Terroir

An Integrated Wine Science Publication

A BIODYNAMIC APPROACH
science and spirituality collide

SULFITES ON BOARD
what a headache!

AGING GRACEFULLY
saving time with irradiation

YEAST TO LEAST
lowering alcohol content

HOLD ME CLOSER TINY CANCER
the big impact of nanoparticles

PUSHING THE BORDERS
options for growing colder

THE ICEWINE COMETH
global production of a delicacy

CHIPPING IN
roll out the flavour barrels

GAME OF DRONES
eye in the sky

"JUST ONE WORD – PLASTICS"
a case for the alternative

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WINE SCIENCE

HOW CAN SOMETHING THAT TASTES SO GOOD BE SO INTERESTING SCIENTIFICALLY?

Working in small groups, students in ISCI 3A12 examine the science behind wine making, from the art of viticulture to its eventual consumption by the public. Students perform literature-based reviews and original research in order to understand the wine industry and its complexity; including the environmental requirements and consequences of winemaking, the short- and long-term health effects of drinking wine, and what factors contribute to the quality, aroma, and taste of wine. This research was formatted to resemble a publicly accessible scientific article, and compiled to create each Vintage of *Terroir*.

This Publication is written by members of the ISCI 3A12 class of 2019

Cover Image: The barrel cellar at Jackson-Triggs Winery Estate. Russ Ellis. 2019
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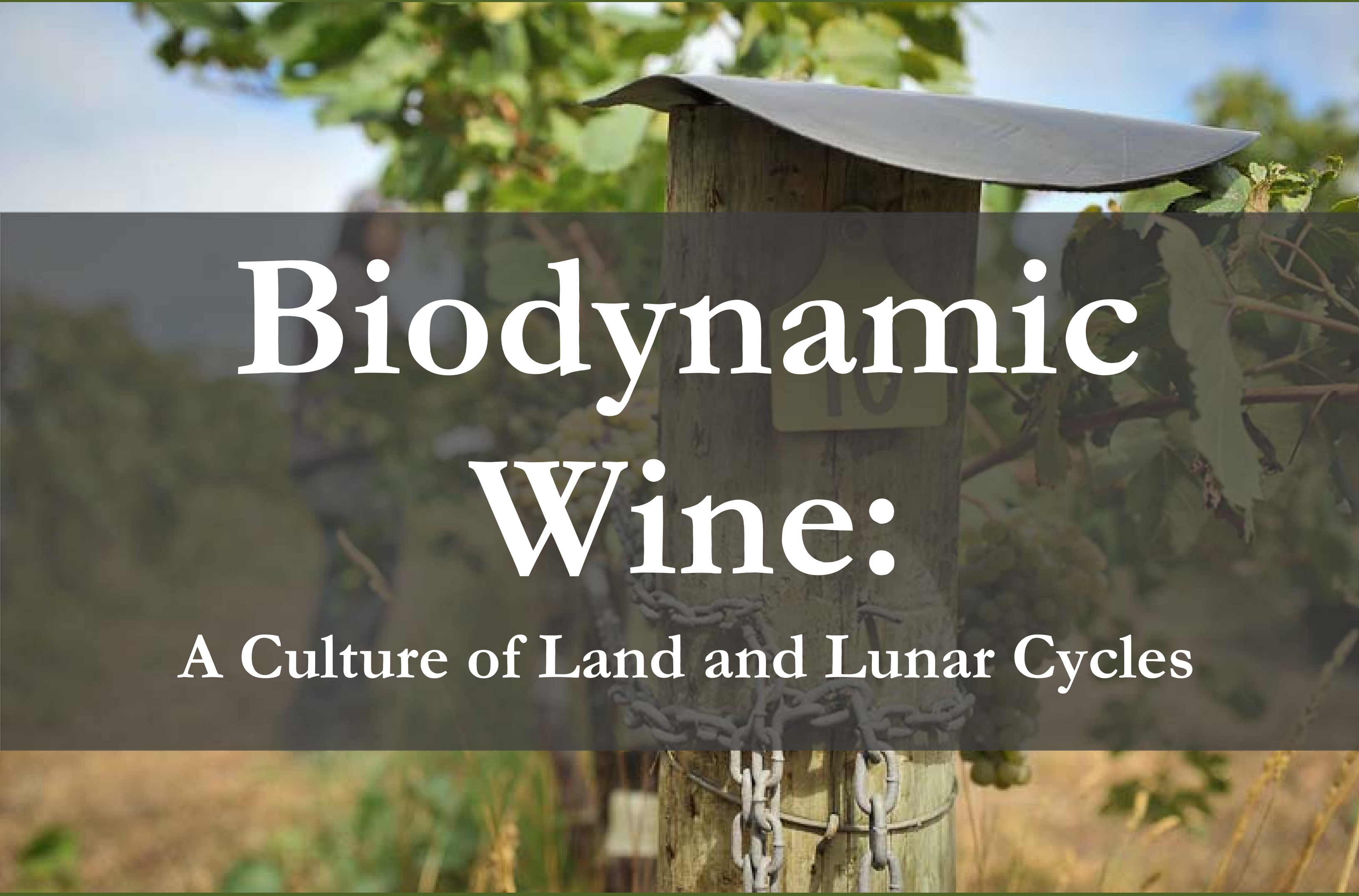
EDITOR: Russ Ellis



Biodynamic Wine:

A Culture of Land and Lunar Cycles

Serena Formenti, Mary Anne Schoenhardt, Caitlin Reintjes, Ishita Paliwal, Maggie Wilberforce

Biodynamics is an alternative farming technique developed by Rudolf Seiner in the 1920s. While many of its aspects overlap with those of organic farming, such as the lack of synthetic fertilizers and use of composts, other techniques required for biodynamic certification involve adherence to lunar cycles, use of homeopathic soil additives, using dead pests to ward off living ones, and a phenomenon known as geo-acupuncture. These treatments are supposed to help unite all the aspects of the farm, increasing not just the health of the crops, but that of the soil and all other living organisms on the farm. What do these techniques really do? Do they have any scientific validity?

In recent years, biodynamics has been adopted by many small-scale wineries, creating a niche market within the wine industry. In this article, we aim to identify how the proportion of biodynamic wine varies from the conventionally produced wine. Is a bottle of biodynamic wine the drink of the Gods, or does it have the future of the City of Troy?

INTRODUCTION

Since the early 1990s, there has been increasing public concern about climate change, leading to a "green" consumer phenomenon[1]. This, along with heightened competition across agricultural markets, has led to an increase in sustainable and environmentally friendly agricultural products, including wine[1–4] Consequently, shifting to biodynamic viticulture has become increasingly attractive, systematically influencing the international wine industry[3,4].

Biodynamic agriculture is often compared with organic agricultures, which consists of farming without the use of synthetic pesticides and fertilizers, although they are not equivalent. In reality, biodynamic viticulture is a subgroup of organics, but not all organic farming methods can be classified as biodynamics[3]. Biodynamic viticulture, like organics, does not use any synthetic materials, including chemical fertilizers and pesticides[2,4]. However, it requires additional elements, including nine preparations that are added to soils and composts to enhance crop quality[2,4]. These preparations, as will be discussed further, include: silica, flowers of yarrow, chamomile, dandelion and valerian, oak bark, and stinging nettle[2]. In addition to these nine biodynamic preparations, this form of viticulture must follow synodic and astrological rhythms, while also being self-sufficient[2,4].

Synodic: Cycles relating to lunar phases[7].

The philosophy behind biodynamic viticulture is rather abstract. It essentially follows a Goethean perspective, meaning that the entirety of the universe is considered to give off an interconnected resonance, and in order to produce quality wine, a vigneron, also known as a winegrower, must balance this resonance between the earth, the stars, the vine, and the human[5]. It is rooted in the anthroposophical theory that human beings are somewhere in between the rhythms of the earth and the cosmos, and, thus, bring the spiritual and material realms together[1]. Vignerons who believe in this philosophical approach aim to produce wine in a holistic way, taking into account natural resource use, sustainability, use of biodynamic preparations, and lunar and planetary cycles, among other things[1,3]. The ultimate goal is to treat the vineyard as if it were a living entity, using preemptive measures to ensure the vines remain in a state of natural balance and health[6]. It has even been said that the vigneron's personal involvement and creativity in implementing the biodynamic method is just as, if not more, important than the actual method itself[6].

The first implementation of biodynamic preparations was in Arlesheim, Switzerland in the fall of 1923[6]. Here, Rudolf Steiner (see Figure 1[7]), who is considered the father of anthroposophy, showed a group of farmers and doctors how to prepare and utilize these eight biodynamic preparations[6]. He then presented eight lectures on the topic in June 1924 at the estate of Count Carl von Keyselingk in Koberwitz, Germany[1,4,6]. He explained the theory behind his eight biodynamic preparations, along with their application according to the seasons and astrological positions[6]. Despite his efforts to establish this process as a "spiritual science", Steiner was dismissed due to the lack of empirical data and scientific support for his methods[6]. Nonetheless, biodynamics were introduced to the United States by Ehrenfried Pfeiffer before the Second World War[2]. Today, the practice is carried on by Marie Thun, who, with the development of the Thun theory, has continued to explore the applications of Steiner's principles to agriculture, specifically further exploring the applications of lunar cycles and astrology in biodynamic agriculture techniques[8].

FIGURE 1: RUDOLF STEINER Photograph of Rudolf Steiner, the father of anthroposophy and the founder of biodynamic farming, taken in 1907[7].

In 1928 a certification program was put in place for biodynamic foodstuffs under the trademark known as "Demeter"[2]. This certification requires biodynamic cultivation sites to go through a three-year conversion period after already being certified organic[3]. After conversion they must meet the extensive requirements of Demeter International, including those for biological diversity, disease, insect and weed control, use of biodynamic preparations, water conservation, livestock integration, soil fertility management, and composting, among others[9].

This article will dive deep into the world of biodynamic viticulture, exploring its soil preparations, astrological calendars, pest-ashing, menhir rock placement, sensitivity testing, and marketing. The goal is to determine if there is a scientific basis behind biodynamic viticulture and the ultimate value of biodynamics in the wine-making community.

WHAT DOES DIRT HAVE TO DO WITH ANYTHING? MY SEDIMENTS EXACTLY.

Rudolf Steiner's initial parameters for biodynamic agriculture included a specific set of biodynamic (BD) soil preparations, numbered 500 through 508, to fulfill the anthroposophical elements of his farming practice (see Figure 2)[10]. These preparations work to create a connection between the spiritual and physical worlds. While he was both a scientist and philosopher, Steiner's guidelines for soil preparation were based more in philosophy than scientific theory. However, more recent literature has recorded some positive agricultural impacts in biodynamic farming that may be the result of some of the soil preparations. These practices are used today in biodynamic viticulture and must be followed in the vineyard for a winery to be certified biodynamic[10].

The first biodynamic soil preparation, BD preparation 500, is horn manure. This involves fermenting manure inside a cow's horn and subsequently leaving it buried underground throughout the fall and winter[10,11]. The fermented manure is then significantly diluted with water and sprayed on top of the soil. In theory, this will stimulate root growth and the formation of humus, the component of soil made up of organic material. According to Steiner, the shape of the cow's horn channels lunar energies and transfers them to the manure within, fortifying the soil and transmitting this benefit to the fruit[10].

BD preparation 501 also involves burying a filled cow's horn during the fall and winter[10,11]. In this case, however, it is filled with powdered quartz. This preparation is used as a

Foliar Spray: Ideal for nutrient deficiencies in soil, foliar sprays are applied directly to leaves or as liquid manures as a supplementary means of delivering nutrients to plants[10].

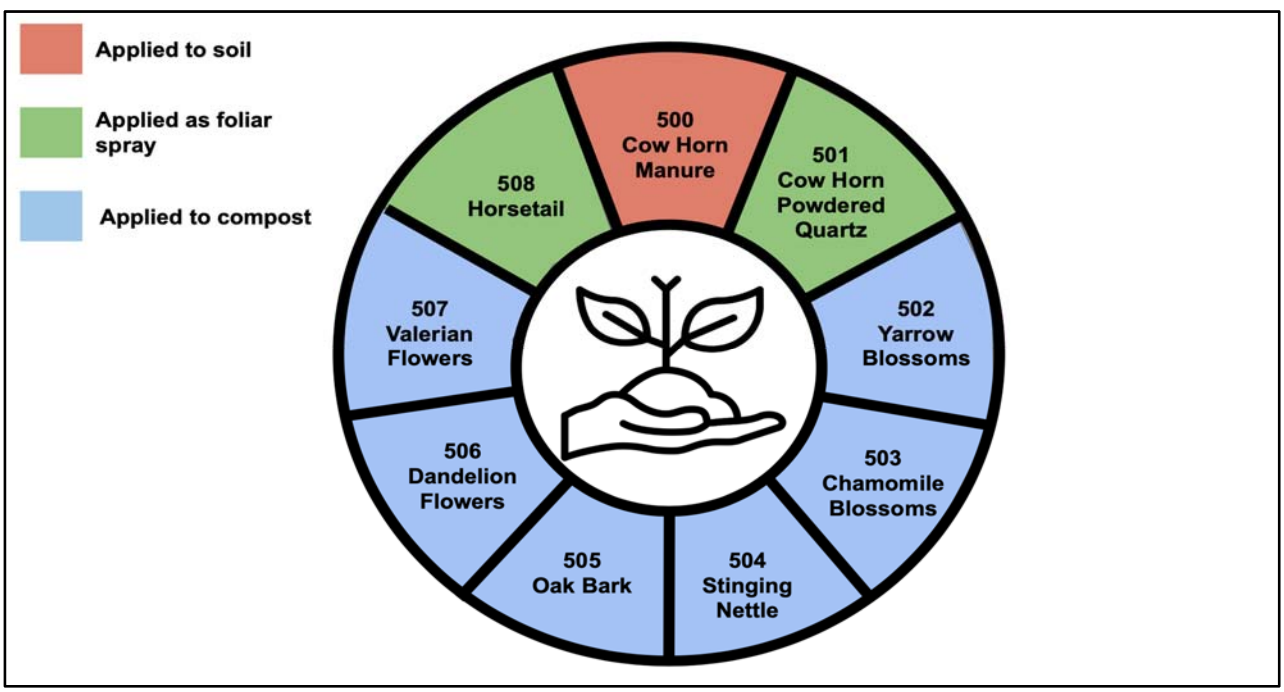


FIGURE 2: BIODYNAMIC SOIL PREPARATIONS. These nine soil preparations are those suggested by Rudolf Steiner[8]. Preparation 500 is applied directly to the soil as a manure spread. Preparations 501 and 508 are applied as foliar spray. Finally, preparations 502 to 507 are added in homeopathic quantities to compost, which is then used as a soil fertilizer (diagram created in Google Slides with central icon from thenounproject.com).

foliar spray to stimulate plant growth. Similarly, BD preparation 508 involves the use of a silica-rich horsetail foliar spray. Here, horsetail serves the purpose of suppressing fungal diseases in plants[10].

Finally, preparations 502 to 507 are compost additives[10,11]. Homeopathic quantities of plant extracts are added to compost before being applied to biodynamic crops. These extracts are designed to strengthen and enhance the life forces present on the farm. In other words, the use of these compost additives are intended to vitalize the individual organisms present on the farm, enhancing and balancing the relationships between them and the land, forming a healthier farm in its entirety. These various compost preparations are made by inserting about one teaspoon of each extract to a depth of 0.5 meters inside a series of large compost piles, which are separated from one another by 1.5 to 2 meters in a grid-like pattern[10].

Homeopathic Quantities:
Quantities that are incredibly dilute. For example, around one teaspoon of each preparation is added to a seven to ten-ton pile of compost[10].

Microbial health is of the utmost importance in producing healthy soils[12]. Soil microbes are necessary for recycling nutrients in the soil, decomposing plant matter, and fixing nitrogen[12]. While there is still uncertainty in the scientific literature as to the effects of biodynamic soil preparations, an increase in soil microbes is reported in many studies[2,13]. Furthermore, a study found that when comparing composts with and without biodynamic treatments, the former showed increased temperatures. This result is suggestive of an increase in thermophilic microbial activity. Interestingly, this effect was not suggested by Steiner in an explanation of his methods, yet it is possible that the increased number of microbes could produce the high nutrient levels that he hypothesized[13]. That being said, increased nutrient levels have not been reported consistently in scientific literature examining biodynamic preparations.

The literature agrees that silica unequivocally enhances plant health and crop yield in agricultural settings, both when added to in the soil and sprayed on foliage[14]. BD preparations 500, 501, and 508 take this approach and are assumed to increase yield and promote plant growth, as suggested by Artyszak in 2018[15]. Although silica is not essential for plant growth, it strengthens cell walls, helps to protect plants against environmental stresses, improves water uptake, and enhances protection against fungal disease[14]. The addition of either powdered quartz, a silica rich mineral, or horsetail provides a suitable means of biodynamic silica fertilization[13,16]. Nevertheless, while silica is known to increase plant health and crop yield, these two results have not been confirmed by studies specific to biodynamic practices.

Aside from these results, the impacts of biodynamic treatments on crops, and specifically viticulture, are quite uncertain. Studies tend to agree that when compared with organic viticulture practices, biodynamics do little to affect grape yield, cluster numbers, or fruit weights[11,13]. Some have found biodynamic grapes to have higher levels of phenols[13] while others have found increased levels of enzymatic activity[17,18], though neither of these two results are consistent throughout the literature. Still, any differences that are observed are minute, and likely make little to no impact on the final quality of the wine[11].

LUNAR AND ASTROLOGICAL CYCLES - LOOK TO THE SKY!

Planting according to the lunar cycle, part of Steiner's biodynamic methods, was first proposed by philosopher Francis Bacon[8]. Some studies on the topic, in the mid and late 1900s, showed an increase in germination rate, water absorption, and metabolism when following this method, but did not show a significant effect on crop yield[8].

In the 1950s, Marie Thun tested Steiner's principles by growing radishes on her farm in Germany and using this research to develop her own theories[19]. Although Steiner advised adhering to the synodic cycle and ecliptic constellations in his biodynamic method, Thun suggested using tropical zodiac signs instead[8]. According to the 1956 Thun Theory, crops are differentiated into four categories: root, leaf, flower, and fruit/seed. These categories can be linked to the four elements (earth, water, air, and fire), as well as to the elements of the zodiac constellations under which the crop should be sown for optimal growth, as seen in Table 1[8].

TABLE 1: THUN THEORY 1956. Division of crops based on category, element, and astrological zodiac sign according to Thun theory[19].

Crop Category and Examples	Element	Astrological Zodiac Sign
Root (Ex. Carrots, Potatoes, Radish)	Earth	Taurus Virgo Capricorn
Leaf (Ex. Cabbage, Rhubarb, Lettuce)	Water	Scorpio Pisces Cancer
Flower (Ex. Broccoli, Cauliflower, Artichokes)	Air and Light	Libra Aquarius Gemini
Fruit/Cereal (Ex. Oats, Barley)	Warmth and Fire	Aries Leo Sagittarius

To obtain optimal agricultural outcomes, Thun's theory identified two important methods to be followed, without giving an explanation as to why they function. First, germination of crops must happen on the day of sowing[8]. Second, a descending moon is appropriate for plant care and cutting, while an ascending moon is appropriate for harvesting and grafting[19].

Various experiments from the 1970s to the 1990s were conducted by researchers, to test whether sowing crops according to their crop and zodiac category, as suggested by Thun, actually increased yield. This was done for fruit crops, baley and oats, on fruit days and root crops, radish and carrots, on root days. Results varied from no significant difference to 7 – 30 % excess yield[8]. These experiments were mainly conducted on fruit and root crops and less so on leaf and flower crops[8].

More recently, a study was conducted to measure the impact of planting according to the lunar cycle on professional perception of pinot noir quality[4]. The theory was that the wines should taste better on flower and fruit days, when the moon is moving through the last six astrological zodiac constellations in Table 1, as it is supposedly fresher and more aromatic[4]. Contrarily, it would taste less good on leaf and root days, when the moon is moving through the first six astrological zodiac constellations (Table 1), as the wine is more tannic and bitter. However, the study showed that the wine taste was not influenced the lunar calendar[4].

BURNING PESTS: A FIRE HAZARD OR PEST CONTROL?

Pest-ashing, another aspect of biodynamic agricultures, involves the spread of a pest's ashes to be used as a natural pesticide[11]. The biodynamic theory believes in the ability of water to transfer life forces and the ability of fire to mediate death forces[20]. It is, therefore, thought that the energy of the dead pests acts as a repellent and prevents reproduction of succeeding generations of pests in this area. Steiner thought pest-ashing would allow crops to be more pest and disease resistant, leading to a reduced need for synthetic pesticides[13].

The first step in pest-ashing is to burn the pests of interest. If the pest is a vertebrate, Steiner advises burning the animal's skin during the alignment of the planet Venus and the constellation of Scorpio[20]. If the pest is an insect, Marie Thun advises the burning of 50 to 60 specimens in a wood-burning stove under different constellations for different species, of which an example is seen in Figure 3[21,22]. If the pest is a weed, it should be burned when the moon is aligned with the constellation of Leo or during a full moon, as this is alleged to favour seed development[23]. The ash is then stirred in a mortar for an hour, alternating directions every ten seconds[21].

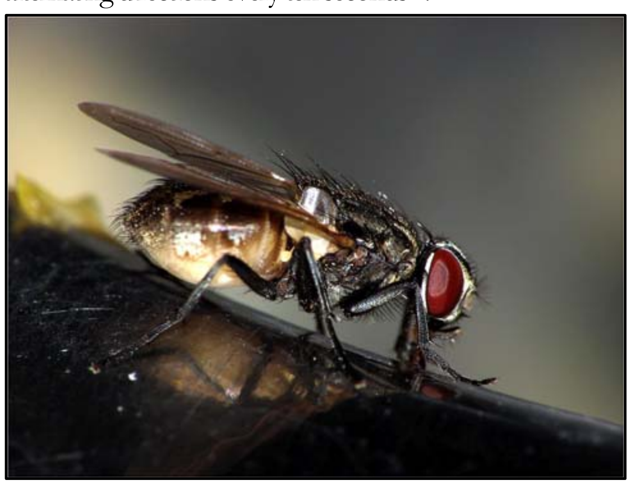


FIGURE 3: PEST-ASHING OF A HOUSEFLY According to Steyn in 2016[20], a pest, such as a housefly, is burnt during a water constellation because it spends most of its life in a watery environment[22].

Once mixed, the ash is made into a spray or dry ash mixture with sand and is applied to the soil of the crops[21]. According to Steiner, it is best to apply these preparations as soon as they are made. Thun also advises that mist sprays should be applied three times at short intervals, such as on three consecutive days. It is important to note that, based on biodynamic principles, a substance such as pest ash supposedly becomes more potent as it is diluted[21]. Usually, a total of eight dilutions are made. The first dilution, D1, uses one-part ash and nine parts ethanol and is stirred for three minutes[24]. The dilutions continue with one part of the previous dilution and nine parts ethanol to make D2 and then D3, which is stored. When ready for use, D3 is made into D8 through subsequent dilutions following the aforementioned procedure[24].

To test the scientific basis of pest-ashing in New Zealand, Eason and Hickling tried using the technique to prevent damage done to crops by invasive Brushtail possums and the spread of bovine tuberculosis in 1992. However, compared to control sprays using sand, water, ethanol, and wood ash, there was no significant difference in possum mean daily food consumption, body weights, or bait taken[20].

MENHIRS: NOT WHISKY, BUT WINE ON THE ROCKS

Another unique aspect of biodynamic preparations is the use of menhirs in the field. Menhirs are large standing rocks with unique shapes[25]. They are typically uneven and taper towards the top, as shown in Figure 4[11,26]. Menhirs date back to the late Neolithic or Bronze Age in Europe (around 1700 BC), and were used in fertility rituals[25]. Those who adhere to biodynamic practices believe that these rocks channel cosmic energy via geo-acupuncture[13]. While mentions of these menhirs can be found in the literature, there have been no studies evaluating their efficacy as part of biodynamic practices, and it has not been stated what exactly their usage accomplishes for biodynamic vineyards.

Geo-acupuncture: While not explicitly defined in any literature, the roots of this word imply that it is a form of acupuncture for the earth.

FIGURE 4: MENHIRS IN SWEDEN Image of menhirs in a field in Fjärâs, Sweden[26].

SENSITIVE TESTING: PAIRING WINE AND COPPER CHLORIDE

In his attempt to establish biodynamics, Steiner wanted to study different chemicals that would distinguish inorganic from organic and biodynamic life[24]. With the aid of Pfeiffer, they developed sensitive testing in which wine is added to a copper chloride solution. Upon evaporation of this solution, a pattern of crystals is produced that represents the vital energies of the wine[25]. Organically grown samples may be more homogenous, uniform and congruent, while conventionally grown samples may be more complex and intricate, as seen in Figure 5[24]. These identification criteria were established on the basis of experiments on wheat crops in organic and conventional farms; however, the mechanism of pattern creation is not yet understood[24].

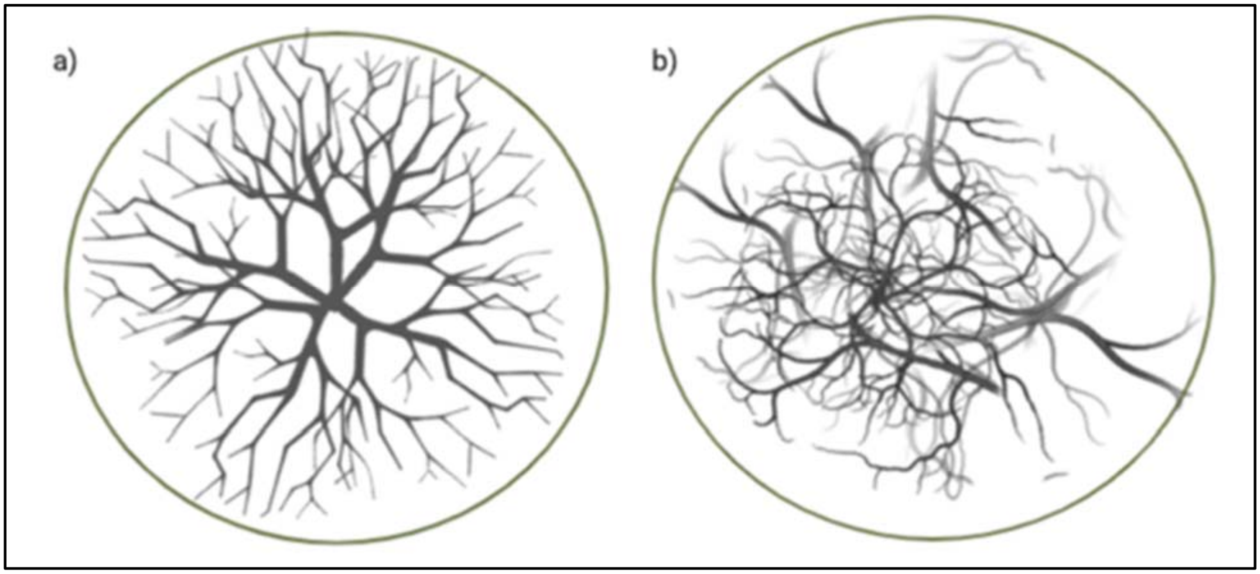


FIGURE 5: EXAMPLE RESULTS FROM SENSITIVE TESTING. An artistic representation of results from sensitive testing after adding wine to a copper chloride solution (image created using Biorender.com and adapted from Steyn (2016))[24]. Organic samples tend to produce more homogenous, uniform, and congruent crystallization patterns (a). Conventional samples are more complex and intricate (b)[24].

The advantage of sensitive testing is the ability to make reproducible crystallization patterns can differentiate between samples from different farming techniques and processing steps[27]. This is done through standardized computer-supported texture, structure, and visual image evaluation. Fundamentally, this technique is used by organic food markets to authenticate the organic nature of the food[27].

MARKETING BIODYNAMICS? WINE NOT?

It has become evident that biodynamic viticulture and winemaking is a unique practice. As a consequence, questions are raised in regards to how such a product is marketed to the general public, and whether or not biodynamic wine is economically viable. In a 2019 interview with Marie Cundari, the director of New World and Ontario wines at the LCBO, she stated that the company has faced challenges marketing biodynamic wines in the area. A general lack of public understanding and awareness surrounding biodynamic practices has made it difficult to market the products to consumers. For this reason, biodynamic wines are not marketed differently from other wines retailed in Ontario given that they fit into a very small niche market[28]. However, the literature does suggest that there may be important aspects to consider if special marketing were to occur.

Marketing this wine can be difficult because biodynamic wineries are competing with wineries that use conventional methods. Although some believe that it is easier to target consumers that typically buy organic foods and products, many of these people are also biased against beverage alcohol, believing it to be unhealthy[1]. This means that biodynamic wineries must direct their marketing towards "typical" consumers and thus, are in direct competition with conventional wineries[1]. This can be challenging, especially since a study suggests that using organic methods to manufacture wine increases the likelihood of purchase for only 27% of surveyed consumers[29].

This suggests that one strategy biodynamic wineries can use in marketing is to emphasize the uniqueness of their product, by considering biodynamic wine to be a new niche within the already niche organic market[30]. While some wineries market 'green' practices as a method of product differentiation[1], other wineries have switched to biodynamic practices mainly for ethical reasons, with only 23 % wanting to differentiate their product from competitors[30]. One study of biodynamic wineries differentiated business strategies on a Likert scale. Factors were evaluated on a range from one to five, where one was considered "not important at all" and five was "very important"[30]. While quality was a top factor considered in marketing (4.86), promotion of the product had a lower ranking of importance as a business strategy, with a value of 3.52. Furthermore, this study indicated that nearly 43 % of these wineries are not advertising their product as biodynamic at all[30].

From this, the literature suggests that better communication and promotion of their practices needed by biodynamic wineries, which can be done by emphasizing biodynamic certifications on wine labels and highlighting any benefits of their practices.

SPIRITUAL "SCIENCE" OR ROOTED IN EVIDENCE?

Through examining biodynamic wine from environmental and economic lenses we have found that while some aspects are rooted in scientific principles, not all are supported by the literature. Biodynamic farming processes such as the use of menhirs and pest-ashing do not seem to have any concrete scientific support, while the application of soil treatments, lunar cycles, and sensitive testing have yielded some positive results on growth and yields in scientific studies.

In terms of pest-ashing, there are distinctions made in the method of how the ash should be prepared relative to each pest, though there are no explanations as to why these differences are scientifically valid. Additionally, it was stated that more dilutions lead to a greater potency of the spray[21], which is illogical. Likewise, the use of menhirs in the vineyard has no scientific support in the literature.

While some the biodynamic soil treatments do not have any scientific backing, there is support for any processes in which silica is added to the soil[14]. However, whether the observed benefits arise from the treatments themselves, or simply the use of overlapping organic practices and the lack of industrial chemicals is questionable as some studies have found very little difference when compairing biodynamic and organic practices. Additionally, sowing crops according to lunar and astrological cycles did increase germination rates and metbolism, potentially allowing for better crop yield[8]. Nevertheless, the mechanisms for these changes have not been explained. Despite these observed correlations, the principles of when crops should be maintained and when a wine should be tasted, are solely based on anthroposophical principles, which lack scientific basis.

Lastly, sensitive testing uses scientific principles by use of a reaction between copper chloride and wine to analyze the characteristics of these wines[23]. The documentation of different characteristics for organic versus conventional samples, along with the reproducibility of the crystallization patterns, allows for both scientific and anthroposophical analysis.

With all of this in mind, there are still lingering questions regarding how to best advertise and market biodynamic wine. Given that only about half of biodynamic wineries are actually taking steps to market their product, it is clear that more can be done to promote it to consumers. A good first step would be increasing public awareness of these practices, which is frequently cited as a major barrier in the biodynamic sector. Specifically, we recommend that wineries educate and advise consumers on the potential benefits and unique aspects of biodynamic wineries to increase the popularity of the product.

Based on our observations, biodynamic methods are relatively prevalent in the wine industry when compared to other agricultural industires due to the high economic value of the wine-grape industry and the formation of a niche market. While this is the case, much of the research on biodynamic techniques is not applied specifically to wine-grapes or the wine industry itself. Given our results, it is likely that any effects of biodynamic practices are minimal, and thus not beneficial enough to apply to large scale agricultural practices. The largest impact that likely occurs from biodynamic practices is for the farmers themselves and their relationship with the land. This is one of the underlying benefits that was suggested by Steiner; by vitalizing this relationship, the land and crops will inherently be better cared for. The spiritual principles of land and human health are not something that can be currently be measured by a scientific study.

MORE TO EXPLORE

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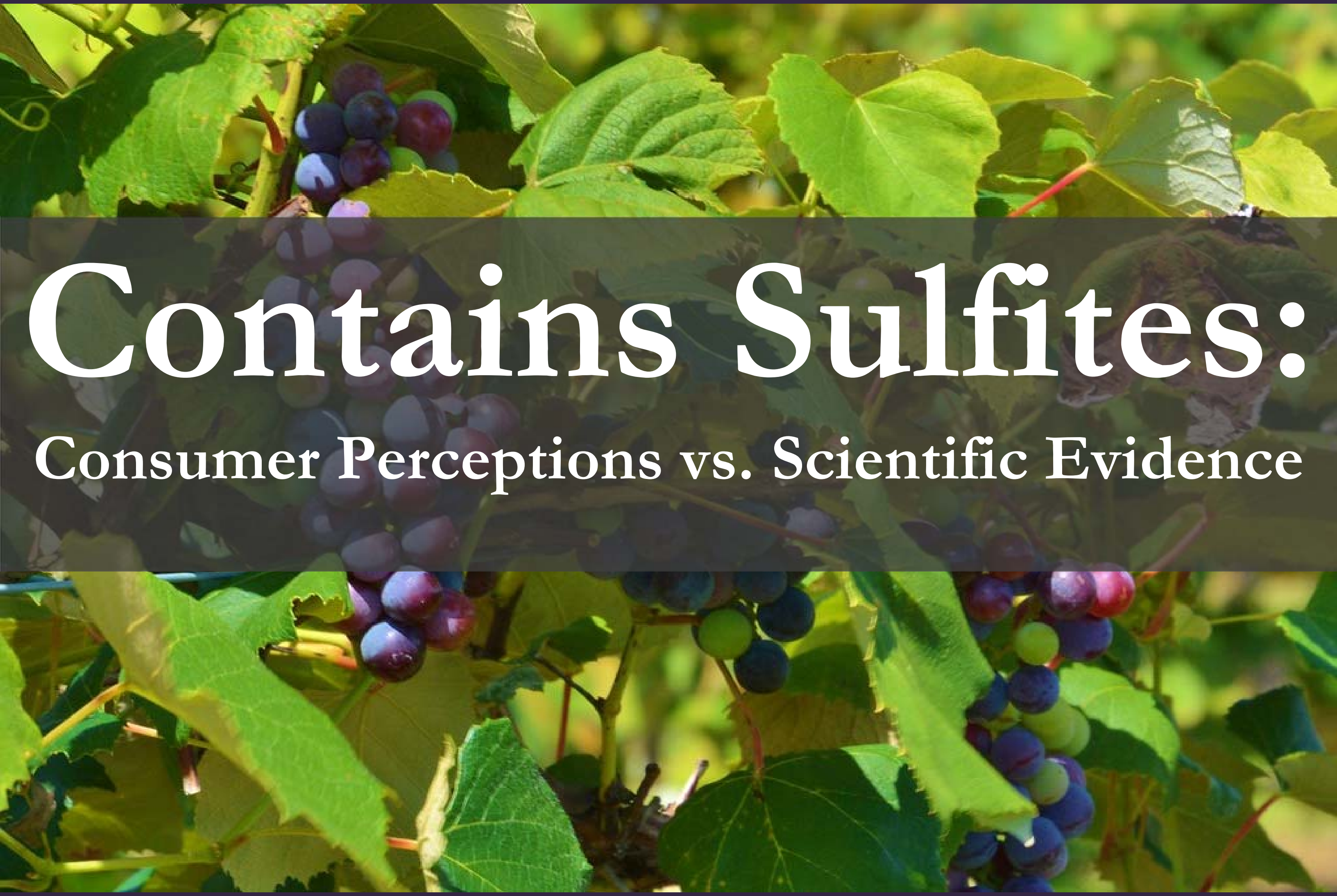
COVER PAGE PHOTO CITATION

Private photo collection.Gruner Veltliner Grapes being harvested at Hahndorf Hill in the Adelaide Hills wine region during harvest 2013 [Internet]. 2009 [cited 2019 Nov 22]. Available from: https://commons.wikimedia.org/wiki/File:Gruner_Veltliner_being_harvested_at_the_biodynamic_vineyard_of_Hahndorf_Hill.jpg?fbclid=IwAR0H9RnDdLrYawQGcn06_OwrwANrZCwlhsdBt7TWeaJ0NN2y5rlTiMElg8U



Contains Sulfites:

Consumer Perceptions vs. Scientific Evidence

Gemma Barber, Katarina Sacka, Dominique Tertigas, Isla Turcke, Jonathan Zaslavsky

When picking up a bottle of wine, one of the first words you are likely to read on the label is "sulfites". This term, which refers to both a naturally evolved compound and synthetic additive, plays a vital role in winemaking and the preserving of wine characteristics. However, the ubiquity of sulfites in wine raises a number of questions, such as how they are regulated and why they are found on labels in the first place. Interestingly, this is rooted in understanding how sulfites in wine affect health. Our aim is to disseminate the evidence surrounding these health effects, namely involving the associations of sulfites with asthma and headaches, in order to demonstrate the importance of informed marketing with appropriate labelling.

WHAT ARE SULFITES?

Sulfites can be found naturally in the human body and in certain foods, however many sulfites are used as common additives in food and wine for their preservative and antioxidative properties. The most prevalent sulfites include potassium bisulfite/potassium metabisulfite and sodium bisulfite/sodium metabisulfite.[1] Compounds containing at least one sulfite ion are known as sulfites. This ion is composed of one sulfur atom with a charge of 4+ and three oxygen atoms each with a charge of 2-, giving the ion an overall charge of 2- (refer to Figure 1).[2]

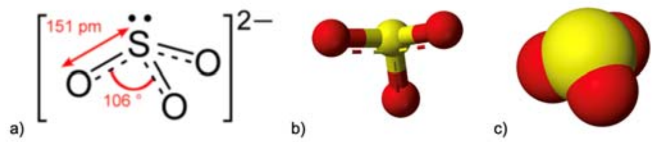


FIGURE 1: MODELS OF THE SULFITE (IV) ION: SO_3^{2-}. a) Skeletal model illustrating the bonding of oxygen to the sulfur atom. b) Ball and stick model with oxygen atoms depicted as red spheres and sulfur as a yellow sphere. c) Space-filling/calotte/CPK model giving an accurate representation of how much space each atom takes up in the molecule. Adapted from PubChem.[2]

If wine is left to age too long or is not treated with the proper preservatives, it is in danger of turning into vinegar, a much less palatable beverage that lacks the desired intoxicative effects of wine.[3] This process occurs in two steps. First, a genus of bacteria called *Acetobacter* converts ethanol, the alcohol in wine, into acetaldehyde. Increased levels of acetaldehyde cause wines to brown and develop aromas and flavours described as grassy, nutty, or "ripe apple-y". In the second step, acetaldehyde reacts with oxygen and is oxidized to acetic acid, which is vinegar.[4]

Sulfites are ideal additives for wines, as they have both antimicrobial and antioxidative properties, helping mitigate both steps of the conversion from wine into vinegar.[5] Small quantities of sulfites are naturally found in wine due to their presence in grapes, and additional sulfites are formed during wine fermentation. However, this natural concentration is quite low and more often than not **vintners** will introduce additional sulfites to prevent both bacterial and oxidative spoilage.[6] Sulfites and sulfur dioxide are used in fruit juices, dried fruits, and wines to limit the growth of spoilage microorganisms, which could cause these products to expire.[5]

Vintner: A winemaker, or a person involved in winemaking.

Sulfur dioxide, or SO_2, exists naturally as a colourless gas that is soluble in water and toxic if inhaled.[7] While sulfur dioxide is not a sulfite itself but a closely related chemical oxide, it is a powerful preservative added to many foods and wines, and as such is commonly referred to as a sulfite.[7]

Oxidation, the second step in the degradation of wine into vinegar, occurs when wine is exposed to too much air. As previously mentioned, oxygen in the air interacts with the acetaldehyde molecules in wine, creating acetic acid.[4] When in contact with wine, oxygen also reacts with **polyphenols**, forming undesirable compounds called quinones.[3] Quinones result in the loss of fruity aromas, the development of brown colouration, and can escalate the oxidation process by reacting with additional polyphenols.[8] While this sounds like a wine lover's worst nightmare, sulfites come back to save the day once more. They block the oxidative reactions from occurring by attaching themselves to the molecules to which oxygen would normally bind. Once the majority of the target molecules are bound by sulfites, the remaining sulfites are free to combat bacterial oxidation and other microbial threats.[5]

Polyphenols: A category of organic compounds that occur naturally in plants including grape vines.

There are a number of factors that influence which quantity of sulfites should be added to a wine. Typically, more sulfites need to be added to white wines because red wines naturally contain antioxidants from the grapes being in contact with their skins during fermentation.[9] The molecular SO_2 required for stability in white wine is approximately 0.8 ppm while it is 0.5 ppm for red wines.[10] As well, sweeter wines receive larger doses of sulfites because the sugar present in the wine can bind to the sulfites added, so a larger dose is required to reach the same concentration of free sulfites.[9] Furthermore, pH has an important effect on how much added SO_2 is required to maintain the same quantity of free sulfites.[10] A wine with a lower pH, or a more acidic wine, will need a smaller quantity of sulfites added to prevent spoilage and oxidation.[11] For example, if a wine has a pH of 3.0 it will need 13 ppm of free sulfites in white wine and 8 ppm in red wine. On the contrary, a wine with a pH of 3.8 would need 79 ppm of free sulfites added for white wine and 49 ppm for red wine (refer to Figure 2).[10] This is because the predominant species at a low pH is molecular sulfur dioxide (SO_2) while at a higher pH (3.0 – 3.8, the average pH range of wine), the predominant species is the bisulfite anion (HSO_3^-).[1] Molecular SO_2 exhibits germicidal properties while the bisulfite anion acts as an antioxidant.[1]

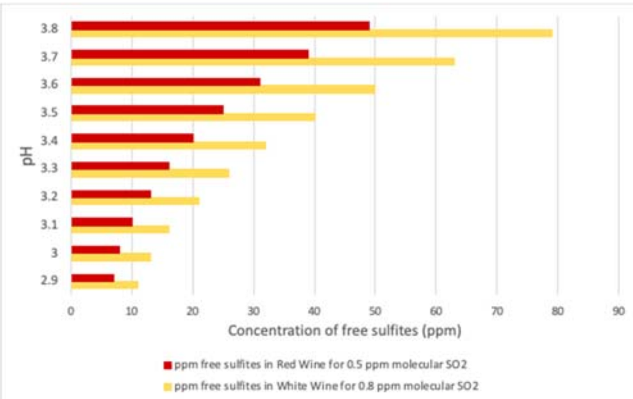


FIGURE 2: WINE COMPARISONS OF pH AND PPM. Recommended quantity of sulfites to be added to white and red wines based on the pH of the wine in order to achieve 0.8 ppm molecular SO_2 for white wine and 0.5 ppm molecular SO_2 for red wine.[10]

The role of sulfites in wine is clearly of importance; however, sulfites in wine are often blamed for negative impacts of wine consumption such as headaches and asthma. This review aims to put sulfites on trial and evaluate how the industry may respond to the negative outlook of sulfites.

SULFITES AND WINE-RELATED HEADACHES

As an alcoholic beverage, understanding the health effects of wine is crucial, where the association of wine with headaches has been an area of significant consideration. Most notably, the notion of a so-called red wine headache or red wine syndrome has pervaded consumer attitudes towards wine and health.[12] In brief, this phenomenon describes the onset of a headache or migraine after consuming red wine, with the caveat that it can occur after a single glass. Typically, the headache is accompanied by nausea as well as flushing of the face.[12] However, due to the highly complex nature of red wines and the variable reactions of individual consumers, the condition is poorly characterized.[13] In effort to better understand the **etiology** of the red wine headache and identify individuals that may be more susceptible to it, the key compounds in red wine are subject to scrupulous study.

Etiology: Cause of disease or the study of causation.

Red wine contains a number of substances in varying abundance (refer to Figure 3).[14] Beyond wine's principal constituents, the content of biogenic amines such as histamine, tyramine, or phenylethylamine, in addition to flavonoid phenols, 5-hydroxytryptamine, and sulfites are all thought to confer some influence on the adverse effects of red wine.[13,15]

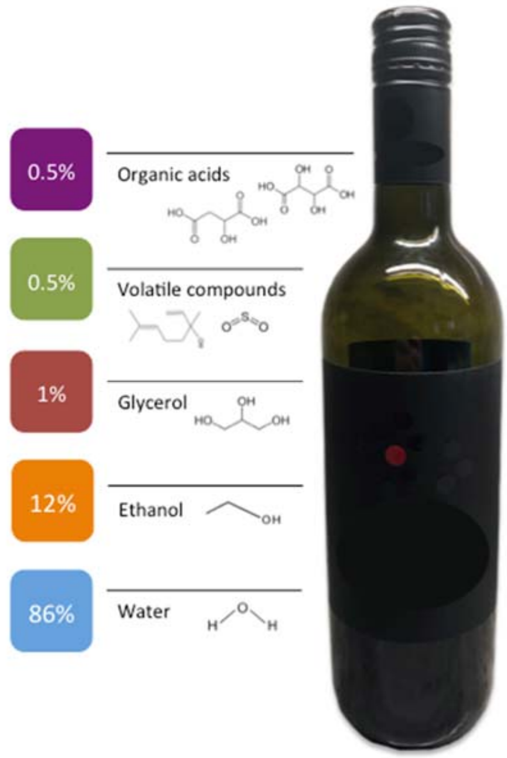


FIGURE 3: THE CHEMISTRY OF AN AVERAGE BOTTLE OF RED WINE. The percent composition of each major component is given. Information adapted from Markoski et al.[14]

Sulfites in particular have been a longstanding purported culprit for red wine headaches. Having been deeply entrenched in the marketing of wines, sulfites are arguably one of the most well-known ingredients of the beverage.[13] However, the direct incidence of wine-related headaches as a result of sulfites is tenuous, given the concentration of sulfites in red wine in relation to other foods.[15] For example, white wines have almost double the sulfite concentration of red wines, and dried fruits can contain up to ten times greater sulfite concentrations, reaching approximately 1000 ppm, when compared to wine.[13,15] Since red wines are exclusively implicated with the eponymous red wine syndrome, and other foods containing greater sulfites are not associated or known to elicit headaches, there is a need to elucidate the role of sulfites in red wine as they relate to inducing headaches.

Several studies have focused on the incidence of headaches after wine consumption in individuals with a history of wine-related headaches. A cornerstone study by Kalish and Kaufman (1981) found that red wine provokes headaches, and that the ingestion of aspirin prior to consumption of red wine was ineffective for preventing its onset.[16] Subsequent studies investigating the amine contents of red wine found that red wines contain migraine-provoking agents excluding alcohol and tyramine, and that the activity of enzymes like phenosulphotransferase P, which is involved in phenol metabolism, diminished as headache occurrence increased.[13,17] While these studies were preliminary and limited, they do point to the importance of selecting a study population that is susceptible to wine-related headaches. A study carried out by Silva et al. (2019) specifically sought to assess how sulfite concentrations affect headache occurrence.[18] A sample of 80 young adults aged 18 to 25 years old were administered minimum and maximum concentrations of sulfites in wine according to body weight. It was found that individuals with a previous history of headaches had a risk 2.266 times greater for developing headaches when consuming maximum sulfite concentrations in wine. Furthermore, the risk of presenting headaches increased by 6.232 times for individuals with a history of constant headaches as opposed to those with sporadic headaches, suggesting that there is substantial variation even within individuals with a history of wine-induced headaches .[18]

Although sulfites are not conclusively the triggering factor for wine-related headaches, it is posited that headaches arise through their interaction with the myriad of other compounds in wine. The prevailing hypothesis for red wine headaches is that they are largely based on an immunological response, whereby nitric-oxide mediated vasodilation, accumulation of phenols, and the release of histamine are all suggested mechanisms.[13,15,18] This is further compounded by evidence of sulfite-sensitive individuals, and individuals with a deficiency of sulfite oxidase, which may be contributing factors to the condition.[19] The lack of definitive scientific evidence for a sole headache-causing agent is rooted in the intricate composition of wine. Given the breadth of inconclusive information regarding sulfites as an established trigger for headaches, the red wine headache phenomenon is difficult to attribute to any single compound and warrants continued research into its particular causes.

SULFIITES AND WINE-INDUCED ASTHMA

Sulfite induced asthma is a major factor influencing the regulation of sulfites in many products. It has been suggested that 3 to 10% of asthmatics experience sulfite sensitivity.[20] Kochen (1973) published one of the first papers describing respiratory irritation as a result of sulfite ingestion.[21] Many papers on this topic soon followed, as well as numerous reportings of adverse reactions to sulfites in the early 1980s, many of which were asthmatic reactions.[20] At the time, sulfites in the air, food and drinks, cosmetics, and medications were all of concern. In 1986, the FDA began to regulate the use of sulfite additives. However, cases of sulfite sensitivities continued to occur sporadically.[20]

Several asthmatic patients have reported induced asthma as a result of drinking wine. Despite the complexity of wine and the numerous compounds it contains, sulfites have received the blame for this phenomenon,[22] likely a result of the reports from the 1980s. A number of studies attempt to determine whether sulfites in wine actually play a role in triggering asthma, either independently or in combination with other compounds, or whether it plays no role at all.

A study by Dahl, Henriksen and Harving (1986) suggests sulfites are the major factor contributing to asthma induced by red wine,[23] while Cardet et al. (2014) believe alcohol in all wine types is responsible, not sulfites.[24] Three studies published by a group at the University of Western Australia between 1999 and 2007 recruited self-reported wine sensitive asthmatics as their study group.[22,25,26] While the methods differ between these three studies, in general, participants ingest varying concentrations of sulfites in wine and are monitored for an indication of respiratory health, such as **forced expiratory volume (FEV)**. For all studies, a lack of positive outcomes in response to low and high sulfite concertation wines was a common result. Despite this, a smaller group of participants from the studies show evidence of asthmatic response that can be attributed to increased sulfite concentrations.[22,25,26] The results are ultimately not definitive; therefore neither wine nor sulfites in wine can be confidently determined to be the sole cause of asthma.

FEV: A test that measures how much a person can exhale during a forced breath.

Nevertheless, patients associate wine with asthma, which means either this relationship has been overestimated, patient history is unreliable, or the study methods mask the true relationship.[22,25] The studies recognize certain limitations which may lead to the lack of positive responses. Prior to discussing the potential confounding variables, it is important to note that participants often experienced asthmatic symptoms even in the absence of a measurable positive response.[22,23] A psychological impact appears to be unlikely due to the lack of placebo responses.[22,25] A more probable explanation for the lack of measurable asthmatic responses is that during the study, participants are consuming wine in a controlled environment where their asthma is stable. The criteria regarding asthma stability and severity may limit the study size and ultimately the number of detectable positive responses.[25] Further, a smoky environment or seasonal pollen may lead to unstable asthma and increase the likelihood of an asthmatic response when exposed to sulfites in wine.[22,23,25]

This prompts the question: do these individuals react to wine when exposed to other irritants or does wine increase the likelihood of an asthmatic reaction to the other irritants?[25] Future studies should continue to mitigate method limitations in order to determine if wine actually induces asthma and if so, what compounds present in wine are responsible.

A select group of individuals in the previously mentioned studies did display evidence of wine induced asthma as a result of sulfites. Due to the prevalence of sulfite induced asthma since the 1980s, many mechanisms have been proposed. One potential mechanism includes insufficient activity of sulfite oxidase which leads to an accumulation of sulfites which is then exposed to the airways.[20,27] Immune system response mechanisms that are **IgE** or non-IgE mediated have also been suggested.[20,23] It is important to recognize that these mechanisms of sulfite induced asthma are not necessarily wine specific. While these mechanisms are plausible explanations, the numerous other compounds and contaminants in wine may influence these mechanisms or have mechanisms of their own. Based on variable response severity, these mechanisms may differ between asthmatics.

IgE: A type of antibody produced by the immune system, which is often associated with an allergic response.

Despite no obvious pattern in the role or mechanisms of sulfites in wine induced asthma, it can be agreed that this subgroup of asthma has a complex etiology. Based on existing evidence of sulfite sensitivities, and the small but present group of asthmatics that show sensitivity to sulfites in wine, sulfites in wine should continue to be studied and regulated.

ROLE AND REGULATION OF SULFITES IN WINE

Once sulfites are added to the wine, they can exist either in the free or bound form. Only the free sulfites react and exhibit antioxidative or germicidal properties.[1] The bound sulfites on the other hand react with other molecules (such as acetaldehyde, pyruvic acid and a-ketoglutaric acid) and have a decreased preservative activity.[28] Acetaldehyde is the biggest contributor to bound SO_2 in both red wines and white wines. Pyruvic acid is a higher contributor than α-ketoglutaric acid in white wines, with the opposite true for red wines.[28]

Although added sulfites have antioxidative properties, it has become a priority for some wineries to try to reduce the amount of sulfites present in wine due to their potential for detrimental impacts on health. One way to accomplish this is by adding other preservatives in combination to reduce the quantity of sulfites added. These include lysozyme, an antimicrobial agent, and dimethyl di-carbonate, a preservative active against yeasts.[29] These compounds have been found to reduce harmful biogenic amines that can occur in wine as a result of the added sulfites, however they must be used in combination with SO_2 as they lack antioxidative activity.[29]

Reducing the amount of sulfites in wine can also be done by reducing the amount of compounds that bind to SO_2 in wine. If there are fewer compounds for SO_2 to bind to, more SO_2 will be found in the free form and less will need to be added to achieve the same concentration of free sulfites. For example, it has been found that late alcoholic phase yeast metabolism (LAPYM) contributed to a decrease in acetaldehyde levels.[28] This decrease in acetaldehyde concentration in the wine will lead to less SO_2 being added (refer to Figure 4).

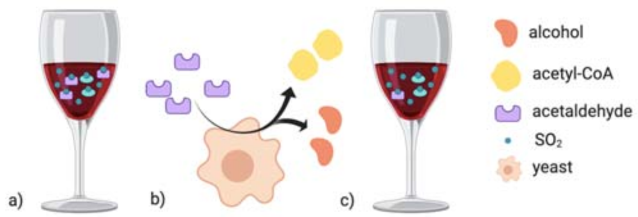


FIGURE 4: LAPYM CAN DECREASE ACETALDEHYDE LEVELS IN WINE. a) Wine without LAPYM has the majority of sulfites bound, and many are bound to acetaldehyde. b) Yeast in the wine undergoes metabolism using acetaldehyde to produce alcohol and acetyl-CoA. c) Wine after LAPYM has a lower concentration of acetaldehyde, meaning there is a higher ratio of free to bound sulfites.[28] Image created using BioRender.

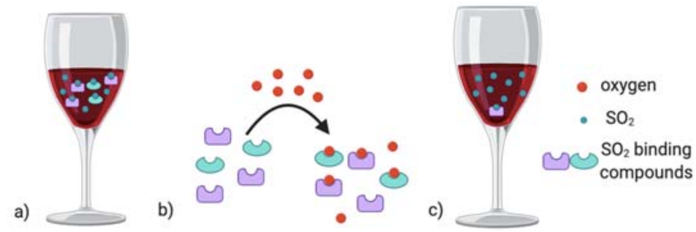


FIGURE 5: THE PROCESS OF HYPEROXYGENATION TO INCREASE THE QUANTITY OF FREE SULFITES IN WINE. a) Wine without hyperoxygenation. This glass has equal amounts free and bound sulfites. b) Hyperoxygenation introduces the wine to oxygen, which binds to the SO_2 binding compounds. These compounds then precipitate out of solution and can be removed. c) Hyper-oxygenated wine has fewer available SO_2 binding compounds, and thus has more free SO_2 than non-hyper-oxygenated wine.[32] Image created using BioRender.

Another method used by some winemakers involves intentionally exposing **must** to oxygen, thereby initiating reactions between the oxygen and compounds in the wine, causing the wine to become **hyperoxygenated.**[30] The vintners at Rosewood Estates winery in the Niagara Region of Canada use this method; they add little to no sulfites to their wine, yet it has the same preservative properties as those with added sulfites.[31] This is because the addition of oxygen allows some precursors of polyphenolic compounds to be oxidized, forming polymers of high molecular weight which are precipitated out of solution and removed before alcoholic fermentation.[32] Polyphenolic compounds are the compounds most susceptible to oxidation in mature wine, so the removal of these compounds at an early stage removes the risk of the wine oxidizing in the future, thereby also removing the need for additional sulfites to prevent oxidation of the wine (refer to Figure 5). Zironi, Celotti, and Battistutta (1997) demonstrated that the addition of the same amount of SO_2 (50 mg/L) to both hyper-oxygenated wines and "traditional technique must" wines resulted in much higher concentrations of free sulfites in the hyper-oxygenated wines compared to the traditional wine.[33]

Must: Freshly crushed grape juice that contains the skins, seeds, and stems of the fruit.

Hyperoxygenated: Has been exposed to and reacted with an excess amount of oxygen. In wine, this causes certain compounds to be precipitated out of the wine.

Regardless of whether or not a wine has reduced amounts of sulfites, sulfite levels in wine must be measured to ensure the correct amount was added to have appropriate preservative activity and to ensure that the wine meets the legal limits set out by each country. Furthermore, the amount of sulfites in wine may be of interest to consumers, so this has become a potential area for marketing.

MARKETING WINE WITHOUT ADDED SULFITES

Regardless of whether or not a wine has reduced amounts of sulfites, sulfite levels in wine must be measured to ensure the correct amount was added to have appropriate preservative activity and to ensure that the wine meets the legal limits set out by each country. Furthermore, the amount of sulfites in wine may be of interest to consumers, so this has become a potential area for marketing.

By regulation, in Canada and many other countries, it is mandatory to label wine bottles with 'contains sulfites' once the amount of sulfites exceeds 10 ppm.[11] Additionally countries will place a maximum on the amount of sulfites wine can contain. This gives the opportunity to suppliers who create no-sulfite added wines to label them as 'sulfite-free' or 'no-sulfite added.' However, these labels that advertise the absence of an ingredient have been shown to sell less effectively to consumers as compared to those labeling the presence of an ingredient.[34] Additionally, absence labels are only informative to consumers who are knowledgeable about the absent ingredient. Thus a customer who may not know a lot about sulfites in wine, may not know how to interpret the 'sulfite-free' label.[34] However, as people become increasingly interested in the processes by which their food is made,[35] perhaps more people will become knowledgeable in the area of wine and sulfites.

Currently, it has been found that the largest impact on a consumer's decision to buy wine has nothing to do with labels, but rather the price of the wine and the quality.[6] The increased quality of the wine has been shown to increase the probability of purchase by 5.71%.[6] However, there has been found to be a select group within the general population that is willing to pay a premium for sulfite-free wine: those who believe that sulfites are the cause of their headaches after a moderate consumption of wine.[6,36] This select group is willing to pay almost double the premium compared to those who do not associate headaches with wine, or who do not get headaches after drinking wine.[36] To hold their place in the market, wines without added sulfites must maintain the same quality as their competitors.[6]

As explained earlier, the literature in this area shows that there is currently no definitive science to support the belief that sulfites cause headaches. It is likely that the negative connotation associated with sulfites comes from the 1980s when people were told that sulfites could be the cause of allergic reactions.[6] Alternatively, because the containment of sulfites in wine must be labeled, it is possible that people link their negative experiences with wine directly to the ingredient that has been explicitly labeled.[6]

It has been found that when a label explicitly shows that the product contains a specific ingredient and there is negative information associated with that ingredient or no information associated with it at all, then the label can significantly decrease the amount consumers are willing to pay for the product[35] (for an example label see Figure 6). Additionally, if the label says it is free of an ingredient that has negative connotations, there will be a significant increase in the willingness to pay for the product.[35] Thus, the negative connotations with sulfites likely increases the willingness of consumers to pay for sulfite-free wine. If it is scientifically proven that sulfites are not the cause of headaches, it is speculated that the market for sulfite-free wines could disappear.[6]

Finally, it was found that those who experience headaches from wine are more likely to pay a premium for no-sulfite added wine rather than organic wine, regardless of the fact the organic wines are also sulfite-free.[6] This occurs for two reasons: (1) from the consumer's perspective, the difference between 'no sulfite added' and 'organic' is ambiguous,[37] and (2) many are unaware that organic wines cannot have added-sulfites.[6]

Due to this confusion, there is a niche market that forms, as it is cheaper to produce sulfite-free wines than organic wines and there is a specific group that would be willing to buy them over organic wines.[36] However, it is recommended to organic wine producers to also label their wines as sulfite-free, as this would make it explicit to buyers.[6]

FIGURE 6: EXAMPLE OF A WINE LABEL. This wine contains sulfites and how that is communicated to consumers is illustrated on the label. The label is found on the back of the wine bottle.

CONCLUSION

While the health effects of sulfites remain inconclusive, it is clear that sulfite content in wine is a growing concern for certain consumers. Individuals have reported symptoms of asthma and headaches caused by sulfites in wine, however there are no conclusive findings in the literature, which complicates the social attitudes associated with this ingredient. This requires both winemakers and marketers to adapt their practices to suit the industry demands. Provided with this knowledge, wine consumers have the power to be their own jury and decide whether or not sulfites are beneficial or of harm.

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The Future of Wine Aging:

Irradiation and other techniques

Abhigyan Dwivedi, Jonah Gautreau, Matthew Pocrnic, Sarah Scott

The winemaking industry serves as one of the oldest industries, with the ancient beverage planting its roots early in human history. Despite its longevity, advancement has remained stagnant, with many of its methods remaining largely unchanged throughout most of its history. One of these methods is the long barrel aging process. In a rapidly advancing world abounding in technology however, new manufacturing techniques have emerged to potentially accelerate this aging process, thus opening new avenues for economic growth. In order for this to occur, consumer acceptance of these techniques is key. Within this article, the techniques of irradiating wine, as well as applying alternating current electric fields and ultrasound waves to the wine aging process are investigated. They are analyzed in terms of their applicability to the industry, functionality, health benefits, and likely consumer acceptance. This will address the feasibility of implementing these techniques in the modern wine industry, with a focus on irradiation.

INTRODUCTION

Wine, without a doubt, has a very deep-rooted history. This history is still expressed today through the preservation of culture and tradition in many wineries. However, as scientific knowledge accumulates over generations, the wine making process has become more founded in science as well. The wine making process began around 7000 years ago as a series of trial and error experiments, before the world of microorganisms had been discovered, and has since evolved into a precise science that involves inoculating wines with pure, selected strains of yeast[1]. The aging process, however, has remained more or less traditional over the years, with wooden barrel aging being most common[2]. This, without argument, is one of the difficulties of making wine. The beverage is stored in vast amounts, and matured for long periods of time, often years, before it can be sold and enjoyed. Thus, not only do wineries have to hold on to large amounts of capital for long periods, but in the case of a new variation of wine, there is an associated uncertainty with how the wine will taste. However, some of the problems associated with the aging process may be mediated, as promising new techniques have shown potential to speed up the wine aging process. Within this article, we will explore some of these techniques, their functionality, and how they may change the future of the wine industry. These techniques include artificially accelerating wine aging via irradiation, applying an alternating current electric field, and using ultrasound waves.

ECONOMICS OF WINE AGING

One of the fundamental principles of economics is efficiency, entailing the speed at which one can manufacture and release a product to the market. Doing so allows one to beat competitors to market, produce more wine, and to yield a greater profit. However the wine business fails to abide by this fundamental principle. Regardless of the variant of the grapes used, the crushing process, and duration of maceration or skin contact, the constant amongst winemakers is the aging process, which lasts from 2 to 50 years for some vintages[3]. There is an intricate balance of sugar, acids, and phenolic components which must be maintained to yield the desired product, yet the only variable a winemaker can control once wine has been placed in a barrel is time[3]. The use of artificial aging techniques, such as radiation allows winemakers to experiment with, and modify the components of wine and thus produce a tailored product with greater efficiency, resulting in more economic value than the current practices. Alternatively, it provides winemakers with a viable option to accelerate the development of new variations so as to not waste the tremendous resources required to store and maintain the product for many decades in the hope of a desirable result.

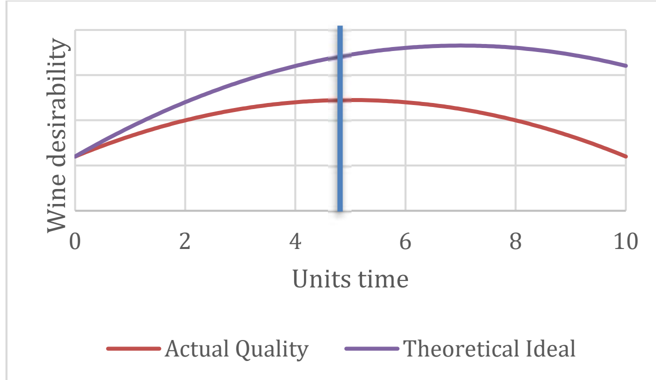

FIGURE 1: The theoretical ideal shows the potential for wine desirability increasing over time based on the winemaker's estimates. The actual quality shows the desirability of the wine though time as tasted by collectors. The blue line shows the point at which a given type of wine is no longer a prudent investment as the value begins decreasing.

Wine goes through two main phases of maturation: cask maturation and in-bottle maturation. Cask maturation ages the wine and adds characteristics of the wood. Oak characteristics are a fundemental and desirable aspect of wine. The second phase occurs in bottles, and often takes place over a longer period of time[4] In-bottle maturation increases both the value and character of the wine. However, wine desirability is not on a constant upward trend, as it hits a point where the quality begins to diminish and the value drops sharply as seen in Figure 1[4] This drop occurs due to the dual nature of wine purchases, to drink or as an investment, as the quality starts dropping investors lose interest, which accounts for a drop in demand and value. There is value to artificial wine aging for both industry and private collectors, as well as casual consumers to be able to speed up either aging process. The process of barrel aging is time consuming, lasting up to 5 years or longer. Additionally, barrels are expensive and require vast amounts of space for storage. Barrels may also become contaminated as time passes, and result in a large financial sink due to loss of wine from evaporation[2] Reducing the time needed to store wine in a barrel would reduce the overall amount of spending on barrels, storage cost, and evaporation loss. This provides a benefit to the profit margins of the winery, and could serve to lower consumer costs, while maintaining wine integrity[2] In terms of bottle aging there are two perspectives in which this could be beneficial. In terms of the traditionalist view of winemaking and the market view. Winemaking carries with it many traditions, and many individuals are not in favor of the artificial aging of wine. However, guesswork may be removed from traditional wine aging by taking a snapshot of how wine will behave with analogous artificial aging process. Some true purists who enjoy wine collecting as a hobby may be of the opinion that removing the guesswork removes the rich traditions of creating wine, however, for those who are procuring a collection as an investment a snapshot of the aged product would allow for better investment. The other view is that of the market, as these techniques provide an option for wineries to release more reasonably priced wines at faster rates. While this most likely will not replace collections for traditionalists, it could open up a new level of wine quality for the consumer. Overall there are a range of financial and practical benefits to the use of artificial wine aging technologies, especially if any of them provide a good analog to the typical aging process.

IRRADIATION OF WINE

One of the most interesting and promising new methods of wine aging is the application or exposure of the wine to a source of radiation. Upon first glance, this method seems rather shocking, questionable, and dangerous, so allow us to explore the features of this method, what it has to offer, and why it is indeed much safer than it sounds. Despite the method's abstract nature, wine is not nearly the first consumable product to be irradiated as the technique is not new to the food and beverage industry. The FDA, WHO, CDC, and USDA have all endorsed irradiated foods due to a series of benefits, including sterilization, preservation, prevention of foodborne illness, pest control, and delaying the onset of ripening[5]. Within Canada, three methods of food irradiation have been approved: gamma ray, X-ray, and electron beam radiation[6]. Some of these same methods have also been applied to wine, and UV sources have also been studied. An interesting 2015 investigates the effect of UV exposure on grapes from a natural source – the sun. This was achieved through constructing a treatment using a polycarbonate sheet to absorb UV radiation while still allowing sunlight to pass, and another treatment involving an acrylic sheet that allowed for all wavelengths of UV to transmit[7]. Coupling this with two other treatments in which the grapes are exposed to sunlight, while the others are shaded by branches allowed the researchers to understand the sole effects of UV radiation. In comparison to the treatment with a lack of UV radiation (polycarbonate sheet), the treatment with UV transmission and sunlight led to significantly higher sugar content, pH, total pigment and colour density, total phenolics, as well as tannin and anthocyanin[7]. Therefore, this generates interest in introducing artificial radiation to bring out more of these components present in aged wines.

It is important to understand why radiation would be used in the first place, or the physical process that explains the results we observe. Irradiation involves high energy photons such as UV photons from the Sun or gamma photons from radioactive decay, that have the potential to ionize target matter[8]. This means that radiation can fracture molecular bonds, producing free ions that can bond amongst others and produce new molecules[8] . In terms of wine, radiation can accelerate break down of certain compounds that are more desirable and those that are naturally produced in the aging process. In terms of gamma irradiation, the most frequent source used is the decay of Cobalt-60[9,10]. Cobalt-60 is a non-naturally occurring isotope with high cost effectiveness, ease of use, and reliability. It is commonly used across a variety of fields including food irradiation and cancer treatments, representing approximately 70% of radiation cancer treatments globally[11]. For a further understanding of the gamma irradiation process, see figure 2 below.

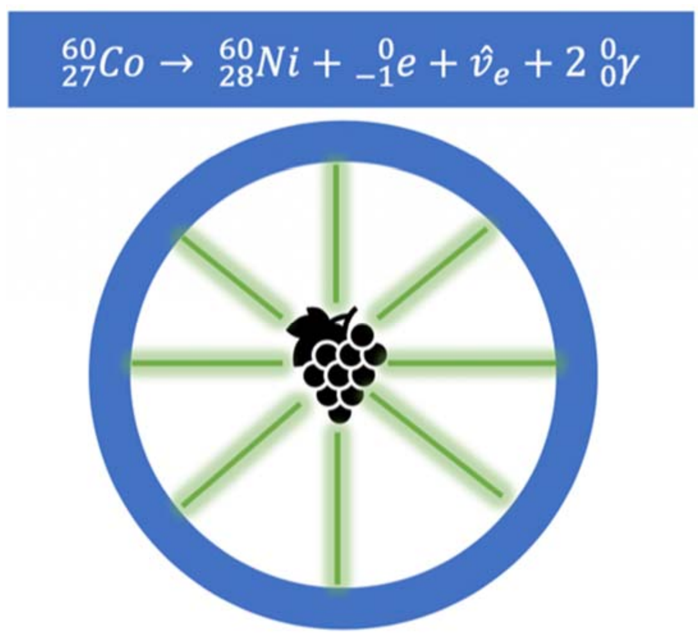

FIGURE 2: Above is a simplified diagram of a panoramic gamma irradiator used to expose grapes to the gamma photon source of the cobalt-60 decay. In blue the reaction is shown,

which occurs in the cylindrical source rack (illustrated here as the blue ring). The gamma photons produced in the reaction then travel inwards to irradiate the grapes. This setup ensures a very uniform dosage[8].

In terms of its effectiveness, a 2019 study found that irradiating the grapes prior to fermentation produced Merlot wines with higher concentrations of anthocyanins and fruity-floral aroma compounds with radiation doses of up to 2 kGy. When the dose was increased to 2.7 kGy, anthocyanins showed their highest concentration, as well as furfural and furfuryl alcohol increased in concentration leading to toasty caramel qualities. Therefore, it was concluded that gamma irradiation could serve as a suitable method to age wine[9]. Future research surrounding gamma irradiation is required to confirm these findings as not all of the current literature agrees. Caldwell and Spayd, 1989 found using very similar doses that anthocyanin content decreased. Sensory evaluation produced no significant differences between treatments, but colour density still increased[12]. However, this study involved irradiation post-fermentation, therefore, it may be reasonable to claim that future research should primarily focus on gamma exposure prior to the fermentation period for a greater likelihood of success.

In terms of safety, doses up to 7 kGy have been shown not to compromise the safety of food products. Interestingly, Chingzu Chang, 2003 studied the effect of radiation on the aging of rice wine for doses up to 0.8 kGy. Upon testing their wine, they found no radioactive products present in any of the treatments proving the wine safe. They also conducted a sensory evaluation with tasters, which yielded increasingly positive results with higher radiation doses [10].

Additionally, electron beam is another form of irradiation. It is a widely utilized cold pasteurization technique, traditionally used to control microbial populations in fruits and vegetables as it preserves all characteristics yet destroys microbes[13]. In essence, electron beam irradiation utilizes an electron accelerator which concentrates a highly charged stream of electrons capable of single or multiple beams from 360° with the sample placed in the middle. The grapes used were treated with 0.5, 1, and 10 kGy of radiation[13]. 10 kGy dosage was found most effective and found to be the most desirable for aging purposes as sensory analysis showed that it yielded a much more fruity aroma and produced less reductive notes and had less astringency and tanninicity, all of which are regarded as undesirable[13].

APPLICATION OF AC FIELDS TO WINE

Another technology that has been applied as a wine aging accelerator is that of alternating current (AC) electric fields. Immediately, the AC field treatment has an advantage over irradiation due to the increased public familiarity of electricity compared to irradiation. The motivation for this method comes from bioengineering research that has shown applied electric fields can alter protein molecules[14]. Additionally, the technique also functions on the basis of electroporation, which is the changing in cell permeability and even the break down of cell membranes when exposed to electric fields. The result of this effect is potential extraction of compounds from grape cells into the wine, absorption of molecules into cells, as well as potentially killing unwanted microorganisms[15]. However, the efficacy of the method is what is truly in question. The application of AC electric fields to wine has produced varying results, both positive and negative[2]. A 2008 study found an optimal treatment for accelerating the aging of young Cabernet Sauvignon wine which involved applying a field of 600 V/cm for 3 minutes[14]. This created an optimal wine according to both sensory evaluation, which reinforces the safety of the method (since the wine was safely consumed by testers), and the chemical evaluation, which revealed lower amounts of bulkier alcohols and increases in the amounts of esters and free amino acids, leading to a higher quality wine[14]. The application of an AC field to wine has already been used in some Chinese wineries[2]. Therefore, this technology has perhaps one of the greatest potentials to become a commonplace technique in accelerating the aging process of wines.

ULTRASOUND AGING TECHNIQUE

Ultrasound provides an interesting option for aging wine, particularly because the technology is accessible, widespread, and is already considered safe by the public. Ultrasound is capable of providing a fast and simple alternate method of extracting mature volatile compounds from young wine[16]. There are different methods by which ultrasonic treatments can be administered. The first of which is an ultrasonic bath, in which a vessel containing wine can be placed for treatment. Another is an ultrasonic probe, which is dipped into the wine[17]. There are several parameters that affect the efficacy of ultrasound treatments in improving wine character. These parameters include: frequency, amplitude, bath temperature, bath duration, and probe diameter. At lower values for each of these factors, with correct probe diameter where applicable, treatments were shown to have a favorable impact on the phenolic, aromatic, and visual characters of red wine[17]. This is a promising result; any benefit to wine characteristic is desirable, especially in a compressed time frame that avoids the need for aging. Next, it must be determined what parameters are most important and whether or not it is possible to alter parameters to create a more significant aging effect. When testing this, it was found that increasing all parameters resulted in a decrease in overall positive effects. This unfortunately means there is a limit to how much ultrasound can be used as a replacement for traditional methods, at least in variations that have been considered thus far[17]. The most influential characteristics for ultrasonic bath are frequency, temperature, then amplitude. For the probe, they are probe diameter, followed by treatment duration, and then amplitude[17]. The current research into ultrasonic aging gives little hope to replace traditional aging methods but does show some promise in terms of positive benefit to wine character, for this reason it could be used as a supplemental treatment in addition to other methods. There are also factors that would need to be tested before this technique is applied in industry. In current research, experimentation has been done on small volumes of wine, and the size of the vessel was not tested as a potential impact on the effect of ultrasound. Both the size and the material of the vessel are important variables to consider, as it would be ideal for wineries to be able to use a probe method in places they already store wine, such as barrels or fermenting vats. However, this would be a much higher volume, compared to bottles, and the vessel would not be glass. Ultimately, this is a relatively new concept and testing has been minimal, though it does show some promise as a supplemental aging technique.

CONSUMER ACCEPTANCE OF IRRADIATED WINE

While it can be established that there is value to irradiating wine for decreasing aging time to put the product to market sooner, there must also be consideration of the market for such wine. The process of irradiation commonly has negative connotations for consumers, and for the use of irradiation to be of value to wine producers, there must be a demand for such wines. As such, an analysis of the consumer acceptance must be performed.

Studies into consumer acceptance of irradiated food products have existed for many years. In 1988, the World Health Organization (WHO) published a paper considering consumer acceptance. It states lack of acceptance being a major drawback to generalized use of irradiation techniques, especially by governments not providing public endorsement. Radiation has commonly been associated with danger, contamination, or war, and thus provides the basis for many negative public attitudes. The WHO stated that:

"There is apparently wide public misunderstanding of what the process is, how it works, and what it will and will not do. A major misconception is that food processed by radiation becomes radioactive"[18].

Considering this, it takes little imagination to understand that at the time, consumer acceptance was very low. The World Health Organization subsequently created a series of 'Frequently Asked Questions' to disprove several of these beliefs[18].

Currently, all irradiated products must be labelled, in order to provide full information to consumers. Moreover products must support public information campaigns regarding irradiation. Both of these tactics must occur in order to improve general market acceptance. However, this may in fact be holding back market acceptance due to the negative connotations with the process, as well as many languages having little difference between "irradiated" and "contaminated." Therefore, a push for more informational and educational campaigns for the public was urged[18]. In 1995, a study was performed that found most recognized health authorities in a wide range of countries considered irradiated foods to be safe. The public knowledge was determined limited, with science-based information only just reaching North American consumers at that time. The consensus was for health professionals to present accurate information to the public in order to increase consumer acceptance[19]. Most recently in 2016, it was determined that foods processed by a range of irradiation techniques are subject to drawbacks including consumer choice, purchasing behaviour, and acceptance of irradiated foods. Gamma irradiation in particular is believed to be hazardous by the public, posing a major barrier to commercialization. It has been demonstrated that communication, labels, and education improve public perception, and that well-informed individuals are less likely to

Irradiated: exposure to measured amounts of radiation

Radioactive: actively emits radiation

reject irradiated food[20]. Nonetheless, there is still confusion regarding the terms "irradiated" and "radioactive"[21].

Although there appears to be slow improvements to public perception based on the wide timing of studies, significant improvements in consumer acceptance must exist before irradiated food can be commonly accepted. As these studies are limited to food products in general, more efforts must be made to directly study wine irradiation. Currently, studies are being conducted in order to test the colour, aroma, and taste values of irradiated wine versus normally aged wine. These studies are imperative to the success of irradiated wine as they will likely lead to more widespread consumer acceptance. Success in studies by Chang (2003) and Lukić et al. (2019) as well as others, has worked to mitigate changes to these qualities in order to improve consumer acceptance. Further studies will be necessary to continue this progress.

CURRENT PROGRESS IN WINE IRRADIATION

While irradiation has been approved for use in many countries for over 30 years, wine is not an commonly approved product. The Canadian Food Inspection Agency has approved potatoes, onions, wheat, flour, whole wheat flour, whole and ground spices, and dehydrated seasoning preparations for irradiation. All irradiated foods are labelled with the international radiation symbol. Other countries have various regulations, although the use of irradiation on wine is not commonly approved[6]. The method of irradiation for wine aging is still under rigorous study. Irradiation on grapes as compared to liquid wine, type of wine and grapes, as well as specifics of the irradiation technique in terms of method, length of exposure, and more, are all being study to determine optimal conditions for aging. Additionally, phenolics, aroma, colour, and taste are being tested to ensure wine quality does not decrease during irradiation treatments[2,17].

In addition to irradiation techniques, other methods are also under study for similar details and results. Ultrasound and AC electric fields may be considered more acceptable by the public, but the optimal treatments must still be determined. AC electric fields are the most advanced technique to date, as they have been introduced into some Chinese wineries[2]. However, in Canada, this method has yet to be approved, as well as it is yet to catch on in other markets.

HEALTH BENEFITS OF IRRADIATED WINE

Radiation: the word itself has been linked to a plethora of issues all of which misconstrue its true meaning. In truth, irradiation is a widely utilized technique in multiple industries and has been shown in wine to increase concentrations of certain polyphenols with a wide range of health benefits. Resveratrol, the main naturally-occurring product of wine aging that furnishes these health benefits, is found in many berries and fruits[22]. However, its concentration in these products is clinically insignificant and since it does not have a compounding effect, its bioavailability is low[23]. As such, resveratrol is present in traditionally aged wines, however artificially UV-aged wines tend to have a significantly higher concentration of resveratrol, and thus, a greater bioavailability and potentially greater health benefits but the reasons are unclear[24].

Furthermore, as our body ages, the ends of our chromosomes (telomeres) shorten. This is directly linked to a decrease in efficiency of ATP synthase, the pump in cells that produces energy. Due to telomere shortening, the efficiency of these pumps decreases and they produce more Reactive Oxygen Species (ROS)[23]. ROS cause cell death which is linked to cancer, coronary heart disease and cerebral ischemia, as well as a variety of other aging susceptible maladies[22]. An accumulation of ROS also acts as a positive feedback loop and further promotes telomere shortening, and ATP synthase pump inefficiency, and more ROS release[22]. Resveratrol is a highly effective antioxidant. It operates through various mechanisms but its most effective mechanism of action is to neutralize ROS, terminating its signaling pathway and preventing cell membrane damage and cell death[22]. This most notably prevents cancer proliferation and coronary heart disease.

CONCLUSION

Overall, the future of wine aging is an exciting and dynamic field filled with possibilities. Due to the current economics of wine aging, and the potential for improvement via decreased aging times, irradiation and other methods may dominate the future market. While the AC electric field method has been employed in some Chinese wineries, none of the other methods have been scaled to market. More studies need to be performed to find the optimal treatments for each method before they can be implemented. Another major factor is consumer acceptance, which must be improved in order for these methods to be feasible for wineries. Most health organizations agree upon the safety of these methods, and thus improvements in government support, educational awareness, and benefits must be made. Once these changes occur, wine aging can step forward into current times.

MORE TO EXPLORE

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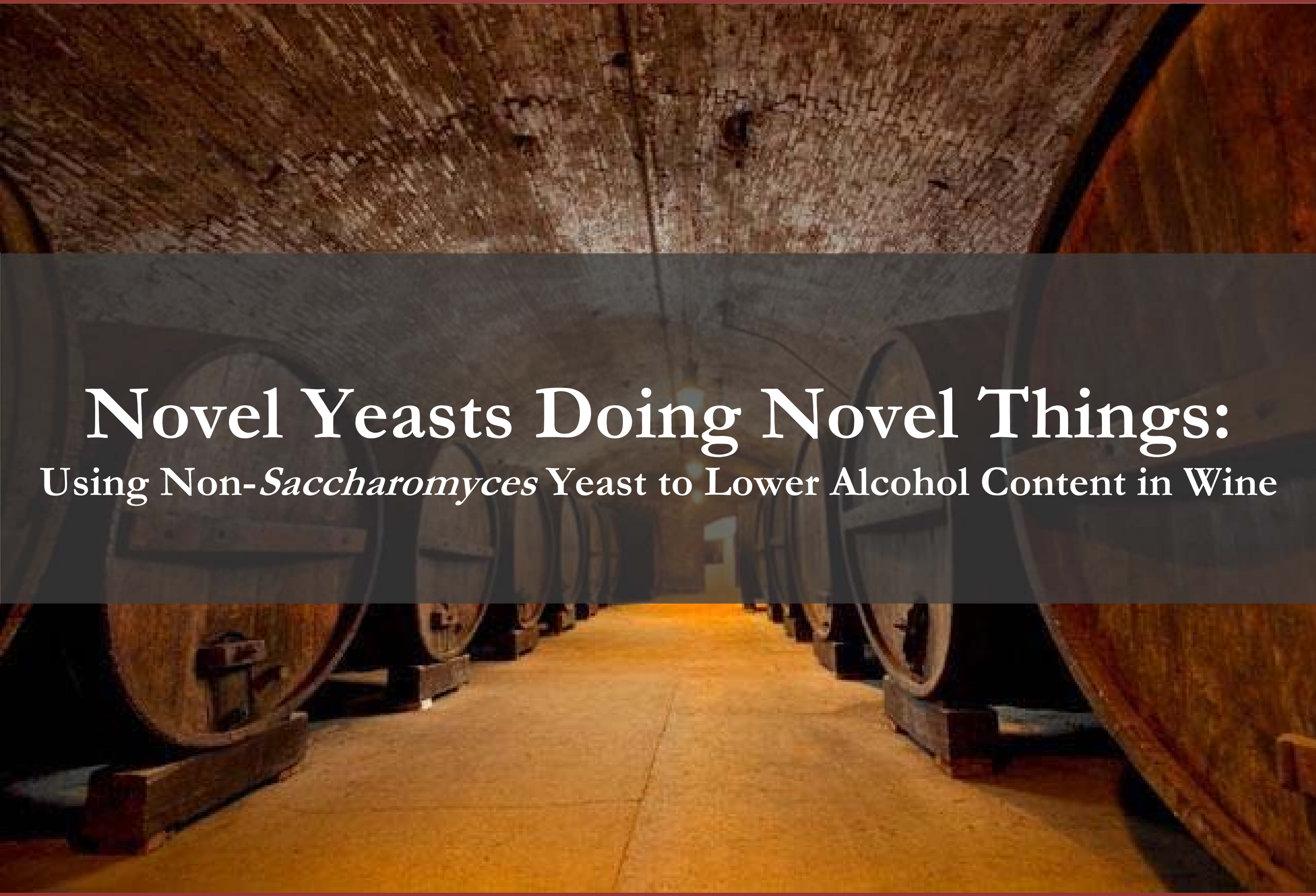

Novel Yeasts Doing Novel Things:

Using Non-*Saccharomyces* Yeast to Lower Alcohol Content in Wine

Meea Fogal, Kate Kim, Alexander McGrath-Santowski, and Yona Tugg

The wine industry has seen an increase in alcohol content due to consumer preferences for more full-bodied wines. However, consumers are also looking for lower alcohol wines because of increased health consciousness. Therefore, the wine industry is faced with the dilemma of producing lower alcohol wines without compromising the integrity of the flavour. Current methods for alcohol reduction are expensive and work at the expense of the sensory profile. As such, research has turned to strategies that reduce alcohol content during fermentation itself. One promising strategy involves the use of non-*Saccharomyces* (NS) yeast as opposed to using only the traditional *Saccharomyces cerevisiae*. Different factors affect the alcohol percentage in wines made with NS yeasts and *S. cerevisiae* and must be considered when attempting this strategy. Current knowledge of wine-yeast interactions and the control of different NS yeasts is limited. While NS yeasts are currently used in the industry, it is not for the purpose of reducing alcohol content. It is important to acknowledge the potential of using NS yeast in the fermentation process to provide the low alcohol wines consumers demand without sacrificing sensorial properties, and as such, more research into this method should be done.

INTRODUCTION

Over the last 30 years, the wine industry has seen an increase in alcohol (ethanol) content of about 2% (v/v) due to several factors[1]. One of which being increased consumer preferences for wine styles that are rich, more full-bodied and contain fruity flavours[2]. These wine styles are produced from riper grapes with increased phenolic maturity and aromatic complexity [1]. As grapes ripen, concentrations of sugars such as glucose and fructose increase. In the alcoholic fermentation process, yeast converts the sugars present in grapes to ethanol, carbon dioxide and other metabolic by-products[3]. As a result, higher concentrations of sugar within grapes correspond to an increased fuel source for fermentation and thus a higher resultant alcohol concentration[3]. Additionally, the onset of climate warming has also resulted in variations in grape sugar content and is expected to further increase this trend in the wine industry[4]. This increase in alcohol concentration comes with disadvantages to winemakers as producing wines with higher alcohol content results in taxation in some regions of the world where tax rates typically increase with alcohol percentage[1].

Climate Change and Sweeter Grapes

Climate change poses detrimental effects to many human activities on earth including the winemaking process. Increases in temperature quicken the ripening process and are associated with over-ripened fruit with low acid, low anthocyanin and high sugar content[4]. As a result, it can be expected that wines produced from these grapes will have altered flavour, colour, mouthfeel and higher alcohol content[4].

At the same time, there is a demand from consumers to produce alcoholic beverages with reduced alcohol content due to increased health consciousness[7]. Health advantages of low alcohol content beverages include reduced caloric intake, and decreased risk of illnesses related to alcohol consumption[7]. In fact, more than 60 different medical conditions today are related to a high level of alcohol consumption such as liver disease and cancer[7]. From a consumers' perspective, low alcohol-content beverages are also more socially acceptable in that consuming lower quantities of alcohol allows for productivity and proper functioning after consuming, allowing individuals to perform tasks such as driving without legal consequences[7]. Though varied considerably between countries, wines with reduced alcohol content have four main classifications as demonstrated in Figure 1: alcohol free, low alcohol, reduced alcohol and lower alcohol.

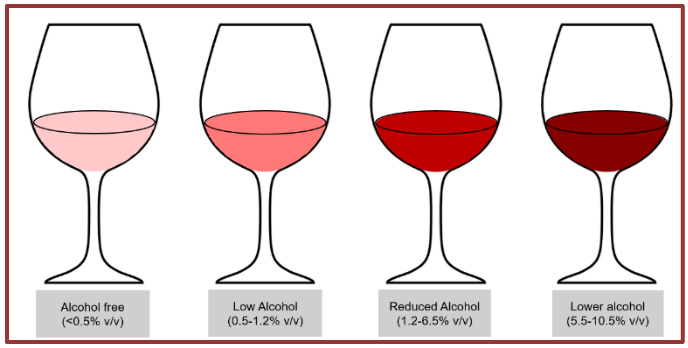

FIGURE 1: CLASSIFICATION OF LOW ALCOHOL CONTENT WINE based on alcohol percentages. Image adapted from Bucher et al., 2015[8]. Wineglass image obtained from Qpad, 2012 [9].

These emerging trends in the wine industry pose challenges for winemakers in the reduction of alcohol content in wine to meet consumer demand and avoid taxation, without compromising levels of phenolic ripeness and flavour profile.

A wide array of methods are currently being employed to reduce alcohol concentrations in wine that target several different steps within the winemaking process as shown in Figure 2. One such method employs changes in viticulture practices to directly alter the properties of the fruit itself[10]. Alteration of sugar concentrations within the fruit is commonly performed through basal leaf removal, decreasing the leaf area to fruit mass ratio – a determinant of the rate of sugar accumulation within the fruit[2]. By reducing leaf area through removal of leaves, one can delay berry ripening to decrease its final sugar concentration at the time of harvest[2]. However, the associated costs of this labour-intensive method are quite high, and reduction of

The Phenolics of Wine

Phenolic compounds are of interest in assessing sensory properties of wines. They are classified as either flavonoids or non-flavonoids[5]. Flavonoids include leaf area poses challenges in maintaining photosynthetic capacity of the vine which may have

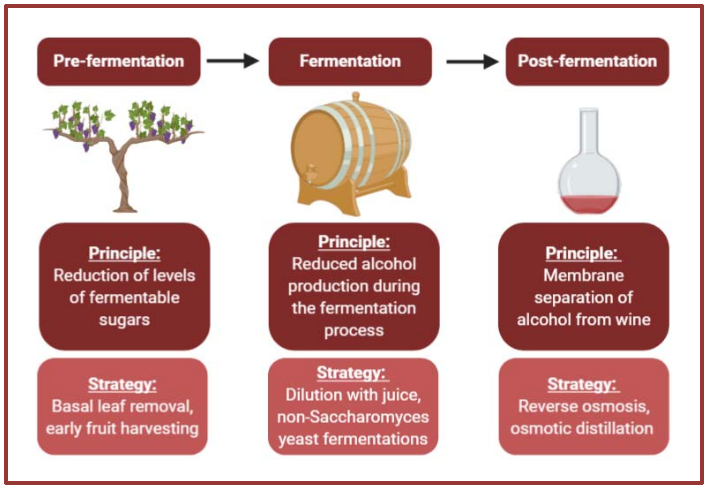

FIGURE 2: STAGES OF THE WINEMAKING PROCESS in which efforts have been made to reduce alcohol concentrations in wine. Each stage has unique processes that operate on different principles. Several strategies have been employed at each stage. Image adapted from: (Schmidtke, 2012)[11].

impacts on fruit and thus wine quality and taste[2,10].

Efforts have also been made to remove ethanol from wine at the post-fermentation stage through the physical removal of ethanol[10]. Such processes include the use of semi-permeable membrane-based systems including reverse osmosis[10]. In this process, the use of pressure pushes wine through a fine porous membrane that is strictly permeable to alcohol and water[10]. The blend of alcohol and water removed from the wine can then be distilled into its individual components to allow for re-addition of alcohol at desired concentrations back into the wine[10]. However, this process may lead to reduction of free volatile compounds when alcohol content is reduced greater than 2% (v/v)[2]. Such compounds in wine are responsible for sensory profile such as the intensity of attributes such as cherry and spicy[10]. Additionally, the high capital cost of this method makes it more favourable for larger-scale wineries[10].

While both pre and post-fermentation methods have all been investigated for their ability to reduce ethanol content in wine, research continues to assess other strategies that influence the fermentation process itself. *Saccharomyces cerevisiae (S. cerevisiae)* is the main microorganism responsible for fermentation in traditional wines and also produce several metabolites that positively influence wine's sensory profile[12]. A recently emerging and propitious method involves the use of non-*Saccharomyces* (NS) yeast in the fermentation process[12]. Such yeasts which are not as capable as *S. cerevisiae* in completing alcoholic fermentation or do so less efficiently. This is often performed in co-fermentation with conventional *S. cerevisiae* wine yeast strains[12]. The use of *S. cerevisiae* and NS yeast co-fermentations are of interest in the winemaking field as they are not only promising in the reduction of ethanol levels, but also for producing wine with enhanced sensory properties and unique flavours[13].

NON-*SACCHAROMYCES* METABOLISM FOR ETHANOL REDUCTION

A typical grape has over 40 species of yeasts present within its juice[14]. NS yeasts consist of all yeast species other than *S. Cerevisiae*, each of which has varying effects on alcoholic fermentation when used individually or with *S. cerevisiae*[14]. In the past, NS yeasts were considered "spoilage yeasts" and were thought to negatively impact the quality of the wine. Some NS yeasts are still considered spoilage yeasts for a variety of reasons, such as producing too much volatile acid that ruins the flavour of the wine[14]. New research in the last decade has shown,

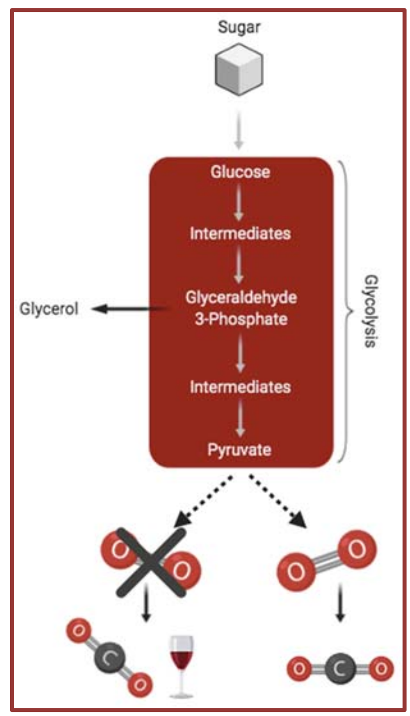

FIGURE 3: METABOLIC PROCESSES OF SUGAR. Yeasts undergo different metabolic processes to consume sugar depending on environmental conditions. After completing glycolysis and converting sugar to pyruvate, depending on whether oxygen is present, yeasts have the ability to make either ethanol and carbon dioxide or just carbon dioxide.

however, that not all NS are spoilage yeasts. It was shown that some can actually result in better flavour profiles by producing various secondary metabolites or different alcohol levels[14,15]. These variations are due to differences in the metabolic processes that occur during fermentation[12,13,16].

Yeasts create ethanol through the consumption of glucose[13]. Yeasts undergo many different metabolic pathways while consuming glucose, as summarized in Figure 3[13]. The first metabolic pathway that occurs is glycolysis. In the presence of oxygen, glucose enters glycolysis and leaves as pyruvate[13,15,16]. Pyruvate is converted to acetyl-CoA, which then undergoes oxidative phosphorylation, a process that makes ATP, or energy, with only carbon dioxide (CO_2) as a by-product[13]. Without oxygen present, or in anaerobic conditions, glucose still enters glycolysis and leaves as pyruvate; however, pyruvate is converted to ethanol and CO_2 to make energy, instead of only CO_2[12,13].

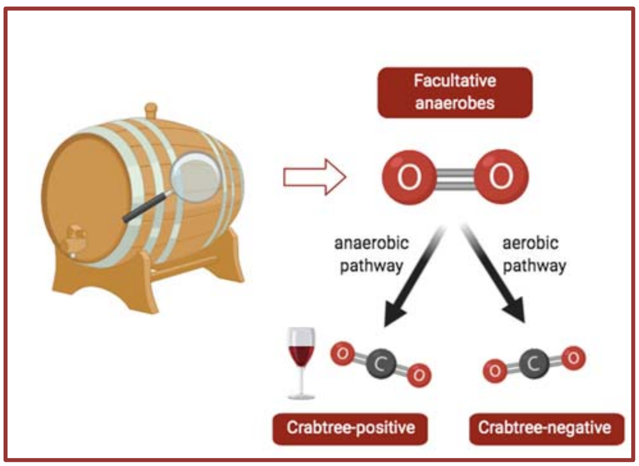

FIGURE 2: YEAST CLASSIFICATION. Facultative anaerobes can consume sugar both with and without oxygen present[13]. They can be further classified as Crabtree-positive or Crabtree-negative, depending on how likely they are to consume sugar by producing ethanol even if oxygen is present[13,15,16].

Many yeast species, including *S. cerevisiae*, are facultative anaerobes, meaning that in the presence of oxygen they can follow either the aerobic or anaerobic pathway[13,16]. Certain yeasts are more likely to follow the anaerobic pathway than others[13]. This phenomenon is known as the Crabtree effect; a metabolic trait seen in some yeasts that makes them preferentially consume sugars by aerobic or anaerobic fermentation[13,15,16]. As shown in Figure 4, Crabtree-positive yeasts are more likely to undergo the anaerobic pathway in the presence of oxygen and produce ethanol, while Crabtree-negative species are more likely to produce carbon dioxide in the presence of oxygen.

While *S. cerevisiae* is a Crabtree-positive yeast strain, a large amount of diversity within NS yeast species means that some NS yeasts are Crabtree-positive, while others are Crabtree-negative[13]. It is these Crabtree-negative NS yeasts that are of interest when attempting to reduce alcohol content during the fermentation process[16].

An issue that arises with many NS yeasts is that many are unable to consume all of the sugar present in grapes and thus cannot fully finish the fermentation process, resulting in a phenomenon called stuck fermentation[12-14]. Stuck fermentation occurs because NS yeasts are generally sensitive to ethanol, and therefore unable to continue the fermentation process once a certain level of ethanol is reached. The level of ethanol at which stuck fermentation occurs differs depending on the specific NS yeast strain used[12-15]. Stuck fermentation is a predicament for winemakers because if the fermentation process is not completed, the resulting wine contains too much sugar and the flavour profile is ruined. A technique called co-inoculations is used to help combat this issue. Co-inoculations work by starting fermentation with NS yeast species present and then adding *S. cerevisiae* after a predetermined amount of time[16]. Co-inoculations diminish the stuck fermentation effect, as *S. cerevisiae* is capable of finishing the fermentation and has demonstrated a very high tolerance to ethanol[12,14,16]. In wine-making, the fermentation process usually occurs in a closed environment where oxygen is not present[13]. In order to allow the aerobic pathway to produce only CO_2, rather than the anaerobic pathway to produce both ethanol and CO_2, the container where fermentation is taking

Aerate:

Exposing the wine to air, and therefore oxygen[13].

place would need to be aerated when only NS yeast is present[12,13,15].

Ideally, an effective co-inoculation with NS yeast and *S. cerevisiae* would use a NS yeast that has a low ethanol yield but consumes enough glucose that a significant impact on the ethanol concentration is attained[13]. Additionally, the NS yeast must be compatible with *S. cerevisiae*; incompatibility between the two yeasts would lead to an inability to complete the fermentation process as competition between the two yeasts would result in stuck fermentation[12,13]. As the metabolic processes of fermentation are incredibly sensitive to environmental conditions, the selection of NS yeast strains to use in co-inoculations with *S. cerevisiae* must be carefully considered.

FACTORS INFLUENCING ALCOHOL REDUCTION

When using *S. cerevisiae* and NS yeasts, there are a number of factors that can be manipulated to affect the final alcohol content of wine. These factors are the fermentation temperature and the time between co-inoculation of NS yeast and *S. cerevisiae*[17-21]. Through testing, it was found that pH and the size of the initial inoculum had no effect on the alcohol concentration[17,18]. A timeline of how these factors

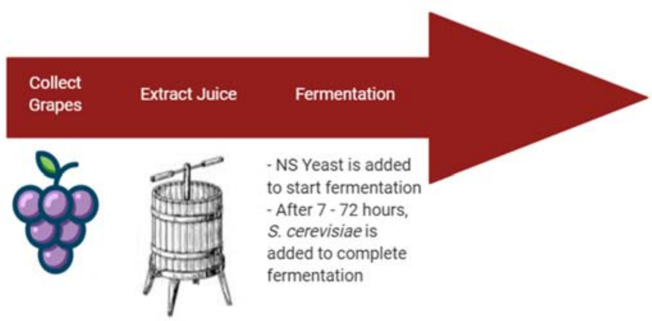

FIGURE 5: OVERVIEW OF THE BEGINNING OF THE WINEMAKING PROCESS showing when the time between co-inoculation comes into play to affect ethanol concentration[22,23].

affect alcohol concentration is shown in Figure 5. Their effects on alcohol concentration, as well as why they affect alcohol concentration, will be examined.

One factor that affects alcohol concentration is the temperature at which the wine completes fermentation[17,19]. Several studies have tested how the ethanol production changes with different temperatures, including between 10ºC and 30ºC for a study done by Gao and Fleet in 1988, and between 15ºC and 25ºC for a study done by Maturano et al. in 2019. Although the exact results, temperature ranges, and types of yeasts differed between the two studies, they both found that the extreme low and high temperatures tested resulted in the lowest alcohol percentages, respectively.

The study by Maturano et al. directly analyzed the alcohol percentage (v/v) after fermentation with two yeasts, *Hanseniaspora uvarum* and *Candida membranifaciens*, each sequentially inoculated with *S. cerevisiae*[17]. It found that higher temperatures resulted in wines with the lowest alcohol percentage[17]. The study by Gao and Fleet found that at temperatures of 10ºC and 30ºC NS yeasts *Candida stellata* and *Kloeckera apiculata* tolerated a lower ethanol percentage[19]. Ethanol tolerance is the maximum concentration of ethanol that the yeast can survive and ferment sugar in, so a lower tolerance means that the yeast will not survive in low ethanol percentages[19]. The study found that in general, at the highest and lowest temperatures tested, the yeasts tested had the lowest ethanol tolerance[17, 19]. Following this, the grape juice can then be treated with *S. cerevisiae* to complete fermentation, and since the initial inoculum produced less ethanol, the final wine will have a lower alcohol percentage because there is less sugar available for *S. cerevisiae* to complete fermentation with[19].

Ethanol tolerance of NS yeasts is substantially enhanced at lower temperatures, therefore allowing the yeast to make a stronger contribution to wine fermentation[17]. Temperature is an important factor because it directly affects microorganism growth and membrane composition[17]. At low temperatures, the membrane is less fluid, which reduces membrane transport, therefore reducing sugar transport[17]. After the NS yeast cannot survive in the grape juice, it must be co-inoculated with *S. cerevisiae*.

The second factor that can affect alcohol percentage is the time between *S. cerevisiae* and NS yeast inoculation[17,18,20,21]. The same study by Maturano et al. tested the relationship between co-inoculation time and alcohol percentage, in which times between 24 and 72 hours were tested. Through experimental trials and mathematical modelling, they found that for *H. uvarum*, the ideal time between co-inoculation was 48 hours and 37 minutes, and for *C. membranifaciens* the ideal time was 24 hours and 15 minutes to reduce alcohol concentration[17]. Similarly, a study by Canonico et al. found that when testing co-inoculation times of 48 and 72 hours, the longer time gave rise to the lowest alcohol percentage for three out of the four NS yeasts tested - *Starmerella bombicola*, *Metschnikowia pulcherrima*, and *Hanseniaspora osmophila*, with *Hanseniaspora uvarum* showing a lower alcohol percentage after 48 hours[21]. Another study by Englezos et al. had similar results to that of Canonico et al.; when they tested co-inoculation for *Starmerella bacillaris* and *S. cerevisiae* at times between seven and 48 hours. They found that the longer the time between co-inoculation, the lower the alcohol percentage, in general[20].

At longer co-inoculation times, the amount of sugar that the yeast needed to produce 1% (v/v) of ethanol increased, which caused ethanol production to decrease over time[20]. The lower ethanol levels can also be associated with high sugar consumption by the NS yeasts at the start of fermentation, which diminishes available sugar for fermentation by *S. cerevisiae*[17].

LIMITATIONS OF THIS TECHNIQUE

Current research has shown that NS yeasts can be used without spoilage, which is encouraging winemakers around the world to start shifting their views towards using NS yeasts[24]. The knowledge on wine-yeast interactions is still developing spontaneously as more research is being done. However, a limitation in such knowledge still exist regards to controlling different NS yeasts in terms of producing specific sensorial compounds[24].

Although there was an increase in focus and interest in NS yeasts over the past ten years, there is still a lack of data compared to *S. cerevisiae*[25]. For using naturally existing NS yeasts along with artificially added NS yeast, high degree of complexity behind properly using the method still exist. There is also great qualitative and quantitative variability found in NS yeasts in various stages of fermentation, which adds to the complexity[24]. As a result, it is extremely difficult to be completely knowledgeable on how to control all kinds of NS yeasts. Thus, it is important to acknowledge the potential of NS yeasts and conduct further research for winemakers to start using this technique for the purpose of decreasing alcohol content[24].

THE CURRENT STATUS OF NON-*SACCHAROMYCES* YEAST IN THE WINE MARKET

Lack of success with low-alcohol wine in the market mainly occurs due to a perceived decrease in sensory quality associated with other alcohol reduction methods[26]. Other techniques used, including dilution with juice and reverse osmosis, often lead to a reduction in wine quality by changing the composition of volatile and sensorial compounds[26].

Using NS yeasts in a co-inoculation with *S. cerevisiae* provides an opportunity for winemakers to meet the sensorial demands of consumers while still providing a wine that is not too high in alcohol[27]. This increases the viability of NS yeasts as a method of producing wine that is competitive on the market. Furthermore, promoting the use of naturally occurring yeast during fermentation, NS yeasts, may attract those concerned with their health[26]. Specifically, it was shown that younger generations tend to have greater attention to their well-being and have an increased interest in lower alcohol wines across the United States, China, Germany, and the United Kingdom[26].

However, winemakers are still uncertain about whether NS yeasts will provide unexpected risks or an opportunity to improve wine quality[24], mainly due to NS yeasts' historical reputation as "spoilage yeasts". To reduce this concern, there are currently wines on the market using NS yeasts, although not necessarily for the purpose of reducing the alcohol content[28]. Rosewood Estates Winery and Meadery, located in Beamsville, Ontario, uses naturally occurring wild yeasts in the production of their wine. Although they aim to capture a unique flavour in their wine rather than reduce alcohol content, their method includes several NS yeast species, rather than the typical addition of *S. cerevisiae* used in many other wineries[28].

CONCLUSION

Winemakers in the industry are currently faced with the challenge of creating wine with low-alcohol content that still maintains sensorial properties associated with more mature and thus higher-alcohol content wines[1]. Based on their ability to maintain and even introduce unique sensorial properties, while significantly reducing alcohol content, NS yeasts appear to be a viable technique available to winemakers[17,18,20,21]. However, the complexity of the interactions between several different NS yeasts and *S. cerevisiae* has not been as thoroughly explored, posing a challenge for implementation. Regardless, given that this technique poses potential for a unique marketing strategy and the available niche in the market for low-alcohol content wines, there is great potential for its application in the winemaking process[26].

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Fighting Cancer With Wine:

Nanoparticle-Conjugated Resveratrol Particles in Wine as Potential Chemotherapeutic Agents

Aakanx Panchal, Maria Romano, Jalen Singh, Armaan Somani, Mateas Winter

Wine has been found to have certain health benefits, but can wine be used in the future as medicine? A glass of wine as treatment for cancer might sound like a fairy-tale but there is some real potential to this. Select compounds found in wine have shown clear anticancer effects, enabling their use for cancer prevention and treatment. This article explores the possibility of using wine as a treatment for cancer by adding nanoparticles to elevate the benefits of the pre-existing compounds found in wine. Wine contains something unimaginably powerful, fostering an exciting area of research key to the future of cancer therapy.

INTRODUCTION

Wine is consumed globally by adults of all ages, backgrounds, and social classes. It is a beverage of choice for many, and as there are a multitude of wines with different flavour profiles, an individual is bound to find one that caters to their needs. As with all alcoholic beverages, there is a stigma around wine for having negative health impacts. While this is rooted in some facts such as the mental and social health impacts, there may actually be some health benefits to wine[1].

The correlation of health benefits with wine was first observed in the French population and was subsequently termed the French Paradox. This was the observation of low coronary heart disease mortality rates in the French population relative to Americans despite the high intake of dietary cholesterol and saturated fat in the French diet[1,2]. In 1991, Professor-Serge Renaud suggested that moderate wine consumption may lower the risk of coronary heart disease[2]. This was based on the premise that the French consume significantly more wine than the Americans.

Expanding on the French Paradox, scientific research has discovered specific compounds within wine that provide these health benefits. This research is primarily based on the idea that polyphenols like phenolic acids, particularly in red wine, confer additional health benefits[3]. These compounds are natural anti-oxidants and as such can reduce the incidence of cardiovascular and coronary heart diseases[4].

Polyphenols:
Micronutrients that we get through certain plant-based foods[3]. Phenolic acids are a type of polyphenol found in fruits that can exert anti-oxidant activity[3].

Anti-oxidants:
Compounds that inhibit oxidation, a chemical reaction that can damage the cells of organisms[4].

Recently, there has been a lot of research on one of these anti-oxidant compounds in particular—resveratrol (RSV). A phytoalexin found in grapes, RSV has been shown to protect against cardiovascular disease[5,6]. Furthermore, current studies have shown that RSV may be an effective compound in preventing and/or treating cancer[7]. A common type of cancer is lung cancer, a complex disease in which cells in the lungs divide uncontrollably to form a tumour. The symptoms of lung cancer are normally not seen until it has spread to other parts of the body[8]. Lung cancer is currently the leading cause of cancer-related death in the United States, among both men and women[9]. While RSV may be effective in preventing many cancer types, this article will explore RSV's potential role in wine to prevent and treat lung cancer.

Phytoalexins:
Substances that are produced by plant tissues in response to contact with a parasite[6].

MECHANISMS OF ACTION

RSV has numerous health benefits in the human body[10]. It acts as an anti-oxidant through the conversion of reactive oxygen species (ROS) to unreactive compounds. ROS are produced by the body during normal uses of oxygen in respiration and cell-mediated immune functions. Unfortunately, ROS at normal levels are able to damage critical biomolecules including lipids, proteins, and nucleic acids[11]. This may cause DNA damage that can lead to DNA mutations that play a role in cancer development[11,12]. These ROS include O^{2-} and OH^- among others[13,14]. Luckily, the body does have cellular constituents that break down these ROS, called anti-oxidant defenses. These constituents include anti-oxidant enzymes; food constituents such as RSV aid in the bodies natural method of ROS breakdown. In recent years, natural anti-oxidants have been sought after and added to food products for their health benefits[11]. Numerous *in vitro* and *in vivo* studies have been done to further understand the anticancer effects of RSV[10].

RSV exhibits anticancer properties through two biochemical mechanisms, prevention and treatment, as overviewed in Figure 1. Preventative mechanisms are visible in healthy cells while treatment mechanisms are illustrated in cancerous or damaged cells.

The preventative mechanism relies on RSV's anti-oxidant ability. RSV and other anti-oxidants are able to give electrons to free radicals to stabilize them, without becoming destabilized themselves[12]. This allows it to neutralize harmful ROS that can cause DNA damage and lead to cancer. The second way RSV can serve as an anti-oxidant is by increasing the activity of cellular anti-oxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase[13,15]. The increase in these enzymes acts to directly decrease ROS levels, and the risk of ROS damage is significantly reduced.

Apoptosis:
The process of cell death which occurs as a normal part of an organism's growth and development[18].

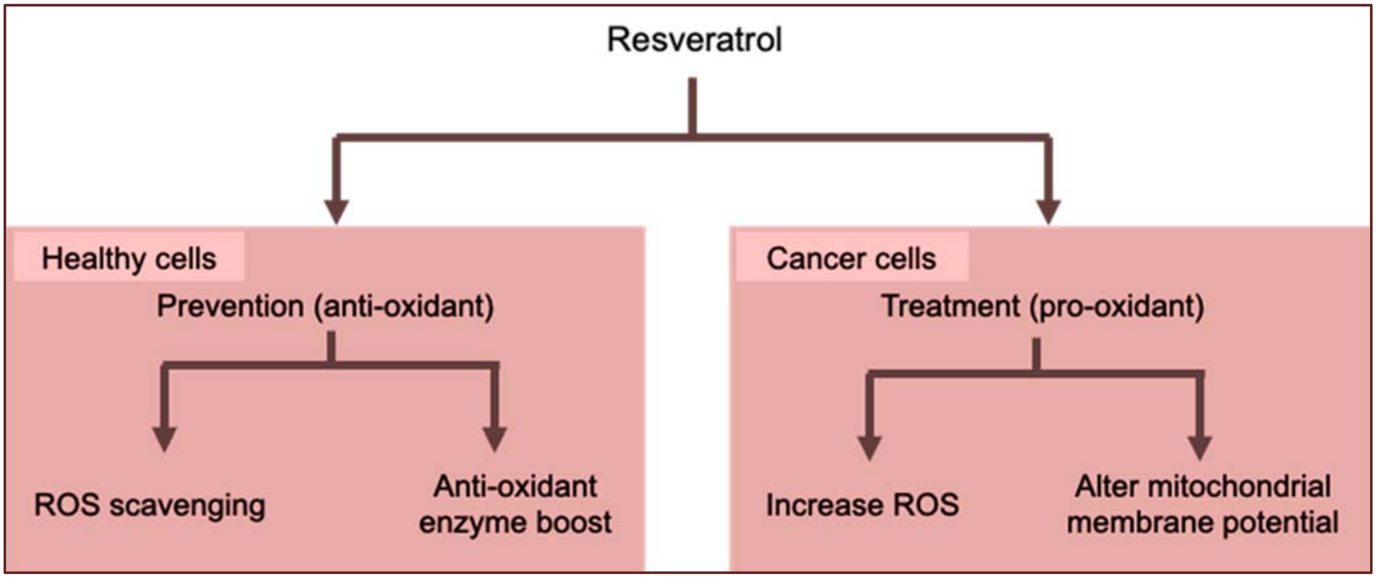

FIGURE 1: OVERVIEW OF MECHANISMS OF ACTION Resveratrol utilizes two main pathways to have anticancer effects on cells in the body. It acts to both maintain healthy cells and damage cancer cells.

On the other hand, RSV's treatment mechanism is a pro-oxidant mechanism, as demonstrated in a study that looked at the activity of nanoparticle-conjugated RSV particles in Non-Small Cell Lung Cancer (NSCLC) cells[16]. It was found that RSV has the ability to kill cancerous cells through the generation of ROS[16]. When RSV increases the ROS concentrations in damaged or cancerous cells it helps the body fight against or prevent cancer. A hallmark of cancer cells is the ability to evade apoptosis, so with RSV being able to selectively target damaged cells and increase ROS, the elevated ROS levels can induce significant DNA damage, activating an apoptotic cascade[16]. In this case, although RSV acts as a pro-oxidant, it is still helping to prevent cancer in adjacent cells and tissue[16].

In addition to interacting with ROS levels, RSV increases permeability of the mitochondrial membrane. Mitochondria have long been understood to be one of the most important organelles in regulating apoptosis. An increase in membrane permeability causes the mitochondria to leak substances into the cell, inducing cytochrome c to activate caspases that again trigger apoptotic signaling cascades[22]. Other apoptotic mechanisms of RSV include chromatin condensation, nucleus fragmentation and apoptotic body formation, further solidifying RSV as a compound with significant anticancer potential[23].

To expand on the ROS mechanism, the DNA damage caused by RSV through an increase in ROS works by interacting with a three-stage collection of cell signaling pathways known as DNA damage response (DDR), as summarized in Figure 2[17]. The first stage of DDR involves sensing DNA damage by ROS. The second stage activates p53, a tumor suppressor protein responsible for transmitting signal to downstream effectors by upregulating a chemokine receptor known as CXCR2[18,19]. The chemokines that bind to CXCR2 cause cell cycle arrest[19]. The third stage decides the fate of the damaged cell; the cell will be repaired if the DNA damage is minimal, and

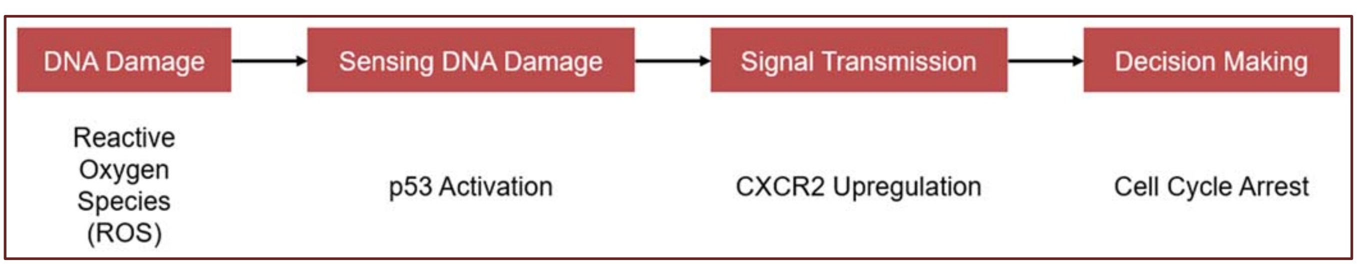

FIGURE 2: DNA DAMAGE RESPONSE Part of the pro-oxidant program of resveratrol's mechanism of action, this pathway can lead to cell cycle arrest, neutralizing and thereby preventing proliferation of cancer cells.

apoptosis will be triggered if it is not repairable[20,21]. Thus, RSV leads to cell cycle arrest.

ENHANCING ANTICANCER EFFECTS

Nanoparticles

Despite RSV's ability to destroy cancer cells, its use in medicine is limited due to its poor water solubility, membrane transport, and bioavailability[24]. Rapid metabolism of free RSV inhibits its biological effects and by extension, clinical utility. However, various approaches have been developed to combat these limitations. For instance, nanoencapsulation in lipid nanocarriers or liposomes, nanoemulsions, insertion into polymeric particles, solid dispersions, and nanocrystals have all significantly increased bioavailability after oral administration[24].

Karthikeyan et al. (2013) have demonstrated that nanoencapsulation by polymeric nanoparticles, designed to attribute further structural integrity to RSV, increases anticancer efficacy of RSV in lung cancer cells. *In vivo* pharmacokinetic assays of RSV encapsulated in gelatin nanoparticles (GNPs) revealed a longer half-life than free RSV, and thus increased bioavailability. Due to this logistic upgrade, RSV-GNPs had greater anticancer efficacy in NSCLC cells. For instance, RSV-GNPs exhibited more rapid cellular uptake, which allows greater amounts of RSV to be transported to the core of cancer cells. Here, RSV-GNPs showed greater antiproliferative effects through increased generation of ROS, DNA damage, increased mitochondrial membrane permeability and leakage, and subsequent apoptotic incidence.

Pharmacokinetic:
Pharmacokinetic is understood in scientific circles as "what the body does to the drug," whereas pharmacodynamic would imply "what the drug does to the body."

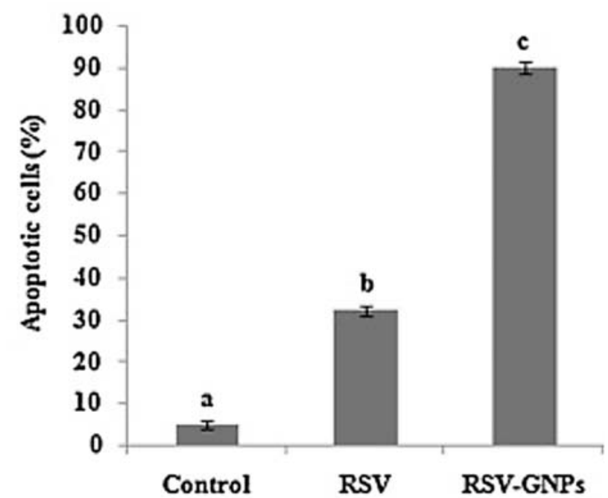

FIGURE 3: EFFECT OF RSV-GNPs ON CELL DEATH RSV-GNPs increase apoptosis in Non-Small Cell Lung Cancer (NSCLC) cells compared to RSV alone.

It is important to keep in mind that appending nanoparticles to RSV should not elicit unwanted toxic responses in healthy cells. Fortunately, an erythrocyte aggregation assay showed that RSV-GNPs did not demonstrate destructive hemolytic properties, supporting specificity of toxic effects towards cancer cells only[16]. The biocompatibility of the GNP nanoparticle carrier system, coupled with biodegradability and chemical modification potential, suggests a greater therapeutic index for the RSV-GNP system compared to free RSV. Altogether, the health benefits and non-toxicity of conjugating nanoparticles to RSV suggest that this unique duo could soon be one of the special ingredients in your next bottle of wine.

Combination Treatments

Several studies have also examined the effects of RSV in combination with other compounds. Simultaneous administration of RSV and Erlotinib in several lung cancer cell lines synergistically reduced cell viability and apoptosis[25]. Increase in ROS production, inhibition of anti-apoptotic protein expression, promotion of p53 expression, and increased caspase-3 activity were observed, making the combination more effective overall at inhibiting cancer cell proliferation[25]. In addition, cancerous lung fibroblasts treated with RSV, in combination with growth factor TGF-β and chemokine CXCL12, showed repressed conversion of fibroblasts to transdifferentiated fibroblasts that inhibit internal wound healing and enhance angiogenesis, again inhibiting cancer cell proliferation[26]. As well, treatment of Gefitinib-resistant cells with a combination of RSV and Gefitinib synergistically inhibited proliferation by inducing apoptosis, autophagy, cell cycle arrest, and senescence[27]. Increased intracellular accumulation of Gefitinib after RSV combination resulted in inhibition of EGFR phosphorylation, and increased expression of CYP1A1, ABCG2, cleaved caspase-3, LC3B-II, p53, and p21, leading to anticancer effects[27]. The ability to target chemotherapy-resistant tumour cells significantly ameliorates RSV's profile of health benefits as drug resistance has gradually become an increasing issue in the treatment of various cancers.

Furthermore, not only does RSV enhance the effects of other chemical compounds, some of which are currently used in cancer drugs, but it also improves some experimental methodologies of cancer treatment, which may be utilized in the future. Cells transfected with an oncolytic adenovirus, when done alongside RSV treatment, increased cytotoxicity[28]. In addition, cells treated with RSV and followed by ionizing radiation treatment also synergistically enhanced an apoptosis-independent mechanism resulting in more senescent cells and increased double-stranded DNA breaks[29]. Therefore, viral- and radiation-coupled administration of RSV also have significant anticancer effects.

LIMITATIONS TO APPLICATION POTENTIAL

The majority of the research discussed in this paper is composed of *in vitro* studies or isolated organism *in vivo* studies. RSV as an anticancer agent is still in early research stages, hence trials are only beginning to transition into animal *in vivo* research. *In vivo* research is not used at the beginning because it is impossible to have complete control over the complex system, and usually the side effects of the therapy are unknown. *In vitro* research offers a superior research method at the beginning of trials due to the more complete control and decreased risks associated with it. However, as a result of this control, it is difficult to extrapolate data from these experiments. Since *in vitro* studies are done under over-simplified physiological conditions, trial results may not accurately represent the effects of a given drug in the natural environment of the test subjects. It stands to reason then that although the studies discussed in this article have indicated positive anticancer impacts of RSV, further research needs to be done *in vivo* (beginning in animals) before it can be decided whether this polyphenol could be used as a chemotherapeutic agent for treatment against lung cancer in humans.

Moreover, while including nanoparticle-conjugated RSV particles within wine may display promise as an anticancer therapy, research demonstrating the feasibility of this option in the context of the wine industry is currently lacking. It is unknown how the incorporation of these nanoparticles might affect the flavour, colour, texture, or other features of wines. This opens up an entirely new field of research. If these therapeutic wines pass all the different trial stages, decisions regarding public availability would have to be made and would most likely be based upon the side effects of the treatment and the level of regulation required. Most likely, the wines would be available based on prescriptions by medical professionals, and there would be little need to market towards the general public. Pre-market surveys and post-market monitoring should be employed as the public perception of wine-soluble RSV-GNPs is currently unknown, and this therapy may thus face some backlash from the general public.

CONCLUSION

Although alcohol may be surrounded by negative stigma, wine has been proposed to have certain health benefits centered upon anti-oxidant and pro-oxidant activity in the polyphenols within it. RSV is one such polyphenol that under normal conditions converts potentially damaging ROS in human cells to various unreactive compounds. Neutralizing these compounds aids in cancer prevention by reducing DNA and cell damage. Conversely, RSV has also demonstrated direct anticancer effects in specific concentrations by increasing the levels of ROS in malignant cells. RSV used in combination therapies with current anticancer chemotherapeutic agents has displayed increased apoptosis of tumour cells. Unfortunately, the concentrations required to visualize significant anticancer effects are around 100 times higher than those normally found in wine.

Distinct research has illustrated promise in conjugating nanoparticles to various drugs to increase bioavailability and distribution of the drug, theoretically minimizing the required effective dose. The use of RSV-GNP has shown great promise through utilizing relatively small doses of RSV to generate greater apoptotic incidence in cancerous cells while reducing off-target effects. Research done on this subject is limited, and further studies should advance towards more *in vivo* experimentation such that one may better characterize the positive and negative effects in a full system. This is required before one can accurately and confidently extrapolate data. Further research should also address whether consumption of wine can serve as a mode of administration of RSV-GNP. Nevertheless, the potential for prescribed chemotherapeutic wines to help treat lung cancers is definitely an exciting prospect for the future.

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Branching Out

Pushing the Boundaries of Viticulture in Ontario

Elysia Fuller-Thomson, Dana Price, Pamela Schimmer and Lelia Weiland

There are many challenges associated with viticulture in northern climates. Currently, the main Ontario vineyards are located in southern Ontario. However, with increased urbanization and population growth, paired with the concept that grapes thrive when challenged, there is a need to consider expanding viticulture further north, where climatic conditions are more challenging. Climate and soil characteristics determine the particular challenges and advantages of growing wine in that region. Factors such as grape varietals and vineyard techniques provide new opportunities to produce high quality wines in various regions that were previously thought to be too extreme to grow the quality of grapes needed to support wine. Some vineyards in Ontario have trailblazed the way towards expanding the wine industry in Ontario through both trial and error and innovative practices. Through the exploration of these factors we can identify the challenges associated with expanding viticulture regions and identify methods for overcoming these challenges, in the hopes that one day great wine will be synonymous with not only the Niagara region, but all of Ontario.

INTRODUCTION

Since 1970, the wine industry in Ontario has grown from being essentially non-existent to a fully-fledged market with an international presence.[1] Currently, Ontario has been able to develop the wine industry into a major business with over 6000 jobs and 724 million dollars in expenditures.[1] Wine growing in Ontario has been extensively limited to three primary regions: Lake Erie, Niagara and Prince Edward County.[2] By examining Ontario's climate and soil, together with new grape varietals and alternative viticulture practices, a better understanding of the limitations to viticulture regions in Ontario outside of these typical regions can be gained. The examination may also identify future locations of growth and expansion in the wine industry.

CLIMATE'S EFFECT ON VITICULTURE

Climate conditions contribute to a region's ability to support viticulture and govern the ability of vieyards to produce great wines; wind, storm events, slope, elevation and sunlight hours all have a distinct effect on wine production. However, only temperature, and to a lesser extent, precipitation have a significant effect on Ontario's winegrowing capabilities.[34] From a global perspective, wines are typically grown in two regions, the warmer Mediterranean climate of dry, summer heat with low humidity, and the cool regions that are typical of central Europe's climate.[3] Similar to central Europe, Ontario is a cold-climate viticulture region, which is beneficial because cool-climate wines are said to be of the very highest quality, particularly due to their lower alcohol content.[5] Since yeasts convert sugar into ethanol, a lower quantity of sugar in the grapes will result in a lower overall alcohol content in the wine. The low temperature during autumn in cool climate regions allows a slower ripening of sugars and a lower pH, which are all desirable for high quality wines.[4]

Table 1: the Köppen climate model categories

Category	Letter	Description
Primary (broad)	A	**Equatorial zone**
	B	Arid zone
	C	Temperate zone
	D	Snow zone
	E	Polar zone
Secondary (moisture)	s	Dry summer
	w	Dry winter
	f	Fully humid
Tertiary (temperture)	a	Hot air temperature
	b	Medium air temperature
	c	Cool air temperture

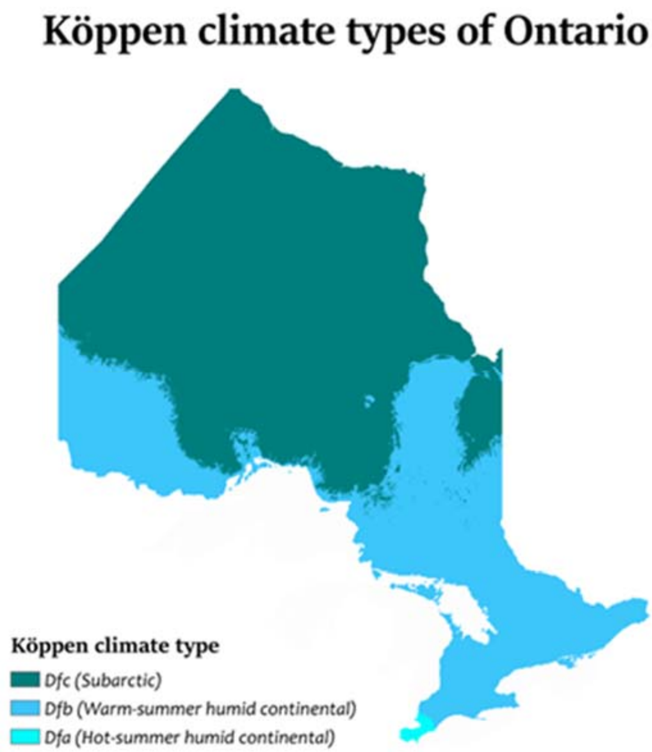

FIGURE 1: THE KÖPPEN CLIMATE MODEL Ontario lies in the D continental climate zone that is fully humid and ranges all three temperature zones.[9] Current wine regions exist in Dfa and Dfb.

The most pertinent limiting factor to expanding the wine industry in Ontario beyond the current viticultured area is the harsh climate. Ontario lies at the very low end of the range of temperatures that support grape growing. Climate environments are often described using the Köppen climate model, which groups climate by the preferences of broad vegetation zones and has continued to be very beneficial in mapping and understanding global climate regions (Table 1).[6] The most famous and well-known wine regions are located in the C climatic type, which is typically temperate and humid, such as central Europe which falls in the Cw category or the Mediterranean which falls in the Cs category.[3] Ontario lies firmly in a more variable range of temperatures in the D continental zone, which has greater seasonal variation. Ontario spans three climate zones, Dfa, Dfb and Dfc, which includes hot continental climates near the American border to subarctic climates in the Hudson Bay lowlands (Figure 1). Due to the more continental climate, Ontario has more frequent frosts and more intensely cold temperatures than central Europe. With

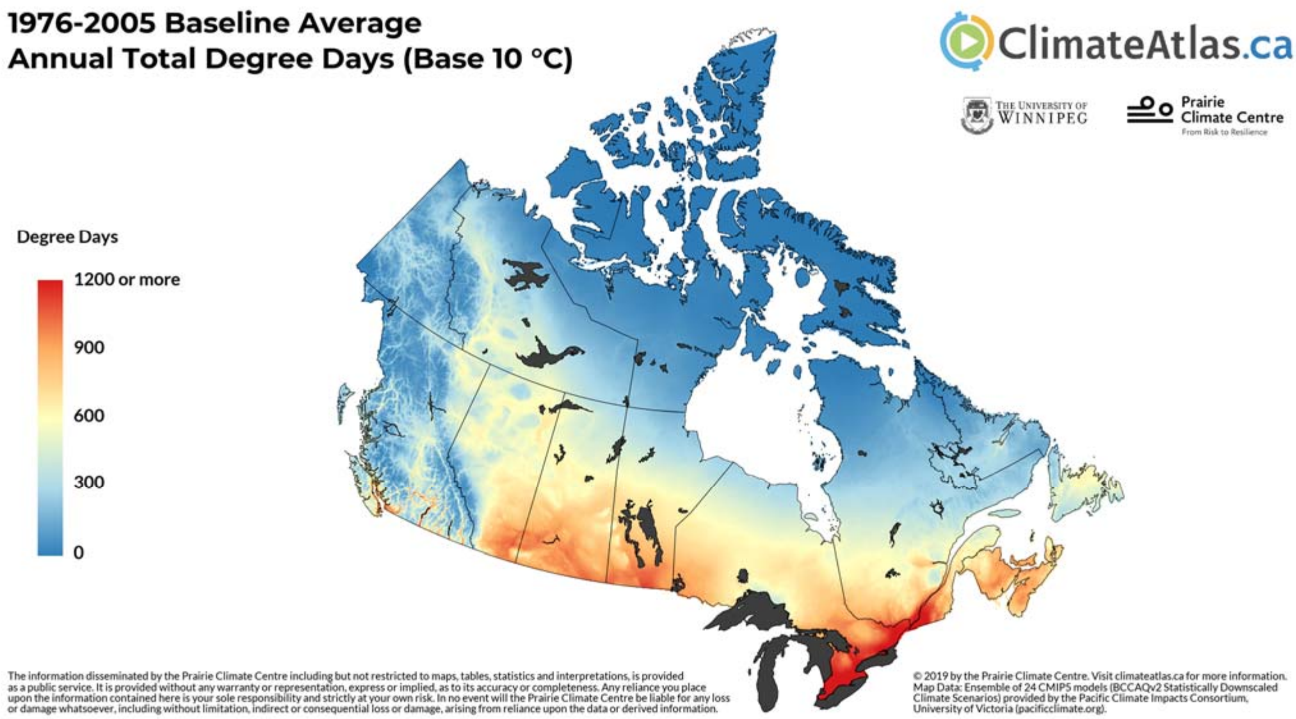

FIGURE 2: GROWING DEGREE DAYS Only the very most southern tip of Ontario would fall in Region II of the Winkler Index, whereas a little larger area falls in both Region I and II. By examining the map, it appears that potentially future areas of expansion could be the Ottawa valley and Eastern Georgian bay, which both benefit from high Region II Winkler Indexes, which contain highly valuable wine.[16]

more extreme cold, grape vines are damaged in the winter and grapes may not fully ripen before the first frost arrives. Since the cold is so extreme in the Dfc regions, those areas would be unlikely to produce high quality wines, and so future expansion of the wine industry should focus on the Dfa and Dfb regions, which have already supported wine vineyards in Ontario.

Nearby water bodies are important to mitigate the more extreme continental climatic conditions, since they serve as heat resevoirs and moderate the temperature. Whereas the Niagara region, Lake Erie and Prince Edward Island have their temperatures moderated by Lake Ontario which absorbs excess heat in the summer and releases this heat in the winter, the rest of Ontario's freshwater typically freezes in winter and cannot provide as extensive a moderating effect, which is part of the reason vineyards have not expanded to border the Georgian Bay, or Lake Superior shorelines.[7,8]

While many climatic factors are important to the creation of great wine, temperature is the single most important variable in vine development.[10] Many viticulturists use the degree day model to simulate the relationship between agricultural ripening and temperature in a simple linear relationship; the ripening of grapes can be modelled by the number of days above a certain temperature.[10] There are certain temperatures below which grapes do not ripen, which is typically thought to be 10°C.[3] At temperatures above 10°C, grape growth increases linearly with temperature.[3] To compute the degree day model, the difference between 10°C and the mean temperature of the day is calculated for every day, and summed for the entire year.[10] This value estimates the amount of ripening potential an area has (Figure 2).

In viticulture, the Winkler index which was developed in the 1940s relates degree days with a region's ability to produce wine and is still used in the modern day.[5] A minimum of 850 degree days (Region I), is needed for the very earliest-ripening grapes, and it is in Region II, which has a minimum of 1111 degree days, that the best wines are typically produced. From this model, the most suitable potential areas for growth of the wine industry can be predicted. Region II, classified as the area that has between 1111 and 1388 degree days, grows some of the very highest quality wine grapes, especially in warmer years, but these do not grow in large quantities.[4] Grapes in Ontario can also be grown in both Region but the vines tend to produce low yields and can suffer from cold winters that can damage the grape vine (Figure 2).[4,11] For future growth of the wine industry in Ontario, areas in Region II should be prioritized.

There are some severe limitations to the degree day model. Not only is mean temperature important for winegrowing, but temperature extremes are a severe and problematic issue for viticulturists. In winter, periods that consistently remain below -23°C damage the most common grape varietals, and only hybrids can withstand these extreme temperatures.[8] Temperatures below -4°C will cause young leaves to die off and under -2°C will cause flower buds to die, meaning that late spring frosts must be extremely rare to create a profitable grape growing areas.[3] Unfortunately, most of Ontario typically experiences seasonal temperatures that are within these ranges, and thus it is crucial to take advantage of novel grape hybrids that can withstand these temperatures to be successful. If traditional varietals are used, they must be buried to protect them from the cold, however this is a costly, time-consuming and potentially damaging process to the grapes themselves.[12]

PRECIPITATION

Precipitation is another climatic condition that dictates a grape growing region's productivity. Without water, yield and growth are dramatically reduced, and a large drought can cause acid, colour and sugar reduction.[3] Too much rain, and the burden of disease and pests can become too great. A typical winery needs their grapes to receive 500 to 700 mm of rainfall during the growing season, however these limits are flexible, as great wine regions span areas of widely varying annual rainfall.[3] The precipitation that falls with snowfall aids in replenishing soil water and insulating vines from the harsh winter conditions.[8] Generally, most of Ontario consistently receives over 700 mm of rain annually as shown by its 'fully humid classification' on the Köppen climate model (Figure 3). Due to the large amounts of precipitation, most wineries would not need to invest in costly irrigation procedures that often decreases the quality of the wine.[3,13] However, the combination of high rainfall amounts, along with Canada's bedrock that lies so close to the surface causes problems of soil saturation, which can prevent root growth and cause rot.[14,15] Rainfall can quickly saturate the ground surface as there is very little internal drainage on top of the impermeable bedrock. Ontario viticulturists have employed the use of dykes, tile drains or drain pipes, which have been highly effective in eliminating this concern.[14]

LIMITATIONS OF SOIL ON GRAPE GROWING

Soil quality is crucial to wine production, and grape berry development.[17] While roots do best in an ideal environment where soil is deep and rich, such as in Niagara, it is often thought

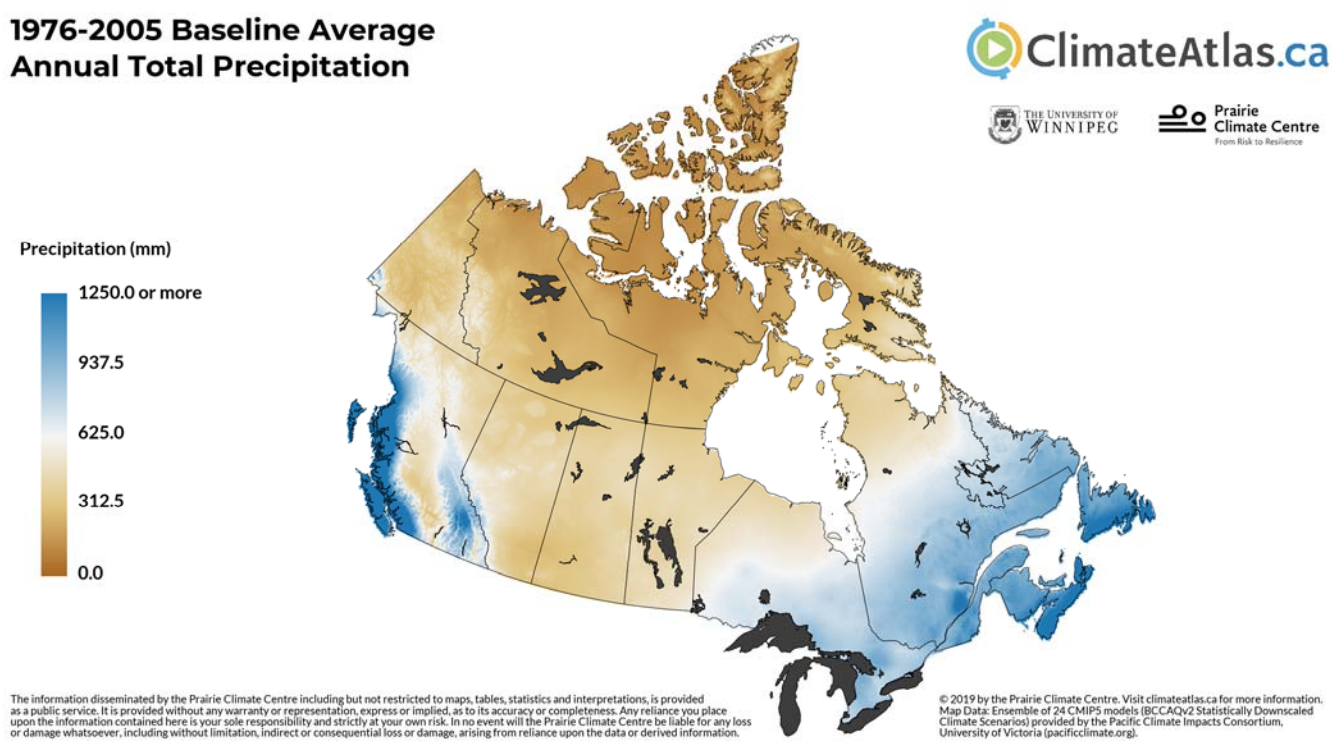

FIGURE 3: PRECIPITATION Drought or low precipitation is not a concern for most of Ontario, which lies in the centre of Canada above the great lakes and below the Hudson bay. All of Eastern Ontario receives over 650 mm of rain, providing enough water for the grapes to grown unhindered. [16]

in the

winemaking industry that soils considered to be agriculturally poor produce higher quality wines.[17] Harsher conditions in other regions of northern Ontario where soils tend to be less than ideal could not only provide a suitable environment for viticulture, but may even yield higher quality wines. The soil tends to be shallow more north along the Canadian Shield, providing for a harsh soil climate.[18] When considering the cold hardiness of grapes, the concepts of soil geology, mineral content, and water content should be examined. soils tend to be less than ideal could not only provide a suitable environment for viticulture, but may even yield higher quality wines. The soil tends to be shallow more north along the Canadian Shield, providing for a harsh soil climate.[18] When considering the **cold hardiness** of grapes, the concepts of soil geology, mineral content, and water content should be examined.

SOIL GEOLOGY

Soil originates from the underlying bedrock (Figure 4). Although in some regions, the type of soil sediment relates to wine quality, in general, there seems to be very little correlation between the bedrock and wine for most regions.[17] For example, in France, both high, and low quality wines are grown on the same type of geology.[17] Since other factors, such as varying microclimate and soil depth, play a large role in high quality wine production, it is difficult to determine the impacts of soil geology on wine quality. In any case, it appears that vineyards farther north in Ontario should not be affected by the particular geology of the region, and the bedrock composition poses no obvious barrier to wine production.[17]

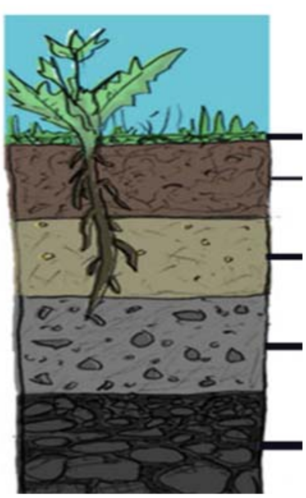

FIGURE 4: SOIL Image of soil layers as they progress from the source, the original bedrock.[19]

SOIL MINERAL CONTENT

Soil having an impact on the character of wine is controversial, however soil undoubtedly plays a role in grape development. When it comes to soil chemistry and trace elements, there is no one consensus regarding the benefits. All the same, there seems to be a general understanding that even if soil chemistry has an effect, it is not agriculturally significant. This unimportance can be attributed to the relatively high tolerance of roots and vine stocks to variable soil chemical environments.[20]

Despite the general unimportance of minerals in soil, a key ingredient for wine growth has been identified as nitrogen.[21] which is often added to soils by viticulturists all over the world.[22] Nitrogen is necessary for grape growth and development, and too little nitrogen is detrimental to berry growth. It was once generally accepted in the grape growing community that increased amounts of nitrogen in soil fertilizer correlates with a reduction in cold hardiness. Upon looking at the evidence, however, it appears that the results are not all in agreement.[22] One study conducted in 1993 in Washington by Wample et. al found that when nitrogen was added pre-bloom, and then a second time post-bloom before veraison, the cold hardiness of grapes is not significantly reduced.[2]

Although having adequate nitrogen is essential for grape growth, too much nitrogen in soils is less than ideal.[21] If too much nitrogen is added to the soil, photosynthesis is increased in leaves, resulting in too much vegetative growth. This causes shading from the leaves, reducing sunlight to the grapes.[21] In cold climates, nitrogen can be added to the soil without impacting the ability of the grape to survive the cold conditions, as long as the methods outlined above in the 1993 Washington study are used, where nitrogen is added in two phases.[22] It appears the limiting factor on how much nitrogen can be added without compromising the grape quality in cold climates is not the effects of nitrogen on cold hardiness, rather the general effects of nitrogen on berry health.[21]

SOIL WATER CONTENT

Aside from nitrogen, another factor surrounding soil as it pertains to grape quality is soil drainage.[20] From a physical point of view, soil needs to have the right porosity to produce grapes. While deep soil is advantageous, it is irrelevant if the soil is too dense, and cannot drain well. Alterations that can be made involve irrigation techniques, and utilizing slopes and microrelief.[21]

Berry ripening occurs more rapidly with decreased water content, potentially due to a decreased berry size.[17] In cold climates north of Niagara, it would be beneficial to reduce water exposure, either by not irrigating, or by developing more artificial solutions.

VINEYARD TECHNIQUES FOR COLD CLIMATES

Growing grapes for the production of wine is a complex process, and challenging grape vines during their growth leads to the production of a higher quality of wine.[17] However, vines can only be challenged to a certain extent before the vines begin to die or reallocate their resources to different locations. This reallocation can dramatically impact the flavour of the resultant wine, often in a non-favourable manner, when vines prepare for winter before the fruit has fully matured.[23] Traditionally, grapes are grown in moderate climates with mild seasons, precipitation, and temperatures. The vines of esteemed European vineyards often never see snow even in the height of winter. As such, it is important to account for the cold hardiness of the vines when one is planning to start a vineyard in a cooler climate.

Grape vines have a natural mechanism to avoid cold damage. As fall progresses and the days grow shorter and colder, vines redistribute the water within their cells to the space between cells, preventing freezing damage from occurring as easily.[24] This process is called **acclimatization**. There is, however, a limitation to a vine's effectiveness in cold tolerance, and below a critical temperature that is specific to each grape varietal, the vine will become damaged. Rapid temperature drops early in the winter can damage the vines since they have not had adequate time to acclimate. It can also be damaging if there is a temperature increase during the winter because the grapes will begin to **deacclimate** and will not be prepared for a return to cold temperatures.[25]

Vitis vinifera vines produce grapes that are used to make wines. There are many different types of *V. vinifera* vines that, based on differing physical characteristics, can survive different temperatures. The most tender of the *V. vinifera* vines including merlot, sauvignon blanc, and zinfandel can survive temperatures of -15ºC and -20.5ºC.[26] While southern regions of Ontario surrounding the Great Lakes do not often go beyond these temperatures, the majority of the province does. In order to expand the vineyard regions of Ontario, we need to establish grape varieties that could survive the harsh northern climate.

To protect their vines from being damaged by the cold, vintners will often take measures to create a warmer, milder microclimate within their vineyard. Heaters are sometimes used, though the cost associated with constant vineyard heating is quite extensive.[27] Alternatively, fans can be used to transfer higher up, warm air downwards over the vines. Another effective technique involves spraying a fine layer of mist over the vines so it will solidify and freeze.[27] The water is intended to transfer heat into the vines as it freezes, preventing them from experiencing the true external temperature.

FIGURE 5: VINYARD FAN Tall posts with rotating blades direct warm air down toward the vines[28].

Vines tend to be more cold hardy when they are a hybrid of *V. vinifera* and another species of grape. Usually *V. vinifera* is hybridized with *Vitis riparia* and *Vitis labrusca*.[23] Two such hybrids are Frontenac and Marquette, which together represent the majority of cold hardy grape varietals used in northern North American viticulture; they can survive temperatures as low as -30ºC.[29] These two hybrids were developed by the University of Minnesota, which devotes many resources to the development of cold hardy grape varietals. The Frontenac is a hybrid of Landot 4511 and *V. riparia* #89 varietals.[23] The

Marquette was created from hybridizing two hybrid grape vines; MN 1094 which is a complex hybrid of *V. riparia*, *V. vinifera*, and other *Vitis sp.* and Ravat.[20,23,26,27] The Marquette grapes, while tending to take longer to develop, produce higher sugar content juice than the Frontenac, which is considered desirable when producing wines, and could be an effective grape varietal for the harsher Ontario environment.[23]

To protect vines from being damaged during the cold winters that are common in most of Ontario, there are structural strategies that vintners can take advantage of. **Shoot vigour** is one aspect that can influence a vine's cold hardiness. Vigorous shoots are large and appear in high quantity. Vigorous shoots are more easily damaged by cold temperatures than low-vigour shoots and they contribute to a denser canopy that will provide undesirable shade for the rest of the plant.[25] To increase vine hardiness, one should decrease vigorous shoots by pruning them and by **deficit training** the vines so that excessive vigour does not occur.[25] It is important to prune vines in winter once the vines are already acclimated to avoid making the vines more susceptible to cold damage.

WINERIES BEYOND ONTARIO

There are many other wineries throughout the world which are expanding the ability to grow grapes and produce quality wine in places that were originally thought to be impossible grape growing areas. Familia Schroeder in Patagonia Argentina is a winery which lies in a challenging area for wine growth due to unpredictable climatic condition.[32] Located on a slope and in a valley, they have access to wide temperature ranges, glacial melt water, and low humidity, allowing them to overcome the typical difficulties associated with wine production in this region.

In Telemark Norway, there is a vineyard called Lerkekåsa Vineyard which is the world's northernmost vineyard.[33] Although low temperatures are an issue, this region receives a lot of sunlight and has mineral rich soils and is supplied by glacial meltwaters. By situating the vineyard in a valley, it is protected from most harsh winds and temperatures.[34]

There are many other wineries throughout the world which have overcome varying challenges. They inspire others to explore new regions in which to develop wineries and embrace new challenges which may arise.

CURRENT WINERIES PUSHING THE BOUNDARIES

While investigating the factors that influence the location of wineries and where we might be able to push the boundaries of wineries in Ontario, multiple wineries were discovered which have begun to test the extent of Canadian winemaking. Two of these wineries were KIN Vineyards in the Ottawa Valley and Potter Settlement Winery in Tweed (Figure 5).

FIGURE 6: WINERIES BEYOND THE NIAGARA REGION A map showing the locations of Potter Settlement, and the KIN Vineyard in Ontario and the Niagara Region where most winemaking is conducted in Ontario[32].

Potter Settlement is a winery in Tweed, located just on the edge of the Canadian Shield, in the Dfb climate region.[30] This unique location provides the vineyard with an immense amount of minerals in the soil, which the vintner believes produces a unique **terroir**. The major impediment to viticulture in this area is the cold temperatures. Over the winter, many vines die if not protected from extreme cold. To solve this issue, vines are buried under layers of soil, until spring, when they are "**dehilled**".[30] This keeps them warm over the winter, but has a high potential to damage the vines when dehilling. Additionally, Potter Settlement uses unique grape varietals to withstand the cold temperatures of the area. The main varietal is the Marquette: a French-Canadian grape, intended for cold

> "If I were to start this winery from scratch, I would win the lottery first!"
>
> — *Brian Hamilton, Owner of KIN Vineyards*

Quebec winters.[30] Two others are the Frontenac and Itasca. Overall, this winery in Tweed is moving forward in huge steps towards having an award-winning winery in a more extreme environment than would be expected.

KIN Vineyards is located in the Ottawa Valley, also in the Dfb climate region.[31] Most issues that they face are similar to those of Potter Settlement. The cold weather is also prevalent in this location, and bury their grapes over the winter. The winery is located on a fault, the Hazeldean Fault, which produces very interesting and variable soils. They have three distinct blocks of soil which each contribute different flavours in wines. These include the sandy loam soil nearest to the surface, loose limestone in the middle, and loam and clay near the river at the bottom. The presence of limestones allows for quality production of chardonnay and pinot noir.[30] The biggest issue that they have found is the expense of maintaining the vines. Growing extreme cold grape varietals and hiring workers to bury them for the winter is very expensive.[31] Creating a winery from nothing great capital outlay and is not always entirely rewarding. However the challenges in the Ottawa Valley are all possible to overcome with the right amount of time and effort.

GLOSSARY

Term	Definition
Cold Hardiness	the ability of grapes to tolerate cold weather
Veraison	the start of the berry ripening process
Acclimate	the process triggered by low temperatures through which vines prepare for winter by decreasing the water content of the plant cells
Deacclimate	the reversal of acclimation that occurs once the weather becomes warm again
Shoot Vigour	the quantity and speed with which new shoots grow
Deficit Training	strategically depriving vines of a growth requirement, typically water, nutrients, or sunlight, in order to create a particular quality in the resultant wine
Microrelief	slight irregularities in the slopes and elevation in land
Terroir	a combination of all the environmental factors associated with growing grapes which then contribute to the unique flavour of a resulting wine.
Dehilled	in the spring, grape vines which have been buried for winter protection must be dug out of the ground that they may begin growth again. This is a tedious and expensive process.

CONCLUSION

Exploring areas for wine production outside of the typical Niagara Region in southern Ontario is beneficial for many reasons. Many vintners look for challenges which would produce new flavours in their wines and provide them with novel marketing opportunities. Moving winemaking further north will allow for new forms of terroir to be expressed in wine and will make use of land that has not yet been used in the wine industry. By exploring the properties that grapes require to grow, specific areas where grapes can survive can be identified. While cold temperatures will continue to be a challenge to grape growing in Ontario, soil quality does not extensively limit the expansion of the wine industry in Ontario. By using grape varietals that can withstand more extreme temperatures and learning from vineyards already expanding in Ontario, the wine industry will be able to expand outside of its traditional regions. Additionally, novel viticulture practices, such as burying grape vines, allow for some of these challenges to be overcome, extending the area of potential growth even further. As the climate changes, many vintners will be on the hunt for new areas to host their wineries and Ontario is proving to be one of those. Overcoming the challenges of growing wine in Ontario remains an exciting element that will shape the wine industry in Canada.

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Breaking the Ice(wine):

The challenges associated with global Icewine production

Luke Buckler, Maha Dogar, Emily Heming, Angela Pollinzi & Megan Tu

Icewine is a unique, high-quality delicacy that has been around for 200 years. Over the past few decades, Canada has become the world's top producer of Icewine due to its optimal climate. The prestige of Icewine is directly associated with the challenges posed by the fermentation process. As the grapes are frozen naturally on the vine, the stress that is induced on the yeast presents various issues. Vintners Quality Alliance has set regulations that must be met within Canada in order for the wine produced to be considered Icewine, and the high sugar content is the root cause of the majority of the challenges. These difficulties have led to a great amount of research into technologies that can be implemented internationally in order to improve the production of Icewine.

INTRODUCTION

Icewine (or Eiswein in German) is thought to have originated from Germany in the late 1700s, following the sudden onset of freezing weather that came before the grapes were harvested.[1] The creation of Eiswein was serendipitous; the wine-maker decided to harvest and press the frozen grapes on the vines to not waste the vintage and ended up creating a unique, sweet wine. Given the positive reception, Germany and Austria continued to produce Eiswein, but the central European conditions were not ideal for its production.[1] German immigrants brought the tradition with them to Canada in the 1970s and continued Icewine production.[1] The climatic conditions present in Canada, with the warm summers allowing for the grapes to ripen and the cold winters allowing for natural grape freezing, have led the country to becoming the largest and highest quality producer of Icewine worldwide.[1,2] Currently, Ontario is Canada's largest exporter of Icewine with over $19.4 million exports compared to second-most of $1.8 million exported by British Columbia.[2]

What is Icewine? - A dessert wine made from grapes that are naturally frozen on the vine and pressed while frozen to create a sweet and flavourful juice, which is then fermented in order to create a unique delicacy.[1]

VQA - Vintners Quality Alliance was established in Ontario and British Columbia in order to regulate wine production and protect the integrity and prestige of Icewine in Canada[2].

Must - The liquid product obtained by crushing frozen grapes that has an actual alcoholic strength by volume of not more than 0.5 per cent at a temperature of 20°C.[3]

Brix (°Bx) - The quantity of dissolved solids expressed as grams of sucrose in 100 g of grape juice or grape must at 20°C.[3]

The conditions required for the production of Icewine make this style of wine unique and prestigious. This has led the Vintners Quality Alliance (VQA) to establish regulations in 1999 for the production of Icewine in Canada in order to protect the integrity and authenticity of Canadian Icewine.[2] In this article, we will discuss the production and regulations of Icewine in Canada, delve deeper into the challenges associated with fermentation and consider current technologies that have been implemented internationally to improve the production of Icewine.

PRODUCTION & REGULATIONS

In order for an icewine in Canada to be labelled as 'Icewine', it must meet the standards set by the VQA. These are legislated, regulated, monitored, and enforced from the early stages of grape growth in the vineyard all the way to the bottling of the wine.[1,2] These regulations have made the production of Icewine fairly standard across all wineries in Ontario and British Columbia.[1]

Calling it 'Icewine', 'icewine', or 'ice wine' depends on the standards met or the country it is produced in. For simplicity, it will be referred to as 'Icewine' for the remainder of the article, as this is the standard in Canada.

To begin, the grapes intended for Icewine must be made from *Vitis vinifera* grape varieties, or the hybrid variety Vidal Blanc, in order to meet VQA standards.[1] Leading up to the harvests, viticulturists employ bird netting to prevent birds from eating the grapes.[1] During the harvest, the grapes must be naturally frozen on the vine and picked at a sustained temperature of -8°C or lower, and then immediately pressed.[3,4] This allows for grape dehydration; the frozen water remains with the grape skins when pressed, and a juice with a very high sugar concentration is collected.[1] Due to the differences in climate conditions between the regions producing Icewine, the harvest time is variable and changes from year to year, typically ranging from early November to late March.[4] Traditionally, hand harvesting is completed by opening the bottom of the bird netting, allowing for the grapes to fall into harvest bins.[4] For VQA regulations, pressing has to be carried out immediately after harvest and must be a continuous process at a lower temperature.[4] This is to ensure that the minimum 32°Bx is being met before transferring the juices to a fermentation vessel.[3] After the juice is pressed, it can then be put into a centrifuge in order to speed up the process of settling and clarification.[4] Once the juice has settled adequately, it is then inoculated at 15-17°C with yeast for fermentation.[4] Due to the high sugar content, the yeast is put under osmotic stress that can create many challenges when producing wine, which will be discussed later. Filtration is then conducted, followed by early bottling in order to preserve varietal intensities.[4] The final product must not have an alcohol level of less than 7% or greater than 14.9%. Additionally, during any part of the process, no frozen concentration of juice, must, or wine can be added.[3] The finished Icewine should be produced from must that has an average no lower than 35°Bx. The residual sugar in the bottling stage must be from the natural sugar of the grapes and cannot be lower than 100 g/L. Additionally, the ethanol present can only be as a result of the fermentation of the natural sugars.[3] This process is summarized in Figure 1 below.

YEAST METABOLISM

Wine enthusiasts have come to know that Icewine is considered to be a delicate and prestigious product. The cost of this delicacy is directly associated with the challenges associated with its fermentation.[5] The organism responsible for turning the grape juice into wine is yeast.

Yeasts are microorganisms that convert sugars to ethanol during the metabolic process of fermentation[5] In the case of wine production, the grapes are harvested, crushed and their musts are subsequently exposed to these yeasts. The yeasts then begin fermentation, metabolizing the sugar for growth and creating alcohol in the process.[5] However, Icewine is very different from table wine and this alters their metabolic processes in a number of ways.[6] As previously mentioned, in order to cultivate grapes for Icewine, there are very specific growing and harvesting conditions[3] The cold conditions cause the water in the grapes to freeze, but allow for the juices to remain liquid. This creates an extreme environment for yeasts in which the musts have extremely high sugar concentrations.[3] In the same way that we bundle up when we go out in the winter, yeasts are required to call upon different strategies in order to survive high sugar concentrations.

The high sugar content results in hyperosmotic stress; a condition that causes yeast cells to shrink, induces water loss, and may even result in death.[6] However, as with any other organism, yeasts will employ different mechanisms to counteract this stress, mainly through adjustment of resource allocation during metabolism.[5] This alteration of the traditional metabolic pathway has implications in Icewine production, creating roadblocks to meeting VQA standards by stunting ethanol production, prolonging the duration of fermentation, and elevating volatile acidity (VA) concentrations.[2]

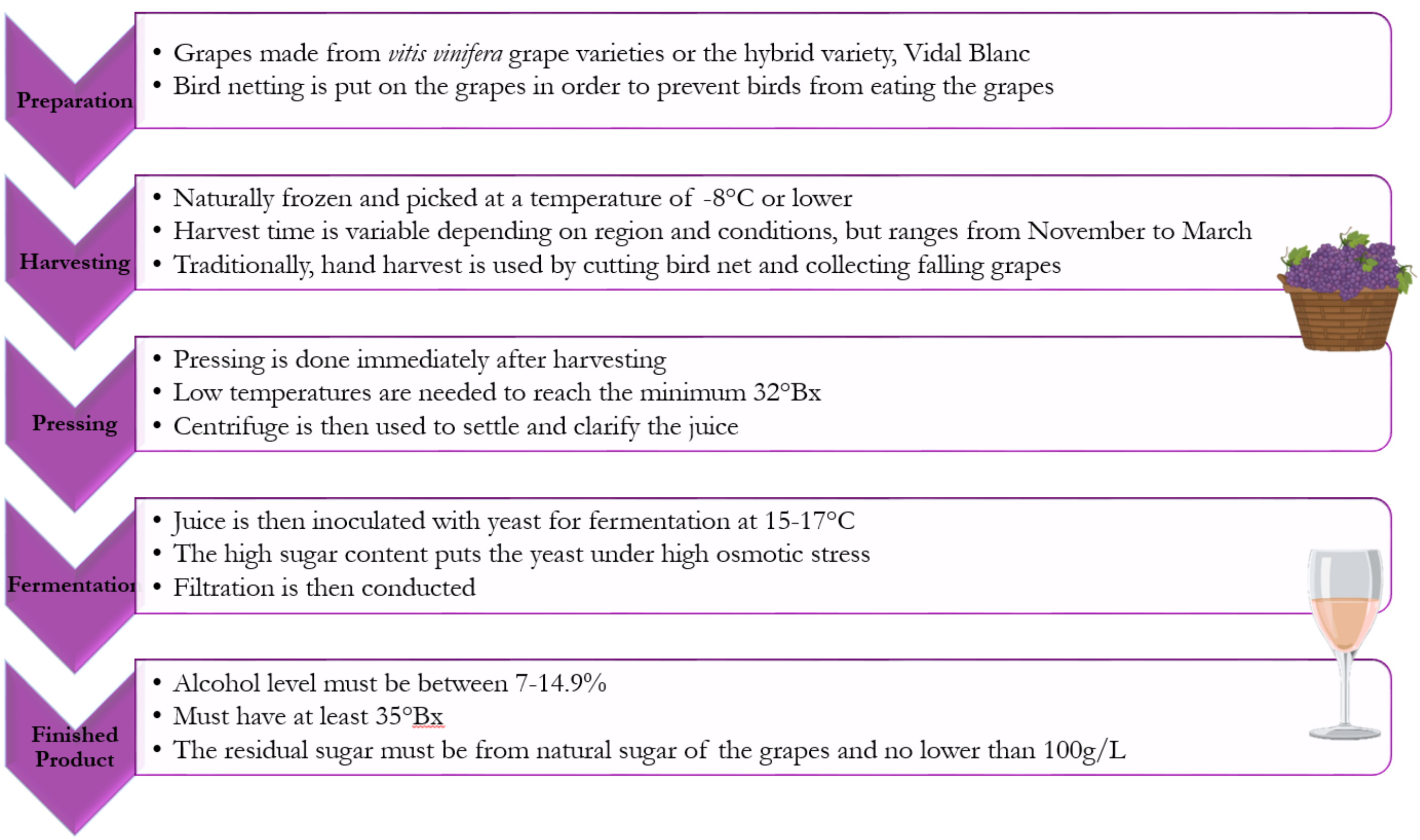

FIGURE 1: An infographic summarizing the process required in order to produce VQA regulated Icewine in Canada (created with BioRender.com).

Volatile Acidity (VA) is the concentration of steam distillable acids present in a wine. Acetic acid is typically the top contributor[3].

High levels of acetic acid are undesirable in all wine as the compound induces spoilage. In Canada, the highest concentration of acetic acid that is permissible in Icewine under VQA standards is 2.1 g/L.7 Moreover, acetic acid is a precursor to ethyl acetate, another undesirable compound due to its nail polish like aroma.[6]

Using the model yeast *Saccharomyces cerevisiae*, one can see that at the cellular level, the High Osmolarity Glycerol (HOG) pathway is activated, helping combat the adverse effects of high stress during grape must fermentation (Figure 2).[8] While the HOG pathway responds to stress in a number of ways, for the purpose of this article we will be focussing on the production of glycerol. For those wine enthusiasts, glycerol is the compound responsible for the formation of tearing on your wine glass as your twirl your drink. At a cellular level, glycerol is produced in order to balance osmotic pressure and maintain cell wall integrity caused by high sugar concentrations. This is a redox reaction in which NADH is oxidized to NAD+.[5] The NAD(H) cofactor system can be explained analogously, in which the hydrogen atom that is responsible for the oxidation and reduction of compounds, can be thought of as a bank loan. When NADH donates a hydrogen to a compound, this can be thought of as a bank loaning an individual money. The bank becomes NAD+, and once the individual pays back the loan, or another compound donates a hydrogen, the bank becomes NADH once again.[6] This redox reaction continues and aids the cell in returning to homeostasis, but at a price. Glycerol is continually produced, which in turn results in high NAD+ concentrations. However, cells need both NADH and NAD+, and most importantly, yeasts need NADH to produce glycerol. Thus, in order to continue to combat hyperosmotic stress, there is a diversion in metabolism- instead of ethanol production, the focus becomes acetic acid production.[9] Although this allows for regeneration of NADH, and consequently glycerol, this also increases VA concentrations in wine, and reduces Icewine quality, as shown in Figure 2.[5]

Acetic acid is formed as a result of a yeast's lack of transhydrogenase to convert between the two redox systems NAD(H) and NADP(H).[6]

In order to form acetyl-CoA, there is a need for acetyl-CoA synthetases, and these enzymes are encoded by the downregualation of ACS genes.[6] A study conducted by Heit et al. (2018), discovered downregulation of ACS1 and ACS2 genes by 19-fold and 11.2-fold in Icewine juices versus diluted juices. [6]

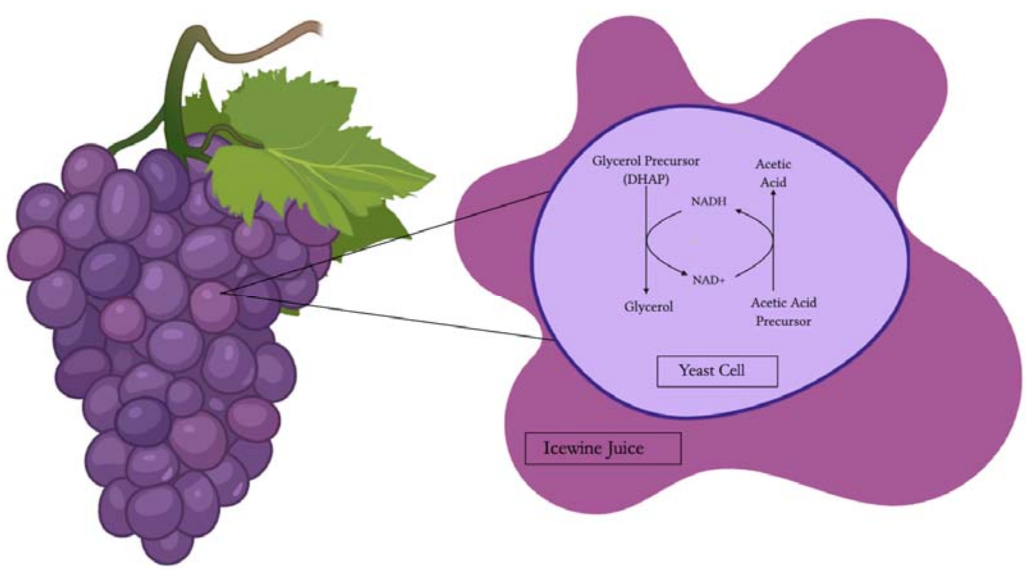

FIGURE 2: A simple diagram showcasing the metabolic processes involved in Icewine fermentation, specifically the HOG pathway. To off-set the hyperosmolarity, glycerol is produced from dihydroxyacetone phosphate (DHAP), and this reaction is oxidized by NAD(H) coupling system. NADH is converted into NAD^+, resulting in NAD^+ pools. To combat this, acetic acid is produced, oxidizing NAD^+ to NADH. The precursor for acetic acid is not specified, as acetic acid is produced by multiple pathways. [10]

High VA content is not just as a result of the HOG pathway.[6] The pyruvate dehydrogenase (PDH) pathway is also responsible for the production of acetic acid. A study conducted by Heit et al. (2018) suggests that acetic acid is consumed less in Icewine production than that of table wine production, resulting in the elevated levels at the end of fermentation.[6]

High sugar content causes different expression of yeast cytosolic aldehyde dehydrogenases (ALD3 and ALD6) and glycerol-3-phosphate dehydrogenase (GPD1).

Enzyme expression is also different in Icewine fermentation than table wine fermentation as a result of hyperosmotic stress.[11] The differing expression patterns can be correlated to metabolites in Icewine, such as acetic acid, acetaldehyde, and glycerol. In one study completed by Pigeau and Inglis (2005), they fermented Icewine juice, diluted Icewine juice, and diluted sugar juice with sugar levels equal to Icewine juice in triplicate and assessed the production of the three metabolites in question.[7] Acetic acid and glycerol production increased by 7.1-fold and 1.8-fold respectively in Icewine versus that of diluted juice. This coincided with the upregulation of certain enzymes and downregulation of others.[11]

In general, Icewine fermentation is not a black and white process due to the innate complexity of the grapes, yeast, and all other factors that go into production. Understanding these factors as both winemakers and buyers is important for transparency in the industry. The induction of the HOG pathway poses many challenges for winemakers, and this is where research and comprehension of the process is essential. Viticulturists and vitners dedicate their lives to investigating the prime conditions to reduce the hyperosmotic stress yeasts experience in order to optimize wine production. A consensus has established that Vidal blanc or Riesling wine grapes are the optimal choice due to their naturally thicker skin, which allows them to thrive in the cold harvesting conditions.[12] However, there are many other areas to be researched to examine, including yeast inoculation times, strains of yeast used, and varying sugar concentrations.[5,12,13]

To reiterate, the high sugar concentrations of the musts (35° to 42°Bx) poses challenges in reaching desired ethanol and VA levels.[13] The yeasts can only consume approximately half of the sugar concentration in the grape juice, producing a wine that is high in sugar content, but the high sugar content is balanced by the high volatile acidity. In one study, using S. cerevisiae and V idal Icewine juice, the effects of yeast inoculation rate, acclimatization, and nutrient addition were examined to see what conditions would result in the optimal ethanol content of 10 %. It was determined that lower yeast inoculation rates were insufficient, regardless of the method used to inoculate or if nutrients were added to assist the fermentation. On the other hand, the addition of nutrients during yeast rehydration did reduce fermentation time. The research also investigates the differences between stepwise versus direction inoculation, the latter producing more ethanol. These are some important questions wine buyers may want to consider the next time they visit a winery.[13]

Commercially, determining the highest concentration at which yeasts are still able to successfully ferment Icewine juices is extremely important.[5] This is because it provides a standard maximum Brix level at which wineries know fermentation will no longer be possible. This both improves the quality of the wine and ensures that industry standards are met. In a study performed by Pigeau et al. (2005), yeasts were exposed to different concentrations of Riesling Icewine juice, and their ability to ferment the must to the desired alcohol concentration was examined.[5] Researchers tested Brix levels ranging from 40°Bx to 46°Bx, allowing the yeasts to ferment at each concentration and testing the levels of acetic acid post-fermentation. They determined that above 42°Bx, yeasts cannot reach an alcohol level of 10 % v/v and beyond 52.5°Bx, the juices are unfermentable. This is a result of metabolic diversion to glycerol and acetic acid production rather than alcohol.[5] This research is pivotal in Icewine production as it gives a benchmark at which sugar concentrations pose an inhibitory effect on the ability to meet VQA standards and therefore, create a satisfactory beverage.

The complexity does not stop there. Wine drinking is a sensory experience, therefore wine buyers may want to know which grape and strain of yeast produced the best wine for them.[12] Unfortunately, research has shown that the odour active compounds in Icewine vary between yeast strains, cultivars, and vintages. In other words, it is not possible to produce the exact same bottle of wine from a different cultivar or different vintage again.[12]

CURRENT TECHNOLOGIES

The Icewine industry is highly regulated across the globe in order to maintain its authenticity and prestige. The strict production process of Icewine makes it very difficult to introduce new technologies that improve it. However, the challenges are still present and as such researchers continue to investigate ways to advance the production process. Currently, there are four major technologies that have been explored: interspecies hybridization, CRISPR-Cas9, high throughput sequencing, and chromatography-olfactometry.

INTERSPECIES HYBRIDIZATION

In recent years, research has focused on the hybridization of different yeast strains and their superior ability to cope with high-sugar concentrations.[14] The response of yeast to hyperosmotic stress during fermentation of high-sugar substrates is a very complex area of research as it involves multiple genes. *Saccharomyces* interspecies hybrids can be formed through conjugation, also referred to as natural sexual hybridization. These offspring show advantageous characteristics in comparison to their parent strains, which have the ability to imporve the production process of Icewine.[15] However, there are challenges with interspecies hybridization as the hybrids are sterile. Research has thus focused on removing this post-zygotic barrier through mutations in cell replication. Mutations that occur in mitosis and meiosis can break down the sterility barrier allowing for the hybrid species to reproduce. However, these mutations cause future generations of offspring to become highly unstable and susceptible to losing additional chromosomes.[14] As a result, unpredictable proportions of the parental gene pools occur and the phenotypic characteristics become difficult to control. Further research has focused on stabilizing the hybrid strains and exploring the solution of mating the hybrid strain with an additional species.[14] These three-species strains are more stable and have shown transgressive phenotypic traits; however, their response under high-sugar stress has not been researched. Overall, as a result of the hyperosmotic stress Icewine grapes experience, hybridizing two sepcies to make a novel strain that can better tolerate such stress would be massively beneficial to the Icewine production process.

> In Hungary, a hybrid of *S. cerevisiae* and *S. uvarum* displayed phenotypic characteristics for robust fermentation in high-sugar concentration as well as the production of wines with low volatile acidity, both ideal characteristics for wine producers and buyers.[14]

CRISPR-CAS9

Furthermore, additional research has been conducted in identifying genes of particular importance in Icewine fermentation using CRISPR-Cas9.[15] CRISPR-Cas9 is a unique technology designed to edit components of the genome by adding, deleting, or altering sections of the DNA sequence. Editing the genome of yeast strains has been highly effective in understanding the importance and mechanism by which specific genes cope with hyperosmotic stress. This method enables rapid strain engineering using time and cost-saving strategies.

> CRISPR-Cas9 was used to demonstrate that Stl1p, a H+/glycerol symporter, in S. cerevisiae plays a key role in coping with hyperosmotic stress.[15]

The idea of genetically manipulating yeasts is a hesitant concept for Icewine producers and buyers. Producers do not want to risk affecting the corresponding prestige and authenticity that comes with Icewine, whereas buyers do want to consume products associated with genetic manipulation. However, interspcies hybridization and CRISPR-cas9, can provide novel yeast strains that can contribute to a greater complexity of flavours and aromas in Icewine.

HIGH THROUGHPUT SEQUENCING

Another area of research that is being explored is the use of high-throughput sequencing in order to determine the composition of the microbiota involved in the spontaneous fermentation of Icewine.[16] High-throughput sequencing is a technique which involves a sample of must being taken during the fermentation process, followed by a comparison of the genetic makeup of all the organisms present. A profile of what types of organisms are contributing to the fermentation at the time of the sample extraction is then given. This is a very useful tool as it can help determine

> High throughput sequencing was used to analyze the diversity of yeasts and other microorganisms involved in the process of fermenting Icewine and has successfully been able to identify other types of fungus which appear to play a role in the fermentation process.[16]

what combinations of yeasts are effective in fermenting inoculated Icewines and improve the rate at which Icewine ferments.

CHROMATOGRAPHY-OLFACTORY

As mentioned earlier, wine-drinking is a sensory experience, and therefore a very popular area of research is the characterization of odour active compounds. This involves determining which compounds present in Icewine are contributing to the aroma of the beverage, an aspect highly valued among wine enthusiasts. The most common methodology used for this type of research is gas chromatography-olfactometry. Gas chromatography is a common technique in analytical chemistry that separates a mixture of compounds into its respective constituents, and olfactometry is a technique used to determine if a substance meets the odour detection threshold. By combining these two techniques, one can separate Icewine into its individual components, and then determine which are contributing to the resultant aroma.[17] This type of research is very valuable in the early stages of Icewine development as the results from these studies can be used for further research. For example, if there is a compound which contributes a large aromatic profile, then further research can be done on what can be done to change the fermentation process in order to alter the levels of this compound to produce a desired aroma for Icewine.

This technology was in fact used to accurately characterize the aroma of a Chinese Icewine and to mimic the aroma with a volatile solution of 44 different chemicals.[18]

CONCLUSION

All over the world, novel technologies are continually being explored and developed to improve the production of Icewine while simultaneously improving its quality. The strict, regulated production process of Icewine and its value on the market as a prestigious product makes it difficult to implement technological findings from current research being conducted. The reaction from wine enthusiasts towards the implementation of these technologies is unknown; however such research has the ability to advance the Icewine industry, resulting in an enhanced delicacy for the wine enthusiast and a higher profit for the winery.

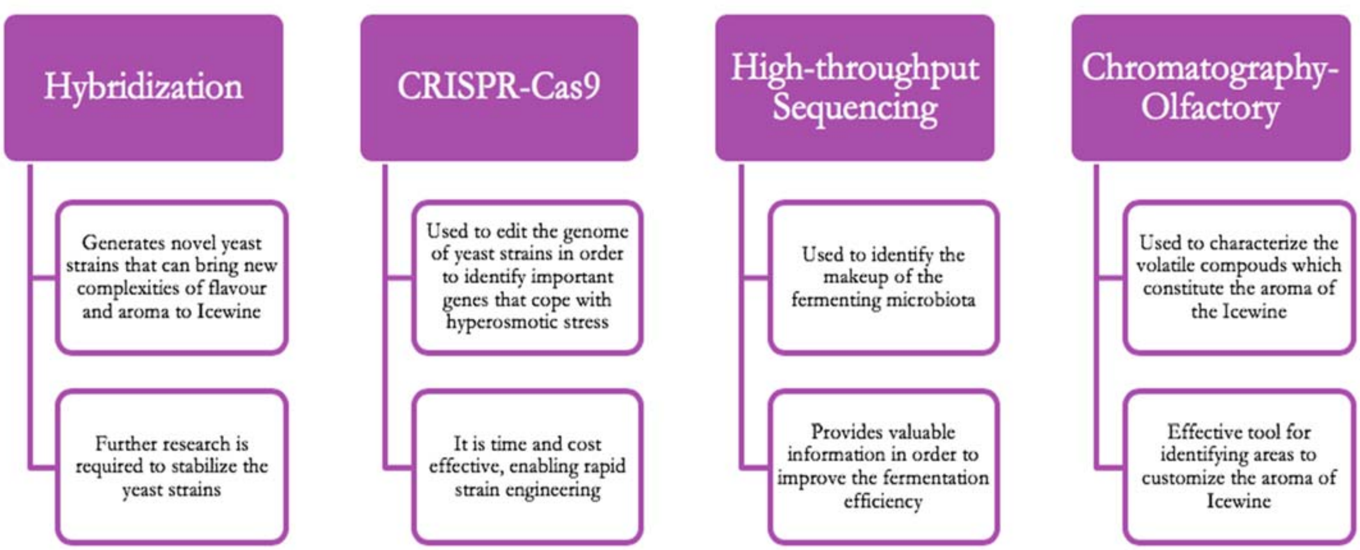

FIGURE 3: A summary of the four current technologies used around the world to enhance the Icewine production process.

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Oak Chips:

A Novel Technique in the Wine Aging Process

Chelsea Aristone, Diego Covarrubias, Lucas Eckert, & Amir Mortazavi

Wine aging by oak wood has long been utilized to adorn our wine experience. This technique prunes the sharp acidity and unpleasant bitterness out of young wine while enhancing the flavour complexity and colour stability. Thus, aging wines using oak has become an inseparable step in the wine making process.

This article tells the story of oak aging, beginning with the historic use of oak barrels and the benefits pertained to them. It moves on by discussing the limitations of barrels that might push winemakers to seek alternative aging techniques. The last section introduces aging wines with oak fragments and compares the two techniques.

A BRIEF HISTORY

Oak wood has been used in the wine aging process for millennia and continues to be used to this day. The most conventional method of using oak in the aging process is in the form of barrels, which are thought to have been introduced by the Celts in the third century B.C. when they became the main form of wine transportation[1,2]. The type of wood that is used for barrels is essential for its functionality in the wine aging process. Species of wood that are used to construct barrels must not contribute negatively perceived flavours to the wine[3]. Additionally, the wood itself should not have any defects (such as splits, fractures, knots, etc.) and be pliable when heated to shape the wood into a barrel[3]. Oak is a very resilient, flexible material that is also mostly impermeable to liquids. These characteristics are all influenced by the natural anatomy of the oak wood itself[4]. Due to these natural properties of oak, it became the most common type of wood used for barrel making in the 16th century, even before the benefits of wine aging with oak were well understood[3].

Types of Wood Historically Used in Wine Making:

Many different types of wood have been used in addition to American and French Oak.

In America:
White oak, red oak, chestnut oak, red or sweet gum, sugar maple, yellow or sweet birch, white ash, Douglas fir, beech, black cherry, sycamore, redwood, spruce, bald cypress, elm, and basswood[5].

In Europe:
Chestnut, fir, spruce, pine, larch, ash, mulberry, as well as species brought in from Australia, South America, and Africa[6].

BENFITS OF BARRELS

Oak barrels provide many benefits to the final wine product during the aging process. Overall, the use of oak barrels produces a wine that is more stable in colour, has a more complex aroma, and has higher clarity[7]. There are two main factors that produce these results: chemical diffusion and oxygen diffusion. The effects of the factors are summarized in Figure 1.

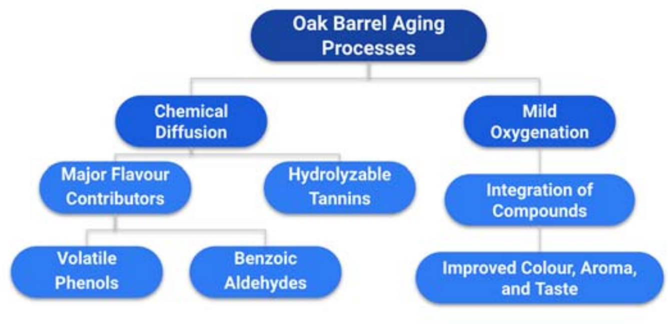

FIGURE 1: THE BENEFITS OF BARRELS: A summary of the effects that oak has in the wine aging process.

Many chemical changes happen during the wine aging process. These may be changes in compounds that are extracted from the wood or from reactions within the previously existing wine compounds[8]. For the purposes of this article, we will be focusing on the former which related directly to contributions of the oak. There is a wide variety of compounds that are extracted from the oak wood including the major contributors to flavour, volatile phenols and benzoic aldehydes[2,9]. Volatile phenols contribute to horsey, smoky, and spicy aromas in the wine[2]. The hydroxybenzoic aldehydes include syringaldehyde and vanillin. These two compounds promote a sweet aroma with syringaldehyde producing a balsamic-floral aroma and vanillin producing an aroma similar to vanilla[2]. Hydrolysable tannins from the oak are a group of compounds that give the wine astringency and stabilize pigments[2,10]. The combination of all these chemicals which diffuse into the wine from the oak improve the overall quality and characteristics of the wine.

A Different Wine Aging Technique - Clay Pots:

Wood barrels, despite their current popularity, are not the only vessels that have been traditionally used to age wine. In fact, the first known method of wine aging was accomplished in clay vessels, a technique used by Egyptians, Assyrians, Greeks, and Romans alike[11].

These clay vessels come in various shapes and sizes. and contributed reduced acidity, insulation, and oxygen diffusion to the wine[11]. They are still used at some wineries today.

Since the oak is permeable to gases, oxygen is able to slowly infuse into the barrel to interact with the wine[2]. This movement of oxygen occurs through the pores of the oak wood[12], allowing for mild oxygenation to take place. Mild oxygenation is known to improve the integration between the compounds that are released from the wine and those released by the wood[13]. It should be noted that while this oxygenation has positive effects of red wine, it can have negative effects on white wine causing the colour to turn brown and a loss of fruity aroma[14]. Overall in red wines, the mild oxygenation improves the colour, aroma, and taste of the final wine product for a higher quality product[2, 12, 15].

DRAWBACKS OF BARRELS

The barrel aging technique has been used for thousands of years and continues to be very popular among winemakers today. The persistence of this technique is due to the complexity and quality that oak lends to the wine as well as a strong sense of tradition amongst winemakers. However, the use of barrels is not without its limitations, most of which are associated with the high cost and limited lifespan of oak barrels.

As previously discussed, oak lends many benefits to the wine aging process, most significantly the addition of compounds such as phenols, lactones, and vanillin[2]. In addition, the porous quality of the wood allows for the diffusion of gases into and out of the barrel. However, both of these benefits decrease significantly with repeated use of the barrels. Each additional use diminishes the amount of chemical extracts present in the wood[16] and causes clogging of the wood pores, decreasing the rate of diffusion[17]. This issue is clearly shown in Figure 2, displaying the amount of extraction of phenols with repeated use of the barrel. Additionally, reused barrels may develop a buildup of unwanted microbes such as *Brettanomyces*, causing the production of undesirable flavours[18]. For these reasons, oak barrels are typically used around three times before they are disposed of or resold[19].

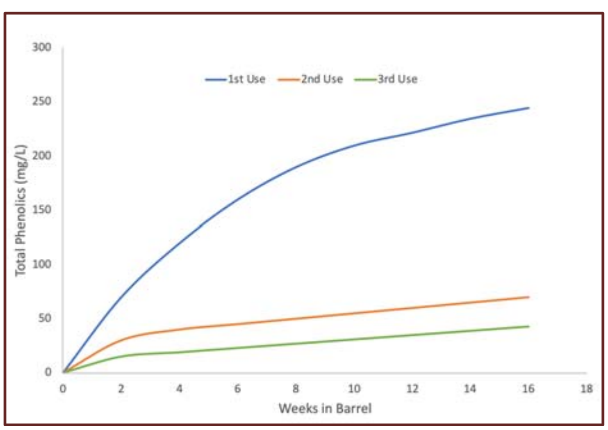

FIGURE 2: LIMITED LIFESPAN: The amount of phenols found in wine aged in the repeated use of a French oak barrel[20].

The other significant drawback of barrel aging is the high cost of this technique. The cost of a single barrel of French or American oak is anywhere from $800 - $4000 USD, depending on the quality of the wood[21]. In addition, there is usually a significant cost of shipping associated with the barrels. The high initial price presents a more significant issue when combined with the limited lifespan of oak barrels. The need to constantly replace old barrels accounts for an ongoing cost for a winery. The use of barrels also results in a significant loss of product by means of evaporation of wine through the wood[22]. As the wine evaporates out of the barrel it must be constantly replenished in order to avoid unwanted oxidation. It is estimated that up to 10% of the initial volume of wine will be lost to evaporation, accounting for a significant loss of product and income[22]. Finally, other labour costs are also associated with barrel aging such as maintenance, topping up the lost volume, and cleaning in between use. All in all, using barrels to age wine is an expensive venture, with wineries typically spending more on barrels than grapes, labour, or shipping[23].

NEW TECHNOLOGIES

In lieu of the high cost and limited lifespan of barrels, it comes as no surprise that some wineries have been shifting towards the use of other technologies in the wine aging process. The use of stainless-steel tanks for wine aging is growing in popularity due to the low relative cost, however these tanks may not provide all of the same flavour and complex qualities as barrel aging. Other more inventive techniques show promise, such as the use of irradiation to accelerate certain chemical processes[24]. One aging technique that has been adopted by many winemakers around the globe, and aims to provide the same quality and complexity as barrels while being faster and cheaper, is the use of oak chips.

Oak chips are broadly described as fragments of oak ranging anywhere from whole staves (wooden pieces used to construct barrels) to a fine powder[19]. These fragments are usually put into a "teabag" or infuser (Figure 3) before being added to the wine aging vessel, typically a stainless-steel vat, and are meant to provide the same flavour quality as that obtained from barrel. By using small fragments of wood, rather than a barrel, the effective surface area of the wood is increased, allowing for more rapid extraction of chemicals from the wood. Chips can be made from the same wood used to make barrels. In addition, the diffusion of gases can be controlled with micro oxygenation technologies, to mimic or even improve upon the diffusion associated with barrels[25].

FIGURE 3: OAK INFUSER: An oak chip infuser being used in an old barrel.

The use of oak chips in the aging process was not well accepted initially by the winemaking community. This technique was illegal in the United States until 1993 and illegal in the European Union until 2006[26], likely due to a strong sense of tradition amongst winemakers and a stigma of lesser quality associated with oak chips. Since its introduction, however, the use of oak chips has been slowly and steadily gaining popularity due to lower cost and shorter aging times.

BARRELS VS CHIPS

With the global legalization of oak chip usage not occurring until 2006, it is still a very novel technique in comparison to that of traditional barrel aging, meaning that research is still underway to fully determine the long-term differences between the two. Based on current understanding of both methodologies it is possible to compare their influence on the quality of the wine produced, as well as costs and time differences, in order to evaluate their effectiveness in the ageing process.

Due to oak's phenolic characteristics, as well as its permeability to oxygen, it is the optimal material for wine storage during fermentation[27]. The first obvious difference between barrels and chips is the size and surface area of wood that is exposed to the liquid. Chips vary considerably in size; the smallest particles with no discernible shape are generally referred to as shavings. Oak pieces longer than 10 cm are called staves, and anything in between is usually referred to as an oak chip; these are the most commonly used fragment size in the wine industry[28]. When put in contact with wine for an extended period of time, extractable compounds from the wood seep into the liquid, providing the wine with its depth and complexity of flavour[29]. Furthermore, the porous nature of oak, as well as the spacing between the barrel's staves, allows oxygen gas to diffuse through and react with phenolic compounds in oxidation reactions, which in essence are the main factors that allow wine to improve, in terms of its phenolics and aromatics over time[29]. While there is no difference in the types of compounds that oak barrels or chips contain, since they are from the same wood genus, the rate at which these are released into the wine varies significantly. Due to a much larger surface area to weight ratio of the oak chips, these are able to extract their compounds much faster than barrels can, which decreases the amount of time that the wood needs to be in contact with the wine[30]. A specific example of this is the benzoic aldehyde vanillin, which contributes to the distinct vanilla flavour of wine. This compound achieved its maximum concentration in the wine after only one month using oak chips, yet achieved the same concentration after a year of barrel aging[28].

Colour is one of the characteristics of wine that is judged by tasting experts, since it serves as a first impression of the wine[29]. This characteristic can be significantly influenced by the usage of oak chips and barrels. Colour intensity (CI) of wine is a measure of its absorbance of red, violet, and green wavelengths of visible light, and this is produced by the micro oxygenation of the wine provided by oak wood[28]. In both the usage of chips and barrels, colour intensity increases rapidly after sealing, in approximately two months[30]. However, the CI values of wine aged in barrels, and particularly new barrels, are always greater than that of wine aged with chips, leading to a deeper, darker, and more desirable colour[29]. As with the release of phenolic compounds, colour complexity evolves faster in oak chip-treated wines compared to barreled ones[31].

When it comes to the development of the sensory profile of a wine, usually categorized by a combination of aromatics, feel, and taste, there is a notable difference in quality and timing between chips and barrels. It is important to note that determining sensory quality is a highly subjective affair, as personal preferences are always prevalent when tasting wines. As previously mentioned, the uptake of phenolic compounds is much faster in chip-treated wines than barreled ones, and because of this, the resultant sensory profiles are of higher quality after two months of aging for those with oak chips[29]. Specifically, the increased absolution of anthocyanins and tannins after a short amount of time results in a higher quality wine[31]. However, it has been found that these aromatic compounds are reduced after the contact time with oak chips surpasses three months[27]. While the wines aged in barrels experience a slower dissemination of phenolic compounds, the resulting wine has a deeper and more complex flavour profile than those produced with oak chips, which develop astringency factors, resulting in a bitter taste that is generally not desired[31].

Furthermore, oak chip aging allows for customizability of oxygen-derived reactions in the aging process. Unlike barrel aging, wines that are being aged using oak fragments can be stored in stainless steel tanks with adjustable oxygen inputs. Hence, the oxygen diffusion into the wine can be controlled to suit the specific wine making technique or grape type[35]. Additionally, utilizing this method eliminates the wine lost due to evaporation through the barrel staves[22].

TABLE 1: SUMMARY OF COMPARISON: Benefits and drawbacks of using barrels and oak chips in wine aging.

	Pros	Cons
Barrels	Significant phenolic extraction after long periods of aging Oxygenation through inter-stave space Deeper development of colour intensity after long periods of aging	High cost Limited lifetime of use Low customizability to the wine Longer aging times
Chips	Cheaper than barrels Higher surface area to weight ratio Faster extraction of compounds Faster development of colour intensity Allows for higher customizability of wine, especially in steel vats	Weaker flavour profile after long periods of aging Chips become fully saturated with wine after time; phenolic release stops

Lastly, it should be noted that the usage of oak chips is much cheaper than barrels, since the purchase of both American and French oak barrels turns out to be the second most expensive step in the wine-making process[23]. In comparison (see Table 1), oak chips are relatively inexpensive, and can even be bought as by-products of other manufacturing processes. In general, it is clear to see that using oak chips in wine aging results in a cheaper alternative which produces a higher quality wine after a short amount of time relative to normal fermentation times[27].

CONCLUSION

As discussed, oak chip aging, appears to contribute to wine complexity, stability and perception in comparable levels to barrel aging. Studies have repeatedly reported that barrel aging alternatives using oak fragments contribute the same desirable compounds to the wine. However, the extraction of these chemicals occurs at different rates and amounts across the two techniques[30, 31]. The high surface area to weight ratio of the fragments allows for a higher extraction rate of the compounds, as the entire surface area of each chip is immersed in the wine, providing 60% more surface contact than that of barrels alone[32]. Consequently, in regards to the aging time, the organoleptic factors of the wine grow very similarly when using barrels versus oak-fragments in the first months, yet diverge after a certain period[30]. Regarding oak usage, as presented previously, barrel aging is known to be a very costly technique. In contrast, oak-chips require a smaller amount of oak due to their high surface area to volume ratio, which allows for a cost reduction up to 10 times compared to barrel aging[33]. Moreover, wines aged using oak chips reveal no significant differences in quality when aged in old versus new barrels, which improves the reusability of barrels[34]. Therefore, the higher rates of compound uptake by the wine containing oak fragments have an economical significance as they reduce the aging time and the quantity of oak used[33].

As previously mentioned, oak chips offer adjustability of the oxygen exposure in the aging process. This will allow the wine maker to experiment with different oxygen diffusion rates to achieve their desired oxidative properties in the wine. This customisability is also beneficial for more modern wineries who often deviate from the conventional methods to discover novelties in the wine experience while keeping the costs to the minimum.

Despite the numerous benefits of aging with oak pieces, there are limitations attributed to this method. As discussed, this method is most suitable for short-term aging. An ideal oak-chip aging period of 2 to 3 months for adequate colour stability, compound diffusion and other aging-related processes has been proposed [30,31,36]. However, these favourable characteristics do not meet the quality standards achieved by barrel-aged wines in longer periods of time. Thus, wine aging by oak fragments contributes less to the complexity and stability of wine compared to barrels[30,31].

In summary, alternatives to barrel aging, in particular the use of oak chips and oak fragments, are recently developed techniques that are undergoing a refinement period as more research is conducted in this field. Aging wine with oak chips provides winemakers with the opportunity to produce inexpensive, mid-range wine that still carries organoleptic and phenolic properties. This is while barrel aging remains the prevalent method to produce a high-quality wine.

MORE TO EXPLORE

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Game of Drones:

Drones and Artificial Intelligence in Viticulture

Geetha Jeyapragasan, Frances Lorenz, Brittany Mascarenhas and Peipei Wang

Viticulture practices have been developed for centuries, requiring extensive human labour and skill throughout the wine making process. Crop management is an especially difficult task for winemakers, as the practices currently in place to manage and protect crop yields are quite tedious and difficult to execute. Some threats are even impossible to detect with the human eye. In just the past 20 years, technological advancements have developed at such a rapid pace, allowing us to pose the question: is there a way we can we use these advancements to make the lives of winemakers easier?

Within the agricultural sector, several technologies have been implemented to help farmers with their crop yields. Agricultural drones allow for a top down view of the entire crop, and when equipped with the right technology, they can gather large amounts of information through digital images. However, sometimes the sheer volume of information and the time necessary to analyze it manually is far too unrealistic. The incorporation of artificial intelligence (AI) in conjunction with drone technology can tremendously benefit winemakers, using computers to analyze the information gathered, and tell farmers exactly what issues their crop yields are facing. Does the future of wine-making lie in technology? Let's explore!

NO MATTER HOW GRAPE YOUR PROBLEMS, WE'VE GOT SOLUTIONS

From wine-carrying vessels dating back to 3500 BC, to Romans traipsing through Europe with vine cuttings on their backs, viticulture is a branch of horticulture strongly rooted in history.[1] With its evolution spanning six millennia, viticulture involves the cultivation, preservation, and harvesting of grapes for the purpose of winemaking.[12]

Methods of vineyard management are meticulous and mostly universal, resulting from centuries of development.[3] Due to the complexity of this process, a variety of methods are enlisted in order to produce the highest quality wines. These methods encompass both vine and grape health, and necessitate the allocation of specific treatments and strategies for both variables.[4] These include pruning treatments, specific irrigation methods, and canopy monitoring in regard to vine management, and measurement of crop load and fruit sampling for grape assessment.[45] Pest reduction and disease detection are also necessary as slight perturbations have the ability to destroy whole vineyards.[6]

Current viticulture practices are largely based on human labour.[4] For instance, in vine management, shoot and grape cluster (Figure 1) thinning is performed manually, with traditional modes of leaf removal requiring around 60 hours of labour per hectare of vineyard.[5] With an average of 46 acres per vineyard in Ontario, winemakers invest a significant amount of time and resources removing excess shoots for preventative measures against pest and disease.[5]

Thinning Crops:

Shoots: Thinning is the removal of excess non-fruitful shoots from the vines[5]. This is done to improve the air circulation, increase sunlight exposure, and reduces the opportunity for pest and disease infestation[5].

Grapes: To maximize quality and quantity of grapes, farmers manage the fruit to vegetation ratio based on a vine's capacity to ripen fruit. This is most common in areas that are water stressed, nutrient limited, or have a pest or disease infestation[5].

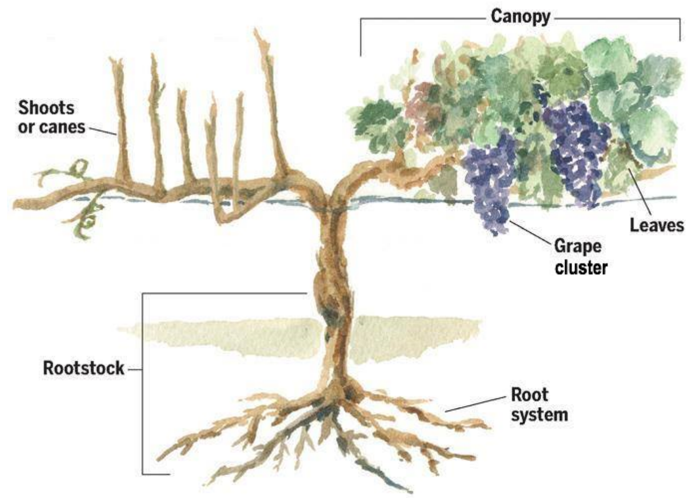

FIGURE 1: STRUCTURE OF GRAPEVINE Structure of a typical grapevine plant below and above ground. Winemakers must ensure the root system is resistant to disease and pests, while harvesting desirable grape cultivars[4].

In assessing fruit composition, grapes are sampled by using the individual berry or the whole cluster. Berry sampling involves the extraction of approximately 100 grapes per acre, with removal from different areas of the cluster.[4] Whole cluster sampling has a higher efficacy in assessing ripeness than the former, but requires the removal of 20 bunches per harvest block, which reduces yield.[4] These forms of sampling can be done systematically or randomly.[4] Reducing vulnerability to pests and disease is accomplished through visual inspection, physical control processes (e.g. mass trapping pests), careful consideration of rootstock (Figure 1) selection, and direct application of pesticides.[7]

Overall, advancements in viticulture science have allowed for the birth of a variety of techniques, aiming to increase production and efficiency.[1] Despite this, traditional methods of vineyard management may be invasive, and require high labour inputs, and time-consuming efforts. Overall, this can result in reduced accuracy.[8] The variability in vineyard management is often treated uniformly, which reduces the efficiency of the employed strategies.[6] Additionally, attention to distribution of human resources is largely overlooked in this sector, as there is an increasing demand for skilled viticulture technicians and a lack of adequate training opportunities.[9] In lieu of these limitations, a unique niche is formed involving the usage of remote sensing through unmanned aerial vehicles (UAVs) and Artificial Intelligence (AI).[8]

UAVs, also called drones, are platforms that can be controlled remotely or can work autonomously through flight control sensors.[10] Different types of sensors can be attached to the platform, allowing for a multitude of management operations to occur.[10] UAVs equipped with multispectral cameras are often used to gather information based on various colour indices.[8] These methods allow for an overview of grapevine characteristics such as shape, size, and vigour, measuring the variability present in the vineyard.[8]

In general terms, AI is the use of computers to simulate human intelligence processes. These systems are able to perform various tasks such as learning, problem solving, categorization, and predictions in very short periods of time.[11] This allows the large amounts of data collected by UAVs to be analyzed quickly, assessing an entire crop for potential or current threats that will affect the yield quality or magnitude.[12] To understand the general processes of AI, we can think of the development of an AI system as similar to students studying for a test. First, the AI begins with a training set to learn how to identify patterns and work within specified parameters, similar to how students learn. Next, a validation dataset fine tunes the model, and finally, a test determines the accuracy of the model.[11]

The use of UAVs and AI may provide solutions to the limitations present in viticulture by improving wine quality and the efficiency by which it is produced, while lowering impacts on the environment.[10] These technologies allow for high resolution spatial visualization of vineyards and impart management improvements related to quality, production methods, and sustainability.[10] Through implementation of these targeted strategies, resources ranging from time input to labour intensity can all be economized.[3]

With the increasing applications of these new technologies, one must consider the impact of drones and AI on viticulture, specifically in regard to their effects on general crop management and their overall feasibility for implementation.

To consider all of this, let's explore the role of UAVs and AI in viticulture, starting with vines and ending with grapes.

TIME FOR SOME VINES

Detecting Disease

Vine health is absolutely crucial to the success of a vineyard, laying the foundation for grape growth and wine production year after year. Thus, it is unsurprising that disease detection and vine management are big areas of focus in viticulture. Luckily, new AI and UAV technologies are emerging to make these processes a lot easier! Disease detection in this article will refer to the detection of diseases that infect grapevines, which can originate from bacteria, fungi, viruses, or other microorganisms.[13] Although invisible to the naked eye, these pests physically affect the plant in unique ways and can be detected with different types of AI models. UAVs are useful in this scenario as they can precisely assess the health of individual vines on a large scale and can even be tailored to selectively treat diseased areas of the vineyard.[14]

Recent research has shown that deep learning models are particularly effective for plant disease detection.[15] Deep learning is a subset of machine learning under the broad scope of AI; it involves networks that can undergo unsupervised learning and focuses on applications with repeating patterns in different locations of the modelling space, perfect for image recognition.[16] So how does it work? One study trained convolutional neural networks (CNNs), a type of deep learning model, with 87,848 images containing 25 different plant species, including grapes. These networks dramatically outperformed conventional methods of disease detection, such as in the identifying plants infected with grapevine yellows.[15] The training required only 5.5 days, and once trained, classifying each image required about 2 milliseconds on average with an accuracy of 99.53%. However, it must be noted some images could not be identified by the CNNs. Misclassification was either due to partial shading on the leaves or images containing too many non-leaf objects.[15] Thus, to overcome both of these issues, effort should be put towards developing an open-source database that wine producers can contribute leaf images to in compliance with formatting guidelines.[14] This will create a tailored set of images to train models for vine leaf or grape recognition. Ultimately, the goal of this would be to implement this model alongside UAVs to achieve continuous real-time vine monitoring and dynamic disease management.

Water we waiting for?

We've discussed how AI and UAVs can effectively detect disease, but what about general vine management? A vital practice for maintaining healthy vines is precise irrigation through monitoring vineyard water levels.[17] Water levels can vary significantly throughout a vineyard, making classical methods to estimate water stress almost impossible due to their labour and time intensive nature.[18] An alternative lies in the use of UAV thermal infrared remote sensing, which is cost and time effective for detecting and monitoring water stress.[17] Remote sensing involves reflectance spectroscopy, which is an optical method that measures the reflection of incoming electromagnetic radiation at varying wavelengths.[10] The correlation of leaf stomatal position and the surface temperature of the leaves is focused upon for water stress assessment,[18] and can be quantified using thermal sensors equipped to UAVs.[10] This strategy, coupled with machine learning algorithms, can provide highly accurate assessments of vine water status and help manage irrigation systems.[18]

HOW GRAPE IS OUR YIELD?

The roles of AI and UAV in managing vine health have been discussed, however even the healthiest vines are useless if they fail to yield high quality grapes. AI and UAV technologies promise to reduce the headache involved with grape management by efficiently assessing vine vigour, estimating grape yield, and identifying grape morphology.[19]

Vine Vigour:

Growth rate of the vine. This can be affected by numerous factors including water and nutrient availability, climate, and soil composition.[20]

Assessing grape quality is highly time-consuming as it can vary greatly over large land areas.[19] Luckily, UAV technology is effective for surveying entire vineyards to assess and map grape characteristics. This brings up the question, which characteristics are most effective for assessing grape quality? In particular, vine vigour is considered a strong indicator of grape quality and yield and can be assessed by UAV and AI technology with high accuracy.[20]

Multispectral Imaging:

An image is produced via sensors which measure the wavelength of light reflected.[19]

The goal of this technology is to achieve the following: (1) precisely identify vines via remote imaging, and (2) predict and map vine vigour to assess grape quality.[19] To achieve these goals, first a UAV employs multispectral remote sensing to produce an image of the vineyard in question. Next, an image processing software analyzes each pixel and determines whether it contains vine foliage.[19] Vines are then highlighted and assigned a Normalized Difference Vegetation Index (NDVI): a measure of the wavelength of light reflected

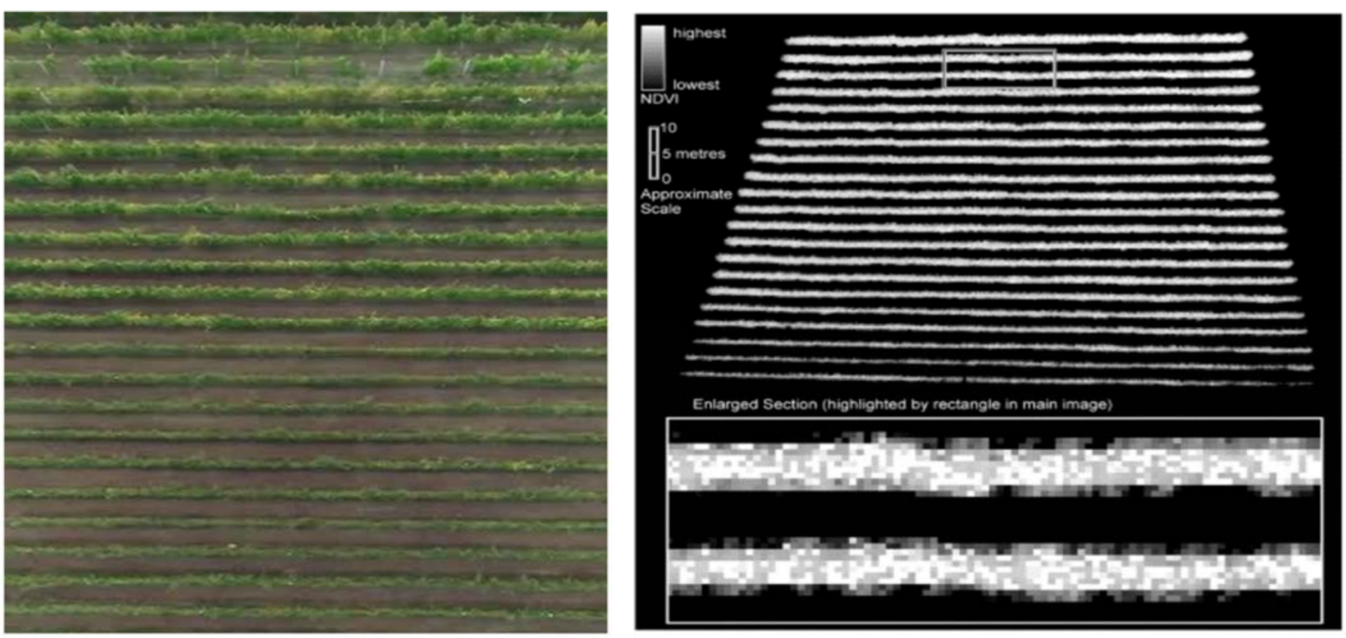

FIGURE 2: ASSESSING GRAPE QUALITY. An aerial image of a vineyard obtained by multispectral remote sensing. Through image processing, vine canopies have been identified in white and grey and assigned an NDVI value. From the enlarged section, the white pixels are areas of high density, while grey pixel clusters are areas of low density. [19]

by the vegetation to determine foliage density.[21] Lower NDVI values have been found to correspond to lower vine vigour, and vice versa. Finally, the image processor creates a map of the vineyard which highlights the vine canopies and illustrates vine vigour using a colour gradient (Figure 2).[19] This will allow vineyard magaers to effectively assess crop yields, and in turn make more economic decisions as well as predict profit margins.

A similar methodology can be used to efficiently estimate grape yield. To begin, a more sensitive image capture system is used to identify berry clusters, such as RGB imagery which differentiates objects based on their colour.[22]

Rather than obtaining an aerial image, the UAV will fly between rows for an up close and personal look. Image processing is then used to estimate the size and number of grapes within clusters (Figure 3). Finally, statistical analyses are performed to estimate grape output.[22] This method has already been proven to be reliable and cost-effective, with one study by Di Gennaro et al. estimating crop yield with 85% accuracy! [22]

Now, what if a vineyard needs to double check the morphology of different grape yields so they know what they're working with? Traditionally, vineyards have relied on the hiring of specialized technicians to manually classify grape varieties.[23] This process is costly, slow, and at times impossible to implement due to a lack of access to experienced technicians.[23] Thankfully, new UAV and AI technologies offer accessible, reliable, and efficient grape classification methods. The following steps outline how this can be done:

1. RGB imagery is used to obtain photos of grape vines.
2. Image processing determines the colour and shape of different grape clusters. This data is then compared to thousands of reference grapes and their corresponding morphologies and classification to obtain a classification for the grape cluster.[24]
3. Through machine learning, the image processor identifies grape morphology with increased accuracy over time (Figure 3).[24]

Current technologies can reach success rates as high as 87% in morphology classification![24]

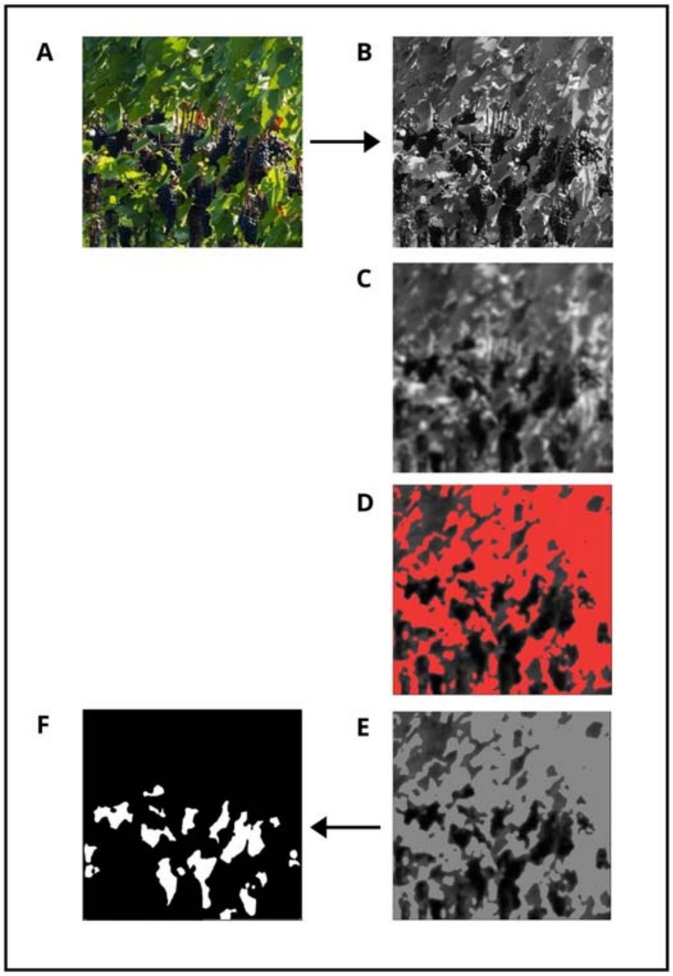

FIGURE 3: IMAGE PROCESSING FLOWCHART. A workflow schematic of image processing. An image displaying a similar perspective to one obtained from a UAV was chosen (A). The final output (F) accurately estimates the size of the grape clusters, depicted in white, allowing for effective extrapolation of vineyard grape yield data (Created by: Brittany Mascarenhas; ImageJ.)

MANAGING PESKY PESTS

Vines versus Spider Mites

When it comes to maximizing grape quality and yield, pest management is another area which could significantly benefit from the incorporation of AI and UAV. Pest management in this article differs from disease detection, a previously mentioned section, in that the former refers to pests that are only visible to the naked eye. This includes invertebrates, such as spiders, and vertebrates such as birds. Not only can AI and UAV replace manual labour methods within pest management in a cost-effective manner, it has also shown to be more efficient and accurate, which in turn drives down the overall cost required for pest management.[25,26]

Common pests such as spider mites are found in vineyards worldwide and usually reside on the underside of leaves to feed on mesophyll cells, causing leaves to lose chlorophyll, brown early, and even prematurely fall.[27] This not only affects the quality of the grapes produced on these vines, but also the ability for the plant to survive. The use of drones for selective spraying of miticides (substances that kill mites) has been studied with some success, but chemical control of spider mites is becoming less and less effective because of their increased resistance.[13] Due to their short life cycle, high reproduction rate, and high fertility, spider mites have been found to resist around 9 active ingredients.[13] Furthermore, no matter how selective a model is in miticide application, it will always have adverse effects on the populations of beneficial natural predators. The increase in resistance and loss of these natural predators demonstrates that it is not feasible to implement UAV with AI models for chemical control of spider mites.[27]

However, emerging research on dispensing predatory mites shows promise.[28] UAVs can be used to release natural enemies onto crops to both promote sustainable pest management and overcome the previous issues faced by miticides. Previously, the distribution of predatory mites for spider mite management was thought to be infeasible due to the limited ability to spread throughout a vineyard manually.[13] AI and drones offer a unique solution to this limitation, since manual selective spraying is too costly and uniform spraying will lead to large amounts of wasted predator mite application. Drones with remote sensing have the ability to detect hotspots where spider mites are likely concentrated.[28]

Remote sensing detects physical characteristics of an area by measuring the radiation that is reflected an emitted by the area. For vineyards, this allows for detection of physical changes within a leaf; usually due to biotic stress that interferes with photosynthesis or plant structure, both of which are affected by spider mites.[29] In turn, UAVs with the ability to model the effect of wind and other flight conditions on the spatial distribution of predatory mites create an effective closed-loop system of pest management (Figure 4).[28] So how does this model work? First, the model analyzes how different wind speeds, drone speeds, and altitudes of releases affect the distribution of predatory mites. Next, the model adjusts the drone accordingly to optimize the speed and altitude at which predatory mites are released.[28] With recent low-cost and autonomous multirotor UAVs, this pest management method is cost-effective and has the potential to revolutionize predatory mite release.[16]

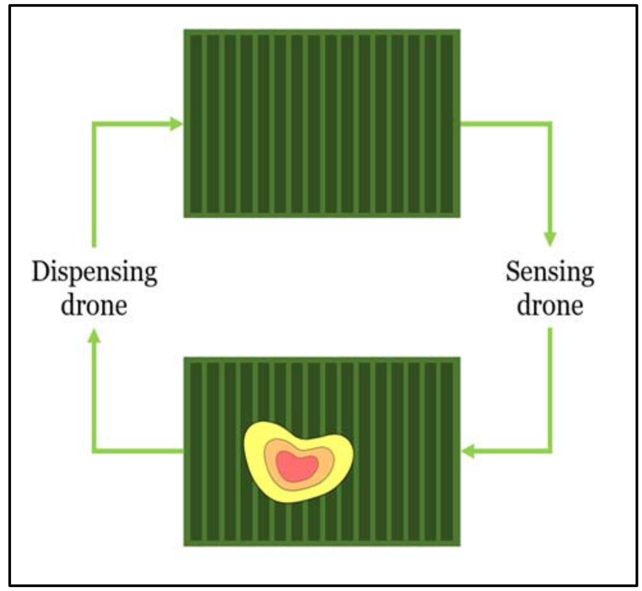

FIGURE 4: TARGETTING SPIDER MITES. A closed loop system of using drones and artificial intelligence to detect and release predatory mites for spider mite control in a vineyard. Adapted from Matese et al.[29]

Other significant pests common in viticulture are birds, which can cause severe damage and lead to an average of 9.4% harvest loss per producer if unmanaged.[30] Conventional methods include nets, acoustic devices, and manual scaring. These methods are unfortunately ineffective, temporary, or very costly to implement. For example, a cheap bird control method such as alarm calls are only effective for up to two weeks before birds acclimate to them.[30] This results from birds habituating to scaring methods once they learn no real threat is present. Once again, the answer to such limitations can be found in UAVs and AI!

A recent study by Wang et al. found that a UAV capable of emitting distress calls with a crow taxidermy fastened to it has lasting effects in bird management.[26] The use of distress calls and crow taxidermy together create a scenario of genuine danger to the birds which they will not quickly acclimate to. Currently, only set routes for drone flight and manual control of the drones have been tested, but the strategy shows great promise.[26] The radius of influence (which is the nearest distance between birds and the drone to observe a response) reached as high as 300 m. In addition, the use of a single drone has been shown to protect a vineyard up to 250,000 m in size by utilizing both danger sounds and unique flight paths.[26] The use of drones for bird management is relatively novel and is currently being studied further, with future goals of making the entire process autonomous as well as incorporating sensors for bird detection in conjunction with path planning algorithms.[29] This study also used commercial multirotor drones, which do not require a launch mechanism and are accessible to inexperienced operators, thus making it a viable option for bird management in vineyards.[26]

CLOSING THOUGHTS

AI and UAV technology is still relatively new in the world of viticulture, but it is quickly evolving. Some of the technologies discussed are still in the testing phase, and many are close to being field ready. The question becomes, is AI and UAV technology actually practical? In short, yes. The technologies are surprisingly user friendly, can be implemented and trained within days, and no coding experience is necessary past the set-up phase. Additionally, technology after technology have proven cost effective and a significant improvement to the time intensive manual labour currently required in viticulture. It must be noted that the initial set-up of the technologies requires extensive coding knowledge, however, this could be easily avoided by creating market-ready AI or UAV for vineyards to purchase. At the rate technology seems to be moving, the future of viticulture could lie in AI and UAV sooner than we think.

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Plastic Makes Perfect:

Considering the Use of Plastic Vessels in the Wine Industry

Christy Au-Yeung, Riddhi Bhatt, Juliette Froelich, Alun Stokes

In general, the process of wine fermentation occurs in stainless steel vessels and aging in oak barrels that impart various flavors on the final product. However, shortages of oak barrels and high costs of necessary materials have led to the exploration of alternative vessel materials such as plastics. The use of plastic tanks in the fermentation and aging processes have been employed by novice winemakers and professionals alike, and have been increasing in popularity in recent years. In this article, the viability of plastic tanks in the process of fermentation and aging was assessed and its performance was compared with its traditional counterparts. We explored the paths the wine industry may take from here given the effects plastics have on the taste of wine, and the environmental and marketing considerations.

OAK BARREL SUPPLY: AT AN ALL TIME MERLOT

As the demand for wine and other alcoholic beverages increases, so does that of oak barrels, which have traditionally been used in the process of fermenting and aging[1]. Oak barrels have been preferred by wine-makers due to their ability to make wine taste smoother due to the oxygen ingress and impart various flavors due to naturally occurring compounds migrating from the wood to wine[2]. Unfortunately, the use of oak barrels to produce various alcoholic beverages, ranging from wine to whisky and bourbon, has caused multiple shortages historically, such as in the wet and long winter in 2014 to 2015[3]. However, the problem doesn't stop here, with average barrel prices on an increasing trend (see Figure 1). Thus, alternatives to oak barrels must be explored. Some alternatives include tanks made of concrete, clay or stainless steel, which have been used for fermentation[4]. One of the promising but less explored options is plastic which is cost- and space-efficient, while still allowing for oxygen and flavor consistency control[5,6].

Plastic vessels have been employed since around 2006 and its use has been increasing in novice winemakers and wineries, alike[5,6]. Typically, these

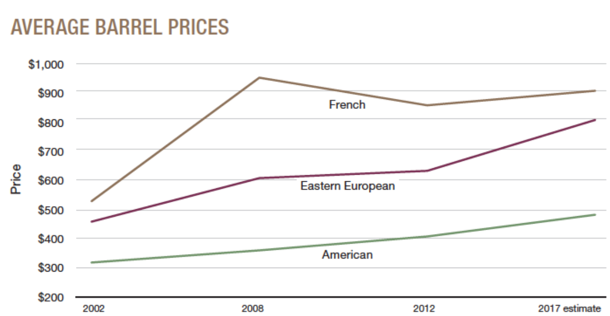

FIGURE 1: INCREASE IN AVERAGE BARREL PRICES from 2002 until an estimate of 2017. Prices seem to steadily increase for Eastern European and American oaks, with the exception of French oaks which have a slight dip before increasing as well[1]

vessels are made of food-grade plastics like high-density polyethylene (HDPE), polyethylene terephthalate (PET), or ethylene vinyl alcohol (EVOH) (FlexTank, 2019). Flextank, one of the main domestic suppliers of plastic vessels, reported in 2013 that they've seen a yearly 20% increases in sales since 2006[4]. Notable advantages of using plastic vessels includes their ability to stack, reduced evaporation reducing the need to top off barrels and durability[6]. Plastic tanks are considerably cheaper, half the price of steel vessels and a third of the price of oak barrels (Figure 2)[6]. The debate about the use of plastics in the wine industry revolves around the impacts it can have on the overall quality of wine, environmental impacts of their life cycle from production to disposal, public perception and marketing.

FIGURE 2: A PLASTIC VESSEL that can be used in place of oak barrels or stainless steel tanks. Commercially produced "Stacker Dexter Maturation Tank" that holds 120 gallons (~454 litres) of wine[6].

FERMENTATION: THE YEAST OF YOUR PROBLEMS

Fermentation is the process where sugars are converted to ethanol and carbon dioxide, as well as additional byproducts, through yeast[7]. Fermentation first occurs in aerobic conditions, which allows the yeast to multiply, with little alcohol product. Once the oxygen in the tank has been consumed by the yeast, the anaerobic conditions prevent the yeast from multiplying, instead directing energy towards ethanol formation[7]. Fermentation tanks are typically composed of stainless steel or wood, though plastic is also a viable, though less commonly used, material[8]. The material of the vessel can impact both the rate of fermentation and the composition of the wine through several factors, such

Aerobic vs. Anaerobic:

In aerobic conditions, oxygen is present. In anaerobic conditions, oxygen is not present.

as temperature, nitrogen content, and oxygen porosity.

Fermentation is a heat catalyzed reaction, the temperature of which must be monitored to ensure it remains within an ideal range. At an insufficient temperature, the rate of fermentation decreases and may halt altogether due to the yeast dying[9]. As well, the rate of fermentation increases at warmer temperatures, though the rapid rate sacrifices the synthesis of the particular aromatic compounds that provide the finished product with its unique sensory characteristics. Extremely warm temperatures will also kill yeast cells[9]. The conversion of sugar to ethanol and carbon dioxide by yeast fermentation is an exothermic reaction. As yeast ferments, it raises the temperature within the vessel; this residual heat can raise the temperature out of the ideal range. To maintain this ideal range, winemakers may manually alter the temperature of the vessel by increasing or decreasing the heat of the surroundings (Figure 3)[10]. The thermal conductivity of the vessel impacts the internal fermentation temperature of the vessel by retaining or releasing heat. Metal has a greater thermal conductivity than plastic[11], and so less of the heat generated by the fermentation reaction will be retained in the vessel; plastic would retain more of this heat, and so more energy would be required to externally cool the vessel to maintain the ideal temperature range. In addition, the ideal temperature range varies between wine styles. White wines should be fermented at lower temperatures to preserve fruity and volatile aromatic compounds (8-15ºC), while red wines should be fermented at higher temperatures to improve colour and increase tannin extraction (21-29ºC)[9]. A fermentation vessel composed of plastic would be more suitable for red wine fermentation, as less external cooling would need to be applied, while metal vessels are more suitable for the cooler temperatures of white wine fermentation.

The Difference between Heat Catalyzed and Exothermic Reactions:

Heat catalyzed reactions need heat to react quickly, while exothermic reactions release heat upon reacting.

FIGURE 3: EXTERNALLY CONTROLLING TEMPERATURE through the use of a heater connected to a temperature stick within the fermentation vessel. The heater can also be switched out for a cooling unit (not pictured)[12].

The nitrogen content of fermenting wine does not impact the taste of the finished product, but it is crucial for the rate of fermentation. Yeast requires nitrogen for growth and fermentation kinetics. Without sufficient yeast assimilable nitrogen, a combination of free-amino nitrogen, ammonia and ammonium[13], the stress of depletion lowers the rate of fermentation and results in the formation of sulfur dihydride, a powerful and undesirable odour compound[14]. To avoid this byproduct, winemakers often supplement wines undergoing fermentation with additional sources of nitrogen[15]. In a study comparing the final composition of Koshu white wines fermented in wooden barrels and plastic tanks, the total concentration of nitrogen in wine did not differ significantly between the wine fermented in barrels and the wine fermented in plastic tanks; however, the concentration of the amino acid proline was consistently lower in plastic-fermented wine (Table 1)[16]. Proline is the dominant amino acid in grape juice, which contains an amine group. Proline is not considered a source of yeast assimilable nitrogen, as it is poorly assimilable by yeast under anaerobic conditions by which most wine is fermented. However, once all other nitrogen sources have been depleted, uptake of proline is possible only if oxygen is present[15]. The lower concentrations of proline in

Assimilability refers to the ability of nitrogen to be used by a biological organism.

Fun Fact:

While long thought to be exclusively of European origin, Koshu wine is actually a hybrid of grapes from both European and Asian vitis plants[16].

plastic-fermented wine from the Koshu white wines study may indicate that the plastic fermentation conditions better facilitate the assimilation of proline by yeast; this would reduce the quantity of supplementary nitrogen in wine, diminishing the additional resources necessary in fermentation. The possible increased proline uptake may be a result of the oxygen permeability of plastic.

Month, Year	Vessel Material	Total Nitrogen (mg/L)	Proline (mg/L)
Mid-Oct, 1988	Plastic Tank	138	765
	Wooden Barrel	137	808
Mid-Oct, 1989	Plastic Tank	184	787
	Wooden Barrel	172	902
Mid-Oct, 1990	Plastic Tank	184	1071
	Wooden Barrel	174	1132
Late-Oct, 1990	Plastic Tank	162	760
	Wooden Barrel	162	858
Mid-Oct, 1991	Plastic Tank	113	613
	Wooden Barrel	137	746
Late-Oct, 1991	Plastic Tank	130	739
	Wooden Barrel	153	854

TABLE 1: COMPARING TOTAL NITROGEN AND PROLINE CONCENTRATIONS in plastic tank- and wooden barrel-fermented wine[16].

The oxygen permeability of the vessel is important to consider as, despite the anaerobic conditions of ethanol production, some oxygen is necessary for fermentation. Adding small quantities of oxygen to fermenting wine prevents stuck fermentations, where the yeast becomes dormant before fermentation is completed, by increasing the viability of the yeast cells which increases the fermentation rate[17]. The limited presence of oxygen is required for lipid and sterol biosynthesis, which impact the aroma of the wine, and the uptake of proline as a nitrogen source. To meet this requirement, winemakers often pump over oxygen into fermentation tanks, a process referred to as "topping up"[17, 18]. Plastic fermentation tanks are more permeable to oxygen, allowing the gas to diffuse directly into the tank (Figure 4), eliminating the need for topping up, while stainless steel tanks are impenetrable to oxygen and the permeability of wooden barrels depends on the type of wood and the age of the barrel[18]. As well, the oxygen permeation of plastics can be decreased with the addition of oxygen scavenger compounds to the plastic, which also eliminate oxygen within the tank[19].

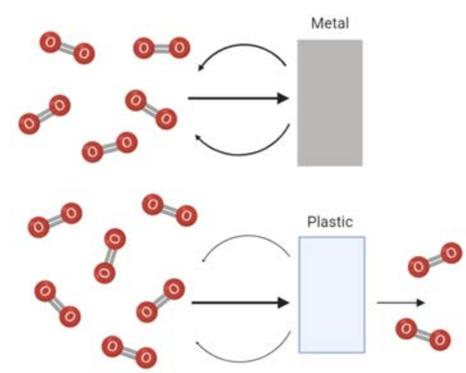

FIGURE 4: OXYGEN PERMEABILITY OF VESSELS while metal is largely impermeable to oxygen, plastic is more permeable to oxygen and the gas can diffuse directly into plastic fermentation tanks.

Depending on the style of wine, plastic tanks may be an adequate fermentation vessel that would decrease resources and labour, such as external cooling energy and oxygen pumping.

ONLY AGING IN OAK? OAKY BOOMER

Perhaps the most contentious part of the winemaking process when it comes to the use of plastics is that of aging. Aging is a step in which winemakers are less likely to be willing to take risks given the extended period of time which the wine will be in contact with its container, and the importance of the choice of container on the final character of the wine[20]. In general, wine is aged in an oak barrel. There is some variety here with the oak's origins - both in terms of geography of the wood and its previous uses - having a profound effect on the wine. One choice to make is between French and American oak (Figure 5), and a further choice is between new barrels, and previously used. Even further is the choice of what may have been stored in that barrel previously, with barrels used to age, for example, whiskey or scotch imparting different flavour profiles on the wines they store[21]. As a result, the suggestion of using a material like plastic to store wine for a long-term is offensive to many in the industry, but is it in any way viable?

FIGURE 5: OAK BARRELS are used in both the process of fermentation and again, and serve as both a vessel to hold the wine, and a source of flavour[22].

While in the process of fermentation, winemakers are concerned with the effects of microbes on the plastic - but here winemakers are solely concerned with the sorts of chemical reactions that happen on the surface of the plastic. These include both those that happen due to contact with the wine, or those that happen spontaneously[22]. It should be noted that there are many food products that are stored in plastic, but wine is more sensitive for a couple reasons. The first reason is its acidity: wine is below neutral at a pH between 4.5 and 5.5, which allows it to catalyze a higher degree of reactions as well as certain reactions that a more neutral solution wouldn't be able to[23]. This is especially concerning due to the more volatile nature of plastic compared to materials like glass. Another is the highly varied number of compounds found in wines. When compared with other beverages such as soft drinks, the diversity of organic compounds is much higher in even a simple wine[24]. The problem here is two-fold: the high number of unique compounds leads to a high number of possible reaction products, with the total characterization of how all these interact together being nigh-on impossible. As well, with the complex flavour profile of the wine being determined by the specific and particular ratios of the compounds to one another, the wine is highly liable to changes in its palatability by even minute changes in this system[25]. As a result, it is important to know, at a moiety and functional level, how wine-compounds interact with plastics.

Moiety:

A distinct part of a chemical compound, such as a hydroxyl group.

There are four principal compounds important for the flavour of wine: anthocyanins, tannins, flavan-3-ols, and flavanols[26]. The specific amount of each of these in relation to one another gives a wine its characteristic taste. As such, there are two problems when storing wine: introducing new compounds, and degrading desirable compounds. The use of plastic containers challenges both of these. Most importantly is the introduction of undesirable compounds by the plastic. This is caused by the leaching of additives from the plastic into the wine[27]. These compounds, generally acting as plasticizers, are not covalently bonded into the structure of the plastic, allowing them to leave the structure with relative ease. Given winemakers are interested in food-grade plastics[28], none of these such compounds would be toxic - but they would have a significant effect on the taste of the wine. Unfortunately, these compounds are rather critical to the function of the plastic, so simply not including them is not an option. Without them, the plastic would not function as intended, and would be less useful as a result[29].

The second of the two problems is the degradation of flavour compounds in the wine - and this generally happens by two main methods: interactions with the surface of the plastic container, and chemical degradation due to the presence of oxygen and UV light. One of the most concerning reactions that can occur in a plastic bottle is transesterification. This is a process by which alcohol in the wine can react with the surface-plastic of the bottle to depolymerize PET, leaving products including dimethyl terephthalate - a known irritant. Depending on the alcohol reacting - of which there are several in wine - the products will be varied, and their effects on humans not necessarily well characterized. Aside from this concern is that of oxidative and photolytic degradation. The permeability of plastic to both oxygen and UV radiation is different than other materials like glass. While PET is notoriously resilient to carbon dioxide diffusion, it readily allows the passage of oxygen[23]. This is concerning, as one of the primary goals in the production of wine is to minimize its contact with oxygen. In addition, but to a lesser extent, the exposure of aging wine to UV light can have a drastic effect on the chemical ratios of flavor compounds[20].

> **Transesterification:**
>
> An organic reaction between an ester and an alcohol.

In light of the numerous detractions, and with no identifiable benefits save for those mentioned in previous sections, it would seem inadvisable that this stage of wine production sees a shift toward the use of plastics.

ENVIRONMENTAL CONSIDERATIONS: SIP, SWIRL, SAVE THE EARTH

When considering a change to plastic materials in the wine industry the environmental impact must be considered. The comparison between plastic tanks and their traditional counterparts can be assessed using a standardized life cycle assessment protocol. This assessment allows for a holistic analysis of every stage of a product's life from raw material, extraction, processing, manufacture, distribution, use and disposal, to calculate various environmental impact categories.

In the fermentation process, a comparison between the commonly used stainless steel and its alternative, plastic tanks, was inconclusive due to contradictory results from the limited number of studies. Two studies completed by Manuilova et al. (2003) and Ernst & Young Accountants LLP, Rietveld and Hegger (2014) compared the life cycle impact of steel and high-density polyethylene (HDPE) containers, with volumes 225 L and 208 L, respectively. Arguably, one of the most important impact factors is global warming potential, which scales different greenhouse gas (GHG) emissions in terms of carbon dioxide (CO_2)[31]. However, the two studies have differing results in terms of the numerical values of the global warming potential. When assessing other impact categories plastic and steel are each superior in three categories (Table 2).

> **For Example:**
>
> Methane emissions are 25 times more potent in trapping atmospheric heat; one gram of methane is equivalent to 25 grams of CO_2[31].

HDPE Plastic	Steel
-Ozone depletion (kg CFC-11 equivalent) -Smog (kg O_3 equivalent) -Eutrophication (kg of nitrogen equivalent)	-Fossil fuel depletion (Megajoules surplus) -Human toxicity (comparitive toxic unit equivalent) -Ecotoxicity (comparative toxic unit equivalent)

TABLE 2: LCIA (LIFE CYCLE IMPACT ASSESMENT) IMPACT CATEGORIES Identification of impact categories where a 208 L tank made of either plastic of steel have lower environmental impacts Plastic tanks (high density polyethylene- HDPE) assessed in this study were made of either new (virgin) pellets or post-consumer resin recycled (recycled) plastics, or reconditioned plastic. Steel tanks assessed in this study were either reconditioned or new[31].

Thus, looking beyond impact categories there are several other factors to consider. In terms of lifetime and recycling rates steel is superior, with a typical lifetime of ten years compared to five for plastics[32]. Both plastic and steel are recyclable, but steel has higher recycling rates due to the ease of separating it among other materials based

> **Fun Fact:**
>
> According to the Environmental Protection Agency, only 12.9% of plastic gets recycled in the United States[31].

on its magnetic properties. There are several challenges in recycling plastic due to low market demand, limited collection systems and different infrastructures required for processing different types of plastics[33]. On the other hand, plastic production requires less energy consumption, plastic can be a viable option if the recycling is improved. The energy required to produce a 225 L container from plastic is 460 megajoules (MJ), compared to 625 MJ for steel[32]. It is difficult to determine which of these containers would be more environmentally beneficial (Table 3); however, a shift towards plastic can be justified if better recycling techniques are adopted.

	Steel	HDPE Plastic
Global Warming Potential	Contradictory results	
LCIA Impact Categories	Inconclusive	
Lifetime	10 years	5 years
Recycling Rates	Easier to recycle and sort	Difficult to recycle due to lack of plastic recycling system
Energy to Produce	More energy required (625 MJ)	Less energy required (460 MJ)

TABLE 3: SUMMARY OF ENVIRONMENTAL IMPACTS based on studies completed by Manuilova et al., 2003 and Ernst & Young Accountants LLP, Rietveld and Hegger, 2014[31,32].

Following fermentation, wine undergoes aging, which is conventionally completed in oak barrels. Plastic tanks (HDPE) were compared with oak barrel and have been demonstrated to have considerable environmental benefits. Due to the limited data available, only four impact categories could be assessed: global warming, acidification, eutrophication and ozone depletion (see Figure 6)[31,34]. However, in all four of these categories, plastic tanks had lower potential environmental impacts. Some factors contributing to the high environmental impact caused by oak barrel production include wood treatment processes and significant energy consumption associated with wood cutting to produce staves and heads of oak barrels[34].

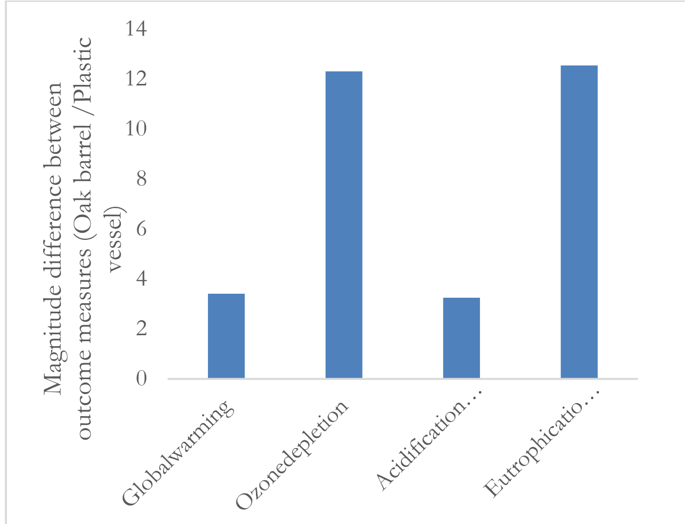

FIGURE 6: A COMPARISON OF IMPACT CATEGORIES Magnitude of difference between average oak barrel impact and average of HDPE plastic tanks in impact categories: global warming (kg CO_2 equivalent),ozone depletion (kg CFC-11 eq.), acidification (kg SO_2 eq.), eutrophication (kg PO_4^- eq) . Oak barrel values calculated from average impacts from oak barrels made from three types of oak (Q. alba, Q. Robur and Q. petraea). HDPE plastic tank values calculated from average impacts of virgin, PCR (post-consumer resin), and reconditioned plastic. In all four categories, oak barrels had higher impacts on the environment compared to HDPE plastic tanks[31,34].

Overall, the use of plastics in fermentation and aging process has the same, if not fewer, the environmental impacts compared to the use of stainless steel vessels or oak barrels.

PUBLIC PERCEPTION AND MARKETING: ARE PLASTIC VESSELS IN POUR TASTE?

In addition to the environmental impacts of plastic, the public opinion of plastic use in the wine industry should be considered as this is the consumer target. A 2019 Australian study asked a sample group about concerning environmental issues creating a questionnaire that covered environmental issues surrounding the current use of plastic. Almost 70% of participants ranked plastic in the oceans as a 9 or 10 on a severity scale from 1-10[35]. This shows that plastic is an important issue that people consider to be detrimental to the environment. The study also found that as environmental awareness increases, the overall dislike of plastic also increases. People perceive plastic to be a large contributor to waste in the environment, but also enjoy the convenience it provides[35]. This shows that environmental issues are not the only influencer of attitudes towards plastic, but convenience and affordability are also an influence.

Understanding the perception of plastic and how it varies from person to person is important when marketing products. It is extremely important for winemakers to understand the numerous consumers within the wine market in order to make products for each group. The groups include two major types of consumers; ones that have favourite products and are consistent, and then ones that are either trend-setters or purchase based on price[36]. Customers that buy the same brands and wines that they have for years are hard to target as they are consistent in their tastes. However, trend-setters would be easier to influence and encourage as their purchases depend on price or current trends. Despite having found a target consumer, the public perceptions of environmental implications also need to be considered. But, this may not be as big a challenge as anticipated as it is important to consider the varying levels of public engagement towards climate change. A 2007 study analyzed the barriers of public engagement in regards to climate change and found that even with cognitive understanding, there is still a lack of behavioural change to people's lifestyles[37]. This barrier in engagement might allow for the consumption of plastic-fermented or -aged wine despite the negative environmental connotations surrounding plastic.

Fun Fact:

The trend-setter group is comprised of millennials who are spontaneous and enjoy trying new things. They tend to spend more money on quality wine and value quality over quantity[36].

A secondary obstacle to incorporating plastic into wine-making is the tradition surrounding the process. For example, the cultural nuances around wine are important to acknowledge and many people would not think plastic in wine-making to be acceptable. Wine labelling would play a role in the public knowing how their wine was produced. Currently, the regulations enforced by the Canadian Food Inspection Agency require labels to include information such as winery name, vintage, grape variety, and certifications but not necessarily whether the wine was aged in French or American oak (Figure 7)[38]. This means that Canadian wine-makers could produce wine using plastic tanks without having to advertise this process, according to current requirements. Regulations would need to be changed to require wineries to include wording such as "aged in plastic" or other production information.

FIGURE 7: CANADIAN WINE LABEL REQUIREMENTS are enforced by the Canadian Food Inspection Agency through the Food and Drug Act of 1985[38].

In order for the wine market and wine consumers to consider alternatives to oak in aging and glass bottles in packaging, people would need to eschew tradition in favour of more contemporary options. To do so is to make a move towards what is perhaps a more environmentally friendly future where wine production has a reduced impact on the environment.

PLASTIC IN VITICULTURE? THEY MAY HAVE THEIR RIESLINGS

Wine making is a rigorous process with years of tradition informing it. Change in this historical process is not accepted easily[39]. Proposing the use of plastic within wine-making is therefore a risky proposal. The two major steps of wine making considered in this analysis are fermentation and aging. As the two lengthiest steps, the final is heavily influenced by what happens during these steps.

Chemical analysis of wine in plastic found various results. Wine fermentation in plastic is an interesting approach that does not have negative impacts on the result and is a feasible practice. Wine aging in plastic, however, does impart negative compounds onto the final product. Aside from the chemical composition of the wine, it is also important to consider the environmental connotations. While the environmental impacts of steel and plastic tanks in fermentation were not significantly different, the environmental impact of oak aging in comparison to plastic is significant and could be reduced by incorporating plastic tanks. Having these varying results between the chemistry and environmental analysis means that an environmental incentive is not sufficient enough for viticulturalists to incorporate plastic into wine making.

Aside from environmental incentives, economic feasibility is a secondary effect that could be used to justify the use of plastic in wine-making. For wine-makers that are starting out in the industry, plastic vessels are cheaper than their oak counterparts. Additionally, the choice to use plastic tanks should depend on whether the end goal is a wine with complex flavour profiles or a wine that is cheaper to produce. Each winery has different philosophies and goals. Wine-makers are recommended to make an educated decision regarding their specific priorities, as there is no definitive answer in the debate of plastic in wine-making.

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