

# Volatilities of Short-Chain Fatty Acids in a Fermented Milk Model System as Affected by Stabilizers

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## ABSTRACT

A fermented milk model system was employed to investigate the volatilities of butyric, caprylic, and caproic acids as affected by low, intermediate, and high levels of carrageenan, guar and xanthan gums. Carrageenan exhibited no effect on fatty acid volatility. Intermediate levels of guar gum increased the volatility of caprylic acid. Low and high levels of guar gum had no effect on free fatty acid volatility. At low and high levels, xanthan gum had no effect on volatilities of fatty acids. The intermediate level of xanthan gum increased volatilities of caproic and caprylic acids. The effects of viscosity were potentiated with increased fatty acid chain length.

**Key Words:** aroma, dairy, fatty acids, chromatography, hydrocolloid

## INTRODUCTION

THE DEVELOPMENT OF REDUCED-FAT PRODUCTS FREQUENTLY involves the replacement of fat with hydrocolloid stabilizers to achieve desirable sensory characteristics. Although such stabilizers may mimic certain characteristics of lipids, studies have shown that hydrocolloid stabilizers can bind or inhibit the release of aroma-active compounds (Voilley et al., 1990; Rankin and Bodyfelt, 1996).

Nonvolatile food components such as proteins and carbohydrates may alter the volatility of flavor compounds. Casein was observed to strongly bind esters (Voilley et al., 1990), acetate, and acetone (Thanh et al., 1992). Hansen and Heinis (1991) reported that the presence of casein decreased the sensory perception of vanilla. Certain carbohydrates were found to bind alcohols, aldehydes, esters (Thanh et al., 1992) and diacetyl (Rankin and Bodyfelt, 1996). Interactions between volatiles and carbohydrates may occur via ionic interactions, hydrogen bonding, hydrophobic effects, formation of inclusion complexes, and impedance of molecular mobility due to increased viscosity (Voilley et al., 1990).

Desirable aroma in fermented dairy products is directly related to the presence and availability of aroma compounds including volatile free fatty acids (VFA). Butyric, caproic and capric acids were identified as potent aroma volatiles in 3-yr-old Cheddar cheese (Christensen and Reineccius, 1995). VFA's also impart the rancid flavor characteristic of dry hard Italian cheeses (Eaton, 1994). Although many compounds and classes of compounds have been evaluated for interaction and binding to stabilizer gums, no research has been published on the potential for VFA's to be affected by such interactions. Our preliminary research demonstrated that hydrocolloid stabilizers can influence the volatility of VFA's in aqueous media (Chen, 1997). The objective of this study was to further characterize the effects of hydrocolloid type, level, and viscosity on volatilities of

VFA's in a fermented milk model system under dynamic headspace sampling conditions.

## MATERIALS & METHODS

### Reagents

Butyric, caproic, caprylic, and valeric acids, carrageenan (type 1 commercial grade), xanthan (practical grade) and guar gums were purchased from Sigma Chemical Co. (St. Louis, MO). GC/MS-grade eluting solvent, CS<sub>2</sub>, was obtained from Sigma-Aldrich (St. Louis, MO).

### Dynamic headspace analysis

Model systems sampled under dynamic conditions were prepared using carrageenan, and xanthan and guar gums at concentrations of 0.025%, 0.1% and 0.4% (wt/wt) by dispersing dry hydrocolloid into commercially pasteurized and homogenized skim milk under constant agitation using a magnetic stir bar in 250-mL glass vials with teflon-lined screw-top caps. Skim milk with no added stabilizer was used as a control. All experimental units were vat-pasteurized at 70°C for 10 min in a circulating water bath. Samples were allowed to cool at room temperature to ~50°C at which point a 300-μg aliquot containing butyric, caproic, and capric acids was added. The final concentration of each VFA in the model system solution was 1.00 mg/g. Each experimental unit was stored overnight at 4°C to complete stabilizer hydration and to allow VFA binding to reach equilibrium. The final pH was 5.2 ± 0.1.

A 10-g sample was transferred to a 20-mL round-bottom flask with a universal inlet adapter. Each flask was immersed in a 50°C circulating water bath and purged for 15 min with nitrogen at 800 mL/min. Effluent analytes were trapped using a Carbotrap solvent desorption tube (20/40 mesh, ORBO 101, Supelco, Inc., Bellefonte, PA). A submersible stirrer with teflon starburst stirring head was used to provide sample agitation. Teflon tubing was used for all connections following the gas filters. A new trap was used for each analysis.

Following sample purge, traps were immediately desorbed by placing the trap adsorbent bed into a 2-mL vial and eluting with 150 μL HPLC grade CS<sub>2</sub> containing 40 μg/g valeric acid.

Gas chromatographic analysis was conducted on a Hewlett-Packard 6890 GC with a flame ionization detector. A relatively short fused silica capillary column (Rtx-Wax, 10m, 0.53 mm i.d., 1.0 μm film thickness, Restek Corporation, Bellefonte, PA) was employed to effect adequate resolution of the underivatized fatty acids in ~15 min. Chromatographic parameters were as follows: injector and detector temperatures, 220°C; oven temperatures: initial 100°C, hold 3 min; ramp 10°C/min; final 180°C; hold 2 min. Helium carrier gas was used with a column flow rate of 2.2 mL/min. A 2-μL sample was injected in splitless mode. Relative analyte quantities were determined using valeric acid as an external standard.

### Viscosity measurement

Viscosity was determined using a Brookfield Model RV rotary

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viscometer (Brookfield Engineering Laboratories, Inc. Stoughton, MA). Randomly selected samples were analyzed at 4°C. For statistical data evaluation, viscosity data were normalized using  $\log_{10}$  transformations.

### Experimental design and statistical analysis

Analysis of variance was used with a  $3^3$  factorial design to evaluate the influences of fatty acid chain length, gum type, and gum level on volatilities of the fatty acids (Minitab Vs. 8, Minitab, Inc., State College, PA). The complete data set was analyzed using the model:

$$\text{Peak Area}_{\text{standardized}} = T + L + F + TL + TF + LF + TLF$$

where T=gum type, L=gum level, F=fatty acid type, followed by interaction terms; each of the main effects was treated as a fixed term. Significance was defined at  $P \leq 0.05$ . Fisher mean comparisons were conducted where appropriate. Relationships between volatility and viscosity data were evaluated using regression techniques. Each experiment was completely replicated on three different days.

## RESULTS & DISCUSSION

### Fatty acid volatility

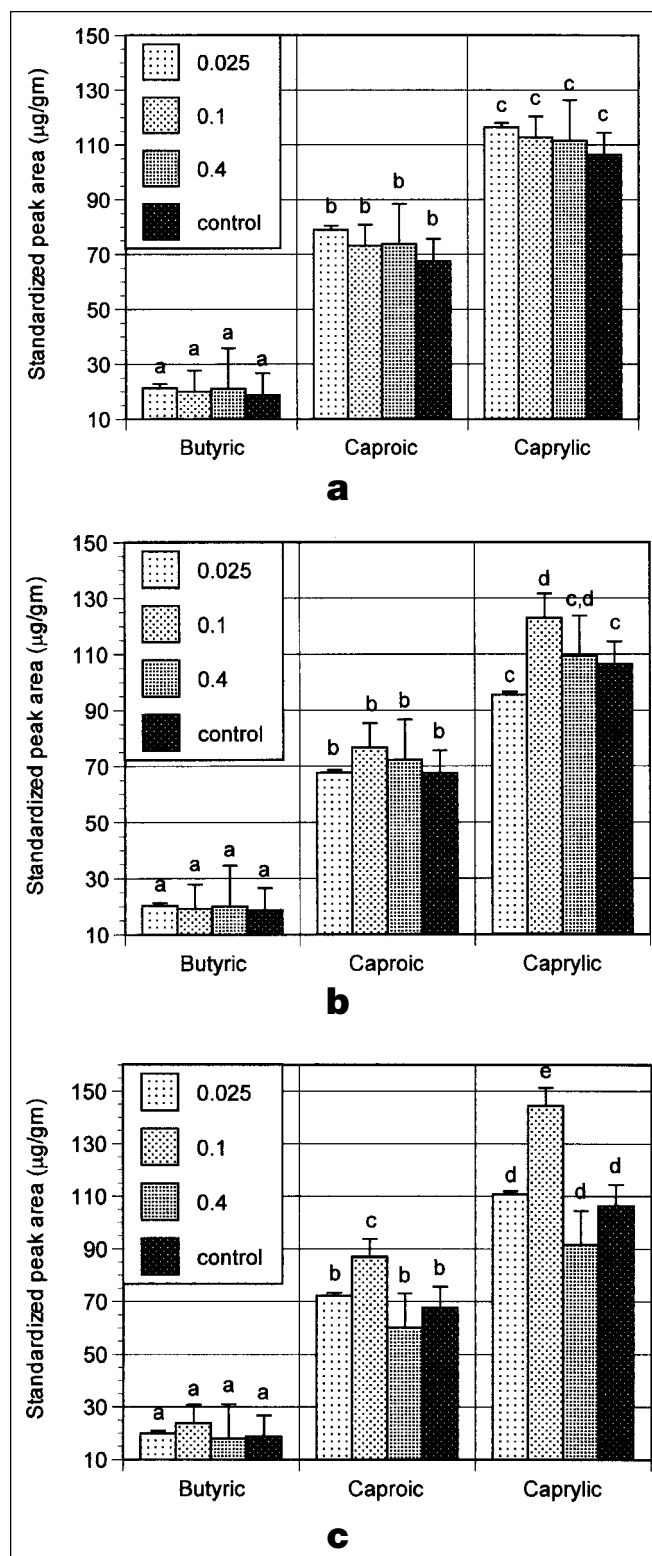
Fatty acid type affected VFA volatility ( $P \leq 0.05$ ). Overall recoveries were 1.86, 6.75, and 10.6% for butyric, caproic, and caprylic acid, respectively. Our results confirmed previous work that has shown an increase in volatility for higher molecular weight compounds of a homologous series in aqueous systems (Buttery et al., 1969).

Hydrocolloid type had no effect on VFA volatility. However, a gum type  $\times$  gum level interaction warranted further evaluation. An analysis of values from carrageenan gum-containing samples across gum level demonstrated that carrageenan had no effect on VFA volatility at any of the three levels within fatty acid type (Fig. 1a) relative to the control. This was contrary to much of research on polysaccharide/volatile compound interactions (Solms, 1986). However, our data demonstrated that an anionic hydrocolloid such as carrageenan and the resulting viscosities may have no effect on VFA volatility. The source of carrageenan we used contained lesser amounts of  $\lambda$ -carrageenan, but was predominantly comprised of  $\kappa$ -carrageenan. Carrageenan has been reported to exhibit strong reactivity with milk proteins (Schmidt and Smith, 1992). This suggests that much of the intermolecular binding potential of carrageenan is complexed with casein proteins, and thus becomes less available as a binding ligand for aroma compounds.

Evaluation of data from guar-containing samples demonstrated that the presence of 0.025 to 0.4% guar gum had no effect on volatilities of butyric or caproic acid. For caprylic acid, there was a slight increase in VFA volatility at the intermediate level of guar gum (Fig. 1b). This novel finding suggests that hydrocolloid gums have the potential to de-solubilize volatile compounds from aqueous media. The uncharged galactomannan structure of guar gum and tolerance of ionic species such as salts and acids (Whistler and Daniel, 1985), may relate to its ability to alter the volatility of VFA's.

Xanthan gum-containing samples demonstrated that at low and high levels no changes in volatilities were detected. Similar to the other gums, xanthan had no effect on volatility of butyric acid at any concentration. The intermediate level of xanthan gum increased the volatility of caproic and caprylic acids as compared to the control (Fig. 1c). The increase in volatility was least for caproic acid (+28%) and greatest for caprylic acid (+36%) suggesting that the increase in volatility was a function of hydrophobic interactions between the gum and the nonpolar regions of the fatty acid molecule.

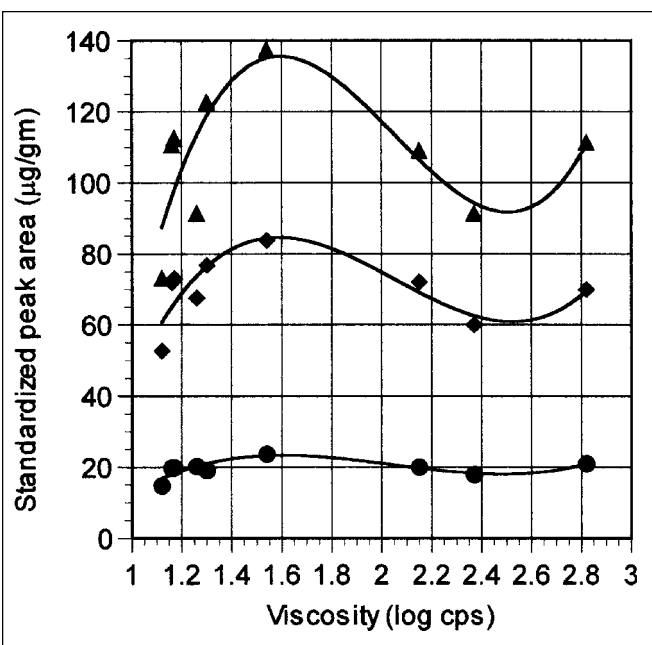
Viscosity data were sigmoidal (Fig. 2); best fits were obtained with the following polynomial models with corresponding correlation coefficients:



**Fig. 1—Effects of chain length and level of carrageenan (a), guar (b), and xanthan (c) gums. Error bars represent one standard mean error. Different letters within each gum represent significantly different means at the  $P \leq 0.05$  level.**

$$\begin{aligned} \text{butyric} \quad f(x) &= 15.4x^3 - 94.5x^2 - 184x - 92.5 & r^2 &= 0.68 \\ \text{caproic} \quad f(x) &= 58.2x^3 - 354x^2 + 700x - 354 & r^2 &= 0.67 \\ \text{caprylic} \quad f(x) &= 116x^3 - 713x^2 + 1390x - 738 & r^2 &= 0.60 \end{aligned}$$

From viscosities of 1.1 to 1.6 log cps, the volatilities increased, then decreased from 1.6 to 2.5 log cps, and then increased again.



**Fig. 2—Effects of viscosity on butyric (●), caproic (◆), and caprylic (▲) acid volatility as determined by dynamic headspace sampling of aqueous solution containing gum and nonfat dry milk powder.**

Several mechanisms of binding ligand-VFA interaction appeared to influence VFA volatility, including the reduction of mass transfer rates as a function of viscosity and potentially competing VFA des-

olubilizing effects. Additionally, the ability of this model matrix to both increase and decrease the volatility of VFA's was ( $P \leq 0.05$ ) potentiated with increasing chain length suggesting that viscosity-influenced volatilities were affected by hydrophobic interactions.

## CONCLUSION

GUM HYDROCOLLOIDS MAY HAVE NO EFFECT, INCREASE OR decrease the volatilities of VFA's. Formulations designed to replace lipid with fat mimetics should consider the effects that gums may have on the volatilities of aroma-active compounds. Further studies are needed to evaluate the effects of re-formulated food matrices on sensory properties of food systems.

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Ms received 9/3/98; revised 12/21/98; accepted 1/10/99.