The Effect of the Barley Variety, Location and Year Crop on the Haze of Congress Wort

Vratislav PSOTA[1], Zuzana SKULILOVÁ[2] and Jiří HARTMANN[3]

[1]Research Institute of Brewing and Malting, PLC, Malting Institute, Brno, Czech Republic; [2]Department of Food Technology, Faculty of Agronomy, Mendel University of Agriculture and Forestry Brno, Brno, Czech Republic; [3]Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic

Abstract

Psota V., Skulilová Z., Hartmann J. (2009): **The effect of the barley variety, location and year crop on the haze of congress wort**. Czech J. Food Sci., **27**: 158–164.

The study set consisted of eleven barley (*Hordeum vulgare* L.) varieties grown at four different locations for a three-year period. The malting characteristics were determined in all samples. The results obtained using the traditional visual evaluation of wort clarity were compared with those obtained nephelometerically. The results indicate a considerable effect of a variety on the wort haze (30–40%). The effect of location on the wort haze varied from 13–22%, the effect of year was small. The level of wort haze or wort clarity split the set of the varieties under study to clearly distinguishable groups. The worts from the varieties Annabell and Nordus inclined to form wort haze. Statistically significant or highly significant correlation was found between the wort haze and clarity and the relative extract at 45°C, diastatic power, final apparent attenuation, and saccharification time. The relationship between the wort haze or clarity and other technological parameters was not statistically significant in most cases. The correlation coefficient between the haze at 15° or 90° (nephelometric method) and wort clarity (visual method) was 0.89. The nephelometric determination of wort haze completely replaces the traditional subjective evaluation of wort clarity.

Keywords: clarity; *Hordeum vulgare*; malting quality; variety

Raw materials for the beer production (malt, hop, water, yeasts) occur in liquid and solid states at the beginning of the production process. During their processing, a number of various reactions take place and the final product as well as the intermediates and wastes from the brewing technologies are prevailingly in the form of liquid dispersions. Generally, these dispersions contain haze-forming particles of different sizes, from large particles of spent grains (a millimeter in diameter) to micron-size rough and fine particles and yeasts and further to submicron-size colloids of tannin and protein nature and other unfermented extract residues.

The size, quantity and solubility of haze-forming particles in the intermediates and in the final product change during the beer production. This is used for the visual assessment of the individual technological steps and for the evaluation of physical and chemical or even biochemical aging of beer (Bamfort 1999).

Various methods are used for the haze measuring. The visual method is the most frequently used

Suported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 6019369701.

and at the same time the oldest technique for the determination of wort clarity (Basařová *et al.* 1992; MEBAK 1997). This method can be easily subjectively influenced. For this reason, other objective methods for the wort haze determination were searched for. The nephelometric method belongs to the objective assays of the wort haze determination (EBC 1998; Prokeš & Hartmann 2001).

Haze is an optical characteristic of solid, gaseous, and liquid substances. The haze of substances is given by the presence of various kinds and quantities of particles below and above micron size causing light scattering. Therefore, light scattering is also used for the determination of the haze extent of the studied substances. The haze extent in liquids depends on the properties of the haze-forming particles in relation to the properties of the measuring light which scatters through the hazed environment. This dependence is generally non linear (Sladký & Dienstbier 2000).

Despite the relatively good general knowledge of the causes of colloidal beer hazes formation and possibilities of its reduction using various stabilisation methods and materials, considerable attention in the brewing research has been devoted even today to study on colloidal instability in beer (Papp *et al.* 2001; Siebert & Lynn 2003; Stewart 2004; Douglas *et al.* 2006), mainly in terms of optimising the production processes and improving the quality of the final product in compliance with the demanding requirements of the modern market. The brewing liquor composition, mashing system, times, and temperatures of the primary fermentation and storage, and especially the selection of the barley variety for the malt production have high importance for colloidal beer stability.

The present study deals with the problems of the barley variety effect on the haze of wort, first intermediate in the beer production.

MATERIAL AND METHODS

Barley samples. Two sets of barley samples obtained in 2002–2004 were used for this study. Each year, the grain samples were collected from four testing locations of the Department of Varietal Testing of the Central Institute for Supervising and Testing in Agriculture. The testing locations were chosen in dependence on the content of nitrogenous substances in grain so that the negative effect of significantly low or significantly high contents of nitrogenous substances on the technological parameters could be avoided.

The first set contained eleven varieties of spring barley included in the list of recommended varieties in the Czech Republic (LRV). The composition of this set was not changed during the whole monitoring period (Table 1). Totally, 132 samples were evaluated within this set.

The second, large set of 824 samples of spring and winter barley contained varieties assigned to the registration procedure in the Czech Republic (R) in 2002–2004. Approximately one third of this set was changed each year.

The effect of variety, location, and year on wort haze was studied in the set of 11 varieties of spring malting barley (LRV).

Comparison of the visual and nephelometric methods of wort haze determination was performed in a large set of 824 samples of spring and winter barley (R), and also in the set of 11 varieties of spring malting barley (LRV). The relationship between the clarity or haze and other technological parameters was determined in the R set.

Micromalting and malt analysis. Malting of 0.5 kg of samples was performed in the micromalting plant of KVM (CR). The modified MEBAK method which is traditionally used in the RIBM was applied for laboratory malting (Psota *et al.* 2008):

Steeping: Temperature of water and air during the air rests was 14.5°C. The duration of steeping: 1st day – 5 h; 2nd day – 4 hours. On the third day, the water content in the germinating grain was adjusted to 45.5% by steeping or spraying.

Germination: The temperature during the process was 14.5°C. Total time of steeping and germination was 144 hours.

Kilning: One-floor electrically heated floor. Total time of kilning was 22 h, prekilning proceeded at 55°C, the temperature of kilning was 80°C for 4 hours.

A number of technological parameters were determined in barley grain, malt, and wort (Table 2) using the EBC (1998) and MEBAK (1997) methods.

Measurement of clarity and haze. The wort clarity (MEBAK 1997, 4.1.4.2.6) and haze (EBC 1998, 9.29) were measured in the congress wort (EBC 1998, 4.5). For the needs of statistical evaluation, the level of wort opalescence was expressed in figures (1 – clear, 2 – weakly opalescent, 3 – opalescent, 4 – hazed). The wort haze was measured with the hazemeter, type MZN-93-MC2 (Charles University, Prague, CR). The measurement was done simultaneously at angles of 90° and 15°.

Table 1. Basic description of barley varieties studied ("LRV")

Variety	Malting quality index[a]	Pedigree	Breeder/Maintainer
Amulet	6	HE 2591 × Salome	Selgen, a.s., Czech Republic
Annabell	7	ST 900 14DH × Krona	Nordsaat Saatzuchtgesellschaft, GmbH, Germany
			CEZEA – Šlechtitelská stanice, a.s., Czech Republic
Diplom	9	(Ditta × Cooper) × Krona	Nordsaat Saatzuchtgesellschaft, GmbH, Germany
			SAATEN – UNION CZ, s.r.o., Czech Republic
Jersey	9	Apex × Alexis	Limagrain Advanta Nederland B.V., Netherlands
			Limagrain Central Europe Cereals, s.r.o., Czech Republic
Kompakt	8	Galan × KM-A10	Hordeum, s.r.o., Slovak Republic
			OSEVA, a.s., Czech Republic
Malz	6–7	Famin × Scarlet	Limagrain Central Europe Cereals, s.r.o., Czech Republic
Nordus	9	845-87 × Krona	Nordsaat Saatzuchtgesellschaft, GmbH, Germany
			CEZEA – Šlechtitelská stanice, a.s., Czech Republic
Philadelphia	7	(ML-i × 2,51784) × Krona	Lochow-Pektus, GmgH, Germany
			SOUFFLET AGRO, a.s., Czech Republic
Prestige	9	Cork × Chariot	Société RAGT 2n, France
			RAGT Czech, s.r.o., Czech Republic
Scarlett	7	(Amazone × Breun St. 2730 e) × Kym	Saatzucht J. Breun, GdbR, Germany
			RAGT Czech, s.r.o., Czech Republic
Tolar	7	HE 4710 × HWS 78267-83	Limagrain Central Europe Cereals, s.r.o., Czech Republic

[a]Based on index (Psota & Kosař 2002): 1 = no malting quality and 9 = best malting quality

The wort clarity and haze were determined immediately after the end of laboratory mash filtration, i.e. after 60 min from the beginning of filtration when all wort was already filtered.

Statistical evaluation of results. The experimental data were assessed using multifactorial analysis of variance. The significance of differences between the years, locations, and varieties was tested with 5% least significance difference (LSD) (the REML system) (Robinson *et al.* 1996). The relationship between the determination of clarity by the visual method and the nephelometric method was expressed using the frequency distribution tables according to the level of frequency and the used angle of haze with the program STATGRAPHICS Version 7.

RESULTS AND DISCUSSION

Beer, similarly to all colloidal solutions, tends to form hazes. The trade with beer in casks and bottles has high requirements for the shelf life, which is given by the time from packaging the beer in a brewery to the formation of a perceivable haze. Therefore, the measurement of the haze of beer and its intermediates during brewing is an integral part of the beer production, first of all of the quality control and shelf life of the final product (Hlaváček & Lhotský 1966).

The formation of haze is influenced by a lot of factors, first of all by the malt quality and wort production technology. The laboratory wort for our research was prepared using the EBC method (1998).

The wort quality depends on the properties of the barley variety used. Malts differ in the contents of polyphenols, polysaccharides, and further substances affecting the wort haze formation.

High polyphenol levels are found e.g. in varieties of winter barley. The behaviour of specific flavonoids is more important than the absolute level of polyphenols (Dadic *et al.* 1976). A few years

Table 2. Correlation between clarity and haze of congress wort and technological parameters ("R")

	Units	References	Clarity		Haze 15°		Haze 90°	
Total nitrogen of barley	%	EBC 1998 3.3.2	0.050	NS	0.041	NS	0.071	NS
Extract of malt, congress mash	%	EBC 1998 4.5.1	–0.101	NS	–0.068	NS	–0.101	NS
Relative extract (45°C)	%	MEBAK 1997 4.1.4.11	–0.219	*	–0.195	*	–0.214	*
Kolbach index	%	EBC 1998 4.9.1	–0.163	NS	–0.150	NS	–0.166	NS
Diastatic power	WK	EBC 1998 4.12	–0.355	***	–0.356	***	–0.345	***
Final attenuation of laboratory wort	%	EBC 1998 4.11.1	–0.392	***	–0.444	***	–0.467	***
Friability	%	EBC 1998 4.15	0.084	NS	0.155	NS	0.108	NS
β-glucans in unhopped wort	mg/l	EBC 1998 4.16.2	–0.093	NS	–0.146	NS	–0.116	NS
Total nitrogen of malt	%	EBC 1998 4.3.2	0.073	NS	0.074	NS	0.102	NS
Total soluble nitrogen of wort	mg/l	EBC 1998 4.9.1	–0.095	NS	–0.077	NS	–0.072	NS
Total soluble nitrogen of malt	%	EBC 1998 4.9.1	–0.095	NS	–0.774	NS	–0.071	NS
Viscosity of laboratory wort	mPa.s	EBC 1998 4.8	–0.069	NS	–0.092	NS	–0.065	NS
Colour of malt, visual method	EBC u.	EBC 1998 4.7.2	0.110	NS	0.126	NS	0.131	NS
Saccharification rate	min	EBC 1998 4.5	0.192	*	0.262	**	0.179	*
Glassy corns	%	EBC 1998 4.15	0.135	NS	0.155	NS	0.122	NS
Partly unmodified grains	%	EBC 1998 4.15	0.120	NS	–0.163	NS	–0.125	NS
Homogeneity (by friabilimeter)	%	Baxter & O'Farrell (1983)	0.110	NS	0.151	NS	0.116	NS

NS = not significant; *, **, and *** indicated value is significantly different at P = 0.05, 0.01, and 0.001, respectively

ago, in the Czech Republic (Prokeš & Špunarová 1996) and abroad (Erdal 1995) barley varieties with low anthocyanogen contents were bred. Beer made from these varieties had a higher colloidal stability. However, these varieties did not spread due to their worse agronomic characteristics. Breeding in this area continues with the aim to develop new varieties that would be interesting for both barley growers and the brewers (Ponton 1988).

Barley varieties differ markedly in their β-glucan contents in wort which is given by the content of this polysaccharide in a barley caryopsis and capacity of the enzymatic apparatus of the caryopsis to degrade β-glucans in the course of malting (Psota & Jurečka 2001, 2002). High molecular weight β-glucans slow down the access of the enzymes to the cells thus negatively affecting the rate of the grain modification during malting. Furthermore, they form high viscose solutions that impede the wort filtration and can be a cause of non biological beer hazes. A homogeneous modification of the cell walls and associated reduction of β-glucan content enables the degradation of starch during mashing without problems (Krottenthaler 1997). This is an important prerequisite for the production of clear wort. The wort production method can affect the formation of wort hazes. The congress mashing method used in this study employing a longer time at 45°C provides more time for β-glucan degradation, and immediate warming to 70°C gives a short time for the activity of β-glucan solubilase; thus the differences between varieties can be reduced to a certain extent. If isothermal mashing at 65°C was used, the differences would probably be more pronounced. At the mashing temperature of 60°C to 65°C, high molecular weight β-glucans bound in the cell walls are massively released by β-glucan solubilase. These high molecular β-glucans are not degraded because the endo-β-glucanases responsible for this process are inactivated at 52°C. Very probably, worts acquired by isothermal mashing at 65°C will be more susceptible to the haze formation (Back 2005).

In the eleven spring barley varieties studied, wort haze was affected by the variety to 30–40%. The effect of the location was at the level of approximately 13–22%. The effect of the year was negligible. The purposeful selection of locations

Table 3. Analysis of variance ("R") for haze 15°, haze 90°, and clarity of wort

Analytical method	Mean square	d.f.	*F* Ratio	*P*	Estimated components of variance	
					abs.	rel. (%)
Haze 15°						
Year	21.93	2	9.891***	0.0001	0.611	10.05
Location	17.38	8	7.840***	0	1.373	22.61
Variety	24.14	10	10.886***	0	1.823	30.01
Residual	2.22	111			2.266	37.33
Haze 90°						
Year	15.30	2	6.725**	0.0018	0.258	4.33
Location	14.98	8	6.586***	0	0.974	16.35
Variety	30.90	10	13.584***	0	2.379	39.94
Residual	2.27	111			2.346	39.38
Clarity of wort						
Year	0.30	2	1.347[NS]	0.2642	0.000	0.00
Location	0.98	8	4.357***	0.0001	0.053	13.10
Variety	1.76	10	7.813***	0	0.128	31.31
Residual	0.22	111			0.227	55.59

[NS]not significant; *, **, and *** indicated values is are significantly different at $P = 0.05$, 0.01, and 0.001, respectively

so that the nitrogenous substances content in the barley grain was close to optimum (10.8–11.2%) was probably the cause of a low impact of locations and year on the haze formation. However, the value of the residual error is high, which means that the values of haze were affected by numerous other effects unknown and not studied by us (Table 3).

The set of the varieties studied (LRV) was split into three groups, based on the measurement of haze at 15° or 90° and the wort clarity (Table 4). In the worts made from the varieties Prestige, Jersey, Diplom, Malz, Amulet, and Tolar, haze wort did not occur or occurred only exceptionally. On the contrary, in worts made from the varieties Nordus

Table 4. Multiple Range Analysis for Haze 15° ("LRV")

Variety	Average[a]		
	haze 15°	haze 90°	clarity of wort
Prestige	0.29[a]	0.27[a]	1.00[a]
Jersey	0.34[a]	0.38[a]	1.00[a]
Diplom	0.35[a]	0.34[a]	1.00[a]
Malz	0.46[a]	0.42[a]	1.00[a]
Amulet	1.00[a]	0.96[a]	1.09[a,b]
Tolar	1.10[a,b]	1.08[a]	1.01[a]
Philadelphia	2.25[b,c]	2.49[b]	1.42[b,c]
Kompakt	2.33[c]	2.85[b]	1.42[b,c]
Scarlett	2.71[c,d]	2.91[b]	1.51[c]
Nordus	3.60[d,e]	4.33[c]	1.76[c,d]
Annabell	4.30[e]	4.43[c]	2.01[d]

[a]Average values followed by different letters are statistically different ($P = 0.05$)

Table 5. Reliability Interval for wort haze scattering ("R") with probability 95% (α = 95%)

	Clear	Weakly opalising	Opalising
Haze 90°			
Reliability interval for average	0.95–1.07	4.44–5.18	7.35–8.77
Reliability interval for scattering	0.57–0.70	2.74–4.75	5.69–11.64
Haze 15°			
Reliability interval for average	0.93–1.03	4.03–4.72	6.92–8.26
Reliability interval for scattering	0.39–0.48	2.46–4.27	5.02–10.26

and Annabel, haze wort occurred frequently. The highest risk of the haze formation was in the worts made from the variety Annabell. Prokeš and Hartmann (2001) suggest a similar risk for the variety Krona. The variety Krona is one of the parents of the varieties Annabell and Nordus (Psota & Jurečka 2001, 2002). Therefore, it seems that the tendency to the haze formation may be a genetically conditioned trait. However, to confirm this hypothesis, further research will be necessary.

Bamforth (1999) did not possess information whether a particular barley variety had a high or low level of the haze forming proteins. It is supposed that a higher level of nitrogen fertilisation can enhance the level of the haze-forming proteins. Prokeš and Hartmann (2001) suggested that clear wort is in relation to the content of soluble nitrogen. Weakly opalescent wort was associated with the wort viscosity, difference between fine and coarse milling, and β-glucan content in wort. Opalescent wort was in relation to friability only. The results achieved in this study cannot therefore provide evidence for the relationship of wort and increased or decreased contents of nitrogenous substances in the caryopsis or malt because only

Table 6. Relationship of wort clarity to wort haze at 15° and 90° in the "LRV" and"R" set

	C	S.l.	H15	S.l.
"LRV" set				
Haze 15°	0.888	***		
Haze 90°	0.892	***	0.95	***
"R" set				
Haze 15°	0.873	***		
Haze 90°	0.868	***	0.95	***

S.l. – significant level; C – unhopped wort clarity; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

the locations providing more or less optimum nitrogenous substance contents in non-malted barley caryopses were chosen.

In the set studied (R), statistically significant but weak relationships between the clarity or haze of wort and activity of amylolytic enzymes, relative extract at 45°C, and wort quality were determined. The level of haze declined with the increasing value of these parameters. On the contrary, the possibility of the haze formation increased with the prolonged saccharification time. This suggests that an increased enzymatic activity of malt and a good wort quality support the reduction of the haze formation (Table 2).

According to Prokeš (1999), the most reliable measuring of haze is that at 90°, which was also confirmed by Wackerbauer *et al.* (1992). The nephelometric evaluation of wort at 90° (15°) showed that, with 95% probability hazes can be regarded clear at the measured values of haze 0.95–1.07 (0.93–1.03) EBC units, i.e. on average 1.01 (0.98) EBC units. Haze values for weakly opalising wort ranged between of 4.44–5.18 (4.03–4.72) EBC units (95% probability), the average being 4.81 (4.37) EBC units. For the wort samples evaluated as opalising worts, the wort values varied from 7.35–8.77 (6.92–8.26) EBC units and the average was 8.06 (7.59) EBC units (Table 5).

It was found out that the visual method could be replaced by the nephelometric method. In the LRV set (132 samples), the relationship between the haze at 15° or 90° and wort clarity was high (0.89) (Table 6). In the large R set it slightly declined (r = 0.87) (Table 6).

CONCLUSION

In this study, the effects were followed of the barley variety, location, and year on the wort haze. Further, the results achieved with the visual method and those obtained with the nephelometer were compared.

It was found that the visual method can be replaced by the nephelometric method as the correlation coefficient between the haze at 15° or 90° (measured nephelometrically) and wort clarity (measured visually) was 0.89.

Based on the values of haze and clarity, the set of the studied varieties of spring barley was split into clearly differentiated groups. Subsequently, according to these values two varieties (Annabell, Nordus) with a marked tendency to form wort hazes were detected.

Wort haze was significantly affected by the variety (30–40%). The effect of the location varied from 13–22% while the effect of the year was small. The values of haze were also largely affected by numerous other influences unknown.

The relationship between the wort clarity or haze and the other technological parameters in most cases was not statistically significant. Only in relative extract at 45°C, diastatic power, apparent final attenuation and saccharification time, it was statistically significant or highly significant. With respect to the complexity of the technological parameters studied, this relationship was markedly weak.

References

Back W (2005): Ausgewählte Kapitel der Brauereitechnologie. Fachverlag Hans Carl, Nürnberg.

Bamforth C.W. (1999): Beer haze. Journal of the American Society of Brewing Chemists, **57**: 81–90.

Basařová G. (1992): Pivovarsko-sladařská analytika. 1 díl. Merkanta, Praha.

Dadic M., Van Cheluwe J.E A., Valyi Z. (1976): Alkaline steeping and the stability of beer. Journal of the Institute of Brewing, **82**: 273–276.

Douglas P., Meneses F.J., Jiranek V. (2006): Filtration, haze and foam characteristics of ferment wort mediated by yeast strain. Journal of Applied Microbiology, **100**: 58–64.

EBC Analysis Committee (1998): Analytica-EBC. Verlag Hans Carl Getränke-Fachverlag, Nürnberg.

Erdal K. (1986): Proanthocyanidin-free malt in malting and brewing – centenary review. Journal of the Institute of Brewing, **92**: 220–224.

Hlaváček F., Lhotský A. (1966): Pivovarství. SNTL, Praha.

Krottenthaler M. (1997): Praxistauglichkeit von Wintergerstenmalz. Brauindustrie, **82**: 25.

MEBAK (1997): Brautechnische Analysenmethoden. Band I, Freising-Weihenstephan.

Papp A., Winnewisser W., Geiger E., Briem F. (2001): Influence of (+)-catechin and ferulic acid on formative of beer haze and their removal through different polyvinylpolypyrollidone-types. Journal of the Institute of Brewing, **107**: 55–60.

Ponton I. D. (1988): Proanthocyanidin-free malt. Ferment, **1**: 33–39.

Prokeš J., Hartmann J. (2001): Evaluation of turbidity in malt of Czech origin. Monatsschrift für Brauwissenschaft, **54**: 237–241.

Prokeš J., Špunarová M. (1996): Zlepšená surovina pro výrobu sladu a piva. Kvasný Průmysl, **42**: 209–214.

Psota V., Jurečka D. (2001): Registration of spring barley varieties in the Czech Republic in the year 2001. Kvasný Průmysl, **47**: 154–158.

Psota V., Jurečka D. (2002): Registration of spring barley varieties in the Czech Republic in 2002. Kvasný Průmysl, **48**: 154–159.

Psota V., Horáková V., Kopřiva R. (2008): Barley varieties registered in the Czech Republic in 2008. Kvasný Průmysl, **54**: 186–192.

Robinson D.L., Mann A.D., Digby P.G.N. (1996): REML. Biomathematics and Statistics. Scotland, Edinburg.

Siebert K.J., Lynn P.Y. (2003): Effects of alcohol and pH on protein-polyphenol haze intensity and particle size. Journal of the American Society of Brewing Chemists, **61**: 88–98.

Sladký P., Dienstbier M. (2000): Modulární turbimetr pro praktickou výuku v pivovarství a nápojovém průmyslu. Kvasný Průmysl, **46**: 280–283, 321–324.

Stewart G.G. (2004): The Chemistry of beer instability. Journal of Chemical Education, **81**: 963–968.

Wackerbauer K., Zufall C., Holscher K. (1992): Der Einfluss von Hammermühlenschrot auf die Würze, Brauwelt, **29**: 1366–1374.

Received for publication September 8, 2008
Accepted after corrections May 19, 2009

Corresponding author:

Ing. Vratislav Psota, Výzkumný ústav pivovarský a sladařský, a.s., Sladařský ústav Brno, Mostecká 7, 614 00 Brno, Czech Republic

tel.: + 420 545 578 703 kl. 27, fax: + 420 545 321 225, e-mail: psota@brno.beerresearch.cz