

## A NEW DEVICE FOR STABILISATION OF WHITE WINES THROUGHOUT A CONTINUOUS FLOW SYSTEM

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### Introduction

Proteins occurring in wine originate from several sources during the winemaking process. Most of them accumulate in grape as a consequence of bio-synthesis during berry development; furthermore, they are produced by yeasts metabolism during fermentation, and released in wine during the yeast cells lysis.

Protein content in wines ranges from traces up to hundreds of mg/L, and may cause physical instability in white wine, inducing haze formation, clouding, and precipitation. However, despite the large number of scientific studies on the matter, the molecular features involved and reaction mechanisms underlying the protein instability have not fully elucidated; protein haze formation is generally considered as a two-steps process, during which wine proteins unfold in response to stimuli (e.g. temperature), then aggregate and flocculate to form a visible haze (Dufrechou et al., 2010).

Wine proteins can be described based upon size and electrical charge, and both attributes are responsible for their reactivity. Research studies have highlighted a prominent role of Pathogenesis-Related Proteins (PRP) in wine instability. PRP are low-molecular weight proteins (10 – 35 kDa) which are bio-synthesized in plants in the event of a pathogen attack and do not alter their chemical structure during the winemaking process; among them, chitinases, thaumatin-like proteins (TLPs),  $\beta$ -1,3 glucanase and lipid transfer proteins are major low-molecular reactive proteins occurring in wines (Waters et al., 1996; Pocock et al., 2000; Marangon et al., 2014), together with others small polypeptides that can be involved in the process (Sauvage et al., 2010; Meier et al., 2016; Perutka et al., 2018). The occurrence of PRP can induce flocculation and formation of haze and cloudiness under thermal stress conditions, regardless their concentration.

White wines are especially prone to protein instability, since they lack sufficient tannins to cause initial protein precipitation; hence, when subjected to temperature fluctuation (i.e. during wine handling and storage), residual PRP could aggregate to other small proteins or minor quantities of reactive phenols, then flocculate and precipitate.

Based on this, it can be stated that the removal of pathogenesis-related proteins is a crucial issue for winemakers, in order to guarantee long-term stabilization of commercial wines. The most common technological approach consists of the addition of fining adjuvants which selectively react with PRP precipitating them from grape must/wine. Among adjuvants, bentonite is the most widespread product exploited to reach wine stabilization; it is a clay-based fining agent acting as a cation exchanger, to produce electrostatic interaction with proteins. Despite this material is able to produce a long-term stabilization of wine after treatment, it requires time-consuming procedures after which the adjuvant need to be removed

(by raking and filtration); the main consequences of wine fining with adjuvant are the production of a large volume of waste for disposal, and the loss of significant volumes of wine during filtration.

In recent years many studies have been conducted with the aim of finding novel fining agents, and to gain innovative and more sustainable approaches for the stabilization of wines on an industrial scale.

In this view, nanotechnology might represent a valid approach for the design of innovative and tunable absorbing materials. The captivating aspect involves the unique properties of nanomaterials; in fact, at the nanoscale level the physical and chemical properties of a material are completely different compared to the bulk, and they can be tuned according to size, shape and distribution of nanometric features; furthermore, nanostructures present an extraordinary high specific surface (Khan et al., 2017).

This scientific work, named “Steady Wine” project, refers to the development of a patent-pending technology (Patent Number 102018000004721), and it was aimed to evaluate the capacity of novel, food-grade ceramic nanomaterials to adsorb PR proteins in wine. Selected white wines were used in case study, to assess the impact of the proposed treatment on the physical-chemical parameters and long-term stability of white wines.

### **Experimental methods**

Two selected, food-grade ceramic materials MCs ((hereinafter labelled MC1 and MC2) were tested as micro-powders (grains dimension >800 nm); the same materials were tested as nano-powders (MC1A and MC2A) to assess the improved efficiency of nanostructures in protein absorption.

The ceramic powders were added in batch at different concentration level (ranging 5 to 200 mg in 50 mL volume) to experimental unstable white wines (Chardonnay and Moscatello). The following parameters were analysed on a daily basis: pH, color at 420 nm, total proteins content (PT), total polyphenols (PFT), and thermal accelerated aging (5 days at  $35 \pm 1^\circ\text{C}$ ).

Protein stability was further assessed using the heat-test, which measures the evolution of wine turbidity when subjected to thermal shock. A detailed information on the removal of targeted PR proteins was achieved using the sodium dodecyl sulfate polyacrylamide gel electrophoretic method (SDS-PAGE). All experiments were performed in duplicate; results were subjected to statistical analysis using ANOVA and the Tukey HSD *post-hoc* test ( $\alpha \leq 0,05$ ). The material providing best performances in terms of selectivity of PR-protein removal and preservation of the wine quality was used to functionalize an inert substrate; the production of a nano-structured surface was aimed to increase the active binding sites also avoiding swelling in must or wine. To achieve this, the MC1A nano-powder was subjected to high-temperature sintering cycles (up to  $550^\circ\text{C}$ ) to obtain a thin, nanostructured layer of functional material, with high specific surface available for PRP absorption. SEM analyses was used to evaluate the occurrence of nanostructures at the surface, coupled to the EDS analysis which was performed to verify the absence of metal contaminants.

### **Discussion of results**

Analyses performed on the experimental wine during the batch treatment with MC materials showed a generic stability of the quality parameters of wine. In more detail, the pH value was not affected by the addition of powders (**Table 1**)

pH	CN	5	25	50	100	200
MC1	2,93	2,91	2,94	2,93	2,93	2,92
MC1A	2,89	2,89	2,92	2,92	2,92	2,93
MC2	2,89	2,91	/	2,93	/	2,94
MC2A	2,93	2,95	/	2,96	/	2,93

Table 1. The impact of MC addition in the pH of wine after 5 days of batch treatment, at the different concentration levels.

The polyphenolic compounds were generally not affected by MC. When statistically significant reduction occurred (MC1 and MC1A, for instance), it referred to losses ranging 5 to 20 mg/L (**Figure 1**); these values are comparable to polyphenolic reduction following batch treatments with bentonite, and do not alter the quality of wine.

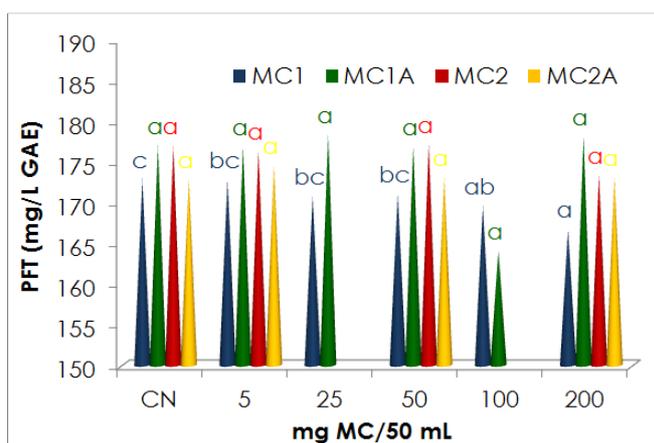


Figure 1. Total polyphenol content (PFT) after 5 days of batch treatment with MC, at the different concentration levels.

The d.o. 420 nm (browning index) is a routinely-used parameter to assess the occurrence of chemical oxidation in wine. Chemical oxidation is catalysed in wine by transition metals which occasionally occur in wine as contaminants; for this reason, novel materials providing a prolonged contact with wine need to be tested for the oxidative stability. In this work, the browning index was monitored during the treatment with MCs under standard conditions (5 days, room temperature) and accelerated aging conditions (5 days at  $35 \pm 1^\circ\text{C}$ ) to exclude potential catalytic activity of the novel material in wine. The optical density was not affected by MCs addition both in standard and accelerated aging conditions (data not shown), thus excluding the release of metal contaminants during the treatment.

Studies on the protein fraction in wine confirmed that the MC1A material was particularly prone to remove PR proteins and to produce stable wines. **Table 2** reports the total protein content

after the batch treatment; in 5 days, a significant amount of proteins was reduced, and the effectiveness increased with the concentration of active material. The SDS-page profile of wine before and after the treatment with MC1A confirmed the removal of proteins with molecular weight < 35 kDa (**Figure 2**). These results showed that MC1 steadily interact with small wine proteins; first evidences showed that the interaction MC – PRP is based on a simple electrostatic mechanism, nevertheless detailed studies on the specific interactions involved are still ongoing.

PT (mg/L BSA)	CN	5	25	50	100	200
MC1-5	66d	63cd	63cd	56bc	39b	38a
Δ		4,5%	4,5%	15,3%	25,2%	42,3%

Table 2. The reduction of the total protein content following batch treatment with nanometric MC1 at the different concentration levels.

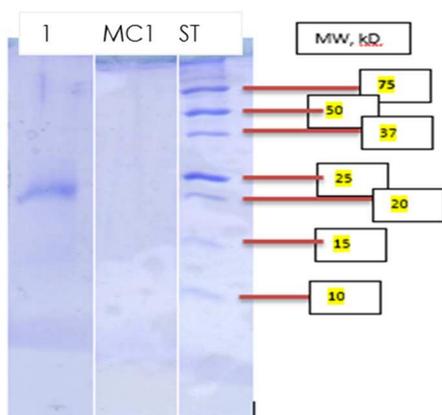


Figure 2. SDS-PAGE of experimental wine before (1) and after (MC1) the batch treatment with nanometric MC1. The ST column refers to the molecular marker used as a reference standard.

Results showed that it is possible to obtain an effective protein stabilization through the maximization of the surface available for absorption.

Nanotechnology offers the possibility to design materials with appropriate active surfaces, through the extent and dimensional modulation of nanometric features. Among others, the sintering process is a cost-effective method to produce large-scale materials; nanoparticles with variable dimensional distributions (range 10-100 nm) can be dispersed in appropriate medium solvents and deposited onto inert surfaces; the resulting layer is subjected to a proper thermal treatment during which the medium is evaporated, and the nanoparticles merge in a bulk material producing nanometric pores ('mesomeric material', **Figure 3**). A mesomeric structure exhibits the properties of a nanometric material but it doesn't produce swelling and can be handled as a bulk material; these characteristics comply with the safety requirements expected for a material used as a food adjuvant.

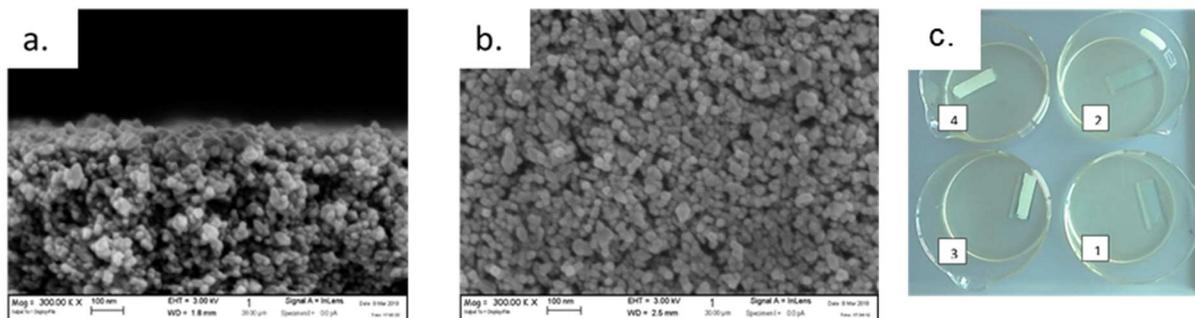


Figure 3. SEM images of the mesomeric material after sintering: Section (a.) and surface (b.) of the active layer obtained after sintering. On the right, the mesomeric materials deposited onto inert glass slides (c.).

Experiments were performed allowing the wine to flow over the active material under standard conditions (number of cycles, duration). Different active surface/wine volume ratios were applied to obtain a provisional model, and samples were subject to the heat-test to assess stability. The decrease in turbidity following thermal stress (Delta NTU) as a function of the active surface involved in the treatment informed about the surface amount requested to achieve full wine stabilization in case study (Figure 4a, b).

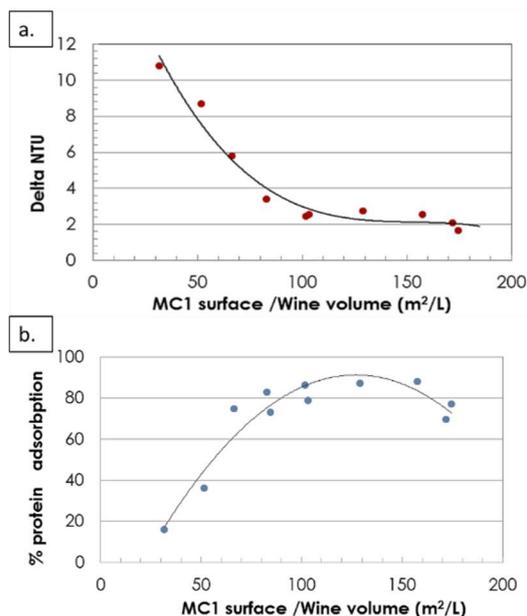


Figure 4. Experiments of wine stabilization performed using the mesomeric material at different exposed surface levels. (a.) Exponential decrease of Delta NTU (increasing stabilization) increasing the active surface of MC1. (b.) Protein adsorption (%) increasing the active surface of MC1. In both curves a steady-state point can be observed, followed by saturation of active sites and protein desorption (b.).

## Conclusions and future perspectives

In this work two major objectives were pursued: firstly, a screening of novel ceramic materials were evaluated on the basis of their efficacy to resolve physical instability involving proteins in wine; in particular, the nanometric powder labelled 'MC1' showed effective removal of pathogenesis-related proteins without altering the physical-chemical parameters of white wines and was selected for further studies.

In the second step, the MC1 material was engineered to obtain a bulk mesomeric material, characterised by porosity on the nanometric scale (thus reproducing the properties of MC1 powder), which can be easily handled and safely used as a food-grade adjuvant.

A further application of the mesomeric MC1 provides its use in a continuous-flow system that enables fast stabilisation and the possibility of regenerating the material for several treatments. A laboratory-scale device is currently available, and optimal parameters for the flow treatments, including volumetric flow rates and life cycles of the material, are still object of study in an industrial scaling-up perspective.

The present work was developed as part of a patent-pending technology (Patent Number 102018000004721).

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