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KHT cold stabilization: A scanning electron microscopy study of the formation of surface deposits on stainless steel in model wines

by

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S u m m a r y : The incidence of yeast cells and wine polysaccharides and polyphenols in the formation of adherent KHT crystals on stainless steel surfaces during cold stabilization was investigated by scanning electron microscopy. Additives were responsible for differences in the deposit configuration, the crystal shape and size as well as in the KHT crystallization kinetics.

Yeast cells act as heterogeneous primary nucleation germs for KHT crystal formation. Colloids from wines interacted with KHT crystal faces and affected growth. It was confirmed that polyphenols strongly inhibit the crystallization and result in small crystals with a unidimensional growth. In contrast, with polyphenols, cubic crystals were obtained when wine polysaccharides were associated with yeast cells.

K e y w o r d s : KHT crystal, cold stabilization, yeast, inhibition, polysaccharides, polyphenols.

Introduction

Potassium hydrogen tartrate (KHT) is one of the major causes of wine instability. It is largely influenced by the wine composition, e.g. by polysaccharides and polyphenols (BALAKIAN and BERG 1968; CORREA-GORROSPE *et al.* 1991; ADGUEGUEN and BOULTON 1993; GERBAUD *et al.* 1996, 1997).

Crystallization naturally develops during wine fermentation and storage, or is provoked by cold treatment to stabilize wine before bottling. It may result in the formation of adherent tartrate deposits on the inner surfaces of tanks which are difficult to remove by winemakers. Thorough studies have led to an improved understanding of this fouling process (CORREA-GORROSPE *et al.* 1991; ABGUEGUEN and BOULTON 1993).

In the present work, adherent crystals formed on stainless steel surfaces from model wines were investigated by scanning electron microscopy.

Material and Methods

M o d e l w i n e s : Five model wines were prepared. Wine 1 was composed of K_2SO_4 (1.5 g·l^{-1}), tartric acid (2 g·l^{-1}), acetic acid (0.48 g·l^{-1}), lactic acid (1 g·l^{-1}), $Na_2S_2O_5$ (0.05 g·l^{-1}) and ethanol (10 %). Wine 2 was similar to wine 1 but yeast (10^5 cells·ml^{-1}) was added. Wine 3 A was similar to 2, polyphenols (2.88 g·l^{-1}) were added. As in wine 3A there was no crystallization, in wine 3 B the concentrations of K_2SO_4 (3 g·l^{-1}) and tartric acid (4 g·l^{-1}) were increased twofold. Wine 4 was similar to wine 2, polysaccharides were added (0.475 g·l^{-1}).

Saccharomyces cerevisiae INRA 7013 was provided by the Institut National de la Recherche Agronomique. Yeast was grown in a 0.15 M sucrose solution for 15 h at 30 °C, then recovered by centrifugation (10 min at 7,000 *g*) and washed twice in demineralized water.

Fractions of wine polysaccharides and polyphenols were isolated from a Carignan Noir red wine (1996, Pech-Rouge Enology Experimental Unity, INRA, Gruissan, France). Separation of wine phenolics was achieved from wine free alcohol by adsorption chromatography on a vinyl-divinyl benzen resin. The resin was then rinsed with one bed volume of distilled water. The unretained fractions, containing wine polysaccharides, were recovered.

Elution of phenolics was achieved with 5 bed volumes of a 5/95 (v/v) water/ethanol solution. The polyphenolic fraction was thus concentrated under vacuum at 28 °C and dried by atomization. It contained cinnamic acids (26 %), anthocyanins (19.1 %), flavonols (2.3 %) and tannins (52.6 %).

The polysaccharide fraction was further purified by successive ultrafiltration and diafiltrations is contained by arabinogalactanproteins (AGP: 51.8 %), mannoproteins (MP: 35.8%), rhamnogalacturonans (RG-I: 2.6 %, and RG-II: 9.8 %).

F o r m a t i o n o f K H T c r y s t a l s o n s t a i n l e s s s t e e l : Solids included AISI 304 stainless steel with 2B finish. Tartrate precipitates on stainless steel sheets (39 mm x 45 mm x 1 mm) were obtained by cold stabilization of model wines, carried out with a cooling device (Fig. 1). It was placed in a 30 l tank filled with a model wine and the temperature of the cooling liquid (water/glycerol) was lowered to -4 ± 0.5 °C.

The KHT crystallization in model wines was followed daily through conductivity measurements. Three parameters

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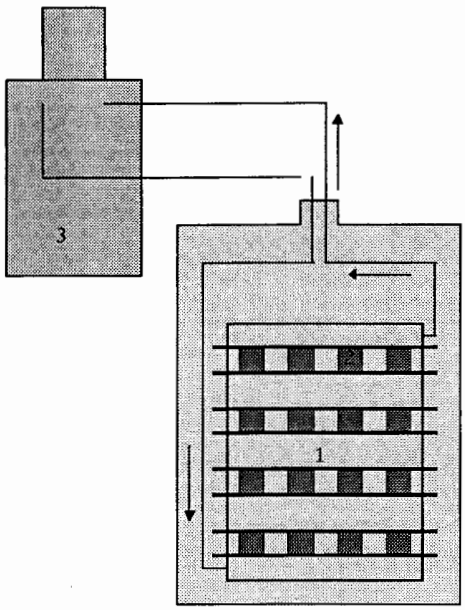

Fig. 1: Experimental device used to obtain KHT precipitates on the stainless steel surfaces. **1**: stainless steel support; **2**: stainless steel sheets; **3**: cooling system.

were determined: (i) the induction time, corresponding to the time required to detect a decrease in the solution conductivity; (ii) the precipitation rate, estimated from the decrease in conductivity; (iii) the maximal decrease of conductivity.

Scanning electron microscopy (SEM): The deposits were air-dried for 24 h and then sputter-coated with gold in a Balzers (Liechtenstein) chamber. The deposits were observed in a DSM 960 (Zeiss, Germany) SEM. The KHT crystal size and shape were observed after 24 h of cold treatment and at the end of the treatment.

Results

SEM observation of the KHT deposits on stainless steel: Crystals formed in wine 1 were typically biphenoidal or prismatic, final size around 1000 µm (Table), as shown in a previous study (RODRIGUEZ-CLEMENTE and CORREA-GORROSPE 1988).

The deposit structure was modified when yeast was added. Yeast cells were linked to the deposit. Initially, the surface was covered with yeast (Fig. 2 A), crystals grew on this layer (Fig. 2 B). At the end, the deposit was made up of successive layers of yeast and crystals (Fig. 2 C). Adding yeast also modified the crystal's shape. In this case, crystals were thinner and larger, indicating a unidimensional growth (Table).

Crystal growth was strongly impeded when polyphenols were added. At the end of the cold treatment, small crystals (length: up to 150 µm, thickness: 5 µm) were obtained (Table). Again, yeast cells were largely present in the deposit structure and involved in its formation.

Similar modifications in KHT crystals were not noticed if polysaccharides were added (wine 4). Large cube-shaped KHT crystals which firstly developed on stainless steel (Table) were always linked to adherent yeast. When the model wine was stabilized, large and cubic crystals (400-600 µm) adhering on surfaces were combined with smaller polyhedral ones (50-150 µm).

Crystallization kinetics in model wines: The induction time for crystal formation varied from 1 to 5 d depending on the composition of the model wine (Table). In wines 1, 3 B and 4, crystals on stainless steel were observed after 24 h of cold stabilization though no conductivity decrease could be detected. This indicated the onset of the crystallization process, however, no significant variation in the wine conductivity was observed. The shortest induction time was observed in model wines containing yeast (wine 2) or polysaccharides (wine 4); this is in contrast with polyphenols (wine 3 B) which strongly inhibited KHT crystallization. In wine 3 B, the concentrations of tartric acid and potassium had to be increased twofold to obtain crystallization.

Discussion

Changes in the deposit, the crystal shape and size and the KHT crystallization kinetics were induced by additives. The lack of yeast and wine molecules in wine 1 resulted in heterogeneous nucleation on the metallic surface. Crystal growth thus led to the formation of an adherent crystalline deposit with both large and small crystals. Adding yeast

Table

Parameters associated with the precipitation kinetics and KHT crystals

Solutions	Induction time (d)	Rate of conductivity decrease (%·d^{-1})	Maximal conductivity decrease (%)	Crystals size after 24 h (µm)	Crytals size after cold stabilization (µm)	Crystal shape
[1] KHT	2	1.4	4.8	300	700-1000	prismatic
[2] KHT + yeasts	<1	1.2	6.8	100-200	150-350	prismatic
[3a] KHT + yeasts + polyphenols	>10	0	0	-	-	-
[3b] KHT + yeasts + polyphenols	5	2.7	2.7	50	50-50	thin plates
[4] KHT + yeasts + polysaccharides	<1	3	12	300-400	400-600	cubic

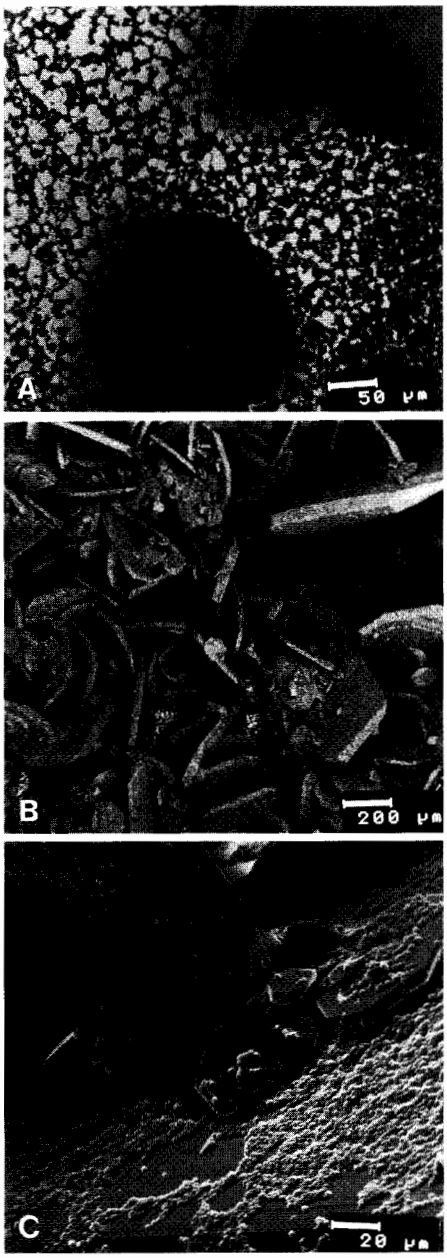

Fig. 2: **A**: KHT precipitates obtained after 24 h cold treatment of wine 2 (M = 200). **B**: Final KHT precipitates obtained after cold treatment of wine 2 (M = 50). **C**: Final KHT precipitates obtained after cold treatment in wine 2 (M = 500).

cells dramatically modified both the formation of KHT deposits and KHT crystallization kinetics. The distinct shortening of the induction time induced by yeast indicated that yeast can act as a heterogeneous primary nucleation germ and was favourable to the KHT crystallization. This was supported by SEM observations which showed crystal growth from yeast adhering on stainless steel.

This mechanism of deposit formation, implying successive layers of yeast cells and KHT crystals, was not modified by the addition of wine polysaccharides and polyphenols. However, the KHT crystallization was influenced differently by polyphenols and polysaccharides. BALAKIAN and BERG (1968), MAUJEAN *et al.* (1985) and GERBAUD *et al.* (1997) have shown that the KHT crystallization was strongly inhibited by wine phenolics, resulting in significant changes in crystal shape and size. These modifications in the crystal morphology can be related to the adsorption of wine phenolics on crystal sides, impeding the growth process (RODRIGUEZ-CLEMENTE and CORREA-GORROSPE 1988); consequently, crystals appeared to be thin plates. On the contrary, the KHT crystallization was enhanced by wine polysaccharides. Previous work dealing with the relative impact of wine polysaccharides on KHT crystallization in model solutions (GERBAUD *et al.* 1996) showed that RG-I and RG-II had a specific concentration-dependent behaviour: at low concentrations KHT nucleation and growth were stimulated whereas at high concentrations they were inhibited. Under our experimental conditions, with concentrations of RG-I and RG-II being medium, crystallization was weakly inhibited. However, it should be noted that a comparison was difficult because (1), the fraction used was a mixture and did not consist of pure macromolecules, and (2), the polysaccharide effect could be modified by yeast.

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