LabWorks APPNOTE

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Aroma Analysis of Yogurt by Solvent-Assisted SBSE (SA-SBSE) and Multivariate Analysis - Comparison with Conventional SBSE Results in Terms of Compounds Detected and Sensory Differences

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Keywords

LabWorks Platform, Solvent-assisted SBSE (SA-SBSE), FLEX-Twister®, yogurt, aroma analysis, polar compounds, sensory evaluation, GC-MS, Aroma Office, multi-variate analysis.

Abstract

Stir Bar Sorptive Extraction (SBSE) and Solvent Assisted Stir Bar Sorptive Extraction (SA-SBSE) are firmly established techniques for extraction of compounds from various matrices, with the latter technique offering significant increased capacity for concentration of hydrophilic/polar compounds. In this application note this difference is explored by applying both SBSE and SA-SBSE to a berry-flavored yogurt followed by GC-MS after liquid desorption of the stir bars. The sequential combination of MassHunter Unknowns Analysis and Aroma Office database search on the resulting data files was used to identify and document the compound profile differences. Four plain (non-flavored) yogurts were extracted by SA-SBSE and both GC-MS and sensory evaluations performed on the back extracts after liquid desorption. Multivariate analysis was then performed on the samples from the list of aroma compounds indicated, profiling them graphically in combination with the sensory characteristics. Finally, both SBSE and SA-SBSE were performed on one of the plain yogurts to investigate the sensory differences in the back extracts. Results indicated that the increased extractive power of SA-SBSE resulted in an aroma profile closer to that of the original product than that produced by SBSE alone.

Introduction

Yogurt is appreciated as a healthy fermented dairy product and its consumption is widespread. In addition to containing aroma compounds derived from raw materials and the fermentation process, commercial yogurt products with added fruits and flavors are also popular. The analysis of aroma compounds in dairy products such as yogurt can be performed by headspace methods (SHS, DHS), solid-phase microextraction (SPME, HS-SPME), stir bar sorptive extraction (SBSE, HSSE), and solvent-assisted flavor evaporation (SAFE), among other extraction techniques. However, due to the relatively large influence of the sample matrix, including milk fat content and non-fat milk solids, it is difficult to extract trace amounts of hydrophilic/polar compounds using these methods.

To improve the extraction efficiency of hydrophilic/polar compounds, and to overcome the difficulties associated with conventional SPME and SBSE, solvent-assisted SBSE (SA-SBSE) was developed in 2016 [1]. This uses a solvent swollen PDMS extraction phase. By swelling PDMS with dichloromethane (DCM), ether, or other suitable solvent, its polarity and volume can be increased to improve the extraction rate of hydrophilic/polar compounds while maintaining the original characteristic of high affinity for hydrophobic compounds.

Here we introduce an example of applying SA-SBSE to the whey obtained by centrifuging a berry-type yogurt and provide an analytical comparison with SBSE. Four types of plain yogurt with-

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out any addition of fruits and flavorings were further analytically investigated by SA-SBSE followed by sensory evaluation of the SA-SBSE back-extracted solutions. In the case of one of these samples (Sample A) sensory evaluation of the back-extracted solutions from both SBSE and SA-SBSE was performed for qualitative sensory comparison.

Several references [1-3] are available for additional information on SA-SBSE.

Experimental

Instrumentation

The thermal desorption (TD)-GC-MS analysis was performed using the GERSTEL LabWorks Platform installed on an Agilent 7890A gas chromatograph with a 5977 single quadrupole MS (QMS).

Sample

The yogurt samples were berry-type yogurt containing fruits and flavorings (milk fat < 0.5%, nonfat milk solids < 10%) and four plain-type yogurt samples (milk fat 3-4%, nonfat milk solids 8-15%) that did not contain any fruits and flavorings. The berry-type sample, which is rich in flavor compounds, was diluted twice with ultrapure water and centrifuged (10 min @ 3000 rpm) to obtain the supernatant. Plain type samples with fewer aromatic compounds were centrifuged (10 min @ 3000 rpm) 2 or 3 times without dilution. 5 mL of the supernatant, which corresponds to whey, was placed in a 10 mL HS vial, salted (30% NaCl), and extracted using SA-SBSE and SBSE.

SA-SBSE and SBSE

A FLEX Twister with 63 µL PDMS (1 cm length × 1.0 mm thickness) (Part No.: 021075-010-00) is used for both SA-SBSE and SBSE.

Before SA-SBSE, solvent swelling of the FLEX-Twister was done in a 2 mL-vial. First, using a syringe, 105 µL of 1/1 dichloromethane (DCM)/diisopropyl ether (DIPE) mixed solvent was added into the 2 mL-vial containing the FLEX-Twister. The sealed vial was laid on its side and left for more than 30 min. The solvent swollen FLEX-Twister can typically be stored in the 2 mL vial at room temperature for a week.

Both individual SA-SBSE and SBSE extractions were performed at room temperature (25 °C) for 60 min while stirring at 800 rpm. After extraction, both stir bars were removed with a magnetic rod (Twister taking tool, Part No.: 013820-000-00) and forceps, rinsed 10 seconds in ultrapure water, and dried with a lint-free tissue.

For liquid desorption (solvent back extraction), each stir bar was placed in a sealed 10 mL HS vial containing 0.5 mL of acetone. The stir bars were stirred at room temperature (25 °C) for 30 min at 800 rpm. After solvent back extraction, the acetone extract was transferred to a 2 mL vial. The sealed 2 mL vial was placed in the MPS robotic pro tray.

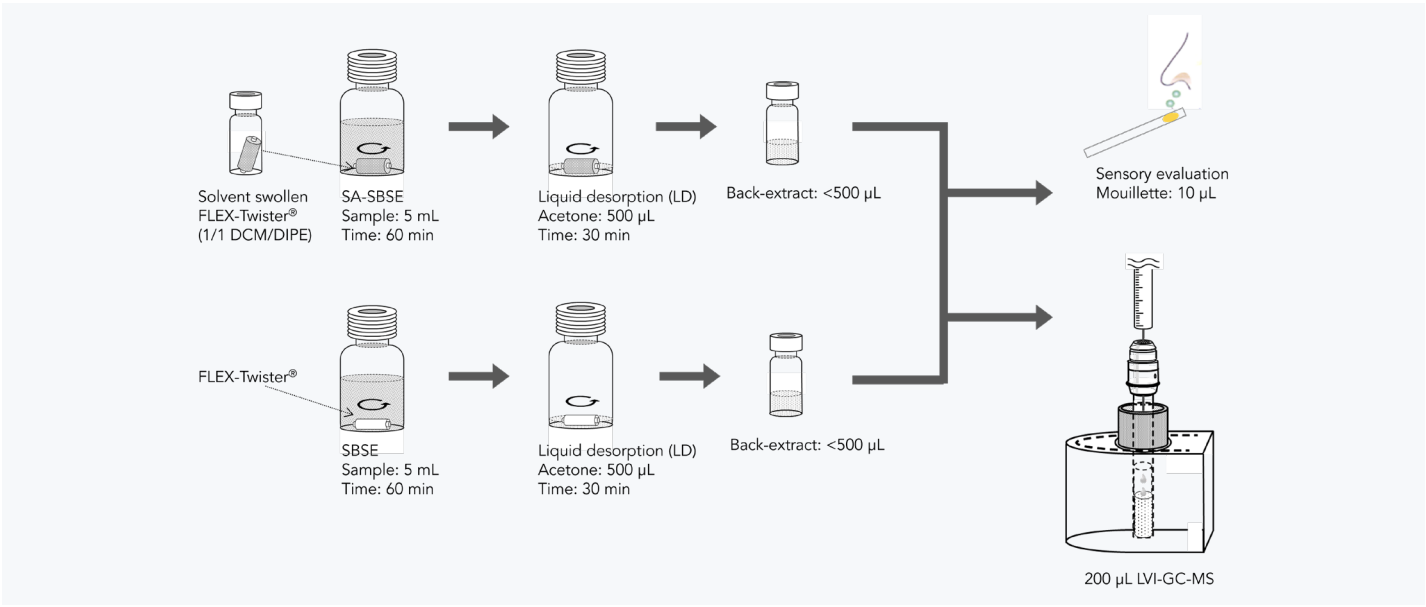

Figure 1: Schematic of SA-SBSE/SBSE-LD-LVI-GC-MS and sensory evaluation.

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Analysis Conditions

LabWorks Platform

MPS robotic^{pro}	200 µL, with 0.85 µL/s
TDU 2	splitless 30 °C (0.5 min), 140 °C/min to 80 °C (7 min)
CIS 4	Tenax TA liner solvent venting, low split vent: 100 mL/min until 0.01 min split: 1:3 @ 0.02 min 20 °C (0.5 min), 12 °C/sec to 240 °C (hold)

GC Agilent 7890A

Column	20 m DB-WAX UI (Agilent), d_i=0.18 mm, d_f=0.30 µm
Pneumatics	backflush @ 240 °C (10 min)
Temperature	40 °C (3 min), 5 °C/min to 240 °C (7 min)

MSD Agilent 5977

SIM/Scan	28.7 to 300 amu

Data Analysis

MassHunter Unknowns Analysis version B.10.0 (Agilent), and Aroma Office database version 7.00.01 (Gerstel KK) were used for data analysis. Aroma Office contains the most comprehensive database of aroma compounds available (>116,000 entries). This software is a searchable database which contains (linear) retention indices (RI) information for a wide range of aroma compounds from many literature references. For identification of aroma compounds, the cross-search function "Aroma Search", using both MassHunter Unknowns Analysis and Aroma Office, was performed combining deconvoluted mass spectra and RI data. Required agreement of mass spectral hit with expected RI value for any compound offers a very secure identification protocol.

Results and Discussion

Comparison of SA-SBSE and SBSE in berry-type yogurt

Figure 2 shows a comparison of total ion chromatograms (TIC) of berry yogurt with SA-SBSE and SBSE. Figure 3 shows the relative intensities of representative aroma compounds at log K_{ow} values from -0.36 to 3.55 (area values of characteristic ions are used). When using SA-SBSE, the peak intensities over the entire TIC are higher than those achieved using SBSE, with significant peak profile differences, especially at retention times (RTs) greater than 20 min. Fatty acid and benzoic acid peaks, for example, are saturated. In addition, there are many compounds for which the peak intensities resulting from SA-SBSE are approximately 10 times higher than those achieved using SBSE. Many of these have (1) log K_{ow} values of less than 2.0, (2) hydrogen bonding capability, and (3) multiple functional groups with hetero atoms, which results in significantly lower affinity for the PDMS stationary phase and particularly in even lower actual extraction rates for SBSE than the theoretical values (4). Therefore, extraction rates tend to be greatly enhanced by SA-SBSE. Furthermore, whey samples are slightly cloudy and their solids content can cause a reduction in SBSE extraction efficiency, whereas SA-SBSE, which involves extraction with a small amount of solvent (swollen in PDMS), tends to be less affected by sample matrix, such as solids. Polar aroma compounds with low log K_{ow} values such as (5) acetoin and (7) ethyl lactate (-0.36 and -0.18, respectively) were detected only in SA-SBSE. Interestingly, (17) ethyl 4-acetoxyhexanoate (log K_{ow} 2.30) and (21) linalool hydrate (log K_{ow} 2.37) with moderate log K_{ow} values were also detected only in SA-SBSE. This would be due to their bifunctional diester or diol structures, resulting in significantly reduced affinity with PDMS. On the other hand, terpenoids and esters have high affinity with PDMS leading to relatively high extraction rates being observed with SBSE.

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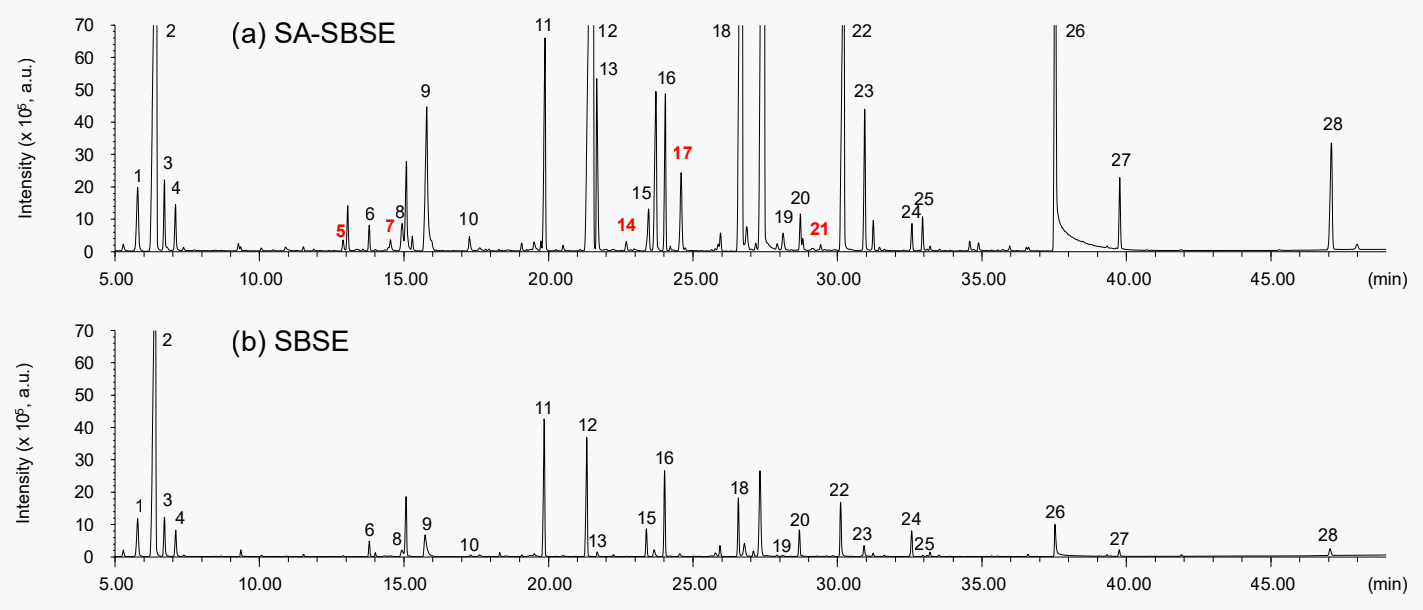

Figure 2: Comparison of TIC of berry-type yogurt obtained by SA-SBSE and SBSE followed by LD-LVI-GC-MS. (a) SA-SBSE, (b) SBSE, 1. Isobutyl acetate, 2. Ethyl butyrate, 3. Ethyl 2-methylbutyrate, 4. Ethyl 3-methylbutyrate, 5. Acetoin, 6. 4-Hexen-1-yl acetate, 7. Ethyl lactate, 8. Hexanol, 9. cis-3-Hexenol, 10. Acetic acid, 11. Linalool, 12. Ethyl levulinate, 13. Butyric acid, 14. 2-Methyl butyric acid, 15. α-Terpineol, 16. Benzyl acetate, 17. Ethyl 4-acetoxyhexanoate, 18. Hexanoic acid, 19. Phenethyl alcohol, 20. cis-Jasmone, 21. Linalool hydrate, 22. Ethyl maltol, 23. Octanoic acid, 24. γ-Decalactone, 25. Nonanoic acid, 26. Benzoic acid, 27. Vanillin, 28. Raspberry ketone. Compounds detected only in SA-SBSE are written in red.

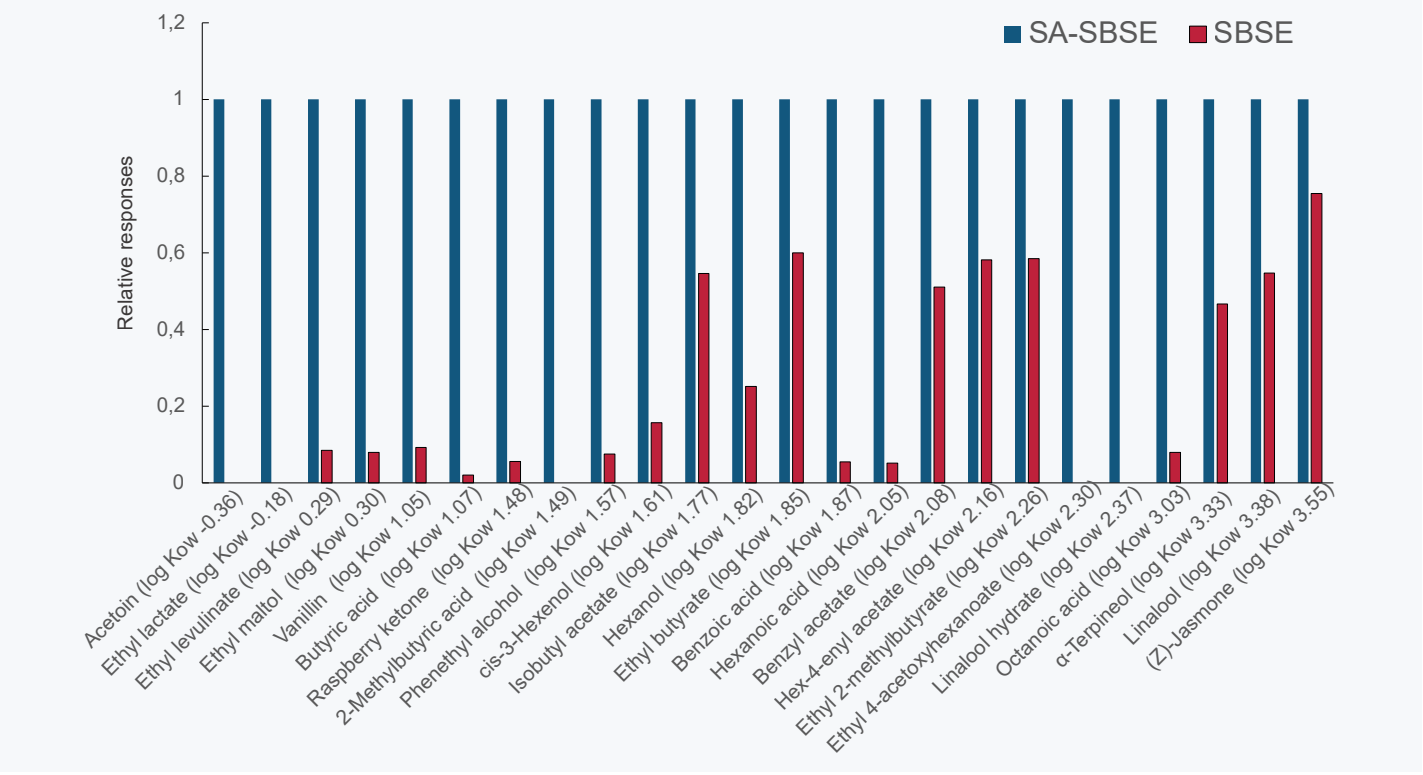

Figure 3: Relative responses for a range of selected compounds detected by both methods.

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Sensory evaluation of SA-SBSE/SBSE back-extracts from plain yogurt

Plain yogurt A with 100% raw milk was extracted with SA-SBSE and SBSE followed by solvent desorption (LD) with acetone, and the resulting back-extracts were compared by sensory evaluation. Ten micro-liters of the back extract was placed on a mouillette (a strip of filter paper, often used in perfume testing) using a micro-pipette. The desorption solvent (acetone) was briefly dried off by simply waving the mouillette in the air and the olfactory evaluation was immediately performed. The SBSE back-extract gave only a buttery aroma with a short afternote, which was different from the original product aroma, and gave the impression of being quite light. The SA-SBSE extract, on the other hand, left long lasting notes of cheesecake, milk candy, creamy, and buttery aromas, which seemed more concentrated than they were in the original product aroma. These aroma characteristics adequately reproduced the aroma derived from 100% raw milk yogurt, indicating a highly comprehensive extraction power.

Multivariate analysis of four plain-type yogurts

Figure 4 shows a comparison of TICs of four plain yogurts by SA-SBSE (first data in triplicate analysis). In order to obtain as much qualitative information as possible, in addition to searching for characteristic flavor compounds, the "Data Analysis Workflow using Aroma Office" introduced in Application Note No. 227 [5] was applied.

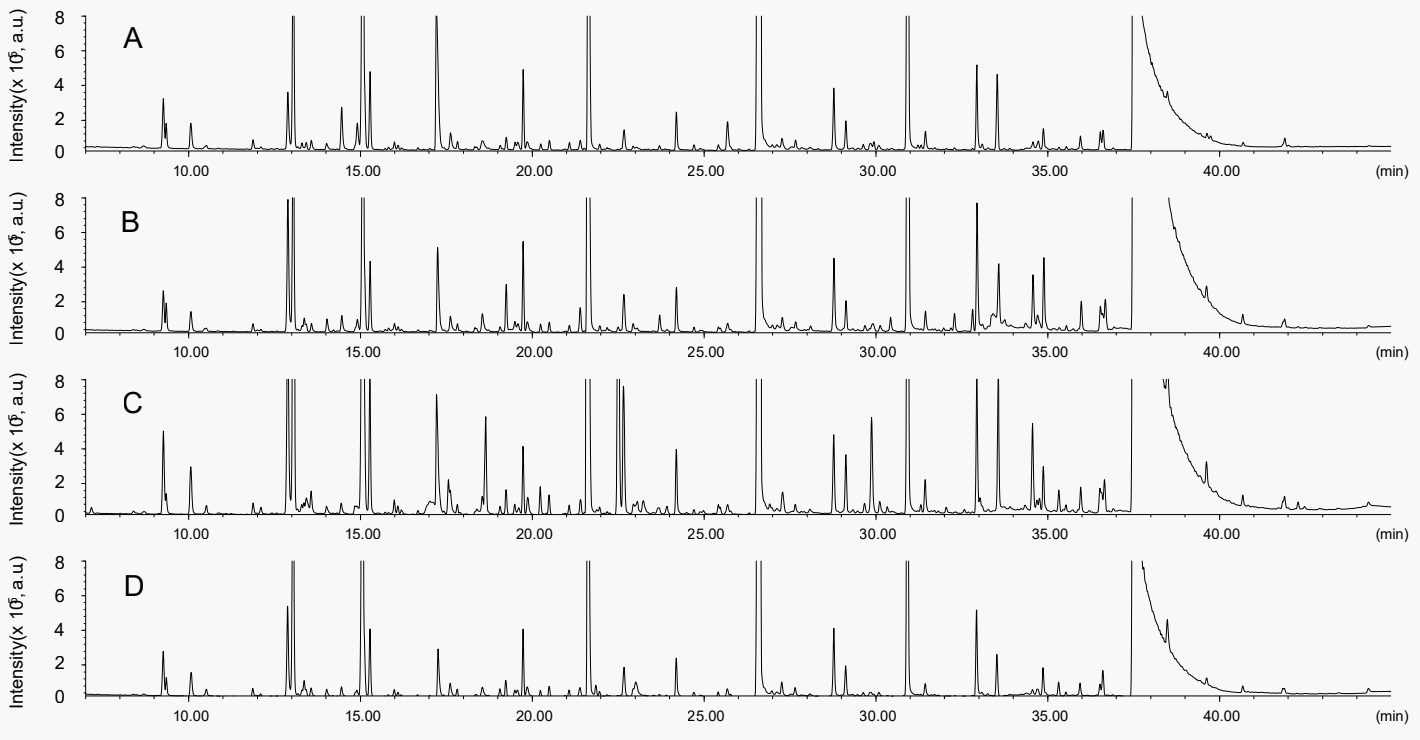

Figure 4: Analysis of four types of plain yogurt by SA-SBSE-LD-LVI-GC-MS.

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Figure 5 shows the data analysis workflow comprising these three steps:

1. Perform deconvolution and mass spectral library search on the batch data file by Agilent MassHunter Unknowns Analysis.
2. Run "Aroma Search" of Aroma Office (Ver. 7) on the batch data file after the library search to match with the RI values in the database and list only aroma compounds.
3. From the list of aroma compounds obtained by Aroma Search, transfer the characteristic ions (m/z values) for each compound, the area value, etc. to multivariate analysis software such as Agilent Mass Profiler Professional (MPP).

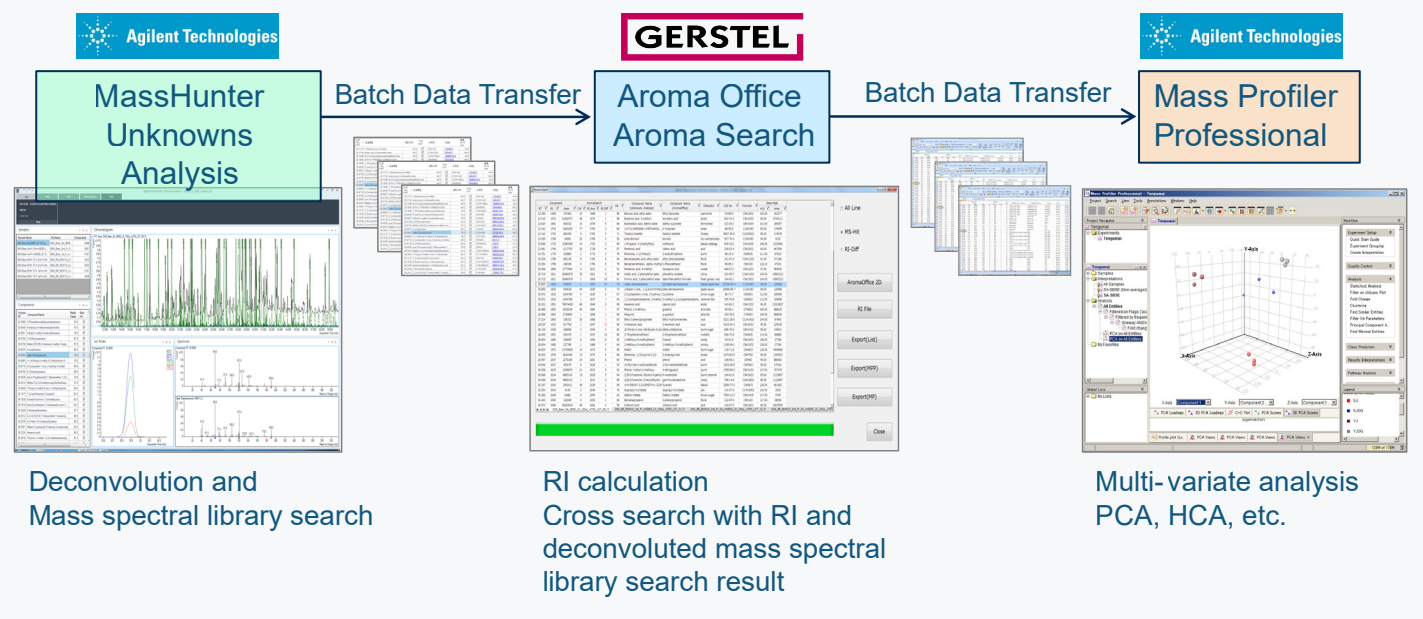

Figure 5: A data analysis workflow using Aroma Office and Unknowns Analysis for secure identification and multi-variate analysis of aroma/flavor compounds.

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Figure 6 shows the Aroma Search results (first data for sample A). The Aroma Search list includes RT, RI, average RI in the database, library search match, compound name/conventional name, aroma description, CAS number, formula, the m/z values of characteristic ions, and their area values. The number of tentatively identified aroma compounds in each sample was around 80. Table 1 details the aroma compounds in Sample A.

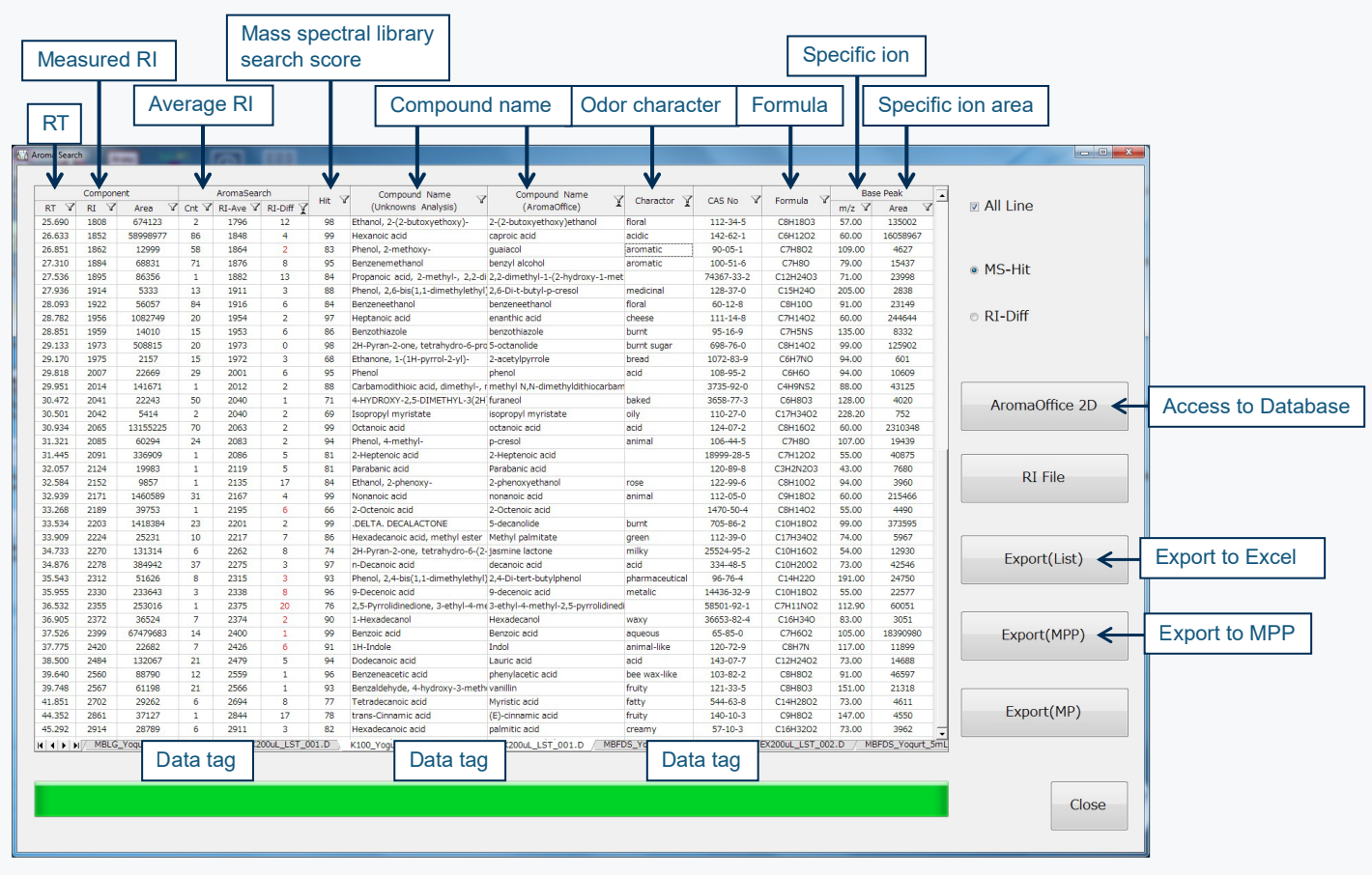

Figure 6: Aroma Search results for triplicate analyses of the four plain yogurt samples using the deconvoluted mass spectra and RI.

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Table 1: Aroma compounds found in Sample A.

Compound name	RI	Average RI	MS Library Match	Character
Diacetyl	981	977	86	butter
3-Buten-2-one, 3-methyl-	994	986	96	-
Thiophene	1007	1009	58	garlic
4-Methylpentan-2-one	1007	1000	91	fruity
Hexanal	1007	1021	83	apple
2,3-Pentanedione	1058	1057	95	bitter
3-Penten-2-one, 4-methyl-	1133	1128	89	almond-like
2-Heptanone	1183	1180	96	blue cheese
Pyrazine	1212	1209	57	earthy
3-Buten-1-ol, 3-methyl-	1254	1247	77	herbaceous
Ethyl pyruvate	1258	1257	66	vegetable
Acetoin	1286	1283	99	butter
3-Methyl-2-butenol	1328	1324	90	gassy fruity
3-Hydroxy-2-pentanone	1344	1338	89	caramel-sweet
2-Hydroxy-3-pentanone	1361	1361	83	buttery
1-Hexanol	1362	1357	86	alcoholic
2-Nonanone	1391	1389	86	baked
Nonanal	1395	1392	94	aldehyde
Acetic acid	1449	1449	99	acetic
2-Ethylhexanol	1500	1495	96	comparatively mild
Benzaldehyde	1522	1520	93	almond
2-Methytetrahydrothiophen-3-one	1529	1539	98	chlorine
Propanoic acid	1540	1533	95	acidic
iso-Butyric acid	1570	1567	96	acid
Pivalic acid	1581	1579	96	acid
5,5-Dimethyl-2(5H)-furanone	1623	1608	72	-
Methyl benzoate	1623	1623	64	flowery
Butanoic acid	1628	1627	99	aged cheese
Ethanone, 1-phenyl-	1652	1651	96	floral
2-Furanmethanol	1666	1662	77	burned
2-Methylbutyric acid	1673	1671	94	acidic
1-Methyl-2-pyrrolidone	1688	1680	81	fish-like
Heptadecane	1700	1697	73	perfume
3-Methyl-2(5H)-furanone	1718	1712	97	-
Benzyl acetate	1731	1738	76	floral herbal
Pentanoic acid	1740	1736	98	acid
Methyl p-toluate	1748	1756	67	spicy
2(5H)-furanone	1754	1749	63	-
5-Ethyl-2(5H)-furanone	1760	1754	65	rice
1-Methyl-1-phenylethanol	1764	1767	70	-
2-Butenoic acid	1774	1769	81	-

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Table 1: Aroma compounds found in Sample A (cont.).

Compound name	RI	Average RI	MS Library Match	Character
Octadecane	1803	1799	60	fresh
Ethanol, 1-(2-butoxyethoxy)-	1808	1800	97	-
Hexanoic acid	1852	1849	99	acidic
guaiacol	1862	1862	83	aromatic
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	1882	1882	86	burnt
Benzenemethanol	1884	1880	95	aromatic
Benzeneethanol	1922	1919	84	floral
Heptanoic acid	1956	1955	97	cheese
Benzothiazole	1959	1954	86	burnt
5-Octanolide	1973	1972	98	burnt sugar
2-Acetylpyrrole	1975	1972	68	bread
2H-Pyran-2,6(3H)-dione	1994	1995	80	-
Phenol	2007	2001	95	acid
Ethyl maltol	2026	2031	61	burnt sugar
Furaneol	2041	2039	71	baked
Isopropyl myristate	2042	2040	69	oily
Octanoic acid	2065	2061	99	acid
p-Cresol	2085	2084	94	animal
2-Heptenoic acid	2091	2086	81	-
Parabanic acid	2124	2119	81	-
Nonanoic acid	2171	2168	98	animal
2-Octenoic acid	2189	2195	66	-
5-Decanolide	2203	2200	99	burnt
Hexadecanoic acid, methyl ester	2224	2220	87	green
Jasmine lactone	2270	2262	74	milky
Decanoic acid	2278	2274	97	acid
9-Decenoic acid	2330	2338	96	metalic
1-Hexadecanol	2372	2374	90	waxy
Benzoic acid	2399	2398	99	aqueous
1H-Indole	2420	2424	91	animal-like
Dodecanoic acid	2484	2482	94	acid
Ethyl vanillin	2524	2539	83	vanilla-like
Phenylacetic acid	2560	2560	96	bee wax-like
Vanillin	2567	2566	93	fruity
Tetradecanoic acid	2702	2696	77	fatty
Hexadecanoic acid	2914	2914	82	creamy

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After exporting the Aroma Search results (Figure 6) by "MPP Export" button, Agilent MPP was used to perform multivariate analysis. After filtering, alignment, and baseline processing, principal component analysis (PCA) was performed. Figure 7 shows the PCA score plots and loading plots obtained from the triplicate measurements of the four plain yogurts. Sample A and D are classified according to the positive principal component 1, against the other samples. Sensory evaluation of the back-extract shows that samples A and D have a cheesecake-like aroma. Samples B and C exhibit similar cheese-like rich aroma in the sensory evaluation, but Sample B is classified against the more sourly Sample C by the positive principal component 2.

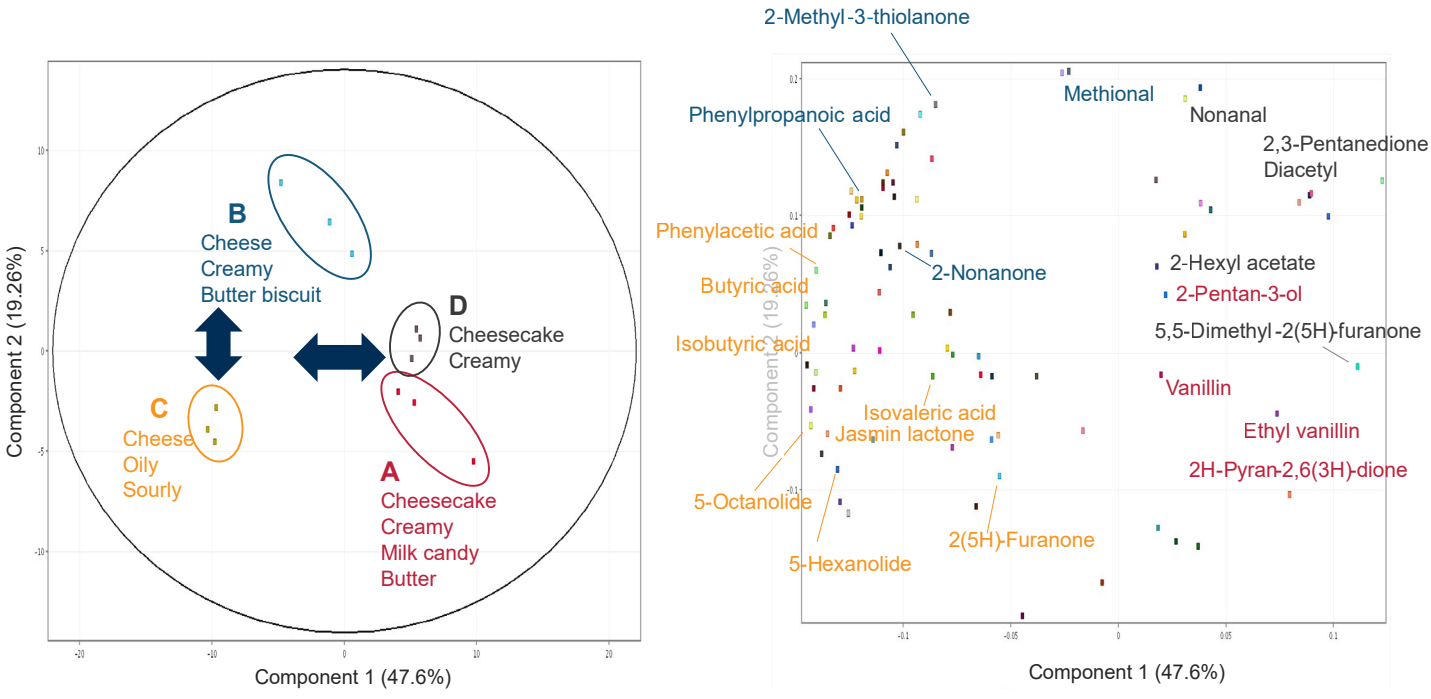

Figure 7: PCA score plot using PC1 and PC2 and the corresponding loading plot for Sample A, B, C, and D.

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From the loading plot, aroma compounds that seemed to contribute more to each sample were selected, and the relative intensities were compared using the area values of specific ions (Figure 8). Sample A, which has a cheesecake-like and milk candy-like sweet aroma, tends to have high intensities of vanillin, ethyl vanillin, furanones, and pyrans, while sample B, which has a cheese-like and butter biscuit-like aroma, has 2-alkanones as well as methional, 2-methyl-3-thiolane, and other sulfur compounds with high intensity. On the other hand, sample C, which tends to be cheese-like and has a strong sour taste, tends to have a high intensity of fatty acids and lactones.

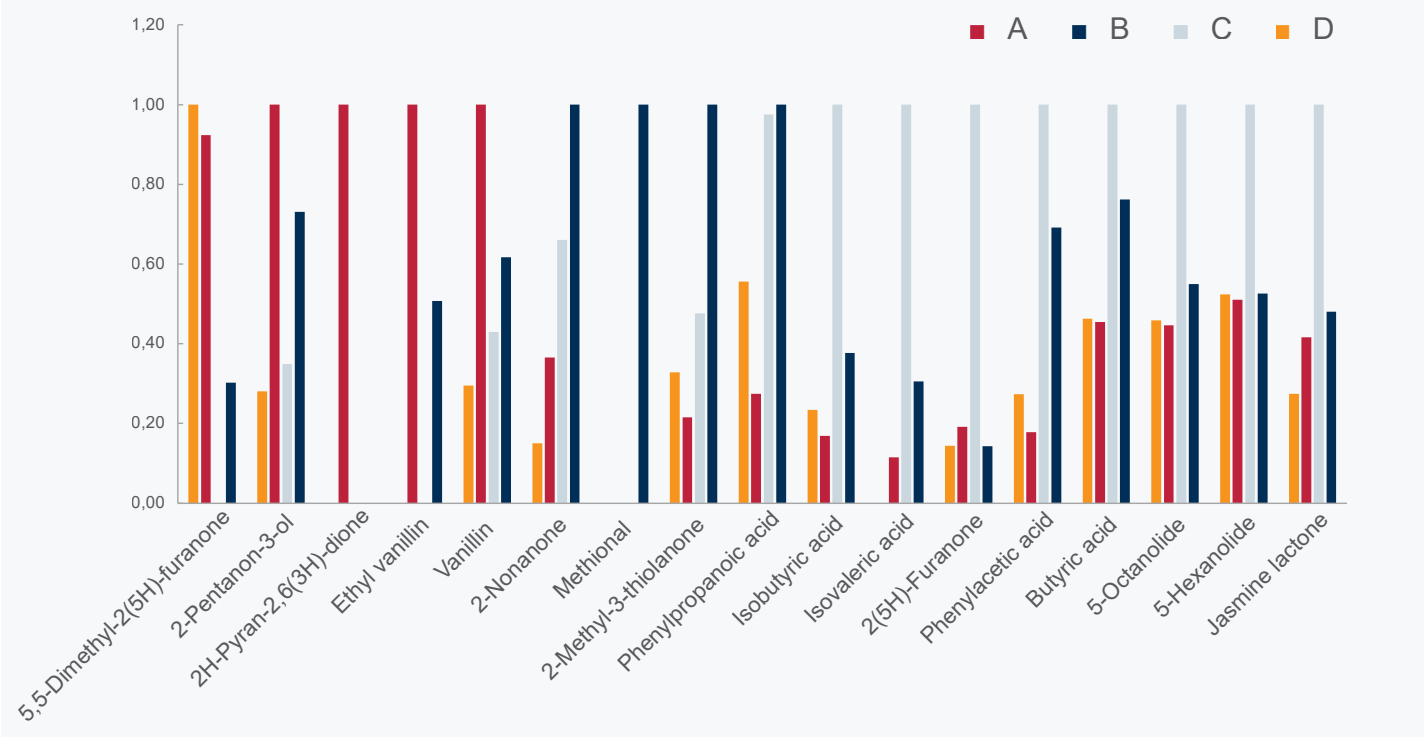

Figure 8: Comparison of the average relative responses for the selected compounds.

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Conclusion

SA-SBSE using solvent swollen PDMS phase (FLEX-Twister®) enhances the extraction efficiency of hydrophilic/polar compounds, which are relatively difficult to extract using conventional SBSE. SA-SBSE thus extends coverage in aroma analysis.

In the application to berry-type yogurts, the extraction efficiency of hydrophilic/polar aroma compounds with (1) log K_{ow} values of less than 2.0, (2) hydrogen bonding capability, and (3) multiple functional groups with hetero atom were all improved approximately 10-fold. Some aroma compounds were detected only when using SA-SBSE.

In addition, in the sensory evaluation of plain yogurt back-extract using a mouillette, SA-SBSE allowed us to recognize and characterize the entire original product aroma, which was difficult with SBSE.

Applying the SA-SBSE-based analysis to four types of plain yogurt enabled classification of the samples by principal component analysis, and the contribution of the aroma compounds that characterized each sample was also suggested.

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