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Are your Cornflakes Stale? Hexanal Formation in Grain Products

Application Note - Environmental

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Abstract

As grain products begin to age, they oxidize. One of the products of this oxidation is Hexanal. Hexanal contributes to the green, grassy odor in food and can ruin a good morning when breakfast takes on an unpleasant taste. The amount of Hexanal in foods in one indicator of the expiration date. This study will examine the formation of Hexanal over time as cereal ages.

Introduction:

Hexanal is known in the food and flavor industry for its green, grassy odor and can be present at very low concentrations in assorted foods. However, due to its extremely low odor threshold, the unwanted formation of Hexanal in grain products can be of concern. As cereal ages it oxidizes and as it oxidizes it forms Hexanal. Consequently, the longer cereal sits in the pantry opened, the more Hexanal forms. Static headspace sampling is a common technique for the determination of Hexanal. Recently, Solid Phase Micro Extraction (SPME) has also been employed to verify Hexanal formation. These two sampling techniques will be compared for their sensitivity and accuracy.

Discussion:

Both static headspace and SPME sample an aliquot of headspace in a closed system. Furthermore, both analyses need to be optimized in order to allow the analyte(s) of interest to partition into the headspace efficiently. During static headspace analysis, a sample is heated and/or agitated in order to lower the partition coefficient and effectively bring the sample to equilibrium. Next, an aliquot of the headspace is sampled and injected onto the column of the Gas Chromatograph (GC) for separation while a Mass Spectrometer (MS) is used for the analysis.

SPME, on the other hand, utilizes a coated fiber to sample the analytes. Much like static headspace sampling, the sample is brought to equilibrium. However, during SPME, a coated fiber is inserted into the sample headspace and the extracted analytes adhere to the coated fiber. Next, the fiber is inserted into the GC inlet and the analytes are desorbed onto the GC column. The SPME fiber selection is dependent upon the analytes to be extracted.

Experimental:

Single portions of corn flakes were purchased from the market. Each portion was opened and labeled with the date. The cereal samples were opened on different dates over the course of three months. Next, several one gram samples of the cereal were placed in headspace vials and sealed. The corn flakes were left whole or were crushed. When comparing the crushed to the whole flakes, it was determined that the crushed flakes provided better Hexanal recoveries. Then, the samples were run at different temperatures, agitations, and times in order to optimize experimental parameters for both techniques. Once the parameters were established, the “aged” cereal samples were examined for Hexanal.

The sampling system used for this analysis was the EST Analytical FLEX autosampler. A 2.5ml syringe was used for the static headspace sampling while a PDMS/DVB fiber was used for SPME. The Agilent 7890 GC fitted with a Restek Rxi-5 Sil MS 30m x 250mm x 0.25μm column was employed for analyte separation and the Agilent 5975 MS was used for the analysis. Refer to Tables 1 and 2 for the experimental sampling and analysis parameters.

FLEX Headspace	
General	
Method Type	Headspace
Incubate Agitate	
Incubation Temperature	120°C
Incubation time	20.1 min
Agitation Speed	100%
Agitation Delay	0.1 min
Agitation Duration	20.0 min
Sample Fill	
Syringe Temperature	Ambient
Syringe Needle Depth	100%
Sample Depth Speed	100%
Sample Volume	80% (2.0mL)
Sample Fill Delay	1.0 sec
Injection	
Needle Depth Speed	100%
Needle Depth	100%
Injection Rate	100%
Injection Volume	80% (2.0mL)
Pre-Injection Delay	1.0 sec
Post Injection Delay	1.0 sec
Sweep Needle	
Needle Temperature	Ambient
Sweep Needle	0.0 min
Syringe Pumps	2
Syringe Volume	90% (2.25mL)
Syringe Pump Speed	100%

FLEX SPME	
General	
Method Type	SPME
Incubate Agitate	
Incubation Temperature	60°C
Incubation time	10.1 min
Agitation Speed	100%
Agitation Delay	0.1 min
Agitation Duration	10.0 min
Extraction	
Fiber Guide Depth	100%
Sample Vial Fiber Depth	100%
Extraction Time	5.0 min
Fiber Extraction Agitate	NO
Wait	
Wait On Input	GC Ready
Desorbtion	
Fiber Guide Speed	100%
Fiber Guide Depth	100%
Fiber Insertion Speed	80%
Fiber Insertion Depth	100%
Fiber Desorbtion Time	5.0 min%
Injection Start Output	Start

Table 1: FLEX Autosampler Experimental Parameters

GC/MS	Agilent 7890/5975 (Headspace)	Agilent 7890/5975 (SPME)
Inlet	Split/Splitless	Split/Splitless
Inlet Temp.	250°C	250°C
Inlet Head Pressure	9.905 psi	9.905 psi
Split	40:1	NA
Purge Flow to Split Vent	NA	10ml/min at 5 min
Injection Pulse Pressure	NA	20psi until 5 min
Liner	Restek SKY liner, Splitless, 2mm x 6.5mm x 78.5mm	Supelco Inlet Liner, Direct (SPME), Straight Design (unpacked), 78.5mm x 6.5mm x 0.75mm
Column	Rxi-5Sil MS 30m x 0.25mm I.D. x 0.25μm film thickness	Rxi-5Sil MS 30m x 0.25mm I.D. x 0.25μm film thickness
Oven Temp. Program	45°C hold for 0.5 min, ramp 5°C/min to 100°C hold for 1.0 min, 12.5 min run time	45°C hold for 5.0 min, ramp 5°C/min to 100°C hold for 1.0 min, 17 min run time
Column Flow Rate	0.8ml/min.	0.8ml/min.
Gas	Helium	Helium
Total Flow	43.8ml/min	13.8ml/min
Source Temp.	230°C	230°C
Quad Temp.	150°C	150°C
MS Transfer Line Temp.	280°C	280°C
Solvent Delay	0.7 min	5.0 min
Acquisition Mode	Scan	Scan
Scan Range	m/z 35-265	m/z 35-265
Sampling Rate	3.12 scans/sec	3.12 scans/sec

Table 2: GC/MS Experimental Parameters

The corn flake samples were crushed into pieces that were approximately two to three millimeters in diameter. One gram of sample was placed into a twenty milliliter headspace vial and sealed. Each sample was run five times in order to ensure reproducibility and to determine the percent relative standard deviation of the results. This procedure was done for both the SPME and the Static Headspace sampling. Results are displayed in Figures 1 and 2 and listed in Tables 3 and 4. Chromatograms of the Static Headspace and SPME Hexanal found in the 10 week old samples are displayed in Figures 3 and 4.

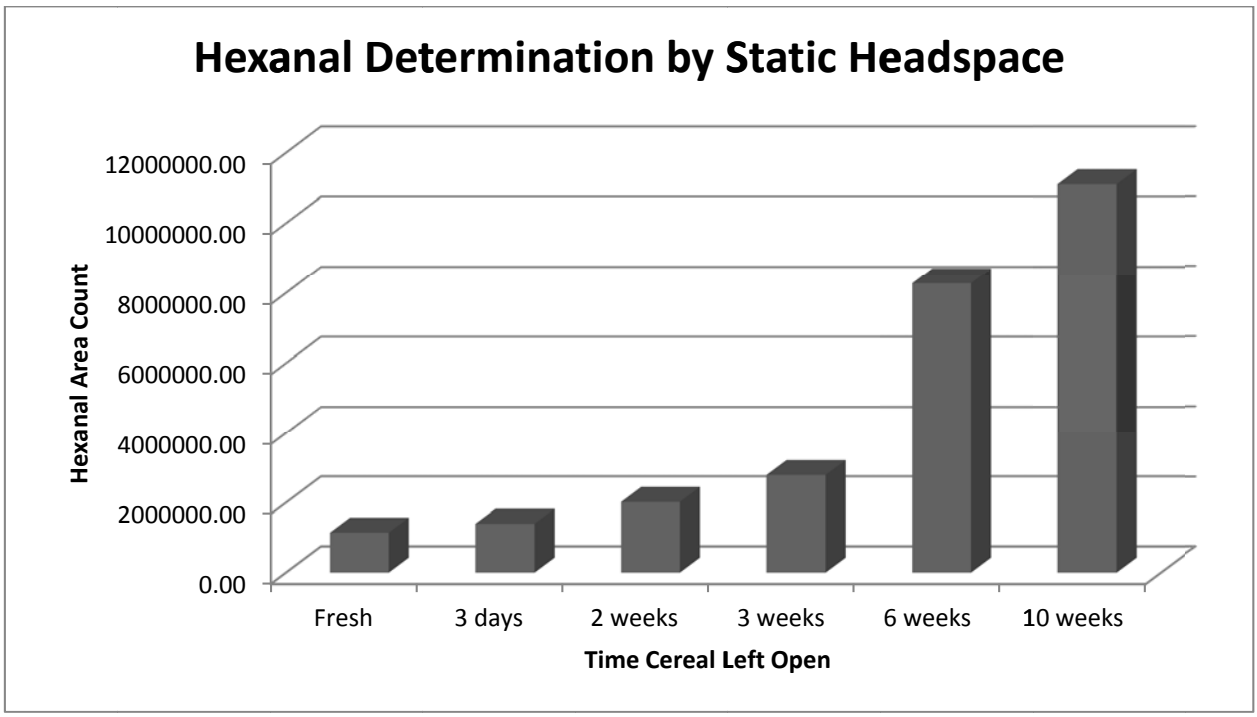

Figure 1: Static Headspace Results Bar Chart

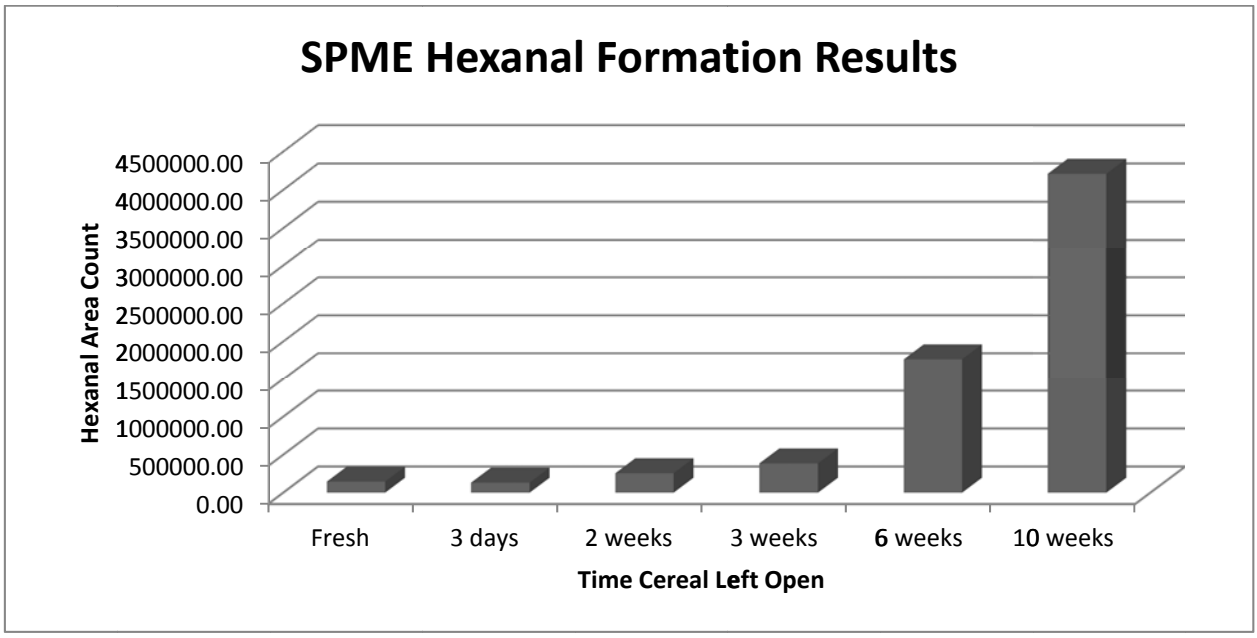

Figure 2: SPME Results Bar Chart

Headspace Results		
Time Cereal Left Open	Average Hexanal Area Count	%RSD
Fresh	1143537.00	2.33
3 days	1396456.20	6.83
2 weeks	2020036.40	8.89
3 weeks	2789457.40	6.86
6 weeks	8277591.50	5.92
10 weeks	11107211.8	6.04

Table 3: Static Headspace Results

SPME Results		
Time Cereal was Left Open	Average Hexanal Area Count	%RSD
Fresh	146412.60	4.74
3 days	131618.50	9.82
2 weeks	256225.00	7.22
3 weeks	385243.60	3.22
6 weeks	1765669.75	8.55
10 weeks	4209690.40	7.03

Table 4: SPME Results

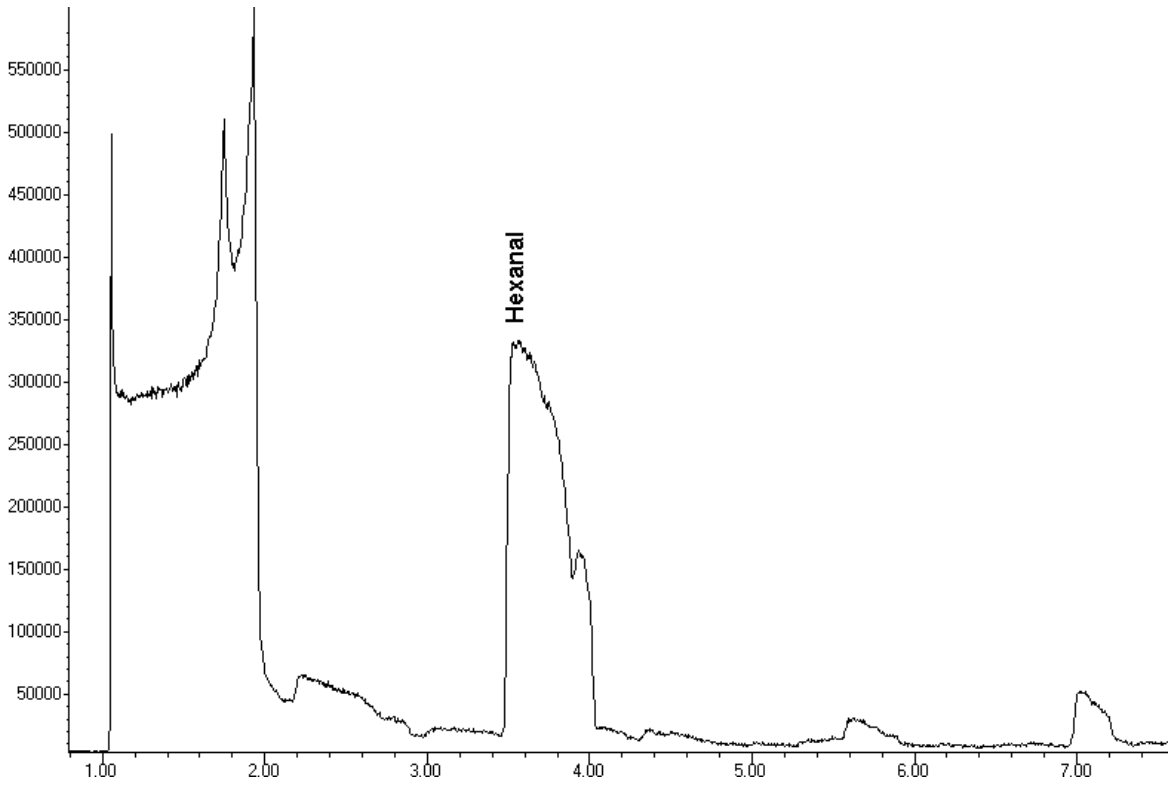

Figure 3: Chromatogram of Hexanal by Static Headspace Sampling of Ten Week Old Cereal

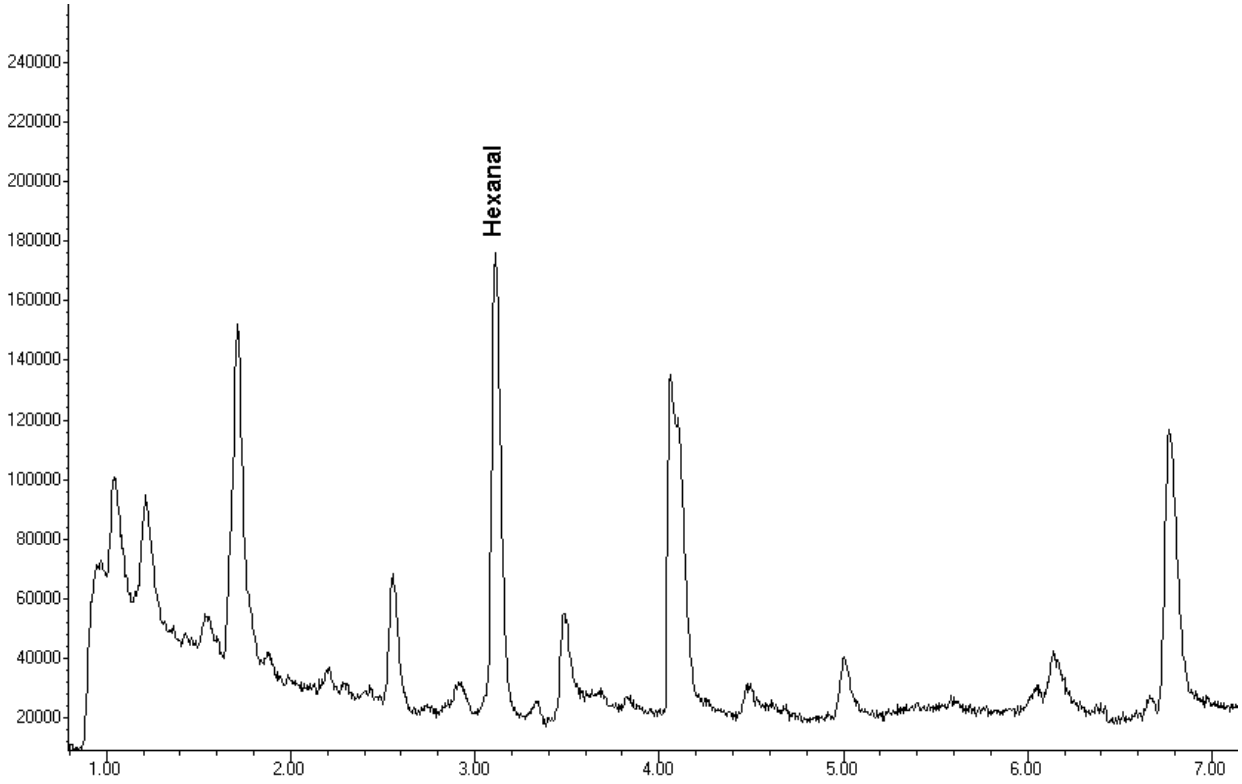

Figure 4: Chromatogram of Hexanal by SPME of Ten Week Old Cereal

Conclusions:

As the cereal samples were left open, Hexanal formed. The longer the samples were left exposed the larger the Hexanal area. Both SPME and Static Headspace provided quick and easy sampling methods for the determination of Hexanal in cereal. The precision and accuracy of SPME and Static Headspace was also comparable at less than 10% RSD for the area count results. However, the SPME chromatography provided a sharp defined peak for Hexanal while the Static Headspace chromatogram displayed a broad, imprecise peak. For this reason, sampling Hexanal in cereal by SPME is the optimum sampling technique.

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