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Composition of Tartrate Precipitates in White Wines used for Making Spanish Sparkling Wine

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ABSTRACT

The composition of tartrate crystals, deposited in various containers that had held white wines used in sparkling wine production, was studied. An improved understanding of the composition of the substances that inhibit KHT precipitation would help achieve higher yields during stabilization of the tartrates in wines. X-ray diffraction and X-ray fluorescence analyses were performed, together with elementary analysis, to determine the crystal phases existing in and the elementary composition of the precipitates, and ultrafiltration was carried out to determine the nature and size of the organic compounds present in the precipitates. The results indicate that the main salt precipitated was in all cases potassium bitartrate. Small quantities of calcium tartrate were also present in the precipitates collected from the containers used for storage or fermentation of the wines. The different stoichiometric balances detected, according to the differing origins of the samples, suggest that there was interference by negatively charged substances in the case of

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precipitation in the containers used for the storage and fermentation of the wines and by positively charged substances in the case of precipitation in the containers used for cold stabilization. The proteins, peptides, and polyphenols of varying degrees of polymerization that had been adsorbed onto the faces of the crystals were separated by ultrafiltration.

INTRODUCTION

Young wines can be regarded as supersaturated solutions of potassium bitartrate (KHT), which may give rise to precipitation of crystals in bottled wines and consequent rejection by consumers. The differing behaviour of this salt in wines and in model solutions is due to the presence in the wines of substances capable of interacting with the KHT. In the case of white wines, the observed differences have been attributed to high-molecular-weight compounds, especially proteins, although the complexity of the phenomenon means that the possible role of other substances cannot be ruled out.

Most studies dealing with KHT precipitation in wines have been carried out by examining the influence of various treatments (decolorization, passage through ion-exchange resins, the use of fining agents, different conditions of filtration, etc.) on precipitate yield and reduction of KHT supersaturation in the wines (Pilone & Berg, 1965; Colagrande *et al.*, 1985; Maujean *et al.*, 1985).

Studying the composition of the KHT crystals that form in wines makes it possible to determine which substances precipitate out together with the KHT. Such substances may include crystallization inhibitors. A better knowledge of the substances that inhibit KHT precipitation could translate into higher yields during the stabilization methods applied to wines.

The present paper examines the tartrate precipitates from different stages of the manufacture of white wines used in the production of Spanish sparkling wines ('cava'). X-ray diffraction was employed to determine the crystal phases present, and X-ray fluorescence analysis and elementary analysis were employed to determine the stoichiometry of the precipitates. The chemical composition and the degree of polymerization of the compounds present in the tartrate lees were also analysed.

MATERIALS AND METHODS

Samples

Tartrates were from containers that had been utilized to hold wines that were used in the production of sparkling wines; the origin of the samples is summarized in Table 1.

TABLE 1
Origin of the Tartrates Analysed

Sample No.	*Source description*
1	Tartrates deposited in stainless steel (AISI 316) tanks used for the storage of white wines.
2	Tartrates deposited in tanks of reinforced concrete lined with ceramic tiles used for raw white wines.
3	Tartrates from various sources collected during drying.
4	Tartrates from oak casks containing white wines of more than one vintage.
5	Tartrates deposited in stainless steel tanks used for fermenting and storing white wines.
6	Tartrates deposited in stainless steel tanks in which precipitation of KHT had been induced by storing white wines at −4°C for 10–12 days.
7	Tartrates deposited in lined concrete tanks in which precipitation of KHT had been induced by storing white wines at −4°C for 10–12 days.

X-ray diffraction

A Philips model PW 1771/00 diffractometer employing CuKα radiation and a model PW 1710/00 control unit were used.

The resulting data were processed using the program developed by Appleman & Evans (1973) as adapted for personal computers by Benoit (1987), which calculates the parameters of the unit cells on the basis of the crystal system, the reference values of the unit cell parameters, and peak spacing.

Mixtures of KHT (Merck, Darmstadt, FRG) and 5, 10, and 15% $CaT.4H_2O$ (Fluka, Buchs, Switzerland) were used as the standards for the quantitative analyses.

X-ray fluorescence analysis

Sample preparation
The sample was prepared according to Barba *et al.* (1979).

Experimental conditions
The determinations were carried out using a model PW 1410/20 Philips spectrophotometer. Intensities were converted to concentrations by applying a procedure for complete correction of the matrix effects using the experimental correction coefficients of Franzini *et al.* (1975). A KHT standard (Merck, Darmstadt, FRG) was used to verify the analytical results.

Analysis of carbon and hydrogen
These analyses were performed on the solid samples of the tartrates using a Heraeus model CHN-O-RAPID elementary analyser.

Nitrogen determination
Kjeldahl method.

Sample solution
The sample solutions were prepared by grinding the solid samples to a fine, homogeneous powder in an agate mortar and dissolving the powders in 0·1N HCl in the proportion of 10 g/litre.

Tartaric acid
Colorimetric reaction of the sample solutions was done with ammonium metavanadate; readings were taken at an absorbance of 500 nm (Vidal & Blouin, 1978).

Malic and oxalic acids
Enzymatic tests were those of Boehringer Mannheim (Mannheim, FRG, Cat. No. 139068 and 755699) of the sample solutions.

Free reducing sugars
Reducing power was measured in alkaline copper solutions prepared from the sample solutions.

Total reducing sugars
Acid hydrolysis was carried out and free reducing sugars were detected.

Pectin analysis
The presence of pectins was determined through the reaction of the uronic acids and carbazole in a hot sulphuric acid medium, with absorbance measured at 530 nm. The calibration curve was constructed using galacturonic acid (Merck, Darmstadt, FRG).

Total polyphenols
Colorimetric reaction of the polyphenols was done with phosphotungstic acid and phosphomolybdic acid using the sample solutions, with absorbance measured at 670 nm (Singleton & Rossi, 1965).

Protein analysis
The analysis of the proteins was carried out using the sample solutions dialyzed against tap water with a membrane with a pore size of 3500 daltons (Spectrum Medical Industries, Los Angeles, CA, USA). The dialyzate was concentrated 60 times and the protein content determined by reaction with Coomassie Brilliant Blue G-250 according to the method of Bradford (1976).

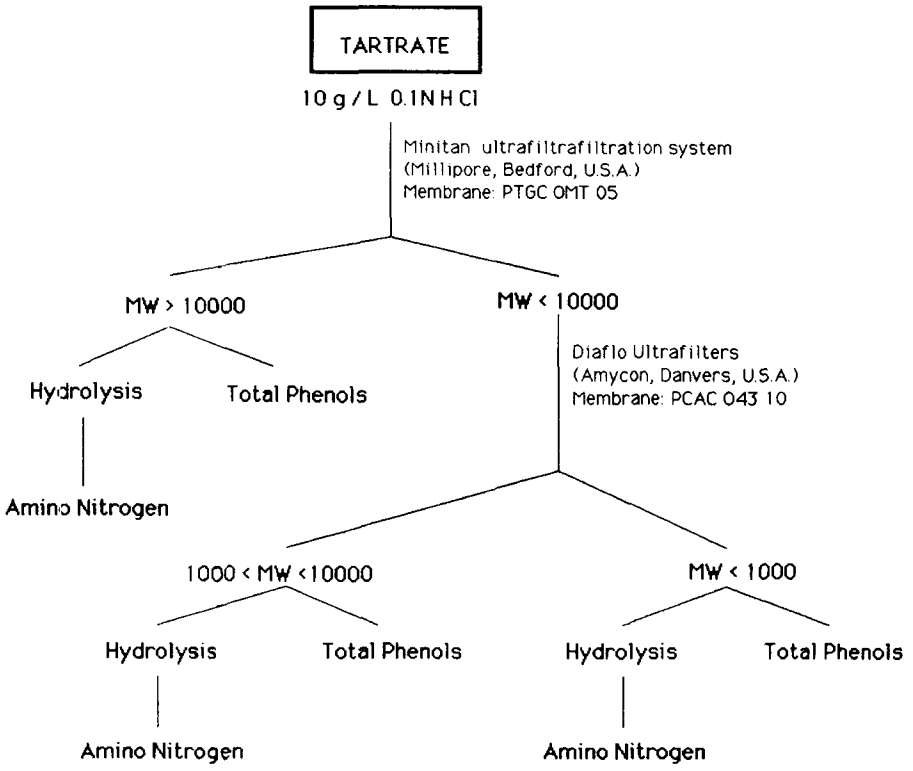

Fig. 1. Diagram of the ultrafiltration procedure used in the fractionation of the tartrate samples.

The calibration curve was constructed using bovine serumalbumin (Merck, Darmstadt, FRG).

Fractionation of tartrates by ultrafiltration
The method summarized in Fig. 1 was employed.

Hydrolysis of proteins and peptides. The hydrolysis reaction was carried out under vacuum in glass vials in a 6N hydrochloric acid medium at 110°C for 24 h. The hydrolysates were concentrated in a rotary evaporator and washed several times in water to eliminate the excess hydrochloric acid prior to determination of the amino acid nitrogen.

Determination of amino nitrogen. This determination was carried out according to Saifer *et al.* (1960). Leucine was used as the standard for constructing the calibration curve. The proline content was not evaluated under these conditions and hence is not included in the amino nitrogen values.

RESULTS AND DISCUSSION

Study of the crystal phases present in the tartrates

The X-ray diffraction diagrams indicate that the main salt precipitated was KHT in all samples. The presence of CaT in the precipitate was only detected in the samples from fermentation or/and storage samples (Nos 1–5 in Table 1). Processing of the data from diagrams using the program of Appleman & Evans (1973) has shown that the values of the dimensions of the unit cells to be calculated for the crystal phases were quite similar to those calculated for the standard and listed on the corresponding ASTM (American Society for Testing Materials) cards (No. 25-644 and 26-330). This indicates that the substances that precipitate out with the KHT and the CaT are not actually part of the crystal structures themselves but are instead adsorbed on the crystal faces, as demonstrated by the morphogical studies (Rodriguez-Clemente & Correa-Gorospe, 1988). The absence of CaT in the tartrate precipitates induced by cold stabilization suggests that precipitation of $CaT.4H_2O$ takes place in the early stages of wine-making with the formation of alcohol. The drop in temperature that occurs during cold stabilization did not cause precipitation of this salt.

Elementary composition of the tartrates

The results of the analysis of the chemical elements present in the tartrates are given in Table 2. Potassium was the major cation in all the samples. The calcium content determined by fluorescence was in all cases greater than that determined by X-ray diffraction; hence it would appear that there existed

TABLE 2
Elementary Composition of the Tartrates (percentage w/w)

Sample	*C*	*H*	*K*	*Ca*[a]	*N*	*Si*	*Na*	*Ca*[b]
KHT	25·53	2·71	20·87	0·04	—	0·03	0·01	—
1	24·14	3·14	18·51	2·86	0·10	0·01	0·01	0·69
2	24·42	3·10	18·10	3·21	0·12	0·01	0·04	2·09
3	22·39	3·22	19·64	1·17	0·16	0·02	0·03	0·80
4	23·74	3·37	18·20	2·52	0·18	0·10	0·02	1·58
5	21·90	3·24	19·13	2·04	0·24	0·03	0·07	1·38
6	26·62	2·90	20·75	0·10	0·28	0·03	0·04	0·00
7	26·44	3·02	20·29	0·09	0·25	0·03	0·02	0·00

[a] X-ray fluorescence.
[b] X-ray diffraction.

amorphous precipitates of calcium as well, in the form of $CaT.4H_2O$ or as a consequence of flocculation in association with the charged colloids.

The tartrates also contained nitrogen, and the nitrogen content was highest in the samples obtained from the residues left after cold precipitation. Concentrations of less than 0·01% of aluminium, iron, magnesium, phosphorus, and copper and somewhat higher concentrations of sodium and silicon were also detected. The silicon may have been remnants of the fining agents after clarification of the wines.

Since the samples were solids that had to be dissolved for analysis, it was not possible to analyse the bitartrate and tartrate ions separately, in view of the dependence of the concentration of such ions on the pH. Therefore, the stoichiometric formula of the precipitate was calculated from the data on the carbon, hydrogen, potassium, and calcium contents as assessed by elemental analysis and X-ray fluorescence analysis given in Table 2. The other elements were not considered, because they represented less than 0·6% of the total sample. The theoretical amounts of carbon and hydrogen needed for stoichiometric precipitation of the potassium and calcium cations were calculated on the basis of the molecular formulae of the two tartaric salts, $C_4H_5O_6K$ and $C_4H_4O_6Ca\ 4H_2O$ (Table 3).

Samples 1 to 5 would appear to contain too little carbon, indicative of precipitation of other negatively charged, anionic substances together with the cations of potassium and calcium. Such anionic substances contributed, on average, around 10% of the charge of the total precipitated carbon. In contrast, in samples 6 and 7, which came from containers used for cold stabilization, there was an excess of carbon, or, in other words, too little

TABLE 3
Stoichiometric Ratios of the Most Important Elements

Sample	*C*	*H*	*K*	*Ca*	*C**	*H**	$(C-C^*)$ (%)	$(H-H^*)$ (%)
1	28·71	44·86	6·71	1	30·84	45·55	−7·42	−0·69
2	25·50	38·75	5·75	1	27·00	40·75	−5·88	−5·16
3	62·33	107·33	16·67	1	70·68	95·35	−13·40	+11·16
4	33·00	56·17	7·83	1	35·32	51·15	−7·03	+8·94
5	36·60	64·80	9·80	1	43·20	61·00	−18·03	+5·86
6	888·00	1 160·00	212·00	1	852·00	1 072·00	+4·05	+7·59
7	977·78	1 342·22	231·11	1	928·44	1 167·00	+5·05	+13·05

C*, H*: stoichiometric carbon (C) and hydrogen (H) needed to bring about precipitation of potassium and calcium.
C–C*, H–H*: excess (+) or deficit (−) of the experimental value with respect to the stoichiometric value.

potassium, in the precipitate. This suggests that other positively charged components precipitated along with the tartrate and bitartrate anions.

Organic compounds

Along with the primary component, tartaric acid, polyphenols and proteins were detected in all the tartrate samples used in this study (Table 4). Malic acid, oxalic acid, and pectins, on the other hand, were not detected in any of the samples. Using electron microscopy Silva *et al.* (1985), on the contrary, did observe crystals of malate and oxalate in the crystallic lees of wines. Moreover, free reducing sugars were not detected in these samples, although small quantities of more highly polymerized forms of these sugars were recorded. Samples 6 and 7, collected from containers used for cold stabilization, had higher phenol contents than the samples collected from containers used for the fermentation or storage of the wines.

Fractionation of high-molecular-weight compounds by ultrafiltration

In order to evaluate the distribution of the molecular weights of the nitrogen and polyphenol fractions, fractionation was carried out using ultrafiltration according to the procedure illustrated in Fig. 1. Table 5 presents the distribution of the total amino nitrogen (after hydrolysis) and of the polyphenols in each of the fractions. The group of samples consisting of the tartrates from fermentation (samples 1 to 5) was, with the exception of the nitrogen fraction in sample 4, rather homogeneous but differed from the samples composed of the tartrates from cold stabilization (samples 6 and 7). Peptides (with molecular weights of between 1000 and 10 000) were the main

TABLE 4
Organic Compounds Present in the Tartrates (% w/w)

Sample	*Tartaric acid*	*Total phenols (gallic acid)*	*Protein (BSA)*	*PN 100/[a] TN*
1	75·07	0·120	0·012	3·52
2	70·56	0·210	0·051	6·83
3	75·93	0·110	0·035	1·31
4	78·82	0·130	0·053	4·72
5	72·35	0·130	0·011	0·75
6	73·62	0·360	0·064	3·64
7	74·00	0·350	0·058	3·72

[a] PN = Protein nitrogen.
TN = Total nitrogen.

TABLE 5
Percentage Distribution of the Total Nitrogen and Total Polyphenols in the Tartrates According to Molecular Weight (MW)

Sample	*Amino nitrogen*			*Total phenols*		
	MW < 1 000	*1 000 < MW < 10 000*	*MW > 10 000*	*MW < 1 000*	*1 000 < MW < 10 000*	*MW > 10 000*
1	52·5	37·4	10·4	90	8·7	1·0
3	52·9	38·6	8·6	84·7	12·1	2·3
4	34·7	57·7	7·6	78	15·9	6·0
5	53·1	36·3	10·6	82·2	16·4	1·4
6	31·6	57·1	10·3	67·5	26·5	5·9
7	39·8	54·9	5·2	68·9	24·5	6·6

nitrogen fraction in these last two samples. The share of compounds with molecular weights higher than 10 000 (proteins) in the nitrogen fraction was comparable in all the samples. The main nitrogen compounds in wines have molecular weights of less than 1000 (Usseglio-Tomasset & Di Stefano, 1978; Colagrande & Silva, 1981), which indicates more intense precipitation of the more highly polymerized nitrogen compounds along with the KHT.

The molecular weights of the main polyphenol fraction were less than 1000 for all the samples of tartrates studied. The percentage share of the polyphenols with molecular weights of between 1000 and 10 000 was somewhat higher in the tartrates collected from the containers used for cold stabilization than in the rest of the samples.

These results would suggest that nitrogen compounds with molecular weights of between 1000 and 10 000 daltons and polyphenols with molecular weights of less than 1000 tend to precipitate out with the KHT during cold stabilization; and the inhibition of the growth of the KHT crystals that form during the quick-chilling procedure to which these wines were subjected can therefore be attributed to them. The results of the X-ray diffraction analysis indicate that such compounds are not true components of the crystal lattice but are adsorbed, thereby slowing the attachment of new growth units to the crystals.

CONCLUSIONS

KHT was the primary salt detected in the tartrates analysed. Small amounts of calcium tartrate were also detected in the samples of the precipitates that had formed during fermentation and/or storage of the wines. A portion of the calcium present in the tartrates was amorphous. Polyphenols, proteins, peptides, and polypeptides were also present. The share of peptides and polyphenols with molecular weights of between 1000 and 10 000 was greater in the precipitates that were formed as a result of cold stabilization than in the precipitates that had formed spontaneously during fermentation and/or storage. None of these elements or compounds was actually incorporated into the structure of the crystals of KHT or CaT; rather, they were adsorbed onto the crystals, thereby inhibiting further growth. This phenomenon was responsible for the stoichiometric anomalies detected.

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