Technological University Institute of Lille “IUT 1” Department of Biological Engineering Nutrition and Dietetics option Academic year: 2016-2017.

Comparative nutritional study of several "fermented" drinks : Fassbrause, Kombucha, Cider, Water Kefir. Fabrication, Analysis(Rate of alcohol, Proteins, minerals), Sensory analysis(descriptors), Microbiology analysis.

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GENERAL INTRODUCTION:

One of the activities that open up great opportunities for a Nutritionist is related to the development and study of fermented beverages. An inherent aspect of a Nutritionist's activity is the permanent concern for the quality and nutritional contribution of the product, being a variable determinant of the products in the face of the requirements of competitiveness in the globalized markets. Fermentation is a millennial method of preserving foods while increasing their nutritional qualities. It can also be applied to beverages, not just those containing alcohol such as wine, beer or cider. There are dozens of drinks with natural fermentation, which, while being delicious to drink, have in the same time a lot of health benefits. They increase the nutritional qualities while bringing precious probiotics that strengthen our immune system and nourish our intestinal flora. This chemical process occurs when certain plants and fruits of high content in glucose are allowed to stand for a long time and at an appropriate temperature. Under these circumstances, the microorganisms in these products convert the sucrose into alcohol. These beverages receive a treatment that involves growth and activity of microorganisms such as molds, bacteria or yeasts. With the term "fermentation", we refer to a process aimed at causing a chemical change to a complex organic compound by the action of one or more enzymes that have been produced by microorganisms. This process serves humanity from the beginnings of civilization to today.

The objective of our study is to compare several fermented drinks, with or without alcohol. The drinks that we worked with were Beer, Fassbrause, Cider, Kombucha and Water Kefir. In order to compare the fermented drinks we realized several analyses (Microbiological, Sensory, Protein, Alcohol and Mineral analyses). I have to mention that we realized analyses in fermented drinks that we created in our own in the laboratory (except of cider). Our purpose is to define the benefits, as well as, the disadvantages or adverse effects, of each of the fermented drinks for our health. For this reason, we need to understand the composition of each drink in bacteria, yeasts, macronutrients and micronutrients (calories, sugars, proteins, fats, minerals etc.) Throughout our study, we worked in a microbiology laboratory, in a biochemical laboratory and in a brewery, in order to prepare each drink and continuously, to realize the different analyses.

Bibliographic Introduction:

KOMBUCHA:

General Informations:

Kombucha tea is a fermented drink made with tea, sugar, bacteria and yeast. Although it's sometimes referred to as kombucha mushroom tea, kombucha is not a mushroom — it's a colony of bacteria and yeast. Kombucha tea is made by adding the colony to sugar and tea, and allowing the mix to ferment. The resulting liquid contains vinegar, B vitamins and a number of other chemical compounds.

Proponents claim kombucha tea can stimulate the immune system, prevent cancer, and improve digestion and liver function. However, there's no scientific evidence to support these health claims.

There have, however, been reports of adverse effects, such as stomach upset, infections and allergic reactions in kombucha tea drinkers. Kombucha tea is often brewed in homes under nonsterile conditions, making contamination likely. If ceramic pots are used for brewing, lead poisoning might be a concern — the acids in the tea may leach lead from the ceramic glaze.

In short, there isn't good evidence that kombucha tea delivers on its health claims. At the same time, several cases of harm have been reported.

Biological :

A kombucha culture is a symbiotic culture of bacteria and yeast (SCOBY), similar to mother of vinegar, containing one or more species each of bacteria and yeasts, which form a zoogleal mat known as a "mother." The cultures may contain one or more of the *yeasts Saccharomyces cerevisiae*, *Brettanomyces bruxellensis*, *Candida stellata*, *Schizosaccharomyces pombe*, and *Zygosaccharomyces bailii*.

The *bacterial component* of kombucha comprises several species, almost always including *Gluconacetobacter xylinus* (*G. xylinus*, formerly *Acetobacter xylinum*), which ferments alcohols produced by the yeasts into acetic and other acids, increasing the acidity and limiting ethanol content. The population of bacteria and yeasts found to produce acetic acid has been reported to increase for the first 4 days of fermentation, decreasing thereafter. *G. xylinum* has been shown to produce microbial cellulose, and is reportedly responsible for most or all of the physical structure of the "mother", which may have been selectively encouraged over time for firmer (denser) and more robust cultures by brewers.

Chemical :

Sucrose is converted, biochemically, into fructose and glucose, and these into gluconic acid and acetic acid, and these substances are present in the drink. In addition, kombucha contains enzymes and amino acids, polyphenols, and various other organic acids; the exact quantities of these items vary between preparations. Other specific components include ethanol (see below), glucuronic acid, glycerol, lactic acid, usnic acid (a hepatotoxin, see above), and B-vitamins. Kombucha has also been found to contain vitamin C.

The alcohol content of the kombucha is usually less than 1%, but increases with fermentation time.

Nutritional value:

Kombucha Calories: The black- or green-tea base of kombucha does not have significant calories, but the yeast and sugar do. The calorie count of your kombucha will depend on how much of those ingredients you use. Sugar has 16 calories per teaspoon, and yeast contains about 13 calories per teaspoon. Store-bought kombucha may have about 30 calories per cup.

Fermentation

If yeast and bacteria are sitting alone with nothing around, they'll starve. When you give them nourishment, like black tea and sugar, the process begins.
The main components in the final brew include:

Ethanol

About 0.5%. By comparison, regular beer generally has about 5%.
Ethanol and acetic acid have been reported to have antimicrobial activity against pathogenic bacteria, which can provide protection against kombucha brew contamination.
The alcohol content in raw kombucha brew can increase after bottling. Sometimes it can reach levels of 2 to 5%.

Carbon dioxide/carbonic acid

This carbonates kombucha brew, making it fizzy.

Sugar

Added to feed yeasts and bacteria.

Vitamins

Mainly B and C vitamins.

Lactic acid

This is a byproduct of the fermentation process. It might have a laxative effect.

Caffeine

Small amount from tea.

Nutritional Facts:

Homemade Kombucha 1 serving/250ml contains:

Calories	596	**Sodium**	636 mg
Total Fat	19 g	**Potassium**	0 mg
Saturated	4 g	**Total Carbs**	58 g
Polyunsaturated	9 g	**Dietary Fiber**	0 g
Monounsaturated	4 g	**Sugars**	0 g
Trans	0 g	**Protein**	48 g
Cholesterol	8 mg		
Vitamin A	0%	**Calcium**	0%
Vitamin C	2%	**Iron**	0

Table 1 Kombucha-Nutritional facts

Kombucha sells for about $3 per bottle and annual sales may soon hit the $100 million mark.

WATER KEFIR:

WHAT IS WATER KEFIR?

Water kefir is a probiotic beverage made with water kefir grains. Water kefir grains can be used to culture sugar water, juice, or coconut water. A powdered Kefir Starter Culture may also be used to culture coconut water or fruit juice.

What are water kefir grains?

Water kefir grains consist of bacteria and yeast existing in a symbiotic relationship. The term "kefir grains" describes the look of the culture only.
Water kefir grains contain no actual "grains" such as wheat, rye, etc.
Water kefir grains are grown in filtered water and organic sugar.
Water kefir contains fewer strains of bacteria and yeasts than milk kefir. On the other hand, water kefir contains far more than other cultured products, like yogurt or buttermilk.

BACTERIA AND YEASTS :

Water kefir grains consist of a complex polysaccharide matrix, in which live a combination of live bacteria and yeasts existing in a symbiotic matrix. Kefir grain make-up can vary depending on culturing location and conditions, resulting in a highly variable community of lactic acid bacteria and yeasts.

BACTERIA

Species Lactobacillus
L. brevis
L. casei
L. hilgardii
L. hordei
L. nagelii
Species Leuconostoc
L. citreum
L. mesenteroides
Species Acetobacter
A. fabarum
A. orientalis
Species Streptococcus
S. lactis

YEASTS

Hanseniaospora valbyensis
Lachancea fermentati
Saccharomyces cerevisiae
Zygotorulaspora florentina

OTHER FACTS:

Water kefir grains do not contain gluten. Water kefir grains are reusable. Once a batch of water kefir is finished culturing, simply remove the water kefir grains and place them in fresh sugar water, juice, or coconut water. If cared for properly, water kefir grains have an unlimited life span and can be used repeatedly to make water kefir. Kefir made with a powdered kefir culture (direct-set) can often be recultured from 2-7 times. The exact number of successive batches will depend on the freshness of the kefir and hygienic practices employed.

WHAT DOES WATER KEFIR TASTE LIKE?

The taste of finished water kefir varies greatly, depending on the sugar used and the culturing time. Water kefir can be fairly sweet and may have a flat taste unless bottled. Most people prefer to add flavoring to water kefir before consuming. For example, coconut water can be used to make water kefir. Finished water kefir can be stored as follows:

- At room temperature: 3 to 4 days
- In the refrigerator: 2 to 3 weeks
- In the freezer: 1 to 2 months or longer
- Storage recommendation: Refrigerate

HOW MUCH ALCOHOL DOES WATER KEFIR CONTAIN ?

As with all cultured and fermented foods, a small amount of naturally occurring alcohol is typically present in the finished product. Although the amount will vary from batch to batch, for the typical brewing period, the amount should be quite low.

NUTRITIONAL FACTS:

Home Made - Water Kefir

1 Serving or 250 ml Contains:

Calories	5	**Sodium**	0 mg
Total Fat	0 g	**Potassium**	0 mg
Saturated	0 g	**Total Carbs**	1 g

Polyunsaturated	0 g	**Dietary Fiber**	0 g
Monounsaturated	0 g	**Sugars**	0 g
Trans	0 g	**Protein**	0 g
Cholesterol	0 mg		
Vitamin A	0%	**Calcium**	0%
Vitamin C	0%	**Iron**	0%

Table 2 Water Kefir-Nutritional Facts

CIDER

*Considering the cider, we did not produce our own in the laboratory and our analysis is based in a commercial drink.

Cider is is an alcoholic beverage made from the fermented juice of apples.

The juice of any variety of apples can be used to make cider, but cider apples are best. The addition of sugar or extra fruit before a second fermentation increases the alcoholic content of the resulting beverage.

Cider is popular in the United Kingdom, especially in the West Country, and widely available. The UK has the world's highest per capital consumption, as well as its largest cider-producing companies. Cider is also popular in other European countries including Ireland, Portugal , France (in particular Brittany and Normandy), northern Italy, and Spain. Cider alcohol content varies from 1.2% to 8.5% or more in traditional English ciders, and 3.5% to 12% in continental ciders. In UK law, it must contain at least 35% apple juice (fresh or from concentrate). In the US, there is a 50% minimum. In France, cider must be made solely from apples. In 2014, a study found that a pint of mass-market cider contained five teaspoons (20.5 g) of sugar, nearly as much as the WHO recommends as an adult's daily allowance of added sugar.

The commercial cider that we used in our analysis

PRODUCTION:

Apples grown for consumption are suitable for cider making, though some regional cider-makers prefer to use a mix of eating and cider apples (as in Kent, England), or exclusively cider apples (as in the West Country, England). There are many hundreds of varieties of cultivars developed specifically for cider making. Once the apples are gathered from trees in orchards they are *scratted* (ground down) into what is called *pomace* or *pommage*. Historically this was done using pressing stones with circular troughs, or by a cider mill. Cider mills were traditionally driven by the hand, water-mill, or horse-power. In modern times, they are likely to be powered by electricity. The pulp is then transferred to the cider press and built up in layers known as *cheeses* into a block. Traditionally the method for squeezing the juice from the apples involves placing sweet straw or haircloths between the layers of pomace. This will alternate with slatted ash-wood racks until there is a pile of ten or twelve layers. The *set* is then subjected to increasing degrees of pressure until all the 'must' or juice is squeezed from the pomace. This juice, after being strained in a coarse hair-sieve, is then put into either open vats or closed casks. The pressed pulp is given to farm animals as winter feed, composted, discarded or used to make liqueurs. Fermentation is carried out at a temperature of 4–16 °C (40–60 °F). This is low for most kinds of fermentation but is beneficial for cider as it leads to slower fermentation with less loss of delicate aromas. Fermentation can occur due to natural yeasts that are present in the must or some cider makers add yeast, such as Saccharomyces bayanus. Shortly before the fermentation consumes all the sugar, the liquor is "racked" (siphoned) into new vats. This leaves dead yeast cells and other undesirable material at the bottom of the old vat. At this point, it becomes important to exclude airborne acetic bacteria, so vats are filled completely to exclude air. The fermenting of the remaining available sugar generates a small amount of carbon dioxide that forms a protective layer, reducing air contact. This final fermentation creates a small amount of carbonation. Extra sugar might be added.

Apple-based juice may also be combined with fruit to make a fine cider; fruit purées or flavourings can be used, such as grape, cherry, raspberry, and cranberry.

The cider is ready to drink after a three-month fermentation period, though more often it is matured in the vats for up to three years.

FRANCE:

French *cidre* (French pronunciation: [sidʁ]) is an alcoholic drink produced predominantly in Normandy and Brittany. It varies in strength from below 4% alcohol to considerably more. Cidre Doux is a sweet cider, usually up to 3% in strength. 'Demi-Sec' is 3–5% and Cidre Brut is a strong dry cider of 4.5% alcohol and above. Most French ciders are sparkling. Higher quality cider is sold in champagne-style bottles (*cidre bouché*). Many ciders are sold in corked bottles, but some screw-top bottles exists. A *kir Breton* (or *kir normand*) is a cocktail apéritif made with cider and cassis, rather than white wine and cassis for the traditional *kir*. The Domfrontais, in the Orne (Basse-Normandie), is famous for its pear cider (poiré).

NUTRITIONAL FACTS:

Serving Size: 1 cup/250 ml
Calories Per Serving: 117Kcal

	% Daily Values
Total Fat 0.27g	**0**%
Saturated Fat 0.047g	**0**%
Polyunsaturated Fat 0.082g	
Monounsaturated Fat 0.012g	
Cholesterol 0mg	**0**%
Sodium 7mg	**0**%
Potassium 295mg	
Total Carbohydrate 28.97g	**10**%
Dietary Fiber 0.2g	**1**%
Sugars 27.03g	
Protein 0.15g	
Vitamin A 0%	**Vitamin C** 4%
Calcium 2%	**Iron** 5%

Table 3 Cider-Nutritional Facts

BEER:

Beer is the world's oldest and most widely consumed alcoholic drink; it is the third most popular drink overall, after water and tea.The production of beer is called brewing, which involves the fermentation of sugars, mainly derived from cereal grain starches—most commonly from malted barley, although wheat, maize (corn), and rice are widely used. Most beer is flavoured with hops, which add bitterness and act as a natural preservative, though other flavourings such as herbs or fruit may occasionally be included. The fermentation process causes a natural carbonation effect, although this is often removed during processing, and replaced with forced carbonation

INGREDIENTS:

The basic ingredients of beer are water; a starch source, such as malted barley, able to be saccharified (converted to sugars) then fermented (converted into ethanol and carbon dioxide); a brewer's yeast to produce the fermentation; and a flavouring such as hops. A mixture of starch sources may be used, with a secondary starch source, such as maize (corn), rice or sugar, often being termed an adjunct, especially when used as a lower-cost substitute for malted barley.

Water

Beer is composed mostly of water. Regions have water with different mineral components; as a result, different regions were originally better suited to making certain types of beer, thus giving them a regional character.

MALT:

Malted barley – a primary mash ingredient

The starch source in a beer provides the fermentable material and is a key determinant of the strength and flavour of the beer. The most common starch source used in beer is malted grain. Grain is malted by soaking it in water, allowing it to begin germination, and then drying the partially germinated grain in a kiln. Malting grain produces enzymes that convert starches in the grain into fermentable sugars.Different roasting times and temperatures are used to produce different colours of malt from the same grain. Darker malts will produce darker beers.

Nearly all beer includes barley malt as the majority of the starch. This is because its fibrous hull remains attached to the grain during threshing. After malting, barley is milled, which finally removes the hull, breaking it into large pieces. These pieces remain with the grain during the mash, and act as a filter bed during lautering, when sweet wort is separated from insoluble grain material. Other malted and unmalted grains (including wheat, rice, oats, and rye, and less frequently, corn and sorghum) may be used. Some brewers have produced gluten-free beer, made with sorghum with no barley malt, for those who cannot consume gluten-containing grains like wheat, barley, and rye.

Hops

Hop cone.

Flavouring beer is the sole major commercial use of hops.The flower of the hop vine is used as a flavouring and preservative agent in nearly all beer made today. The flowers themselves are often called "hops".

Hops contain several characteristics that brewers desire in beer. Hops contribute a bitterness that balances the sweetness of the malt; the bitterness of beers is measured on the International Bitterness Units scale. Hops contribute floral, citrus, and herbal aromas and flavours to beer. Hops have an antibiotic effect that favours the activity of brewer's yeast over less desirable microorganisms and aids in "head retention", the length of time that a foamy head created by carbonation will last. The acidity of hops is a preservative.

Yeast

Yeast is the microorganism that is responsible for fermentation in beer. Yeast metabolises the sugars extracted from grains, which produces alcohol and carbon dioxide, and thereby turns wort into beer. In addition to fermenting the beer, yeast influences the character and flavour.

The dominant types of yeast used to make beer are the top-fermenting *Saccharomyces cerevisiae* and bottom-fermenting *Saccharomyces pastorianus*. *Brettanomyces* ferments lambics,and *Torulaspora delbrueckii* ferments Bavarian weissbier.

Before the role of yeast in fermentation was understood, fermentation involved wild or airborne yeasts. A few styles such as lambics rely on this method today, but most modern fermentation adds pure yeast cultures.

Clarifying agent

Some brewers add one or more clarifying agents to beer, which typically precipitate (collect as a solid) out of the beer along with protein solids and are found only in trace amounts in the finished product. This process makes the beer appear bright and clean, rather than the cloudy appearance of ethnic and older styles of beer such as wheat beers.

BREWING:

The process of making beer is known as brewing. A dedicated building for the making of beer is called a brewery, though beer can be made in the home and has been for much of its history. A company that makes beer is called either a brewery or a brewing company. Beer made on a domestic scale for non-commercial reasons is classified as homebrewing regardless of where it is made, though most homebrewed beer is made in the home. The purpose of brewing is to convert the starch source into a sugary liquid called wort and to convert the wort into the alcoholic beverage known as beer in a fermentation process effected by yeast. The first step, where the wort is prepared by mixing the starch source (normally malted barley) with hot water, is known as "mashing". Hot water (known as "liquor" in brewing terms) is mixed with crushed malt or malts (known as "grist") in a mash tun. The mashing process takes around 1 to 2 hours, during which the starches are converted to sugars, and then the sweet wort is drained off the grains. The grains are now washed in a process known as "sparging". This washing allows the brewer to gather as much of the fermentable liquid from the grains as possible. The process of filtering the spent grain from the wort and sparge water is called *wort separation.* Some modern breweries prefer the use of filter frames which allow a more finely ground grist.

Most modern breweries use a continuous sparge, collecting the original wort and the sparge water together. However, it is possible to collect a second or even third wash with the not quite spent grains as separate batches. Each run would produce a weaker wort and thus a weaker beer. This process is known as second (and third) runnings. Brewing with several runnings is called parti gyle brewing.

The sweet wort collected from sparging is put into a kettle, or "copper" and boiled, usually for about one hour. During boiling, water in the wort evaporates, but the sugars and other components of the wort remain; this allows more efficient use of the starch sources in the beer. Boiling also destroys any remaining enzymes left over from the mashing stage. Hops are added during boiling as a source of bitterness, flavour and aroma. Hops may be added at more than one point during the boil. The longer the hops are boiled, the more bitterness they contribute, but the less hop flavour and aroma remains in the beer.

After boiling, the hopped wort is now cooled, ready for the yeast. In some breweries, the hopped wort may pass through a hopback, which is a small vat filled with hops, to add aromatic hop flavouring and to act as a filter; but usually the hopped wort is simply cooled for the fermenter, where the yeast is added. During fermentation, the wort becomes beer in a process which requires a week to months depending on the type of yeast and strength of the beer. In addition to producing ethanol, fine particulate matter suspended in the wort settles during fermentation. Once fermentation is complete, the yeast also settles, leaving the beer clear.

Fermentation is sometimes carried out in two stages, primary and secondary. Once most of the alcohol has been produced during primary fermentation, the beer is transferred to a new vessel and allowed a period of secondary fermentation. Secondary fermentation is used when the beer requires long storage before packaging or greater clarity. When the beer has fermented, it is packaged either into casks for cask ale or kegs, aluminium cans, or bottles for other sorts of beer.

VARIETIES:

While there are many types of beer brewed, the basics of brewing beer are shared across national and cultural boundaries.The traditional European brewing regions—Germany, Belgium, England and the Czech Republic—have local varieties of beer.

Top-fermented beers are most commonly produced with *Saccharomyces cerevisiae*, a top-fermenting yeast which clumps and rises to the surface, typically between 15 and 24 °C (60 and 75 °F). At these temperatures, yeast produces significant amounts of esters and other secondary flavour and aroma products, and the result is often a beer with slightly "fruity" compounds resembling apple, pear, pineapple, banana, plum, or prune, among others. After the introduction of hops into England from Flanders in the 15th century, "ale" referred to an unhopped fermented beverage, "beer" being used to describe a brew with an infusion of hops.

Pale ale

Pale ale is a beer which uses a top-fermenting yeast and predominantly pale malt. It is one of the world's major beer styles.

Stout

Stout and porter are dark beers made using roasted malts or roast barley, and typically brewed with slow fermenting yeast. There are a number of variations including Baltic porter, dry stout, and Imperial stout.

Mild

Mild ale has a predominantly malty palate. It is usually dark coloured with an abv of 3% to 3.6%, although there are lighter hued milds as well as stronger examples reaching 6% abv and higher.

Wheat

Wheat beer is brewed with a large proportion of wheat although it often also contains a significant proportion of malted barley. Wheat beers are usually top-fermented (in Germany they have to be by law). The flavour of wheat beers varies considerably, depending upon the specific style.

Lambic:

Lambic, a beer of Belgium, is naturally fermented using wild yeasts, rather than cultivated. Many of these are not strains of brewer's yeast (*Saccharomyces cerevisiae*) and may have significant differences in aroma and sourness. Yeast varieties such as *Brettanomyces bruxellensis* and *Brettanomyces lambicus* are common in lambics. In addition, other organisms such as *Lactobacillus* bacteria produce acids which contribute to the sourness.

Kriek, a variety of beer brewed with cherries, and a sweet taste.

Lambic, a beer of Belgium, is naturally fermented using wild yeasts, rather than cultivated. Many of these are not strains of brewer's yeast (*Saccharomyces cerevisiae*) and may have significant differences in aroma and sourness. Yeast varieties such as *Brettanomyces bruxellensis* and *Brettanomyces lambicus* are common in lambics. In addition, other organisms such as *Lactobacillus* bacteria produce acids which contribute to the sourness.

Lager

Lager is cool fermented beer. Pale lagers are the most commonly consumed beers in the world. Lager yeast is a cool bottom-fermenting yeast (*Saccharomyces pastorianus*) and typically undergoes primary fermentation at 7–12 °C (45–54 °F) (the fermentation phase), and then is given a long secondary fermentation at 0–4 °C (32–39 °F) (the lagering phase). During the secondary stage, the lager clears and mellows. The cooler conditions also inhibit the natural production of esters and other byproducts, resulting in a "cleaner"-tasting beer.

Paulaner dunkel – a dark lager

Colour

Beer colour is determined by the malt. The most common colour is a pale amber produced from using pale malts. *Pale lager* and *pale ale* are terms used for beers made from malt dried with the fuel coke.

In terms of sales volume, most of today's beer is based on the pale lager brewed in 1842 in the town of Pilsen in the present-day Czech Republic. The modern pale lager is light in colour with a noticeable carbonation (fizzy bubbles) and a typical alcohol by volume content of around 5%. The Pilsner Urquell, Bitburger, and Heineken brands of beer are typical examples of pale lager, as are the American brands Budweiser, Coors, and Miller.

Dark beers are usually brewed from a pale malt or lager malt base with a small proportion of darker malt added to achieve the desired shade. Other colourants—such as caramel—are also widely used to darken beers. Very dark beers, such as stout, use dark or patent malts that have been roasted longer. Some have roasted unmalted barley.

Strength

Beer ranges from less than 3% alcohol by volume (abv) to around 14% abv, though this strength can be increased to around 20% by re-pitching with champagne yeast, and to 55% abv by the freeze-distilling process. The alcohol content of beer varies by local practice or beer style. The pale lagers that most consumers are familiar with fall in the range of 4–6%, with a typical abv of 5%. The customary strength of British ales is quite low, with many session beers being around 4% abv. The alcohol in beer comes primarily from the metabolism of sugars that are produced during fermentation. The quantity of fermentable sugars in the wort and the variety of yeast used to ferment the wort are the primary factors that determine the amount of alcohol in the final beer. Additional fermentable sugars are sometimes added to increase alcohol content, and enzymes are often added to the wort for certain styles of beer (primarily "light" beers) to convert more complex carbohydrates (starches) to fermentable sugars. Alcohol is a by-product of yeast metabolism and is toxic to the yeast; typical brewing yeast cannot survive at alcohol concentrations above 12% by volume. Low temperatures and too little fermentation time decreases the effectiveness of yeasts and consequently decreases the alcohol content.

BEER AND NUTRITION:

	Regular Beer	**Light Beer**	**Bud Light**	**Michelob Ultra**
Water	327.4g*	335.8g	336.3g	337.7g
Calories	153*	103	110	96
Alcohol	13.9g*	11.0g	11.7g	11.3g
Protein	1.6g*	0.9g	0.9g	0.6g
Carbohydrate	12.6g*	5.8g	6.6g	2.6g
Fat	0.0g	0.0g	0.0g	0.0g
Cholestrol	0.0g	0.0g	0.0g	0.0g
Calcium	14mg	14mg	11mg	14mg
Magnesium	21mg	18mg	25mg	14mg
Phosphorous	50mg	42mg	39mg	28mg
Potassium	96mg	74mg	92mg	60mg
Sodium	14mg	14mg	11mg	11mg
Niacin	2mg	1mg	N/A**	N/A**
Folate	21mcg	21mcg	N/A**	N/A**

Table 4 Beer- Nutritional Facts per serving/350ml

*Include ales, lagers, porters, premium beers and stouts. All other nutrients based on lager samples

FASSBRAUSE:

Fassbrause literally "keg soda", is a non-alcoholic or alcoholic (depending on the brand) German drink made from fruit and spices and malt extract, traditionally stored in a keg. *Fassbrause* is a speciality of Berlin, where it is sometimes called *Sportmolle*. (*Molle* used to be a term for "beer" in the Berlin dialect.) *Fassbrause* is about the same color as some beers, and usually tastes like apples. The taste is strongly reminiscent of the Austrian drink, *Almdudler*, except that *Fassbrause* is less sweet, and not quite as spicy.

A variant of *Fassbrause*, the so-called *Rote Fassbrause* ("red keg soda"), which is available in some of the new states, but not in Berlin itself, appeared in the 1950s. This variant was available in the German Democratic Republic (GDR) prior to German Reunification and tastes like raspberries. The procedure of making a beer without alcohol (Fassbrause) is the same with the one of a classic beer, instead of the final step where bacteria are added in the place of the yeasts for the fermentation. I have to mention that, in all our analysis, we used an equivalent of Fassbrause, Bionade, as we couldn't found the label of Fassbrause in French commerce.

Bionade- Sureau

Nutritional facts for 250 ml of drink	
Energie	237,5KJ/55Kcal
Fats	<1,25g
-From which Saturated Fats	<0,25g
Carbohydrates	12,50g
-From which Sugars	12,50g
Proteins	<1,25g
Salt	<0,025g

Table 5 Bionade-Nutritional Facts

MATERIALS AND METHODS:

KOMBOUCHA:

RECIPE OF KOMBOUCHA:

EQUIPMENT:

- Quart-Size Glass Jar
- Plastic or Wooden Stirring Utensil
- Tight-Weave Cloth or Paper Coffee Filter
- Something to secure the cover to the jar (rubber band or canning jar rings work well)

INGREDIENTS:

- Water (1L)
- White Sugar (70gr)
- Tea Bags *or* Loose Tea (2 tablespoons)
- Starter Tea *or* Distilled White Vinegar
- Active Kombucha SCOBY

Kombucha Scoby

INSTRUCTIONS:

1. Combine hot water and sugar in a glass jar. Stiruntil the sugar dissolves. *The water should be hot enough to steep the tea but does not have to be boiling.*
2. Place the tea or tea bags in the sugar water to steep.

NOTE: Using a metal tea ball to contain loose tea for making kombucha is acceptable. The tea ball should be removed before adding the SCOBY and starter tea, so the tea ball will not come into contact with the SCOBY. Cool the mixture to 20-30 degrees. *The tea may be left in the liquid as it cools or removed after the first 10-15 minutes. The longer the tea is left in the liquid, the stronger the tea will be.*

3. Remove the tea bags or completely strain the loose tea leaves from the liquid.
4. Add starter tea from a previous batch to the liquid. *If you do not have starter tea, distilled white vinegar may be substituted.*
5. Add an active kombucha SCOBY.
6. Cover the jar with a tight-weave towel or coffee filter and secure with a rubber band.

7. Allow the mixture to sit undisturbed at 20-30 degrees, out of direct sunlight, for 7-30 days, or to taste. *The longer the kombucha ferments, the less sweet and more vinegary it will taste.*
8. Pour kombucha off the top of the jar for consuming. Retain the SCOBY and enough liquid from the bottom of the jar to use as starter tea for the next batch.
9. The finished kombucha can be flavored and bottled, if desired, or enjoyed plain. (We did not flavored our kombucha.)

WATER KEFIR :

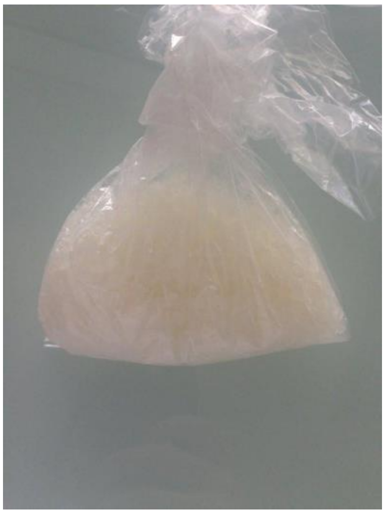
RECIPE OF WATER KEFIR (ORANGE FLAVORED):

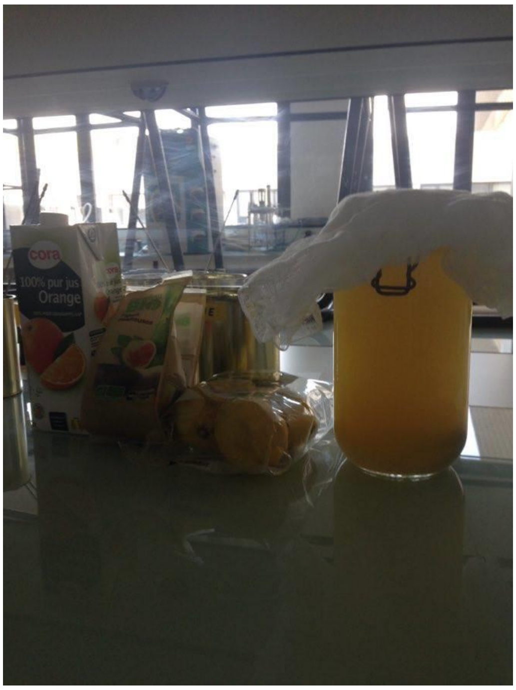
EQUIPMENT :

- A glass jar
- A plastic or wood stirring utensil
- A towel, butter muslin or paper coffee filter to use as a covering for the jar
- A band to secure the covering such as a rubber band or jar ring.
- A fine mesh strainer for removing the grains from the finished water kefir

INGREDIENTS :

- Rehydrated Water Kefir Grains (60gr)
- 1/4 cup of sugar
- 1L Water (free of chlorine and fluoride)
- 2 dry figs
- 2 slices of lemon
- 200ml of orange juice





Water Kefir grains

INSTRUCTIONS :

1. Pour 1/4 cup sugar in to the jar.
2. Add ½ L of hot water.
3. Swirl to dissolve the sugar.
4. Add ½ L of room temperature or cool water.
5. Check the temperature of the liquid to make sure it's room temperature (20-30 degrees.)

6. Add the water kefir grains, the 2 dry figs, the 2 slices of lemon and the 200ml of orange juice.
7. Cover the jar and place in a warm spot, 20-30 degrees, to culture for 24-48 hours.
8. After culturing is complete, separate kefir grains from the finished water kefir.
9. Place kefir grains in the new batch of sugar water.
10. The finished water kefir is now ready to consume, bottle, or store in the refrigerator.

Water Kefir grains in sugared water

FASSBRAUSE :

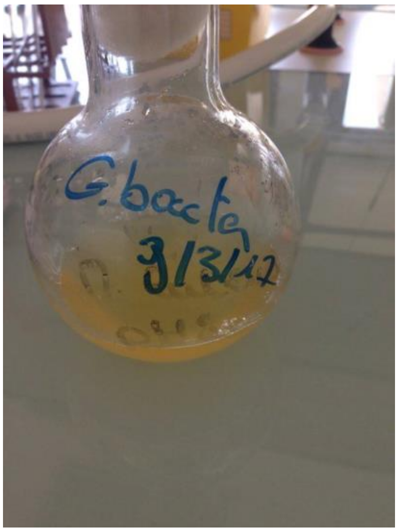
RECIPE OF FASSBRAUSE:

INGREDIENTS :

1. 8Kg malt blade7 EBC
2. Water 12L
3. Hop of bitterness 30g
4. Bacteria : Gluconobacter

COMPOSITION OF BACTERIA GLUCONOBACTER (Procedure that we followed in the laboratory of microbiology):

In a nutritious bottle we added a little of glucose in order to promote the development of the bacteria (Gluconobacter). We continue by adding a few drops from the old culture of Gluconobacter. Due to the fact that the Gluconobacter is an aerobic bacteria we have to put the sample in an agitator machine for 24 hours in 30 degrees celsius. After this procedure the sample was ready to be added in our beer.



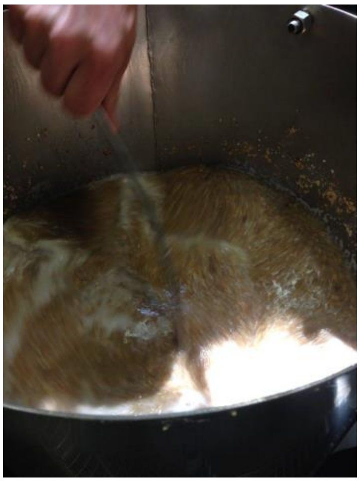
Gluconobacter

BREWING :

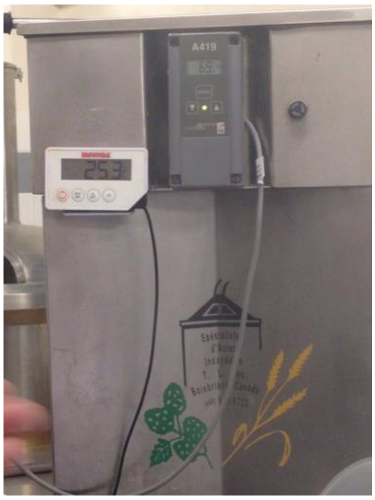
Weighing of malt

Mashing of the malt grains

The first step, where the wort is prepared by mixing the starch source (normally malted barley) with hot water, is known as "mashing". Hot water (known as "liquor" in brewing terms) is mixed with crushed malt or malts (known as "grist") in a mash tun.The mashing process takes around 1 to 2 hours, during which the starches are converted to sugars, and then the sweet wort is drained off the grains. The grains are now washed in a process known as "sparging". This washing allows the brewer to gather as much of the fermentable liquid from the grains as possible. The process of filtering the spent grain from the wort and sparge water is called *wort separation*. The traditional process for wort separation is lautering, in which the grain bed itself serves as the filter medium.



Mixing hot water with malt



Testing the temperature while brewing

Most modern breweries use a continuous sparge, collecting the original wort and the sparge water together. However, it is possible to collect a second or even third wash with the not quite spent grains as separate batches. Each run would produce a weaker wort and thus a weaker beer. This process is known as second (and third) runnings.

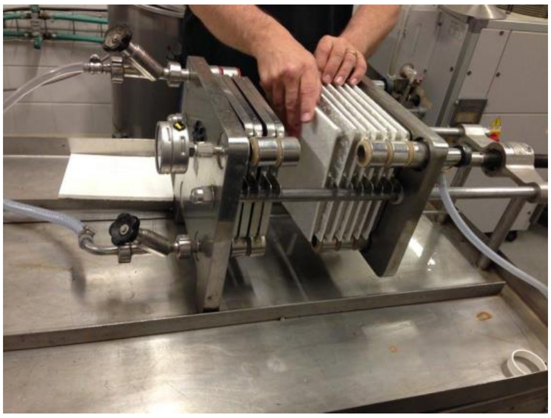
The sweet wort collected from sparging is put into a kettle and boiled, usually for about one hour. During boiling, water in the wort evaporates, but the sugars and other components of the wort remain; this allows more efficient use of the starch sources in the beer. Boiling also destroys any remaining enzymes left over from the mashing stage. Hops are added during boiling as a source of bitterness, flavour and aroma. Hops may be added at more than one point during the boil. The longer the hops are boiled, the more bitterness they contribute, but the less hop flavour and aroma remains in the beer.

Adding hops in our beer

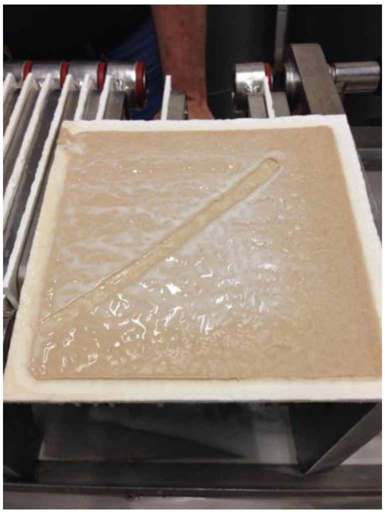
After boiling, the hopped wort is now cooled, ready for the bacteria. We added the bacteria and in the same time oxygen due to the fact that our bacteria is aerobic.

Adding bacteria (Gluconobacter) in beer in the same time with oxygen.

The day after adding the bacteria we continue with filtration and pasteurization of our beer in order to stop the fermentation and the production of ethanol. Moreover, pasteurization is necessary to avoid the acidification of the drink.




Filtration




Filter

Pasteurization

The result of filtration in our beer

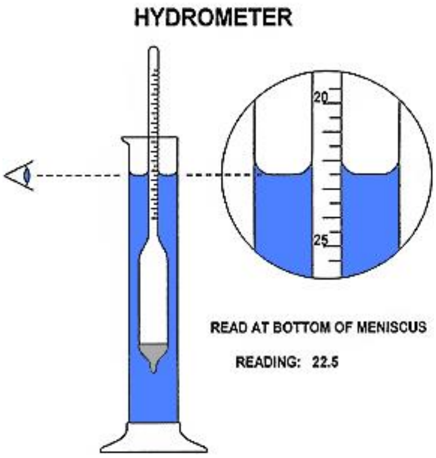
DEVELOPMENT :

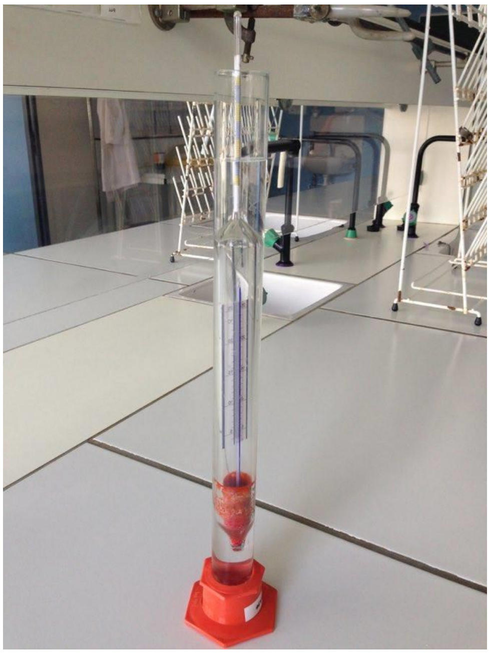
ANALYSIS OF ALCOHOL, MINERALS, PROTEINS, SENSORY AND MICROBIOLOGY ANALYSIS.

ANALYSIS OF ALCOHOL:

*In the alcohol analysis, we worked only with the alcoholic drinks such as beer and cider.

In order to measure the amount of ethanol in the alcoholic drinks (beer, cider) we used the method of distillation and thanks to a hydrometer, we defund the percentage of alcohol (% abv). A hydrometer is a measurement tool for determining the specific gravity of a liquid. Typically, a hydrometer is made of glass and has a weighted bulb on the bottom filed with lead so that the tool will float in a liquid. The hydrometer has measurement increments on the upper half for taking readings when the hydrometer reaches its equilibrium point in the liquid. When the hydrometer is floating properly, the bottom of the meniscus will read the correct specific gravity value of the liquid trying to be measured. The proper method to read a hydrometer is illustrated in the picture below. This specific gravity can also be known as relative density, because the density of the measured liquid is determined by its density compared to the density of water.





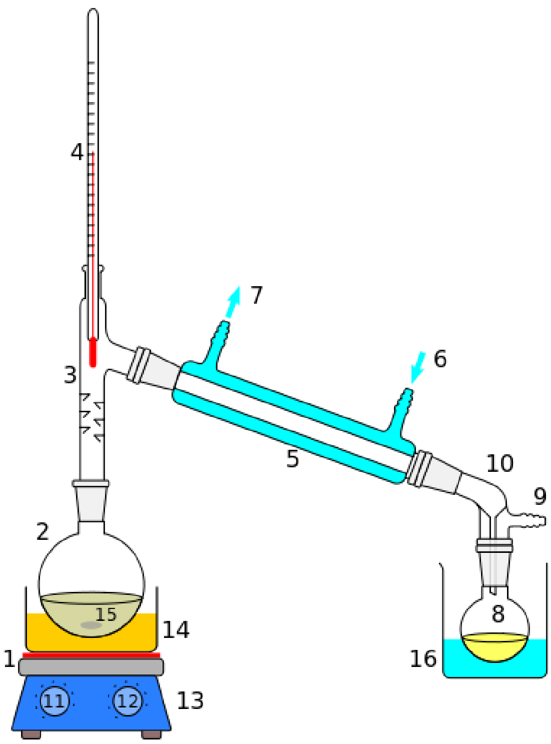
Hydrometer in laboratory

Distillation is the process of separating the component or substances from a liquid mixture by selective evaporation and condensation. Distillation may result in essentially complete separation (nearly pure components), or it may be a partial separation that increases the concentration of selected components of the mixture. In either case the process exploits differences in the volatility of the mixture's components. In industrial chemistry, distillation is a unit operation of practically universal importance, but it is a physical separation process and not a chemical reaction.

Commercially, distillation has many applications. For example:

- In the fossil fuel industry distillation is a major class of operation in obtaining materials from crude oil for fuels and for chemical feed stocks.
- Distillation permits separation of air into its components — notably oxygen, nitrogen, and argon — for industrial use.
- In the field of industrial chemistry, large amounts of crude liquid products of chemical synthesis are distilled to separate them, either from other products, or from impurities, or from unreacted starting materials.
- Distillation of fermented products produces distilled beverages with a high alcohol content, or separates out other fermentation products of commercial value.

Picture 2 : Laboratory display of distillation

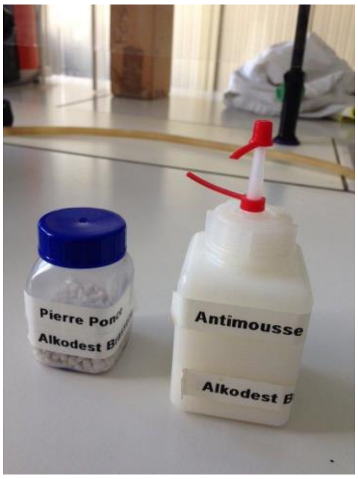


***1:** A source of heat **2:** Still pot **3:** Still head **4:** Thermometer/Boiling point temperature **5:** Condenser **6:** Cooling water in **7:** Cooling water out **8:** Distillate/receiving flask **9:** Vacuum/gas inlet **10:** Still receiver **11:** Heat control **12:** Stirrer speed* *control **13:** Stirrer/heat plate **14:** Heating (Oil/sand) bath **15:** Stirring means e.g. (shown), boiling chips or mechanical stirrer **16:** Cooling bath.*

Distillation display in our laboratory

PROCEDURE:

1. We added in the distillator 100ml of our sample (beer).
2. We added a pumice.
3. We added 2 drops of anti-foam (in order to avoid the excessive foam of the sample).



Pumice and anti-foam in laboratory

4. We opened the water source.

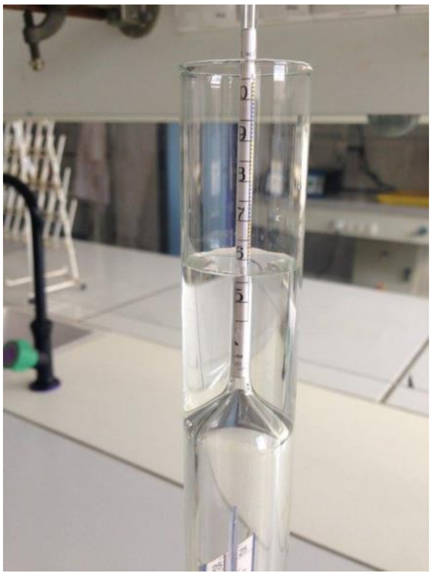
5. We turn on the heat source and the sample started to boil. The distillation started.

6. After the distillation, we collected only water and ethanol in the receiving/ distillate flask.

7. In the distillate flask, we added a little bit of water and we versed all the contained in the hydrometer.

8. We read in the hydrometer the percentage of alcohol which was 8,5% (abv) at 25 degrees Celsius.

We followed the same procedure for the cider and the results was 6% abv at 25 degrees Celsius.



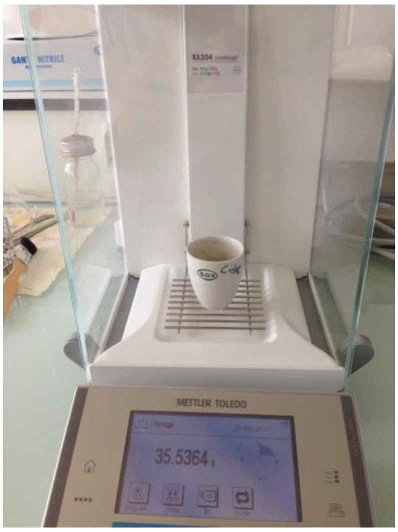
Rate of alcohol of cider (5%)

MINERAL ANALYSIS

PROCEDURE:

We realized a mineral analysis in the following drinks: bionade (a commercial beer without alcohol similar to the fassbrause one), beer, water kefir and cider.

Firstly, we weighted, in a high accuracy weight scale, approximately 20g of each drink in order to use them as samples in our analysis (19,96g of bionade, 20,33g of beer, 20g of water kefir and 20,03g of cider). Then, we put all the sample in the oven at 60 degrees Celsius for at least 24 hours.



The samples in the oven at 60 degrees

The day after we collect the dry matter of each sample as all the water had evaporated. The dry matter contains the mineral but also others macronutrients such as proteins, sugars and fats. In order to isolate only the minerals, we had to put the dry matter of each different sample in 550 degrees Celsius for 1 hour. The results that we collected are presented in the table below:

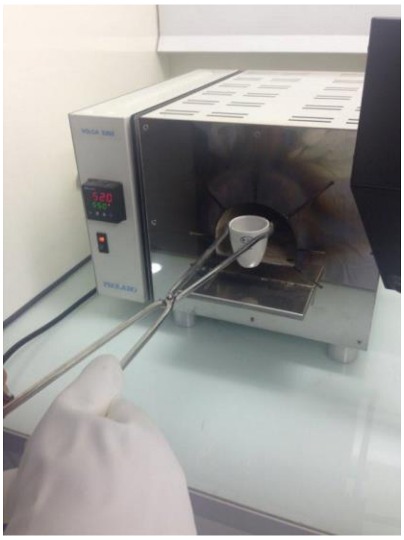
	BIONADE	**BEER**	**WATER KEFIR**	**CIDER**
ORIGINAL WEIGTH	19,96g	20,33g	20g	20,33g
DRY MATTER	1,2307g	1,5662g	0,9764g	0,6946g
MINERALS	0,0132g	0,0457g	0,0167g	0,06g
% DRY MATTER	**0,06154%**	**0,07704%**	**0,04882%**	**0,03467%**
%MINERALS	**0,00066%**	**0,00224%**	**0,00083%**	**0,00299%**

Table 6 Results of Mineral Analysis

CONCLUSION:

We can easily define that cider and beer, between the other drinks, contains the highest amount of minerals. Instead, bionade, contains the lowest amount of minerals though, with little differences with water kefir.

Dry matter of cider



Entering the sample in the oven

The samples after 1h at 550 degrees

SENSORY ANALYSIS

In order to realize a sensory analysis we create a questionnaire for the 4 drinks Komboucha, Water Kefir, Cider, Bionade Ginger-Orange flavor (beer without alcohol). The questionnaire was answered by my colleague Ana Machado Leon.

QUESTIONNAIRE FOR THE DRINKS KOMBOUCHA, WATER KEFIR, CIDER AND BIONADE:

1.How you would characterize the color of the drink?

KOMBOUCHA: dark orange

WATER KEFIR: pale yellow

CIDER: transparent yellow

BIONADE: orange

2.Does the drink has an acid taste? (1=not, 5=too much)

KOMBOUCHA: 1 2 ***3*** *4 5*

WATER KEFIR: 1 2 3 ***4*** *5*

CIDER: 1 2 3 4 ***5***

BIONADE: 1 ***2*** *3 4 5*

3.Does the drink has a bitter taste?

*KOMBOUCHA: **1** 2 3 4 5*

*WATER KEFIR: 1 **2** 3 4 5*

*CIDER: 1 **2** 3 4 5*

*BIONADE: **1** 2 3 4 5*

4.Does the drink has a sweet taste?

*KOMBOUCHA: 1 2 3 4 **5***

*WATER KEFIR: 1 **2** 3 4 5*

*CIDER: **1** 2 3 4 5*

*BIONADE: 1 2 **3** 4 5*

5.Does the drink has a salted taste?

*KOMBOUCHA: **1** 2 3 4 5*

*WATER KEFIR: **1** 2 3 4 5*

*CIDER: **1** 2 3 4 5*

*BIONADE: **1** 2 3 4 5*

6.Does the appearance of the drink I pleasant for you?(1=not at all, 5=yes)

*KOMBOUCHA: 1 2 3 **4** 5*

*WATER KEFIR: 1 **2** 3 4 5*

*CIDER: 1 2 3 4 **5***

*BIONADE: 1 2 3 4 **5***

7.Does the drink is clear and transparent?

*KOMBOUCHA: 1 2 3 **4** 5*

*WATER KEFIR: 1 **2** 3 4 5*

*CIDER: 1 2 3 **4** 5*

BIONADE: 1 2 3 4 ***5***

8.How you would describe the odor of the drink?

KOMBOUCHA: Sweet, pleasant and light.

WATER KEFIR: Strong, acid and peculiar.

CIDER: Strong, acid.

BIONADE: Fresh, sweet and light.

9.Does the drink is homogeneous?

KOMBOUCHA: 1 2 3 ***4*** *5*

WATER KEFIR: ***1*** *2 3 4 5*

CIDER: 1 2 3 ***4*** *5*

BIONADE: 1 2 3 4 ***5***

10.Does the drink is gaseous?

KOMBOUCHA: ***1*** *2 3 4 5*

WATER KEFIR: ***1*** *2 3 4 5*

CIDER: 1 2 3 4 ***5***

BIONADE: 1 2 3 4 ***5***

11.Does the drink has a fruit flavor?

KOMBOUCHA: 1 2 ***3*** *4 5*

WATER KEFIR: 1 2 3 ***4*** *5*

CIDER: 1 ***2*** *3 4 5*

BIONADE: 1 2 3 ***4*** *5*

12.Does the drink has an astringent flavor?

KOMBOUCHA: ***1*** *2 3 4 5*

WATER KEFIR: ***1*** *2 3 4 5*

*CIDER: 1 **2** 3 4 5*

*BIONADE: **1** 2 3 4 5*

13.Does the drink has a malt flavor?

*KOMBOUCHA: **1** 2 3 4 5*

*WATER KEFIR: **1** 2 3 4 5*

*CIDER: **1** 2 3 4 5*

*BIONADE: **1** 2 3 4 5*

PROTEIN ANALYSIS (KJELDHAL METHOD)

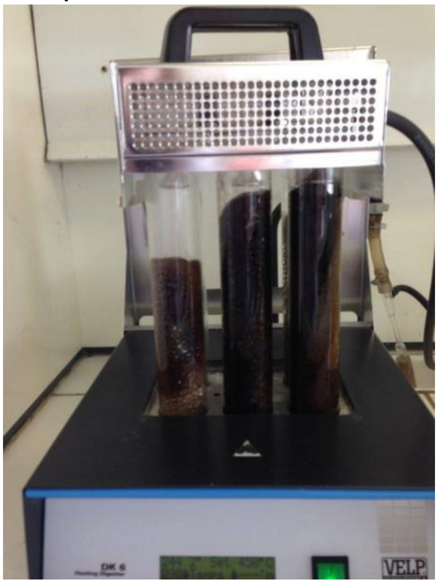
We realized a protein analysis in the following drinks: Beer, Beer without alcohol (Bionade), Water Kefir and Komboucha. The protein analysis contains the following 3 stages: **Mineralization, Distillation, Dosage.**

Mineralization:

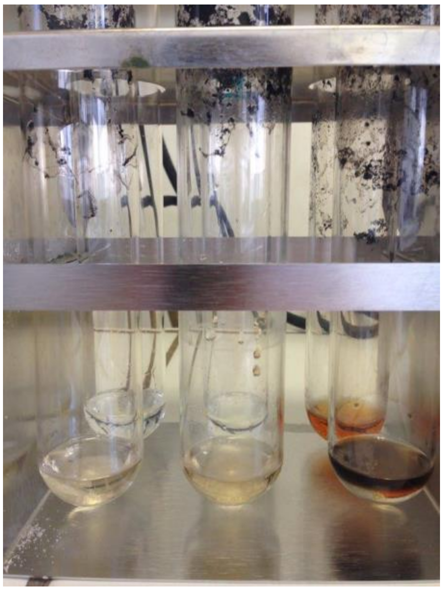
We weight 20ml of each drink and put them in 4 different big tubes. We also weight 100g of casein which was our standard sample. We added in each sample 15ml of H_2SO_4 .

Adding H_2SO_4 in each sample

I have to mention that the total tubes were 6 as we had also an empty tube (0) with only 15ml of H_2SO_4 in it. In continue, we burnt all the samples at 425 degrees Celsius for 30 minutes. After that, the receiving substance in our samples was NH_4^+.



Burning the samples at 425 degrees



Receiving substances in samples

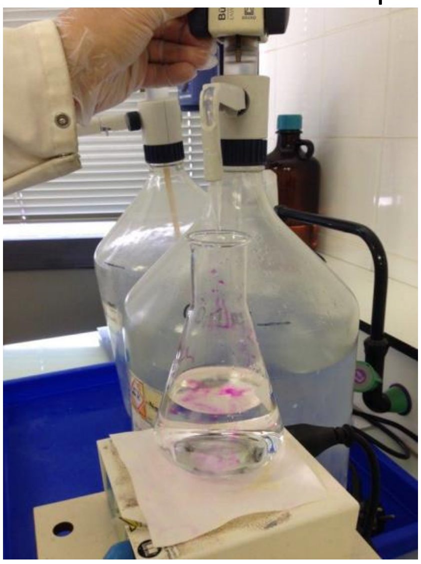
Distillation:

The aim of this stage is the transformation of NH_4^+ in NH_3 through NaOH. At each sample we added approximately 25 ml of H_2SO_4 0,05 M and put the tube in the distillation machine for about 5 minutes. The machine send in the tube 10 ml of water, 30 ml of NaOH and steam. The contain of each final recipient is NH_4^+. We keep on this procedure for all 6 samples.


Distillation

Dosage:

In the last stage of dosage we added in each recipient of NH_4 some drops of phenolphthalein. Finally, we added in each recipient NaOH 0,1 M until we noticed the change of the color (light pink). We followed this procedure for each of the different recipients.


Dosage

RESULTS: The exact amount of H_2SO_4 and NaOH that we measured for each sample is depicted in the table below:

	0	**CASEINE**	**BEER**	**BIONADE**	**WATER KEFIR**	**KOMBOUCHA**
H_2SO_4 0,05M (ml)	25,22	25,03	25,07	25,07	25,07	25,01

NaOH 0,1M (ml)	28,63	19,53	17,31	27,38	27,03	28,60

Table 7 Kjeldhal Method

CALCULATION OF PROTEINS:

In order to define the exact amount of proteins in each drink we used the following calculations:

n(number of mol N_2 measured)= (ml NaOH of the 0 sample – ml NaOH of each sample)* Molarity of NaOH (0,1mol/ L)

%age of N_2= [(n*1,4007)/ml of the original sample]*100

%age of proteins= %age of N_2*6,25

CASEINE:
n= (28,63ml-19,53)*0,1mol/L=0,91mol N_2
%age of N_2=[(0,91*1,4007)/100g]*100=1,27% N_2
%age proteins=1,27*6,25=**0,00794%**
BEER:
n N_2=0,16mol
%age of N_2= 1,12%
%age proteins= **0,007%**
BIONADE:
n N_2=0,125mol
%age of N_2=0,88%
%age proteins=**0,0055%**
WATER KEFIR:
n N_2=0,16mol
%age of N_2= 1,12%
%age proteins= **0,007%**
KOMBOUCHA:
n N_2=0,003mol
%age of N_2=0,02%
%age proteins=**0,0013%**

CONCLUSIONS:

It's more than obvious from the results that Komboucha, from all the drinks, contains the smallest amount of proteins (0,0013%). Bionade, contains the second smallest amount of proteins (0,0055%).Finally, it seems that Water Kefir and Beer contain approximately the same amount of proteins (0,007%), which is the biggest amount between all the other drinks.

MEASUREMENT OF SACCHAROSE:

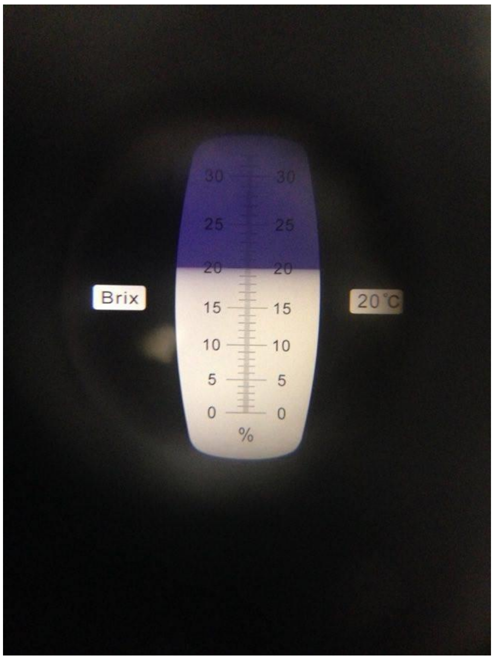
We realized the measurement of saccharose in 3 of our drinks, that is Cider, Komboucha and Water Kefir. In order to measure the rate of saccharose in our drinks we used a traditional handled refractometer. A traditional handheld refractometer is an analog instrument for measuring a liquid's refractive index. It works on the critical angle principle by which lenses and prisms project a shadow line onto a small glass reticle inside the instrument, which is then viewed by the user through a magnifying eyepiece. In use, a sample is placed between a measuring prism and a small cover plate. Light traveling through the sample is either passed through to the reticle or totally internally reflected. The net effect is that a shadow line forms between the illuminated area and the dark area. It is where this shadow line crosses the scale that a reading is taken. Because refractive index is very temperature dependent, it is important to use a refractometer with automatic temperature compensation. In our study, we used the refractometer to measure the specific gravity before fermentation to determine the amount of fermentable sugars which will potentially be converted to alcohol. We also measured the brix of pure water and pure alcohol to compare the results. We collected the following results:

	WATER	ALCOHOL	CIDER	KOMBUCHA	WATER KEFIR
BRIX	0	20	6	7	3,9

Table 8 Results of Saccharose Measurement

CONCLUSIONS:

We can easily observe that Komboucha is the drink with the largest amount of fermentable sugars. On the other hand, Water Kefir is the drink with the lowest amount of fermentable sugars.



Refractometer in laboratory

Watching through the refractometer (20 brix)

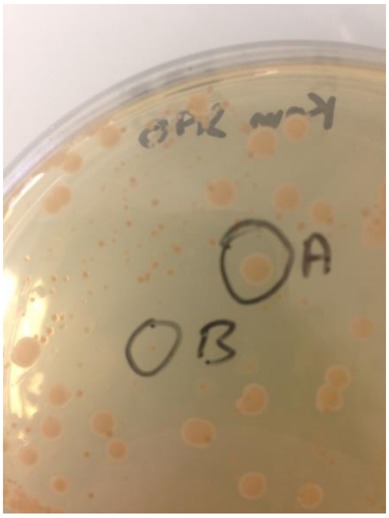
MICROBIOLOGY ANALYSIS:

We realized a microbiology analysis in the Komboucha Scoby and the Water Kefir grains. In our study we used the following mediums: BHI(Brain Heart Infusion which is used to cultivate bacteria), SAB (Sabouraud Agar which is used to cultivate yeasts), MRS(Man Rogosa Sharpe wich is used to cultivate bacteria), GNO (Nutrient Agar or Ordinary Nutrient Agar is a medium non-selective isolation).

KOMBUCHA:

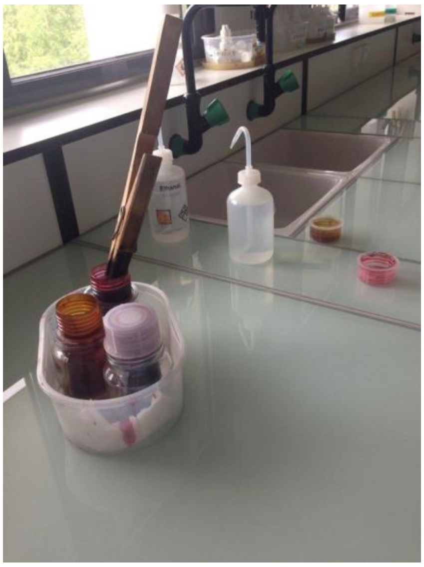
We started by adding some drops of the kombucha Scoby in 2 different tubes (one with BHI medium and one with SAB medium) in order to identify the existed yeasts and the bacteria. We put the tubes at 24 degrees Celsius for 48 hours.

After 48 hours we realized the isolation of the 2 different tubes in the Petri dishes. In microbiology, the term isolation refers to the separation of a strain from a natural, mixed population of living microbes, as present in the environment, in order to identify the microbe of interest.We waited for 48 hours for the colonies to growth in the dishes at 24 degrees Celsius. After 2 days we noticed 2 different colonies A, which was the big one and B, the smaller one.



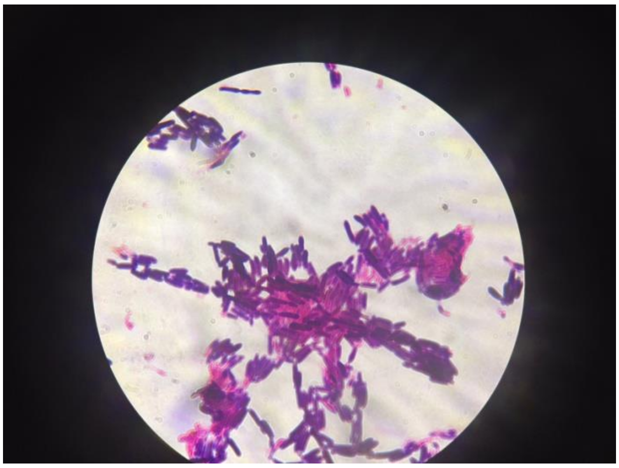
In order to observe each one of them in separate we isolate A and B colony in 3 new Petri dishes with the following mediums: MRS, SAB, GNO. We put all the 6 Petri dishes at 24 degrees Celsius for 48 hours and waited for the colonies to growth. Continuously, we realized a gram staining for each one of them.

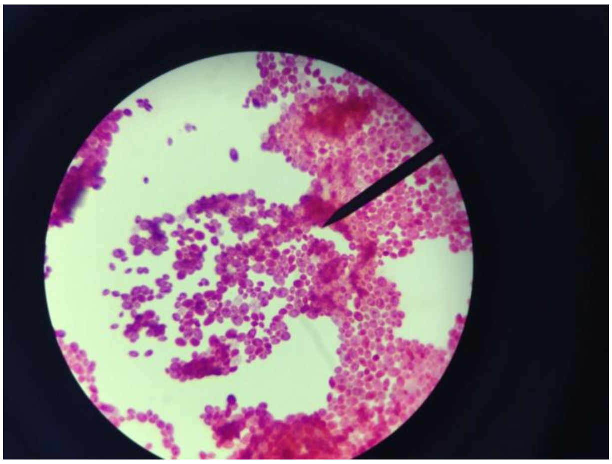
Gram staining: We selected a few colonies from the A colony MRS dish and with a blade we put it in a microscope slide. We put a few drops of ethanol for fixation and we sterilized the slide in the fire. Continuously, we dived the slide in crystal violet for 1 minute. We washed out with distil water and dived the slide in lugol for 30 seconds. We washed out with distil water and ethanol and dived the slide in safranin for 1 minute. We washed out with distill water. After this procedure the slide is ready for the microscopic observation. We followed the same procedure with the rest 5 Petri dishes.



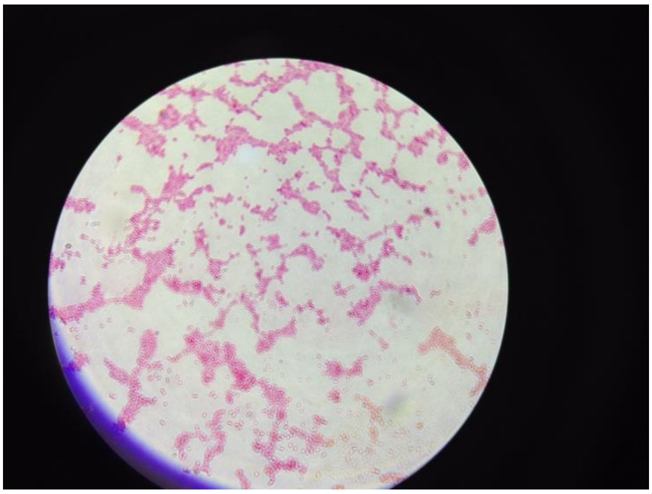
Microscopic observation: We continue with the observation in the microscope of the 6 grams.

A Colony: We concluded that A Colony is a mixture of different yeasts (in the microscope the yeasts appeared mush bigger and circular than bacteria) which was light violet and deep violet. We also noticed bacteria with a light pink color (gram-).



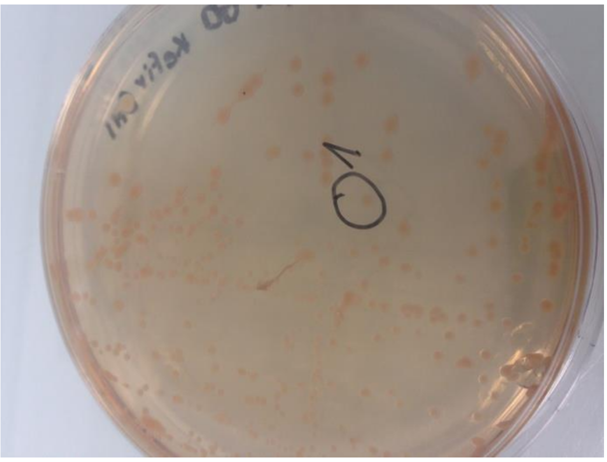


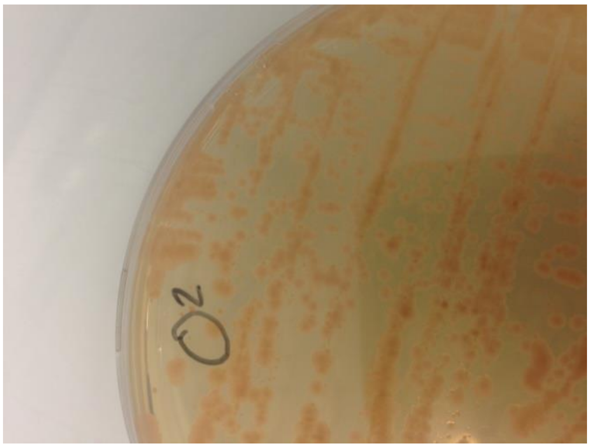
B Colony: We concluded that B Colony was mainly consisted of bacteria with a light pink color. (gram-)

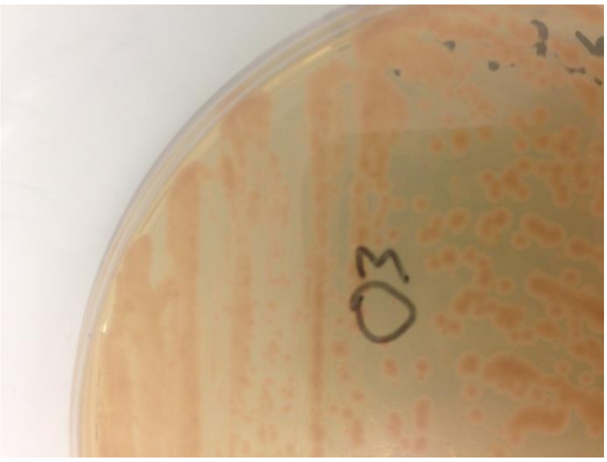


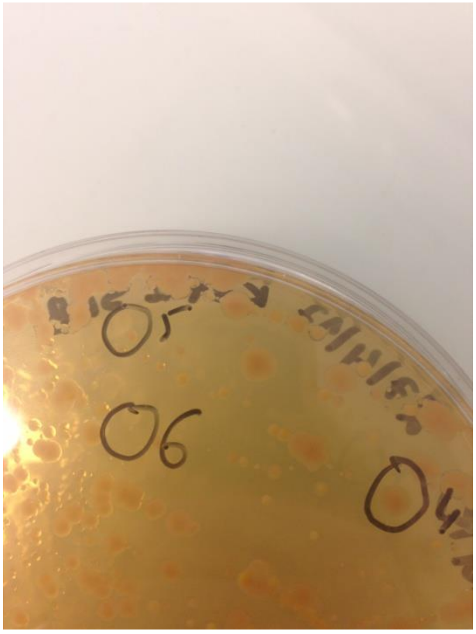
WATER KEFIR:

We started by adding some kefir grains in 2 different tubes (one with BHI medium and one with SAB medium) in order to identify the existed yeasts and the bacteria. We put the tubes at 24 degrees Celsius for 48 hours. After 48 hours we realized the isolation of the 2 different tubes in the Petri dishes. We waited for 48 hours for the colonies to growth in the dishes at 24 degrees Celsius. After 2 days we noticed 6 different colonies: 1,2,3,4,5 and 6.



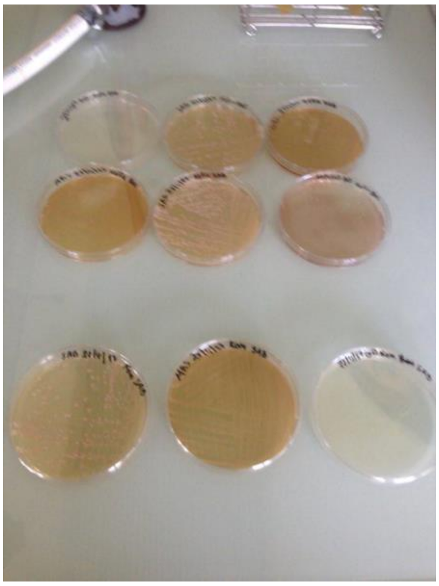






In order to observe each one of them in separate we isolate 1,2,3,4,5 and 6 colony in 3 new Petri dishes with the following mediums: MRS, SAB, GNO. We put all the 18 Petri dishes at 24 degrees Celsius for 48 hours and waited for the colonies to growth. Continuously, we realized a gram staining for each one of them.

Gram staining: We selected a few colonies from the 1 colony MRS dish and with a blade we put it in a microscope slide. We put a few drops of ethanol for fixation and we sterilized the slide in the fire. Continuously, we dived the slide in crystal violet for 1 minute. We washed out with distil water and dived the slide in lugol for 30 seconds. We washed out with distil water and ethanol and dived the slide in safranin for 1 minute. We washed out with distill water. After this procedure the slide is ready for the microscopic observation. We followed the same procedure with the rest 17 Petri dishes.

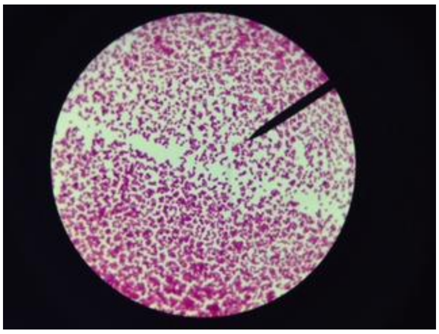


Petri dishes after 48h

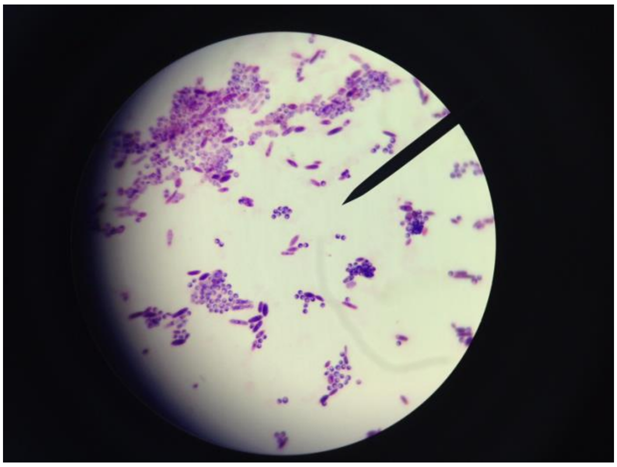
Microscopic observation: We continue with the observation in the microscope of the 18 grams.

Microsope slides and petri dish

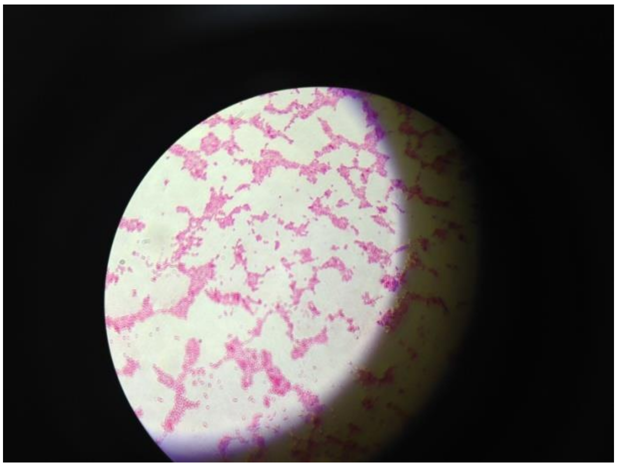
CONCLUSIONS: We concluded that kefir grains is a mixture of lactic bacteria and yeasts, a symbiotic effect. Specially, in the 4 Colony we noticed lactobacillus gram+ bacteria (in the microscope appeared in violet color).



In the 2 Colony we noticed yeasts and bacteria as well gram+.



We noticed bacteria gram- as well in the 5 Colony(in the microscope were colored light pink.)



OXIDASE AND CATALASE TEST:

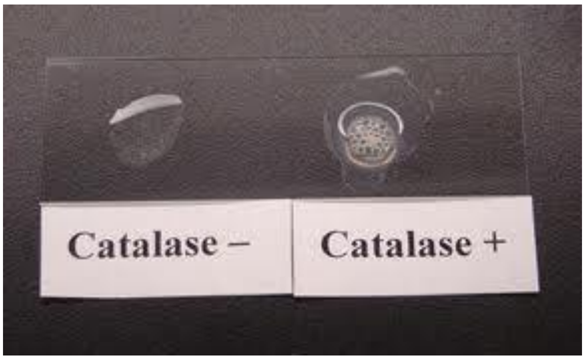
We realized oxidase and catalase tests in the water kefir colonies.

The oxidase test is a test used in microbiology to determine if a bacterium produces certain cytochrome c oxidases. It uses disks impregnated with a reagent such as *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) or *N,N*-dimethyl-*p*-phenylenediamine (DMPD), which is also a redox indicator.

The reagent is a dark-blue to maroon color when oxidized, and colorless when reduced. Strains may be either oxidase-positive (OX+) or oxidase-negative (OX-)

OX+ normally means the bacterium contains cytochrome c oxidase and can therefore use oxygen for energy production by converting O_2 to H_2O_2 or H_2O with an electron transfer chain.

OX- normally means the bacterium does not contain cytochrome c oxidase and, therefore, either cannot use oxygen for energy production with an electron transfer chain or employs a different cytochrome for transferring electrons to oxygen.



Catalase test is also one of the tests used by microbiologists to identify species of bacteria. Catalase-positive bacteria include strict aerobes as well as facultative anaerobes. They all have the ability to respire using oxygen as a terminal electron acceptor. Catalase-negative bacteria may be anaerobes, or they may be facultative anaerobes that only ferment and do not respire using oxygen as a terminal electron acceptor (Streptococci).

Procedure of Catalase test:

1. Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick.
2. Place a drop of H_2O_2 on to the slide and mix.
3. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling.
4. A negative result is no bubbles or only a few scattered bubbles.

RESULTS:

After realizing the Oxidase and Catalase test we collected the following results presented on the table below:

WATER KEFIR	**OXIDASE**	**CATALASE**
COLONY 1	-	+
COLONY 2	-	+

COLONY 3	+	+
COLONY 4	-	-
COLONY 5	-	+
COLONY 6	+	-

Table 9 Oxidase and Catalase Tests Results

CONCLUSIONS

KOMBUCHA:

Kombucha has been promoted with claims that it can treat a wide variety of human illnesses, including AIDS, cancer, and diabetes, and that it provides other beneficial effects such as stimulation of the immune system, boosting the libido, and reversal of gray hair.People drink it for its many putative beneficial effects, but evidence of kombucha's beneficial effects in humans is lacking. According to testimonials, kombucha brew can:

- Detoxify the body
- Reduce cholesterol
- Reduce atherosclerosis
- Reduce blood pressure
- Reduce inflammatory problems
- Alleviate arthritis, rheumatism and gout symptoms
- Promote liver function
- Normalize intestinal activity, balance intestinal flora, and cure hemorrhoids
- Reduce obesity, regulate appetite, enhance metabolism
- Prevent/heal bladder infection and reduce kidney calcification
- Stimulate the glandular system
- Protect against diabetes
- Increase resistance to cancer, counteract aging problems
- Antibiotic effect against bacteria, viruses and yeasts
- Enhance the immune system
- Relieve bronchitis and asthma
- Reduce menstrual disorders and menopausal hot flashes
- Improve hair, skin and nail health
- Reduce craving for alcohol
- Reduce stress and nervous disturbances, headaches, insomnia
- Improve eyesight

Adverse effects:

Reports of adverse effects related to kombucha consumption are rare. It is unclear whether this is because adverse effects are rare, or just underreported.

Adverse effects associated with kombucha consumption include severe hepatic (liver) and renal (kidney) toxicity as well as metabolic acidosis. At least one person is known to have died after consuming kombucha, though the drink itself has never been conclusively proved a cause of death.

Some adverse health effects may be due to the acidity of the tea, which can cause acidosis, and brewers have been cautioned to avoid over-fermentation. Other adverse health effects may be a result of bacterial or fungal contamination during the brewing process. Some studies have found the hepatotoxin usnic acid in kombucha, although it is not known whether the cases of damage to the liver are due to the usnic acid contamination or to some other toxin.

Due to its microbial sourcing and possible non-sterile packaging, kombucha is not recommended in people with poor immune function, in women who are pregnant or nursing, or in children under 4 years old.

WATER KEFIR:

Water Kefir Health Benefits :

Here is a list with the most common conditions benefiting from water kefir:

- Arthritis - Fungal infection is not, according to the medical world, a frequent cause of arthritis. Medicine admits that the number of cases is on the rise, with Candida infection as a specific rising cause. The truth is, that there might be a lot more cases of fungal infection caused arthritis than believed. Because fungal infections are more likely to affect people with higher age, arthritis can be easily blamed on the age.
- Irritable Bowel Syndrome - Joint pain is most commonly a result of inflammation. Inflammation which normally is a natural healthy process to heal tissue, it is not beneficial if it lasts too long. Latest scientific research shows that Candida is linked to abnormal inflammation responses, and it promotes this artificial inflammation because this helps the colony grow faster. IBS is an inflammatory condition.
- Migranes without any apparent explication - migraines are linked to ear infections caused by Candida Albicans. Fighting the infection will make the migraines go away.
- Chronic fatigue - chronic fatigue is linked to over growth of Candida Albicans, and it looks like diet is an efficient way to control the infection

and reduce the symptoms. I would say, add probiotics in your diet, and prebiotic dietary fiber.

- Weight gain - Candida thrives on sugar, and it will modify your body to make you crave sugar. This leads to over consumption. In normal circumstances the amount of sugar you eat while on a Candida craving would make you a bit sick. Not when this is dictated by the yeast overgrowth.
- Depression - It is not sure how probiotics helps curing depression, but it does. Anecdotic evidence shows water kefir very efficient in treating depression.
- Eczema - There are a few yeasts that can cause eczema. However, atopic dermatitis, is an inflammation of the skin that is caused by an allergen such as food, and there is no known cure for it. Eczema sufferers are hypersensitive to various triggers. Many people have found that probiotics work for them, reducing the hypersensitivity, and the eczema outbreaks.
- ADHD - ADHD is thought to be an allergic hypersensitivity disorder. As such, if the allergic reaction is diminished, the symptoms will diminish as well. There is an entire underground current with people using special diets, and probiotics to cure ADHD.
- Acne - Acne is an apparently trivial problem but very difficult to treat. The use of probiotics both on the skin and taken orally, can help with acne.

Side Effects of Water Kefir

Many people who drink water kefir will swear by it and say that there are absolutely no side effects. The reality is that if consumed in high quantities, there could be serious side effects, from severe constipation, to stomach cramps, and even urinary tract infection. The most common side effects are flatulence and lose stool. If you have one of these more than a couple of days, lower the dose. If the side effects still don't go away, stop taking it temporarily. Be advised, start slowly, with a tablespoon per day, and slowly increase your dose. Kefir is not recommended for people with Niemann-Pick Disease both types A and B.

BIONADE, FASSBRAUSE:

Health Benefits:

Bionade does have plenty of benebits in it: calcium and magnesium, for instance. A litre of Bionade will supply half the daily requirements of these minerals. The drink contains the right balance of the two minerals so that one doesn't cancel out the other (magnesium uptake inhibits the body's uptake of calcium, and vice versa). They will even take effect faster than if delivered in tablet form because Bionade is isotonic, which is to say it has a chemical resemblance to blood and so can be easily absorbed. But Bionade is not one of the drinks in which lots of supposedly healthy things have been crammed. Instead, it's low in sodium and phosphorous-free.

CIDER :

Health Benefits:

Like apples, apple cider has many health benefits. The health benefits of apples and apple cider come from the fruit's phytochemicals. Phenolics, flavonoids and carotenoids from apples may play a key role in reducing instances of some chronic diseases. People who consume apples regularly in the diet are at lowered risk of cardiovascular disease, asthma, diabetes and some forms of cancer. Apples are also a source of antioxidants, which protect the body from damage caused by free radicals in the environment. Furthermore, they have found that it’s packed with chemicals that help protect against cancer, heart disease and other agerelated conditions.Cider, one of England's oldest alcoholic drinks, has long been thought to be beneficial to health, but there was no proof. Now scientists have confirmed there are high levels of health-enhancing antioxidants in cider.Half a pint delivers the same amount of antioxidants as a glass of red wine, recognised as having health benefits. Antioxidants 'mop up' substances called free radicals, naturally produced in the body but linked to damage to cells. However, we don’t have to forget that cider is an alcoholic drink, that’s way we have to be careful with the consumtion.

BEER:

Health Benefits:

Health benefits of beer become prominent if it is consumed in moderate amounts. Some of these interesting health benefits include the following:

Anti-Cancer Properties: A flavonoid compound called Xanthohumol is found in the hops commonly used in brewing beer. It has been seen to play a major role in the chemoprevention of cancer, including prostate cancer. According to Biomedicine, it is also a good source of polyphenols, due to the grains used for fermentation. It has been proven effective in fighting cancer, just like red wine.

Reduced Risk of Cardiovascular Diseases: Beer contains vitamin B6, which protects against heart diseases by preventing the build-up of a compound called homocysteine. It has a thinning effect on the blood and prevents the formation of clots, which cause blocks in the coronary arteries. Moderate consumption also reduces the risk of inflammation, the root cause of atherosclerosis, which is cholesterol and plaque building up on the blood vessels and artery walls.

Increased Bone Density: Moderate intake is shown to increase bone density, thereby preventing the risk of fractures and osteoporosis.

Diabetes: Studies have linked moderate beer consumption to a lower prevalence of type 2 diabetes.

Prevention of Anemia: Beer is a good source of vitamin B12 and folic acid, a deficiency of which may lead to anemia. Vitamin B12 is also essential for maintaining normal growth, good memory and concentration.

Hypertension: Regular beer drinkers have been found to have lower blood pressure, compared to people that consume similar amounts of wine or other spirits.

Anti-Aging Properties: Beer increases the potency and impact of vitamin E, which is a major antioxidant in the body. It is an important part of the maintenance of healthy skin, while also slowing down the aging process.

Gallstones: Regular consumption of moderate amounts of beer affects the cholesterol levels and decreases bile concentration, leading to a reduced risk of developing gallstones.

Prevention of Dementia and Coronary Disease: Beer consumption also boosts the level of “good cholesterol” by 10-20%, thus reducing the risk of dementia and cardiovascular diseases.

Aids Digestive System: Beer is shown to possess a number of digestive properties, which include the stimulation of gastrin, gastric acid, cholecystokinin and pancreatic enzymes.

Kidney Stones and Osteoporosis: Beer has been found to be high in potassium and low in sodium. It is a rich source of magnesium, which results in a reduced risk of kidney stones. The silicon present in it is also readily absorbed by the body, further explaining the protective effect of beer against osteoporosis.

Stress Buster: Like other alcoholic drinks, beer is shown to reduce stress and facilitate sleep.

Diuretic: Beer acts as a diuretic, and significantly increases urination. This facilitates the increased removal of toxins and waste materials from the body.

Side effects of beer:

1. Interferes With The Blood Sugar Level: Beer drinking can actually interfere with your body's blood sugar levels. The liver converts glycogen stored in it into glucose and releases it into the blood stream. Alcohol in beer actually interferes with this process. It can create hunger pangs and will leave you gorging on more food. This can pave way to weight gain. This can be countered by taking a proper meal before gulping down beer.

2. High In Calories: Commercial beer brands contain fewer amounts of nutrients, but come loaded with calories. This makes them less than ideal for people who are trying to lose excess weight. It makes your body burn fewer calories than what it would do normally. The alcohol in beer is converted into acetate by the liver. The body then burns acetate for energy and the excess fat remains stored in parts like hips and belly.

3. Gluten Insensitivity: A majority of beer variants found in the market contain malted barley. Barley contains gluten, a type of protein. Some people are found to be sensitive to gluten. If you are among them, opt for beers made with gluten free compounds.

4. May Be Bad For Cardiovascular Health: Some studies have shown that drinking beer can actually be good for the heart, but that happens when you drink in limited amounts. Besides, someone who has an existing cardiovascular ailment will not benefit from drinking beer at all. In fact, it will worsen their heart health.

5. Can Raise Blood Pressure Level: If you take several glasses of beer a day, it can lead to a spike in blood pressure level.

6. Can Lead To Heartburn: Beer contains some stimulants that work with gastric acid, which may lead to the onset of gastro oesophageal reflux and result in heartburn.

7. Leads To Intoxication And Hangover: Like all forms of alcohol, excess beer consumption does affect your nerves and motor skills. This can lead to accidents.

8. Interacts With Certain Medications: Sedatives and Erythromycin can interact with beer and can be bad for your health. Several antibiotics too interact with beer and can lead to side effects like headache and vomiting. The same holds good with a few pain medications.

GENERAL CONCLUSION:

Fermented foods are no doubt beneficial for our health. Essential for good digestion, fermented foods give our bodies the probiotics that they need, boosting immunity, and even improve our brain functions and mental health. In fact, fermentation is one of the oldest forms of food preservation; it is no wonder that fermented foods have long been a staple of almost ever diet around the world. The microbes involved in fermentation have got-friendly probiotic effects. In addition, fermentation creates beneficial by-products, such as B vitamins. While some people assert that these fermented drinks carry additional benefits, such as improved immunity and even cancer prevention, there is little evidence in human studies to back these claims. On the other hand, it should be said that most of the health benefits touted by pro-probiotic advocates are controversial. The European Food Safety Authority (EFSA) has found insufficient evidence to validate any of the claims made regarding the benefits of probiotics. Furthermore, there are hundreds of probiotic strains, and studies always focus on only one strain at a time.

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