THE EVALUATION OF *HUMULUS LUPULUS* UTILISATION DURING WORT BOILING AND IN BEER

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ABSTRACT

Beer brewing is an intricate process encompassing mixing and further elaboration of four essential raw materials, including barley malt, brewing water, *Humulus Lupulus* **(**hops) and yeast. Particularly hops determine to great extent typical beer qualities such as bitter taste, hoppy flavour, foam stability and bacteriostatic properties. The bitter taste of beer is derived from hop added to the wort during brewing. In the wort boiling process, the hop α-acids are isomerised into the bitter-tasting iso-α-acids and dissolve in wort. Utilisation of *Humulus Lupulus* in brewing process depends from duration and temperature of boiling process, pH value and wort extract, varieties, ages and added amount of hops. During the wort boiling process, *Humulus Lupulus* are added at different times and in various amounts. Therefore for determination the desired value of beer bitter taste is very important to study the utilisation of *Humulus Lupulus* in beer. The goal of this research was to determine the *Humulus Lupulus* utilisation by adding the various amounts of hops at the different stages during wort boiling process with different extracts. The investigation was performed in the real production process: boiling wort duration of 20, 60 and 90 minute, pH value from 5.2 to 5.8 and wort original gravity of 10.5, 13 and 15 %. The varieties of Aurora (Super Styrian) and Styrian Golding hops were added in various amounts into the wort during wort boiling process. Beer bitterness was estimated according to European Brewery Convention, 2004, Analytica-EBC, 9.8. Utilisation of *Humulus Lupulus* was determined by three unknown linear equations system. Based on the obtained results it was seen that the boiling duration of *Humulus Lupulus* and original wort gravity have sensitive impact in bitterness intensity.

Keywords*: beer, brewing, Humulus Lupulus utilisation, alpha acids, bitterness.*

INTRODUCTION

Beer is one of the most extensively consumed beverages world-wide. A pleasant and consistent bitterness is an essential flavour attribute of beer. The bitter taste of beer is derived from hops (*Humulus Lupulus*) or hop extracts added to the wort during brewing (Hough *et al*., 1999). These products are not only responsible for the bitter taste of beer, but they also play an essential role in enhancing the foam stability of beer and have antimicrobial properties which help to extend product shelf-life (Simpson *et al*., 1994; Lajçi *et als*., 2012). Hops, *Humulus Lupulus*, are essential and unique as a raw material in beer production. The lupulin glands of the female hop flowers (hop cones) contain resins comprising principally hop acids, hop essential oil and particular, prenylated hop polyphenols. The hop acids, part of the soft resin fraction, consist of α-acids and β-acids.

α-Acids are however by far the most important constituents of hop resins. They represent a mixture of three major analogues, namely cohumulone, humulone, and adhumulone. The most important chemical conversion occurring during wort boiling process is the thermal isomerisation of the α-acids, which are almost tasteless, into the bitter tasting iso-α-acids via an acyloin type ring contraction. The degree of bitterness imparted by hops depends on the degree of isomerisation of the insoluble α-acids during the wort boiling (Jaskula *et al*., 2009). β-acids (lupulone, colupulone, and adlupulone) contribute significantly less to overall bitterness than α-acids (Keukelerie *et al*., 2009). Quantitative analyses of the α-acids reveal rather high concentrations in the wort but only low levels in beer (Haseleu *et al*., 2010). This is because during wort boiling the most important chemical conversion of the brewing of beer occurs: the thermal isomerisation of the α-acids (humulone, cohumulone and adhumulone) to the iso-α-acids (isohumulone, isocohumulone, and isoadhumulone) via an acyloin-type ring contraction (Keukeleire, 1999). Isomerisation in a boil is not very efficient, perhaps no more than 50 % of the α-acids are isomerised and less than 25 % of the original bittering potential survives in the beer (Bamforth, 2000). Utilization (%) is defined as the ratio of the amount of iso-α-acids found in the finished beer to the amount of hop α-acids added to the wort (McMurrough *et al*., 1986).

When considering only the wort boiling step, hop bittering appears to be difficult to control since, next to the low solubility of α -acids and losses by adsorption of bittering substances on the protein break, the yield of iso-α-acids in the kettle is influenced by numerous variables, such as temperature, length of boil, pH of the wort, specific gravity, hopping rate, type of hop product used, and kettle design (Benitez *et al*., 1997). α -acids isomerisation is highly time and temperature dependent and low yields are obtained when a short boiling process or a relatively low temperature are applied (Malowicki and Shellhammer, 2005), increasing wort gravity shows a negative effect on isomerisation yields.

The aim of this work was to investigate the effects of added amount of hops during various stages of wort boil, original wort and beer gravity and length of boil that may impact hops α-acids isomerisation and utilisation in wort and beer.

MATERIAL AND METHODS

Material

The evaluation of *Humulus Lupulus* utilisation during wort boiling and in beer was investigated at the Beer Factory, Sh. A. "Birra Peja". The experiments were conducted using two hops varieties: Aurora (super styrian) (α-acids 8.5 %) and Styrian Golding (α-acids 5 %) of the forms "Regular Hop Pellets Type 90". HMEZAD exim d.d. Žalec.

Aurora (super styrian) has an intense and pleasant hoppy aroma. It is a Slovenian favourite hop, accepted by brewers as variety contributing excellent aroma harmonically combined with its moderate bitterness. It contains 7 % - 13 % of α-acids (22 - 26 % of cohumulone). The alpha/beta-acid ratio is around 2.7. The essential oil content varies from 0.9 % to 1.6 % (ml/100 g cone dry basic). This variety has very good storage stability. Beer prepared with this variety has good organoleptical scores. The yield of α-acids is very good when brewing with this variety. Aurora is very suitable for extraction and for combination with other varieties in the brewing process. It is used in both Pilsener and lager brewing.

Styrian Golding is a traditional Slovenian hop variety belonging to the Fuggle ecotype. It's a world-renowned aroma hop with widespread usage in both ale and lager brewing. Typical beer styles include English and Belgian-style ales, Lagers and Pilsners. Styrian Golding is an aroma hop variety, its aroma is very mild and it excels by noble hoppy flavour. It contains 2.8 % - 5.5 % of α -acids (27 % - 31 % of cohumulone). The alpha/beta-acid ratio is around 1.7. The yield of α - acids is good when brewing with this variety. The essential oil content varies from 0.6 % to 1.2 % (mls/100g of dry hops). Styrian Golding has very good storage stability. Beer prepared with this variety has very good organoleptical scores for its bitterness, taste and aroma.

Beer and wort samples

The samples of wort and beer for evaluation of hops utilisation during wort boiling and in beer were taken from normal production process at the Beer Factory, Sh. A. "Birra Peja".

Regents and apparatus

For determination the wort and beer bitterness intensity it was used a spectrophotometer type Agilent 8453 UV-visible Spectroscopy System. The reagents used for this investigation were p.a. quality: Hydrochloric Acid 6M (Sigma, P/N 258148), 2,2,4 Trimethylpentane (isooctane)(Sigma, P/N 155012) and bi-distilled water.

Experiment protocol

The laboratory work was organized in two stages. In the first stage the bitterness was measured in wort while in the second stage in the beer. Samples were prepared from beer with the original wort extracts of 10.5 %, 13 % and 15 %. Beer with original extracts of 13 % and 15% from the beer filling phase was diluted with water to the 10.5 % original extract. For each case, were prepared three samples with different amount of added hops.

During wort boiling process, the hops were added in three doses. Styrian Aurora hop was added in the first and second stages of wort boiling process while in third stage the Styrian Golding hop was added into wort. The first stage of wort boil was 90 minutes (hop was added at the beginning of wort boiling), the second stage of wort boil was 60 minutes (hop was added 30 minutes before boil ended) and third stage was 20 minutes (hop was added 20 minutes before boil ended). The amount of added hops at three stages of wort boiling process, the content of α-acids in hop and wort boiling time are presented in table 1.

Table 1. The amount of added hops into the wort, measured α-acids in wort and boiling time.

Type of beer	Beer with original extract 10.5%			Beer with original extract 13%			Beer with original extract 15%		
Wort boiling time, min	90	60	20	90	60	20	90	60	20
α- acids, %	8.5	8.5	5.0	8.5	8.5	5.0	8.5	8.5	5.0
Sample I, added hop, kg	10	5	15	15	10	15	20	10	20
Sample II, added hop, kg	10	8	10	15	8	15	20	15	20
Sample III, added hop, kg	15	5	15	15	10	20	20	15	15

Methods

Methods for analyses of bitterness in wort and beer used in experiments consist of determination of α-acid concentration by spectrophotometer according to method EBC 2004, (European Brewery Convention, 2004, Analytica-EBC, 9.8 and Analytica-EBC, 8.8). The traditional and internationally approved method for bitterness determination in beer involves the extraction of iso-α-acids from acidified beer into iso-octane, followed by a centrifugation step, and photometric measurement at a wavelength of 275 nm against a reference of pure iso-octane.

Bitterness compounds mainly iso-α-acids was determined by extracting of iso-α acids from acidified samples into iso-octane. First the samples of beer were decarbonised without losing beer foam. A 10 ml sample of degassed beer was pipetteted (5 ml of wort samples for determination bitterness in wort) and acidified with 0.5 ml HCl 6 M, and mixed with 20 ml pure iso-octane. The tubes were shaken for 15 minutes. Samples then left to stand for 30 minutes before measurements. The spectrophotometer was calibrated with pure iso-octane. The absorbance of the iso-octane layer was measured in a 1 cm cuvette optical path at optical density of 275 nm against the pure referent iso-octane. The bitterness of wort and beer were determination by UV- VIS spectrophotometer type Agilent 8453 UV-Visible. The optical density of the acidified solvent extract is multiplied by a factor to produce an analytical value, measured as International Bitterness Units (IBU) = Optical Density at 275 nm x 100 (Verzele *et al*., 1999). Bitterness is expressed in IBU (International Bittering Units), with one IBU equating to 1 mg of dissolved alpha acids. For most beers, the range of bitterness was once between 20 and 60 bitterness units (IBU), but for the majority of beers today it is between approximately 6 and 30 IBU.

Determination of bitterness in wort and beer

Bitterness units for wort (IBU) = $100 \cdot A_{275}$

Bitterness unit for beer (IBU) = $50 \cdot A_{275}$

Extension was measured at 275 nm, A_{275}, against pure iso-octane referent, where A_{275} is absorbance at 275 nm.

Degree of utilization

The degrees of utilization of the bitterness potentials in the wort and beer were calculated through the system with three linear equations with three unknowns and the solution of the system according to the Cramer formula.

The general form of three linear equations system with three unknowns is:

$$a_{11}x_1+a_{12}x_2+a_{13}x_3=b_1$$

$$a_{21}x_1+a_{22}x_2+a_{23}x_3=b_2$$

$$a_{31}x_1+a_{32}x_2+a_{33}x_3=b_3$$

where a_{ik} numbers (i, k=1, 2, 3) are the coefficients of the amount of alpha acids dosage and numbers b_i (1, 2, 3) are free restrictions of this system, measured bitterness value, $D=\det[a_{ik}]_1^3$ is called the main determinant, while D_1, D_2 and D_3 are called characteristic determinants of the system.

RESULTS AND DISCUSSIONS

During wort boiling process insoluble α-acids are converted to soluble and bitter iso-α-acids. To increase conversion of α-acids it must be controlled the wort boiling parameters: slightly alkaline conditions, divalent metal ions (especially Mg^{2+}) as a catalyst and boiling times.

In the context of wort boiling, conditions are much more difficult to control and many other reactions can occur. Humulones have many double bonds and harsh conditions such as boiling in the presence of air results in a number of reactions and a complex mixture of products, some of which are bitter tasting and some are not. The obtained results of laboratory experiments carried off by the absorbance for determination the bitterness of wort and beer are shown in tables 2 and 3.

Table 2. The bitterness values, IBU, of wort with original extracts of 10.5, 13 and 15 %.

Samples	First experiment	Second experiment	Third experiment
Wort with original extract 10.5 %	30.64	32.73	39.07
Wort with original extract 13.0 %	37.31	35.46	40.24
Wort with original extract 15.0 %	41.0	45.57	44.80

From the results in table 2 it is seen that the bitterness of wort was higher in the wort with higher wort extract and that is higher in the second experiment.

Table 3. The bitterness value, IBU, of beer with original extract of 10.5, 13 and 15 %.

Samples	First experiment	Second experiment	Third experiment
Beer with original extract 10.5 %	20.16	21.25	25.24
Beer with original extract 13.0 %	21.95	20.55	23.22
Beer with original extract 15.o %	21.20	23.93	22.92

From the results in table 3 it is seen that the bitterness of beer depend on the amount of added hops and the length of wort boiling.

The most important step in wort boiling process is the isomerisation of α-acids to iso-α-acids which retain the bittering quality of the α-acids and are soluble in beer (unlike the original α-acids). The amounts of iso-α-acid were measured by spectrophotometry.

Determination the degree of hops utilization

The degree of hops utilisation of the bitterness potential, measures the percentage of α-acids added to the wort which was actually utilised were calculated as:

% hops utilization = (iso-α-acids in beer) / [(α-acids added into wort) X 100]

The obtained results of isomerization of α-acids to iso-α-acids during wort boiling process and in beer are presented in the tables 4 and 5.

Table 4. The hops utilisation values in wort as a function of boiling length.

Wort with original extracts (%)	Hops utilisation in wort (%)		
	Boiling time, 90 min	Boiling time, 60 min	Boiling time, 20 min
10.50	55.10	51.43	32.26
13.00	45.15	42.23	26.32
15.00	39.90	37.24	23.30

Our results in table 4 indicate that for the first twenty minutes of the wort boil the isomerisation of α-acids remains low, but rapidly increases after this time. Maximum isomerisation occurs around 60 minutes. The isomerisation degree of α-acids is lower with the increase the original wort extracts.

Table 5. The hops utilisation values in beer as a function of boiling length.

Beer with original extracts (%)	Hops utilization in beer (%)		
	Boiling time, 90 min	Boiling time, 60 min	Boiling time, 20 min
10.50	35.875	33.484	20.993
13.00	32.825	30.699	19.137
15.00	30.028	28.035	17.538

From the results in the table 5 it is seen that degree of hops utilisation in beer increases with the increase the wort boiling time but are lower with increasing the original wort extracts.

The isomerisation value of α-acids to iso-α-acids (hop compound in wort and beer), hop utilisation values, at pH value of 5.1-5.8, as function of original wort extracts (10.5, 13.0, and 15 %), and wort boiling time (20, 60 and 90 minutes), at wort boiling temperature about 100°C are shown in figures 1 and 2.

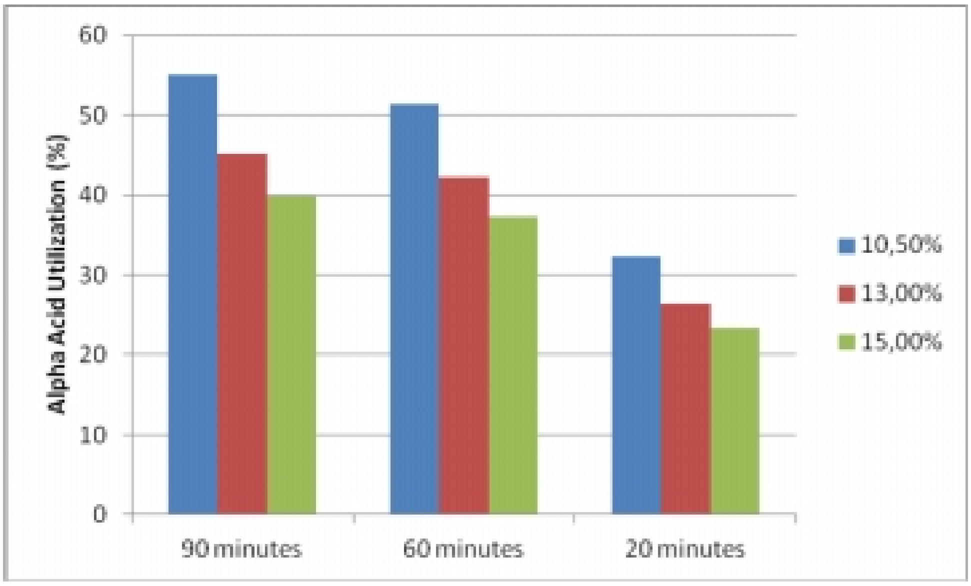

Figure 1. Hops utilization in wort as a function of wort boil time and original extracts.

The hops utilization values in wort were increased with the increasing of wort boiling length, but it was higher with the wort extract 10.50 % and lower with the wort extract of 15.00 %.

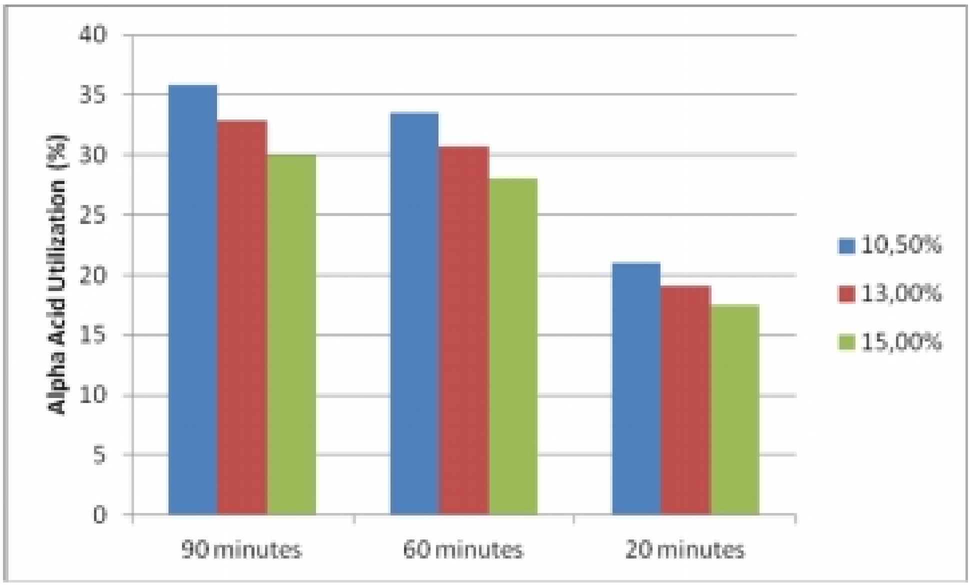

Figure 2. Hops utilization in beer as a function of wort boil time and original extracts.

From figure 2 it is seen that degree of hops utilisation in beer increases with the increase the wort boiling time but are lower with increasing the original wort extracts.

CONCLUSIONS

In this paper, the hops utilisation during wort boiling process and in beer has been studied as a function of original wort and beer extracts, length of boiling time and dosages of hops into wort. The hops utilisation in wort for the first 20 minutes of the boil was low, but rapidly increases after this time. The hops utilisation rate in wort lowered after about 60 minutes. It is depend also from the original wort extract and it was higher with lower wort extract, and increased with boiling time.

It is very important that during the beer production processes to know the degree of hops utilisation for production the beer with desired bitterness values. For the beer with original extracts of 10.5, 13 and 15 % the hop utilisation for two types of used hops, various added dosage of hops and various length of boiling time were found as following:

- The hops utilisation for the beer with original extract of 10.5 % for the first added dosage was about 35.87 %, for the second dosage was 33.4 % and for the third dosage was 20.99 %.
- The hops utilisation for the beer with original extract of 13 % for the first added dosage was about 32.8 %, for the second dosage was 30.7 % and for the third dosage was 19 %.
- The hops utilisation for the beer with original extract of 15 % for the first added dosage was about 30 %, for the second dosage was 28 % while for the third dosage was 17.5 %.

The improving hop utilisation is very important for beer quality and financial performances of the brewing.

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