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PRODUCTION OF WINE FROM FRUITS AND GRAINS WITH LOW GYLCEMIC LOAD INDEX using *Saccharomyces cerevisiae*

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Abstract: Wine holds immense cultural and historical importance across societies. This study developed a wine formulation using readily available ingredients: pumpkin, papaya, and millets. During fermentation, organic acids in fruits and alcohol production caused the wine's pH to shift from alkaline to slightly acidic, enhancing taste and preventing contamination. Sugar content, crucial for alcohol production, decreased significantly after fermentation, indicating efficient conversion. Compared to a sweet red wine, the samples had lower sugar levels. Protein concentration, vital for wine analysis, varied across formulations, affecting clarity, stability, and sensory attributes. Total acidity, pivotal for taste and quality, was monitored over five weeks in different wine formulations, informing production and labeling decisions. Total phenolic content, contributing to color, flavor, aging potential, and health benefits, varied among formulations. Flavonoid content, with potential health benefits, differed across formulations. The study also isolated *S. cerevisiae* yeast during fermentation, indicating suitability in formulations 1 and 2 but inhibition in formulation 3 due to high alcohol content. This comprehensive analysis offers valuable insights into the chemical composition and characteristics of diverse wine formulations, highlighting the role of natural ingredients in wine production.

Key words: Fortification, yeast, volatile acidity, total acidity, TPC, TFC

1. INTRODUCTION

Wine has deep cultural and historical roots in various societies. It has been associated with rituals, celebrations, religious ceremonies, and social gatherings for centuries. The production and consumption of wine have played a significant role in shaping traditions and customs around the world (Harutyunyan & Malfeito-Ferreira., 2022). It is a complex mixture, consisting of both organic and inorganic compounds, including esters, high alcohols, fixed acidity (malic, tartaric and citric acid), sugars, aldehydes, tannins, pectins, vitamins and minerals. It can be defined as an alcoholic beverage made from grape juice or other fruits through fermentation of must by wine yeasts (Nemzer *et al*., 2021). Most wines have a total acidity content ranging from 0.3 to 0.55% (as tartaric acid and acetic acid). The Wine can be classified as table wine, sparkling wine, fruit wine, fortified wine, dry wine or sweet wine (Harutyunyan & Malfeito-Ferreira., 2022). They may also be classified on the basis of the countries of origin or fruit type from which they were obtained. For example red table wines are made from black grapes while white wines are made from black or white grapes. It is now known that it can be produced from other fruits such as oranges, bananas, mangos, pineapples, lemons, etc. and the wine so produced bears the name of the fruit used in its production (Vilela., 2019). Most wines consumed in the world are completely fermented, aged, bottled and imported ones. The temperature restriction of most grapes to temperate regions predisposes this trend. Imported products are costly now due to high duties paid on them. This had made imported wines too expensive to local consumers and for these reasons, there arose the need for more wine from other sources (Ohana-Levi & Netzer., 2023).

Mixed fruit wines, which are wines made from a blend of different types of fruits, have gained popularity in recent years. They offer a unique and diverse flavor profile compared to traditional grape wines. They offer a wide range of flavors and aromas that can't be achieved with grape wines alone (Zhu *et al*., 2023). Different fruits contribute their unique characteristics, creating exciting and novel taste experiences. People with varying taste preferences might find mixed fruit wines more appealing than traditional wines. These wines can be sweeter and fruitier, catering to those who enjoy a milder taste. It can be a great option for individuals who are allergic to grapes or who have dietary restrictions related to grape products (Chang *et al*., 2019). Like grape wines, some mixed fruit wines contain antioxidants that can be beneficial for health. Antioxidants help in resisting oxidative stress and inflammation in the body. Fruits used in mixed fruit wines are rich in vitamins and nutrients (Zhu *et al*., 2023). Depending on the fruit blend, these wines might provide vitamins such as vitamin C, vitamin A, and various B vitamins. Some fruits used in mixed fruit wines, such as berries, are associated with potential cardiovascular benefits. They might help improve heart health by reducing the risk of certain heart-related condition (Basu *et al*., 2010). Many fruits used in mixed fruit wines contain polyphenols, which are compounds with potential health-promoting effects. These compounds have been studied for their role in supporting various bodily functions. Some fruits contain dietary fibers that can aid in digestion and promote gut health. However, it's important to note that the health benefits of mixed fruit wines are generally similar to those of other wines and fruits (Cory *et al*., 2018). Moderation is key; excessive alcohol consumption can have negative health effects, and the sugar content in some mixed fruit wines might be higher than in traditional grape wines (Zhu *et al*., 2023). As with any alcoholic beverage, it's essential to consume mixed fruit wines responsibly.

In tropical areas, papayas, sometimes known as papaws or pawpaws, flourish. They have a pleasant flavour and a vivid colour. They provide a wide variety of health benefits which make them a popular fruit that includes reducing the risk of diabetes, cancer, heart attack, improving blood sugar regulation in diabetics, lowering blood pressure, aiding wound healing, and improving digestion (Basu *et al*., 2010). It is a fruit growing in a polygamous herbaceous plant and bears fruits throughout the year in a good tropical climate. The fruit remains in clusters near leaves, and after maturation and ripening, the fruits turn yellow or red (Zhu *et al*., 2023). It contains a lot of sugar (59%). The papaya fruit is a nutrient-dense diet high in vitamins, phytonutrients, and minerals. In contrast with different fruits like banana, guava, and apples, papaya has a lot more carotenes which facilitate the reduction of oxidative stress. Papaya wine is also regarded as a promising neutraceutical and an effective antioxidant (Santana *et al*., 2019).

Pumpkin (*Cucurbita moschata*) stands out as a notable fruit abundant in antioxidants, boasting a rich composition of active compounds, including beta-carotene, flavonoids, vitamin C, and vitamin E. These compounds, renowned for their antioxidant properties, exhibit the capability to counteract the actions of free radicals during instances of oxidative stress (Men *et al*., 2020). Remarkably, flavonoids exhibit dual potential, aiding in the reduction of insulin resistance while enhancing insulin sensitivity, thereby manifesting a hypoglycemic effect (Mokhtar *et al*., 2021). The pivotal role of pumpkin's antioxidant content in managing blood glucose levels becomes evident. Furthermore, this antioxidant arsenal plays a pivotal role in thwarting the accumulation of malondialdehyde (MDA) (Hussain *et al*., 2022).

Horse gram is a leguminous crop with high nutritional value in terms of protein, carbohydrates, dietary fiber, vitamins like thiamine, riboflavin and niacin and most of the minerals like calcium, phosphorous, molybdenum, zinc, iron and various types of phytochemicals and low fat (Prasad *et al*., 2014). Legumes in particular are considered as low glycemic index (GI) foods as they have beneficial effects in the management of diabetes (Basu *et al*., 2010). Reducing the glycaemic index of carbohydrate rich foods in diet may in decreasing the metabolic risk of various non-communicable diseases like obesity, cardiovascular diseases and diabetes (Prasad *et al*., 2014). Legumes can significantly reduce blood glucose, insulin, serum cholesterol and low density lipoprotein (LDL) cholesterol due to high total dietary fibers, soluble fiber, amylose, anti-nutrients and slow digestibility of starch in legumes (Amoah *et al*., 2023). Slow rate of starch digestion of legumes is however, considered the most important determinant of low glucose and insulin response to diabetes (Zhang *et al*., 2010).

Finger millet grains are rich in dietary fibers, protein, micronutrients and phenolic compounds, which make it more nutritious when compared to other most commonly, consumed cereals including rice and wheat (Devi *et al*., 2014). Finger millet grains can provide various health advantages to habitual finger millet consumers due to their antioxidant, hypoglycemic, hypocholesterolemic, anticancer, antibacterial, antilithiatic, anticataractogenesis, antiulcerative, and cardioprotective qualities (Hassan *et al*., 2021). Finger millet is considered as an ideal diet for people who are suffering from malnutrition, celiac disease and osteoporosis Antidiabetic properties of finger millet are mainly attributed to its high amounts of dietary fiber and phenolic compounds (Gowda *et al*., 2022). Phenolic compounds of finger millet have been recognized as effective inhibitors of α-amylase and α-glucosidase enzymes (Devi *et al*., 2014). It has been reported that the intake of dietary calcium and magnesium helps in reducing the risk of type II diabetes mellitus (Hassan *et al*., 2021). The objective is to create an affordable, diabetes-friendly wine with a low glycemic index using readily accessible natural ingredients such as papaya, pumpkin, and sprouted grains (finger millet and horse gram).

2. METHODOLOGY

2.1 Materials Procurement: Fresh papaya, pumpkin, and sprouts were acquired from a local market situated near K R Puram, Bengaluru. These items were transported to the laboratory using clean cellophane bags and an ice box to maintain their freshness during transit.

2.2 Starter Culture Activation: Dried yeast powder was procured from a local market in K R Puram, Bengaluru. The starter culture was prepared by dissolving yeast granules in lukewarm water and allowing them to sit at room temperature for 30-40 minutes.

2.3 Wine Formulation: Papaya (A) and Pumpkin (B) were meticulously washed with hot water to disinfect them. Subsequently, they were sliced and their juices were extracted. The extracted juice was then heated along with half the volume of sterilized water at 121°C for 2 minutes. Sprouted grains, having been washed with hot water for 30 seconds, were added to their respective jars in accordance with the various formulations described below.

1. Sprouted finger millet (D) +Sprouted horse gram(C) +Papaya (A)

2. Sprouted finger millet (D) +Sprouted horse gram(C) +Pumpkin (B)

3. Sprouted finger millet (D) +Sprouted horse gram(C) +Pumpkin (B) +Papaya (A)

2.3.1 Formulation 1: 1.2 kg of papaya were meticulously weighed, thoroughly washed, and completely peeled. Subsequently, 400 milliliters of juice were extracted, followed by the addition of 10% water to the must. The mixture was briefly boiled for 5 seconds at 135°C. Sprouted grains C and D were introduced to the must, along with 100μg of potassium metabisulfite (KMS) and Saccharomyces sp. The fermentation process was initiated and maintained at a temperature range of 28-30°C under anaerobic conditions (Niraj *et al*., 2022).

2.3.2 Formulations 2: 1.5 kg of pumpkins were weighed, thoroughly washed, and their juice (400 milliliters) extracted. To the pumpkin must, 10% water was added and the mixture was briefly boiled for 5 seconds at 135°C. After cooling to room temperature, sprouted grains C (50 grams) and D (80 grams) were incorporated, along with 100μg of potassium metabisulfite (KMS) and Saccharomyces sp. The fermentation process was conducted at a controlled temperature of 28-30°C under anaerobic conditions (Niraj *et al*., 2022).

2.3.3 Formulation 3: The papaya and pumpkin followed by the extraction of their respective juices. To the resulting juice, 10% water was added and the mixture was briefly boiled for 5 seconds at 135°C before being cooled to room temperature. Subsequently, both sprouted grains C (50 grams) and D (80 grams) were introduced, along with 100µg of potassium metabisulfite (KMS) and *Saccharomyces* sp. The fermentation process was initiated and maintained at a temperature range of 28-30°C under anaerobic conditions (Niraj *et al*., 2022).

2.4 Fermentation: The formulated mixture was combined with the starter culture, and potassium metabisulfite (KMS) was added to prevent contamination. The jars were sealed airtight and stored in darkness at room temperature for a period of 21 days, during which fermentation occurred. Following the 21-day fermentation period, the contents were filtered using a muslin cloth, with the solid residue being discarded and the supernatant collected in a separate jar. These jars were then set aside for aging. After a subsequent 60-day aging period, the jars were opened, and further analyses were conducted.

2.5 Physical-chemical characterization of wine: The wines resulting from different formulations underwent evaluation for their physico-chemical characteristics. After fermentation, the pH of all the wine samples was determined using digital pH meter. Total reducing sugars were determined spectrophotometric ally using dinitrosalicylic acid (DNS) method. Protein content was estimated by Bradford's method using bovine serum albumin as standard. Total titratable acidity (TTA) and Volatile acidity (VA) was also determined.

2.6 Total Phenolic content: Total polyphenols content was determined by the Folin–Ciocalteu method (Pincemail *et al*., 2019). Appropriately the wine samples (3.6 mL) were mixed with 0.2 mL Folin–Ciocalteu reagent and 3 min later, 0.8 ml sodium carbonate (20% w/v) was added. The mixture was incubated for 40 min. Later, the absorbance at 750 nm was measured. Using gallic acid (GA) as a standard, results were expressed as mg gallic acid equivalents/par liter (GAE) L^{-1}.

2.7 Total flavonoid content: Total flavonoids were determined using the aluminium chloride colorimetric method. The absorbance was measured at 510 nm and results were expressed as mg of quercetin equivalents for wines (Aryal *et al*., 2019).

2.8 Isolation of microorganisms from the formulation: Microbial analysis of the fermentation broth was performed using Nutrient Agar (NA), MacConkey Agar (MA) and Potato Dextrose Agar (PDA). The nutrient agar used was treated with fulcin (50 mg/20 ml of NA) to suppress fungal growth while the PDA was treated with chloramphenicol. The cultured plates were inoculated with 0.1 ml of formulated wine samples and incubated at room temperature. The pure cultures of the colonies were obtained by streaking and identified based on colonial characteristics, microscopy, biochemical reactions and carbohydrate utilization. The fungi were identified only by their cultural characteristics and microscopy.

3. RESULTS AND DISCUSSION

3.1 Physical-chemical characterization of wine

3.1.1 Determination of pH: During course of fermentation pH of the wine decreased due to two main reasons that is presence of organic acids (citric acid, malic acid and tartaric acid) in fruits and production of alcohol (Mendes Ferreira & Mendes-Faia., 2020). Hence, the pH readings were recorded before fermentation and after fermentation (Table. 1). According to pH values the wine before fermentation was alkaline in nature but after fermentation the pH values decreased making it slight acidic. This acidic condition makes the wine tastier and also prevents the contamination.

Sample	pH	
	Before fermentation	After fermentation
Formulation 1	7.5	6
Formulation 2	9.1	5
Formulation 3	8	5.4

Table.1: pH of the wine

3.1.2 Determination of Sugar content: Sugar content was measured by using the dinitro salicylic acid method and results were noted (Fig. 1). According to which the Formulation 1 had a low sugar content of 140µg/ml, formulation 2 had a highest sugar content of 600µg/ml, whereas formulation 3 had a moderate sugar content of 450µg/ml before fermentation. This information is useful in predicting the amount of alcohol that could be produced through fermentation, as the sugar content is a primary factor in the production of alcohol.

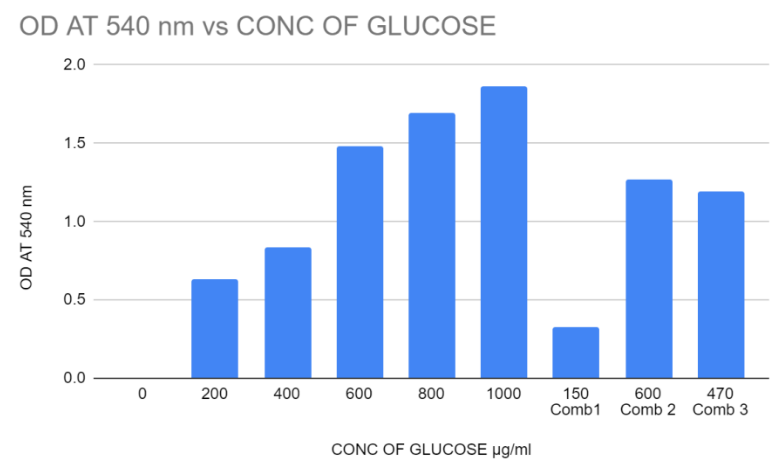

Fig. 2: Concentration of reducing sugar before fermentation

Later after conducting fermentation, it was found that most of the sugars had been converted, leaving only small quantities in the samples (Fig. 2). Formulation 1 had a low sugar content of 20µg/ml; formulation 2 had 25µg/ml, while formulation 3 had 30µg/ml. This indicates that the fermentation process was effective in converting the sugars present in the formulation. The sugar content of a sample was compared to that of a commercially available sweet red wine and found to be lower. The sample had a sugar content of 140µg/ml. This information provides insight into the relative sweetness of the two products and can be useful in making informed decisions when selecting a wine.

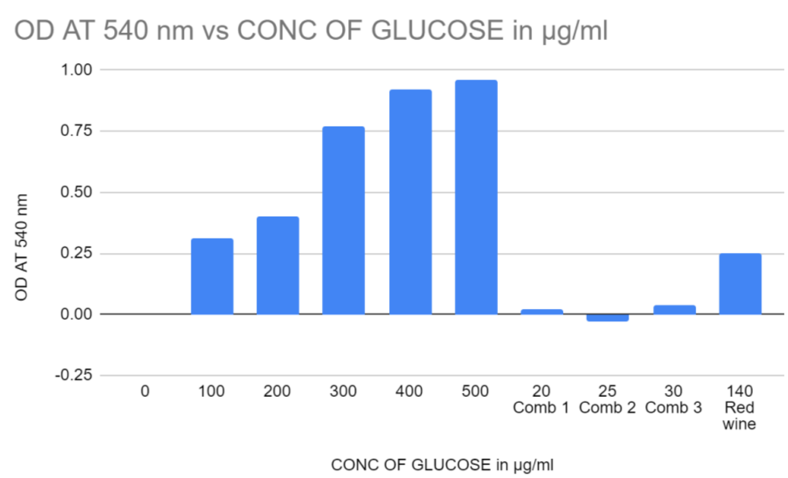

Fig. 3: Concentration of reducing sugar after fermentation

3.1.3 Determination of protein content: Presence of proteins in wines adds to their nutritional quality and therefore, increases their caloric value. Elevated protein levels can lead to wine haziness and unsightly cloudiness due to interactions with phenolic compounds and tannins. This can mar the wine's visual appeal, necessitating monitoring and adjustments for improved clarity and stability. Proteins can bind with aromatic compounds, influencing the wine's aroma and flavor profile (Cosme *et al*., 2020). Skillful control of protein levels preserves the wine's intended sensory attributes. In the resent study, the protein concentration was measured using Bradford's technique and results were recoded and compared with standard (Fig 4). According to which the protein concentration for formulation 1 was found to be 1850 µg/ml, for formulation 2 it is 2500 µg/ml and Formulation 3 is 3200 µg/ml.

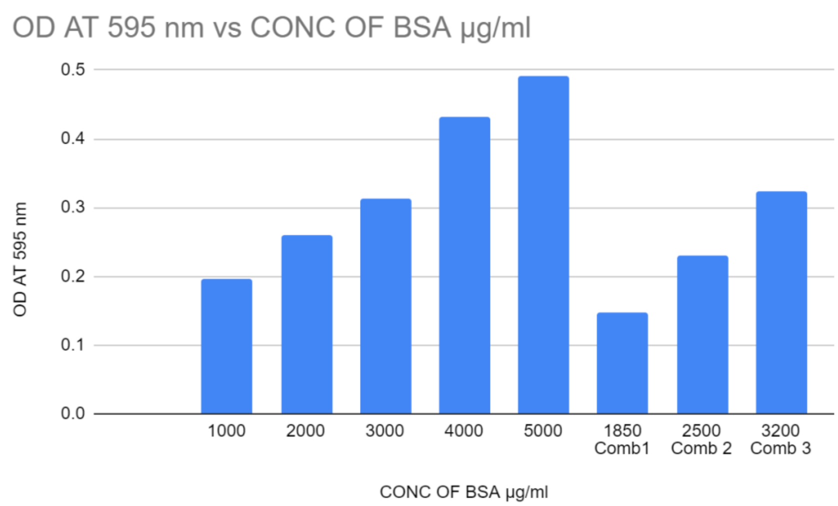

Fig. 4: Results for protein concentration

3.1.4 Determination of Total and Volatile Acidity: Total acidity of wine is an important factor that can significantly influence its taste, stability, and overall quality. It refers to the sum of all the acids present in the wine, primarily tartaric, malic, and citric acids. Volatile acidity (VA) in wine refers to the presence of volatile acids, primarily acetic acid (vinegar-like acid), in the wine. While some level of volatile acidity is natural and can contribute to a wine's complexity and aroma, excessive levels of volatile acidity are considered a fault and can negatively impact the wine's quality (chidi *et al*., 2018). Hence, in the present study over a period of five weeks, the total acidity of wine samples was measured to identify the acidity levels of three different formulations and a commercially available wine (Table. 2). Using a 4:6 ratio of 4 ml of wine and 6 ml of water, the total acidity present in the wine was determined. The results of the study provide important information about the acidity levels of different formulations of wine, which can help inform the production and labeling of these products.

Samples	Number of weeks	% of volatile acidity (acetic acid)	% of total acidity (tartaric acid)
Formulation 1	1	0.052	0.111
	2	0.052	0.113
	3	0.054	0.116
	4	0.068	0.121
	5	0.069	0.125
Formulation 2	1	0.056	0.145
	2	0.061	0.149
	3	0.068	0.155
	4	0.072	0.159
	5	0.077	0.166
Formulation	1	0.061	0.127

3	2	0.061	0.127
	3	0.061	0.128
	4	0.068	0.137
	5	0.075	0.145
Red wine	1	0.099	0.123
	2	0.099	0.123
	3	0.099	0.123
	4	0.101	0.128
	5	0.108	0.130

Table. 2: Results for total and volatile acidity of wine

3.2 Determination of total phenolic content: Total phenolic content is an important chemical parameter in wine analysis that measures the concentration of phenolic compounds in the wine. Phenolic compounds are a diverse group of naturally occurring organic compounds found in wine and other plant-based foods. They play a significant role in the sensory characteristics, aging potential, and health benefits of wine (Gutiérrez-Escobar *et al.*, 2021). Therefore samples were diluted in a ratio of 1:3 (sample: water) in order to identify the phenolic compounds present in the formulation (Fig. 5). This dilution likely allows for a more accurate analysis of the sample, as it helps to minimize any interference from other substances that may be present. The results revealed that the formulation 3 had more phenolic content compared to others.

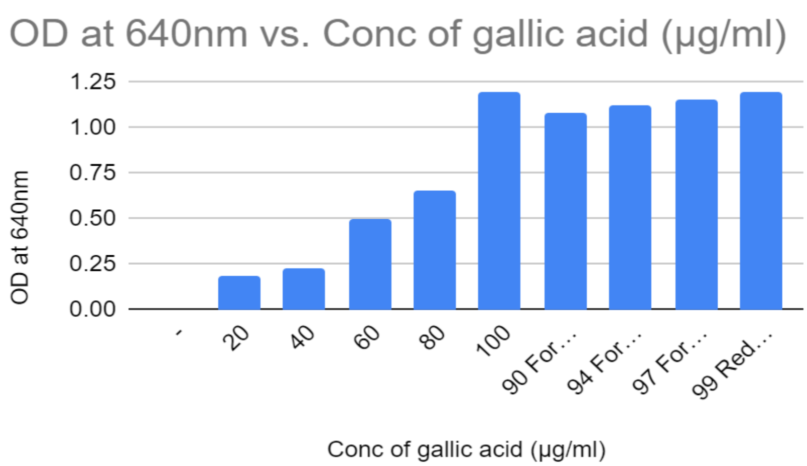

Fig. 5: Results of TPC for the formulation

3.3 Total flavonoid content: Flavonoids are a group of polyphenolic compounds found in wine, primarily in the skin, seeds, and stems of grapes. They contribute to the color, flavor, and health benefits of wine. Flavonoids are a subclass of phenolic compounds, and they are one of the most significant groups of naturally occurring antioxidants in wine. The presence and concentration of flavonoids can vary depending on grape variety, terroir, and winemaking methods and they contribute to the diverse range of wine styles available to consumers (Panche *et al.*, 2016). In the present study, the aluminum chloride method was used to estimate the total flavonoid content in the samples (Fig. 6). A dilution ratio of 0.5:3 (formulations: distilled water) was employed. Among the different formulation, the TFC was found high in formulation 3. Flavonoids are important substances that exhibit anti-diabetic properties.

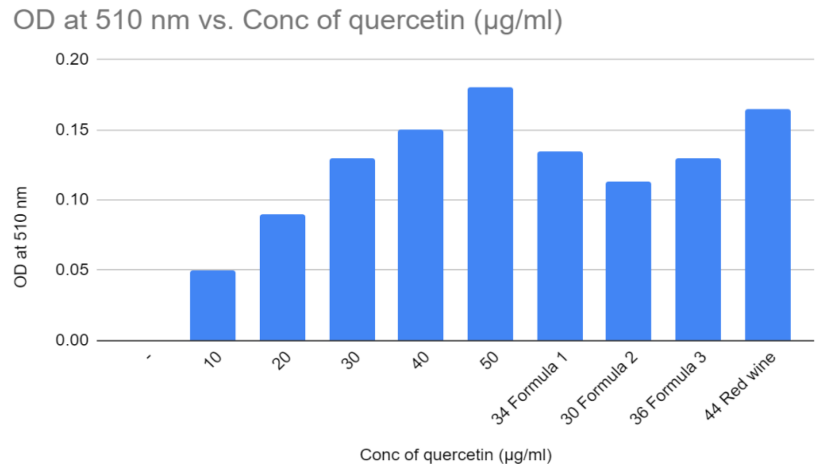

Fig. 6: Results for TFC for the formulation

3.4 Isolation of microorganisms from the formulation:

In the present investigation, the test fermentation yeast (*S. cerevisiae*) was the only organism isolated from formulation 1 and 2. This is an indication of good quality. The findings can be related to the wines' low pH values, high acidity, and high alcohol concentrations, which are known to hinder pathogen growth and provide fermenting yeast with a competitive edge in the natural environment, as described by Reddy and Reddy (2005) and Chilaka *et al*. (2010). The absence of the growth of the yeast in formulation 3 could be due to the high alcoholic content which exceeded the ethanolic tolerance level of the yeast used for fermentation.

4. CONCLUSION: In conclusion, this study delves into the intricate world of wine production and analysis, exploring the effects of natural ingredients on various chemical parameters and the suitability of fermentation conditions. The findings provide significant insights into the composition and characteristics of different wine formulations. The shift in pH from alkaline to slightly acidic during fermentation underscores the transformative nature of the winemaking process. This change not only enhances the wine's taste but also acts as a safeguard against contamination, ensuring the final product's quality. The analysis of sugar content before and after fermentation reveals the effectiveness of the process in converting sugars into alcohol. Comparing these results with a commercially available sweet red wine suggests that the formulations created in this study contain lower sugar levels, which may cater to consumers seeking wines with reduced sweetness. Protein concentration assessment underscores the importance of monitoring and controlling protein levels in wine production. This factor influences clarity, stability, and sensory attributes, necessitating careful management to achieve the desired visual and taste qualities. The monitoring of total acidity over a five-week period provides valuable data for both production and labeling considerations. This information ensures that acidity levels align with quality standards and consumer preferences. The examination of total phenolic content highlights the potential of different wine formulations in terms of color, flavor, aging potential, and potential health benefits. Variations among formulations open doors to diverse wine styles and applications. Similarly, the evaluation of flavonoid content offers insight into the potential health benefits of these compounds and their presence in different wine formulations. Lastly, the isolation of *S. cerevisiae* yeast during fermentation demonstrates the suitability of the fermentation conditions in formulations 1 and 2, while formulation 3 poses challenges due to excessive alcohol content. Collectively, these findings shed light on the complexity of wine production and the role of natural ingredients in shaping wine characteristics. They provide a foundation for further research and innovation in winemaking, offering opportunities to craft wines with diverse attributes to cater to a broad range of consumer preferences and needs.

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Conflict of interest: None

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