Influence of Uronic Acids on the Spontaneous Precipitation of Calcium L-(+)-Tartrate in a Model Wine Solution

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Polygalacturonic acid has been found to inhibit the spontaneous precipitation of calcium tartrate in a model wine solution. The monouronic acids, galacturonic and glucuronic, do not affect the crystallization of calcium tartrate. Using a calcium ion selective electrode, the binding of calcium to polygalacturonic acid was found to reach a maximum at pH 4 with significant complexation occurring between pH 3.0 and 3.5. The polygalacturonic acid also affects the membrane asymmetry potential of the calcium ion selective electrode, and an appropriate correction must be applied. A study of the concentration of polyuronic acids (chain length >8) in commercial wines by the *m*-hydroxybiphenyl method showed that they are present at sufficient concentration to inhibit the calcium tartrate precipitation in wine. Sparkling wines have a much lower concentration of polyuronic acids than still white table wines, and this difference may be responsible for the calcium tartrate stability of white table wines.

Keywords: *Calcium tartrate; spontaneous precipitation; polygalacturonic acid; wine; calcium ion selective electrode; membrane asymmetry potential*

INTRODUCTION

The spontaneous precipitation of calcium tartrate (CaT) from wine, unlike that of potassium hydrogen tartrate (KHT), cannot be predicted using a simple cold stability test (Watson, 1988). This is of particular concern as calcium tartrate instability occurs randomly within a batch of wine, and the precipitation can be slow, often occurring after the wine is bottled. If precipitation occurs at this stage, the wine must be recalled and the precipitate removed. Although the presence of calcium tartrate precipitation in wine is not a health concern, its presence because of aesthetics can lead to a loss of consumer confidence in the wine. The problem primarily occurs in sparkling wines and only rarely in still white wines.

The crystallization kinetics of calcium tartrate have been reported in aqueous (Grases et al., 1993) and model wine solutions (Abguéguen and Boulton, 1993) through the use of seed crystals. The spontaneous precipitation of calcium tartrate has recently been studied in our laboratory using a model wine solution (McKinnon et al., 1994). The first step in calcium tartrate precipitation was found to be the formation of a soluble calcium tartrate molecule, which then aggregates to form a precipitate. In addition, we have investigated the influence of various wine components on calcium tartrate precipitation in model wine solutions (McKinnon et al., 1995) and found that carboxylic acids containing additional adjacent carboxyl and/or hydroxyl functional groups, such as malic and citric acids, were efficient inhibitors of the spontaneous crystallization of calcium tartrate.

For many years, the wine industry has searched for effective inhibitors of tartrate crystal growth (Clutton, 1974). Stocké and Görtges (1989 a,b) found that carboxymethyl cellulose (CMC) was effective at inhibiting calcium tartrate precipitation at an addition level of 50 mg/L. However, CMC is not a legal additive to wine, although it has been demonstrated by numerous studies to be a safe additive in foods and is approved for food use by the World Health Organization (Wucherpfennig et al., 1984).

All fruits contain polyuronides or polysaccharide polymers, known as pectic substances or pectins (Robertson et al., 1980), which are recognized as strong binders of calcium salts (Kohn, 1975). Pectic substances are composed of polymers of galacturonic acid, linked 1,4 with side chains of covalently linked neutral sugars, typically rhamnose, arabinose, mannose, and galactose (often the carboxyl groups of the uronic acids are methylated). Pectic substances are termed pectic or uronic acids when only a small percentage of the carboxyl groups are present in the methyl ester form. Uronic acids are naturally occurring compounds in wines and have the structural requirements for inhibiting calcium tartrate crystallization (McKinnon et al., 1995). Their structural similarity to CMC coupled with the relatively high concentration of pectic substances in wines (compared to that necessary for CMC inhibition of precipitation) indicates that they are potentially effective inhibitors of calcium tartrate precipitation. In this study, a number of commercially available pectic substances were investigated with respect to their ability to inhibit crystallization and to establish if pectic substances have the potential to inhibit calcium tartrate precipitation.

MATERIALS AND METHODS

Model Solutions. Standard model solutions contained 130 mg/L of calcium, 2 g/L of tartaric acid, and 11% ethanol at a pH of 3.5 and were prepared as described previously (McKinnon et al., 1994). All water used was purified by a MilliQ water system. Polygalacturonic acid (sodium salt) was obtained from Sigma Chemical Co. All other reagents were the highest purity available. Spontaneous precipitation experi-

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ments were performed in a double-walled, water-jacketed glass vessel thermostated to 15 °C, with continuous stirring at an approximate speed of 950 rpm.

The measuring system for ionized calcium concentrations consisted of an Orion 93-20 calcium ion selective electrode (Ca ISE) and an Orion 90-01 single-junction silver/silver chloride reference electrode coupled with an Orion 520A millivolt meter. The Orion Ca ISE has been shown to be independent of any pH effect above pH 3 and is stable in aqueous ethanol solutions (Cardwell et al., 1991). The millivolt output was converted to the ionized calcium concentration using a calibration graph prepared from calcium standards in the concentration range $10^{-2}-10^{-4}$ M. Standards were prepared containing 11% (v/v) ethanol at the appropriate pH and ionic strength for the supersaturated solution under study. When it was required, the ionic strength was adjusted by the addition of 4 M sodium chloride. The potentials of the calcium calibration standards were determined in the model reaction vessel at 15 °C, with stirring, allowing 20 min of stabilization time.

When polygalacturonic acid was present in the model, calibration curves were adjusted for the effect of asymmetry potential (McKinnon, 1994). To determine the magnitude of the asymmetry potential, calcium calibration standards were prepared containing $10^{-2}-10^{-4}$ M calcium and 11% (v/v) ethanol at pH 2 with an ionic strength appropriate for the polygalacturonic acid model solution under study; from the resulting calibration curve, the corresponding calibration equation was obtained. The known total calcium concentration, for the model solution under investigation, was entered into this calibration equation and the expected E_0(calculated) value obtained. The E_0 value of the model solution containing polygalacturonic acid was determined experimentally by obtaining a calibration curve for a series of calcium standards with polygalacturonic acid present. The difference between the calculated E_0 and the experimental E_0 was the asymmetry potential. This asymmetry potential was subtracted from all subsequent potentials obtained in solutions containing polygalacturonic acid so that the correct ionized calcium concentration could be determined.

Total calcium concentrations were monitored throughout the precipitation process by removing 1−2 mL samples at regular intervals, filtering the sample through a 0.45 μm cellulose acetate filter (Schleicher and Schuell, FP 030/2), and acidifying to prevent further precipitation. Calcium was determined using a Hitachi Z6000 flame atomic absorption spectrophotometer (AAS) by the method of Hart et al. (1984).

Calcium binding curves were obtained by determining the total and ionized calcium concentrations at various pH values and plotting these as a function of pH. The concentration of calcium retained by a 0.45 μm filter was calculated by determining the amount of calcium in the filtered solution (flame AAS) and subtracting this from the initial calcium concentration.

Crystallization Curve Calculations. Crystallization curves were generated by plotting either the total calcium concentration or the ionized calcium concentration as a function of time. Induction was taken as the first detectable decrease in calcium concentration. In spontaneous precipitation experiments where ionized calcium concentrations were monitored, the onset of precipitation caused an immediate change in the electrode potential, which was used to identify the induction period. The rate of crystal growth was estimated from the slope of the crystallization curve. The rates quoted correspond to the maximum change in concentration with time for each model (McKinnon et al., 1994, 1995).

Polyuronic Acid Determination. Water-soluble polysaccharides were separated from wines, prior to colorimetric analysis, by following the method described by Silacci and Morrison (1990). Wine samples (15 mL) were adjusted to approximately 80% ethanol by the addition of 75 mL of absolute ethanol and stored in the refrigerator overnight (4 °C). The resulting precipitate was collected by filtration through a 0.2 μm cellulose acetate membrane (Sartorius, 11107-47N), washed with additional 80% ethanol, and then redissolved in 75 mL of water.

Table 1. Effect of Uronic Acids on the Induction Period and Crystallization Rate of Calcium Tartrate at pH 3.5

standard model plus	induction period, (min)	crystallization rate (mg/min) ionized Ca	crystallization rate (mg/min) total Ca
no addition	7	2.78	4.57
0.5 g/L galacturonic acid	11	1.93	3.95
0.5 g/L glucuronic acid	6	2.68	5.74
0.5 g/L polygalacturonic acid	510	0.10	
0.125 g/L polygalacturonic acid	80	0.47	
0.0625 g/L polygalacturonic acid	44	0.68	

Total polyuronic acid concentrations were determined colorimetrically by the *m*-hydroxybiphenyl method (Blumenkrantz and Asboe-Hansen, 1973). The sample was mixed with sodium tetraborate and heated for 5 min. After cooling, *m*-hydroxybiphenyl (Aldrich) was added and the resulting absorbance determined at 520 nm. Samples were analyzed in duplicate with appropriate blanks.

RESULTS AND DISCUSSION

Crystallization curves have been shown to be particularly useful in evaluating the effect of a wine component on the precipitation of calcium tartrate (McKinnon, 1994; McKinnon et al., 1995) as they provide both the time for (i.e. induction) and the rate of crystallization. The reproducibilities of the induction period and the crystallization rate are reasonably high (25% rsd and 18% rsd, respectively, for the model in the absence of any inhibitor) (McKinnon et al., 1995). Changes in the induction period and the crystallization rate indicate that a potential inhibitor is affecting the calcium tartrate nucleation process (induction) or crystal growth (crystallization rate).

Monouronic Acids. Galacturonic acid is the most abundant uronic acid present in wines, with reported levels in German white table wines (Sponholz and Dittrich, 1984) ranging from 0.15 to 1.0 g/L. Galacturonic acid, added at 0.5 g/L to a standard pH 3.5 model solution, had no significant effect on the induction period but decreased the crystallization rate (Table 1), implying that galacturonic acid has a slight inhibitory effect on crystal growth. With a 0.5 g/L glucuronic acid addition to the standard pH 3.5 model, which is 10 times the mean glucuronic acid concentration in wines (Sponholz and Dittrich, 1984), there was no effect on either the induction period or the crystallization rate. Experiments using ionic strength buffered model solutions (McKinnon et al., 1995) containing 0.5 g/L of monouronic acid showed no binding between calcium and either glucuronic acid or galacturonic acid at pH 3.5, confirming earlier data (Kohn et al., 1968). This absence of calcium−monouronic acid binding explains why these monouronic acids have little effect on the induction period.

Polyuronic Acids. It has been previously reported that calcium binds strongly with polyuronates at a neutral pH (Kohn et al., 1968; Kohn and Larsen, 1972). To determine whether calcium is capable of binding with polyuronic acids at the pH of wine, a calcium−polyuronic acid binding curve was prepared. Polygalacturonic acid was used, as it is the most abundant uronic acid present in wine (Sponholz and Dittrich, 1984). The amount of ionized calcium was determined in a model solution containing 0.5 g/L polygalacturonic acid in the absence of tartaric acid over a range of pH values. The resulting binding curve in Figure 1 shows that the concentration of ionized calcium at pH 3.5 was 77% of

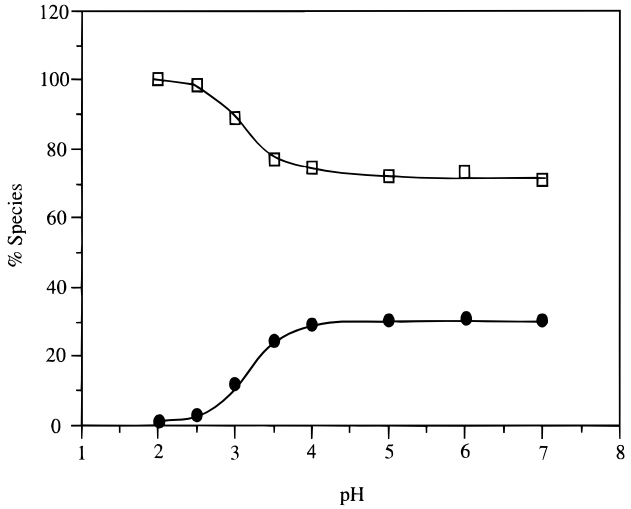

Figure 1. Binding curve showing the pH dependence of calcium in a model solution containing 0.5 g/L of polygalacturonic acid: (□) ionized calcium determined by Ca ISE adjusted for asymmetry potential; (●) calcium retained by a 0.45 μm filter determined by flame AAS.

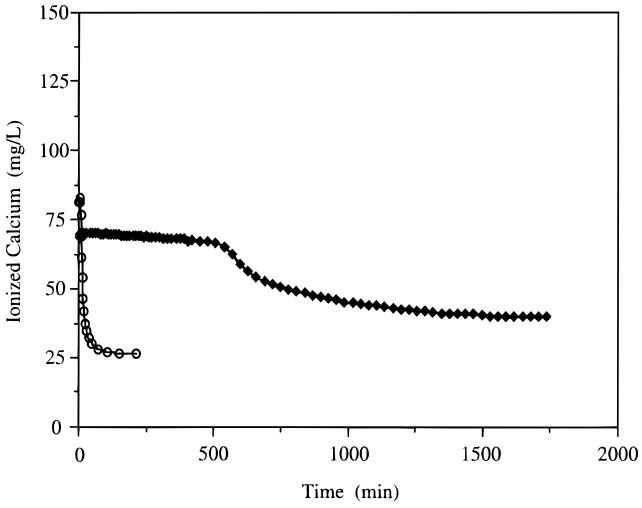

Figure 2. Crystallization curves for the standard model (○) and the model containing 0.5 g/L of polygalacturonic acid at pH 3.5 adjusted for asymmetry potential (♦).

the total present and that 23% of the calcium present was bound. The binding curve also shows that the pH of the model wine is important to the amount of calcium binding, with minimal binding occurring at pH 2.5 and near-maximum binding at pH 4.0. The percentage of retained (bound) calcium is also displayed in Figure 1. If the ionized calcium concentration and the retained calcium concentration are combined, then the total calcium concentration is obtained, signifying that all the bound calcium in the model is retained by a 0.45 μm filter and therefore must exist as a high molecular mass species (probably as a gel (Rolin and De Vries, 1990)).

Ionized calcium determinations in model solutions containing polygalacturonic acid were affected by an asymmetry potential interference (McKinnon, 1994). This interference is caused by contaminants such as proteins (Dürselen et al., 1988) influencing the surface behavior of the ion selective electrode, resulting in a shift in the standard cell potential (E_0) when changing from aqueous standard solutions to samples containing interfering compounds. It was found that a constant E_0 shift occurred in the presence of polygalacturonic acid which was independent of concentration; thus, reliable ionized calcium concentrations were obtained from the Orion Ca ISE after correction for asymmetry potential effects.

The ability of polygalacturonic acid to influence the calcium tartrate precipitation process was investigated with a 0.5 g/L polygalacturonic acid addition to the standard pH 3.5 model. The resulting crystallization curve is shown in Figure 2 along with the standard pH 3.5 model and it is apparent that the polygalacturonic acid has a large effect on both the induction period and the rate of crystal growth. With an induction period of 510 min (Table 1), the polygalacturonic acid containing model shows a greater than 70-fold increase over the mean induction period for the standard pH 3.5 model (7 min). The polygalacturonic acid model (0.5 g/L addition) shows a 5-fold increase over the mean induction period for the malic acid (2 g/L addition), demonstrating that polygalacturonic acid is a more effective inhibitor of both nucleation and crystal growth than any of the organic acids present in wine (McKinnon, 1994). At a pH of 3.5, calcium binding curves have showed that 7% of the calcium is bound in a model containing 2 g/L malic acid (McKinnon, 1994), whereas 23% of the calcium is bound in a model containing 0.5 g/L pf polygalacturonic acid (Figure 1).

Earlier studies have shown that calcium binds more strongly with polygalacturonic acid than monogalacturonic acid (Kohn, 1975; Kohn et al., 1968; Kohn and Larsen, 1972) as a consequence of intermolecular chelate binding (Kohn and Luknár, 1977) in concordance with the "egg-box" binding model described by Rees and co-workers (Grant et al., 1973). The intermolecular binding involves the interaction of Ca^{2+} with two carboxyl groups belonging to two different polyuronic acid chains. The strongest binding occurs with polyuronic acid chain lengths above 15 - 20 units (Kohn and Luknár, 1977; Yalpani, 1988).

Uronic acid concentrations in wine have been reported (Arndt and Thaler, 1974; Tusseau and Laer, 1993) to fall between 10 and 2300 mg/L. The influence of various polygalacturonic acid concentrations on the spontaneous precipitation of calcium tartrate in the model at pH 3.5 was therefore examined, and the results are presented in Table 1. As expected, the inhibitory effect on nucleation and crystal growth decreases as the concentration of polygalacturonic acid in the model solution is reduced. There is, however, a significant inhibitory effect observed even at a polygalacturonic acid concentration as low as 62.5 mg/L; the degree of inhibition is greater than that observed with a 2 g/L lactic acid addition to the model solution (McKinnon et al., 1995).

Determination of Polyuronic Acid Levels in Wine. While the concentration of uronic acids has been determined for European wines (Arndt and Thaler, 1974; Sponholz and Dittrich, 1984; Tusseau and Laer, 1993), no data are available for Australian wines. A survey of Australian still white and sparkling wines was undertaken to establish if polyuronic acids were present in any of the samples in sufficient concentration to influence calcium tartrate precipitation.

The determination of pectic substances in grapes and wines has been made by colorimetric assays using either carbazole (Dubourdieu et al., 1981; Robertson, 1979) or *m*-hydroxybiphenyl (Mourgues, 1981; Mourgues et al., 1984; Robertson, 1979; Robertson et al., 1980; Silacci and Morrison, 1990). Both of these colorimetric methods were investigated for the determination of pectic substances in wine. The traditional carbazole procedure has the disadvantage that neutral sugars interfere due

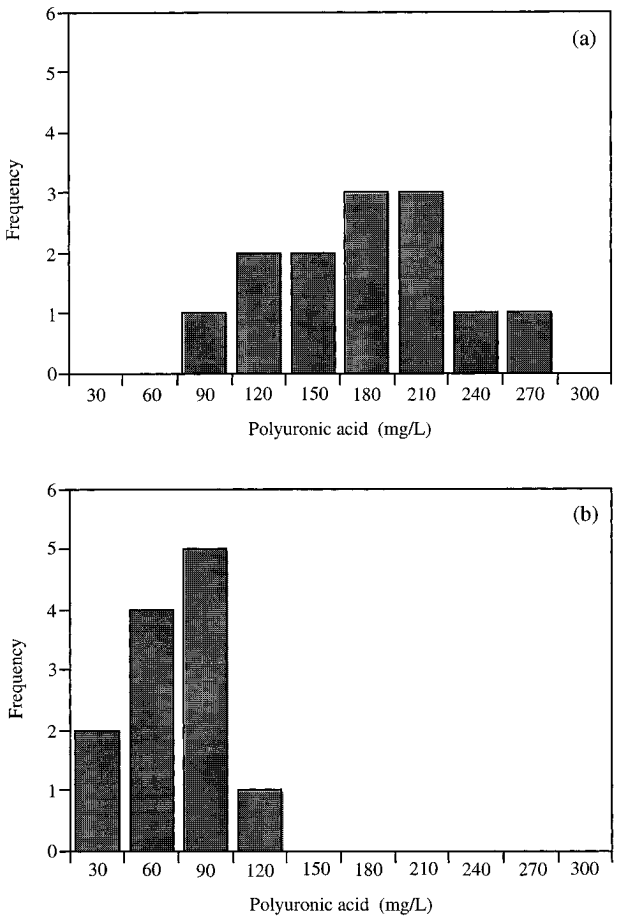

Figure 3. Histograms of polyuronic acid concentrations in surveyed Australian wines: (a) still white wine; (b) sparkling wine.

to the inability of the carbazole reagent to differentiate between uronic acids and hexoses (Dische, 1947; Robertson, 1979), although this neutral sugar interference can be eliminated by the use of a modified carbazole method (Galambos, 1967). The *m*-hydroxybiphenyl method, which involves the formation of a chromogen, is specific for uronic acids and is unaffected by relatively high concentrations of neutral sugars (Blumenkrantz and Asboe-Hansen, 1973). An additional advantage of the *m*-hydroxybiphenyl method was that it was not affected by variations in tartaric acid concentration, the major wine acid (data not shown), and with aqueous polyuronic acid standards, prepared from polygalacturonic acid, a linear calibration graph was obtained in the 0−100 mg/L concentration range (correlation coefficient 0.998).

A survey of 25 Australian wines was carried out using the *m*-hydroxybiphenyl method. Wine samples were fractionated with ethanol prior to analysis to isolate the polyuronic acids with chain lengths greater than approximately eight monomer units (Whistler and Sannella, 1965). The results of the wine survey are shown in Figure 3. The mean level of polyuronic acids found in sparkling wines is similar to the lowest level of polygalacturonic addition used in this study (62.5 mg/L). It is therefore reasonable to expect inhibition in wine similar to that observed in the model as reported in Table 1. However, it must be recognized that the model contains a single polyuronic acid of reasonably well defined composition, whereas little is known about the structure and chemical composition of these compounds in the wines studied here.

From the survey results, the white table wines have approximately 3 times the polyuronic acid content of sparkling wines. On the basis of the data in Table 1, it is reasonable to assume that the inhibition of calcium tartrate crystallization will increase as the concentration of the polyuronic acids in wine increases. This observation offers a possible explanation as to why white table wines are stable at calcium and tartrate concentrations similar to those of unstable sparkling wines; that is, white table wines contain higher amounts of effective inhibitors.

It is not fully understood why white table wines have higher polyuronic acid concentrations than sparkling wines. A possible explanation is that grape maturity may influence the polyuronic acid concentration, as sparkling wine grapes are picked earlier than grapes intended for still white wines. Some workers have reported that the polyuronic acid concentration changes with berry ripening (Mourgues, 1981; Silacci and Morrison, 1990), whereas others have noted no change (Robertson et al., 1980). These apparently conflicting reports do not allow any definite conclusion to be drawn about the relationship between berry ripeness and polyuronic acid concentration. The variation in the polyuronic acid content seen in Figure 3 may be linked to grape processing. Preliminary results from the polyuronic acid concentration survey has shown that premium sparkling wines have less polyuronic acids than lower quality sparkling wines and that bag-in-box white wines have the highest polyuronic acid concentrations. Minimal skin contact is common practice in the preparation of base wines for sparkling wine production, and whole bunch pressing is frequently used for juice extraction in the production of premium sparkling wines. Further, the juice used in base wine preparation is either "free run" or that obtained from pressing at low pressure. In table wine production, especially those made in large volumes (i.e. bag-in-box style wines), some maceration may occur during grape processing and higher proportions of pressed juice may be used. Thus, it is possible that as the extent of grape processing is increased, the extraction of pectic substances, including polyuronic acids, from berries is also increased.

ABBREVIATIONS USED

AAS, atomic absorption spectrophotometry; Ca ISE, calcium ion selective electrode; CaT, calcium tartrate; CMC, carboxymethyl cellulose; E_0, standard electrode potential; KHT, potassium hydrogen tartrate.

LITERATURE CITED

Abguéguen, O.; Boulton, R. B. The crystallization kinetics of calcium tartrate from model solutions and wines. *Am. J. Enol. Vitic.* **1993,** *44*, 65−75.

Arndt, W.; Thaler, H. Determination of galacturonic acid in wine and fruit juices. *Mitt. Klosterneuburg* **1974,** *24*, 325−340.

Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973,** *54*, 484−489.

Cardwell, T. J.; Cattrall, R. W.; Mrzlijak, R. I.; Robins, L. M.; Scollary, G. R.; Sweeney, T. Determination of Ionized and Total Calcium in White Wine Using a Calcium Ion-Selective Electrode. *Electroanalysis* **1991,** *3*, 573−576.

Clutton, D. W. Tartrates in wine—a review. *Process Biochem.* **1974,** 25−28.

Dische, Z. A new specific color reaction to hexuronic acids. *J. Biol. Chem.* **1947,** *167*, 189−198.

Dubourdieu, D.; Hadjinicolaou, D.; Ribéreau-Gayon, P. Soluble polysaccharides of must: simple method of estimation;

development in the course of ripening; effect on the prefermentation operations. *Connaiss. Vigne Vin.* **1981,** *15*, 29−40.

Dürselen, L. F. J.; Wegmann, D.; May, K.; Oesch, U.; Simon, W. Elimination of the asymmetry in neutral-carrier-based solvent polymeric membranes induced by proteins. *Anal. Chem.* **1988,** *60*, 1455−1458.

Galambos, J. T. The reaction of carbazole with carbohydrates 1. Effect of borate and sulfamate on the carbazole colour of sugars. *Anal. Biochem.* **1967,** *19*, 119−132.

Grant, G. T.; Morris, E. R.; Rees, D. A.; Smith, P. J. C.; Thom, D. Biological interactions between polysaccharides and divalent cations: the egg-box model. *FEBS Lett.* **1973,** *32*, 195−198.

Grases, F.; Melero, G.; March, J. G. Kinetics of calcium tartrate crystal growth from supersaturated solutions. *Colloids Surf.* **1993,** *71*, 115−121.

Hart, L.; Mylonas, G.; Scollary, G. Calcium in wine: some thoughts on its determination. *Aust. Grapegrower Winemaker* **1984,** *244*, 29−32.

Kohn, R. Ion binding on polyuronates—alginate and pectin. *Pure Appl. Chem.* **1975,** *42*, 371−397.

Kohn, R.; Furda, I.; Haug, A.; Smidsrød, O. Binding of calcium and potassium ions to some polyuronides and monouronates. *Acta Chem. Scand.* **1968,** *22*, 3098−3102.

Kohn, R.; Larsen, B. Preparation of water-soluble polyuronic acids and their calcium salts, and the determination of calcium ion activity in relation to the degree of polymerization. *Acta Chem. Scand.* **1972,** *26*, 2455−2468.

Kohn, R.; Luknár, O. Intermolecular calcium ion binding on polyuronates - polygalacturonate and polyguluronate. *Collect. Czech. Chem. Commun.* **1977,** *42*, 731−744.

McKinnon, A. J. An examination of factors affecting calcium tartrate precipitation in wine. Ph.D. Thesis, University of Melbourne, 1994.

McKinnon, A. J.; Scollary, G. R.; Solomon, D. H.; Williams, P. J. The influence of wine components on the spontaneous precipitation of calcium L(+)-tartrate in a model wine solution. *Am. J. Enol. Vitic.* **1995**, *46*, 509−517.

McKinnon, A. J.; Scollary, G. R.; Solomon, D. H.; Williams, P. J. The mechanism of precipitation of calcium L(+)-tartrate in a model wine solution. *Colloids Surf. A* **1994,** *82*, 225−235.

Mourgues, J. Changes in pectic substances and non cellulosic neutral polysaccharides. *Sci. Aliment.* **1981,** *1*, 377−388.

Mourgues, J.; Flanzy, C.; Bourzeix, M. Non cellulosic polysaccharides evolution during anaerobic metabolism of grape berry. *Sci. Aliment.* **1984,** *4*, 257−272.

Robertson, G. L. The fractional extraction and quantitative determination of pectic substances in grapes and musts. *Am. J. Enol. Vitic.* **1979,** *30*, 182−186.

Robertson, G. L.; Eschenbruch, R.; Cresswell, K. J. Seasonal changes in the pectic substances of grapes and their implication in juice extraction. *Am. J. Enol. Vitic.* **1980,** 31, 162−164.

Rolin, C.; De Vries, J. Pectin. In *Food Gels*; Harris, P., Ed.; Elsevier Science: London, 1990; pp 401−434.

Silacci, M. W.; Morrison, J. C. Changes in pectin content of cabernet sauvignon grape berries during maturation. *Am. J. Enol. Vitic.* **1990,** *41*, 111−115.

Sponholz, W. R.; Dittrich, H. H. Galacturonic, glucuronic, 2- and 5-oxogluconic acids in wines, sherries, fruit and dessert wines. *Vitis* **1984,** *23*, 214−224.

Stocké, R.; Görtges, S. Retardation of Calcium Tartrate Crystallization. *Weinwirtsch., Tech.* **1989a,** *3*, 30−32.

Stocké, R.; Görtges, S. Retardation of Calcium Tartrate Crystallization. *Weinwirtsch., Tech.* **1989b,** *4*, 24−28.

Tusseau, D.; Van Laer, S. Study of macromolecules in champagne wines. *Sci. Aliment.* **1993,** *13*, 463−482.

Watson, B. Stability testing for commercial wine production. *Wine Advisory Board Res. Rep.* **1988,** *7*, 9−12.

Whistler, R. L.; Sannella, J. L. Fractional precipitation with ethanol. In *Methods in Carbohydrate Chemistry;* Whistler, R. L., Ed.; Academic Press: New York, 1965; Vol. V (General Polysaccharides), pp 34−36.

Wucherpfennig, K.; Dietrich, H.; Götz, W.; Rötz, S. Influence of colloids on tartrate crystallization. *Weinwirtsch., Tech.* **1984,** *120*, 13−23.

Yalpani, M. *Polysaccharides—Syntheses, Modifications and Structure/Property Relationships*; Elsevier: Amsterdam, 1988.

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