BIOGENIC AMINES IN WINE

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DECLARATION

“I, Jacques Lombard, declare that this report is my own, unaided work. It is submitted in partial fulfilment of the requirement for the diploma of Cape Wine Master to the Cape Wine Academy. It has not been submitted before for qualification of examination in this or any other educational organisation.”

Signed: ……………………………………. Date: 30 January 2024

ABSTRACT

This dissertation explored the biochemistry involved in the formation of biogenic amines in wines, the most prevalent biogenic amines in wines, and their health effects on consumers. A literature review examined the factors influencing biogenic amine formation across the entire wine production process, while an exploratory study shed light on consumer perceptions and knowledge. The current international regulations and some countries' recommendations related to levels of biogenic amines in wine were described. This report provided winemaking mitigating strategies to wine producers including prevention, limitation and removal of biogenic amines.

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LIST OF FIGURES AND TABLES

A List of the figures and tables presented are given below. For ease of reference the first digit refers to the chapter of the dissertation in which the table is presented, with the subsequent digits indicating the order in which they appear in that chapter.

LIST OF ABBREVIATIONS

AA	Amino Acid
AAB	Acetic acid bacteria
AF	Alcoholic fermentation
DAO	Di-amine oxidase
HDC	Histidine decarboxylase
LAB	Lactic acid bacteria
MAO	Monoamine oxidase
MAOIs	MAO inhibitors
MLF	Malolactic fermentation
O. oeni	*Oenococcus oeni*
ODC	Ornithine decarboxylase
S. cerevisiae	*Saccharomyces cerevisiae*
SO_2	Sulphur dioxide
TDC	Tyrosine decarboxylase
TSO_2	Total sulphur dioxide

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Keywords: Amino acids, decarboxylase enzymes, biogenic amines, winemaking process and factors, toxicology

1. INTRODUCTION

1.1. BACKGROUND AND JUSTIFICATION FOR THE RESEARCH

Biogenic amines are nitrogenous, and organic compounds and can be found in some fermented foods such as cheese, sausage, fermented vegetables, fish, and wine. Furthermore, biogenic amines are natural contaminants of wine that originate from decarboxylase microorganisms found in grapes and the winemaking environment. These microorganisms are also involved in fermentation processes.

Biogenic amines in food or wine, in high concentrations, can result in negative health effects for consumers. The toxicity symptoms caused by biogenic amines in wine are similar to the symptoms experienced by individuals with wine intolerance, such as headaches, flushing, rashes and nausea.

This, together with the negative impacts biogenic amines can have on wine quality, make biogenic amines an important area of research for wine consumers and producers. It is important to understand what knowledge consumers may have of this area, to provide an outline of the formation of biogenic amines in wine which may benefit consumers and/or producers and to investigate possible mitigations to the production of biogenic amines in the winemaking process.

1.2. METHODOLOGY

A literature review was carried out to determine what is currently known about the subject, with an emphasis on outlining the factors that affect the formation of biogenic amines across the entire wine production process. An exploratory study was conducted on a sample of wine consumers to establish what they understood about biogenic amines and their health effects.

1.3. OBJECTIVES OF THE RESEARCH

The specific objectives of this dissertation were to:

i. Provide a background on the most common biogenic amines found in wines and a description of their formation.
ii. Describe the various factors influencing the formation of biogenic amines.
iii. Provide an overview of the main health effects of biogenic amines on wine consumers.
iv. Outline the current regulations and recommendations relating to the maximum amounts of biogenic amines in wines.
v. Investigate mitigating strategies available to winemakers to control the amount of biogenic amines in their wines.
vi. Investigate wine consumers' perceptions about biogenic amines and the effect of wine consumption through the use of an exploratory survey.

1.4. CHAPTER SUMMARIES

The remainder of the dissertation is set out as follows.

i. Chapter 2 outlines the most prevalent biogenic amines in wines.
ii. Chapter 3 briefly describes the biochemistry involved in the formation of biogenic amines.
iii. Chapter 4 gives a general overview of the health effects of biogenic amines, specifically describing histamine, tyramine, putrescine and cadaverine.
iv. Chapter 5 describes in more detail the factors influencing the formation of biogenic amines. The microorganisms involved, matter of geography, cultivar, vintage, viticulture practices, the role of pH, the winemaking process and the levels of biogenic amines in different styles of wine.
v. Chapter 6 briefly describes the current regulations and recommendations related to levels of biogenic amines in wine.
vi. Chapter 7 explains the mitigating strategies that could be implemented by winemakers to avoid excessive amounts of biogenic amines in their wines.
vii. Chapter 8 sets out the exploratory survey of wine consumers' perceptions about biogenic amines and the effect of wine consumption and analyses the results using descriptive statistics and tests of significance as appropriate.
viii. Chapter 9 describes the main conclusions of the research and makes recommendations that winemakers may be able to follow to avoid excessive biogenic amines in their wines.

2. THE MAIN BIOGENIC AMINES IN WINE

An amine refers to a chemical compound that contains nitrogen. Biogenic amines are nitrogen-containing compounds that are produced, and broken down by the metabolic processes of various living organisms, including microorganisms, humans, animals and plants (Smit et al., 2008).

The main biogenic amines associated with wine are cadaverine, putrescine, histamine and tyramine. They are followed in minor concentrations by phenylethylamine, spermidine, spermine, agmatine and tryptamine, all of which serve as markers of the wine's quality.

During the different stages of wine production and storage, various microorganisms can cause the formation of biogenic amines in wine (Constantini et al., 2019). The table below (Table 2.1) contains the main biogenic amines with their functions and toxicity symptoms.

Table 2.1: Biogenic amine functions and symptoms

Biogenic amines	Functions	Toxicity symptoms
Histamine	Neurotransmitter and vasodilator in the central nervous and cardiovascular systems. Dilates peripheral blood vessels, capillaries and arteries. Excites smooth muscles of the uterus and gastrointestinal tract, enhancing acid secretion from the gastric mucosa. Stimulates mucus secretions and causes endothelial permeability of the airway. Releases adrenaline and noradrenaline.	Migraine/headache, vertigo, hypotension, flushing, pruritus (itching), urticaria (hives), arrhythmia, tachycardia. Nausea, vomiting, dysmenorrhea, cramps, stomach ache, diarrhoea. Nasal congestion, rhinorrhoea sneezing, wheezing, bronchoconstriction, anaphylaxis.

Table 2.1: Biogenic amine functions and symptoms *continued*

Tyramine	Promotes catecholamine efflux from the sympathetic nervous system and the adrenal medulla.	Increase in mean arterial blood pressure and heart rate by peripheral vasoconstriction – migraine/headache, hypertension.
	Dilates pupils and palpebral tissue, causes lacrimation and salivation, accelerates respiration and increase blood sugar content.	Has been linked to development of Parkinson's disease, schizophrenia and mood disorders.
Phenylethylamine	Regulates monoamine neurotransmission. Neurotransmitter in human central nervous system.	Hypertension, headache/migraine.
Polyamines – putrescine, cadaverine	Involved in cell proliferation. Formation of carcinogenic nitrosamines by reaction between nitrite and secondary amines (putrescine, cadaverine, agmatine).	Carcinogenesis, tumour invasion.

Adapted from Maintz and Novak (2007) and Smit et al., (2008)

3. BIOCHEMISTRY OF BIOGENIC AMINE FORMATION IN WINE

In wine biogenic amines are primarily formed through the chemical process of decarboxylation of their precursor amino acids (AA) (Bartowsky, 2009). Microorganisms in the wine environment produce enzymes known as decarboxylases, which specifically catalyse this reaction. Microbes that are related to wine, such as bacteria and yeast, which can be found in grape must and wine, have their origin from the vineyard, grapes and winery processing equipment. The natural microflora contains various types of yeast, with the most common being *Saccharomyces cerevisiae (S. cerevisiae).* The only bacterial families detected in grape must and wine are lactic acid bacteria (LAB) and acetic acid bacteria (AAB). Among them are two dominant genera of AAB, *Acetobacter* and *Gluconobacter,* as well as four dominant genera of LAB, including *Lactobacillus*, *Leuconostoc, Oenococcus* and *Pediococcus* (Bartowsky, 2009).

Because amino acids are acquired by the bacterium from the extracellular medium, in this case wine, it is believed that decarboxylase enzymes operate together with a transporter protein (Lucas et al., 2005). The presence of a net charge difference between the precursor molecule, such as monovalent histidine and the end product, divalent histamine, causes the transporter to generate a membrane potential during the uptake of AAs (Smit et al., 2008).

Typically, the production of biogenic amines arises from the existence of bacteria with the capacity to perform amino acid decarboxylation (Figure 3.1). Specifically, histidine decarboxylase (HDC) produces histamine from histidine, ornithine decarboxylase (ODC) catalyses the decarboxylation of ornithine to putrescine and tyrosine decarboxylase (TDC) results in the creation of tyramine using tyrosine as the precursor (Costantini et al., 2019). Cadaverine is formed through the direct decarboxylation of L-lysine catalyzed by lysine decarboxylase (Weichao et al., 2017).

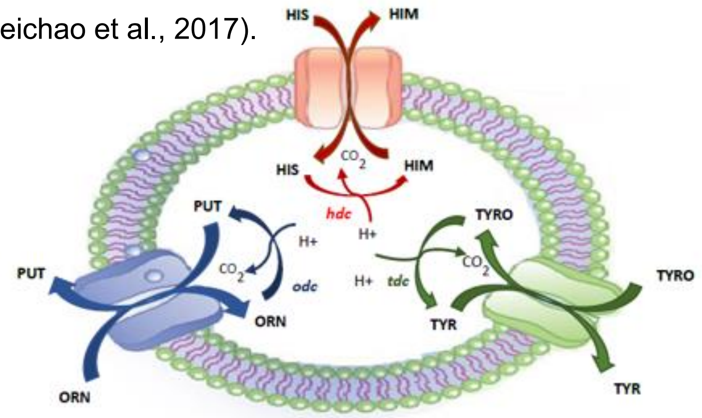

Figure 3.1 The formation of biogenic amines from their precursor amino acids and their subsequent intake and uptake by the transmembrane antiporter (Costantini et al., 2019).

Decarboxylase activity can be affected by the sugar levels present in the medium; specifically, a deficiency of sugars has frequently been linked to increased production of biogenic amines. The reason behind this correlation can be demonstrated by the fact that the decarboxylative pathway transport system creates metabolic energy (Costantini et al., 2019).

Landete et al., (2008) noted that in the oenological LAB (*Lactobacillus hilgardii, Pediococcus parvulus* and *Oenococcus oeni*), higher concentrations of fructose and glucose resulted in a gradual inhibition of histamine formation. Additionally, the expression of the HDC gene was decreased by malic acid and citric acid, while ethanol increased the activity of the HDC enzyme. It was demonstrated that the synthesis of HDC and ODC strongly relies on the existence of elevated levels of free AAs in the growth medium.

4. THE HEALTH EFFECTS OF BIOGENIC AMINES IN WINE

Although there has been extensive research (Maintz and Novak, 2007; Smit et al., 2008; Martuscelli and Mastrocola, 2018; Coton et al., 2010; Constantini et al., 2019) on biogenic amines in wine, neither the industry nor consumers are knowledgeable about their importance concerning product quality or health implications. Knowledge of the level of amines in wines can be highly beneficial due to their widely recognised association with causing intolerance to wine. This has been noted by the European Food Safety Authority (Constantini et al., 2019).

In the following sub-sections, the health effects will be elaborated on.

4.1. PHYSIOLOGICAL FUNCTIONS OF BIOGENIC AMINES IN THE HUMAN BODY

A variety of metabolic processes in the human body are regulated by biogenic amines as shown in Table 4.1.

Table 4.1: Some functions of biogenic amines in the human body

System	**Functions**
Nervous	Regulates body temperature, brain activity, transmitting messages/ neurotransmitter, effect of tyramine is the indirect stimulation of the release of noradrenaline from the sympathetic nervous system.
Digestive	Regulates stomach volume, stomach acidity
Immune	Regulating the immune system, immune response, allergic reactions
Respiratory	Regulating the immune system, immune response, allergic reactions
Cardiovascular	Maintaining heart health; expansion of blood vessels, capillaries and arteries in the outer parts of the body
Endocrine	Controlling sleep cycles.

Adapted from: Ten Brink et al., 1990; Maintz and Novak, 2007; and Stratton, Hutkins, and *Taylor, 1991.*

Usually, when a person consumes biogenic amines in small quantities, their body will rapidly break them down using amine oxidases. The process of oxidative deamination of biogenic amines involves amine oxidases that generate an aldehyde, hydrogen peroxide and ammonia as the end products (Gardini et al., 2005).

Conversely, if a large quantity of biogenic amines is consumed or if the usual processes of breaking them down are blocked or lacking due to genetics, various physiological conditions can arise. Histamine and tyramine are commonly known as the most harmful biogenic amines to human health if consumed beyond the threshold levels that can be metabolised (Gardini et al., 2005).

The body possesses an inherent mechanism to remove low levels of biogenic amines when they are consumed. Biogenic amines are restricted from being fully absorbed by the body due to the intestinal mucin layer and the closely connected epithelial cells. However, the body also counteracts this by detoxifying biogenic amines with the help of the enzymes HNMT6 and di-amine oxidase (DAO) (Maintz and Novak, 2007).

DAO plays a major role in the breakdown of metabolic processes of histamine but it is not located in the brain, which might be significant for headaches caused by biogenic amines. Females tend to have lower levels of DAO compared to males, which aligns with informal observations of a higher incidence of wine intolerance among women (Jarisch et al., 1996).

Ethanol is one of the most powerful amine oxidase inhibitors that can hinder the body's enzymatic breakdown of biogenic amines (Zimatkin and Anichtchik, 1999). Hence, the levels of biogenic amines present in wine are even more important. The susceptibility to biogenic amines consumed through diet is influenced by reduced amino oxidase function due to medication, hereditary predisposition (histamine intolerance), digestive tract ailments, suppression by liquor, acetaldehyde and additional biogenic amines like putrescine and cadaverine (Martuscelli and Mastrocola, 2018).

Histamine, tyramine, putrescine, cadaverine, phenylethylamine and tryptamine are a set of naturally occurring active compounds that possess significant physiological impacts on the human body (Moreno-Arribas et al., 2010). Histamine is the biogenic amine known to trigger headaches, low blood pressure and digestive complications. Tyramine and phenylethylamine are linked to migraines and high blood pressure when ingested in significant concentrations. Both psychoactive or vasoactive effects could be produced by them. Psychoactive amines influence the nervous system by acting on neural transmitters, whereas vasoactive amines act on the vascular system (Lovenberg, 1973).

The group of vasoactive amines comprises presser amines such as tyramine, tryptamine and phenylethylamine. By narrowing the blood vessels and increasing the heart rate, they elevate blood pressure and force the heart to contract. On the other hand, vasodilatation is caused by histamine which results in a decrease in blood pressure (Smith, 1981).

Consuming too many biogenic amines orally can result in adverse reactions such as nausea, headaches, rhino-conjunctival (disorder of the nose characterised by itching, nasal discharge, sneezing and nasal airway obstruction) symptoms, high or low blood pressure, flushing, skin irritations and abnormal heartbeats. These are caused by the intricate pharmacological impacts that are linked with both the histamine and neurotransmitter systems (Maintz and Novak, 2007).

In alcoholic drinks the amount of histamine which can be harmful is usually between 8 to 20 mg/L whereas for tyramine it is between 25 and 40 mg/L. Negative physiological effects can occur from as little as 3 mg/L phenylethylamine (Soufleros et al., 1998). Kanny et al., (2001), stated that an average person can handle 120 mg/L of histamine consumed orally before any signs of discomfort appear. However, the tolerance level drops down to just 7 micrograms when administered intravenously. Nonetheless, some research (Kanny et al., 1999, 2001; Jansen et al., 2003) suggests that consuming biogenic amines orally does not lead to wine intolerance as per each individual toxicological capacity. Instead, these writers suggest that wine might have certain substances such as ethanol and acetaldehyde that encourage the discharge of histamine within the human system.

In addition to their toxic impact, certain biogenic amines may bring about other unfavourable outcomes, primarily affecting the sensory qualities of wine and the economic repercussions. It is crucial to emphasise that the negative consequences that arise from ingesting food and drinks containing high levels of biogenic amines will only occur if these amines manage to enter the bloodstream (Smit et al., 2008). The body typically averts this by means of a defensive mechanism positioned in the digestive system (Moreno-Arribas et al., 2010). Biogenic amines have varying threshold levels of safety depending on the individuals' physiological conditions and tolerance.

Amines ingested via food and alcoholic beverages are broken down through the enzymatic action of mono- and di-amino oxidases (MAO and DAO) that are present in the intestines. Alcohol, certain antidepressants and hypotensive medications, known as MAO inhibitors

(MAOIs), can inhibit the action of MAO and DAO in the intestines (Martuscelli and Mastrocola, 2018). Diamine substances such as putrescine and cadaverine, along with polyamine substances like spermine and spermidine, can intensify the toxicity of histamine, tyramine, tryptamine and phenylethylamine. This results because they compete with these substances in the metabolic processes carried out by MAO and DAO, which causes a decrease in detoxification (Martuscelli and Mastrocola, 2018). Patients with histamine intolerance (a disorder associated with an impaired ability to metabolize ingested histamine), with those taking MAOI drugs or individuals on new generation RIMA (reversible inhibitors of MAO-A drugs) treatment should be aware that the risk of toxic reactions is heavily influenced by the composition of the entire meal, rather than solely the consumption of wine (Martuscelli and Mastrocola, 2018).

Besides causing allergic reactions, biogenic amines can also lead to severe human conditions such as the development of cancer and the invasion of tumours, which can be caused by ornithine-derived polyamines and histamine (Smit et al., 2008). Additionally, the immune response and neurological disorders can also be the result of histamine exposure. The reaction between nitrite and secondary amines, including putrescine, cadaverine and agmatine, can lead to the formation of carcinogenic nitrosamines hindering cellular metabolism. Furthermore, tyramine and phenylethylamine can cause migraines and hypertension, while tyramine alone can lead to Parkinson's disease, Schizophrenia and mood disorders (Smith, 1980; Ten Brink et al., 1990; Silla Santos, 1996; Medina et al., 1999).

The red wine headache is also a well-known type of headache syndrome. After consuming red wine, the occurrence of its effects may develop within a few minutes and is frequently linked to the amount that was consumed. The headache initially becomes very intense after around two hours, then subsides, but recurs after about eight hours, with even stronger intensity (Kaufman, 1992).

4.1.1. HISTAMINE

Histamine facilitates several important functions in the human body (refer to Table 2.1). It plays a role as a mediator during allergic responses (Taylor, 1985), and is stored in unique granules found in mast cells and basophils in the blood. When an allergic reaction occurs, these granules release histamine into the bloodstream. The effects can be similar when histamine is released through the degranulation of a mast cell or consumed through food and alcoholic beverages. The impact of histamine occurs through attaching to receptors located on the membranes of cells (Brink et al., 1990).

Humans have two kinds of histamine receptors, called the H1 and H2 receptors (Taylor, 1986). The cardiovascular system contains both of these receptors. When histamine interacts with these receptors, it causes the expansion of blood vessels, capillaries and arteries located in the outer parts of the body (Stratton, Hutkins, and Taylor, 1991). In addition, there is an increase in the ability of capillaries to let substances pass through, causing plasma to leak out into the surrounding tissues and resulting in an increase in blood concentration. Histamine can also be responsible for the increase in heart-rate and beat strength.

Histamine receptors can be found in different secretory glands. It regulates the secretion of gastric acid by binding to H2 receptors found on parietal cells. Histamine triggers the production of fluids by the pancreas, intestine and bronchi. It has the capability to release adrenaline and noradrenaline from the suprarenal gland, which in turn has a direct impact on the stimulation of the heart (Joosten, 1988; Shalaby, 1996). It stimulates the smooth muscles of the uterus, intestines and respiratory tract through the H1 receptors. Moreover, there is a likelihood that histamine functions as a neurotransmitter within the central nervous system. The H1 receptors are responsible for stimulating both sensory and motor neurons (Taylor, 1986; Moreno-Arribas et al., 2010).

Typical symptoms that impact the skin system consist of skin rash, hives, swelling and inflammation in a specific area. The presence of gastrointestinal symptoms is indicated by feelings of queasiness, vomiting, diarrhoea and discomfort in the abdominal area (Taylor, 1985). Additional indications consist of low blood pressure, headaches, irregular heartbeats, numbness, reddening and burning feeling in the mouth. In severe instances, there have been reports of shock, constriction of the bronchioles, asphyxiation and intense difficulty with breathing. Nonetheless, the illness typically exhibits a gentle nature and the indications are of short duration (Moreno-Arribas et al., 2010).

Research by Maintz and Novak (2007) states that around 1% of subjects suffer from a medical condition known as histamine intolerance or enteral histaminosis. This results in an uneven build-up of histamine in the body and a lack of ability to break it down efficiently. These subjects fall under the category of histamine sensitive individuals, which is distinct from histamine poisoning brought on by ingesting dangerous concentrations.

According to a study done by Rohn et al., (2005), skilled wine tasters can detect high levels of histamine in some commercially produced wines. In the study, mouthfeel attributes such as "discomfort in the back of the throat" and "tingling sensation on the tongue" were used to describe the texture of the food or drink. Histamine does not have a distinct or particular taste.

4.1.2. TYRAMINE

Tyramine is typically found in small concentrations and is not a significant by-product in the body, unlike histamine. The primary effect of tyramine is the indirect stimulation of the release of noradrenaline from the sympathetic nervous system. The most notable consequence of this action causes blood pressure to rise due to the constriction of the cardiovascular system and an increase in cardiac output (Joosten, 1988; Stockley, 1973). Tyramine also dilates the pupils and eyelids. It also triggers tear production and excessive saliva flow. Additionally, it boosts respiration. An increase in blood sugar levels can occur if there are high concentrations present (Franzen and Eysell, 1969; Joosten, 1988).

The main reason why tyramine in food and wine is significant is because it has toxicological implications. Tyramine is inherently poisonous, but it also combines with MAOI medications to cause a sudden and severe rise in blood pressure, known as a hypertensive crisis (Shalaby, 1996). MAO has the role of eliminating dangerous amines, particularly tyramine, to prevent them from reaching the bloodstream, by functioning in the liver and intestine. Using MAOI medications to treat depression removes this cleansing process from the body. This results in the accumulation of large concentrations of the pressor amines obtained from food and wine in the bloodstream (Moreno-Arribas et al., 2010).

4.1.3. PUTRESCINE AND CADAVERINE

Histamine and tyramine appear to have much higher pharmacological activity than putrescine and cadaverine. Living cells require essential compounds such as putrescine, cadaverine, spermidine and spermine in order to function properly. These amines have a significant role in regulating nucleic acid (nucleic acids are large biomolecules that play essential roles in all cells and viruses) function and protein synthesis, as well as potentially stabilising membranes. The detrimental impacts are noticeable solely when consuming exceedingly high quantities of putrescine and cadaverine. Reported symptoms include low blood pressure, slow heart rate, difficulty breathing, stiff muscles in the jaw and weakness in the limbs (Moreno-Arribas et al., 2010).

4.2. RELATIONSHIP BETWEEN BIOGENIC AMINE DOSAGE AND RESPONSE

The following factors need to be taken into account when assessing the relationship between dosage and response:

i. Individual vulnerability: The capacity to detoxify the body of biogenic amines differs from person to person due to genetic factors or gastrointestinal conditions that hinder the performance of enzymes and/or the ability to absorb these toxins (Maintz and Novak, 2007).

ii. Inhibition of DAO/HNMT: The ability of certain substances to discourage the activity of DAO and HNMT (Zimatkin and Anichtchik, 1999). Ethanol is considered as one of the inhibitors that actively competes with DAO. The functionality is also reduced by acetaldehyde and specific DAO/HNMT-blocking medicines (known as DAOIs/MAOIs). Antidepressants constitute the most extensive category within the DAOIs/MAOIs group. The literature indicates that one out of every five people in Europe takes DAOI/MAOI antidepressants on a regular basis (Ruiz-Capillas and Herrero, 2019).

iii. The combined enhancing impact of biogenic amines: Some types of biogenic amines, especially polyamines, have been demonstrated to enhance the effects of histamine and tyramine when combined. Bulushi et al., (2009) found that the existence of cadaverine and putrescine resulted in the suppression of the enzymes responsible for breaking down histamine. The presence of biogenic amine co-potentiators caused an increase in histamine toxicity, as they inhibited the oxidation of histamine.

5. FACTORS INFLUENCING BIOGENIC AMINE LEVELS IN WINE

The quantity of biogenic amines created in wine is primarily determined by the number of AA precursors present in the medium, because generally speaking, the level of biogenic amines rises alongside that of AAs. The number of AAs present in wine can vary depending on the winemaking process, type of grape, the region where it was produced and the year in which it was harvested, as indicated by several studies (Lonvaud-Funel and Joyeux, 1994; Soufleros et al., 1998; Moreno Arribas et al., 2000).

Some factors boost the concentration of precursor AAs, while others have an impact on the development and enzyme functioning of microorganisms that have the ability to create biogenic amines. Furthermore, to achieve significant production of biogenic amines, three main requirements must be met (Parker-Thomson, 2020).

These include:

i. The availability of free amino acids or protein.
ii. The presence of microorganisms that possess decarboxylase genes.
iii. Favourable conditions for microbial growth and decarboxylase synthesis activity.

The physiochemical properties of wine, which include factors like pH, temperature, SO_2 levels and various fermentation substrates and products, can have an impact on the abundance and variety of microorganisms that are present in the wine. Additionally, these properties can also influence the activity of decarboxylase enzymes and the expression of genes related to wine production. The presence of the HDC gene in wine does not guarantee that the enzyme will function properly and produce histamine (Coton et al., 1998).

It appears that when growing conditions are unfavourable, particularly in situations where the substrate supply, such as malic acid and glucose, is limited, the production of histamine by LAB is consistently amplified (Lonvaud-Funel and Joyeux, 1994). Still, the existence of glucose and malic acid can enhance the process of decarboxylation. The activation of arginine catabolism by the LAB, *O. oeni*, and subsequent increase in putrescine production were discovered to be significantly influenced by the presence of malic acid (Smit et al., 2008). There are various elements that significantly impact the amount and variety of microbes in wine, as well as alter the function of decarboxylase.

5.1. MICROORGANISMS ASSOCIATED WITH BIOGENIC AMINE FORMATION IN GRAPES AND WINES

Microorganisms can enter the winemaking process at different stages and have the ability to impact the final product's quality. The initial stage involves the grapes. The winery equipment (crusher, presses, tanks, pipes, pumps, filtration units, etc.) is in contact with the grapes, if the equipment is not cleaned and sanitised properly, it can contaminate the grape juice as a source of infection (Du Toit and Pretorius, 2001).

The grapes that are brought to a winery are not all in good and healthy condition, which can have an impact on the variety of living microorganisms that exist naturally in the juice. When examining AAB, it was found that *Gluconobacter oxydans*, which exists in concentrations of 10^2 colony-forming unit per gram (cfu/g), is the primary species present on healthy grapes. *Botrytis cinerea*-infected grapes contain mainly *Acetobacter aceti* and *Acetobacter pasteurianus* totalling to 10^6 cfu/g with fewer *Gluconobacter oxydans* cells (Drysdale and Fleet, 1988; Fugelsang, 1997). In addition to the acetic bacterial genera, other spoilage microorganisms include yeast genera (*Brettanomyces, Candida, Hanseniaspora, Pichia, Zygosaccharomyces,* etc.) and lactic acid bacterial genera (*Lactobacillus, Leuconostoc, Pediococcus*, etc.).

The wine faults caused by the spoilage microorganisms include astringency and off-aromas and off-flavours (buttery, geranium tone, mousiness, vinegary and ester taint) and off-conditions regarding appearance such as sediment, film formation, turbidity and viscosity (Du Toit and Pretorius, 2001). Natural microorganisms that are present can be indirectly influenced by external factors, such as the various grape cultivars, the physical condition of grapes during the time they are harvested (e.g. damage due to birds, insects, harvesting and mould), as well as factors such as temperature, rainfall, soil type, use of insecticides and fungicides, and other methods related to grape growing and viticulture (Du Toit and Pretorius, 2001).

Healthy grapes contain a small amount of LAB, which typically decreases during the process of alcoholic fermentation. LAB can also be found in winery equipment and their population can be quite substantial (Wibowo et al., 1985). *O. oeni* remains the most prevalent type of bacteria that endures throughout the alcoholic fermentation process and primarily facilitates MLF. Nonetheless, when the pH level of the wine exceeds 3.5, certain species of *Pediococcus* and *Lactobacillus*, which are typically linked with spoilage, could survive and reproduce to quantities ranging from 10^6 to 10^8 cells/mL. These microorganisms might engage in opposing actions in relation to *O. oeni* (Wibowo et al., 1985; Lonvaud-Funel, 1999).

Extensive research has been conducted to establish a connection between the formation of biogenic amines in wine and the types of LAB utilised to perform successful MLF during winemaking. Historically, spoilage microorganisms, particularly *Pediococcus spp*., such as *Pediococcus cerevisiae*, were blamed for the creation of histamine in wine (Delfini, 1989). Landete et al.'s (2007) findings suggest that while the number of *Pediococcus* bacteria capable of generating histamine may be minimal, certain strains of *Pediococcus parvulus* in this study, could still cause elevated levels of histamine production.

Prior to 2003, there were no confirmed reports on the involvement of *Leuconostoc* strains in the creation of biogenic amines in wine. Moreno-Arribas et al., (2003) demonstrated that certain strains of *Leuconostoc mesenteroides* have a considerable capacity to generate tyramine. Landete et al., (2007) discovered another strain of *Leuconostoc mesenteroides* in wine that has the ability to produce a considerable amount of histamine (Smit et al., 2008). More recently it has been observed that biogenic amine-related pathways in LAB are determined by the strain rather than the species. It has been suggested that horizontal gene transfer is responsible for the genes involved in *O. oeni*'s putrescine production, *Lactobacillus hilgardii*'s histamine production and *Lactobacillus brevis*'s tyramine production (Smit et al., 2008).

In 2006, a study was conducted by Martín-Álvarez, et al., to analyse the variations in biogenic amines throughout the production of 55 batches of red wine in an industrial setting. Throughout the production process, the origin of biogenic amines that are connected to must, alcoholic fermentation, MLF, SO_2 addition, wine ageing, as well as the interactions between amines and their corresponding AAs and pH, were statistically analysed in samples from the same batches. The authors' conclusion was that certain biogenic amines, like putrescine, cadaverine and phenylethylamine, can arise either in the grape or during the initial stages of winemaking (refer to Table 5.1). Additionally, during alcoholic fermentation, yeasts are capable of producing ethylamine and phenylethylamine. However, the amount of these biogenic amines present during these stages is very low. During MLF, it was observed that there was a rise in levels of histamine, tyramine and putrescine, which corresponded with a reduction in the AAs that serve as precursors to LAB (Moreno-Arribas et al., 2010).

Yeasts have the potential to have a significant impact on the production of biogenic amines by LAB, as they influence the amino acid makeup either during the process of alcoholic fermentation (Soufleros et al., 1998) or through autolysis (Moreno-Arribas, Pueyo, Polo, and Martín-Álvarez, 1998; Villamiel, Polo, and Moreno-Arribas, 2008). During MLF and the ageing of wine, these AAs have the potential to serve as building blocks for the creation of biogenic amines (Marques, Leitao, and San Romao, 2008; Martín-Álvarez et al., 2006). Decarboxylase enzymes, which might contribute to biogenic amine production, could potentially be derived from yeast and bacteria lees (Marcobal et al., 2004). Although certain yeasts have been discovered to have the ability to produce biogenic amines, the overall impact of their contribution to biogenic amine production is insignificant. They only generate insignificant quantities of the most harmful biogenic amines (Herbert et al., 2005, Marcobal et al., 2006). It is widely accepted that yeasts have a lower impact on the production of biogenic amines in wine compared to LAB. Certain authors have discovered that there is no significant rise or even a decline in the levels of biogenic amines during the process of alcoholic fermentation when utilising either natural or commercially produced yeast strains (Herbert et al., 2005, Marcobal et al., 2006, Landete et al., 2007).

Garcia-Marino et al., (2010) showed that the introduction of yeast mannoproteins led to a rise in biogenic amines. The cause of this was an increase in nitrogen compounds, which can be used as precursors for AAs. If must or wine remains in contact with grape skins for a long time and there are microbes that have the ability to produce biogenic amines, it is possible for biogenic amines to be formed because grape skins contain AAs.

Several research studies have revealed that specific yeasts are capable of generating biogenic amines; however, histamine and tyramine were either undetectable or present at a minimal concentration (<4 mg/L) in all cases (Buteau et al., 1984, Goñi and Ancín-Azpilicueta 2001, Torrea and Ancín 2002, Caruso et al., 2002, Granchi et al., 2005, Tristezza et al., 2013). The production of biogenic amines by *S. cerevisiae* greatly depends on its strain, as some strains have the capability to produce ethanolamine and agmatine in concentrations as high as 10 mg/L (Caruso et al., 2002).

The yeasts and LAB involved in the process of wine fermentation necessitate specific nitrogen nutrients. *Saccharomyces* species have the ability to use ammonium ion (NH_4) and free alpha AAs as nitrogen sources. Nevertheless, once ammonium is exhausted, yeasts will utilise AAs exceedingly well if they are available. Different types of yeast display varying preferences for absorbing specific AAs and they are also capable of releasing specific AAs into the wine (Bely et al., 1990). Typically, yeasts need a minimum amount of 140-150 mg per litre of nitrogen in order to avoid stuck fermentation. However, it is advised to have at least 200 mg of nitrogen per litre to prevent the development of undesirable flavours (Ribérau-Gayon et al., 2006). Due to the fact that ammonium alone does not fulfil all the nutritional needs of yeast, several wine yeast producers suggest the utilisation of advanced yeast nutrients that incorporate an additional nitrogen supplement (González-Marco et al., 2006; Hernández-Orte et al., 2006; Smit et al., 2012). Bach et al., (2011) demonstrated that including yeast nitrogen compounds in wine results in a rise in the overall number of biogenic amines.

Among yeasts, *Brettanomyces bruxellensis* stands out as an exception due to its ability to produce the greatest quantities of biogenic amines, making it a spoilage yeast (Caruso et al. 2002, Granchi et al., 2005). In the two studies where it was considered, *B. bruxellensis* had the highest overall concentration of biogenic amines compared to other yeasts. In both situations, 2-phenylethylamine emerged as the dominant biogenic amine (Caruso et al., 2002, Granchi et al., 2005).

Table 5.1: Microorganisms associated with biogenic amines' production

Species	**Role**	**Biogenic amine**
Saccharomyces cerevisiae	Fermenting wine yeast	Histamine
Brettanomyces bruxellensis	Spoilage yeast	Agmatine, phenylethylamine, ethanolamine
Kloeckera apiculta, Candida stellata, Metschnikowia pulcherrima	Natural wild yeast	Agmatine, phenylethylamine, ethanolamine
Botrytis cinerea	Noble late harvest wines fungi	Tyramine, putrescine, cadaverine, phenylethylamine, spermidine
Lactobacillus **spp.**, *Pediococcus* **spp.**	Fermenting and spoilage lactic acid bacteria	Histamine (histidine decarboxylase) Tyramine (tyrosine decarboxylase) Putrescine (ornithine decarboxylase) Phenylethylamine
Oenococcus oeni	MLF	Histamine (histidine decarboxylase)

Source: Adapted from Moreno-Arribas et al., 2010

5.2. GEOGRAPHICAL AREA AND TERROIR

Terroir is a term that refers to the complete natural surroundings of a vineyard location (Robinson, 2015). The essential elements that make up terroir include the soil and surrounding topography, as well as their interdependence with one another and their interplay with the macroclimate (temperature, rainfall and wind), which ultimately impacts the meso-climate and microclimate of the vine. Each site is believed to possess a distinct terroir that is a result of a harmonious blend of all its elements. This terroir is consistently reflected in the wines produced annually, despite the differences in viticulture and winemaking techniques to some extent. Therefore, each individual piece of land, as well as broader regions, may possess unique attributes that define their particular wine style, which cannot be exactly duplicated elsewhere. These conditions will thus contribute to the various levels and concentrations of biogenic amines found in wine. The level of putrescine present in wine might be affected more by the type of grape and geographical location rather than winemaking methods (Landete et al., 2005).

Furthermore, the following factors also play a significant role along with the geographical area and terroir:

i. A correlation has been found between low levels of potassium in soil and higher levels of putrescine in plants (Adams, 1991).
ii. The final product's biogenic amine levels may also be affected by the type of soil and the grape's level of maturity (Glória et al., 1998). This could play a key role in determining the ultimate level of biogenic amines that are present or generated by microorganisms during the winemaking process (Smit et al., 2012).
iii. The lack of water does not affect the biogenic amine content in grapes and wines (Bover-Cid et al., 2006).
iv. The composition of AAs found in grapes was altered due to varying weather conditions and grape harvest seasons (Ortega-Heras et al. 2014).

5.3. CULTIVAR

Grape berries frequently contain amines, specifically polyamines like putrescine, which are a result of plant metabolism (Halász et al., 1994; Bover-Cid et al., 2006). For instance, the pericarp of Cabernet Sauvignon berries contains high concentrations of putrescine, cadaverine and spermidine (Glória et al., 1998). High levels of putrescine, cadaverine and spermidine have also been discovered in grape berry seeds (Kiss et al., 2006). The grape cultivars that contain higher levels of assimilable AAs have been shown to produce the greatest concentration of biogenic amines (Herbert et al., 2005).

Cecchini et al., (2005) conducted a study to investigate the impact of red grape varieties on the levels of biogenic amines present in wines. Merlot, Syrah, Sangiovese and Cabernet Franc were studied. The research revealed that there was a notable contrast in the individual and total biogenic amine contents in wines that were produced from various types of grape cultivars. From the cultivars that were examined, Cabernet Franc had the largest amount of total biogenic amine content by a considerable margin.

Numerous studies have revealed that Pinot noir grapes possess elevated concentrations of biogenic amines compared to Cabernet Sauvignon. Ough (1971) noted that Pinot noir grapes in California had noticeably higher levels of histamine than Cabernet Sauvignon grapes, whereas Glória et al., (1998) discovered that Pinot noir grapes from Oregon had significantly greater concentrations of total biogenic amines than Cabernet Sauvignon grapes. Soleas et al., (1999) noticed higher biogenic amine concentration levels in Pinot noir originating from Ontario (Canada), in comparison to other types of red wine produced from the same area (Smit et al., 2008).

5.4. VINTAGE

The amount of free AAs and amines in must and wine can also be influenced by the vintage of the wine and the area where it was produced (Herbert et al., 2005). The concentrations of amino acid precursors can vary greatly over vintage years. The fluctuation in biogenic amine levels in wine can also be associated with the variety of naturally selected microorganisms present in the wine, which in turn depends on the pH of the grapes and wines, that may vary with different vintages (Martín-Álvarez et al., 2006). These researchers discovered that Spanish Tempranillo wines originating from a particular area contained significantly higher levels of biogenic amines in 2001 compared to the year 2002. The amine levels in Aszu wines were observed to be affected by the year of production and showed significant variations among the vintages of 1993, 1997 and 1998 (Sass-Kiss et al., 2000). Contrarily, Glória et al., (1998) discovered that there were no variations in amine levels between two vintages, 1991 and 1992. This literature indicates that different vintages may vary regarding biogenic amine accumulation depending on the vintage conditions and the winemaking process as described in section 5.7.

Additional factors that can impact the levels of biogenic amines in grapes include the stage of ripeness and maturity of the fruit, along with the application of irrigation. Martínez-Pinilla et al. (2013) displayed that the levels of biogenic amines in red wines made from Monastel, Tempranillo and Maturana Tinta de Navarrete grape varieties differed from year to year. The researchers noticed that wines from the 2009 harvest had a higher biogenic amine content compared to wines from the 2010 harvest, which caused a shift in their amine composition. These findings are consistent with previous studies. The authors provided an explanation for the variations in amine content, suggesting that the diversity of wine microorganisms, which naturally undergo different selection processes every year, is likely influenced by climatic conditions.

5.5. VITICULTURE PRACTICES

The application of nitrogen fertilisers can result in a rise in the levels of AAs and biogenic amines in grapes. In particular, fertilisation significantly increased the number of biogenic amines in must, whereas in wine, fertilisation had a relatively minor impact on amine content in comparison to the contribution of microorganisms (Smit et al., 2008). The quantity of AAs present is affected by the level of maturity, as in the case of grapes or musts where the amount is seen to increase from veraison to the harvest. The growth of berries slows down and the production of proteins decreases during their maturation, which might be the reason for the buildup of free AAs. According to the same authors, the alcoholic fermentation process was not influenced by irrigation in terms of the development of nitrogen compounds, but it did affect the level of maturity in certain AAs tested. There was no direct correlation found between biogenic amines and either irrigation or degree of maturity (Costantini et al., 2019).

5.6. pH LEVELS IN GRAPES AND WINE

There is a strong relationship between pH and biogenic amine levels. When the pH is high, there are usually a greater number of biogenic amines, whereas lower pH leads to a decrease in the production of biogenic amines (Lonvaud-Funel and Joyeux, 1994, Gardini et al., 2005, Martín-Álvarez et al., 2006). pH is an important factor to consider when trying to predict the amino biogenic activity of wine. One theory suggests that microorganisms may engage in the synthesis of biogenic amines as a metabolic response to acidity in the environment, or as a means of obtaining alternative energy (Cotter et al., 2003).

Histamine levels were observed to show a significant increase by Landete et al., (2005) when the pH level exceeded 3.6. Increased pH levels result in a higher variety of bacterial microorganisms, including spoilage bacteria and an increased chance of growth and survival of decarboxylase-positive bacteria. If the pH of wine drops below 3.3, it can become unsuitable for most LAB, excluding *O. oeni*, making MLF a challenge. This may result in lower levels of biogenic amines, although there is no surety. In addition, when the pH of wine elevates to above 3.6, it can result in the promotion of LAB growth, specifically *Lactobacillus, Leuconostoc* and *Pediococcus* (Du Toit and Pretorius, 2001).

Cilliers and Van Wyk (1985) observed that all the red wines containing a considerable amount of histamine (>10 mg/L) had a pH level higher than 3.7 (Smit et al., 2008). Although ethanol as a parameter was not analysed independently, investigations have revealed that when high ethanol concentrations (>12% abv) are combined with either low pyridoxal 5-phosphate9 concentrations (plays a key role in amino acid metabolism) or low pH levels, the resulting biogenic amine levels are lower (Gardini et al., 2005). Lonvaud-Funel and Joyeux (1994) demonstrated that wines with high pH and low ethanol had the greatest biogenic amine concentrations, yet in every circumstance, pH played the most significant role.

5.7. WINEMAKING PROCESS

When it comes to how winemaking methods and technology can affect the formation of biogenic amines in wines, it's important to focus on certain practices that are commonly used to improve the aromas and flavours of wines. However, these same practices can also raise the levels of AAs that lead to biogenic amine formation (Martín-Álvarez et al., 2006). The production of biogenic amines is influenced by various aspects of wine such as pH-, ethanol- and SO_2 levels. These factors play a significant role in the range of microorganisms present, as well as the activity of decarboxylase enzymes and the expression of decarboxylase genes (Moreno-Arribas et al., 2010).

Based on the findings of other authors, the impact of a winery appears to be noteworthy, irrespective of its geographical location. A predominance of decarboxylating positive microbiota can occur in some wineries, even if the fermentation is carried out by commercial starter strains (Moreno-Arribas et al., 2010). The possibility of contamination can be brought on by improper hygiene procedures related to grape cultivation and equipment handling (Zee et al., 1983; Ten Brink et al., 1990; Shalaby, 1996; Leitão et al., 2005), as mentioned in section 5.1.

For some time now, winemakers have been working to decrease the levels of amines in wines and enhance their quality and safety. An investigation of approximately 700 Spanish wine samples exhibited that the average concentration of histamine in red, rosé and white wines had reduced from 2010 to 2015. Furthermore, none of the samples had a histamine content exceeding 10 mg/L (Moreno-Arribas et al., 2010).

There are two primary stages of fermentation involved in the production of wine from grapes. The process of turning grape sugar into ethanol and CO_2 through alcoholic fermentation typically involves the use of *S. cerevisiae*, a type of wine yeast. LAB from the genera

Lactobacillus, Leuconostoc, Pediococcus and *Oenococcus* conduct MLF in red wines and some white wines such as Chardonnay. This process of MLF mainly aims to convert malic acid into lactic acid to lessen the perceived acidity of the wine. The process of MLF also guarantees the wine's microbial stability and changes its taste by creating additional bacterial by-products (Lonvaud-Funel, 1999). The growth of microorganisms like acetic acid bacteria and *B. bruxellensis* during the process of fermentation or storage can result in the production of compounds that give undesirable qualities to the wine. In addition to the main primary metabolic products and various flavour elements (favourable or not), that are created during alcoholic fermentation and MLF, certain microbes also generate secondary metabolic products that can impact the overall wholesomeness and quality of the wine. One such group of compounds is the biogenic amines (Smit et al., 2008).

5.7.1. COLD SOAK AND MACERATION

The process of grape skin maceration enhances the extraction of various grape ingredients like phenolic compounds, proteins, AAs and polysaccharides. Before the process of alcoholic fermentation, grape juice remains in contact with the grape skins at a low temperature, which is known as cold maceration or soaking. Red wines typically undergo the process of alcoholic fermentation while in contact with the skins of grapes. To prolong the extraction process, extended maceration can be employed at lower temperatures subsequent to the completion of alcoholic fermentation (Smit et al., 2008).

During the process of maceration and fermentation, the grape skins and seeds contribute to the extraction of grape phenolics as well. Many chemical reactions in grape must and wine are attributed to these compounds. They participate in oxidation reactions (working as strong antioxidants) and impact the taste and appearance of wine (Smit et al., 2008).

Martín Álvarez et al., (2006) determined that the inclusion of pectolytic enzymes in grapes at a rate of 2 g/100 kg did not stimulate the buildup of biogenic amines in wine. Some individuals (Bauza et al., 1995; Martín Álvarez et al., 2006; Smit et al., 2008) discovered that the length of time for skin maceration is a crucial factor influencing the levels of biogenic amines in wines. They found that a longer maceration period could potentially promote higher production of biogenic amines. Grape musts are supplemented with pectolytic enzymes in order to enhance the juice production, clarify the must or wine, extract additional grape-based compounds like phenols and aid in the pressing and filtration processes (Smit et al., 2008).

The contact between the grape skins and the juice during the process of making red wine, which cannot be avoided, contributes to the typically elevated levels of biogenic amines in red wines (Smit et al., 2008). The majority of studies investigating maceration duration discovered a direct relationship between the time and the buildup of biogenic amines (Bauzá et al., 1995, Martín-Álvarez et al., 2006, Kovačević Ganić et al., 2009). Kovačević Ganić et al., (2009) examined the effect of skin contact on white wines as a factor. Out of three distinct winemaking processes (free run, press wine and macerated wine), it was found that macerated wine contained the highest levels of biogenic amines.

5.7.2. LEES AGEING AND AUTOLYSIS

The presence of yeast lees during the ageing process of wine contributes to the rise in biogenic amines after fermentations. The growth and activity of LAB are promoted by the release of vitamins and nitrogenous compounds, which is facilitated by yeast autolysis. LAB have the capability to break down proteins and peptides and utilise the resulting AAs as a source of nutrients or energy. The precursors of biogenic amines may be included in these AAs (Lonvaud-Funel and Joyeux, 1994).. Bauza et al., (1995) noticed that there is a rise in the production of tyramine and putrescine in wines when bacteria are introduced by adding lees.

Practices like ageing wines with lees or leaving them to macerate for longer periods of time should be particularly monitored (Martín-Álvarez et al., 2006). Numerous researchers have discovered that a lees ageing period of more than three months enhances the concentration of biogenic amines (Martín-Álvarez et al., 2006, Gonzalez-Marco and Ancín-Azpilicueta 2006, Marques et al., 2008). Lees stirring raised the level of biogenic amines in wines, particularly histamine in red wines and tyramine in Chardonnay. The greatest concentrations were attained through weekly stirring (Alcaide-Hidalgo et al., 2007, Gonzalez-Marco and Ancín-Azpilicueta, 2006). The action of lees stirring increases biogenic amine concentrations because amino acids are released by yeast and bacteria autolysis.

Several studies have discovered that there is an elevation in the levels of biogenic amines during the maturation phase after MLF and after bottling (Herbert et al., 2005, Gerbaux and Monamy 2000, Jiménez-Moreno et al., 2003, Henríquez-Aedo et al., 2018, Marques et al., 2008). Coton et al., (1998) showed that the activity of enzymes can continue for several months after the population of bacteria diminishes during the process of lees ageing. This indicates that biogenic amines can potentially build up in wine even if viable LAB is not present (Ancín-Azpilicueta et al., 2008). Polo et al., (2011) discovered that as the population of LAB survived longer throughout the ageing process, the levels of biogenic amines increased progressively.

5.7.3. MALOLACTIC FERMENTATION

Wine has been found to contain various types of biogenic amines produced by *Oenococcus, Leuconostoc, Lactobacillus* and *Pediococcus* bacteria (Smit et al., 2008). *Lactobacillus hilgardii* and *Pediococcus parvulus* species have been recognised as the most abundant histamine producers, with the ability to generate concentrations of up to 200 mg/L. Furthermore, *Lactobacillus hilgardii* is also known for its significant production of tyramine (Landete et al., 2005).

It is thought that while yeasts can play a role in the overall levels of biogenic amines in wines (Caruso et al., 2002; Torrea & Ancín, 2002), the majority of biogenic amine contamination in wine occurs during MLF. This is because MLF involves the presence of LAB strains that create enzymes that convert the precursor AAs into biogenic amines. Because LAB must possess particular genes necessary for producing biogenic amines, and because the presence of these genes varies depending on the species and strain of LAB, the ability of LAB to produce biogenic amines varies.

The outcome of the MLF process, which is lactic acid, was discovered to hinder the activity of HDC. Conversely, it seems that lactic acid does not hinder the activity of ODC (Mangani et al., 2005). HDC inhibition can also be caused by citric acid. Furthermore, there is a slight occurrence of TDC activity at typical levels found in wines following MLF (Rollan et al., 1995; Moreno-Arribas and Lonvaud-Funel, 1999). Additional substances discovered to hinder TDC function to varying degrees encompass glycerol, β-mercaptoethanol, lactic acid and ethanol. However, according to Moreno-Arribas and Lonvaud-Funel (1999), they suggest that the concentrations of these compounds found in wine, even at their maximum levels, will not be enough to inhibit the creation of tyramine. Using *O. oeni* starter cultures that cannot generate biogenic amines can be a suitable strategy for managing and reducing the presence of these compounds in wine (Smit et al., 2008).

O. oeni is the predominant species of LAB found in wine and is exceptionally well-suited for conducting MLF under the challenging conditions of winemaking. In winemaking, the MLF phase is generally regarded as a highly significant factor for biogenic amine production. Multiple authors have provided evidence suggesting that during the MLF phase, the majority of biogenic amines are formed. According to Landete et al., (2005), LAB involved in the process of MLF was responsible for the primary presence of tyramine, histamine and putrescine in red wines, although the levels of cadaverine and tryptamine remained unaffected. On the other hand, Bauza et al., (2007) noticed an elevation in the levels of putrescine as fermentation progressed from must to alcoholic fermentation to MLF, which concurred with Soufleros et al.'s (1998) findings.

A common belief is that LAB's participation in MLF implies that MLF is responsible for the highest levels of biogenic amines (Parker-Thomson, 2020). Consequently, many studies have focused on investigating how MLF affects the levels of biogenic amines in wine. However, research confirms that the capacity of LAB to generate biogenic amines is primarily found in non-*O. oeni* LAB species that do not typically engage in MLF. Hence, the emphasis on MLF appears to be theoretically misdirected; MLF plays a role, but it is not necessary for substantial buildup of biogenic amines in wine (Parker-Thomson, 2020). Martín-Álvarez et al., (2006) examined the impact of LAB inoculation and noted a notable decrease in biogenic amines concentration in the wines that were inoculated. Furthermore, Schneider et al., (2011) found that inoculating with *O. oeni* during MLF in German wineries led to a decrease in histamine concentration compared to spontaneous MLF.

Lonvaud-Funel and Joyeux (1994) demonstrated that the process of histidine decarboxylation is increased when malic acid is excluded from the environment, indicating that MLF did not take place. Therefore, although MLF's metabolic process does not directly generate biogenic amines, the circumstances required for MLF promote the production of all LAB capable of producing biogenic amines.

Research indicates that naturally occurring MLF results in biogenic amine levels that are statistically higher compared to MLF that is intentionally introduced (Martín-Álvarez et al., 2006). The reason for this is that other types of LAB besides *O. oeni* are responsible for this and commercial cultures used for MLF are carefully chosen to exclude LAB that can produce harmful decarboxylase enzymes. During the alcoholic fermentation process, the inoculated culture can potentially overpower and suppress the growth of the natural LAB population, resulting in a reduced likelihood of undesired bacterial activities (Smit et al., 2008; Smit et al., 2012).

In a study by Smit et al., (2012) the production of a substantial amount of tyramine was only detected in the treatments that were inoculated with *L. hilgardii* strain 1. This strain has been confirmed to be a tyramine producing strain through a PCR test. It is noteworthy that this strain exhibited a higher production of tyramine when it was inoculated in a sequential manner, as opposed to simultaneous inoculation, in both cultivars (Shiraz and Pinotage). Furthermore, this outcome emphasised the significance of using a starter culture that lacks the ability to generate biogenic amines during the winemaking process. Of all the amines that were assessed, putrescine had the highest concentration.

Winemakers may take the step of introducing additional nutrients to sluggish spontaneous MLF, or they may opt to allow the MLF to naturally progress after incorporating yeast nutrients with intricate properties during the alcoholic fermentation process. There is a question about the potential existence of residual precursor AAs derived from complex nutrients in wine, which the natural LAB flora, including decarboxylase positive strains, may utilise (Smit et al., 2012).

5.7.4. POST-MALOLACTIC FERMENTATION AGEING, VARIOUS VESSELS UTILIZED AND WINE STORING TEMPERATURE

According to Gerbaux and Monamy (2000), the level of histamine progressively rises in Pinot noir and Chardonnay wines between four to eight months after undergoing MLF. In another study (Herbert et al., 2005), it was found that histamine levels in both red and white wines continued to rise 18 months after MLF, while tyramine and putrescine levels appeared to increase right after MLF in red wines in this particular study.

One possible explanation for the initial rise in levels after the MLF process is that the introduction of SO_2 into the wine doesn't entirely inhibit all biochemical reactions and enzyme activity. Furthermore, because many wines have a high pH level, the effectiveness of SO_2 decreases, resulting in a possible increase of biogenic amines in SO_2 added wines as they age (Gerbaux and Monamy, 2000).

Moreno and Azpilicueta (2004) conducted a comparison of the levels of biogenic amines in wines that had been aged in barrels for a duration of 243 days, both in their filtered and unfiltered forms. Diatomaceous earth used as a wine filter has the ability to absorb positively charged AAs and proteins, which can have an impact on the development of biogenic amines over time. Unfiltered wine may contain remnants of grape skins that can be abundant in amino acid building blocks. However, it was discovered that the level of turbidity did not have any impact on the buildup of biogenic amines as the wine aged. Furthermore, the variety of barrels used (specifically American oak, French Allier oak, and French Nevers oak) had no impact on the levels of biogenic amines present in the content. In a research study, the wines that underwent MLF in tanks rather than barrels, and then aged with regular lees stirring either on a weekly or monthly basis, exhibited the highest levels of histamine (Smit et al., 2008).

Typically, storing wine at high or inconsistent temperatures can lead to undesired chemical, microbial, or enzymatic reactions within the wine's components, resulting in a significant decline in product quality. Nevertheless, it was discovered that the influence of wine storage temperature on amine concentration is minimal (Alcaide-Hidalgo et al., 2007). The levels of histamine were observed to slightly rise after wines were kept for 105 days, especially at a temperature of 20°C compared to the more extreme temperatures of 4°C or 35°C. During the initial 45-day storage period for all temperatures examined, the occurrence or breakdown of amines in wine predominantly happened, as a result of the presence of remaining decarboxylase activity following alcoholic fermentation and MLF (González Marco and Ancín Azpilicueta, 2006).

Vidal-Carou et al., (1991) discovered that there was no rise or development in biogenic amines (specifically histamine or tyramine) in wines stored under unfavourable conditions for a period ranging from 93 to 125 days, despite being exposed to temperatures between 4°C to 35°C. The biogenic amine content only showed changes in histamine and tyramine, which decreased regardless of temperature (Smit et al., 2008).

5.7.5. CHOICE OF FINING AGENTS

The utilisation of various clarification substances and oenological enhancers, such as bentonite or polyvinylpolypyrrolidone (PVPP), in typical winemaking additives, may impact the levels of biogenic amines in wines (Alcaide-Hidalgo, Moreno-Arribas, Polo, and Pueyo, 2008). The mentioned substances possess the capacity to absorb certain amines.

The presence of biogenic amines in wines is also a result of the different techniques used during the winemaking process. Some of these techniques promote the synthesis of amines, such as treating the wine and must with yeast mannoproteins or proteolytic enzymes. Conversely, other techniques, like the use of clarification substances and oenological additives such as bentonite and PVPP, are capable of absorbing biogenic amines and reducing their levels in the final products (Martuscelli and Mastrocola, 2018).

Moreover, the presence of mould in grapes has considerable effects on the initial concentration of biogenic amines found in grape must. However, the level of these amines cannot be effectively reduced by the fining agents currently used in the wine industry (Martuscelli and Mastrocola, 2018). Research has demonstrated that the use of bentonite can effectively decrease biogenic amines by means of absorption (Schneyder 1973). Nonetheless, there is a scarcity of available research on this topic. In addition, negative consequences can arise when using bentonite, including the loss of colour in red wines (Schneyder, 1973).

5.8. BIOGENIC AMINE LEVELS IN DIFFERENT STYLES OF WINE

The quantity of biogenic amines present in wines is crucial for assessing their quality and safety, along with their concentration of polyphenols and polysaccharides (Martuscelli and Mastrocola, 2018). Red wine has been found to contain a greater number of amines, as explained below, than white or rosé wine.

According to the literature (Zee et al., 1983), it is expected that white wines will have lower levels of biogenic amines (refer to Table 5.2) because they have fewer AA precursors, a lower pH resulting from no skin contact during fermentation and generally do not undergo MLF. Zee et al., (1983) pointed out that white and rosé wines that underwent MLF had biogenic amine levels similar to those found in red wine after MLF.

Various methods are employed to make red, white and rosé wines, leading to distinct chemical and physical-chemical properties that are also associated differently with their levels of biogenic amines (Plotka-Wasylka et al., 2018). Polyamines in wines may come from grapes or yeast breakdown, so the reason for the low levels of spermidine in rosé wines could be attributed to the fact that yeasts are not capable of releasing significant concentrations of polyamines (Broquedis et al., 1989).

White wine often contains higher levels of SO_2 to maintain its colour stability, especially during the post-malolactic fermentation process and prior to bottling. As a result, the total concentration of SO_2 can vary, but it must always remain within the legal limits of the country of origin (Moreno-Arribas et al., 2022).

The higher pH levels in red wines, compared to white or rosé wines, result inter alia from the process of MLF (explained in section 5.7.3) (Moreno-Arribas et al., 2022). Furthermore, Buteau et al., (1984) explained that the reason for the higher levels of biogenic amines observed in red wines is because they do not go through bentonite treatments (which absorb amines), and instead, cellular amines are released by yeast cell autolysis in lees during MLF.

Table 5.2: Typical biogenic amine levels (mg/L) in red and white wine in different countries

Wine	Biogenic amine	Canada	USA	South Africa	France	Switzerland	Spain	Germany
Red	Histamine	3.7	7.3	1-18	8.1	2.0	4.1	0-4
	Tyramine	4.3	8.6	0-16	7.3	2.8	3.0	0-5
	Putrescine	2.2	5.5	0-331	7.6	21.4	-	0-12
White	Histamine	1.9	3.6	0.1	4.4	1.5	0.8	-
	Tyramine	-	3.2	0-9	6.5	7.5	1.5	-
	Putrescine	1.3	1.7	0-42	2.3	11.1	-	-

Source: Unpublished data

6. REGULATION OF BIOGENIC AMINE LEVELS IN WINE

To properly assess the dangers of consuming biogenic amines through wine, it is essential to take into account that a long-term exposure scenario can be equivalent to a person consuming 150 mL (one glass) of wine per day. Additionally, in the context of the Mediterranean diet, it is common for women and men to consume approximately 100-200 mL of wine with meals (Martuscelli and Mastrocola, 2018).

It is essential to recognize that consumers need a favourable message about the product to spark their desire to purchase. However, it is equally important for consumers to be well-informed about any potentially harmful substances, taking into consideration their physical condition and any existing illnesses. (Martuscelli and Mastrocola, 2018).

Numerous countries such as Greece, Spain, Hungary, the USA (Oregon), South Africa, Argentina, Canada, Portugal, Italy and France have investigated the presence and concentration of biogenic amines in their wine (Bover-Cid et al., 2006; Gerbaux & Monamy, 2000; Gloria et al.,1998; Herbert et al., 2005; Soufleros et al., 1998; Moreno-Arribas et al., 2010). Wine contains a wide range of biogenic amine levels, from none at all to very high concentrations (factors explained in chapter 5).

The practice of using histamine levels as a gauge of wine safety and quality may have an impact on wine imports and exports to European nations. In Europe, the maximum limits for histamine are as follows: Switzerland and Austria (10 mg/L), France (8 mg/L), Belgium (6 mg/L), Finland (5 mg/L), Holland (3 mg/L) and Germany (2 mg/L) (Moreno-Arribas et al., 2010). These biogenic amines will not only impact on wine flavour and quality, but may also have a physiological effect on sensitive individuals.

While there currently do not appear to be any legal limits, certain countries have recommended maximum limits on histamine levels (mg/L) applied to imported wines (refer to Table 6.1). This can cause commercial import and export difficulties. The wine industry does not have established regulations by the European Union (EU), as the EU has only recommended “safety threshold values”. Certificates of analysis regarding the quantity of biogenic amines in wines are often provided when wines are imported into the EU, even if there are no EU regulations regarding the permissible levels of these substances in wines (Martuscelli & Mastrocola, 2018).

Table 6.1: Recommended biogenic amine levels in different countries

Country	**Histamine level (mg/L)**
Germany	2
Holland	3
Finland	5
Belgium	6
France	8
Switzerland	10
Austria	10

Adapted from Moreno-Arribas et al., 2010

Olivia Poonah (personal communication, January 2024), from the Wine and Spirit Board (Stellenbosch), indicated that there are no regulatory requirements or limits relating to biogenic amines in South African wines. Thus, the level of biogenic amines in South African wines currently only impact the potential commercial values of wines to be exported.

Should the wine label include details regarding the presence and quantity of biogenic amines, it would inform the consumer about the actual risk involved. This would serve two purposes: first, it would help the consumer avoid any unpleasant or potentially harmful health effects, and second, it would enable them to choose wines that have low or non-toxic amine content (Martuscelli and Mastrocola, 2018). These authors suggested that it would be beneficial to create a regulatory framework for wines, similar to the existing system for sulphites, that enables the disclosure of the levels of amines present.

This could be done after thoroughly gathering toxicological data and conducting an informative media campaign. Alternatively, and perhaps preferably, a label could be introduced declaring the wine to be histamine-free. Like any such regulation, however, it would be important to strike a balance between providing sufficient clear and understandable information to the consumer as opposed to swamping them with excessive amounts of information of limited use.

7. MITIGATING STRATEGIES TO DECREASE BIOGENIC AMINE LEVELS IN WINE

Enhanced biogenic amines can cause sensory alterations that may remain hidden (such as a loss of varietal character) or become apparent (such as a musty odour resembling tuna).
The existence of biogenic amines in wine is now more significant than ever to both the wine industry and consumers, as it poses a potential threat to human health and may also have trade repercussions. Understanding the levels of biogenic amines present in wines is pertinent to both producers and consumers, given their inherent significance. The surveillance of biogenic amine concentrations in wines can present a significant advantageous edge in marketing strategies (Martuscelli and Mastrocola, 2018).

Concerns about the wines that are currently produced have also been raised as a result of recent improvements in techniques for locating and measuring biogenic amines. Although the presence of biogenic amine concentrations in wine is usually not seen as a concern, it is suggested that the combination of ethanol and acetaldehyde could potentially intensify the harmful effects of these amines (Vinci et al., 2021).

It is particularly important to intervene early in wines that may have biogenic amines, by quickly identifying the bacteria responsible for their formation. This way, certain strains of bacteria can be eliminated and replaced with established commercial starter cultures that do not possess the specific decarboxylase genes. Once wine has matured, it becomes challenging to eliminate or minimise the amines that are present in it. It can be challenging to detect any changes in the flavour or smell of a product caused by amines since, in general, they usually do not impact these characteristics (Moreno-Arribas et al., 2010).

Currently, it is extremely challenging to find wines that do not contain biogenic amines and still preserve their organoleptic properties. Even if we could manipulate the important technological factors, especially the microorganisms in the fermentation process, it would still be difficult to achieve this. However, there is a chance to produce wines with low or moderate levels of biogenic amines that are safe for consumer' health (Vinci et al., 2021).

Prof M. Du Toit (personal communication, February 2024), Department of viticulture and oenology, University of Stellenbosch, indicated that reducing the levels of harmful LAB is the most important factor as it will lower the risk of the toxicological biogenic amines which are more harmful than those found in grapes. The biogenic amines from grapes mainly affect flavour and are not a risk to humans.

A substantial collection of information obtained from multiple production sites and varied vintage years is imperative to create biogenic amine profiles as a wine fingerprint. This will establish a scientific approach and basis for managing the winemaking process to guarantee safe levels of biogenic amines (Martuscelli and Mastrocola, 2018).

7.1. TO PREVENT AND DECREASE BIOGENIC AMINE FORMATION

The only viticultural methods that can be applied to control the build-up of biogenic amines in grapes are regulating the soil and vine nutritional status. However, it is also possible to control their existence in musts or wine by decreasing winemaking practices that result in amino acid extraction, such as grape skin maceration and lees contact (Smit et al., 2008).

The most viable approach to managing the potential creation of biogenic amines involves suppressing the growth of decarboxylase-positive indigenous bacteria and other microorganisms that contribute to this transformation. SO_2 is the conventional antimicrobial treatment employed in wine cellars to eradicate undesirable yeasts and bacteria during subsequent stages (Smit et al., 2008).

Vidal-Carou et al., (1990) discovered that red wines with low levels of SO_2 contain the highest concentrations of biogenic amines. Additionally, Vidal-Carou et al., (1990) observed a connection between higher levels of SO_2 and a decrease in the concentration of histamine and tyramine.

During the process of wine maturation, industrial studies have revealed that the introduction of SO_2 to red wines, at permissible concentrations that effectively lessen bacterial growth, serves as a preventive measure against the formation of biogenic amines throughout the ageing of the wine (Marcobal, Martín-Álvarez, et al., 2006). These findings suggest that it is advantageous to promptly introduce SO_2 after malic acid degradation in order to rapidly eliminate LAB and thus, inhibit the production of biogenic amines. Because numerous wines have elevated pH levels, the effectiveness of employing SO_2 can decline. As a consequence, there are situations where the levels of amines can elevate in wines as they mature (Smit et al., 2008).

The co-inoculation of *O. oeni* starter cultures with alcoholic fermentation appears to have greater potential in reducing the formation of biogenic amines compared to conventional inoculation during MLF after the alcoholic fermentation process is finished (Smit et al., 2008). The most widely employed approach to manage the production of amines during the process of winemaking is through the utilisation of malolactic starter cultures that possess specific attributes, such as a significantly reduced or non-existent ability to generate biogenic amines (Smit, 2007; Van der Merwe, 2007).

To assess the effect of various scenarios of MLF inoculation on the concentration of biogenic amines, the formation of biogenic amines during MLF was observed. The simultaneous inoculation of MLF and alcoholic fermentation has been proven to decrease the formation of biogenic amines in wine in comparison to the traditional method of inoculating them sequentially (Van der Merwe, 2007; Smit and Du Toit, 2011). It appears that this tool could effectively control the occurrence of biogenic amine contamination. The suggested method to reduce the buildup of biogenic amines is to introduce LAB starters right after the completion of alcoholic fermentation, or simultaneously inoculate rather than relying on natural occurrence of MLF (Martín-Álvarez et al., 2006).

In 2007, Alberto et al., carried out the initial research to explore the potential impact of phenolic compounds on the growth, metabolism and malolactic activity of LAB in wine. It had been previously acknowledged that these compounds could either inhibit or stimulate these functions. This study aimed to determine whether phenolic compounds also played a role in the production of biogenic amines by wine LAB with particular emphasis on how phenolic compounds impact the agmatine metabolism of *L. hilgardii* X1B (Smit et al., 2008). Phenolics have strong antioxidant properties and the decrease in putrescine production when phenolics are present can be credited to the capability of the phenolics to safeguard cells from oxidative stress (Alberto et al., 2007). The process of phenolic decarboxylation can also act as a rival to an enzyme that is responsible for transforming agmatine into putrescine, known as N-carbamoyl putrescine decarboxylase. Consequently, this hinders the creation of putrescine. According to this study, the presence of phenolic compounds could pose a natural solution to reduce putrescine formation in red wine (Smit et al., 2008).

Lysozyme is an enzyme capable of causing the breakdown of cell walls in Gram-positive bacteria, which also includes LAB that are found in wine. The effectiveness of lysozyme remains intact even in wines with higher pH levels. When used with SO_2, it has the ability to effectively hinder the growth of various LAB (Delfini et al., 2004; Ribérau-Gayon et al., 2006). Antimicrobial peptides called bacteriocins are created by certain types of LAB (Smit et al., 2008). The conclusion is thus that lysozyme addition will hinder and inhibit LAB growth and therefore controlling the formation of biogenic amines.

7.2. TO REMOVE ALREADY FORMED BIOGENIC AMINES

In the conventional method, SO_2 has been utilised in winemaking to manage undesirable micro-organisms. Typically, it is applied to bins containing machine-harvested grapes as well as after the process of MLF. In wine, SO_2 serves dual purposes as an antimicrobial substance and an antioxidant (Romano and Suzzi, 1993). Nevertheless, numerous types of bacteria can withstand high levels of SO_2. Filtration of juice or wine can be employed to physically eliminate microorganisms. Nevertheless, filtration is primarily employed before wine is bottled, so it is not typically utilised to eliminate microorganisms throughout the winemaking process. Additionally, in recent times, some winemakers have been avoiding filtration as they believe it may have a negative impact on the taste of the wine. As a result, alternative methods (refer to Table 7.1) such as chemical inhibitors and physical techniques have been sought after in order to prevent bacterial spoilage in wine (Bartowsky, 2009).

Table 7.1: Approaches to limit or halt bacterial growth in wine

Controlling agent	**Mechanism of action**
Traditional	
Sulphur dioxide	Inhibits the development of bacteria
Filtration	Physical removal of bacteria from wine
Chemical	
Dimethyl dicarbonate (DMDC)	Reacts irreversibly with the amino groups on active sites of enzymes
Natural products	
Lysozyme	Disrupts cell wall synthesis causing cell lysis
Bacteriocins	Alters cell wall components causing cell lysis
New physical technologies	
Ultrahigh pressure	Causes damage to cytoplasmic membrane and inactivates enzymes
High power ultrasound	Sound waves cause thinning of cell membranes, localized heating and production of free radicals
UV irradiation	Damages DNA
Pulsed electric fields	Dielectrical breakdown of cell membranes

Source: Bartowsky, 2009

7.3. MEASURING BIOGENIC AMINES

Techniques have been created to evaluate both the levels of biogenic amines and the potential danger posed by the natural wine microflora. The quantitative analytical techniques are fast, precise and sensitive enough to be used routinely. In the future, infrared spectroscopy could also potentially serve as an additional technology for screening purposes. The initial investigation showed that this technology possesses immense possibilities (Smit, 2007). It is recommended to employ PCR tests to detect the presence of genes responsible for decarboxylase enzymes in wines prior to MLF (Moreno-Arribas et al., 2010). To guarantee a high-quality product, it is crucial to repeat the qRT-PCR test at significant stages of fermentation, as it is the most preferable method for assessing and managing potential risks. This method is also a valuable means to measure the quantity of cells capable of synthesising biogenic amines (Moreno-Arribas et al., 2010).

PCR tests can identify lactic acid bacteria that produce biogenic amines in wine, which helps determine the potential risk of histamine, tyrosine, cadaverine, and putrescine formation.

There are several ways to measure biogenic amines – qualitatively through screening and enzymatic methods, semi-quantitatively via thin-layer chromatography, or quantitatively using liquid chromatography, capillary electrophoresis, or gas chromatography (Smit et al., 2008).

8. CONSUMER KNOWLEDGE OF BIOGENIC AMINES – A SURVEY

An exploratory (online) survey of wine consumers was carried out, the goals of which were to:

i. Estimate whether South African wine consumers had any knowledge about, in particular, biogenic amines and the health effects of wine consumption.
ii. Provide an indication of wine consumers' beliefs regarding the causes of health effects of wine consumption.

In addition to capturing the demographics of the sample (see section 8.1), the questionnaire contained questions aimed at establishing some of the preferences of the respondents (section 8.2), their knowledge about biogenic amines etc. (section 8.3), and their perceptions about the side-effects of wine consumption (e.g. headaches) and the causes thereof (section 8.4).

8.1. METHODOLOGY

The survey was carried out through Google Forms – see Appendix 1 for the Google Forms Questionnaire and response options. Defined response options were offered, with no option for respondents to provide free-form responses to questions.

The survey was done in the following way:

i. To ensure that the survey would target wine consumers, the sample population was drawn from the database of active wine consumers of an online and telephone-sales based wine outlet based in the Western Cape with national sales ("Winebrands Pty Ltd").
ii. The survey was conducted during June 2023. 151 customers from the company's database were asked to complete the survey and 109 responses were received (i.e. a response rate of 72%).
iii. The responses were downloaded to an Excel file in which the subsequent analysis was carried out.
iv. Descriptive statistics have been used in sections 8.2 – 8.4 to present the results of the survey. Statistical analysis was carried out relating to the questions covered in 8.5 below.
v. Chi-squared tests were carried out to determine whether statistically significant differences were observed in the data compared to the expected outcomes, i.e. whether any observed differences were due to random chance as opposed to a genuine relationship existing between the (categorical) variables in question. A "p-

value" was calculated for each such test and compared to the significance level (usually 0.05, representing a 5% level of significance). If the calculated p-value is less than or equal to 0.05 the null hypothesis (of no difference being present between the observed and expected outcomes) is rejected in favour of the alternative hypothesis (that a genuine difference does exist). A p-value above 0.05, while meaning that the null hypothesis cannot be rejected at the 5% level of significance, may still suggest that there is a trend towards an underlying difference.

None of the respondents could be regarded as experts in the field of winemaking. Three of the respondents could be classified as having more expertise than the general wine-buying population as they work as distribution agents. It was decided to retain these three responses in the survey analysis, as a number of wine consumers in the country will have similar knowledge and so excluding them could have made the sample less representative.

8.2. THE SAMPLE DEMOGRAPHICS

The demographics of the respondents is summarised in the table below:

Table 8.1: Demographic distribution of respondents by sex and age group

	Age range			TOTALS (by sex)
Sex	Below 40	40 - 60	60 and above	
Males	8% (9/109)	28% (31/109)	14% (15/109)	50% (55/109)
Females	13% (14/109)	25% (27/109)	12% (13/109)	50% (54/109)
TOTALS (by age group)	21% (23/109)	53% (58/109)	26% (28/109)	100%

This represents a good split across sexes and across age groups.

8.3. RESPONDENTS' PREFERENCES

Several questions in the survey targeted respondents' wine preferences, including: Preferred wine producing region; preferred price bracket; preferred packaging (e.g. glass bottle); preferred wine type (red, white/rosé, sparkling); whether foods such as aged cheeses were often eaten when consuming wine; and whether wines with no added Sulphates were preferred. These questions were aimed at helping confirm that the sample was a reasonable representation of the wine-consuming population in South Africa.

The results were as indicated below:

8.3.1. PREFERED WINE REGION

Table 8.2: Preferred wine region

Wine Region	No. of respondents
Stellenbosch	30% (33/109)
Robertson	13% (14/109)
Durbanville	9% (10/109)
Franschhoek	6% (7/109)
Paarl	6% (7/109)
Other	15% (16/109)
No specific favourite	20% (22/109)
TOTAL	109

This shows that the survey respondents, while having a preference for wines of the Stellenbosch region, enjoyed wines from a wide variety of regions. 20% of respondents did not single out one preferred region. This suggests that the sample is a reasonable representation of wine consumers. No conclusion could be drawn from this.

8.3.2. PREFERRED TYPE OF PACKAGING

Table 8.3: Preferred type of packaging

Type of packaging	No. of respondents
Glass bottle with cork	61% (67/109)
Glass bottle with screw cap	34% (37/109)
Bag in box	5% (5/109)
TOTAL	109

There was a preference for glass bottles with a cork closure. However, there was again a spread of responses indicating that the sample covered a range of wine consumers.

8.3.3. PREFERRED PRICE RANGE

Table 8.4: Preferred price range

Price range	No. of respondents
< R80	8% (9/109)
R80 – R130	57% (62/109)
R130 – R200	29% (32/109)
> R200	6% (6/109)
TOTAL	109

The vast majority (86%) preferred wines in the R80 – R200 price range. There were some, however, above and below this range.

8.3.4. PREFERRED TYPE OF WINE

Table 8.5: Preferred type of wine

Type of wine	No. of respondents
Red	62% (68/109)
White/Rosé	29% (32/109)
Sparkling	8% (9/109)
TOTAL	109

There was a preference in the sample group for red wine. However, there was again a spread of responses indicating that the sample covered a range of wine consumers.

8.3.5. FOODS CONSUMED WITH WINE

The surveyed individuals were asked whether they enjoyed eating aged cheese, cured meats or processed fish when consuming wine. 90% (98/109) respondents indicated that they did, with 10% (11/109) indicating that they did not. This could be relevant, in that these foods can contribute to higher levels of biogenic amines in individuals.

8.3.6. PREFERENCE FOR WINES WITHOUT ADDED SULPHATES

The surveyed individuals were asked whether they preferred purchasing wines that stated that they contained no added sulphates. 36% (39/109) indicated that this was the case, with 64% (70/109) indicating that this was not the case.

8.4. RESPONDENTS' KNOWLEDGE ABOUT BIOGENIC AMINES, ETC.

The survey included questions aimed at understanding the level of knowledge which wine consumers believe they have relating to the wine industry and biogenic amines in particular. It is difficult to assess someone's true knowledge of such things through this type of survey. However, the respondents' answers should provide an indication of their belief about whether they have such knowledge.

The results were as indicated below:

8.4.1. KNOWLEDGE ABOUT THE WINE OF ORIGIN CLASSIFICATION

50% (55/109) of respondents indicated that they were aware of the Wine of Origin classification system in South Africa. The remaining 50% (54/109) indicated that they were not aware of the classification. It is interesting to note that only half of the wine consumers in the survey were aware of this standard classification system.

8.4.2. KNOWLEDGE OF THE TERM BIOGENIC AMINES

Only 18% (20/109) of the survey respondents indicated that they had heard of the term biogenic amines. The remaining 82% (89/109) were not even aware of the term, let alone what their impact might be relating to the side-effects of wine consumption.

8.4.3. KNOWLEDGE ABOUT HISTAMINE, TYRAMINE, ETC.

26% (28/109) of respondents indicated that they knew what the physiological functions in the human body are of histamine, tyramine, putrescine and cadaverine (the main biogenic amines). 74% (81/109) indicated that they did not know their functions. Interestingly, the proportion claiming to be aware of the physiological functions of these main biogenic amines was much higher than the proportion who had heard of the term biogenic amines.

8.4.4. KNOWLEDGE OF LACTIC ACID BACTERIA

36% (39/109) of respondents indicated that they knew what the function of LAB is in winemaking. The remaining 64% (70/109) indicated that they did not know what the function is.

8.4.5. KNOWLEDGE ABOUT THE INFLUENCE OF pH IN WINE

52% (57/109) of the respondents answered that they were aware of the influence of pH in wine. The remaining 48% (52/109) indicated that they were unaware of its influence.

8.5. RESPONDENTS’ OPINIONS ABOUT CERTAIN SIDE-EFECTS OF WINE CONSUMPTION

Finally, the survey asked respondents questions pertaining to their own experience of the side-effects of wine consumption and their beliefs about the causes thereof.

The results were as indicated below:

8.5.1. EXPERIENCED SIDE-EFFECTS OF WINE CONSUMPTION

57% (62/109) of the survey respondents indicated that they had experienced headaches/flushes which they believed were caused by wine consumption. 43% (47/109) indicated they had not experienced such side-effects relating to wine consumption. This is a large proportion of the wine consuming population which emphasizes the importance of determining the underlying causes of such side-effects. Considering the cross-tabulation of sex against headaches/flushes experienced from wine consumption we notice the following:

Table 8.6: Cross-tabulation of sex against headaches/flushes

	Experienced headaches/flushes	
Sex	Yes	No
Male	51% (28/55)	49% (27/55)
Female	63% (34/54)	37% (20/54)
TOTAL	62/109	47/109

Performing a chi-squared test (with 1 degree of freedom) on the above data, with the null hypothesis that there is no difference in the likelihood of experiencing headaches/flushes from wine consumption based on sex, we obtain a p-value of 0.20. As this value is above the 0.05 p-value which would have indicated statistical significance at the 5% level, the null hypothesis (of no difference based on sex) cannot be rejected.

However, the data does suggest a trend towards a higher proportion of females experiencing such side-effects compared to males. This is supported by other studies (Jarisch et al., 1996) and could usefully be retested using a larger sample size. Considering the cross-tabulation of age group against headaches/flushes experienced from wine consumption we notice the following:

Table 8.7: Cross-tabulation of age group against headaches/flushes

	Experienced headaches/flushes	
Age group	Yes	No
Below 40	70% (16/23)	30% (7/23)
40 – 60	55% (32/58)	45% (26/58)
60 and above	50% (14/28)	50% (14/28)
TOTAL	62/109	47/109

Performing a chi-squared test (with 2 degrees of freedom) on the above data, with the null hypothesis that there is no difference in the likelihood of experiencing headaches/flushes from wine consumption based on age group, we obtain a p-value of 0.34. As this value is above the 0.05 p-value which would have indicated statistical significance at the 5% level, the null hypothesis (of no difference based on age group) cannot be rejected.

However, the data does suggest a trend towards individuals below age 40 experiencing more headaches/flushes related to wine consumption. This may, of course, be due to higher levels of wine consumption at younger ages, which was not surveyed.

8.5.2. CONTRIBUTORS TO SIDE-EFFECTS OF WINE CONSUMPTION: WINE TYPE

The individuals in the survey were asked to indicate which types of wine (red, white/rosé or sparkling) they believed would result in the greatest number of headaches.

The responses are summarised in the table below:

Table 8.8: Wine type believed to be most likely to cause headaches

Type of wine	No. of respondents
Red	40% (44/109)
White/Rosé	31% (34/109)
Sparkling	28% (31/109)
TOTAL	109

Respondents clearly have different experiences and beliefs about the impact of different types of wines on wine headaches. Considering the cross-tabulation of preferred wine type against the type of wine believe to cause headaches/flushes relating to wine consumption we notice the following:

Table 8.9: Cross-tabulation of preferred wine type against wine type believed to cause headaches

	Wine type believed to cause most headaches		
Preferred type of wine	Red	White/Rosé	Sparkling
Red	31% (21/68)	35% (24/68)	34% (23/68)
White/Rosé	59% (19/32)	19% (6/32)	22% (7/32)
Sparkling	44% (4/9)	44% (4/9)	11% (1/9)
TOTAL	44/109	34/109	31/198

Performing a chi-squared test (with 4 degrees of freedom) on the above data, with the null hypothesis that there is no difference in the type of wine believed to cause headaches /flushes from wine consumption based on the respondents' preferred type of wine, we obtain a p-value of 0.05. This is just sufficient to indicate statistical significance at the 5% level (and is certainly so at the 10% level of significance), suggesting that the null hypothesis (of no difference based on age group) can be rejected in favour of the alternative hypothesis that there is a difference. The data suggest that consumers prefer the type of wine which they believe is least likely to cause headaches/flushes from wine consumption, which is not unreasonable.

The difference is most notable for the respondents' who prefer white/rosé wine, where 59% believe red wine is most likely to cause such side-effects, compared to 22% for sparkling wine and only 19% for white/rosé wine. The proportions for sparkling wine are also quite distinct, but the number of individuals who preferred sparkling wine is very low.

8.5.3. CONTRIBUTORS TO SIDE-EFFECTS OF WINE CONSUMPTION: SULPHATES

39% (43/109) of the respondents indicated that they believe that sulphates in wine contribute to headaches related to wine consumption. The remaining 61% (66/109) did not believe this. Considering the cross-tabulation of the wine type believed to most likely cause headaches/flushes following wine consumption against the belief that wine containing sulphates causes headaches we notice the following:

Table 8.10: Cross-tabulation of wine type believed to cause headaches against the belief that sulphates cause headaches

	Sulphates in wine cause headaches	
Wine causing headaches	Yes	No
Red	36% (16/44)	64% (28/44)
White/Rosé	53% (18/34)	47% (16/34)
Sparkling	29% (9/31)	71% (22/31)
TOTAL	43/109	66/109

Performing a chi-squared test (with 2 degrees of freedom) on the above data, with the null hypothesis that there is no difference in the belief that sulphates in wine cause headaches based on the type of wine believed to cause most headaches, we obtain a p-value of 0.12. As this value is above the 0.05 p-value which would have indicated statistical significance at the 5% level, the null hypothesis (of no difference based on wine type believed to cause headaches) cannot be rejected.

However, the data does suggest a trend towards individuals who believe that white/rosé wine is most likely to cause headaches having more belief that sulphates cause headaches than is the case for those believing that other wine types are more responsible for headaches. This is consistent with the proportion of added sulphites in red wine generally being lower than that in white/rosé wines, due to the greater natural protection against oxidation afforded to red wines by their higher tannins.

8.6. CONCLUSION OF THE STUDY

It is interesting to note that while 39% of respondents were of the opinion that sulphates in wine contribute to headaches after wine consumption, only 18% were even aware of biogenic amines – let alone their potential to contribute to such side-effects. The fact that many people have experienced side-effects relating to wine consumption means that it is important to research and publicise the contributors to such side-effects. The lack of knowledge of biogenic amines makes this an important area for research and publication.

Respondents clearly have different experiences and beliefs about the impact of different types of wines on wine headaches. 40% believed red wine was the main contributor, 31% believed it was white/rosé wines and 29% believed it was sparkling wines. This suggests that wine consumption impacts different people in different ways.

The relationship between individuals' preferred wine type and the type of wine believed to cause headaches/flushes (described in 8.5.2) is an important one. This suggests that consumers prefer the type of wine which they believe is least likely to cause headaches/flushes from wine consumption. Improving consumers' knowledge of what causes such side-effects is thus an important issue.

It should be noted that the survey did not include the amount of alcohol consumed by the respondents. Further studies should include this factor, as it is likely to be a significant factor in the experiencing of side-effects from wine consumption. A larger sample size could have also contributed to the power of the statistical test, making it more likely to find statistically significant relationships.

9. CONCLUSION

Biogenic amines are naturally occurring contaminants in wine that stem from the action of decarboxylase microorganisms during the fermentation process which includes alcoholic- and malolactic fermentation. They pose significant health risks to consumers when present in high concentrations, manifesting symptoms akin to wine intolerance such as headaches, flushing, rashes, and nausea. These adverse effects, coupled with the impact on wine quality, underscore the importance of ongoing research in this area for the benefit of both consumers and producers.

The literature review conducted in this dissertation has provided valuable insights into the formation of biogenic amines in wine and the factors influencing their production. The synthesis of biogenic amines in wine is a complex interplay of various factors, predominantly influenced by the availability of amino acid precursors. The quantity of these precursors is subject to variation, affected by the viticultural and oenological parameters such as grape variety, regional characteristics, vintage differentiations, and specific winemaking practices. This dissertation thus highlights the importance of measuring the genes responsible for decarboxylase enzymes involved in the formation of biogenic amines.

The exploratory study of wine consumers carried out suggested that consumers did not have great knowledge of biogenic amines and their effects. The relationship found between individuals' preferred wine type and the type of wine believed to cause headaches/flushes suggests that consumers prefer the type of wine which they believe is least likely to cause headaches/flushes from wine consumption. Improving consumers' knowledge of what causes such side-effects is thus an important issue. The findings emphasize the critical role of consumer education regarding biogenic amines and their potential effects. The wine industry must balance the need for positive consumer perceptions with responsible communication about potential health hazards, especially for individuals with specific health conditions.

The review of current regulations and the examination of mitigation strategies highlight the need for continued efforts to control biogenic amine levels in wine. These mitigation strategies comprise of the prevention and removal of biogenic amines. Furthermore, the study advocates for the implementation of mitigation measures during winemaking, such as the use of selected microbial strains and the optimization of fermentation conditions to minimize biogenic amine production. By adopting these strategies, winemakers can produce wines with lower levels of biogenic amines, ensuring a safer and higher quality product for the consumer.

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APPENDIX 1 – SURVEY OF WINE CONSUMERS

A survey of wine consumers was carried out using the Google Forms platform. The questionnaire contained the following questions, with response options as indicated.

1. Gender
    - Male
    - Female

2. Age group
    - Below 40
    - 40-60
    - 60 and above

3. Where is your favourite wine production area from which you purchase most of your wine? i.e. Paarl, Robertson, etc.

4. Are you aware of the wine of origin classification system in SA?
    - Yes
    - No

5. In which packaging format do you purchase wine?
    - Bag in box
    - Tetra pack,
    - Glass bottle with screw cap
    - Glass bottle with cork

6. Have you ever heard of the term biogenic amines?
    - Yes
    - No

7. Do you think wine that states “contain sulphates” is the cause of headaches?
    - Yes
    - No

8. In which price bracket do you buy most (more than 50%) of your bottled wine?
    - Commercial (≤R80)
    - Average price class (R80-R130)
    - Premium wines (R130-R200)
    - Fine wines (≥R200)

9. Which wine class would you think would give more wine headaches and body discomforts?
    - Sparkling Cap Classique
    - Dry white and rosé wine
    - Red wine

10. What style of wine do you mostly consume?
    - Sparkling cap Classique
    - Dry white and rosé
    - Red wine

11. Do you enjoy aged cheeses, cured meats and processed fish with your wine?
    - Yes
    - No

12. Do you know the physiological functions in the human body of histamine, tyramine, putrescine & cadaverine?
    - Yes
    - No

13. Do you ever experience headaches/flushing from wine?
    - Yes
    - No

14. Do you know what the function is of lactic acid bacteria during winemaking?
    - Yes
    - No

15. Do you prefer to buy wines that states “no added Sulphates”?
    - Yes
    - No

16. Are you aware of the influence of pH in wine?
    - Yes
    - No