P-05

Correlation of free amino nitrogen (FAN) and amino acids in wort on finished beer flavor stability

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Abstract

One of the largest problems persisting in modern brewing is beer staling. A primary portion of beer staling is the result of amino acids reacting with alpha dicarbonyls to form Strecker aldehydes. These staling compounds can then affect sensory perception of finished beer throughout shelf life. By understanding the sources and composition of the nitrogenous material in wort, breweries can further control the specific ways that beer forms staling compounds by manipulating the raw ingredients of wort.

Introduction

Wort is a complex matrix containing metals, proteins, amino acids, free amino nitrogen, fermentable carbohydrates, among many other compounds. The dynamic chemical reactions of all these constituents throughout the brewing process can lead to undesirable sensory changes in packaged beer over time, whether it's through lipid oxidation, Maillard reactions, degradation of hop acids, hydrolysis of glycosides, Strecker degradation, or another mechanism. There are two pathways that Strecker aldehydes can be formed. The first pathway is a reaction in which the oxidative deamination and decarboxylation of an alpha amino acid takes place in the presence of an alpha dicarbonyl compound, resulting in an aroma active aldehyde. Alpha dicarbonyls are compounds that are introduced through the malt and the brewhouse. The second pathway, less common in beer, is the oxidation of higher alcohols. The most important factor that affects the accumulation of Strecker aldehydes is the concentration of the precursor amino acid, although factors such as iron, pH, oxidation, and higher alcohol content also play a role (1). Strecker aldehyde formation also significantly increases with malt modification. The higher amount of soluble nitrogen present in malt provides an increase in amino acid concentration, thereby increasing the potential for greater Strecker aldehyde concentrations in the finished product (2). Strecker degradation has been extensively researched and results in numerous aroma active compounds associated with different off aromas and flavors including bready, oniony, meaty, and vegetal (3). Many craft brewers brew all malt beers using relatively low adjuncts which results in a large concentration of Free Amino Nitrogen (FAN) in the wort. It is important to note that not all FAN is utilized by the yeast during fermentation, resulting in an excess concentration of amino acids in the finished beer, which can increase the potential for the Strecker degradation reaction to occur. Due to Strecker aldehydes having relatively low flavor thresholds, these are associated with some of the aged attributes that can be perceived by consumers.

Table 1: Characteristics and threshold values of Strecker Aldehydes present in beer

Amino Acid	Strecker Aldehyde	Characteristic	Absorption and Quality of Nitrogen Source	Threshold (ppm)
Alanine	Acetaldehyde	Pungent, green, sweet	Vital and slow, poor quality of nitrogen source	10
Cysteine	Mercaptoacetaldehyde acetaldehyde, H2S* ammonia*	Meaty, cabbage Green apple, rotten egg Pungent, solvent		various
Glycine	Pyrazine Formaldehyde*	Nutty, green bell pepper, tomato pickle like odor, new house	Vital and slow, poor quality of nitrogen source	0.002 25
Isoleucine	2-methylbutanal	Roasted cocoa or coffee-like, fruity, almond, apple-like, malty	Vital and moderate, good quality of nitrogen source	1.25
Leucine	3-methylbutanal (isovaleraldehyde)	Acrid-pungent, fruity, malty, chocolate, cherry, almond	Intermediate, good quality of nitrogen source	0.6
Methionine	Methional	Onion-meat-like, cooked potato, wort-like	Intermediate, good quality of nitrogen source	0.25
Phenylalanine	Phenylacetaldehyde Benzaldehyde	Pungent, floral, sweet, roses Almond, cherry, stone fruit like	Vital and slow, poor quality of nitrogen source	1.6 0.5
Valine	2-methylpropanal (isobutyraldehyde)	Green apple, fruit/banana, grainy, varnish	Vital and moderate, good quality of nitrogen source	1
Proline	Pyrrolidine*, 1-pyrroline*	Important intermediates for bread like aromas	Largely unabsorbed	20

Methodology

- FAN was measured via Thermo Scientific Alpha-Amino Nitrogen by OPA (NOPA) using a Thermo Scientific Gallery Plus. Amino acids were measured via UPLC-MS (Ultrahigh Pressure Liquid Chromatography – Mass Spectrometry).
- Sensory evaluations were performed by a trained internal panel of 8-15 panelists (on average) at different sites and during different years. For the hazy IPA, each panelist wrote descriptions with word intensities, which were summarized by a sensory scientist and translated to an 8-point scale.
- For the imperial IPA and lager, attributes selected by sensory scientists were scaled by panelists using an 8-point scale. The timepoint evaluations for Lager Month 0-2 used descriptions instead of scaling and can't be directly compared with Month 3-6. For Hazy IPA and Lager, each timepoint was the same batch of beer at different ages by different panelists. For Imperial IPA, each timepoint was a different batch and a different age, scaled on the same day by the same panelists.

Free Amino Nitrogen	Amino Acid	
• Thermo Gallery Discrete Analyzer*	• Ultra-performance liquid chromatography-Q-tof mass spectrometry	• Waters Acquity Premiere T3 column,100 x 2.1 mm, with 1.7-micron particle size

* FAN measurements are made up of amino acids and small peptides, with ammonia and proline excluded

Results: Free Amino Nitrogen

Table 2: Breakdown of Sources of Free Amino Nitrogen and S/T Ratio

Beer Type	Source	PPM FAN/hL	Malt Breakdown	S/T
Imperial IPA	Malt	100.83	99.5% pale 0.5% black	*43%
	Added Nutrients	3.64		
	Hops	0.008		
Hazy IPA	Malt	190.83	85% pale 8% oat 7% wheat	42%
	Added Nutrients	1.82		
	Hops	0.003		
Lager	Malt	226.51	100% pale	43%
	Added Nutrients	1.82		
	Hops	N/A		

*No S/T value for the black malt

Table 3: Process Stages and Definitions

Process Stage	Definition
1st wort	Undiluted extract from the mash
Begin boil	Diluted extract from the mash
CAW	Cold aerated wort with added nutrients
SFV	Post fermentation, cold crash
FP	Packaged product

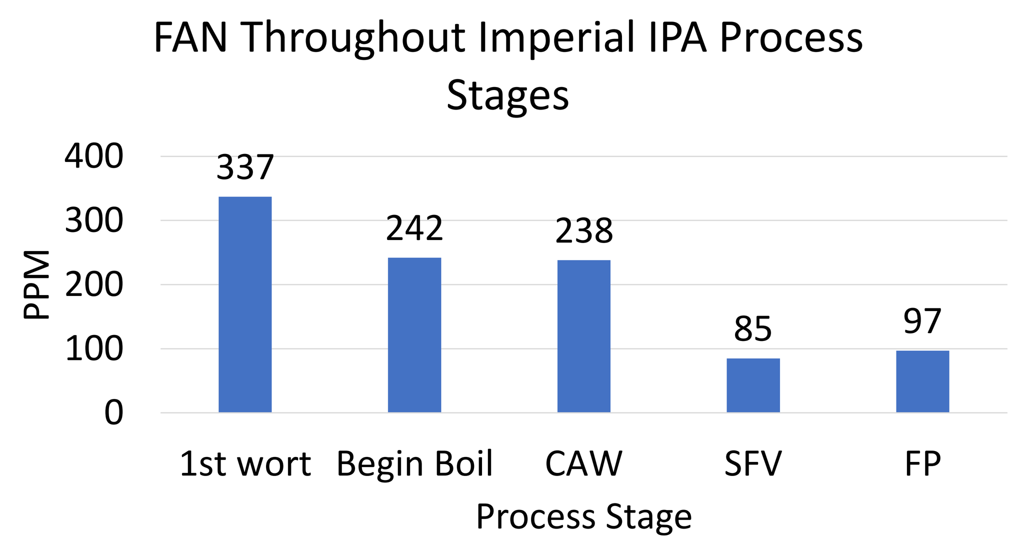
Table 4: Recommended FAN and Starting Gravity

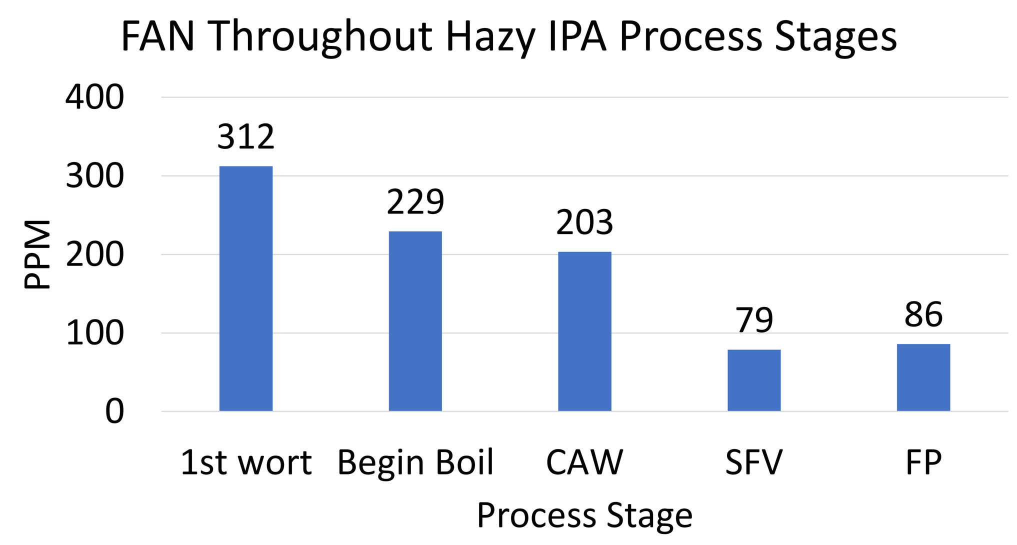
Beer Type	Starting Gravity	Recommended FAN
Imperial IPA	20.34 °P	* 264.42 ppm
Hazy IPA	17.73 °P	*230.49 ppm
Lager	12.71 °P	*165.23 ppm

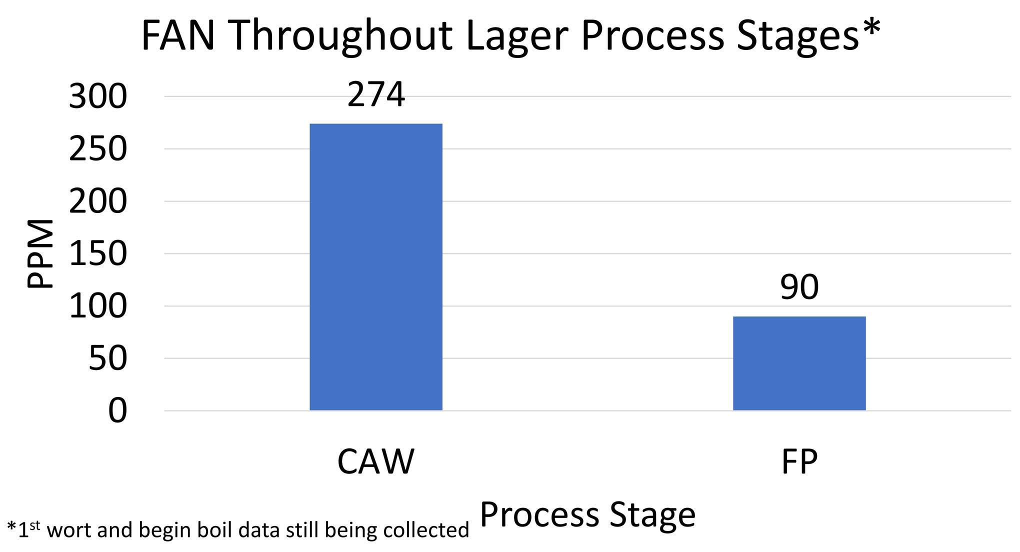
*These are estimates based on sale gravity and lager beers, high gravity FAN recommendations need to be determined

Takeaways:

- In the Imperial IPA, 64% of FAN is utilized
- In the Hazy IPA, 61% of FAN is utilized
- In the Lager, an estimated 67% of FAN is utilized
- Roughly 36% of FAN remains unutilized by yeast







*1st wort and begin boil data still being collected

Results: Amino Acids

All amino acids were tested, but only the precursors for Strecker Aldehydes are included in this report

Imperial IPA:

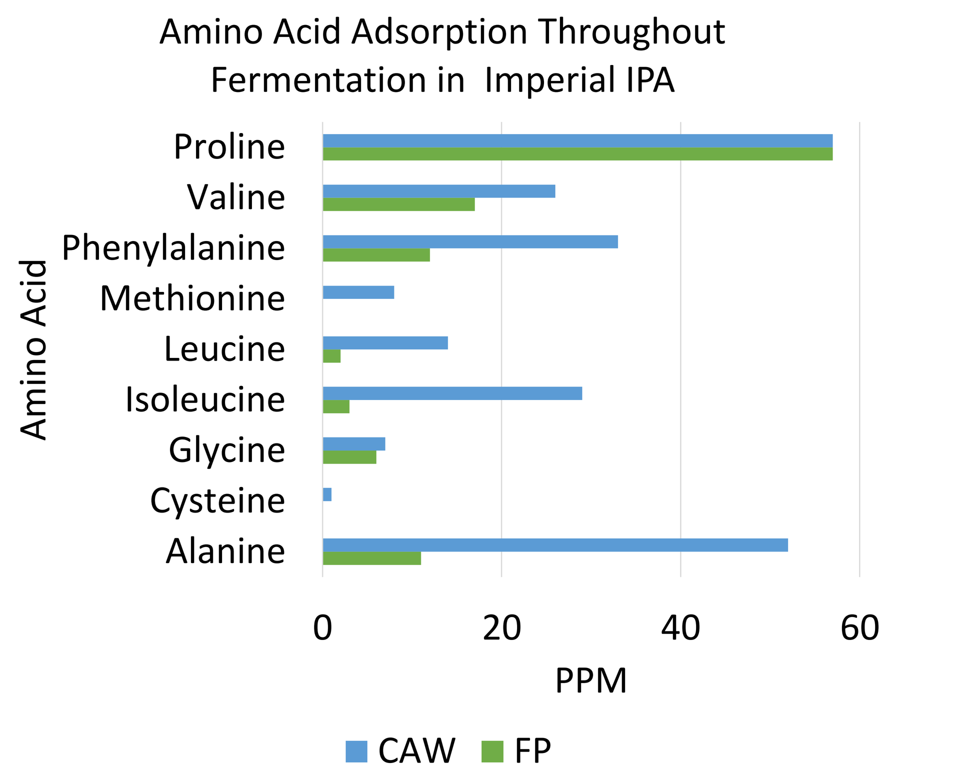
- Proline remains unchanged from CAW to FP
- 17 ppm of valine unutilized in FP
- 12 ppm of phenylalanine unutilized in FP
- 2 ppm of leucine unutilized in FP
- 3 ppm of isoleucine unutilized in FP
- 6 ppm of glycine unutilized in FP (unabsorbed)
- 11 ppm of alanine unutilized in FP

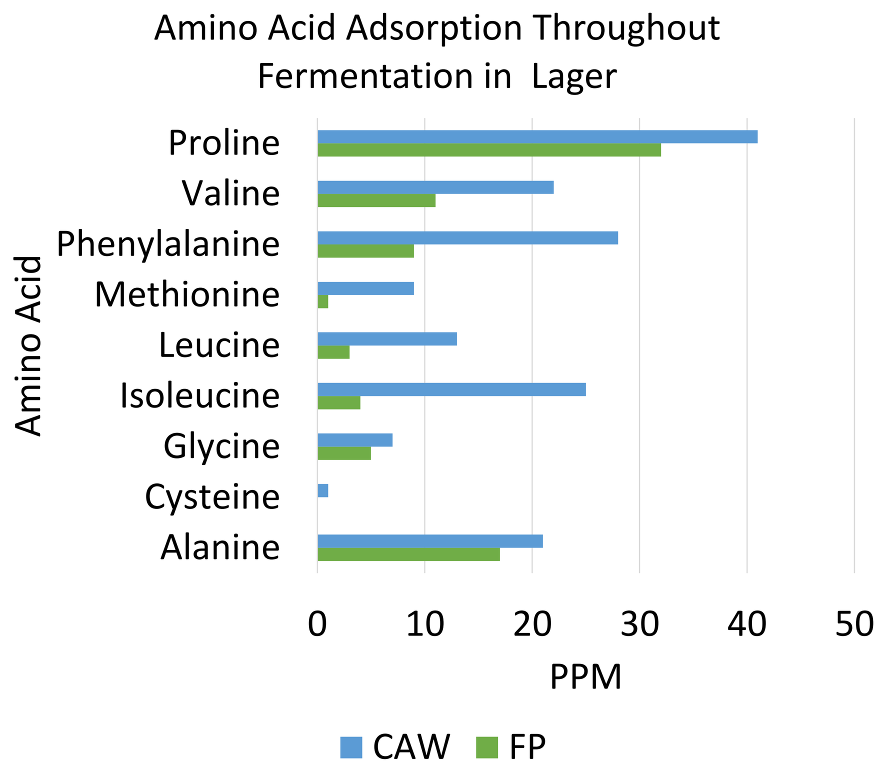
Hazy IPA:

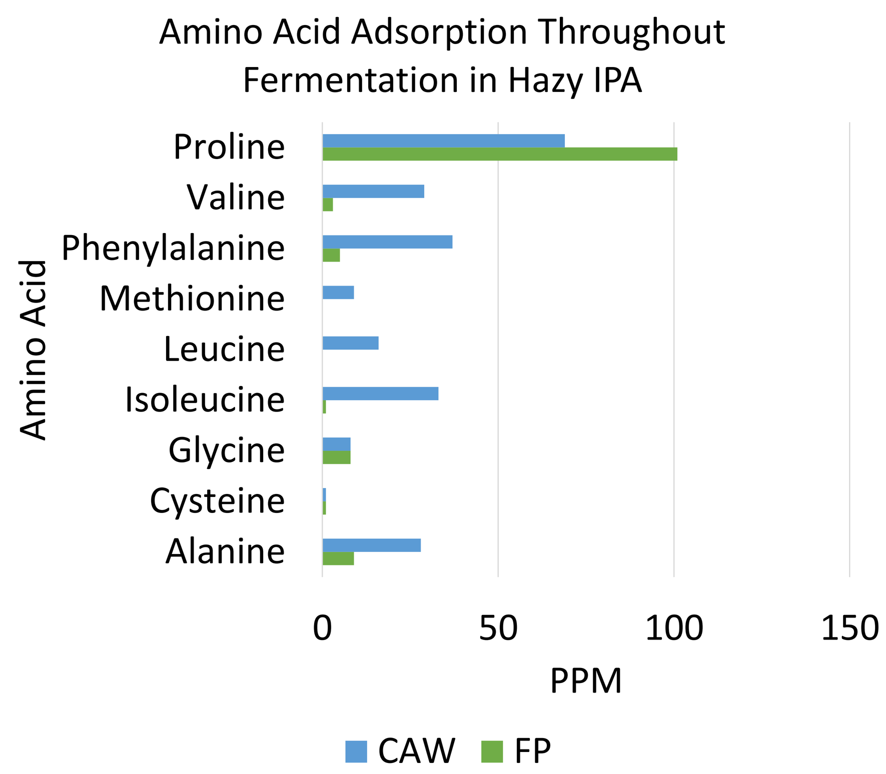
- Proline increases from 69 to 101 ppm from wort to FP
- 3 ppm of valine unutilized in FP
- 5 ppm of phenylalanine unutilized in FP
- 1 ppm of isoleucine unutilized in FP
- 8 ppm of glycine unutilized in FP (unabsorbed)
- 9 ppm of alanine unutilized in FP.

Lager:

- Proline decreases from 41 to 32 from wort to FP
- 11 ppm of valine unutilized in FP
- 9 ppm of phenylalanine unutilized in FP
- 1 ppm of methionine was unutilized in FP
- 3 ppm of leucine unutilized in FP
- 4 ppm of isoleucine unutilized in FP
- 5 ppm of glycine unutilized in FP (unabsorbed)
- 17 ppm of alanine unutilized in FP (unabsorbed)





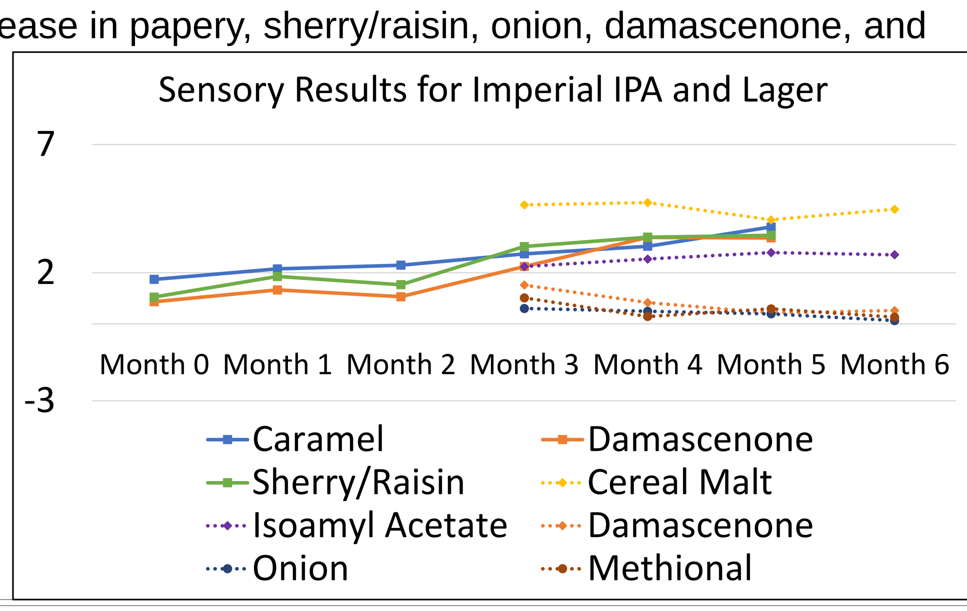


Results: Sensory

Imperial IPA: Caramel, damascenone, and sherry/raisin increased over the shelf life and were statistically significant (shown in solid lines in graph). Attributes with no significant differences (pine, onion, isovaleric, methional, mercaptan, papery) are not shown (6).

Hazy IPA: Over the shelf life, panelists noted an increase in papery, sherry/raisin, onion, damascenone, and methional (7).

Lager: Shelf life was relatively stable over the 6-month evaluation. Attributes with minor statistically significant differences (cereal malt, isoamyl acetate, damascenone, onion, methional) shown with dotted lines in graph. Attributes with no significant differences over shelf life (corn chips, sulfitic, bready, honey, pear, white wine, grassy, sherry/raisin, papery, mercaptan, caramel, isovaleric) are not shown (8).



Takeaways

- **In the three beer types studied, there are high levels of residual FAN. This contributes to the unutilized amino acids in FP. In the sensory results for the hazy and imperial IPAs, there are trends of increasing undesirable attributes associated with Strecker aldehydes.**
- Unutilized precursor amino acids could lead to potential Strecker aldehyde formation (Ex: valine to 2-methylpropanal, etc), thereby increasing potential for undesirable sensory attributes.
- Higher malt modification leads to increased amino acid concentrations, therefore Strecker aldehydes (3).
- One method to lower the concentration of amino acids unutilized by yeast is by using less modified malt and/or use malt with a lower S/T value. This includes variations to the mash regime, as well as processing of the raw barley itself.
- Aldehyde measurements could potentially be used to predict the aging potential of fresh beer (4).
- Aldehydes can further condense to adols, leading to the formation of furans, pyrazines, pyrroles, oxazoles, and thiazoles, which are flavor active compounds (10).
- Increase in FAN, as well as proline, from SFV to FP could be attributed to dry hopping additions.
- The Imperial IPA, due to inclusion of black malt, could potentially form more Strecker aldehydes due to potential higher concentration of alpha dicarbonyls from the Maillard reaction.
- Low concentrations of cysteine in CAW could mean less aldehyde-cysteine adduct formation, causing less stable beer, as well as increased formation of furfural (9).

Next Steps

- **Determine FAN recommendations for all malt, high gravity beers.**
- Study needs to be conducted in beer to determine correlations between concentrations of precursor amino acid and it's corresponding Strecker aldehyde concentration in various conditions (ex: correlation of deeper roasted malts and higher degree of Strecker aldehyde formation due to increased alpha dicarbonyls).
- Amino acid and FAN contribution of hops added at various process stages needs to be further studied.
- Amino acid and FAN contribution of various hop varieties needs to be further studied.
- Explore yeast uptake and regulation of amino acids in all malt, high gravity beers with various yeast strains.
- Investigate what the unutilized crucial and rapid amino acids mean for yeast health and fermentation success (ex: arginine and histidine - not included in this poster but data was collected).
- Study the impact that unutilized amino acids have on haze formation - particularly proline, phenylalanine, and glutamine (5).
- Need to correlate packaged oxygen levels with results due to Strecker Aldehydes increasing in the presence of oxygen (4)

Citations

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