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DEVELOPMENT OF A SENSITIVE LC-MS COMPATIBLE ANALYSIS METHOD FOR AMINO ACID DETERMINATION IN HONEY

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In case of some foods and drinks, their amino acid content is demonstrated to correlate with the botanical and/or geographical origin of the plant.

In the present work a method for amino acid analysis in honey was developed and validated. This is a first method development for a contemporary all inclusive (sample preparation, derivatization, analysis, quantitation, validation) method to determine amino acids in honey, which would allow MS detection. The method consists of sample preparation (including solid phase extraction), derivatization with diethyl ethoxymethylenemalonate and liquid chromatographic analysis. This is the first report of application of SPE to honey amino acid analysis. SPE cartridges with polymer-based (styrene–divinylbenzene) strong cation exchange sorbent were used. The analytes were eluted with 2.5 M ammonium hydroxide containing 10% of acetonitrile.

The derivatization procedure of the new and promising derivatization reagent diethyl ethoxymethylenemalonate (DEEMM) was under close testing (Fig. 1). It appeared that derivatized samples must be analysed not earlier than 24 hours and not later than 48 hours after derivatization. Derivatized samples are stable if refrigerated.

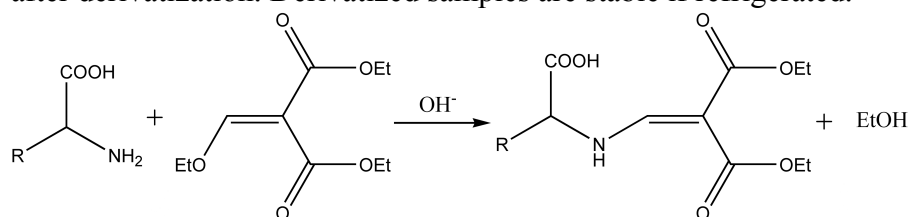


Fig. 1. Reaction of amino acids with DEEMM [1]

Full separation of 23 amino acids was achieved by extensive testing of different columns and gradient programs.

Limits of quantitation (LoQ) values are in the range 0.05 to 0.72 mg kg⁻¹ (except Proline – 10.25 mg kg⁻¹). Recoveries between 80 and 100% were obtained with the exception of Proline, Methionine and Tryptophan.

Major advantage of the developed method is that it is suitable for ultraviolet (UV) and mass spectrometric (MS) detection. Therefore, all steps in optimized amino acid analysis method are MS compatible. Regarding the proposed procedure as generic method for amino acid analysis in different matrices MS and especially MS/MS detection would allow lower detections limits compared to UV detection.

The developed method has been applied to honey samples from Estonia to determine relationship between amino acid composition and botanical and geographical origin. [2]

References

1. R. Chicón, I. Herminos, D. Cabezudo. *Tecnología del Vino*, **2001**, 1 95-100.
2. Rebane, R; Herodes, K. Evaluation of the botanical origin of Estonian uni- and polyfloral honeys by amino acid content. *J. Agric. Food Chem.* **2008**, 56, 10716-10720.