



# Free amino nitrogen in sweet wort made from barley varieties tested in the Czech Republic

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## Abstract

The effect of the variety on the free amino nitrogen (FAN) content in wort was studied in the set of 24 varieties grown for a three-year period at four different testing sites. FAN was determined by the EBC 4.10 method. The highest average FAN was exhibited by the variety KWS Amadora (193 mg/l). The content of FAN was mostly affected by the year (24%), followed by the site (15%); the effect of the variety was the lowest (13%). Nearly 50% of the samples exhibited low FAN, slightly over 40% of the samples had medium FAN and 12% of the samples exhibited high FAN.

**Key words:** barley, sweet wort, free amino nitrogen, malting quality

## 1 Introduction

Quality of malt has a significant effect on the brewing process and the final product quality. Most of the characters assessed in malt, and namely sweet wort, can be split into three groups. These characters describe the result of the activity of cytolytic, proteolytic and amyolytic enzymes.

Proteolytic modification describes degradation of nitrogenous substances and their conversion to low-, medium- and high-molecular soluble forms. Too low degradation of nitrogenous substances causes insufficient supply of yeast with nitrogen compounds, which results in insufficient yeast proliferation and formation of undesirable fermentation by-products (eg diacetyl). Too high protein degradation leads to strong degradation of high molecular weight proteins. The lack of high molecular weight proteins as well as the excess of medium-molecular compounds and amino acids lysine, arginine and histidine has a negative effect on foam stability. Furthermore, sweet worts and beers made from over-modified malts are more intense in colour (Back, 2005).

The content of soluble nitrogen, Kolbach index, and free amino nitrogen (FAN) are the principal parameters characterizing proteolytic modification.

The importance of nitrogenous substances for malt and beer production and their quality is significant (Pierce, 1987). The total content of nitrogenous substances in barley grain is mainly affected by growing conditions at the given locality in a particular year. The quantity of nitrogenous substances in malt and content of the total soluble nitrogen in sweet wort is affected by the content of nitrogenous substances in non-malted grain, variety and malting technology. Only part of nitrogen passes from the malt to the sweet wort. Total soluble nitrogen affects the technological process of beer production and the sensory properties of the final product. The values of the soluble nitrogenous substances usually range from 650 to 950 mg/l.

Kolbach index informs about the ratio of nitrogen that comes from the malt to the laboratory wort. Kolbach index is the most commonly used parameter characterizing proteolytic modification. It expresses the ratio of soluble nitrogen substances determined in wort to the total content of nitrogenous substances in malt. The values of this character usually range from 36 to 44%.

Brewer's yeast receives nitrogen in the form of amino acids, peptides, and ammonium ions produced by proteolysis of barley nitrogenous substances (Clapperton, 1971;

O'Connor-Cox and Ingledew, 1989). Amino acids, ammonium ions and peptides (di- and tripeptides) required for the synthesis of cell proteins and other yeast cell compounds are referred to as free amino nitrogen (FAN) (Pugh *et al.*, 1997). FAN should form approximately 21–22% of the total soluble nitrogen, its content in wort most often ranges from 120 to 200 mg/l (Kofroň *et al.*, 2006; Psota *et al.*, 2018).

FAN helps to ensure a sufficient activity of hydrolytic enzymes and a necessary level of fermentation. The levels of FAN in sweet wort inform on the level of malt proteolysis, since amino acids are released mainly during malting. FAN and its composition are considered an important parameter for predicting the fermentation intensity and sensory quality of beer (Inoue and Kashihara, 1995; Baldus *et al.*, 2018).

The amount of the individual amino acids that the FAN contains is also the source of a number of fermentation by-products, including diacetyl and acetolactate, which may affect the resulting taste and sensory stability of beer (Mändl and Wagner, 1978; Pickerell, 1986; Sablayrolles and Ball, 1995; Malfli *et al.*, 2008; Lei *et al.*, 2012; He *et al.*, 2014; Meier-Dörnberg *et al.*, 2017; Ferreira and Guido, 2018). FAN not consumed during fermentation can serve mainly as a nutrient for the development of undesirable microorganisms in non-pasteurized beer.

## 2 Material and methods

The effect of the variety on the free amino nitrogen content in sweet wort was studied in 2014–2016 in the set of 24 varieties of spring malting barley (Table 1). Every year this set of varieties was obtained from four various testing sites. The testing sites were selected so that the content of nitrogenous substances in the samples of non-malted grain of the studied varieties was around 11%. A total of 288 barley grain samples were assessed.

The summary of the studied parameters is given in Table 2 including the references to the used methods. FAN was determined by the method 4.10 Free amino nitrogen of malt by spectrophotometry (EBC Analysis committee, 2010).

After harvest, malt was produced from the studied varieties. The samples graded on 2.5 mm sieve were malted. Malting of the samples (0.5 kg) was conducted in the micro-malting plant of the KVM company (CR). Laboratory malting was carried out by the method traditionally used in RIBM, which is almost identical with the MEBAK method (2011). Steeping was conducted in the steeping box. The temperature of water and temperature of air were kept at 14.0 °C.

**Table 1** Spring barley varieties

Variety	Year of registration	Country of origin
Bojos	2005	CZE
Francin	2014	CZE
Kampa	2015	FRA
Kangoo	2008	NLD
KWS Amadora	2015	DEU
KWS Irina	2014	DEU
Laudis 550	2013	CZE
Leenke	2017	DEU
LG Monus	2017	CZE
Libuše	2016	DEU
Malz	2002	CZE
Manta	2016	DEU
Octavia	2017	GBR
Odyssey	2014	GBR
Overture	2014	GBR
Petrus	2013	CZE
Pop	2017	FRA
Remark	2017	DEU
Sebastian	2005	DNK
Soulmate	2017	DNK
Sunshine	2012	DEU
Tango	2016	FRA
Vendela	2013	DEU
Xanadu	2006	DEU

### Note

CZE - Czech Republic

DNK - Denmark

GBR - United Kingdom of Great Britain

FRA - France

NLD - Netherlands

DEU - German

Length of steeping: 1st day – 5 hours; 2nd day – 4 hours. On the third day the water content in germinating grains was adjusted to the value of 45% by steeping or spraying.

Germination was conducted in the germination box. The temperature during germination was 14.0 °C. The total time of steeping and germination was 144 h. Kilning was performed on a one-floored electrically heated kiln. The total kilning time was 22 h, prekilning at 55 °C, kilning temperature was 80 °C for 4 hours.

Many quality parameters were assessed in the produced malt and sweet wort (EBC Analysis committee, 2010; MEBAK, 2011; Baxter and O' Farrell, 1983) (Table 2). Notes to some parameters given in the table: Wort clarity is assessed as follows: 1 = clear, 2 = weakly opalizing, 3 = opalizing, 4 = cloudy. Malt yield in dry matter (%) = weight of deculmed malt in dry matter / (weight of barley in dry matter / 100). Respiration losses (%) = 100 - (malt yield in dry matter + rootlet losses). Rootlet losses (%) = 100 - weight of deculmed malt / (weight of non-deculmed malt / 100) (Briggs, 1998).

**Table 2** Correlation and regression coefficient of FAN

Method	Unit	Reference	r	reg	Sign.
Bulk density of barley d. m.	kg		0.04	0.01	NS
Protein content of barley (factor 6.25)	%	EBC, 2010	0.08	0.00	NS
Starch content of barley	%	NIR	0.17	0.01	**
Degree of steeping 1	%		0.01	0.00	NS
Degree of steeping 2	%		0.01	0.00	NS
Extract of malt, congress mash	%	EBC, 2010	0.32	0.02	***
Mash method according to Hartong and Kretschmer VZ 45 °C	%	MEBAK, 2011	0.41	0.08	***
Kolbach index	%	EBC, 2010	0.41	0.07	***
Diastatic power	WK	EBC, 2010	0.13	0.39	*
Final attenuation of laboratory wort from malt	%	EBC, 2010	0.14	0.01	*
Friability	%	EBC, 2010	0.13	0.04	*
High molecular weight $\beta$ -glucan content of malt, FIA	mg/l	EBC, 2010	-0.21	-1.01	***
Protein content of malt (factor 6.25)	%	EBC, 2010	0.13	0.00	*
Total nitrogen of malt, Kjeldahl method	%	EBC, 2010	0.13	0.00	*
Soluble nitrogen of wort, Kjeldahl method	mg/l	EBC, 2010	0.58	1.65	***
Soluble nitrogen of malt, Kjeldahl method	%	EBC, 2010	0.58	0.00	***
Viscosity of laboratory wort from malt	mPa.s	EBC, 2010	-0.30	0.00	***
Total polyphenols in wort	mg/l	EBC, 2010	0.12		*
Colour of malt, visual method	EBC	EBC, 2010	0.16	0.00	**
Glassy corns	%	EBC, 2010	-0.04	0.00	NS
Partly unmodified grains	%	EBC, 2010	-0.08	-0.01	NS
Homogeneity (by friabilimeter)	%	Baxter a O'Farrell, 1983	0.08	0.01	NS
Appearance (clarity) of wort		MEBAK 2011	-0.06	0.00	NS
Haze of wort (90°)	EBC	EBC, 2010	-0.15	-0.01	*
Haze of wort (12°)	EBC	EBC, 2010	-0.10	0.00	NS
Respiration losses d. m.	%	Briggs, 1998	0.21	0.00	***
Rootlet losses d. m.	%	Briggs, 1998	0.23	0.00	***
Malt yield d. m.	%	Briggs, 1998	-0.28	-0.01	***

**Note**

Sign. – significant level  
*r* – correlation coefficient  
*reg* – regression coefficient  
 NS non significant

\*\*\* *P*=0.001  
 \*\* *P*=0.01  
 \* *P*=0.05

The set was evaluated by a three-way analysis variance and the set structure was described by a frequency distribution table and a histogram. A Bonferroni test was used to create homogeneous groups. Furthermore, correlation and regression coefficients between FAN and other studied traits were determined.

### 3 Results and discussion

FAN content in sweet wort is mainly affected by the malting technology used, content of nitrogenous substances in non-malted barley grain and variety. Leach et al. (2002) found that, using different malting conditions, malts with a significant difference in the contents of FAN, soluble ni-

trogen, and different activity of hydrolytic enzymes could be produced from the same barley sample. The Canadian two-row barley varieties processed in the pilot malt house were found to have a FAN content of 185–223 mg/l. However, the same varieties processed in industrial malting facilities showed a FAN content of 160–197 mg/l. The difference was definitely caused by different malting conditions and different content of nitrogenous substances in the non-malted barley grain (Li and Egi, 2004). In addition, Back and Narziß (1997) demonstrated that the length and temperature of germination and degree of steeping markedly affected the FAN content.

Within this experiment we tried, through the selection of testing sites in which the grain of the studied varieties showed optimal values of nitrogenous substances

**Table 3** Analysis of variance and estimated components of variance of a) free amino nitrogen, b) soluble nitrogen of wort, c) ratio FAN in soluble nitrogen**a) free amino nitrogen**

Source of variation	d.f.	Mean square	Significant level	F ratio	Estimated components of variance		
					abs.	rel. (%)	s.e.
Year	2	8257	***	24.66	132	20	140
Site	7	3517	***	10.51	98	15	61
Variety	23	1305	***	3.90	81	13	52
Residual	255	335			336	52	30

**b) soluble nitrogen of wort**

Source of variation	d.f.	Mean square	Significant level	F ratio	Estimated components of variance		
					abs.	rel. (%)	s.e.
Year	2	42010	***	27.41	637	12	670
Site	7	43714	***	28.52	1780	33	987
Variety	23	18259	***	11.91	1394	26	449
Residual	255	1533			1534	29	136

**c) ratio FAN in soluble nitrogen**

Source of variation	d.f.	Mean square	Significant level	F ratio	Estimated components of variance		
					abs.	rel. (%)	s.e.
Year	2	50	***	11.03	1.0	16.4	1.0
Site	7	16	**	3.45	0.3	5.8	0.3
Variety	23	5	NS	1.12	0.0	0.8	0.1
Residual	255	5			4.5	77.1	0.4

**Note**

d.f. degrees of freedom  
rel. relative value  
\*\*\* P=0.001

abs. original value NS non significant  
s.e. standard error  
\*\* P=0.01

(11.0% or similar values) and using a uniform malting technology, to suppress in statistical evaluation the effect of nitrogenous substances and malting technology and highlight the effect of varieties on the FAN content in sweet wort. Despite this effort, the FAN content of the sweet wort in the studied set of varieties was influenced from 20% by the year, 15% by the site and only from 13% by the variety. Non-monitored sources of variability formed more than 50% (Table 3). The low influence of the variety on the FAN content was probably due to the small variability of the set. The studied set contained only good quality spring barley malting varieties. Most samples (63%) ranged from 151–195 mg/l. 30% of the samples exhibited the FAN content in the range from 90–150 mg/l. Only 7% of the samples had higher FAN content (196–240 mg/l) (Figure 1). According to the average FAN content, the set of the studied spring barley varieties was divided into four homogeneous groups that overlapped each other (Table 4). Only the varieties Kampa and Petrus exhibited the average FAN content up to 150 mg/l. All other varieties had an average FAN content in the range from

151–193 mg/l. None of the studied varieties showed an average value higher than 195 mg/l (Table 4).

Requirements of breweries for the FAN content may vary significantly. Varieties suitable for breweries that add non-malted grain of barley or other cereals to malt, are bred in the USA. For this reason, they require barley varieties that provide malt with a higher FAN content, a high level of diastatic power and Kolbach index (Hertrich, 2013). Pitz (1990) reported the FAN content in North American two-row varieties in the range from 180 to 220 mg/l and in six-row varieties from 160 to 210 mg/l. Hertrich (2013) reported FAN values of up to 235 mg/l in North American barley varieties. European varieties have lower FAN levels. The American Brewers Association (2014) associating craft breweries reports that the FAN content should be at least 150 mg/l. The requirements of the craft breweries for FAN content ranged from 120 to 200 mg/l.

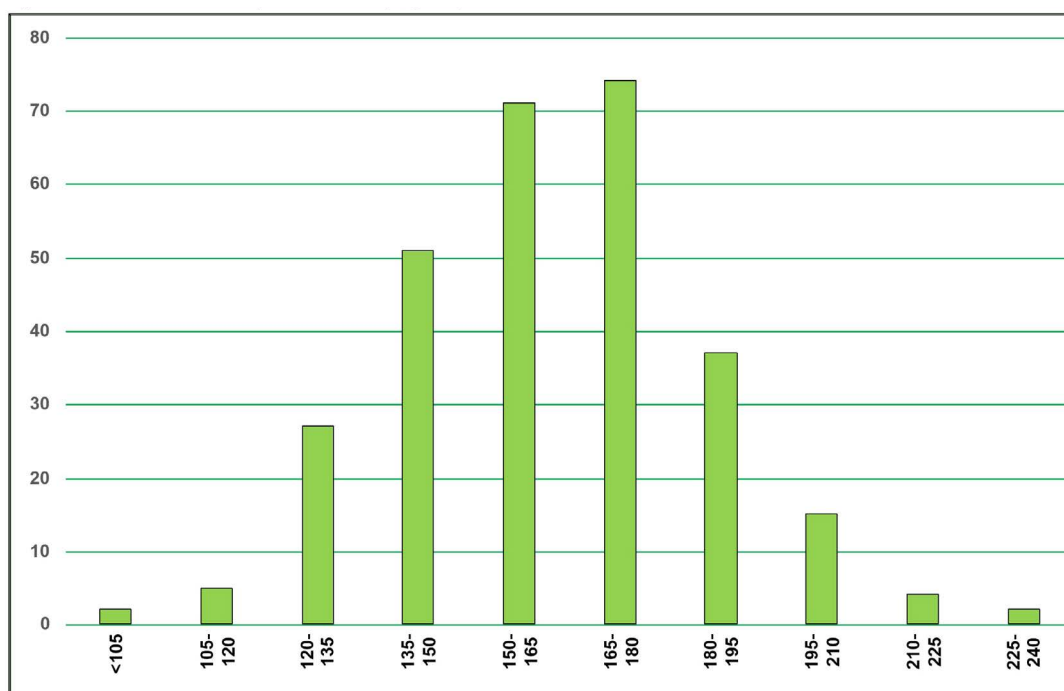
The absolute value of the FAN content has been shown to be less important than the FAN to soluble nitrogen ratio (Back and Narziß, 1997). The ratio of FAN in soluble nitrogen varied between 12.31% and 26.01% in the

**Table 4** Multiple range analysis for free amino nitrogen in sweet wort (mg/l)

Variety	Mean	Homogeneous groups			
Kampa	144	a			
Petrus	147	a	b		
Octavia	151	a	b	c	
KWS Irina	155	a	b	c	
Vendela	156	a	b	c	
Manta	156	a	b	c	
Kangoo	156	a	b	c	
LG Monus	157	a	b	c	
Francin	157	a	b	c	
Laudis 550	157	a	b	c	
Sebastian	158	a	b	c	
Bojos	158	a	b	c	
Leenke	159	a	b	c	
Odyssey	159	a	b	c	
Pop	164	a	b	c	
Tango	164	a	b	c	
Overture	165	a	b	c	
Soulmate	166	a	b	c	d
Malz	168	a	b	c	d
Remark	169	a	b	c	d
Libuše	173	a	b	c	d
Sunshine	175		b	c	d
Xanadu	176			c	d
KWS Amadora	193				d

**Table 5** Multiple range analysis for FAN ratio in soluble nitrogen (%)

Variety	Mean	Homogeneous groups
Leenke	19.4	a
Kangoo	19.6	a
Bojos	19.6	a
Laudis 550	19.6	a
Petrus	19.7	a
Octavia	19.8	a
Sunshine	19.9	a
Pop	20.0	a
KWS Irina	20.0	a
Francin	20.1	a
Kampa	20.1	a
Malz	20.2	a
Libuše	20.4	a
Overture	20.4	a
Vendela	20.4	a
Sebastian	20.4	a
Manta	20.5	a
Remark	20.6	a
Odyssey	20.7	a
Soulmate	20.9	a
Tango	20.9	a
LG Monus	21.0	a
Xanadu	21.2	a
KWS Amadora	22.3	a

**Figure 1** FAN distribution in a set of samples of monitored spring barley varieties

studied set of varieties. The lowest average ratio of FAN in soluble nitrogen was reported by Leenke (19.37%). The highest ratio of FAN in the soluble nitrogen content was recorded in KWS Amadora (22.34%). However, there was no statistically significant difference between the varieties (Table 5). It was found that for good fermentation and yeast multiplication and standard level of fermentation by-products (Gibson et al., 1985).

FAN in sweet wort should form 20–22% of the soluble nitrogen (Mändl and Wagner, 1978; Back and Narziß, 1997); this was fulfilled by the studied varieties.

The FAN content was in weak to moderate relationship with the parameters characterizing, in a certain way, mainly the proteolytic and cytolytic modification of the caryopsis endosperm. The relationship between the FAN content and content of soluble nitrogen ( $r = 0.58$ ) and also Kolbach index and relative extract at 45° C ( $r = 0.41$ ) was moderately strong (Table 2). The correlation between FAN and relative extract was also reported by Back and Narziß (1997).

The relationship between the FAN content and extract content ( $r = 0.32$ ) was weak. A negative weak relationship was recorded between the contents of FAN and beta-glucans in sweet wort ( $r = -0.21$ ) and viscosity ( $r = -0.30$ ). With decreasing cytolytic modification, FAN content decreased. A similar situation occurred in losses by malting. Increasing respiration losses ( $r = 0.21$ ) and deculming losses ( $r = 0.23$ ) are mostly an indicator of a higher level of modification; FAN content increases with a higher level of modification. The opposite situation obtains in the case of malt yield ( $r = -0.28$ ). FAN content decreases as yield increases. Other traits were very weakly related to the FAN content. Although FAN affects yeast activity, no significant relationship between FAN content and achievable degree of fermentation was observed (Table 2). Conversely, with increasing malt yield ( $r = -0.28$ ) the content of FAN decreases. The relationship of the other studied parameters to the FAN content was weak. Although FAN affects the activity of yeast, no more significant relationship between the FAN content and apparent final attenuation was detected (Table 2).

#### 4 Conclusion

The present study was performed on a collection of 24 spring barley malting varieties. The free amino nitrogen content was studied in the sweet wort made from these varieties.

In the studied set, the FAN content in sweet wort was influenced from 20% by the year, 15% by the site and only from 13% by the variety. The average FAN content up to 150 mg/l was recorded in Kampa and Petrus, the

other varieties had an average FAN content in the range of 151–193 mg/l. The ratio of FAN in the soluble nitrogen content in the studied set of varieties moved in the range of 12.31% to 26.01%. Moderately strong relationship was detected between the contents of FAN and soluble nitrogen ( $r = 0.58$ ) and Kolbach index and relative extract at 45° C ( $r = 0.41$ ), i.e. parameters characterizing proteolytic modification. FAN significantly affects fermentation and its composition affects the resulting taste and sensory stability of the final product.

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