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Prevention of Tartrate Crystallization in Wine by Hydrocolloids: The Mechanism Studied by Dynamic Light Scattering

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Supporting Information

ABSTRACT: Young wines are supersaturated in potassium bitartrate, which induces rather uncontrolled crystallization processes. Delayed crystallization may occur even after bottling of the young wines, which is undesirable because it gives rise to a visual defect in the wine. Colloids such as mannoproteins, metatartaric acid, and carboxymethylcellulose are available on the market and may be added to delay crystallization. It has been a matter of debate whether such hydrocolloids prevent nucleation, growth of crystals, or both. It was the objective of this investigation to study the crystallization event by a new approach using dynamic light scattering and to clarify the mode of action of these hydrocolloids. To achieve this, model solutions and standardized wines were enriched with potassium bitartrate (KHT) to trigger crystallization. In this way, it was possible to distinguish between the influence of the hydrocolloids on nucleation and on crystal growth. It was found that the hydrocolloids do not prevent KHT nucleation. Instead, these compounds delay or even arrest the outgrowth of the crystals to a macroscopic, visual size.

KEYWORDS: *mannoproteins, potassium hydrogen tartrate, potassium bitartrate, metatartaric acid, carboxymethylcellulose, KHT nucleation, tartrate crystals, DLS, wine, colloids, yeast polysaccharides, crystallization*

INTRODUCTION

It is a well-known fact that after alcoholic fermentation all wines are supersaturated in potassium and tartaric acid, which leads to the spontaneous but slow crystallization of potassium hydrogen tartrate (KHT).[1,2] Unfortunately, this spontaneous crystallization is highly unpredictable because of the stochastic nature of the nucleation burst and is therefore characterized by a lag time that can last several months. If spontaneous crystallization occurs after bottling of the wine, a deposit of crystals is clearly visible, a phenomenon that winemakers wish to avoid as it is perceived negatively by most consumers. The delay of crystallization is partly caused by the presence of wine colloids, such as proteins, polysaccharides, and polyphenols,[3,4] and the duration of the delay depends on their respective concentrations and molecular structure, as well as on the wine pH, the concentration of tartaric acid, the concentration of potassium, and other cations. The storage conditions of the wine, especially the temperature, play a determining role. The role of colloids was first demonstrated from the observation that wines aged on lees and stirred regularly (technique known as bâtonnage in traditional winemaking) have a higher resistance against the spontaneous crystallization of KHT, which was attributed to the release of mannoproteins (MP) from yeast cell walls.[5]

One traditional method of overcoming unpredictable crystallization of KHT is to perform a cold stabilization, i.e., to simultaneously cool the wine to −4 °C, seed with KHT crystals, and keep the wine at this temperature for several days.[6] This process enables depletion of the supersaturation but at the same time is detrimental to wine quality, due to oxidation and loss of aroma, and some loss of unstable (hydro)colloids, and a change in pH and acid composition.[7] Removal of colloidal substances from the wine by fining generally accelerates the spontaneous crystallization of KHT,[3] but such "overfining" is undesirable from an organoleptic point of view, because the colloidal substances significantly contribute to the mouthfeel of the wine. In addition, this process is costly and environmentally unfriendly because of the requirement of cooling energy.[8] More recently, electrodialysis was introduced as a membrane method for decreasing potassium and tartaric acid concentrations in the wine.[9] This method gives very good results in terms of tartrate stability. Because it requires a substantial financial investment and some water and energy input, it is more suitable for large wineries.

A second approach consists of the addition of stabilizing substances to the wine, which inhibit crystallization. The polymers metatartaric acid (MT), carboxymethylcellulose (CMC), and *Saccharomyces cerevisiae*-derived mannoproteins (MP) are examples of hydrocolloids that are known as effective

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stabilizers with respect to KHT crystallization.[10,11] MP are naturally present at significant concentrations in wine as they are produced by yeasts during alcoholic fermentation. Whether MP and other colloidal substances prevent nucleation has been a matter of debate,[12] or if they rather influence the growth of crystals.[13,14] Many authors mention the inhibition of both nucleation and growth of crystals.[15−17] Excellent overviews of tartrate crystallization in wine have been published.[7,18] Here we report a study of the initial stage of the crystallization process by employing dynamic light scattering (DLS, also termed photon correlation spectroscopy). Our results reveal new insights into the crystallization process at submicrometer length scales. This gives more information about the mechanism of action of wine stabilizers.

The effect of wine stabilizers was studied by a dedicated investigation using dynamic light scattering (DLS). DLS[19,20] is known as being very accurate in detecting hydrodynamic diameters of particles in the size class of interest, i.e., a few nanometers to the micrometer size regime, and tracking the time evolution thereof. This method is frequently used in colloid science and has been applied to different fields of application. Ample examples can be found in the literature: formation and growth of crystal nuclei of zeolite A from an amorphous gel,[21] protein crystal growth,[22] nanoencapsulation of epigallocatechin 3-gallate by protein,[23] size distribution of casein micelles in milk,[24] and aggregation of the casein micelles in milk as mediated by acidification and pectin adsorption.[25] Furthermore, DLS has been used successfully for the study of aggregation of polyphenols and protein polyphenol interactions in wine[26,27] and for the study of wine protein haze formation.[28−30] To the best of our knowledge, DLS has not yet been used to study the crystallization events in wine. It is the goal of this study to clarify whether MP, CMC, and MT inhibit the nucleation of KHT in wine, the growth of such crystals, or both.

MATERIALS AND METHODS

Commercial Wine Stabilizers. Metatartaric acid (MT) with a degree of esterification of 33/34 was purchased from La Littorale (Servian, France, commercial name "Solutartre"). Carboxymethylcellulose (CMC) with a degree of substitution between 0.65 and 0.9 was purchased from Acatris (commercial name Blanose 7LF). Mannoprotein (MP) products that were highly effective as protective colloids against KHT crystallization (commercial name Claristar, Oenobrands, Montpellier, France) described hereafter as Mannoprotein B as well as an experimental batch of MP fractions with low effectiveness on tartrate stabilization described hereafter as Mannoprotein A (liquid formulation containing 20% dry matter) were produced by DSM Food Specialties (Delft, The Netherlands).

Test Solutions for Investigating the Effects of Wine Colloids. Two media were studied: a "model solution" prepared from a solution of ethanol in ultrapure water (10 vol % ethanol) and "standardized wine" prepared from deionized Chardonnay wine (composition in Table SI1 of the Supporting Information). Deionization was performed by electrodialysis at the Unité Expérimentale de Pech Rouge (INRA). The solutions were prepared starting from KHT from Sigma-Aldrich dissolved either in the model solution or in the standardized wine. The solvents were filtered using a 0.2 μm polyvinylidene fluoride (PVDF) filter. To ensure the complete dissolution of KHT, the solutions were stirred at 27 °C for 20 min.

Four solutions at different concentrations were prepared (see Table SI2 of the Supporting Information), and four samples per concentration were measured to verify the reproducibility. Additionally, to efficiently induce nucleation within a short period of time even in the presence of wine stabilizers, a dedicated procedure (procedure 1) was developed making use of the antisolvent property of ethanol for KHT. KHT (2.5 g/L) was dissolved in standardized wine at 20 °C on a magnetic stirring plate; the KHT-enriched standardized wine was then filtered through a 0.2 μm PVDF filter, and subsequently, a commercial wine stabilizer, i.e., CMC or MT (50 or 100 mg/L, respectively, according to common practice in the winery industry), was added. The obtained standardized wine solution was then split into two portions. To one portion was added ethanol after being filtered on a 0.2 μm PVDF filter resulting in 4% additional ethanol. The second portion to which no ethanol was added was used as a control sample.

Procedure 1 was tested on a blank solution, i.e., without the addition of a wine stabilizer to verify the robustness of the method and the sensitivity of DLS. Procedure 1 was also used to investigate the effect of MP on nucleation. In that case, 200 mg/L MP were added to a KHT-enriched standardized wine solution.

To further investigate the effect of MP on the growth of KHT nuclei, a dedicated procedure (procedure 2) was employed. Also for procedure 2, 2.5 g/L KHT was dissolved in standardized wine at 20 °C on a magnetic stirring plate, and the solution was filtered on a 0.2 μm PVDF filter. However, to promote nucleation of KHT, additional 4% ethanol was added to the wine solution directly after being filtered on a 0.2 μm PVDF filter.

The two mannoprotein preparations, Mannoprotein A (low effectiveness) and Mannoprotein B (Claristar, high effectiveness), were added to the wine solution at a concentration of 200 mg/L. Continuous DLS measurements were recorded for an observation time of 800 min. From each distribution, the mean sizes were collected and the size variation in time was evaluated from the plot of size versus time.

Preparation of KHT Crystals for the Investigation of Growth. KHT crystals were nucleated and grown in four media: (a) ultrapure water, (b) ultrapure water supplemented with Mannoprotein B, (c) standardized wine, and (d) standardized wine supplemented with Mannoprotein B. For each medium, 2.5 g/L KHT was dissolved at 20 °C in a volume of 40 mL. The solutions were then filtered through 0.2 μm PVDF filters. Nucleation was then initiated by adding a proper amount of ethanol, which was 10 vol % to water solutions and 4 vol % to wine ones. After storage at 0 °C for a couple of days, the crystals were large enough to settle down. The samples were filtered, and the crystals were analyzed by light microscopy and X-ray diffraction (XRD).

Dynamic Light Scattering (DLS). DLS experiments were performed by using a Malvern Instruments (Malvern, U.K.) Zetasizer Nano ZS particle size analyzer. The refractive index (RI) of the scattering species was set at 1.450. This is a fair estimate for wine colloids (proteins) and does not differ that much from the RI of KHT (1.511). This estimate will not give errors of more than a few percent. The solvent (water + 10 vol % ethanol) RI and viscosity were set at 1.330 and 0.8872 cP, respectively. Using the Zetasizer Nano ZS instrument, we measured fluctuations in the scattered intensity at a scattering angle of 173°. At 25.0 ± 0.1 °C, the effect of wine stabilizers on nucleation was investigated. A temperature of 4.0 ± 0.1 °C was chosen to study the effect of MP on crystal growth. Samples were measured (undiluted) in 10 mm Poly (methyl methacrylate) PMMA sizing cuvettes and allowed to equilibrate in the Zetasizer instrument for 120 s prior to measurement. The number and duration of the runs were fixed. The particle size (we report diameters throughout this work) and size distribution were derived from a cumulant fit of the intensity autocorrelation function,[19] using the general purpose (normal resolution) analysis model.

Light Microscopy. A Leica Microsystems Wetzlar GmbH microscope (type 020-520.007 DM/LP) fitted with objectives for 25, 50, 100, 200, and 500-fold enlargement factors was used (Leica, Wetzlar, Germany). The images were captured by a Kappa (Gleichen, Germany) model CF 11 DSP analogue video camera, while a software application from Optik, Elektronik & Gerätetechnik (Frankfurt a/d Oder, Germany) and a Falcon (Frankfurt a/d Oder, Germany) model 761202 hardware board allowed us to grab the picture as an uncompressed bitmap file or compressed jpeg file.

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X-ray Diffraction (XRD). The XRD patterns were recorded on a D8 ADVANCE powder diffractometer from Bruker (Karlsruhe, Germany), equipped with a VANTEC array detector, with the divergence slit angle set as 0.3°. Diffraction angle 2θ ranged from 2° to 60°, with a step increase of ~0.007°, and the count time was 1 s/step. The samples were carefully loaded onto a normal sample holder (cavity diameter of 2.5 cm). To allow good statistics, the sample rotation was set to 15 rpm. Eventually, the recorded patterns were background subtracted.

RESULTS AND DISCUSSION

Influence of Wine Colloids on KHT Nucleation. First, the effect of wine colloids on the solubility of KHT was investigated (see Figure SI1 of the Supporting Information for details). As expected, the solubility of KHT was not affected by the presence of colloids. To study the influence of added colloids on KHT nucleation, we developed a method based on DLS that allows the detection of particles between one nanometer and a few micrometers, e.g., the size range in which a nucleus is stable enough to develop into a crystal but too small to be detected by the naked eye or by optical microscopy.

To properly use the technique for our purpose, it was necessary to develop a method that can account for wine colloids with sizes between 10^{-3} and 10^{-1} μm that are already present in wine and to distinguish them from KHT crystals. In addition, the method should be efficient enough to induce nucleation during the experimental observations even in the presence of wine stabilizers. To account for the presence of wine colloids, the particle size distribution of wine colloids in standardized wine was assigned (see Figure 1). The distribution

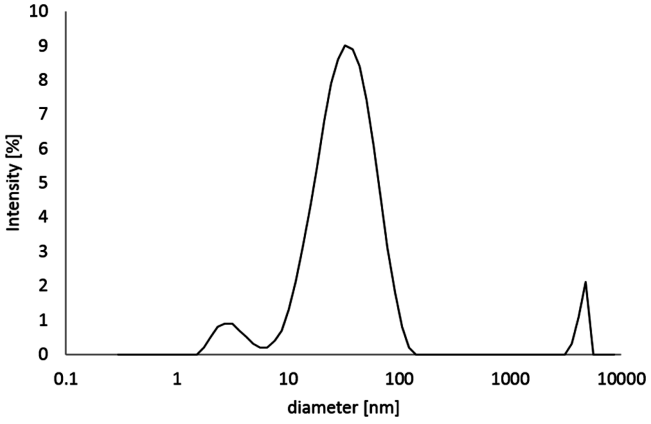

Figure 1. Intensity-weighed particle diameter distribution as a reference for standardized wine to assign the wine colloid size distribution. Scattered intensity I_0 = 117 kcps.

plotted in Figure 1 was used as a reference during the investigation. This reference scattered intensity is termed I_0. In the legend, the value for I_0 is indicated [in kilocounts per second (kcps)]. Below, we will present the intensities measured as dimensionless relative intensity values defined as $I_{rel} = I/I_0$. Note that we plot intensity-weighed size (diameter) distributions.

The DLS measurements revealed that when a KHT-enriched standardized wine was stirred at 20 °C and filtered on a 0.2 μm PVDF filter, a clear and stable solution was obtained. We reached this conclusion because of the good correspondence between the various size distributions (see Figure SI2) upon comparison of the particle distribution of the wine solution directly after preparation and 5 days later (stored at 3.5 °C) with the reference one. Relative intensity $I_{rel} = I/I_0$, where I_0 = 117 kcps (see Figure 1), also did not change (see the values in the legends of Figure SI2). Upon addition of ethanol to the wine solution, KHT nuclei were detected as a significant additional peak in the size distribution in a size class larger than the wine colloids (>100 nm) (dashed curve in Figure 2).

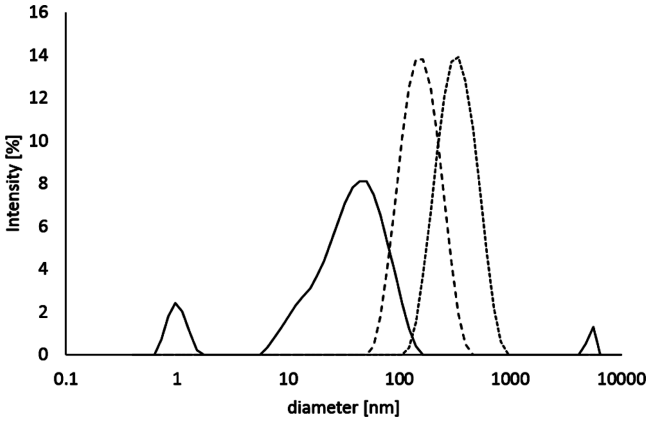

Figure 2. Particle size distribution after the addition of ethanol from 10 to 14 vol % (---; I_{rel} = 5.24) and upon storage at 3.5 °C for 5 days (···; I_{rel} = 5.87). The comparison with the wine colloid reference (—) reveals the formation of nuclei and their subsequent outgrowth.

It has to be pointed out that although not visible as a peak, the wine colloids are still present at smaller size classes in the dashed line and in the dotted line curves in Figure 2. This is due to the normalization of all scattered intensities to 100% (the unit of the ordinate is defined such that integrating all scattered intensities gives 100%). Hence, the scattering of the wine colloids is negligible because $I_{rel} \gg 1$. In particular, it appears that the scattered intensity contribution of the KHT nuclei is much larger than the contribution of the wine colloids to the scattered intensity.

Influence of Carboxymethylcellulose and Metatartrate on KHT Nucleation. Subsequently, the method was applied to commercially known wine stabilizers; in particular, the influence of CMC at a concentration of 50 mg/L and that of MT at a concentration of 100 mg/L were studied by adding them to the wine solutions before additional ethanol was added (procedure 1 in Materials and Methods). Particle size distributions were measured right after sample preparation, and after storage for 3 and 6 days at 3.5 °C. The results for CMC are plotted in Figure 3. It is noted that the relative scattered intensity starts at I_{rel} = 1.42 directly after ethanol addition, I_{rel} = 4.95 after storage for 3 days, and I_{rel} = 5.62 after storage for 6 days.

These results clearly show that the addition of ethanol brings about the formation of nuclei despite the presence of CMC (solid curve in Figure 3) and that they grow larger after 3 days at 3.5 °C (dashed curve in Figure 3). DLS measurements after 6 days at 3.5 °C revealed only a slight peak shift in the size distribution toward larger sizes and a slight increase in scattered intensity, hinting that the nuclei were still growing but at a much slower rate (dotted curve in Figure 3) than during the first 3 days. Similar outcomes were obtained from the runs with MT (see Figure SI3). Also in this case, the addition of ethanol resulted in the appearance of nuclei (solid curve in Figure SI3; I_{rel} = 1.61), which grew upon storage at 3.5 °C (dashed and

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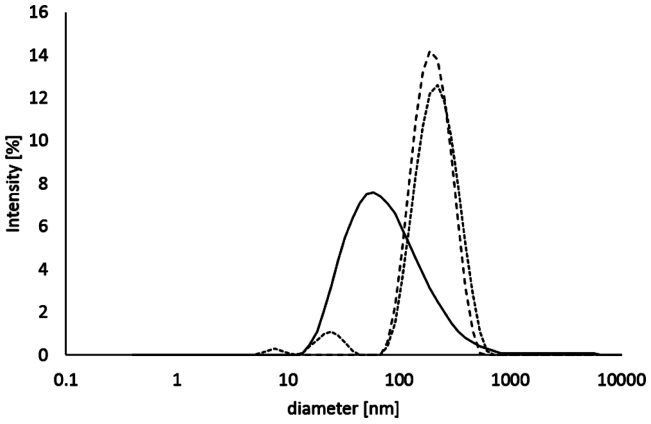

Figure 3. Time evolution of the particle size distributions of wine solutions with CMC after the addition of ethanol. I_{rel} = 1.42 directly after ethanol addition (—). I_{rel} = 4.95 after storage for 3 days (---). I_{rel} = 5.62 after storage for 6 days (···).

dotted curves in Figure SI3; I_{rel} = 4.79 and 5.42 after storage for 3 and 6 days, respectively). The peak shift after 6 days is larger than that from the previous runs, suggesting that in the presence of MT the KHT nuclei seem to keep on growing faster as compared to the samples with added CMC. From the observations mentioned above, we conclude that both CMC and MT do not inhibit nucleation of KHT; instead, they hinder growth.

Influence of Mannoproteins on KHT Nucleation. Next, we investigated the influence of mannoproteins on the initial crystallization process of KHT by applying the same method that was used for CMC and MT. To this end, 200 mg/L MP fraction B were added to a wine solution prepared according to the procedure described previously. Once again, particle distributions were measured directly after the addition of ethanol, and 3 and 6 days later (storage at 3.5 °C). While CMC and MT are present in solutions as polymeric chains, MP are present in wine as branched proteoglycans and can be regarded as colloidal particles. With this notion in mind, a distribution of MP in a wine solution (standardized wine enriched with KHT) was first assigned before the addition of ethanol. The solid curve in Figure SI4 shows the MP size distribution.

Also in the case of MP, the DLS measurements revealed that KHT can nucleate if ethanol is added to the wine solution (dashed curve in Figure SI4) and that those nuclei further grow during storage at 3.5 °C (dotted curve in Figure SI4). This conclusion agrees with the finding that the scattered intensity (I_{rel}) increases to 2.45 after ethanol addition and to 7.31 after storage for 3 days. However, the complete overlap of the peak after 6 days with that after 3 days and the nearly constant scattered intensity after storage for 3 (I_{rel} = 7.31) and 6 days (I_{rel} = 7.44) indicate that no further outgrowth occurred. In summary, the results presented thus far show that, under conditions extremely favorable to nucleation, neither random MP, CMC, nor MT can prevent KHT nucleation. Tiny KHT crystals do still form and grow under the experimental conditions. However, while in the presence of CMC and MT the nuclei continue to grow for a period of >3 days, in the case of MP the nuclei stop growing earlier. Hence, we conclude that the functionality of MP, MT, and CMC is to slow rather than to prevent crystallization. It is important to stress that our findings came to light only via the creation of extreme conditions for nucleation (i.e., adding ethanol to a KHT-rich wine solution), and by employing DLS, which is sensitive enough to detect particles in the submicrometer size range.

Further Investigation of the Influence of Mannoproteins on Crystal Growth. The investigation so far revealed that neither MP nor CMC and MT are preventing the formation of KHT nuclei. To explain the stabilizing activity of MP, we investigated in more detail the mechanism that leads to the prevention of crystal growth by MP. With the notion that MP are a large family of molecules, their stabilization activity might be exerted at different degrees according to the size and structure of the molecules. To account for the variation in the degree of stabilization, two largely different fractions (A and B)

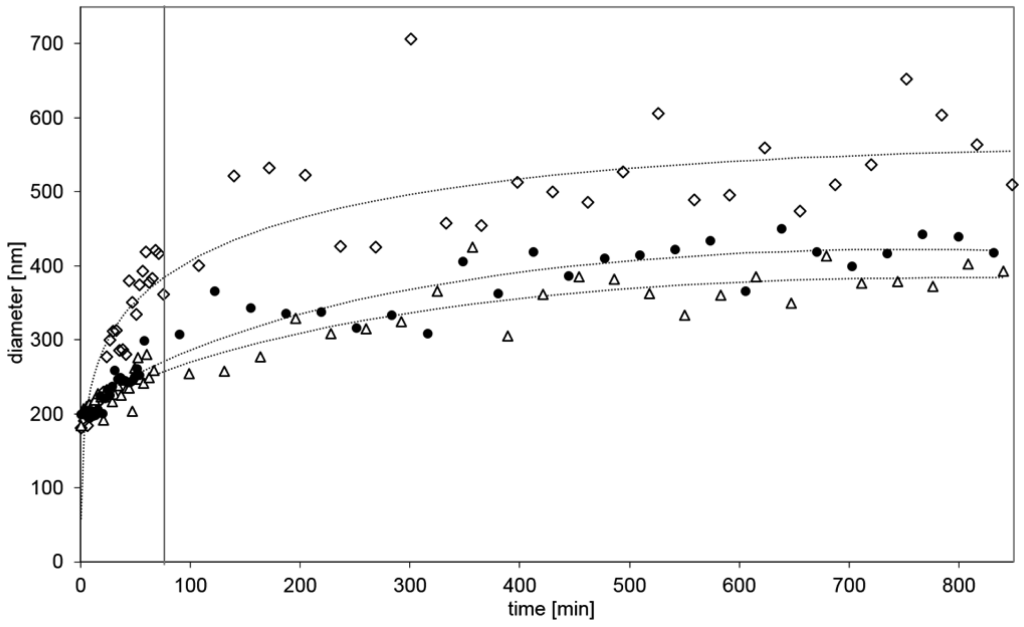

Figure 4. Size variation (diameter) over time of KHT nuclei grown in a wine solution (diamonds), in a wine solution with fraction A (circles), and in a wine solution with fraction B (triangles). Full curves are plotted to guide the eye. These curves are the result of polynomial fits.

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were chosen. Among other structural differences, fraction A is characterized by a very high carbohydrate content (90%) versus the protein moiety as compared to fraction B that has a carbohydrate content close to 70%. The average molecular weight of the mannoproteins in both fractions was determined to be approximately 400 kDa by means of size exclusion chromatography.

A cold stabilization test was performed to assess the degree of stabilization of the two fractions with respect to a rather unstable Chardonnay wine; i.e., visible KHT crystals appear in this wine overnight in the official test of the International Enological Code (Office International de la Vigne et du Vin). The concentration of tartaric acid was 2.5 g/L, and the concentration of K was 838 mg/L; no ethanol, K, or tartaric acid was added. The two fractions A and B were spiked at concentrations of 100 and 200 mg/L (dry matter MP per liter of wine) to assess their effect on the stability by the visual test at −4 °C. The wine solutions with additional MP fractions A and B were stored in a refrigerator together with control samples (to which no MP were added) at a constant temperature of −4 °C. Each day the solutions were visually inspected to monitor the appearance of KHT crystals. Generally, it is believed that a wine is stable toward crystallization of KHT when no crystals are detected by the naked eye during the stabilization test for a minimum of 6 days. Mannoprotein fraction A, when added at a concentration of 200 mg/L, induced a delay in the appearance of visible crystals by an additional 2 days, which is a significant improvement, but not enough to stabilize the wine. Mannoprotein fraction B at the same concentration delayed the appearance of visible crystals for 15 days, from which we could conclude that B was a high-performance fraction with respect to tartrate stability. The wine added with fraction B can be considered as stable for tartrate crystals. The results of this visual test are presented in Table 3 of the Supporting Information.

Next, DLS measurements were designed to follow the time evolution of particle size distributions of KHT nuclei in wine solutions. To stimulate the outgrowth of nuclei, the temperature of the DLS was set as low as possible (i.e., 4.0 ± 0.1 °C). The samples were prepared from standardized wine according to procedure 2 mentioned in Materials and Methods. It should be noted that, in contrast to the investigations of nucleation reported in the previous two sections, MP fractions A and B were now added after the formation of particles (after the addition of ethanol), ensuring that we were observing the effect on outgrowth exclusively. It has to be stressed that also in this case extreme conditions were imposed, so that the effects, if any, could be captured within the observation interval. A blank without MP (diamond data set in Figure 4) was run and used as a reference for the tests with the chosen MP fractions (circle data set for fraction A and triangle data set for fraction B in Figure 4). Large scattering affected the data especially in the long run, complicating a precise size determination. The full curves in Figure 4 are added as a guide to the eye and are the result of a polynomial fit. From the fit, we can determine the plateau value at $t \rightarrow \infty$, given in the text below. For each run, similar trends were found: a noticeable size increase at the beginning followed by a stabilization (plateau regime).

Already after 80 min, a large size difference was detected compared to the initial size: nuclei from the blank reached a diameter of ∼383.1 ± 26.0 nm, and the diameter of the nuclei grown in the presence of MP were ∼252.1 ± 21.5 nm for fraction A and ∼249.3 ± 15.0 for fraction B. In the plateau regime, the nuclei fluctuated around a final value that varied according to the solution composition: a diameter of ∼563.4 ± 56.2 nm for nuclei grown in the blank, ∼417.3 ± 18.0 nm in the presence of fraction A, and ∼378.7 ± 12.4 nm in the presence of fraction B. This observation led us to an important conclusion: MP fraction B drastically reduces growth rates of KHT nuclei in the submicrometer size class already at the onset of crystallization.

A close look at the runs with MP fraction B confirmed the results from the stabilization test; i.e., different performances are expected from different fractions. This emerges when we take a close look at the sizes obtained from DLS measurements on the run with fraction B (triangles in Figure 4) that reveal growth rates slower than those with fraction A (circles in Figure 4). This implies that these two fractions have a different influence on delaying the KHT crystallization kinetics. According to crystallization theory, if a foreign body influences the growth rate of a crystal, this should macroscopically result in a habit modification of the fully developed crystal and would not affect its lattice.[31] With this notion in mind, KHT crystals were intentionally grown to a microscopic size (millimeter size class range, as described in Materials and Methods) to be observed via light microscopy (for morphology recognition) and to be analyzed by XRD (for crystal structure identification). In line with the expectations, the crystals appeared to be noticeably different as determined from the light microscope images in Figure 5. In particular, four different morphologies were identified.

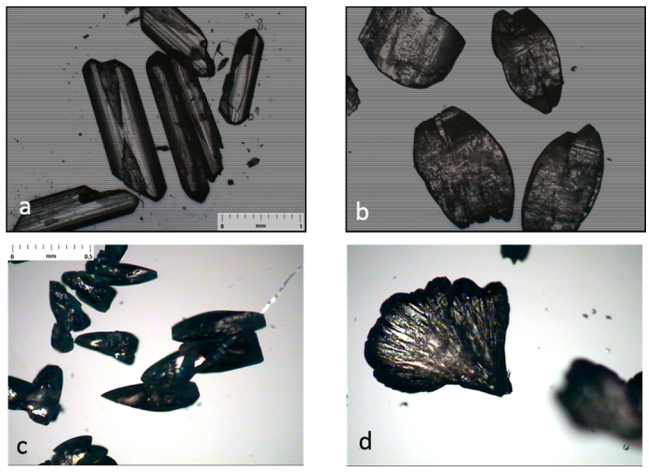

Figure 5. Light microscopy images of KHT crystals grown in four different media: (a) KHT crystals in ultrapure water (reference), (b) KHT crystals in ultrapure water with MP fraction B (effect of MP), (c) KHT crystals in standardized wine (effect of wine colloids), and (d) KHT crystals in standardized wine with the MP fraction (effect of wine colloids and MP).

XRD spectra were recorded. These XRD spectra reveal a close to perfect correspondence of reflections upon comparison of the reference (KHT from ultrapure water) with the observation samples, confirming that the crystal lattice was rather similar in spite of the morphological differences (see Figure SI5). The observations reported here confirmed the conclusions drawn above that mannoproteins affect KHT crystal growth in the sense that they delay the development of the crystal faces.

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On the basis of our findings, we propose a mechanism of action for MP. The mannoproteins probably adsorb to the surface of the KHT crystals, thereby interfering with further face advancement. The interference results at least in modification of the crystal morphologies. Crystal outgrowth is probably stopped when the adsorbed MP cover the surfaces, so further growth on all growth centers of the crystals is prevented. Additionally, in light of these observations, the results from previous investigations[32] of wine colloids in standardized wine can be better interpreted. In particular, KHT was also nucleated in standardized wine at low concentrations; the crystals did, however, not grow to a detectable size, from which the wrong conclusion was drawn, that wine colloids might inhibit the nucleation. At low KHT concentrations, the KHT−solution surface area is small, so the wine colloids can arrest crystal outgrowth at a very early stage.

In summary, we have reported the development of a method based on DLS to monitor KHT crystallization in wine. The methodology developed was shown to be a powerful tool for investigating the effect of wine stabilizers on KHT crystallization. The mode of action of the inhibition of KHT crystallization could be unambiguously unravelled by intentionally creating extreme conditions to promote nucleation. In particular, experimental procedures tailored for nucleation or outgrowth enabled us to discriminate between the two phenomena and to come in a straightforward way to the conclusion that the wine colloids mannoproteins (MP), carboxymethyl cellulose (CMC), and metatartaric acid (MT) do not prevent KHT nucleation. Their mechanism of action is rather the inhibition of crystal growth. It was found that initially tiny crystals still form and grow under the experimental conditions described in this study, which were intentionally extreme. At a later stage, however, further outgrowth of the crystal is severely slowed by wine colloids.

From this investigation, an important point came to light: when the traditional inspection is performed by the naked eye, the submicrometer-sized crystals are not detected, giving rise to misinterpretations of the occurring phenomena (growth inhibition is interpreted as an antinucleation effect). In light of this outcome, the so far accepted mechanism of action of wine stabilizers should be reconsidered. While in the presence of CMC and MT the nuclei continue to grow for more than 3 days, the nuclei stopped growing earlier when mannoproteins with the appropriate structure were used. Those mannoproteins were found to drastically reduce the growth rate at the very beginning of the formation of nuclei. Additionally, we found that the slowing of crystal outgrowth depends on the type of MP as follows from studying wine stored at −4 °C. The appearance of crystals, visible to the naked eye, can also differ strongly between different MP fractions. Hence, discrimination of low and high effectiveness can be performed, and MP fractions dedicated to wine stability, meaning with high effectiveness, can be isolated. It goes without saying that the DLS method presented here may be used for the study of the mode of action of other crystallization inhibitors, such as the recently proposed polyaspartate,[33] as well. It must be mentioned here that in this study only one unstable Chardonnay wine was investigated. Because wine is a product with a very complex and variable composition, the possibility that in other types of wine the mode of action of the hydrocolloids is different cannot be ruled out completely. It could be that for other types of wine, for instance, very surface active, compounds may interfere with the mechanism uncovered here. Such compounds are, therefore, not expected to be present in other wines. The methodology based on DLS measurements is generally applicable, independent of the type of wine or crystallization inhibitor studied, and can be used in future studies to detect whether such wine variations result in different mechanisms.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b01854.

Influence of colloids on the solubility of KHT (Figure SI1), composition of the deionized wine (Tables SI1 and SI2), visual crystallization test after addition of mannoproteins (Table SI3), particle size distribution in wine after the addition of KHT (Figure SI2), after the addition of ethanol in the presence of MT (Figure SI3), and after the addition of ethanol in the presence of MP (Figure SI4), and XRD spectra of KHT crystals (Figure SI5) (PDF)

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Notes

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■ DEDICATION

This paper is dedicated to the memory of Prof. C. G. (Kees) de Kruif (1943−2016), who contributed several seminal papers to this Journal. With his combined insights into colloid science, thermodynamics, scattering, and rheology, he contributed new insights into food science.

■ ABBREVIATIONS USED

MP, mannoproteins; MT, metatartaric acid; CMC, carboxymethylcellulose; DLS, dynamic light scattering; KHT, potassium bitartrate; PVDF, polyvinylidene fluoride; PMMA, poly(methyl methacrylate); RI, refractive index; XRD, X-ray diffraction

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