Fabien Krebs, Martin Zarnkow and Frank Endres

Investigation of beer foam with X-ray photoelectronspectroscopy: first insights

This first investigation of beer foams using XPS enables a deeper insight than conventional analytics into the composition of beer foams as well as indications of criteria for good beer foam (NIBEM > 300 s; foam index, SKZ > 115). Due to the high sensitivity of this method, even the smallest amounts of residues can be detected in survey spectra. For an initial assessment, the total nitrogen content can be determined comparatively quick and easy, whereby a high value is obviously characteristic of good foams. The N 1s spectra can be employed to determine the quantity and size of proteins which are considered to be foam positive. A high O=C-N/C-NH_2 ratio might indicate long peptide chains and seems to be foam positive, as the results reveal. Other groups can also be identified, such as polyphenols, whose influence on the foam is also assessed. Furthermore, the results indicate that appropriate reference measurements could provide a more precise determination of the proteins and influencing factors.

Descriptors: XPS, protein fractions, beer foam, metals

1 Introduction

One of the most important criteria in assessing the quality of a beer is the foam, which is analyzed primarily for foam retention [1]. Foam determination according to NIBEM and the SFT foam tester have been established for this purpose [1, 2]. However, both methods do not allow any statements to be made about the causes for the classification into good (+) and bad (-) foams. The composition of proteins (> 12,000 Da), glycoproteins, isohumulones, beta-glucans and heavy metals have a considerable influence on the quality of the foam [1–8]. Especially proteins are described as a foam-forming and stabilizing fraction, while carbohydrates are said to have a stabilizing effect [6]. Thereby the protein Z (MW 40,000) was firstly associated with foam stability [1]. In addition, there are numerous other species that also have an influence on the foam, although the basic interaction of all species in the foam is largely unexplained. A promising method for analyzing the foam is X-ray photoelectron spectroscopy (XPS), which has been used to fundamentally investigate the chemical nature of the foam in the framework of the present study. Six beers, which were previously divided into beers with good (NIBEM > 300 s; foam index, SKZ > 115) and bad foam (NIBEM < 220 s; SKZ < 100) using NIBEM and SFT foam testers, were be used to test the suitability of this method. These are commercially available beers whose brand is not mentioned here, for legal reasons.

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X-ray photoelectron spectroscopy is a spectroscopic measurement method that provides qualitative and quantitative information about the elemental composition of a sample and its binding conditions, which allows the determination of chemical bonds and identification of molecules. For this purpose, photoelectrons that are emitted when a sample is irradiated with X-rays due to the photoelectric effect are detected as a function of their kinetic energy. The binding energy of the photoelectrons can be determined from the kinetic energy, considering the energy of the X-rays used. The result of this measurement is a spectrum in which the count rate is plotted against the binding energy. A distinction is made between so-called survey spectra, which resolve the entire energy range of the X-rays, and detailed spectra, which depict the specific range of an element with a higher resolution. These spectra usually contain several superimposed peaks, which can be differentiated by deconvoluting them into individual peaks of different intensity and peak shape. During the deconvolution it is possible to distinguish different bonds of an atom as each peak represents a chemical state. The measurements are carried out under ultra-high vacuum conditions, which is why volatile substances such as water and alcohol are removed before the measurement.

As part of these investigations, the causes of good and bad foams should be determined with XPS. For this purpose, different beer foams are characterized in order to clarify their composition and get an insight to the chemical compounds.

2 Material and methods

In this study, in total six beers of the type of lager beer, pilsener beer and export beer were analyzed, which were previously divided into beers with good (+) and bad foam (-) according to the NIBEM and SFT foam testers. For each type of beer, one good and one bad foam is analyzed. To ensure that the conditions for analyzing the foams are as reproducible as possible, all foams are produced in

the same way using a foam frit. First, 300 mL of a chilled beer was carefully poured into an 800 mL beaker so that the beer foams up as little as possible. A foam frit flushed with CO_2 was then held in the beaker for 5 seconds to foam the beer. The gas pressure used was identical for all beers. Using tweezers, a gold substrate, which serves as a measuring base, was passed through the foam twice from bottom to top. Gold was used as the substrate as it is inert to the medium being analyzed and is usually not present in the foam. The substrate was mounted on a stainless-steel sample holder and transferred to the ultra-high vacuum system. In between samples the beakers, foam frit and tweezers were cleaned with ethanol and then thoroughly rinsed with tap water and dried. The foam frit was additionally dried by passing CO_2 through it.

2.1 Experimental

SPECS Phoibos 150 hemispherical analyzer was used to perform the X-ray photoelectron spectroscopy (XPS) measurements. A SPECS XR50 M monochromatic AlK_α source (1486,6 eV) was used to generate the X-rays. The monochromatic X-rays protect the samples from damage. The XP survey spectra and detail spectra were recorded with a constant analyzer pass energy of 20 eV. The energy resolution (full width at half maximum, FWHM) of the system at the pass energy of 20 eV for the used AlK_α excitation for the Au $4f_{7/2}$ level was about 0.9 eV.

After the samples have been introduced into the vacuum, they were degassed for about 30 minutes until the pressure normalized.

The XPS-peaks were fitted using CASA XPS software. A Gaussian-Lorentzian GL(30) line shape was applied for all components. For the analysis of all peaks a Shirley-type background was used. Some of the spectra had to be corrected for charging. Charging occurs if the holes which are created during the emission process cannot be filled by electrons from the sample. This happens especially with non-conductive or poorly conductive samples such as organic samples. By correcting the BE using a defined reference, the spectra are shifted during evaluation. The peaks are referenced to Au 4f or F 1s and C-C peaks.

3 Results

The foams were firstly analyzed for impurities and unusual substances using the survey spectra. The survey spectra, which provide an overview of the present elements, are considered for this purpose. In addition to the main components carbon, nitrogen and oxygen, the survey spectrum of the export beer with bad foam (Fig. 1 b) also shows small amounts of chlorine, sulfur and copper. A possible source of chlorine could be the brewing water, while the chlorine content in brewing water depends on the used water source like whether spring water, tap water or mixed water is used. The type of water treatment in the brewery can also influence the chlorine content. Chlorine could possibly also originate from residues of cleaning agents. Cleaning agents and disinfectants that remain in the beer due to insufficient rinsing process can reduce the surface tension and have a negative effect on the foam [1, 9–11]. Another source could be $CaCl_2$, which is sometimes added to adjust the brewing water [1, 12]. Chlorine can only be found in the poor foams of export and pilsner beers. Copper is recognizable in the spectra of all three beers with poor foam, although metal ions, including copper, are usually described as foam promoting [3, 9–11]. For Cu^{2+}, a cross-linking of iso-α-acids is assumed, which strengthens the bubble film [1, 13, 14]. The observed copper could originate from pipes and vessels used during the brewing process. While modern brewhouses are made of stainless steel, traditional brewhouses were made of steel and copper and might be a possible source of copper. In addition, copper can also be introduced into beer through barley and hops, whereby copper can accumulate due to its natural content in the soil or using plant protection products. In order to better evaluate the influence, it is necessary to analyze a larger series of measurements. Foam-reducing sulphites are also conceivable [3, 9–11], but further differentiation based on the detailed spectra is required here. The observed fluorine is a typical impurity of the system used, but the associated species are

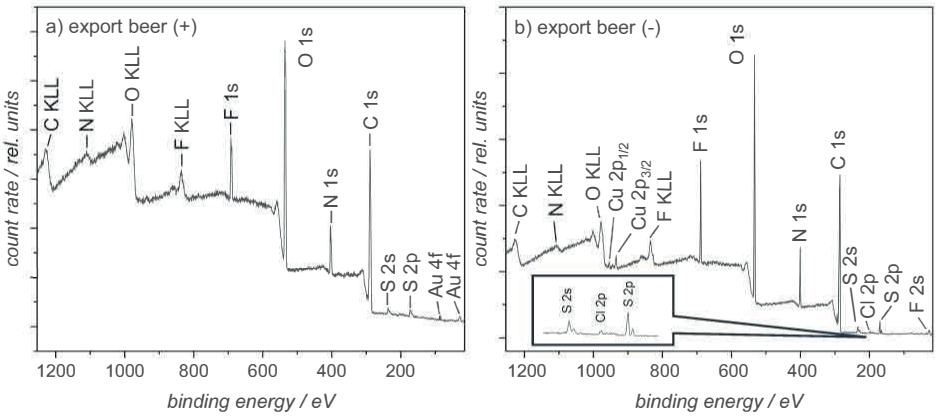

Fig. 1 Survey spectra of export beers with a) good foam and b) bad foam

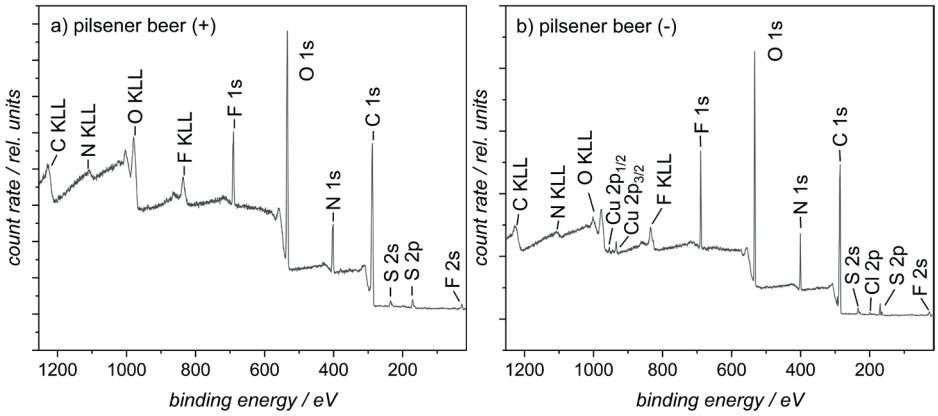

Fig. 2 Survey spectra of pilsener beers with a) good foam and b) bad foam

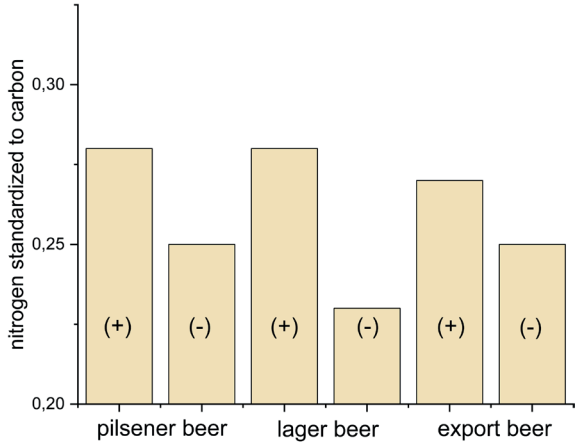

Fig. 3 **Ratios of the total nitrogen content to the total carbon content (N 1s/ C 1s) in the six foams (pilsener, lager, export) from the survey spectra**

sufficiently stable so that their influence is negligible. In contrast, the export beer with good foam (Fig. 1 a) shows no hints of chlorine and copper and the remaining composition seems quite similar. The Au 4f peaks are caused by the underlying gold substrate and indicating a thin coverage.

The comparison of the two pilsener beers (Fig. 2) shows that copper and chlorine are also present in the beer with bad foam, which is why both chlorine and copper seem to have a negative effect on the foam and appear to have a significant influence on the quality of foam.

In addition, the survey spectra can be used to determine the total nitrogen content (Fig. 3) of the foams. A high total nitrogen content is usually an indicator of a good foam [3, 6–11]. The methods according to Kjeldahl (EBC) and the combustion method according to Dumas (EBC) are usually used for this determination [6]. The total nitrogen contents are normalized to the respective total carbon content to eliminate the influence of fluctuating intensities and fluorine impurities of the six measurements. All analyzed beer types with good foams have higher total nitrogen contents than the beers with the bad foams (Fig. 3), whereas the absolute amount of nitrogen in all good foams is similar. This could indicate a certain ratio of carbohydrates and proteins that is present in good foams.

In principle, however, beer contains numerous different nitrogen compounds such as amino acids, peptides, proteins, nucleic acids and derivatives, ammonium salts, nitrate, aliphatic and aromatic amines and heterocycles, of which peptides and proteins are of particular interest for the foam [6]. Medium and higher molecular weight nitrogen compounds, often referred to as foam proteins, are described as foam-promoting [6, 15], which is why it is also necessary to differentiate the nitrogen compound. Both species are described as important for the stability and flavor of the foam [6]. For this purpose, the nitrogen (N 1s) and carbon (C 1s) spectra are analyzed, and the foam proteins are primarily examined for their molecular size. However, the foam proteins must be first identified in the spectra. Generally, proteins are peptide chains, which are made up of amino acids linked by peptide bonds. The reaction equation below (Fig. 4) shows the formation of a peptide bond (O=C-N) from two amino acids in basic form.

To identify the foam building proteins, the foams are analyzed for the characteristic peptide bonds using XPS. In the case of higher molecular weight nitrogen compounds, especially for the foam proteins, the proportion of peptide bonds should be high due to the long chain length. At the same time, the terminal amines (-NH_2), whose proportion should be as low as possible compared to the peptide bonds, can be analyzed.

The detail spectra indicate a different composition of the foams depending on the type of beer. While the spectra of one beer type are very similar, the different beer types each had different peak shapes (Fig. 5 – 8), which is why the same beer types are used to compare with each other in the analysis. The N 1s spectra of the two pilsner beers show a peak at 399.8 eV, which is presumably caused by the peptide bond [15, 17]. The pilsner beer with good foam has a larger proportion of peptide bonds as the comparison of the peak areas shows. At the same time, less of the terminal primary amine (-NH_2) is present in the good foam at 398.6 eV [15, 17]. An identical observation is made for the lager beer and export beer types, with supposedly longer peptide chains present in the good foams.

The peak at 401.7 eV can be assigned presumably to the species C-NH^+ and C-NH_3^+ [15, 17], which can originate from amino acids such as glycine, aspartic acid, glutamic acid or histidine [15, 17]. The amino acids can be bound or unbound in the peptide chains. Unbound, smaller species can be formed during the cleavage of foam proteins by peptidases [18]. Honno et al. revealed that the amino acids arginine, lysine and histidine inhibit lacing [16]. The fourth peak (NR_4^+, N-O) could be quaternary ammonium compounds such as vitamin B1 or nitrogen-oxygen bonds.

In the C 1s detail spectra (Fig. 6), a higher proportion of peptide bonds is recognizable in the good foam, which is consistent with the

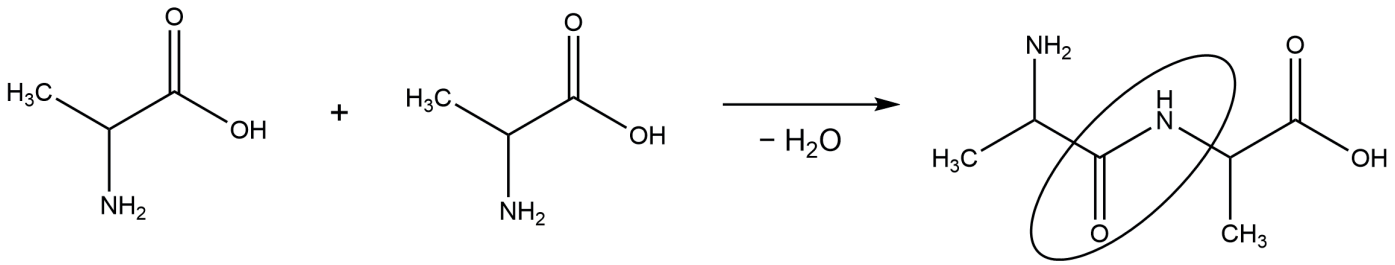

Fig. 4 **Schematic drawing of the formation of a peptide bond (encircled) from two amino acids**

observations of the N 1s spectra. In addition, a considerably higher proportion of C-C bonds is present in the poor foam, which could indicate foam-reducing lipids or detergents [8]. Considered together with the π-π* shake up, which is possibly present at 292.1 eV and is characteristic of carbon in aromatic compounds, the C-C bonds could also indicate foam-reducing polyphenols. In shake-up processes, the ion does not remain in the ground state but in an excited state. The kinetic energy is reduced by the difference to the ground state and the binding energy is correspondingly higher.

The N 1s spectra of both lager beer (Fig. 7) show similar components, although the ratios are different. The comparison of both lager beers with good and bad foams reveals also a higher content of peptide bonds in case of the good foam, while the ratio of O=C-N and C-NH_2 is smaller indicating longer peptides.

The spectrum of lager beer with good foam (Fig. 7 a) exhibit charging effects of the sample, which leads to a doubling of the peaks in each case. Thereby the C-NH^+ Peak is overlayed by C-NH_2, NR_4^+ by O=C-N and both dashed lines are due to the doubling of C-NH^+ and NR_4^+. Figure 7 b shows broadening of peaks due to charging. Presumably the charging is caused because of too thick foam covering.

The C 1s spectra show in case of the lager beer with good foam a higher ratio of O=C-N/C-NH^+, which also might indicate long peptides. The π-π*-Peaks (Fig. 8 a) could be also caused by charging of the sample. The spectra of lager beer (Fig. 8 b) with bad foam show higher C-C content, which could be assigned to foam-reducing polyphenols, fatty acids, fats or detergents.

In principle the N 1s spectra of pilsener and lager beer reveal a different composition of each foam. Due to the charging effects of the lager beer samples, there is no exact differentiation possible, but pilsener beers in this comparison seem to have a higher peptide bond content. On the other hand lager beers seem to have higher C-NH^+ and C-NH_3^+ content, which may be caused by amino acids like glycine, aspartic acid, glutamic acid or histidine.

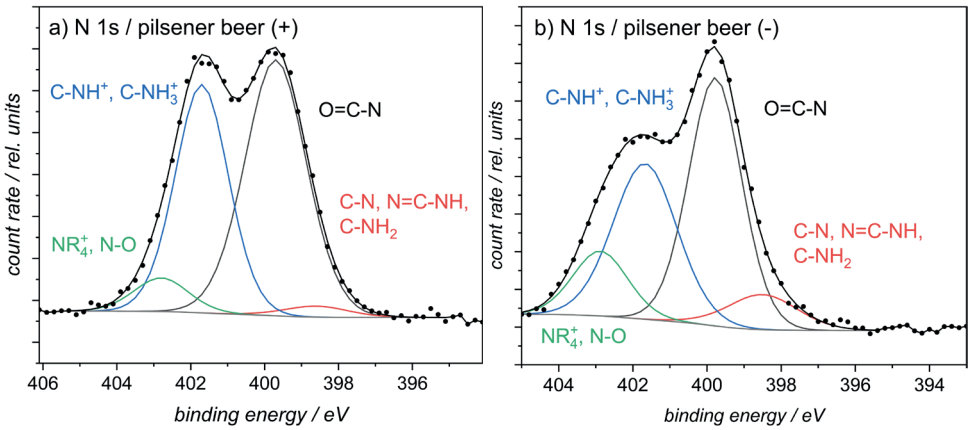

Fig. 5 **N 1s spectra of pilsener beer with a) good foam and b) bad foam**

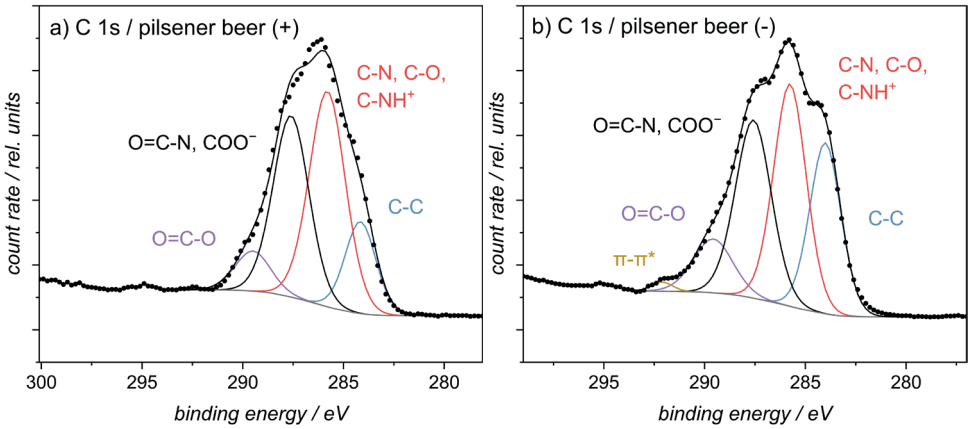

Fig. 6 **C 1s spectra of pilsener beer with a) good foam and b) bad foam**

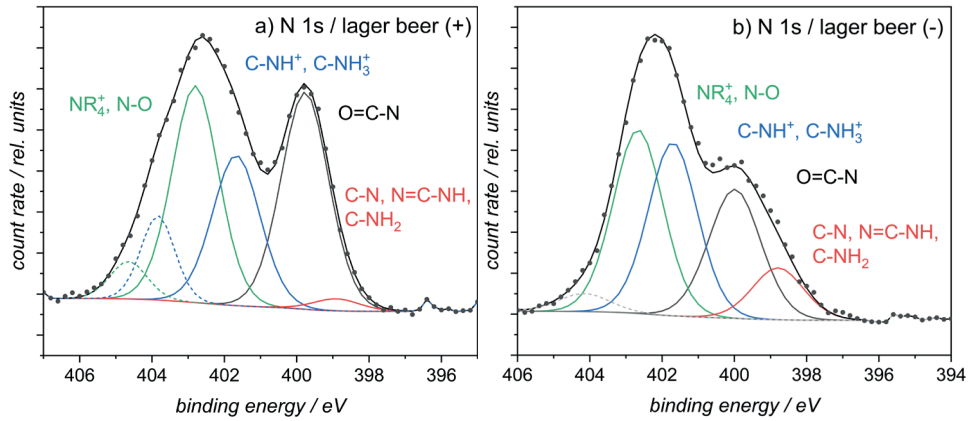

Fig. 7 **N 1s spectra of lager beer with a) good foam and b) bad foam**

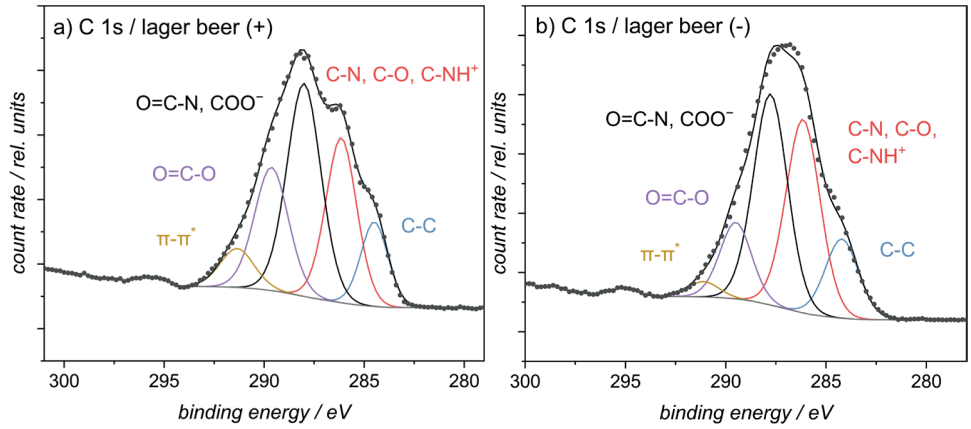

Fig. 8 **C 1s spectra of lager beer with a) good foam and b) bad foam**

4 Conclusion

These first investigations show that XPS is a promising method for analyzing beer foams and it is suitable for determining the reasons for good and bad beer foams. Using the survey spectra, the foams can be analyzed for foreign substances, whereby chlorine and copper, for example, were present in the bad foams. The analysis of the total nitrogen content, which is described in the comparative literature as an indicator of good foams (high contents), shows that the good foam of a beer type always has a higher total nitrogen content than the bad foam. Determination of total nitrogen content in this way appears to be considerably faster and simpler than using conventional methods according to Kjeldahl and Dumas. Further investigations could clarify whether there is an optimal ratio of nitrogen to other species. As higher molecular weight nitrogen compounds are described as foam promoting, the detailed spectra were used to differentiate the nitrogen compounds. The spectra indicate different compositions of the foams of different types of beer, although the foams of one type are quite comparable. The length of the peptide chains of the foam proteins in the tests proposed to be a criterion for the quality of the foam. This can be estimated via the ratio of peptide bonds to primary amines. The spectra also show a large proportion of functional groups that could indicate amino acids. Generally, the higher this ratio, the longer the peptide chains and the better the foam, which is confirmed by the investigations. Through further differentiation and comparison with the beer types, further criteria could be derived from this, and the foam proteins could be characterized more precisely. In addition, the detailed carbon spectra contain indications of fats, fatty acids and polyphenols, which also have an influence on the foam quality.

The significance of the promising results and the further potential of XPS should be proven in a larger project framework.

5 References

1. Evans, D.E. and Sheehan, M.C.: Don't Be Fobbed Off: The Substance of Beer Foam – A review, J. Am. Soc. Brew. Chem., **60** (2002), no. 2, pp. 47-57.
2. Klopper, W.J.: Measurement of foam stability, Brew. Dig., **52** (1977), no. 1, pp. 51-53.
3. Narziss, L.; Back, W.; Gastl, M. and Zarnkow, M.: Abriss der Bierbrauerei, Wiley-VCH, 2017.
4. Neumann, L.: Trade Marks of beer-exploitation of analyses during a 5 years period, Monatsschrift für Brauerei, **31** (1978), no. 3, pp. 86-101.
5. Schur, F.: Rolle der Polyphenole beim Brauen, Brauerei und Getränkerundschau, **98** (1987), no. 3, pp. 37-43.
6. Jacob, F: MEBAK: Brautechnische Analysenmethoden; Würze, Bier, Biermischgetränke; Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommission, MEBAK, 2012.
7. Kaersgaard, P. and Hejgaard, J.: Antigenic beer macromolecules, an experimental survey of purification methods, J. Inst. Brew., **85** (1979), no. 2, pp. 103-111.
8. Bamforth, C.W.: The physics and chemistry of beer foam: a review, European Food Research and Technology, **249** (2023), no. 1, pp. 3-11.
9. Belitz, H.D. and Grosch W.: Lehrbuch der Lebensmittelchemie, Springer-Verlag, 1992.
10. Kunze, W.: Technologie Brauer & Mälzer, VLB Berlin, 1994.
11. Heyse, K.U.: Handbuch der Brauereipraxis, Fachverlag Hans Carl GmbH, 1994.
12. Lewis, M.J. and Young, T.W.: Brewing, Springer, 2001.
13. Hughes, P.S. and Simpson, W.J.: Interactions between hop bitter acids and metal cations assessed by ultra-violet spectrophotometry, Cerevisia Biotechnol., **20** (1995), no. 2, pp. 35-39.
14. Wietstock, P.C.; Kunz, T.; Perreira, F. and Methner, F.-J.: Metal Chelation Behavior of Hop Acids in Buffered Model Systems, BrewingScience – Monatsschrift für Brauwissenschaft, **69** (2016), no. 9/10, pp. 53-63.
15. Stevens, J.S.; De Luca, A.C.; Pelendritis, M.; Terenghi, G.; Downes, S. and Schroeder, S.L.M.: Quantitative analysis of complex amino acids and RDG peptides by X-ray photoelectron spectroscopy (XPS), Surf. Interface Anal., **45** (2013), no. 8, pp. 1238-1246.
16. Honno, E.; Furukubo, S.; Kondo, H.; Ishibaski, Y.; Fukui, N. and Nakatani, K.: Improvement of foam lacing of beer, Tech. Quart. Mast. Brew Assoc. Am., **34** (1997), no. 1, pp. 299-301.
17. Artemenko, A.; Shchukarev, A.; Stenclova, P.; Wagberg, T.; Segervald, S.; Jia, X. and Kromka, A.: Reference XPS spectra of amino acids, Mater. Sci. Eng., **1050** (2021), no. 1, p. 012001.
18. Dreyer, T.; Biedermann, K. and Ottesen, M.: Yeast Proteinase in Beer, Carlsberg Res. Commun., **48** (1983), pp. 249-253.

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