Characterization of two Unknown Compounds in Methanol Extracts of a Rosemary Oil

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Rosemary (\textit{Rosmarinus officinalis}) oil is rich in phenolic diterpenes, of which carnosic acid has the highest antioxidant activity. However, carnosic acid appears to be unstable, e.g. during frozen storage. Determination and quantification of the breakdown products is dependent on the source and the extraction method, and is hampered by the lack of available standards. In this study, two unknown compounds in a rosemary oil were identified and characterized. A commercially available rosemary oil, coated on a vegetable oil and containing 3\% carnosic acid, 0.3\% carnosol and 0.03\% methylcarnosate, was extracted in methanol. After purification and analysis by reversed-phase HPLC and LC-MS a high recovery of carnosic acid was obtained, but no carnosol and methylcarnosate was found. However, two unknown compounds with a molecular mass of 330.2 g/mol and 302.2 g/mol respectively were consistently found. From additional LC-MS-MS and \textsuperscript{1}H-NMR analyses, it became clear that the first compound (MW = 330.2 g/mol) could not be carnosol. We hypothesised that it originated from the breakdown of the intramolecular bound of carnosol in the acidic analytical conditions, followed by the addition of a water molecule. Possibly, after dehydratation, an unsaturated double bound was formed.

Assuming that rosmanol was present in the sample due to the oxidation of carnosol, the second compound (MW = 302.2 g/mol) may have resulted from the breakdown of the intramolecular bound of rosmanol and its isomers. Similarly, an unsaturated double bound may have been formed and after splitting off carbon dioxide, a detectable molecule with a MW of 302.2 g/mol was observed.

No carnosic acid or metabolites could be detected when rosemary oil was mixed with a feed, with linseed oil or with soy protein. Hence, further optimisation of the extraction and analytical procedure is necessary to determine carnosic acid and its metabolites in protein and/or fat rich matrices.