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Comparative Analysis: Effects of Souring and Aging Beer

Abigail Clement[1], Ally Gowman[1], Rachel Vincent[1] , Ping Wu[1,2]

1. School of Engineering, University of Guelph, Guelph, ON, Canada, N1G 2W1
2. Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada, N1G 4Y2

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ABSTRACT Sour beer is a popular type of beer typically found in Belgium and Germany. It produces a sour flavour from the presence of lactic acid in the beer, which is a byproduct of the fermentation process. Different strains of *Lactobacillus, L. delbrueckii and L. brevis* were tested to determine lactic acid concentration, ethyl acetate concentration, diacetyl concentration as well as acetic concentration. It was found that the strain of *Lactobacillus* used to sour the beer not only has a profound impact on the flavour of the final product, but also the chemical composition. In general, no discernible amounts of acetic acid, ethyl acetate or diacetyl were found in any of the samples of beer. Furthermore, the different strains of bacteria as well as the aging of the beer had an impact on both the lactic acid concentrations as well as the general acidity of the beer.

Keywords: *Lactobacillus* bacteria, sour beer, brewing, fermentation, pH, lactic acid

INTRODUCTION Beer is a well-established beverage that has become popular all over the world. This alcoholic beverage has been produced since 5 BC, and the brewing process has since been constantly improved. Modern brewing has utilized many engineering principles to achieve greater efficiency and brewhouse standards. These improvements in quality have allowed for the production of many styles of beer, including sour beer. Sour beer is a category of beer that is popular in European countries such as Germany and Belgium. This specialty beer has a sour taste due to the presence of lactic acid. Some types of sour beer include Lambic, Gose, Flanders Red Ale, and Berliner Weisse.

As previously stated, sour beer gets its unique bitter flavour through the presence of lactic acid bacteria. Under anaerobic conditions, lactic acid bacteria use glycolysis metabolic pathways to order to convert sugar into lactic acid. This conversion reduces the pH level of the beer, subsequently reducing the isoelectric point and enzyme activity within the beverage. These chemical reactions contribute to the sour flavour of the beer.

Recently, sour beer has become increasingly popular in North America due to its unique flavour. However, an issue with current brewing methods is that there has yet to be identified strains of bacteria that produce the specific desired sour flavours. The purpose of this experiment is to quantify the amount of lactic acid and other chemical compounds that the bacterium responsible for souring the beer produces. This will allow brewers to have a better understanding of the bacteria and the flavours it could produce.

Various strains of *Lactobacillus* will be used to test their effects for the fermentation of sour beer. The strains to be tested are *L. delbrueckii* and *L. brevis. L.delbrueckii* is homofermentative, meaning the bacteria can only secrete lactic acid. *L. brevis* is heterofermentative, meaning it can produce both lactic acid and ethanol. Once fermented, the secretions from each of these strains are to be tested for acetic acid, lactic acid, ethyl acetate, and diacetyl. From this, conclusions regarding the souring capabilities and the concentrations of off-flavours that each strain produces can be made. This study addresses the need for more experimental research related to the brewing industry to be performed. The study will ensure that multiple strains of lactobacillus bacteria are tested and that the resulting concentrations of lactic acid are quantifiable and can be replicated in the industry. Samples will also be taken from each lactobacillus batch and aged in Chardonnay soaked oak chips in order to determine the effect that aging has on the flavour and acid composition of sour beers.

METHODOLODY The process used to brew the sour beer varies from traditional brewing methods. The typical initial malting, mashing and lautering steps were not performed by the team, as the beer was primarily extract based. The brewing process per one batch is as follows:

1. All of the equipment must be washed and then soaked or sprayed with a solution of water and food grade sanitizer. Sanitization is the most important step in the brewing process as it allows for more accurate results.
2. Soak each two-ounce bag of oak chips in individual food-grade plastic bags of 200 mL of chardonnay.
3. Boil 2 gallons of filtered water and add to the primary fermenter. Allow the water to cool to room temperature

4. Specialty grains needs to be steeped before being added into the wort. In this case, acidulated malt was placed in a grain bag and steep in 3.8L of filtered water at 70 degrees Celsius for 45 minutes. Next, the acidulated malt was rinsed with 2L of filtered water. Finally, allow the bag of malt to drip into the brewing kettle for 15 minutes. The spent grain can be disposed of.

Figure 1: The grain being steeped in the appropriate amount of water.

Figure 2: The draining of the steeped grain.

5. The wort can now be prepared. Since this recipe is extract based, 3 gallons of filtered water were added to the brewing kettle and brought to a boil. The proper amounts of wheat and light pilzen extracts were added to the kettle very slowly in order to avoid clumping or burning.

Figure 3: Preparation of the concentrated wort using the extracts.

6. Allow the wort to cool to 38 degrees Celsius.
7. Wait for the pH to drop to 4.3 or lower. The acidulated malt added earlier will facilitate this. However, if the pH is dropping too slowly, lactic acid can be added.
8. Pitch the strain of lactobacillus (if being used). Cover the kettle and keep it warm (36°C). The ideal temperature for lactobacillus growth is 47-48°C, so do not expect the maximum souring rate to occur. As we did not have the technology of an industrial brewer, some ingenuity was needed to keep the wort warm. A heating pad was set to high and placed underneath the kettle. Blankets were then securely wrapped around the kettle in order to insulate it and prevent heat loss. Wait until the pH drops to at least 3.4.

Figure 4: The lactobacillus culture being pitched.

Figure 5: The souring wort being insulated by blankets.

Figure 6: The pH of the wort being approximated. The colour shown relates to a pH of approximately 4.0.

9. Uncover the kettle and bring to a boil. The total boiling time is 90 minutes. 6-8 International Bittering Units (IBUs) worth of hops should be added for the entirety of the boil. The sample calculations below were used to determine the amount of IBUs that were added to the beer. The yeast can be activated at around this time.

Figure 7: The hops (in a small hop bag) being steeped in the boiling wort.

10. The wort should be chilled as quickly as possible to room temperature (21°C), which can be done by placing the covered kettle into an ice bath. For this experiment it took approximately 45 minutes for the kettle to cool to room temperature.
11. Aerate the wort by pouring it back and forth between the fermenter and the kettle a few times.
12. Once aerated, keep the wort in the fermenter. Pitch (pour) the activated yeast into the fermenter. Keep the fermenter at the desired temperature.
13. After a week the primary fermentation should be complete. This can be confirmed by taking specific gravity readings 2 days in a row. Specific gravity is the ratio of the density of the beer to the density of water. If the specific gravity readings are the same, then the primary is complete. In this case a specific gravity of 1.004 was the target.
14. Thaw and then refreeze frozen raspberries. This will break down their cell walls and make it much easier for their flavour to be incorporated into the beer. Make sure to sanitize the bags and a fine nylon bag. Empty the frozen raspberries into the nylon bag and close it securely

with a sanitized zip tie. The nylon bag of raspberries can now be placed inside the fermenter.

15. Allow the beer to ferment for at least another week. Take specific gravity readings as previously outlined to determine whether or not the secondary fermentation is complete.
16. The beer can now be primed and bottled. Priming involves making a concentration water and sugar solution being added to the beer. This sugar solution allows the remaining yeast to create more carbon dioxide in the bottle. Since the bottles are tightly sealed the resulting built up pressure cause the CO_2 to be forced into the liquid, thus carbonating it. For the batches that were aged, 15g of the chardonnay-soaked oak chips per 350mL of beer should be put in the specific bottles before being capped.

Figure 8: The bottling bucket with attached spigot and bottle filler (left).

Figure 9: A growler being filled with the beer (middle).

Figure 10: A 355mL bottle of beer being capped using a dual hinge capper.

RESULTS

Sensory Evaluation

Tasting

The qualitative analysis was performed by a group of volunteers using a scale of 0-5. In this case, a 0 meant that the specific flavour profile was not at all present in the beer, whereas a 1 meant that the flavour was just barely present and a 5 meant that the flavour was extremely prominent. The data was compiled for a sample size of 21 participants (n=21), and the average for each category was calculated.

Table 1: The average ranking of each flavour for each beer sample. The number in parenthesis denotes which batch the beer was from. Del refers to lactobacillus delbrueckii, brevis refers to lactobacillus brevis and plain refers to the beer that was not soured with lactobacillus bacteria.

Beer Type / Flavour	Beer 1 Del. (2)	Beer 2 Plain (2)	Beer 3 Del. (2)	Beer 4 Brevis (2)	Beer 5 Brevis (2)	Beer 6 Aged Brevis (2)	Beer 7 Del. (1)	Beer 8 Brevis (1)	Beer 9 Del. (1)
Cocoa	0.19	0.19	0.19	0.10	0.10	0.33	0.20	0.05	0.18
Bitter	1.29	1.19	1.10	1.19	1.10	1.05	0.95	1.43	1.17
Acid	2.24	1.19	2.19	2.19	2.14	1.67	2.62	2.62	2.24
Burnt	0.24	0.52	0.24	0.14	0.19	1.62	0.05	0.10	0.05
Fruity	3.00	2.19	3.48	2.67	2.62	1.76	2.62	2.31	2.29
Nutty	0.14	0.43	0.24	0.38	0.33	0.86	0.10	0.10	0.29
Earthy	0.52	0.67	0.24	0.29	0.52	1.90	0.19	0.24	0.38
Astringent	1.33	0.67	1.43	0.90	1.14	0.29	0.71	0.52	0.57
Colour	3.52	2.00	3.61	2.52	2.43	2.90	3.00	2.90	2.86
Clarity	2.67	2.29	3.19	2.72	1.62	1.48	2.60	2.29	2.95

Triangle Testing

Triangle testing is a common method used for sensory evaluation in the food industry. The testing determines whether or not a single ingredient makes a difference in the overall quality of the product. For the purpose of this experiment 3 rounds of testing were underwent; soured vs. un-soured, aged vs. un-aged and finally, one strain of *lactobacillus* versus the other. A sample size of 36 participants (n=36) was used for this test. For the first round of testing (soured vs. un-soured) the majority of the participants (90.91%) were able to distinguish the difference between the two categories. For the aged vs. un-aged triangle test, 77.27% of the test group were able to distinguish the difference between the two products. In the final category of *lactobacillus brevis* and *lactobacillus delbrueckii*, only 50.09% of the test group were capable of discerning the difference between the sour beers.

Chemical Analysis

In order to determine the basic chemical composition of the beer, several tests had to be performed. The presence of lactic acid, acetic acid, diacetyl, and ethyl acetate were being carefully monitored. To begin, the presence of acetic acid was tested using a basic sodium bicarbonate test. Only the samples that were tested from the aged groups showed trace amounts of acetic acid. The amounts present in the beer were determined to be negligible in terms of flavour profiles. In order to determine the lactic acid concentration, a pH meter was used. All samples were tested at room temperature to ensure that there would not be any discrepancies in the testing. The pH levels were consistent across all three batches. From each batch 10 samples were tested to ensure that the pH for that batch is uniform. For the un-soured wheat beer, the pH was averaged to 3.7. The pH values for the beers soured with *lactobacillus brevis* and *lactobacillus delbrueckii* were 2.7 and 3.1 respectively. Aging dropped the pH by roughly 0.2-0.3 for each sample that was tested. It is important to note that the samples aged for two weeks had the same pH as the samples merely aged for a week. It can thus be concluded that the maximum effects of the aging process were achieved after a single week. Ethyl acetate and the precursor to diacetyl, alpha-acetolactate, were also tested for. It was determined that none of the tested samples contained any discernible concentrations of either compound.

DISCUSSION

Sensory Evaluation

Tasting

The results from the tasting are seemingly accurate. The volunteers were, for the most part, able to distinguish the key differences between the different variations of the beer. Both the aged beer and the un-soured beer were rated much lower is terms of acidity. The two soured beers ranked similarly for acidity. However, it is important to note that nearly all of the participants that were able to differentiate between the two soured beers noted that the way in which the acidity of the beer could be tasted was different. The *delbrueckii* soured beer was a very traditional, tangy and long-lasting lactic acid flavour that focused on the back of the palate, whereas the *brevis* was a stronger, but short lasting sour flavour that was primarily tasted using the front of the tongue. Other flavour profiles that were analysed were the earthiness and burnt-like flavours. These flavours are definitely not associated with either plain wheat beer or even sour beer. The results from this experiment confirm this fact. Although, the sample that was aged was the only sample that had these tasting notes. These flavours can be attributed to the charred oak chips that were added during the aging process. It was also observed that the beers that were soured retained the red colouring and fruity flavour from the raspberries much better than the control (un-soured). The cause of this preservation and flavour of the fruit can be primarily based on the higher acid content of the sour beer. Finally, an astringent type flavour was noted in all the samples. This is a cloying flavour that has the effect of drying out the mouth and is often associated with the presence of ethyl-acetate in beer. Since no ethyl-acetate was detected during the chemical analysis it can be determined that the astringent taste may have been mistaken for either bitterness or acidity since many people are not accustomed to what astringent flavours.

Triangle Testing

The triangle testing was useful for analysing the qualitative differences between the different samples. Since for each trial, the majority of the participants were able to pick the sample that was different out of the three, it can be concluded that each added ingredient to the beer allowed for a unique and distinguishable flavour. For the first trial of soured versus un-soured, the difference between the samples was apparently blatant since the un-soured beer was much less vibrant in colour than the plain wheat beer. The difference between the samples in the second trial was the aged beer's unique smokiness and earthy undertones. Finally, for the last trial of the *lactobacillus debrueckii* versus the *lactobacillus brevis* soured beers also led to some key differences in the flavours derived from the bacteria to be discovered. Primarily, that the samples of *lactobacillus brevis* beer had much more intense upfront sour flavours, that did not linger very long on the front of the tongue. Whereas the *lactobacillus delbrueckiii* samples had a much more balanced and tangy sour flavour that lingered towards the back of the tongue. These key differences in the flavour allow for the two beers to be distinguished from each other.

Chemical Analysis

The chemical analysis that was performed on the variations of beer allowed the results obtained via qualitative evaluation to be confirmed using standard laboratory techniques. The lack of diacetyl in the beer is not surprising due to the fact that it is rather simple to avoid the presence of this compound when making beer. Diacetyl forms when the yeast used to in the fermentation process is no longer able to perform respiratory processes. Thus, the yeast can no longer metabolise the butter flavour component. Often in brewing, a tart, apple-like or vinegar type smell can be present in beer. This type of smell can be attributed to the presence of acetic acid. None of the beer that was produced for this experiment contained acetic acid since the process was highly controlled. Acetic acid usually arises when the beer is infected with wild strains of bacteria such as *pediococcus*. Ethyl acetate is the ester of acetic acid and ethanol. This product forms when acetyl CoA is condensed with ethanol. Since the appropriate yeast strain was selected and the temperature of the process was carefully monitored, no discernible amount of ethyl acetate was detected. Additionally, the wort was well aerated prior to fermentation. This allows for acetyl CoA to be properly metabolized rather than congregate in the wort. Finally, the variations in lactic acid concentration can be related to the different types of acid flavour that the two strains of lactic acid bacteria created. Although, further testing would need to be performed in order to determine whether or not the flavour differences were due to the difference in lactic acid concentrations or by other flavour compounds present in the beer.

CONCLUSION It was found that different strains of *Lactobacillus* did in fact play a role in different flavour compounds found in sour beer. The different strains had different amounts of lactic acid, and pH that resulted in the formation of different compounds. In the future more testing with different strains could be done to determine the best strain for the desired flavour outcome. It is recommended that more testing of different strains is done, resulting in a more robust flavour analysis. The testing can easily be scaled up using industrial equipment and by increasing the recipe. HLPC testing would also be recommended for more in depth flavour analysis.

NOMENCLATURE

HLPC: high performance liquid chromatography

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