Structures of storage-induced transformation products of the beer's bitter principles, revealed by sophisticated NMR spectroscopic and LC-MS techniques

Besides undesirable changes in the attractive aroma, a significant decrease in the intensity of the bitterness as well as a change of the taste into a lingering, harsh bitterness has long been known as a shelf-life limiting factor of beer. Multiple studies have demonstrated that the aging of beer induces a decrease of the total amount of cis- and trans-iso-alpha-acids, the well-known bitter principles of beer. Although the trans-iso-alpha-acids exclusively, not the cis-iso-alpha-acids, were found to be degraded upon storage of beer, the key transformation products formed exclusively from the trans isomers in beer are not known. In the present study, suitable model experiments followed by LC-MS/MS and sophisticated NMR spectroscopic experiments, including the measurement of residual dipolar couplings (RDCs) in gel-based alignment media as well as a novel broadband and B(1)-field-compensated incredible natural abundance double-quantum transfer experiment (INADEQUATE) pulse sequence, enabled the identification of a series of previously unknown trans-specific iso-alpha-acid transformation products, namely, tricyclocohumol, tricyclocohumene, isotricyclocohumene, tetracyclocohumol, and
epitetracyclocohumol, respectively. HPLC-MS/MS analysis of these compounds, which exhibit the aforementioned harsh lingering bitter taste and have threshold concentrations ranging from 5 to 70 micromol L\(^{-1}\), confirmed their generation during aging of beer and, for the first time, explained the storage-induced changes of the beer's bitter taste on a molecular level.