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Analytical Products

Application Note

The use of SPME-GC/MS to determine the best packaging material for preventing light-induced off-flavors in milk

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Abstract

Light induced off-flavors (LIOFs) can be an issue when milk is packaged in high density polyethylene (HDPE) plastic jugs instead of glass bottles. In this study, we used solid-phase microextraction (SPME) followed by GC/MS to investigate the effectiveness of a range of plastic materials as barriers to the production of off-flavors. Headspace SPME with a Carboxen®/PDMS (CAR/PDMS) coated Nitinol fiber retains low molecular weight analytes which can be detected from samples at concentration levels of less than 1 ng/mL (ppb) allowing accurate analysis of these small flavoring analytes.

Introduction

Light induced off-flavors (LIOFs) in milk became an issue when dairies began to package milk in high density polyethylene (HDPE) plastic jugs instead of glass bottles. There are several types of LIOFs with the most common coming from oxidation of lipids and degradation of sulfur containing amino acids. Light induced lipid oxidation occurs from free radicals reacting with unsaturated fatty acids in milk. The free radical reaction cleaves the double bond and forms hydroperoxides that degrade predominately to aldehydes and, to a lesser degree, ketones and alcohols. The most common light activated analytes in this class are hexanal and pentanal primarily induced from linoleic acid.

The mechanism for the breakdown of sulfur containing amino acids in whey protein is not fully understood, but most common breakdown products in this class are dimethylsulfide (DMS), methanethiol (MT) and dimethyldisulfide (DMDS). Due to the high volatility of DMS and MT, this study focused primarily on DMDS.

It is well documented that UV rays do not easily penetrate glass but have been known to penetrate various types of plastic materials. Milk is commonly sold in HDPE jugs, some of these jugs contain white or colored pigments to increase the effectiveness of the plastic to serve as a barrier to UV light. The goal of this study was to evaluate various types of plastics to determine which type provides the best barrier for preserving the integrity of the milk.

Several analytical methods have been used for the analysis of LIOFs in milk. In this study we chose solid-phase microextraction (SPME) to analyze the various milk samples. This technique is sensitive, easy to automate, and is accurate with good precision.

Materials & Methods

Milk containing 2% fat was purchased from a local farm dairy and was stored in ½ gallon glass jugs with a wall thickness of approximately 5 mm. The plastic sealing cap was immediately covered with aluminum foil upon purchase and the milk was stored at 4 °C in the dark.

Different types of plastic containers were obtained from various sources throughout the lab. Each of the plastic containers contained a symbol indicating type of plastic. Effort was taken to find containers with similar surface areas and volumes. The wall thickness of each container was measured with calipers. The containers were filled to 93 ±1% of the internal volume. The purpose was to keep the void volume of the containers consistent since the shape of the containers varied. The caps and container necks were wrapped with aluminum foil to prevent UV permeation through the cap. The container materials and dimensions are shown in **Table 1**.

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Table 1. Container materials and dimensions used in milk light exposure study

Container Material	Wall Thickness (mm)	Base shape	Total surface area (mm^2)	Volume of milk in container (mL)	Internal volume of container (mL)	Percent of fluid volume
PETE[1]	0.60	Circular	10241	55	59	93%
HDPE[2]	0.80	Circular	9864	65	71	92%
PP[3]	1.32	Circular	9694	50	54	93%
White HDPE[4]	1.50	Rectangular	10400	67	72	93%
Glass bottle	2.00	Circular	11327	75	80	94%

[1]PETE - polyethylene terephthalate ether
[2]HDPE – high density polyethylene
[3]PP-Polypropylene
[4]White HDPE – HDPE impregnated with white opaque pigment

A 500 mL volumetric flask was filled with cold milk and spiked with an internal standard, hexanal-d12, at 5 µg/L. The milk was immediately dispensed into containers at the volume levels listed in Table 1 and into 2 glass vials sealed and placed in the refrigerator at 4°C. Caps were covered with aluminum foil to reduce UV permeation. The containers were placed in a foil-lined tray about 10 cm beneath Sylvania Octron 32 w fluorescent lights as a UV light source. The exposure time was 2 hours.

After the milk was exposed, the containers were placed in the refrigerator at 4 °C for 1 h to cool the milk and prevent rancidity. During the time the milk samples were being cooled, ten empty 10 mL vials were placed in a Peltier-cooled vial tray holder set at 4 °C on the Gerstel MPS II multi-purpose sampler. The sampler was also equipped with a needle conditioner to clean the fiber and an agitator for sample mixing.

5 mL of milk was transferred in duplicate into 10 cooled vials. The 2 vials containing the spiked fresh milk in the refrigerator were also added to the tray. A CAR/PDMS fiber on a Supelco® Nitinol core was used to extract the samples. The extraction conditions used in the study are shown in **Table 2.**

Table 2. SPME sampling conditions

Auto sampler:	Gerstel MPS II
Sample:	5 mL cooled milk
Fiber:	Carboxen®/PDMS (CAR/PDMS) on Nitinol core (57907-U)
Incubation:	50 °C for 1 min with agitation
Agitation:	250 rpm
Extraction:	Headspace for 15 min at 50 °C with agitation at 250 rpm
Desorption:	3 min at 300 °C
Post desorption:	2 min at 280 °C in needle cleaner

The samples were analyzed with an Agilent 7890B GC connected to a 5977 A MSD. The conditions used to analyze the desorbed analytes are shown in **Table 3.**

Table 3. GC/MS Analysis conditions

GC	Agilent 7890
GC column:	VOCOL® 30 m x 0.25 mm ID, 1.5 µm df
Oven program:	45 °C (2 min) to 100 °C at 8 °C/min to 140 °C at 12 °C/min to 180 °C at 16 °C/min (0.2 min)
Carrier Gas:	Helium at 1 mL/min constant flow rate
Inlet:	300 °C with 0.75 mm ID liner
Injection port:	Splitless for 0.75 min then vent at 20 mL/min
Transfer line:	250 °C
Detector:	MSD quadrupole, m/z 40-150
Quantitation ions:	pentanal-44; hexanal-56; dimethyldisulfide-94; hexanal-d12 -64

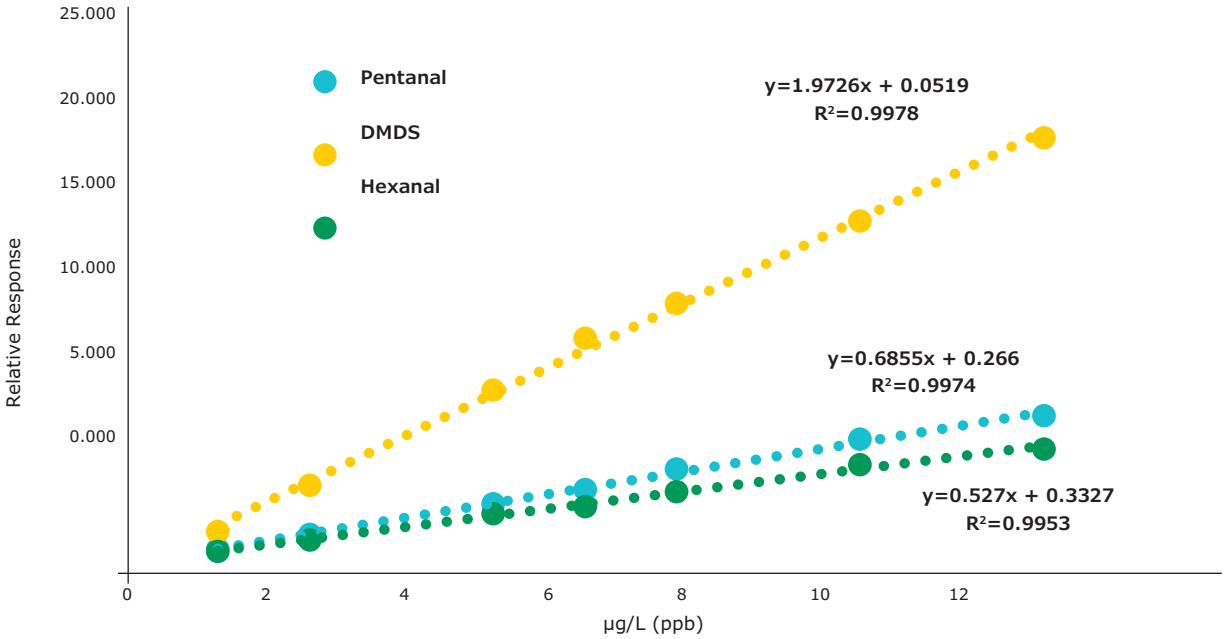
Results

The CAR/PDMS fiber on the Nitinol core is an excellent choice for this application due to the small micropores of CAR/PDMS. These pores are ideal for extracting small and midsized analytes. The Nitinol core is very durable and inert. The coating process is produced with state-of-the-art coating equipment that assures good reproducibility by constant monitoring of the coating thickness.

The addition of sodium chloride does increase recovery of these analytes in water but not in milk containing fat. The responses in milk samples were higher with better precision without added salt; therefore, salt was not added to the samples. Various extraction times were evaluated, but it was determined that 15 minutes enabled samples to be quantified below µg/L concentration levels.

A calibration curve was generated by spiking 7 fresh milk samples with a standard of the LIOF analytes from 1-10 µg/L sample concentration and with hexanal-d12 at 5 µg/L. Another vial of fresh milk was only spiked with hexanal-d12 at 5 µg/L. The samples were extracted and analyzed according to the methods listed in **Tables 2 and 3**. The relative responses of each analyte were calculated and the relative responses from the sample not spiked with LIOF standard were subtracted from the 7 LIOF spiked samples.

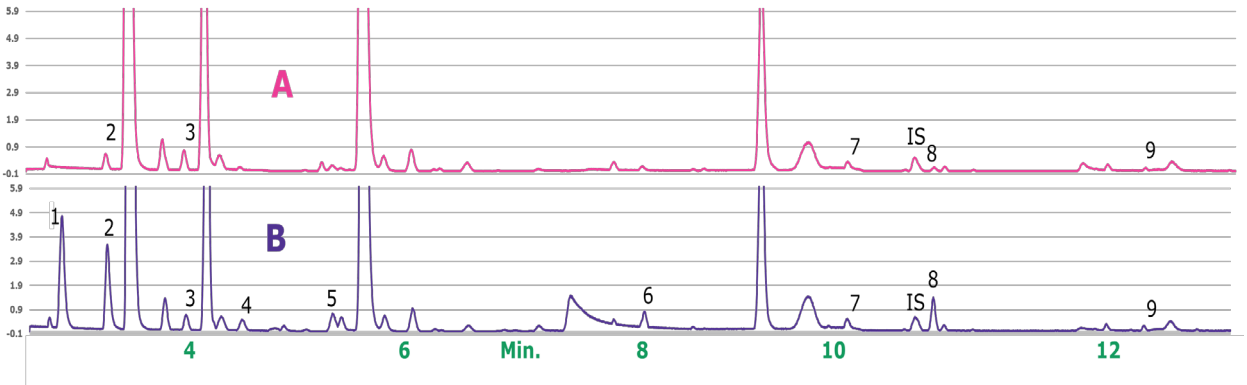
Figure 1. Calibration Curve of Relative Responses of LIOFs with background subtraction



The responses from the 3 analytes have regression coefficient values in excess of 0.99 and low y intercept values. These results were obtained in full scan mode, so greater sensitivity could be obtained using SIM mode if needed.

Chromatograms of milk not exposed to light spiked with the hexanal-d12 internal standard (IS) (A), and milk exposed to light in a polypropylene container (B) are shown in **Figure 2**.

Figure 2. Chromatograms of milk spiked with hexanal-d12 IS not exposed to light (A); Milk spiked with hexanal-d12 IS exposed to light stored in a polypropylene container (B)



1. Pentane
2. Isopropanol
3. Dimethylsulfide
4. n-Hexane
5. 2-Butanol
6. Pentanal,
7. Dimethyldisulfide, IS. Hexanal-d12
8. Hexanal
9. Heptanal. (See **Table 3** for run conditions)

The comparison of the chromatograms shows that light exposure in the PPE container increased the response of many of the analytes. Both chromatograms are at the same scale and the hexanal d12 internal standard, responses were similar in both plots. Even though this study focused on 3 analytes, other analytes are generated from the light exposure or some other mechanism. Two small volatile analytes, pentane and isopropanol, have much larger responses on the light exposed samples. Note that the samples were run in duplicate and the responses of duplicate samples were similar.

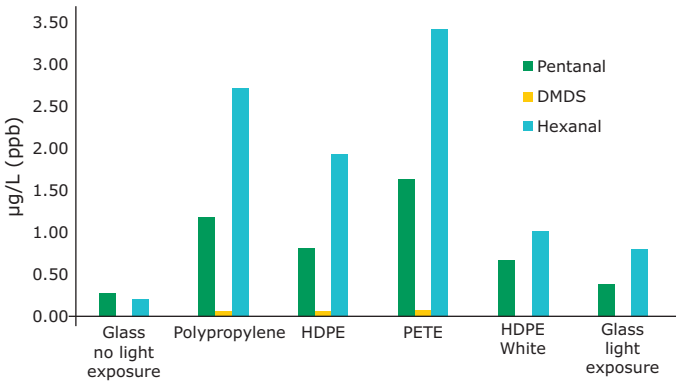
To calculate the concentration level of the selected LIOFs obtained from milk exposed in the various containers, the average of duplicate relative responses for each analyte was calculated. The average relative responses are shown in **Table 4**.

Table 4. Relative responses of LIOFs in milk after exposure to light in various containers

	No Light	PPE	HDPE	PETE	HDPE White	Glass
Pentanal	0.206	0.826	0.572	1.142	0.470	0.282
DMDS	0.000	0.172	0.170	0.196	0.000	0.000
Hexanal	0.122	1.454	1.027	1.826	0.551	0.438

The average relative responses for each analyte obtained from the no light exposed milk samples were subtracted from the average relative responses obtained from the various containers. The background subtracted relative responses were divided by the slope of the line as listed in **Figure 1**. **Figure 3** shows the calculated results.

Figure 3. Concentration in µg/L of LIOFs in milk with background subtraction



The results show that two of the plastics, PETE and PP, were the least efficient barrier to UV light. The PETE container had the thinnest wall of the containers which may have contributed to the barrier properties. PP had the thickest wall of any plastic but the formation of LIOFs were quite high. The addition of white pigment to the HPDE plastic made it a much better barrier to UV light. Its properties were similar to glass. The thickness of the glass does affect the barrier properties as we demonstrated in a separate study.

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Conclusions

The type of material used to store milk can be critical in the prevention of lipid peroxidation. This study shows that glass is still the best barrier to UV light, but HDPE impregnated with a pigment is a good option. In this case white pigmentation helped to reduce LIOF formation, but studies have shown that yellow or pink pigments may be better.

The CAR/PDMS fiber on the Nitinol core was able to retain the small flavoring analytes. The micropores retain and release these analytes efficiently. In addition, the Nitinol core is highly inert and extremely durable. This fiber is a viable alternative to this coating on a fused silica core.

Featured Materials

Description	Cat. No.
Carboxen® PDMS (CAR/PDMS) on Nitinol Core	**57907-U**
VOCOL® 30m x 0.25mm ID, 1.5 µm df	**24205-U**
Inlet Liner, Direct (SPME) Type, Straight Design (unpacked)	**2637501**
Hexanal-d12	**732338**
Valeraldehyde (Pentanal) analytical standard	**42272**
Dimethyl disulfide analytical standard	**68986**
Hexanal analytical standard	**18109**

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