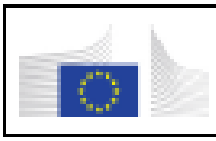

EUROPEAN COMMISSION
Research Executive Agency
SME Actions

Project No: 314903

Project Acronym: STABIWINE

Project Full Name: USE OF BIOPOLYMERS FOR SUSTAINABLE STABILIZATION OF QUALITY WINES

Final Report

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Dr. Cristina Michaloni

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ASSOCIAZIONE ITALIANA PER L AGRICOLTURA BIOLOGICA*AIAB

Final Report

PROJECT FINAL REPORT

Grant Agreement number:	314903
Project acronym:	STABIWINE
Project title:	USE OF BIOPOLYMERS FOR SUSTAINABLE STABILIZATION OF QUALITY WINES
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Final Report

Please note that the contents of the Final Report can be found in the attachment.

4.1 Final publishable summary report

Executive Summary

The European project STABIWINE (FP7-SME-AG, n. 314903) developed a new technology for wine tartaric stabilization that presents significant qualitative, technological, environmental and economic advantages on practices currently applied by the European wine industry.
To avoid the formation in the bottle of crystals of tartaric acid and potassium, two natural components of grape, that form a deposit disliked by the consumers, winemakers stabilize most wines before bottling. Two main stabilization strategies are applied by EU wineries: subtractive approach through cold treatment, electrodialysis and exchange resins, or use of additive inhibiting crystallization. Subtractive techniques have high environmental and economic costs, and none of the additives presently available can be efficiently used on any type of wine.

The solution was found in the use of potassium polyaspartate, a polyaminoacid whose study highlighted several positive characteristics:
- is more efficient than all other additives in inhibiting the formation of tartrate crystals;
- it works in all wines of different types, origins and instability levels;
- has no effect on taste or aroma of wines, even at very high dosages;
- is stable over time, contrary to metatartaric acid;
- does not cause color instability as for carboxy-methyl-cellulose in some red wines;
- does not show fouling effect at membrane filtration
- is produced from natural and renewable row materials
- is totally biodegradable and constitute no danger for the operators.

The practice has the lowest carbon and water footprint among all tartrate stability treatments; its cost of the treatment is very low, and it can be easily adopted even by the smallest wine producers.
For what concerns human safety, potassium polyaspartate is considered a harmless substance: studies within Stabiwine project showed total absence of toxicity, immune-stimulation and mutagenic effects.
The procedure for authorization of industrially produced potassium polyaspartate as wine additive has been already started at OIV, EU and national level: once completed, the polyaspartate will become a revolutionary alternative for wine stabilization, and will allow very significant economic and environmental saving by wine producers, including micro-wineries not having access to expensive technologies.

A second pillar of the project aimed to find an alternative to bentonite fining for protein stabilization. A small residue of grape proteins is present in many wines, and can it form haze in the bottle if accidental heating occurs during distribution: prudentially, all wineries willing to avoid the phenomenon treat the wine before bottling, adding a natural clay able to absorb the proteins and drag them to the bottom of the tank to be eliminated by racking. Albeit very efficient and economic, bentonite is not liked by many winemakers for the loss of flavor and product linked to the treatment.
The project developed a new polymer, based on natural ingredients like starch derivates and grape organic acids, having absorbing efficacy similar to bentonite, and respectful of wine quality.
The phases of industrial scale-up of the polymer synthesis process, and of elimination of any practical problems potentially occurring with its use in commercial wineries, are presently ongoing.

Summary description of project context and objectives

The goal of STABIWINE project was 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 was 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.

Polyaspartate: a biopolymer for sustainable tartrate stabilization of wines
A polymer of aspartic acid can substitute wine refrigeration in the phase of stabilization of wine against precipitation on tartrate crystals in the bottle; this is the innovative finding of the European project STABIWINE.
Naturally, wine contains tartaric acid and potassium, absorbed by the vine from the soil into grapes. These two elements are normally present in concentration above saturation: if the wine is not stabilized, crystals of potassium tartrate can form during storage, giving origin to deposits in the bottle that are completely harmless, although not appreciated by consumers.
Presently, most wines are stabilized by mean of subtraction method: they are chilled at few C° degrees below zero and kept in these conditions for some days, in order to induce formation of crystals that will be then removed by filtration. This method requires significant investments in a powerful refrigeration system and insulated tanks; in addition, the energy needed to cool down the wine and kept its temperature below zero significantly contributes to the carbon footprint of a winery. Moreover, refrigeration and filtration are critical steps for the wine quality, that can be drastically reduced in case of incorrect management of these phases.
Polyaspartate added to wine even in very low dosage has been found to inhibit the formation of tartrate crystals; the natural wine composition is not altered by subtraction of salts, and no refrigeration or other physical process are required.
Currently, other winemaking practices follow the same additive method: addition of metatartaric acid, carboxy-methyl-cellulose or yeast mannoproteins. Nevertheless, because of limited efficacy of the former two additives, or of cost of the third, these technologies are far to be universally applied.

Microsponges: a reticulated polymer for protein stabilization of wines
Many wines, especially whites and rosé, have a natural content of proteins that, in case of accidental heating during storage and transportation, can give origin to haze negatively perceived by consumers. In particular, two types of proteins (TLP and chitinase) produced by the vine in response to pathogen infections, seems to be the most unstable fractions.
Presently, protein stabilization is reached in wine production by addition of bentonite, a clay able to absorb positively charged molecules – like unstable proteins at wine pH – between its layers and to drag them down while settling. The lees are then eliminated by racking or centrifugation, living a stable wine ready for filtration and bottling. Although effective, inexpensive and widely adopted worldwide, bentonite fining is not beloved by many winemakers claiming that this non-specific treatment is reducing wine sensory quality. Sustainability performance of bentonite fining is also weak: together with lees, a significant amount of product is wasted (3-10% of volume) causing economical damage, and inter-continental freight of high amount of bentonite are at the origin of relevant GHG emission.
Stabiwine project aimed to developed a new processing aid able to absorb the unstable fractions of wine proteins, alternative to bentonite, obtained through renewable sources and without organoleptic effect on wine.

Description of main S & T results/foregrounds

Microsponges: hyper-reticulated polymers for elimination of wine instable proteins
During the project, significant activity on biopolymers for removal of unstable proteins from wine have been performed, leading to the development of an hyper-reticulated polymer based on harmless ingredients like starch derivate and organic acids, able to absorb the wine protein fractions causing haze formation in wine (i.e. TLPs and chitinases).
Initially, several types of polymers were synthetized, and the efficacy was evaluated by using the "heat test", an empirical but inexpensive and reliable test largely used in wineries QC, that assesses

the turbidity induced by heating the wine in standard conditions.
The first phase of the research was devoted to the identification of ingredients with no toxicity effect, in view of the authorization procedures that the new processing aid must overcome to be commercially exploitable. After several attempts, it was finally obtained a hyper-reticulated polymer from the following food additives as ingredients:

• #-cyclodextrin, a cyclic compound composed by 7 molecules of glucose, obtained from corn starch
• LineCaps, a linear maltodextrin with spiral tertiary structure, obtained from pees
• Citric acid, a tri-carboxylic organic acid naturally present in wine, acting as cross-linker

Such polymer, washed and grinded, gives a powder that in lab conditions demonstrates its ability to interact with wine proteins; when the powder is added to wine - in dosages comparable to those used with bentonite i.e. 500-2000 mg/L - and removed after few hours of contact by filtration or centrifugation, the treated wine contains significantly lower amounts of haze forming proteins.
The following phases of activities were devoted to:

- transfer the polymer synthesis from the lab scale (production of 1-2 grams of powder) to the pilot scale (production of few hundred grams of powder), in order to
- test the new processing aid on larger quantities (50-100 liters) of different wines, to evaluate the practical feasibility and the sensory effect of the treatment
- assure that the newly synthetized polymer doesn't show any toxic or allergenic effect in preliminary in-vitro tests

The scale-up of polymer production was performed initially in UNITO labs, then the industrial partner Roquette Italia (supplier of #-cyclodextrins and exclusive producer of LineCaps) was involved in the development through an agreement reached with ESSECO and UNITO. Both UNITO and Roquette produced several lots of polymer, promptly tested by IFV on standard unstable wines.
This step presented several unexpected troubles, due to the increase of volume of starting solution (up to 2 liters): even by introducing the improvements of acting under vacuum, and dividing the solution in several trays, the reaction time necessary to obtain efficient polymer turned out to be above 100 hours, heavily compromising the environmental and economic sustainability of the process. Moreover, the grinding and washing phases of preparation of the powder become more elaborated and long.
Nevertheless, some hundred grams lots of efficient powder were produced and distributed to several project partners to be tested on different juices and wines. This new series of experiments showed some negative traits of microsponges:

- while in some wines the treatment have confirmed the capability to reduce the haze formation after heating, in other wines the turbidity increased after microsponge treatment, in some cases even in the wine before heat test
- the microsponges were settling down very slowly, in a timeframe 5-10 times longer than bentonite
- the sediment created after treatment and settling has a gelatinous texture, and the clear surnatant wine is more difficult to separate than when bentonite is used.

In parallel, this pilot lot of microsponges was used for toxicology and allergenic essay in WP3, and the sustainability evaluation was based on parameters and values issued from the process at the actual stage of development.
In the last months of activities several trials to understand the causes of the practical problems and to find a solution were carried out.
Thus, it was demonstrated that the cause of turbidity is a soluble compound released into wine from microsponges, and not corresponding to one of its ingredients; therefore it probably is a fraction of polymer constituted by few molecules of saccharides linked with citric acid, with enough negative charge at wine pH to react with other non-identified wine components – when present in sufficient concentration – to form insoluble macromolecules.
It was verified that this compound at the origin of turbidity is not the product of chemical degradation of the polymer, but diffuses from the microsponges cavities into the liquid. Cyclic washing of microsponge powder before use, even by different solvents, is unable to eliminate the release of haze forming compounds, that probably need long times to slowly diffuse from the small cavities of the internal polymer.
The tentative of use of microsponges in combination with other processing aids (gelatin, albumin, silica sol, and even bentonite) to avoid turbidity creation in critical wines and to speed up sedimentation of less was unsuccessful: the settling dynamic did not improve, at the contrary even the other processing aids stayed longer in suspension after treatment.
In order to modify the gelatinous texture of the microsponge sediment after treatment, several lots of

polymer were produced by changing temperature and duration of reticulating reaction; unfortunately, it was demonstrated by this set of trials that a certain degree of swelling property in wine is required to effectively remove unstable wine proteins. The swelling characteristic of the polymer is particularly undesired as it totally impedes further developments of the new technology in a column treatment, that would offer very interesting environmental and economic advantages.
To summarize, the results of the projects are:
- the knowledge on the nature (ingredients, type of cross-link, reactions parameters etc.) of a hyper-reticulated polymer able to interact and absorb the wine protein fractions responsible for haze formation after heating (TPL and Chitinase)
- the evidences of null absorption from human gut, and of lack of toxic and allergenic effects on in-vitro models of the polymer;
- knowledge on the effect of the microsponges on wine quality (result: no advantages on bentonite treatment)
- knowledge of the environmental and economic impact of a wine treatment making use of microsponges at this stage of development (result: clear disadvantages compared to bentonite treatment)
None of these new knowledge has been judged by the Steering Committee to need IPR protection, in addition to the patented background.

Potassium polyaspartate: the new and best practice for tartaric stabilization of wine

The first project period was used to identify the best polyaminoacid for wine treatment, to characterize its behavior in wine and to lay the foundations of the authorization procedure.
An initial market research has been done to identify the worldwide industrial producers of polyaminoacids. This phase showed that:
- Polyglutamate is commercially available on the market but its trading price is too high to envisage its use in winemaking
- Polyaspartate is produced by only two companies in western countries, each one in different variants (sodium and potassium salts, liquid or powder, with different average molecular weight).
Asian producers have very bad reputation concerning consistency of the product, and were not taken in consideration.
From a bibliography search on toxicology studies on polyaminoacids already performed, polyglutammate resulted to be authorized as food additive by American FDA but not in Europe.
Several unofficial studies on polyaspartate demonstrated its substantial safety for human health and environment, nevertheless no authorization procedure was started in Europe.
A dozen of different types of polyaminoacids were recovered and tested for their efficiency in inhibiting formation of potassium tartrate crystals in wine during shelf life.
Polyaspartates showed a very good performance in wines with different instability. Polyglutamate appeared less efficient: these data, together with the cost consideration, soon eliminated polyglutamate from the screening.
Numerous comparison tests on different wines, demonstrated that:
- All forms of polyaspartate (powder or liquid, sodium or potassium salts, different molecular weight, different producers) have similar performances in terms of tartaric stability
- At similar dosages, polyaspartate efficiency is comparable with the one of metatartaric acid and carboxymethylcellulose, the two main additives presently used in winemaking for the same purpose;
- Polyaspartate, unlike metatartaric acid, is not chemically degraded in wine over time, and its stabilization effect have a longer duration
- Unlike carboxymethylcellulose, polyaspartate does not destabilize color components in red wines; among all types, low and medium MW polyaspartate seems to be the most ineffective on color stability; these results have been confirmed by IFV in its trials;
- The addition of PAA to wines, even at high dosages, do not causes modification of wine aroma and differences are not perceived by an expert panel of tasters
Toxicology assays showed no mutagenesis neither immune-stimulation effect in all types of polyaspartates, although in liquid forms the carrier (water) can be contaminated by immune-stimulating compounds. The bibliographic search done on the compounds revealed that sodium salts can produce inflammatory phenomena at urinary system in male rats, but no other toxicity issue has been found in the available studies.

On the basis of these results, the best polyaminoacid for wine use was identified in a potassium salt

of polyaspartate of low/medium molecular weight.

Meanwhile, a partner producer, whose active and collaborative attitude was essential in the preparation of the toxicology dossier, was identified in the North American company Nanochem Solutions Inc.

INTOX, a GLP certified laboratory, was contracted to run toxicology tests: all in-vitro and in-vivo tests foreseen in Tier 1 of the EFSA guidelines for submission for food additive evaluation were performed. The final INTOX report demonstrated the total lack of toxicity of A5DK-SD.
The dossier "Application for registration of a new Food Additive (EU Reg No 1333/2008)" for the product A-5D K/SD was composed and officially submitted on February 2015 to EFSA (European Food Safety Agency – Panel on Food Additives and Nutrient Sources added to Food ANS); the expression of EFSA Scientific opinion is expected on February 2016.

Since March 2013, the use of polyaspartate for tartaric stabilization of wine was introduced to the Technology Commission of OIV. Results on technological interest, toxicology assays and analytical methods were presented in several sessions, and the resolution is under evaluation by national groups for a final decision in 2016.
Special derogation ex art. 4 of Reg. CE n. 606/2009 were obtained by competent national offices, in order to have the possibility to run demonstrative trials of tartaric stabilization by addition of KPA (A-5D K/SD) in commercial wineries and in real conditions.
Derogations were obtained in 5 EU countries:
- In Spain, by Aragon Government, on Sept 2014, with obligation to distill the treated wine
- In Italy on Dec 2014, with allowance of commercialization of treated wine within EU
- In Greece on May 2015, with allowance of commercialization of treated wine within EU
- In Portugal on July 2015, with allowance of commercialization of treated wine within EU
- In France on November 2015, with obligation to distill the treated wine

These authorization allowed to carry out twelve comparative trials on large volumes of wines (from 10 to 130 hL), with the aim of verify the absence of practical problem already registered at experimental level, and to enlarge the case history of the product.

Meanwhile, additional studies and controls were performed in lab and experimental winery conditions by RTDPs and ESS, in order to deepen the knowledge of KPA (potassium polyaspartate) behavior in wine and to verify by replication some of the most important characteristics.

Additional trials in experimental facilities and on real scale, in commercial wineries on volumes of 10-130 hL, were carried out. Globally, more than 50 wines, of different type and origin, were treated with KPA. All added wines – including red wines - resulted stable for tartrate precipitation, and with dosages equal or lower than for metatartaric acid. In all cases monitored for one year or more, the stability effect was persistent. No color changes were reported in red wines even after long period after addition of potassium polyaspartate. Only in one red wine (< 5%) a slight increase of turbidity after addition was observed, easily eliminated by preliminary bentonite fining or gum Arabic addition.
A detailed sustainability study showed that addition of KPA is by far the stabilizing practice with lowest environmental impact, both in terms of carbon and water footprint. Moreover, the treatment has a cost comparable to the cheapest current technology - i.e. metatartaric acid addition – and do not require investments neither specialized personnel to be performed.
For all these reasons, the new practice will probably see a very wide diffusion in the European wine industry, especially in the small and medium wineries that represent the great majority of the sector.

Potential impact and main dissemination activities and exploitation results

From the technical standpoint, potassium polyaspartate (KPA) resulted to be the best practice presently available for tartrate stabilization of wines:
- KPA is able to avoid the formation of crystals in white, rosé and red wines, even with very high instability
- doses of 5-10 g/hL are sufficient to stabilize most of the wines, higher doses might be needed only for very young wines
- KPA does not degrade in wine environment, and maintains its stabilizing properties for years
- KPA does not have any significant wine sensory impact, nor it causes modification in volatile

composition, even when added at very high dosages
- Membrane filterability of white and red wines is not affected by the addition of KPA, that can therefore be used immediately before final filtration and bottling
- On red wines, the addition of KPA does not modify the color of wine, neither induces modification in phenolic composition
- KPA does not have general stabilizing effect on wine colloids, and its addition cannot substitute bentonite fining of gum Arabic addition in unstable wines
- Carbon footprint and water footprint of KPA stabilization practice are several times lower than present subtractive technologies (cold, electrodialysis and resin treatments) and the lowest even among additive technologies (metatartaric acid, carboxy-methyl cellulose, mannoproteins, gum Arabic)

From the marketing standpoint, the practice of potassium polyaspartate (KPA) addition has additional advantages on all present technologies for tartrate stabilization:
does not require initial investments for equipping the facility (insulated tanks, refrigeration systems, cooling piping and exchangers, electrodialysis equipment, resins exchanger etc.)
does not need particular expertise of personnel for its implementation in the wine production process
treatment with KPA is the most inexpensive practice, with a cost comparable to metatartaric acid addition

Potassium polyaspartate is already manufactured at industrial scale, and presently available on the international market for uses other than wine.
The type of potassium polyaspartate characterized by Stabiwine project and object of all authorization procedures, A-5D K/SD, is produced by the company Nanochem Solution Inc., based in Peru, IL, USA; the supplying company has signed a cooperation agreement with Esseco srl, that contemplates active collaboration in the authorization procedure and reciprocal worldwide exclusivity at commercialization, taking in account all obligations foreseen in project Consortium Agreement.

EFSA opinion is expected for February 2016. If positive, EU Commission can activate the procedure for adding Potassium Polyaspartate A-5D K/SD in the positive list of wine additives, taking in account OIV resolution.
Final authorization of KPA use for winemaking in Europe is expected for the end of 2017.

The adoption of the new practice will bring significant improvements for the EU wine industry, both in terms of economics and of environmental impact: it was estimated that a hypothetical complete substitution of the practices presently used with the KPA addition technology could bring to savings of GHG emission for 90,000 tCO2e/y, of water footprint for 163 Mm3/y, of costs for 200 M€/y.

Address of project public website and relevant contact details

The project presentation, activity history and outcomes are available on the project website www.stabiwine.eu
The website provides contacts for scientific and practical exploitability of foreground.
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Project Consortium

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3	CONSEJO REGULADOR DE LA DENOMINACION DE ORIGEN CARINENA	CRC
4	ENTAV-ITV FRANCE	IFV
5	CONSIGLIO PER LA RICERCA E SPERIMENTAZIONE IN AGRICOLTURA	CRA
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12	VINIDEA SRL	VIN
13	ESSECO SRL	ESS
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