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Quality of base and sparkling wines as influenced by the type of fining agent added pre-fermentation

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Abstract

A comparison of the effect on base wines of pre-fermentative clarification between a fining mixture (potassium caseinate, bentonite and cellulose microcrystalline) and bentonite was carried out. Fining agents were added to two grape juices from different cultivars: Macabeo and Parellada. These varietal wines are used for making sparkling wine within the appellation (certified brand of origin) Cava. Vinification was done in parallel on an industrial scale (100 000 l). The use of the fining mixture in grape juice made the fermentations more complete and gave wines with less browning ability, more foam stability time and a lower content of nitrogenous fraction, polyphenols and some volatile compounds than the wines treated with single bentonite. Sensorial analysis showed that the wines produced with the use of different fining agents had different organoleptic characteristics. In addition, the effect of fining agent added pre-fermentatively was observed on some components of sparkling wines.

1. Introduction

Fining agents increase the efficiency of settle juice and make the precipitation of suspended solids easier. Moreover, their use increases the stability of wine against changes of temperature and oxidation during aging and commercialization. The fining agent can also modify wine quality. Wehrung (1996) suggested that fining agents, in white vinification, should be added to juice since the process is less drastic in juice than in wine. Furthermore, when fining agents are part of the fermentation process, they act as insoluble solids that promote yeast growth, causing fermentation to finish faster and more completely (Groat and Ough, 1978; Sims et al., 1995).

The juice colloids and fining agents employed to treat juice bind components of juice such as flavor compounds, so enabling the aroma to be changed (Voilley et al., 1990; Lubbers et al., 1993; Landy et al., 1996). Moreover, Andrés-Lacueva et al. (1996) indicate that the kind of fining agent added before fermentation influences foam characteristics since the fining agent affects colloids and other components that are decisive for foam quality.

Wine composition can depend on the kind of fining agent (organic or inorganic) added to the juice. In white vinification, the fining agents most commonly added to the grape juice are bentonite and potassium caseinate. The main effect of bentonite is protein precipitation by adsorption and neutralization charge (Dal Cin, 1988; Manfredini, 1989a) so that oxidative enzymes are removed. It also has a further effect on other nitrogenous compounds such as polypeptides and amino acids (Saners and Ziemelis, 1973; Goristein et al., 1984; Dal Cin, 1988; Manfredini, 1989a). Potassium caseinate mainly affects polyphenol composition and for this reason browning reactions occur less often in white wine and the color of the white wine is more stable (Amati et al., 1979; Giacomini, 1987; Manfredini, 1989b).

The current study was carried out on base wines, used to make the sparkling wine Cava, obtained from grape juices added pre-fermentation with two fining agents (bentonite and a mixture of potassium caseinate, bentonite and microcrystalline cellulose). Bentonite is the fining agent most widely used in certified brand of origin wines from Spain, and the caseinate mixture is a

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commercial product recently introduced to the market. The fining mixture has some advantages, according to its manufacturer (AEB, 1989), versus single bentonite; wines with less color, more flavor and more stability can be obtained. The microcrystalline cellulose contained in Microcel adsorbs alcohol, fatty acids and esters, thus avoiding a halt in fermentation, and, enables wines with minor residual sugars to be produced. The composition, browning stability and sensorial properties of wines from juices treated with bentonite or the caseinate mixture were compared in order to find out if the mixture enables higher quality wines to be produced. The foam capacity of these wines was also studied since this is an important parameter for base wines that are used to make sparkling wines. The fining agents were added to two varietal juices (Macabeo and Parellada cultivars) and the wines were produced in parallel on an industrial scale. Sparkling wines (with 5, 7, 9, 12, 15, 18, 21, 24 and 30 months of ageing with yeast) from the Parellada base wines were considered in order to establish if the juice treatment had an effect on composition, although second fermentation in bottle and the ageing with yeast has also an effect.

2. Materials and methods

2.1. Samples

The grapes used were of two different white varieties (Parellada and Macabeo) from the Penedès area of certification. Grape juices were obtained at an industrial winery with a Wilmes pneumatic press (<0.2 bar) and treated with SO_2 (*ca.* 70 mg l^{-1}). Both juices were settled for 24 h and each juice was divided into two batches of 100 000 l. To one batch bentonite was added (at 20 g/100 l dose) and to the other caseinate mixture (at the 100 g/100 l dose suggested by the manufacturer) was

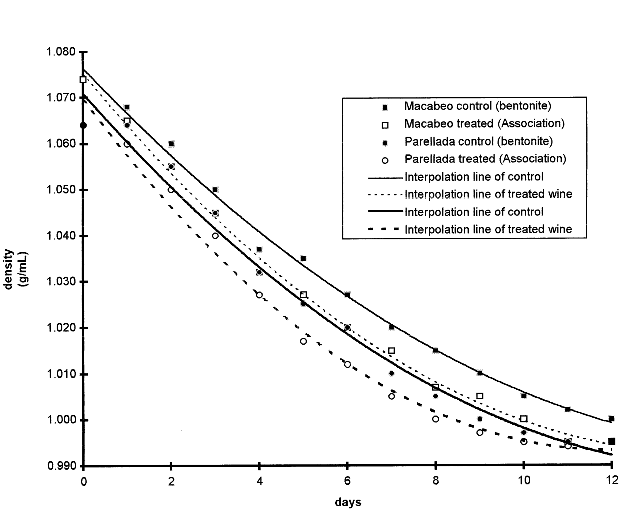

Fig. 1. Ratios (density vs days) of fermentations.

added. The juices were then fermented in parallel with 10^6 cells ml^{-1} of *Saccharomyces cerevisiae* (selected strain). Fermentation took place in a commercial winery in stainless steel tanks (100 000 l) at 15-16°C. Fermentation lasted 10 days (Fig. 1). After two rackings for settling, the wines were filtered through diatomaceous earth and the base wines were obtained. Wines from the grape juice treated with the caseinate mixture were considered treated wines, and those from bentonite were the controls.

Sparkling Cava wines from Parellada (treated and control samples) were also considered in order to establish differences resulting from a second fermentation and ageing with yeast. Nine points of ageing with yeast were taken (5, 7, 9, 12, 15, 18, 21, 24 and 30 months of ageing) for both series of sparkling wine (treated and control).

2.2. Fining agents

2.2.1. Bentonite suspension

1 kg sodium bentonite ASB-60-S (ECC International, France) in 10 l distilled water was prepared.

2.2.2. Microcel suspension

50 kg Microcel (*Microcel*®, AEB Ibérica SA, Spain) in 200 l distilled water was prepared. Microcel composition was potassium caseinate (50% w/w), bentonite (45% w/w), and microcrystalline cellulose (5% w/w).

2.3. Analytical methods

Conventional enological parameters such as alcohol content (% v/v), pH, titratable and volatile acidities, and free and bound sulfur dioxide were measured according to Office International de la Vigne et du Vin methods (OIV, 1990) (Table 1).

Galacturonic acid, sugars (glucose and fructose) and glycerol were determined by high-performance liquid chromatography (HPLC) (López-Tamames et al., 1996).

2.3.1. Fatty acids

Hexanoic, octanoic, decanoic, dodecanoic, tetradecanoic, hexadecanoic and saturated, mono, and unsaturated octadecanoic acids were determined by gas chromatography as methyl ester derivatives. 20 ml wine (with tridecanoic acid (1.5 mg l^{-1}) added as an internal standard) was extracted with 3×5 ml of pentane and concentrated to 1 ml with nitrogenous gas. Concentrates were derivatized with 1 ml 3% H_2SO_4 in methanol at room temperature for 3 h. 2 µml of the organic phase was injected into a Perkin-Elmer Sigma 3B gas chromatograph equipped with an FID detector and a 15% DEGS-PS (WAW 80/100, 2 m×1/8 µm) column. Oven temperature was kept at 50°C for 10 min then pro-

Table 1
General parameters of juices and wines

	Macabeo variety			Parellada variety		
	Juice	Wine		Juice	Wine	
		Control[b]	Treated[a]		Control[a]	Treated[b]
Ethanol (%)	9.86[c]	11.09	10.90	9.53[c]	9.55	9.55
Sugars ($g\,l^{-1}$)	165.9	0.70	0.23	160.4	1.35	1.15
pH	3.10	3.06	3.10	3.33	3.07	3.06
Titratable acidity ($g\,l^{-1}$)	4.7	5.2	5.0	3.4	5.1	5.0
Volatile acidity ($g\,l^{-1}$)	—	0.3	0.3	—	0.3	0.3
Free SO_2 ($mg\,l^{-1}$)	3	8	7	3	8	8
Total SO_2 ($mg\,l^{-1}$)	86	70	73	96	77	80

[a] Bentonite as fining agent.
[b] Mixture of potassium caseinate, benonite and microcrystalline cellulose.
[c] Predicted value.

grammed to reach 175°C at 3°C/min and remain at 175°C for 15 min. Injector and detector temperatures were both 225°C.

2.3.2. Polyphenols

Total phenols were determined following Singleton and Rossi (1965), flavonoids and non-flavonoids were determined according to the method described by Kramling and Singleton (1969) and *o*-diphenols were determined following Flanzy and Aubert (1969).

2.3.3. Color and accelerated test for browning capacity

50 ml samples of the wines were sealed in the presence of air in 100 ml glass bottles. Bottles were maintained at 40°C for two weeks. Browning was measured at 0, 7 and 15 days as the absorbance at 420 nm, as recommended by Singleton and Kramling (1976).

2.3.4. High volatile compounds

Acetaldehyde, methyl acetate, ethyl acetate, methanol, propanol, isobutanol and isoamylic alcohols were determined. Wine samples containing 4-methyl-2-pentanol ($50\,mg\,l^{-1}$) as an internal standard were directly injected (2 µml) on a Perkin-Elmer Sigma 3B gas chromatograph equipped with an FID detector and a Seelcosteal Alcohol TC (Carbowax 1500) (4 m×1/8 cm i.d.) column. Oven temperature was kept at 45°C for 1 min then programmed to reach 80°C at 2°C/min and then remain at 80°C for 45 min. Injector and detector temperatures were both 180°C.

2.3.5. Less volatile compounds

Isoamyl acetate, hexyl acetate, isoamyl alcohols, ethyl hexanoate, hexyl alcohol, ethyl lactate, ethyl octanoate, linalool, ethyl decanoate, diethyl succinate, α-terpineol, nerol, *cis* and *trans*-pyranic linalool oxid, 2-phenethyl acetate, ethyl dodecanoate, geraniol, benzyl alcohol, 2-phenethylethanol, 2,6-dimethyl-3,7-octadiene-2,6-diol and ethyl tetradecanoate were isolated by discontinuous liquid-liquid extraction with three portions of pentane. 1-Heptanol ($0.500\,mg\,l^{-1}$) was added to 100 ml of wine before extraction as an internal standard. The aroma extracts were dried with sodium sulfate and concentrated to 0.2 ml with nitrogen flow. 1 µml of this extract was injected in split mode (1:60) into a Hewlett-Packard 5890 series II gas chromatograph equipped with an FID detector and a Supelcowax 10 (30 m×0.25 mm i.d.) column. Oven temperature was kept at 50°C for 10 min then programmed to reach 240°C at 2°C/min and remain at 240°C for 25 min. Injector and detector temperatures were both 250°C.

2.3.6. Sensorial analysis

A triangular trial (Roessler et al., 1978) was performed by a panel of seven expert judges to establish differences between wines from the treated and control juices for each variety. Quality evaluations were performed using scorecards made by the panel. Wines stored at 4°C were evaluated in triplicate 3 months after fermentation and were randomly presented to the experts. 30 ml samples were presented in coded, clear, 170 ml tulip glasses covered with Petri dishes. At each session the judges evaluated the overall color, aroma and taste of each sample on a 0-10 point quality scale for each characteristic. The judges scored the samples according to their sensory knowledge, training and experience. Evaluations were conducted at 21–23°C in an isolated room under white light.

2.3.7. Nitrogenous substances

Total soluble proteins was determined by the Bradford method (Bradford, 1976), and whole free amino acids (Cya, Hyp, Asp, Ser, Glu, Asn, Gly, Gln, Thr, Ala, Pro, Arg, His, GABA, Tyr, Val, Cys, Met, Ile, Leu, Phe, Orn, Trp and Lys) and ethanolamine were determined by the HPLC method (Puig-Deu and Buxaderas, 1994). Peptids were determined by molecular exclusion into a Merck-Hitachi HPLC system (L-5000 LC

Table 2
Characteristics of varietal control (bentonite) and treated (caseinate association) wines: ANOVA results p = degree of significance

	Macabeo wines		Parellada wines		Fining agent	Interaction (fining agent/variety)
	Control[a]	Treated[b]	Control[a]	Treated[b]		
Hexanoic acid (mg l^{-1})	2.30	2.41	2.25	2.43	—	—
Octanoic acid (mg l^{-1})	14.46	13.98	11.53	14.01	—	—
Decanoic acid (mg l^{-1})	4.49	4.10	3.63	4.31	—	—
Dodecanoic acid (mg l^{-1})	0.13	0.19	0.09	0.08	—	—
Miristic acid (mg l^{-1})	0.12	0.13	0.04	0.04	—	—
Palmitic acid (mg l^{-1})	0.12	0.14	0.04	0.04	—	—
Stearic acid (mg l^{-1})	0.07	0.10	0.01	0.03	—	—
Oleic acid (mg l^{-1})	0.06	0.11	0.01	0.03	—	—
Linoleic acid (mg l^{-1})	0.04	0.09	0.04	0.07	—	—
Ethyl hexanoate (μg l^{-1})	1152	1146	1367	907	0.0003	0.0036
Ethyl octanoate (μg l^{-1})	2712	2410	2652	2041	0.0001	0.0301
Ethyl decanoate (μg l^{-1})	1091	1001	863	608	0.0089	—
Ethyl dodecanoate (μg l^{-1})	134	114	76	23	0.0012	—
Ethyl tetradecanoate (μg l^{-1})	109	12	5	3	0.0001	0.0001
Acetaldehyde (mg l^{-1})	28.44	29.98	30.73	27.47	—	—
Methyl acetate (mg l^{-1})	1.36	0.92	1.17	1.00	—	—
Ethyl acetate (mg l^{-1})	12.59	7.62	14.60	11.57	0.0003	—
Butyl acetate (mg l^{-1})	12	7	5	3	0.0136	—
Isoamyl acetate (μg l^{-1})	2108	1785	3061	1630	0.0001	0.0009
Hexyl acetate (μg l^{-1})	234	214	427	240	0.0001	0.0003
2-Phenetyl acetate (μg l^{-1})	514	489	569	538	—	—
Linalool (μg l^{-1})	1	8	7	3	—	—
α-Terpyneol (μg l^{-1})	37	30	27	14	0.0005	—
cis-Pyranic-linalool oxid (μg l^{-1})	83	40	11	8	0.0002	0.0004
trans-Pyranic-linalool oxid (μg l^{-1})	92	58	14	10	0.0001	0.0031
Nerol (μg l^{-1})	290	154	66	30	0.0004	0.0247
2,6-dimethyl-3,7-octadien-2,6 diol (μg l^{-1})	14	5	1	—	0.0001	0.0008
Geraniol (μg l^{-1})	85	76	45	28	0.0001	—
γ-Butyrolactone (μg l^{-1})	22	13	25	4	0.0005	0.0287
Diethyl succinate (μg l^{-1})	199	173	233	240	—	—
Ethyl lactate (μg l^{-1})	638	490	1122	1247	—	—
Glycerol (mg l^{-1})	5.07	5.72	4.95	4.13	—	—
Methanol (mg l^{-1})	27.45	40.67	23.09	26.11	0.0001	0.0001
Isobutanol (mg l^{-1})	13.24	23.08	14.43	10.29	—	—
Propanol (mg l^{-1})	10.99	10.85	14.65	14.98	—	—
Isoamilic alcohol (mg l^{-1})	148.92	81.29	122.85	133.83	—	—
Hexanol (μg l^{-1})	421	419	972	680	0.0003	0.0003
cis-3-Hexenol (μg l^{-1})	69	78	276	209	—	—
Benzyl alcohol (μg l^{-1})	75	59	11	17	—	—
2-Phenylethanol (μg l^{-1})	9997	10059	8762	12465	—	—
Cysteic acid (mg l^{-1})	0.17	0.31	0.98	0.93	—	—
Aspartic acid (mg l^{-1})	3.14	4.45	9.79	10.37	—	—
Hydroxyproline (mg l^{-1})	2.62	3.82	3.90	5.42	0.0001	—
Glutamic acid (mg l^{-1})	9.73	6.81	18.92	16.66	0.0048	—
Serine (mg l^{-1})	3.37	3.68	6.55	6.61	—	—
Asparagine (mg l^{-1})	3.36	2.83	7.78	6.06	0.0034	—
Glycine (mg l^{-1})	2.77	2.31	5.39	4.40	0.0001	—
Glutamine (mg l^{-1})	4.99	2.18	5.32	3.25	0.0001	—
Threonine (mg l^{-1})	1.32	1.37	5.75	4.14	—	—
Alanine (mg l^{-1})	6.62	9.95	9.71	13.07	0.0006	—
Histidine (mg l^{-1})	1.58	1.45	5.62	3.07	0.0001	0.0001
Proline (mg l^{-1})	289.11	286.94	292.21	292.51	—	—
Arginine (mg l^{-1})	9.91	7.97	23.96	15.33	0.0010	—
Tyrosine (mg l^{-1})	7.85	2.56	8.50	9.23	—	—
Valine (mg l^{-1})	5.88	2.63	4.16	3.69	0.0001	0.0001
Metyonine (mg l^{-1})	1.79	—	—	—	0.0001	0.0001
Isoleucine (mg l^{-1})	1.44	1.63	3.99	3.63	—	—
Leucine (mg l^{-1})	8.66	5.49	17.36	18.71	—	—
Phenylalanine (mg l^{-1})	3.25	2.12	9.53	7.90	0.0001	—

(table continued on next page)

Table 2—*contd.*

	Macabeo wines		Parellada wines		Fining agent	Interaction (fining agent/variety)
	Control[a]	Treated[b]	Control[a]	Treated[b]		
Ornytine ($mg\,l^{-1}$)	1.06	1.12	1.71	3.17	—	—
Tryptophan ($mg\,l^{-1}$)	2.70	1.67	2.12	1.65	0.0001	0.0079
Lysine ($mg\,l^{-1}$)	4.39	4.96	14.31	16.02	—	—
GABA ($mg\,l^{-1}$)	5.48	4.21	4.50	5.51	—	—
Total polyphenols ($mg\,l^{-1}$)	165.90	157.81	163.70	160.84	0.0003	—
Non-flavonoids ($mg\,l^{-1}$)	143.06	135.90	135.43	129.07	0.0001	—
o-Diphenols ($mg\,l^{-1}$)	40.76	37.21	27.55	27.39	0.0137	0.0293
Colour (420 nm)	0.055	0.057	0.067	0.060	—	—
Proteins ($mg\,l^{-1}$)	8.66	6.02	7.93	5.63	0.0001	0.0139
Ethanolamine ($mg\,l^{-1}$)	7.55	6.44	24.91	19.18	0.0036	0.0250
Peptids F-I (area count)	1.51	1.80	1.25	2.34	—	—
Peptids F-II (area count)	13.95	12.35	10.92	17.39	—	—
Peptids F-V (area count)	1.10	0.77	1.19	2.05	—	—
Galacturonic acid ($g\,l^{-1}$)	0.44	0.34	0.38	0.36	0.0012	0.0119
HM (mm)	147.0	51.0	152.0	109.3	0.0001	0.0270
HS (mm)	28.0	24.7	27.7	23.7	0.0002	—
TS (s)	27.3	35.0	70.0	153.3	0.0035	0.0094

[a] Bentonite as fining agent.
[b] Mixture of potassium caseinate, bentonite and microcrystalline cellulose.

Controller, 655A-12 liquid chromatograph equipped with a L-4200 UV-VIS detector). 1 ml of centrifuged sample (1800 **g**, 5 min.) was passed through two consecutive cartridges: anionic exchange (Bond Elut SAX, Varian International, Switzerland) and C_{18} (Sep-Pack, Waters). Peptides were eluted with 1 ml 90% tetrahydrofurane in water. 20 µml of the sample was injected into a TSK-GEL G2000PW column, 300×7.5 mm, 10 µm particle size (Supelco Inc.). The mobile phase was water acidified with acetic acid (pH 2.5) and the flow rate was $1.5\,ml\,min^{-1}$. Detection was performed at 210 nm. Four fractions were separated by molecular weight: I > 1000; II, 800-600; III, 600-400; IV, 400-250 ($g\,mol^{-1}$, expressed as an area count).

2.3.8. Foam capacity

Foam capacity was determined by the Mosalux procedure (Maujean et al., 1990). Three parameters were measured:

(a) HM: the maximum height reached by the foam after carbon dioxide injection through a glass frit, expressed in mm; this represents foamability, i.e. the wine's ability to foam.
(b) HS: the foam stability height during carbon dioxide injection, expressed in mm; this represents the foam stability, i.e. the wine's ability to produce stable foam or the persistence of a foam collar.
(c) TS: the foam stability time until all bubbles collapse when CO_2 injection is interrupted, expressed in s; this represents the foam stability time once effervescence has decreased.

2.4. Statistical procedures

Statgraphics 7.0 software (STSC Inc. and Statistical Graphics corporation, USA) was used to apply ANOVA analysis. Qualitative variables were the fining agent used (bentonite or caseinate mixture) and grape variety (Macabeo or Parellada). ANOVA results (Table 2) were expressed as degree of significance (p) ($p < 0.05$ was considered a significant result). For several compounds, the variety and the fining agents interact. In these cases, the fining agent effect is studied separately for each variety. A principal component analysis (PCA) was carried out on the results of the 5, 7, 9, 12, 15, 28, 21, 24 and 30 month aged sparkling wines.

3. Results and discussion

Juices treated with the caseinate mixture fermented more completely (Fig. 1) than bentonite treated ones. In addition, the wines treated with the caseinate mixture had a lower residual sugars (glucose + fructose) content than wines treated with bentonite (Table 1). Microcrystalline cellulose in the caseinate mixture could absorb inhibitor substances and thus fermentations were more complete, just as the manufacturer indicates. However, wine composition in terms of fatty acids (Table 2) and the ethanol content (v/v %) (Table 1) of base wines was not affected by the fining agent.

The polyphenol fraction (total and non-flavonoid polyphenols, and *o*-diphenols) was less concentrated in wines whose juice was clarified with the caseinate mixture (Table 2). This characteristic of potassium case-

Table 3

Methanol ($mg\,l^{-1}$)	27.45	40.67	23.09	26.11	0.0001	0.0001
Isobutanol ($mg\,l^{-1}$)	13.24	23.08	14.43	10.29	—	—
Propanol ($mg\,l^{-1}$)	10.99	10.85	14.65	14.98	—	—
Isoamilic alcohol ($mg\,l^{-1}$)	148.92	81.29	122.85	133.83	—	—
Hexanol ($\mu mg\,l^{-1}$)	421	419	972	680	0.0003	0.0003
cis-3-Hexenol ($\mu mg\,l^{-1}$)	69	78	276	209	—	—
Benzyl alcohol ($\mu mg\,l^{-1}$)	75	59	11	17	—	—
2-Phenylethanol ($\mu mg\,l^{-1}$)	9997	10059	8762	12465	—	—
Cysteic acid ($mg\,l^{-1}$)	0.17	0.31	0.98	0.93	—	—
Aspartic acid ($mg\,l^{-1}$)	3.14	4.45	9.79	10.37	—	—
Hydroxyproline ($mg\,l^{-1}$)	2.62	3.82	3.90	5.42	0.0001	—
Glutamic acid ($mg\,l^{-1}$)	9.73	6.81	18.92	16.66	0.0048	—
Serine ($mg\,l^{-1}$)	3.37	3.68	6.55	6.61	—	—
Asparagine ($mg\,l^{-1}$)	3.36	2.83	7.78	6.06	0.0034	—
Glycine ($mg\,l^{-1}$)	2.77	2.31	5.39	4.40	0.0001	—
Glutamine ($mg\,l^{-1}$)	4.99	2.18	5.32	3.25	0.0001	—
Threonine ($mg\,l^{-1}$)	1.32	1.37	5.75	4.14	—	—
Alanine ($mg\,l^{-1}$)	6.62	9.95	9.71	13.07	0.0006	—
Histidine ($mg\,l^{-1}$)	1.58	1.45	5.62	3.07	0.0001	0.0001
Proline ($mg\,l^{-1}$)	289.11	286.94	292.21	292.51	—	—
Arginine ($mg\,l^{-1}$)	9.91	7.97	23.96	15.33	0.0010	—
Tyrosine ($mg\,l^{-1}$)	7.85	2.56	8.50	9.23	—	—
Valine ($mg\,l^{-1}$)	5.88	2.63	4.16	3.69	0.0001	0.0001
Metyonine ($mg\,l^{-1}$)	1.79	—	—	—	0.0001	0.0001
Isoleucine ($mg\,l^{-1}$)	1.44	1.63	3.99	3.63	—	—
Leucine ($mg\,l^{-1}$)	8.66	5.49	17.36	18.71	—	—
Phenylalanine ($mg\,l^{-1}$)	3.25	2.12	9.53	7.90	0.0001	—
Ornytine ($mg\,l^{-1}$)	1.06	1.12	1.71	3.17	—	—
Tryptophan ($mg\,l^{-1}$)	2.70	1.67	2.12	1.65	0.0001	0.0079
Lysine ($mg\,l^{-1}$)	4.39	4.96	14.31	16.02	—	—
GABA ($mg\,l^{-1}$)	5.48	4.21	4.50	5.51	—	—
Total polyphenols ($mg\,l^{-1}$)	165.90	157.81	163.70	160.84	0.0003	—
Non-flavonoids ($mg\,l^{-1}$)	143.06	135.90	135.43	129.07	0.0001	—
o-Diphenols ($mg\,l^{-1}$)	40.76	37.21	27.55	27.39	0.0137	0.0293
Colour (420 nm)	0.055	0.057	0.067	0.060	—	—
Proteins ($mg\,l^{-1}$)	8.66	6.02	7.93	5.63	0.0001	0.0139
Ethanolamine ($mg\,l^{-1}$)	7.55	6.44	24.91	19.18	0.0036	0.0250
Peptids F-I (area count)	1.51	1.80	1.25	2.34	—	—
Peptids F-II (area count)	13.95	12.35	10.92	17.39	—	—
Peptids F-V (area count)	1.10	0.77	1.19	2.05	—	—
Galacturonic acid ($g\,l^{-1}$)	0.44	0.34	0.38	0.36	0.0012	0.0119
HM (mm)	147.0	51.0	152.0	109.3	0.0001	0.0270
HS (mm)	28.0	24.7	27.7	23.7	0.0002	—
TS (s)	27.3	35.0	70.0	153.3	0.0035	0.0094

[a] Bentonite as fining agent.
[b] Mixture of potassium caseinate, bentonite and microcrystalline cellulose.

inate was previously reported by Amati et al. (1979), Manfredini (1989b) and Puig-Deu et al. (1996). The removal of polyphenols, especially *o*-diphenols, by caseinate is greater in Macabeo wines than in Parellada wines. Although juices treated with the caseinate mixture produce wines with a lower polyphenol content, the color of the wine (expressed as absorbance at 420 nm) is not affected (Table 2). However, these wines are more stable to oxidation. The increase in absorbance produced by accelerating the browning test was less in wines whose juices were treated with the caseinate mixture. The treated wines browned 79 and 58% less than the control wines (for the Macabeo and Parellada varieties, respectively).

Wines whose juice had been treated with the caseinate mixture had a lower concentration than the controls of some volatile compounds, i.e. ethyl esters, acetates (ethyl, butyl, isoamyl and hexyl acetates), some terpenyl compounds (nerol, geraniol, linalool, α-terpineol, 2,6-dimethyl-3,7-octadiene-2,6-diol, *cis*-pyranic linalool oxid and *trans*-pyranic linalool oxid) and γ-butyrolactone (Table 2). Furthermore, the concentration of alcohols formed before fermentation and thus before the addition of any fining agent (methanol, hexanol and cis-3-hexenol) was less in the treated wines. Potassium caseinate, which is part of the caseinate mixture, did not increase aroma composition, although some authors

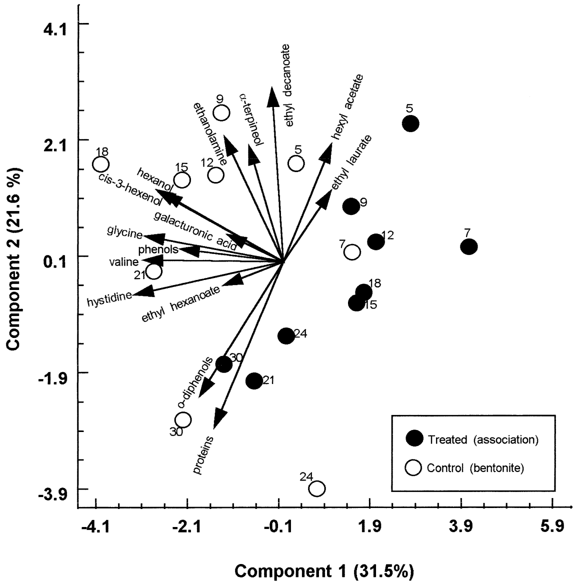

Fig. 2. Principal component analysis of some compounds of sparkling wines (aged for 5, 7, 9, 12, 15, 21, 24 and 30 months with yeasts), which are different for control (bentonite) and treated (caseinate mixture) sparkling wines.

(Amati, 1986; Giacomini, 1987) suggest it does. This may be because of the large dose of bentonite added with the mixture; the bentonite would adsorb aroma compounds and/or their precursors. The alcohols mainly formed during fermentation (glycerol, isobutanol, propanol, isoamyl alcohol, bencyl alcohol and 2-phenylethanol) do not seem to be affected by the type of fining agent used (Table 2). Different aroma profiles were perceived by the sensorial panel (under a triangular trial, treated wines and controls showed differences ($p<0.001$), all judgements ($n=21$) were in agreement). However, the score card (Table 3) did not record the kind of fining agent used. The smaller amounts of some volatile compounds in wines whose juice was treated did not result in a reduction of organoleptic quality. The larger or smaller amounts of these compounds in the wines resulted in wines of different organoleptic characteristics, but not necessarily of worse or better quality.

The concentration of proteins was lower in wines whose juice was clarified with the caseinate mixture than in those treated with bentonite alone (Table 2). The quantity of bentonite added with the caseinate mixture (100 g/100 l, as suggested by the manufacturer), which is composed of 45 g/100 l bentonite + 50 g/100 l potassium caseinate + 5 g/100 l microcrystalline cellulose, was 2.25 times more than the quantity of bentonite added as a single fining agent (20 g/100 l). This high level of bentonite could lead to a higher adsorption of proteins. However, the amount of peptides detected did not change on addition of a higher amount of bentonite (Table 2). As Hsu and Heatherbell (1987) suggest, bentonite adsorbs peptides of higher molecular weight (30 000-45 000) than that of the peptides in the current study (250-5000). Some amino acids (Glu, Asn, Gly, Gln, His, Arg, Val, Met, Phe and Trp) and ethanolamine were present in lower concentrations in treated wines; other amino acids (Asp, Ser, Thr, Pro, Tyr, Ile, Leu, Orn, Lys, Gaba and cysteic acid) did not different in amounts in the differently treated wines. Hyp and Ala contents were higher in the wines whose juices were treated with the caseinate mixture than in the bentonite controls. Although bentonite can adsorb amino acids (Goristein et al., 1984; Manfredini, 1989a), the changes observed in the amino acid content of the wines may be the result of the assimilation and excretion process of yeast during fermentation.

Table 4
Results of sensory analysis

Variety	Fining agent	Mean ($n=7$)	95% confidence for mean	n	F ratio	Significance level
Parellada	Control[a]	7.6	7.2 – 8.0	21	0.996	0.3322
	Treated[b]	7.3	6.9 – 7.7	21		
Macabeo	Control[a]	7.0	6.6 – 7.4	21		
	Treated[b]	6.9	6.5 – 7.3	21		

[a] Bentonite as fining agent.
[b] Mixture of potassium caseinate, bentonite and microcrystalline cellulose.

The effect on wine composition of the fining agent used pre-fermentation has an influence on foam (Table 2): wines treated with the caseinate mixture have less foamability (HM) and height stability of foam (HS) than the ones treated with bentonite. Stability time of foam (TS) is greater in the wines treated with the caseinate mixture. These results are similar to those obtained by Andrés-Lacueva et al. (1996), who compared the foam capacity of 44 white wines from the AOC Penedès. These authors found a positive relationship between foam formation (HM and HS) and residual sugars, proteins, ethanolamine, proline and glutamine. According to the current study, the concentrations of these compounds are higher in the control wines than in these treated wines. Lower levels of galacturonic acid, proteins, amino acids and ethyl acetate favours a greater stability time of foam (TS) so the fact that these compounds were in lower quantity in treated wines (Table 2) explains the greater TS of these wines.

The PCA of some components of sparkling wines aged with yeast (5, 7, 9, 12, 15, 18, 21, 24 and 30 months) is shown in Fig. 2. The differences observed for base wines are also shown. Proteins, *o*-diphenols, total phenols, histidine, valine, ethanolamine, glycine, hexanoate and decanoate ethyl esters, hexanol, *cis*-3-hexenol, α-terpineol, hexyl acetate and galacturonic acid were chosen to establish differences between the two series of sparkling wines (treated and control). Both series of sparkling wines come from their respective base wines but have been vinified by a different fining agent added to the grape juice (bentonite or the caseinate mixture). Differences were observed although the aging with yeast also affects.

4. Conclusions

Fining mixtures may be advantageous for pre-fermentation use in wine making. Wines treated with the mixture of potassium caseinate, bentonite and micro-crystalline cellulose had a lower polyphenol content, which implies less browning capacity. The use of this mixture in juices produces wines with minor residual sugars and so more complete fermentation. If these wines are then used to make sparkling base wine, it was found that use of the caseinate mixture produces wines with higher foam stability time; however, wines treated with the caseinate mixture have less foamability and persistence of foam collar. The different aroma profile obtained with caseinate mixture does not result in any difference in wine quality. Even when aged the characteristics of sparkling wines depend on the kind of fining agent added to the grape juices rather than the ageing process used.

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