

Supporting information for: Occurrence of Perillaldehyde and Other (Suspected) Genotoxic Flavoring Substances in Water-Based Beverages Consumed by Belgian Children

Alexandre Dusart^{a,b*}, Els Van Hoeck^a, Séverine Gosciny^a, and Sonia Collin^b

^a Department of Chemical and Physical Health Risks, Sciensano, Ixelles 1050, Belgium ;

^b Unité de Brasserie et des Industries Alimentaires, Louvain Institute of Biomolecular Science and Technology (LIBST), Faculté des Bioingénieurs, Université catholique de Louvain, Louvain-la-Neuve 1348, Belgium.

* Corresponding author: Alexandre.Dusart@sciensano.be (Alexandre Dusart): +32 2 6425188

Supporting Information 1: Sample description

All samples are described in Table S1 based on food product labeling information. The order of the ingredients was unchanged from labeling, and irrelevant data to this work was not included here (e.g., vitamins, preservatives, sugars).

Table S1: Sample description.

N°	Sample description (main relevant ingredients, flavorings in bold)
Citrus and citrus-based juices	
1	Hand-squeezed orange juice
2	Fresh orange juice
3	Orange juice from concentrate
4	Orange juice from concentrate
5	Orange juice from concentrate
6	Orange juice from concentrate
7	Orange juice from concentrate
8	Orange juice from concentrate
9	Hand-squeezed orange juice
10	Water, fresh lemon juice
11	Lemon juice from concentrate
12	Water, fruit juice from concentrate (orange, lime, lemon), natural flavorings
13	Fruit juices from concentrate (Apple, orange, pineapple, mandarin, maracuja, lemon), fruit purees (apple, banana, nectarine, pear, mango, guava, apricot).
14	Water, fruit juice from concentrate (orange, guava, apricot, mango, passion fruit)
15	Water, fruit, and vegetable juices from concentrates and purees (apple juice, orange juice, carrot juice, lemon juice, pineapple juice, apricot puree, passion fruit juice, grapefruit juice, banana puree, guava puree, mango puree, grape juice, kiwi juice)
16	Water, fruit juices from concentrate (orange, apple, lemon, grape, peach), flavorings
17	Fruit juices from concentrate (apple, white grape, orange, mango, acerola, lemon, carambola), flavoring
18	Water, fruit juices from concentrate (apple, pear, orange, passion fruit, pineapple), fruit puree (banana, peach, mirabelle plum, pear, mango)
19	Fruit juices from concentrate (apple, white grape, orange, mango, passion fruit), natural flavoring
20	Fruit juices (orange, grape, pineapple, apple, lemon), fruit purees (apricot, peach, banana, guava, mango, passion fruit, pear)
Apple, apple-based fruit juices, and other noncitrus fruit juices	
1	Fresh apple juice
2	Apple juice from concentrate
3	Apple juice from concentrate
4	Apple juice from concentrate
5	Apple juice from concentrate

6	Apple juice, fruit juices from concentrate (pear, cherry), natural flavoring
7	Fruit juices from concentrate (apple, cherry)
8	Fruit juices from concentrate (apple, orange, pineapple, passion fruit, pear, lemon, grape), fruit purees (banana, nectarine, mango, papaya, guava)
9	Water, cranberry juice from concentrate, vegetable concentrate (cranberry and carrot), natural flavoring.
10	Water, juices from concentrate (cranberry, lemon)
11	Water, guava coulis, fruit juices from concentrate (lemon, blackcurrant)
12	Pineapple juice from concentrate
13	Red grape juice from concentrate
Lemonades	
1	Spring water, fruit juices from concentrate (orange, apple, acerola), natural orange flavoring
2	Spring water, fruit juices from concentrate and purees (apple, orange, acerola, passion fruit, mango, banana), natural orange flavoring with other natural flavoring
3	Spring water, fruit juices from concentrate (orange, apple, acerola, passion fruit, mango), natural orange flavoring with other natural flavoring
4	Spring water, fruit juices from concentrate (orange, apple, passion fruit, mango), flavoring
5	Spring water, fruit juices from concentrate (orange, apple, passion fruit, mango), flavoring, natural flavoring
6	Water, fruit juices concentrate (orange, pineapple), orange extract; flavorings
7	Spring water, fruit juices from concentrate (orange, apple, passion fruit, mango), flavoring
8	Spring water, fruit juices from concentrate (orange, apple, grapefruit, passion fruit), carrot concentrate, natural flavorings, natural orange flavoring
9	Spring water, fruit juices from concentrate (orange, apple, passion fruit, mango), flavoring
10	Spring water, fruit juices from concentrate (lemon, pineapple, orange, mandarin, lime), natural flavoring
11	Spring water, fruit juices from concentrate (lemon, orange, apple, pineapple, banana, kiwi, passion fruit), natural flavoring
12	Spring water, fruit juices from concentrate (lemon, orange, passion fruit, pineapple, apricot), natural flavoring
13	Sparkling water, natural lemon-lime flavorings
14	Sparkling water, natural lemon flavoring with other natural flavorings
15	Sparkling water, citrus juices from concentrate (orange, lemon, grapefruit), natural flavorings (including citrus)
16	Sparkling water, natural flavorings
17	Sparkling water, orange juice from concentrate, natural orange flavoring with other natural flavorings, carrot concentrate
18	Sparkling water, orange juice and other citrus fruit concentrates (orange, lemon, mandarin, grapefruit), pulp (orange, mandarin), orange zest extract, natural orange flavorings
19	Sparkling water, natural lemon-lime flavoring with other natural flavorings
20	Sparkling water, orange extracts, natural citrus flavorings with other natural flavorings
21	Water, fruit juices from concentrate (mandarin juice, orange juice, clementine juice), natural flavorings
22	Natural mineral water, fruit juices from concentrate (orange juice, passion fruit juice, mango juice), natural flavoring
23	Sparkling water, natural lemon-lime flavoring
24	Sparkling water, orange juice from concentrate, flavorings
25	Sparkling water, orange juice from concentrate, natural orange flavoring with other natural flavorings
26	Water, fruit juices from concentrate (raspberry, cherry), flavoring
27	Water, fruit juices from concentrate (apple, blackberry, blueberry), flavoring
28	Spring water, fruit juice concentrate (apple, blackcurrant, elderberry, raspberry), natural flavorings
29	Sparkling water, concentrated fermented milk, skimmed milk powder, strawberry juice from concentrate, flavorings, concentrated cranberry juice
30	Sparkling natural mineral water, fruit juice (lemon, strawberry, raspberry, casis), concentrates (carrot, safflower), natural flavorings

31	Sparkling water, flavoring , blackcurrant and carrot concentrates
Iced teas	
1	Water, lemon juice from concentrate, tea extract, natural lemon-lime flavoring
2	Green tea infusion (water, concentrated green tea infusion), lime juice from concentrate, green tea extracts, natural lemon-lime flavoring , mint extract, natural mint flavoring with other natural flavorings
3	Sparkling water, tea extract, lemon juice from concentrate, flavoring
4	Sparkling water, tea extract, lemon juice from concentrate, flavoring
5	Sparkling water, fruit juices from concentrate (white grape, apple, pear), natural fruit extracts, concentrated lemon juice, green tea extract, natural flavorings , ginkgo biloba extract (<1%)
6	Sparkling water, lemon juice from concentrate, tea extracts, natural flavorings
7	Sparkling water, lemon juice from concentrate, tea extract, natural flavoring
8	Sparkling water, tea extract, flavoring , lemon juice concentrate
9	Green tea infusion, raw cane sugar, lemon juice from concentrate.
10	Water, green tea extract, natural flavoring
11	Water, green tea extract, flavorings
12	Water, green tea extract, flavorings
13	Black tea infusion (water, concentrated black tea infusion), peach juice from concentrate , natural peach flavoring , natural flavorings , hibiscus extracts
14	Water, peach juice from concentrate, 0.1% tea extract, flavoring
Colas	
1	Sparkling water, lemon juice concentrate, flavorings (including caffeine and natural plant extracts)
2	Sparkling water, flavorings (including caffeine)
3	Sparkling water, natural flavorings (plant extracts) including caffeine
4	Sparkling water, flavorings (including caffeine and plant extracts)
5	Sparkling water, natural flavorings, caffeine flavoring
6	Sparkling water, flavor (caffeine), natural flavor
7	Sparkling water, natural flavorings (including plant extracts), flavoring (caffeine)
8	Sparkling water, natural flavoring
9	Sparkling water, natural flavorings (plant extracts) including caffeine
10	Sparkling water, natural flavorings
11	Sparkling water, flavorings (including plant extracts)
Sports beverages	
1	Water, natural orange flavoring with other natural flavorings
2	Water, natural lemon flavor with other natural flavor
3	Water, flavoring
4	Water, natural flavorings
5	Water, flavorings

Supporting Information 2: Mass spectral data and retention indices of monitored compounds

Table S2: Mass spectral data and retention indices of monitored compounds.

N°	Compound	RI (CP-Sil 5 CB)	Molecular ion	Major ions m/z (intensity %)
1	2-Ethyl-5-methylfuran	791 ^a	110	43 (5), 95 (100), 110 (16)
2	2-Octylfuran	1283 ^a	180	29 (13), 39 (13), 41 (22), 53 (22), 81 (100), 82 (30), 95 (24), 123 (8), 180 (9)
3	1-(2-Furyl)-2-propanone	925	124	29 (5), 39 (15), 41 (8), 51 (5), 53 (24), 81 (100), 82 (21), 124 (12),
4	2-Acetylfuran	887	110	38 (5), 39 (15), 43 (10), 95 (100), 110 (42)
5	2-Pentylfuran	981	138	39 (5), 41 (6), 53 (13), 81 (100), 82 (26), 95 (5), 138 (18)
6	3-Acetyl-2,5-dimethylfuran	1062	138	43 (54), 53 (8), 81 (24), 95 (6), 123 (100), 124 (7), 138 (72)
7	2-Heptylfuran	1184	166	41 (9), 43 (6), 53 (16), 67 (7), 81 (100), 82 (30), 95 (21), 109 (10), 123 (11), 166 (23)
8	2-Hexanoylfuran	1281	166	123 (9), 95 (70), 110 (100)
9	2-Acetyl-5-methylfuran	1011	124	43 (11), 53 (15), 81 (6), 109 (100), 110 (5), 124 (34)
10	2-Acetyl-3,5-dimethylfuran	1072	138	39 (9), 41 (12), 43 (39), 67 (9), 112 (6), 113 (6), 123 (100), 124 (7), 138 (40)
11	2-Butylfuran	882	124	27 (11), 39 (11), 41 (6), 53 (20), 81 (100), 82 (22), 124 (16)
12	2-Butyrylfuran	1075	138	39 (18), 95 (100), 110 (55), 138 (6)
13	1-(2-Furyl)-3-butanone	1043	138	43 (44), 53 (14), 67 (21), 68 (11), 81 (92), 82 (16), 95 (78), 96 (10), 123 (9), 138 (100)
14	3-Methyl-2(3-methylbut-2-enyl)furan	1089 ^a	150	77 (16), 79 (27), 82 (15), 91 (36), 95 (27), 105 (16), 107 (30), 135 (100), 150 (98)
15	2-Pentanoylfuran	1178	152	27 (6), 39 (17), 95 (92), 110 (100)
16	2-(<i>s</i> -Butyl)-4,5-dimethyl-3-thiazoline	1222;1238;1240	171	55 (6), 82 (10), 87 (20), 100 (9), 114 (100), 171 (11)
17	4,5-Dimethyl-2-ethyl-3-thiazoline	1061;1074	143	42 (10), 53 (11), 68 (32), 69 (19), 73 (6), 82 (12), 87 (29), 102 (46), 114 (100), 143 (60)
18	4,5-Dimethyl-2-isobutyl-3-thiazoline	1215;1221	171	68 (35), 71 (28), 87 (33), 100 (15), 101 (20), 110 (18), 114 (100), 115 (17), 138 (29), 171 (38)
19	4-Methyl-5-vinylthiazole	1010	125	39 (10), 45 (12), 58 (33), 59 (6), 71 (6), 97 (42), 98 (24), 124 (14), 125 (100)
20	Ethone	1783	190	118 (6), 133 (25), 161 (100), 162 (11), 190 (21)
21	Vanillylidene acetone	1795	192	43 (41), 51 (35), 77 (34), 78 (25), 89 (38), 117 (49), 145 (98), 177 (96), 191 (35), 192 (100)
22	Homo ethone	1783	204	90 (7), 118 (5), 133 (17), 161 (100), 162 (11), 204 (28)
23	2-Phenylcrotonaldehyde	1246	146	51 (11), 63 (10), 65 (10), 78 (11), 91 (27), 115 (99), 116 (31), 117 (100), 118 (16), 146 (66)
24	5-Methyl-2-phenyl-2-hexenal	1474	188	41 (62), 43 (80), 91 (60), 103 (37), 104 (76), 115 (82), 117 (100), 188 (65)
25	4-Methyl-2-phenyl-2-pentenal	1357	174	41 (57), 43 (100), 71 (47), 77 (45), 91 (70), 103 (89), 131 (51), 174 (91)
26	2-Phenyl-2-pentenal	1327	160	91 (57), 103 (17), 115 (46), 116 (25), 117 (51), 128 (16), 129 (29), 130 (13), 131 (49), 145 (33), 160 (100)
27	Menthallactone	1474	166	39 (20), 41 (24), 67 (52), 81 (42), 95 (32), 109 (44), 110 (23), 137 (53), 138 (32), 166 (100)
28	Hex-2-eno-1,4-lactone	997	112	27 (57), 28 (28), 29 (36), 39 (18), 55 (63), 57 (19), 83 (100), 84 (15)
29	3-(2-furyl)acrylaldehyde	1081	122	38 (12), 39 (62), 40 (18), 63 (15), 65 (77), 66 (65), 68 (21), 94 (80), 121 (26), 122 (100)
30	4-(2-Furyl)-3-buten-2-one	1158	136	39 (31), 43 (54), 65 (74), 93 (17), 94 (46), 121 (100), 136 (65)
31	3-(2-Furyl)-2-methyl-2-propenal	1167	136	39 (40), 51 (41), 53 (23), 68 (38), 77 (68), 79 (96), 80 (25), 107 (38), 108 (58), 136 (100)
32	3-(5-Methyl-2-furyl)-2-propenal	1202	136	43 (31), 53 (10), 65 (27), 77 (18), 93 (12), 94 (58), 107 (20), 121 (71), 135 (19), 136 (100)
33	<i>delta</i> -Damascone	1354	192	41 (29), 69 (100), 81 (19), 123 (26), 192 (10)
34	<i>alpha</i> -Damascone	1378	192	41 (14), 69 (100), 81 (18), 123 (20), 192 (16)
35	Perillaldehyde (<i>p</i> -mentha-1,8-dien-7-al	1257	150	39 (47), 41 (42), 53 (45), 67 (54), 68 (100), 77 (36), 79 (60), 93 (35), 107 (43), 150 (32)
36	2,4-Pentanedione	760	100	27 (7), 42 (6), 43 (100), 72 (5), 85 (34), 100 (22)
37	3-Acetyl-2,5-dimethylthiophene	1232	154	43 (21), 45 (8), 59 (18), 67 (15), 77 (11), 111 (25), 139 (100), 154 (46)
38	Furan-2(5 <i>H</i>)-one	864	84	27 (12), 29 (5), 38 (5), 39 (12), 54 (20), 55 (100), 84 (56)

^a = RI from NIST database

Supporting Information 3: Validation of the method

The method was validated for 30 flavoring substances out of the 38 initially targeted. The validation confirmed the accurate and versatile character of the SAFE technique. The different validation parameters are described in the following sections.

S3.1. Linearity

Linearity was assessed at six concentration levels (i.e. 0.25; 2.5; 5; 7.5; 10, and 15 mg.L⁻¹). N-decane was used as an external standard, spiked at 12.5 mg.L⁻¹ in each vial. The relative response of each compound was plotted against relative concentration levels and submitted to a Mandel's Fitting test ($R^2 > 0.99$). Linearity was confirmed for all compounds within the concentration range.

S3.2. Stability

Compound stability was verified by comparing chromatograms of fresh standard solutions to those stored at -80 °C and -20 °C. Peak areas were identical for both storage temperatures.

S3.3. Matrix effect

Standard addition with an internal standard inherently took into account the matrix effect. Four reference samples underwent the standard addition procedure to use their slopes to quantify similar products. Standard addition curves were submitted to a Mandel's Fitting test ($R^2 > 0.99$) to ensure linear responses.

S3.4. Selectivity, limits of detection (LOD) and quantification (LOQ)

The pre-SAFE L/L extraction, followed by the SAFE extraction and GC-MS(SIM) allowed for high selectivity of the method. LOD and LOQ were determined by the signal-to-noise approach to estimate practical values for these two parameters (S/N ratio of 3 and 10 respectively). The limit of quantification (LOQ) was estimated by spiking standards at low concentration (i.e. 10 µg.kg⁻¹) in the samples before the extraction, and measuring the S/N after analysis. The estimated LOQ were confirmed by GC injection of standards (in dichloromethane) at the estimated LOQ. The LOD was calculated as the LOQ divided by three. LOD and LOQ values ranged from 0.01 – 0.2 µg.kg⁻¹ and 0.03 – 0.6 µg.kg⁻¹, respectively. To limit the risk of compound misidentification (false positive), more than two ions were monitored per analyte in the SIM program. For each analyte, the abundance ratios between the quantifier ion (100% intensity) and the different qualifier ions were interpreted according to EC guidelines.

S3.5. Precision, measurement uncertainty and recoveries

Recoveries were assessed with standard addition and an external calibration, both with an external standard (EST, n-decane) using the following equation: X absolute recovery (%) = slope from standard addition of X relative to the EST × (1/slope from calibration of X relative to the EST) × (1/concentration factor). As already reported in our previous work, alkylfurans (**5**), (**7**), (**11**) and thiazolines (**16**), (**17**), and (**18**) did not pass the validation criteria as their standard addition slopes were not linear. They were semi-quantitatively analyzed, as well as compounds (**20**) and (**21**) which were poorly recovered. For all the remaining compounds (#28), coefficients of variation on repeatability and within-lab reproducibility were respectively below 14% and 23%. The measurement of uncertainties ranged between 7–46% (7-20% for eighteen compounds, 20-46% for ten compounds). Absolute recoveries were between 60 and 84%.