New Strategy for the Analysis of Aroma Compounds in Cocoa and Chocolate

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Introduction

The success of an aroma analysis is largely depending on the sample preparation technique and its ability to transfer an authentic image of the original aroma profile into the GC for analysis. Conventional sample preparation techniques very often include strongly discriminating steps especially when volatile aroma compounds are separated from the non-volatile chocolate/cocoa matrix by any type of evaporation and collection from the gas phase (headspace, SPME, SAFE, thermodesorption). Many less volatile aroma compounds are still very important to the aroma. The most negative consequence of this discrimination is that the relevance of these compounds will be significantly underestimated in GC-Olfactometry.

Multiple Step Extraction Approach (MSEA)

MSEA is based on the idea of liquid-liquid extraction but is using several cycles and different solvents. The method is quite universal but is adjusted to the requirements of the actual sample matrix. In the case of chocolate/cocoa the GC sample is prepared from an aqueous slurry and the aroma compounds are extracted several times with different protic and aprotic solvents (water, methanol, ethanol, dichloromethane, diethyl ether and pentane). This procedure ensures that compounds of any volatility and a very broad range of polarity are extracted with a minimum of discrimination. Residual particles and fat are removed by centrifugation and/or filtration. In order to increase the concentration in the final extract for GC injection, the solvent of the final extract is dried over Na_2SO_4, concentrated and injected into the GC.

Comparison of Multiple Step Extraction Approach (MSEA) to Solvent Assisted Flavour Evaporation (SAFE)

40 g of a regular chocolate market sample (99% cocoa content) was extracted following the principle of MSEA. In parallel the same amount of chocolate was extracted by SAFE (standard analytical procedure relying on high vacuum distillation). Both extracts were dried over Na_2SO_4, concentrated and injected into the GC. Peak areas of important aroma compounds were determined on two different column polarities, a non-polar (DB5) and polar column (FFAP).
The relative extraction efficiency of MSEA and SAFE was determined for a wide range of different compounds by calculating corresponding peak area ratios (see Table 1).

Results

Multiple Step Extraction Approach (MSEA) showing significantly higher peak areas of aroma compounds of medium to low volatility compared to Solvent Assisted Flavour Evaporation (SAFE). Table 1 displays results of selected aroma compounds.

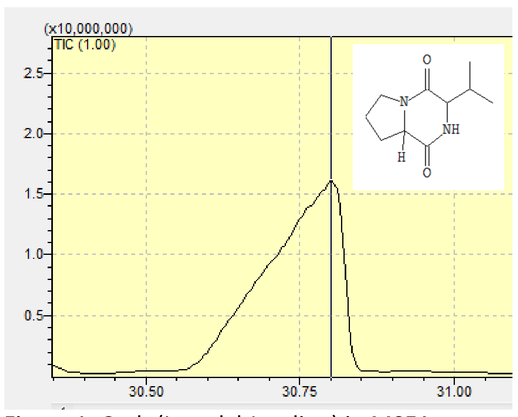

Figure 1. Cyclo(L-prolyl-L-valine) in MSEA extract.

Bitter Compounds

MSEA is able to detect a range of diketopiperazines, which are known to contribute to the bitter taste of cocoa including the most relevant Cyclo(L-prolyl-L-valine), figure 1. Identification is based on their mass spectrum and RI value.

Conclusion

The MSEA is showing very obviously benefits regarding the recovery of aroma compounds of medium to low volatility. This implies that the contribution and relevance of aroma compounds has to be reassessed new by GC-Olfactometry and that most likely other compounds than conventionally assumed have to be also considered for an analytical description of cocoa/chocolate aroma. Very interestingly, even bitter tasting diketopiperazines like Cyclo(L-prolyl-L-valine) can be easily quantified together with aroma compounds. It has to be noted that the method is also very well suited to detect highly volatile compounds with a recovery comparable to that of conventional techniques.

Table 1. Comparison of the the extraction efficiency of MSEA to SAFE.

Compound; CAS	Volatility	Peak area MSEA/SAFE	Column
3-Methylbutanal; 590 - 86 - 3	high	1	DB5
2-Methylbutanal; 96 - 17 - 3		1	DB5
Hexanoic acid; 142 - 62 - 1		1	DB5
Benzaldehyde; 100 - 52 - 7		1	DB5
Phenylacetaldehyde; 122 - 78 - 1		1	DB5
Linalool; 78 - 70 - 6		1	DB5
2-Ethyl-6-methylpyrazine; 13925 - 03 - 6		2	DB5
Trimethylpyrazine; 14667 - 55 - 1		3	DB5
2-Ethyl-3,6-dimethylpyrazine; 13360 - 65 - 1		3	DB5
Tetramethylpyrazine; 1124 - 11 - 4		4	DB5
delta-Hexalactone; 823 - 22 - 3		5	DB5
Phenol; 108 - 95 - 2		5	FFAP
2-Acetyl-6-methylpyrazine; 22047 - 26 - 3		6	DB5
2-Acetyl-3,5-dimethylpyrazine; 54300 - 08 - 2		6	DB5
2-Phenylethanol; 60 - 12 - 8		7	DB5
2-Acetylpyrrole; 1072 - 83 - 9		8	DB5
p-Cresol; 106 - 44 - 5		9	FFAP
Maltol; 118 - 71 - 8		10	FFAP
(Z)-2-delta-Octenolactone; 16400 - 69 - 4		13	DB5
Furaneol; 3658 - 77 - 3		20	FFAP
Octanoic acid; 124 - 07 - 2		23	FFAP
Nonanoic acid; 112 - 05 - 0		23	FFAP
Decanoic acid; 334 - 48 - 5		25	FFAP
Massoia lactone; 54814 - 64 - 1		64	FFAP
Sulfurol; 137 - 00 - 8		132	FFAP
Vanillin; 121 - 33 - 5		153	FFAP
Sotolone; 28664 - 35 - 9		clear peak / no peak	FFAP
Piperonal; 120 - 57 - 0		clear peak / no peak	FFAP
Eugenol; 97 - 53 – 0	low	clear peak / no peak	FFAP