

Application of Citrus Bioadsorbents as Wine Clarifiers

J. García Raurich¹, A. Vázquez Ricart², M. Pallarès Andreu³, P. Monagas Asensio⁴,
M.P. Almajano Pablos⁵

CRESCA (Research Centre in Food Control and Security of the UPC). ESEIAAT Colom 1, 08222-Terrassa

Abstract— *In recent years, reuse and recycling has taken on an increasingly important role in our society. As a result, there has been an increase in research and development of sustainable technologies. The experience acquired by the CRESCA team in the study of the revaluation of orange peels and lemon have allowed him to have a vision of this by-product as a raw material that, with the opportune treatments, can be origin of products of high added value. In this sense, very satisfactory results have been achieved for different fields of application such as:*

- a) *Agricultural: As water adsorbents, obtaining better results than conventional products (silica gel).*
- b) *Wine: As an alternative wine clarifier to products currently used (gelatin, potato protein, egg albumin, etc.)*
- c) *Treatment of wastewater with high metallic load: As heavy metal adsorbents (Ni, Cu, Pb, etc.)*
- d) *Wastewater Treatment of textile industry: as adsorbent of organic dyes.*

This paper proposes the use of orange peel and lemon, after being subjected to a process physicochemical, as clarifiers of wine and compared the results with those obtained with vegetable protein, gelatin and bentonite.

Keywords— *absorption, lemon, orange.*

I. INTRODUCTION

The Spanish law 24/2003 of the Vine and the wine (BOE of 11 July 2003), defines the wine as the natural food obtained exclusively by the alcoholic fermentation, total or partial, of fresh grapes, crushed or not, or of grape must. One of the biggest problems in the manufacture of wine is the residues of grapes, microorganisms and fermentation remains that are deposited in the bottom of the bottle.

Limpidity and stability are achieved both by physical procedures (cold, filtering, centrifugation, racking) and chemical (clarification). While the physical procedures allow to extract or eliminate the particles that cause the turbidity and sedimented microorganisms, thus obtaining the biological stability, the chemists allow to achieve the desired physicochemical stability.

Artificial (or provoked) clarification consists of introducing certain colloidal substances to the wine, which, flocculating, increase their size and deposit themselves in the bottom of the vessels, dragging with them (by adsorption and partly by action Mechanical) the particles scattered in the wine (Hidalgo 2003).

The clarifying agents are selected depending on the item you want to remove. If an excess of astringent and/or drying polyphenolic compounds is detected in the wine, it is advisable to add high molecular weight proteins (such as long-chain gelatines or egg albumin). These will adsorb unwanted compounds and Eliminated by getting a smooth effect on the final wine. On the contrary, if the wine has a protein instability accused it is advisable to add inorganic compounds (such as bentonite or silica gel) so that during the process drag this excess protein and achieve the stability sought (Ribéreau-Gaiusn et al., 1982).

The use of clarifying agents is regulated by Regulation (EC) No 606/2009, which determines the substances that can be used. Currently, they are allowed: food gelatine, protein materials of vegetable origin from wheat, pea and potato; Casein; Fish tail; Potassium caseínatos; Egg albumin; Bentonite; Silicon dioxide in the form of a gel or colloidal solution; Kaolin; Tannins Pectolytic enzymes and enzyme preparations of betaglucanase.

On the other hand, there is a wide variety of materials available in large quantities that have been proposed as adsorbents: natural products, agricultural waste of food industries, among others. In many cases, these residues have been processed to obtain active charcoal, for example, coconut residues (Selomuya et al., 1999) or sugarcane (Mohan and Singh, 2002).

The current trend is the use of agro-industrial waste as an alternative for the preparation of biosorbent materials, since they are cheap and effective in the elimination of heavy metal ions (Fu and Wang, 2011). When processed by physicochemical methods (Vijayaraghavan and Balasubramanian, 2015), cation exchange is the mechanism accepted in the case of the removal of metal ions.

Another area of use of bioadsorption as an alternative process (economic and with acceptable environmental impact) is that of wastewater from the textile industry. Traditionally, these wastewaters have been treated with physical and chemical processes that are costly to eliminate the colorants present. These processes incur operating and maintenance expenses that most small industries are unable to absorb (Lu et al., 2010) (Simphiwe et al., 2012). It should be noted that synthetic dyes are widely used in different types of industries: textiles, paper, pharmaceutical, food, cosmetics, etc., using, approximately 10,000 dyes and pigments of which almost 70% are type azo dyes.

The structural complexity of these xenobiotics compounds translates into a low percentage of elimination of the same in conventional treatment plants, which is why they are discharged without being treated (Gupta and Sahas, 2009). In this way, they provoke different impacts on the environment, producing variations in the waters in terms of suspended solids, ionic load, toxicity, dissolved oxygen concentration, color.

Adsorption is a new treatment option (Wang and Li, 2007) (Afsin, 2007) because it is a substance separation operation, which is done by putting in contact a fluid with a solid adsorbent. This is a surface phenomenon by which the sorbate is retained on the outer surface and the inner pores of the solid (Wang and Zhu, 2007). The superficial retention of these organic molecules is explained by a four-stage mechanism: diffusion of the dye towards the surface of the bioadsorbent; diffusion of the dye through the pore of the bioadsorbent; start of the dye bioadsorption process and final dye bioadsorption process. (Sivakumar and Palanisamy, 2010).

For its part, the chemical procedure of clarification is a process of attraction between the positive loads of the clarifying agents and the negatives of the impurities of the wine so that, by attraction, conglomerates are formed that precipitate at the bottom of the deposits in the form of flocs. This process is carried out after the malolactic fermentation, when the wine presents the highest concentration of solid materials in suspension. The doses used depend on the clarifying agent used and the type of wine treated.

II. MATERIAL AND METHOD

It was determined the behavior of bioadsorbents obtained by physicochemical treatment of orange peel and lemon and was compared with those of bentonite, vegetable protein and gelatin, products that have been used for many years to reduce proteins present in the wine; astringency due to the presence of tannins, or components that can easily oxidize.

The bentonite was supplied by Agrovín (Alcazar de San Juan, Ciudad Real, Spain), combining a good clarifying action with a high capacity of protein elimination. It is presented as a beige granular.

Laffort España S.L. supplied the vegetable protein used with the name of Vegcoll. It is a clarifier based on vegetable proteins extracted from the potato that is presented in a pulverulent form of beige color. It has a high clarification capacity, a high sedimentation speed and a high elimination of astringent tannins. Gelatine was also supplied by Laffort España S.L. With the name of Gecoll, it is a liquid gelatin of porcine origin. It guarantees a specific action in the elimination of the tannins responsible for the astringency.

The orange and lemon peels were obtained from the local trade. Both were subjected to a physicochemical process through which all sugars contained in the albedo were extracted, as well as the sufficient amount of pectins to achieve a degree of useful consistency to obtain a final product with characteristics of cationic exchanger.

Following the recommendations of the suppliers, the doses of the selected clarifying agents were 18 mg of bentonite; 8mg of gelatin and protein 8 mg in 100 mL of wine. For this reason, the behavior of Bioadsorbents was determined in the following concentrations: 8mg; 13 mg and 18 mg in 100 mL of wine.

2.1 Physicochemical treatment of citrus peels

The treatment begins with the collection of orange and lemon peels, followed by a wash with soap and water to remove the wax and resins that are applied to ensure a better appearance for sale.

Once the wash is applied, the shells are dried in an air current at 50 ° C until constant weight, and then the grinding with an ice crusher is obtained, until obtaining particles with a particle size between 500-1000 µm.

With this particle size, chemical treatment is proceeded. It should be noted that lignocellulosic materials are mostly formed by cellulose, hemicellulose, pectin and lignin. These polymers of long, branched or linear chains, are present in the cell walls of plants and are the main responsible for the adsorption of both metal ions (Galant et al., 2014) and macromolecules (Xu et al., 2013).

The chemical treatment begins with a process of acidification of the shells by hydrochloric acid, aimed at the extraction of pectin. Although obtaining pectin from orange peels has been studied extensively (Fishman et al., 2003), (Msebahi et al., 2005), (Liu et al., 2006), (Yeoh et al., 2008), extraction of pectins by conventional methods is carried out at close temperatures at 90 ° C for at least one hour in acidic aqueous solutions (Fishman and Cooke, 2009), so that the pectins are extracted that are not sensitive to calcium. After a while, the resulting solution is removed from the non-soluble solid by filtration. It then mixes with alcohol and precipitates pectin (Claus, 2002).

The extraction of pectin is necessary because it forms colloids and has the property to absorb a large amount of water and, if not, the final product would not have the degree of consistency enough to be used as bioadsorbent. In conjunction with pectin, acid hydrolysis involves solubilization and degradation of carbohydrates, especially xylan and hemicellulose since glucomannan is relatively stable in acidic medium (Van Buren, 1991).

In this study, heat treatment was replaced by ultrasound treatment (US) in order to have a significantly faster methodology (Casas-Orozco et al., 2015) (Sundaraman et al., 2016). For this a US Elmasonic model LC 30 H bath was used with a fixed frequency of 37kHz and regulation of time and temperature, for a period of 45 minutes.

The acid treatment was repeated until obtaining that the remaining solid is free of sugars and soluble pectin, as well as of the fraction of hemicellulose soluble in these experimental conditions. Usually, it is enough with a repetition to get the test of reducing sugars (Fehling method) to be negative. It should be remembered that carbohydrates are components of the cell walls and are part of the structural matrix (Carpita and Mc Cann 2000).

Extracted pectin and hemicellulose soluble in acid medium, as well as reducing sugars, a treatment with distilled water was carried out, in order to remove the excess hydrochloric acid used initially. Next, it was treated in basic medium. In this way, the saponification (demethoxylation) of the non-soluble pectin in acid medium (which still remains in the shell), as well as the solubilization of the soluble fraction of hemicellulose in alkaline medium (Grace et al., 1996), is proceeded. Saponification is usually done with a treatment with NaOH 0.2 M (pH 10-11) under slow agitation for two hours at room temperature. The solid fraction is then filtered and dried is filtered and dried at 50° C.

Once Saponification is produced, the solid fraction is treated with CaCl₂ 0.2M at room temperature, for twenty-four hours keeping it in gentle agitation. This process allows to cross the polymer, to produce the formation of three-dimensional meshes in the internal part in order to increase the mechanical stability of the material.

It is proven (Arjona et al., 2018) that the saponification and cross-linking process can be carried out in a single stage with the use of a dissolution of Ca (OH)₂ 0.2M.

Analogous to what was done in acid treatment, the saponification and crosslinking process performed in this study was carried out in the US bath for a period of 45 minutes. After this treatment, the excess of Ca (II) was eliminated by means of washing with distilled water.

The biopolymers obtained were dried at 105°C and milled until obtaining a final product (bioadsorbent orange based and bioadsorbent lemon based) of particle size between 500-1000 µm.

The verification of the efficacy of this chemical treatment was carried out from the determination of the IR spectrum of the initial shells and the final bioadsorbents.

In Figure 1, the spectra go before and after the chemical treatment is shown, as far as the orange is concerned, made with a spectrophotometer Perkin Elmer model Paragoni 500.

In both spectra, the presence of the functional groups is confirmed: OH, C=O, C-O-C and C-O, as well as C-H and CH₂ before and after chemical treatment, being appreciable the disappearance of a single peak attributable to the extraction of acid-soluble pectin.

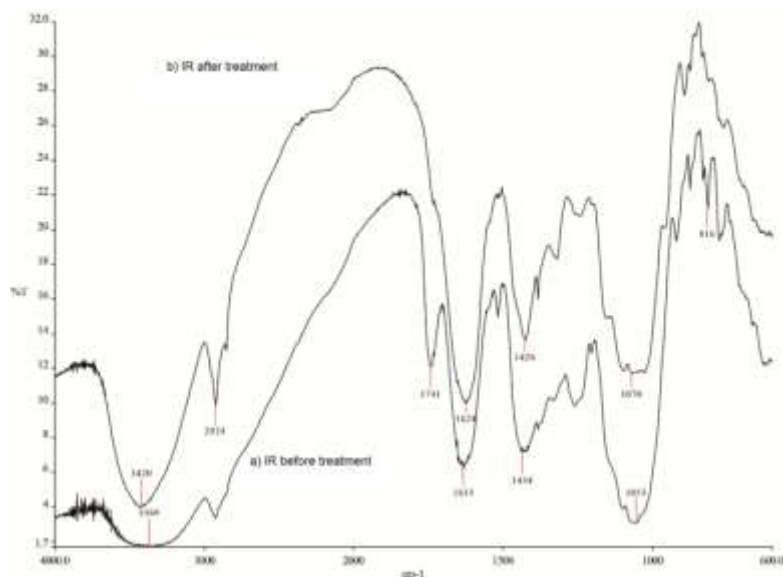


FIGURE 1. a) IR spectrum of Orange peel, b) IR spectrum of the final bioadsorbent

The broadband signal between ($3600 - 3200 \text{ cm}^{-1}$) centred at 3369 cm^{-1} in the case of orange peel before chemical treatment and at 3420 cm^{-1} after such treatment is attributable to the hydroxyl group. This group is found in cellulose, pectin, absorbed water, hemicellulose and lignin present in the shell both before and after chemical treatment. The presence of cellulose as the main component of citrus peels makes the elimination of hemicellulose not observable, for practical purposes.

On the other hand, the peak observed at 2924 cm^{-1} corresponds to the group C-H and the peaks that appear centered in 1741 cm^{-1} and in 1633 cm^{-1} that appear in the spectrum of the orange peel, before its chemical treatment are attributable to the carbonyl group (C=O) as indicators of esterified carboxylic groups and free. In fact, the disappearance of the peak at 1741 cm^{-1} in the orange peel spectrum once subjected to chemical treatment indicates the disappearance of the high-methoxyl pectins.

Finally, the peaks centered around 1430 cm^{-1} justify the presence of aliphatic and aromatic (C-H) and the peaks centered around 1060 cm^{-1} correspond to the C-O group, present in both alcohols and carboxylic acids, and with the ether group (C-O-C).

2.2 Spectral characteristics of red wines

Red wines have a maximum absorption at 520 nm , where the colour is intense, due to anthocyanins. Between 280 and 520 nm (2 maximums) there is a minimum (around 420 nm), yellow color zone. As the wine ages, the differences between the two values are diminishing, because the red color disappears and the yellowish shades appear.

The rapid method recommended by the Office International de la Vigne et du Vin (OIV, 2018) determines that the colour intensity (IC) of a wine is the sum of the absorbances of the wine, in a 1 cm thick cuvette, corresponding to the wavelengths of 420 nm , 520 nm and 620 nm . For this reason these wavelengths were chosen to carry out the study. Complementarily, the content of polyphenols was determined with absorbance at 220 nm .

The determination of the absorbance was carried out with a molecular absorptive spectrophotometer Agilent model Cary 60 and the experimental results of both bioadsorbents were compared with those obtained with the clarifiers previously selected (bentonite, vegetable protein and gelatin).

III. RESULTS

3.1 Characteristics of cationic exchangers of bioadsorbents

First, we proceeded to check the efficacy of these bioadsorbents as cationic exchangers. To this end, it was verified the elimination of Cu (II) in synthetic dissolutions of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ of different concentrations ($5, 10, 30, 100 \text{ ppm}$ of Cu^{2+}). To this end, 25 mL of each of the four dissolutions of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ were introduced in two test tubes (provided with a screw cap) in which, previously, 0.5 g of the corresponding bioadsorbent was added and kept in gentle agitation during an hour.

The determination of Cu (II) was performed with an Atomic absorption spectrometer Varian AA24OFS. The results shown in table 1 make it possible to affirm that both bioadsorbents have a high elimination capacity of Cu (II) and, consequently, present characteristics of cationic exchangers.

TABLE 1
%BIOADSORPTION OF CU (II) DEPENDING ON THE INITIAL CU (II) CONCENTRATION.

Bioadsorbent	[Cu(II)] initial mgL ⁻¹	[Cu(II)] final mgL ⁻¹	Cu (II) retained (%)
Orange	5	1,25	75
	10	1,15	88,5
	30	2,69	91
	100	3,64	96,4
Lemon	5	0,78	84,4
	10	0,82	91,8
	30	1,64	94,5
	100	2,22	97,8

3.2 Clarifying properties of Bioadsorbents

Proven to be effective as cationic exchangers of both bioadsorbents, it was determined its ability to remove organic molecules with electrical load through mechanisms of electrostatic attraction (hydrogen bridges, forces of Van Der Waals...). To this end, we acted on two red wines, one bottled and marketed and another still without bottling.

Experimentally, the centrifugation of a sample of 1L of each type of wine selected by means of a centrifuge Sorvall RC 3b Plus was proceeded. In this way, the suspended solids were eliminated, with a significantly higher sediment in the case of unbottled wine.

The liquid fraction, free of suspended solids, was stored in hermetically sealed glass jars inside a refrigerator at a temperature of 7 °C until the time of use.

The behavior was determined as clarifiers of the bioadsorbent depending on the particle size. This was done by a set of tests with different concentrations and particle sizes of bioadsorbents. The concentrations were: 8mg; 13mg and 18mg in 100mL of wine and particle size: less than 250 µm; between 250-500 µm and higher than 500 µm. Systematically, all the samples were filtered by a Millex filter of 0.45 µm to avoid the possible interference of solids in suspension, proving that the best results were obtained with the particle size between 250-500 µm. Table 2 collects the values of the reduction of absorbance obtained with bottled wine and wine without bottling for the particle size between 250-500 µm.

TABLE 2
PERCENTAGES OF REDUCTION OF ABSORBANCE OBTAINED WITH BIOADSORBENTS WITH PARTICLE SIZE BETWEEN 250-500 µm (AVERAGE VALUES OF THREE DETERMINATIONS)

Type of wine	Bioadsorbent	Wavelengths			
		220 nm	420 nm	520 nm	620 nm
Bottled	[Orange]	220 nm	420 nm	520 nm	620 nm
	8 mg/100 mL	10,89 %	1,75 %	2,26%	3,39%
	13 mg/100 mL	18,61 %	2,51 %	3,21 %	4,19 %
	18 mg/100 mL	19,80 %	3,82 %	4,44 %	6,34 %
	[Lemon]	220 nm	420 nm	520 nm	620 nm
	8 mg/100 mL	12,00 %	5,31 %	3,99 %	2,31 %
	13 mg/100 mL	20,29 %	5,54 %	4,25 %	3,51 %
	18 mg/100 mL	21,74 %	7,57 %	5,86 %	5,13 %
Non Bottled	[Orange]	220 nm	420 nm	520 nm	620 nm
	8 mg/100 mL	4,94 %	3,91 %	5,16 %	5,09 %
	13 mg/100 mL	5,38 %	5,91 %	7,37 %	7,75 %
	18 mg/100 mL	6,78 %	7,00 %	8,27 %	9,36 %
	[Lemon]	220 nm	420 nm	520 nm	620 nm
	8 mg/100 mL	6,40 %	5,28 %	6,41 %	6,43 %
	13 mg/100 mL	6,87 %	8,07 %	9,78 %	7,06 %
	18 mg/100 mL	7,54 %	12,48 %	10,67 %	7,77 %

From the results collected in this table it comes off:

- In all cases, the percentage of absorbance reduction is proportional to the concentration of bioadsorbent, both from orange and from lemon.
- In all cases, the percentage of absorbance reduction is higher when the bioadsorbent is the product obtained from lemon.
- In the area of the visible, this percentage is more pronounced in the unbottled wine.
- In the UV zone of the spectrum, this percentage is significantly more pronounced in bottled wine.

3.3 Comparison of the behavior of bioadsorbents with respect to Clarifiers

Once the clarifying capacity of both bioadsorbents was verified, they were compared with vegetable protein, gelatin and bentonite. Selected the Clarifiers and their dosage, the following protocol was followed:

- Preservation of the clarifiers in hermetically sealed containers, under the conditions described by the supplier.
- Preparation of solutions or suspensions of clarifiers.
- Incorporation of the clarifying agent in a progressive way in the wine sample.
- Action of the clarifying agent for long enough, avoiding exceeding 10 days. (During this period of time, the wine remained at absolute rest and at a constant temperature of 7°C inside a refrigerator).
- Careful separation of the sediment from the liquid, just before determining the absorbance, followed by a filtration of the liquid fraction with a Millex filter of 0.45 µm.
- Spectrophotometric determination of the absorbance decrease in the four pre-selected wavelengths.

3.3.1 Behavior of biosorbents with respect to vegetable protein Vgecoll

First, the behavior of biosorbents compared to the protein Vgecoll was compared, maintaining a constant concentration of the three products studied in 8mg/100 mL of wine.

In the case of non-bottled wine, systematically, the percentage of reduction of the absorbance obtained with the bioadsorbent from the orange peel was lower than that obtained with the bioadsorbent from the lemon peel and with Vgecoll. This behavior was also observed with bottled wine, with the exception of the wavelength of 620 nm in which the three clarifiers presented a behavior without significant differences.

The concentration of the bioadsorbents was then increased to 18 mg/100 mL of wine (maximum recommended concentration for bentonite). In these conditions, the percentage of reduction of the absorbance of bioadsorbents was higher than that of Vgecoll (8mg/100mL of wine), except in the UV zone of the spectrum in the case of unbottled wine.

3.3.2 Behavior of biosorbents with respect to Gecoll gelatin

In an analogous way to the plant protein, the concentration of the three products studied in 8mg/100 mL of wine was maintained in the first place. In these conditions, in all cases, the percentage of reduction of the absorbance obtained with Gecoll was higher than those obtained with the bioadsorbents.

When the concentration of the bioadsorbents was increased to 18 mg/100 mL of wine, the predominance of the gelatine Gecoll remained, although in a little significant way in the case of bottled wine. On the contrary, in the case of non-bottled wine, bioadsorbents showed values of percentage of absorbance reduction slightly higher than 420 and 620 nm.

3.3.3 Behavior of biosorbents with respect to Bentonite

On this occasion, the concentration of the three studied products was fixed directly at 18 mg/100 mL.

Bentonite presented the highest percentage of absorbance reduction in the UV zone, surpassing significantly the vegetal protein, gelatin and bioadsorbents, both in bottled wine and non-bottling. As far as the area of the visible spectrum is concerned, only in the case of unbottled wine, both bioadsorbents surpassed the bentonite in the wavelength of 620 nm and the bioadsorbent of lemon at 520 nm.

Table 3 shows the set of experiences with bottled wine and table 4 shows the group of experiences made with unbottled wine.

TABLE 3
PERCENTAGES OF REDUCTION OF ABSORBANCE OBTAINED IN BOTTLING WINE

λ	concentration	Bioadsorbent			Vgcoll	Gecoll	Bentonite
		Orange	Lemon				
220 nm	8mg/100mL	10,89%	12,00%		13,48%	25,00 %	
	13mg/100mL	18,61 %	20,29 %				
	18mg/100mL	19,80 %	21,74 %				<u>38,92%</u>
420 nm	8mg/100mL	1,75 %	2,31 %		2,86 %	<u>6,84%</u>	
	13mg/100mL	2,51%	3,51%				
	18mg/100mL	3,82 %	5,13 %				5,86%
520 nm	8mg/100mL	2,26 %	2,99 %		3,94 %	6,66 %	
	13mg/100mL	3,21%	4,25%				
	18mg/100mL	4,44%	5,86%				<u>8,48 %</u>
620 nm	8mg/100mL	3,39 %	3,42 %		3,68 %	<u>12,96 %</u>	
	13mg/100mL	4,19%	5,54%				
	18mg/100mL	6,34%	7,57%				8,22%

TABLE 4
PERCENTAGES OF REDUCTION OF ABSORBANCE OBTAINED IN NON-BOTTLE WINE

λ	concentration	Bioadsorbent			Vgcoll	Gecoll	Bentonite
		orange	lemon				
220 nm	8mg/100mL	4,95 %	6,40 %		9,84%	9,52 %	
	13mg/100mL	5,38 %	6,87 %)				
	18mg/100mL	6,78 %	7,54 %				<u>27,63%</u>
420 nm	8mg/100mL	3,91 %	5,28 %		6,98 %	6,39 %	
	13mg/100mL	5,91 %	7,06 %				
	18mg/100mL	7,00 %	7,77 %				<u>11,56 %</u>
520 nm	8mg/100mL	5,16 %	6,41 %		7,37 %	<u>11,38 %</u>	
	13mg/100mL	7,47 %	9,78 %				
	18mg/100mL	8,27 %	10,67 %				7,21 %
620 nm	8mg/100mL	5,09 %	6,43 %		6,37 %	8,58 %	
	13mg/100mL	7,75 %	8,07 %				
	18mg/100mL	9,36 %	<u>12,48 %</u>				7,21 %

IV. DISCUSSION

The biosorption is a physicochemical process that includes the phenomena of adsorption and absorption of molecules and ions. The biosorption occurs if the molecules or cations of the metals are joined by electrostatic interactions to the anionic sites found in the biosorbents. These sites that serve as active centers for the biosorption are located in the functional groups: carboxyl, hydroxyl, amino, sulfonic, which are part of the structure of most of the polymers of natural origin by different mechanisms (physical adsorption, complexation, ionic exchange, etc.) (Veglió and Beolchini, 1997; Volesky, 2001; Davis et al., 2003; Gravilescu, 2004; Baytak and Turkes, 2005; Zhang and Banks, 2006).

Biosorbents are natural materials available in large quantities, or certain residual products from industrial or agricultural operations, which can be used for the purpose of capturing contaminants due to their low cost (Vargas et al., 2010).

It has studied a wide range of low cost materials and potential to be used in the biosorption: Wood, clay, ashes, activated sludge, orange peel and banana (Namasivayam et al., 1996).

The lignocellulosic materials (agricultural, agro-industrial and forestry wastes) can present in their composition up to 50% m/m of cellulose, and for that reason have been quite used to obtain that biopolymer and its derivatives, for the production of papers or compounds of high commercial value, such as glucose, ethanol and others (Saha, 2003).

Some of the solids that have been employed are: barks and leaves of conifers, rice husks, walnut, peanut, orange peel, banana peel (Annadurai et al., 2002), Grapefruit husks (Hameed et al., 2008), algae, fungi, nopal, olive bones, etc. (Gupta and Suhas, 2009). However, adsorbents prepared from citrus peels are those that present higher adsorption capacities (Lu et al., 2009).

On the other hand, the clarifying agents must be free from undesirable contaminants and must comply with the applicable legislation. They must be kept in a cool and dry environment, in sealed containers or in other recommended conditions of preservation, according to the manufacturers own suggestion. The quantity of the clarifier used shall be that which corresponds to the lowest level necessary in order to achieve the desired result and in no case must exceed the applicable legislation and standards in force.

The addition of a clarifying agent to wine usually responds to three objectives (resolution OIV, 2014):

- Soften or reduce its astringency and/or bitterness;
- Clarify and eliminate proteins that produce turbidity
- Stabilize and reduce color by adsorption and precipitation of polymerized phenolic compounds and tannins.

The turbid aspect of a wine is due to the presence of scattered particles in it that intercept the luminous radiation that comes from one direction and reflects it in different directions, making them look opaque and turbid. This group of particles that cause turbidity are substances that can be found in the wine or formed during the vinification process (colouring material in colloidal state, potassium tartrate crystals, precipitates of phenolic compounds, proteins...).

Although in the time the decantation can occur in a natural way, in the first months, finished the elaboration, the sedimentation is difficult due to the amount of CO₂ that contains the wine.

The proteins of the wine, contribute to the sensation of unctuousity in the wine, to the stabilization of the foam in sparkling wines (Cava), and they fix the aromas. However, they also provoke the so-called protein bankruptcies. Unstable wine proteins are those of low molecular weight between 12.6-30 kDa and low isoelectric point between 4.1-5.8 (Waters et al., 1992). The process of protein turbidity is caused by denaturation, binding and subsequent flocculation of proteins with other wine compounds in suspension, eventually leading to precipitation in bottled wines (Waters et al., 2005).

As a result of the problems caused by mad cows, there was a movement aimed at substituting the gelatin of animal origin with vegetable proteins.

The protids or proteins are nitrogen substances of complex constitution that in the water give colloidal dispersions. With the pH values of the wine, between 2.8-4 units, they act as electropositive colloids. Employees as clarifiers, are added to the wine in a state of colloidal dispersion, and when coagulating and sedimentation occurs limpidity (Ribéreau-Gaiusn et al., 1984).

The reactions of tannins and protein correspond to flocculation, i.e. the association of particles between them and the formation of flocs which are assembled and precipitated. Proteins that do not react with tannins can be combined with suspended particles or colloidal solution that are negatively charged (most turbid particles in wines have negative charges), producing the Mutual flocculation of the two colloids (neutralization of loads).

In the case of white wines the only really effective system to avoid protein haze is the elimination of unstable proteins, which can only be achieved with the treatment with bentonite or ultrafiltration (Ribéreau-Gaiusn et al., 1999a) (Hsu ET AL., 1987). However, the clarification with bentonite is a process that affects the sensory quality of the wine. By eliminating most of the proteins, the wine loses structure and unctuousity (Ledoux and Dubourdiou, 1994). In addition, bentonite seriously affects the aroma of wine, since it absorbs directly or indirectly aromas (Guillou et al., 1998), taking into account that proteins are fixatives of aromas and when they are eliminated from the wine, they drag with them some of the aromas (Lubbers et al., 1993) (Lubbers et al., 1996). In addition, proteins are surface molecules and have been found to be very positive factors for foamability and the persistence of foam of sparkling wines (Pueyo et al., 1995) (Brissonet and Maujean, 1993). The alternative of ultrafiltration also significantly affects the aroma and unctuousity of wine (Ribéreau-Gaiusnet.al., 1999b) (Flores et.al., 1991).

The bioadsorbents proposed in this study are constituted by a cellulosic base containing the fraction of pectin not soluble in acid medium. Cellulose is a hygroscopic material, insoluble in water due to its high molecular weight, but it can swell in that medium, being also insoluble in dilute acids, as well as in most organic solvents (Nishinari, 2006). The subsequent alkaline treatment leads to the possibility of a physisorption mechanism because pectin and cellulose have high bonding capacities with the calcium cation.

V. CONCLUSION

The bioadsorbents obtained from the orange and lemon peels have good clarification capacities, especially the bioadsorbent of lemon.

It cannot be said that there is a universal clarifier, valid for the different components that are determined in the different wavelengths selected

As, chemically, a wine is a complex matrix, the behavior of each clarifier must be considered depending on the type of wine to be clarified.

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