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Bentonites
Interaction with wine proteins and fermentation aromas

Despite its technological importance, much is still to be clarified about the constitution, the behaviour and the evolution of colloidal substances in wine products. In fact, the subject is very complicated and it is one of the knottiest questions to be solved in oenology. As well known, the composition of the grapes include some colloidal substances, generally with complex structure, whose molecular weights range in prevalence between 10,000 and 20,00 Dalton, with peaks that can reach the million. The nitrogenous colloids with average of high molecular weight and glucidic colloids, or "protectors" are essential for the structure and the colloidal stability of the wines. First of all, it is important to highlight that the former ones, i.e. proteins, are subject to easy coagulation and flocculate and, therefore, can give way to turbidity and sedimentations in the wine, while the latter ones, i.e. glucidic colloids, like the relevant hydrolysis products, rather seldom insolubilise. Therefore, the danger of alterations of the limpidity derives almost exclusively from proteinic colloids (1). Besides concentration, the instability of the wines depends also on the nature of proteins. Most authors agree thinking that the proteins with lower molecular mass (between 15,000 and 35.000 Dalton) and lower isoelectric point are those most responsible for the formation of particulate (2; 3). Only a part of the proteinic fraction seems to the involved in colloidal instability, a phenomena including also a series of non-proteinic compounds, such as polysaccharides and polyphenols, that can modify the efficiency of the stabilization treatments. Since the pH of the wine is lower than most isoelectric points of the proteins, they have positive charges and can interact with various agents with opposite charge to bind with each other and then precipitate. In most cases, the stabilization of rosé or white wines as to the proteinic class, is obtained through bentonite, whose mechanism of action is mainly based on the principles of above-described electrostatic interaction. Bentonite is a natural clay-based mineral belonging to the group of the montmorillonites (aluminium hydrate silicates) swelling in water, getting more or less large,

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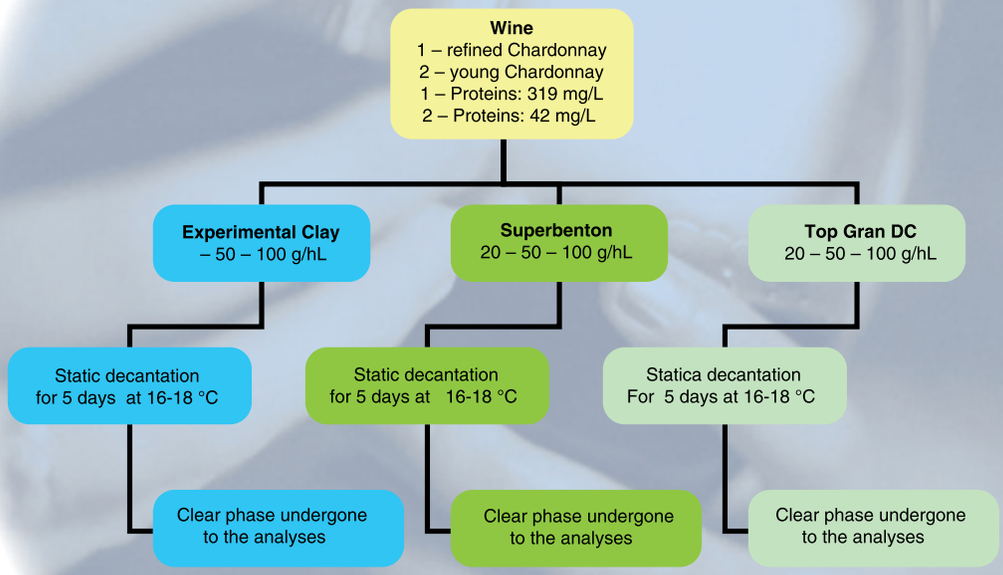
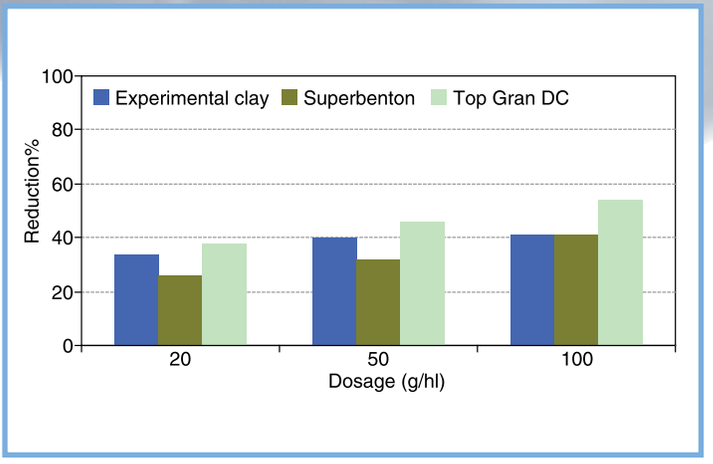


and jellifying. From the morphological point of view, it has a lamellar structures alternated with exchangeable cations and hydration water. The nature of ions, prevalently calcium and sodium, strongly influences some properties of clay, like, for instance, available surface and exchange capacity (1). Being electronegative, the lamellas of bentonite can interact with the proteinic molecules with positive charge to the pH of the wine; as a consequence of that, the proteins combined are eliminated from the liquid through precipitation with the particles of bentonite (4). As cation exchanger, bentonite is not specific for the proteins only, yet it removes also other charged species or aggregates. Therefore, high additions of them can induce a reduction in the organoleptic properties of the wines debasing the configuration of their aromatic compounds (5). Studies demonstrated that some macromolecules of the wine play an important role in the maintenance of the aromatic balance of the product; however, said macromolecules can be removed in more or less important quantity during the stabilization treatment. The removal can be ascribed to both a direct interaction between bentonite and the aromatic compounds and an indirect removal linked to the elimination of proteins (6). So, it is interesting to understand more details of the interaction of bentonites with proteins having different molecular weight that are naturally contained in white wines and evaluating the possible impact on the aromatic compounds originated from the fermentation.



Research

The experimental tests are part of a wide research project developed in cooperation between the Institute of Oenology and Agrofood Engineering of the Università Cattolica del Sacro Cuore di Piacenza and Azienda *Dal Cin Gildo S.p.A.* di Sesto San Giovanni (MI).

Material and methods

The study foresees the acquisition of technical-scientific information on some characteristics of the materials and the interaction of them with the must and the wine. In particular, the tests concerned the evaluation of three types of bentonites produced by Azienda Dal Cin: two of them, Superbenton (in powder) and Top Gran DC (granular), used in oenology for a long time, and a third one called “experimental clay” with a high content in montmorillonites. The tests were prepared on laboratory scale and included the use of two white wines both deriving from the variety Chardonnay, but having very different characteristics: product 1, taken after 6 months’ refining on the lees, is characterized by a high content in proteins, and product 2, a young wine, taken immediately after the alcoholic fermentation, has a reduced content in proteins. The clays have been used after re-hydration in a water volume ten times their weight, as summarized in the scheme of picture 1. The following parameters were evaluated on all samples:

• deproteinizing capacity of the material, stated through an analysis of the proteins and their fractionation by molecu-

lar weight, by using techniques of high performance liquid chromatography;
• interaction of bentonite with odorous molecules, by monitoring the main markers of he fermentative aroma of wine through chromatography in gaseous phase.

Results

The percent reduction of the protein in comparison with the content of the sample after static sedimentation was calculated to evaluate the deproteinisation done by the clays. Figure 2 shows the data of the percent reduction for every dose of bentonite used in the case of wine 2, the young Chardonnay. It is bentonite Top Gran DC that in terms of global reduction gives the best performances at all dosages of treatment. By using a quantity of 50 g/hl, the proteinic reduction is higher that that attainable with other products at the dosage of 100 g/hl. At the maximum level of treatment, experimental clay and Superbenton show the same effectiveness, while at lower levels (20 and 50 g/hl) the experimental preparation has a slightly higher deproteinisation capacity. It is worth observing that the proteins with a molecular weight between 17,000 and1.350 Daltons, representing the most important fraction, mirror the effectiveness of the treatment in terms of total proteins. On the contrary, wine 1 showed less important deproteinisation even at the dose of 100 g/hl. This fact can be due to a "matrix effect" that in wine we concerns the type and the charge of the proteins involved, for instance the mannoproteins released by the yeasts during the refinement of the wine on the yeasts. Besides, in Chardonnay 1 there is a higher level of protector colloids and a higher pH, which could interfere with the action of bentonite. As to the interaction with the odorous substances of fermentative origin, all volatile substance determined have been gathered in three homogeneous families including respectively the compounds with fruity/floral characteristics, vegetal/herbaceous and sweet/juicy notes. Therefore the wines object of the experiment were subdivided according to these characteristics: wine 2, the youngest Chardonnay, is a product with freshness notes of the scents; wine 1, more structured, besides a smaller fraction of fruity and herb aromas, presents a more accentuated juicy and sweet note. Examining the percent reduction in comparison with the sample, it can be observed

TABLE 1 – PERCENT REDUCTION CAUSED BY TOP GRAN DC ON THE HERB – VEGETAL NOTES TOWARDS THE CONTENT OF THE SAMPLE

Dosage Top Gran DC	Wine 1	Wine 2
20 g/hl	-26	-65
50 g/hl	-49	-67
100 g/hl	-51	-70

TABLE 2 – PERCENT REDUCTION CAUSED BY BENTONITES AT THE DOSAGE OF 100 G/HL ON THE JUICY AND SWEET NOTES TOWARDS THE CONTENT OF THE SAMPLE

Type of bentonite	Wine 1	Wine 2
Experimental clay	-62	-87
Superbenton	-32	-80
Top Gran DC	-37	-73

that the removal of the herbaceous/vegetal notes (table 1) is more contained through Top Gran DC bentonite in both wines, even if used at low dosage. As for the volatile compounds contributing to the characteristics of juiciness and sweetness of wine (table 2), the reduction to the dose of 100 g/hl gives way to very varied progresses in both products and it is more accentuated in wine 2. The volatile substances with fruity/floral character (table 2) are removed in different percentage according to the type of wine, of bentonite and of the dosage used. In particular, in wine 2, the experimental preparation produces a reduction always lower at all dosages, while in wine 1 Superbenton results to be the less invasive. For those clays, a removal of small quantity at the dosage of 20 g/hl, reaching 50% at 100 g/hl, is observed in both wines. The results of the first year experimentation showed that the "wine matrix" affects the effectiveness and the affinity of bentonite. For instance: in case of a product rich in macromolecules deriving from the yeasts, the deproteinising effect of the clays is less important, probably because the colloidal system reduces their effectiveness and because par of the macromolecules can have the same charge of the adjuvant. The less invasive effect on the wine with fuller body than the younger wine concerns also the aromatic component; in fact, reductions that can be compared with those obtained on the younger product are obtained with higher dosages of bentonite. Besides it can be observed that the experimental bentonite is more effective on the more complex wine, while Top Gran DC seems to better adapt to the younger wine. As to the behaviour towards the volatile fraction of fermentative origin of the wine, it was proved that the removal is not non-specific, yet it differs depending on the family of molecules involved and the type of bentonite used. This fact, according

TABLE 3 – PERCENT REDUCTION DONE BY EXPERIMENTAL CLAY AND SUPERBENTON ON THE FRUITY – FLORAL NOTES TOWARDS THE CONTENT OF THE SAMPL

Dosage	Experimental clay on Wine 2	Superbenton on Wine 1
20 g/hl	-20	-9
50 g/hl	-34	-36
100 g/hl	-45	-49

to the type of product, can promote an improvement of the pleasantness and fineness of the bouquet when, for instance, the herbaceous and juicy notes are mitigated and there is a moderate reduction of the floral and fruity notes.

Who is who

The company was established in Milan by Mr Gildo Dal Cin, in September 1949, first to deal with research and industrial manufacturing of wine chemicals. In 1954 they moved to Sesto San Giovanni and in 1957 Dal Cin opened their present plant. In the Sixties, they opened mines of kieselgur in Tuscany and bentonite in the isle of Ponza, recording patents about use of perlite and bentonite in many industrial processes. In 1979 the new plant of Foggia (Southern Italy) was completed, later enlarged in 1986 and 2001. Today the main production of Dal Cin (bentonites, filter media, pesticides) takes place here. In the Nineties Dal Cin developed a multinational group of companies (in the UE and Australia), and joint-ventures with Italian and international partners. The company boasts today 35.000 m² of total industrial area (half is roofed), with two manufacturing plants, in Milan and Foggia, with 12.000 of tons sold every year. In Italy, five branches and many warehouses assure the best distribution of goods, while abroad a network of sole agents and retailers works in five Continents. Dal Cin is today a multi-business group of companies successfully dealing with chemicals applied to agriculture and industry, with particular attention to food & beverage field: in the wine world, Dal Cin were the very first in Italy to deal with research and manufacturing of a wide range of wine-making specialities, deeply marking the history of oenology: from the use of bentonite for fining purposes (1949) to meta-tartaric acid for wine stabilization (1956), to "physical activators" of alcoholic fermentation (1985). Dal Cin also means filtration, thanks to a manufacturing experience of more than forty years about precoat media and filter sheets, successfully used in many different fields. As far as bentonite is concerned, Dal Cin have a unique experience that has made the company leader in beverage fining (wine, juices, etc.), waste water treatment, animal feeding, ceramics, pharmaceutical and cosmetic industries.

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