Short communication: Comparison of 3 rapid methods for analysis of vitamin degradation compounds in fluid skim milk

A. N. Schiano, D. M. Benoist, and M. A. Drake*

Department of Food, Bioprocessing and Nutrition Sciences, Southeast Dairy Foods Research Center, North Carolina State University, Raleigh 27695

ABSTRACT

Vitamin fortification of dairy products, including fluid milk and fortified whey protein beverages, is an industry standard but can lead to the development of off-flavor compounds that are difficult to extract and detect by instrumental methods. Previous work has identified these compounds and their specific role in off-flavors in skim milk, but efficient extraction and quantification of these compounds remains a challenge. Three rapid methods (stir bar sorptive extraction, solvent-assisted stir bar sorptive extraction, and solid-phase microextraction) were compared for their ability to effectively recover vitamin degradation volatiles from fluid skim milk. The performance of the 3 methods for detecting and quantifying vitamin degradation-related volatile compounds was determined by linear regression of standard curves prepared from spiked standards of 5 vitamin degradation volatiles, the reproducibility on the same day and between days as measured by the average relative standard deviation of each standard curve, and the limits of detection and quantitation. Measurement of vitamin degradation compounds in commercial pasteurized fortified skim milks was also conducted using each method. Detection of selected vitamin degradation volatiles was linear in skim milk (0.005–200 µg/kg). Coefficient of determination values differed between methods and compounds. Within-day and between-day percentage of relative standard deviation also varied with compound and method. Limits of detection and quantitation values for all methods except solid-phase microextraction were lower than concentrations of selected volatile compounds typically found in commercial milk. Solvent-assisted stir bar sorptive extraction with a 10-mL sample volume provided the most consistent detection of selected compounds in

commercial milks. Based on linearity, relative standard deviation, and limits of detection and quantitation, cyclohexane solvent-assisted stir bar sorptive extraction with 10-mL sample volume is recommended for the quantitation of vitamin degradation-related volatiles in fluid skim milk.

Key words: milk, vitamin degradation product, solid-phase microextraction, stir bar sorptive extraction

Short Communication

The flavor of high-quality fluid milk is often described as pleasant or bland, posing a particular challenge for milk manufacturers as any off-flavors present will be noticeable by consumers. Flavor variation in milk can be attributed to varied factors such as the diet of the cow, the breed of the cow, the processing methods used, and the macronutrient content of the milk. Recent studies have shown that other milk processes, such as vitamin fortification, can also create noticeable off-flavors detected by consumers (Yeh et al., 2017). According to the Nutrition Labeling Act of 1990, skim and reduced-fat milks sold in the United States are legally required to be fortified with enough vitamin A to be nutritionally equivalent to whole milk (DPC, 2001).

Vitamin A can degrade under conditions typically experienced by commercial fluid milk (Whited et al., 2002; PHS/FDA, 2015). During light exposure, singlet oxygen free radicals are created through photosensitization of riboflavin and other naturally occurring tetrapyrroles (Airado-Rodríguez et al., 2011). These free radicals can result in lipid and protein oxidation, leading to light-oxidized off-flavors in milk (Lee and Min, 2009; Wold et al., 2015). Free radicals can be stabilized by the polyene chain of carotenoids such as retinyl palmitate; however, this cleaves the carotenoid at an unpredictable carbon along the polyene chain (Palace et al., 1999; Rodríguez-Amaya, 2016). Following this cleavage, the vitamin is destroyed and downstream reactions can lead to the development of vitamin degradation compounds (Palace et al., 1999; Rodríguez-Amaya, 2016). Furthermore, the premixes themselves also contain deg-

Received August 12, 2018.

Accepted January 28, 2019.

*Corresponding author: mdrake@ncsu.edu

radiation compounds. The addition of some vitamin A premixes to fluid skim milk imparted a floral or caroty flavor, which was attributed to vitamin A degradation compounds present in the premixes themselves (Yeh et al., 2017). Yeh et al. (2017) established that these vitamin A degradation compounds were only an off-flavor issue in skim milks and that not all vitamin A premixes contributed to this off-flavor. Specific flavor-active vitamin degradation compounds were identified in skim milks, but consistent and accurate quantitation was not possible due to the semivolatile nature of these specific compounds.

Reliable detection and quantification of aroma-active compounds related to vitamin degradation in fluid milk is challenging. Commonly used methods for analyzing volatile aroma-active compounds in fluid milk include solid-phase microextraction (SPME) and solvent-assisted flavor evaporation performed on direct solvent extracts (McGorin, 2007). Although SPME is effective at recovering a wide range of target compounds, the technique is not sensitive enough to efficiently recover vitamin degradation-associated volatiles from fluid milk, which are present in small concentrations and are often semivolatile (Yeh et al., 2017). The solvent-assisted flavor evaporation extraction process is sensitive and able to extract heavy and semivolatile compounds; however, the process is time consuming and requires large volumes of solvent, which can pose health hazards and disposal issues (Jeleń et al., 2012).

Stir bar sorptive extraction (SBSE) uses small magnetic stir bars encased in glass and coated in sorbent material to extract volatiles. Stir bars are added to fluid samples or may be suspended in the headspace of a solid, liquid, or gaseous sample during the absorption step. Volatiles from the stir bars are typically thermally desorbed and cryofocused, followed by GC-MS (Baltussen et al., 1999; McGorin, 2007; Prieto et al., 2010). Stir bar sorptive extraction has been used for volatile compound analysis of a variety of products, including hormones, veterinary residues, acid and alcohol volatiles, and sulfur volatiles in fluid milk (McGorin, 2007; Huang et al., 2009; Zabbia et al., 2012; Ochiai et al., 2016). Like SPME, SBSE operates on the basic principle of partitioning coefficients, and the ratio of the mass of analyte in the stir bar and the mass of analyte originally in the matrix can be expressed as follows:

$$\frac{m_{\text{SBSE}}}{m_0} = \frac{\left(\frac{K_{O/W}}{\beta} \right)}{1 + \left(\frac{K_{O/W}}{\beta} \right)},$$

where m_{SBSE} is the mass of the analyte in the stir bar, m_0 is the mass of the analyte originally in the matrix, $K_{O/W}$ is the octanol water partition coefficient, and β is the phase ratio (the ratio of the volume of the matrix to the volume of the polydimethylsiloxane (PDMS); Baltussen et al., 1999). A typical SPME fiber contains roughly 0.5 μL of PDMS, resulting in a suboptimal extraction for all compounds without a high $K_{O/W}$ value; SBSE, on the other hand, allows for quantitative extraction of compounds with $K_{O/W}$ values over 500. As such, SBSE offers potential significant resolution improvement over traditional SPME methods.

Like other extraction methods, SBSE results are heavily influenced by the sample matrix and specific extraction protocols, including pH, extraction time, salt addition, stirring speed, extraction temperature, sample volume, and stir bar coating. Adding salts such as sodium chloride can improve extraction of polar analytes but reduce extraction of nonpolar analytes (Prieto et al., 2010). Choosing an ideal sample volume is also a balancing act. For milk, volumes of 5 to 100 mL are typically selected (León et al., 2003).

Solvent-assisted SBSE (SA-SBSE) is a modified SBSE technique using sorptive stir bars swollen in solvent that was developed as a way to circumvent the need to manufacture in-house custom coatings for stir bars (Ochiai et al., 2016). The PDMS stir bars are “bloated” in solvent before volatile compound extraction. The solvent bloating increases diffusion into and out of the PDMS coating as well as partitions into the aqueous matrix to allow for increased recovery of aqueous phase (polar) analytes (Ochiai et al., 2016). Solvent-assisted SBSE has been shown to improve peak resolution and extraction efficiency for both polar and nonpolar analytes (Ochiai et al., 2016).

The SBSE technique has been used for flavor research on dairy products including milk, yogurt, cream cheese, and dried dairy ingredients (Hoffman and Heiden, 2000; Park and Drake, 2016) and has demonstrated significant improvement over SPME for detecting and quantifying sulfur and carbonyl compounds in aseptically processed fluid milk (Zabbia et al., 2012). Solvent-assisted SBSE, a newer technique, has not been widely applied to dairy research. The objective of this study was to compare 3 methods (SA-SBSE, SBSE, and SPME) to rapidly and effectively recover vitamin degradation volatiles from fluid skim milk.

The performance of the 3 methods (SA-SBSE, SBSE, and SPME) for detecting and quantifying vitamin degradation-related volatile compounds was evaluated by linear regression of standard curves prepared from spiked standards, the reproducibility on the same day and between days as measured by the average relative

standard deviation (**RSD**) of each standard curve, and the limit of detection (**LOD**) and limit of quantitation (**LOQ**). Reagent-grade solvents (cyclohexane, methanol, and dichloromethane) and pure chemical standards (2-methyl-3-heptanone, α -phellandrene, β -cyclocitral, β -damascone, β -ionone, and α -irone) were purchased from Sigma-Aldrich (St. Louis, MO). These compounds were selected because they were identified by Yeh et al. (2017) as key aroma-active vitamin degradation compounds contributing to vitamin-related off-flavors in fluid milk. We purchased HPLC-grade water from Fluka (Seelze, Germany), and unfortified raw skim milk (<0.5% fat) was obtained from the North Carolina State University Dairy Enterprise System (Raleigh, NC). Skim milk was HTST pasteurized at 72°C for 15 s using a Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) with a 2-stage homogenizer (GEA Niro Soavi, Parma, Italy). A stock solution of vitamin degradation compound standards was prepared by dissolving 0.25 g of each compound into 5 mL of methanol (Sigma-Aldrich). The stock solution was diluted to 25,000 $\mu\text{g/kg}$ for each compound and added directly to the HTST pasteurized skim milk in varying amounts to achieve the desired concentration. Commercial HTST and ultrapasteurized (**UP**) skim milks ($n = 5$) were purchased locally (Raleigh, NC) and were in mid to late shelf life (10–12 d for HTST milk; 35–50 d for UP milk). All milks were prepared with overhead lights off to prevent light oxidation.

The headspace SPME–GC–MS system was an Agilent 7820 GC with a 5975 MSD (Agilent Technologies Inc., Santa Clara, CA) with a ZB5-ms (5% phenyl-arylene stationary phase, 30-m length $\times$ 0.25-mm i.d. $\times$ 0.25- μm film thickness; Zebron, Phenomenex, Torrance, CA). The sample preparation and SPME GC–MS method were modified from the method reported by White et al. (2013). Samples were injected using a CTC Analytics CombiPal Autosampler (Zwingen, Switzerland). Five milliliters of milk with varying concentrations of vitamin degradation compound standards was added to 20-mL amber glass SPME vials (Microliter Analytical, Suwanee, GA) in triplicate. Vials were equilibrated for 25 min at 40°C with 4-s pulses of 250-rpm agitation. A 1-cm SPME fiber (divinylbenzene/carboxen/polydimethylsiloxane; Supelco, Bellefonte, PA) was used for all analyses. The fiber was retracted and injected at 5.0 cm in the GC inlet for 5 min. Initial GC oven conditions were 40°C for 3 min with ramp rates of 10°C/min to 90°C, 5°C/min to 200°C held for 10 min, and 20°C/min to 250°C held for 5 min. A combination of scanning from 40 to 200 mass:charge ratio (m/z) and selective ion mode for ions 93 (α -phellandrene), 121 (α -irone), 137 (β -cyclocitral), and 177 (β -damascone,

β -ionone) was performed to identify the compounds of interest. Samples were evaluated in triplicate. The entire analysis was repeated on a second day.

The SA–SBSE and SBSE analyses were performed using an Agilent 7890B series GC/Agilent inert 5977A MSD equipped with a ZB5-ms column (5% phenyl-arylene stationary phase, 30-m length $\times$ 0.25-mm i.d. $\times$ 0.25- μm film thickness; Zebron, Phenomenex). The SBSE GC–MS program was modified from the method used by Park and Drake (2016). Stir bars were injected using an autosampler (MPS Autosampler, Gerstel Inc., Linthicum, MD) and desorbed on a thermal desorption unit (Gerstel Inc.) at 250°C for 10 min. Volatile compounds were cryogenically focused at -120°C and analyzed using GC–MS. Initial GC oven conditions were 40°C for 3 min with ramp rates of 10°C/min to 90°C, 5°C/min to 200°C held for 10 min, and 20°C/min to 250°C held for 5 min. Purge time was set to 1.2 min using helium as the carrier gas at a constant flow rate of 1 mL/min. A 3-min solvent delay was included in the MS acquisition parameters. A combination of scanning from 40 to 200 m/z and selective ion mode for ions 93 (α -phellandrene), 121 (α -irone), 137 (β -cyclocitral), and 177 (β -damascone, β -ionone) was performed to identify compounds of interest. Magnetic stir bars (10 $\times$ 0.5 mm) coated in PDMS as well as thermal desorption unit tubes (Gerstel Inc.) were conditioned before analysis for 1 h at 300°C. Stir bars were cleaned following desorption by soaking the bars 3 times in a 50:50 (vol/vol) methanol:dichloromethane solution for 1 h, after which the stir bars were allowed to dry overnight before thermal conditioning as previously described.

For SBSE, stir bars were added to 5 or 10 mL of milk spiked with varying concentrations of vitamin degradation compounds in a 20-mL amber glass vial (Microliter Analytical) and rotated on a magnetic stir plate at 900 rpm for 60 min at 25°C. Two sample volumes (10 and 5 mL) were used to determine the effect of sample volume on efficiency of vitamin compound extraction. After sample extraction on the stir plate, stir bars were rinsed with HPLC-grade water and dried briefly before thermal desorption as described previously.

For SA–SBSE, batches of 10 stir bars were submerged in 20 mL of cyclohexane (99.9% purity) for 30 min before being briefly dried and added to 5 mL of milk spiked with varying concentrations of vitamin degradation compounds as previously described. Bloat time was determined by bloating stir bars in cyclohexane and weighing them periodically; the ideal bloat time was the time after which the stir bars no longer significantly increased in weight. Previous literature (Ochiai et al., 2016) suggested that cyclohexane would be an appropriate solvent for this application; this was veri-

fied by preliminary work testing the efficacy of cyclohexane, hexane, diethyl ether, and dichloromethane. Preliminary experiments were conducted with stir bars bloated in each solvent followed by recovery of vitamin degradation compounds in spiked samples and detection of compounds in commercial HTST and UP milks. Following preliminary experiments, cyclohexane was selected.

To determine that the relationship of vitamin degradation compound concentration and signal from the instruments was linear for each extraction method, the 5 vitamin degradation compounds were spiked into unfortified HTST pasteurized skim milk over a wide range of concentrations (0.005–200 µg/kg) and extracted and quantified by each of these methods. The concentrations were prepared by making a stock solution of known concentration of the vitamin degradation compounds and then making dilutions over the previously stated range. Each concentration was evaluated in triplicate by each extraction method. The entire experiment was repeated on a second day. Measurement of vitamin degradation compounds in commercial HTST and UP pasteurized vitamin A fortified skim bovine milks was conducted using all 3 methods. Three conventional HTST pasteurized milks packaged in translucent high-density polyethylene plastic containers, 1 conventional UP milk and 2 organic UP milks packaged in opaque cardboard cartons, were used. Concentrations of vitamin degradation compounds extracted using these methods were determined using relative response factors previously determined with calibration standards during linear regression of standard curve experiments.

All data were analyzed using XLSTAT software (version 2018.1, Addinsoft, New York, NY). Solvent uptake by stir bars to determine maximum bloom time was analyzed by ANOVA with means separation using Tukey's honestly significant difference test at 95% confidence. Vitamin degradation compound recoveries from commercial skim milks were also evaluated by ANOVA with Tukey's honestly significant difference test at 95% confidence. Linearity of the standard curves for each method was determined using linear regression. The same-day reproducibility was determined by the RSD between peak areas of compounds from spiked unfortified milks analyzed on the same day. The between-day reproducibility was determined by RSD between peak areas of compounds from spiked unfortified milks analyzed on different days. The LOD and LOQ were defined as signal:noise ratios of 3:1 and 10:1, respectively, for each vitamin degradation compound in skim milk. The LOD and LOQ determinations were generated from spiked unfortified skim milk to account for background interference to be calculated into these limits.

Three vitamin degradation compounds (β -damascone, β -ionone, and α -irone) produced a signal in unfortified skim milk, likely due to residual breakdown products of endogenous retinol found in whole milk before separation (MacGibbon and Taylor, 2006). The LOD and LOQ were determined in these signal-producing compounds using the AOAC International (2016) alternative definition of the LOD and LOQ. The LOD was interpreted as the blank (unfortified) milk value plus 3 times the standard deviation of the blank, whereas the LOQ was interpreted as the blank milk value plus 10 times the standard deviation of the blank.

Detection of selected vitamin degradation volatiles was linear in skim milk (0.005–200 µg/kg). Coefficient of determination (R^2) values differed between methods and compounds (Table 1). α -Phellandrene curves were associated with the lowest R^2 values regardless of method, whereas β -damascone was associated with the highest R^2 values regardless of method. Of the 3 methods evaluated, SA-SBSE with a 10-mL extraction volume and SPME had the most consistently high R^2 values. Solvent-assisted SBSE with 10-mL extraction volume was the only method with R^2 values ≥ 0.90 for all compounds tested, indicating that all compounds can be reliably extracted across this range.

The R^2 values for the 5-mL extraction volume SBSE methods were distinctly lower than the R^2 values of the other methods regardless of stir bar bloating. Increasing the volume of matrix in an SBSE extraction can result in poorer recovery in some cases as the phase ratio (ratio of the volume of the matrix to the volume of the PDMS) is inversely related to recovery (Prieto et al., 2010). However, a sample volume that is too low can also result in very poor recovery of analytes at concentrations near their LOD (Prieto et al., 2010). For milk, sample volumes of 5 to 100 mL are typically selected (León et al., 2003). The headspace in the amber glass vials used for sample extraction could also have contributed to the increased linearity resulting from a 10-mL extraction volume. Holding the vial size consistent while decreasing the sample volume results in greater headspace within the vial. Volatile compounds are partitioned between the headspace and the sample matrix, leaving less in the sample matrix to partition into the absorbent stir bar.

Slopes of the standard curves varied by compound regardless of the method used, in some cases varying by orders of magnitude (Table 1). The variability of slopes indicates that using a single internal standard to quantify vitamin degradation compounds in fluid milk, especially at the levels typically found in commercial milks, will provide less accurate results than standard curves prepared with the compound of interest. Of the

Table 1. Slopes of linear regression equations, R^2 values, limits of detection (LOD), and limits of quantitation (LOQ) for selected spiked vitamin degradation compounds in skim milk¹

Item	Method ²	SA-SBSE		SBSE		SPME
		10 mL	5 mL	10 mL	5 mL	5 mL
α -Phellandrene	Slope	0.000181	0.000089	0.000322	0.000439	0.000003
	R^2	0.885	0.439	0.679	0.482	0.831
	LOD	1.00	5.00	5.00	5.00	2.50
	LOQ	5.00	5.00	25.00	25.00	5.00
β -Cyclocitral	Slope	0.000033	0.000030	0.000153	0.000216	0.000006
	R^2	0.930	0.676	0.927	0.870	0.987
	LOD	0.01	0.01	0.01	0.01	1.00
	LOQ	0.05	0.05	0.05	0.05	2.50
β -Damascone	Slope	0.0000203	0.0000198	0.0000396	0.0000541	0.0000068
	R^2	0.986	0.634	0.975	0.960	0.989
	LOD	0.34	0.46	0.73	0.83	2.50
	LOQ	0.59	1.00	1.57	1.60	2.50
β -Ionone	Slope	0.000013	0.000015	0.000019	0.000024	0.000007
	R^2	0.986	0.727	0.989	0.894	0.934
	LOD	0.10	0.12	0.13	0.20	2.50
	LOQ	0.11	0.18	0.23	0.35	2.50
α -Irone	Slope	0.000038	0.000047	0.000049	0.000065	0.000017
	R^2	0.980	0.798	0.987	0.743	0.938
	LOD	0.21	0.31	0.38	0.71	2.50
	LOQ	0.26	0.49	0.66	1.55	5.00

¹Results are averaged across samples evaluated in triplicate on 2 different days. SA-SBSE = solvent-assisted stir bar sorptive extraction; SBSE = stir bar sorptive extraction; SPME = solid-phase microextraction.

²LOD and LOQ values are expressed in micrograms per kilogram.

methods tested, SPME had the least variability in slopes of the standard curve, indicating that quantification with a single internal standard would be more likely to produce accurate results with SPME than a stir bar method, provided that concentrations are within LOD.

Within-day percentage of RSD varied with compound and method (7–52%; Table 2). The bloated stir bar method with 10-mL sample volume was the only method to produce <20% RSD for all selected compounds. Between-day percentage RSD values tended to be higher than within-day values (Table 2).

The LOD and LOQ values for all methods except SPME were lower than concentrations of selected volatile compounds typically found in commercial milk (Table 1). All 3 methods tested were able to detect selected volatile compounds at concentrations lower than the consumer detection threshold for the compounds in fluid skim milk (Yeh et al., 2017). The LOD and LOQ values obtained with SPME were higher than the other methods tested, and the LOD and LOQ values obtained with the 2 stir bar methods were largely similar within the range of compounds tested. α -Phellandrene,

Table 2. Within- and between-day percentage of relative standard deviation (RSD) for selected vitamin degradation compound recovery by method (n = 6 replicates across 2 d)

Item	Method	SA-SBSE		SBSE		SPME
		10 mL	5 mL	10 mL	5 mL	5 mL
α -Phellandrene	Within-day RSD	18.3	52.1	24.9	24.0	12.6
	Between-day RSD	35.0	90.2	79.5	50.2	16.7
β -Cyclocitral	Within-day RSD	19.7	13.7	16.7	13.8	7.4
	Between-day RSD	33.8	43.9	22.7	20.0	6.9
β -Damascone	Within-day RSD	13.3	30.7	15.7	21.3	12.3
	Between-day RSD	26.8	70.6	30.1	33.4	25.5
β -Ionone	Within-day RSD	7.9	16.1	12.7	11.1	17.8
	Between-day RSD	23.1	39.7	28.7	12.2	30.5
α -Irone	Within-day RSD	8.9	17.8	20.9	14.6	14.0
	Between-day RSD	21.2	37.5	47.3	16.9	13.0

¹SA-SBSE = solvent-assisted stir bar sorptive extraction; SBSE = stir bar sorptive extraction; SPME = solid-phase microextraction.

Table 3. Quantification of selected vitamin degradation compounds in commercial pasteurized vitamin A fortified skim milks¹

Method	SA-SBSE		SBSE		SPME
	10 mL	5 mL	10 mL	5 mL	5 mL
α-Phellandrene	ND ²	ND	ND	ND	ND
β-Cyclocitral	0.08 ^b	0.05 ^b	0.23 ^a	0.05 ^b	ND
β-Damascone	0.68 ^a	NQ ³	NQ	NQ	ND
β-Ionone	0.12 ^a	0.19 ^a	NQ	NQ	ND
α-Irone	3.98 ^{ab}	6.72 ^a	5.99 ^{ab}	2.17 ^b	ND

^{a,b}Means within a column not sharing a common superscript are statistically different ($P < 0.05$).

¹Values presented are an average of the 5 commercial milks tested in triplicate (μg/kg). SA-SBSE = solvent-assisted stir bar sorptive extraction; SBSE = stir bar sorptive extraction; SPME = solid-phase microextraction.

²Not detected.

³Not quantified. Compounds were detected but measured below limit of quantitation.

the most polar compound tested, showed considerable improvements in LOD and LOQ when SA-SBSE was used over SBSE, consistent with previous work (Ochiai et al., 2016).

Average concentrations of selected compounds in commercial milks are presented in Table 3. Concentrations of selected compounds varied with the commercial milk tested, as expected (data not shown). Average concentrations across the samples tested are presented to demonstrate the effect of method on compound detection rather than the effect of sample. α-Phellandrene was not detected in commercial skim milks by any of the methods. Although β-damascone and β-ionone were consistently detected by some of the methods (SA-SBSE and 10 mL/5 mL SBSE, respectively), other methods did not detect the compounds consistently enough to result in an average across all 5 milk samples above the previously determined LOQ.

Solvent-assisted SBSE with a 10-mL sample volume provided the most consistent detection of selected compounds in commercial milks, although the levels of compounds detected were not consistently higher than other methods ($P > 0.05$). The SA-SBSE method provided more consistent detection of the selected compounds than SBSE. None of the selected compounds were detected in any of the 5 commercial milks tested using SPME, consistent with previous findings (Yeh et al., 2017).

Rapid methods are able to reliably extract and quantify vitamin degradation-related volatile compounds in fluid milk at low concentrations; however, the method used can greatly affect results. The SPME method produced linear standard curves and low RSD values, but the LOD and LOQ offered by the method were not

low enough to accurately track concentrations of vitamin degradation compounds in fluid milk, making the method unsuitable for this application. The reduced sensitivity of SPME is likely due to the lower volume of PDMS coating on a fiber versus a stir bar, resulting in suboptimal extraction of compounds without a high partition coefficient between the headspace and the fiber (Baltussen et al., 1999). However, if the expected concentrations of these volatiles are higher, SBSE or SA-SBSE offer no significant improvement over SPME for analysis. Based on linearity, RSD, LOD, and LOQ, cyclohexane SA-SBSE with 10-mL sample volume is recommended for the quantitation of vitamin degradation-related volatiles in fluid skim milk.

ACKNOWLEDGMENTS

Funding was provided in part by the National Dairy Council (Rosemont, IL). The use of trade names does not imply endorsement or lack of endorsement of those not mentioned.

REFERENCES

- Airado-Rodríguez, D., N. Intawiwat, J. Skaret, and J. P. Wold. 2011. Effect of naturally occurring tetrapyrroles on photooxidation in cow's milk. *J. Agric. Food Chem.* 59:3905–3914.
- AOAC International. 2016. *Official Methods of Analysis*. 20th ed. AOAC International, Gaithersburg, MD.
- Baltussen, E., P. Sandra, F. David, and C. Cramers. 1999. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: Theory and principles. *J. Microcolumn Sep.* 11:737–747.
- DPC (Dairy Practices Council). 2001. DPC 53: Guidelines for vitamin A and D fortification of fluid milk. 2001 revision. Accessed Jun. 15, 2018. <https://www.dairypc.org/catalog/vitamin-a-and-d-fortification-of-fluid-milk>.
- Hoffman, A., and A. Heiden. 2000. Determination of flavor and off flavor compounds in dairy products using stir bar sorptive extraction (SBSE) and thermal desorption GC/MSD/PFPD. AppNote 5/2000. Gerstel Inc., Baltimore, MD.
- Huang, X., N. Qiu, and D. Yuan. 2009. Simple and sensitive monitoring of sulfonamide veterinary residues in milk by stir bar sorptive extraction based on monolithic material and high performance liquid chromatography analysis. *J. Chromatogr. A* 1216:8240–8245.
- Jeleń, H. H., M. Majcher, and M. Dzidas. 2012. Microextraction techniques in the analysis of food flavor compounds: A review. *Anal. Chim. Acta* 738:13–26.
- Lee, J. H., and D. B. Min. 2009. Changes of headspace volatiles in milk with riboflavin photosensitization. *J. Food Sci.* 74:C563–C568.
- León, V. M., B. Alvarez, M. A. Cobollo, S. Munoz, and I. Valor. 2003. Analysis of 35 priority semivolatile compounds in water by stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry. I. Method optimisation. *J. Chromatogr. A* 999:91–101.
- MacGibbon, A. K. H., and M. W. Taylor. 2006. Composition and structure of bovine milk lipids. Pages 1–42 in *Advanced Dairy Chemistry*. Vol. 2: Lipids. 3rd ed. P. L. H. McSweeney and P. F. Fox, ed. Springer, New York, NY.
- McGorin, R. J. 2007. Flavor analysis of dairy products. Pages 23–49 in *Flavor of Dairy Products*. 1st ed. K. R. Cadwallader, M. A.

- Drake, and R. J. McGorin, ed. American Chemical Society, Washington, DC.
- Ochiai, N., K. Sasamoto, F. David, and P. Sandra. 2016. Solvent-assisted stir bar sorptive extraction by using swollen polydimethylsiloxane for enhanced recovery of polar solutes in aqueous samples: Application to aroma compounds in beer and pesticides in wine. *J. Chromatogr. A* 1455:45–56.
- Palace, V. P., N. Khaper, Q. Qin, and P. K. Singal. 1999. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. *Free Radic. Biol. Med.* 26:746–761.
- Park, C. W., and M. A. Drake. 2016. Condensed milk storage and evaporation affect the flavor of nonfat dry milk. *J. Dairy Sci.* 99:9586–9597.
- PHS/FDA (Public Health Service/Food and Drug Administration). 2015. Grade “A” Pasteurized Milk Ordinance. US Government Printing Office, Washington, DC.
- Prieto, A., O. Basauri, R. Rodil, A. Usobiaga, L. A. Fernández, N. Etxebarria, and O. Zuloaga. 2010. Stir-bar sorptive extraction: A view on method optimisation, novel applications, limitations and potential solutions. *J. Chromatogr. A* 1217:2642–2666.
- Rodriguez-Amaya, D. B. 2016. Food Carotenoids: Chemistry, Biology, and Technology. 1st ed. IFT Press, Hoboken, NJ.
- White, S. S., K. M. Fox, S. M. Jervis, and M. A. Drake. 2013. Influence of heating and acidification on the flavor of whey protein isolate. *J. Dairy Sci.* 96:1366–1379.
- Whited, L. J., B. Hamond, K. Chapman, and K. J. Boor. 2002. Vitamin A degradation and light-oxidized flavor defects in milk. *J. Dairy Sci.* 85:351–354.
- Wold, J. P., J. Skaret, and T. K. Dalsgaard. 2015. Assessment of the action spectrum for photooxidation in full fat bovine milk. *Food Chem.* 179:68–75.
- Yeh, E. B., A. N. Schiano, Y. Jo, D. M. Barbano, and M. A. Drake. 2017. The effect of vitamin concentrates on the flavor of pasteurized fluid milk. *J. Dairy Sci.* 100:4335–4348.
- Zabbia, A., E. M. Buys, and H. L. de Kock. 2012. Undesirable sulphur and carbonyl flavor compounds in UHT milk: A review. *Crit. Rev. Food Sci. Nutr.* 52:21–30.