Getting Rid of the Stinky Taint Problem

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It is estimated that 2-7% of all wines are returned or rejected due to cork taint (Fuller 1995). The negative impact that cork taint has on the wine industry is currently unknown, but it could easily be an influence on unearned negative judgments about a restaurant's or winery's quality. Current estimates may be as high as four out of ten bottles produced contain taint due to cork contamination depending on the batch. Cork Taint has the ability to ruin a bottle of wine as well as ruin the reputation of the winery from which it came or the establishment that served it. It is estimated that the global lost revenue due to spoiled or tainted wine is over $10 billion annually (Fuller 1995). 2 4 6-Trichloroanisole (TCA) is the chemical responsible for the taint, and it may be transmitted into a bottle of wine via a contaminated cork, wood barrels, and other winery surfaces. Once a wine has been tainted there is no way to correct the problem. The most effective way to stop TCA from contaminating a wine is to ensure that only non-contaminated cork and other source materials are used.

In an effort to protect the reputations of the wine producers as well as on-premise providers, this research used e-beam irradiation to reduce or remove molds that produce TCA. In addition experiments were conducted to test the integrity of the post-irradiated corks including a long-term storage project.

Problem Statement

2 4 6 trichloroanisole (TCA) forms when 1 or more of the 11 Fungal strains know to cause TCA comes into contact with 2 4 6 trichlorophenol (TCP) and through O methylation turns TCP into TCA. The 11 fungal strains known to have the ability to convert TCP into TCA are *Acremonium stricum, Chrysonilia sitophila, Cladosporium oxysporum ,Fusarium oxysporum, Mortierella alpine, Mucor plumbeus, Paecilomyces viridis , Penicillium chrysogenum, Penicillium citregenum, Penicillium decumbens, Penicillium purpurogenum, Trichoderma longibrachiatum, Trichoderma viride, Verticillium psallitae.* These 11 fungal strains have been found in cork oak *Quercus suber* which is used to make natural cork stoppers. Wine closures contaminated with one or more of the 11 fungal strains can then come into contact with TCP and through O methylation convert to TCA. The only way to prevent TCA contaminated corks is to ensure that the 11 mold strains never come into contact with TCP.

Literature Review

Consumer wine consumption

Wine consumption among adults with a household income of more than $70,000 has been steadily rising since the year 2000. Most consumers are unaware of TCA and the role it plays in tainting a wine. Consumers often associate the off-putting taste of TCA with the quality of the establishment that sold or served it. Such association can have an adverse affect on the restaurant's bottom line. Guests who have received a tainted bottle of wine are likely to seek out an alternative beverage or even stop frequenting the establishment that served the tainted wine all together. With wine having the second highest markup in a restaurant second only to liquor, it is important for restaurants to serve wine free from cork taint.

Cork

Natural cork stoppers are a punched cylinder or composite chip formed into a cylinder from the cork oak, *Quercus suber*. Cork is produced from the suberized outer tissues of the cork oak that is at least thirty years of age and can only be harvested every 9 years. It is estimated that 12 to 13 billion bottles of wine annually are packaged with cork stoppers (Aidan, 2007). This means that there are 12 to 13 billion chances for wine to become contaminated by cork taint. The most common and powerful cork taint is 2,4,6-trichloroanisole (TCA) .

Contact with cork stoppers is the most common way for wine to become contaminated with TCA. There have been reports of rare occasions where wine has become contaminated through wooden barrels infected with TCA as well as contaminated winery equipment (Liegh, 2003). When wine becomes contaminated before entering the bottle it is easily detected (Careri, 2001). The wine is then discarded and never makes it to the consumer. The problem lies in the fact that it is difficult and costly to detect a contaminated cork before insertion into the bottle.

Currently 83% of the world's cork supply comes from Portugal and Spain. Portugal produces the lion's share of the corks at 55% of all corks produced. (Liegh, 2003) Due to high levels of cork contaminated with cork taint, as many as 40% in reported cases, a search for alternative wine bottle closures has substantially grown in the past 15 years. (Mark A. Sefton, 2005) This has had a large economic impact on cork growing countries such as Portugal.

2,4,6-Trichloroanisole (TCA)

2,4,6-Trichloroanisole is the most common and odiferous of the 6 cork taints. It has been reported that TCA is responsible for at least 80% of all wine contaminated with cork taint (Silva, 2005). TCA is formed when 1 or more of 11 known mold strains found naturally in cork come into contact with 2,4,6 – trichlorophenol (TCP) and through O Methylation converts TCP into TCA. (L.Maggi, 2008) If the mold strains that are known to contribute to the formation of TCA were eliminated or drastically reduced, the insanities of TCA in cork stoppers would be eliminated or reduced. It is estimated that the world wide cost due to wine contaminated with TCA is $10 billion annually. (Aidan, 2007) Once a wine has become contaminated with TCA there is no known way to remove the contaminants. Consumers are often unaware of TCA cork taint and assume the offensive aroma of TCA as a flaw in the wine and not the cork. This perceived wine flaw often prevents the consumer from repurchasing the same wine a second time. TCA produces a musty aroma commonly described as moldy newspapers or wet dog. This smell will overpower the aroma that the wine drinker was meant to enjoy. While unpleasant, TCA is not dangerous to consume. TCA can be detected in wine as low as 1.4 ppt (parts per trillion). To put this into perspective, the sensory threshold of 1.4 ppt is roughly the equivalent of dropping a sugar cub into a body of water the size of 5 Olympic swimming pools and being able to detect a sugar taste. This low threshold of detection is one of the main reasons TCA is the largest offender all the cork taints.

Current cork sterilization methods

The cork industry has been aware of cork taint for many years. A report titled *Identification of 2,4,6-Trichloroanisole as a potent causing cork taint in wine* was one of the first articles to point the finger at the cork industry for tainted wine. Before scientific proof of cork taint came about it was believed TCA was just a wine flaw that needed to be tolerated. The cork industry soon took notice and started cleaning up production facilities. One method

employed by many cork producers was to use bleach sterilize equipment. This actually increased the occurrence of contaminated corks. Bleach helps to form TCP, a chemical required for the production of TCA. Bleach is rarely used in modern day cork manufacturing facilities.

Today hot water baths are used instead of chemical cleaning agents. The hot water, unless changed after ever washing cycle, can actually contaminate non-contaminated batches of cork with mold as well as TCA. This is similar to cross-contamination in commercial restaurants.

There are two notable cork sterilization methods that have come about in recent years. The first is the ROSSA system which uses high pressure hot water in the attempt to sterilize cork chips used in composite corks. The second is low pressure, high volume C02, again used for cork chips. Low pressure, high volume Co_2 is the method used to de-caffeinate coffee. Both processes have shown little or no positive effects in reducing cork taint. In fact the ROSSA system has actually been shown to increase the occurrence of TCA in corks.

Ionizing radiation

Ionizing radiation produces electrically charged particles or ions by removing

electrons from an atom (Neal, 2008). Ionizing radiation has higher energy than non-ionizing radiation such as light, microwaves, and radio waves. The three most common types of irradiation used today are gamma rays, x-rays and electron beams.

A 1988 Portuguese study found that after exposing mold strains commonly found in cork to gamma radiation of 20 kGy the mold strains showed no signs of life. (Davis, 1982) There are significant draw backs to this study since some molds not proven to cause TCA were tested. The test was carried out using plated samples, not solid cork or composite stoppers.

Electron beams are produced by linear accelerators which are powered by electricity and can generate and accelerate electrons to 99 % of the speed of light. When these high energy electrons penetrate a thin foil of metal such as tungsten, x-rays are produced. (Neal, 2008) The advantage of using E-beam over gamma radiation is the speed in which the items can be processed. E-beam irradiation has been approved by the FDA for use on food products such as ground beef, poultry and spices. This form of food sterilization has been proven effective in several ground breaking experiments. E-beam has been proven to destroy such microorganisms as *Escherichia coli* O157:H7 in spinach (Neal, 2008) with no detectable damage to the product being irradiaated. Similar studies have been conduted on cantaloupe in which a sensorty panel could detect no differences betweeen irradoated and non-irradiated items (Fields 2007). This sugeest E-beam irradiation will be able to significantly reduce microflora in cork while leaving the structure of the cork unchanged.

Purpose and Objectives

The overall goal of this project was to determine the effectiveness of electron beam irradiation for the reduction of TCA producing mold on wine corks and investigate the effect on the cork. This goal will be accomplished by fulfilling the following objectives:

1. Determine if e-beam irradiation can reduce or eliminate TCA producing mold.
2. Using Scanning Electron Microscopy (SEM), determine if e-beam irradiation causes any structural changes in cork.

Significance of the Study

Electron beam irradiation has never been used on corks to reduce or eliminate TCA producing molds. If this study is successful, irradiated corks could become the new standard for the wine industry. A significant reduction in tainted corks, which would result in a reduction of tainted wine, would help reduce a $10 billion industry-wide annual loss in revenue due to contaminated wines. No longer would a restaurant's reputation be influenced by tainted wine.

Hypothesis

In order to achieve the goals of this study the following hypotheses will be tested.

H1: E-beam irradiation will reduce or eliminate TCA producing mold in sold cork stoppers.
H2: Irradiated corks will be structurally identical to non-irradiated corks.

Methodology

Microbial Cultures

The microbial cultures *Paecilomyces viridis* CECT 20427, *Paecilomyces glabrum* CECT 20558, *Paecilomyces chrysogenum* CECT 2306, *Mucor racemosus* CECT 2670,*Trichoderma viride* CECT 20721, *Aspergillus oryzae* CECT 2095 COLECCIÓN ESPAÑOLA DE CULTIVOS TIPO (CECT) Spain were received in dehydrated form. The cultures were rehydrated and propagated according to guidelines set forth by the supplier. Cultures were transferred to sterile tubes and centrifuged at 2500 rpm for 10 min in Jouan Centrifuge Model B4 (Winchester, VA). After discarding the supernatant, the resulting pellets were re-suspended in 9.9 ml 0.85% sterile saline, vortexed and recentrifuged. This process was completed twice before dispensing 0.5 ml of the resulting culture into 17 x 60 mm screw cap vials containing 4.5 ml 0.85% sterile saline.

Cork

Cork samples consist of 144 premium Grade A natural cork stoppers, 44 x 24 mm. 144 Grade A natural cork stoppers, 44 x 24 mm. 144 Grade A 1+1 stoppers, 44 x 23.5 mm. 144 Grade a composite cork stoppers 44 x 24 mm. Cork stoppers were supplied by Carolina Wine Supplies.

Inoculation

Corks were inoculated with 1 cc of mold solution injected into the center of each cork using a sterile 30G .3 X 13 mm hypodermic needle. A new sterile needle was used for each cork. Standard aseptic practices were followed. 10 grams of inoculated corks were placed in small stomicer bags and sealed. Three small sealed stomicer bags were then placed in a larger stomicer bag and sealed. The sealed inoculated corks were then made ready for overnight shipping to the irritation facility.

Application of irradiation treatment

Inoculated cork samples were placed in a single layer in cardboard boxes on a conveyer and treated with an average absorbed dose of either 5 kGy , 10 kGy, or 15 kGy of electron beam irradiation. Control samples remained in storage at 24ºC at the Food Microbiology Laboratory located at the University of Houston Conrad N. Hilton College. Appropriate attenuation and rate of process were used for each dose as per dose mapping completed prior to the experiment described above. Bare standards were measured on the processing day at the beginning, middle and end of treatment of samples to verify consistent energy output by the electron beam. To minimize variations in dose absorption, all samples were placed in the cardboard trays in an identical attenuation configuration and geometry.

Sampling cork for microbial enumeration

Corks sample were separated by type as well as irradiation doses received. Cork samples were then pulverized in a Waring lab blender model 31bl92(7ull) with 90ml of peptone. Samples were plated on previously prepared potato dextrose and malt plates. Plating was done in triplicate and in a 6 dilution sequence. Plates were then incubated and monitored for growth in 24, 36, and 48 hour increments. Plates were then analyzed for microbial colony formation. Plate counts were taken with a numeric clicker to determine an accurate count of viable microorganisms. No visible colony forming units were detected.

Scanning Electron Microscopy

Irradiated cork sample were viewed at 1k-20k times magnification under a scanning electron microscope and visually compared side by side. No structural differences were found between irradiated and non irradiated corks.

Results

This experiment has shown that e-beam irradiation can be used to significantly reduce or eliminate mircoflora known to cause TCA in cork stoppers, thus preventing a negative consumer perception of a winery or on-premise provider being associated with tainted wine. Analyses of inoculated corks post irradiation showed no evidence of microorganisms. Corks analyzed by SEM showed no damage associated with the irradiation treatment. Further studies will be conducted to determine consumer detection of wine bottled with irradiated corks.

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