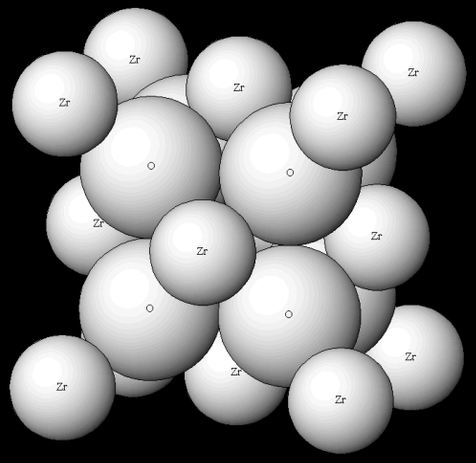
This thesis presents a novel industrial approach for protein stabilizing in white wine by a continuous process of packed column adsorption using zirconium oxide as the adsorbent material.
The author also wishes to add that the reader can find information on the importance of the protein in wine and how to avoid the protein haze occurring after bottling.
The effects on the total polyphenols and proteins contents, physicochemical properties, phenolic profile, protein fractions and sensory analysis are compared in the wine when zirconia and bentonite are used as fining agents.
Some techniques are described that are associated with characterization of porous materials. BET surface area, FTIR, X-ray diffraction, SEM and z-potential, which have been used to study the structure, morphology and surfaces properties of zirconia.
From the results obtained in this study we can predict promises applications of this new method on an industrial scale to stabilize white and sparkling base wines as well as wine filtration improvement comprising the new continuous process with a crossflow microfiltration.

White wine continuous protein stabilisation: industrial viability

Fernando N. Salazar G.

White wine continuous protein stabilisation

Industrial viability



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ISBN: 978-84-691-2703-2/DL.T.388-2008

Department of Chemical Engineering

Universitat Rovira i Virgili

Spain

WHITE WINE CONTINUOUS PROTEIN STABILISATION

Industrial viability

Thesis submitted by

FERNANDO NOÉ SALAZAR GONZÁLEZ

To obtain the degree of

Doctor from Universitat of Rovira I Virgili

Tarragona, December 2007

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ISBN: 978-84-691-2703-2/DL.T.388-2008

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Certify: That the doctoral thesis entitled "White wine protein stabilization: Industrial viability" by Fernando Noé Salazar González to obtain the degree of doctor from Universitat i Virgili has been carried out under my supervision at the Department of Chemical Engineering of the Universitat Rovira i Virgili.

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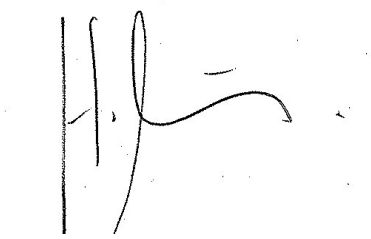

Dr. Francisco López Bonillo

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Acknowledgements

I would like to sincerely express my gratitude and appreciation to my supervisor Dr. Francisco López for giving me the valuable opportunity to work with him during four successful years. I thank Francisco for understanding me and for believing in me and most of all giving me his whole trust during the most critical and complicated periods. Thank you to all members of Food Technology Research Group (GITA) for their friendship, enthusiasm, encouragement, advice and assistance during my doctoral studies.

I would also like to thank the Alban Programme (European Union Programme of High Level Scholarships for Latin America, Nº LX-3769-80-DKPXAHWUYT) for the PhD scholarship during the three first years, the AGAUR programme (Agencia de gestió d'Ajuts Universitari i de Recerca, Beques per a estades per a la recerca fora de Catalunya BE 2005, Nº 2005 BE 00198) for the financial support for my research stay in France. My thanks to the Universitat Rovira i Virgili for the scholarship that enable me to finish my doctoral studies (1355 U07 E20 N-2005SGR-01066) and the financial supports received from the Spanish Agency for International Cooperation by the projects AECI A/0212/03 and AECI A/4726706 and from the Spanish Ministry of Education and Science by the Project N° AGL2006-07034/ALI.

I would like to express my appreciation to Dr. Vladan Milisic and Dr. Martine Mietton-Peuchot for their appreciated support and cooperation during my research stay in France at the Faculté d'Oenologie of the Université Victor Segalen Bordeaux 2. Furthermore, I would like to thank Dr. Elizabeth Waters and Dr. Kenneth Pocock for teaching me a lot of things about wine protein and

ISBN: 978-84-691-2703-2/DL.T.388-2008

for their lovely support during my stay in Australia as visiting researcher at the Australian Wine Research Institute.

I would also like to say thanks to all those people who made this wonderful dream a reality.

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Thesis summary

Heat-unstable soluble proteins in grapes, grape juices and wines may become insoluble and precipitate causing the formation of undesirable hazes or deposits in white wines after bottling and during storage.

Proteins are commonly prevented from forming hazes with bentonite, though this technique does have drawbacks: for example, the sensory properties of the wine are affected adversely because flavour compounds are removed and wine volume is lost as lees because of the swell and settling of the bentonite. In addition, the handling and disposal of spent bentonite continues to be a concern, because it involves high labour input and the associated costs, occupational health and safety issues, and the wine industry's environmental responsibilities and legislative requirements. It is estimated that the cost of bentonite fining to the wine industry worldwide is in the order of US$300-500 m per annum. Alternative fining technologies to bentonite, which are economically viable and maintain wine quality, are currently being sought. However, no successful techniques have been developed to date: all the attempts so far have either affected the quality of the wine or not been economically viable under standard winemaking conditions. Therefore, research on the implementation of new practices that have a less negative impact on the environment and are economically viable is particularly challenging.

For this reason the aim of this thesis was to study the industrial viability of an alternative technology to bentonite fining which enables unstable proteins to be removed from white wines using zirconia as the adsorbent material. We also attempted to develop a continuous process, which stabilizes wine protein

ISBN: 978-84-691-2703-2/DL.T.388-2008

without having any negative effects on the physicochemical and sensory properties of the wine. Likewise we tried to make the process have a lower environmental impact by testing various regenerative treatments of the adsorbent material.

First we studied the structure, morphology and surface properties of the zirconia and its capacity to remove unstable proteins from white wine versus thermal and chemical regeneration treatments. Subsequently we compared the physicochemical and sensory properties of a white wine fined by zirconia and bentonite.

To further our understanding of the effect on membrane fouling and wine protein stability, we developed a hybrid process consisting of in-column adsorption with crossflow microfiltration. We also applied this new method to stabilize the base sparkling wine and compared the results with the conventional method of using bentonite as the fining agent to see the effects of the treatments on the foam quality and protein fractions. Finally, we applied the new method on an industrial scale by packing zirconia into a fixed bed column and by using the batch and continuous systems.

The results show that the zirconia can be regenerated by thermal and chemical treatments, and that its physical, morphological and chemical properties are not altered. In fact its protein adsorption capacity can increase probably because some compounds or active centres derived from wine proteins are absorbed.

The hybrid process was used to increase the permeate flux during crossflow microfiltration and stabilize wine proteins. We observed that proteins were reduced when the zirconia column adsorption was used during the crossflow microfiltration. Therefore both processes may act together.

By comparing the physicochemical and sensory analyses of white wine proteins stabilized by zirconia and bentonite, we found that results were best when zirconia was used.

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The continuous protein stabilization of white wines by zirconia may also be useful for stabilizing proteins in base sparkling wines. Treating base sparkling wines with zirconia definitely gives better foam quality than with bentonite.

Finally the results obtained in our industrial scale experiment showed that white wine continuous protein stabilization with zirconia as the adsorbent material is not only viable in both the continuous and batch systems, it also leaves the quality of the wine unchanged.

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Resumen

Las proteínas térmicamente inestables que están presentes en uvas, jugos de uva y vinos podrían llegar a ser insolubles y precipitar causando la formación de turbidez o precipitados indeseables en vinos blancos después del embotellado durante el almacenamiento.

La turbidez proteica en vinos blancos es evitada tradicionalmente, mediante la adición de bentonita, aunque esta técnica presenta algunas desventajas tales como efectos negativos sobre las propiedades sensoriales del vino debido principalmente a la remoción de componentes aromáticos o gustativos y por la merma de vino, debido al gran aumento de volumen y poder de sedimentación de la bentonita. Además, el recurso humano, los tiempos de proceso y la descarga de residuos al ambiente sigue siendo una inmensa preocupación, debido a los significativos costos asociados a la salud y seguridad laboral, y así como también las responsabilidades y obligaciones legales de la industria en material de impacto ambiental. Es estimado que los costos del uso de la bentonita en la industria del vino a nivel mundial son del orden de los 300-500 millones de dólares por año. Por lo tanto es necesario el desarrollo de nuevas tecnologías alternativas a la bentonita que sean económicamente viables y que mantengan la calidad del vino, así como también la generación de un menor impacto ambiental. No obstante, nuevas técnicas exitosas a nivel industrial aun no han sido desarrolladas, por que afectan la calidad del vino o porque su aplicación no es viable económicamente bajo normales condiciones de operación de producción de vino. Por lo tanto, es muy atractivo investigar la viabilidad de nuevas prácticas que tengan un menor impacto sobre el ambiente y sean económicamente viables.

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Por esa razón la principal motivación de nuestra investigación ha sido estudiar la viabilidad industrial de una tecnología alternativa al uso de la bentonita, la cual permita remover proteínas inestables de los vinos blancos usando zirconia como material adsorbente. Además, nosotros nos hemos concentrado en el desarrollo de un proceso continuo que permita conseguir vinos estables proteicamente sin afectar sus propiedades fisicoquímicas y sensoriales, probando diferentes técnicas de regeneración del material adsorbente.

Primeramente nosotros hemos estudiado la estructura, morfología y propiedades superficiales de la zirconia, así como también su capacidad de adsorción para remover proteínas inestables de vinos blancos, aplicando tratamientos regenerativos químicos y térmicos. Después hemos comparado las propiedades fisicoquímicas y sensoriales de un vino blanco estabilizado proteicamente mediante zirconia y bentonita.

Además, nosotros hemos desarrollado un proceso híbrido integrando un proceso de adsorción en columna y un proceso de microfiltración tangencial de vino, para conocer los efectos de este nuevo proceso sobre el ensuciamiento de la membrana y la estabilidad proteica del vino.

Por otro lado, nosotros también hemos aplicado un nuevo proceso de estabilización proteica de vino base para cava, comparando los resultados con el método tradicional usando bentonita como agente estabilizante y observando los efectos de ambos tratamientos sobre la calidad de la espuma y las fracciones proteicas del vino. Finalmente, hemos aplicado el nuevo proceso de estabilización proteica a escala industrial, empacando zirconia sobre una columna fija y realizando el proceso mediante sistema continuo y discontinuo.

Los resultados demuestran que la zirconia puede ser regenerada química o térmicamente, que sus propiedades físicas, morfológicas y químicas no son alteradas y que incluso su capacidad de adsorción proteica podría ser aumentada

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probablemente producto de la adsorción de algunos componentes o centros activos derivados de las proteínas del vino.

A través del proceso híbrido ha sido posible conseguir vinos estables proteicamente y aumentar la densidad de flujo del permeado durante la microfiltración del vino. De hecho, hemos observado que la reducción de proteínas mediante la adsorción en columna usando zirconia también ocurre durante la microfiltración tangencial, por lo tanto ambos procesos pueden actuar conjuntamente en la reducción y estabilización proteica.

Por otro lado, comparando las propiedades fisicoquímicas y sensoriales de vinos blancos estabilizados proteicamente mediante zirconia y bentonita, podemos afirmar que los mejores resultados son conseguidos usando zirconia como agente estabilizante.

La estabilización proteica de vinos blancos en continuo también puede ser útil para estabilizar vinos base para cava, ya que comparando la calidad de la espuma de un vino base para cava tratado con zirconia y bentonita, los resultados demuestran que la calidad de la espuma de aquellos vinos bases es mejor usando zirconia, ya que la adicción de bentonita produce considerables efectos negativos sobe la calidad de la espuma.

Finalmente, los resultados obtenidos a escala industrial, muestran que es viable la estabilización proteica de vinos blancos usando zirconia como material adsorbente ya sea mediante un sistema continuo o discontinuo sin afectar la calidad del vino inicial.

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Chapter 1

INTRODUCTION

1.1 Introduction

Heat-unstable soluble proteins present in grapes, grape juices and wines may become insoluble and precipitate causing the formation of undesirable hazes or deposits in white wines after bottling during the storage or when different white or rosé wines are blended. This protein haze may be intrinsically or extrinsically induced by changes, such as pH, storage temperature and the amount of polyphenols (Siebert, 1996; Sarmento et al., 2000; Mesquita et al., 2001) and is almost certainly due to slow denaturation of the heat-unstable wine proteins (Tattersall et al., 2001).

The haze-forming proteins commonly is prevented using bentonite, though this technique present some drawbacks such as adverse effect on the sensory properties of wine due to the removal of flavour compounds and loss of wine volume as lees due to greatly swell and settling of the bentonite (Miller et al., 1985; Rankine 1987; Tattersall et al., 2001). Moreover the bentonite fining is laborious spending long work time and presenting waste disposal problem (Høj et al., 2000). Therefore it is very important research new fining technologies alternatives to solve this “old” problem of white wine instability (Waters et al., 2005). There are several studies on the reduction of unstable protein from wine such as the application of ultrafiltration (Hsu & Heatherbell, 1987b; Peri et al., 1988; Flores et al., 1990), used of proteolytic enzymes (Lagace & Bisson, 1990) and flash pasteurization (Hsu & Heatherbell, 1987a). However, news successful

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techniques have not been development to date, since the wine quality is affected or because it is not viable economically its application under standard winemaking conditions. Therefore, it is very exciting to research on the implementing of new practices that have less negative impact on the environment and economically viable (Waters et al., 2005).

The research described in this thesis aims to study a new technique alternative to bentonite, which allow the removal of the unstable proteins from white wines selectively by a continuous process whilst with less environmental impact.

1.2 What are the proteins?

Proteins also called polypeptides are large molecules with molecular weight above 10 kDa. Proteins are complex structures defined as linear polymers of amino acids linked by peptides bonds. Proteins are constituted basically for a set of 5 atoms; carbon (C), hydrogen (H), oxygen (O), nitrogen (N) and sulfur (S). The main subunits of a protein are amino acids. An amino acid consist of a central carbon atom (C_α) and an amino group (NH_2), a hydrogen atom (H), a carboxyl group (COOH) and a side chain (R) which are bound to the α-Carbon atom (see Figure. 1.1). In nature, there exist twenty different types of amino acids. According to the structure of the side chain, the amino acid can be grouped into apolar, polar, uncharged, or charged side chains. Proteins are essential structural and functional macromolecules of all living organism.

The proteins function as catalysts, transport and storage of other molecules such as oxygen, mechanical support and immune protection, also generate movement, transmit nerve impulse and control the growth and differentiation. Four levels of structure are usually distinguished: the primary, secondary, tertiary and for some proteins also quaternary structure. The primary structure is

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the sequence of the amino acids in the polypeptide chain. The secondary structure is the bending and hydrogen bonding of a protein backbone to form repeating patterns. The elements of the secondary structure are subdivided into α-helices and β-sheets (see Figure 1.2). The way in which twist or bends of whole polypeptide are folded together is called the tertiary structure (see Figure 1.2).

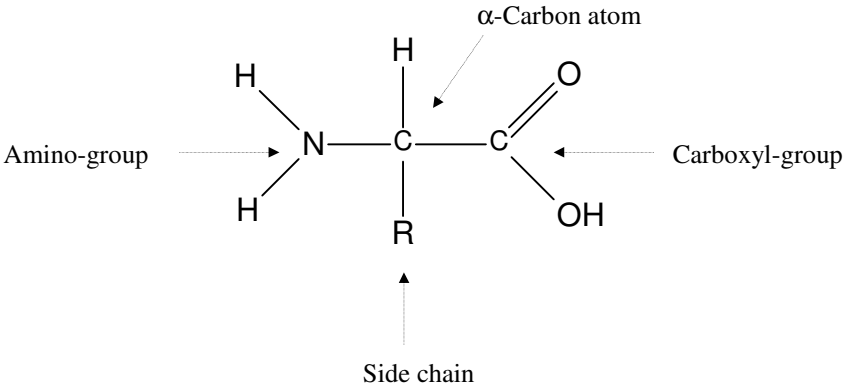

Figure 1.1 Basic unit of a protein conformed for an amino acid consisting of amino group (NH_2), carboxyl group (COOH) and the side chain (R).

The association of proteins by non-covalent bonds in order to form larger units is termed quaternary structure. Many globular proteins ('monomers') form dimmers, trimers or larger aggregates. Secondary, tertiary and quaternary structures together determine the conformation of the protein. The proteins in general are amphoteric molecules, since they contain both acidic and basic moieties, and amphiphilic molecules, since contain also both hydrophilic and hydrophobic moieties. Hydrophilic amino acid side groups reside predominantly at the surface, and hydrophobic ones in the core of the structure. This chemical particularity allows that the proteins can be absorbed on wide range of different surfaces (Martin, 2003; Engel, 2004; Rezwan, 2005).

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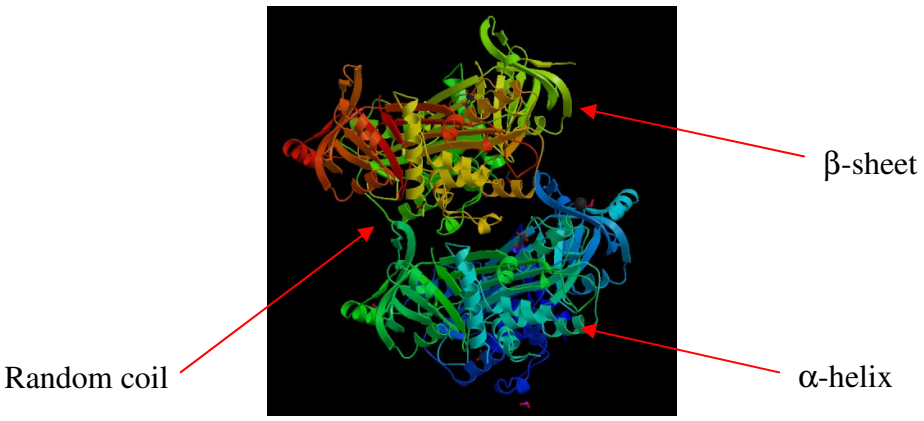

Figure1.2 Crystal structure of uncleaved chicken egg albumin at 1.95 Å resolution (Stein et al., 1991).

Wine proteins have long been considered as a mixture of grape proteins and proteins from autolyzed yeast and are typically present in low concentration in wines. Although proteins are the minor components of nitrogen fraction in must and wines, they play a extremely important role in different operations carried out in wineries (filtration, clarification and cold stabilization) and in different aspects concerning the wine's stability (Moreno-Arribas et al., 2002; Ferreira et al., 2002).

1.3 Haze in white wines

An important aspect of quality in white wines is its clarity, because it is a visual attribute valued for the consumer. The measurement of clarity is related with the turbidity, which corresponding to optical phenomenon known as the Tyndall effect, caused by the presence of particles in suspension that deflect light from its normal path (Ribéreau-Gayon et al., 2000).

It is possible to find different types of hazes in bottled wine, which can have microbiological or chemical origin. The haze induced by microorganism can

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occur due to the action of spoilage yeast (*Zygosaccharomyces* and *Brettanomyces*) and bacteria (lactic acid bacteria, acetic acid bacteria and *Bacillus* species), causing off-flavours and changes in the wine quality (Rankine & Pilone, 1973; Van de Waters, 1985). The haze induced by chemical factors can occur by crystallization of metal salts (iron and copper), including potassium bitartrate (Dunsford & Boulton, 1981; Rodriguez-Clemente et al., 1990; Lubbers et al., 1994) and calcium tartrate (Clark et al., 1988, Mínguez & Hernández, 1998). Polysaccharides and polyphenols are also involved in the induced-haziness, producing color changes and precipitates (Somers & Ziemelis, 1985; Siebert et al., 1996; Waters et al., 1996; Pellerin et al., 1994). However, the most abundant haze problem in white wine is still due to precipitation of unstable protein in bottle (Waters et al., 2005).

Figure 1.3 Example of protein haze formation in white wine. On the left side a white wine protein unstable and on the right side a white wine clarified and stabilized.

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1.4 Heat-unstable white wine proteins

An early investigation on wine protein carried out by Berg & Akioshi (1961) showed that there is not correlation between the total protein concentration of must and the turbidity formed by heating as result of protein instability. Later Bayly & Berg (1967) supported this assertion saying that proteins in must are not all equally heat-sensitive.

At the beginning several studies on wine protein concluded that protein fractions of low isoelectric point are responsible of haze formation in wines (Moretti & Berg, 1965; Bayly & Berg, 1967). Later other studies has supported these works suggested that within the low isoelectric point fraction of proteins, those with low molecular weight, contributed most to heat instability and theses unstable protein fractions also are presents in white wine haze (Hsu & Heatherbell, 1987a). Hsu and Heatherbell (1987a, 1987b) by electrophoresis on polyacrylamide gel isolated heat-unstable protein from Gewürztraminer and Riesling grapes with molecular weight between 12.5 and 30 kDa, and isoelectric points ranging from 4.1 to 5.8. Paetzold et al. (1990) obtained similar results from Sauvignon Blanc must by fractionating proteins between 13 and 67 kDa, and isoelectric points ranging from 4 to over 7, though most of the proteins separated by this way have molecular weight between 20 and 30 kDa. Waters et al. (1991, 1992) showed that heat-unstable proteins in Muscat of Alexandria wines presents a molecular weight between 24 and 32 kDa. Nevertheless some wine proteins give more haze than others, and these are really the low molecular weight proteins and low isoelectric point (Waters et al., 1991; Waters et al., 1992).

Overall proteins responsible for instability in white wine come exclusively from grapes and have relatively low molecular weight between 12 and 35 kDa,

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though according to the grape variety, can differ the type of proteins, as well as isoelectric point, degree of glycosylation and even its heat sensitivity.

Recent studies have reported that haze-forming proteins of white wines are unstable in long term and very stable in short to medium term, and resistant to proteolysis under conventional winemaking conditions (Feuillat & Ferrari, 1982; Waters et al., 1992; Waters et al., 1995). These heat-unstable proteins have been identified as pathogenesis-related (PRs) proteins, specifically thaumatin-like proteins and chitinases presents in grape berry of *Vitis vinifera* (Waters et al., 1996; Waters et al., 1998; Pocock et al., 2000; Høj et al., 2000, Ferreira et al., 2004)

1.4.1 Pathogenesis-related proteins

Pathogenesis-related proteins are now widely regarded as a rich source of allergens. The PRs are defined as proteins that are encoded by the plant genome and induced specifically in response to infections by pathogens such as fungi, bacteria, or viruses, or by adverse environmental factors (Stintzi et al., 1993; Edreva, 2005; Kiba et al., 2007).

Pathogenesis-related proteins do not constitute a super-family of proteins but represent a collection of unrelated protein families which function as part of the plant defence system. Today, PRs are classified into 14 families. Many important plant food allergens are homologues to proteins that are members of PR families (Van Loon & Van Strien, 1999; Hoffmann-Sommergruber, 2002). The family 5 of PRs comprises unique proteins with diverse functions. Because of the sequence homologies between PR-5 proteins and thaumatin, an intensely sweet tasting protein isolated from the fruits of the West African rain forest shrub *Thaumatococcus daniellii*, members of this family of proteins are referred to as thaumatin-like proteins (TLPs). TLPs can be classified into three groups,

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(i) those produced in response to pathogen infection, (ii) those produced in response to osmotic stress, also called osmotins, and (iii) antifungal proteins present in cereal seeds. The TLPs are generally resistant to proteases and pH- or heat-induced denaturation (Breiteneder, 2004).

In grapevines, the synthesis of the PR proteins occurs predominantly in the skins of grapes and is regulated in a developmental and tissue-specific manner (Monteiro et al., 2001; Waters et al., 2005,).Thaumatin-like proteins and chitinases, which are pathogenesis-related proteins are the major soluble protein components of grapes from *Vitis vinifera* (Pocock et al., 2000; Hayasaka, et al., 2001) and whilst the major wine haze forming proteins (Waters et al., 1996; Waters et al., 1998; Tattersall et al. 2001). The pathogenesis-related proteins are presents in infected grapes and are also produced rapidly in grapes damaged during harvesting (Pocock & Waters, 1998; Pocock et al., 1998; Høj et al., 2000).

1.5 Protein fining treatments

The mechanisms of fining wine are based on the charge cancellation between the suspended particles and fining agent particles, allowing the colloid suspension to agglomerate and flocculate by gravity. Other mechanism can be the absorption of the suspended particles on the surface of the fining agent.

Commonly the fining agents used in the winemaking are proteins of animal origin such egg albumin, blood albumin, casein, isinglass and gelatins. However, some fining agents as bentonite and siliceous earth are also used in process of clarification and stabilization of the wines. For example, the activated carbon can be used to reduce colour intensity and to remove bad odours from wine. Particularly the activated carbon in white wine, it reduces the

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oxidized brown or yellow colour, or the pink colour in "blanc de noir" wine (Ribéreau-Gayon et al., 2000).

1.5.1 Bentonite fining

The bentonite is still universally employed in the wine industry to prevent the protein haze-forming in white wines. This material is montmorillonite clay, which contains exchangeable cations. The adsorption of wine proteins onto the bentonite is due to the cationic exchange capacity of this clay and dependent on the amount of displacement of aluminium ions by sodium, calcium, or magnesium ions. Wine proteins are positively charged at wine pH, and thus can be exchanged onto bentonite, which carries a net negative (Blade & Boulton, 1998; Ferreira et al., 2000). The sodium bentonites present a more effective protein adsorption than calcium bentonites (Blade & Boulton, 1988).

Some studies reported that the bentonite fining of white wines had no effect on the wine aroma and palate (Leske et al., 1995; Pocock et al., 2003). In contract other study showed a significant reduction of aromas to juice, must, and wine (Miller et al., 1985). Other studies on bentonite fining in sparkling wines demonstrated a less of sensory and foamability quality (Martinez-Rodriguez & Polo, 2003; Vanrell et al., 2007). However, it is absolutely sure that an excessive bentonite adding can cause a negative effect on wine and flavour.

A commercial process for bentonite regeneration does not currently exist, and thus bentonite is only used once before being discharged. Therefore this is still a great environmental problem (Waters et al., 2005).

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1.5.2 Alternatives techniques or other fining agents

Several alternative techniques to bentonite fining as well as the use of others fining agents to reduce the removal of haze-forming wine proteins have been studied. Some alternative techniques studied have been ultrafiltration (Hsu et al., 1987; Flores, et al., 1990), addition of proteolytic enzymes (Feuillat & Ferrari et al., 1982; Waters et al., 1992), flash pasteurization (Francis et al., 1994; Pocock et al., 2003) and use of others adsorbents materials such as resin (Sarmento et al., 2000) and metal oxides (Pashova et al., 2004a; Pashova et al., 2004b; Salazar et al., 2006; Salazar et al., 2007).

The addition of polysaccharides such as mannoproteins also has been used as alternative to reduce the protein haze in white wine (Waters et al., 1991; Waters et al., 1994a; Waters et al., 1994b) and even polysaccharides extracted from seaweeds (Cabello-Pasini et al., 2005) as well the use of immobilized phenolic compound such as proanthocyanidins (Weetall et al., 1984; Powers et al., 1988).

The results obtained to date have not been successful on industrial scale and thus the bentonite is still the only commercially acceptable practical solution to avoid protein haze in bottled white wine (Waters et al., 2005).

1.6 Scientific and technological objectives

The main objective of this thesis has been to study the industrial viability of a technology alternative to bentonite fining which allows remove unstable protein from white wines using zirconia as adsorbent material by a continuous process in packed column adsorption.

Firstly we have studied the structural, morphology and surface properties of the zirconia and its adsorption capacity to remove unstable proteins from white

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wine against thermal and chemical regeneration treatments. Afterwards we have compared the psychochemical and sensory properties of a white wine fined by zirconia and bentonite.

Furthermore, we have integrated this new method of protein stabilization with a crossflow microfiltration process to know its effect on the membrane fouling and wine protein stability.

On other hand we also have compared the foam parameters and protein fractions of a base sparkling wine fined by zirconia and bentonite. And finally, we have applied this new method on industrial scale using zirconia as fining agent by means batch and continuous system.

1.7 Innovation & novelty of the project

The zirconia is a ceramic material widely studied as catalyst, porous and support material in membranes technology and liquid chromatography from many years ago. However its use as adsorbent material in the food industry has still not been fully explored. Its innocuousness, mechanical and chemical resistance are a support for its industrial use.

The zirconia can be packed in column and regenerated due to physical, chemical and mechanical resistances. These facts are the most important advantages on the traditional process of white wine protein stabilization applied currently by bentonite.

Besides, the application of a new hybrid process comprising column adsorption and crossflow microfiltration could allow us increase the permeate flux whilst the wine protein stability. Therefore, the white wine protein stabilization could be as result of the protein reduction achieved by adsorption in column and the

ISBN: 978-84-691-2703-2/DL.T.388-2008

crossflow microfiltration. Furthermore, this research has allowed improve the knowledge about the effect of the protein and polyphenol content on the fouling or the permeate flux decline. On the other hand, nowadays in the winemaking there are a great accumulation of wastes derivate mainly from wine clarification stage due the use of bentonite and diatomaceous earth. Therefore the application of this new hybrid process might be a constructive technology alternative.

Finally, studying the properties of the zirconia and its application as fining agent for the removal of unstable protein present in base sparkling wines, could release a promises alternative for improving the foam quality of sparkling wines fined currently by bentonite.

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UNIVERSITAT ROVIRA I VIRGILI
WHITE WINE CONTINUOUS PROTEIN STABILISATION: INDUSTRIAL VIABLITY
Fernando Noé Salazar González
ISBN: 978-84-691-2703-2/DL.T.388-2008

Chapter 2

CHARACTERIZATION OF ZIRCONIA AS AN ABSORBENT MATERIAL FOR STABILIZING UNSTABLE PROTEINS IN WHITE WINE

2.1 Abstract

The unstable proteins present in white wine can cause haze and deposit in the bottle during storage. This problem is often avoided by using bentonite fining during the winemaking, even though it can cause changes in wine quality.

For this reason, in the last eight years we have been using zirconia to remove the unstable proteins from white wine by means of a continuous adsorption process in a packed column. The results show that the wine quality is not affected. However, we still do not fully understand the properties and the protein adsorption capacity of this material. In the present paper, we study the characterization of the zirconia when it is used as an adsorbent material for stabilizing unstable proteins in white wine.

The zirconia was characterized by a variety of techniques (BET surface area, FTIR, XRD, ESEM and ζ-potential) in order to determine its structure, morphology and surface properties when it is used as an absorbent material for removing unstable proteins present in white wine. The adsorption process was performed by the batch system and the adsorbent material was regenerated by means of thermal and chemical treatments.

The structural and morphological properties of the zirconia were unaltered by the regenerative treatments. Nevertheless, its protein adsorption capacity

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improved which may be related to changes in its functional groups or surface charge. These last results were shown by FTIR spectra and the ζ-potential behavior at different pHs.

Keywords: Zirconia; wine unstable proteins; adsorbent material; surface properties.

2.2 Introduction

The zirconia is a metal oxide of special interest due to their surface properties, which allow it to be employed commonly as catalyst or support material (Mallick et al., 2006; Stichert & Schuth, 1998). Zirconia has also been widely used as refractory material, ceramic material and even as biomechanical support in medical implants due to their harmlessness, high hardness, low coefficient of friction, high elastic modulus, low thermal conductivity, chemical inertness and as well as for its thermal and mechanical resistances (Rovira-Bru et al., 2001; Sharma et al., 2002; Liu et al., 2005; Ray et al., 2006; Chevalier, 2006).

Pure zirconia can be crystallized in cubic, tetragonal or monoclinic structures. The monoclinic form of zirconium dioxide is usually found at room temperature, it undergoes a reversible martensitic phase transformation at 1473 K to a high temperature tetragonal phase (Garvie, 1978; Portinha et al., 2003) which remains unaltered by thermal treatment until 2673 K, whereupon it transforms to a cubic phase which is stable to the melting point at 2973 K (Chadwick et al., 2003). Even these properties can be modified by doping with other material or elements (Xie et al., 2000; Liu et al., 2005; Jia et al., 2006).

Also the zirconia is used in liquid phase chromatography as a stationary phase support due to its great mechanical stability, its resistance to high temperatures and its resistance to extreme pH. Moreover, this oxide has amphoteric

ISBN: 978-84-691-2703-2/DL.T.388-2008

hydroxide groups on its surface which could be used to generate electro-osmotic flow inside the capillary. Moreover, the acid-base features and support of different pores size to uphold their applications in protein separation by high performance liquid chromatography (Millot, 2003; Sarkar et al., 2005). In this case the protein adsorption mechanism is controlled meanly by surface charge and the pore size of the adsorbent material as well as electrostatic, van der Waals and solvation forces of the proteins (Novak et al., 1995; Roth et al., 1996; Griffith et al., 1997; Hoth & Rivera, 2005).

For the last eight years we have been using zirconia to remove the unstable proteins in white wine by means of a continuous adsorption process in a packed column. The results show that the wine quality is not affected (Pachova et al., 2002; Pashova et al., 2004a; Pashova et al., 2004b; Salazar et al., 2006; Salazar et al., 2007). However, we still do not fully understand the properties and the capacity for protein adsorption of this material. For this reason it is very interesting to make a detailed analysis of the properties of zirconia in order to evaluate how its morphology, structure and functional properties can be affected by it being used as an adsorbent material and regenerated by thermal or chemical treatments.

2.3 Materials and methods

2.3.1 Wine protein adsorption and regeneration treatments

Malvasia wine with an initial protein content of 31 mg/L (vintage 2006, Tarragona, Spain) was treated in a batch adsorption system using zirconia in powder and pellet form (Saint-Gobain, Ohio, USA). A total of 20 experiments were performed using twenty 500-mL bottles, which contained 5 g of zirconia and 200 mL of wine (ten bottles with zirconia in pellets and ten bottles with

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zirconia in powder form). All the bottles were placed on a shake plate at room temperature. The adsorption process was carried out until to get the equilibrium. Each 12 or 24 hours were taken aliquots of wine to protein analysis and once finalized the process approximately 0.5 g of zirconia dirty was taken to storage at -20 ºC to analyze later. The protein content was determined by Bradford's method and the protein stability according to heat test at 80 ºC for 2 h and at 4 ºC for 2 h, with $\Delta NTU < 2$ taken to mean stability. The first six samples were regenerated at 300, 400 and 500 ºC for 6 and 12 h. Sample 7 was regenerated by 2% NaOH at 50 ºC for 2 hours and 0.7% HNO_3 at 90 ºC for 2 hours and finally washed with Milli-Q water until neutralization. Sample 8 was regenerated by 4% NaOH and 1.4% HNO_3. Samples 9 and 10 were treated in the same way as samples 7 and 8 but for 4 h. The same treatments were applied to zirconia in pellet and powder form.

2.3.2 BET surface area

Surface area and pore size distribution of the zirconia were determined by Brunauer-Emmett-Teller (BET) method by nitrogen adsorption and desorption isothermal at 77K in a Micromeritics ASAP 2000 surface analyzer, assuming a cross-sectional area of 0.162 nm^2 for nitrogen. Before the adsorption measurements were taken, the samples were outgassed under a vacuum of 0.001 mbar at 120 ºC.

2.3.3 Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded in order to identify the functional group present in the adsorbent material, using a spectrometer Bruker Equinox with a detector MCT-DRODTS. The spectrum of FTIR is to range of 400 a 4000 cm^{-1} with 4 cm^{-1} of resolution and 200 scanners for sample.

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2.3.4 Power X-ray diffraction (XRD)

XRD analyses were performed to investigate the morphology and crystalline structure of the zirconia. The XRD diffraction was conducted with a SIEMENS D5000 diffractometer using a nickel-filtered Cu $K\alpha_1$ radiation to produce X-rays with a wavelength of 1.5418 Å by a cooper tube operated at 45 kV and 30 mA. The angular 2θ diffraction range was from 10 to 70º at a scan rate of 4º min^{-1} with a 0.05º data interval. The crystallite size was determined by the Scherrer equation.

2.3.5 Environmental scanning electron microscopy (ESEM)

All the zirconia was crushed into powder and placed on a graphite adhesive fixed and analyzed by a FEI Quanta 600-ESEM under vacuum due to low conductivity of the zirconia. The identification of elements was achieved by a mapping procedure using cobalt spectrum as standard.

2.3.6 Determination of ζ-potential

The electrostatic surface charge of the zirconia was deduced from its electrophoretic mobility using a Malvern Zetasizer 3000 HAS (Malvern Instruments Inc., U.K.). The electrophoretic mobilities were converted into ζ-potential using Smoluchowski's equation and the software PCS V1.41 Malvern Instruments, 1992.

The ζ-potential measurements were carried out on dilute zirconia suspensions of 0.1 g/L concentration using Mili-Q water. The pH was adjusted in the ranges of 2 to 11 by titration with 0.1 M HCL and M 0.1 NaOH. Also was determined the ζ-potential and the particle size distribution of the wine. All the measurements were performed at a temperature of 25 ºC. The protein-zirconia suspensions

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were allowed to equilibrate for 12 hours prior to ζ-potential measurements being taken.

The electrophoretic mobilities were converted into ζ-potential internally by the software PCS V1.41 (Malvern Instruments, 1992) and according to Helmholtz-Smoluchowski equation:

$$\zeta = \frac{U \cdot \eta}{E \cdot \varepsilon_0 \cdot \varepsilon_r} \tag{2.1}$$

where ζ is the zeta potential, U is the electrophoretic velocity, η is the viscosity of the medium, E is the electric field strength, and ε_0 and ε_r are the permittivity of free space and the relative permittivity, respectively.

2.4 Results and discussions

The untreated wine with a total protein content of 31 mg/L, expressed as BSA was protein unstable according to heat test showing a turbidity difference of 9.1 NTU. After a first adsorption using zirconia in pellet and powder form without any previous treatment, the protein stability of all wines was improved, though the wines were not completely stable by the zirconia in pellet form. However, a second adsorption using zirconia used previously at the first adsorption and regenerated by thermal or chemical treatments allowed obtain wines protein stable with a ΔNTU < 2 to all cases.

During the first adsorption the adsorbed protein from wine at 72 h was around 60 and 70% with zirconia in pellet and powder form, respectively. However, during the second adsorption to some cases the protein adsorption from wine was higher than first one achieving even up to 70 and 80% with zirconia in pellet and powder form, respectively. The zirconia in pellet shows a less protein

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adsorption capacity which could be related with the effective contact area (see Figure 2.1.

Perhaps the regenerative treatments improved the protein adsorption capacity of the zirconia or some element or compound from wine changed the surface properties of the zirconia. Probably this fact could explain the improvement of the wine protein stability to some cases.

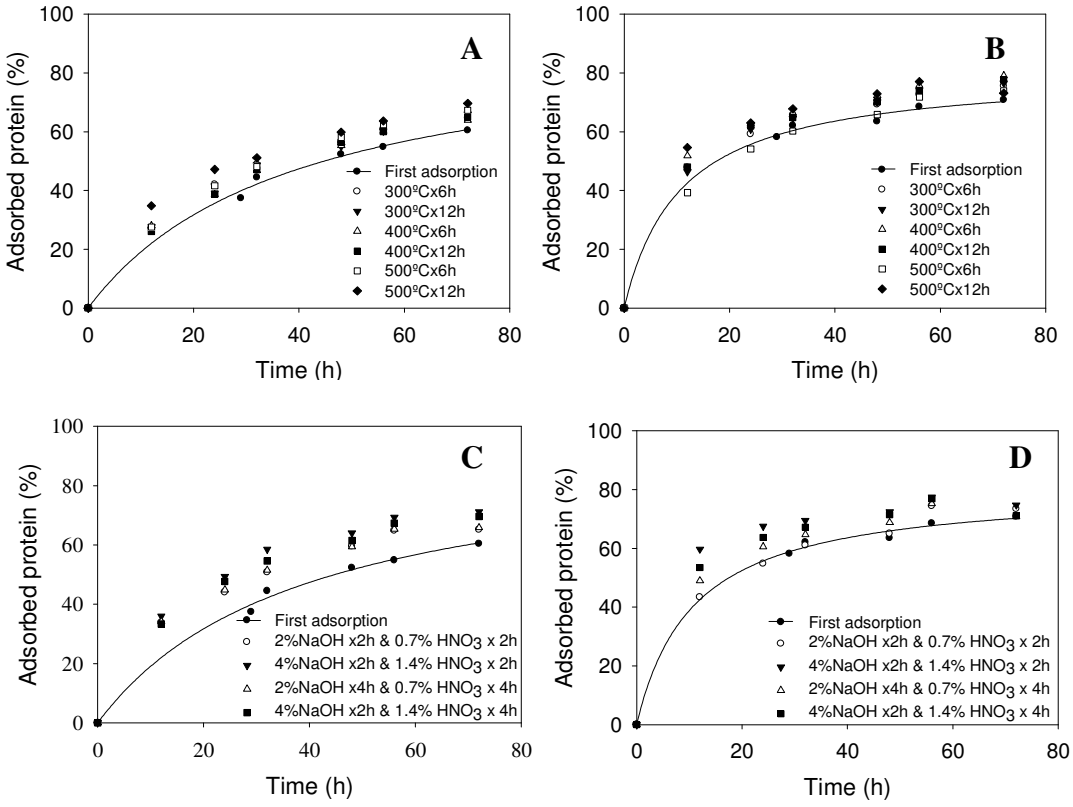

Figure 2.1 Protein adsorption capacity of pure and regenerated zirconia (A and B, thermal regeneration of zirconia in pellet and powder, respectively; C and D, chemical regeneration of zirconia in pellet and powder, respectively)

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The surface area and average pore size of the zirconia were not affected by regenerative treatments which were 159 ± 6 m^2/g and 46 ± 2 nm, respectively. The morphology and structure of the pure zirconia was not modified, because the XRD pattern shows characteristic tetragonal nanoparticles in all cases (Figure 2.2). The mean crystal size of the pure and treated zirconia was 5.6 ± 0.2 nm.

Table 2.1 Heat-protein stability of Malvasia wine treated with zirconia (some examples)

Treatments	Heat-protein stability (ΔNTU)			
	first adsorption [a]		second adsorption [b]	
	pellet	powder	pellet	powder
300 ºC x 12h	2.2	1.9	1.6	0.3
400 ºC x 12h	4.6	0.7	1.3	0.5
500 ºC x 12h	4.0	1.7	1.8	0.2
2% NaOH & 0.7% HNO_3 x 2 h	3.3	1.5	0.3	0.4
2% NaOH & 0.7% HNO_3 x 4 h	3.9	0.8	0.5	0.7
4% NaOH & 1.4% HNO_3 x 4 h	5.8	1.3	1.9	0.3

[a] zirconia without any previous treatment

[b] regenerated zirconia by thermal or chemical treatment.

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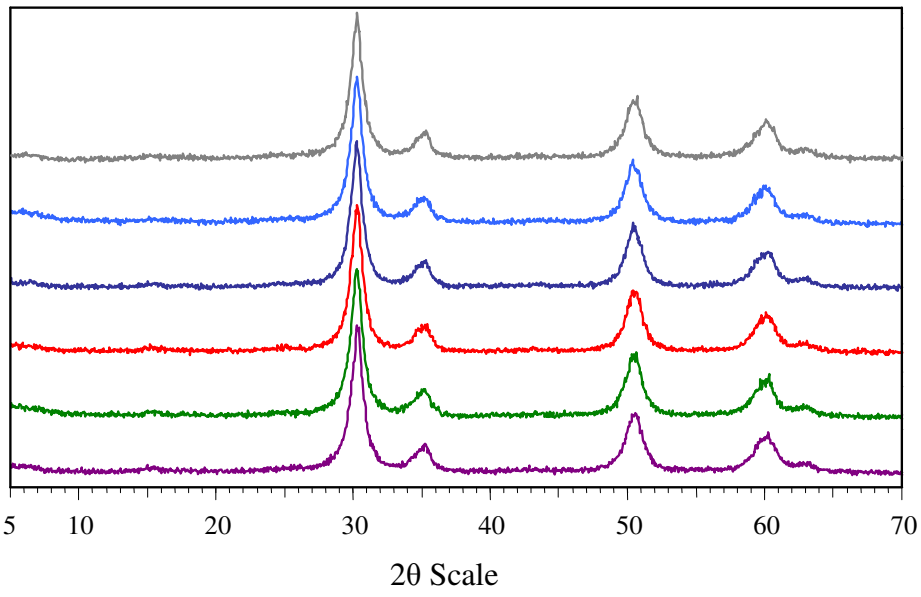

Figure 2.2 XRD patterns of pure and regenerated zirconia (—: pure zirconia; —: 500 ºC x 12 h without use; —: 300 ºC x 6 h; —: 500 ºC x 12 h; —: 2% NaOH x 2 h & 0.7% HNO_3 x 2 h; —: 4% NaOH x 4 h & 1.4% HNO_3 x 4 h)

The results obtained by FTIR show a new peak in the spectrum at around 1200-1000 cm^{-1}. This only occurs in the used and regenerated zirconia (Figure 2.3). A certain compound come from the wine into zirconia which produce a change in the functionality groups of the zirconia, shows clearly by the FTIR spectrum.

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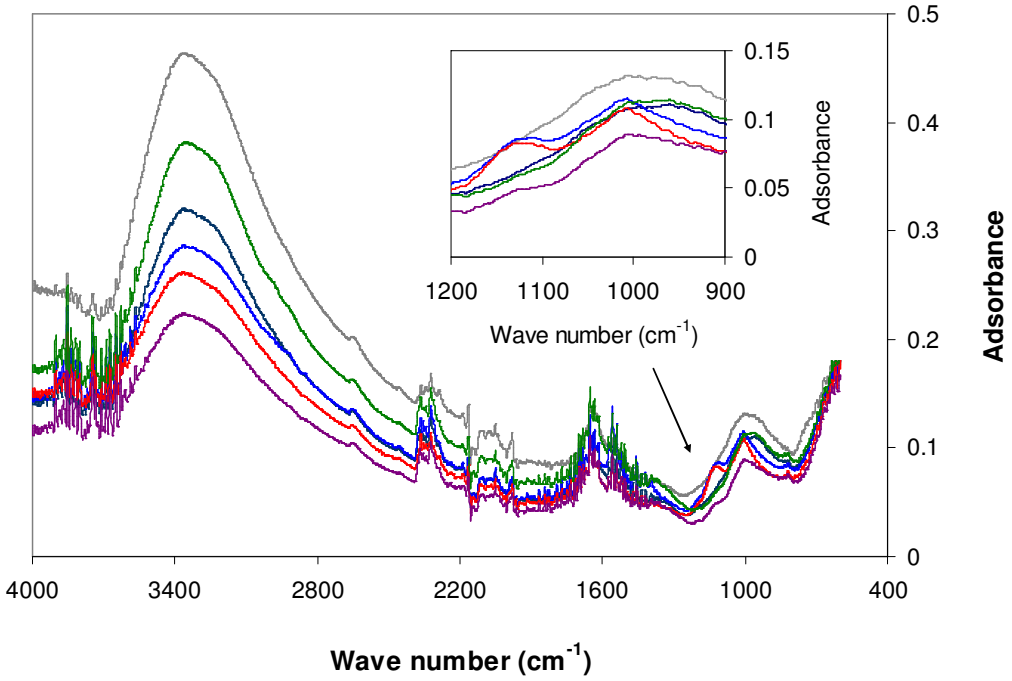

Figure 2.3 FTIR spectrum of pure and regenerated zirconia (—: pure zirconia; —: 500 ºC x 12 h without use; —: 300 ºC x 6 h; —: 500 ºC x 12 h; —: 2% NaOH x 2 h & 0.7% HNO_3 x 2 h; —: 4% NaOH x 4 h & 1.4% HNO_3 x 4 h)

ESEM mapping shows an adsorbent material consisting mainly of Zr and Si elements. It was not possible to detect other elements in the used or regenerated material (Figure 2.4). Moreover, the microphotography shows that the morphology is similar to that of pure and regenerated zirconia.

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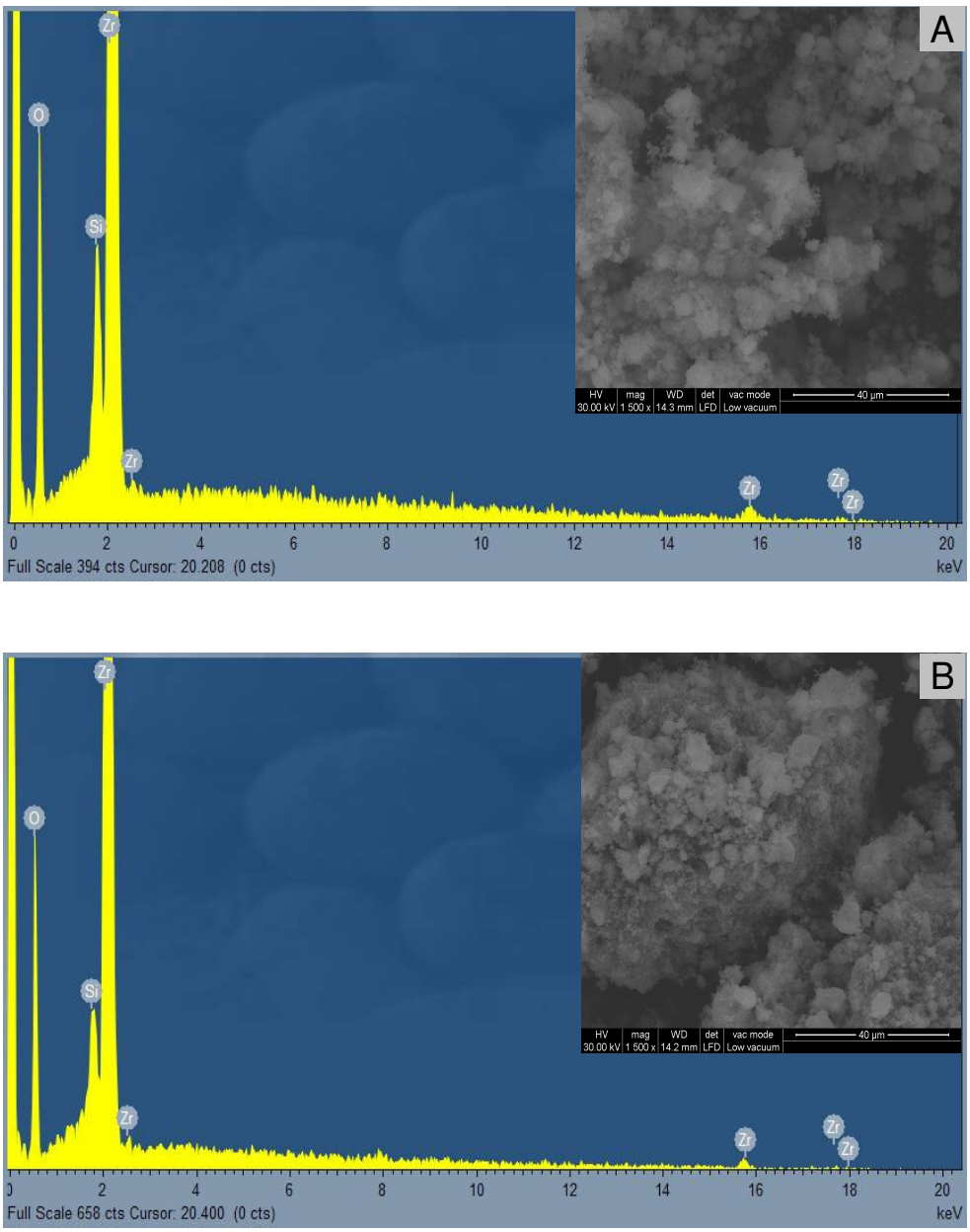

Figure 2.4 Spectrum and micrograph by ESEM (A: pure zirconia; B: used and regenerated zirconia at 500 ºC for 12 h)

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The pure zirconia is amphoteric with an isoelectric point around 7.9. In contrast, the used or regenerated material was more electronegative with an isoelectric point around 2.5. This may be related to the active site of the protein adsorbed (Figure 2.5).

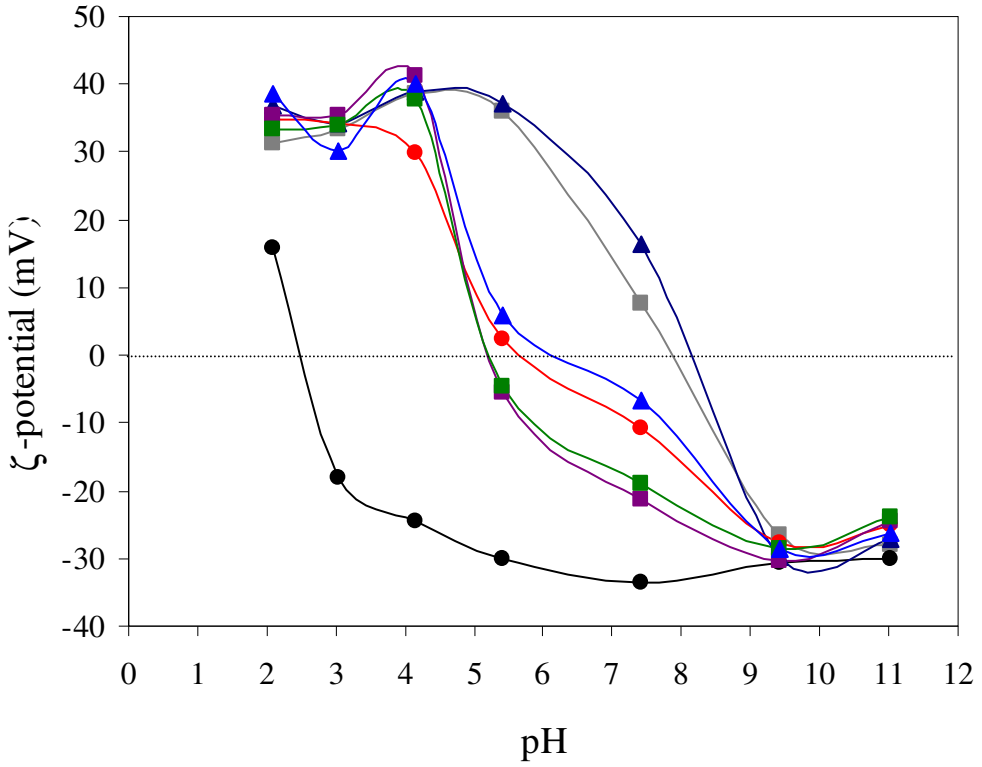

Figure 2.5 ζ-potential of the pure, used and regenerated zirconia (—■—: pure zirconia; —●—: used zirconia; —■—: 2% NaOH x 2 h & 0.7% HNO_3 x 2 h; —▲—: 500 ºC x 12 h without use; —▲—: 300 ºC x 6 h; —■—: 500 ºC x 12 h; —■—, 4% NaOH x 4 h & 1.4% HNO_3 x 4 h.

The results indicate that is viable to use zirconia as an adsorbent material in white wine protein stabilization. It has also been shown that this adsorbent material can be regenerated chemically and thermally without having any negative effects on its physical properties or wine protein adsorption capacity.

ISBN: 978-84-691-2703-2/DL.T.388-2008

Acknowledgements

We would like to thank the Spanish Ministry of Education and Science for funding (Project Nº AGL2006-07034/ALI). We are also grateful for the cooperation of Alβan (Nº LX-3769-80-DKPXAHWUYT) and AGAUR (Nº 2005BE00198) programmes. We would also like to thank the technicians Nicolas Guidolin and Lydia Raison of the Université Bordeaux I for their assistance with the Zetasizer equipment.

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Chapter 3

COMPARATIVE STUDY OF PROTEIN STABILIZATION IN WHITE WINE USING ZIRCONIA AND BENTONITE: PHYSICOCHEMICAL AND WINE SENSORY ANALYSIS

3.1 Abstract

A semi-industrial application of the continuous stabilization of white wine protein using a column packed with zirconia was studied and compared to the traditional bentonite treatment using a Macabeu white wine. Physicochemical and wine sensory properties were evaluated using a rating system and triangle tests. Continuous protein stabilization was analyzed in three residence times and the equivalent of 300 BV of wine was used for both treatments. Wine protein content was reduced by 21 %, 40 % and 42 % using the continuous process with residence times of 7.5, 15 and 30 minutes respectively, and by 61.4 % using the bentonite treatment. The wines obtained from the packed column were protein stable up to 25, 75 and 175 BV for residence times of 7.5, 15 and 30 minutes, respectively. The amount of polyphenol removed was less than 10%, and similar amounts were removed from the wine regardless of residence time while 20.6% of polyphenol was removed using bentonite. The physicochemical and sensory properties of wine treated with bentonite were similar to those of wine treated with zirconia.

Keywords: Unstable wine protein; wine sensory analysis; adsorption; bentonite; zirconia

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3.2 Introduction

One of the major factors that influences the quality of white wine is its protein stability. In the winemaking process, proteins are stabilized because wine proteins are adsorbed by bentonite. However, the selectivity of these discontinuous processes is low and their environmental impact high. This affects the quality of the wine and also means that some products are lost. For all these reasons, it is desirable to develop new alternatives, which are economically viable and maintain wine quality (Waters et al., 2005).

The stabilization of wine protein by bentonite is a discontinuous process, which takes some considerable time during the preparation and the gravity-settling steps. In winemaking, bentonite must be completely hydrated before it is added to wine, and the dose must be appropriate to prevent harming the wine's organoleptic properties (Stanković et al., 2004; Weiss et al., 2001).

In previous laboratory-scale studies, we have demonstrated that the continuous stabilization of white wines (Chardonnay and Muscat) is viable by means of a packed column using zirconia as the adsorbent material (Pashova et al., 2004a; Pashova et al., 2004b). Furthermore, the environmental impact of this continuous stabilization by zirconia is lower than that of the usual bentonite treatment because the chemical and mechanical resistance of this material (among its other physical properties) enables it to regenerate (Pachova et al., 2002; Pashova et al., 2004a; Pashova et al. 2004b; Salazar et al., 2007). However, the effect of this new continuous process on wine sensory properties has still not been demonstrated, nor do we know what differences, if any, there are between the results obtained using this process and those obtained from the conventional bentonite method of wine protein stabilization.

ISBN: 978-84-691-2703-2/DL.T.388-2008

Therefore, we were interested in comparing the new continuous stabilization of wine protein using zirconia with the conventional bentonite treatment in terms of its physicochemical and wine sensory properties. We were also interested in determining the operating conditions required to achieve protein-stable wines without affecting wine quality.

3.3 Materials and methods

3.3.1 Wine samples

Samples of Monovarietal Macabeu white wine were obtained from the Mas dels Frares winery (the 2005 vintage, Tarragona, Spain). This wine was produced with must clarified by settling. The fermentation was controlled at 18 ºC on an industrial scale and the wine samples were used immediately after fermentation with no additional treatment.

3.3.2 Physicochemical properties of the wine

The physical and chemical properties were determined by an infrared technique using WineScan^{TM} FT120 Basic (Foss, Denmark).The total protein and polyphenol content was determined by Bradford's and Folin's methods, respectively.

3.3.3 Stabilization of wine protein using zirconia and bentonite

Wine protein stability was studied using a Turbiquant 1000IR turbidimeter (Merck KGaA, Germany) and a thermal test described by Moine-Ledoux & Dubourdieu, 1999. The difference in turbidity between the initial wine and the wine after the thermal test was proportional to protein instability. The wines were considered stable if this difference did not exceed 2 NTU.

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The wine proteins were stabilized by means of a continuous process using zirconium oxide packed in a fixed bed column and through a discontinuous process by sodium bentonite (Laffort, France). In both treatments the volume of the wine treated was 25 L.

The treatment with bentonite included a preliminary test that used a variety of different doses (5, 10, 20, 30 and 40 g/hL) so that the most appropriate dose could be determined.

The continuous adsorption process was carried out in a 165 mm-high packed column with an internal diameter of 40 mm using 100 g of granulated zirconia with a particle size of 1-2 mm (Saint-Gobain, USA). The wine was pumped by up-flow mode through the column using a peristaltic pump (Watson Marlow 101 U/R, UK). The volume of wine treated was equivalent to 300 BV and three residence times were considered (7.5, 15 and 30 minutes). After each treatment the adsorbent material was regenerated at 500 ºC for 12 hours.

3.3.4 Physical properties of zirconia

The surface properties of zirconia were studied with Brunauer-Emmett-Teller (BET) model adsorption with liquid N_2 using a surface analyzer (Micromeritics ASAP 2000, USA) and assuming a cross-sectional area of 0.162 nm^2 for nitrogen. Before the adsorption measurements were taken, the samples were outgassed under a vacuum of 0.001 mbar at 120 ºC. The morphology of the material was studied by X-ray diffraction (XRD) using a nickel-filtered Cu $K\alpha_1$ radiation (λ=1.5418 Å) in the 2θ range 10-70º through a SIEMENS D5000 diffractometer.

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3.3.5 Cold stabilization and microfiltration of the wine

Once the wine protein stabilization treatments had concluded, samples of the wine were taken to evaluate its tartaric stability using conductivity measurements at a temperature near 0 ºC and Boulton's test (Boulton, 1983) using a Crison CM35 conductimeter (Crison Instruments, Barcelona, Spain).All wines were treated with 4 g/L of potassium hydrogen tartrate (KHT) and stored for 2 to 3 weeks at 5 ºC until tartaric stability was achieved. Finally, all the wines were filtered by tangential microfiltration using a ceramic membrane of zirconium oxide with a pore size of 0.45 µm (Tami, France). Filtration was effected at room temperature with a transmembrane pressure of 150 kPa and a tangential velocity of 2 m/s.

3.3.6 Wine sensory analysis

Macabeu wine treated with zirconia and bentonite was subjected to sensory evaluation by means of a triangle test in accordance with ISO 4120:2004. Additionally, both non-treated and treated wine was tested using rating system tests according to Office International de la Vigne et du Vin, Resolution ENO 2/94 OIV standard for the international wine competitions. Both tests were performed in a wine tasting room in compliance with standard NF V09-105 AFNOR. The triangle test was conducted by seventeen untrained wine tasters involved in enology research and the rating system test was conducted by a panel of six experienced wine tasters.

The main objective of the triangle test was to determine any significant overall differences between wine samples treated with zirconia and those treated with bentonite. To this end, we prepared four individual sensory triangle tests of the

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treated wine. These individual triangle tests were carried out using random formation and placement.

The sensory rating system test was carried out on non-treated and treated wines in order to confirm the results of the first test and to determine which wine protein stabilization treatment rated higher among experienced tasters. The wine samples were placed in random formation and the tasters performed the sensory test in no predetermined order.

3.4 Results and discussions

3.4.1 Physical properties of zirconia

The zirconia have a BET surface area of 164 m^2/g, an average pore diameter of 44 nm, a tetragonal morphology and higher mesoporosity according to the BET analysis. The material was crushed into small 1-2 mm particles to increase its contact surface area during the continuous adsorption process of the wine protein.

3.4.2 Stabilization of wine protein using zirconia and bentonite

In this study, the wines treated presented a low total protein concentration of 17.92 ± 0.87 mg/L BSA. However, all the samples were unstable in terms of protein, with a turbidity of 11.94 ± 1.86 NTU.

We tested five different doses of bentonite in the Macabeu wine (5, 10, 20, 30 and 40 g/hL) to determine the minimum dose that stabilized the wine protein and least changed the quality of the wines studied. The best results were obtained with a dose of 20 g/hL.

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Figure 3.1 shows the effect of residence times on the amount of protein adsorbed during the continuous protein stabilization of Macabeu wine. The data show that for the first accumulated 25 BV there is no difference in the amount of protein adsorbed. However, above these bed volumes, the total protein adsorbed depends considerably on the residence time, and the protein is reduced by 21 %, 40 % and 42 % for residence times of 7.5, 15 and 30 minutes, respectively, and by 61.4% using the bentonite treatment.

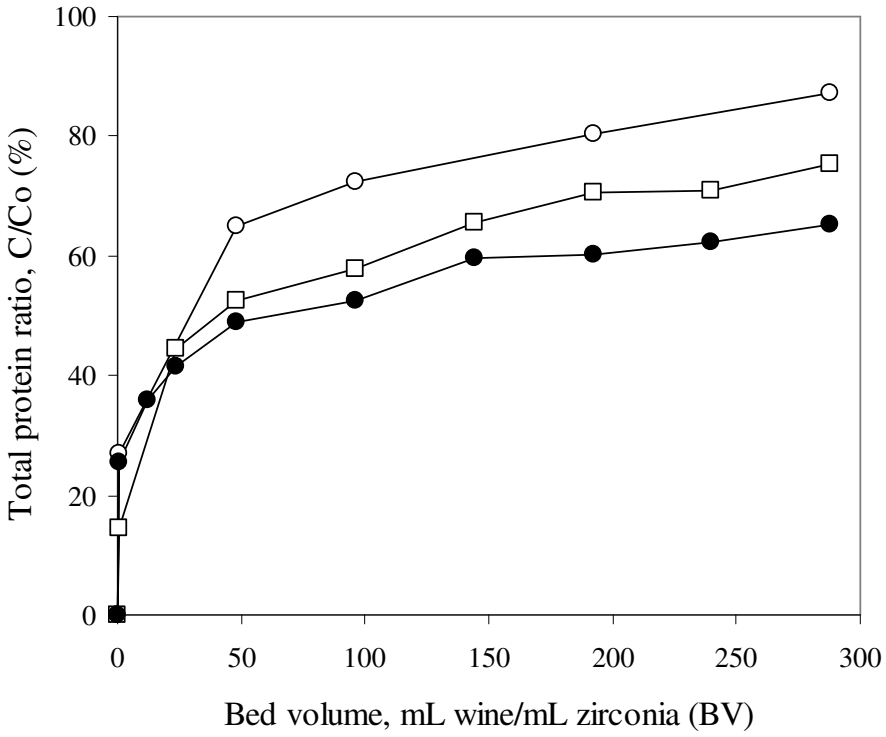

Figure 3.1 Total protein adsorbed for different residence times during continuous wine protein stabilization (○: residence time of 7.5 min; □: residence time of 15 min; ●: residence time of 30 min)

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The results of the treated with zirconia shown Table 3.1 and Table 3.2 are of the accumulated 300 BV. The total polyphenol content in the non-treated wines was 219 ± 11 mg/L gallic acid and the amount of polyphenol adsorbed through the treatment with zirconia was less than 10 % and similar for the three residence times and 20.6 % in the bentonite treatment (Table 3.1). Therefore, according to these results, zirconia has greater selectivity for wine protein adsorption than polyphenol compounds, as reported by Salazar et al. (2007). Zirconia adsorbed a smaller amount of polyphenols from Macabeu wine than the bentonite treatment. Therefore, stabilization of wine protein using zirconia results in stable wines and does not greatly affect their polyphenol content (Table 3.1).

Table 3.1 Total protein and polyphenol content in the wine after protein stabilization and bottling [a]

Wine condition	Total protein (mg/L BSA)		Total polyphenol (mg/L gallic acid)		Protein stabilization (ΔNTU)	
	AA	AB	AA	AB	AA	AB
Zirconia/ residence time						
7.5 min	13.73±0.18	11.08±0.11	206±1	203±2	4.50±0.46	0.95±0.12
15 min	11.28±0.21	10.31±0.11	198±1	195±1	2.28±0.31	0.65±0.18
30 min	10.03±0.33	9.14±0.06	201±1	197±1	1.10±0.33	0.49±0.11
Bentonite (20 g/hL)	8.45±0.07	7.58±0.22	174±2	170±2	0.20±0.10	0.16±0.05

[a] All the values are presented as means and standard deviation of at least two independent experiments. AA, wine after protein stabilization treatment; AB, wine after bottled (wine cold stabilized, filtered and bottled)

The amount of zirconia required to treat considerable volumes of wine during the continuous stabilization of wine protein indicates that this process may be promising for use on an industrial scale.

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Table 3.2 shows that the physicochemical properties of Macabeu wine were not greatly affected by either treatment, in agreement with previous results from other wines (Pashova et al., 2004a; Pashova et al., 2004b; Salazar et al, 2007). The one exception was the absorbance at 420 nm both in wines treated with the continuous process for a residence time of 30 min and those treated with bentonite.

Table 3.2 Physicochemical properties of nontreated and bottled wines [b]

Parameter	nontreated wine	Zirconia residence time (min)			Bentonite dose (20 g/hL)
		7.5	15	30	
pH	3.22±0.07	3.17±0.00	3.18±0.01	3.19±0.01	3.17±0.01
Total acidity, g/L tartaric acid	3.77±0.02	3.53±0.01	3.56±0.04	3.49±0.00	3.52±0.01
Volatile acidity, g/L acetic acid	0.26±0.02	0.19±0.01	0.18±0.01	0.20±0.00	0.19±0.01
Total dry extract, g/L	16.10±1.39	16.20±0.14	16.15±0.42	15.70±0.28	15.78±0.13
Sugar reducers, g/L	1.33±0.25	2.03±0.05	1.82±0.04	1.96±0.21	2.12±0.11
Glycerol, g/L	7.06±0.75	6.35±0.07	6.48±0.10	6.35±0.07	6.30±0.00
Gluconic acid, g/L	0.36±0.03	0.38±0.00	0.38±0.01	0.37±0.00	0.38±0.01
Malic acid, g/L	1.01±0.13	0.98±0.01	0.95±0.02	0.94±0.01	0.94±0.01
Tartaric acid, g/L	3.32±1.14	1.89±0.00	1.89±0.04	1.96±0.01	1.92±0.05
Absorbance at 420 nm	0.060±0.004	0.064±0.001	0.062±0.002	0.042±0.000	0.041±0.003

[b] All the values are presented as means and standard deviation of at least two independent experiments and corresponding to results of non-treated and bottled wines.

The wine obtained at the exit of packed column can be considered protein stable up to 25, 75 and 175 BV for residence times of 7.5, 15 and 30 minutes,

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respectively (Figure 3.2). However, when we analyzed the accumulated volume of the wine (equivalent to 300 BV), the turbidity values were lower and in some case just slightly higher than 2 NTU for residence times of 15 and 30 minutes. Therefore, these wines are practically stable.

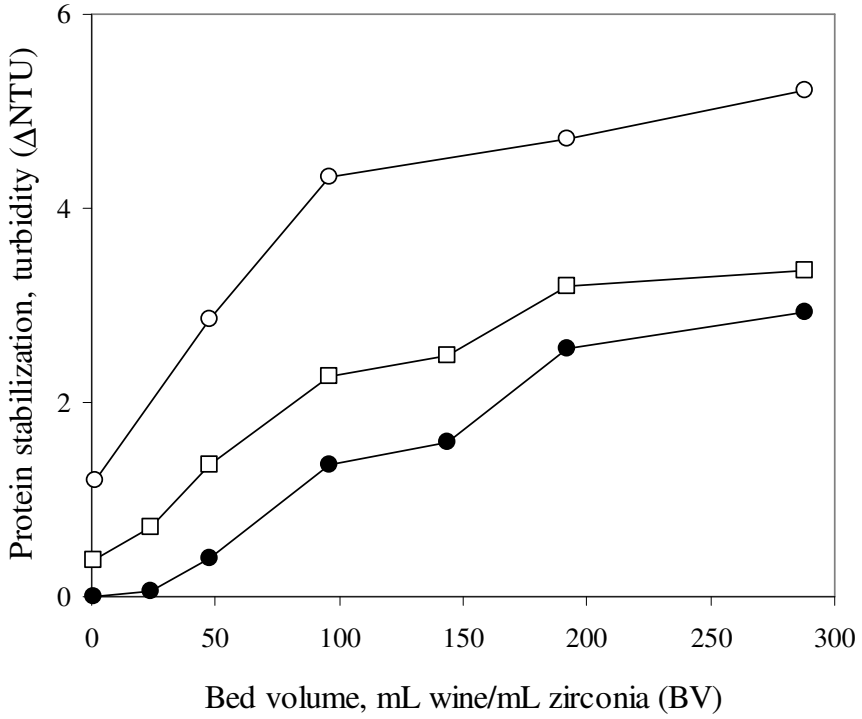

Figure 3.2 Wine protein stability for different residence times during the continuous wine protein stabilization (○: residence time of 7.5 min; □: residence time of 15 min; ●: residence time of 30 min)

It should also be pointed out that, from an economic perspective, industrial-scale processes should consider a residence time of 15 minutes, as wine protein stabilities were similar at residence times of 15 and 30 minutes. Therefore, a residence time of 15 minutes may be sufficient for the protein stabilization of 300 BV of Macabeu wine on an industrial scale using zirconia. Furthermore, a

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subsequent clarification by crossflow microfiltration may improve wine protein stabilization, as is shown in Table 3.1 and in accordance with that reported by Salazar et al. (2007).

3.4.3 Wine sensory analysis

The untrained wine tasters were not able to distinguish significant differences between the wines treated with bentonite and those treated with zirconia using a triangle test ($p < 0.005$). Figure 3.3 illustrates the results obtained from the sensory rating system test for the wines.

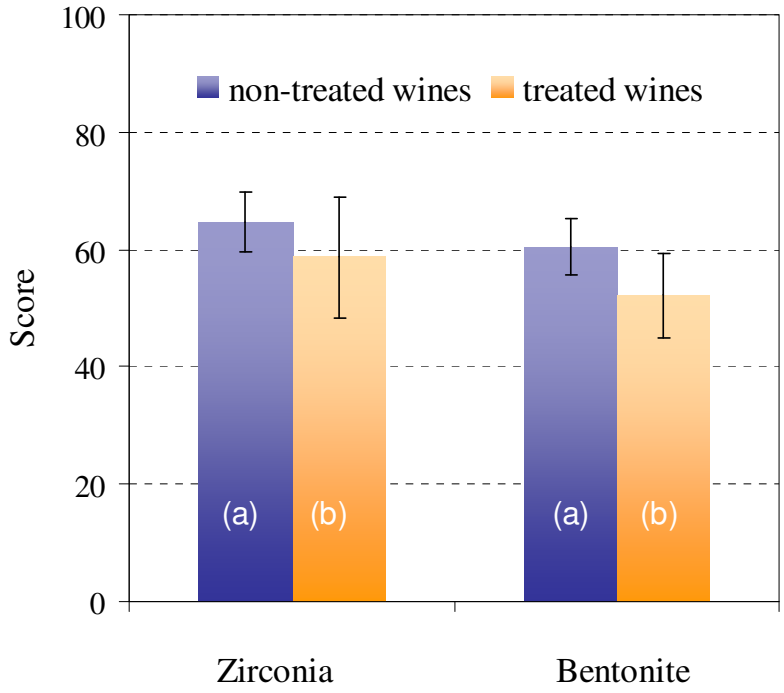

Figure 3.3 Results of the sensory rating system test for the wines (a) before treatment with zirconia (retention time 15 min) and bentonite, and (b) after treatment (protein and cold stabilized, and microfiltrated) and bottling.

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There were no significant sensory differences between the wines treated with zirconia and those treated with bentonite. However, the wines stabilized with zirconia were scored slightly better than wines treated with bentonite according to the results of the rating system test.

Abbreviations used

BV, bed volume (volumetric ratio between the wine treated and the zirconia used at process time determined); NTU, nephelometric turbidity units; ΔNTU, difference of nephelometric turbidity units between the wine before and after thermal test applied.

Acknowledgement

The author would like to thank the Alβan programme for a pre-doctoral scholarship (No.LX-3769-80-DKPXAHWUYT) and CEVIPE (Centre Vinícola del Penedès, Spain) for supplying the WineScan equipment. Financial support provided by the Spanish Ministry of Education and Science Project Nº AGL2006-07034/ALI.

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UNIVERSITAT ROVIRA I VIRGILI
WHITE WINE CONTINUOUS PROTEIN STABILISATION: INDUSTRIAL VIABLITY
Fernando Noé Salazar González
ISBN: 978-84-691-2703-2/DL.T.388-2008

Chapter 4

IMPROVEMENT OF WINE CROSSFLOW MICROFILTRATION BY A NEW HYBRID PROCESS

4.1 Abstract

A hybrid process comprising column adsorption and crossflow microfiltration was applied to reduce the unstable proteins of Pinot Noir wine and improve the permeate flux during wine microfiltration. The process was performed using a packed column with zirconium oxide and a ceramic membrane. Proteins and polyphenols were reduced by bentonite and activated carbon addition in order to evaluate their influence on permeate flux decline. The column adsorption process achieved a better protein stability and increased the permeate flux by 15-20 %. The molecular weight range of 20-70 kDa could be related to unstable proteins or cause membrane fouling. The phenolic composition of wine was not affected by adsorption with zirconium oxide or the microfiltration process.

Keywords: Wine clarification; crossflow microfiltration; fouling; adsorption; protein stabilization

4.2 Introduction

Crossflow microfiltration is becoming widely extended in the winemaking industry, mainly as a clarification and microbiological stabilization technique. However, a major limiting factor in microfiltration is the decline in the permeate flux over time due to membrane fouling. Membrane fouling is mainly

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due to the accumulation of macromolecular or colloidal compounds (such as proteins and polyphenols) in wine treatment (Czekaj et al., 2001; Vernhet & Moutounet, 2002). Studies of the effects of the membrane material, pore size and operating conditions on permeate flux decline have also demonstrated that crossflow microfiltration did not significantly modify the sensory quality of wines. Moreover, they have shown that it is possible to reduce membrane fouling by adjusting operating parameters such as transmembrane pressure and crossflow velocity to optimum values. However, permeate flux decline is still an important problem for the process yield (Vernhet et al., 2003; Palacios et al., 2002).

The new concept of a hybrid process or integrated system has recently been proposed to improve the selectivity and yield of waste separation processes. This concept involves the coupling of two or more unit operations that provide a more efficient separation than each operation, i.e. adsorption and membrane separation, alone (Mavrova et al., 2003; Roux et al., 2005; Konieczny & Klomfas, 2002). A hybrid process can be applied to fields such as gas separation, biosorption and the removal of heavy metal from wastewater (Juang et al., 2004; Reddad et al., 2003; Zhang et al., 2003; Kwang-Sup et al., 2004). The main advantage of this type of hybrid process is a high yield combined with high separation efficiency. In the adsorption step, it is important to use an adsorbent material with the highest area of contact. The use of small particles must be avoided if the adsorption is by means of a packed column because the pressure drop and, therefore, the pumping fluid costs are higher (Mavrova, et al., 2003; Kwang-Sup et al., 2004; Klomfas & Konieczny, 2004). Also, small particles could pass to the treated fluid and risk being incrusted on the membrane pores during the filtration step. It is important, therefore, to propose or develop an adsorbent material that is easy to package, has a high separation

ISBN: 978-84-691-2703-2/DL.T.388-2008

efficiency and can be regenerated to avoid the accumulation of waste and damage to the environment.

In winemaking, the most common procedure for clarifying and stabilising wine proteins is to add bentonite, let it settle by gravity and then remove the solid suspension by filtration with diatomeaceous earth or membranes. However, bentonite and diatomeaceous earth are considered a hazardous waste that implies disposal costs. Bentonite has a wide range of protein adsorption but a low selectivity, so using it inappropriately can cause important variations in wine quality (Main & Morris, 1994; Martínez-Rodriguez & Polo, 2003; Stanković et al., 2004). Moreover, due to its high adsorption capacity, a wrong separation of the bentonite during the settling step could increase the risk of membrane fouling. For these reasons, it is extremely important to apply other kinds of adsorbent materials that are more selective and have a low interaction with the membrane during the filtration process. Sarmento et al. (1999, 2000) studied the adsorption of bovine serum albumin (BSA) on different adsorbent materials and sodium bentonite in a model wine solution. They achieved positive results of protein adsorption using a packed column with ion-exchange resins but the protein adsorption capacity was lower than with bentonite. They were also unable to evaluate the wine protein stabilisation against the final wine quality and the effect or interactions that could generate the compounds of real wines, such as ethanol, phenols and proteins.

Previous studies have shown that zirconium oxide packed in a column can be used to reduce the unstable wine proteins and preserve the physicochemical properties of wine (Pachova et al., 2002; Pashova et al., 2004a; Pachova et al., 2004b). It could be interesting, therefore, to integrate an adsorption process with crossflow microfiltration because, if it is possible to reduce the unstable proteins, it may also be possible to improve the microfiltration step by

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increasing the permeate flux due to less membrane fouling. This last aspect is attractive because it implies lower production costs mainly thanks to an increase in the membrane's useful life time and microfiltration yield.

4.3 Materials and methods

4.3.1 Wine samples

Samples of the Monovarietal Pinot Noir wine were obtained from the Mas dels Frares winery, 2004 harvest, Tarragona, Spain. This wine was produced with must clarified by settling. The fermentation was controlled at 16 ºC on an industrial scale and the wine samples were used immediately after fermentation without additional treatment.

4.3.2 Adsorption process

The adsorption process was carried out in a 165 mm high packed column with an internal diameter of 40 mm using 250 g of zirconium oxide in pellet with a particle size of 3-6 mm in length (Mel Chemicals, England). The wine was pumped by up-flow mode through the column using Watson Marlow 101 U/R peristaltic pump. The volume of wine treated was 3.5 L. The volumetric flow rate was kept constant at 4 mL/min during each experiment. Residence time was 1 hour and total process time was around 14.5 h.

4.3.3 Regeneration of adsorbent material

The adsorbent material was regenerated at 500 ºC for 12 hours before and after each adsorption experiment. To evaluate the stability of zirconia, the textural properties and crystallinity structure were determined. The surface area and pore size distribution were evaluated using the Brunauer-Emmett-Teller (BET)

ISBN: 978-84-691-2703-2/DL.T.388-2008

method by nitrogen adsorption and desorption isothermal at 77 K in a Micromeritics ASAP 2000 surface analyzer and assuming a cross-sectional area of 0.162 nm^2 for nitrogen. Before the adsorption measurements were taken, the samples were outgassed under a vacuum of 0.001 mbar at 120 ºC. Crystallinity was carried out by X-ray diffraction (XDR). The X-ray powder diffraction (XDR) of the zirconia was carried out on a Bruker D5Adbance X-ray diffractometer using a nickel-filtered Cu K_α radiation (λ=1.5418 Å) in the 2θ range 10-70º. The crystallite size was determined by the Scherrer equation.

4.3.4 Crossflow microfiltration

The filtration was performed in a crossflow microfiltration pilot plant using a ceramic membrane of zirconium oxide with a pore size of 0.2 μm (Tami, France). The membrane configuration is a tubular module of 25 cm in length, with an outer diameter of 10 mm and three inner channels each of 3.5 mm diameter and a total surface area of 0.0094 m^2. The operating conditions were a transmembrane pressure of 150 kPa, a feed flow rate of 3.7 L/min, a crossflow velocity of 2.2 m/s and a temperature of 20 ± 2 ºC.

The filtration experiments were carried out in a continuous mode for a period of 30 min. Permeate was collected and measured every 60 seconds by an electronic microbalance (Denver Instruments XL 3100) with a data acquisition system. The membrane was cleaned after each run according to the manufacturer's recommendations with 1.5% NaOH and 0.4% HNO_3 until the original miliQ-water permeate flux was achieved.

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4.3.5 Analytical methods

The physico-chemical parameters, such as pH, total acidity, volatile and fixed acidity, volumetric alcohol degree and chromatic characteristics, were measured according to the Official Newspaper of the European Communities (1990).

4.3.6 Total protein & protein fraction

The total protein concentration was measured by Bradford's method using Coomasie brilliant blue reagent and reading absorbance at 595 nm on a spectrophotometer (Cecil CE2021, England) after 5 minutes of incubation (Bradford, 1976). The protein content was expressed as mg/L of bovine serum albumin (Sigma, cat. No. A-3803).

The wine protein fractions before and after each treatment were evaluated by gel permeation chromatography, using the method described by Czekaj, López, and Güell (2001). Gel filtration was carried out at 25 ºC using a HPLC Agilent 1100 series equipped with a UV detector at a wavelength of 220 nm. The column system consisted of a TSK-Gel G2000SW column (TosoHaas GmbH, Stuttgart Germany, 7.5 x 300-mm) with a Guardcolumn SW (TosoHaas GmbH, 7.5 x 75-mm). Bovine serum albumin (Sigma, cat. No. A-3803, MW 67 kDa), chicken egg albumin (Sigma, cat. No. A-5503, MW 45 kDa) and lysozyme (Sigma, cat no. L-2879, MW 14.5 kDa) were used as molecular weight standards.

4.3.7 Total polyphenol & phenolic profile

Total polyphenols were determined by the Folin-Ciocalteau method (Folin & Ciocalteu, 1927). Phenolic compounds before and after each treatment of adsorption, microfiltration and hybrid process were determined using liquid

ISBN: 978-84-691-2703-2/DL.T.388-2008

chromatography according to the method described by Betés-Saura, Andrés-Lacueva, and Lamuela-Raventós (1996). A HPLC Agilent 1100 Series equipped with a column Supelcosil LC-18 and a diode array detector at 280, 320 and 365 nm was used. The phenolic compound was identified by comparing the spectra and retention time of each peak with standards previously studied by the same method.

4.3.8 Protein heat stability test

After each adsorption, microfiltration and hybrid process, a 20 mL sample of wine was filtered through a cellulose nitrate membrane filter with a pore size of 0.45 μm (Whatman, cat. No 7184009, England), heated for 2 h at 80 °C in a bath equipped with a digital control immersion thermostat (Digiterm 100 model). It was then incubated for 2 h at 4 °C. Finally, the turbidity was measured by nephelometry (Turbiquant 1000 IR turbidimeter) and expressed in nephelometric turbidity units (NTU). The difference in turbidity between the initial wine and the wine after the thermal test was proportional to protein instability. The wines were considered stable if this difference did not exceed 2 NTU (Moine-Ledoux & Dubourdieu, 1999).

To identify the range of molecular weight of the proteins responsible for wine instability, a sample of wine was treated by the thermal test described above. The precipitated proteins were removed by centrifugation (Hettich, EBA21, Germany) and the supernatants of wine were analysed by HPLC.

4.3.9 Effect of protein and polyphenol content on the permeate flux

Three different treatments were applied to the wine using very high doses of bentonite and activated carbon. These treatments were intended to dramatically

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reduce protein and polyphenol contents. 0.2% sodium bentonite (Laffort, France) was used for the first treatment, 1% activated carbon (Martin Vialatte OEnologie, Spain) was used for the second treatment and a mixture of both was used for the third treatment. The bentonite had been hydrated for 24 hours before use.

4.3.10 Statistical analysis

The effect of adsorption, microfiltration and the hybrid process on the physico-chemical parameters and chromatic characteristics of the wine was analysed by one-factor ANOVA. The effect of the protein and polyphenol contents on the permeate flux during the microfiltration was evaluated by Spearman and Kendall's methods using the WinSTAT program with a significance level of $p \leq 0.05$.

4.4 Results & discussions

4.4.1 Influence of protein and polyphenol content on the permeate flux

Fig. 4.1 plots fluxes against time for treated and untreated wine. Permeate flux is expressed by a relation between the permeate flux of wine (J) with respect to the initial permeate flux with miliQ-water (Jo). The permeate flux profile shows the typical flux decline of crossflow microfiltration.

The microfiltration of wine previously treated with zirconium oxide (hybrid process) led to an increase of 15-20% in the permeate flux. Therefore, a previous adsorption with zirconium oxide using a packed column was very useful for enhancing microfiltration. Moreover, the adsorption process with zirconium oxide reduced the total protein concentration from 24.5 to 13.0 mg/L and the total polyphenol index from 6.0 to 5.1 (see Table 4.1). These results

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therefore show that the improvement in permeate flux decline could mainly be due to the reduction in protein. Figure 4.1 also shows that the increases in permeate flux were mainly due to the strong reductions in proteins and polyphenols achieved by treatment with bentonite, activated carbon or a mixture of the two. Adsorption by zirconium oxide led to a lower reduction in the total polyphenol content but a fair reduction in the total protein content. Moreover, there was a greater selectivity of bentonite for proteins and activated carbon for polyphenols (see Table 4.1).

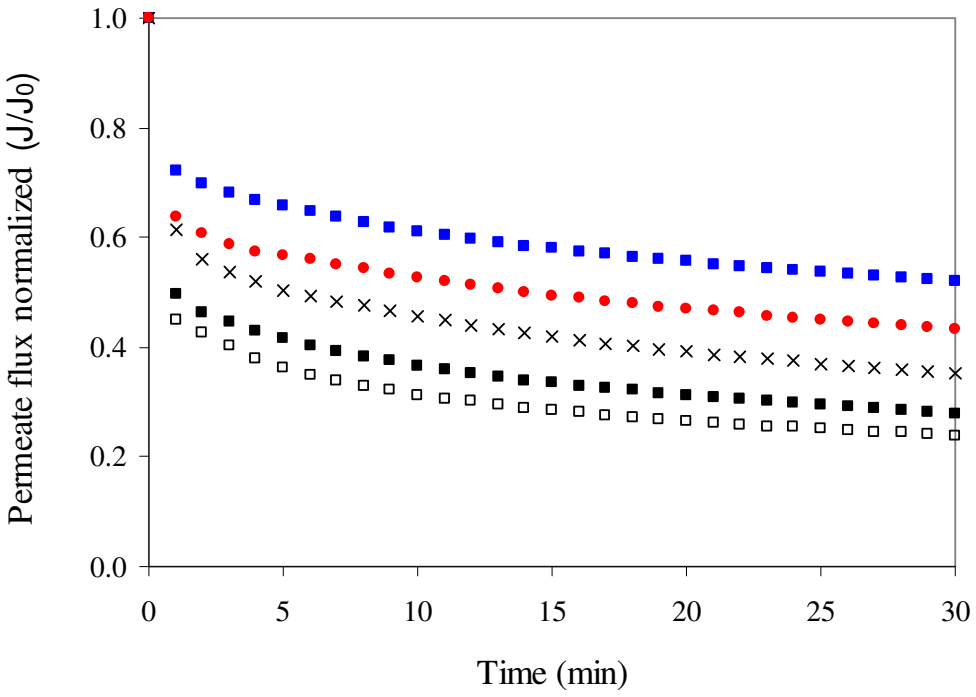

Figure 4.1 Influence of protein and polyphenol content on the wine permeate flux during microfiltration using different treatments. □: microfiltration; ■: microfiltration (zirconium oxide); ×: microfiltration (activated carbon); ●: microfiltration (bentonite); ■: microfiltration (bentonite & activated carbon).

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According to Spearman (-0.9286) and Kendall's tau (-0.8095) correlation coefficients using a confidence interval of 99% (α=0.01), there is an inverse relationship between total protein concentration, total polyphenol index and the permeate flux decline. Therefore, decreases in protein and polyphenol contents lead to an increase in permeate flux.

An increase in residence time during the adsorption process with zirconium oxide could help to improve protein retention even further. This would increase wine protein stability and reduce membrane fouling. This will be covered in a future study, however.

Table 4.1 Reduction in protein and polyphenol content after different treatments

Treatment	Total protein (mg/L)		Total polyphenol (TPI)	
	before	after	before	after
Bentonite	24.5 ± 2.2	6.3 ± 0.6	6.0 ± 0.1	4.8± 0.1
Activated carbon	24.5 ± 2.2	8.3 ± 0.4	6.0 ± 0.1	3.8± 0.0
Bentonite & activated carbon	24.5 ± 2.2	4.8 ± 0.1	6.0 ± 0.1	3.3± 0.0
Zirconium oxide (adsorption process)	24.5 ± 2.2	13.0 ± 0.2	6.0 ± 0.1	5.1± 0.2
Microfiltration	24.5 ± 2.2	22.5 ± 2.4	6.0 ± 0.1	5.2± 0.0
Microfiltration (bentonite)	6.3 ± 0.6	4.7 ± 0.1	4.8 ± 0.1	4.4± 0.0
Microfiltration (activated carbon)	8.3 ± 0.4	6.5 ± 0.2	3.8 ± 0.0	3.5± 0.1
Microfiltration (bentonite + activated carbon)	4.8 ± 0.1	2.5 ± 0.5	3.3 ± 0.0	2.9± 0.0
Microfiltration (zirconium oxide)	13.0 ± 0.2	11.8 ± 0.1	5.1 ± 0.2	4.9± 0.2

4.4.2 Physicochemical properties of wine

Table 4.2 presents the physicochemical properties of treated and untreated wine. Our results showed that there were only significant differences in total, volatile

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and fixed acidity. The adsorption process reduces the acidity, which may be considered a positive effect. Ugarte et al. (2005), for example, presented a study in which they applied integrated systems by adsorption and reverse osmosis to reduce acidity and separated undesirable compounds from impaired wines or from wines that increased their acidity during storage or ageing.

The only difference in the chromatic characteristics of the wine was the colour intensity after the microfiltration and hybrid process (see Table 4.3). This was due to the lower turbidity of the wine clarified by the microfiltration. Similar results have been obtained with Chardonnay wine, as reported by Pashova et al. (2004a).

Table 4.2 Physicochemical properties of Pinot Noir wine

Analytical parameter	Initial wine	Microfiltration	Adsorption	Hybrid Process
pH	3.04	3.02	3.05	3.08
Total acidity, g/L tartaric acid	6.53	6.47	5.89	5.31[a]
Volatile acidity, g/L acetic acid	0.48	0.43	0.38[a]	0.36[a]
Fixed acidity, g/L tartaric acid	6.33	6.38	5.73[a]	5.19[a]
Volumetric alcohol Degree, % v/v	9.8	9.7	9.6	9.7
Reducing sugar, g/L	2.0	1.80	1.90	1.78
Total dry extract, g/L	20.6	---	---	---
Sulfates, g/L K_2SO_4	< 0.7	< 0.7	< 0.7	< 0.7
Free sulphurous, mg/L SO_2	21	---	---	---
Total sulphurous, mg/L SO_2	83	---	---	---

[a] There were significant differences due to treatment, according to analysis of variance (One–factor ANOVA) to $p \leq 0.05$

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Table 4.3 Chromatic characteristics of Pinot Noir wine after different treatments

Treatment	Chromatic characteristics				
	Absorbance (λ, nm)			Colour intensity (CI) [b]	Tonality (N) [c]
	420	520	620		
Initial wine	0.086	0.092	0.011	0.188	0.934
Microfiltration	0.075	0.087	0.007	0.168[a]	0.861
Adsorption	0.079	0.093	0.013	0.185	0.849
Hybrid process	0.067	0.082	0.007	0.155[a]	0.822

[a] There were significant differences due to treatment, according to analysis of variance (One–factor ANOVA) to $p \leq 0.05$; [b] Summing absorbance at 420, 520 and 620 nm; [c] Corresponding to relation between 420 and 520 nm

4.4.3 Wine protein stabilisation

Table 4.4 shows that the wine processed by the hybrid process achieved better protein stability than wine treated by conventional microfiltration. Although protein stability was greater than 2 NTU, these results are positive in the case of young wines, where storage and sale time are short. We also demonstrated that a soft adsorption treatment with zirconium oxide significantly improved wine protein stabilisation and avoided the environmental impact caused by using bentonite as adsorbent material.

Table 4.4 Protein stabilisation of Pinot Noir wine

Treatments	Nephelometric turbidity units (NTU)		
	Before	After	Difference
Initial wine	0.65	24.05	23.40±0.52
Microfiltration	0.25	19.46	19.21±0.71
Adsorption (zirconium oxide)	1.50	11.39	9.90±1.39
Hybrid process	0.22	5.61	5.40±0.57

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4.4.4 Protein fraction

We used HPLC to investigate the molecular weight ranges of the proteins that cause wine instability and membrane fouling. First, a mixture protein solution was prepared to identify the retention time of each standard protein (Figure 4.2). Samples of initial and treated wines were then analysed, taking as reference the retention time of standard proteins.

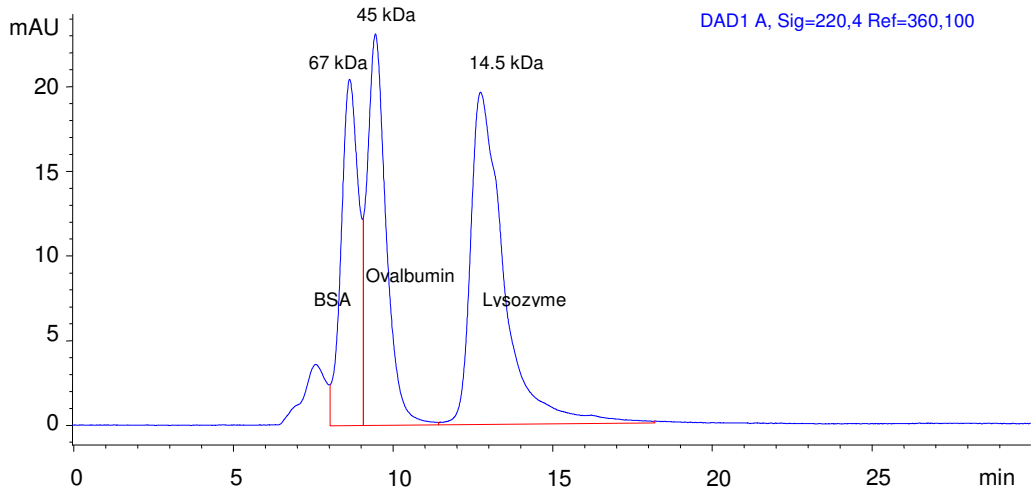

Figure 4.2 Retention time of a mixture of protein standards

The amount of protein removed during each treatment was determined qualitatively, taking as a reference the value of each peak obtained in samples of initial wine without treatment (Figure 4.3).

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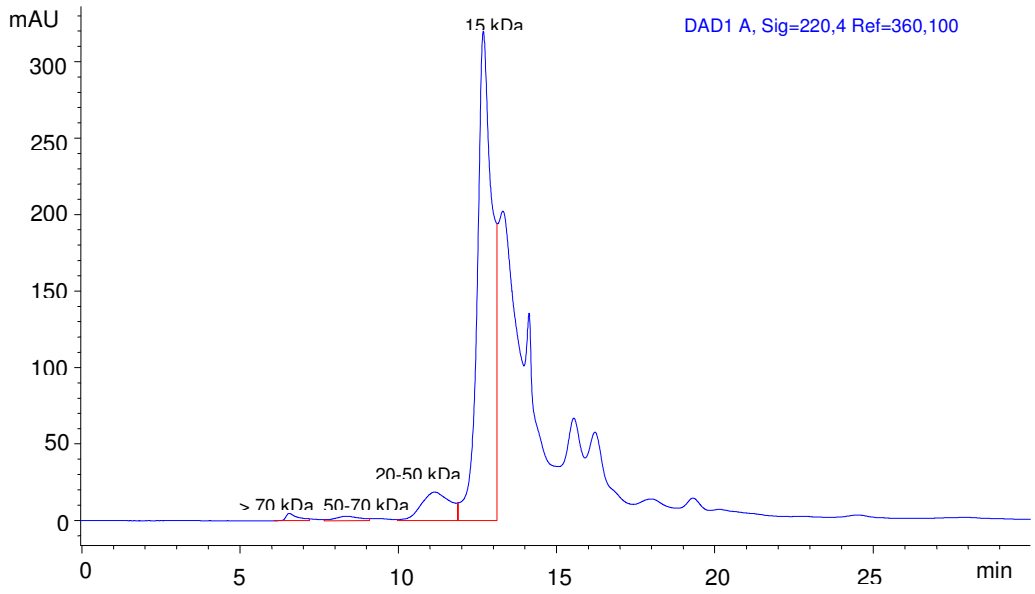

Figure 4.3 Protein molecular weight ranges of initial wine sample

We assumed that the proteins removed by the thermal test and the microfiltration treatments were the unstable proteins and the proteins causing the membrane fouling, respectively. From Figure 4.4 and these assumptions, we can see that the order of protein fraction removed was 20-50 kDa, 50-70 kDa, >70 kDa and 15 kDa. The molecular weight ranges of the unstable protein and the protein that causes membrane fouling were 20-50 kDa and 50-70 kDa (Pashova et al., 2004a; Pashova et al., 2004b)

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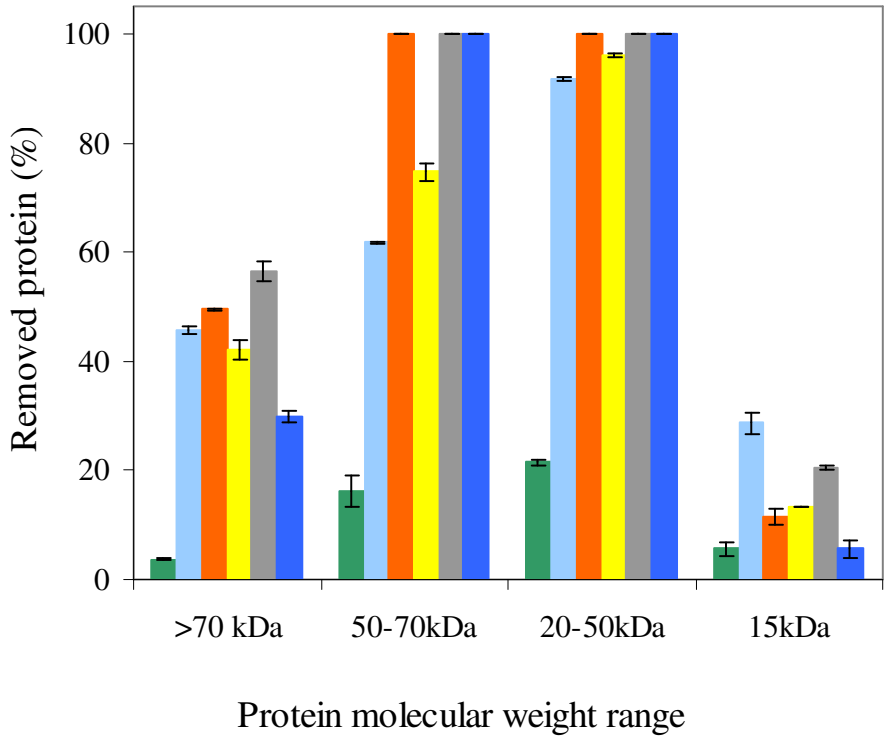

Figure 4.4 Percentage of proteins removed from wine after each microfiltration and thermal test. ■: microfiltration; ■: microfiltration (zirconium oxide); ■: microfiltration (bentonite); ■: microfiltration (activated carbon); ■: microfiltration (bentonite & activated carbon); ■: thermal test.

4.4.5 Phenolic profile

By studying the phenolic profile, we identified 9 phenolic compounds and showed that adsorption, microfiltration and the hybrid process had little effect on the reduction of phenolic compounds. This is in agreement with the total polyphenol index results obtained by Folin's method. Treatment with bentonite and activated carbon changed the phenolic compounds in the initial wine considerably. Some compounds, such as (-) epicatechin and ferulic acid, even

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disappeared when activated carbon was used (results not shown). On the other hand, when we evaluated the samples of treated wine by thermal test we found no loss of phenolic compounds and in some case the amount detected was higher (see Table 4.5).

Table 4.5 Identification of phenolic compounds in the initial wine and after each treatment

Compound	Concentration (mg/L)				
	Initial wine	Zirconium oxide	Microfiltration	Microfiltration (zirconium oxide)	Thermal test
Gallic acid	7.43±0.76	6.52±0.01	7.65±0.06	7.03±0.03	11.55±0.28
Tyrosol	12.27±0.05	10.96±0.14	12.18±0.71	11.32±0.03	17.02±0.29
Vanillic acid	0.98±0.02	0.59±0.02	1.06±0.19	0.94±0.01	2.09±0.08
Syringic acid	1.18±0.10	0.99±0.17	1.22±0.17	0.86±0.01	1.64±0.04
(-)Epicatechin	2.25±0.03	2.64±0.12	2.50±0.52	1.99±0.00	2.98±0.11
Chlorogenic acid	1.48±0.01	1.50±0.18	1.38±0.01	1.32±0.00	1.45±0.01
Caffeic Acid	19.86±0.13	17.82±0.26	18.93±0.17	16.75±0.08	20.59±0.23
p-Coumaric acid	0.32±0.00	0.32±0.00	0.31±0.01	0.26±0.00	0.40±0.01
Ferulic acid	0.18±0.00	0.13±0.00	0.18±0.01	0.15±0.02	0.17±0.00

4.4.6 Adsorbent material regeneration

Regeneration treatment did not affect the textural properties of zirconia, such as surface area and average pore diameter (see Table 4.6). Similar results were obtained by Pashova et al. (2004a and 2004b). The XRD patterns of the original and regenerated zirconia shown in Figure 4.5 confirm this result. The diffraction pattern of original zirconia is characteristic of nanoparticles of monoclinic zirconia (Southon, 2000; Liu et al., 2003). The monoclinic phase was 95% before and after the regeneration. The mean crystal sizes of original and regenerated zirconia were 9.6 ± 0.2 and 10.8 ±0.2 nm, respectively.

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Table 4.6 Physical properties of adsorbent material used during adsorption process

Material	BET surface area (m^2/g)	Micropore area (m^2/g)	Mesopore external surface (m^2/g)	Average pore diameter (nm)	Pore size distribution (%)	
					Micropore	Mesopore
ZrO_2-1	74.2±2.0	5.3±0.9	69.0±1.1	12.0±0.7	7.1±1.0	92.9±1.04
ZrO_2-2	64.2±1.4	5.9±0.9	58.4±0.1	13.7±0.0	9.2±1.13	90.8±2.23
ZrO_2-3	67.5±3.3	5.8±0.1	61.8±2.4	12.8±0.7	8.5±0.22	91.5±2.69

ZrO_2-1: Zirconium oxide without thermal treatment; ZrO_2-2: Zirconium oxide regenerated after the first adsorption process; ZrO_2-3: Zirconium oxide regenerated after the second adsorption process

The protein content in wine after treatment with original and regenerated zirconia was 13.1 ± 0.3 and 12.9 ± 0.1 mg/L. The capacity of protein removal is therefore statistically the same with both types of zirconia.

When we used a hybrid process comprising an adsorption step with zirconium oxide in a packed column followed by wine crossflow microfiltration, the permeate flux in Pinot Noir wine microfiltration increased by 15-20%. Moreover, the protein stabilisation of the wine was better with the hybrid process than with conventional microfiltration and did not affect the colour or phenolic compounds of the wine.

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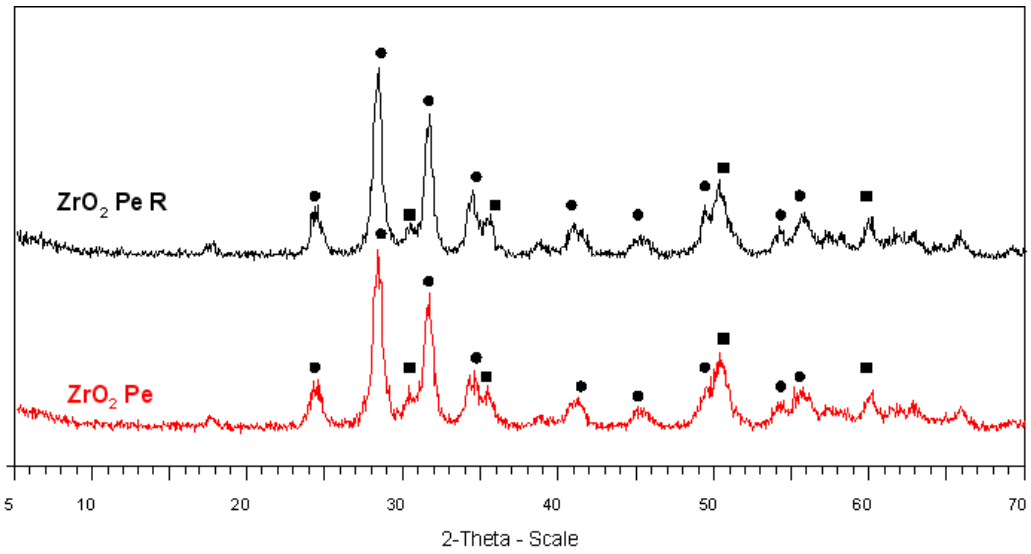

Figure 4.5 X-ray diffraction of pure (ZrO_2 Pe) and regenerated (ZrO_2 Pe R) zirconia (●: monoclinic; ■: tetragonal)

The role of proteins and polyphenols on permeate flux has not been fully explained since it was not possible to selectively reduce polyphenols and proteins using activated carbon and bentonite, respectively. However, we have demonstrated the importance of polyphenol and protein reductions on the improvement in permeate flux. The protein molecular weight range of 20 to 70 kDa may be related to unstable proteins and/or cause membrane fouling. The phenolic compounds are not significantly affected by adsorption with zirconium oxide. This is an advantage over other adsorbent materials such as bentonite. The regeneration step for the zirconium oxide did not affect its capacity for protein removal.

Acknowledgements

This work has been funded by AECI (Spanish Agency for International Cooperation), Nº A/0212/03 and Alβan programme (European Union

ISBN: 978-84-691-2703-2/DL.T.388-2008

Programme of High Level Scholarships for Latin America), Nº LX-3769-80-DKPXAHWUYT.

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Chapter 5

PROTEIN STABILIZATION IN SPARKLING BASE WINE BY ZIRCONIA AND BENTONITE: INFLUENCE ON THE FOAM PARAMETERS AND PROTEIN FRACTIONS

5.1 Abstract

Foam is an important organoleptic attribute in sparkling wines. It may be related to the nature and concentration of proteins presents in the base wines. The foam quality and protein fractions present in a Chardonnay base wine fined by zirconia and bentonite were compared in this study. Fining experiments using a continuous system of zirconia packed in a column at three retention times 7.5, 15 and 30 minutes and a discontinuous process using sodium bentonite with three added dosages of 20, 40 and 60 g/hL were performed. The wine foam quality according to maximum height (HM) and stability height (HS) parameters obtained by gas-sparging method shows a significant reduction using bentonite fining. Those wines fined by zirconia had better foam quality and in some cases was no significant difference between the unfined and fined wines. Gel filtration by FPLC shows that both fining treatments remove mainly protein fractions of 20−30 kDa, followed by 60 kDa. The fractions more than 100 kDa showed no significant reduction. The protein fractions of 20−30 kDa are related with unstable proteins which may generate haze in the white wines after bottling. This has also been demonstrated in this study since all the wines fined by bentonite were protein stables and the fractions of 20−30 kDa were

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almost removed totally. Probably the difference in foam quality of base wines fined by zirconia and bentonite could be due to the protein fractions of 20–30 kDa and 60 kDa since by the zirconia fining there was less reduction of these fractions whilst the wine foamability was better.

Keywords: Foam; sparkling base wine; zirconia; bentonite; Mosalux; FPLC.

5.2 Introduction

Always the winemakers have been focused in understanding the factors that affects the foam quality of the sparkling wines, because the foam is an important visual attribute observed by consumers.

The foamability capacity of sparkling wines can be related with the grape varieties, the harvest and even with the winemaking techniques used during fermentation process (Robillard et al., 1993; Andrés-Lacueva et al., 1996; López-Barajas et al. 1998; Puig-Deu et al., 1999; Girbau-Sola et al., 2002).

The maximum height (HM), stability height (HS) and stability time (TS) are important parameters to evaluate the foam quality in sparkling wines, which currently are studies mainly by Mosalux® gas-sparging method (Maujean et al., 1990; Robillard et al., 1993; Gallart et al., 1997). Some authors have studied the influence of polysaccharides and nitrogen compounds on these parameters, reporting that there is a direct relationship between the proteins and polysaccharides content and the foamability capacity of base and sparkling wines (Brissonnet et al., 1991; Brissonnet & Maujean, 1993; Pueyo et al., 1995; López-Barajas et al., 2001; Cilindre et al., 2007; Vanrell et al., 2007).

Often, bentonite fining is used to remove the heat-unstable proteins present in white wine that can cause hazes and deposit after bottling. However some

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studies have been demonstrated that the use of this fining agent could produce negatives effects on the foam characteristics of base wines chosen to produce sparkling wines (Andrés-Lacueva et al., 1996; Puig-Deu et al., 1999; Vanrell et al., 2007).

The last eight years we have been studying a new method to remove the unstable proteins from white wine by means of a continuous adsorption process in packed in column using zirconium oxide as adsorbent material (Pachova et al. 2004; Salazar et al., 2006; Salazar et al., 2007). Our results indicate that this new method allow to get stable wines with less negatives effects on the wine quality than bentonite (Salazar et al., 2006). Therefore currently our challenge is to know if this new method also could keep the foam quality of base wines used to make sparkling wines. For this reason the aim of this study has been to compare the protein stabilization of a base sparkling wine by zirconia and bentonite in order to how are affected the foam parameters and which could be the soluble protein fractions responsible of the foamability in a base sparkling wine.

5.3 Materials and methods

5.3.1 Wine samples

A monovarietal sparkling base wine was used in this study corresponding to Chardonnay wine, vintage 2006 made by the Bellvei Agricultural Cooperative, Tarragona, Spain. The wine samples were used immediately after fermentation and settling stages with no additional treatment.

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5.3.2 Protein haze and fining experiments

The wine protein stability was studied by a heat test at 80 °C for 2 hours, with a ΔNTU < 2 taken to mean stability such as describes Salazar et al. (2007). The difference in turbidity between the initial wine and the wine after heat test is proportional to protein instability or protein haze. The turbidity readings were done using a Turbiquant 1000IR turbidimeter (Merck KGaA, Germany).

All the fining experiments were done in duplicated. The wine was fined with zirconia by a continuous process packing zirconium oxide (Saint-Gobain, USA) on a fixed bed column. This continuous adsorption process was carried out in a 165 mm high packed column with an internal diameter of 40 mm using 40 g of granulated zirconia (tetragonal morphology, pore size of 44 nm and a BET surface area of 144 m^2/g), with a particle size of 1-2 mm. The wine was pumped by up-flow mode through the column using a peristaltic pump (Watson Marlow 101 U/R, UK). The volume of wine treated was equivalent to 100 BV or 4 L, and three residence times were considered 7.5 (T1), 15 (T2), and 30 (T3) min. After each treatment, the adsorbent material was regenerated at 500 °C for 12 h. The wine fined with bentonite was performed using powder sodium bentonite (Microcol Laffort, France). Bentonite hydrated in water at 50 g/L had been prepared 24 h before use. The treatment with bentonite considered 4 L of wine and three different doses 20 (T1), 40 (T2), and 60 (T3) g/hL.

The sparkling base wine samples untreated and treated with zirconia and bentonite were filtered by cellulose acetate membrane of 0.45 μm and after that bottling. All the bottles were stored at 4 °C until the corresponding analysis.

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5.3.3 Measurement of foam parameters

Before to measure the foam parameters the samples were degassed by magnetic stirrer for 15 min and centrifuged a 4,000 *g* at 4 °C for 5 min. The foam parameters were determined by Mosalux method such as describes Maujean et al. (1990). A glass cylinder placed on a glass-frit was filled with 100 mL of sparkling base wine. Carbon dioxide was injected into the glass cylinder through the glass-frit with a constant gas flow of 115 mL/min under a constant pressure of 100 kPa. Foam height was measured in millimeters, was controlled by photoelectric cells (infra-red beams). Each sample was analyzed in triplicated. The foam parameters measured in this study corresponded to the maximum height reached by the foam (HM) and stable height of the foam (HS). HM represented the foamability and HS the persistence of the foam collar or the wine's ability to produce stable foam. Some authors also use to measure another parameter, TS that correspond to time needed for the foam to collapse after the gas flow has stopped (Maujean et al., 1990). This parameter also represents the foam stability but we have not determined it due to the imprecision of its measurement.

5.3.4 Protein fractions

The protein fraction presents in untreated and treated wine were analyzed by fast protein liquid chromatography (FPLC) according to Canals et al. (1998). Samples of sparkling base wine previously degassed were placed on dialysis tubes of a molecular weight cut-of of 12 kDa (SIGMA, dialysis tubing-cellulose membrane, D-9652) to remove salts and other low molecular weigh compounds. The dialyzed samples were lyophilized and stored at -20 °C until these were analyzed by FPLC. Previously the FPLC analysis the lyophilized samples were suspended in 600 µL of 0.3 M ammonium acetate solution

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adjusted at a pH 7.0 and after that the samples centrifuged at 12,000 *g* at 4 °C for 2 min. The supernatant obtained was used to evaluate the protein fractions by FPLC. Afterwards each sample was separated by a Superdex 75 PC 3.2/30 column using a FPLC system (Smart system, Pharmacia, Uppsala, Sweden). The operating condition considered a sample injection of 50 µL and a flow rate of 40 µL/min using 0.3 M ammonium acetate solution as eluent. The column eluent was continuously monitored at 280 nm using a µPeak Monitor (Pharmacia, Uppsala, Sweden). Three different fractions (F1, F2 and F3) could be collected such as describes Vanrell et al. (2007). The fraction F1 corresponding to proteins with molecular weight above 100 kDa and the fractions F2 and F3 to proteins of 60 and 20-30 kDa, respectively.

5.3.5 Statistical analysis

The effect of zirconia and bentonite fining on the foam parameters of the sparkling base wine was analyzed by one-factor ANOVA using the SPSS 12 software with a significance level of $p \leq 0.05$. The results are shown as mean ± standard deviation.

5.4 Results and discussions

5.4.1 Total protein concentration and heat-protein stability

The total protein concentration present in the unfined base sparkling wine was 16.76 ± 0.81 BSA mg/L with a mean heat-protein stability of 10.31 NTU according to heat test at 80 ºC for 2 hours. Table 5.1 show these values, including also those base sparkling wines protein stabilized by zirconia and bentonite.

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Table 5.1 Effect of fining trials on the total protein concentration and heat-protein stability [a]

Wine condition	Total protein (mg/L BSA) [b]	Heat-protein stability (ΔNTU)
Unfined control wine	16.76±0.81	10.31±0.96
Zirconia/residence time		
7.5 min (T1)	14.80±0.05	4.19±0.66
15 min (T2)	13.28±0.85	3.47±0.04
20 min (T3)	10.50±0.66	1.30±0.11
Bentonite/dosage		
20 g/hL (T1)	8.25±1.65	0.13±0.11
40 g/hL (T2)	6.34±0.33	0.06±0.06
60 g/hL (T3)	6.06±0.52	0.06±0.04

[a] All the values are presented as means and standard deviation of at least two independent experiments. [b] The total protein concentration was determined by Bradford's method

According to the amount of protein removed, the base wines fined by zirconia show a protein reduction of 12, 21 and 37 % with residence times of 7.5, 15 and 30 min, respectively, while those wines fined by bentonite a higher protein reduction of 50, 62 and 64 % using dosages of 20, 40 and 60 g/hL, respectively. On the order hand according to heat-protein stability all those wine fined by bentonite were stable. In contract, the zirconia fining improved the heat-protein stability of the wines, achieving stables wines with a mean stability < 2 NTU only by a residence time of 30 min. However, in this case we only wanted determined the effect of zirconia and bentonite fining on the foam parameters whilst to know the protein fractions involves. Therefore more or less protein stability it is not really important in this case. The most important in this study has been to reveal information on the protein fractions and its possible effect on the foam parameters.

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5.4.2 Foam parameters

The foam parameters HM and HS of untreated base sparkling wines and fined by bentonite and zirconia are shown in the Figure 5.1 and 5.2, respectively. In general terms, the wine foam quality according to maximum height (HM) and stability height (HS) parameters obtained by gas-sparging method shows a significant reduction using bentonite fining (Figure 5.1 and Figure 5.2). However, those base wines fined by zirconia had better foam quality and in some cases was no significant difference between the unfined and fined wines, mainly regarding to persistence of the foam (Figure 5.2). Therefore, in this study has been demonstrated that bentonite fining can affects seriously the foam quality of the base sparkling wines, which has also been previously reported by Puig-Deu et al. (1999) and Vanrell et al. (2007).

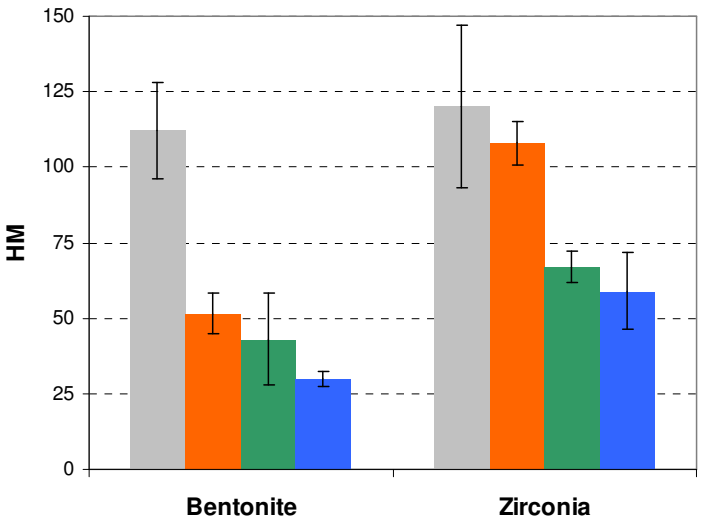

Figure 5.1 Foamability (HM) for both unfined base sparkling wine and fined base sparkling wines by bentonite and zirconia (■: unfined control wine; ■: T1; ■: T2, ■: T3).

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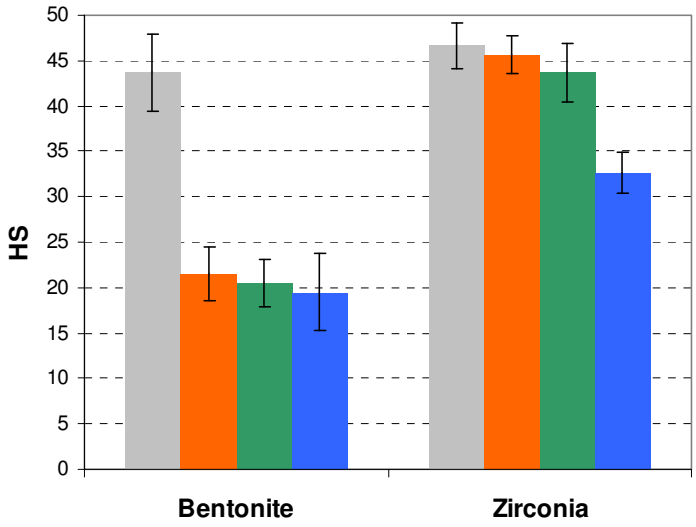

Figure 5.2 Persistence of the foam (HS) for both unfined base sparkling wine and fined base sparkling wines by zirconia and bentonite (■: unfined control wine; ■: T1; ■: T2, ■: T3).

Furthermore, there was a proportional relationship between the total protein content and the foam quality of the base wines, which to agree with the published by López-Barajas et al. (2001), Dambrouck et al. (2005), Cilindre et al. (2007). This last observation can be explained comparing the foam quality and total protein concentration of the wines treated by zirconia and bentonite, since all the wines treated by zirconia shows a higher total protein concentration whilst a better foam quality than those wines treated by bentonite (Table 5.1). Concretely an excessive reduction of the total protein concentration of a base sparkling wine can affect drastically its foamability.

5.4.3 Protein fractions

The effect of both fining trials on the protein fractions of base sparkling wine are shown in the Figures 5.3, 5.4 and 5.5. Overall gel filtration by FPLC shows

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that both fining treatments remove mainly protein fractions of 20-30 kDa, followed by 60 kDa. The fractions greater than 100 kDa showed no significant reduction. The protein fractions of 20–30 kDa may include pathogenesis-related proteins such as Thaumatin-like proteins and Chitinases, unstable proteins that can generate haze in the white wines after bottling (Waters et al., 1996; Waters et al., 1998; Pocock et al., 2000; Høj et al., 2000; Ferreira et al., 2004). In fact the mentioned above has also been demonstrated in this study since all the wines protein stables showed a meaningful reduction of the fractions of 20–30 kDa (see Tables 5.1 and 5.2, and Figures 5.3, 5.4 and 5.5).

The foam quality of the base sparkling wines against the removal of protein fractions by bentonite and zirconia could make known which are the protein fractions responsible of the foamability.

From a first approach we could believe that the foam quality in the base sparkling wines (Figures 5.1 and 5.2) is associated with the protein fractions of 20–30 kDa and 60 kDa. Since these fractions were greatly removed by both fining trials (Table 5.2). Nevertheless, even though these protein fractions also were removed by the zirconia fining, the base sparkling wines treated with zirconia showed a better foam quality. Therefore it is not awfully clear whether the protein fractions of 20–30 kDa could are also related with the foamability of base sparkling wine. Perhaps the protein fractions most important could be those of 60 kDa.

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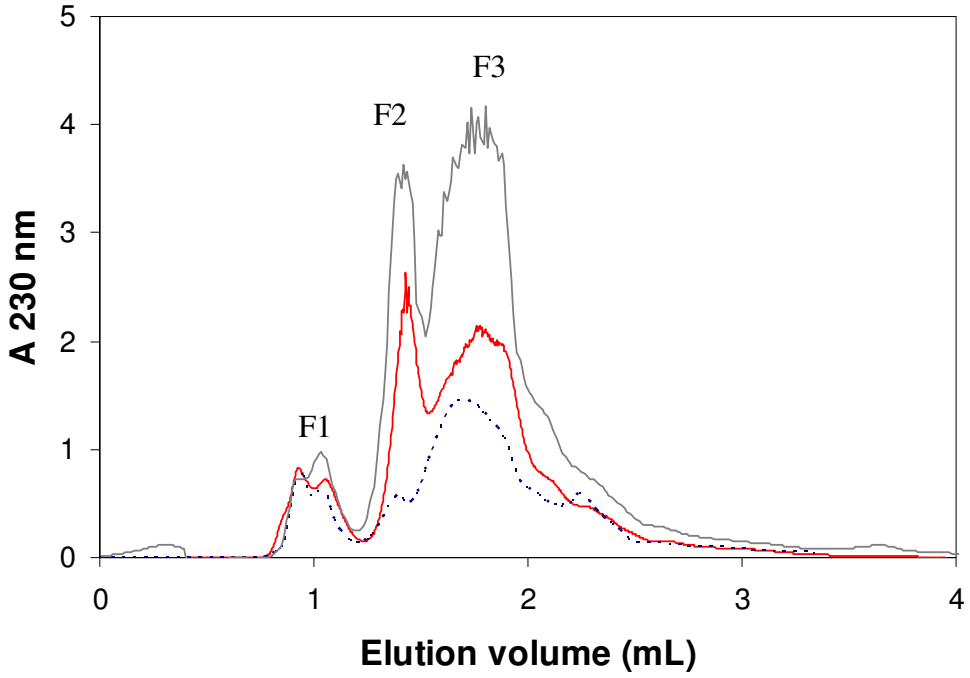

Figure 5.3 Gel filtration chromatogram of base sparkling wine (–, unfined base wine; –, base wine fined by zirconia at a residence time of 7.5 min; ---, base wine fined by bentonite with a dosage of 20 g/hL)

Otherwise, analyzing particularly the bentonite fining we can observer that with a bentonite dosage of 20 g/hL the protein fraction F2 of the unfined base wines was reduced almost completely and even undetectable in those base wines treated by bentonite dosages of 40 and 60 g/hL. Under this assumption we could suppose that the fraction F2 play a roll most important on the foamability than the fraction F3. However this fact has not been absolutely clarified by this study, we believe that should be applied another study on a proteins analysis more detailed.

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Definitely the fraction F1 was not affected significantly by both fining trials. Probably this fraction is constituted mainly for proteins uncharged electrically at pH of the wine, being electrically repulsed by both fining agents (Vanrell et al., 2007). This fraction could be composed majority for polysaccharides or mannoproteins (Waters et al., 1994), which evidently also play an important roll on the wine foamability (Brissonet & Maujean, 1991 and 1993).

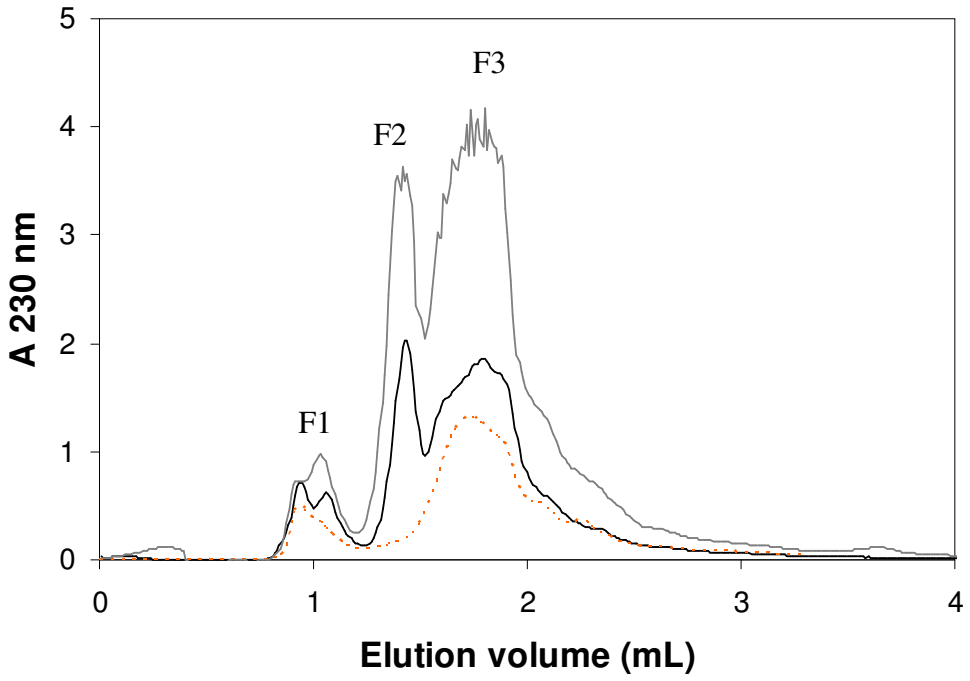

Figure 5.4 Gel filtration chromatogram of base sparkling wine (–, unfined base wine; –, base wine fined by zirconia at a residence time of 15 min; ---, base wine fined by bentonite with a dosage of 40 g/hL)

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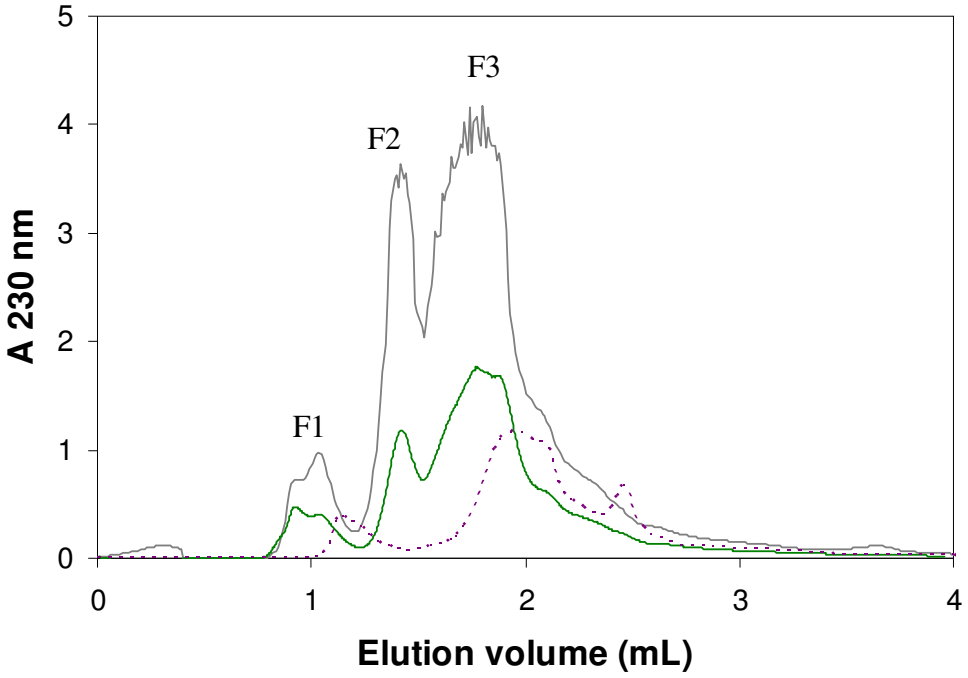

Figure 5.4 Gel filtration chromatogram of base sparkling wine (–, unfined base wine; –, base wine fined by zirconia at a residence time of 30 min; ---, base wine fined by bentonite with a dosage of 60 g/hL).

In conclusion the bentonite fining reduces majority protein fractions of 60 kDa, followed by 20-30 kDa. Unlike the zirconia fining reduces mainly protein fractions of 20-30 kDa followed by 60 kDa and definitely the base sparkling wines treated by zirconia shows a better wine foamability.

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Table 5.2 Effect of bentonite and zirconia fining on protein fractions of base sparkling wine [c]

Protein fraction (mg/L BSA)	Unfined control wine	Bentonite fining			Zirconia fining		
		20 g/hL	40 g/hL	60 g/hL	7.5 min	15 min	30 min
F1	0.39±0.04	0.42±0.08	n.d.	n.d.	0.45±0.11	0.39±0.02	0.32±0.03
F2	0.50±0.06	0.44±0.04	n.d.	n.d.	0.45±0.09	0.38±0.06	0.28±0.02
F1+F2	0.89±0.07	0.86±0.13	0.68±0.06	0.53±0.06	0.90±0.20	0.33±0.08	0.60±0.01
F3	2.55±0.16	0.56±0.08	0.14±0.02	0.12±0.02	1.74±0.20	1.41±0.05	0.96±0.17
Total Protein	4.32±0.29	2.28±0.17	0.82±0.08	0.65±0.60	3.53±0.60	2.95±0.22	2.17±0.20

[c] All the values are shown as the mean ± s.d. of at least two independent experiments. n.d., the protein fractions F1 and F2 were evaluated jointly.

Abbreviations used

BV, bed volume (volumetric ratio between the wine treated and the zirconia used at process time determined); NTU, nephelometric turbidity units; ΔNTU, difference of nephelometric turbidity units between the wine before and after thermal test applied. F1, high molecular weight protein fraction (MW > 100 kDa); F2, intermediate molecular weight protein fraction (MW = 60 kDa); F3, low Intermediate molecular weight protein fraction (MW = 20–30 kDa).

Acknowledgements

The authors are grateful for the financial support provided by the Spanish Ministry of Education and Science (AGL2006-07034/ALI) and The Spanish Agency for International Cooperation (AECI A/4726/06)

ISBN: 978-84-691-2703-2/DL.T.388-2008

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ISBN: 978-84-691-2703-2/DL.T.388-2008

Chapter 6

WHITE WINE CONTINUOUS PROTEIN STABILIZATION: PRELIMINARY RESULTS ON INDUSTRIAL SCALE

6.1 Abstract

In this work are shown the first results obtained on industrial scale of the white wine continuous protein stabilization using zirconia as adsorbent material. A monovarietal Chardonnay wine, vintage 2006 was used in this study. The results have demonstrated that is viable to stabilize this wine by batch and continuous system packing zirconia on a fixed bed column. The protein removed was around 40-45 %, achieving stables wines with a mean stability < 2 NTU and least effect on their physicochemical properties.

Keywords: Heat-unstable wine protein; packed column; adsorption; zirconia; continuous process, batch process.

6.2 Materials and methods

6.2.1 Wine samples and adsorbent material

A monovarietal white wine was used in this study corresponding to Chardonnay wine, vintage 2006 made by the Bellvei Agricultural Cooperative, Tarragona, Spain. The wine samples were used immediately after fermentation and settling stages with no additional treatment.

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The zirconia was supplied by Saint Gobain NorPro (Staw, OH, USA). In this occasion was tested a new material made exclusively for this study. The main difference was regarding to the shape and morphology. Concretely the current material were small disks with a diameter of 3 mm and a thickness of approximately 1 mm, a pore size of 3.6 nm, a surface area of 227.4 m^2/g and with amorphous morphology.

6.2.2 Wine protein adsorption on industrial scale

The wine was protein stabilized by continuous and batch system placing 6.5 L of zirconia on a fixed bed column (12.9 cm of internal diameter and 50 cm of high).

In this occasion, the first experiments were unsuccessful since the material showed a low capacity for removing protein from wine. Therefore we have decided treat thermally the material at 500 ºC for 12 hours before use, considering that the material showed an amorphous structure. The physical properties of the material before and after thermal treatment were performed by BET method and X- ray diffraction (see below in the point 6.2.5).

6.2.3 Continuous and batch protein adsorption on fixed bed column

A first treatment of the wine (Test 1) was carried out by continuous system with a flow rate of 20 L/h using a residence time of 20 min. The volume of wine treated was 550 L equivalent to 85 times the volume of the column (85 BV). A second treatment of the wine (Test 2) was performed by batch system with feedback of 300 L/h. The volume of wine treated was 1000 L. Figure 6.1 shows a schematic diagram of wine protein adsorption on a fixed bed column by continuous and batch system.

ISBN: 978-84-691-2703-2/DL.T.388-2008

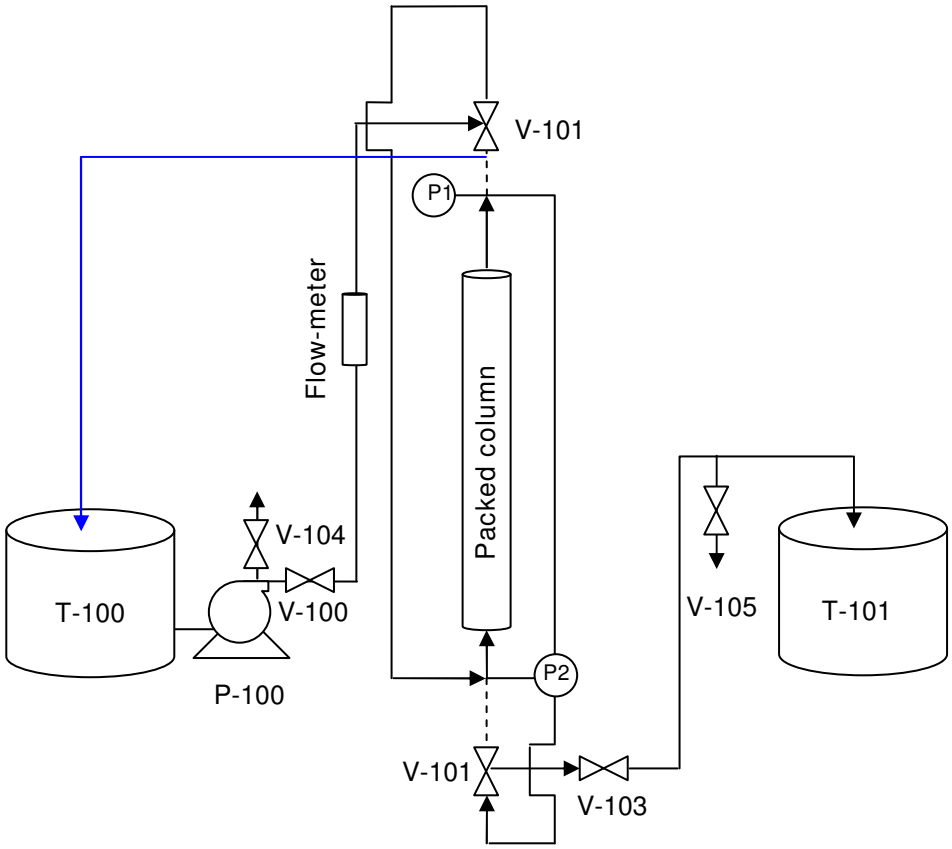

Figure 6.1 Schematic diagram of the wine protein adsorption on a fixed bed column by continuous and batch system. The blue line shows the feedback way.

6.2.4 Heat-protein stability and physicochemical properties of the wine

The wine protein stability was studied by a heat test at 80 °C for 2 hours, with a ΔNTU < 2 taken to mean stability such as describes Salazar et al. (2007). The difference in turbidity between the initial wine and the wine after heat test is proportional to protein instability or protein haze. The turbidity readings were done using a Turbiquant 1000IR turbidimeter (Merck KGaA, Germany).

The physicochemical properties of wine were determined by an infrared technique using WineScan^{TM} FT120 Basic (Foss, Denmark) and the total protein by Bradford's methods.

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6.2.5 Physical properties of zirconia

The surface properties of zirconia were studied with Brunauer-Emmett-Teller (BET) model adsorption with liquid N_2 using a surface analyzer (Micromeritics ASAP 2000, USA) and assuming a cross-sectional area of 0.162 nm^2 for nitrogen. Before the adsorption measurements were taken, the samples were outgassed under a vacuum of 0.001 mbar at 120 ºC. The morphology of the material was studied by X-ray diffraction (XRD) using a nickel-filtered Cu $K\alpha_1$ radiation (λ=1.5418 Å) in the 2θ range 10-70º through a SIEMENS D5000 diffractometer.

6.3 Results and discussions

6.3.1 Physical properties of zirconia

The thermal treatment of zirconia at 500 ºC for 12 hours affects significantly the physical properties of zirconia, such as surface area and average pore diameter. After thermal treatment at 500 ºC for 12 hours the zirconia decreased its surface area from 227.4 m^2/g up to 108.5 m^2/g. Unlike the average pore size was increased from 3.6 nm up to 6.2 nm.

The adsorption isotherm shows as an increase of the average pore size improved the adsorption capacity of zirconia, which occur also later during the wine protein adsorption (Figure 6.2).

The morphology of material also was modified. According to XRD the original zirconia was characterized as nanoparticles amorphous and the zirconia treated thermally as tetragonal (91.8 %) and monoclinic phases (8.1 %).

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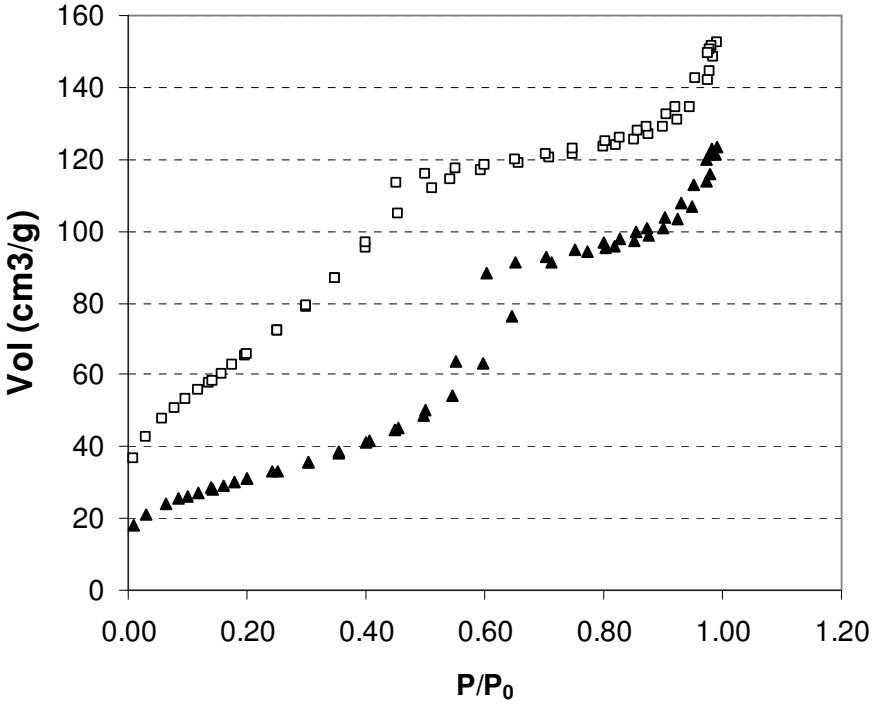

Figure 6.2 N_2 adsorption and desorption isotherms at 77K of original zirconia and treated thermal thermally at 500 ºC for 12 hours (□, original zirconia; ▲, zirconia treated thermally).

6.3.2 Wine protein adsorption on industrial scale

Figure 6.3 shows the protein concentration of treated wine by continuous process. A total protein concentration of 18 mg/L present in the unfined wine was reduced up to 10 mg/L by this continuous system achieving wine protein stable with a mean stability < 2 NTU.

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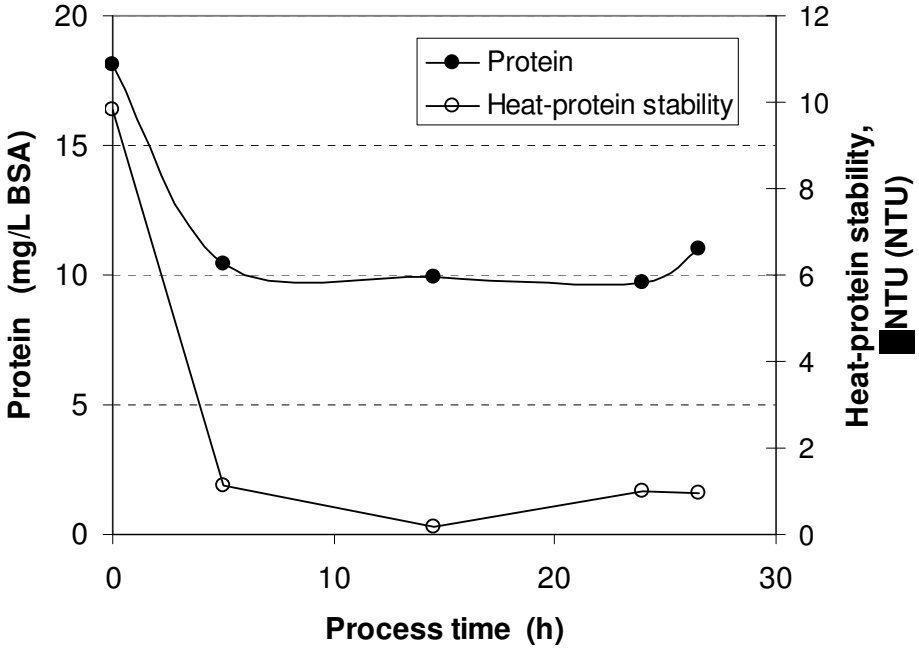

Figure 6.3 Total protein content and heat-protein stability of treated wine by continuous process

Figure 6.4 shows the protein concentration of treated wine by batch process. In this case was necessary reduced up to 12 mg/L the initial protein concentration of unfined wine and process time of 70 hours to achieve wine protein stable with a mean stability < 2 NTU.

The comparison between both treatments shows similar process times considering that to stabilize 550 L of wine by continuous process it is necessary around half an hour and to stabilize 1000 L by discontinuous process approximately the double time around 70 hours.

ISBN: 978-84-691-2703-2/DL.T.388-2008

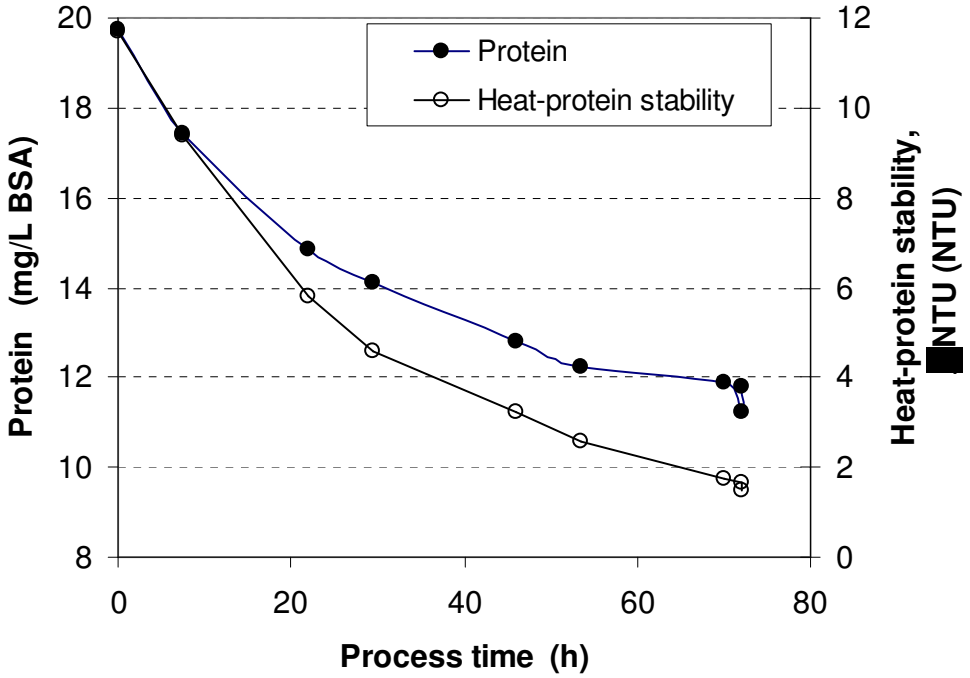

Figure 6.3 Total protein content and heat-protein stability of treated wine by batch process

6.3.3 Physicochemical properties of wine

The parameters physicochemical of wine was not affected by both treatments, although insignificant changes were observed concerning to color, total and volatile acidity, which are in concordance with the results obtained previously on laboratory and pilot scale (see Table 6.1).

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Table 6.1 Physicochemical properties of the Chardonnay wine treated by continuous and batch system

Parameter	Unfined control wine	Test 1	Test 2
pH	3.33	3.37	3.37
Total acidity, g/L H_2SO_4	3.98	3.77	3.82
Volatile acidity, g/L acetic acid	0.38	0.34	0.37
Volumetric alcohol degree, % v/v	12.51	12.53	12.52
Sugar reducers, g/L	1.99	1.65	1.94
Density, g/L	0.9903	0.9903	0.9903
Total SO_2, mg/L	62	51	60
Free SO_2, mg/L	8.5	8.0	8.0
Malic acid, g/L	0.90	0.79	0.82
Gluconic acid, g/L	0.075	0.070	0.075
Total polyphenols, mg/L gallic acid	177	167	176
Absorbance at 420 nm	0.132	0.126	0.122

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ISBN: 978-84-691-2703-2/DL.T.388-2008

Chapter 7

CONCLUSIONS

The main conclusion of this thesis is that an industrial scale continuous process of white wine protein stabilization using zirconium oxide (zirconia) as the adsorbent material packed in column is viable and a real alternative to traditional bentonite fining.

We also conclude that:

- Zirconia can be regenerated by thermal and chemical treatments, since its physical, morphological and chemical properties are not altered. Even its protein adsorption capacity can be increased probably because some compounds or an active centre derived from wine proteins are adsorbed.

- The removal of heat-unstable proteins from white wine can be controlled better by the continuous process using zirconia than by the discontinuous process using bentonite.

- The continuous process using zirconia removes fewer polyphenolic compounds from white wine than bentonite fining.

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- The physicochemical properties of white wines fined by the continuous process using zirconia are similar to those of white wines fined at low bentonite rates.

- The sensory properties of white wines fined by the continuous process using zirconia are similar to those of white wines fined at low bentonite rates. However, the white wines stabilized by zirconia score slightly better than wines fined by bentonite according to the rating system test.

- A hybrid process consisting of column adsorption (with zirconia) and crossflow microfiltration (with a ceramic membrane) reduces the heat-unstable proteins in wine and increases the permeate flux during wine microfiltration. Furthermore, the phenolic composition of wine is not affected by zirconia adsorption or the microfiltration process. The proteins with a molecular weight range of 20–70 kDa can be related to heat-unstable proteins or cause membrane fouling.

- White wine continuous protein stabilization by zirconia can also be useful for stabilizing proteins in base sparkling wines. As far as the foam quality of base sparkling wines is concerned, zirconia gives better results than bentonite fining.