IAA Journal of Scientific Research 11(2):40-51, 2024.
©IAAJOURNALS
https://doi.org/10.59298/IAAJSR/2024/112.4051
www.iaajournals.org
ISSN:2636-7319
IAAJSR:112.4051

Investigating the Impact of Acetaldehyde Accumulation on Beer Quality: Metabolic Pathways, Yeast Health, and Mitigation Strategies

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ABSTRACT

Acetaldehyde is the immediate precursor to ethanol in fermentation. Like diacetyl, acetaldehyde is found in large quantities during early fermentation as the yeast produces it early in its metabolic cycle. If there is a high amount of dissolved oxygen present in the young beer, then the oxygen could react with ethanol and oxidize it back into acetaldehyde. The content of acetaldehyde in beer varies from 1 to 20 mg/L depending on many processing factors. Higher concentrations of this metabolite not only induce unpleasant "young" or "green" off-tastes but also participate with phenolics in the formation of beer haze. Acetaldehyde is present in all beers and, in lower concentrations, can contribute positively to a beer's sensory character. When the concentration of acetaldehyde remains above or well above its sensory threshold, it becomes an off flavour. The concentration of acetaldehyde is tied to yeast health and is formed during the beginning and middle of beer fermentation. Its concentration will normally decrease towards the end of a healthy fermentation and maturation process. Acetaldehyde can rise during prolonged warm maturation when yeast loses viability. Typical sensory descriptors associated with acetaldehyde include green (Granny Smith) apples, pumpkin flesh/seed, unripe avocado, and latex paint. Acetaldehyde is somewhat unique in this regard; the "character" of its aroma can change as its concentration changes. The sensory threshold in pale lager beer is typically 1-5 mg/L but this can vary with beer style, in some cases ranging from 5 – 15 mg/L. Acetaldehyde is formed by all yeast. As yeast undergoes fermentation, glucose is turned into pyruvate and then acetaldehyde, and finally ethanol. Yeast utilizes this metabolic pathway of glycolysis to maintain levels of ATP, adenosine triphosphate, aka the energy currency of the cell. From the study entitled investigating the possible causes of the high levels of acetaldehyde off flavour in beer during fermentation in cellars about the suspected Aber system inconsistency, the obtained results portrayed a very abnormal acetaldehyde production level. Three samples were studied for each brand and it was observed that all showed higher acetaldehyde level diversion. For NLS, all the samples studied were found to have acetaldehyde levels of 24.07, 22.01, and 20.87 ppb against the expected maximum levels of 10 ppb. For CPL, the levels were at 24.05, 20.00, 23.50, and finally 25.04, 26.24, and 27.01 for all the ELX brands.

Keywords: Acetaldehyde; Off-flavour; Yeast pitching efficiency; Fermentation tanks; Nile Breweries

INTRODUCTION

Beer making is a complex process that involves the use of ingredients such as barley, hops, water, and yeast to create beer [1–3]. The malting department is responsible for receiving barley and transforming it into malt, which is then used in the brewing department. Malt is a friable package of husk, starch, proteins, flavor precursors, and nutrients, along with a balanced supply of enzymes [4, 5]. The malting department is divided into dry and wet ends, and barley goes through several processes before becoming malt. The brewing department (BREW HOUSE) is where the brewing takes place, divided into brew houses B and C. Brew house B is used for local beer brands like ELD, ELO, and ELX, while brew house C is used for premium brands like CP, CT, and NLS. The major ingredients used in brewing include water, malt & adjuncts, hops, calcium sulfate, and calcium hydroxide. The fermentation process involves using Brewer's yeast, a single-celled fungus that acts as a catalyst in converting sugar to alcohol. Yeast is found everywhere in the environment and can turn sugars into alcohol and CO2 under oxygen-free conditions[6, 7]. Under aerobic conditions, yeast can respire normally and produce CO2 and water. It needs oxygen for cell wall production and reproduces by budding [8, 9]. Yeast pitching involves introducing yeast into a unit tank containing aerated wort from the brew house at 9-10oC to avoid shocking the yeast cells[1]. Proper

aeration of the yeast ensures maximum fermentation intensity is achieved after 48 hours. The primary fermentation takes 9 days, is monitored by measuring gravity, and stops when the gravity drops to LE (limit of extract) for a particular brand. Secondary fermentation follows, with the remaining sugars helping to fuel it. After the required fermentation level is reached, temperatures are lowered from 20 0C to 4 0C in a process known as chill back [10]. The maturation period depends on the beer brand and takes 21-22 days depending on the brand [11]. Brewers' claret is added during maturation to help with faster sedimentation of yeast and other suspended solids from the beer. Beer packaging involves packaging beer in bottles of varying volumes (500-300 ml) and kegs (30 litters). Bottles such as Euro and Amber are used for beer packaging, and the whole process is joined and moved using a conveyor system. Aber, a technology invented by Alber Instruments in 1988, is applied mainly in yeast pitching at NBL to control the pitching efficiency of yeast into the fermentation tanks [12, 13]. The system runs from the yeast tank to the fermentation vessel through pipes and is controlled by a PLC. The Aber probe only detects and counts viable cells, leaving out dead ones. The study aims to investigate the possible causes of high levels of acetaldehyde off flavor in beer during fermentation in cellars in relation to the suspected Aber system inconsistency. High levels of acetaldehyde across all beers are lowering the Brewery Sensory Support Index (BSSI) score below the brewery target. This project aims to study the causes of the Aber system inconsistency and its effects on the BSSI score and improve beer quality.

METHODOLOGY

Analytical

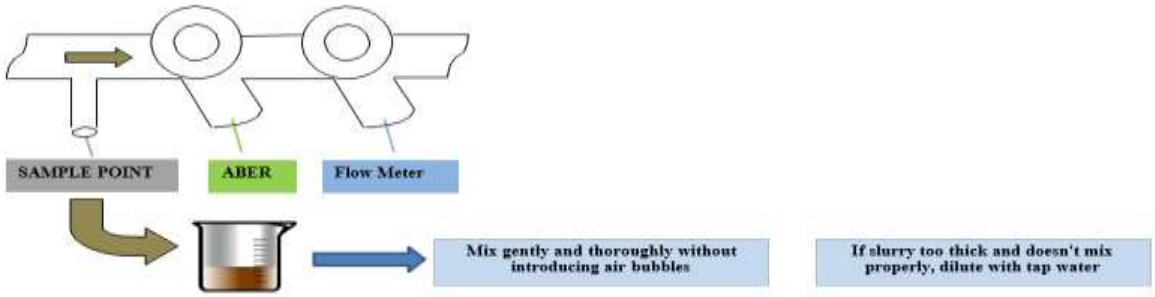
The Aber verification test was carried out on different samples of yeast obtained during the pitching of yeast across the Aber monitor to verify if the Lab tests tallied with the Aber monitor readings for different brands mainly NLS, ELX, and CPL.

Apparatus and Equipment

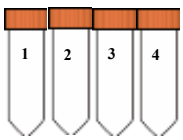
The materials for the experiment include a sampling bucket, yeast sample, tap water, centrifuge tubes, weighing scale, centrifuge machine, glass rod, porcelain spot plate, haemocytometer, micro-haematocrit capillary pipette, disposable Pasteur pipette, 5M ammonium hydroxide, physiological saline test tube, and Methylene blue.

NB: For each sample obtained, the Aber verification procedure was carried out as stipulated in the **ACADIA** following the following steps.

1. One liter of yeast was collected from the Aber sample point. The yeast slurry was found to be too thick to be stirred in a verivent, it was diluted with tap water.

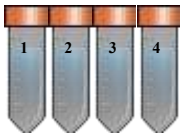


2. Four centrifuge tubes were taken and labeled 1-4. Each of the empty centrifuge tubes was weighed and the results were recorded as below



Tube number	Weight of empty centrifuge tube
1	43.22
2	43.75
3	42.97
4	43.1

3. Each of the centrifuge tubes were filled with yeast slurry and reweighed. The results were then recorded in the table below for the different tube numbers.

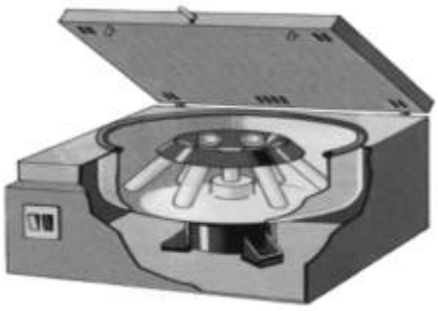


Tube number	Weight of empty centrifuge tube
1	122.03
2	122.07
3	122.09
4	122.07

Therefore, the weight of the yeast slurry contained in each tube was obtained as a difference in weight between the tubes containing the yeast slurry and the empty tubes and recorded respectively for each tube as shown in the table below.

Tube number	Weight of Yeast slurry
1	78.81
2	77.32
3	79.22
4	78.97

4. The tubes containing the yeast slurry were then centrifuged at 4000rpm for 10 minutes or 2500 rpm for 15 minutes.



4000 rpm for 10 min
OR
2500 rpm for 15 min

5. The supernatant was discarded for each tube and the residual yeast pellets were re-suspended in 5M or 17.5% ammonium hydroxide. The ammonium hydroxide mainly dissolves the proteins and Trub particles in the pellet. This step was mainly to improve accuracy for calibration.

6. The tubes were centrifuged again at 400rpm for 10 min or 2500rpm for 15 min.

4000 rpm for 10 min
OR
2500 rpm for 15 min

7. The supernatant was discarded and the tubes left upside down to dry.

8. The tubes containing the now dry pellets were weighed and so the weight of the centrifuge tubes + the dry pellets obtained and recorded as shown in the table as shown below for the different tube numbers.

Tube number	Weight of centrifuge tube + pellets
1	123.86
2	124.19
3	125.02
4	124.35

Therefore, the weight of the dry pellets was obtained from the difference between the weight of the tubes + dry pellets and the weight of the empty tubes, and then results were recorded as shown in the table below.

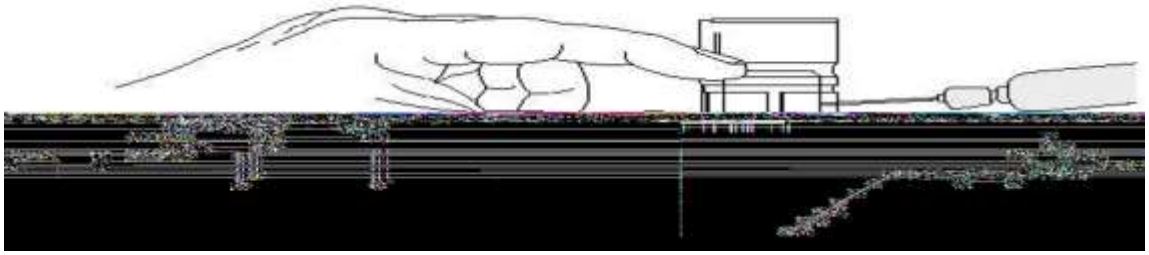
Tube number	Weight of Dry Pellet
1	63.79
2	63.85
3	64.40
4	65.45

Therefore, the average total % spun solid concentration of the yeast slurry was obtained from;

$$\frac{Weight\ of\ dry\ pellets}{\text{Weight of yeast slurry}} \times 100\%$$

i.e. 37.89

9. A yeast viability count was then performed using the original sample and the result obtained progressively recorded as a percentage.



The yeast viability test was progressively and continuously carried out as follows;

The yeast sample was mixed thoroughly, and then transferred into a test tube of saline to create a suspension. Two drops of the yeast suspension were added to a spot plate, and two drops of methylene blue solution were added. The mixture was allowed to stand for one minute before being placed on a counting chamber. A small amount of the mixture was removed from the spot plate and examined under ×400 bright-field magnification. The cells were observed and counted, with the number of cells in the same area-stained blue being noted. Buds or cells less than half the size of the mother cell were progressively ignored during counting. The yeast viability was continuously obtained from the cells.

$$\frac{number\ of\ colourless\ cells\ in\ the\ area}{total\ number\ of\ cells\ in\ the\ same\ area}\times 100$$

i.e. 95%

Therefore, the % viable spun solids concentration of the yeast slurry was obtained and progressively recorded from;

(Viability)× (Total spun solids)

i.e. 36%

Interviews

Also conducted a multitude of interviews with different people mainly the Operators around the Cellars section about the Aber system which gave me a delightful overview of the whole system. The operators were so resourceful to me.

RESULTS

The different samples studied were keenly followed up and the results for the acetaldehyde levels of the respective samples at the end of fermentation were obtained. The tabulated results of the acetaldehyde levels were continuously obtained from the laboratory record book and recorded against their respective sample numbers.

Table 1. Results obtained during the pitching of yeast into the Fermentation tanks for the different brands that is Eagle Lager Extra (EXL), Club Pilsner (CPL) and Nile Special (NLS)

Sample number	*Brand pitched*	***yeast generation***	***Yeast consistency (%)***	***Yeast viability (%)***	***Expected pitching rate (million cells/ml)***	***Aber monitor value (%SS)***	***Aber verification value(lab) [%VSS]***	***Acetaldehyde levels <10ppb***	***Yeast counts at full volume***
I	NLS	3	55.24	95	22.5	31.99	52.48	24.07	
2	NLS	8	47.56	87	22.5	27.73	41.36	22.01	
3	NLS	5	53.81	90	22.5	34.67	48.70	20.87	47
1	CPL	3	59.57	94	21.5	29.29	56	24.05	
2	CPL	4	53.18	93	21.5	33.62	49.46	24.00	
3	CPL	4	52.93	95	21.5	31.76	50.28	23.50	45
1	ELX	7	49.86	90	33	34.8	44.87	25.04	
2	ELX	7	50.54	87	33	28.17	43.97	26.24	
3	ELX	7	50.53	83	33	30.71	42.14	27.01	40

Graphs relating the expected pitching rates, the Alber monitor readings, the Lab Aber verification values, and yeast counts at full volume during yeast Pitching for 3 samples of NLS, CPL and ELX

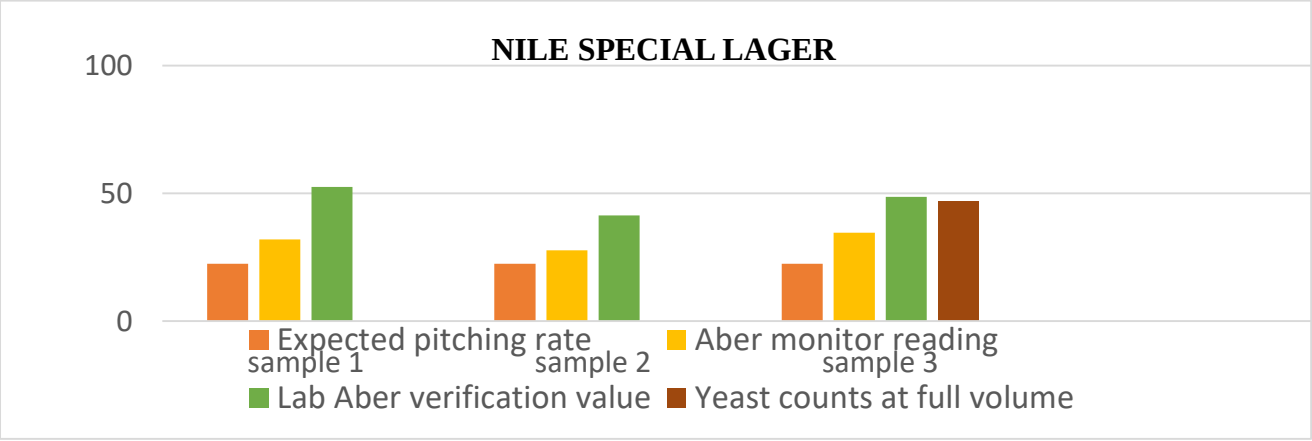


Figure 1: For Nile Special Lager

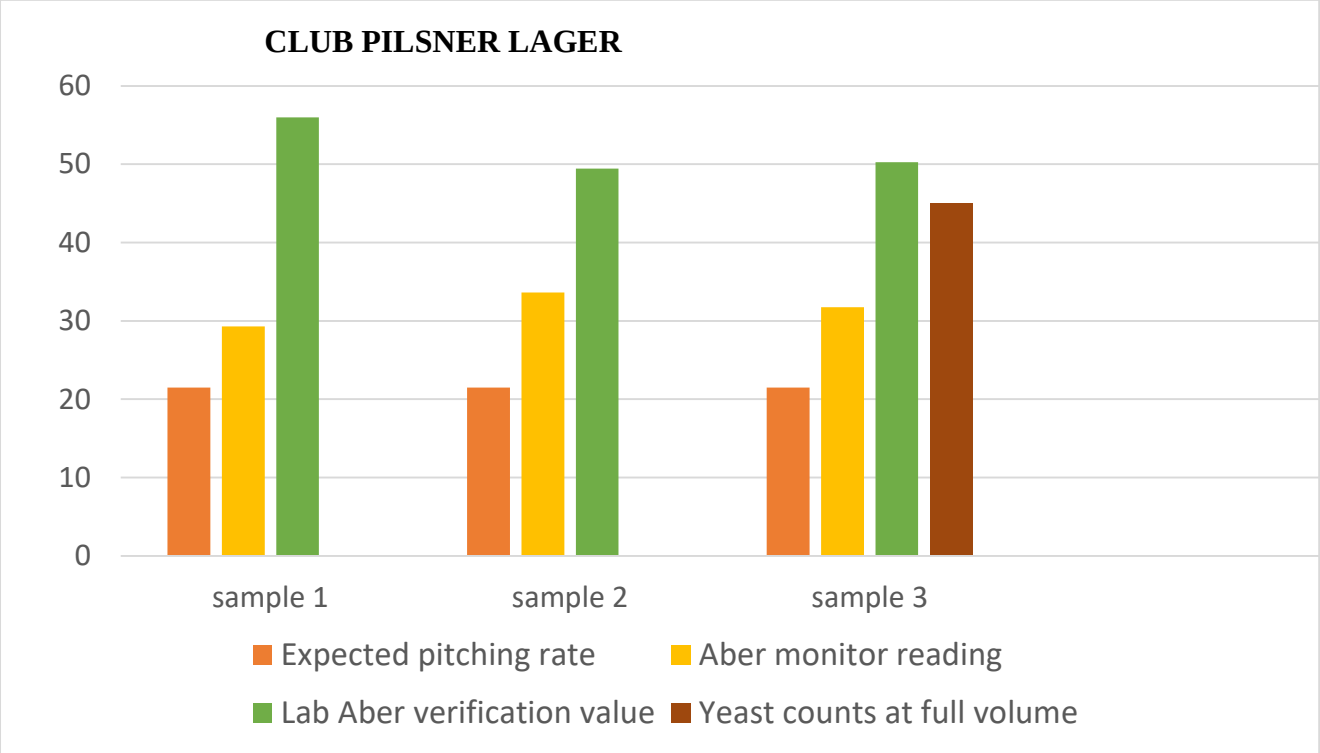


Figure 2: For Club Pilsner Lager

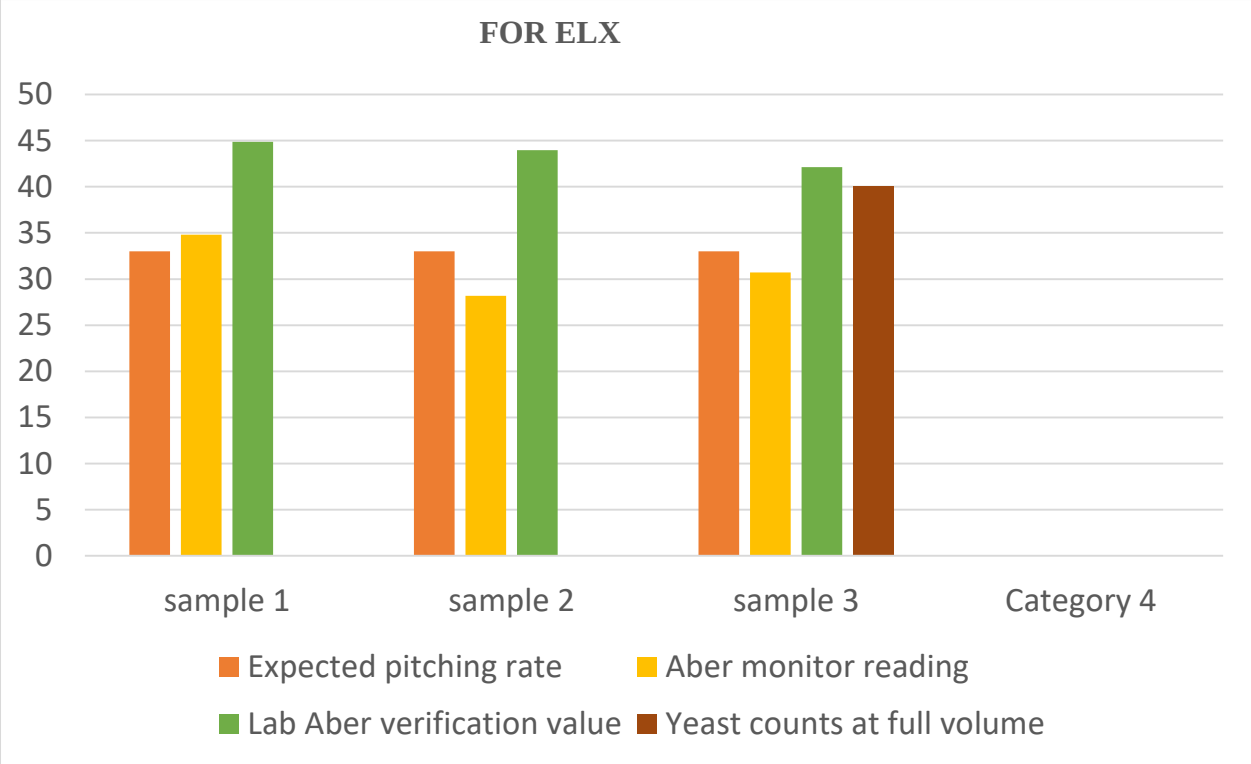
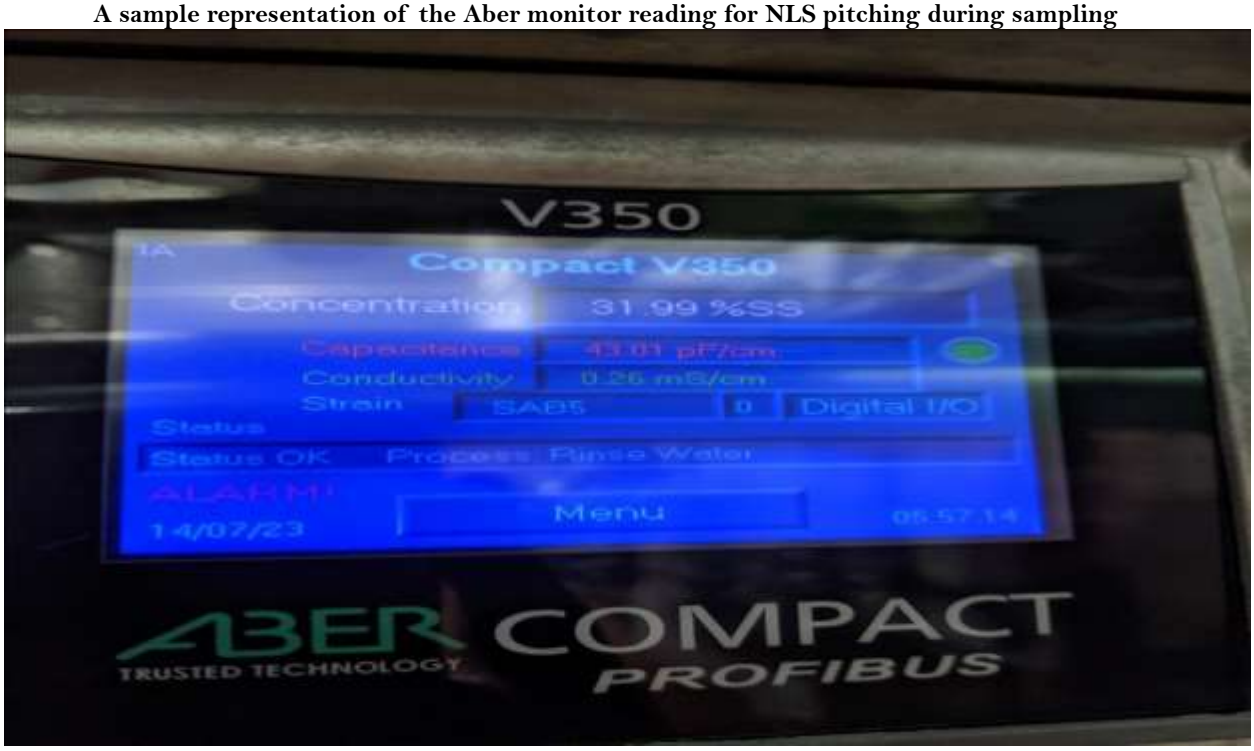


Figure 1: For Eagle Lager Extra

A sample representation of the Aber monitor reading for NLS pitching during sampling



Sample illustration graphs showing the relationship between the Abler verification values and Acetaldehyde levels variation for NLS and CPL

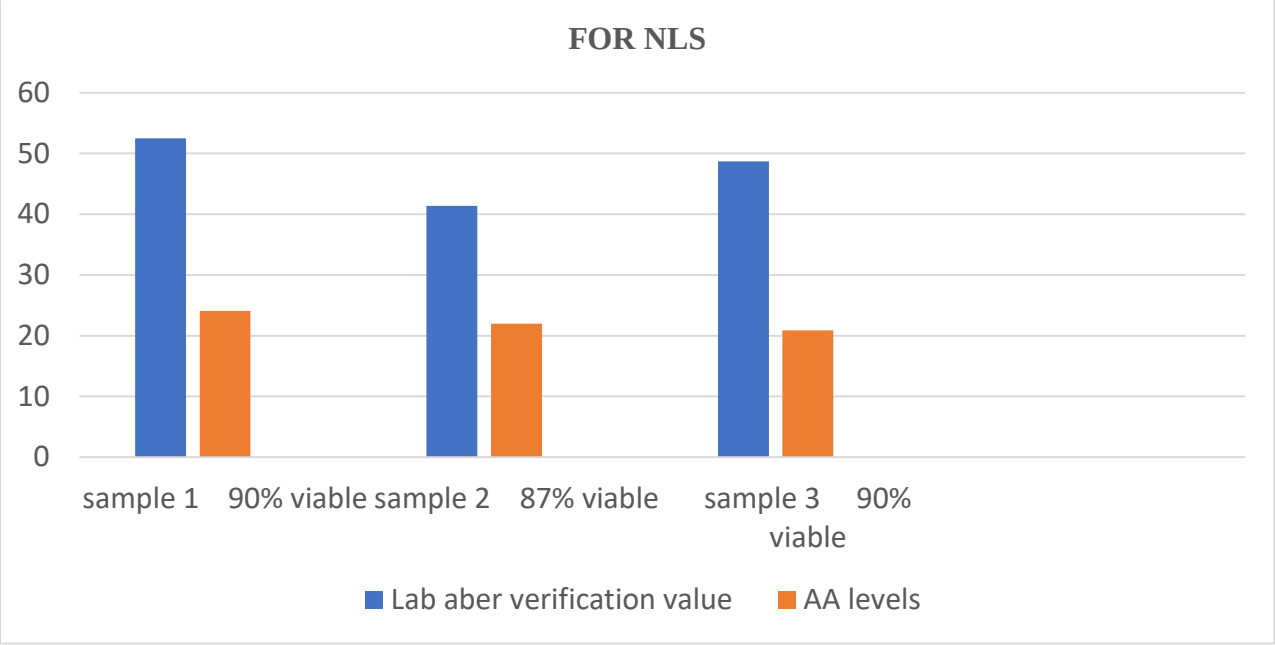


Figure.2: A Graph of Nile Special Lager

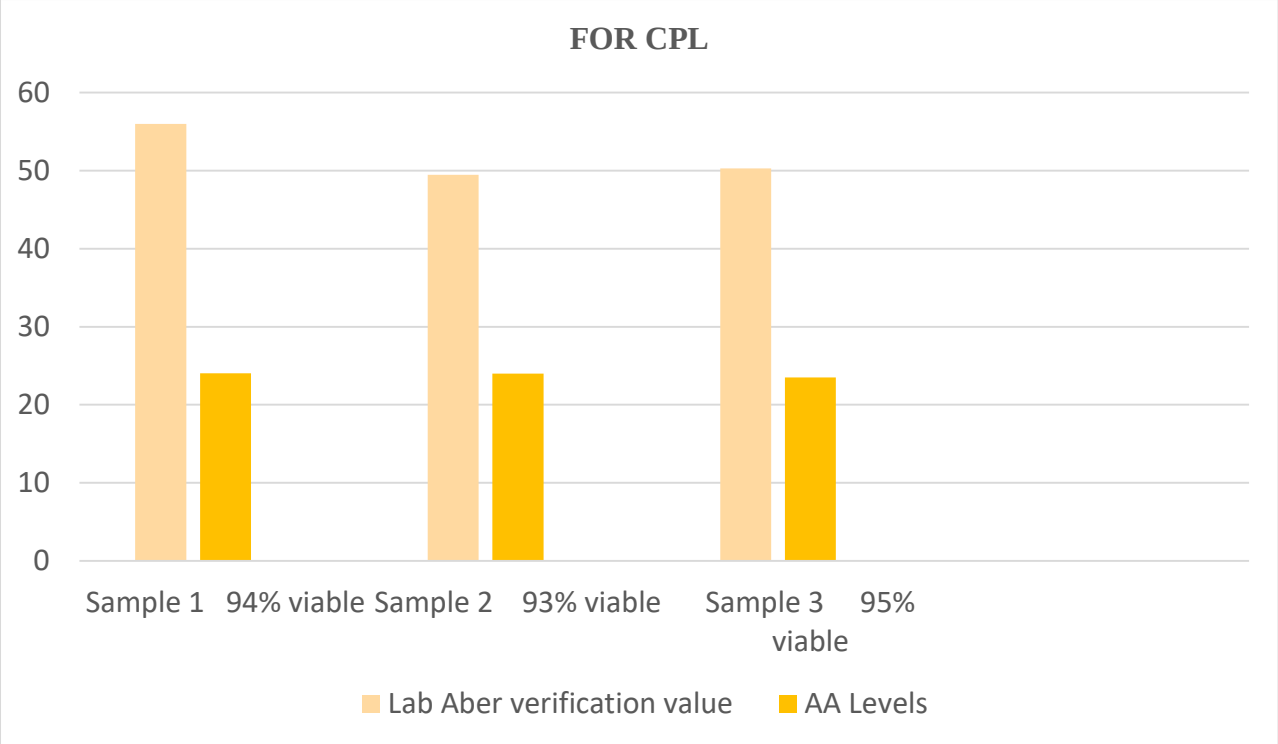


Figure 3: A Graph for Club Pilsner Lager

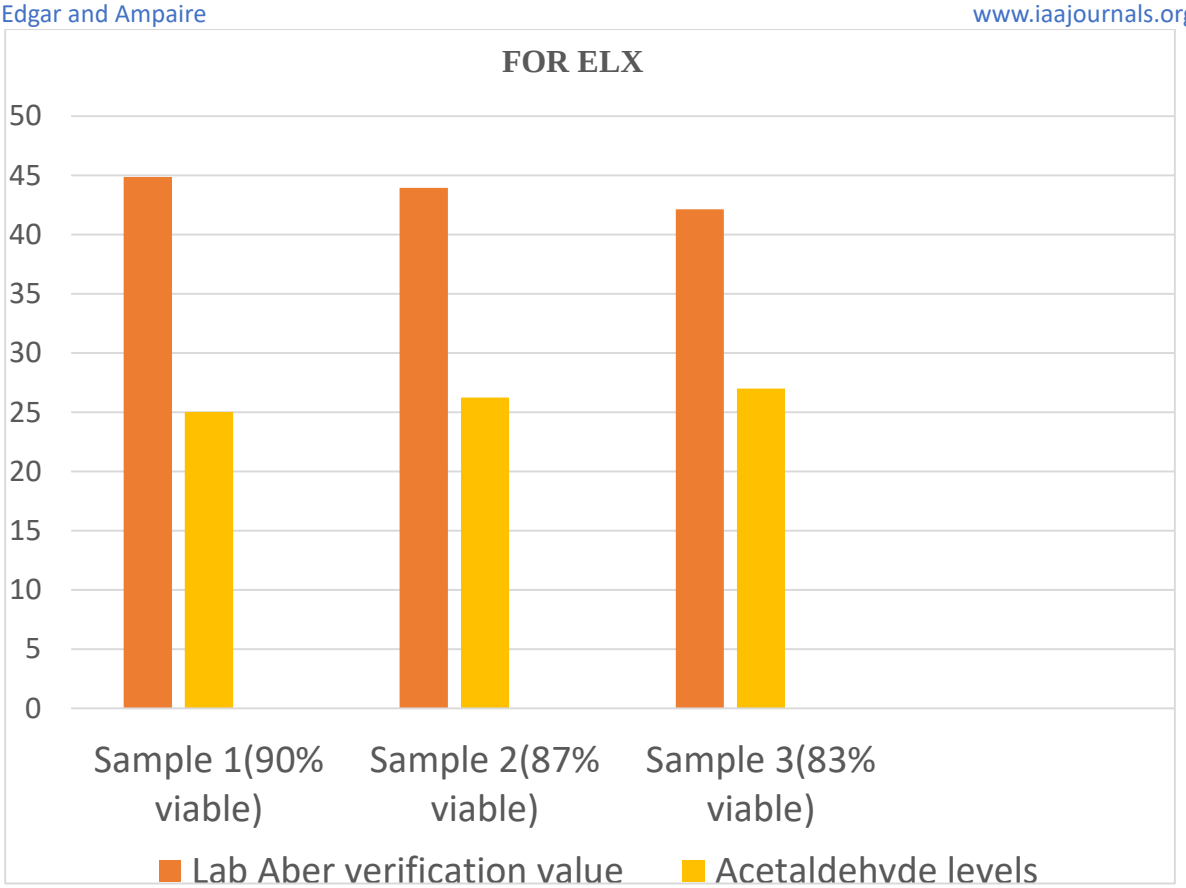


Figure.4: For Eagle Lager Extra

DISCUSSION

According to the acquired data, there was a notable discrepancy among the anticipated pitching rate, the Aber monitor rate, and the Aber verification value as a result of the inconsistent performance of the Aber system [13, 14]. The Aber verification results consistently exceeded both the Aber monitor readings and the projected pitching rate for all brands. There was a significant association observed between the Aber verification values and the yeast counts at full volume for all brands. The discrepancies detected in the Aber system indicated a problem with excessive yeast pitching [13, 15]. The excessive pitching was caused by the inconsistent and poor calibration schedules for the Aber system, resulting in the buildup of defects inside the system. The presence of these faults diminished the system's sensitivity and its capacity to accurately distinguish between viable and non-viable yeast cells during pitching. Precise yeast inoculation is crucial in brewing as it directly impacts fermentation efficiency and the quality of the final product[16–18]. Furthermore, a significant direct correlation was observed between the rise in acetaldehyde concentrations and the Aber verification readings. However, the viability of yeast remained consistent across all brands. Elevated quantities of acetaldehyde were found in certain sample tanks, specifically the ELX samples. This rise was caused by both excessive pitching of yeast and low yeast viability. The Aber verification values for yeast pitching in the ELX samples were somewhat lower compared to the CPL and NLS samples. This difference can be explained by the increased yeast production and lower yeast viability employed in the ELX samples.[19, 20] .

CONCLUSION

There are very high levels of acetaldehyde off flavor in the three beer brands studied which is an outstanding detractor for the set Brewery Sensory Support Index (BSSI). Acetaldehyde indicates a problem with the fermentation, and generally shouldn't be in a beer at levels where it is constantly noticeable. Taking beer with high acetaldehyde levels is in a way dangerous since some of the acetaldehyde enters blood, damaging membranes and possibly causing scar tissue. It also leads to a hangover and can result in a faster heartbeat, a headache or an upset stomach. The brain is most affected by acetaldehyde poisoning. It causes problems with brain activity and can impair memory. Future research should furthermore consider the impact of other flavor groups to identify specifically problematic compounds with regard to the perception of alcohol-free beverages.

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CITE AS: Magezi Edgar and Ampaire Wycliffe (2024). Investigating the Impact of Acetaldehyde Accumulation on Beer Quality: Metabolic Pathways, Yeast Health, and Mitigation Strategies. IAA Journal of Scientific Research 11(2):40-51. https://doi.org/10.59298/IAAJSR/2024/112.4051