FLUORIDE INHIBITION OF WINE YEASTS

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ABSTRACT

Stuck or slowed fermentations are costly in time and money to winemakers. There are many variables that can interrupt fermentation. One of the lesser known factors is the effect of fluoride on grape juice fermentations. Winemakers in California have had problems with slow or stuck fermentations with grapes that have been treated with the insecticide Cryolite, which contains fluoride.

A selection of 6 yeasts, 3 commercial strains and 3 natural strains, commonly associated with winemaking were used in this study. Preliminary experiments investigated a wide range of fluoride challenge with different pH and cell densities on solid and liquid media. The effectiveness of fluoride was compared between sodium fluoride and Cryolite, as the fluoride source. The effect of fluoride was more potent with sodium fluoride, as the fluoride source. The minimum inhibitory concentration of fluoride for the yeast strains was recorded. The most sensitive commercial yeast was *Saccharomyces cerevisiae* RS1, the most resistant commercial yeast was *Saccharomyces bayanus* RS2. The most sensitive yeast overall was *Hansenula saturnus* AWRI-354.

The next stage examined the effect of fluoride on the selected yeast in small scale grape juice fermentations. Within this investigation the effect of different media sources and heat treatments was included. Fluoride concentrations reflected levels of fluoride found in grape musts and wines.

During this study we found that the effect of fluoride on yeasts is increased with lower pH and lower cell densities. The effect of fluoride on yeast growth and fermentation was also strain dependent.

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INTRODUCTION

Grape juice is a challenging medium for microbes to grow in. It is sweet, acidic and sometimes contains added sulphur dioxide. Only microorganisms which can tolerate these restrictive conditions can grow. This group is comprised of yeasts and certain lactic and acetic acid bacteria.

The yeasts which grow readily in grape juice are the fermenting yeasts, mainly *Saccharomyces,* and these are added as pure culture. Other wild yeast such as *Kloeckera* species may also initiate fermentation.

Pure starter cultures are chosen for their supreme tolerance of the harsh environment of the grape juice medium. An effective fermentator i.e. a yeast which efficiently converts sugars to ethanol and carbon dioxide, but is tolerant to grape juice conditions, saves money and time. The quick and reliable completion of alcoholic fermentation allows the winemaker to improve productivity.

The use of viticultural antiparasitic products are essential to the success and economic yield of grape crops. These products are applied to all parts of the plant, whether intentionally or not where they adhere and are either gradually washed away by rain, eliminated by evaporation, or destroyed by the sun's rays. Residues from these antiparasitic products have important implications on the suitability for human consumption of the wine and table grapes produced, and also may affect the process of the winemaking.

Scientific knowledge in the latter part of the twentieth century has allowed winemakers greater control over biological pests. Cryolite (Kryolith; Ice spar; sodium aluminium fluoride, (AlF_6Na_3)) is a natural compound that is used as an insecticide against three main pests, that is; the Omnivorous Leaf Roller, Western Leaf Skeletoniser and Orange Tortrix. These insects can cause considerable damage to the vine, leaves and berries of the grape plant. The Omnivorous Leaf Roller attacks the berries. This damage attracts vinegar flies, and other secondary feeding insects, that carry spoilage organisms which can initiate bunch rot. The Orange Tortrix feed on berries and stems, causing berry drop and stem girdling. The Skeletoniser eats the lower epidermidis and green part of the leaf, interfering with photosynthesis.

There are several advantages of using Cryolite. It is a natural compound, and is found in large mineral deposits in Greenland and the Urals, but it can also be easily synthesized. The consequence of Cryolite's commonality is that it is cheap. It is also very effective on the targeted insects, and has no adverse effect on beneficial insects such as bees. A single application of Cryolite is typically used due to the long term residual effect.

Recent literature (Andris, 1990., Kunkee, 1991., Wahlstrom *et al* , 1991., Wahlstrom *et al,* 1996.) shows that vineyards using Cryolite, however, have had problems with slowed or stuck fermentations, whereby the alcoholic fermentation is slowed down below the normal rate or fermentation does not start at all. Cryolite is commonly used in California, more specifically Big

Valley and to a lesser extent Napa Valley. Residues of Cryolite have been detected in the grape must and in wines from California, at levels of 10mg/L. Other independent vineyards report levels at 15 to 20 mg/mL and at these levels objectionable flavours and smells are noted (Morenzoni,1991 personal communication).

Fluoride residues are also becoming undesirable in products due to their affect on ethyl carbamate production in wine. It is known that certain wines contain urea which is a precursor to the production of ethyl carbamate, a suspected carcinogen (Andris, 1990., Famuyiwa and Ough, 1991., Henschke and Jiranek, 1993., Kunkee, 1991., Ough *et al*, 1989., Trioli and Ough,1989). Recently the Japanese introduced an enzyme containing an acid urease which rapidly removes urea from wine therefore halting the production of ethyl carbamate. Researchers since then, have shown that fluoride inhibits the activity of this enzyme which reduces the likelihood for ethyl carbamate elimination (Andris, 1990., Famuyiwa and Ough, 1991).

The investigation of residues of fluoride in wine therefore, is important as these residues may have health implications to the consumer, as well as an organoleptic effect on the wine. It is also very significant with respect to the pecuniary effectiveness of the making of the product as stuck or delayed fermentations cost the winemaker both time and money.

The most significant source of fluoride in grape must appears to be the fluoride component of the Cryolite compound. The fluoride ion is apparently translocated into the grape berries and therefore into the grape juice (Kunkee, 1991). The antimicrobial action of fluoride is well documented in dental literature, for example, in the inhibition of streptococcal enolase in oral microflora (Belli *et al.*, 1995). The inhibition of yeast enolase by fluoride is also well recorded (Bunick and Kashket, 1982., Curran *et al*, 1994., Kaufmann and Bartholmes,1992., Lebioda *et al,* 1993). Fluoride acts via a number of different mechanisms against oral microflora (Kashket and Kashket, 1985., Marquis, 1995., Marquis, 1989., Marquis, 1977.,Villar-Palasi and Larner, 1970) but the effect of fluoride, by residual action on yeast during winemaking, is not well known.

The purpose of this research project was to investigate the effect, if any, the fluoride ion has on yeast involved in the process of winemaking. In this context different yeast, both commercial starter cultures and wild yeasts, were used to determine this effect. The range of fluoride was broad and included concentrations similar to fluoride levels found in grape must and wines as previously reported (Andris, 1990., Kunkee, 1991., Morenzoni, 1991, personal communication, Wahlstrom *et al* , 1991., Wahlstrom *et al*, 1996.). The conditions were intially optimal for the effect of fluoride to be demonstrated. Experimental parameters then focused on the reconstruction of "real world" conditions. From there a clearer idea of the effect of fluoride on yeast may be obtained.