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Analytica Chimica Acta 458 (2002) 197–202

ANALYTICA CHIMICA ACTA

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Removal of iron, copper and manganese from white wines through ion exchange techniques: effects on their organoleptic characteristics and susceptibility to browning

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Received 20 June 2001; accepted 30 October 2001

Abstract

Ion exchange techniques have been used to reduce the content of iron, copper and manganese in white wines. Two exchanger resins have been compared, a chelating resin, the active group of which is iminodiacetate and a Dowex, acidic cation exchange resin.

The results obtained show that the technique of using exchanger resins is extremely effective in lowering the metal content of wines, although on occasions, their use alters the organoleptic characteristics of the wine. Treated wines present lower polyphenolic and aromatic profiles than the untreated wines. Polyphenolic and metallic reductions would explain why treated wines present a notably reduced susceptibility to browning.

Keywords: Metals; White wine; Browning; Ion exchange

1. Introduction

The phenomenon called "browning" in white wines constitutes one of the principal enological problems for wine producers. In organoleptic terms, this phenomenon translates into a process of continuous oxidation, a loss of aromatic freshness and, in the final stages, in the appearance of precipitates of condensed phenolic material in the bottled wine.

It is known that there are certain species which participate significantly in the destabilization of wines and in their oxidative evolution [1,2]. Notable among these species are oxygen (the initiator of the process), polyphenols (the oxidizable matter), and certain metal ions, such as Fe, Cu and Mn (activators of the process) [3–5], which are also present. There are various enological techniques in use that seek to avoid the browning of white wines by acting on one or more of these species [6,7].

Given the proven participation of the metals present in the wine in its browning, and the variability in the concentrations in which they are found in particular wines, many studies have been directed towards determining these concentrations but also in minimising them. Notable among the techniques that seek to reduce the metal content of wine is the use of potassium hexacyanoferrate(II), which when added to the wine eliminates part of the iron content and significantly reduces the content of other metals that also participate in the browning of white wines and are highly

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0003-2670/02/$ – see front matter
PII: S0003-2670(01)01499-4

contaminating (Mn, Cu, Zn, etc.) [8]. The use of this agent carries with it the danger of the possible transformation of any excess into highly toxic cyanide [9].

In recent years, the search has begun for alternative methods to enable the metal content of white wine to be reduced without altering its organoleptic characteristics. To this end, and taking into account of the bibliographical antecedents available [10–12], two exchanger resins have been compared, one a chelating resin, the active group of which is iminodiacetate and a Dowex, acidic cation exchange resin.

2. Material and methods

Ion exchange: A total of 12 white wines have been subjected to ion exchange treatment. Two resins have been used, a chelating resin (capacity 1.1 meq. ml^{-1}; Sigma–Aldrich) the active group of which is iminodiacetate and Dowex 50WX8-100 (capacity 1.7 meq. ml^{-1}; Sigma–Aldrich) an acidic cation exchange resin. Both resins were activated with 10% HCl after which Milli-Q water was passed through them until the eluate is neutral. In both cases 90 ml was used as the resin bed volume. The volume of wine treated in all cases was 1.5 l.

Metal content: The metal content was determined by means of inductively coupled plasma-atomic emission spectrometry (ICP-AES, Philips PU7000) and atomic absorption spectrometry (AAS), i.e. flame AAS, FAAS UNICAM 939 atomic absorption spectrometer and graphite furnace AAS (GFAAS, Philips PU92000X with PU9380X autosampler and PU9390X electrothermal atomizer). Instrumental parameters for analysis were:

- *ICP-AES (Na and K)*: sample uptake rate: 1.0 ml min^{-1}, argon flow: 13 l min^{-1}, pressure of the nebulizer: 40 psi. Samples, in duplicate, were diluted 10 times with Milli-Q water.
- *FAAS (Fe, Cu, and Mn)*: the following conditions were used for determining Fe, Cu, and Mn in an acetylene-air flame: wavelengths of 248.3, 324.8, and 279.5 nm; lamp currents of 15, 10, and 12 mA; warm-up of 75, 90, and 75%; and bandpass of 0.2, 0.5 and 0.2 nm, respectively. Flow rates of acetylene and air were 1.0 l min^{-1}. Accessory for copper: atom trap (STAT).
- *GFAAS (Fe and Cu)*: the temperature program for the graphite furnace was: char at 1000 °C (Fe), and 450 °C (Cu); atomize at 2000 °C (Fe), and 1800 °C (Cu). Volume of sample injected: 10 μl.

The determinations of Fe, Cu and Mn carried out by means of FAAS were done directly, without the need for dilution, whereas those carried out by GFAAS required dilution (10 times with Milli-Q water, in duplicate).

The standard solutions were prepared by dilution of 1000 mg l^{-1} stock solutions (Merck), using ethanol (15% v/v) and Milli-Q water.

Polyphenol profile: 80 μl of wine after filtration (0.45 μm pore size) were analysed by liquid chromatography (LC) in duplicate. The elution phases used were solvent A (95% water, 5% methanol) and solvent B (95% methanol, 5% water) at pH 2.5 (extra pure sulfuric acid). The elution gradient was from 100 to 85% solvent A in 5 min; from 85 to 50% solvent A in 40 min; and isocratic elution for 35 min. The analyses were carried out using a C_{18} column (Lichropher 100 RP-18, 250 mm × 3 mm, 5 μm particle size) at a flow rate of 0.5 ml min^{-1} and detection at 280 and 320 nm.

The various polyphenolic compounds present were identified by comparison with a library of DAD spectra and retention times of standards. Commercial standards were purchased from Fluka (Buchs, Switzerland) and Eastman Kodak (Rochester, NY). Caftaric and coutaric acids were isolated by the method described by Singleton et al. [13]. Each compound was quantified by use of a calibration graph obtained with the corresponding standard, except GRP (2-S glutathionyl caftaric acid), which was quantified as caftaric. Polyphenolic compounds used in this study were gallic acid, protocatechuic acid, caftaric acid, *cis p*-coutaric acid, *trans p*-coutaric acid, GRP, tyrosol, *p*-hydroxybenzaldehyde, chlorogenic acid, catechin, epicatechin, vanillic acid, protocatechualdehyde, caffeic acid, syringic acid, *cis p*-coumaric acid, *trans p*-coumaric acid, *i*-ferulic acid and ferulic acid.

Susceptibility to browning: Quantities of 120 ml of wine were subjected, in duplicate, to a process of electrochemical oxidation using equipment devised by our research group [14]. In this, the susceptibility to browning is quantified as the increase in absorbance recorded at 420 nm (y) against the electrical current (x) applied [15]. This equipment allows the

susceptibility of a wine to browning to be determined rapidly and reliably, without having to wait the length of time taken for this phenomenon to evolve naturally.

Aromatic profile: The aromatic profiles were determined in duplicate, using a prior stage of continuous rotary liquid–liquid extraction for 150 min (0.8 rpm.). The extraction was performed on 100 ml of wine diluted to 200 ml with water. The mixture was saturated with NaCl, at which time 50 μl of the internal standard, 4-methyl-2-pentanol, was added. A mixture (2:1) of ether–*n*-pentane (90 ml) was used as the organic extractant. The extract obtained was concentrated under an atmosphere of N_2 in a TurboVap II (Zymarck) station until reaching a final volume of 2 ml.

Subsequently, the extract obtained was subjected to gas chromatography using a HP 5890 series II gas chromatograph with flame ionisation detection (FID). The injection volume was 1 μl with splitless for 0.5 min. The column used was a JW-DBWAX of 60 m and 0.25 mm internal diameter. Split flow was 30 ml min^{-1} and purge flow was 1.5 ml min^{-1}. The carrier gas used was helium (column head pressure of 14 psi). The temperature of the detector during the analysis was 250 °C, while the injector was held at 200 °C. The temperature gradient used began at 45 °C for 20 min, and was raised to 95 °C at 10 °C min^{-1}. After 1 min, it was increased to 130 °C (2 °C min^{-1}). This temperature was held for 1 min, and then increased to 210 °C (1 °C min^{-1}) and held at this temperature for 20 min.

A Voyager (Thermoquest) gas chromatograph with a mass detector (electronic impact and quadrupole) was used for the identification of the various signals obtained. The signal was recorded and processed with Masslab software supplied with the Wiley 6.0 MS library. Chromatography conditions were as before. Peak identification was carried out by analogy of mass spectra and confirmed by retention indices of standards when they were available or by retention data from the literature. Quantitative data from the identified compounds were obtained by measuring the relative peak area in relation to that of 4-methyl-2-pentanol, the internal standard. The concentration of those compounds for which there was a standard available was obtained by means of a calibration graph. For those compounds whose standard was not available, the relative peak area was used.

Statistical treatment: Variance analyses and principal component analysis were performed on the metal content, polyphenol and volatile data from the replicated samples using the Statgraphics Statistical Computer Package "Statgraphics Plus 3.1" for Windows95.

3. Results and discussion

The data in Table 1 give the contents found for Fe, Cu and Mn in the various wines studied. The range of concentrations found in the untreated wines was fairly wide (6.07–0.830 mg l^{-1} for Fe, 0.359–0.014 mg l^{-1} for Cu, and 1.29–0.111 mg l^{-1} for Mn). Both tested resins demonstrated the ability to reduce dramatically the metal content of the treated wines, as can be appreciated from Table 1. The reductions in the content of Fe and Mn reached around 90% of the original values, while in the case of Cu, the scale of reductions was somewhat less.

The variance analysis conducted on these reductions in metal content (Table 1) revealed significant differences between the two treatments in respect of the Mn content removed from the wines ($P = 0.0052$). The resin with the iminodiacetate chelating group removed a greater quantity of Mn from the wines. There were no significant differences between the resins in respect of the quantities of iron and copper removed from the wines. It would appear therefore, that the two treatments could be employed with equal success in obtaining white wines with a reduced metal content and with less tendency to undergo browning.

Fig. 1 shows the behaviour observed under the accelerated browning test by two of the wines studies, before and after being treated with the ion exchange resins. It can be observed that the samples that exhibit less susceptibility to browning are those corresponding to the treatment with the chelating resin. The wines treated with the Dowex resin show an intermediate degree of susceptibility. Similar results were obtained for the rest of the wines considered in this study.

At this stage we proceeded to assess the effects of these treatments on the treated wines organoleptic characteristics. The variance analysis conducted on the data corresponding to the polyphenolic profiles of the various wines considered in this study reveal that the ion exchange techniques applied produced

Table 1
Metal content found for wines before and after resin treatment[a]

Samples		Fe ($mg\,l^{-1}$)	Cu ($\mu g\,l^{-1}$)	Mn ($mg\,l^{-1}$)	Diminution Fe (%)	Diminution Cu (%)	Diminution Mn (%)
Wine 1	Initial	5.41	67.6	0.512			
	Dowex	0.101	33.2	0.042	98.1	50.9	91.8
	Iminodiacetate	0.292	33.2	<0.015	94.6	50.9	97.1
Wine 2	Initial	2.92	48.0	0.451			
	Dowex	0.091	22.4	0.092	96.9	53.3	79.6
	Iminodiacetate	0.290	16.4	<0.015	90.1	65.8	96.7
Wine 3	Initial	1.33	44.0	0.161			
	Dowex	0.020	20.1	<0.015	98.3	54.5	90.7
	Iminodiacetate	0.045	17.6	<0.015	96.6	60.0	90.7
Wine 4	Initial	0.832	187.2	0.111			
	Dowex	0.028	24.8	<0.015	96.6	86.7	86.5
	Iminodiacetate	0.073	22.4	<0.015	91.2	88.0	86.5
Wine 5	Initial	2.59	359.1	0.281			
	Dowex	0.283	74.2	0.035	89.1	79.4	87.5
	Iminodiacetate	0.275	74.3	<0.015	89.4	79.4	94.7
Wine 6	Initial	1.60	37.0	0.762			
	Dowex	0.036	13.5	0.052	97.7	63.5	93.2
	Iminodiacetate	0.132	14.9	<0.015	91.7	59.7	98.0
Wine 7	Initial	1.31	31.3	0.572			
	Dowex	0.044	26.3	0.022	96.6	91.6	96.1
	Iminodiacetate	0.162	17.8	<0.015	87.8	94.3	97.4
Wine 8	Initial	6.07	61.1	1.29			
	Dowex	0.031	27.7	<0.015	99.5	95.5	98.8
	Iminodiacetate	0.042	22.7	<0.015	99.3	96.3	98.8
Wine 9	Initial	1.72	112.1	0.651			
	Dowex	0.321	8.53	0.055	81.3	92.4	91.5
	Iminodiacetate	0.162	24.9	<0.015	90.6	77.8	97.7
Wine 10	Initial	5.41	73.2	0.682			
	Dowex	0.194	14.2	0.071	96.4	80.6	89.6
	Iminodiacetate	0.704	24.9	<0.015	87.0	66.0	97.8
Wine 11	Initial	3.04	32.0	0.481			
	Dowex	0.495	14.9	0.076	83.7	53.4	84.2
	Iminodiacetate	0.276	14.2	<0.015	90.9	55.6	96.9
Wine 12	Initial	3.22	24.2	0.492			
	Dowex	0.158	14.9	0.041	95.1	38.4	91.7
	Iminodiacetate	0.185	10.7	<0.015	94.2	55.8	96.6

[a] Diminution percentage for these metals.

significant losses of polyphenolic compounds from the wines. This has also been observed by other authors [16,17].

These losses were much more severe for the wines treated with the chelating resin. This finding is in agreement with losses of colour observed for the wines following their treatment. This result would explain why the wines treated with this resin presented a notably reduced susceptibility to browning, because their content had previously been significantly reduced in two of the species that determine this tendency-metals and polyphenols.

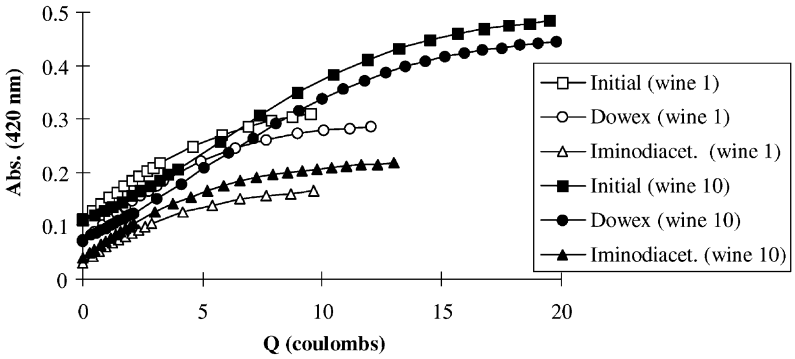

Fig. 1. Electrochemical test for accelerated browning of wines before and after treatment with ion exchange resins.

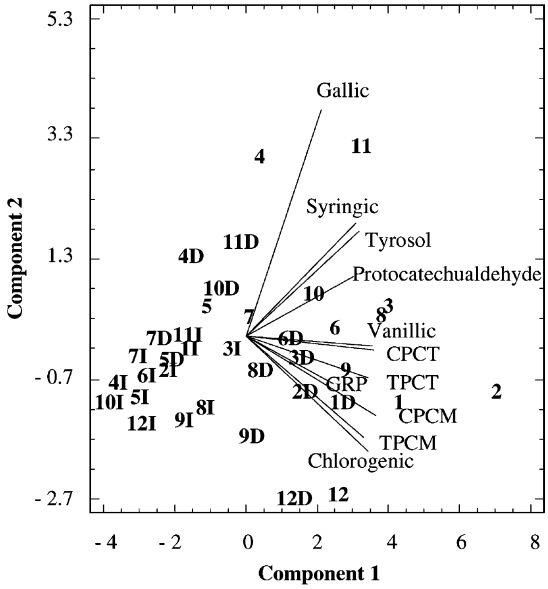

Fig. 2. Principal component analysis performed on phenolic data. Biplot representation of untreated wines (1–12), wines treated with a cation exchange resin (D) and wines treated with a chelating resin (I).

In order to examine the overall effect of ion exchange treatments on the phenolic compounds studied, a multivariate principal component analysis was carried out for those compounds that were significantly affected by these techniques ($P < 0.01$) in comparison with untreated wines. Fig. 2 shows the plane defined by the first two components, which represents the statistical weights of the samples corresponding to the untreated wines and those treated with ion exchange techniques, together with the vectors which reflect the contribution of each phenolic compound.

Component 1, which accounted for the 58.1% of the overall variance, divided the samples into two groups, untreated wines and wines treated with chelating resin. Wines treated with Dowex resin are situated between them. Taking into account that all the phenolic compounds considered contributed with a positive sign to component 1, wines treated with chelating resin were those which led to the largest overall decrease in phenolic compounds. Component 2, accounting for a much lower proportion of the total variance (12.5%) provided no clear results because some phenols contributed to it with a positive sign and others with a negative sign.

Similar studies conducted on the aromatic content revealed that both treatments produced significant reductions (Table 2). These aromatic losses observed from analysis by gas chromatography were corroborated in the tasting tests to which the various treated wines were submitted. In all cases, the wines treated by ion exchange received a lower rating, with those treated with the Dowex resin being found to have excessive acidity. When the pH of these wines treated with the Dowex resin was measured, this was found to be significantly decreased. It is assumed that this resin was supplying the wine with greater quantities of protons as a result of the exchange with other metal ions not subjected to this study. Given that there are considerable concentration of sodium and potassium ions in wine, it was decided to perform a study to determine these elements. All the wines subjected to treatment

Table 2
Variance analysis applied to volatile compounds in the control and treated wines[a]

Compound	Dowex		Iminodiacetate	
	F	*p*	*F*	*p*
Isobutanol	10.08	0.0192*	5.04	0.0658
3-Methylbutanol	8.44	0.0271*	4.77	0.0717
Isoamyl acetate	12.89	0.0115*	16.18	0.0069**
3-Hydroxy-2-butanone	12.12	0.0131*	9.89	0.0199*
Ethyl lactate	11.72	0.0141*	7.98	0.0301*
Unknown 1	86.82	0.0001**	8.84	0.0249*
Acetic acid	14.17	0.0094**	12.90	0.0115*
2,3-Butanediol	10.87	0.0165*	10.87	0.0165*
2-Methylpropanoic acid	10.41	0.0180*	9.82	0.0202*
Butanol	9.73	0.0206*	6.91	0.0391*
3-Methylbutanoic acid	8.12	0.0292*	8.08	0.0295*
Valeric acid	8.25	0.0284*	7.92	0.0306*
Unknown 2	8.98	0.0241*	8.87	0.0247*
Unknown 3	2.34	0.1768	2.18	0.1902
Benzyl alcohol	1.30	0.2983	1.24	0.3081
2-Phenylethanol	16.71	0.0064**	15.66	0.0075**
Ethyl octanoate	4.41	0.0805	4.33	0.0826
3,4-Dimethylpentanol	7.51	0.0337*	7.30	0.0355*
Unknown 4	0.03	0.8607	2.06	0.2011
Unknown 5	4.26	0.0845	3.97	0.0933
Unknown 6	16.81	0.0064**	16.81	0.0064**
Unknown 7	4.02	0.0918	2.10	0.1972
3-Methyl-2-pentanol	10.28	0.0184*	10.16	0.0189*
2-Pentanol	12.41	0.0125*	12.41	0.0125*
n-Propanol	7.73	0.0320*	7.29	0.0356*
2-Phenylethyl acetate	1.29	0.2992	1.29	0.2992
Unknown 8	2.64	0.1553	2.64	0.1553
Unknown 9	2.01	0.2061	1.92	0.2154

[a] Relative area data have been used for unknown compounds.
* Values are significantly different at $P < 0.05$.
** Values are significantly different at $P < 0.01$.

with the Dowex resin were shown to present dramatic reductions in the sodium and potassium content in contrast, the losses of these elements were insignificant in the wines treated with the chelating resin.

4. Conclusions

Both resins were demonstrated to be effective in reducing the content of Fe, Cu and Mn in white wines. The treated wines exhibited a reduced susceptibility to undergo browning. However, in both cases, significant losses were observed in the organoleptic characteristics of the treated wines. The chelating resin with iminodiacetic groups presented the greater capacity for reducing the polyphenolic content of the wines, which would explain that the wines treated with this resin had a very low susceptibility to browning.

Overall, it was considered that neither resin treatment was of practical value in reducing the tendency of white wines to suffer browning without adversely affecting their organoleptic qualities.

Acknowledgements

We gratefully acknowledge the collaboration from Sandeman-Coprimar. This work was supported by Spanish CICYT (ALI 97-0795).

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