Analysis of Yogurt by Dynamic Headspace and SPME

Application Note

Food and Flavor

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Abstract

This application note will compare dynamic headspace and Solid Phase Micro Extraction (SPME) sampling techniques for the detection of the volatile components in plain yogurt. Yogurt has been around for years and the health benefits are well known. With the advent of Greek yogurt, there has been increased interest and consumption of yogurt. Yogurt is a very healthy food. It has the nutrients found in milk like calcium, magnesium, potassium and vitamins B-2 and B-12. Furthermore, yogurt has the benefit of containing bacteria that aids in digestion. With all of these benefits, many food companies have increased interest in selling yogurt products. Thus, analysis of yogurt becomes essential for creating a product that will sell. .

Introduction:

Dynamic headspace sampling is an exhaustive sampling technique that involves sweeping the headspace of a sample onto an analytical trap and desorbing the trap into the Gas Chromatograph/Mass Spectrometer (GC/MS). Dynamic headspace is more sensitive than static headspace due to the fact that the headspace is swept for a programmed period of time and captured on a trap during dynamic sampling while static headspace analysis is the removal of a specific volume of headspace gas in a single sweep which is then injected into the GC/MS without concentration. It is for this reason that dynamic headspace was chosen.

SPME is another type of headspace extraction. SPME is a non-exhaustive sampling method that consists of exposing the sample headspace to a phase coated fiber. The chosen phase coating is based on the analytes of interest for the extraction. Although SPME is a non-exhaustive technique, it can be a better way of sampling volatile compounds due to the selectivity of the phase coating.

During this investigation, dynamic headspace and SPME sampling will be utilized for the sampling of a 10% solution of plain yogurt in de-ionized water. Sample volumes will be compared for each technique. Next, replicate samples will be analyzed in order to determine volatile compound sampling and reproducibility of each sampling method.

Experimental:

An Rxi®-624Sil MS 30m x 0.25mmID x 1.4um column was installed in an Agilent 7890GC/5975MS. For the first portion of the study, the GC/MS system was connected to an Evolution purge and trap concentrator and a Centurion WS autosampler. The Centurion WS autosampler was configured with a specialized two stage needle for the dynamic headspace sampling. The SPME sampling was done with a 2cm long 50/30um Divinyl Benzene/Carboxen/Polydimethyl Siloxane (DVB/CAR/PDMS) coated fiber through the use of the FLEX autosampler installed on the top of the GC. Switching from one sampling technique to the other was easy. Tables 1 and 2, display the Dynamic Headspace and SPME sampling parameters respectively. While table 3 outlines the GC/MS parameters for each sampling technique.

Purge and Trap Concentrator	EST Evolution
Trap Type	Vocarb 3000
Valve Oven Temp.	150ºC
Transfer Line Temp.	150ºC
Trap Temp.	35ºC
Moisture Reduction Trap (MoRT) Temp.	39ºC
Purge Time	20 min
Purge Flow	40mL/min
Dry Purge Temp.	ambient
Dry Purge Flow	40mL/min
Dry Purge Time	1.0 min
Desorb Pressure Control	On
Desorb Pressure	5psi
Desorb Time	0.5 min
Desorb Preheat Delay	15 sec
Desorb Temp.	260ºC
Moisture Reduction Trap (MoRT) Bake Temp.	210ºC
Bake Temp	270ºC
Sparge Vessel Bake Temp.	120ºC
Bake Time	8
Bake Flow	85mL/min
Purge and Trap Auto-Sampler	**EST Centurion WS**
Sample Type	Soil
Sample Preheat Time	10 min
Sample Preheat Temp.	40ºC
Sample Purge (Sweep) Temp.	40ºC
Soil Valve Temp.	85ºC
Concentrator Line Temp	150ºC

Table 1: Dynamic Headspace Parameters

Autosampler	FLEX
General	
Method Type	SPME
GC Ready	Continue
GC Cycle Time	27min
Constant Heat Mode	Yes
Sample Incubate Agitate	
Incubation Temp.	40ºC
Incubation Time	10.0min
Extraction	
Fiber Guide Depth	55%
Sample Vial Fiber Depth	2cm
Extraction Time	20min
Agitate Type	Oscillate
Agitate Delay	0.1 min%
Agitate Duration	19.9min
Wait	
Wait On Input	GC Ready
Desorbtion	
Fiber Guide Depth	55%
Fiber Insertion Speed	40%
Fiber Insertion Depth	2cm
Fiber Desorbtion Time	2min
Injection Start Output	Start

Table 2: SPME Sampling Parameters

GC/MS	Agilent 7890A/5975C inert XL Headspace	Agilent 7890A/5975C inert XL SPME
Inlet	Split/Splitless	Split/Splitless
Inlet Temp.	250ºC	250ºC
Inlet Head Pressure	12.153 psi	12.153 psi
Mode	Split	Pulsed Splitless
Injection Pulse Pressure	NA	50 psi until 2 min
Purge Flow to Split Vent	NA	2ml/min at 2.01 min
Split Ratio	40:1	NA
Column	Rxi-624Sil MS 30m x 0.25mm I.D. 1.4µm film thickness	Rxi®-624Sil MS 30m x 0.25mm I.D. 1.4µm film thickness
Oven Temp. Program	45ºC hold for 1 min, ramp 15ºC/min to 300ºC, hold for 5 min, 23 min run time	45ºC hold for 1 min, ramp 15ºC/min to 300ºC, hold for 5 min, 23 min run time
Column Flow Rate	1mL/min	1mL/min
Gas	Helium	Helium
Total Flow	44mL/min	6mL/min
Source Temp.	230ºC	230ºC
Quad Temp.	150ºC	150ºC
MS Transfer Line Temp.	180ºC	180ºC
Scan Range	m/z 30-350	m/z 30-350
Scans	4.4 scans/sec	4.4 scans/sec
Solvent Delay	0.7 min	0.7 min

Table 3: GC/MS Experimental Parameters

The dynamic headspace samples were prepared in 40mL vials. Each vial was loaded with three grams of sodium chloride. Next, a 10% solution of plain yogurt in de-ionized water was prepared. Five different volumes of the yogurt solution were added to the prepared 40mL vials. The volumes tested were 1, 2, 3, 4, and 5 mLs. Finally, four samples of the 5mL yogurt solution were tested in order to determine the reproducibility of the experiments. The sample volume comparison results are displayed in Table 4. While a bar graph of the experimental results is presented in Figure 1. Table 5 demonstrates the experimental reproducibility of dynamic headspace sampling using a 5mL sample volume. A chromatogram of the experimental results is shown in Figure 2.

Yogurt By Dynamic Headspace					
Compound	Area Count 1ml 10% Sample	Area Count 2ml 10% Sample	Area Count 3ml 10% Sample	Area Count 4ml 10% Sample	Area Count 5ml 10% Sample
acetaldehyde	1687068	2695626	3660514	4289254	4389989
hydrazinecarboxamide	844512	654285	705826	732214	822172
ethanol	123944	105782	110819	104101	148808
acetone	472547	934630	1012455	1246830	1122587
isopropyl alcohol	96403	152904	143700	177548	249141
2,3-butadione	126545	155331	171929	178921	157302
2-butanone	53432	82900	110195	125615	141992
2-pentanone	12715	18145	22702	30889	30369
2,3-pentadione	57310	66885	88289	93837	89521
3-hydroxy-2-butanone	243831	212114	172570	183445	201752
hexanal	26290	40815	31123	36754	36875
3-pentanol	21918	21028	21802	22856	26797
2-heptanone	17659	28610	32902	31145	34410
2-ethyl-1-hexanol	38711	46298	17776	19903	21156
2-ethylhexylester acetic acid	24341	27228	9227	18386	34586

Table 4: Compound Response vs. Sample Volume for Dynamic Headspace Sampling

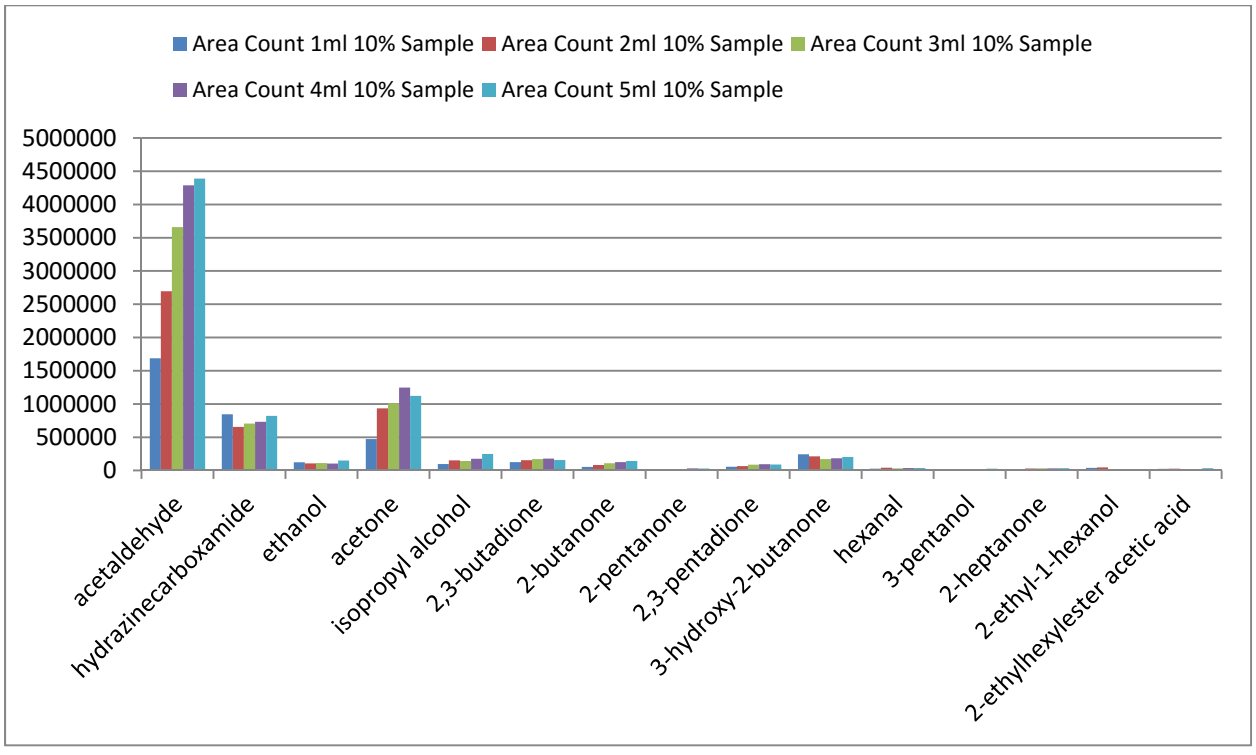

Figure 1: Compound Response vs. Sample Volume Bar Graph for Dynamic Headspace Sampling

Figure 2: Dynamic Headspace Sampling Chromatogram of 5mL Yogurt Solution

Compound	%RSD
acetaldehyde	3.73
hydrazinecarboxamide	6.29
ethanol	11.79
acetone	6.18
isopropyl alcohol	8.57
2,3-butadione	4.37
2-butanone	9.99
2-pentanone	8.69
2,3-pentadione	6.97
3-hydroxy-2-butanone	11.20
hexanal	12.58
3-pentanol	12.42
2-heptanone	7.39
2-ethyl-1-hexanol	6.55
2-ethylhexylester acetic acid	25.48

Table 5: Precision of Dynamic Headspace Sampling

After the dynamic headspace sampling and analysis was finished, the SPME portion of the experiment was initiated. The SPME samples were prepared in 20mL headspace vials. Each vial was loaded with three grams of sodium chloride. As with the headspace samples, a 10% solution of plain yogurt in de-ionized water was prepared. Five different volumes of the yogurt solution were added to the prepared 20mL headspace vials. The volumes tested were 1, 2, 3, 4, and 5 mLs. Next, four samples of the 5mL yogurt solution were tested in order to determine the reproducibility of the SPME sampling technique. The sample volume comparison results are shown in Table 6. A bar graph of the experimental results are presented in Figure 3. Table 7 displays the experimental reproducibility of SPME sampling technique using a 5mL sample volume. Finally, a chromatogram of the experimental results is shown in Figure 4.

Yogurt By SPME					
Compound	Area Count 1ml 10% Sample	Area Count 2ml 10% Sample	Area Count 3ml 10% Sample	Area Count 4ml 10% Sample	Area Count 5ml 10% Sample
acetone	108844	140015	122436	124420	88749
2,3-butanedione	130999	184970	183520	185691	162832
acetic acid	50731	237362	649543	601844	215781
2-pentanone	61239	107089	131869	172291	130687
2,3-pentanedione	277401	397602	525888	658273	544488
3-hydroxy-2-butanone	1993015	1725097	1729522	1627752	1247692
propanoic acid	456435	1174629	1130350	899568	495325
3-methyl-2-butanal	208560	252374	225960	323807	283408
2-methyl-3-pentanol	259288	275488	309658	344554	304228
1-methoxy-pentane	132189	136623	123985	128660	88307
2-heptanone	113557	272105	367848	502721	480090
1-butoxy-2-propanol	121284	115662	93636	151350	118325
Ethanol, 2-(2-ethoxyethoxy)-	89276	153099	136010	156164	168852
2-ethyl-1-hexanol	76886	112159	92015	142992	72850
2-nonanone	225984	304274	353794	511760	536393
nonanal	123981	121354	134187	144739	165451
2-(2-butoxyethoxy) ethanol	68377	66143	84106	63339	28855
1-[2-(2-emthoxy-1-methylethoxy)-1-methylethoxy}-2-propanol	1763985	2772445	2140194	1907612	1734012
Tri(1,2-propyleneglycol), monomethyl ether	93327	137122	116406	96845	93112
Caprolactam	102498	136610	116581	83061	71728
Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	155823	131253	99945	106626	67414
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	176625	157061	97885	109863	77892
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	424599	310707	306163	315805	262694
diethylphthalate	75909	83735	72437	75858	79814

Table 6: Compound Response vs. Sample Volume for SPME Sampling

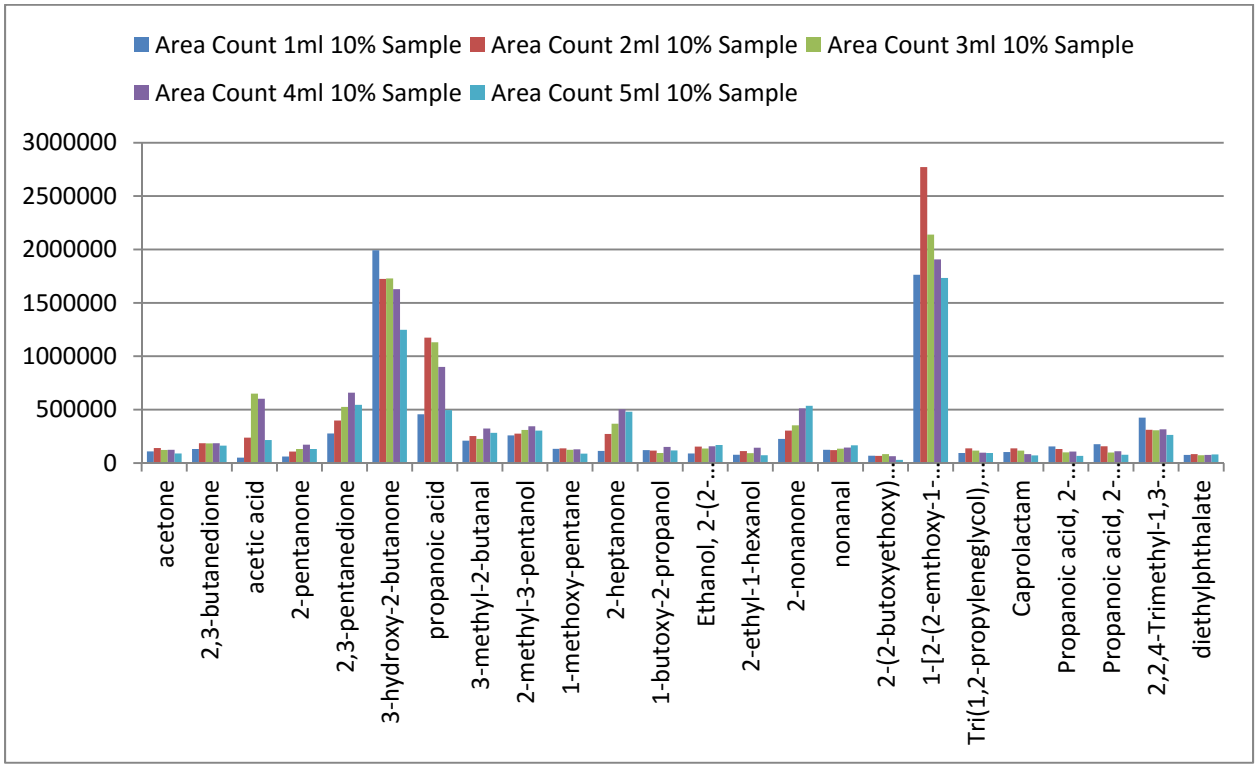

Figure 3: Compound Response vs. Sample Volume Bar Graph for SPME Sampling

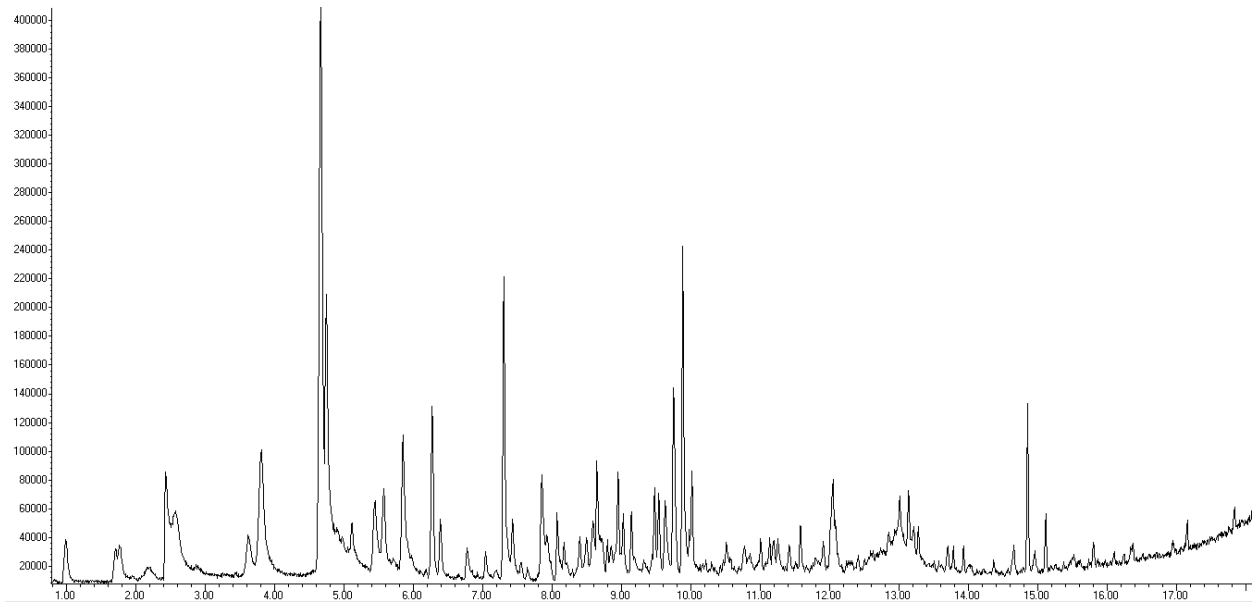

Figure 4: SPME Sampling Chromatogram of 5mL Yogurt Solution

Compound	%RSD
acetone	8.65
2,3-butanedione	6.43
acetic acid	82.54
2-pentanone	7.09
2,3-pentanedione	4.70
3-hydroxy-2-butanone	11.74
propanoic acid	47.97
3-methyl-2-butanal	8.96
2-methyl-3-pentanol	6.21
1-methoxy-pentane	14.95
2-heptanone	6.01
1-butoxy-2-propanol	27.85
Ethanol, 2-(2-ethoxyethoxy)-	9.48
2-ethyl-1-hexanol	22.00
2-nonanone	6.13
nonanal	11.00
2-(2-butoxyethoxy) ethanol	24.08
1-[2-(2-emthoxy-1-methylethoxy)-1-methylethoxy}-2-propanol	44.75
Tri(1,2-propyleneglycol), monomethyl ether	25.56
Caprolactam	50.69
Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	21.67
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	18.58
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	28.18
diethylphthalate	28.44

Table 7: Precision of SPME Sampling

Conclusions:

The dynamic headspace and SPME sampling techniques provided very different insights into the yogurt samples. The dynamic headspace provided a less detailed account of the compounds in the samples. However, the more volatile compounds were better detected with this technique. Due to the fact that yogurt is not miscible in water; the dynamic headspace exhaustive sampling method provided more reproducible results than the non-exhaustive SPME procedure. SPME sampling, on the other hand, provided much more detail on the compounds to be found in the yogurt samples. The 5ml sample volume proved to be much better using dynamic headspace analysis. However, due to the lack of reproducibility using the SPME technique, the optimum sample volume was not as discernable. Since both sampling techniques provide different insights into the yogurt samples' composition, experimental requirements would direct the sampling method chosen.

References:

1. Amarson, Atli. "Yogurt 101: Nutrition Facts and Health Benefits", https://authoritynutrition.com/foods/yogurt/, June 8, 2016.

2. Axe, Josh, "10 Proven Benefits of Yogurt", Food is Medicine, https://draxe.com/probiotic-yogurt/, June 8. 2016.

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