Dynamic Evolution of Bitterness Units in Beer Worts: Modeling and Concerns

Ivi do Nascimento[a], Lucas Calado[b], Ninoska Bojorge[c], Vinícius Silva[d], Fernando Peixoto[e*]

[a,c]*Departamento de Engenharia Química e de Petróleo. Universidade Federal Fluminense. Rua Passo da Pátria, 156 – 24210-240 - Niterói – RJ – Brazil*

[b]*Escola de Química, Universidade Federal do Rio de Janeiro, Cidade Universitária, CEP: 21949-900, Rio de Janeiro, RJ, Brazil*

[d]*Departamento Engenharia de Telecomunicações. Universidade Federal Fluminense. Rua Passo da Pátria, 156 – 24210-240 - Niterói – RJ – Brazil*

[e]*Seção de Engenharia Química do Instituto Militar de Engenharia. Praça Gen. Tibúrcio, 80 - Urca, Rio de Janeiro - RJ, 22290-270 – Brazil*

[a]*Email: ivicnascimento@gmail.com,* [b]*Email: Lucascalado1991@hotmail.com,* [c]*Email: nbojorge@id.uff.br,* [d]*Email: viniciusnhs@id.uff.br,* [e]*Email: fpeixoto@ime.eb.br*

Abstract

Beer bitterness is reported in International Bitterness Units (IBU), which is the concentration (in ppm) of iso-alpha-acids in the product. Such acids result from the isomerization of alpha-acids originally found in hops carried out by boiling the beer wort. This concentration can be measured by HPLC, which led, in the past, to some empirical predictions for the effects of boil time and wort density on the dynamic evolution of IBU in barley worts. Since HPLC is an expensive and time onerous procedure, the organizations devoted to the standardization of procedures in the brewing industry established protocols that indirectly evaluate the IBU by spectrophotometry. This work investigated the dynamic evolution of IBU values as a function of the wort density when evaluated by this standard procedure, which involves the extraction with iso-octane and the UV absorbance measurement in a spectrophotometer.

----

* Corresponding author.

Three barley worts were prepared with dry malt extract (DME) having specific gravities (SG) of 1.030, 1.040 and 1.050. These worts and a fourth sample constituted of distilled water (SG = 1.000) had their pH buffered in 5.2 and were boiled under atmospheric pressure. During the boiling step, Czech Saaz hop pellets were added according to a time schedule (1, 3, 5, 10, 25, 40 and 60 minutes). All reactions were conducted in small (500 ml) aluminum reactors with triplicates for each isomerization time. Finally, the IBU samples were measured and used to fit non-linear empirical models for the IBU evolution in the wort, based on a maximum likelihood statistical criterion and deterministic optimization methods. Results indicated that IBU values, evaluated by the standard procedure, rise to their final value within a few minutes instead of one hour, which is traditionally assumed.

Keywords: iso-alpha acids; spectrophotometer; IBU; mathematical model; beer; bitterness.

1. Introduction

Hops are natural preservatives of beer, contributing with flavor, aroma and bitterness, the last one being produced by the isomerization of alpha-acids inherent to this cannabinaceous flower. Such features are due to the presence of special chemical compounds in the lupulin glands of the hop flower, known as cones [1]. Some of these substances are the α-acids, which are directly related to the bitterness of the final product [2, 3]. There are three types of α-acids relevant for the characteristics of beer, name, humulone, cohumulone and adhumulone [4]. Although other constituents of beer contribute to the final bitterness, it is recognized that the main substance responsible for bitterness is the iso-α-acids derived from hops [5, 6, 7, 8]. Each isomer has two stereoisomers: cis-isohumulone and trans-isohumulone; cis-isocohumulone and trans-isocohumulone; and cis-adhumulone and trans-adhumulone. The main reason that iso-α-acids are more important for beer production, when it comes to bitterness and chemical and bacterial stability is that they are soluble in water and carry their properties to the final product non-isomerized counterparts. For the production of iso-α-acids, the α-acids must undergo an isomerization reaction at high temperatures [9]. The knowledge about such isomerization reaction is crucial to the quality control of the beverage. The observed efficiency of this reaction under different conditions does not exceed the range of 50-60% of conversion. In addition to the efficiency of the isomerization reaction, a term widely used in the brewing industry to refer to the bitterness of the beer is Utilization. The term is defined as the ratio between the amount of iso-α-acid found in the finished beer and the amount of α-acids initially added to the wort. Due to the loss of iso-α-acids in the post-boil, the utilization in the final product is even lower than the efficiency, falling in a range of 30-40% [3, 10]. The low yield of the isomerization reaction is affected by many factors, such as the non-solubility of alpha-acids in water [11], the pH of the wort [12, 13], mass transfer effects related to the technology used for hops pelletization [14, 15], the wort density, the boiling temperature [2, 9], the boiling time [16, 17], and beer storage [18, 19, 8]. Some studies have shown satisfactory results assuming a first-order kinetics model with Arrhenius behavior for the isomerization reaction [9]. The concentration of iso-α-acids in ppm is reported as International Bitterness Units (IBU), and several methods for its determination can be found in the literature, such as High-Performance Liquid Chromatography - HPLC [9, 20], ion-exchange chromatography [21], liquid chromatography with detection of ultraviolet absorbance or with mass spectroscopy [22, 23], electronic tongues [24] and even a low-cost method based on fluorescence [25]. However, the most used methodology for determining the bitterness beer in the industry is recommended by the American Society

of Brewing Chemists (ASBC) and the European Brewing Community (EBC). It involves extracting the iso-α-acids with iso-octane, measuring the extract absorbance at 275 nm, using a spectrophotometer, and multiplying the result by 50, which falls within the IBU scale [26, 14, 27] and practices as an approximation of perceived bitterness of the product. Although this routine is readily available to the brewing industry, it may have to be performed for every batch to ensure the quality of the final product, which naturally involves costs and time to perform the analysis. If one contemplates the development of a new product, several bitterness analyses may be necessary. In this scenario, reliable correlations for estimating IBU in beers are an interesting alternative. Although such correlations may not entirely replace an experimental IBU characterization, it can complement these, especially when designing new products. In this scenario, the present work is devoted to analyzing the IBU of beer wort during a typical boil stage, focusing on the variation with boil time and wort density and consequently presenting a correlation for predicting wort bitterness values. The standard method, proposed by ASBC and EBC was chosen, and the results (as well as the fitted model) showed that even the IBU final values are achieved much faster than what is assumed in the brewing industry.

2. Introduction

2.1. Isomerization

Each wort was prepared with dechlorinated water and enough commercial dry malt extract (DME) to the predefined specific gravity (SG) values of 1.030, 1.040 and 1.050, common in the beer industry. Heat was applied in bulk to ensure maximum solubilization, and the mixture was cooled to room temperature so that the spare malt would settle and separate from the wort. The supernatant was siphoned to another recipient and received a commercial buffer used in beer industry (pH Stablizer) to lock the pH in 5.2 (the usual value). The specific gravity was then determined from measurements made on a Brix refractometer, and the pH was measured with a digital pHmeter. Each wort was then distributed into custom-made 500 ml aluminum mini reactors (a total of 21) equipped with special loose-fit lids to guarantee that the inner pressure was maintained at atmospheric levels and that evaporation losses were minimized. The aluminum reactors were all placed in a heating bath simultaneously, and the internal temperature of each reactor was measured manually. As the temperature of all reactors reached 100ºC, time counting started. On predefined times (1, 3, 5, 10, 25, 40 and 60 minutes), three 500 mg hop loads were placed in sets of three reactors simultaneously to obtain a triplicate sample for each reaction time. Before being added to the respective reactors, the entire hop load was homogenized and crushed in a porcelain mortar. The hop varietal used was the Czech Saaz, from a 2018 harvest, with an α-acid content of 2.9 % (cohumulone 23-26 %). At the end of the experimental run, all reactors were simultaneously cooled by immersion in an ice bath, once α-acid isomerization is reported to be irrelevant under 85 °C [2]. Samples of 20 ml were drawn from each reactor, transferred to 50 ml amber glass flasks to avoid degradation [19, 17, 28] and stored at 5 °C for 15 days to precipitate oxidated forms of α-acid from the solution as these could interfere in the measurements. Luckily, such molecules are way less soluble than the iso-α-acids, especially at low temperatures. The complete procedure was also conducted using pure water (with no DME) for an additional set of experimental points, corresponding to SG = 1.000.

2.2. Extraction and absorbance measurements

The extraction and UV absorbance measurement procedures were based on the recommendations by ASBC and EBC and are detailed by Calado and co-workers [25]. All reagents (iso-octane, 1-octanol and hydrochloric acid) were purchased from Sigma-Aldrich (Sigma-Aldrich Brasil Ltda, São Paulo, Brazil) as P. A. standards, the mechanical shaker was a Wrist Action Shaker Model 75 (Burrell Scientific, Pittisburgh, U.S.A.) and the spectrophotometer was a Bel Spectro S-2000 (Bel Photonics, Milan, Italy). Iso-octane quality was assured by checking whether its absorbance was less than 0.010 at 275 nm [26]. We want to highlight three important aspects of the experiment: the need for an in-depth cleansing of the cuvettes between readings, once some iso-α-acids are easily adsorbed on the cuvette walls [29]; the fact that polymer recipients must be avoided in all steps, once they can interfere in the readings [28]; and the fact that some increase in temperature is expected under UV light, increasing the IBU, which can be avoided by expedite readings [29]. The interference of polypropylene is particularly acute, and its effect was analyzed in a previous work [28], in which an anomalous increase of the IBU was observed in samples that were prepared in tubes made with this polymer in opposition to what was observed in glass tubes. This indicates that, even with small contact times, organic solvents can chemically attack polypropylene tubes and interfere in the final bitterness measurements.

2.3. Modeling and parameter estimation

The experimental data, obtained by varying the density (i.e., specific gravity) and the isomerization (boiling) time as described in section 2.1, was used to fit a non-linear predictive model, described by

$$IBU = U \left(1 - exp(\beta \, t)\right) \frac{\alpha \, m}{V} \qquad (1)$$

where α stands for the percentage of α-acids in hops, informed by the supplier or measured according to specific procedures [30], m is the mass of hops, V is the wort volume, and t is the boil time. The parameter U is an effective utilization factor, which can depend on a variety of factors. It represents the actual fraction of α-acids that will contribute to bitterness in the finished beer. For obtaining the commonly used IBU values, the mass of hops should be entered in milligrams and the wort volume in liters. Equation (1) represents a simple form for calculating the IBU using a simple isomerization model where there is the production of iso-α-acids from α-acids, however, with no degradation of iso-α-acids, which is suitable for the boil times and pressures used in this study. It must be said that, even though this form is commonly used in the brewing industry, no systematic (and scientific) study was conducted and reported in the prevailing literature.

Model parameters were fitted using a Maximum Likelihood criterion, as (carefully) described by Kappel and co-workers [31]. In the present work, the optimization was conducted using a direct Nelder-Mead algorithm [32] and its coherent unbiased estimator gives the model "fundamental" variance:

$$S_y^2 = \frac{1}{n-q} \sum_i \sum_j \left(y_{ij} - \hat{y}_i\right)^2 \qquad (2)$$

where y_{ij} is the experimental (measured) IBU value for the j-th replica of the i-th coordinate (given by the ordered pair {SG, boiling time}), $\hat{y}_i$ is the fitted model prediction for this coordinate, n stands for the total number of experimental points and q is the number of model parameters. The parameter variances and

covariances matrix is given by:

$$\underline{\underline{COV}}\left(\underline{p}\right) = S_y^2 \left(\underline{\underline{J}}^t.\underline{\underline{J}}\right)^{-1} \tag{3}$$

where $\underline{\underline{J}}$ is the Jacobian matrix of the fitted model.

In order to characterize the bitterness variation with wort density, the utilization factor is written in terms of the wort density, using four different forms:

$$U = U_0 \, exp[a\,(1 - SG)\,] \tag{4}$$

$$U = U_0 \, [1\, + a\,(1 - SG)^3\,] \tag{5}$$

$$U = U_0 \, c\left[1 + \frac{2}{\pi}tan^{-1}(a(1 - SG) + b)\right] \tag{6}$$

Where $c = \left[1 + \frac{2}{\pi}tan^{-1}(b)\right]^{-1}$ and U_0 is the value of U for SG = 1. The parameters a and b are determined using the same curve fitting procedure used for Equation (1).

3. Results and Discussion

Table 1 summarizes the resulting IBU values obtained with the experimental procedure described in Section 2.2, for worts with different SG values for different boil times (t). As can be seen, the IBU values generally increase with boil time and decrease for higher SG values.

Fitting the experimentally obtained IBU data to the form described in Equation (1) leads to the parameter values described in Table 2. These results, along with the experimental data (points), are plotted in Figure 1. As one can observe from these results, the utilization factor U has a prominent variation, dropping around 50 %, from the pure water samples to the 1.050 SG samples.

Table 1: IBU of samples with different specific gravity (SG) values for different boil times.

SG	t (min)	Triplicate 1	Triplicate 2	Triplicate 3
1.000	1	12.90	14.15	15.50
1.000	3	13.45	15.60	13.85
1.000	5	19.40	15.90	16.70
1.000	10	14.45	14.65	15.35
1.000	40	15.35	15.60	16.40
1.000	60	18.15	17.60	15.65
1.030	1	10.65	11.20	12.75
1.030	5	12.65	14.20	12.60
1.030	10	14.45	15.00	14.80
1.030	25	15.00	14.00	14.10
1.030	40	13.25	15.25	14.35
1.030	60	14.50	14.80	14.70
1.040	1	10.20	10.15	10.2
1.040	3	9.75	11.15	10.00
1.040	5	12.00	10.80	11.40
1.040	10	13.40	12.90	13.00
1.040	25	13.00	14.25	13.05
1.040	40	12.55	13.15	14.10
1.040	60	11.60	13.00	11.40
1.050	1	5.95	5.75	6.30
1.050	3	6.10	5.95	6.00
1.050	5	6.15	6.05	6.40
1.050	10	6.70	6.45	6.95
1.050	25	7.00	6.70	6.25
1.050	40	6.75	7.60	7.95
1.050	60	6.30	7.35	6.20

Table 2: Calculated model parameters for Equation (1).

SG	U	Error (U)	β	Error (β)	Variance
1.000	0.547870	2.55%	2.19938	25.2%	2.43696
1.030	0.491217	1.56%	1.65609	11.7%	0.735158
1.040	0.423962	2.37%	1.66684	19.3%	1.48075
1.050	0.227798	1.98%	2.36308	23.3%	0.306252

When looking at the time decay parameter (β), the order of magnitude of the calculated β-values indicates that the IBU reaches its final (i.e., maximum) values a few minutes after the beginning of the boil. This is different

from the traditionally expected behavior for bitterness in the finished beer, where smaller time decay parameters are seen. The maximum IBU values are generally obtained around an hour of boil time. These differences may be attributed to the fact that characterization followed the procedure established by ASBC and EBC for product quality control, which is different than the employed, for instance, by Malowicki and Shellhammer [10].
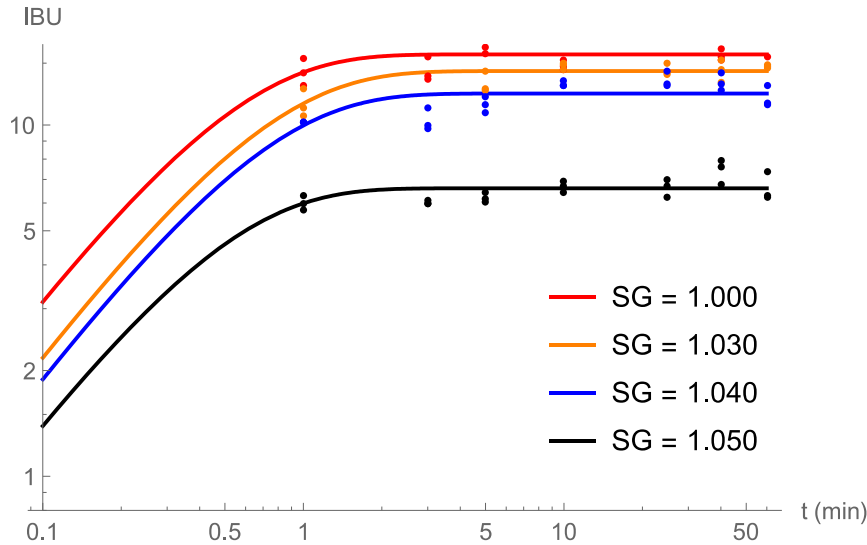
**Figure 1:** Evolution of IBU with boil time: experimental data and fitted models

These authors measured the isomerized alpha acid content by HPLC, which is a much more expensive and complex procedure, almost prohibitive for most breweries. This may indicate that the alpha acids that have not been fully isomerized (which would be absent in the finished beer) may contribute to the bitterness readings when adopting the standard IBU characterization procedure. Regardless of the time decay results, the IBU reduction for increasing wort densities is by the expected behavior. In this sense, the subsequent results are intended to quantify the IBU variation with wort density. Table 3 presents the calculated values for parameters a and b for the models for describing the IBU variation with wort density. In contrast, Figure 2 presents the actual curves for these models along with the experimental data. The points in this figure correspond to the U-values obtained in the previous analysis (Table 2), normalized with the value for SG = 1.000.

Table 3: Calculated model parameters for Equations (4) to (6).

Model	a	Error (a)	b	Error (b)	Variance
Eq.(4)	9.79727	41.37%	-	-	0.035636
Eq.(5)	4415.29	7.49%	-	-	0.00223793
Eq.(6)	120.54	4.23%	5.68122	4.31%	0.0000658787

As seen from these results, the exponential model given by Equation (4), gives decreasing U-values with an asymptote with $U = 0$ for large SG values; however, it does not capture the U-values obtained from the previous analysis with precision. Equation (5), on the other hand, gives a better fit to the U points; nevertheless, it yields negative values for SG > 1.060, which is physically unsound. Finally, Equation (6), gives a fit that accurately

represents the *U* points but has an asymptote going to zero for larger SG values. Finally, a curve fit of the entire data set in the form obtained by the substitution of Equation (6) in Equation (1) is performed. The results are presented in Table 4 and Figure 3. The estimated variance for this fit is 1.19387.
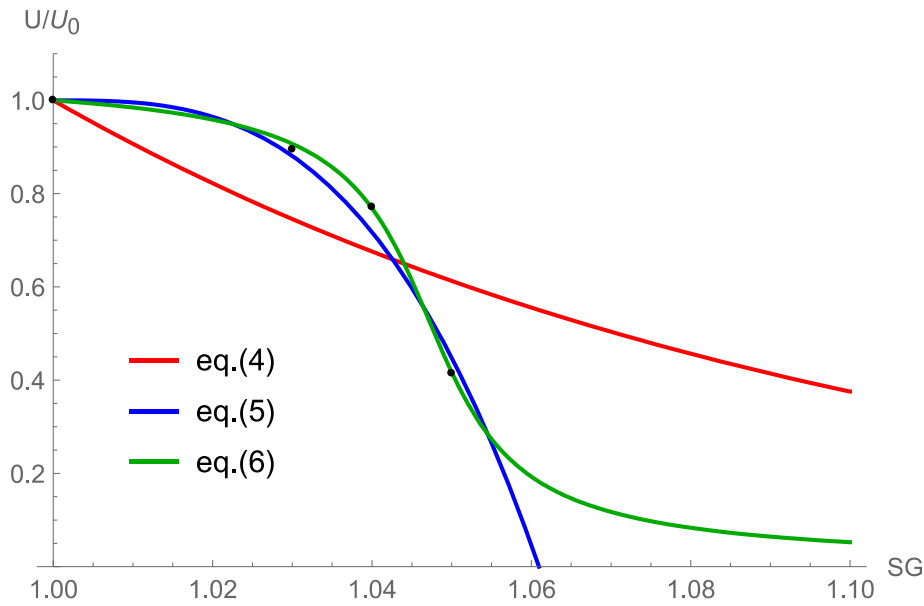
Figure 2: Variation of the utility factor U with wort density for different models

Table 4: Calculated model parameters for the model given by Equations (1) and (6).

Parameter	*a*	Error
a	116.905	9.76%
b	5.50862	10.32%
β	1.87721	9.23%
U_0	0.547697	1.54%
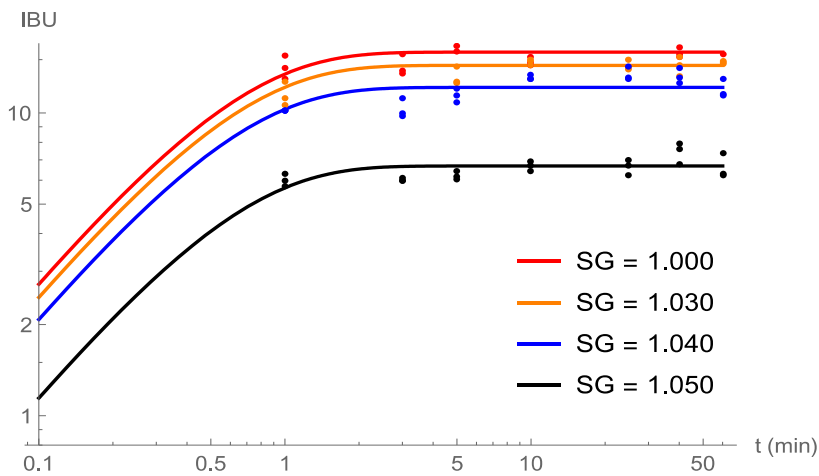
Figure 3: Variation of IBU with wort density for the model given by Equations (6) and (1), along with experimental points.

The low estimated variance indicates that the model presents an adequate adherence to experimental data.

4. Conclusions

The apparatus developed was adequate to conduct the isomerization of the hops alfa-acids controlled, which allowed studying its kinetics. As expected, the IBU bitterness value increased during boiling and decreased when the sugar concentration increased. Nevertheless, the IBU increase with boil time was faster than the usually expected behavior, which indicates that the traditional approach employed can be subject to the interference of other UV absorbing substances in the early stages of the boiling. This can be a particular concern when using late hopping techniques, whirlpool hop additions and/or dry hopping when the hop is added during fermentation and/or maturation. Another limitation of the model is related to the fact that IBU readings were performed in unfermented worts, which misses the loss of bitterness substances during fermentation that can lower IBU values. Besides, the model doesn't take into account the boiling temperature and pH, which interfere in the IBU evolution and can be subject to further studies. Regardless of the behavior, the IBU values stabilized for larger boil times, leading to different quantities for different wort densities. These values were fitted to different models, and the best fit was in the form of an inverse tangent function. This empirical model can estimate the effect of wort density in the IBU, which is of assistance for process simulation and product design.

Acknowledgments

References

[1]. F. Vriesekoop, M. Krahl, B. Hucker, G. Menz. "Bacteria in brewing: The good, the bad and the ugly". Institute of Brewing & Distilling, 118, 335-345, 2012

[2]. H. O. Askew. "Changes in hop α-acids concentrations on heating in aqueous solutions and unhopped worts". J. Inst. Brew., 70, 503-513, 1964.

[3]. B. Jaskula, G. Aerts, L. D. Cooman. "Hop alpha-acids isomerization and utilization: an experimental review". Cerevisia, 35, 57-70, 2010.

[4]. K. Sakamoto, W. N. Konings. "Beer spoilage bacteria and hop resistance". International Journal of Food Microbiology, 89, 105-124. 2003.

[5]. I. Caballero, C. A. Blanco, M. Porras. "Iso-α-acids, bitterness and loss of beer quality during storage". Trends in Food Science & Technology. 26, 21-30. 2012

[6]. J. Christensen, A. M. Ladefoged, L. Norgaard. "Rapid Determination of Bitterness in Beer Using Fluorescence Spectroscopy and Chemometrics". Journal of The Institute of Brewing, 111 (1), 3-10, 2005.

[7]. G. A. Howard, L. R. Bishop, P. A. Martin, J. R. Hudson, J. S. Pierce "Recommended Methods of Analysis. Institute of Brewing Analysis Committee", J. Inst. Brew., 77, 181-226, 1971.

[8].   D. Nibumona, C. A. Blanco, I. Caballero, A. Rojas, C. Andrés-Iglesias. "An approximate shelf life prediction of elaborated lager beer in terms of degradation of its iso-α-acids". Journal of Food Engineering, 116, 138-143, 2013.

[9].   B. Jaskula, P. Kafarski, G. Aerts, L. De Cooman. "A Kinetic Study on the Isomerization of Hop α-Acids". J. Agric. Food Chem. 56 (15), 6408-6415, 2008.

[10]. M. G. Malowicki, T. H. Shellhammer. "Isomerization and degradation kinetics of hop (humulus lupulus) acids in a model wort-boiling system", J. Agric. Food Chem. 53, 4434-4439, 2005.

[11]. B. Jaskula, G. Aerts, L. D. Cooman. "Potencial Impacts of medium characteristics on the isomerization of hop α-acids in the wort and buffer model systems". Food Chemistry, 123, 1219-1226, 2010.

[12]. M. G. Malowicki, T. H. Shellhammer. "Factors Affecting Hop Bitter Acid Isomerization Kinetics in a Model Wort Boiling System". J. Am. Soc. Brew. Chem, 64 (1), 29-32, 2006.

[13]. O. Oladokun, K. Smart, D. Cook. "An improved HPLC method for single-run analysis of the spectrum of hop bittering compounds usually encountered in beers". J. Inst. Brew. 122, 11-20, 2016.

[14]. B. Jaskula, K. Goiris, G. De Rouck, G., G. Aerts, L. De Cooman. "Enhanced Quantitative Extraction and HPLC Determination of Hop and Beer Acids", Journal of The Institute of Brewing, 113 (4), 381-390, 2007.

[15]. B. Jaskula, E. Syryn, K. Goiris, G. De Rouck, F. Van Opstaele, J. De Clippeleer, G. Aerts, L. De Cooman, "Hopping technology in relation to beer bitterness consistency and flavour stability". J. Am. Soc. Brew. Chem. 65 (1), 38–46, 2007.

[16]. H. O. Askew. "Changes in concentration of α and β acids and of iso-compounds on heating extracts of hops in aqueous solutions and unhopped worts", J. Inst. Brew., 71, 10-20, 1965.

[17]. K. Huvaere, M. L. Andersen, L. H. Skibsted, A. Heyerick, D. De Keukeleire. "Photooxidative Degradation of Beer Bittering Principles: A Key Step of the Route to Lightstruck Flavor Formation in Beer", Journal of Agricultural and Food Chemistry, 53 (5), 1489-1494, 2005.

[18]. C. A. Blanco, D. Nimubona, I. Caballero. "Prediction of the ageing of commercial lager beer during storage based on the degradation of iso-α-acids", J Sci Food Agric. 94, 1988-1993, 2014.

[19]. F. Gunst, M. Verzele. "On The Sunstruck Flavor of Beer", J. Inst. Brew., 84, 291-292, 1978.

[20]. S. Kappler, M. Krahl, C. Geissinger, T. Becker, M. Krottenthaler. "Degradation of Iso-α-Acids During Wort Boiling". Journal of The Institute of Brewing, 116 (4), 332-338, 2010.

[21]. M. Kowaka, E. Kokubo. "Composition of Bitter Substances of Hops and Characteristics of Beer Bitterness". ASBC Journal, 35 (1), 16-21, 1976.

[22]. D. Intelmann, O. Demmer, N. Desmer, T. Hofmann. "[18]O Stable Isotope Labeling, Quantitative Model Experiments, and Molecular Dynamics Simulation Studies on the Trans-Specific Degradation of the Bitter Tasting Iso-α-acids of Beer", J. Agric. Food Chem. 57, 11014-11023, 2009.

[23]. G. Vanhoenacker, D. De Keukeleire, P. Sandra. "Analysis of iso-α-acids and reduced iso-α-acids in beer by direct injection and liquid chromatography with ultraviolet absorbance detection or with mass spectrometry". Journal of Chromatography A, 1035(1), 53–61, 2004.

[24]. E. Polshin, A. Rudnitskaya, D. Kirsanov, A. Legin, D. Saison, F. Delvaux, F. R. Delvaux, B. M. Nicolai, J. Lammertyn. "Electronic tongue as a screening tool for rapid analysis of beer". Talanta, 81(1-2), 88–94, 2010.

[25]. L. S. Calado, A. L. F. Lacerda, S. B. Fiaux, L. A. Sphaier, V. N. H. Silva, F. C. Peixoto. "Low-cost fluorescence-based method for beer bitterness measurement". Journal of Food Engineering, 262, 9-12, 2019.

[26]. G. A. Howard. "Estimation of the bitterness of beer". J. Inst. Brew., 74, 249-251, 1968.

[27]. O. Oladokun, A. Tarrega, S. James, K. Smart, J. Hort, D. Cook. "The impact of hop bitter acid and polyphenol profiles on the perceived bitterness of beer", Food Chemistry, 205, 212–220, 2016.

[28]. I. Nascimento, L. Calado, M. E. Duncan, B. Trindade, L. Sphaier V. N. H. Silva, F. C. Peixoto. "Interference in beer bitterness measurements caused by polymer tubes", Journal of Food Engineering, 275, 1-4, 2020.

[29]. J. Philpott, D. M. Taylor, D. R. Williams. "Critical Assessment of Factors Affecting the Accuracy of the IoB Bitterness Method". Journal of the American Society of Brewing Chemists, 55 (3), 103-106, 1997.

[30]. ASBC "Method of Analysis. American Society of Brewing Chemists. α- and β-Acids in Hops and Hop Pellets". The Society. St Paul, MN, U.S.A, 2011.

[31]. M. A. A. Kappel, F. C. Peixoto, G. M. Platt, R. P. Domingos, I. N. Bastos. "A study of equivalent electrical circuit fitting to electrochemical impedance using a stochastic method", Applied Soft Computing. 50, 183-193, 2017.

[32]. T. F. Edgar, D. M. Himmelblau, L. S. Lasdon. "Optimization of Chemical Processes", McGraw-Hill, Nova York, N. Y., U.S.A., 2001.