10.1515/umcschem-2015-0021

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. LXX, 2 SECTIO AA 2015

Isomerization of bitter acids during the brewing process

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Beer is the world's oldest and most widely consumed alcoholic beverage. The transformation of bitter acids to iso-α and iso-ß-acids is recognized as the key step of beer production. The paper shows and discusses the transformation kinetics of α- and ß-acids into their iso-form. Following the performed experiments, the largest amounts of the bitter acids isomers are formed in the brewing process carried out at 80°C for more than 100 minutes. The presented data are in good agreements with the knowledge of experienced brewers who learned about the brewing process relying on sensory tests.

Keywords: bitter acids, isomerization, brewing process, wort preparation.

1. INTRODUCTION

Beer is one of the oldest and most widespread alcoholic beverages in the world. It is well known, however, that its production is not trivial and involves several steps. Wort preparing is recognized as the key step of beer production. The proper mixing of barley malt with grounded hop cones or hop extract is the essence of this process. The addition of hop not only increases beer durability, but above all, gives the beer a specific

bitter taste which results from the presence of bitter acids being the major organic compounds occurring in hop cones.

Bitter acids can be divided into three basic groups: α-acids, ß-acids and γ-acids. α- acids (co-humulone, n-humulone, ad-humulone) and ß-acids (co-lupulone, n-lupulone, ad-lupulone), called frequently as bitter acids, are primarily of importance to the production of beer. As results from the literature [1–2], these acids, heated in water/alcohol solution, isomerize to iso-α and iso-ß-acids. In brewing process, the isomerization of α- and ß-acids occurs during the addition of hops to the boiling wort. The degree of this isomerization, and hence the bitter flavor intensity of beer, is highly dependent on the temperature and boiling time of the wort preparation [3–9].

The aim of these experiments was to find the effect of temperature conditions on the degree of bitter acids transformation. The bitter acids transformation degree was examined during simulated brewing process i.e. using the model aqueous-alcoholic mixtures of a hop extract. The presented scientific problem seems to be justified as there are not literature data showing the transformation kinetics of α- and ß-acids into their iso-form.

2. EXPERIMENTAL

2.1. Materials

Methanol (HPLC), ethanol 96 % and ammonium acetate were purchased from the Polskie Odczynniki Chemiczne S.A. POCh (Gliwice, Poland). Bitter hop extract (Magnum) was obtained from INS Pulawy. Bitter acids analytical standards Sigma-Aldrich Co. (Germany).

Water was purified on the Milli-Q system from Millipore (Millipore, Bedford, MA, USA).

2.2 Methods

2.2.1. Sample preparation

The investigations of bitter acids transformation were performed by heating under reflux ethanolic solution of bitter hop extract in ethanol/water mixture under reflux.

The ethanolic solution of bitter hop extract contained 1 g of this extract in 10 cm^3 of ethanol. The heated mixture contained 100 mm^3 of

ethanolic solution of bitter hop extract in 100 cm^3 of ethanol/water mixture, 5% *v/v*.

In order to eliminate the possible impact of interferents on the bitter acids isomerization process, the prepared solution contained neither malted barley nor yeast.

Individual solutions were heated for 200 minutes at temperatures: 50, 60, 70, 80°C. Samples were collected every 20 minutes. Subsequently, each obtained solution was subjected to LC-MS-PDA analysis.

The applied LC-MS-PDA equipment was calibrated using humulone and lupulone analytical standards. The working solutions were obtained by serial dilutions of the stock solution with ethanol to obtain the following concentrations of analytes (n = 5):

- co-humulone: 5, 10, 25, 50 and 100 mg/cm^3,
- n+ad-humulones: 5, 10, 25, 50 and 100 mg/cm^3,
- co-lupulone: 1, 2.5, 5, 10 and 25 mg/cm^3,
- n+ad-lupulones: 1, 2.5, 5, 10 and 25 mg/cm^3,
- iso-co-humulone: 0.05, 0.1, 0.5, 1 and 5 mg/cm^3,
- iso-n+ad-humulones: 0.5, 1, 2.5, 5 and 10 mg/cm^3,
- iso-co-lupulone: 0.05, 0.1, 0.5, 1 and 5 mg/cm^3,
- iso-n+ad- lupulone: 0.5, 1, 2.5, 5 and 10 mg/cm^3.

The resultant calibration curves show in all cases very good linearity:

- co-humulone: $R^2 = 0.9968$;
- n+ad-humulones: $R^2 = 0.9957$;
- co-lupulone: $R^2 = 0.9984$;
- n+ad-lupulones: $R^2 = 0.9967$;
- iso-co-humulone: $R^2 = 0.9935$;
- iso-n+ad-humulones: $R^2 = 0.9963$;
- iso-co-lupulone: $R^2 = 0.9945$;
- iso-n+ad- lupulone: $R^2 = 0.9978$.

2.2.2. HPLC measurements

The chromatographic measurements were performed using LC/MS from Finnigan (LCQ Advantage Max) equipped with the ion-trap mass spectrometric system (ThermoElectron Corporation, San Jose, CA). A Gemini C18 column (4.6 × 100 mm, 3 um) (Phenomenex, USA) was employed for chromatographic separation. Chromatographic separation

was performed using isocratic elution 35% A and 65% B. Mobile phase was a mixture of solvent A (20 mM ammonium acetate in water) and solvent B (20 mM ammonium acetate in methanol). Flow rate was 0.65 cm^3/min. During the course of each run, a MS spectra in the range of 100-1000 m/z were collected continuously. The column effluent was ionized by electrospray (ESI).

In all bitter acids solutions, the SIM function was used to better visualize the chromatographic separation. The monitored compounds, their *m/z* ratio and retention times were as follows:

- iso-co-humulone (347 *m/z*, 7.1 min),
- iso-n+ad-humulone (361 *m/z*, 7.9 min),
- iso-co-lupulone (399 *m/z*, 9.25 min),
- iso-n-ad-lupulone (413 *m/z*, 11.3 min),
- co-humulone (347 *m/z*, 15.1 min),
- n+ad-humulone (361 *m/z*, 20.6 min),
- co-lupulone (399 *m/z*, 22.8 min),
- n-ad-lupulone (413 *m/z*, 31.7 min).

3. RESULTS AND DISCUSSION

Fig. 1 presents exemplary chromatogram of bitter hop extract in ethanol/water mixture heated at 80°C for 60 minutes. As appears from the chromatogram, four bitter acids and four their isomers are present in examined extract. They are: iso-co-humulone, iso n+ad-humulone, iso-co-lupulone, iso-n+ad-lupulone (peaks 1-4, respectively) and co-humulone, n+ad-humulone, co-lupulone, n+ad-lupulone (peaks 5-8, respectively). All of them were identified and confirmed on the basis of the retention data of their standards, PDA spectra and MS^2 data. The structures of these compounds are shown in Fig. 2.

As it was mentioned in Introduction, the most desirable in the brewing process is the formation of water-soluble iso-bitter acids. For this reason, two basic and crucial parameters, temperature and heating time of the wort, are optimized in the brewing process. Fig. 3 shows the influence of the time on the concentration changes of bitter acids (Fig. 3 A-D) and their isomers (Fig 3 A'-D') during the simulated wort process carried out at different temperatures. The presented results lead to the following conclusions:

- heating time elongation resulted in the decrease of bitter acid concentration and a simultaneous increase of the content of corresponding isomers (compare proper curves in Figs. 3 A-D and Figs. 3 A'-D'). This phenomenon is obvious and results from the bitter acids isomerization pathway [10];
- the increase of brewing temperature accelerates bitter acid transformation what is connected with the influence of temperature on the reaction rate;
- the greatest concentration changes of the bitter acids and their isomers are observed at 80°C. It should be noted that the concentration of β-acids, after 100 min of heating at 80°C, drops to 0 mg/cm^3 (see Fig 3 D). In consequence, the concentration of the corresponding isomers in the heated wort is constant (see data for iso-n+ad-lupulones and iso-co-lupulone – solid lines with crosses and triangles in Fig. 3 D') or begins to settle down (see data for iso-n+ad-humulones and iso-co-humulone – solid lines with diamonds and squares in Fig. 3 D').

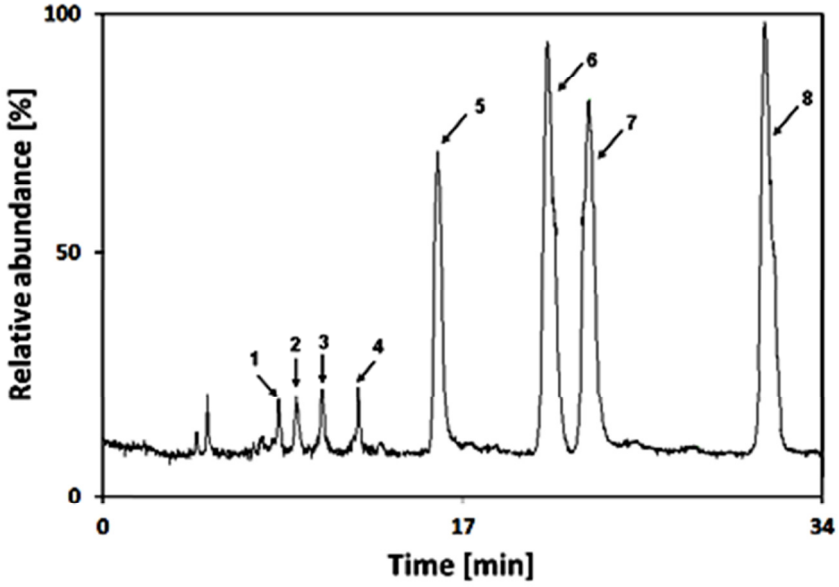

Fig. 1. Exemplary chromatogram of bitter hop extract in ethanol/water mixture heated for 60 minutes at 80°C. The peaks numbers correspond to: iso-co-humulone (peak 1), iso n+ad-humulone (peak 2), iso-co-lupulone (peak 3), iso-n+ad-lupulone (peak 4), co-humulone (peak 5), n+ad-humulone (peak 6), co-lupulone (peak 7), n+ad-lupulone (peak 8).

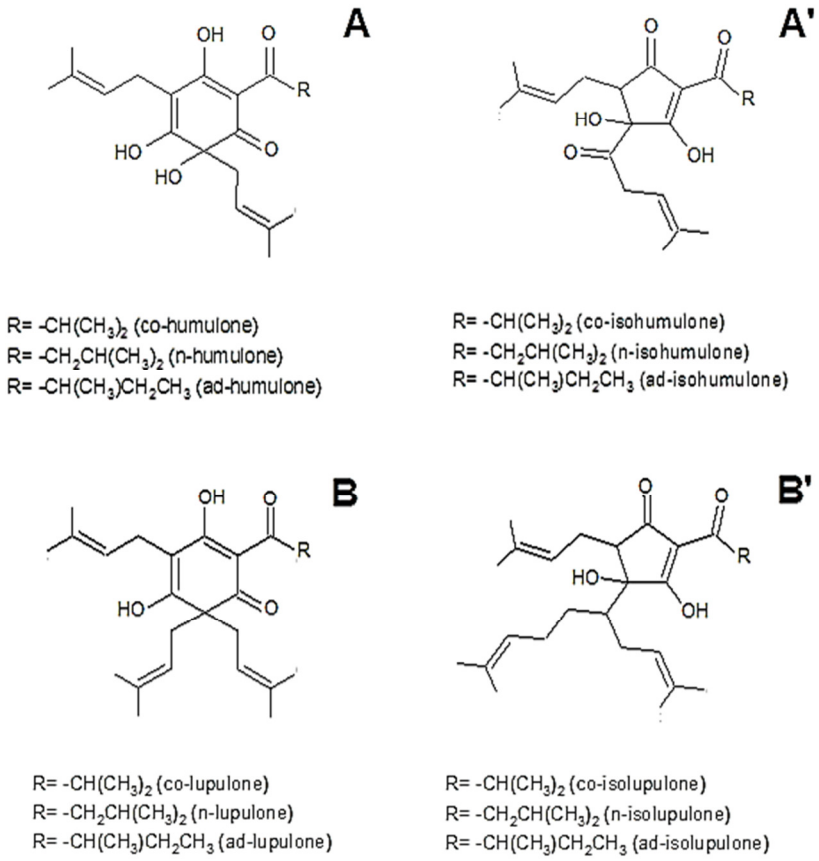

Fig. 2. Structures of identified bitter acids and their isomers.

4. CONCLUSIONS

The bitter flavor intensity of beer is highly dependent on the transformation degree of bitter acids to iso-α- and iso-ß-acids. As results from the performed experiments, the degree of this isomerization is strongly dependent on the temperature and time of the wort preparation. The highest amounts of the bitter acids isomers are formed in the brewing process carried out at 80°C for more than 100 min. These data are in good agreements with the knowledge of experienced brewers who learned about the brewing process based on sensory testing. The shown kinetics of bitter acids transformation is relevant and useful for breweries in which the beer production process is optimized for an industrial scale.

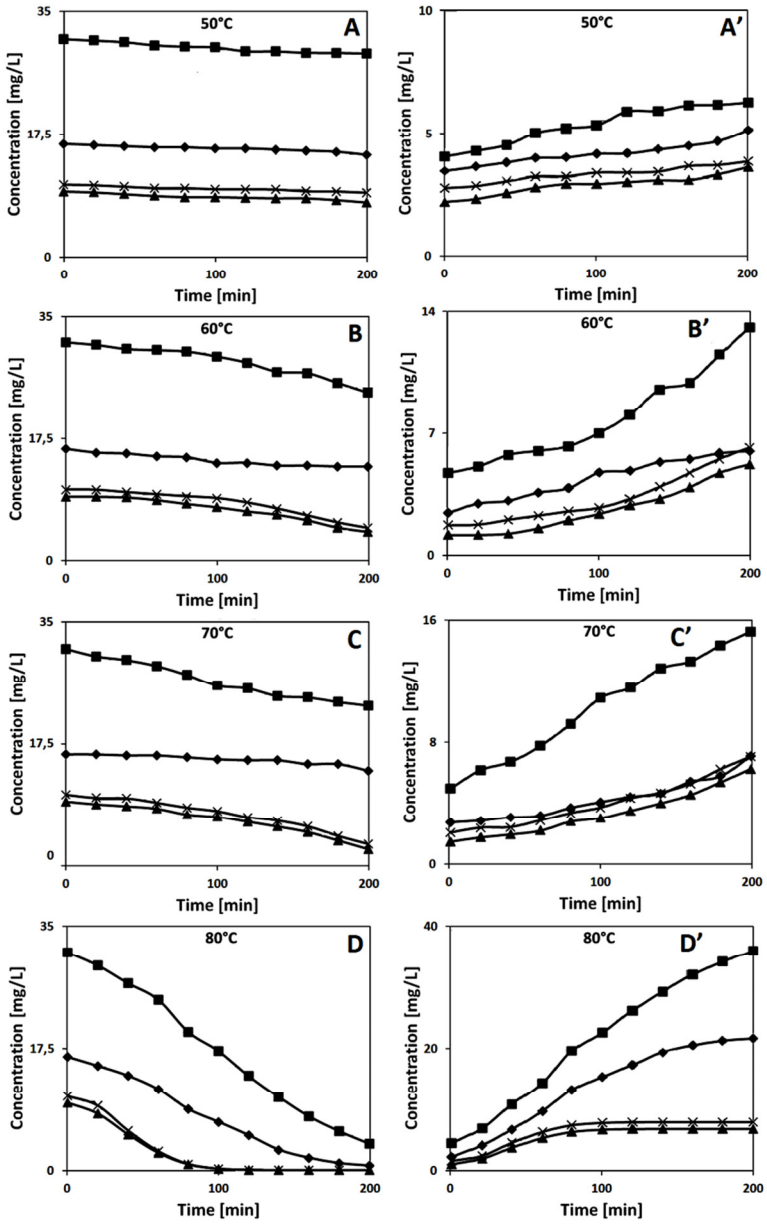

Fig. 3. Influence of heating time and temperature on concentration changes of bitter acids (A-D) and their isomers (A'-D') in simulated brewing process. Line with squares corresponds with n+ad-humulones and iso-n+ad-humulones, line with diamonds corresponds with co-humulone and iso-co-humulone, line with crosses corresponds with n+ad-lupulones and iso-n+ad-lupulones, line with triangles represents co-lupulone and iso-co-lupulone.

REFERENCES

[1] Y. Huang, J. Tippmann and T. Becker, *International Journal of Bioscience, Biochemistry and Bioinformatics*, **3**, 47-52, (2013).
[2] M. G. Malowicki, T. H. Shellhammer, *Journal of Agriculture and Food Chemistry*, **53**, 4434-4439, (2005).
[3] J. Urban, C. Dahlberg, B. Carroll and W. Kaminsky, *Angewandte Chemie International Edition,* **52**, 1553-1555, (2013).
[4] L. Royle, J. M. Ames, C. A. Hill and D. S. J. Gardner, *Food chemistry*, **74**, 225-231, (2001).
[5] A. C. J. Hermans-Lokkerbol, A. C. Hoek and R. Verpoorte, *Journal of chromatography A,* **771**, 71-79, (1997).
[6] R. Hampton, G. Nickerson, P. Whitney and A. Haunold, Phytochemistry, **61**, 855-862, (2002).
[7] C. A. Blanco, A. Rojas, P. A. Caballero, F. Ronda, M. Gomez, I. Caballero, Trends in Food Science & Technology, 17, 373-377, (2006).
[8] M. Goese, K. Kammhuber, A. Bacher, M. H. Zenk, W. Eisenreich, *The Federation of European Biochemical Societies Journal,* **263**, 447–454, (1999).
[9] J. De Keukeleire, G. Ooms, A. Heyerick, I. Roldan-Ruiz, E. Van Bockstaele, D. De Keukeleire, *Journal of Agricultural and Food Chemistry,* **51**, 4436–4441, (2003).
[10] K. Huvaere, M.L. Andersen, M. Storme, J. Van Bocxlaer, L.H. Skibsted, D. De Keukeleirel, *Journal of Agriculture and Food Chemistry*, **53**, 1489-1494 (**2005).**