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IMPROVING THE HOP UTILIZATION IN THE BEER BIOTECHNOLOGY

Mudura Elena , Sevastita Muste, Maria Tofana, Sonia A. Socaci, Veronica Goina

University of Agricultural Sciences and Veterinary Medicine,
Faculty of Agriculture, 3-5 Manastur Street, 400372 Cluj-Napoca,
e-mail: elenamudura@yahoo.com

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Abstract : The rate of isomerization of alpha acids to iso-alpha acids (the bittering compounds in beer) was characterized over a representative pH, wort concentration, time boiling and temperature range during the boiling portion of the brewing process. Because of the complex wort matrix and interfering interactions occurring during real wort boiling (i.e., trub formation and α-acids/iso-α-acids complexation), this investigation on α-acid isomerization was performed in wort solution as a function of time (60−120 min), pH variation (5.1-5.8) and wort original gravity (10-14^{o}Plato). Precise understanding of isomerization kinetics allows improved accuracy in hopping rate calculation to achieve target concentrations of bitter compounds in wort, despite varying pH as the kettle approaches boiling, or as wort encounters a lag time prior to entering a heat exchanger for cooling. Also, understanding of isomerization is essential if novel regimes are to be explored for potential bioactive compounds in final products.

INTRODUCTION

Hops play a significant role in beer flavor and quality. The quality of hop bitterness is a subtle but powerful driver of beer quality and contributes significantly to the "drinkability" of the final product.

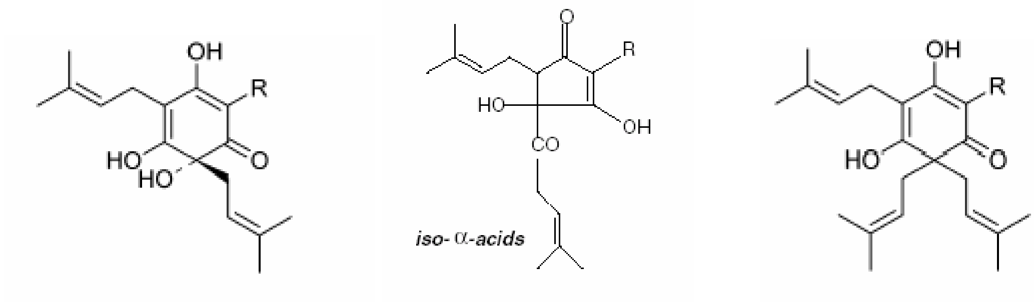
Bitterness in beer is derived primarily from alpha acid isomerization, which occurs during wort boiling. The alpha acids represent a group of chemically similar compounds, the proportions of which vary greatly depending upon variety. The cohumulone content is varietal dependent and it is often used as a quality index in the selection of existing and new hop varieties. A direct relationship between alpha acid composition and bitter quality is speculative, and reports indicate that varieties high in cohumulone lead to an inferior bitter quality in the finished beer. Despite the paucity of scientific evidence, cohumulone levels are still used an index for hop quality. The influence of hop polyphenol on hop bitter intensity, bitter quality (harshness versus smoothness) and tannin astringency is also being explored by brewers. In other food systems, monomers and polymers of flavanols elicit bitterness and astringency depending upon their degree of polymerization yet the sensorial effect of these compounds in beer has not been adequately determined. Dr. Shellhammer has investigated bitter quality in addition to temporal bitter parameters (intensity, duration, etc) of hop-derived polyphenols in beer and discovered that these compounds contribute significantly to beer bitterness and the quality of bitter from hop polyphenols may be varietal-dependent.

Due to their antioxidant power, hop polyphenols may also contribute positively to beer flavor stability. While the majority of the polyphenolic content of beer comes from malt, hop polyphenols contribute up to one third of the total phenolic load in beer and therefore cannot be ignored in regard to their effect on flavor stability and quality.

Beer is the most widely consumed beverage in the world. Of all the herbs that have been used in beer, only the hop (*Humulus lupulus* L., Cannabinaceae) plant has gained widespread acceptance and is regarded as an essential raw material in the brewing industry. Hops are perennial plants grown on trellises, and different varieties are derived from breeding programs. The hop plant is dioecious and cultivated in most temperate zones of the world for its female inflorescences, which are commonly referred to as hop cones or simply hops. The female flower clusters are partly covered with lupulin glands, while male flowers have only a few glands in the crease of their anthers and on their sepals. The resin secreted by these glands contains bitter acids, essential oils, and flavonoids (flavonol glycosides, prenylflavonoids, and proanthocyanidins). Hops or hop products account for the bitter taste and the flavor of beer. In addition, hops have a favorable influence on the stability of beer foam and contribute to the microbiological stability of beer. Brewers may benefit from proper selection of particular hop varieties that add subtle tastes and flavors. Among various hop constituents, hop proanthocyanidins have attracted increasing attention, and have been considered as the most reactive of hop polyphenols.

Antioxidants of plant origin are an important component of food with a positive effect on human health. They are able to eliminate from the organism reactive oxygen and nitrogen radicals that irreversibly damage live tissues and induce serious diseases. Oxidative damage is considered to be the main cause of ageing and of several degenerative diseases, such as cardiovascular disease and cancer. Hop is not a direct food material, but the antioxidants present in hops are of considerable importance in the brewing industry. They act in the course of beer production and storage as protection against the origin of undesirable sensorial active substances of stale flavor and have a favorable health effect on beer consumer. Polyphenols play a key role in hop antioxidant activity as they have antioxidant, antimutagenic, anticarcinogenic, antimicrobial, antithrombotic, and anti-inflammatory effects and in addition they regulate blood pressure and blood glucose levels.

Today the brewing industry uses dozens of market hop varieties differing in content and composition of secondary metabolites, first of all resins and essential oils; therefore differences in their antioxidant properties can be rightfully supposed. The objectives of this paper are to analyze and optimize the extraction and izomeration of active substances from hops during wort boiling in brewing process.



Humulones *Iso-humulones* *Lupulones*

Fig.1 The structure of α- and β-acids

Alpha acids are **humulones**: these have the main bittering potential, though they are formed into isohumulones or iso-α-acids in the wort boiling process. β-acids are **lupulones**. Here is the basic structure of the humulones and lupulones, where R is an alkyl group:

Analogues vary in the nature of the side chain present. The most important examples are

$R = CH_2CH(CH_3)_2$ isovaleryl = humulone (lupulone)

$R = CHCH_3)_2$ isobutyryl = cohumulone (colupulone)

MATERIAL AND METHODS

Material. The experiments were conducted using two hops varieties: Styrian Aurora and Warrior. Styrian – Aurora is a Slovenian favourite hops, accepted by brewers as variety contributing excellent aroma harmonically combined with its moderate bitterness. Alpha acids of Aurora are between 6,5-8,5%. Beer prepared with this variety has good organoleptical scores. The yield of alpha-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.

Warrior is a general-purpose bittering hop that offers a neutral, clean bittering primarily in ale and lager styles. Alpha acids are in the typical to high bittering hop range of 15 to 17%. It has a relative low cohumulone content which contributes to a smooth, pleasing bitterness. This is a relatively new variety that was first bred at Yakima Chief Ranches in USA.

Regents and apparatus. For determination of hops bittering concentration it was used a Titration system wih automatic sample changer from Schott Instruments and UV- VIS spectrophotometer typ UV 1700 from Schimadzu Instruments.

The solvents from extraction were p.a. quality: toluene, methilic alcohol, ethylic alcohol, clorhidric acid, lead acetate and izooctane.

Experiment protocol. The laboratory work protocol is organized in three steps. First step is preparing the three wort samples from malt and water (1:4 ratio), using a infusion mashing regime. The wort concentrations were set at 10, 12 and 14^o Plato. From $12\ ^o$ P wort were prepared another six samples: three with 5.3 pH and three with 5.1, 5.3, 5.8 pH using phosphoric acid(85% technical grade). Hoping rate was setting at 8 g alpha-acid/hl wort.

The second step consist of boiling the wort samples with both varieties of hop: Warrior and Styrian Aurora for 60-120 min at atmospheric pressure. The sample were immediately chill down to 10^oC to prevent microbiological contamination.

Methods for analyses of hop active bittering compound in hop varieties used in experiment consist of determination of alpha acid concentration by Lead Conductance Value (LCV) according to method 7.4 (Analityca EBC 2006). Alpha-acids form lead salts which are insoluble in methanol. A solution of lead acetate / acetic acid is titrated into a methanol solution of hop extract while monitoring conductance; initially there is no change in conductance as a yellow lead salt of the α-acids precipitates. Then the conductance increases: extrapolation of the two straight line curves gives the end point.

The traditional and internationally approved method for bitterness determination in beer involves the extraction of iso-alpha 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 (European Brewery Convention, 2006, Analytica-EBC, 7.8.). The optical density of the acidified solvent extract is multiplied by a factor to produce an analytical value, measured as Bitterness Units (BU): BU = Optical Density at 275 nm x 50.

RESULTS AND DISCUSSIONS

The performances of laboratory trials for determination of LCV value for **Styrian Aurora and Warrior** hop pellets are presented in table 1.

Table 1.

Alpha acid content (w/w) in Styrian Aurora and Warrior hop pellets

Hop Varietes	Moisture content %	Lead Acetate Titer	Mass sample (g)	Volum lead acetate (ml)	Alphaacids (dry matter) (%)	Avarage (%)
WARRIOR	7,59	1,9974	10,00	7,65	17,26	17,35
		1,9974	10,00	7,47	17,26	
		1,9974	10,00	7,65	17,26	
		1,9974	10,00	7,56	17,63	
STYRIAN AURORA	7,03	1,9974	10,00	1,98	4,56	4,79
		1,9974	10,00	1,98	4,93	
		1,9974	10,00	1,98	4,74	
		1,9974	10,00	1,98	4,93	

During wort boiling insoluble α-acids are converted to soluble and bitter iso-α-acids. To increase conversion of α-acids you have to control the wort boiling parameters: slightly alkaline conditions, divalent metal ions (especially Mg^{2+}) as a catalyst, 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 brewing trials were carried off to improve the isomerization of alpha acids into iso-alpha acids during wort boiling process. The results are presented in table 2 and 3.

Table 2

Bitter units content in brewing trial with Styrian Aurora Hop Variety

Hop Variety		Process Parameters	Optical absorbance at 275 nm	Bitter units (BU)	Hop Utilisation %
STYRIAN AURORA	Boiling time	60 min	0,307	15	18,75
		90 min	0,347	17	21,25
		120 min	0,357	18	22,50
	Conc.	10°P	0,338	17	21,25
		12°P	0,396	20	25,00
		14°P	0,406	20	25.00
	pH	5,1	0,436	22	27,50
		5,5	0,547	27	33,75
		5,8	0,625	31	38,75

Table 3

Bitter units content in brewing trial with Warrior Hop Variety

Hop Variety		Process Parameters	Optical absorbance at 275 nm	Bitter units (BU)	Hop Utilisation %
WARRIOR	Boiling time	60 min	0,241	12	15,00
		90 min	0,277	14	17,50
		120 min	0,347	17	21,25
	Conc.	10°P	0,298	15	18,75
		12°P	0,342	17	21,25
		14°P	0,351	18	22,50
	pH	5,1	0,344	17	21,25
		5,5	0,366	18	22,50
		5,8	0,381	21	23,75

The most important step in boiling is the isomerization 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 amount of iso-acid is measured by spectrophotometry.

Hop utilisation measures the percentage of α-acids added to the wort which are actually utilised.

$$\% \text{ utilisation} = (\text{iso-acids in beer}) / (\alpha\text{-acids added to kettle}) \times 100\%$$

Bitterness units in mgL^{-1} of equivalent iso-α-acid give an indication of the total bitterness. Note that because other substances in hops (such as the lupuolones), bitterness units is higher than the actual iso-α-acid content of beer. In traditional brewing in which whole hops are added to wort, only about 25% of the humulones present in hops are converted into beer-soluble bitter substances, the majority of which are iso-humulones.

The results of laboratory experiments demonstrate that the hop varieties with low alpha (Styrian Aurora) acids contents have a better utilization in wort kettle than high alpha hops (Worrior). This yield, increase with boiling time, wort concentration and pH. Unfortunately, the high pH value is not a good decision because a lot of unlikable reactions could be develop for other wort chemical compounds(proteins and poliphenols).

CONCLUSIONS

One of the most important roles of the boil brewers carry out is the production of bittering compounds from the isomerisation of hop alpha acids. Isomerisation is a chemical process which involves molecules being converted from one configuration to another. Alpha acids are dubbed iso-alpha acids once isomerised but they contain the same amount of atoms, merely in a different configuration. The isomerisation reaction is favored by alkaline conditions with a pH of around 9 being optimal, but these conditions are never met during the boil and this explains the notoriously poor level of hop utilization during the brewing process which rarely exceeds 40%. Wort becomes steadily more acidic during the boil due to the formation of break material so the extraction of bittering compounds becomes less efficient as the boil goes on. Along with specific pH conditions, magnesium or another divalent ion and a vigorous boil are required to carry out the isomerisation reaction.

The gravity of the wort can further influence the isomerisation reaction with high gravity worts impeding the progress of the isomerisation step. The loss of precious bittering compounds is bad enough, but the brewers can expect to further lose what little bittering has been achieved through adsorption to yeast and filter material and also some will be scrubbed by CO2 production during fermentation.

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