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# WORLD BREWING CONGRESS 2016

# Analysis and applications of free amino nitrogen in craft beer using the ninhydrin method

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## Overview

Free Amino Nitrogen (FAN) has been studied in beer for over 40 years as it is an essential nutrient for yeast during fermentations. However, the majority of these studies have been conducted on lagers and other traditional beer styles. With craft brewing on the rise, it is important to expand this testing to look at some of the styles that are present in the current beer market.

The purpose of this experiment is:

- To establish baseline averages for FAN levels in popular American craft ales, all of which were fermented with the same strain of ale yeast
- To determine if FAN can be used to measure fermentation efficiency
- To determine if FAN testing is a viable metric to track significant process changes made between batches of beer

## Methods

This study utilizes the internationally recognized Ninhydrin Method (ASBC Method Wort-12A), depicted in **Fig. 1** below, to measure FAN levels. During this ongoing study, wort and corresponding chilled pre-filtered beer samples are collected during the fermentation process.

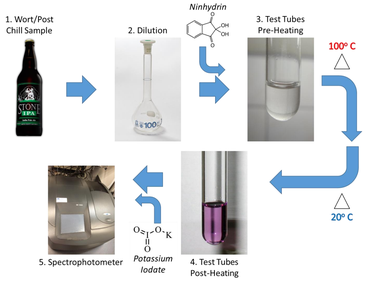


**Figure 1**. A depiction of the internationally recognized Ninhydrin Method used for measuring free amino nitrogen (ASBC Wort-12A)

Some adjustments were made over the course of the experiment in an effort to increase precision of the results.

- The use of a step pipette, in place of serological pipettes, allows for the Ninhydrin Color Reagent and the Dilution Solution to be added much quicker. This improves the precision of the triplicate test tubes and also helps ensure the reagents are added to all tubes in a timely fashion.
- The test tubes are transferred as a group into and out of the water baths. This ensures that all of the tubes are being heated and cooled for the exact same amount of time, in turn, standardizing the reaction.

## Beer Styles

During this study seven popular craft beers were extensively tested, including two India Pale Ales, two double IPAs, a session IPA, a hoppy pale ale and an American strong ale. Importantly, all seven beer styles utilize the same strain of ale yeast. Other specialty beer styles were also analyzed using the same method, however, this data was not included in the study.

**Table 1**. A list of the beer styles that were analyzed during the study, accompanied by a reference code that will be used to identify each specific beer through the study

| Beer Style | Reference Code |
|---|---|
| India Pale Ale | I1, I2 |
| Double India Pale Ale | D1, D2 |
| Session India Pale Ale | S |
| Hoppy Pale Ale | P |
| American Strong Ale | A |

## Results

There are five important statistics to consider when measuring FAN:

- Wort FAN - the amount of FAN in the wort
- Beer FAN - the amount of FAN remaining in the beer after fermentation has finished
- FAN Uptake - the amount of FAN that the yeast consumes during fermentation; calculated as the difference between the Wort and Beer FAN level
- % FAN Uptake - the FAN Uptake expressed as a percentage of the initial wort FAN, which helps to normalize the data when comparing beer styles
- Fermentation Length - for the purpose of this study it is the amount of time from yeast pitching to capping after diacetyl rest

The baseline averages that were developed for each beer style are represented in **Fig. 2**. Both the FAN uptake and the wort FAN levels seem to increase together, which is logical due to the additional malt that is required to brew a Double IPA compared to the amount need to brew a Session IPA.

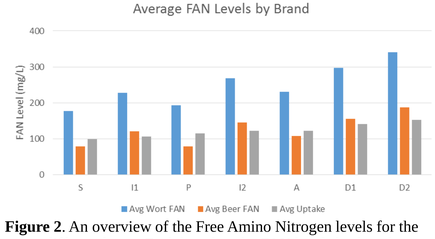


**Figure 2**. An overview of the Free Amino Nitrogen levels for the seven different beers. Each beer has the FAN levels broken down by wort and beer FAN levels, as well as, the uptake. (All values are reported in mg/L)

## Results

In order to determine if FAN can be utilized to determine fermentation efficiency:

- The data must first be normalized. This means that the % FAN uptake statistic is utilized in order to account for the differences in malt quantities
- The fermentation length must be tracked from pitching to capping in order for it to be used as a timeline

For the most part, the beer styles that had the lowest % FAN uptake (**Fig. 3a**), spent the longest time fermenting (**Fig. 3b**). This isn't an identical correlation across the styles, but can be used as a rough estimate. **Fig. 4** depicts how the relationship between % FAN uptake and fermentation length can be applied broadly, as there is a distinct trend, when looking at all of the beers tested. It is important to note, there are other factors that can extend fermentations, so FAN uptake is not the sole cause of long fermentations.

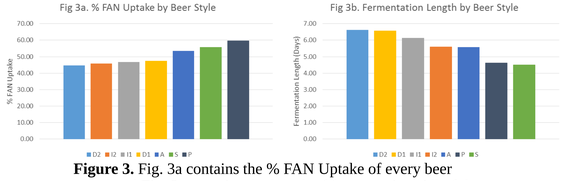


**Figure 3.** Fig. 3a contains the % FAN Uptake of every beer tested and Fig. 3b contains the length of fermentation. All of the beers followed a similar pattern, where the quicker, or more efficient, the fermentation, the higher the % FAN uptake.

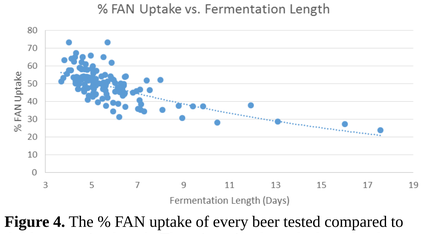


**Figure 4.** The % FAN uptake of every beer tested compared to the fermentation length. There is a visual trend where the shorter the fermentation, the higher the % FAN uptake is. This corresponds to the assumptions made off of the data from **Fig. 3**.

To determine if FAN can be used to monitor process changes in a Double IPA (D1):

- Baseline values were established with the beer preceding the change
- Two groups of fermentations were tracked after the change to determine if the change had a lasting and positive effect

A significant change to the grain bill was made after Group 1, which resulted in Group 2's fermentation length decreasing (**Fig. 5b**) and the FAN uptake increasing (**Fig. 5c/d**). These results were similarly maintained through Group 3's fermentations.

## Results

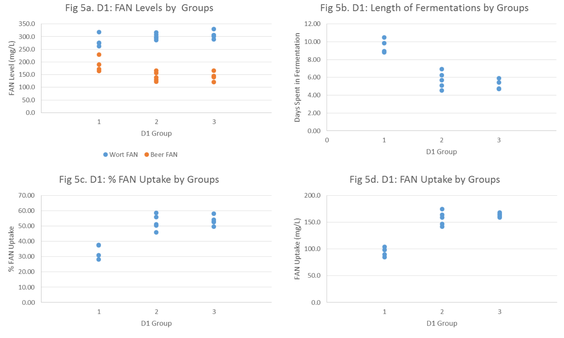


**Figure 5**. Double IPA, Reference Code D1, is brewed in groups and three consecutive groups were tested. Fig. 5a compares the wort and finished beer FAN levels, Fig. 5b compares the length of the fermentations in days, Fig. 5c compares the % FAN uptake and Fig. 5d compares the FAN uptake of each group

## Conclusion

This study was used to develop baseline FAN values for seven American craft ales, which, in turn, were used to determine if FAN testing can be considered a viable tool to help monitor fermentations and evaluate yeast performance and health. Based off of the results depicted in **Figs. 3 and 4**, it has been determined that FAN uptake corresponds to fermentation length and efficiency.

The other goal of this study was to determine if FAN analysis can be leveraged as a primary metric to track significant process changes made between batches. The data that was collected while tracking a significant process change made to a Double IPA, D1, successfully demonstrates that monitoring FAN levels in American craft ales, should be considered a critical control point used to support business decisions.

## References

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