Food Authenticity – HPLC Determination of 16-O-Methylcafestol in Instant Coffee

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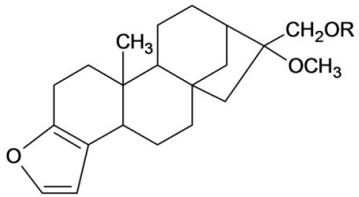
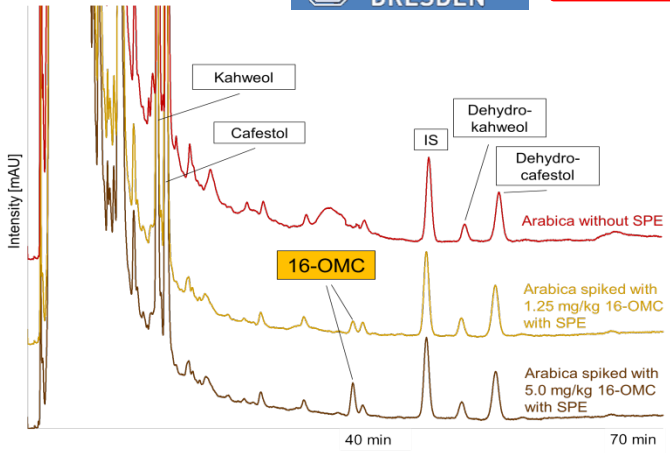
Introduction

The coffee species with the greatest commercial importance are *Coffea arabica* and *Coffea canephora var. robusta*. Within these species, Arabica coffees have a significantly higher market value than Robusta coffees. The botanical origin of coffee can be determined unambiguously by analyzing the concentration of the diterpene 16-O-methylcafestol (16-OMC) [1,2,3]. Whereas Arabica coffees contain no detectable or only very small amounts of 16-OMC (less than 20 mg/kg), the concentrations in Robusta coffees are significantly higher in the approximate range of 800 to 2500 mg/kg [2]. Actually, the determination of 16-OMC in green and roasted coffees is described in DIN method 10779. The NMR analysis is also applied [4].

For instant coffees, no official method is available. Due to the water extraction, instant coffees contain only very small quantities of lipids (0.04 and 0.14%) and, in consequence, of 16-OMC as well, which therefore are not determinable using NMR. In the following, an HPLC method for analyzing 16-OMC in instant coffees based on the DIN 10779 is presented.



R = H: free 16-OMC
R = fatty acid: 16-OMC ester



Methods

After the direct saponification of 5 g instant coffee powder plus 16-oxocafestol (CAS:10864-98-8) as internal standard the unsaponifiable matter is isolated by a two-fold liquid-liquid extraction with tert. butyl methyl ether. The combined ether extracts are concentrated by vaporization and filled up to 10 ml.

In order to reduce impurities caused by the high matrix amount, an additional clean-up via solid phase extraction (SPE) using a silica gel cartridge is introduced. The elution solution is evaporated and the residue is redissolved in acetonitrile/water (50:50).

After membrane filtration, the solution is ready for the HPLC analysis.

5 g Instant coffee

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Saponification

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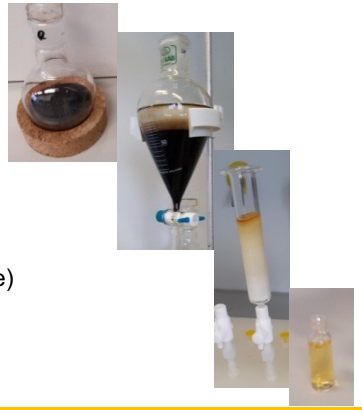
Liquid-liquid extraction

⇩

Clean-up (silica gel cartridge)

⇩

HPLC-UV



Results and Discussion

Due to the expected small quantities of 16-OMC in instant coffees and the high matrix amount, an efficient sample clean-up becomes evident (see red chromatogram above). The developed method using a silica gel cartridge allows for a clear evaluation of the recorded 16-OMC peak in the HPLC chromatograms.

For the validation, an Arabica instant coffee with no detectable 16-OMC was spiked with 1.25 mg/kg, 2.5 mg/kg, 5.0 mg/kg, 10.0 mg/kg, and 20.0 mg/kg 16-OMC prior to the SPE. The resulting recoveries of 16-OMC were in the range between 90% and 98%. The validated method enables a limit of quantitation (LOQ) of 2.5 mg/kg and a limit of detection (LOD) of 1.25 mg/kg.

Therefore, an addition of Robusta can clearly be detected in an instant Arabica coffee declared as 100%.

However, statements concerning the Robusta proportion are not possible because the low 16-OMC contents in instant coffees very strongly depend on the extraction conditions used, such as high pressure and temperature.

Conclusion

The developed HPLC-UV method allows for the clear analysis of the 16-OMC content in instant coffee.

References

[1] Speer K, Tewis R, Montag A (1991), 16-O-Methylcafestol: a quality indicator for coffee. *Proc.14th ASIC Coll.*, ASIC, Paris, 237-244.
[2] Speer K, Kölling-Speer I (2006), The lipid fraction of the coffee bean. Braz. J. Plant. Physiol.,18, 201-216.
[3] DIN 10779: 2011-03
[4] Monakhova YB et al. (2015) The presence of Arabica and Robusta species in coffee using ^{1}H NMR spectroscopy. Food Chemistry 182, 178–184.