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Biochemical Changes in Main and Auxiliary Fermentation in Wine Technology

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Abstract
Wine is an alcoholic beverage (content from 5 to 13) fermented from ripe fruit with a distinctive flavour. The winemaking process goes through many complex stages in which primary fermentation and secondary fermentation are central processes. The yeasts used for these two processes are Saccharomyces cerevisiae and several other yeasts of the same Saccharomyces family. Under the influence of yeast and suitable technological conditions, the sugars and nutrients in the grapes are converted into ethanol and aromatic compounds specific to each grape wine. At the same time, in this process, harmful compounds are also removed.

Keywords: Wine, Grapes, Saccharomyces Cerevisiae, Primary Fermentation, Secondary Fermentation

1. What is wine?

Wine is made from fruit juice, by natural fermentation. To produce fruit wine, people use ripe fruits with special flavors such as grapes, pineapple, strawberry, ...

Wine has characteristics: low alcohol, natural product derived from fruit, high nutritional value, no harmful chemicals.

The most popular type of wine in the world and in Vietnam is grape wine, the main ingredients are ripe grapes: red grapes, white grapes.

Fruit wine is an alcoholic beverage that is naturally fermented from ripe fruits with special flavors such as grapes, bananas, peaches, pineapples, strawberries, etc. Wines usually have an alcohol content of between 5 and 5. 13% and is usually named after the fruit used to ferment. Wine often has a taste similar to that of fresh fruit and can be stored and transported under normal conditions. Because it is fermented from fresh fruit and not through the distillation process, the wine retains almost all the nutrients in the fruit. The nutritional value of wine is even increased by the release of amino acids and other nutrients from yeast during fermentation. Fruit wine contains from 8 - 11% alcohol and 2 - 3% sugar with energy from 70 -90 kcal/100ml.

2. Wine classification

Wine often contains ethyl alcohol, sugar, acids, higher alcohols, tannins, aldehydes, and esters. This is a fermented beverage dating back to ancient times, mentioned in the bible

and in the historical record of several Asian countries. Depending on the soil, the maturity of fruit, chemical composition of fruit, additives used, fermentation technology and age of wine, alcohol and sugar content, wine can be classified into several categories such as natural wines (9-14% alcohol) and aperitifs (15-21% alcohol).

The classification most commonly used by wine professionals is by color of wine: red wine and white wine then rose and sparkling wine.

Grape wine is made from only grapes and in the production, process does not use any other ingredients (except sugar and oak barrels).

Wine can be fermented from many other small basic ingredients, may also be added flavors from fruit, flowers and herbs. These wines are made from the juice of peaches, apples, bananas, pineapples, mangoes, and jackfruits. Cherries are usually made from cherries because they can provide enough acidity to the wine. Plant wine is produced from the juices of plants such as maple, birch, melon, and watermelon, and other garden plants such as rhubarb, parsley, and rose petals. Raisin wine is made from raisins. Multisort wine is produced by mixing different grapes and wine ingredients. Depending on the age of the grape varieties fermenting and the fruit color wines are classified as in red, white and rosé wines.

3. The process of making wine from grapes

Grape wines can be made from green grapes (which make white wine) or red grapes (which make red wine). The process of making wine from grapes includes the main stages: harvesting, crushing, juice collection (for white wine), fermentation, maturation, stabilization, filtration and bottling.

4. Structure and composition of grapes

Grapes are composed of 4 main parts; each part has its own components and characteristics:

- **Wax layer:** the outermost layer has the effect of protecting the fruit, preventing water loss of fresh fruit. As for the winemaking process, it again blocks the entry of enzymes during fermentation.
- **The peel:** usually accounts for about 10% of the fruit weight, the main chemical composition of the peel is pectin, the peel is relatively durable, difficult to be decomposed by enzymes, the peel contains most of the flavoring compounds. color for wine.
- **Fruit pulp:** accounting for about 80% of the fruit weight, containing mainly water, sugar and organic acids. This is the part that is easily broken down by enzymes in the winemaking process.

When performing wine fermentation from grapes, the first thing to do is to break the structure of the fruit, creating the best conditions for fermentation. This process can be carried out mechanically by grinding method. To facilitate the fermentation process, some hydrolytic enzymes such as pectin lyasa can be used.

5. The main fermentation process in winemaking

Wine fermentation is a complex process, influenced by many conditions. The grape itself, on the surface of the skin, contains a very diverse system of fungi, bacteria, viruses and microorganisms.

It can be imagined that the process of wine formation includes the process of nutritional metabolism (glycolysis), assimilation, and conversion of compounds present in grapes to form wine, flavor, taste and color for wine.

Accordingly, yeast converts nutrients from small fruit into ethanol, carbon dioxide. Other compounds such as acids, polyphenols, partly metabolized, partly released from the rind give the wine its flavor. To accomplish this conversion, several strains of wine-fermenting yeast that can be used are:

- **Saccharomyces vini:** These are oval-shaped cells, average size: (3¸8) x (5¸ 12) mm, reproduce by budding and have the ability to form spores. In S.vini juice juice containing more than 80% of total Saccharomyces, they have the ability to produce extracellular invectase to convert sucrose into fructose and glucose. Therefore, S.vini can be used to ferment alcohol from the juice. The fruit has added sucrose at a rather high concentration. S. vini has the ability to tolerate alcohol at high content and can produce 18¸ 19%V of alcohol, the ability to precipitate after finishing fermentation of S.vini is quite fast, making the product fragrant, very distinctive taste.
- **Saccharomyces cerevisiae (also known as: S.cerevisiae var ellipsoideus):** These are elliptical-shaped cells, similar in size to Saccharomyces vini. Many studies have shown that: S.cerevisiae not only has high alcohol tolerance, but also the ability to ferment and create alcohol is much higher than that of some strains such as: S. festinans, S. oviformis. S. cerevisiae can produce 18¸ 20% V alcohol. In addition, the ability of S. cerevisiae to settle and create flavor and characteristic flavor for products is also very good. Therefore, in the production of fermented fruit juice with high alcohol content, people often use S. cerevisiae for fermentation.
- **Saccharomyces oviformis:** has the same shape and size as S.vini but the ability to reproduce and develop at an early stage is slower than that of S.vini, due to its rather good alcohol tolerance, the number of cells still continues. gradually increased during fermentation. S. oviformis does not ferment galactose, and can form a film on the surface of products containing 10% V of alcohol. S. oviformis has the ability to ferment sugar to exhaustion and the alcohol level can reach 18%V, the product has good flavor and taste, so it is often used to produce all kinds of sugar-depleted fermented products.

During the fermentation process, there are many factors that affect the quality and content of wine. Among them should be noted:

Fermentation conditions

1. Calculation function from 12 -16%
2. Weak acidic pH + Fermentation temperature ranges from 18 280C
3. Fermentation time from 1-14 days
4. Fermentation equipment is usually stainless steel or wooden barrels with large volume.

Changes that occur during the main fermentation process

During the main fermentation of wine at relatively high temperature, high concentration of sugar, yeast mainly ferment converts sugar into alcohol and by-products.

$$C6H12O6 \rightarrow C2H5OH + CO2 + Byproduct$$

Factors affecting the main fermentation process

- **Temperature:** Temperature has a direct effect on the reproduction and growth of yeast and the quality of the product. Yeast can survive and grow at temperatures from 4÷450C, but the most suitable temperature for the reproduction and development of yeast is 28÷300C. The lower the temperature, the slower the yeast reproduces, the longer the fermentation time, but limits the infection, and vice versa, the higher the temperature (exceeding 380C), the faster the yeast reproduces., fermentation time is shorter, but the possibility of bacterial infection will be very high.
  On the other hand, temperature also greatly affects the quality of the product; When fermenting at cold temperature, the flavor and taste of the wine will be fruitier and fresher, the volatile acid content will be lower, and the glycerol content will increase more.
- **pH:** Yeast can reproduce and grow in an environment with a pH of 2.5÷7.5, but according to most authors, the pH most suitable for the reproduction and development of yeast is 4 6. Yeast strains can reproduce and grow well at pH 3÷3.5. Therefore, in order to limit the infection and growth of many types of bacteria, in accordance with the natural pH of the raw materials and

the quality of the product, in production, manufacturers always maintain the pH at 3, 2÷4, 0. To adjust the pH of the fermentation broth, people use $NaHCO_3$, $CaCO_3$, tartric acid, citric acid. Too low or too high pH will also change the protein structure of many enzymes directly involved in the wine fermentation process and reduce the enzyme's ability to activate.

- **Alcohol concentration:** Each yeast variety has the ability to tolerate different alcohol concentrations, there are varieties that are tolerant to alcohol at low concentrations such as Hansenula, Anomala and some wild yeasts that can only tolerate 3%V but many strains of fungi. Saccharomyces yeasts can tolerate 9÷12%V of alcohol and ferment up to 14÷16%V. During alcoholic fermentation, the alcohol concentration gradually increases, which inhibits not only the live activities of yeast but also inhibits the activity of many enzymes that convert sugar into alcohol, the ability of yeast to survive. It depends on the temperature and the alcohol concentration in the fermenter.
- **Sugar content:** Yeast reaches the fastest fermentation rate when the sugar content in the fermenter is from 1÷2%, but for maximum alcohol yield/gram of fermented sugar, the maximum sugar concentration must be much higher. Many studies show that: sugar content suitable for fermentation is 12÷20%, while at 25% sugar content inhibits fermentation.
  On the other hand, the ratio of sugars in the fermenter also significantly affects the fermentation speed and efficiency. Research results of many authors have shown that: glucose is the most suitable sugar for the reproduction and development of yeast as well as alcohol production, followed by fructose and sucrose... The higher the reducing sugar content, the more beneficial it is to the fermentation process and the quality of the product.
- **Organic acids:** Little attention has been paid to the influence of non-volatile organic acids on fermentation, because many times the influence of organic acids is nested within the influence of pH. Many research results show that: acid is an important ingredient to reduce pH, inhibiting many types of bacteria. The influence of organic acids on fermentation is not due to the total content of non-volatile organic acids but to the composition and content of some organic acids present in the fermentation broth, fatty, acetic, butyric, propyonic all have a decisive influence on the reproduction and development of yeast.
- **Nitrogen content:** Nitrogen is an important component involved in the formation and development of cells in general. Almost every component of the cell contains nitrogen in varying proportions. Protein accounts for 25÷60% of the dry weight of the cell (in which total nitrogen has 4÷12%), nitrogen in the form of NH_2 accounts for about 75%V. In fact, when breeding yeast, people often add lysine, arginine or $(NH_4)_2SO_4$ at a concentration of 0.6% (calculated by nitrogen) to enhance the reproductive capacity of yeast.
- **Types of mineral salts:** It has been confirmed that the elements N, P, S. play an important role in the formation of cell membranes and nucleus, Cu, Ca, and Mg are the activation centers of many enzymes involved in the cell metabolism. the process of converting sugar into alcohol (Cu is present in polyphenol oxidase, Mg is present in many cofactors of enzymes, in addition to phosphate salts, K, also acts as a buffer solution to keep the pH of the environment stable.
  Sources of mineral salts needed for fermentation usually include: Mg, K, I_2, Ca, Co, Fe, S, P.., in the juice, there are already full components of minerals, the addition of various types. Minerals should be very cautious and depending on the type of yeast, the nature of the juice. any excess of mineral elements can inhibit the fermentation process.
  Some vitamins (B1, biotin, E..) also have a very good effect on the reproduction and development of yeast, yeast also has a very rich vitamin composition in terms of type and content, ending the process. The yeast fission sequence released amino acids and alcohol-soluble vitamins, making the total protein content in the product increase 3-4 times. The presence of amino acids and vitamins in the product has significantly increased the sensory value of the product. Many vitamins also have the effect of enhancing the activation of certain enzymes during alcoholic fermentation.
- **Density of yeast seed:** The lower the content of yeast cells in the fermenter, the longer the propagation and fermentation time will be, and the easier it will be to get bacterial infections. The fermentation time will be shorter, limiting the possibility of infection due to the overwhelming yeast. But too high a yeast content will change the composition of the fermentation medium and will not be beneficial to the fermentation process and the product quality. In general, according to many authors, the content of yeast in the fermenter should only be 4÷10 million cells/1ml of the fermenter, which is appropriate.
- **Disinfectant:** The use of SO_2 as a disinfectant was known in the mid-20th century and is still widely used today in the production of fermented products. SO_2 can be added directly as a gas, or it can be used as several salts: kalimetabisulfite ($K_2S_2O_5$), sodiummetabisulfite ($Na_2S_2O_5$) or metabisulfite (liquid SO_2).
  All forms of SO_2 in this equilibrium are considered free SO_2, and HSO_3^- (bisulfite) can react with aldehydes, dextrins, pectic, proteins, and sugars to form a-hydroxy sulfuric acid (the stable form of SO_2. stable).
  The sterilization property of SO_2 is mainly due to the free form, the sterilization ability of SO_2 is very good for many bacteria, but yeast can be adapted to grow and ferment in an environment with SO_2 in the lower range. high quantity. However, SO_2 has the effect of slowing down fermentation (because SO_2 reduces the oxidizing capacity of the fermenter).
  SO_2 increases the amount of acetaldehyde and glycerol in the fermentation process, and SO_2 also limits the malolactic fermentation, so the use of SO_2 in the fermentation process also needs to be calculated in accordance with technology and common sense. Using SO_2 with a concentration of 75-100 ppm as a biocide and as an antioxidant is appropriate and has very good results.
  In addition to the factors mentioned above that have a great influence on the fermentation process, the presence of tannins, pectins, nutrients... in the fermenting juice also has certain effects on the fermentation process and is usually treated immediately

before fermentation.
- **Oxygen:** Aeration provides oxygen, stirring the environment has an impact on the growth and development of yeast. Therefore, although alcoholic fermentation is an anaerobic process, when fermentation in the early stages, especially in the propagation stage, it is necessary to expose the juice to oxygen of the air.

6. Secondary fermentation

Secondary fermentation is the process of ripening wine, forming flavors, clarifying and stabilizing wine quality.

After the main fermentation is complete, carry out secondary fermentation under stable temperature conditions from 15 - 180C, time from 1 to 3 months in stainless steel tanks or wooden barrels. During the secondary fermentation in wine, yeast continues to function, performing important metabolic processes that increase flavor quality, clarity, and eliminate harmful substances in wine such as:
- Continue alcoholic fermentation at a slower rate
- Removal of aldehydes
- Settle in the constituents that make the wine cloudy
- Continue to form esters that form the fragrance of the product.

6.1 Malolactic and malo-etanolic fermentation

The malolactic fermentation is carried out by lactic acid bacteria such as O.oeni, immediately following the alcoholic fermentation by the yeast S. cerevisiae.

6.2 The process of creating flavor for wine

Compounds in the fermentation fluid make the taste of wine not harmonious, shock like compounds of nitrogen, H2S formed during fermentation, compounds with sulfide odors such as rotten eggs, rotten cabbage. A mixture of several strains of Saccharomyces cerevisiae and several substrains (S.arboricolus, S.cariocanus, S. eubayanus, S. kudriavzevii, S. mikatae, S. paradoxus, S. uvarum and Naumovozyma castellii) can be used. Used to transform aroma and taste, creating a difference for the product.

6.3 Create a harmony between wine and taste

The goal of a winemaker is to produce balanced wines where alcohol concentration, acidity, sweetness, fruitiness and tannins complement each other to create a harmonious taste when drunk. Products containing glycerol-3-phosphate dehydrogenase isozymes have the ability to reduce ethanol content from 15.6% (v/v) to 13.2% (v/v) and 15.6% (v/v) to 12% (v/v) v) in Chardonnay and Cabernet Sauvignon. However, in the process of reducing alcohol, undesirable compounds such as acetaldehyde and acetoin are formed, which cause the smell of bruised apple in wine. To reduce this odor, it is necessary to neutralize the compounds forming 2,3 butanediol using a product containing butanediol dehydrogenase.

These two processes will significantly reduce the formation of acetaldehyde, acetic acid and acetoin.

6.4 Elimination of ethyl carbamate in alcohol production

Ethyl carbamate is a compound produced during fermentation, the product of the reaction of urea with ethanol, especially in blended alcohols. Despite its low content, it is a potentially carcinogenic compound.

To control the ethyl carbamate content, an improved strain of S. cerevisiae DUR1,2 containing urea amidolyase was used, which converts urea to ammonia and carbon dioxide, from removing the ethyl carbamate from the production process. The preparation ECMo01 has been evaluated to be able to remove 90% of the ethyl carbamate in Chardonnay alcohol.

6.5 The formation of flavors from aromatic compounds in wine

The complex mixture of compounds in the wine such as aromatics, aroma compounds and the alteration of chemical compounds from within by microorganisms and oak casks creates distinct flavors for the wines. Alcohol.

The compounds from the small fruit give the wine its basic flavors and make it different for different wines. The compounds that produce the flavor are: aliphatics, benzene derivatives, esters (cis-rose oxide, ethyl acetate, ethyl butyrate, ethyl formate, ethyl exanoate, ethyl propionate, furaneol, isoamyl acetate, isobutyl acetate, methyl cinnamate), lactones, sotolon), norisprenoids (e.g., β-demascenone, β-ionone, 1,1,6-trimethyl-1,2-dihydronaphtalene) hexanoate, ethyl propionate, furaneol, isoamyl acetate, isobutyl acetate, methyl cinnamate), lactones (sotolon)), norisprenoids (e.g., β-demascenone, β ionone, 1,1,6-trimethyl-1,2-dihydronaphthalene), phenols, pyrazines (e.g., 3-isobutyl-2metoxypyrazine), sesquiterpenes (e.g., rotundone), monoterpenes (e.g., linalool, geraniol, hotrienol, nerol, citronellol, cis-rose oxide and α-terpineol) and thiols (e.g., 4-mercapto-4-methyl-pentan-2-one, 3 mercaptohexan-ol, 3-mercaptohexyl acetate, etc.). These compounds act individually, individually, and in combination with other substances to give wines distinctive characteristics. For example, the "bell pepper" flavor of Cabernet Sauvignon is caused by the compound 3-isobutyl-2-methoxypyrazine while the strawberry like character of Pinot Noir is caused by combination of several esters including ethyl acetate, ethyl butyrate, ethyl formate, ethyl hexanoate, furaneol and methyl cinnamate....

From the aroma and flavor compounds from the grapes, it is possible to identify and characterize some wines according to the grape.

The interaction of yeast with compounds from grapes and secondary metabolism (esters, fusel alcohols, carbonyls, volatile fatty acids, sulfur compounds, etc.) gives the wine its character. During primary fermentation, several compounds are biotransformed to form precursors for secondary conversions to volatile compounds. For example, non-volatile cysteinylated grape thiols (Cys-4MMP and Cys-3MH) can be released from cysteine (4MMP and 3MH) and converted to volatile compounds. During malolactic fermentation, malolactic bacteria can decarboxylate to convert malic acid to lactic acid and perform a number of other conversions, such as diacetyl, to give the wine a buttery flavour. Furthermore, when the wine is aged in oak barrels for further fermentation and maturation, a series of chemical transformations lead to a dramatic change in the flavor of the final product. Dry oak is often used to make casks because of the potential for aromatic compounds (vanillin, vanillin- derivatives, volatile phenols and lactones) that can affect the flavor and type of wine. As fermentation continues in oak casks, a number of compounds from the oak pillows will be released into the wine causing a large change in aromatic compounds. Similarly, furfuryl alcohol and furfuryl mercaptan (2-furanmethanethiol) can be formed from oak furfural. The presence of compounds from oak in the wine enhances the flavor of the wine such as coconut, spicy, vanilla.

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8. Conclusion
Wine is a product that contains many antioxidants and is good for health. During the manufacturing process, there are many stages and factors that affect the final quality. Primary fermentation and secondary fermentation are two central processes in winemaking.

Primary fermentation creates the alcohol content of the wine and forms a number of precursors for the secondary metabolism to give the individual flavors to wines from different grape varieties.

Secondary fermentation is the conversion of residues after primary fermentation and secondary metabolites, malolactic fermentation to form aromatic compounds for alcohol. At the same time, this is also a process to remove and metabolize unwanted compounds to ensure a safe level for users' health and create a balanced taste for alcohol products. From there, it is possible to classify alcohol according to the characteristics of the aroma formed.

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