Olive Oil Addition to Yeast as an Alternative to Wort Aeration

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9/26/05

Submitted to Professors Graham Stewart and Brian Eaton

in Partial Requirement of

Postgraduate Diploma/MSc in Brewing and Distilling

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Acknowledgements

First of all I would like to thank the New Belgium Brewing Company for allowing me to conduct this research at their brewery.

Thank-you to Peter Bouckeart for mentoring me throughout this project.

I would like to thank Dr. Ir Guy Derdelinckx for his ideas and direction.

Thank-you to Lauren Salazar for running the flavor panels and to Todd Bennet for performing the GC analysis.

I would also like to thank Jason Trujillo, Simon Ahlgren, Katie McGivney and the rest of the New Belgium quality assurance department for all of their analytical work.

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Abstract

To extend the flavor stability of their beers, many breweries are researching ways of reducing oxygen ingress throughout the brewing process. However, the practice of aerating the wort prior to fermentation is almost universal in the brewing industry because oxygen is necessary for yeast health and growth. Recent studies have shown that alternative methods to traditional wort aeration such as aeration of the yeast prior to pitching or the addition of the unsaturated fatty acid linoleic acid can yield fermentation characteristics similar to wort aeration. It has also been shown that using these alternative methods instead of aerating the wort can reduce oxidation potential. This paper reports the findings of a series of full-scale production tests that were conducted in an operating brewery to evaluate the effects of another type of yeast treatment. By mixing olive oil into the yeast, during storage, instead of aerating the wort, fermentations can be achieved with only minor increase in fermentation time. The beers produced from these fermentations were comparable in flavor and foam retention to beers produced by traditional wort aeration. The ester profile of the beers produced using olive oil addition was significantly higher than the controls and the flavor stability of these beers was significantly improved.

Introduction

In modern breweries a great deal of care is taken to ensure that oxygen is excluded from coming into contact with the beer or unfermented substrate (wort) throughout the brewing process. The one exception to this practice is that the wort is typically injected with air or pure oxygen (aerated) prior to fermentation. Although it is universally accepted in the brewing industry that oxygen contact with beer or wort leads to staling of the product, wort aeration is considered necessary because without it the yeast will not be healthy enough to properly ferment the wort (Kunze, 1999).

The reason the yeast needs oxygen for a proper fermentation is because it needs to synthesize sterols and unsaturated fatty acids (UFAs) for its cell walls. Yeast is typically collected from a fermenter at the end of fermentation and stored in a storage tank. The yeast is then re-used in a subsequent fermentation. The physiological condition of the yeast is usually poor at the time of re-use due to depletion of sterols and unsaturated fatty acids from the previous fermentation (Moonjai, 2003a). Therefore oxygen is typically added to the wort at the start of every fermentation.

One interesting alternative to aerating the wort is to add the UFAs directly to the yeast during storage. Theoretically the yeast should be able to take up the UFAs and use them in a subsequent fermentation without the use of oxygen. This should result in a beer that has better resistance to staling oxidation without adversely effecting fermentation performance or flavor.

The purpose of this research was to compare the effects of adding olive oil to storage yeast vs. traditional wort aeration. The theory is that the oleic acid in the olive oil will provide the UFAs necessary for yeast growth and proper fermentation, eliminating the need for wort aeration.

In this paper we will look at the results of a study in which full-scale fermentations were conducted to evaluate the fermentation performance, flavor, fusel profile, and analytical attributes of beer, which had been fermented with yeast that was treated with olive oil during storage instead of aerating the wort. The aim is to achieve a normal fermentation, and flavor profile while improving resistance to oxidative staling.

Background

Wort Aeration

Yeast cells require oxygen to manufacture the sterols and unsaturated fatty acids needed for cell membrane construction and growth. For this reason oxygen is typically injected into the wort prior to fermentation to bring the total dissolved oxygen content of the wort up to about 8-9 ppm. This addition of oxygen to the wort has traditionally been considered to be essential for yeast growth (Kunze, 1999). If the oxygen content in the wort is insufficient, the yeast cells will be unable to

manufacture the sterols and unsaturated fatty acids necessary for cell membrane health. As a result the yeast cells do not grow and the loss of membrane integrity results in cell death (Hough et. al., 1982).

Stored yeast from a previous fermentation is typically deficient in sterols and unsaturated fatty acids and therefore wort aeration is necessary for yeast health and growth but over oxygenation of the wort should also be avoided as it can make the resulting beer more susceptible to oxidation (Anderson et. al., 2000). The amount of oxygen added to the wort will also effect ester production and subsequently the flavor profile of the resulting beer. Wort that is not properly aerated will not support healthy yeast growth and the resulting beer will have an increased ester content and other flavor defects. If the wort is over oxygenated not only will there be an excess production of yeast during fermentation, causing a decrease in yield, but ester synthesis will also be strongly inhibited (Smart, 2003).

During the short period of time that the oxygen is in contact with the wort, harmful oxidative chemical reactions are taking place that form the precursors of beer staling compounds. Although the yeast is taking up the oxygen from the wort very quickly, the oxidative reactions are taking place at a similar rate. As oxygen ingress into the cold wort is reduced, the formation of these beer staling precursor compounds is also reduced. If wort aeration could be avoided completely, without adversely effecting yeast health or fermentation performance, flavor stability should be improved (Burkert, 2004).

Yeast Aeration

Studies have been conducted to investigate aeration of storage yeast, prior to use, as a means of avoiding wort aeration. The theory is that the oxygen will be taken up into the yeast cells during storage, before the yeast is added to the wort. This should supply the yeast cells with the oxygen they need without oxidizing the wort. There are, however, some problems with this method of aeration.

The correct amount of oxygen must be dissolved into the yeast in order to achieve optimal growth during fermentation. Under aeration will obviously lead to low growth but over aerating can also lead to low growth due to glycogen and trehalose exhaustion (Fugiwara et. al., 2003). The success of yeast aeration can also be

dependent on the yeast strain. Each yeast strain reacts differently to storage aeration so the aeration schedule must be specifically tailored to each strain (Depraetere et. al., 2003).

In the initial lag phase of fermentation the yeast cells begin taking up the oxygen from the wort. Usually at the end of these first few hours all of the oxygen has been taken up by the cells and during this time the cells rely on glycogen for their metabolic activity so it is essential that the cells begin fermentation with all of their glycogen reserves in tact (Hardwick, 1995). For this reason, brewery yeast is typically stored cold and not aerated during storage to minimize metabolic activity so that the yeast cells will have enough glycogen and trehalose to remain vital during this initial lag phase and begin fermentation.

Yeast aeration may not be a practical option for many breweries because oxygen cannot be added to the yeast too early. Yeast storage is a critical step in yeast handling and any storage conditions that increase metabolic activity such as increased temperature, prolonged time of storage, or the presence of oxygen will cause the yeast to deplete its internal glycogen and trehalose reserves. Also studies conducted to replace wort aeration with yeast aeration have shown that in order to replicate the fermentation times from traditional wort aeration it was necessary to increase the amount of yeast by approximately 30% (Smart, 2000).

Addition of Unsaturated Fatty Acids

Research has been done to investigate adding linoleic acid to wort prior to use but the result was a change in flavor quality due to an increase in the acetate esters. Another possible alternative to wort aeration that has been studied is the direct addition of linoleic acid to storage yeast. Yeast stored in a cold stationary phase under fermented beer has been shown to take up unsaturated fatty acids. Additionally, linoleic acid is taken up by the spheroplasts, proving the oil is not just adsorbed to the cell walls (Moonjai, 2003a).

Research done by Nareerat Moonjai at Leuven University has shown that small-scale, non-stirred fermentations in a wort medium using yeast that had been treated with linoleic acid instead of wort aeration could produce normal fermentations without significantly affecting the acetate esters. These findings showed that under typical

production yeast storage conditions it should be possible to get fermentation performance comparable to traditional wort aeration by adding an unsaturated fatty acid such as linoleic acid to the yeast (Moonjai et. al., 2003b).

For this study olive oil was selected as the unsaturated fatty acid (UFA) over linoleic acid because it's much lower cost and wide spread availability make it a more realistic choice for production fermentations. Also the oleic acid in olive oil is an 18-carbon monounsaturated UFA (C18: 1), which is one of the UFA's that S. cerevisiae manufacture. Linoleic acid, being a polyunsaturated 18-carbon UFA (C18: 2) is not naturally produced by yeast (Depraetere et. al., 2003). Also, because these fermentations were carried out with ale yeast at fermentation temperatures starting at 15.5 °C and rising naturally to 24°C, the improved fluidity of the polyunsaturated linoleic acid was not required.

Materials and Methods

Fermentations

The same yeast strain was used for all test and control fermentations. It is an ale yeast, which is pitched during transfer to fermentation. The yeast is then collected from the bottom of the fermenter at the end of fermentation and stored in yeast storage vessels to be re-used on a subsequent fermentation. The wort has a starting density (before fermentation) of 1.057 g/ml and an final density (after fermentation) of 1.010 g/ml. After a fermenter has been cooled the maximum storage times for yeast were 48 hours in the fermenter cone and 72 hrs in the storage tank. Yeast viabilities for these tests and controls were above 85%, with the average being 95%. Yeast pHs were below 4.6. IBU's (ppm isomerized alpha acids) were around 20.

During the first round of testing the temperature of the wort for both the test and the control was 15.5 °C at the start of fermentation. The fermentation temperature was allowed to rise naturally to 20.0 °C during fermentation. After this first round, an unrelated procedural change was made at the brewery to allow all fermentation temperatures to rise to 24 °C. Therefore all tests and controls following this first round had faster fermentation times due to the increased temperature.

In the first round of testing a 360 hl batch size was used. The second round was a 720 hl batch, and the third and fourth rounds were 2100 hl. The 2100 hl batches were kept separate throughout production and were bottled and sold as finished product without being blended with any of the control beer.

The 360 hl batch was blended in the bright beer tank with 1800 hl of regular production beer after determining it was safe to do so based on flavor. A keg of filtered carbonated beer from the test fermenter, which was 100% olive oil test beer, was collected after post filtration. The beer that was pulled after the filter was tested for aromatic compounds, foam retention, and flavor. The second 720 hl batch was blended with 1400 hl of normal production beer in the bright beer tank and again, a keg of filtered carbonated beer from this tank was pulled after the filter for testing aromatic compounds, foam and flavor. The 2100 hl test batches were bottled without blending so that they could be compared directly against packaged control production beers. These beers were tested for initial flavor quality, aromatic compounds, foam, and flavor stability.

Aeration and Olive Oil Addition

All controls were aerated in-line, with micro filtered compressed air, in excess of saturation for the entire duration of the transfer according to the breweries standard operating procedures. The tests were not aerated. For the test fermentations, olive oil was added to the yeast in storage tanks five hours prior to use and the amount added increased with each trial. Due to the variation in yeast slurry thickness the amount of olive oil used was based on the total number of cells instead of mg / L of yeast. In the 360 hl batch the olive oil was added to the yeast at a rate of 1 mg / 67 billion cells pitched (15 mg olive oil / L of yeast assuming a count of 1 billion cells / ml). In the 720 hl trial the concentration was increased to 1 mg / 50 billion cells and in the 2100 hl trials the concentration was increased again to 1 mg / 25 billion cells. Aside from the changes previously mentioned with aeration, olive oil addition and fermentation size, all other aspects of production were carried out identically for both the tests and the controls.

Sensory Analysis

New Belgium Brewing Company's in-house taste panel conducted all sensory analyses. All taste panels were comprised of 12-14 internally trained senior level taste panelists. Qualification for senior level panel requires a minimum of three years training and 20 hrs of training classes. Panelists tasting ability determined by weekly flavor attribute testing. Panelists tasting calibration determined by Senstools General Procrustes Analysis statistical software. Senstools statistical software used for all ANOVA and Principal Component Analysis. All flavor profile analyses were performed on a nine-point scale. Sample randomization and data entry organization performed by Williams complete block method using Compusense sensory software.

Gas Chromatography (GC)

GC was carried out at New Belgium Brewing Company using Perkin Elmer Autosystem XL GC equipped with a Perkin Elmer Turbomatrix headspace sampler, a Perkin Elmer PE-Wax column (60m x 0.25mm id x 0.25um film), and a flame ionization detector (FID). See Appendix 1 for detailed GC procedure.

Paragon Laboratories performed external GC/MS analysis using a Hewlett Packard 5890 GC equipped with an EST Archon auto sampler coupled to an OI 4560 Purge & Trap sample concentrator, a Restek RTX-624 capillary column (60m x 0.25mm id x 1.4um film), and a Hewlett Packard 5971 mass spectral detector.

Analytical Analysis

A Nibem foam analyzer was used to determine the rate of foam collapse according to ASBC standardized method. Yeast health and viability were monitored on a percent viability basis using a hemocyometer and the ASBC standardized method for the methylene blue staining technique. Fermentation speed was measured from the start of the first transfer to the time the temperature on the tank was turned down at the end fermentation. All fermentations were ended when density reduction had stopped and total Vidicinal Keytone (VDK) levels had reached the brewery's specification. VDKs were measured using the ASBC spectrophometric method.

Results

Trial One

The results for the first olive oil test fermentation are shown in Table 1. The olive oil test fermentation was 20% longer than the average aerated fermentation during that time period but it did attenuate completely. Both had good yeast viability. Foam was not significantly affected by the test.

Table 1. Results for the first olive oil test fermentation vs. the control.

Sample	Fermentation Time (hrs)	Density (g/ml)	Yeast Viability	Nibem Foam Collapse (sec)
Olive oil test	140	1.011	94%	272
Control	117	1.011	96%	278

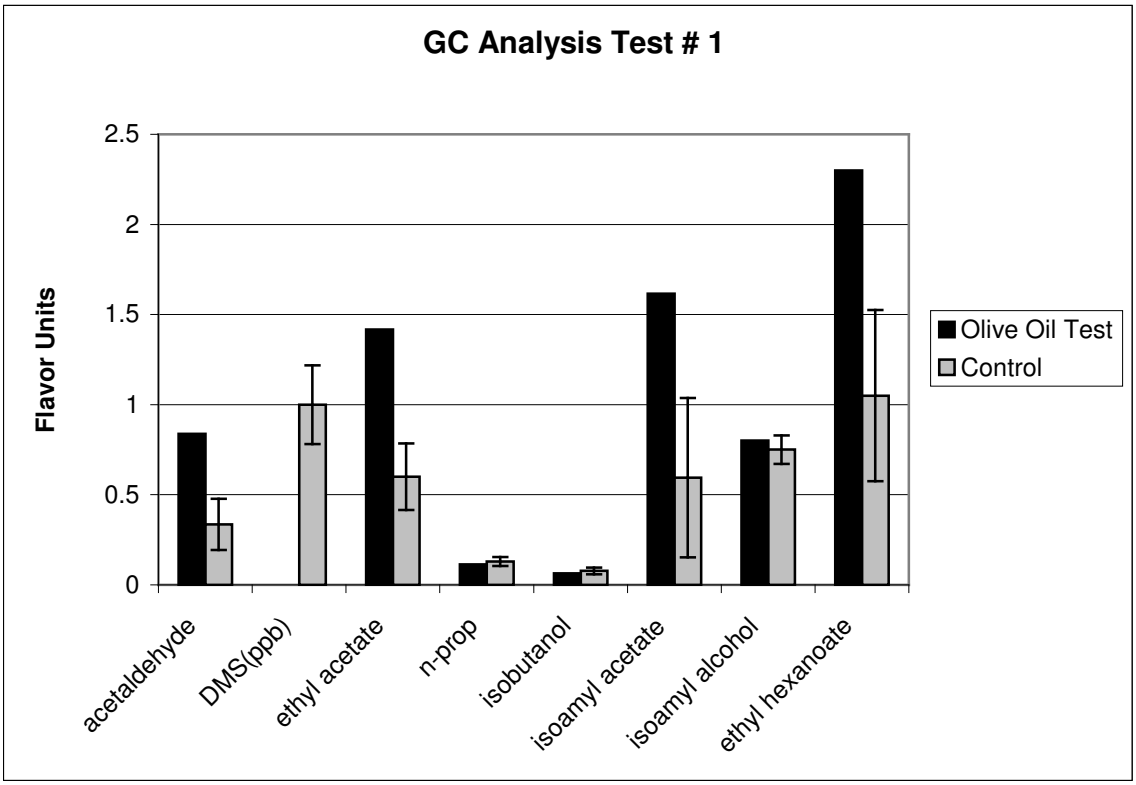
In all of the G.C. figures, the flavor units are the amount of each individual compound, in parts per million (ppm) as determined by G.C. divided by the established flavor threshold for that particular brand. Therefore, any compound with a flavor unit value greater than one is considered to be above flavor threshold for that brand. The control values given in these GC analysis graphs are an average of all of the non-test production beer during that time period. The Y error bars given for each control show the average standard deviation between the control finished product samples during that time period. The test results do not show standard deviation Y error bars because they are single test results not averages.

One anomaly in this first test appears to be the absence of dimethyl sulfide (DMS) in the test sample. This was considered to be an anomaly because it sometimes seen in normal production beers. Total DMS levels are considered to be more of a function of the brewhouse boiling system and not related to fermentation.

The ester profile for the test in Figure 1 was higher than that of the control but not out of the breweries specifications for the brand. Also, in this particular situation, the

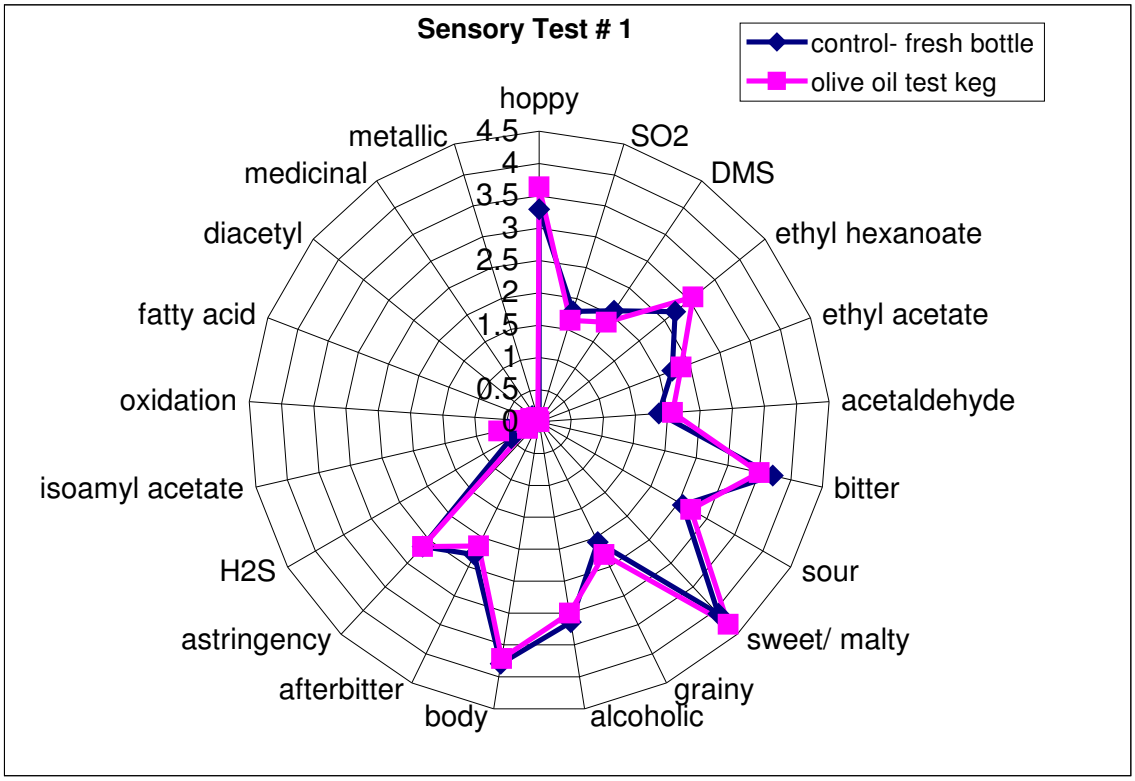
increase in esters was considered by the brewery to be a positive change because of the potential to mask staling flavor compounds. We know from historical data that as this brand ages ethyl hexanoate and iso-amyl acetate both decrease. Ale flavor compounds such as acetaldehyde, ethyl acetate, isoamyl acetate and ethyl hexanoate were all more than double the control (Figure 1).

Figure 1. Gas Chromatography results for the first olive oil test vs. the control beers.



The test beer was then put on the brewery's flavor profile analysis taste panel to see if the brewery's flavor panel could detect the increase in esters shown by the GC results. The sensory results, shown in Figure 2, confirm the increase in acetaldehyde, ethyl acetate, isoamyl acetate and ethyl hexanoate indicated by G.C., however the taste panelists did not perceive the difference as significant so the next round of testing continued. Note that Figure 2 illustrates that an acceptable flavor match was achieved between the test and control beers. The comments from that taste panel indicated an overall preference for the olive oil test.

Figure 2. Sensory results for the first olive oil test vs. the control.



Trial Two

The analytical results for the second test, shown in Table 2, were similar to the first trial. For this and all subsequent rounds of testing the brewery had made a change in the maximum fermentation temperature, increasing it from 20°C to 24°C. This procedural change was unrelated to the olive oil tests. Due to this change all fermentation times for both tests and controls were decreased. It is interesting to note that the difference in fermentation time decreased from 20% in the first test to 14% in the second. The rate of olive oil addition for the second test was 33% greater than in the first.

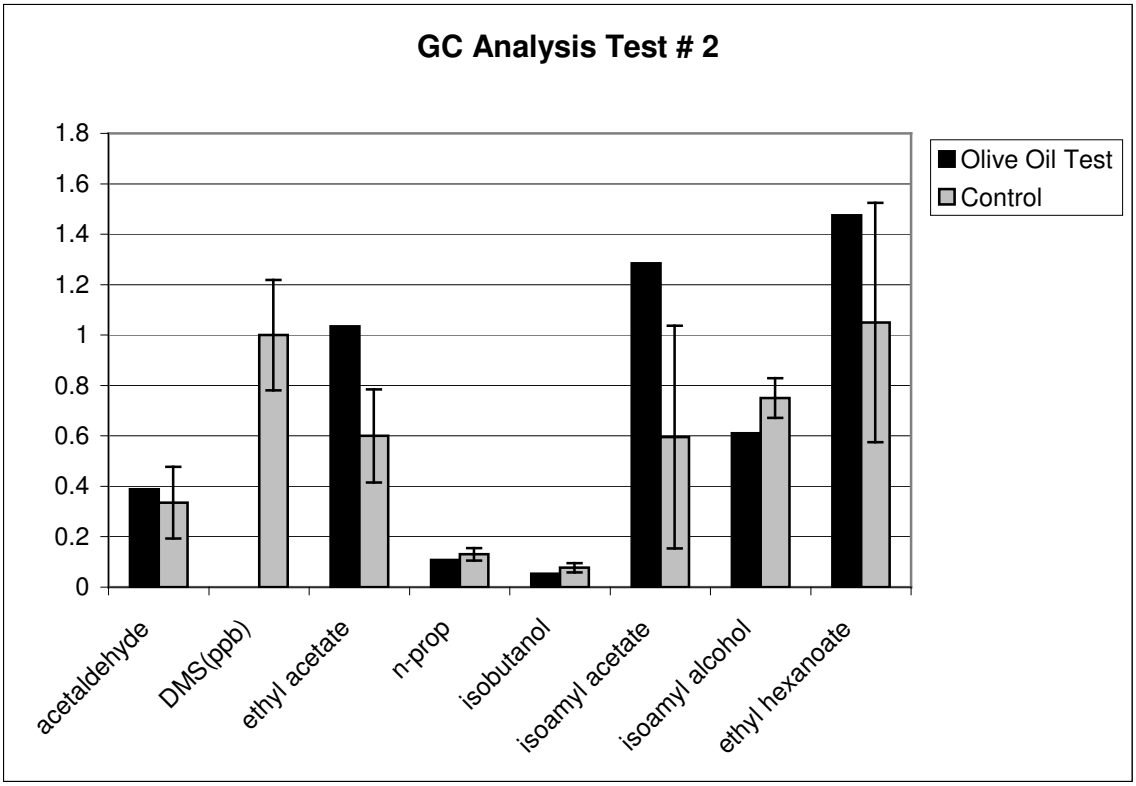
The difference in the post-fermentation density is significant and it is a concern but it is not outside of the breweries specification range (1.010 to 1.014 g/ml). The apparent improvement in foam is also well within the breweries normal variations, which can fluctuate between 230 and 290 seconds on the Nibem. Again viability was considered to be normal for both the test and the control (Table 2).

Table 2. Results for the second olive oil test fermentation vs. the control.

Sample	Fermentation Time (hrs)	Density (g/ml)	Percent Viability	Nibem Foam Collapse (sec)
Olive oil test	90	1.014	96%	277
Control	79	1.012	92%	262

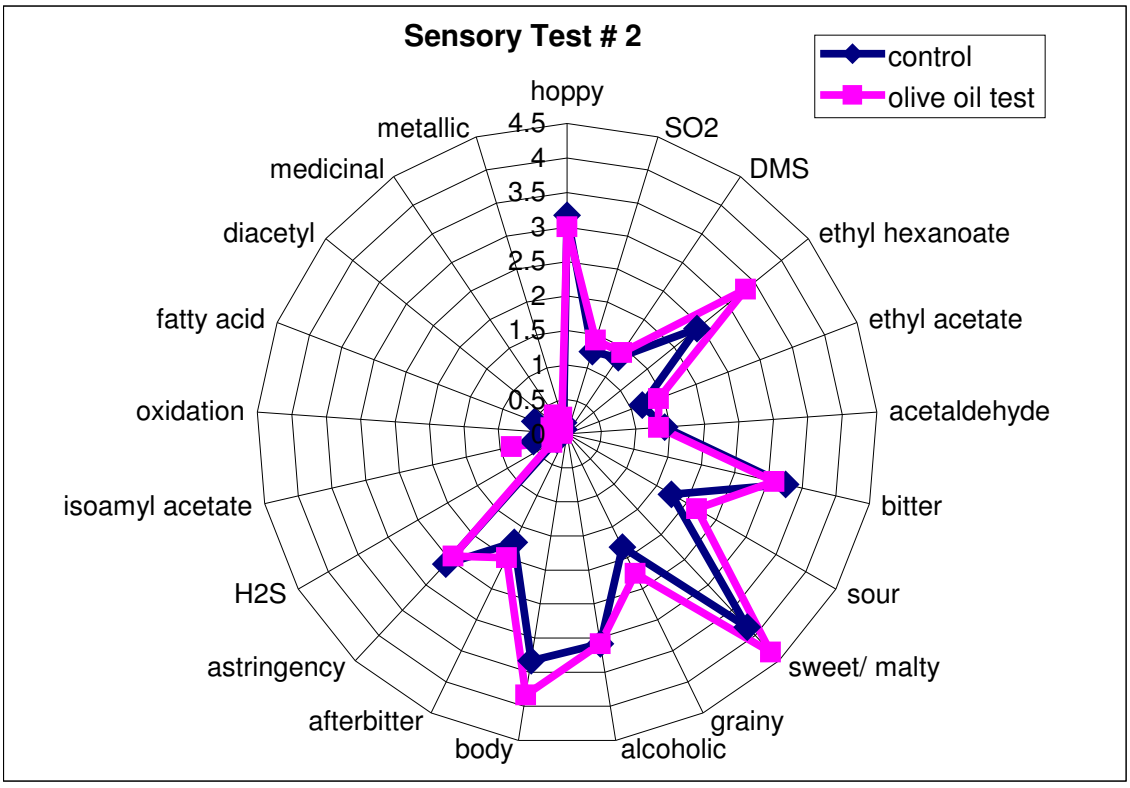
The G.C. results for the second round of testing shown in Figure 3 also indicate that this test was closer to the control than in the first round. Compounds such as acetaldehyde and ethyl hexanoate are actually within the brewery's standard deviation and the remaining fusel compounds such as ethyl acetate and isoamyl acetate are much closer than they were in the previous round of testing (Figure 3).

Figure 3. Gas Chromatography results for the second olive oil test vs. the control.



According to the sensory panel (Figure 4) there was a statistically significant increase in ethyl hexanoate at a 95% confidence level. While there were no other attribute changes that could be called significant to that confidence level, the panel perceived the olive oil test to be higher in other esters such as ethyl acetate and isoamyl acetate. Other flavor attributes that have been linked to these fruity-ester compounds such as perceived body, and sweetness were also rated higher in the test. Despite the increase in ethyl hexanoate shown in Figure 4, the flavor panel approved both rounds of testing for production release.

Figure 4. Sensory results for the second olive oil test vs. the control.



The sensory, fusel, and analytical testing results for the first two rounds of testing were all within the breweries specifications so the decision was made to proceed with the full-scale test. Bringing the test through production as a full scale batch so that all product handling including packaging are done in the same way as the control sample was deemed to be the most appropriate way to be sure that noise from other variables such as the operators, equipment or procedures would be kept to a minimum.

Trial Three

In the first two rounds of testing, esters were increased and higher alcohols were decreased compared to the control indicating low yeast growth in the olive oil test (Fig. 1-4). Supporting this assumption were the reduced fermentation speed and attenuation (Table 1,2). To improve this, the amount of olive oil used in the third round was increased 100% over the previous round.

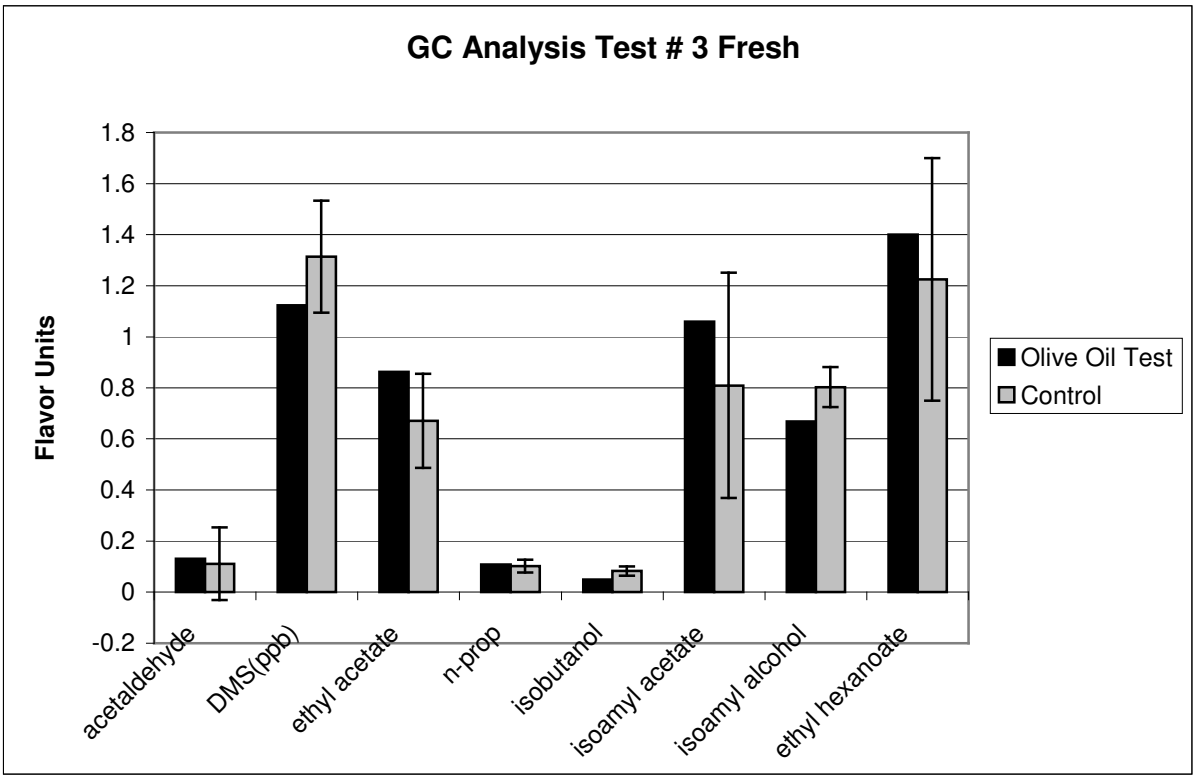
As shown in Table 3, again the difference in fermentation time decreased, but this time only slightly from 14% longer in the second test to 13% longer in the third. Post fermentation density was much better, compared to the control, in this test than in the previous test although again both were well within the brewery's specifications. Foam, attenuation and yeast viability were all within the normal range.

Table 3. Results for the third olive oil test fermentation vs. the control.

Sample	Fermentation Time (hrs)	Density (g/ml)	Percent Viability	Nibem Foam Collapse (sec)	pH
Olive Oil Test	94	1.011	90%	263	4.38
Control	83	1.013	97%	269	4.33

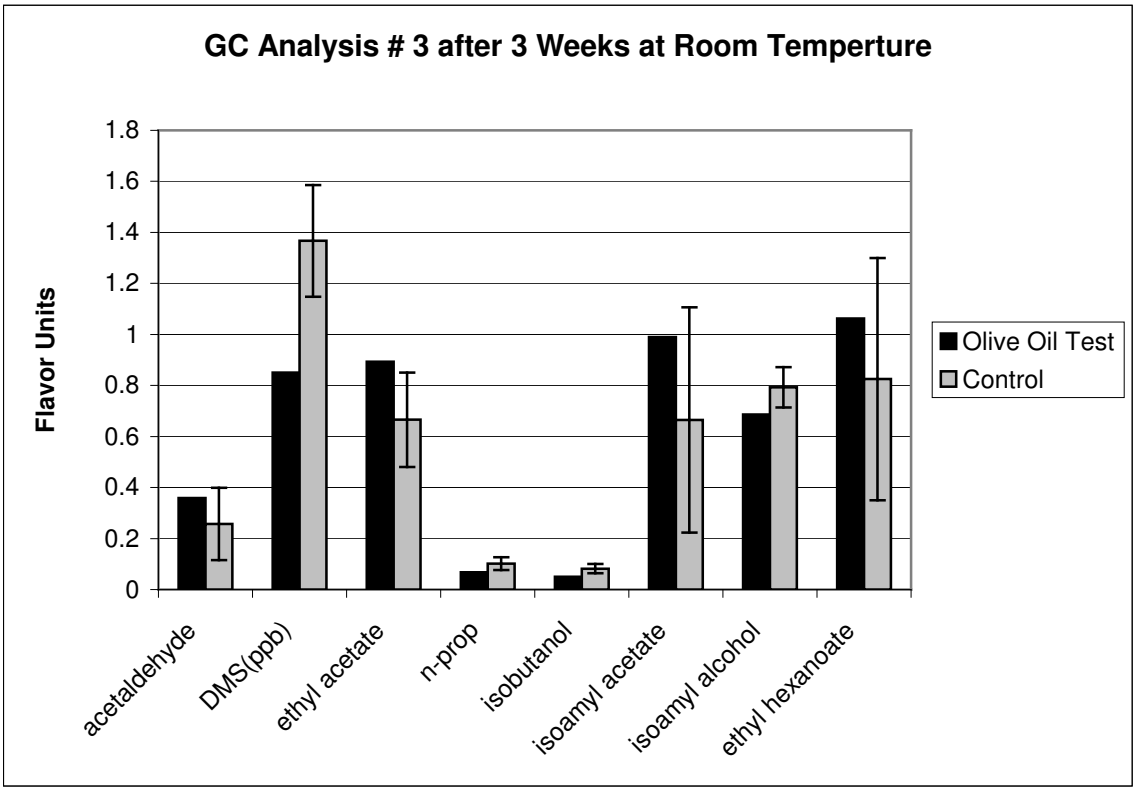
The G.C. flavor component results for this third round of testing show a significant improvement in total esters and higher alcohols (Figure 5). Although there is still an increase in esters and a decrease in higher alcohols, the difference for most of these compounds is within the standard deviation of the average control. The only important beer flavor compound in which the difference exceeds the standard deviation was isoamyl alcohol.

Figure 5. Gas Chromatography results for the third olive oil test vs. the control at fresh.



The bottles from this run were then stored at room temperature for three weeks and the beer was re-analyzed by G.C. (Figure 6). As expected acetaldehyde increased in both the test and control samples although both were well below threshold. Isoamyl acetate and ethyl hexanoate were both reduced during warm storage although the test was less effected than the control and these two compounds were still present at threshold levels after one week warm storage.

Figure 6. Gas Chromatography results for the third olive oil test after 3 weeks of being stored at room temperature.

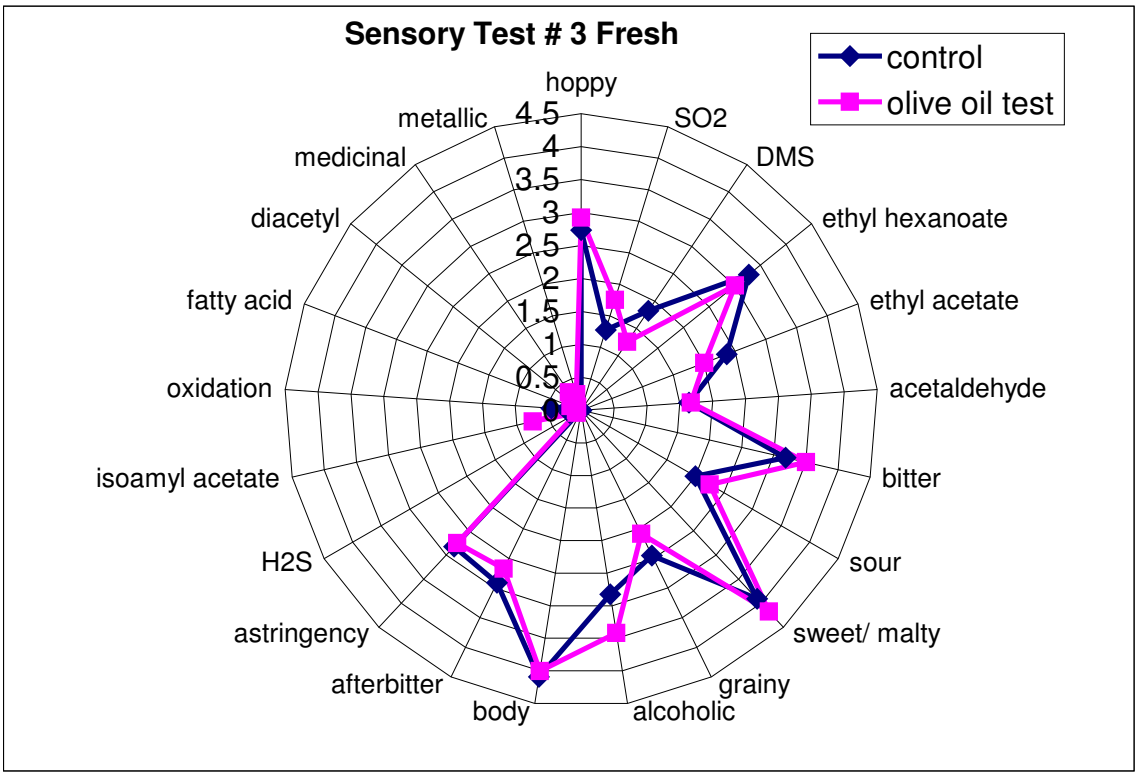


There are some marker compounds that can be used to quantify oxidative changes. Compounds such as acetaldehyde will typically increase during storage as a result of the oxidation of ethanol. Ester compounds such as, ethyl hexanoate, and iso-amylacetate will typically decrease with oxidation. Two test bottles and two control bottles were stored at 30 °C for one week and two test and two controls were stored cold. All of these bottles were then tested by GC/MS for known oxidation markers for this brand. The results from the GC/MS analysis were mixed. Previous oxidation research has shown that when this brand oxidizes compounds such as acetaldehyde, nonanol and furfural increase and other compounds such as myrcene, and esters decrease. Some of the results indicate that the olive oil test was less oxidized. For instance, acetaldehyde was 25% higher in the control beer than in the test, nonanol was 10% higher in the control beer and most of the ethyl esters were higher for the test brew. Some of the results indicate that the control was less oxidized. For example furfural was 10% higher for the test beer and b-myrcene was 30% less in the test beer.

The sensory results for this round of testing also indicated very little difference between the test and control. This round of testing was taken all the way through to finished product and approximately 2100 hl of unblended test finished product were packaged and released into the market by three separate taste screenings (finishing taste release, packaging taste release and the brewery's sensory panel).

The results of the sensory panel are given in Figure 7. Again the test was rated higher in isoamyl acetate and sweetness but the differences were not statistically significant. Some esters such as ethyl hexanoate and ethyl acetate were actually perceived as being higher in the control. Based on the results shown in Figure 7, the goal of this study, which was to achieve a flavor match without aerating the wort, was achieved in this third test.

Figure 7. Sensory results for the third olive oil test vs. the control.

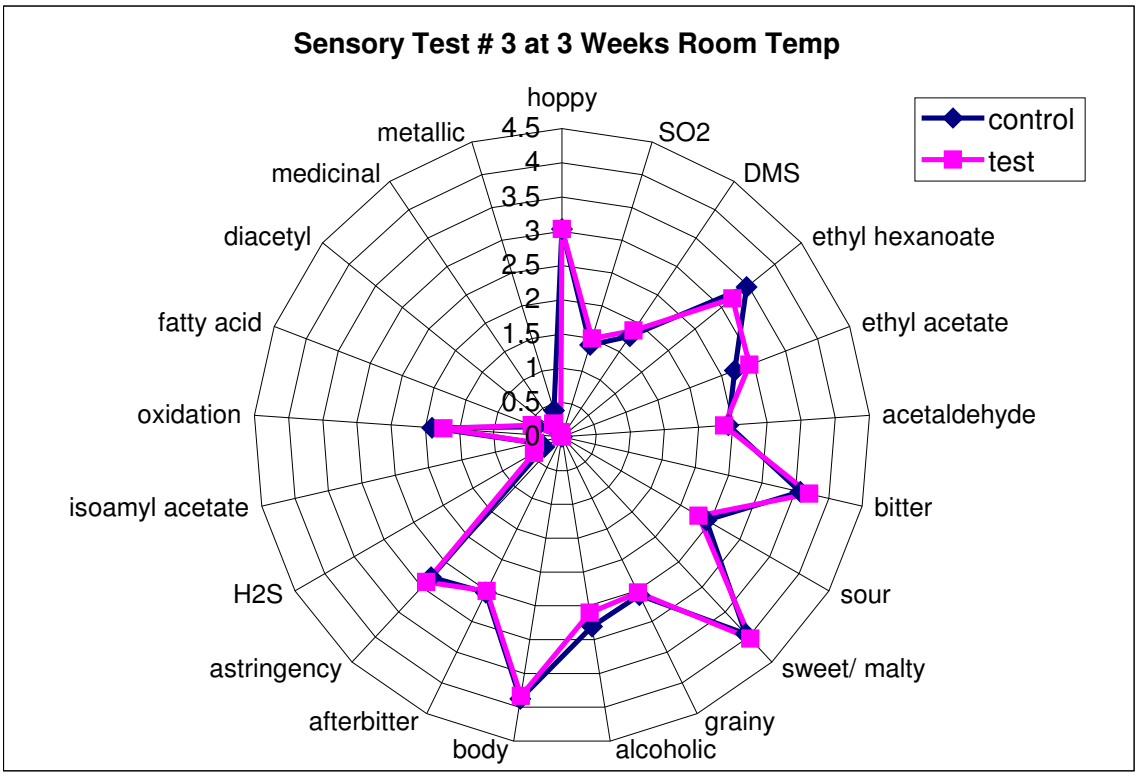


One of the goals of this series of tests was to identify a process change that would improve beer flavor stability without having a significant negative impact on finished product quality or process efficiency. The results shown in Figure 8 do not show that

this process change significantly improves flavor stability. Although the control was rated slightly higher in overall oxidation after 3 weeks at room temp, the difference is obviously not significant.

The comments however, show that eight of the sixteen panelists said that the control tasted oxidized but only four of them commented that the olive oil test was oxidized. Other attributes remained approximately the same as the fresh test and were similar to the control.

Figure 8. Sensory results for the third olive oil test after 3 weeks room temp.



Trial Four

In the fourth round of testing, again the GC results showed an increase in esters such as ethyl hexanoate, ethyl acetate, and isoamyl acetate and a decrease in acetaldehyde, DMS and higher alcohols. The results are shown in Figure 9. Based on past results 2100 hl of this beer was packaged and sold without being blended.

The initial sensory results for this trial, shown in Figure 10, also indicated an increase in ethyl hexanoate. The beer was released for sale by the in-house taste panels. After three weeks warm storage the olive oil test was significantly less oxidized than the control. It was also perceived by the panel as retaining more of the fresh beer attributes such as ester and hop flavors (Figure 11). Overall the olive oil test was preferred to the control.

Figure 9. Gas Chromatography results for the fourth olive oil test vs. the control.

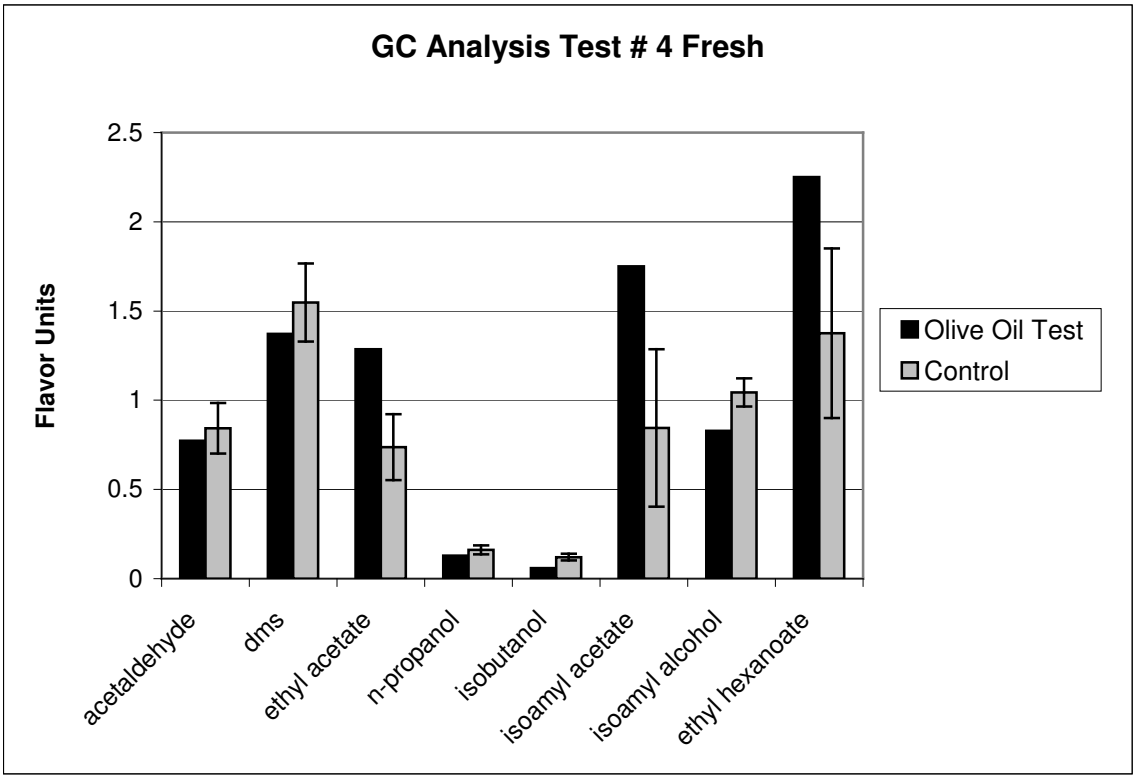


Figure 10. Sensory results for the fourth olive oil test vs. control.

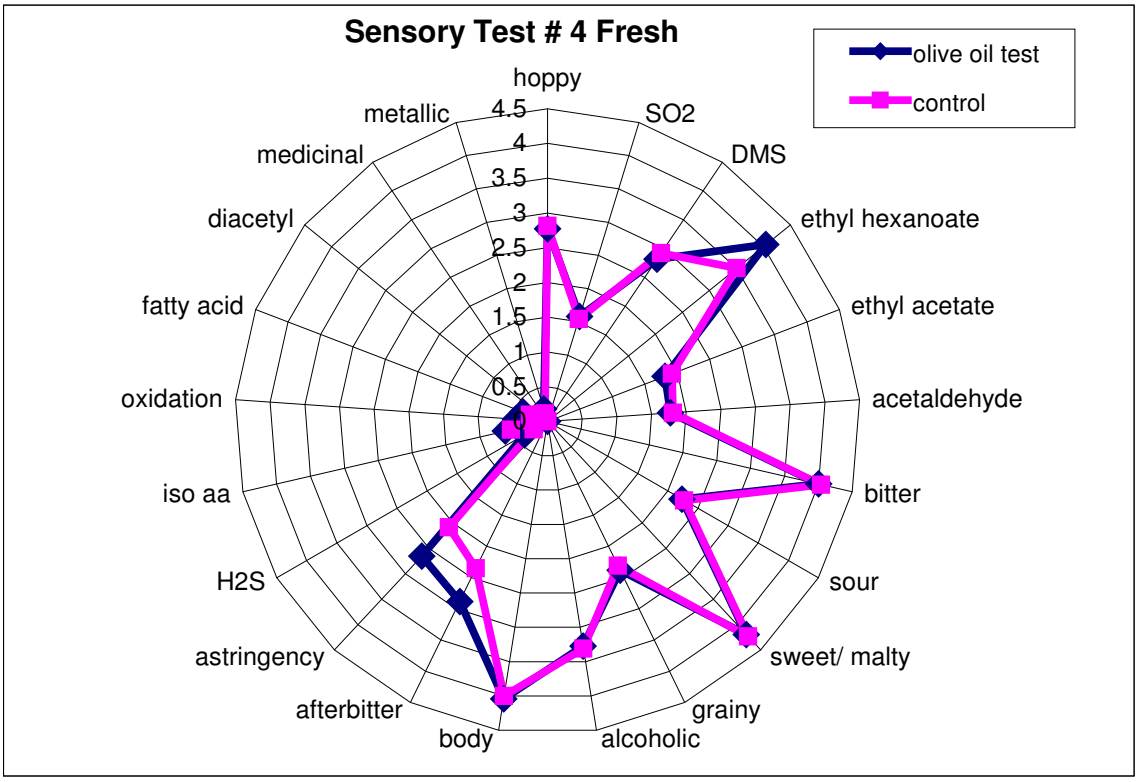
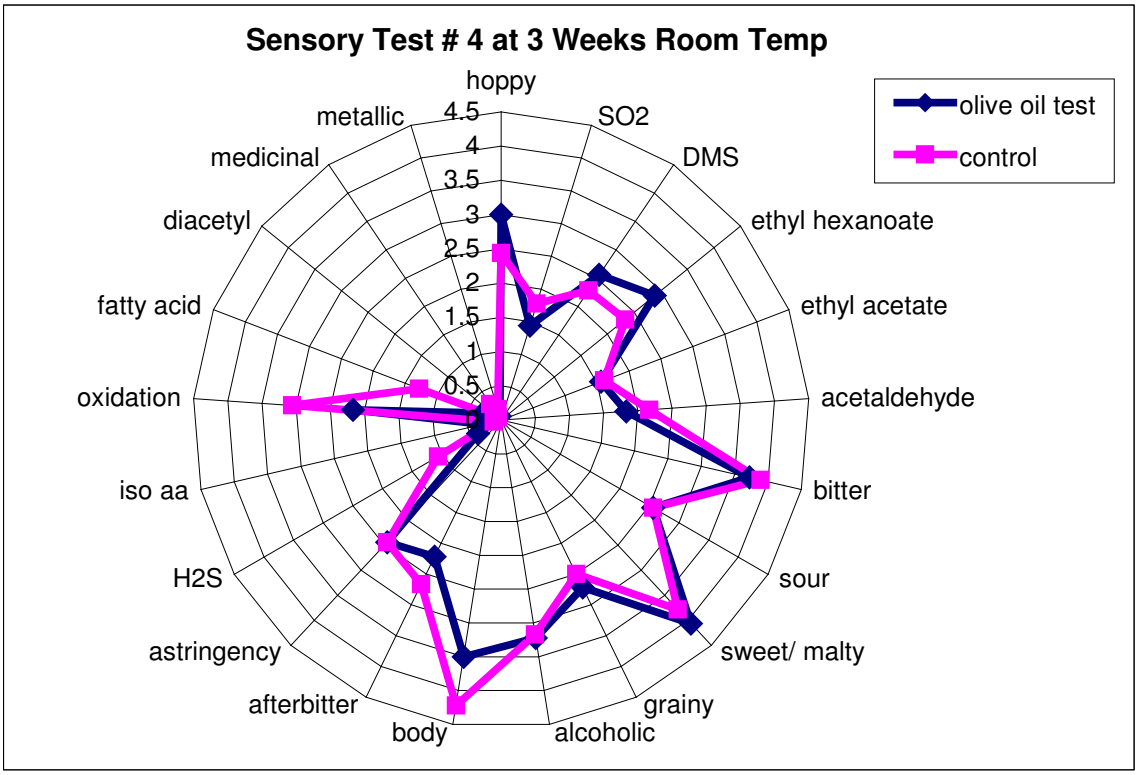


Figure 11. Sensory results for the fourth olive oil test after 3 weeks warm storage.



Discussion

This project set out to investigate the effects of using olive oil addition to storage yeast vs. traditional wort aeration on a full production scale. Many similar studies have been published using similar yeast treatments such as lineoleic acid addition to yeast or yeast aeration (Fugiwara et. al., 2003, Depraetere et. al, 2003, Smart, 2000, 2003 Moonjai et. al., 2003a,b). The use of olive oil was selected for this study because it contains oleic acid, which is the monounsaturated fatty acid naturally produced by yeast cells.

The main areas of interest were fermentation performance, yeast health, ester production and flavor stability. It was expected that if normal fermentations were achieved flavor stability would be improved due to the reduced contact of oxygen with the wort. It was also expected that if the olive oil treatment was unsuccessful, as a means of supplying the necessary fatty acids, yeast growth would be reduced, attenuation and VDK reduction would be incomplete, and that ester production would be significantly increased.

Results

The results of this series of tests showed that normal production wort fermentations could be carried out using yeast treated with olive oil instead of wort aeration. This procedural change effected ester and fusel oil production, fermentation speed, and overall flavor perception. Attenuation, pH, and foam were not affected. Ester production was increased in all tests, although this increase was not deemed to be out of specification for the brand by the flavor profile analysis panel. The rate of attenuation in all trials was slower than the control samples but the fermentations were complete and all final gravities were similar to controls. The overall effect on oxidation potential in the final product was improved in the tests when compared to the controls after three weeks warm storage. In the last round of testing the olive oil test batch was judged by the panel to be significantly less oxidized than the control after a period of warm storage. This method of treating the yeast with olive oil during storage instead of aerating the wort did improve the overall flavor stability of the beer without compromising flavor quality.

Oxidative Effects of Wort Aeration

Previously conducted research has shown that wort aeration causes oxidative reactions to occur in the wort, which form the precursors to beer staling compounds (Burkert, 2004). It is widely understood that minimizing the exposure of beer or wort to oxygen will improve the finished product's resistance to oxidation. During knock out it is traditional practice to completely saturate the wort with air or oxygen, intentionally dissolving 8 to 10 ppm oxygen into the liquid.

The yeast takes up the oxygen very quickly after wort aeration but the oxidative reactions also take place very quickly. Even though wort aeration is a universally excepted practice it seems very logical that eliminating this step would be a significant improvement on the final beer's resistance to oxidation. Therefore it makes sense that reducing the product's exposure to oxygen would improve its flavor stability.

Ester Production and Growth

Another interesting aspect of this test was ester production. Low yeast growth has been shown to cause a decrease in higher alcohols and an increase in esters (Kunze, 1999). Oxygen is added to the wort so that the yeast cells can synthesize sterols and fatty acids necessary for growth so it was expected that removing the oxygen would cause an increase in esters and a decrease in higher alcohols. The GC, and sensory panel results confirmed both of these outcomes. The fermentations however were complete which indicates that the olive oil had a positive effect on the fermentations. As the amount of olive oil was increased with each trial, the fermentation performance improved. It is possible that the rate of fermentation and the ratio of esters to higher alcohols could be improved if the amount of olive oil addition were increased beyond the rate of 1 mg / 25 billion cells. For this brand, the increase in total esters was perceived as preferable by the flavor panel.

Suggestions for Future Work

In order to achieve a healthy, vigorous fermentation yeast requires both sterols and fatty acids. In this study only oleic acid was provided to the yeast. One suggestion

for future work would be to repeat this study adding a combination of ergosterol and olive oil to the storage yeast. This would provide the yeast with both the unsaturated fatty acid and the sterol normally produced by yeast in the presence of oxygen. It would also be interesting to repeat this study adding only ergosterol to observe the differences in its effect on attenuation, growth, esters, and flavor stability compared to olive oil addition.

During storage yeast loses vitality and viability. It becomes vulnerable to conditions that affect its metabolic activity. For this reason it has been proposed by Chris Boulton (Smart, 2000) that any yeast aeration should be done at the start of yeast storage rather than at the end. During storage yeast depletes its glycogen and trehalose reserves, so oxygenating yeast after a prolonged period of storage can trigger metabolic activity, which may be harmful to the yeast. Oxygenating yeast at the beginning of yeast storage may provide the yeast with the sterols necessary for proper fermentation at a time when the yeast is healthy enough to withstand some metabolic activity (Smart 2000). This, together with olive oil addition, may provide the combination of sterols and fatty acids necessary for a proper fermentation. The addition of olive oil and sterols could also be combined with a reduction in wort aeration to achieve a vigorous fermentation with reduced oxygen exposure.

Another suggestion for future work would be to continue with the addition of olive oil to storage yeast but to experiment more with addition amounts and contact time. Increasing the amount of oil addition, oil and yeast contact time, or yeast-pitching rate may improve yeast health and growth, reducing ester formation and achieving fermentations closer to the controls. Also other types of unsaturated fatty acids such as linoleic acid could be used in place of olive oil. Fermentation parameters such as temperature and pressure could also be manipulated to compensate for differences in fermentation.

If the focus of the testing were taken away from flavor stability improvement, there are a number of intriguing possibilities for future study. The addition of olive oil during yeast storage, combined with wort aeration, could be used to test improvements in fermentation performance. Alternatively the olive oil addition could be tested in conjunction with zinc addition and wort aeration to optimize fermenter turn around time and yeast health.

Conclusion

The idea of supplying yeast with the unsaturated fatty acids that it requires for membrane health and growth is an intriguing possible alternative to the practice of wort aeration. It has been proven that during the dormant phase of storage yeast cells will take up fatty acids such as linoleic acid (Moonjai, 2003a). It is commonly accepted that wort aeration is necessary for yeast growth but also that oxygenation of the product reduces flavor stability. If the yeast is supplied with the olive oil during storage, normal fermentations can be achieved with improved flavor stability. The addition of olive oil to storage yeast in this study showed that consistent, complete fermentations of acceptable flavor quality and improved flavor stability can be achieved. However, these test fermentations were slower and produced an increased amount of esters compared to the controls. Although the finished product was significantly higher in ester content, it was not determined to be out of specification and was actually preferred by the internal flavor panel. The goal of improving flavor stability was achieved but at the cost of increased esters and slightly slower fermentations.

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Appendix 1

Fusel Oils by Headspace GC SOP

1.0 Purpose & Scope

This method separates and quantifies acetaldehyde, ethyl acetate, isobutyl acetate, n-propanol, isobutanol, isoamyl acetate, n-butanol, isoamyl alcohol, ethyl hexanoate, and dimethyl sulfide in cold wort, in process beers and finished product.

2.0 Equipment & Chemical Preparation

- Perkin Elmer Autosystem XL gas chromatograph
- Perkin Elmer Turbomatrix headspace sampler
- Perkin Elmer PE-Wax column (60m x 0.25mm id x 0.25um film)
- 20 ml Headspace vials and caps with septa and star springs
- vial crimper
- Ammonium Sulfate, granular (Mallinckrodt chemicals #7725)
- n-propanol (Sigma Aldrich 29,328-8)
- absolute alcohol (Sigma Aldrich
- acetaldehyde
- ethyl acetate
- ethyl hexanoate (ethyl caproate)
- isobutanol (2-methyl-1-propanol)
- isobutyl acetate
- isoamyl acetate
- methyl acetate
- 2-methyl-1-butanol (active amyl alcohol)
- 3-methyl-1-butanol (isoamyl alcohol)
- dimethyl sulfide
- 1-pentanol
- pipettes, 250ul, 1 ml, 5 ml, and 10ml
- Finn adjustable pipette
- 10 100ml volumetrics

- 1 liter volumetric flask
- analytical scale
- shaker table

3.0 Calibration

Stock Solutions

To prepare the stock solutions (FOA, FOB, FOC, and FOD) weigh the volume of each compound given to 4 decimal places, into a 100 ml volumetric flask, approximately half-full with absolute alcohol.

FOA

0.4 mls n-propanol
0.6 mls 2-methyl-1-butanol
0.6 mls 3-methyl-1-butanol
0.5 mls isobutanol

FOB

2.5 mls ethyl acetate
1 ml acetaldehyde (kept in lab fridge, freeze before attempting to pipette, and freeze pipette)
0.1 ml isobutyl acetate
0.4 mls isoamyl acetate

FOC

0.1 ml ethyl hexanoate

FOD

0.1 ml DMS

Working solution FOE

Pipette 20 mls of FOA, 2mls of FOB, 1 ml of FOC, and 0.4 mls of FOD into a 100 ml volumetric flask and make up to the mark with distilled water.

Standard solutions FO1, FO2, FO3, FO4, FO5, and FO6

Pipette the following volumes of FOE and absolute alcohol into separate 100ml volumetrics, approximately half full with distilled water:

1 ml FOE, 3.8 mls absolute alcohol
2 mls FOE, 3.5 mls absolute alcohol
3 mls FOE, 3.3 mls absolute alcohol
4 mls FOE, 3.0 mls absolute alcohol
5 mls FOE, 2.8 mls absolute alcohol
6 mls FOE, 2.6 mls absolute alcohol

Make up to the mark with distilled water and label FO1, FO2, FO3, FO4, FO5, and FO6 respectively.

Internal standard solution

Pipette 1 ml of methyl acetate and 2 mls of pentanol into a 1-liter volumetric flask approximately half full with distilled water. Using a graduated cylinder, add 40 mls of absolute alcohol to the flask and make up to the mark with distilled water. Decant the solution into headspace vials (filling completely), crimp on the caps, and label FO Int Std. Use a fresh vial with each analysis. You may decant some of the internal standard into clean amber sample bottles, fill completely and label. Once this container is open, you should decant the rest into headspace vials as described above.

Calibration check solution

Pipette 14mls of absolute alcohol into a 500ml volumetric flask. Next pipette 25 mls of FOE into the flask and make up to the mark with distilled water. Decant the

solution into clean, dry amber sample bottles (fill completely). Label the bottles as FOCC.

4.0 Procedures

All samples, standards, and controls should be at or near 0C prior to preparation. In process beers that are warm may be placed in the freezer for 10 minutes before pipetting. Controls should be spaced between samples at a rate of approximately 1 control for every 6 samples.

Weigh 5.0 grams ± 0.1g of ammonium sulfate into a 20 ml headspace vial. It is best to prepare around 30 vials at a time. Place the vials in the freezer for at least 30 minutes before using. Pipette 5 mls of sample or standard and 250ul of internal standard into the vial and crimp on the cap. Always check the crimp for a good seal, it should not spin easily. When all the vials have been prepped, transfer them to the rotary shaker in the micro lab. Shake at 250 RPM for 30 minutes. During this time you may log in the samples into the Turbochrome sequence. Run all samples at least in duplicate.

Linear calibration

Perform the linear calibration every six months.

Prepare the standards FO1, FO2, FO3, FO4, FO5, FO6 in triplicate, as described above. The Rsq should be above 0.98, if not the calibration should be repeated.

Calibration Check

Check the linear calibration by analyzing the standard calibration check solution (FOCC) daily at the beginning and end of the run. Treat FOCC as a sample and calculate the concentrations of the individual compounds. The calculated values should fall in line with the known values ± 5%.