Mr Zé, Mrs. Ná and the orange wine

Brief history of this research

The knowledge around the orange wine brewing by a family residing in the town of São Tiago, Minas Gerais, Brazil, came out through the interest of a member of the family, Ronaldo, to understand better the phenomena involved. At that time, he was a student of Physics in the University but grew up watching and participating in the orange wine making process within his family year after year. Motivated by him, we developed an extension project that aimed to investigate the production of this wine and its knowledge insertion into chemistry classes.

In the project, one of us, Daniela, was a student of Chemistry and the other, Paulo, the advisor. At the beginning of the work, Daniela, who was more interested in the educational part of the project always wondered what Mr. Zé and Mrs. Ná (the Ronaldo's parents) could teach and whether it was possible to propose a new way to educate and learn chemistry in school through their knowledge. On his turn, Ronaldo liked to go to the laboratory and participate in the work related to the reproduction of the wine and determination of its density and alcohol content.

However, the most relevant part of the investigation occurred in the backyard of Mr. Zé and Mrs. Ná home, where the wine is usually produced, which involved writing and audio visual documentation of the process. After that, we did a literature research, wrote a text, conducted an interview with the couple, improved the text, interacted with a microbiologist expert on yeast, planned activities and developed classes at a third year high school classroom, starting the work by students´ interaction with a video and a text on the orange wine making.

Mrs. Ná, Mr. Zé and the son Ronaldo in 2002 and 2014

We describe here the preparation of the orange wine according to the knowledge and practice of Mr. Zé and Mrs. Ná alongside its scientific explanations. The material consists of texts and photographs. Good reading and enjoy!

A receipt from many cultures?

Mrs. Maria da Conceição Sousa de Castro and her husband Mr. José Resende de Castro have made the wine of orange for more than thirty years. Both were born and live in the city of São Tiago, in the state of Minas Gerais, Brazil, and are known as "Dona Ná" and "Seu Zé". She was born in October 12, 1948, and he in May 21, 1938. Both studied until the third and fourth years of elementary school, respectively.

Mrs. Ná learned to make the wine with Mr. Zé, who knows the process since childhood as his parents produced the beverage. He told us that it was a lady called Maria Silva, better known as “Dona Inacita”, born in June 27, 1923, and deceased in May 16, 1985, who gave the wine´s recipe to “Dona Mindica”, mother of Mr. Zé.

The couple follow exactly the receipt of Dona Inacita and the result is a delicious wine made with oranges. The beverage produced by them is for their own consumption but also to give as a gift for relatives and friends. They also sell the wine when ordered. However, isn´t the word "wine" exclusively used to refer to an alcoholic drink made with grapes?

The wine is an alcoholic beverage fermented by diffusion, which is generally obtained by alcoholic fermentation of a juice from natural mature fruit, especially the grape (*Vitis vinífera*). Traditionally, it is admitted that the name wine is reserved only for the beverage from grapes. For those produced by alcoholic fermentation, which is not from grape, it must be indicated the name of the fruit, as the current case of the wine of orange. It is possible to produce wine from any fruit that contains considerable levels of sugar with the characteristic flavor of the fruit used (Corazza et al, 2000, p. 449).

The production of wines is ancient and follows the history of civilization. It is believed that they were manufactured in the Caucasus and in Mesopotamia eight thousand years ago and around four thousand years in Greece. The monks were those who improved its manufacturing art in the Middle Ages (Panek, 2003). Curiously, we found several articles in newspapers and periodicals, which were published in the United States in the 19th century regarding the production of orange wines:

- In the edition of December 1st, 1878, it is cited in the *New York Times* the development of experiments with orange juices aiming to replace wine grapes due to the ravages of the European vines by the insect *Phyllocera vastatrix*, which generated a crisis in the international market at that time; four distinct kind of wines made with different oranges were mentioned, with the

following characteristics: "color pleasing to the eye", "perfectly translucid", "has an agreement savor with a slight tinge of acidity and an alcoholic richness of about 15%";

- In the *Chicago Daily Tribune* of May 28, 1879, a small note says "Orange wine is the latest novelty" and "contains 15 per cent of alcohol and is agreeable to the taste", regarding to a wine produced in Spain as alternative to the problem of Phylloxera;

- A wine made with sweet and ripe oranges produced in the state of Florida/USA is mentioned in an article published in the *Los Angeles Times* in 1885, as a "perfectly pure native", "splendid wine", "surpassing in purity any of the European wines", "agreeable favor", "marvelously palatable", "the cleanest wine in the market today", "will at no distant day outrival any of the imported still wines" with its "8.64 per cent. of absolute alcohol and slightly over 5 per cent. of sugar", but "the supply is in no way equal to demand";

- In the *Chicago Daily Tribune* of February 2, 1888, there is a quotation about the establishment of an industry for orange wine production in Florida;

- An article entitled "Manufacture of Orange Wine" was published in the periodic *Southern Cultivator* of June, 1892, where there is a letter of Professor Serge Malyvan describing the production of 1,500 gallons (5678,1 liters) in the County of Bradford, considered by an expert from New York as having excellent quality but requiring the increase of the production for consumption. In the same missive, there is a detail about the time production and others:

"The wine fermentation of oranges takes about six months, the clarification about three months, it is not a baby wine, it is a strong one, very healthy, eighteen per cent. alcohol, rich tasting as sherry, and endorsed by physicians as a first-class cordial. The only trouble for selling is, it is not manufactured in large enough quantities, and not enough advertised".

- Again in *The New York Times*, in 1904, an article mentioned a study of the French pharmacist Mr. Pairault about the wine orange fermentation in the Martinique island, a French colony:

"In the Antilles, orange wine has been made for some time in the following manner: the oranges are peeled and pressed by hand. To the juice thus obtained sugar is added, and it is subjected immediately in a vessel made of glass or earthenware, to spontaneous fermentation, which in general takes place easily, because the ferment which determines it is often found in the oranges themselves".

After the above description, it comes Mr. Pairault's version about the "rational preparation of orange wine":

"After the wine of orange have been sterilized sufficiently there should be added to every quart of the liquid 12.25 to 14 ounces avoir-dupois (350 to 400 grams) of sugar, 0.175 ounce avoir-dupois (5 c.c.) of brewer´s yeast, and 2 ounces of a mixture made of the following proportions: ammonium phosphate, 30 : calcium phosphate, 40 : potassium bitartrate, 40 : magnesium sulfate, 3. When the mixture is cooled fermentation proceeds, and in a few days there results an excellent product. A sweet or dry wine may be made by increasing or diminishing the amount of sugar added".

The news in the media show the production of the orange wine in at least three different places in the 19th century: in USA, Spain and Martinique. The brief descriptions of how the wine was prepared in the County of Bradford and in the French colony suggest similarities to that observed in Brazil. However, the wine of orange neither replaced the wine of grapes nor occupied a place in wine trading. Why did it not happen? Why the orange wine is not known nowadays? Why its industry did not develop as the American media of the 19th and beginning 20th century acclaimed its qualities? Why it is not usual to find this wine in the supermarket shelves? The expression "wine of orange" is known today but it is used differently to refer to a kind of grape wine that has been taking place in the international market with a bright gold to tawny-brown color, which taste is in between those of the traditional red and white wines.

The wine manufactured with oranges is not an industrialized and traded beverage in Brazil either. We know that produced by Mrs. Ná and Mr. Zé and another made on experimental basis

(Corazza et al., 2001), but we know also that there are other producers of this wine in the region. In the following pages, we will see how the wine of orange is made by the couple using two basic ingredients: the sugar syrup and the orange juice.

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“With the black and the white: making the “sugar syrup”

The process of making the wine begins with the preparation of the "sugar syrup". They dissolve two types of sugar in water: the "black" (1/3) and the "white" (2/3), both derived from sugar cane’s juice cooking. This plant belongs to the genus *Saccharum L.*, where six species are known: two pure and four hybrids. Much of the sugar produced in Brazil derives from the hybrid *S. officinarum L.* (Almeida et al., 1995), but why do they use two kinds of sugar?

The “black” sugar

The “white” sugar

The “black” sugar is acquired directly from a local producer, brown in color actually, but they call it “black”. The white is also known as “cristal”, for its large and clear crystals, and is easily found in food shops and supermarkets. The first is the sugar closer to its natural state and the second undergoes controlled crystallization and clarification processes, which usually make use of lime and sulfur. Both have a high content of sucrose (about 90% in the brown sugar), but the content is little higher in the crystal (about 99.5%) (Chemello, 2005; Cruz & Sarti, 2014). The black sugar contains higher amounts of calcium, iron, potassium, magnesium, copper and phosphorus minerals and the vitamins B1, B2, B6, whose levels decrease in crystal sugar due to the industrial purification process (Chemello, 2005).

The syrup, an aqueous solution of sucrose, is prepared using aluminum pans or in a copper pan of around 40 liters of capacity, or using both depending on the amount of wine they will produce. They put the water to heat up in gas or wood stove and add the sugars. They mix them "with high attention to do not let it burble", said Mrs. Ná, taking care to do not let it reach the boiling point. "The fire makes the sugar melt", it helps to dissolve the sucrose. Mr. Zé claimed that this way "it melts more sugar", it increases the water capacity to dissolve more amount of this solute.

The solubility of sucrose in water increases significantly with temperature. At 68° F, for instance, the amount dissolved in 100 milliliters of water is 203.9 grams and 415.7 grams at 212°F. Sucrose is modified at higher temperatures. Between 338 and 374°F it turns to caramel, but this can also occur in lesser extent when moisture is present at temperatures below 212° F. Just below 338°F it starts the merging of sucrose, literally. If its aqueous solution keeps on heating, sucrose’s decomposition can also takes place in a minimum extent since 176° F (Browne, 1912, p. 649, 655-657). Although Mrs. Ná do not have this knowledge, let us remember what she said: "with high attention to do not let it burble", to dissolve sucrose without its decomposition closer to the water boiling point.

The receipt followed by them has a written observation regarding the syrup: "Do not let the syrup boils, must not put the wet hand and is not advised to put the ingredients in greasy containers". We see here that the advice of not boiling the syrup is in the receipt. However, from

what kind of knowledge did it come? How did they prescribe "do not let the syrup boils"? Did it come from scientific information or from observing problems in the wine making? Mr. Zé and Mrs. Ná recommend also that if a foam appears on the surface during the brewing of the syrup, it must be removed through a skimmer.

Before the use of the solution containing sucrose dissolved in the wine making, the sugar syrup, they let it cools. According to Mr. Zé "the best is to prepare it a day before to be sure that it´ll be very cold", as the temperature is an important factor for the growth, metabolism, viability and fermenting action of the microorganisms that will convert the sugar in ethanol (Lima et al., 2005). High temperatures affect their metabolism leading to decrease the ethanol tolerance and produce secondary metabolites such as glycerol (Oliveira, 1998 quoted in Souza, 2009).

Then, the temperature is an important factor both to do not decompose the sugar as to maintain the microorganisms alive. Nevertheless, how who wrote the receipt determined it? In what it is based? As the procedure goes on, after the dissolution of the sugars the syrup is filtered through a cloth to separate impurities.

The sugar syrup

Filtering the syrup

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Peeling, squeezing and talking

Mr. Zé and Mrs. Ná usually prepare the orange wine in May and June, during Brazilian winter, when the oranges are mature. They say it is possible to use any type of orange to produce the wine but they prefer the "sourest". The orange used by them is known as "campista" or "seleta" and is harvested in a family property in the rural area. This variety has fruits with thick skin.

The orange types basically belong to two different species and are classified according to the acid concentration, color of pulp and presence of reproductive organ (seeds). One species, the *Citrus sinensis*, produces sweet oranges such as the "seleta" used by Mr. Zé and Mrs. Ná. Another, the *Citrus aurantium*, produces more acid types, the sour orange.

According to Queiroz-Voltan and Blumer (2005 quoted in Carvalho, 2010), the sweet orange trees are medium-sized plants with up to eight meters tall, show wood with straw-yellow color and treetops rounded shaped with many leaves. The orange-sour trees have other characteristics: usually they are small with many thorns on the branches, have fragrant leaves and the taste of the pulp is not pleasant.

Orange trees

The use of a sweet orange by Mr. Zé and Mrs. Ná, but "sourest", indicates that oranges are harvested before they are wholly ripe, like the observation found in the *New York Times*:

"The first trials made showed that oranges when they have attained their full development, are unfit for the purpose proposed, and they must be selected not when they have become quite mature and superabound in the sugary principle, but before they are wholly ripe and still possess an appreciable amount of citric and malic acids". (Proquest Historical Newspapers, The New York Times, 1904)

This quotation shows the development of experiments searching for the ideal stage of oranges maturity to produce the wine. However, although Mr. Zé and Mrs. Ná did not mention the requirement of an "appreciable amount of citric and malic acids" but their preference for the "sourest" oranges, how did they arrived to this conclusion? The early fruits from orange trees are green. With maturation they acquire the orange color due to progressive loss of chlorophyll and carotenoid compounds production. The ripe fruits contain high percentage of water (85-90%) and many constituents: carbohydrates, organic acids, vitamin C, minerals and small amounts of lipids, proteins, carotenoids, flavonoids and volatile compounds.

The total soluble solids content is between 10-20% of the weight of fresh fruit and consists primarily of carbohydrates (70-80%) and smaller amounts of organic acids, proteins, lipids and minerals. During the ripening there is an acidity decrease, mostly due to the catabolism of citric acid (the main organic acid, others are malic, succinic and isocitric), and an increase of sugars which reaches the maximum level in the ripe fruit (Iglesias et al, 2007). The oranges "sourest", as preferred by Mr. Zé and Mrs. Ná, thereby, contain organic acids in their composition. This will favor the sucrose conversion to ethanol as values of pH between 4.5 and 5 are optimal for alcoholic fermentation and for the growth of microorganisms that will make this conversion (Alcarde, 2014).

To extract the juice, the oranges are peeled with knives until they are "well hurt", without the pericarp, and then are pressed using the hands. The fruit contains two morphologically distinct

regions: the pericarp (peel or rind) and the endocarp (pulp). The pericarp has two parts: 1st) the exocarp (also called flavedo) is the outer colored portion and has about 2/3 of the total mass of the rind, and 2nd) the mesocarp (or albedo), that is the innermost part comprising a spongy white tissue.

The oranges without the pericarp are put in plastic buckets and are squeezed using hands over a second bucket to collect the juice. Mr. Zé said that the juice must be extracted this way otherwise "the wine doesn't turn" and must not be extracted using manual or electric juicers because "the wine doesn't get good", "it can be only the sumo[1] of the orange that disturbs the wine". For Mrs. Ná the "sumo" of the rind must never fall on the peeled oranges, as "the wine gets bad, gets marly". In the pericarp there are several substances that can communicate this taste to the wine, provoke its browning and damage to the fermentation (Hashizume, 1991; Moretto et al, 1998). One of these substances is limonene, 1-methyl-4-(1-methylethenyl)-cyclohexene.

This problem is solved when the oranges pericarp is removed and so the pulp becomes available to extract the juice, which is located inside small buds, vesicles or bags in the endocarp and contains carbohydrates (sugars), fibers, fats, proteins, vitamins, water and mineral salts. However, what they know is that the oranges must be "well hurt".

The "well hurt" oranges and the juice's extraction with the hands

Onde, when we were following the wine making, seven members of the family helped to peel and squeeze the oranges. On that moment there was a great interaction between them. While oranges were being "hurt" and squeezed, they talked about cases, stories, their lives, beliefs, tastes, dreams, concerns and so the work was done and the time passed without our perception.

Note: 1. Portuguese term for the liquid substance in the peel that usually irritates the eyes.

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The mixture fermentation

After the juice extraction, it is filtered through cloth and mixed with the sugar syrup in equal amounts inside a clean and dry plastic bucket. Due to the amount of wine they produce, the mixture is made in large buckets with approximately 50 liters of capacity.

Filtration of orange juice

The biggest concern of Mr. Zé and Mrs. Ná is that the containers are cleaned and dried perfectly. According to them "the science to make the wine is this and cannot change, the thing already came with this requisite, my mother learned this way and we do so as well". The mixture intended for alcoholic fermentation (tecnically know by mosto) "is susceptible to acquire the smell and the taste that by chance may exist in the containers. Those which were in contact with fatty, oily or derived substances such as soap, kerosene, lard, grease, etc., transmit to the mosto its organoleptic properties, i.e., color, smell, taste" (Lima et al, 1975).

After they mixed the juice with the syrup, they put it into glass demijohns of five or 30 liters, the biggest externally coated with cement paste.

Mixture of orange juice with the sugar syrup

The demijohns containing the mixture are then capped with cork stoppers that receive a beeswax layer around. Regarding the sealing of the demijohns Mr. Zé said: "The air is very important thing, it cannot enter, if it enters weakens the wine", it prejudices the fermentation, but for Mrs. Ná: "If the air enters it's possible that doesn't ferment". Dr. Rosane Freitas Schwan, microbiologist expert in yeasts from the Federal University of Lavras in Brazil, told us that fruit wines fermentation is carried out under anaerobic conditions, in the absence of oxygen, but that the presence of this gas in the initial step of the process is beneficial for the microorganisms' growth. She even suggested for Mr. Zé and Mrs. Ná to use a small air pump as the type commonly used in aquariums for the medium initial oxygenation.

The microorganism that is predominantly responsible for fermentation in the orange wine brewing is the *Saccharomyces cerevisiae*, a unicellular fungus widely distributed in nature originally present in the orange and sugar cane juices, which is able to tolerate the medium conditions: high initial sugar concentration, acidity and temperature variations. These fungi survive in both aerobic and anaerobic conditions, but as already mentioned it is under

anaerobiose that they make the fermentation, the process of converting sugar into ethanol (alcohol) with energy production in the form of ATP (adenosine triphosphate) that will be used by the *Saccharomyces* to fulfill many physiological activities as absorption and excretion, for instance, and to the required biosynthesis for life sustaining, such as growth and multiplication. Mrs. Ná said: "it is the ferment that makes it" and Mr. Zé: "it is the bitterness that is there inside", the yeast *Saccharomyces cerevisiae*.

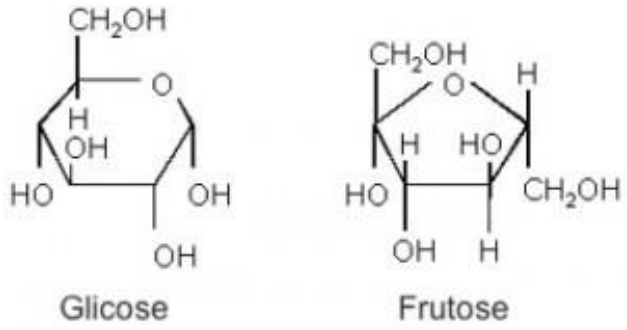
He said that "it boils inside the demijohn to turn wine. It releases a gas that bubbles like when water boils to make coffee". The fermentation produces also the carbon dioxide (carbonic gas, CO_2) and "when it´s cold it boils slower". At lower temperature the fermentative activity decreases producing lesser amount of gas. The word fermentation derives from Latin *fermentare* and was given by Louis Pasteur due the boiling aspect caused by the release of carbon dioxide. Pasteur was also who showed that fermentation is a vital process for some microorganisms. Soon, it became the general name for the *anaerobic degradation of glucose* or other organic nutrients with energy generation and conservation in the form of ATP (Nelson & Cox, 2006). The expression *anaerobic glycolysis* is also known (from the Greek *glykys* = sweet and *lysis* = break).

An aspect of alcoholic fermentation is the mechanism of sugars transportation into the *Saccharomyces cerevisiae* cell, where happens the conversion to ethanol. Due to its size, sucrose cannot penetrate through the cell membrane and is hydrolyzed in the external side with the aid of an enzyme produced by the yeast, the invertase. That produces glucose and fructose:

$$C_{12}H_{22}O_{11}(aq) + H_2O \xrightarrow{\textit{invertase}} C_6H_{12}O_6 + C_6H_{12}O_6$$

Sucrose Glucose Fructose

The glucose and fructose have the same chemical composition, differentiating on the positions of their atoms according the representations below:



They either do not cross the cell membrane easily, requiring the action of carrier proteins: the permeases, which, however, have a higher affinity for glucose. Thus, this molecule is captured faster from the medium and is carried into the cell by means of what is called *facilitated diffusion* (Zastrow & Stambuk, 2000).

The process of converting glucose in ethanol, the alcoholic fermentation, involves a sequence of 12 steps and enzymes. Its clearance involved enormous amount of research and at least 50 scientists during the first half of the twentieth century, with the development of methods, equipment, isolation and purification of substances and discussions that put the science of Biochemistry forward. The research followed two investigation routes: one involving the fermentation of yeast and other directed to the understanding of the lactic fermentation in the muscles, with similarities and interconnections.

Some scientists involved were refugees from Nazi Germany and developed their research in England and the United States. Six of them were awarded with Nobel Prizes for their contributions to the glycolysis steps elucidation, which was completely established in 1940 after a little more than 30 years of studies (Barnett, 2003). The equation showed below displays in a simplified manner the starting reactant (glucose), the enzymes participation and the reaction products: ethanol and carbon dioxide, but does not allow to realize the details, the complexity of the overall process and the intermediaries involved:

$$C_6H_{12}O_6 \xrightarrow{\text{enzymes}} 2\ C_2H_5OH + 2\ CO_2$$

As Mr. Zé mentioned: "it doesn´t have any drop of alcohol in the wine. It's on to ferment that gives the alcohol. We don't put alcohol in the wine. It turns there inside. Its fermentation is that creates this alcohol". When he said "fermentation", he is using the same expression given to the process by the scientist Louis Pasteur. The verb "to ferment" and what Mrs. Ná said: "it is the ferment that makes it" and "If the air enters it's possible that doesn't ferment" are derived.

The final products of the alcoholic fermentation, the carbon dioxide (CO_2) and ethanol (C_2H_5OH), have no use for the yeast (Lima et al, 2005). Because they are small molecules that interact properly with the lipid layer of the membrane cell they cross it by simple diffusion and are released out (Yuan et al., 2000; Zastrow and Stambuk, 2000) as Mr. Zé said: "it boils inside the demijohn to turn wine", "it releases a gas that burbles like when water boils to make coffee".

The mixture remains isolated inside the demijohns for eight days. Why this time? After this period, the cork stoppers are removed and replaced by small bamboo cylinders, measuring four to eight centimeters long, with a diameter that fits the demijohns´mouths. On these, they adjust the end of a plastic little hose of approximately 40 centimeters long and pass beeswax around to seal. They also use pierced cork stoppers rather the bamboo cylinders, adapt the little hose on them and insert in the demijohn´s mouth to cover it.

The free end of the little hoses is placed into a small container with water where the gas produced on fermentation bubbles: "have to put to ferment, if doesn’t put it doesn’t ferment" (Mrs. Ná), "have to put it into the glass of water to do not bring the air inside, the air has only to come out". "The air is very important thing. It cannot come into. If it does it weakens the wine" (Mr. Zé). There is the requirement to ensure anaerobic condition to do not prejudice the fermentation. Therefore, they seal the demijohns' mouth with stoppers containing hoses whose free ends are placed inside a glass with water to the gas exit. The fermentation releases carbon dioxide, but requires the oxygen absence.

The fermentation ends after around two months, where there is substantial decrease in the amount of the gas that bubbles in the container with water. In this step, the quantity of the ethanol produced affects the metabolism of the yeast. The yeast die when the alcohol content is above 16%. In 6% and 15% only 0.25% and 0.05% of the yeasts survive, respectively (Peixoto, 2002, p.302).

As the fermentation ends, the bamboo cylinders or the cork stoppers and the hoses are removed and the demijohns are again capped with normal cork stoppers. The wine is almost ready, but remains in the demijohns for more two months. A dreg is deposited in the bottom of them, which according to Mr. Zé is the "orange dirt or acid" probably composed by dead yeast or solids that were not properly separated by the filtration method used. After this time, they remove the wine by suction of the supernatant liquid using a hose. The wine prepared like that has a honey color with light orange taste. It is stored in glass bottles and it is ready to be tasted during meals and especially in festive days or family celebrations.

It is interesting to observe that the orange wine making by Mr. Zé and Mrs. Ná does not involve any explicit scientific knowledge or advanced technological resource. Surely that Ronaldo, their son, know a little more about what is involved in the production of the wine of their parents and should now see it otherwise. However, will this change the way his family make the orange wine? Will help them to produce a better wine? Will place the orange wine in the supermarket shelves? Does science and technology may alter these knowledge and practices?

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