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A method to determine flavouring substances according to EU 1334/2008 in food by GC-MS

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Background

The incorporation of herbs in foods as flavouring agents dates back to the origins of cooking. In fact, today's society is increasing the demand for food products prepared from natural ingredients. Some flavouring plants and plant extracts may contain substances, whose carcinogenic or genotoxic effects have been demonstrated or are currently under study. One category of toxic plant metabolites is alkenylbenzenes.

Objective

Development and validation of a fast, straightforward and affordable method to determine alkenylbenzenes and other flavouring compounds (Figure 1) in a wide range of food commodities.

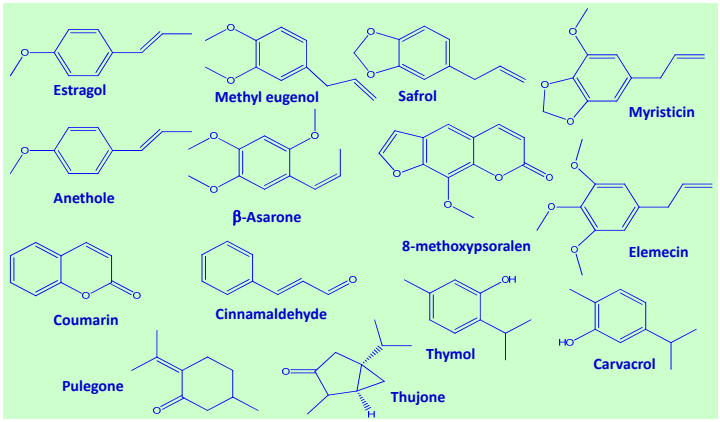

Figure 1. Chemical structure of the alkenylbenzenes and other flavouring substances of interest.

Methodology

- Matrices types: liquids, semi-solids, dry solids and fatty solids.
- Solvent extraction: 2.5 g + 5 mL acetonitrile for 30 min; $MgSO_4$/NaCl (4:1)
- Internal standard: dicyclohexylmethanol
- GC/MS : Restek Rtx®-CL Pesticides (30m x 0.25 mm ID; 0.25μm df)
  60°C (1min), 50°C/min 80°C, 3°C/min 125°C, 10°C/min 300°C (5min)
  1 μL in splitless mode at 250 °C
  MS in EI mode: $T_{ion\ source}$ 250°C, $T_{interfase}$ 280°C
  Acquisition: SCAN (50-250 m/z, scan time: 0.2s) / SIM

Results: in-house validation

- Range: 0.5-200 mg/kg (semi)solids; 0.625-250 mg/kg alcoholic beverages; 0.0625-25 mg/kg non-alcoholic beverages.
- Matrix-matched calibration gave best results.
- LOQs: 0.5 mg/kg (0.0625 mg/kg non-alcoholic beverages).
- Average recoveries all within 70-120%; Repeatability: ≤20%
- Exception: menthofurane; inconsistent results. It may undergo oxidation reaction favoured by the presence of $MgSO_4$ to form menthofurolactone.

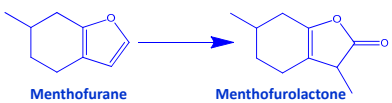

Figure 2. Oxidation of menthofurane [Frerot *et al.*, Flavour Fragr. J. 2002: 17:218-226].

Results: Food survey in the Netherlands

The method was applied to a survey of 61 food products purchased in retail shops in the Netherlands. The survey was designed to cover all the food commodities, for which the EU Regulation 1334/2008 has set regulatory limits.

Table 1. Concentration levels (mg/kg) for the analytes detected in the different food products

Food commodities	Compound	# detected	Mean (mg/kg)	Median (mg/kg)	Highest level (mg/kg)	EU limit (mg/kg)
Dairy products (n = 5)	coumarin	1/5	2.2	2.2	2.2	c.
	myristicin	3/5	6.8	1.0	19	-
	methyl eugenol	1/5	1.4	1.4	1.4	20
	safrol	1/5	2.4	2.4	2.4	-
	elemicin	1/5	1.3	1.3	1.3	-
	trans-cinnamaldehyde	1/5	5.0	5.0	5.0	-
Processed fruits, vegetables, nuts and seeds (n = 5)	coumarin	1/5	6.1	6.1	6.1	-
	myristicin	3/5	1.1	1.2	1.5	-
	methyl eugenol	1/5	1.2	1.2	1.2	-
	estragol	2/5	3.9	3.9	5.2	50
	thymol	1/5	3.5	3.5	3.5	-
	carvacrol	1/5	41	41	41	-
	trans-cinnamaldehyde	1/5	15	15	15	-
Fish products (n = 5)	myristicin	1/3	1.3	1.3	1.3	-
	estragol	2/5	0.9	0.9	1.0	50
	thymol	2/5	4.4	4.4	5.8	-
	carvacol	2/5	2.6	2.6	4.9	-
Meat products (n = 5)	myristicin	2/5	1.9	1.9	2.5	-
	methyl eugenol	1/5	0.6	0.6	0.6	15
	elemicin	1/5	1.2	1.2	1.2	-
Soups and sauces (n = 6)	myristicin	2/5	6.4	6.4	11	-
	estragol	1/5	2.1	2.1	2.1	-
	thymol	1/5	2.0	2.0	2.0	-
	carvacrol	1/5	9.3	9.3	9.3	-
Ready-to-eat savouries (n = 5)	myristicin	3/5	1.2	0.7	2.5	-
	estragol	2/5	2.3	2.3	2.4	-
	thymol	4/5	2.9	1.7	6.8	-
	carvacrol	4/5	20	4.0	70	-
Confectionary (n =5)	pulegone	3/5	3.7	2.6	6.2	250/2000
Alcoholic beverages (n = 6) from *Artemisa* species (n = 2)	*trans*-anethole	5/6	1642	738	5090	-
	coumarin	3/6	23	4	63	-
	thujone	2/6	18	18	30	10/35 (*Artemisa*)
	8-MOP	1/6	2.1	2.1	2.1	-
	thymol	1/6	4.1	4.1	4.1	-
	trans-cinnamaldehyde	2/6	23	23	44	-
Non-alcoholic beverages (n = 5)	*trans*-anethole	3/5	1.3	0.4	3.3	-
	coumarin	1/5	7.1	7.1	7.1	-
	thujone	1/5	2.7	2.7	2.7	-
	estragol	1/5	0.15	0.15	0.15	10
	trans-cinnamaldehyde	2/5	15	15	28	-
Breakfast cereals with muesli (n = 5)	coumarin	2/5	6.9	6.9	11	20
	trans-cinnamaldehyde	2/5	31	31	53	-
Chewing gum (n = 5)	*trans*-anethole	3/5	364	19	1073	-
	pulegone	4/5	75	73	140	350
	trans-cinnamaldehyde	1/5	31	31	31	-
Bakery products (n = 5)	*trans*-anethole	1/5	3.7	3.7	3.7	-
	coumarin	4/5	8.5	9.1	15	50
	myristicin	2/5	3.2	3.2	4.8	-
	methyl eugenol	3/5	1.8	1.1	3.3	-
	trans-cinnamaldehyde	4/5	9.9	10	19	-

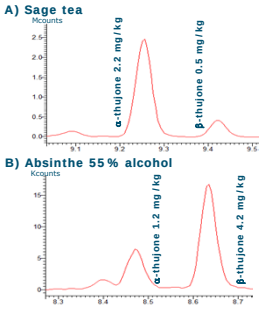

Figure 3. Diasteroimeric distribution of α–thujone and β–thujone in samples.

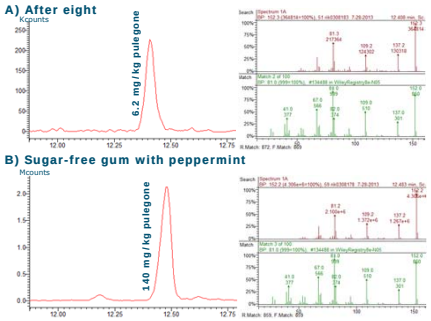

Figure 4. Identification of pulegone in samples.

Conclusions

- All samples were compliant with EU Regulation 1334/2008.
- Levels of coumarin may result in an exposure that would exceed the TDI of 1 mg/kg bw/day for children.
- Myristicin and *trans*-anethole (non-regulated) were the most common alkenylbenzenes detected in food.

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