**Yeast derivatives for precision oenology: emerging and sustainable application for wine production (WInnY)**

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Yeast derivative products (YDPs) have been increasingly used in the wine industry thanks to the many needs they can meet. YDPs can be used as nutrient sources for the fermentation processes, as stabilizing agents and for improving the sensory characteristics of wines. This PhD project aims to find possible relationships between their chemical composition of the YDPs and their function. Several commercial YDPs will be characterized and they will be assayed as fermentation enhancers, stabilizing agents and as antioxidant during the wine aging. Thus, this proposal will provide important information to YDP producers who can improve their production methods and the precision of their products. In this way, the winemakers could benefit of more specific and effective YDPs.

Derivati di lievito per l’enologia di precisione: applicazione sostenibile ed emergente per la produzione enologica (WInnY)

I derivati di lievito (YDP) sono sempre più utilizzati nell'industria enologica grazie alle molteplici esigenze che possono soddisfare. Possono essere utilizzati come fonti nutritive per lo svolgimento della fermentazione, come agenti stabilizzanti e per migliorare alcune caratteristiche sensoriali dei vini. Questo progetto di dottorato mira a trovare le possibili relazioni tra la composizione chimica dei YDP e la loro funzione. Diversi prodotti commerciali verranno caratterizzati e testati come nutrienti per le fermentazioni, come agenti stabilizzanti e come antiossidanti per l’invecchiamento dei vini. Questo progetto potrà fornire importanti informazioni ai produttori di YDP che possono migliorare i loro metodi di produzione e la precisione dei loro prodotti. In questo modo, gli enologi potranno usufruire di YDP più specifici ed efficaci.

# **1. State-of-the-Art**

Yeast derivative products (YDPs) are widely used in winemaking. In particular, YDPs can be used to enhance the fermentation processes (Pozo-Bayón *et al.,* 2009), to promote the tartaric and protein stability (Rigou *et al.,* 2021), to improve the antioxidant capacity (Lambert-Royo *et al.,* 2022), and to ameliorate the sensory characteristics of wines (Ruipérez *et al.,* 2022). Nowadays, the International Organization of Vine and Wine (OIV) has approved different typologies of YDPs, including inactivated yeasts, yeast autolysates, yeast protein extracts, yeast hulls and mannoproteins. They are used in different steps of winemaking depending on their composition and, consequently, their oenological ability. These products can be considered as an effective alternative to the use of chemicals, such as sulfur dioxide or inorganic nitrogen sources, implementing the sustainable production of wine.

Despite the importance of these products, the action mechanisms and the possible correlation between the chemical composition and the technological characteristics of YDPs have never been investigated in deep.

The main goal of this PhD project is the selection of YDPs with specific functions for the further improvement of precision oenology. This goal will be achieved by means of investigating the use of YDPs for specific applications and their mechanisms of action. Therefore, the specific objectives of the WInnY proposal will concern the use of YDPs as (i) nutrient source for both alcoholic [AF] and malolactic fermentations [MLF], (ii) stabilizing agent and (iii) antioxidant considering their impact on the overall composition of wine. Moreover, (iv) a crossover activity regarding a deep and novel characterization of YDPs will be performed in order to evidence, for the first time, a possible correlation existing between their composition and their specific ability.

The expected increase of knowledge will regard the composition and mechanisms of action of YDPs, in order to improve the accuracy of commercial YDPs and to provide directions to winemakers on their properties. These recommendations can also support the YDP producers being able to adequately setting up their production methods allowing to obtain YDPs with more specific functions and improved performances.

# **2. PhD Thesis Objectives and Milestones**

In order to achieve the objectives described above, this PhD project is organized into 4 activities which are in turn divided into sub-activities, according to the Gantt chart reported in Table 1:

A1) **Characterization of YDPs.** Different commercial YDPs (20-25 products) will be characterized in terms of glutathione, cysteine residues (by UPLC-UV prior derivatization with *p*-benzoquinone), total nitrogen content (by enzymatic assays), amino acid profile (by UPLC-FL prior derivatization with *o*-phthalaldehyde) and antioxidant capacity (e.g., DPPH, ABTS, ORAC assays) (A1.1). Advanced characterization techniques such as spectroscopy, calorimetry and relaxometry NMR, will be used to determine the structure of yeast cell walls and their evolution with changes in the hydration level (A1.2).

It is expected to verify the composition differences between the YDPs and to build up a protocol for the application of the advanced techniques on YDPs.

*Milestones: M1.1 – Complete chemical characterization of YDPs (month 9); M1.2 – Complete characterization of cell wall structure (month 17).*

A2) **YDPs as source of nutrients for the fermentations.** YDPs will be used as source of nutrients for both AF (A2.1) and MLF (A2.2). AF trials will be carried out in laboratory scale using 2-4 different musts as medium. The fermentation trend will be monitored as well as the general chemical parameters (residual sugars, ethanol, pH, titrable acidity, organic acid profile, amino acid profile, aroma profile). MLF trials will be carried out in red wines. The concentrations of malic and lactic acids will be regularly determined (e.g., once a week) for monitoring MLF proceeding. Once MLF will be completed, the chemical parameters, the phenolic compounds and the biogenic amines will be determined.

It is expected to build up a link between their ability as fermentation enhancers and their chemical composition.

A3) **Improvement of wine stabilization in a sustainable perspective.** A large number of commercial YDPs (20-25 products) will be screened for tartaric and color stabilization in different wines (A3.1). The YDPs with the best performances will be added at variable amounts in 5-8 wines with different phenolic composition. The impact on tartaric stabilization and color as well as on phenols will be assessed (A3.2). In addition, the interactions between YDPs and phenols will be evaluated by using advanced techniques, as calorimetry and relaxometry NMR (A3.3).

It is expected to verify the effectiveness of YDPs as stabilizing agents, to select YDPs suitable for wine stabilization and to assess the relation between the YDP composition and the stabilizing activity.

*Milestones: M3.1 - Complete screening of YDPs with stabilizing ability (month 6).*

A4) **Exploitation of YDPs for wine aging.** In-bottle fermentation trials will be carried out with 2 white base wines following the *Champenoise* method. YDPs will be added before the fermentations and the sampling will be performed every 6 moths up to 18 months (A4.1). The samples will be subjected to accelerated aging trials (storage at 40 °C for 2 months in the dark) (A4.2). The antioxidant capacity, glutathione, cysteine residues, aromatic compounds (GC-MS) and sensory characteristics (e.g., mapping, quantitative-descriptive analysis) will be determined in the experimental wines.

YDPs are expected to effectively prolong the wine shelf life and enhance the sensory characteristics of the final product.

A5) **Bibliographic research, writing & editing:** PhD thesis, scientific papers, oral and/or poster communication for the dissemination of the results obtained from the WInnY project.

***Table 1***Gantt chart for the WInnY project:

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity Months | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| A1) | ***Characterization of YDPs*** |   |   |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 1) Chemical characterization of YDPs |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 2) Advanced characterization techniques |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
| A2) | ***YDPs as source of nutrients***  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |   |  |   |   |   |  |  |  |  |
|  | 1) Alcoholic fermentations |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |  |  |  |   |   |  |  |  |  |
|  | 2) Malolactic fermentations |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |   |  |   |   |   |  |  |  |  |
| A3) | ***Improvement of wine stabilization***  |   |  |   |   |   |   |  |   |   |   |   |   |  |   |  |  |  |  |   |   |   |   |   |  |
|  | 1) Screening of YDPs |   |  |   |   |   |  |  |   |   |   |   |  |  |   |  |  |  |  |   |   |   |   |  |  |
| 2) Selection of YDPs |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 3) Exploitation of interactions with phenols |   |  |   |  |  |   |  |   |   |  |  |   |  |   |  |  |  |  |   |   |  |  |   |  |
| A4) | ***Exploitation of YDPs for wine aging*** |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |  |   |   |  |  |   |   |
|  | 1) In-bottle fermentation trials |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |  |   |   |  |  |   |   |
|  | 2) Accelerated aging trials |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
| A5) | ***Thesis and Paper writing and editing*** |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |   |   |   |   |   |   |   |

# **3. Selected References**

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