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Department of Chemical Engineering

CHE 3110L Laboratory Simulation of Industrial Product Manufacture

Pre-laboratory Report

(Form CHE 3110L-1)

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Experiment:	**Wine Making**

Objectives of the Experiment

1. Prepare a process flowsheet for the manufacture of chemical product, complete with details in process conditions and stream specification;
2. Monitor overall and component mass and energy flows during the lab-scale implementation of the product manufacture;
3. Calculate component yields in every process step and for the entire process; and
4. Identify critical steps in the process based on laboratory data and the entire experience of generating the product.

Theoretical Background

Wine, from the Latin word, *vinum* is an alcoholic beverage made originally from fermented grapes. Naturally, the grapes contain sugars that are then consumed by the yeast and are then converted into ethanol and carbon dioxide (Ronsheim, Johnson, & Robinson, 1990). The wine is the result of the

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fermentation process and other chemical reactions that took place in its production. Furthermore, various wines can also be made from fermenting other fruits or other plant materials.

The art of winemaking can be traced back to as far as 6000 BC when it was believed that most civilizations have started to create permanent settlements due to the adoption of farming and animal domestication which allowed people to experiment with drinks including wine (Wynnefeb, 2019). This became popular with civilizations such as the Egyptian (Predynastic Era) and the Greeks (3000 BC). However, the Greeks had the most influence worldwide, starting with European countries, creating 300 different grape varieties each with their own distinctive flavors. After the Greeks, the Romans have made major contributions to the science of winemaking (1000 BC). They have invented the wooden wine barrel and have classified many varieties of grapes. Their contributions have paved the way for modern wine making techniques such as aging and corking. Soon after, the use of wine was brought to Catholicism and continued to play a significant role in the Sacrament of Communion - this further enhanced the influence of winemaking throughout Europe, and then worldwide.

Currently, the wine-making industry is worth millions of dollars with France and Italy at the leaderboards for being the largest producers, making about 5 million hectoliters (Zeppa, 2007). Traditional techniques and procedures for winemaking such as aging in barrels, fermentation with yeast, and bottling are still utilized as of today, with few advancements such as the use of enzymes.

The Fermentation Process

Fermentation is a metabolic process of energy generation that utilizes organic compounds as the electron acceptor. In this process, organisms acquire the necessary energy to drive biochemical reactions from the rearrangement of chemical bond energy and the movement of electrons or protons. This process breaks down sugars to create a higher energy molecule, in this case, adenosine triphosphate (ATP). This ATP serves as the major source of energy used for biological work within the cells (Davis, 2018).

Ethanol Fermentation

Ethanol fermentation is biological process which involves the conversion sugars such as glucose, fructose, and sucrose to cellular energy, thus producing ethanol and carbon dioxide. This anaerobic process is also referred to as *alcoholic fermentation*. In this process, yeasts are the agents of fermentation in the absence of oxygen.

Ethanol fermentation is also widely used in the beverage industry. This process is used to produce alcoholic beverages such as beer and wine. According to Waarde et.al, this method of fermentation is also applicable in manufacturing ethanol fuel and in bread making.

Chemistry of Ethanol Fermentation

Alcoholic fermentation converts glucose ($C_6H_{12}O_6$) into carbon dioxide (CO_2) and ethanol (C_2H_5OH). The reaction below shows the stoichiometric equation of the reaction. This process generates ATP molecules as it progresses.

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2$$

Equation 1. Conversion of Glucose to Ethanol and Carbon Dioxide

The reaction equation above summarizes the conversion of glucose. This sugar results from the breaking in the linkage between glucose and fructose in a sucrose molecule. Sucrose is a disaccharide and a dimer of glucose and fructose molecules (Reece, et al., 2014).

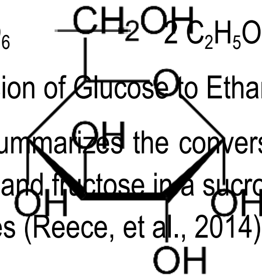


Figure 1. The Glucose Molecule

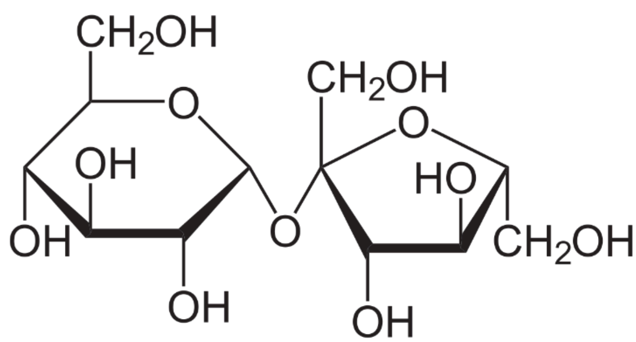


Figure 2. The Sucrose Molecule

In the first steps of fermentation, the glycosidic linkage between the glucose and fructose in a sucrose molecule are cleaved by an enzyme called *invertase* in the presence of water to produce two moles of glucose.

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{invertase}} 2\ C_6H_{12}O_6$$

After two moles of glucose are formed, each glucose molecule is broken down into two Pyruvate (CH_3COCOO^-) molecules in a process called *glycolysis*. The glycolysis reaction is summarized below.

$$C_6H_{12}O_6 + 2\ ADP + 2\ Pi + 2\ NAD^+ \rightarrow 2\ CH_3COCOO^- + 2\ ATP + 2\ NADH + 2\ H2O + 2\ H^+$$

During glycolysis, two molecules of Adenosine Diphosphate (ADP) is converted into two molecules of ATP. It is also evident that there is a reduction reaction since the NAD^+ is reduced into NADH.

Pyruvate
decarboxylase

Consequently, a two-step reaction occurs to convert pyruvate to ethanol and carbon dioxide and to regenerate NAD^+ (Stryer, 1975).

$$CH_3COCOO^- + H^+ \longrightarrow CH_3CHO + CO_2$$

$$CH_3CHO + NADH{+}H^+ \xrightarrow{\text{Alcohol dehydrogenase}} C_2H_5OH + NAD^+$$

During the fermentation process, yeasts transport sugars into their cells and convert these sugars to extract energy. There is a transfer of electrons from the glucose to the electron acceptor, NAD^+, to form pyruvate molecules through a process called glycolysis. The conservation of energy is also manifested in the conversion of ADP to ATP. In the presence of oxygen, there is an optimization occurring in the conversion process of pyruvate thus resulting to more production of ATP.

The fermentation process produces secondary by-products that may enhance the flavor of the wine. Glycerol is among the secondary by-products which enhances the mouthfeel of the wine. Moreover, acetic acid and acetaldehyde are also produced, and these contribute to the quality of the wine whose effects are variable depending on concentration. Some processes may produce aromatic and flavorful esters and alcohols that contribute to the sensory quality of the wine (Styger, Prior, & Bauer, 2011).

Effect of Oxygen

Oxygen is not required in a fermentation process. The presence of oxygen in the process hastens the complete conversion of pyruvate to carbon dioxide and water. If complete conversion to CO_2 and H_2O occurs, it is a sign that cellular respiration is happening instead of fermentation. Hence, yeasts produce ethanol in an anaerobic environment. However, there are yeasts that prefer fermentation to respiration. An example of these yeasts is *Saccharomyces cerevisiae* or commonly known as the baker's yeast.

Low concentrations of oxygen at normal conditions increases the yield of ethanol. This is commonly referred to as the microaerobic effect. If the amount of oxygen surpasses the level allowed for the microaerobic effect, secondary by-products such as acetic acid and glycerol are produced. The production of these secondary by-products reduces the purity of ethanol. If the oxygen concentration increases, the fermentation process will eventually come to a stop. This stop is referred to as the *Pasteur Effect* (UCSB, n.d.)

Effect of pH

All wines are acidic. The fermentation process is carried out by microbes; hence, it is affected by the acidity of the wine. The pH levels are used as indicators of the wine's acidity. Since the pH scale is logarithmic, minor changes in the pH values of the wine affects tremendously on the wine's acidity.

The color of the wine is also affected by the pH levels. Generally, wines with lower pH levels tend to have red and purple colors while wines with higher pH values tend to have various colors and may even be colorless. This is due to the effect of the pH levels on the polyphenolic compounds, anthocyanins, and tannins which are present in the wine (Sensorex, 2017).

The sensory characteristics of the wine such as taste, smell, and astringency are also affected by the pH levels. The astringency and bitterness of the wine is highly associated with the polyphenolic compounds and tannins present in the wine. These compounds are highly affected by changes in pH levels, thus resulting in the alteration of the taste and texture. The sharpness of the wine increases as the pH level decreases. This results to a tarter and stronger wine (Fontoin, Saucier, Teissedre, & Glories, 2008).

Presence of Other Microorganism

Further exposure to oxygen may result to oxidation of the ethanol to acetaldehyde then to acetic acid. These compounds are formed from the oxidative metabolism of ethanol (Adachi, E, K, K, & M, 1978). These allows acetic acid bacteria to thrive in the wine. According to a study conducted by Van keer, acetic acid bacteria are associated with apples and pears. Thus, the presence of these bacteria may form the ethyl ester of acetic acid and ethyl acetate. The formation of these compounds may result to a pungent odor and wine spoilage (Francis & Newton, 2005).

Minimizing the growth of other microorganisms is vital for wine aging. Although it delays wine maturation, storing the wine at temperatures below 15ºC might aid in minimizing the ability of the bacteria to proliferate in the wine. Sterilizing wine containers prior to usage is highly recommended to free the wine container from bacterial contaminants that might hasten spoilage (Ribereau-Gayon, Glories, Maujean, & Dubourdieu, 2006).

Tannins

Tannins are phenolic secondary metabolites that are widely distributed in plants and fruits. Tannins are a class of astringent, polyphenolic biomolecules that bind to and precipitate proteins. Tannins are usually found on seeds, wood, and fruit skin. This metabolite binds with the proteins found in the saliva which in return, imparts bitterness. Due to this, these made tannins a popular molecule in the wine making industry. Aside from its effect on taste and quality, tannins are vital for the protection of the wine. Tannins are natural antioxidants; hence, it could prevent the wine from oxidation by binding with oxygen molecules which is useful for spoilage protection which makes it essential in wine aging (Brandao, et al., 2017).

Sugar Content of Apple

According to the U.S. Department of Agriculture – Agricultural Research Service, every 100 grams of apple contains 10.33 g of sugar. The average weight of an apple approximately weighs 100 grams, hence, for every piece of apple, there is a 10% supply of sugar.

Apples to be used in winemaking may vary in sugar and acid level, as the alcohol level of the final product-compared to the more stringently-prepared cider-can be adjusted with sugar, and the juice will be fermented to dryness (Bader, 2019). However, tannin-rich, “bittersharp” apple varieties are highly recommended for cider and wine-making, due to their composition and properties which are optimized for fermentation and spoilage-prevention.

Materials and Methodology

Materials

8 lbs (~3.6 kg) of fresh red apples (~24 medium pieces)
2 lbs (~0.9 kg) of granulated white sugar
Distilled water enough to make 1 gallon of wine
Wine yeast (follow manufacturer's guide regarding amount)
Tap water for sterilization of apparatus

Methodology

Selection and Preparation of Apples

1. Pick apples that have minimal bruising and unbroken skin. Bruising will cause unwanted alteration in taste of the wine product while broken skin may be an indication of pests that are within the fruit, both of which should be avoided.
2. Rinse the 8 pounds of apples properly with water and set aside. Leave them whole until only when about to be used, this is to prevent browning due to oxidation that may cause unwanted flavors and colors in the final wine product.
3. Cut apples into small pieces and remove the seeds. Seeds contain bitter resins that will leave a bitter taste. Make sure to remove any damaged parts. [*OPTIONAL*: Freeze fruits before juice extraction. Freezing the fruit breaks down the cell membrane walls and result in better extraction. To freeze apples, cut into small pieces and drenched in a mixture of water and lemon juice (1 Tbsp of lemon juice in a quart of water) then freeze. Thaw the frozen fruit before proceeding to juice extraction.]

Extraction of Juice

1. Transfer the chopped apples in the sanitized fermentation vessel (this may be any sanitized vessel made of glass, stainless-steel, or plastic) and fill with enough boiling distilled water to cover the apples. Cover with a new clean cloth, secure it with a rubber band or strap, and leave it for about three to four days to allow the juice of the apple to seep out. Stir twice daily with a sterilized wooden spoon or ladle.
2. Strain out the apples from the apple liquor using a sterilized strainer and discard the apple solids.

Fermentation

1. Add the 2 pounds of granulated white sugar and top up with lukewarm distilled water until it reaches a gallon. Stir the mixture with a sterilized wooden spoon or ladle to ensure that all the sugar is dissolved.
2. Add the baker's yeast (amount depending on manufacturer's instruction), stir, and cover with cloth and tie to secure.
3. Let it ferment in the primary fermentor for at least a week or two, stirring contents 2-3 times a day.

Aging or Maturation

1. Transfer the liquid to another sterilized vessel by siphon using sterilized plastic tubing and funnel. This is done by setting the receiving vessel lower than the vessel the liquid is siphoning from. Avoid disturbing and transferring the sediment from the bottom of the primary fermentor.
2. Top up the newly filled vessel with distilled water until it reaches a gallon, stir, and cover.

3. Store the vessel in a cool and dark spot and allow it to remain undisturbed for at least a month.

Final Clarification, Bottling, and Labeling

1. Strain the wine through a cheesecloth and sterilized strainer into another sterilized vessel and store again for at least two weeks.
2. Transfer the strained wine into the sterilized wine bottles by siphon using sterilized plastic tubing.
3. Finally, seal the wine bottle with a cork and label them accordingly.

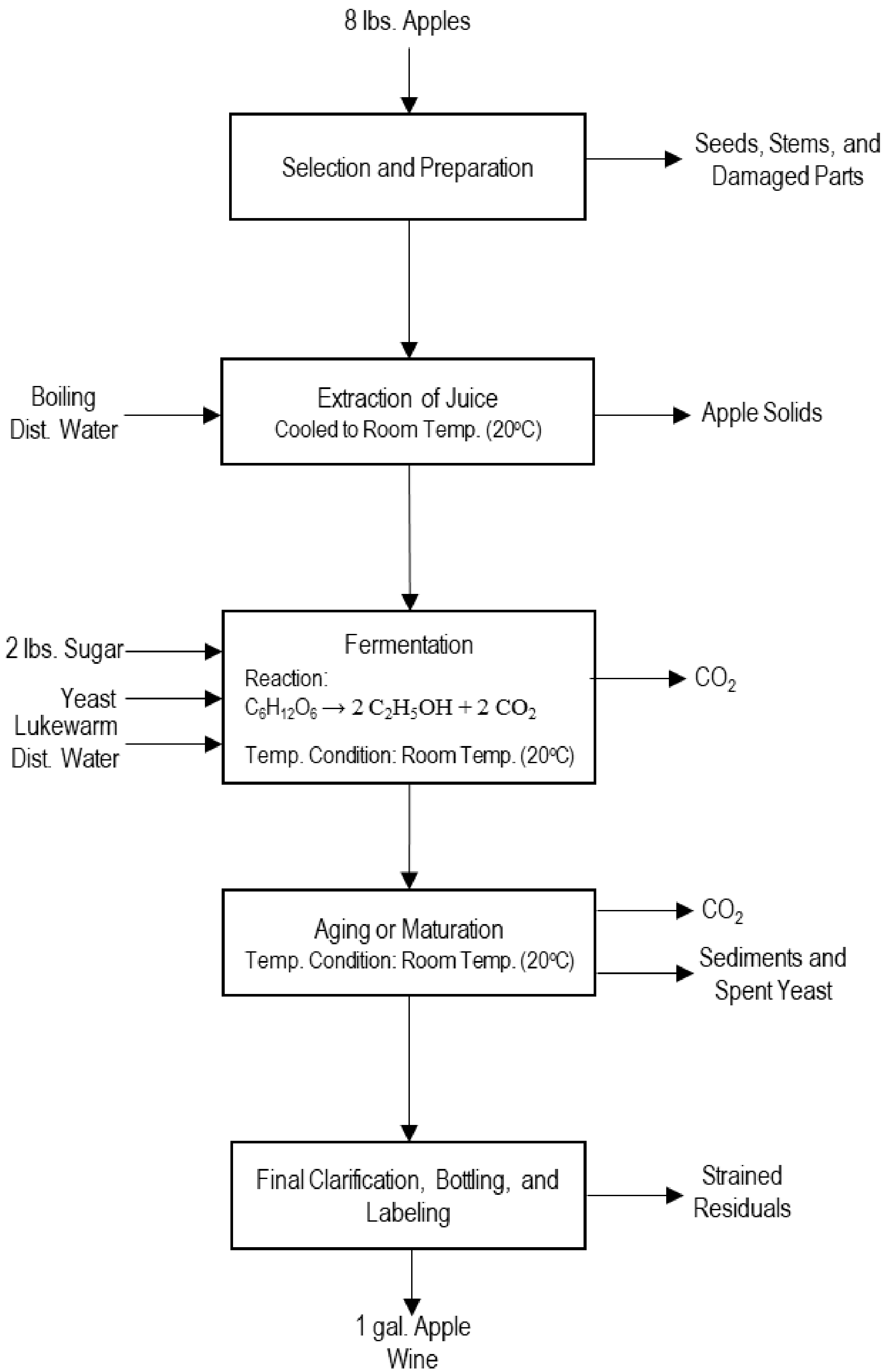
Proper Disposal of Waste

The seeds, stems, and damaged parts of the apples from the selection and preparation of apples and the apple solids from the extraction of juice may be disposed in a compost bin.

The sediments and spent yeast should NOT GO DOWN THE DRAIN. Though homebrewing wine only produces minimal sediments and spent yeast is commonly disposed in the drain, proper practice for the disposal of such is to either reuse it for other fermenting processes such as sourdough making, bulk for onsite composting systems, or used as additive in animal feed.

Other wastes, such as plastic bottles or sachets, must be disposed properly to their respective waste bins. Recyclables must be cleaned properly prior to disposing to recycling bins.

Process Flow Diagram



Cost Estimates

Table 1. Proposed Budget for Materials

Item Description	*Quantity*	*Cost/Unit*	*Subtotal*
[1] Red Apples	24 pcs	₱ 31 / pc	₱ 744.00
[2] Granulated White Sugar	0.9 kg	₱ 60 / kg	₱ 54.00
[3] Distilled Water	5.0 L	₱ 12 / L	₱ 60.00
[4] Wine Yeast	5 g	₱ 0.21 / g	₱ 1.05
[5] Tap Water for Sterilizing	10 L	₱ 0.0152 / L	₱ 0.152
		Total	₱ 859.21

Table 2. Proposed Budget for Packaging and Labeling

Item Description	*Quantity*	*Cost/Unit*	*Subtotal*
[1] Wine Bottle	6 bots	₱ 5 / bot	₱ 30.00
[2] Cork	4 pcs	₱ 6.1 / pcs	₱ 24.4
[3] Labeling (Sticker Paper and Print)	4 pcs	₱ 7 /pc	₱28.00
		Total	₱ 82.00

Table 3. Proposed Budget for Laboratory Equipment/Apparatus Use

Item Description	*Quantity*	*Cost/Unit*	*Subtotal*
[1] Stove Top	½ canister	₱ 120 / canister	₱ 120.00
[2] Weighing Scale	1 unit	₱ 630 / unit	₱ 630.00
[1] Fermenting Vessels	2 pcs	₱ 795 / pc	₱ 1590.00
[2] Cloth (for covering)	1 pc	₱ 25 /meter	₱ 25.00
[3] Rubber band	20 pcs	₱ 0.07 / pc	₱ 1.40
[4] Wooden Spoon/Spatula	1 pc	₱ 59 /pc	₱ 51.00
[5] Strainer	1 pc	₱ 35 / pc	₱ 35.00
[6] Plastic Funnel	1 pc	₱ 22 / pc	₱ 22.00
[7] Vinyl Tubing	5 ft	₱ 12.62 / ft	₱ 63.09
[8] Cheese cloth	1 pc	₱ 36 / meter	₱ 36.00
[9] Knife	1 pc	₱ 34 / pc	₱ 34.00
[10] Chopping Board	1pc	₱ 95 / pc	₱ 95.00
		Total	₱ 2702.40

Table 4. Room Rental Costs

Room	*No. of days*	*Cost/day*	*Subtotal*
[1] ChE Unit Operation Laboratory	60	50	₱ 3,000.00
		Total	₱ 3,000.00

Table 5. Overall Costs

Grand total	₱ 6 643.60

Grand total (with 20% contingency)	₱ 7972.34

Plan for Acquiring Funds

Due to present circumstances and the relatively high costs for a group of four students, each member would make equal contributions to offset the costs. Fund acquisition will be done by making requests to each members' parental benefactors.

The acquired funds would then be wired via G-Cash for convenience to the marketer.

Tasks Designation

a. **Coordinator (Villanueva)** – gives everyone their tasks and makes sure everyone is involved; checks the attendance of members in the activities as well as the materials and equipment needed; and double-checks the outputs before they are submitted.

b. **Researcher (Amaba)** – sees to it that the outputs are properly documented (theoretical background, procedures for the project, product made, journal, written report, etc); conducts research on the best procedures to be undertaken in the experiment; also facilitates in the search for information for preparation of the pre-lab and the post-lab reports.

c. **Marketer and Treasurer (Talandron)** – facilitates the purchase and procurement of materials needed for the activity; handles the budget for the said activity; calculates the materials costing and the product yield

d. **Financier (Yu)** – provides a greater margin of the needed financial resource for the procurement of materials

e. **Videographer (Villanueva)** – ensures the documentation of the activity in video format; edits the quality of the video suitable for submission

f. **Data Processing (Amaba)** – gathers all the raw data collected; processes the data to produce data sets viable for presentation and reporting

g. **Quality Check (Yu)** – inspects the physical appearance of the final product; judges its sensible characteristics such as color, turbidity, scent, and taste

h. **Weekly Monitoring (Talandron)** – checks on the product for the appearance of irregularities; stirs/agitates the product during aging if deemed necessary; monitors the temperature; lists all of the observations in a journal

References

Adachi, O., E, M., K, S., K, M., & M, A. (1978). Purification and Properties of Particulate Alcohol Dehydrogenase from Acetobacter aceti. . *Agric Biol Chem*, 2331-2340.

Bader, S. (2019). *Make Hard Cider and Apple Wine*. Retrieved from Wine Maker Mag: https://winemakermah.com/article/1513-make-hard-cider-apple-wine

Brandao, E., Silva, M., I, G.-E., Williams, P., Mateus, N., Doco, T., & Soares, S. (2017). The role of wine polysaccharides on salivary protein-tannin interaction: A molecular approach. *Carbohydrate polymers*, 117, 77-85.

Brewers Association. (n.d.). *Solid Waste Reduction Manual.* Retrieved from BrewersAssociation.org: https://www.brewersassociation.org/attachments/0001/1529/Sustainability_Manual_Solid_waste.pdf

Davis, U. (2018). *The Fermentation Process* . Retrieved from https://wineserve.ucdavis.edu/industry-info/enology/fermentation-management-guides/winefermentation/introduction

Fontoin, H., Saucier, C., Teissedre, P., & Glories, Y. (2008). Effect of pH, ethanol and acidity on astrigency and bitterness of grape seed tannin oligomers in model wine solution. *Food Quality and Preference*, 19(3), 286-291.

Food Data Central: Apple. (n.d.). Retrieved from U.S. Department of Agriculture: https://ndb.nal.usda.gov/fdc-app.html#/food-details/577849/nutrients

Francis, I., & Newton, J. (2005). Determining wine aroma from compositional data. *Aust J Grape Wine Res*, 114-126.

HomeBrewIt.com. (n.d.). *Making Wine With Fresh Fruit: General Instructions And Tips*. Retrieved from Home Brew It: https://www.homebrewit.com/pages/making-wine-with-fresh-fruit-general-instructions-and-tips

Improving the taste and color of the wine with pH control. (2017). Retrieved from Sensorex: https://sensorex.com/blog/2017/12/06/ph-improve-taste-colorwine

John. (n.d.). *Apple Wine Easy Recipe*. Retrieved from Allotment Heaven: https://allotmentheaven.blogspot.com/2011/07/apple-wine-easy-recipe.html

Kraus, E. (n.d.). *Controlling Oxidation In Your Homemade Wines*. Retrieved from eckraus: https://eckraus.com/contolling-oxidation-in-your-homemade-wines/

Kuzemchak, S. (2016, September 12). *How to Freeze Apples*. Retrieved from stemilt: https://www.stemilt.com/stem-blog/freezing-apples/

Nelson, L., & Cox, M. (2008). Glycolysis, gluconeogenesis, and the pentose phosphate pathway. In *Lehninger Principles of Biochemistry* (pp. 528-546). New York City: W.H. Freeman and Company.

Reece, J., Urry, L., Cain, M., Wasserman, S., Minorsky, P., & Jackson, R. (2014). *Campbell Biology.* Boston: Pearson.

Renou, M. (2020, September 22). *The Best Homemade Apple Wine Recipe From Fresh Apples*. Retrieved from wineladybird: https://wineladybird.com/homemade-fresh-apple-wine-recipe/

Ribereau-Gayon, J., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). Stabilizing wine by physical and physicochemical processes. In *Handbook of Enology: The Chemistry of Wine Stabilization and Treatments* (pp. 369-386). Chichester, UK: John Wiley & Sons, Ltd.

Ronsheim, J., Johnson, H., & Robinson, J. (1990). Vintage: The Story of Wine. *The Antioch Review*, 48.1: 111.

Stryer, L. (1975). *Biochemistry.* W.H. Freeman and Company.

Styger, G., Prior, B., & Bauer, F. (2011). Wine flavor and aroma. *Journal of Industrial Microbiology and Biotechnology*, 38: 1145-159.

UCSB. (n.d.). *UCSB*. Retrieved from WHat effect do varying levels have on Saccharomyces cerevisae's (baker's yeast) production of ethanol from glucose?: http://sciencelin.ucsb.edu/getkey.php?key-199#:text=The%20presence%20of%20oxygen%20at, will%20inhibit%20any%20fermentation%20process.&test=As%20the%20level%20of%20oxygen, of%20the%20ethanol%20are%20reduced

Van Waarde, A., Van den Thillart, G., & Verhagen, M. (1993). Ethanol Formation and pH-Regulation in Fish. *Survivng Hypoxia*, 157-170.

Wynnefeb, R. (2019, February 14). *A History of Wine*. Retrieved from https://www.arenaflowers.com/blogs/news/history-of-wine#:~:text=The%20earliest%20remnants%20of%20wine,sometime%20between%205400%2D5000%20B.C.

Youngblood, J. (2014, September 12). *When Yeast Goes Down the Drain*. Retrieved from The Beer Diaries: http://thebeerdiaries.tv/yeast-goes-drain/

Zeppa, G. (2007). *The science and technology of wine making*. Retrieved from https://www.dairyscience.info/index.php/science-and-technology-of-wine/124-the-science-and-technology-of-wine-making.html