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# Effect of mashing-in temperature on free amino nitrogen concentration and foam stability of beer

**Low mashing-in temperature (37 °C) and long duration of resting (60 min. including mashing-in) and long time of heating up to 63 °C leads to very high amount of FAN – up to 270 mg/l in 12 °P wort, but to very poor foam stability of beer produced (220 seconds in average) due to a low amount of high molecular proteins. When mashing-in temperature was higher (63 °C) the FAN concentrations of 12 °P wort was only 162 mg/l, but foam stability of beer was higher – 268 seconds in average. Mashing-in temperature of 50 °C leads to respectable FAN concentration. Yeasts utilised 104 mg/l of FAN in average using 13.5 °P wort with initial FAN concentration of 235 mg/l, 90 mg/l using 12.5 °P wort (initial FAN – 219 mg/l) and 87 mg/l using 10.5 °P wort (initial FAN – 197 mg/l). During maturation process any decrease of FAN was registered. FAN content was dropped due to blending of beer with deoxygenated water by automatic HGB system for desiderative resultant gravity. FAN content in finished product varied from 85 to 106 mg/l for 12 °P beer, and from 70 to 94 mg/l for 10 °P.**

BC 22 Brewhouse

(Descriptors: Beer, mashing-in temperature, free amino nitrogen (FAN), foam stability, high gravity brewing.

Deskriptoren: Bier, Einmaischtemperatur, freier Aminostickstoff (FAN), Schaumhaltbarkeit, High Gravity Brewing).

## 1 Introduction

Amino acids and ammonium salts are the main source of nitrogen for metabolic processes of brewer's yeasts. High-molecular proteins are degraded during wort boiling. In wort mainly soluble fissile products of proteins remain, which are necessary for yeast propagation and fast fermentation. Yeasts need 100 – 140 mg of nitrogen per litre on average from wort in form of amino acids and lower-molecular peptides for building new cell structures.

Wort consists of a wide range of nitrogenous compounds like proteins, peptides, composite proteins, amino acids, ammonium salts, nitrates, aliphatic and aromatic amines, nucleic acids and their derivatives. Low-molecular compounds like amino acids and ammonium salts are the main source of nitrogen for metabolic processes of brewer's yeasts. The process of yeast propagation, saccharide utilization and forming of secondary fermentation products, which influences the organoleptic properties of beer depends on the quantity and quality composition of the low-molecular nitrogen substances in wort. [1]

A source of nitrogen is required by yeast to form amino acids and nucleotides, so that proteins, nucleic acids and coenzymes can be formed. Usually wort does not contain many ammonium ions and the yeast uses the amino acids instead. Consequently it is not necessary to provide a balanced mixture of amino acids corresponding to the mixture present in the yeast's proteins. Instead, a certain total amount of "free amino nitrogen" must be provided to supply the N atoms needed by the yeast to synthesize the amino acids used to form proteins. Free amino nitrogen (FAN)

is the term used to describe nitrogen in the NH<sub>2</sub> groups of amino acids which are not involved in peptide bonds, together with the nitrogen in any ammonium ion present. Although peptides and proteins contain a free amino group at one end (and a carboxyl group at the other) only amino acids and some very small peptides can be taken into yeast. Consequently the decrease in FAN during fermentation is almost all due to uptake and metabolism of amino acids and FAN in most of the peptides in wort is still present in the beer [2].

Enzymatic breakdown of protein occurs predominantly at 45 to 55 °C, but does not stop even at higher temperatures. With a 45 °C rest more lower molecular weight products are formed, at 55 °C more high molecular weight substances. During protein breakdown, proteins are progressively degraded to high-molecular weight products and these in turn to low-molecular weight products and finally to amino acids. Because the higher molecular weight products formed during protein degradation are subsequently degraded, there is no sense in providing a rest for their formation, especially since foam forming gums are degraded at the same temperature. Therefore a long rest at 50 °C always results in poor foam. [3] In wort the main source of nitrogen is the range of amino acids formed by proteolysis of barley protein. Temperature range from 40 to 60 °C is important for degradation of nitrogenous substances and maximum is reached at 50 °C. Proteolyses decreases with rising temperature and stops at temperatures close to 80 °C. [4]

β-glucanase, protein breakdown and substrate go into solution at 35 °C and so the enzyme is already in the dissolved state at the optimum temperature if mashing-in is performed at 35 °C. These facts argue for mashing-in at 35 °C. [3]

The formation of higher homologues of ethanol is linked with nitrogen, rather than sugar metabolism. Ester formation is therefore also associated with nitrogen metabolism, since esters of higher alcohols are important contributors to the flavour and aroma of beer. For instance, high levels of assimilable nitrogen in wort, rapid fermentation and quick removal of yeast from beer are important. [5]

Most of the higher alcohols are chemically very similar to the amino acids and they are formed either from the amino acids in the wort or during the reactions in which the yeast synthesizes amino acids. Use of large amounts of sugar or low-N adjunct tends to

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decrease higher alcohol and ester formation. [2] The amount of usable (assimilable) N affects the extent of formation of higher alcohols and their esters.

The aim of the work was to study the influence of mashing-in temperature on FAN content and foam stability of produced beer, as well as the utilization process of low-molecular nitrogen substances during wort fermentation of different gravity.

## 2 Materials and methods

### 2.1 Microorganisms

We used an operation strain of brewer's yeast *Saccharomyces cerevisiae* subsp. *uvarum* W 34/70. The culture was kept on slanting wort agar at 4 °C and it was pre-inoculated every three months.

### 2.2 Mashing process conditions

1. Mashing-in temperature was 37 °C, duration of mashing, including rest of the mash was 60 minutes, the consequent heating up to 63 °C took 60 minutes (in range of 45 to 55 °C, 40 minutes) and the mash was held at this temperature for 25 minutes. Finally the mash was heated up to 72 °C. We prepared wort of gravity 12 °P.
2. Mashing-in temperature was 63 °C, duration of mashing, including rest of the mash was 27 minutes. Finally the mash was heated up to 72 °C. We prepared wort of gravity 12 °P.
3. Mashing-in temperature was 50 °C, duration of mashing, including rest of the mash was 20 minutes, the heating up to 63 °C took 13 minutes (1 °C per min.) and the mash was held at this temperature for 10 minutes. Finally the mash was heated up to 72 °C. We prepared worts of gravities 10.5 °P, 12.5 °P and 13.5 °P.

We achieved results from ten measurements in each condition.

### 2.3 Wort fermentation

Primary fermentation ran in cylindro-conical tanks (CCT) of 2170 hl volume, filled to 83 %. Pitching temperature was 9.5 °C and during primary fermentation it was kept at 14 °C. The fermentation process was carried out under carbon dioxide overpressure of 50 kPa maximum.

Yeasts were removed on the 5<sup>th</sup> – 6<sup>th</sup> fermentation day, when the apparent extract decrease was minimal or no decrease was registered. Primary fermentation was stopped when diacetyl concentration decreased to the required level of 0.15 mg/l. Beer maturation ran in classical vessels (horizontal tanks) at approx. 1 °C.

### 2.4 Analytical methods

Low-molecular nitrogen compounds were determined by ninhydrin method. Wort and beer samples were diluted to FAN concentration of 1 to 3 mg/l (wort 150 times, beer 50 times). After measurement of absorbance at 570 nm, the FAN content was calculated as follows:

$$N = \frac{(A_H - A_S) \cdot 2V}{A_{ST}} \quad (\text{mg/l})$$

N free amino nitrogen (mg/l)

$A_H$  absorbance main test at 570 nm

$A_S$  value of blind test (it is deducted only for analysis of dark wort or beer)

V diluted correct coefficient

$A_{ST}$  absorbance of standard glycine dilution

Vicinal diketones concentration was determined as total vicinal diketones after distillation with water stream by EBC recommended spectrophotometrical method [6].

The foam stability was measured in beer bottle as an interval between the foam creation by carbon dioxide and its drop using device NIBEM T (Haffmans).

## 3 Results and discussion

Low-molecular fissile products consist of the smallest components of proteins – amino acids and peptides originated by polymerisation. These fissile protein products are a very important source for yeast nutrition as the amino unit is located in the  $\alpha$ -position. During malting high-molecular proteins have to be degraded into soluble low-molecular fissile products, and due to this process the overall composition of proteins in grain is changed. During germination about 35 – 40% of proteins are transformed into their soluble form (Kolbach index) and low-molecular compounds (amino acids, oligopeptides) are formed by peptidase activity [7, 8].

The ideal FAN content in 12 °P wort is 186 mg/l (calculated from formula, 15.5 mg/l FAN/1 °P) [9], therefore we monitored the influence of temperature on FAN formation during mashing, as well as foam stability of beer produced using wort of gravity 12 °P.

When mashing-in temperature was 37 °C (for details of mashing process, see "Material and methods") FAN concentrations in worts of gravity 12 °P were very high, 220 mg/l on average (Fig. 1). In some worts FAN concentration reached 270 mg/l. FAN concentration of finished 12 °P beers were 116 mg/l in average. Based on triangular test, beers produced had rather an ester flavour. Foam stability of beers was not satisfactory, about 220 seconds on average, which is considered as weak foam (Fig. 2). Process was too long and furthermore there was a problem with foam stability because of great proteins breakdown.

When mashing-in temperature was 63 °C, the FAN concentration was only 162 mg/l in average and in finished 12 °P beer only 75

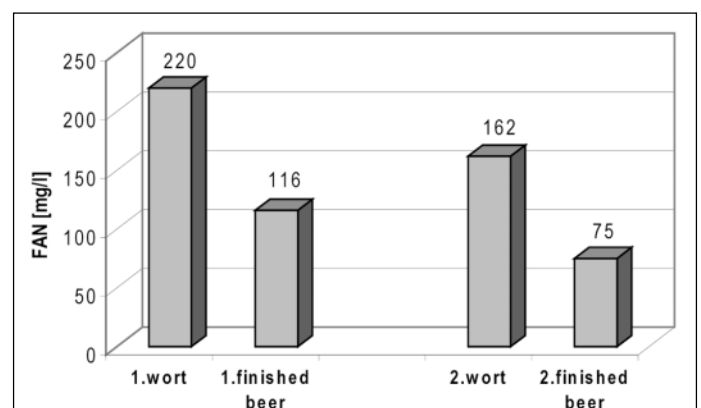
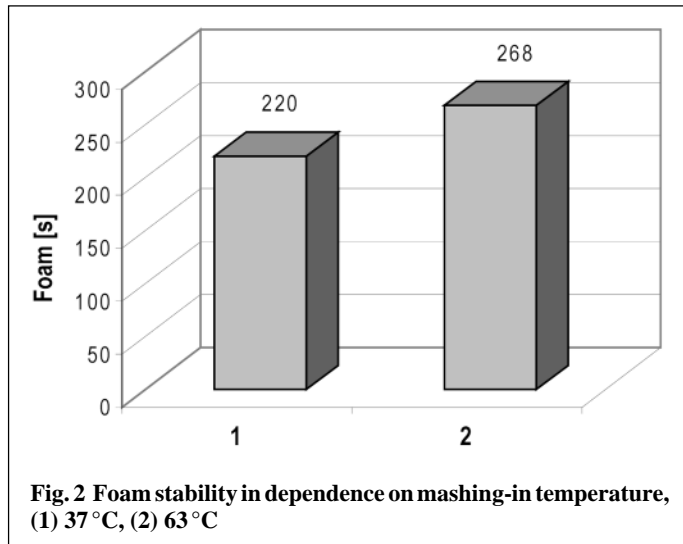


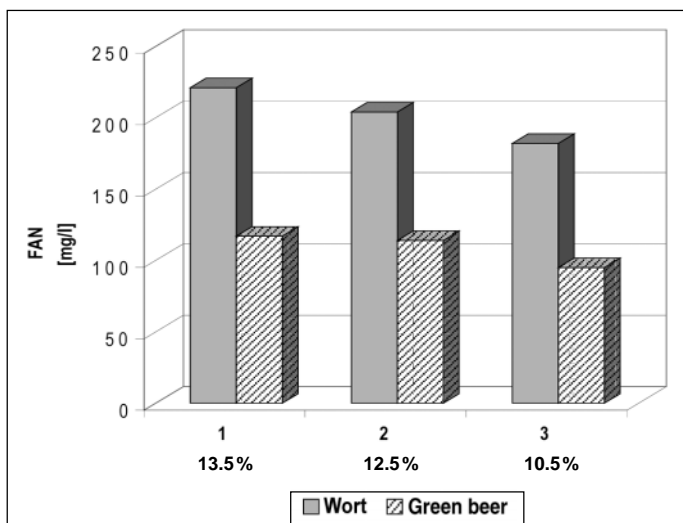
Fig. 1 FAN concentration in wort and finished beer in dependence on mashing-in temperature, 37 °C (1.) and 63 °C (2.)



mg/l (Fig. 1), whereas the foam stability was higher with up to 268 seconds (good foam) and more (Fig. 2). We have not registered any negative changes during fermentation (higher production of diacetyl, slowing down the growth of yeast and fermentation).

**Table 1 FAN concentration in worts and green beers of different gravities (FAN in green beers after yeast harvest)**

FAN (mg/l)					
13.5 % P wort	13.5 % P beer	12.5 % P wort	12.5 % P beer	10.5 % P wort	10.5 % P beer
210	100	184	90	183	88
206	105	200	98	170	81
226	131	216	126	187	95
235	129	208	114	183	103
216	126	202	123	179	98
221	124	190	87	197	108
218	125	214	90	189	113
233	120	206	108	186	93
222	110	219	101	165	80



**Fig. 3 FAN concentration in worts and green beers of different gravities (FAN in young beers after yeast removing)**

**3.2 FAN utilization**

During wort boiling high-molecular proteins are precipitated. In wort mostly soluble fissile protein products remained, which are necessary for yeast multiplication and fast fermentation. Yeast utilization of nitrogenous substances in form of amino acids and lower-peptides from wort is around 100 – 140 mg per litre [7,8]. In HGB technology beer with higher gravity (13.5 °P, 12.5 °P, 10.5 °P) is blended with deoxygenated water for desiderative gravity (12 °P, 10 °P) by automatic blending system. During this stage FAN are thinned and this decreases their amount in the finished product. The experiment was done with wort prepared in mashing conditions 3 (see “Material and methods”, 2.2).

FAN utilization by *Saccharomyces cerevisiae* subsp. *uvarum* W 34/70 during fermentation of different wort concentration is documented in Table 1 and Figure 3. In case of 13.5 °P wort yeast utilised 104 mg/l FAN in average; using wort of concentration 12.5 °P it was 90 mg/l FAN and using 10.5 °P wort 87 mg/l FAN in average.

Further decrease of FAN content in beer was a result of blending beer with water proprietary to the HGB system. Total decrease of FAN – by yeast utilization (1 – 2) and by blending (2 – 3) – in worts of different gravities is shown in Figs. 4, 5 and 6. FAN content in finished beer was in the range of 85 – 106 mg/l for 12 °P beer and 70 – 94 mg/l for 10 °P beer. In 13.5 °P wort maximum FAN concentration was 235 mg/l, minimum 206 mg/l, in 12.5 °P wort maximum FAN concentration was 219 mg/l, minimum 190 mg/l and in 10.5 °P wort maximum was 197 mg/l and minimum 165 mg/l FAN.

**4 Conclusion**

A very low mashing-in temperature (37 °C) and an extended rest and time of heating up to 63 °C resulted in a very high amount of FAN – up to 270 mg/l for 12 °P wort, but the foam stability of the finished beer was very poor due to a low amount of high-molecular proteins.

On the other hand, when mashing-in temperatures were higher (63 °C) the FAN concentration of 12 °P wort was only 162 mg/l, but foam stability of beer was higher with 268 seconds in average, and fermentation took place as usual.

Average decreases of FAN using yeasts were 104 mg/l for 13.5 °P wort, 90 mg/l for 12.5 °P wort and 87 mg/l for 10.5 °P wort.

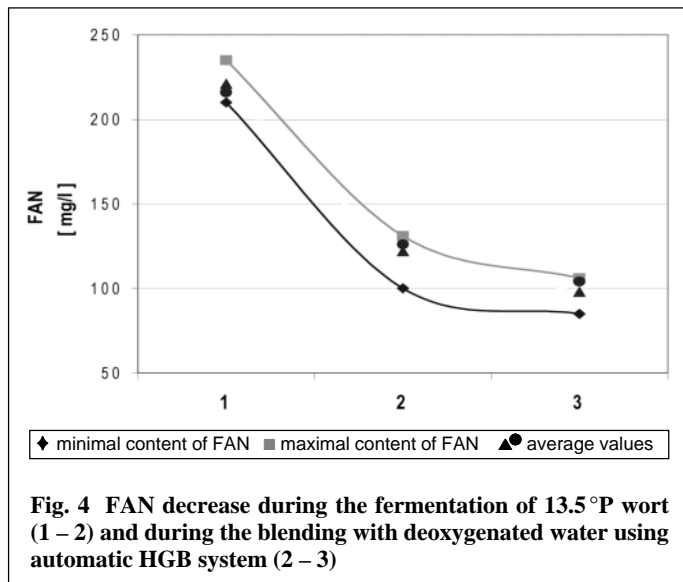
FAN concentrations in finished beer using HGB technology (blending from higher gravity to required gravity) were 85 – 106 mg/l for 12 °P beer, 70 – 94 mg/l for 10 °P beer.

**5 Zusammenfassung / Resumé**

**Cvengroschová, M., Šepeřová, G. und Šmogrovičová, D.: Auswirkung der Einmischtemperatur auf die FAN-Konzentration und die Schaumhaltbarkeit von Bier** — Monatsschrift für Brauwissenschaft 56, Nr. 7/8, 128 – 131, 2003

**BC 22 Sudhaus**

Eine niedrige Einmischtemperatur (37 °C), eine lange Rast (60 min. inklusive Einmischdauer) und eine lange Aufheizphase auf 63 °C führen zu sehr hohen FAN-Konzentrationen von bis zu 270 mg/l in Würze mit 12 °P, aber zu sehr schlechter Schaumhaltbarkeit des hergestellten Bieres (durchschnittlich 220 sec.). Der Grund dafür liegt in dem niedrigen Gehalt an hochmolekularem Eiweiß. Wenn die Einmischtemperatur höher lag (63 °C), betrug die FAN-Konzentration einer 12 °P-Würze lediglich 162



mg/l, aber die Schaumhaltbarkeit des Bieres war besser, nämlich 268 Sekunden im Durchschnitt. Einmaischtemperaturen von 50 °C führten zu beachtlichen FAN-Konzentrationen. Hefen verbrauchten durchschnittlich 104 mg/l FAN bei Würzen mit 13,5 °P und einer ursprünglichen FAN-Konzentration von 235 mg/l. In Würzen mit 12,5 °P (Ausgangswert FAN: 219 mg/l) verbrauchten sie 90 mg/l und 87 mg/l bei einer Würze mit 10,5 °P (Ausgangswert FAN: 197 mg/l). Während des Reifungsprozesses wurde jegliche FAN-Abnahme aufgezeichnet. Der FAN-Gehalt fiel aufgrund des Verdünnens des Bieres mit entgastem Wasser auf den gewünschten Stammwürzegehalt beim HGB-Verfahren. Der FAN-Gehalt im fertigen Produkt schwankte zwischen 85 und 106 mg/l in einem Bier mit 12 °P, und zwischen 70 und 94 mg/l bei 10 °P.

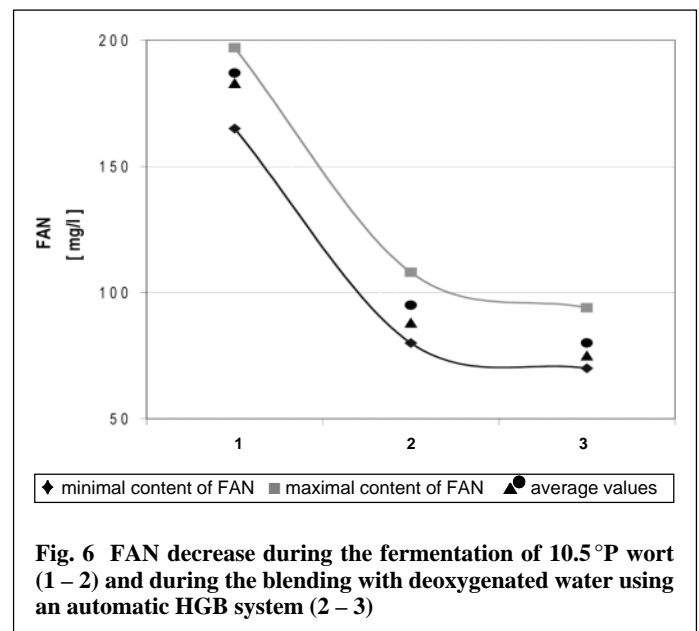
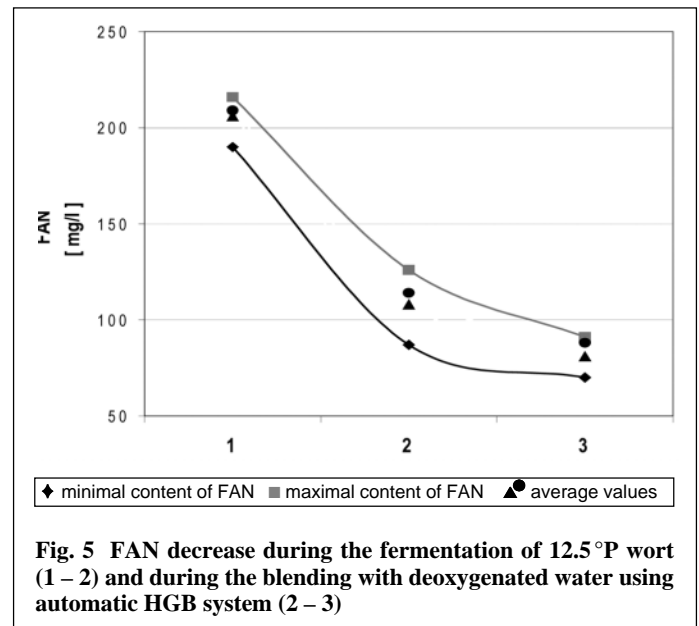
**Cvengroschová, M., Šepeřová, G., and Šmogrovičová, D.: Répercussions de la température d'empâtage sur la concentration des AAL (acides aminés libres) et de la stabilité de mousse de la bière —** Monatsschrift für Brauwissenschaft 56, No. 7/8, 128 – 131, 2003

#### BC 22 Salle à brasser

Une température d'empâtage basse de 37 °C, un palier long (60 min. incluant le temps d'empâtage) et une durée importante de chauffage jusqu'à 63 °C, produisent des moûts à 12 °P avec une teneur très élevée jusqu'à 270 mg/l d'AAL mais conduisent également à une très mauvaise stabilité de mousse des bières produites (moyenne 220 sec.). La cause réside dans la faible teneur en protéines à poids moléculaire élevé. En utilisant une température d'empâtage plus élevée (63 °C), la concentration en AAL d'un moût à 12 °P était seulement de 162 mg/l et la stabilité de la mousse de la bière était meilleure, c'est-à-dire 268 secondes en moyenne. Des températures d'empâtage de 50 °C conduisent à des taux importants d'AAL. Les levures utilisent en moyenne 104 mg/l d'AAL dans des moûts à 13,5 °P qui contiennent initialement une concentration de 235 mg/l d'AAL. Dans des moûts à 12,5 °P (taux d'AAL de départ de 219 mg/l) les levures utilisent 90 mg/l d'AAL et 87 mg/l pour des moûts à 10,5 °P (valeur d'AAL de départ: 197 mg/l). Pendant le temps de maturation, on a enregistré toute diminution d'AAL. La teneur en AAL est réduite par le fait d'une dilution de la bière par de l'eau désaérée à la concentration de la densité du moût primitif désirée, en utilisant la méthode de fabrication de haute densité. La concentration en AAL dans le produit fini varie entre 85 et 106 mg/l dans une bière à 12 °P et entre 70 et 94 mg/l à 10 °P.

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