Table S1. Baseline characteristics of the 14 healthy volunteers

Volunteer	Sex	Age [years]	Size [cm]	Weight [kg]	BMI [kg/m^2]
A	M	24	187	87	24.9
B	M	23	186	84	24.3
C	M	20	158	56	22.4
D	M	18	174	62	20.5
E	F	20	172	65	22.0
F	M	19	188	72	20.4
H	F	20	173	74	24.7
I	F	24	168	52	18.4
J	F	23	170	63	21.8
K	M	21	178	67	21.1
L	M	20	175	67	21.9
M	F	20	174	73	24.3
N	F	22	169	68	23.8
O	F	23	163	50	18.8
Mean		21	174	67	22.1
SD		2	9	11	2.1

M, male; F, female; SD, standard deviation.

Table S2. Composition of the low-fibre and high-fibre breakfasts

	Low-fibre breakfast Baguette, white bread	Butter	Total	**High-fibre breakfast** Orange Juice	Rye bread	Carob paste	Total
Macronutrients							
Portion	80g	14g		200ml	100g	25g	
Energy, kcal	204.8	104.0	308.8	82.0	192.0	142.8	416.8
Carbohydrate, g	41.4	0.1	41.5	17.6	34.9	6.6	59.1
Protein, g	6.8	0.1	6.9	1.4	4.6	1.6	7.6
Lipid, g	1.0	11.5	12.4	1.0	1.3	11.3	13.6
SFA, g	0.2	7.5	7.7	0.1	0.1	3.1	3.3
MUFA, g	0.1	3.0	3.1	0.1	0.1	3.1	3.2
PUFA, g	0.5	0.5	0.9	0.1	0.3	3.8	4.2
Total fibre, g	2.6	-	2.6	0.8	11.0	4.3	16.1
Amino acids							
Isoleucine, mg	287.2	4.1	291.3	12.0	168.0	63.9	243.9
Leucine, mg	494.4	6.7	501.1	22.0	326.4	111.8	460.2
Lysine, mg	160.8	6.2	167.0	16.0	214.8	91.2	322.0
Methionine, mg	107.2	1.7	108.9	6.0	87.0	18.3	111.3
Cystine, mg	126.4	0.6	127.0	8.0	110.4	22.8	141.2
Phenylalanine, mg	344.8	3.5	348.3	14.0	231.6	70.7	316.3
Tyrosine, mg	160.8	2.9	163.7	6.0	126.0	45.6	177.6
Threonine, mg	195.2	2.9	198.1	14.0	180.0	54.8	248.8
Tryptophan, mg	68.8	1.0	69.8	4.0	58.8	18.2	81.0
Valine, mg	333.6	4.8	338.4	18.0	244.2	68.5	330.7
Arginine, mg	264.8	2.4	267.2	78.0	265.2	102.6	445.8
Histidine, mg	149.6	2.0	151.6	6.0	120.0	36.5	162.5
Alanine, mg	218.4	2.2	220.6	26.0	231.6	61.6	319.2
Vitamins							
Vit. A, µg	-	103.4	103.4	9.7	-	0.6	10.3
Retinol, µg	-	94.9	94.9	-	-	-	-
Beta-Carotene, µg	-	51.1	51.1	58.0	-	3.7	61.7
Vit. D, µg	-	0.1	0.1	-	-	-	-
Vit. E, mg	0.4	0.3	0.7	0.2	0.5	4.6	5.3
Vit. K, µg	-	-	-	-	1.2	7.4	8.6
Vit. B1, mg	0.1	-	0.1	0.1	0.1	-	0.3
Vit. B2, mg	0.04	-	0.04	0.1	0.1	-	0.1
Vit. B3, mg	0.9	0.01	0.9	0.6	0.5	0.1	1.2
Vit. B5, mg	0.3	-	0.3	0.3	0.2	0.1	0.5
Vit. B6, mg	0.1	-	0.1	0.1	0.1	-	0.2
Vit. B8, µg	1.6	-	1.6	1.7	2.9	-	4.5
Vit. B9, µg	25.6	0.4	26.0	32.0	40.8	28.0	100.8
Vit. B12, µg	-	-	-	-	-	-	-
Vit. C, mg	-	-	-	78.8	-	-	78.8
Minerals							
Sodium, mg	413.9	2.0	415.9	30.0	1.5	0.1	31.6
Potassium, mg	97.2	2.7	100.0	336.0	255.0	67.8	658.8
Calcium, mg	27.3	2.1	29.4	34.2	19.2	5.3	58.7
Magnesium, mg	16.8	0.2	17.0	20.0	51.9	8.4	80.3
Iron, mg	0.8	-	0.8	0.4	1.5	0.1	2.0
Copper, mg	0.1	-	0.1	0.1	0.2	0.1	0.3
Zinc, mg	0.6	-	0.6	0.1	1.7	0.2	2.0
Manganese, mg	0.4	-	0.4	-	1.2	0.1	1.3
Selenium, µg	1.9	0.1	2.0	0.1	1.9	0.4	2.4
Phosphorus, mg	75.1	3.4	78.4	43.2	174.0	19.6	236.8
Chloride, mg	303.2	2.2	305.4	NA	923.0	NA	NA
Iodine, µg	16.9	0.2	17.1	0.8	-	-	0.8
Nickel, µg	2.9	0.1	3.0	1.2	6.9	13.9	22.0
Fatty acids							
C4:0, g	-	0.4	0.4	-	-	-	-
C6:0, g	-	0.3	0.3	-	-	-	-
C8:0, g	-	0.2	0.2	-	-	-	-
C10:0, g	-	0.4	0.4	-	-	-	-
C12:0, g	-	0.4	0.4	-	-	-	-
C14:0, g	-	1.3	1.3	-	-	0.1	0.1
C16:0, g	0.2	3.4	3.6	0.1	0.1	2.3	2.5
C18:0, g	-	1.2	1.2	-	-	0.4	0.4
C18:2 n-6, g	0.4	0.2	0.7	0.1	0.3	3.8	4.1
C18:3 n-3, g	-	0.2	0.2	-	-	-	-

Macronutrients were quantified using Nubel; amino acids, vitamins, and minerals were quantified using FRIDA; and fatty acids were quantified using FRIDA and Ciqual. - = absent. NA = not available. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

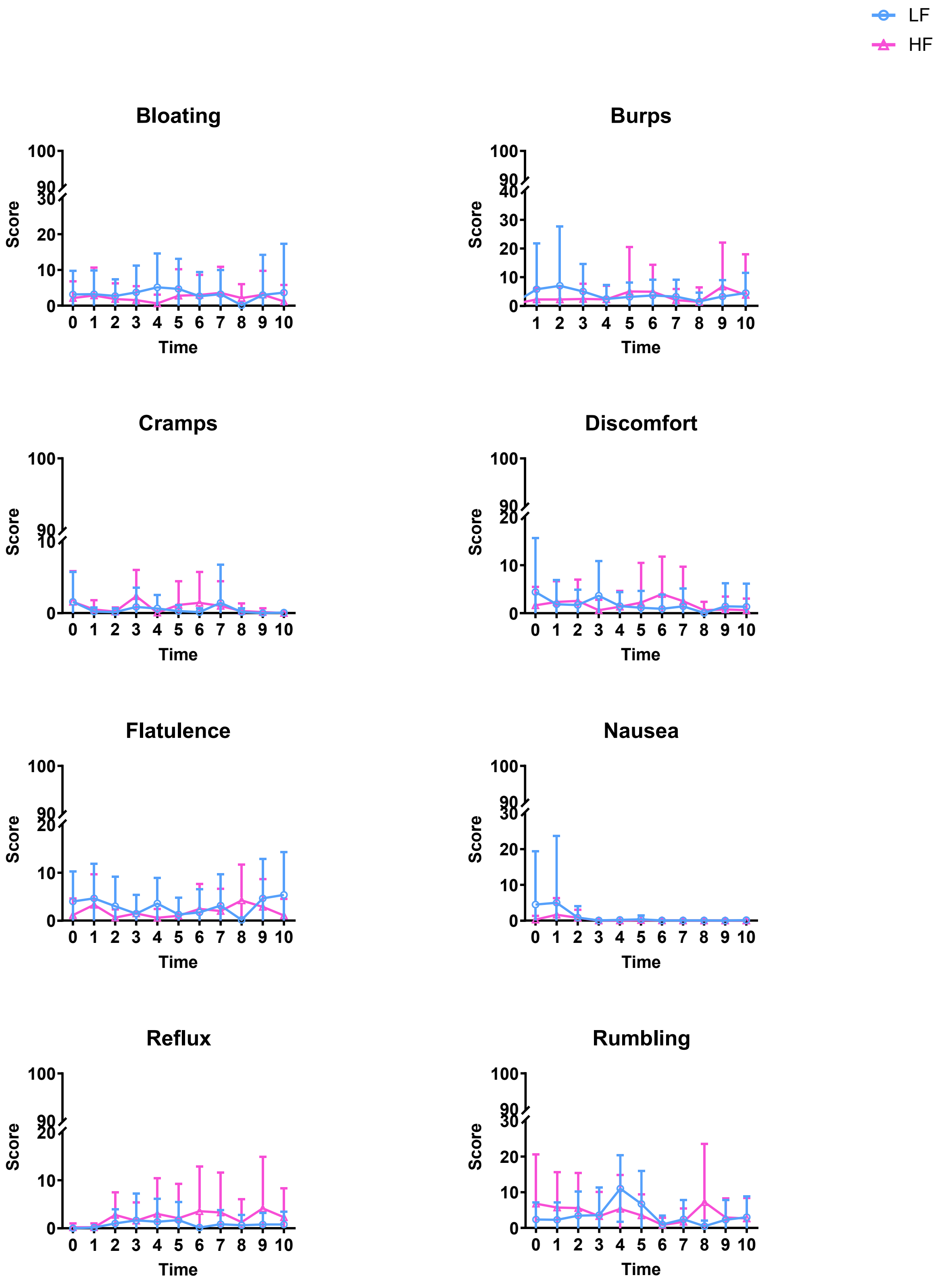


Figure S1. Evolution of 8 gastrointestinal symptoms of 14 healthy volunteers after low-fibre (LF) and high-fibre (HF) breakfast ingestion. Data are not normally distributed and expressed as mean ± standard deviation. Wilcoxon test on net area under the curve (AUC, $p > 0.05$). Šídák test ($p > 0.05$).

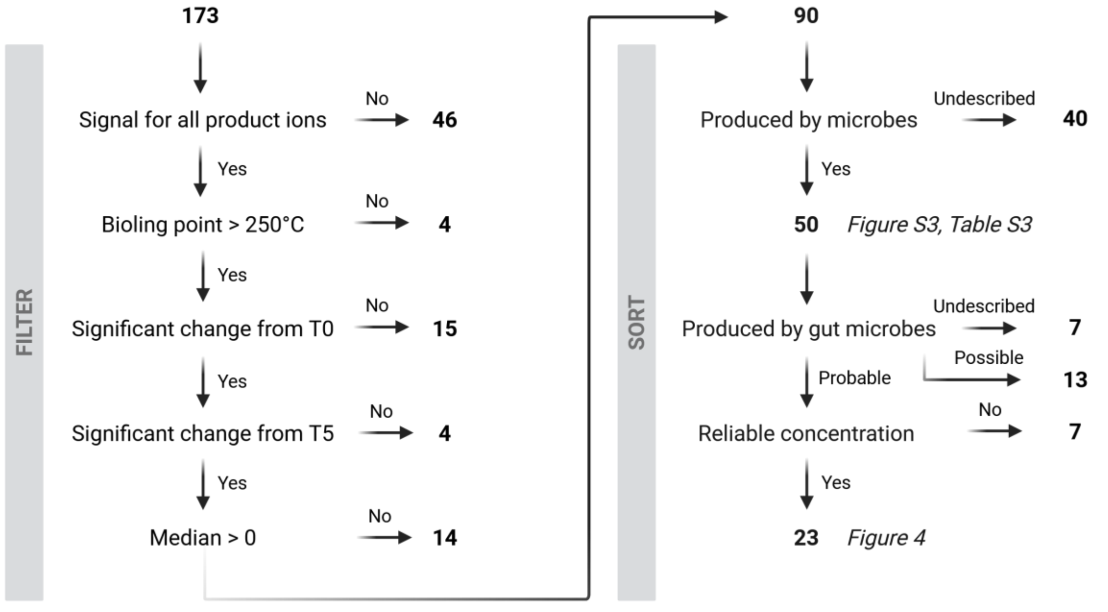


Figure S2. Decision tree showing the steps followed to highlight breath compounds as potential biomarkers of the gut microbiota activity. The 173 initial compounds are the candidates that might have formed one or several of the 30 signal(s) most noteworthy for explaining the difference between breath samples collected between 5 and 10 hours following low-fibre and high-fibre breakfast ingestion. The filtering process led to exclusion of the compounds when (1) the ions they produce during reactions with the reagent ions in the selected-ion flow-tube mass spectrometry (SIFT-MS) device were not detected in the spectra, making their presence unlikely; (2) their boiling point was above 250°C, making their volatilisation in the instrument unlikely; they did not show a significantly different concentration evolution between the two test days when considering (3) all measurement times (Wilcoxon test on net area under the curve (AUC) from T0 to T10, $p < 0.05$) and (4) from 5 hours post-breakfast (Wilcoxon test on net AUC from T5 to T10, $p < 0.05$), indicating their concentration curves over the two test days did not explain the clustering observed in the principal component analysis (PCA); (5) the median difference between their concentration value and that of the helium blank was less than 0 for at least one measurement time, questioning the precision of their quantification with the current method. The 90 compounds that passed the filtering process were sorted based on the probability of their production by microbes, assessed by an extensive literature review. Microbial production of identified compounds was evaluated until 8th January 2025 using the following PubMed query: (compound name OR synonym) AND ("gastrointestinal microbiome"[MeSH Terms] OR ((intestin* OR gastrointestin* OR "gut" OR "enteric") AND ("flora" OR "microflora" OR microb* OR bacteria*))), where the name of the compound was the one used in LabSyft and its synonym was the international union of pure and applied chemistry (IUPAC) name if different. If no results were obtained using this first equation, the following was applied: (compound name OR synonym) AND ("flora" OR "microflora" OR microb* OR bacteria*). Moreover, the name of the compound and its IUPAC name were searched in the MiMeDB [1] (metabolites, microbial sources section) and KEGG [2] (KEGG pathway entry point) databases. When microbial pathways were described in KEGG, enzymes were searched in the BRENDA [3] database to know about microbes able to synthetise the compound of interest. "Probable" means production by bacteria present in the gut and "Possible" means production by bacteria expected (MiMeDB) in the gut, or production by *Saccharomyces cerevisiae* only. Finally, 23 compounds were selected for discussion based on the reliability of their concentration, meaning maximum 2 conflicts and concentration close to that usually described in breath samples.

1. Wishart DS, Oler E, Peters H, Guo A, Girod S, Han S, et al. MiMeDB: the Human Microbial Metabolome Database. Nucleic Acids Res. 2023;51(D1):D611–D20.
2. Kanehisa M, Furumichi M, Sato Y, Kawashima M, Ishiguro-Watanabe M. KEGG for taxonomy-based analysis of pathways and genomes. Nucleic Acids Res. 2023;51(D1):D587–d92.
3. Schomburg I, Chang A, Hofmann O, Ebeling C, Ehrentreich F, Schomburg D. BRENDA: a resource for enzyme data and metabolic information. Trends Biochem Sci. 2002;27(1):54–6.

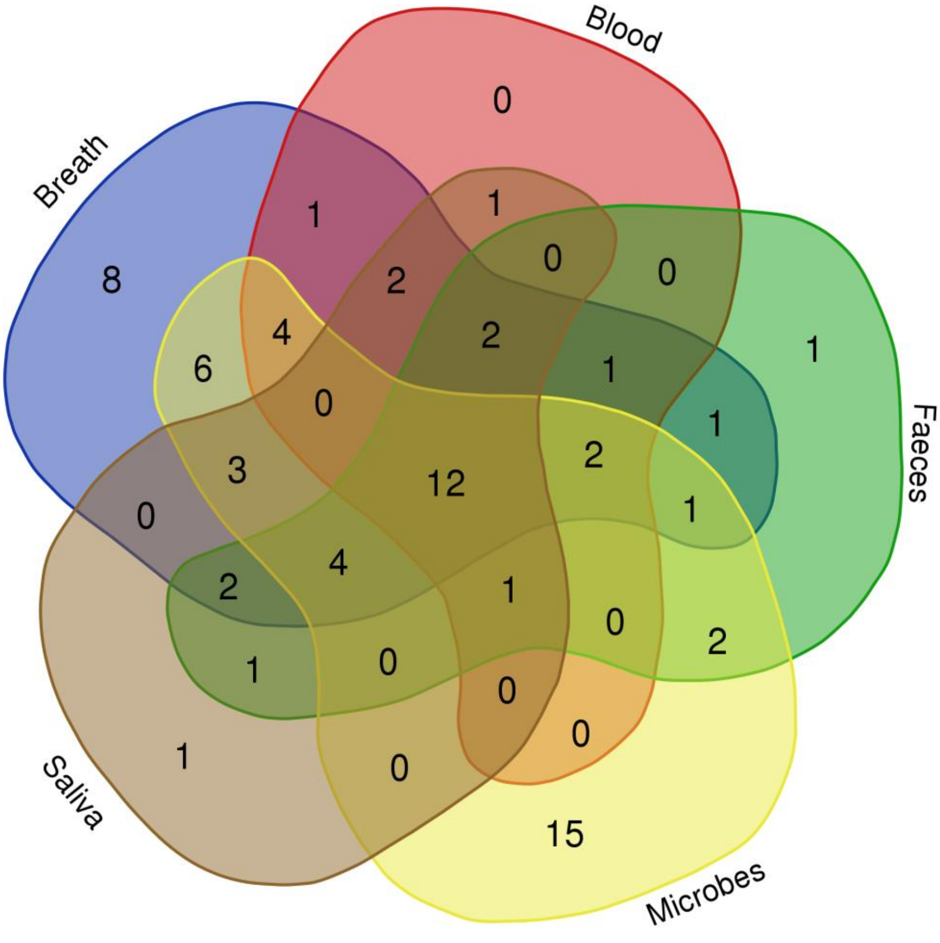


Figure S3. Venn diagram showing the current knowledge about the 90 breath compounds highlighted in the present paper as potential biomarkers of the gut microbiota activity. Out of them, 19 compounds aren't embraced in any of the intersects. Previous measurements in human matrices (focus on blood, breath, faeces and saliva) were established based on [1].

1. Drabinska N, Flynn C, Ratcliffe N, Belluomo I, Myridakis A, Gould O, et al. A literature survey of all volatiles from healthy human breath and bodily fluids: the human volatilome. J Breath Res. 2021;15(3).

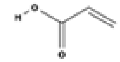
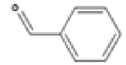
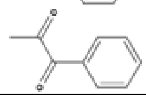
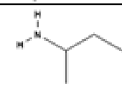
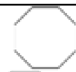
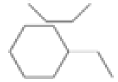
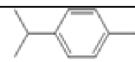
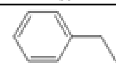
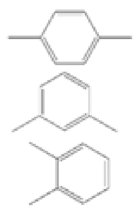
Table S3. Detail of the Venn diagram shown in Figure S3.

Intersection	Total	Compound
Blood ∩ Breath ∩ Faeces ∩ Microbes ∩ Saliva	12	Benzaldehyde [2-5, 10] Cumene [3-5] Dimethyl disulfide [1, 10] Ethylbenzene [2-5, 10] Heptanal [2, 10] Isoamyl alcohol [2, 10] Limonene [3-5, 10] Methanol [2-5] Octane [2] 1-Pentanol [2] Trimethylbenzene [2] Xylene [2-5, 10]
Blood ∩ Breath ∩ Faeces ∩ Microbes	2	Methylcyclohexane [10] Nonane [10]
Blood ∩ Breath ∩ Faeces ∩ Saliva	2	2-Methyl-2-butenal Trimethylsilanol
Breath ∩ Faeces ∩ Microbes ∩ Saliva	4	Cyclohexane [3-5] p-Cymene [2-5, 10] Naphthalene [2] 2-Phenylethanol [2, 10]
Blood ∩ Faeces ∩ Microbes ∩ Saliva	1	Phenylacetaldehyde [2-5]
Blood ∩ Breath ∩ Faeces	1	Ethyltoluene
Blood ∩ Breath ∩ Microbes	4	Chlorobenzene [2-5] 1,1-Dichloroethane [3-5] 1,2-Dichloroethene [3-5] Dimethyl selenide [2]
Blood ∩ Breath ∩ Saliva	2	3-Buten-2-one Methylcyclopentane
Breath ∩ Faeces ∩ Microbes	1	3-Methyl-2-butenal [2-5]
Breath ∩ Faeces ∩ Saliva	2	2-Pentanol 1-Propanethiol
Breath ∩ Microbes ∩ Saliva	3	Dimethyl sulfone [2-5] Guaiacol [2-5] Lactic acid [2-5]
Blood ∩ Breath	1	2,2,4-Trimethylpentane
Breath ∩ Faeces	1	4-Methylbenzaldehyde
Breath ∩ Microbes	6	Acrylonitrile [3-5] Ethylcyclohexane [10] 2-Ethylfuran [2] 2-Hexen-1-ol [2] 1-Phenylethanol [3-5] Propylbenzene [2]
Blood ∩ Saliva	1	Oct-2-en-1-ol
Faeces ∩ Microbes	2	Methional [2] Methionol [2]
Faeces ∩ Saliva	1	3-Pentanol
Breath	8	Bromomethane Cyclooctane N,N-Dimethylaniline 2,2-Dimethylhexane 2-Ethylthiophene 3-Methyl-2(5H)-furanone 2-Nitropropane 1-Pentene
Faeces	1	Cuminal
Microbes	15	Acrylic acid [2-5] Allyl chloride [6] Benzyl mercaptan [2] sec-Butylamine [2] Chloroacetic acid [2] 4-Chlorophenol [3-5] 1,3-Dithiane [7] 4-Hydroxybenzyl alcohol [2] 2-Methylbenzaldehyde [3-5] 3-Methylbenzaldehyde [3-5] 6-Methylhept-5-en-2-ol [9] Nitroethane [2] Peracetic acid [2] 1-Phenyl-1,2-propanedione [8] Styrene oxide [2]
Saliva	1	3-Vinylpyridine
None	19	Benzylamine Benzyl chloride 1-Butanethiol 2-Chloroethyl ethyl sulfide 2-Chlorophenol 3-Chlorophenol Cyclopropanecarboxylic acid 2,5-Dimethylthiophene Epichlorohydrin 1,2-Ethanedithiol 2-Mercapto-3-butanol Methyl acrylate (Methylthio)acetic acid n-Nitrosodi-n-propylamine 2-Pentenal 1-Pentyn-3-ol 1-Pentanethiol 2-Vinylpyridine 4-Vinylpyridine

The compounds are potential biomarkers of gut microbiota activity highlighted in breath samples from 14 healthy volunteers. The presence in human samples was verified based on [11]. The production by bacteria was established based on several references listed in the table.

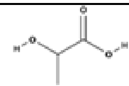
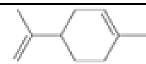
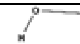
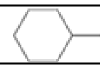
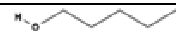
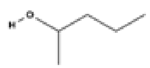
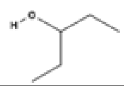
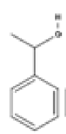
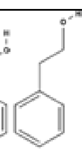
1. Rimbault A, Niel P, Virelizier H, Darbord JC, Leluan G. l-Methionine, a Precursor of Trace Methane in Some Proteolytic Clostridia. Appl Environ Microbiol. 1988;54(6):1581–6.
2. Wishart DS, Oler E, Peters H, Guo A, Girod S, Han S, et al. MiMeDB: the Human Microbial Metabolome Database. Nucleic Acids Res. 2023;51(D1):D611–D20.
3. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28(1):27–30.
4. Kanehisa M, Furumichi M, Sato Y, Kawashima M, Ishiguro-Watanabe M. KEGG for taxonomy-based analysis of pathways and genomes. Nucleic Acids Res. 2023;51(D1):D587–d92.
5. Kanehisa M. Toward understanding the origin and evolution of cellular organisms. Protein Sci. 2019;28(11):1947–51.
6. Zhang M, Ning Z, Guo C, Shi C, Zhang S, Sheng Y, et al. Using Compound Specific Isotope Analysis to decipher the 1,2,3-trichloropropane-to-Allyl chloride transformation by groundwater microbial communities. Environ Pollut. 2023;316(Pt 1):120577.
7. Baek JH, Kim KH, Min Han D, Lee SH, Ok Jeon C. Effects of glutinous rice paste and fish sauce on kimchi fermentation. Food Science and Technology. 2023;173.
8. Gong S, Lan T, Zeng L, Luo H, Yang X, Li N, et al. Gut microbiota mediates diurnal variation of acetaminophen induced acute liver injury in mice. J Hepatol. 2018;69(1):51–9.
9. Bianchi F, Careri M, Mangia A, Mattarozzi M, Musci M, Concina I, et al. Differentiation of the volatile profile of microbiologically contaminated canned tomatoes by dynamic headspace extraction followed by gas chromatography-mass spectrometry analysis. Talanta. 2009;77(3):962–70.
10. Hernandez-Leyva AJ, Berna AZ, Liu Y, Rosen AL, Lint MA, Whiteside SA, et al. The breath volatilome is shaped by the gut microbiota. medRxiv. 2024.
11. Drabinska N, Flynn C, Ratcliffe N, Belluomo I, Myridakis A, Gould O, et al. A literature survey of all volatiles from healthy human breath and bodily fluids: the human volatilome. J Breath Res. 2021;15(3).

Table S4. Overview of the main results of the literature review about the novel potential breath biomarkers of gut microbiota activity.

# CAS	Compound name	Formula	Breath	Structure	Gut microbiota
79-10-7	Acrylic acid	$C_3H_4O_2$	No		Synthetised by gut bacteria, notably belonging to *Bacillota* and *Pseudomonadota* [2].
107-13-1	Acrylonitrile	C_3H_3N	Yes		Synthetised by microbes either from acrylic acid or acrylamide [3].
100-52-7	Benzaldehyde	C_7H_6O	Yes		Synthetised by *Bacillus subtilis* [2].
579-07-7	1-Phenyl-1,2-propanedione	$C_9H_8O_2$	No		Synthetised by *Escherichia coli*, *Citrobacter freundii*, *Clostridium difficile* and *Enterococcus faecalis* [4].
13952-84-6	sec-Butylamine	$C_4H_{11}N$	No		Synthetised by *Bacteroides ovatus*, *Bacteroides thetaiotaomicron* and *Bifidobacterium longum* [2].
292-64-8	Cyclooctane	C_8H_{16}	Yes		Has never been associated with the gut microbiota.
1678-91-7	Ethylcyclohexane	C_8H_{16}	Yes		Synthetised by *Collinsella aerofaciens* [5].
99-87-6	p-Cymene	$C_{10}H_{14}$	Yes		Concentration in the breath of paediatric patients and mice influenced by their gut microbiome [5].
624-92-0	Dimethyl disulfide	$C_2H_6S_2$	Yes		Synthetised by *Clostridium hastiforme*, *Clostridium histolyticum*, *Clostridium subterminale*, and *Clostridium sp. strain DSM 1786* [6].
593-79-3	Dimethyl selenide	C_2H_6Se	Yes		Selenium metabolism influenced by gut microbes [7].
100-41-4	Ethylbenzene	C_8H_{10}	Yes		Increased concentration in the breath of mice mono-colonised with *Bacteroides thetaiotaomicron* and *Ruminococcus torques* compared to germ-free controls [5].
95-47-6 108-38-3 106-42-3 1330-20-7	Xylene	C_8H_{10}	Yes		Increased concentration in the breath of mice mono-colonised with *Akkermansia muciniphila*, *Bacteroides thetaiotaomicron*, *Escherichia coli* and *Ruminococcus torques* compared to germ-free controls [5].

The presence in human samples was verified based on [1]. Compounds likely to be confused are listed on the same row.

Table S4 (continued)

# CAS	Compound name	Formula	Breath	Structure	Gut microbiota
111-71-7	Heptanal	$C_7H_{14}O$	Yes		Synthetised by *Saccharomyces cerevisiae* [2]. Upregulated in active Crohn's disease compared to inactive disease and healthy controls [8]. Increased after administration of lactic acid bacteria to ducks [9].
123-51-3	Isoamyl alcohol	$C_5H_{12}O$	Yes		Synthetised by *Roseateles aquatilis* [10], *Klebsiella oxytoca* and *Citrobacter freundii* [11] isolated from insect gut.
50-21-5	Lactic acid	$C_3H_6O_3$	Yes		Synthetised by lactic acid bacteria [12].
138-86-3	Limonene	$C_{10}H_{16}$	Yes		Synthetised by many microbes found in human faeces [2].
67-56-1	Methanol	CH_4O	Yes		Decreased addition rate to plasma after removing the intestine in rats [13].
505-10-2	Methionol	$C_4H_{10}OS$	No		Synthetised by *Saccharomyces cerevisiae* [2]. Positive correlation between concentration in serum and bacteria *Streptococcus* and *Veillonella* but also with fungi *Pseudeurotium*, *Gibellulopsis*, *Petriella*, *Humicola*, *Cercophora*, *Schizothecium*, *Neurospora*, and *Sordaria* [14].
108-87-2	Methylcyclohexane	C_7H_{14}	Yes		Synthetised by *Collinsella aerofaciens* [5].
111-84-2	Nonane	C_9H_{20}	Yes		Synthetised by *Collinsella aerofaciens* [5].
71-41-0	1-Pentanol	$C_5H_{12}O$	Yes		Synthetised by *Saccharomyces cerevisiae* and *Escherichia coli* [2]. Positive correlation between concentration and family *Pirellulaceae* in patients with autism spectrum disorder and control subjects [15].
6032-29-7	2-Pentanol	$C_5H_{12}O$	Yes		Positive correlation between concentration and *Psychrobacter*, *Pseudomonas*, *Staphylococcus*, and *Shewanella* during bacon production [16].
584-02-1	3-Pentanol	$C_5H_{12}O$	No		Has never been associated with the gut microbiota.
122-78-1	Phenylacetaldehyde	C_8H_8O	No		Synthetised by gut bacteria, mainly belonging to *Pseudomonadota* [2].
98-85-1	1-Phenylethanol	$C_8H_{10}O$	Yes		Synthetised from acetophenone or ethylbenzene by bacteria expected to belong to the gut microbiota [2, 3].
60-12-8	2-Phenylethanol	$C_8H_{10}O$	Yes		Synthetised by *Roseateles aquatilis* [10], *Klebsiella oxytoca* and *Citrobacter freundii* [11] isolated from insect gut.

The presence in human samples was verified based on [1]. Compounds likely to be confused are listed on the same row.

Table S4 (continued)

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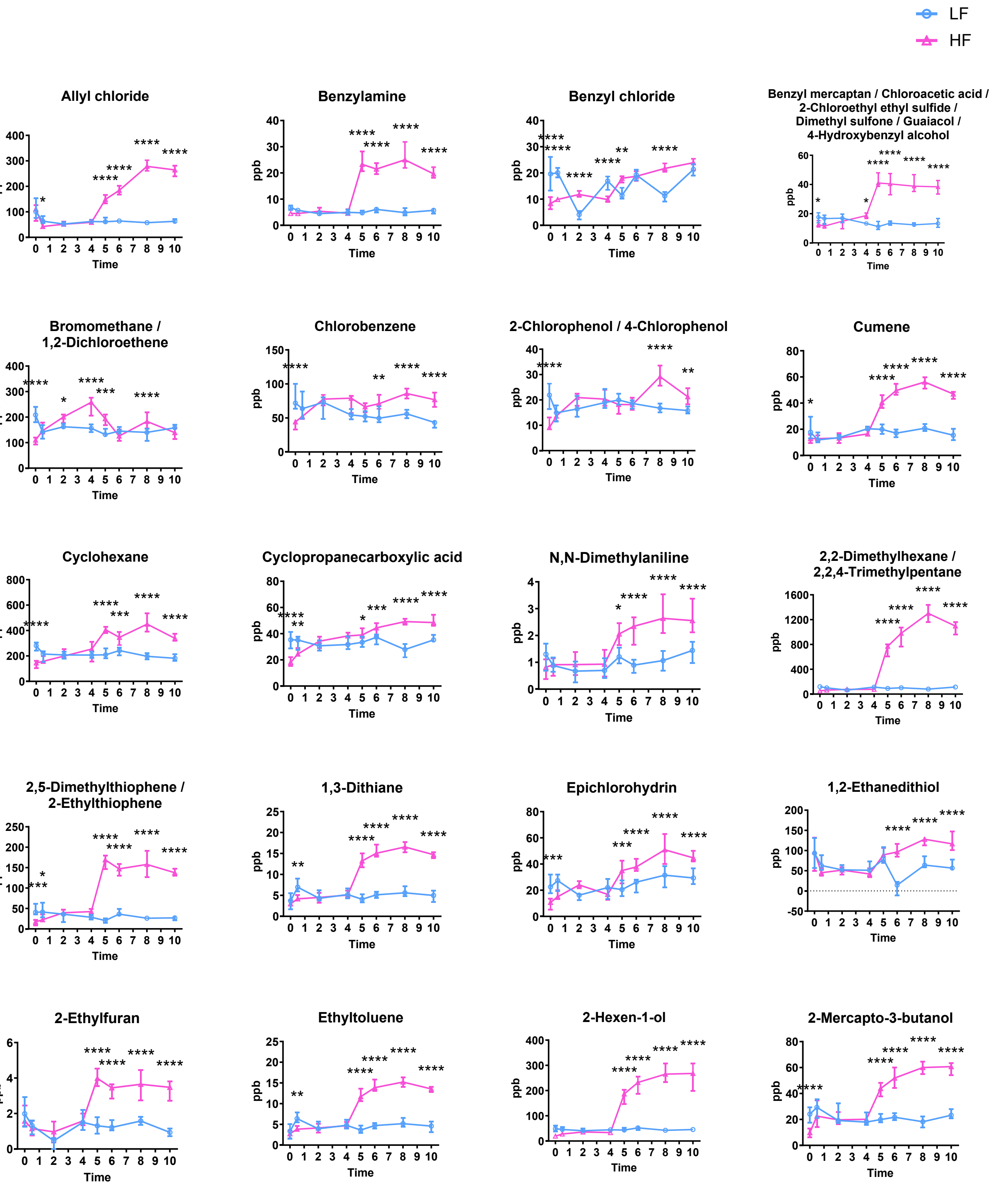

Figure S4. Evolution of the compounds not included in the main Figures, measured in breath samples from 14 healthy volunteers with a higher concentration after high-fibre (HF) compared to low-fibre (LF) breakfast ingestion. The compounds are sorted in alphabetical order. Data are not normally distributed and expressed as median [P25-P75]. Wilcoxon test on net area under the curve (AUC, $p < 0.05$). Šídák test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

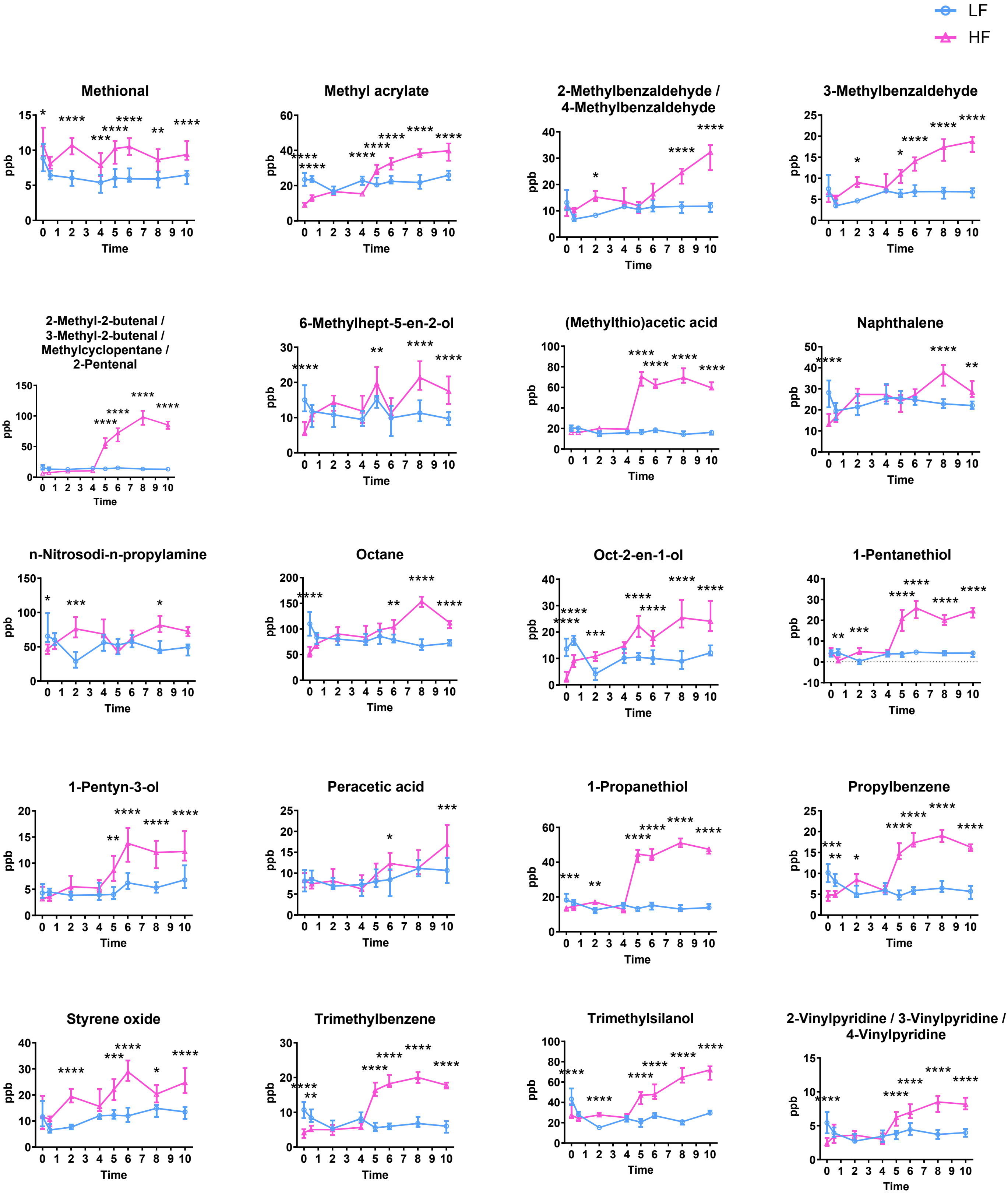

Figure S4 (continued)

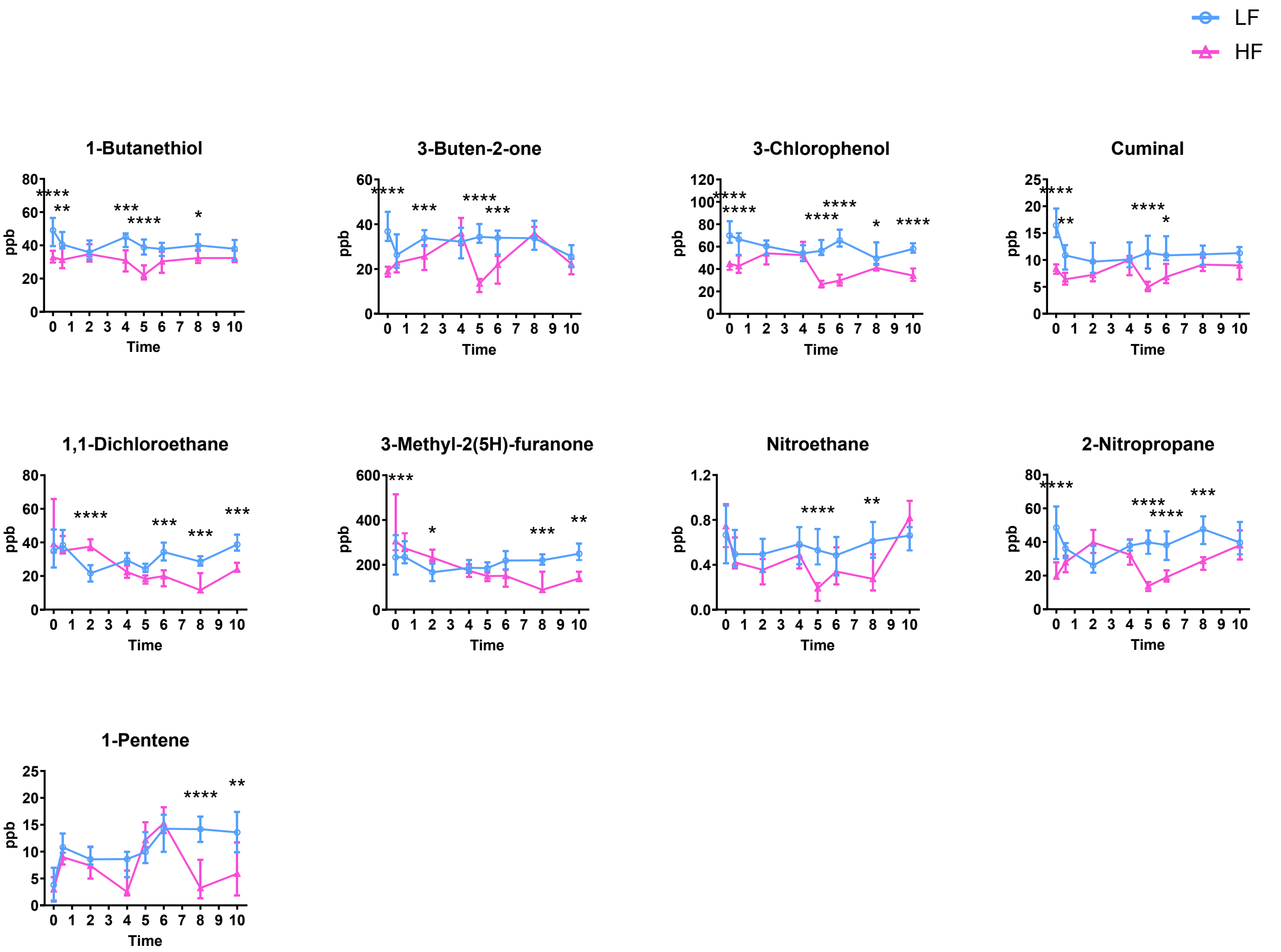

Figure S5. Evolution of the compounds not included in the main Figures, measured in breath samples from 14 healthy volunteers with a higher concentration after low-fibre (LF) compared to high-fibre (HF) breakfast ingestion. The compounds are sorted in alphabetical order. Data are not normally distributed and expressed as median [P25-P75]. Wilcoxon test on net area under the curve (AUC, $p < 0.05$). Šídák test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).