



STABIWINE Report Summary

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Periodic Report Summary 1 - STABIWINE (USE OF BIOPOLYMERS FOR SUSTAINABLE STABILIZATION OF QUALITY WINES)

Project Context and Objectives:

STABIWINE project objective is the development of alternative practices for protein and tartaric stabilization of wine, that are important phases of winemaking, particularly for wines exported and sold through organized distribution that undergo long traveling and long storing periods often in not totally controlled conditions. The stabilization technologies in use, albeit effective, have some negative side impacts on wine quality, production costs and environment, and for many decades the wine producers – in Europe and overseas - have looked for alternatives.

A possible solution is offered by the use of biopolymers, i.e. compounds of natural origin, obtained from secondary products of the agro-food industry and already exploited in pharmaceutical and cosmetic sectors.

The project is composed of two major activity pillars, one devoted to the development of a new processing aid for protein fining, and a second developing a new additive inhibiting the formation of tartrate precipitates in the bottle.

Reticulated polymers for elimination of unstable proteins

In order to eliminate the unstable proteins from wine, every year in Europe about 10.000 tons of bentonite are used. Bentonite is a type of clay, mainly originated from other continents, that is suspended into wine where it absorbs proteins; after settlement, it is eliminated by racking, centrifugation and filtration to obtain a clear and stable wine. Bentonite fining is effective and inexpensive, however wine producers dislike it because it tends to reduce and modify the aroma and the taste of wine, and because the treatment often implies significant product losses. Moreover, the transport and distribution of such quantities of bentonite from continent to continent makes use of significant energetic resources.

Stabiwine project aims to exploit for this purpose the characteristics of polymers based on starch derivates, able to interact with proteins. The elemental bricks of these polymers are molecules of glucose and other sugars, obtained from cereals, and linked together in circular or linear compounds. The dextrins can be chemically bound together into reticulated polymers with different dimension, shape and charge, by varying the ingredients, their proportion and the reaction conditions.

Polyspartate to avoid tartrate precipitation

Tartrate, an organic acid typical of grapes, combines with potassium and calcium, two cations highly present in grape and then in wine, thus originating crystals that precipitate in the bottle and form an unwelcome deposit for consumers: to avoid this phenomenon, before bottling most wines are treated in order to eliminate the excess of tartaric acid and/or potassium.

According to the most popular technique, the wine is kept refrigerated to temperatures below 0°C for several days, thus inducing the formation and precipitation of crystals in the tank, which are finally eliminated by racking, centrifugation and filtration. This practice represents one of the most important point of energetic consumption in the winery, and has some negative effect on wine sensory profile. Furthermore, many small wineries cannot afford the purchase of the required equipment.

The addition of metatartaric acid can represent an alternative to cold treatment, but limited to wines with short shelf life; other additives, such as carboxymethylcellulose (cellulose gum) and mannoproteins are effective only in wines with low instability, and are not allowed in the production of organic wines (increasing percentage of European wines). The same for electrodialysis and resin exchange, whose development in the wine industry is restraint by the high cost and by the related significant water consumption.



Stabiwine project intends to introduce in the wine industry the use of a new additive, able to inhibit the formation of tartrate crystal, thus preserving the original wine composition. The research is focused on polyaminoacids, and in particular on polyaspartate (PAA), a new anti-scaling additive that is seeing an exponential diffusion in other sectors thanks to their positive combination of efficacy, safety and pricing characteristics. Polyaspartate, obtained by polymerization - through simple heating - of aspartic acid, a widespread aminoacid; the compounds obtained has very high surface charge, and peculiar physical and chemical characteristics which makes it ideal for winemaking use.

Project Results:

Reticulated polymers for elimination of unstable proteins

The research activity is focused on the identification of a biopolymer effective for protein removal, without negative effects on wine quality, composed exclusively by harmless and natural components, and with production cost compatible with wine industry standards. A multidisciplinary approach was adopted, where chemists were synthetizing several types of polymers with variable combinations of monomers, cross-linkers, functionalizing agents, catalysts and reaction conditions; the polymers were tested by wine experts on different wines, varying usage modalities, to assess their suitability to become a processing aid for winemaking; toxicologists were advising on potential risk for human health or environment of polymer components; industrial partners were suggesting routes to simplify future industrial production and distribution, and to limit production cost; all these inputs were jointly discussed and originated ideas for further improvement of polymers by the chemist, with the consequent restart of the virtuous loop.

Several dozens of polymers of different nature were synthetized, and evaluated for their effectiveness in real wines.

First, research identified the components able to create polymers with the right charge and shape, able to interact with the specific unstable protein fraction of wine, mainly composed by chitinases and TLP proteins. Progressive improvements led to a polymer exclusively constituted by natural compounds, and with significant and consistent removing action on wine proteins.

In a second step, the proportion among polymer components varied, and this brought to a powder that, once added to wine in a batch treatment, is able to remove wine proteins to an extent comparable to that of a high quality bentonite.

Very interestingly, the treatment with the new polymer, even at high dosage, does not seem to affect the concentration of wine volatile compounds, neither it is perceived by expert sensory panels. Conversely, the polymer might pay a positive role against wine oxidation; moreover, its effectiveness is scarcely dependent from wine characteristics, like pH, so allowing its use on a wide range of wines.

Polyspartate to avoid tartrate precipitation

The research on polyaminoacids started with a screening of the different types available on the market. Several commercial producers have been contacted and polymers of different nature were tested for their ability to reduce tartrate instability in white and red wines. PAAs resulted better than polyglutammates for this specific application; the tests did not show significant differences among polyaspartates, differing for type of salt, molecular weights and production process. The behavior of best performing PAAs for tartaric stability was studied, to fully understand their interaction with wine component and the overall effect on wine quality.

Several wines - different for type, original, age, degree of tartaric and color instability - were added of variable dose on PAAs, in comparison with most popular additives like metatartaric acid, cellulose gum and arabic gum. Wine parameters were assessed just after addition and, in many cases, after some months of storage.

Meanwhile, polyaspartates underwent several in vitro test to verify the absence of immunological or intestinal effects: all tests showed that PAA is well tolerated. Moreover, PAA is weakly digested by gastric and pancreatic enzymes, thus probably are poorly absorbed into the blood through the intestinal wall. These aspects will be further studied in the next months of the project.

These encouraging results allowed the activation of a series of toxicology tests, in collaboration with a GLP certified laboratory, to scientifically validate the overall safety of the polymer for human health.

The procedure toward authorization of PAA addition for winemaking in Europe was started.

The study led to the identification of a PAA type with many significant advantages on existing additives for tartaric stabilization:

- Is effective at low dosage also on very instable wines, both white and red
- Over time, is more stable than metatartaric acid
- $\ensuremath{\cdot}$ Has lower impact on color stability than cellulose gum
- Does not affect wine filtrability
- \bullet Does not affect color, aroma and taste of wine, even at high dosages
- Is easy to solubilize in water and wine



One of the few worldwide producers of commercial polyaspartate was actively involved in the project, and gave a very important contribution to the understanding of the behaviour of polyaspartate in wine by providing samples of different nature, and to the start of the authorization procedure by disclosing key information on production process and quality control.

Potential Impact:

Reticulated polymers for elimination of unstable proteins

The following months of activity will be devoted to the understanding of the mechanisms of interaction of the new polymer with wine proteins, and with other native or added wine components like metals, phenols, other types of proteins, etc. Swelling characteristics and lees volume after batch treatment will be optimized.

The possibility to use the new polymer in a column treatment will also be explored.

The industrial scale-up of the polymer production process, and the activation of the authorization procedure as processing aid for winemaking, will also be an important part of the actions. Additionally, demonstrative trials in different European regions are planned, to evaluate the new practice in real winery conditions. The advantages offered toward better sustainability will be assessed in comparison with bentonite fining.

Results obtained till now encourage the expectation of the wine industry to eventually find an effective and affordable alternative technology to bentonite fining: the use of a reticulated biopolymer, obtained from natural and renewable row matters, specific against unstable proteins, and without alteration of the original wine composition. Possibly, the new processing aid will offer side advantages on wine quality and will reduce wine loss accompanying bentonite fining, with gain in competitiveness for wine producers. Notably, being the new technology very simple and inexpensive, it will be accessible to winery with any size and location around Europe.

Polyspartate to avoid tartrate precipitation

The project research activity of next months is focused on the characterization of the effects of PAA on wine shelf life: indeed, the chelating properties of the polymer against bivalent cations like iron and cupper is well known in other application. This can potentially reduce the sensitivity of wines to oxidation, thus extending their shelf life or opening new routes for reduction in the use of preservatives like sulphites. Another objective of incoming research on PAA is the study of the interaction of this polymer with macromolecules, both naturally present (proteins, peptides, polyphenols, and polysaccharides coming from grape or fermentation) and added for technological purpose (polysaccharides, proteins, tannins).

Moreover, a large number of applicative trials are planned on different types of wine throughout Europe, in order to observe PAA behavior and potential side effects under real conditions.

There is high confidence that the good characteristics of PAA will be confirmed, and that the new additive could be authorized by EU regulation for winemaking.

The technological, economic and environmental characteristics of PAA are providing very significant advantages on all present practices. Once authorized, tartaric stabilization through addition of PAA is expected to become the first choice for a very large number of wine producers in Europe, especially among small and medium size facilities. Total annual volume of wine treated with PAA can rapidly reach 50 Mhl, with a yearly saving of about 90 M€ and 100 GWh.

Even more relevant, mainly small producers - located in rural areas - will benefit of the resulting increase of competitiveness: therefore, the new technology will also have a notable social impact.

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