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The Importance of Free Amino Nitrogen in Wort and Beer

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ABSTRACT

Supplementation of wort (15°P containing 30% very-high-maltose [VHM] syrup) was carried out with five times the natural wort concentration of L-lysine or L-methionine. Control lager fermentations reached target specific gravity (sp gr) within 96 h, while wort supplemented with either methionine or lysine resulted in fermentations that achieved completion within 103 and 48 h, respectively. The vicinal diketone (VDK) concentration in the fermented wort with added lysine was greater compared with that generated at the end of control fermentations. The VDK levels present at the end of fermentations with supplemented methionine were lower than the levels at the end of the control fermentations.

Keywords: amino acids, ammonia, fermentation performance, free amino nitrogen, vicinal diketones, wort supplementations

SÍNTESIS

Se le añadió L-methionina y L-lisina a cinco veces la concentración normal, a un mosto de 15°P que tenía 30% de un jarabe VHM (de muy alto contenido de maltosa). La fermentación (baja) de un control sin los aditivos alcanzó el Ea deseado en 96 h, mientras que el mosto adicionado de methionina o lisina tardaron 103 h y 48 h, respectivamente. La concentración de diketonas vicinales (VDK) en el mosto fermentado con lisina fue mayor que la del mosto de control, mientras que la con methionina fue menor que la del control.

Palabras claves: amino ácidos, amonio, desempeño de fermentación, amino nitrógeno libre, diketones vicinales, suplementos para mosto

Introduction

Brewing yeasts are required to adapt to a highly complex environment in wort, in terms of the great range and variety of nutrients. Wort is a growth medium consisting of fermentable sugars (fructose, sucrose, glucose, maltose, and maltotriose), nitrogenous materials (amino acids, peptides, proteins, nucleic acids, and other malt endosperm degradation products), vitamins, ions, mineral salts, trace elements, and many other constituents (1). Yeast growth involves the uptake of nitrogen, mainly in the form of amino acids, for the synthesis of cellular proteins and other cell compounds (5). In wort, the main nitrogen sources for yeast metabolism are the individual amino acids, small peptides, and ammonium ions formed from the proteolysis of barley malt proteins (2). Brewer's wort contains 19 of the 20 essential amino acids, and as with wort sugars, the uptake of amino acids is ordered. Amino acids have been categorized into four groups in ale yeast on the basis of their assimilation patterns (3) (Table 1). This pattern has not been confirmed for every brewing strain and for lager yeast in general.

The individual wort amino acids and small peptides (dipeptides and tripeptides) are known collectively as free amino nitrogen (FAN) (6). FAN is believed to be a good index for potential yeast growth and fermentation efficiency (7). Adequate levels of FAN in wort ensure efficient yeast cell growth and, hence, a desirable fermentation performance (5). FAN measurement in wort has been used within the brewing industry for historical reasons because of the ease and availability of the analytical methods. FAN is only a general measurement and a "blunt instrument" for setting wort and, ultimately, malt specifications. It is hoped that, as a result of the elucidation of the role of the different nitrogenous wort components on yeast fermentation, it will be possible to set more meaningful malt and wort specifications.

Corresponding author Christoforos Lekkas started his studies in 1996 at the University of Wolverhampton (U.K.), where he received his B.Sc. (Hons.) degree in biological sciences-biotechnology. His final-year research project was based on the investigation of biofilm formation in water purification plant systems and the treatment of the infected water supplies by biofilms and their toxic by-products. He next received an M.Sc. degree in food biotechnology from Reading University (U.K.). Currently, Christoforos is finishing his Ph.D. degree at the University of Heriot-Watt (ICBD) in Edinburgh, working under the supervision of Pr. G. G. Stewart and Scottish Courage Ltd. for a project concerning the importance of nitrogenous materials that may play a significant role in brewing fermentations.

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Materials and Methods

Fermentations

Static fermentations were conducted in sterilized 2-L cylinders, sealed with a rubber bung and a fermentation lock, containing 70% ethanol (Fig. 1). Wort of similar composition was used for all trials (15°P containing 30% very-high-maltose [VHM] syrup). $ZnSO_4$ (0.2 mg/L) and a silicon-based antifoam agent (0.1 mL/L) were also added. The incubation temperature was 18°C for the lager fermentations.

Yeast Strain

The yeast employed for this study was a lager strain of *Saccharomyces cerevisiae* (syn. *S. pastorianus*).

Cell Numbers

Cell counts were determined by using an improved Neubauer haemocytometer (Weber Scientific Int., Middlesex, U.K.) at ×40 magnification with a light microscope. Yeast viability was determined by the methylene violet staining method.

Specific Gravity

The sample was centrifuged for 5 min at 5,000 rpm (5,000 × *g*) and the specific gravity of the supernatant was measured with a DMA 46 calculating digital density meter (PAAR Scientific Ltd., London).

FAN

FAN was determined by a ninhydrin-based method (4).

Ammonia

A spectrophotometric assay was used for ammonia determinations. The calibration standard was ammonium chloride and distilled water. The color reagent used was a mixture of 3.5% phenol and 0.04% sodium nitroprusside. The absorbance of each sample was measured at 665 nm against a distilled water blank.

Table 1. Classification[a] of wort amino acids according to their consumption rate by ale yeast

Group A Fast Absorption	Group B Intermediate Absorption	Group C Slow Absorption	Group D Little or No Absorption
Glutamic acid	Valine	Glycine	Proline
Aspartic acid	Methionine	Phenylalanine	
Asparagine	Leucine	Tyrosine	
Glutamine	Isoleucine	Tryptophan	
Serine	Histidine	Alanine	
Threonine		Ammonia	
Lysine			
Arginine			

[a] Source: Jones and Pierce (3).

Figure 1. Two-liter cylinders used for the fermentations.

Amino Acids

Quantification of individual amino acids was achieved by gradient elution—high-performance liquid chromatography with a fluorescence detector. All amino acids were fully resolved by this method.

Vicinal Diketones (VDK) and Their Precursors

The VDK levels were analyzed by gas chromatography with an electron capture detector (Hewlett Packard, Crawford Scientific, Strathaven, Lanarkshire, U.K.). Samples were preheated at 60°C for 90 min in order to convert the precursors into VDK. Unheated samples were also measured to calculate the concentration of the precursors. Precursor levels were estimated by subtracting the values obtained from the heated samples from the values obtained from the unheated samples.

Results and Discussion

Figures 2, 3, and 4 show FAN reduction, attenuation rate, pH changes, and the suspended cell concentration in ferment-

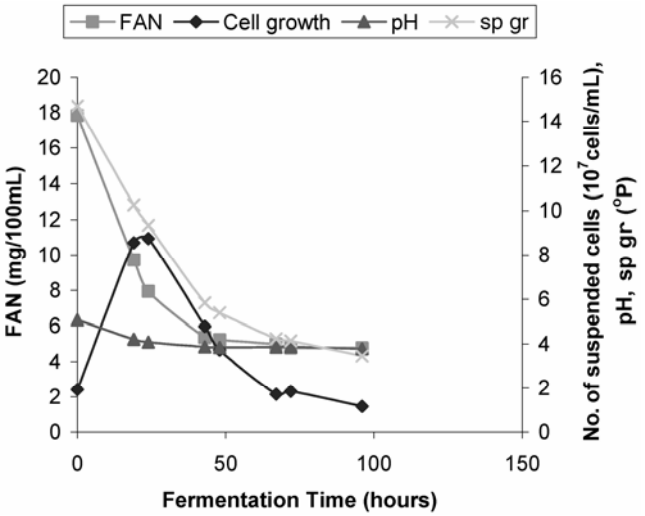

Figure 2. Control fermentation profile. FAN = free amino nitrogen, and sp gr = specific gravity.

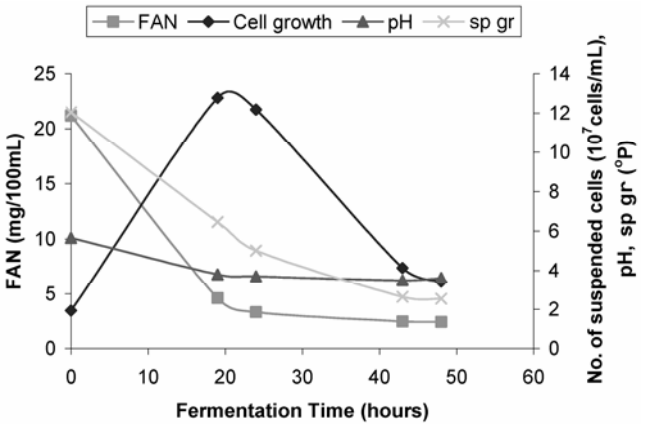

Figure 3. Fermentation profile of wort supplemented with lysine. FAN = free amino nitrogen, and sp gr = specific gravity.

ing wort during the course of lager fermentations. In the control fermentations (Fig. 2), the suspended cell concentration peaked at 9×10^7 cells per mL 24 h into the fermentation. The FAN concentration decreased sharply until 48 h and then followed a constant reduction rate. The pH fell from 5.07 to 3.78 after 48 h of fermentation and then remained more or less constant until the end of the fermentation. Wort specific gravity (sp gr) gradually dropped until the termination of incubation. The sp gr, pH, and FAN concentration decreased in step and are considered to be related values.

In the fermentation supplemented with lysine (Fig. 3), the suspended cell concentration peaked at 13×10^7 cells per mL 19 h from the start of the fermentation. The FAN concentration decreased sharply during the first 24 h and then plateaued. The pH followed the same reduction pattern as FAN, while the sp gr decreased gradually until the fermentation was complete. Lysine was used for supplementation since it was one of the amino acids that was totally removed from the wort by the yeast.

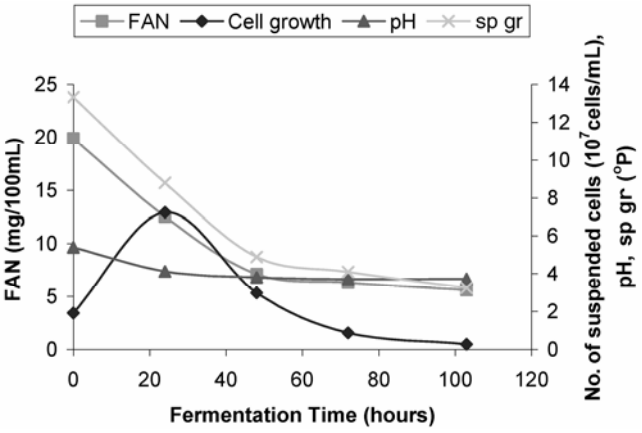

Figure 4. Fermentation profile of wort supplemented with methionine. FAN = free amino nitrogen, and sp gr = specific gravity.

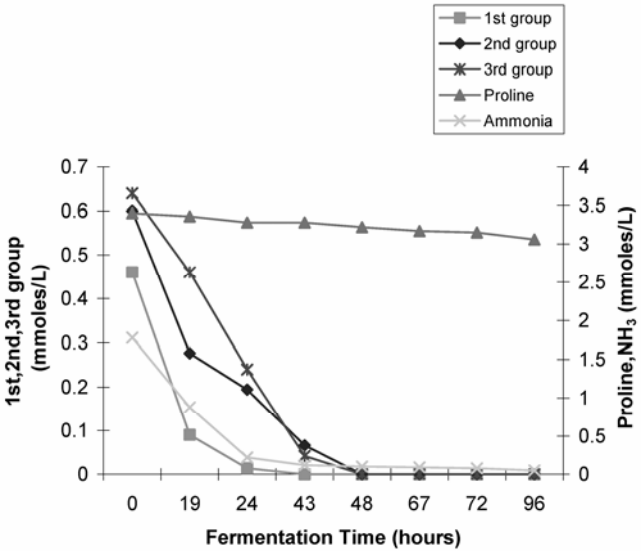

Figure 5. Amino acid and ammonia (NH_3) utilization during control fermentation.

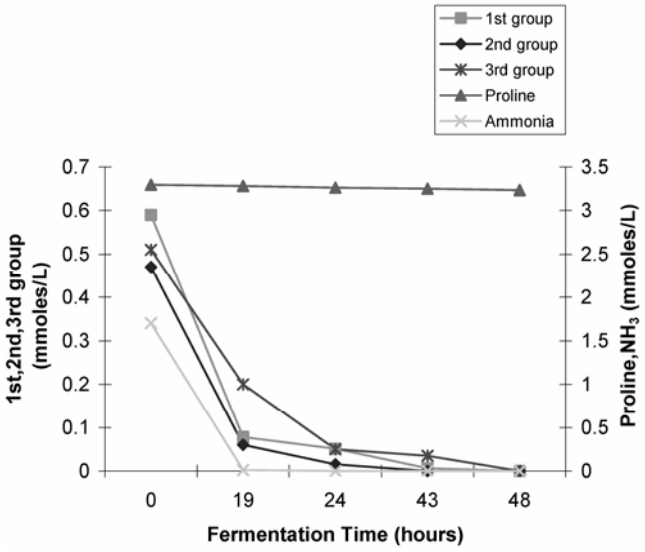

Figure 6. Amino acid and ammonia (NH_3) utilization during fermentation of wort supplemented with lysine.

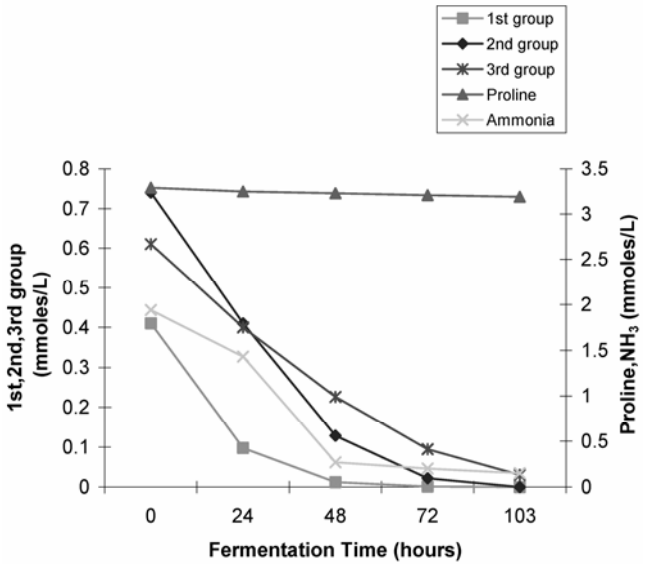

Figure 7. Amino acid and ammonia (NH_3) utilization during fermentation of wort supplemented with methionine.

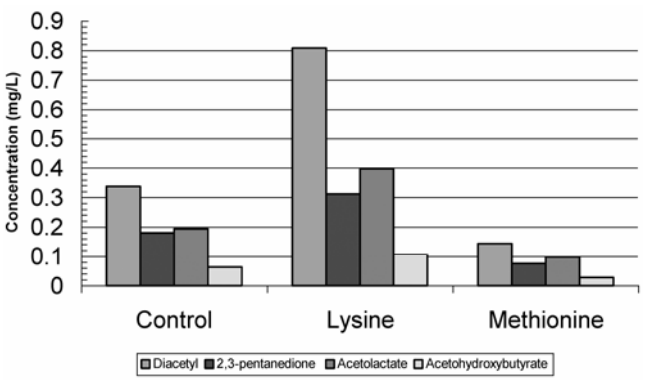

Figure 8. End of fermentation vicinal diketone concentrations.

In the fermentation supplemented with methionine (Fig. 4), the suspended cell concentration peaked at 7×10^7 cells per mL 24 h into the fermentation. The FAN concentration decreased gradually until the end of fermentation. The same observations were also valid for pH and sp gr. Methionine was used for supplementation since it was a group B amino acid that was removed from the wort very rapidly by the yeast.

Figures 5, 6, and 7 illustrate amino acid consumption with time. All of the amino acids from group A in the control fermentations were completely absorbed within the first 43 h (Fig. 5). The amino acids of this group were totally consumed within 43 and 72 h for the fermentations supplemented with lysine and methionine, respectively (Figs. 6 and 7, respectively). Total consumption of group B amino acids in control wort fermentations was achieved in 48 h, while in fermentations with added methionine, it was complete in 103 h. In fermentations with added lysine, all amino acids were taken up by 43 h. The uptake of amino acids belonging to group C was accomplished within 48 h for fermentations supplemented with lysine and for control fermentations. For fermentations supplemented with methionine, the amino acids from this group were not completely absorbed.

As expected, proline exhibited almost no uptake for all the wort fermentations conducted. Wort ammonia was used during the first 19 h of incubation for fermentations with added lysine. In both control and methionine-supplemented fermentations, ammonia utilization was incomplete.

The lysine-supplemented fermentations were considerably faster (completed in 48 h) than were the control and methionine-supplemented fermentations. Such an effect can be explained by the increase in the suspended yeast cell concentration. The reason why lysine stimulated cell growth is unclear, but this amino acid is obviously a 'key' wort amino acid.

Figure 8 shows the VDK and their precursor concentrations produced during wort fermentations. The levels of diacetyl, 2,3-pentanedione, and their precursors formed during the fermentations with added lysine were approximately double those produced during the control fermentations. On the other hand, during methionine-supplemented fermentations, the concentrations of these flavor compounds and their precursors were lower than that of the control.

Conclusion

Supplementation of wort with L-methionine had an inhibitory effect on yeast fermentation and extended the fermentation time from 96 to 103 h. Lower suspended yeast numbers were obtained within the first 24 h of fermentation compared with those of the control fermentations. The presence of added methionine prevented group C amino acid absorption. Diacetyl and 2,3-pentanedione levels in the fermented wort were lower than those in the control fermentations. These lower levels could be because of the elongation of the fermentation time in the presence of an excess concentration of methionine. A decreased level of yeast growth could also provide an explanation for the relatively low levels of VDK encountered at the end of these fermentations.

Addition of L-lysine enhanced yeast metabolic activity and shortened the fermentation time from 96 to 48 h. Such an effect can be characterized as stimulatory, since a great increase in suspended yeast cell numbers also occurred. The majority of the amino acids were taken up within the first 43 h of fermentation, and the same effect was also observed for ammonia, which was taken up by 19 h. The excessive biomass production is likely responsible for the raised VDK levels of the supplemented fermentations.

The work presented here forms part of an investigation into the role nitrogenous wort components (oligopeptides, ammonium salts, and both total and individual amino acids) in brewer's yeast play in fermentation performance. The identification of key "marker" amino acids or other nitrogenous wort constituents that are responsible for triggering and reinforcing yeast fermentative performance is currently under study.

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