Abstract

Nitroso compounds are beer contaminants related to carcinogenicity and can be naturally formed during brewing. They are generally categorized as volatile and non-volatile. Well-characterized and proven carcinogenic compounds are mostly volatile *N*-nitrosamines, whereas specific non-volatile nitroso compounds are nearly unknown even by structure. This deficiency limits improvements in health-risk assessment and control of beer. The present study focused on these barely known compounds in beer, related to their molecule structures and natural occurrence in beer and malt. Since these compounds form by nitrite reactions with naturally occurring compounds in beer or raw materials, the beer sample was parallelly treated by nitrite and isotopically labelled nitrite-^{15}N at acidic conditions. Due to isotopic labelling, formed nitrite-related reaction products were distinguished by gas chromatography with tandem mass spectrometry. By this approach, up to 22 unknown nitrite-related reaction products (N-products) were found and structurally studied from mass spectrometric fragmentation patterns. Besides previously found *N*-nitrosoproline and *N*-nitrosoproline ethyl ester, newly characterized compounds in beer were 4-cyanophenol, pyruvic acid oxime, 2-methoxy-5-nitrophenol, nitrosoguaiacol, and 4-nitrosophenol. Several N-products coincided with the retention time and mass spectra with nitrite-related reaction products of tyrosine or vanillic acid. Also, nearly all N-products were found in nitrosated malts treated with nitrogen oxides and showed great extractability into the wort. The acquired chromatographic and mass spectrometric data led to the development of a specific method for monitoring N-products in commercial beers and malts (up to 200 samples each). The observation of relative responses and frequency of N-products appearance by multivariate analyses distinguished important N-products that could be interesting for future investigations. For instance, synthesizing standard compounds of sufficient purity would lead to accurate N-products determination in beer and studies of their potential health-effect on consumers.