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Solvent Type Affects the Number, Distribution, and Relative Quantities of Volatile Compounds Found in Sweet Whey Powder

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ABSTRACT

This study compares the performance of diethyl ether, methylene chloride, methyl formate, and pentane in the analysis of volatile flavor components in sweet whey powder. Extracts were prepared from sweet whey powder using each solvent. Volatile components were isolated by solvent extraction followed by solvent-assisted flavor evaporation. Gas chromatography-mass spectroscopy, coelution with known standards, and retention indices were used to identify the volatile compounds. Sixty total compounds were either positively or tentatively identified across all 4 solvents, but the number, distribution between the molecular classes, and relative quantities detected depended on solvent type. The highest number, widest distribution, and greatest relative quantities were found using methylene chloride and methyl formate, whereas diethyl ether and especially pentane were noticeably less effective. Results are characterized using molecular-based characteristics of solvents and solutes including dipole moment, dielectric constant, Log P (octanol-water partition coefficient), polarizability, water solubility, and Lewis acidity/basicity. Polarity and acidity/basicity were the primary factors that determined solvent performance. This work establishes a molecular-level basis for the selection of solvents in the analysis of sweet whey powder flavors.
Key words: solvent, volatile, sweet whey powder

INTRODUCTION

The determination of flavor-active compounds in foods is an established area of research. Several different techniques exist to extract volatile compounds from a food matrix including extraction with organic solvents. Solvent choice is a critical factor in determining volatile compound recovery and efficiency of an extraction method. Three solvents that have been widely used in many food extractions include pentane (**PN**), methylene chloride (**MC**), and diethyl ether (**DE**). Diethyl ether has been one of the most frequently used solvents for extraction of volatile compounds over the last decade, but few studies provide a justification for its use. One solvent not typically used in volatile extractions is methyl formate (**MF**). The 4 solvents differ in extraction efficiencies due to differences in chemical and physical properties (Table 1). Diethyl ether is less effective at extracting polar compounds in a variety of foods primarily due to its highly nonpolar character. Methyl formate and MC would predictably extract more polar compounds due to their greater polar character, as expressed by their larger dipole moments and dielectric constants and lower Log P values. Because PN is relatively insoluble in water and very nonpolar, it would predictably extract greater numbers and relative quantities of increasingly nonpolar compounds. The compatibility between the molecular and physical properties of the solvent and volatile solute is critical in selecting an appropriate solvent.

Other factors affecting solvent selection include flammability, toxicity, cost, reactivity, and stability. Understanding the potential hazards (e.g., flammability, toxicity, and stability) during storage is critical because it requires more caution in its use and handling. Using the National Fire Protection Association (**NFPA**) rating system for flammable and combustible liquids, MC has the lowest flammability rating (NFPA = 1) whereas DE (NFPA = 4), PN (NFPA = 4), and MF (NFPA = 4) have the highest flammability rating, because of their high volatility and low ignition temperatures. Solvent toxicity is another determinant in solvent selection for extractions, especially in the case where sensory detection is utilized; for example, GC-olfactometry. Diethyl ether has an oral ($LD_{50(rat)}$ = 1,215,000 μg/kg) and inhalation ($LD_{50(rat)}$ = 73,000,000 μg/kg; 2 h) toxicity that is relatively similar to the oral ($LD_{50(rat)}$= 1,600,000 μg/kg) and inhalation ($LD_{50(rat)}$ = 88,000 μg/kg; 30 min) toxicities of MC (National Research Council, 1995). However, inhalation of high concentrations of DE can cause sedation, unconsciousness, and respiratory paralysis; high concentrations of MC vapor (>500,000 μg/kg for 8 h) can cause less detrimental effects such as lightheadedness, fatigue, and nausea (National Re-

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Table 1. Chemical and physical properties of commonly used solvents amenable to volatile compound extractions

Solvent	Boiling point[1] (°C, 760 mmHg)	Dipole moment[2]	Dielectric constant[3]	Log P[1]	Polarizability[1] ($\pm 0.5 \times 10^{-24}$ cm^3)	Water solubility[1] (g/L, 25°C)	SB[4]	SA[5]
Diethyl ether	33.2	1.15	4.3	0.98	8.85	47.2	0.562	0.000
Methyl formate	32	1.77	8.5	–0.23	5.25	402	0.422	0.000
Methylene chloride	40	1.60	9.1	1.19	6.49	15.8	0.178	0.040
n-Pentane	35.2	0	1.8	3.41	9.99	0.031	0.073	0.000

[1]Values obtained from Advanced Chemistry Development, Inc., 2006; Log P = log of octanol/water partition.

[2]Values obtained from Univ. Southern Maine (http://www.usm.maine.edu/~newton/Chy251_253/Lectures/Solvents/Solvents.html) and Buyong et al., 2000.

[3]Values obtained from ASI Instruments Inc., 2006.

[4]SB = Solvent basicity; values obtained from Catalan et al., 1996.

[5]SA = Solvent acidity; values obtained from Catalan and Diaz, 1997.

search Council, 1995). Methyl formate has an oral ($LD_{50(rat)}$ = 1,500,000 μg/kg) toxicity similar to MC, and a lower inhalation toxicity ($LD_{50(rat)}$ = 50,000,000 μg/kg; 30 min; Chemical Land 21, 2000). Pentane is moderately toxic (oral $LD_{50(rat)}$ >2,000,000 μg/kg; inhalation $LC_{50(rat)}$ = 364 μg/kg; Chemical Land 21, 2000). Inhalation of PN may result in nausea, dizziness, or irritation of the mucous membrane of the nose and throat.

The only solvent that exhibits high hazards during storage is DE. Over time, it has a potential explosive hazard due to the formation of unstable peroxides promoted by exposure to oxygen, light, and strong oxidizing agents. Pentane, MC, and MF exhibit fewer hazards than DE during storage under normal conditions (National Research Council, 1995).

The level and spectrum of volatile compounds obtained after extraction and GC-MS analysis is expected to be highly dependent on the solvent selection. To date, few recent studies have considered the effects of solvent properties on the extraction of volatile compounds from foods. This study will compare the types and relative quantities of volatile compounds extracted from sweet whey powder (**SWP**) by DE, PN, MF, and MC as related to the molecular and physical properties of the solvents and solutes.

MATERIALS AND METHODS

SWP Composition

Commercial SWP (Cheddar cheese whey, US extra grade, Foremost Farms Inc., Baraboo, WI) aged for 4 yr under ambient conditions was selected for analysis. The pH was measured using a pH electrode (Accumet AB15, Fisher Scientific, Pittsburgh, PA) on a 6.5-g SWP sample in 100 mL of distilled water (Sithole et al., 2005). Free moisture content was determined by a standard oven-drying method (Marth, 1985). Fat content was determined by the Mojonnier method (method 989.05; AOAC, 2000). Ash content was determined using a muffle furnace at 535°C (method 935.42; AOAC, 2000). The lactose content was determined using an enzymatic method (984.15; AOAC, 1995). Volhard's method (method 935.43; AOAC, 2000) was used to determine the salt content. The protein content was determined by the Kjeldahl method (methods 930.29, 991.20; AOAC, 2000). Sample color was determined by colorimeter (Colorquest 45/0, Hunterlab, Reston, VA). All analyses were done in triplicate. The average chemical characteristics (± standard deviation) of the SWP samples were as follows: moisture = 4.72% ± 0.12; protein = 13.2% ± 0.03; fat = 1.04% ± 0.10; lactose = 63.9% ± 0.4; ash = 5.39% ± 0.05; salt = 0.34% ± 0.05; and pH = 5.58 ± 0.01. Colorimeter results were L* = 77 ± 0.15, a* = 4.3 ± 0.068, and b* = 28 ± 0.12. Results were consistent with those reported by Banavara et al. (2003); thus, the SWP selected would be considered to be in legal compliance with respect to US compositional standards. The colorimeter values are reflective of whey that has undergone some browning, resulting in a possible reduction in lactose and protein levels.

Preparation of Extracts

Sweet whey powder (8.0 g) was extracted with 25 mL of each solvent in a tightly capped 40-mL vial for 1 h with mild, intermittent, manual shaking every 15 min. The 4 solvents used were DE (Sigma-Aldrich, Inc., St. Louis, MO), MC (Sigma-Aldrich, Inc.), MF (Acros Organics, Morris Plains, NJ), and PN (Acros Organics). At 1 h, the samples were centrifuged at 1,200 × *g* for 2 min. The supernatant was pipetted from the vial and collected in a 100-mL glass vial with a Teflon-lined lid. The SWP was resuspended in an additional 25 mL of solvent and the extraction was repeated as above for a total of 3 × 25 mL of each solvent per SWP sample. The combined extracts (~75 mL) were distilled under