive heating. This is achieved by first isolating these compounds appropriately and then implementing alcohol removal techniques that respect their integrity. The overall process combines membrane separation with microbiological methods, focusing on a gentle and selective treatment that avoids harsh conditions harmful to the wine's desirable characteristics, ensuring the preservation of these compounds in the final partially or totally dealcoholized wine.

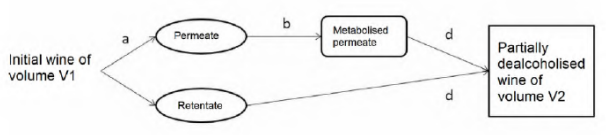

Figure 1. Essential steps of the process for obtaining a partially dealcoholized wine

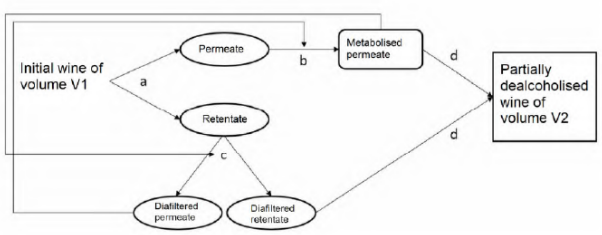

Figure 2. Essential steps of the process for obtaining a totally dealcoholized wine

In essence, the method is a careful balancing act: using membrane separation to isolate the alcohol, using microorganisms to gently remove it, and then recombining the fractions to create a dealcoholized wine that retains the essence of the original.

2.1. Membrane separation of the wine (Step a)

The initial volume of wine (V1) with an alcohol content (ABV) is divided into two fractions using a membrane separation technique (ideally reverse osmosis or nanofiltration): **(1) a retentate fraction:** a concentrated fraction (volume = 1/k * V1) containing most of the wine's flavor compounds, sugars, acids, etc. with an alcohol content of ABV(R). **(2) a permeate fraction:** a fraction (volume = (k-1)/k * V1) mainly composed of water and ethanol and with an alcohol content of ABV(P).

2.2. Microbiological dealcoholization of the permeate (Step b)

Ethanol is removed from the permeate fraction using yeasts in a respiratory state, which means they metabolize the ethanol into water and CO2 under oxygen-rich conditions. The resulting "metabolized permeate" has a lower alcohol content (ABV(P metabolised)) than the original permeate (ABV(P)).

2.3. Optional diafiltration (Step c)

If further alcohol reduction is needed or desired, the retentate can be diafiltered, or washed, with the “metabolized permeate” from step b. This process extracts more alcohol from the retentate, resulting in a "diafiltered retentate" with a lower alcohol content (ABV(R diafiltered)) than ABV(R). The "diafiltered metabolized permeate," which now contains some of the extracted alcohol and have an ABV value greater than ABV(P metabolised), can optionally be treated again with yeasts (step b) for further alcohol removal.

2.4. Recombination (Step d)

Finally, the retentate (or diafiltered retentate) and the metabolized permeate are combined in specific proportions in order to obtain a dealcoholized wine (volume V2) with a reduced alcohol content (ABV(2)) compared to the original wine (ABV(1)).

3. Description of the different steps of the process

3.1. Membrane separation of the wine - Step (a)

This initial step is crucial in the process, as it sets the stage for selective alcohol removal while aiming to retain the wine's valuable components. It involves using a membrane separation method to divide the initial wine volume into two distinct fractions with differing composition: (1) the retentate enriched in the desired flavor compounds and other non-volatile components and (2) the permeate containing most of the water and ethanol (the permeate). This separation facilitates the targeted removal of ethanol while protecting compounds of interest in subsequent steps.

Membrane technology and types: The separation relies on semi-permeable membranes that act as selective barriers. These membranes have a porous structure that allows certain molecules to pass through while retaining others, based on factors such as size, shape, charge, and chemical properties. The choice of membrane is critical for achieving efficient separation and preserving wine quality. Several membrane separation techniques that can be used, including microfiltration, ultrafiltration, nanofiltration or reverse osmosis (RO).

Membrane characteristics and selection: Key parameters for characterizing membrane performance include the rejection rate which is its ability to retain specific solutes - a high rejection rate for a particular compound means the membrane effectively prevents it from passing through, and the retention rate which is complementary to the rejection rate and indicates the proportion of a solute that is retained by the membrane. Ideally, the membrane used in this process should have a high rejection rate (close to 1) for glycerol, organic acids, sugars, polyphenols, flavor compounds, and other desirable wine components to ensure their concentration in the retentate and a have a very low rejection rate (ideally zero) for ethanol, allowing it to pass freely into the permeate.

No single membrane perfectly satisfies both criteria. Therefore, the selection process involves a trade-off, balancing the need for ethanol removal with the desire to retain flavor and quality. In a preferred implementation, the separation in carried out by reverse osmosis and the membrane used has a rejection rate for salts greater than or equal to 0.97 and is an organic membrane, made of polypropylene.

Several **operating parameters** influence the effectiveness of the membrane separation:

- **Pressure:** Applying pressure is necessary to drive the separation process, forcing the permeate through the membrane. The optimal pressure depends on the membrane type and the composition of the wine but will be between 20 and 100 bars, with 50 bars being a typical value.
- **Temperature:** Maintaining a low temperature (ideally between 15°C and 25°C, more preferably between 15°C and 20°C, or even between 15°C and 18°C) is crucial to prevent thermal degradation of the wine's delicate flavor compounds.
- **Atmosphere:** To minimize oxidation of sensitive compounds, the separation process is preferably carried out under an inert atmosphere with low molecular oxygen levels (less than 3%, more precisely comprising less than 3% molecular oxygen, or better less than 2% molecular oxygen, or even less than 1% molecular oxygen).
- **Concentration Factor (k):** This factor determines the ratio between the initial wine volume (V1) and the retentate volume (1/k * V1). A higher concentration factor results in a smaller retentate volume and a greater concentration of non-volatile compounds. The choice of concentration factor depends on the desired degree of alcohol reduction and the characteristics of the wine. The concentration factor k is between 0.1 and 20, preferably between 2 and 10, more preferably between 5 and 10. For example, for the complete dealcoholization of a wine with ABV of approximately 11 vol.%, the concentration factor k is advantageously equal to 8.

Fractions obtained: The membrane separation results in two distinct fractions: (1) **Retentate:** this is the concentrated fraction that is retained by the membrane. It contains water, ethanol, glycerol, organic acids, sugars, polyphenols, flavor compounds, and other non-volatile components of the wine. The retentate volume is 1/k * V1, and its alcohol content is denoted as ABV(R). (2) **Permeate:** this is the fraction that passes through the membrane. It is mostly composed of water and ethanol, with smaller amounts of other low-molecular-weight compounds. The permeate volume is (k-1)/k * V1, and its alcohol content is denoted as ABV(P).

Table 1 presents a numerical example of physical separation of a wine using a membrane, by reverse osmosis, at a pressure of 50 bars and while concentrating the retentate by a factor of 8.

Table 1. Example of physical separation of a dry white wine 11,6 vol.% ABV

Product of fraction	Wine	Permeate	Retentate
Volume	8/8	7/8	1/8
Theoretical ABV of the fractions	11.6 vol.%	9.3 vol.%	28 vol.%
Observed ABV of the fractions (average of 40 tests)		9.7 vol.%	21 vol.%

Post-Separation handling: Following the membrane separation, the retentate is carefully stored, preferably at a low temperature (less than 10°C), to preserve its quality until it is used in subsequent steps.

Pre-reducing permeate ABV (Step a'): An optional step to reduce the alcohol content of the permeate before the microbiological step (b) can be done to prevent the high alcohol from harming the yeasts and can be achieved

through several technics as evaporation, distillation, dilution or membrane contractor.

This **example** illustrates the step of separating a dry white wine into two fractions using reverse osmosis (RO). The wine, with less than 10 g/L residual sugars and an alcohol content of 11.3 vol.%, was processed using a high-pressure pump (up to 60 bars) and a 2.5-inch diameter RO membrane (Alfa Laval RO98pHt with a 26 m² surface area). A total of 516 kg of wine was placed in an inerted feed tank (with oxygen level maintained below 2% using nitrogen). The wine circulated through the RO system via a positive displacement pump, with a strainer to retain crystals and a mass flow meter to measure flow rate and density. A plate exchanger kept the temperature at approximately 15°C, while the process was conducted under a constant pressure of around 50 bars. The separation continued until a volume concentration factor of k=8 was achieved, resulting in a permeate whose ABV increased over time. Ultimately, the process yielded 52,4 kg of retentate with an ABV of 20.5 vol.% and 451 kg of permeate with an ABV of 9,2vol.%.

3.2. Microbiological dealcoholization - step (b)

The microbiological removal of ethanol from the permeate is a core element of the process, where the ethanol present in the permeate is selectively metabolized by yeasts under controlled aerobic conditions and convert into water and carbon dioxide (CO2) [13], thereby lowering the alcohol by volume (ABV) of the permeate from its initial value (ABV(P)) to a significantly reduced value (ABV(P metabolised)). The aim is to reduce the alcohol content of the permeate efficiently and gently, minimizing any negative impact on the wine's desirable flavor compounds for later recombination with the retentate. The removal of ethanol present in the permeate can be partial or total.

Yeast selection: While any yeast that can metabolize ethanol aerobically is potentially suitable, a study [14] of 439 yeast species identified 334 capable of growing on ethanol alone, with 250 showing normal growth. *Saccharomyces cerevisiae* is one such species, along with others like *S. dairensis*, *S. exiguus*, *S. kluyveri*, *S. telluris*, and *S. unisporus*. However, one implementation of the process uses yeasts from the *Saccharomyces* genus.

Pre-Culture and Conditioning (Sub-steps b1, b2): Recognizing the challenging conditions in the permeate (low nutrients – except for ethanol, low conductivity, and acidic pH), the yeasts must be pre-cultured and conditioned to build sufficient biomass to ensure effective ethanol metabolism [15]. This involves (b1) an initial pre-culture : yeasts are pre-cultured at a high concentration (at least 10^6 cells per milliliter) in a growth medium containing a sugar source (40-120 g/kg) under aerobic conditions followed by (b2) a culture volume adjustment : yeasts from the first pre-culture are further cultured in a similar growth medium and the culture volume is adjusted to match the permeate volume that will be treated in the subsequent step, ensuring a sufficient yeast population for the metabolization process. The **growth medium** comprises inositol [16, 17] at a concentration of 2-200 mg/L, and also a source of nitrogen (300-400 mg/L), zinc (3-5 mg/L), vitamins and ions as needed by the yeast strain. Standard microbiology media such as “Sabouraud”, “Yeast Extract Peptone”, “Yeast Nitrogen Base”, with or without amino acids, or even a grape must or a synthetic must [18] can be used. The sugar source has to be from 40 to 120 g/kg such as glucose, sucrose, or a mix. A preferred implementation uses only sucrose at a concentration of 80 g/kg. Yeasts are cultivated in aseptic mode and the pre-culture steps are carried out between 15°C and 38°C while stirring. These sub-steps aim to generate biomass with a good viability, store nutrients (ions, nitrogen, vitamins, lipids) that will be required during ethanol metabolism, and shift yeast metabolism from sugar-based fermentation to ethanol-based respiration. Between each phase, the yeasts are harvested and rinsed and the container changed if needed.

Ethanol metabolization (Sub-step b3): The pre-cultured yeasts are added to the permeate at a high concentration (at least 2 x 10^8 cells per milliliter) under aerobic conditions. This phase corresponds to the phase of metabolizing the ethanol present in the permeate itself. The ethanol metabolism is carried out under strict **aerobic conditions**, requiring a constant supply of dissolved oxygen. This is achieved through continuous equilibration with an atmosphere containing molecular oxygen or continuous injection of a gas flow (air -molecular oxygen [O2] and nitrogen [N2] or oxygen-enriched gas) into the medium. The **oxygen flow rate** is carefully controlled and adjusted based on the amount of ethanol present. **The duration of phase b3** varies depending on the initial ABV(P), the nature and quantity of yeasts added. For example, in order to obtain a partial removal of the ethanol present in the permeate, a duration of 4 to 8 days would be sufficient; for complete dealcoholisation of a permeate with ABV(P) of approximately 6 vol.%, between 24 and 32 days will be necessary, depending on the strain of yeast used. During phase (b3), the weight reduction of the medium can be monitored over time. The flow rate and percentage of CO2 can be monitored at the fermenter outlet, or by sampling and then assaying the ethanol.

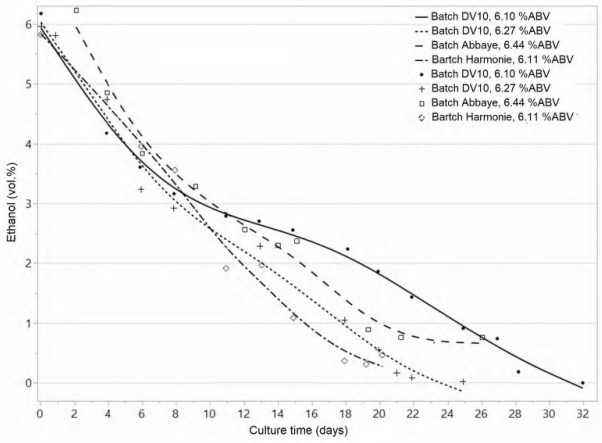

Figure 3. Metabolization, in batch mode of ethanol present in a permeate, from a dry white wine, with an ABV of approximatively 6 vol.%

After partial or total metabolization of the ethanol, the yeasts are removed from the mixture using techniques like filtration or centrifugation. The resulting fraction is referred to as "metabolized permeate" and has a significantly reduced alcohol content (ABV(P metabolised)). If desired, this metabolized permeate may be filtered beforehand in order to remove any undesirable compound.

This **example describes the microbiological dealcoholization** of a permeate obtained from a dry white wine (11.3 vol.%) with an ABV of approximatively 6 vol.%. This ABV was obtained by dilution with a permeate of 0 vol.% from a previous dealcoholization cycle. The permeate was placed in a bioreactor and inoculated with *Saccharomyces cerevisiae* yeast (strain Vitilevure DV10). The yeast was pre-cultured to ensure it was in a suitable state for ethanol metabolism. The process was conducted under aerobic conditions, with a constant supply of oxygen to facilitate the yeast's respiration. Over a period of 24 days, the ethanol content of the permeate was significantly reduced, achieving a final alcohol content of less than 0.5 vol.%.

3.3. Optional diafiltration - Step (c)

The optional diafiltration step refines the dealcoholization process by using the metabolized permeate (from step b) as a “diafiltration agent” to "wash" the retentate (from step a) reducing its alcohol content while retaining desirable compounds and enabling precise ABV control in the final dealcoholized wine.

The **process** combines dilution with membrane separation. It repeatedly adds a diafiltration agent (the metabolized permeate) to the retentate, then uses a membrane (ideally, the same as in the step a) to remove the added liquid along with the unwanted components (ethanol). This "washes" the retentate, removing progressively more of the ethanol. A membrane with a high rejection rate for salts (greater than 0.9) is preferred. The diafiltration is carried out under pressure and the specific pressure depends on the membrane type and process setup. The diafiltration rate, which indicates how many times the retentate volume is diluted and reconcentrated, should be performed at least twice (ideally three times).

Diafiltration produces **two key fractions** (1) **diafiltered retentate** with a reduced alcohol content (ABV(R diafiltered)) compared to the original retentate (ABV(R)). The goal is to achieve an ABV that is sufficiently low for the desired final product. The diafiltered retentate is carefully collected and stored for later recombination with the metabolized permeate; and (2) **diafiltered metabolized permeate** which is the original metabolized permeate now enriched with ethanol extracted from the retentate and has an ABV value greater than ABV(P metabolized). This "diafiltered metabolized permeate" can then be processed again in step (b) to reduce its ABV value.

Critically, using metabolized permeate as a diafiltration agent ensures that only components of the original wine are used, with no exogenous liquids or other input.

This **example illustrates the diafiltration step**. A retentate with an initial ethanol content of 20,5 vol.%) is diafiltered using a metabolized permeate (with ABV of 0 vol.%) as the diafiltering agent. The process is conducted at approximatively 50 bars of pressure and employs a diafiltration rate of 4 to reduce the retentate’s alcohol content about 4 vol.%. Specifically, 64 kg of retentate from step (a) are diafiltered with 256 kg of filtered metabolized permeate from a previous dealcoholization cycle. This results in 64 kg of diafiltered retentate with an ABV of 4,2 vol.% and 256 kg of diafiltered metabolized permeate with an ABV of 5,3 vol.%.

3.4. Fraction combination

This final step involves carefully recombining the fractions previously obtained (1) the retentate (or diafiltered retentate) and (2) metabolized permeate, in specific proportions to achieve the target alcohol content and preserve the desired flavor profile and mouthfeel of the dealcoholized wine.

The key to this final step is accurate proportioning. The proportions are determined by the concentration factor (k) used in the initial membrane separation (step a). Specifically, the volume of metabolized permeate should equal (k-1)/k * V2, and the volume of retentate (or diafiltered retentate) should equal 1/k * V2. For example, if the method is carried out with a concentration factor k=8 (meaning the retentate volume Vr is 1/8 of the original wine volume V1) then a 7/8 of metabolized permeate is combined with 1/8 of retentate.

This combination step is not merely about mixing two fractions. It is a carefully controlled process that aims to create a dealcoholized wine maintaining the character and quality of the original while meeting the desired alcohol reduction target.

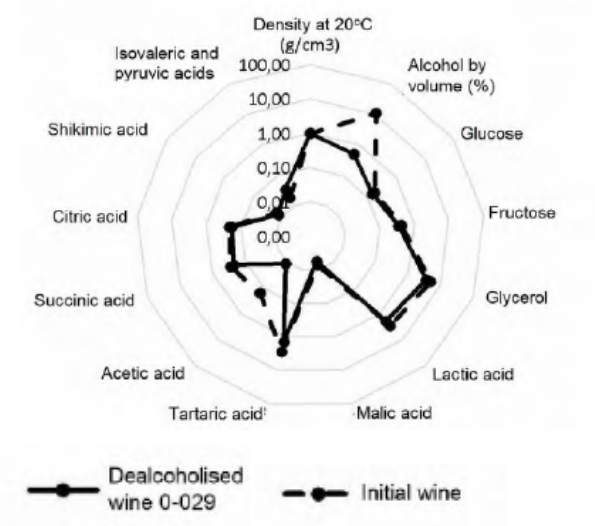

Figure 4. Comparison of dealcoholized wine by the method and initial dry white wine: profile of sugars, organic acids and alcohols (in %)

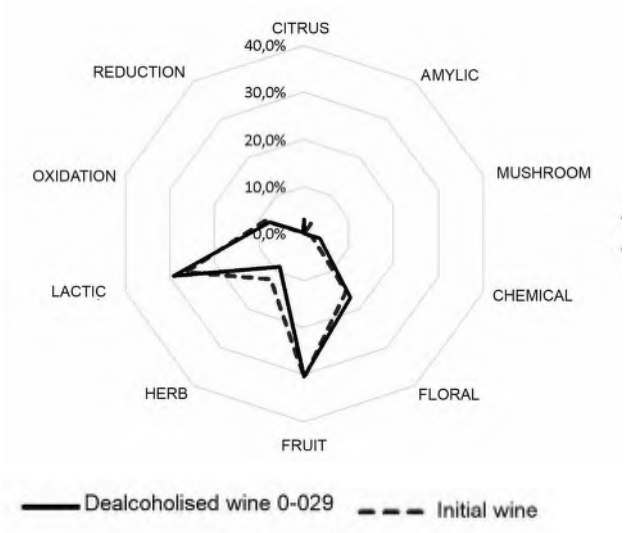

Figure 5. Comparison of dealcoholized wine by the method and initial dry white wine: flavour profile

This **example illustrates step (d) of the process**, where two fractions are combined to produce a dealcoholized wine with an ABV of approximately 0.5 vol.%. In this step, 4.98 kg of diafiltered retentate are mixed with 33.98 kg of metabolized permeate, which has an ABV of 0 vol.%. The mixture is then homogenized and filtered, resulting in 35.21 kg of dealcoholized wine with the targeted ABV of 0.5%.

3.5. Application of the method for the partial or total dealcoholization of a wine

The resulting dealcoholized wine (volume V2) will have a final alcohol content (ABV(2)) lower than the starting wine (ABV(1)). The specific target ABV(2) can be tailored by adjusting parameters such as the concentration factor (k) in the membrane separation step, the degree of alcohol reduction achieved in the microbiological step, the number of diafiltration cycles (if used) and the exact proportions used in this recombination step. For *partial* dealcoholization, the ABV(2) will be reduced by at least 1 vol.%. For *total* dealcoholization, the ABV(2) will be less than or equal to 0.5 vol.%, or even 0 vol.%; if k=8, it's preferable to combine during step (d) a diafiltered retentate with an ABV of 4 vol.% or less with a metabolized permeate with an ABV of 0 vol.%. This ensures the dealcoholized wine maintains the same proportions of the two fractions as initially obtained in the membrane separation step.

3.6. Post-Blending Steps:

After combining the retentate and metabolized permeate, the dealcoholized wine may undergo additional steps such as final filtration (for clarity and stability), adjustment of acidity or other parameters (if needed), bottling, pasteurisation and packaging.

4. Conclusion

This comprehensive process for wine dealcoholization effectively combines membrane separation with microbiological techniques to achieve both partial and total alcohol reduction. A standout feature is the microbiological dealcoholization step, where yeasts metabolize ethanol under controlled aerobic conditions. This approach not only significantly lowers the alcohol content but also preserves the wine's inherent flavor profile and quality by minimizing harsh treatments and exogenous inputs. The integration of microbiological methods ensures a gentle yet efficient transformation, aligning with modern demands for high-quality, low-alcohol wines. This innovative method offers a refined solution to traditional challenges, providing winemakers with the flexibility to tailor alcohol content while maintaining the integrity of the original wine.

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6. Aknowledgements

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