

ABSTRACT

LEKSRISOMPONG, PATTARIN. The Roles of Fat and pH on the Detection Thresholds and Partition Coefficients of Three Compounds: Diacetyl, Delta-Decalactone, and Furaneol in Water, Oil, and Emulsions. (Under the direction of MaryAnne Drake.)

The effect of fat and pH on the best estimate threshold (BET) of 3 prominent dairy product flavor compounds with varying physicochemical properties: diacetyl, delta-decalactone, and furaneol, in water, oil and oil-in-water model emulsions (at 10 and 20 % fat at neutral and acidified pH 5.5) were investigated. The headspace-matrix partition coefficients ($K_{HS/matrix}$) of each compound in the different matrixes were established. The rheology and particle size of the emulsions used in this study were also investigated. The particle size and the viscosity of the emulsions did not affect the BET or the partition coefficients. Reducing fat from 20 to 0 % did not affect the BET value or partition coefficient of diacetyl ($P>0.05$). Increasing fat content increased the BET value and decreased the partition coefficient ($P<0.05$) of the most lipophilic compound in the study, delta-decalactone. Fat did not affect the BET of furaneol ($P>0.05$) but did have an effect on the partition coefficient ($P<0.05$). At pH 7, addition of fat decreased the partition coefficient of furaneol whereas at pH 5.5, addition of fat increased the partition coefficient of furaneol. Adjustment of pH from 7.0 to 5.5 did not impact the BET values of delta-decalactone, but did affect the partition coefficients of furaneol at all fat levels and impacted diacetyl at 0 % fat. The partition coefficient results generally agreed with the BET values on the effect of fat and pH, although, the partition coefficient test was more sensitive to the differences in the matrix composition than a threshold test.

The Roles of Fat and pH on the Detection Thresholds and Partition Coefficients of
Three Compounds: Diacetyl, Delta-Decalactone, and Furaneol, in Water,
Oil, and Emulsions

by
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BIOGRAPHY

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CHAPTER 1

LITERATURE REVIEW

CHAPTER 1. LITERATURE REVIEW

1.1 Roles and Perception of Fat in Foods

Fat plays important roles in human diets and sensory quality of food products (Sinclair and O’Dea, 1990; Richardson, 2000; Folkenberg and Martens, 2003a,b). In foods, fat usually is present in the form of an emulsion within an aqueous phase, either oil-in-water or water-in-oil (Bakker and Mela, 1996; de Roos, 2006). The physico-chemical parameters that control a compound’s release from products are partitioning of the flavor substance between the oil, water and gas phases (partition coefficient) and mass transfer (McGorin, 1996; de Roos, 2006). These parameters may provide information regarding the concentration of volatile compounds in the headspace as a function of concentration of the compounds in the food matrix. The rate of compound flavor release also influences the time required for the sensory threshold concentration to be perceived (Bakker and Mela, 1996).

Studies have demonstrated high hedonic ratings for foods containing fat (Richardson, 1999; Folkenberg and Marten, 2003b). Excessive fat consumption, however, is associated with high-cholesterol, coronary heart disease, stroke, hypertension, diabetes, obesity and cancer (Sinclair and O’Dea, 1990). Attempts to manufacture healthier foods with reduced fat content by using different processing techniques and fat substitutions is increasing in order to create palatable reduced fat foods to fulfill the consumer’s desire and trends for healthier foods. In order to do so, the psychophysics of food lipids including

perception of lipids and how they impact sensory properties of food must be thoroughly understood.

1.2 Effects of Fat on Food Flavor and Model Systems

The interactions between volatile aroma compounds and the food matrix components influence flavor perception in food (Stampanoni-Koeflerli et al., 1996; Wiet et al., 1993; Folkenberg and Martens, 2003b; Prindiville et al., 1999). The level of detection of a flavor component for olfaction is affected by its concentration in the nasopharynx and for gustation, its concentration in the saliva (Kinsella, 1989). Factors such as the rate of release of flavor compounds, the compound's disposition in the food (free, entrapped, adsorbed, complex), the components of the food, the particle size of food components, the extent of mastication, and temperature influence the concentration and partitioning of flavor molecules (Kinsella, 1989). The main reaction that affects olfactory sensory perception is selective binding of flavor compound molecules to specific receptor proteins in the olfactory epithelium (Kinsella, 1989). By changing the conformation of receptor proteins, compound binding activates enzymes such as sodium potassium ATPase and thus initiates a neural response (Schiffman, 1988). To obtain a response, a sufficient concentration of flavor compounds in the vapor phase or aqueous phase must be achieved. The rate of release of vapor compounds depends on the partition coefficient of the compounds, molecular interactions between flavor components, the ambient temperature, the composition and the viscosity of the food matrix, and the binding of flavor compounds to the matrix (Kinsella, 1989).

1.2a Effects of Partition Coefficient on Flavor Release

The partition coefficient, interfacial mass transport and diffusion are the physico-chemical parameters that are often used to indicate the intensity of flavor release from the food matrix to the headspace (air) as well as the predicted effect on sensory perception (Bakker and Mela, 1996; de Roos, 2006). Equilibrium is the point where an aroma compound is allowed to equilibrate between product and air (DeRoos, 2006). At that point, an aroma compound is distributed over the 2 phases according to the air-product partition coefficient K_{ap} . The air-product partition coefficient is defined as the concentration (g/cm^3) of a flavor compound in air over the concentration (g/cm^3) of a flavor compound in the product: $K_{ap}=C_a/C_p$. The air-product coefficient measures the volatility of a compound in the product and is highly dependent on the product composition. When the product consists of oil and water, P_{ow} is expressed for the oil-water partition coefficient. The affinity of aroma compounds to products is affected by the difference in the volatility of aroma compounds in different products (DeRoos, 2006). Therefore, P_{ow} can be used to measure the hydrophobicity of an aroma compound. P_{ow} is defined as the concentration (g/cm^3) of an aroma compound in the lipid phase over its concentration in the aqueous phase:

$$P_{ow}=C_o/C_w.$$

Usually, olive oil is used as reference oil as it is moderately unsaturated. Flavor compound hydrophobicity is usually expressed on a Log scale (Log P), with hydrophilic molecules having a negative Log P and a hydrophobic molecules having a positive Log P (Taylor, 2002). In most foods, oil and water are present in an emulsified form (Taylor,

2002). The partitioning properties of the flavor compounds may be different in emulsions as partitioning is affected by interfacial properties of emulsions. In emulsion systems, the volatility of compounds is calculated by the volume fractions of oil (f_o) and water (f_w) together with the partition coefficients $P_{o/a}$ and $P_{w/a}$:

$$P=C_a/C_p=C_a/(f_o C_o+f_w C_w) = 1/(f_o P_{oa} + f_w P_{wa})$$

Note that this equation only holds for the phases that are not soluble in each other. Also, a food system is dynamic whereas the partition coefficient is calculated at equilibrium, thus, these numbers may not accurately predict the partitioning of compounds in the headspace, but provide an estimate of a system at equilibrium.

Several studies were conducted on relating partition coefficients to flavor release and perceived odor intensity of different compounds at different fat levels (Frost et al., 2005; Guyot et al., 1996). Frost et al. (2005) studied the flavor intensity in ice cream with different fat levels and flavorings to evaluate the relationship between flavor intensity and the logarithm of the n-octanol/water partition coefficient (Log P) level of fat on melting rate, sensory flavor intensity duration and time to maximum sensory intensity (Tmax). Log P is the hydrophobicity index, the higher Log P, the more hydrophobic the flavor compound. The 4 compounds evaluated were beta-ionone (Log P = 3.04), d-nonalactone (Log P=2.67), isopentyl acetate (log P=1.81), and vanillin (Log P=1.09) at fat levels of 3.6 and 12 %. The fat content of the ice cream was the main factor influencing the melting rate. Increases in fat content slowed the perceived melting rate of ice cream. A significant difference in the Tmax was mainly caused by differences in fat level and flavor compounds. Ice creams with lower

fat had lower T_{max} but the effects were not the same for the 4 different compounds. The 3 % fat ice creams with isopentyl acetate and vanillin, the 2 compounds with lower Log P, had significantly lower T_{max} than beta-ionone suggesting that compounds with lower partition coefficients required significantly less time to reach maximum intensity compared to compounds with higher partition coefficients (more hydrophobic) due to greater binding to fat in the matrix. Fat increased the duration of perceived flavor and flavor compound interaction. D-nonolactone and beta-ionone had high hydrophobicity indexes (Log P) but had similar increase rates of flavor intensity to vanillin conveying that fat had no effect on the initial compound perception. However, compounds with high Log P were slower in the decrease rate of perceived intensity or the duration of perceived flavor intensity, indicating that hydrophobicity increased the duration of perceived flavor intensity.

Four different matrixes with different fat contents: water, whole milk (3.8 % fat), reduced fat milk (2.7 % fat), and skim milk (0.033 % fat) were studied using 5 different aroma compounds at 3 different concentrations (Roberts et al., 2003). The sensory nosespace release of volatile compounds from the foods was evaluated by proton transfer reaction mass spectrometry (PTR-MS). There were no significant differences in release of a compound with low lipophilicity, 2,3-butanedione (diacetyl) (Log P=-0.3) across different fat contents. Compounds with higher lipophilicity, benzaldehyde (Log P=1.02), significantly decreased in headspace release from whole milk compared to water. The sensory intensity ratings gave similar results, with low intensity scores for compounds in the highest fat products and high intensity scores for compounds perceived in water. As fat content increased, the release of

lipophilic compounds decreased. This can be explained in that lipid absorbs and solubilizes most volatile flavor compounds (Kinsella,1989). Thus, according to physical properties, lipophilic flavors partition and concentrate in the lipid phase. As a result, the sensory detection threshold of lipophilic compounds is increased in lipid compared to air or water due to less partitioning in the air or headspace (Kinsella, 1989).

Guyot et al. (1996) studied the odor intensity of 3 aroma compounds: d-decalactone, diacetyl and butyric acid, in model emulsions using liquid/liquid partition coefficients and vapor/liquid partition coefficients to evaluate how odor intensity was affected by the oil content of the emulsion in relation to partition coefficient. The liquid/liquid and vapor/liquid partition coefficients were measured by gas chromatography. For sensory evaluation, a 10-point category scale was used by trained panelists (n=20) to document perceived aroma intensity of the headspace of compounds in solution. Gas chromatography equipped with a 1:1 split into a FID and a sniffing port at the end of the capillary column was used for olfactive purity sniffing analysis of compounds. The 3 chosen aroma compounds had different physicochemical properties and behaved differently in different medium compositions. In general, the instrumental and sensory results agreed with each other. The vapor-liquid partition coefficients for each compound at different pH and fat concentration were calculated and measured. The vapor/liquid partition coefficient was higher at pH of 4.5 for butyric acid across all fat levels compared to pH of 5.2, due to higher percentages of the acid presented in protonated form at a lower pH. The partition coefficient of butyric acid at both pH 4.5 and pH 5.2 increased as oil content increased and the odor intensity decreased as

the oil content of the emulsion increased. The same pattern was seen for diacetyl which was also a hydrophilic compound. Hydrophobicity contributed greatly to these effects. The addition of oil in the emulsion increased the gaseous phase concentration as well as the perceived odor intensity. Diacetyl and butyric acid both had higher vapor/liquid partition coefficients and odor intensities when the medium was hydrophobic. On the other hand, d-decalactone, a hydrophobic compound, had lower vapor/liquid partition coefficient and odor intensity when the medium was hydrophobic. The vapor/liquid partition coefficient decreased as the oil content increased. This led to a decrease in odor intensity due to solubilization of the compound in the hydrophobic organic phase and a decrease of the compound in the gaseous phase (Guyot et al., 1996). The binding of flavor compounds to lipid foods is usually related to the relative amount of flavor partitioning in the lipid and water phases (McGorin, 1996). Most fats and oil in foods are in the form of di- and triglycerides which can bind to lipophilic and partly-lipophilic flavor substances. The amount of binding of flavor compounds to fat depends on the chain length and degree of unsaturation of triglycerides (Hyvonen et al., 2003).

1.2b Effects of Mass Transfer on Flavor Release

Under conditions of food being eaten, non-equilibrium and multiphase systems must be considered. This can be acquired approximately by calculating the rate of mass transfer of an aroma compound across different interfacial phases and into the air, often done using an interfacial mass transfer theory such as penetration theory (Harrison et al., 1997). The penetration theory assumes that the liquid phase was agitated and that the element of liquid

was exposed to the interfacial phase for a certain amount of time. During this time, the flavor molecules from the liquid diffused across the interface from the liquid into the gas phase. The rate of mass transfer is affected by the emulsion shear viscosity, oil fraction, size of oil droplets, and the type of emulsifying agents and their interfacial properties (Harrison et al., 1997; Moore et al., 1998). Bakker and Mela (1996) hypothesized that the difference in the viscosity of oil and water affected the rate of transfer from water to air which they found to be greater than that of oil to air. De Roos (2006) attributed a slower flavor release from lipid phase than aqueous phase to higher resistance to mass transfer in fat and oil than in water. The flavor compounds must be released from the lipid phase to an aqueous phase before going to the headspace. The emulsion shear viscosity is affected by the oil fraction such that as oil fraction in an emulsion increases, the emulsion viscosity increases (Harrison et al., 1997).

Bakker and Mela (1996) studied the flavor release of diacetyl in different types of emulsions: water in oil (W/O), oil in water (O/W) and single phases (water vs. oil) using instrumental and sensory perceptions. Sample headspace was analyzed using gas chromatography. Sensory analysis for intensity of specific taste qualities and oral viscosity were evaluated by 10 trained panelists who were instructed to take a full plastic spoon of sample, evaluate them in the mouth for 2-3 sec and expectorate. Instrumental flavor release evaluation found that, under static conditions, diacetyl was released from oil 5 times faster than from water. Time to reach equilibrium was 15 minutes from oil compared to 4 hours from water. They found that the release from both emulsions was faster than from single

phases. The rate of release from O/W was also faster than W/O. Mass transfer between phases of the 2 emulsions may be influenced by the structure of the interphase formed between droplets and the continuous phase (Bakker and Mela, 1996). Diacetyl, being a polar compound, was highly concentrated in the water phase of the emulsion and thus released slower in water and in the O/W emulsion. This finding coincided with the study of Mialon and Ebeler (1997) on polar and non-polar compounds in vegetable oil/water emulsions. Bakker and Mela (1996) found no significant effects on the types of emulsion on flavor intensity. This may be due to dilution with saliva that changed the characteristic of the sample (Bakker and Mela, 1996).

1.2c Effects of Fat on Flavor Release

The presence of fat in food products affects the release of flavor compounds (Hyvonen et al., 2003; Fabre et al., 2006; Mialon and Ebeler, 1997). Different types of fat affected the flavor release and melting of ice cream as they had different chain length and degree of saturation (Hyvonen et al., 2003). Hyvonen et al. (2003) conducted a study using dairy fat and vegetable oil with fat contents of 0-18 % in strawberry ice cream. Lower fat levels increased the rate of flavor release and had the highest flavor intensity while 18 % fat ice creams were the lowest in perceived flavor intensity. When fat was removed from ice cream, greater intensity and sharpness of aroma and flavor were perceived compared to fat-containing samples. The flavor intensities of fat-free ice cream and 18 % vegetable fat ice cream were significantly higher than 18 % milk fat ice cream. This may be explained by the physical properties of the fats. The concentration of flavor released from lipids was affected

by temperature, and the physical state of the lipid (Kinsella, 1989). The amount of solid fat and state of dispersion of fat were influenced by the melting rate and solid fat contents.

Thus, higher solid fat content and slow melting rates of saturated fat in dairy fat released less flavor than the less-saturated vegetable oil (Hyvonen et al., 2003; Fabre et al., 2006).

Fabre et al. (2006) studied the flavor release of aroma compounds from O/W emulsions containing either vegetable fat (a mixture of palm and palmist oils (extract of almond)) or animal fat. These fats differed in polarity, chemical composition and melting behavior. Vegetable fat contained less saturated triacylglycerols, was more polar than animal fat, and melted at a higher temperature than animal fat. Aroma compounds (diacetyl, hexanol and ethyl hexanoate), which were different in hydrophobicity, were used. Flavor release from emulsified samples was quantified by solid-phase microextraction (SPME) coupled with GC-MS analysis. The emulsions were made using 3 % whey protein, 9 % fat and 0.5 % emulsifier (a mixture of mono- and diacylglycerols, guar gum and carrageenan). The vapor-oil partition coefficients ($K_{a/o}$) were determined at 10, 20 and 40 C. SPME results showed that the hydrophobicity of the compounds influenced the rate and intensity of flavor released from emulsions. The nature of the fat affected the flavor release of the compounds, especially with diacetyl (Log P= -2.26) and ethyl hexanoate (Log P= 2.80). Ethyl hexanoate was the most hydrophobic compound out of the 3 compounds and was released most from emulsions with vegetable fat than emulsions with animal fat. With the hydrophilic compound diacetyl, the opposite was observed. The properties of the fat did not seem to influence hexanol (Log P=1.60), the compound with intermediate hydrophobicity (Fabre et

al., 2006). No sensory analysis on the release of aroma compounds from O/W emulsions in different fats was conducted.

Flavor release depends on the flavor compound retention capability and the mechanism of binding of flavor compounds to the food matrix (Plug and Haring, 1993). The rate of flavors dissolved, entrapped, encapsulated and diffusion may be limited by food components (Kinsella, 1989). This depends on the properties of the flavor chemical and the physico-chemical properties of the food components. In fat-containing food, lipophilic flavor compounds are bound to the fat globule by weak Van der Waals bond and hydrophobic interactions (Plug and Haring, 1993). The mechanism of the release is altered when the fat content in food is altered, affecting time-intensity and flavor-release behavior. Previous studies on the effects of fat content in foods and properties of flavor compounds determined that the physico-chemical interactions between the volatile and non-volatile food components can greatly affect the flavor release, flavor intensity and perception of the foods (Hyvonen et al., 2003; Mialon and Ebeler, 1997; Roberts et al., 2003).

Mialon and Ebeler (1997) studied the effects of flavor/matrix interactions using time intensity methods and model systems. Vegetable oil/water emulsions (0, 10 and 50 %) and 2 compounds, a polar aroma compound, vanillin, and a non-polar volatile compound, limonene, were used in the study. For the non-polar compound, limonene, when the fat in the emulsion was reduced, the maximum intensity and total duration of retronasal limonene aroma increased. This finding was concurrent with the findings of Buttery et al. (1973) who found that small amounts (1%) of fat present in a vegetable oil-water matrix caused decreases

in volatility of lipophilic compounds. The total duration of the retronasal aroma of the polar aroma compound studied (vanillin) was slightly higher with higher concentrations of oil although the maximum time and maximum intensity were not affected by the percentage of fat content (Mialon and Ebeler, 1997).

Jo and Ahn (1999) studied the release of volatile compounds in oil-in-water emulsion systems with fat levels of 0, 0.5, 1, 2, 4, or 8 % (w/w) using purge-and-trap dynamic headspace/gas chromatography. The volatile compounds used were hydrocarbons, aldehydes, ketones, and alcohols groups. Increases in oil content decreased headspace concentration of volatile compounds. This finding concurred with Buttery et al. (1973) who studied vegetable oil, water, and water-oil emulsions and found that the increase of oil reduced the rate of air to solution partition coefficient of volatile compounds. The results also showed that compounds containing less than 6 carbons such as propanol, 2 methyl propanol, and 4 methyl-2-pentanone had lower concentrations in the headspace. The author explained that this effect was because short chain length compounds may have been volatilized into the headspace during and after emulsion preparation resulting in lower concentration in the liquid phase of the mixture (Jo and Ahn, 1999). Higher concentrations of hydrocarbons were documented compared to aldehydes, alcohols and ketones. This might be because increased amounts of high molecular weight hydrocarbons (C7-C10) remained in the liquid phase mixture during analysis and were not lost in the preparation steps as with other volatile compounds (C4-C7) (Jo and Ahn, 1999).

Chung et al. (2003) studied the effect of fat on the flavor release behavior of selected

odor-active compounds while ice cream was consumed. Ice creams (12, 6, 3 and 0.7% milk fat) were spiked with 3 flavor compounds: vanillin (vanilla flavor), benzaldehyde (cherry flavor), and hexanal (stale flavor). Volatile profiles were determined by a modified purge and trap apparatus and sensory analysis by time-intensity. Volatiles collected were identified and quantified by GC-MS. As fat level increased, the area under the curve for both cherry and stale flavor decreased and area under the curve increased for vanilla flavor. This result implied that fat suppressed maximum intensity of those 2 lipophilic compounds benzaldehyde and hexanal, but enhanced the duration of the lipophobic compound vanillin. The increase of fat also extended the time to reach maximum intensity and duration of perceived vanilla flavor. The opposite prevailed with benzaldehyde and hexanal. When spiking ice cream with flavor compounds, the time intensity parameters of ice cream flavor were altered. High levels of benzaldehyde increased the duration of perceived flavor, time to reach maximum intensity, and maximum intensity. When spiking the ice cream with low concentrations of benzaldehyde but high concentrations of hexanal or vanillin, ice creams with hexanal were perceived to have stronger cherry flavor and persisted longer as compared to ice cream with high concentrations of vanillin. This result indicated that vanillin was better at suppressing cherry flavor than hexanal.

Instrumental analysis of compound release from ice creams revealed 6 primary compounds: 2-hexanone, hexanal, 2-heptanone, benzaldehyde, 2-nonanone, and nonanal as the main compounds presented. Benzaldehyde and hexanal were spiked compounds whereas methyl ketones and nonanal originated from the ice cream base mix. Increased fat levels

increased the release rate and the amount of methyl ketones in the headspace when measured using a modified purge and trap apparatus. There were no clear trends for aldehydes as the release rate and amount were different at different fat levels and stage. These results were expected for lipophilic compounds like methyl ketones and aldehydes.

1.2d Effects of Fat on Flavor of Dairy Products

The sensory attributes of dairy products correlated with fat included creamy flavor, mouthfeel, sweet taste, sweet odor, buttery, and diacetyl odor (Koeferli et al., 1995; Folkenberg and Martens, 2003a). Folkenberg and Martens (2003a) studied the sensory properties of low fat yogurts and found that increases in fat content enhanced creamy flavor, gel firmness, sweet taste and diacetyl odor. Fat content was inversely related to boiled milk flavor, bitterness, acetaldehyde odor, sour taste, astringent mouthfeel and powdery texture (Folkenberg and Martens, 2003a). This finding coincided with the findings of Stampanoni-Koeferli et al. (1995) on the influence of fat on selected taste, flavor, and texture of vanilla ice cream. They found that fat increased buttery and creamy notes while it decreased coldness and ice-crystal perception in ice cream.

Prindiville et al. (1999) studied the effect of milk fat on sensory properties of chocolate ice cream and found ice cream containing lower fat to have higher cocoa flavor and cocoa aftertaste than ones with higher fat (Prindiville et al., 1999). Similar to previous studies (Wiet et al., 1993), the lower fat ice cream was significantly sweeter than others with higher fat contents. In addition, this study showed that milk fat played a role in stability of texture and flavor of ice cream during storage and prevention of heat shock. After storage,

non-fat ice cream increased in cocoa flavor. After the ice cream was exposed to heat-shock, lower milk fat ice cream showed the greatest change in iciness, smoothness, and mouth coating compared to higher fat ice cream (Prindiville et al., 1999).

Fat affected basic tastes (i.e. sweet, sour and bitter) and flavors in food (Prindiville et al., 1999; Wiet et al., 1993; Elmore et al., 1999; Metcalf and Vickers, 2002). The perceived sweetness in 0, 3, 6, 9, and 18% fat unflavored lipid model systems with different types of sweeteners, aspartame and sucralose, and sucrose were examined (Wiet et al., 1993). They found that increases in fat concentration slightly reduced the perceived sweet taste intensity of both artificial sweeteners, aspartame and sucralose, and sucrose with the change in sweet taste intensity more noticeable at lower sweetener levels. This effect prevailed with aspartame, sucralose, and sucrose. They did not find an effect of fat on sweetness delay or sweet aftertaste. With an increase in fat level, the perceived bitterness was reduced which may be due to the tongue coating effect of the fat (Stampanoni-Koeflerli et al., 1995; Prindiville et al., 1999). Metcalf and Vickers (2002) also found that fat decreased the intensity of bitterness but increased the intensity of 4 basic tastes: sweet, salty, sour, and umami in their studies using O/W emulsions (Metcalf and Vickers, 2002). In contrast, Prindiville et al. (1999) studied the effect of milk fat on the sensory properties of chocolate ice cream and found that ice creams containing the lowest fat (0.5%) were significantly sweeter than the others (4, 6, and 9% fat). Regarding the ice cream studies and the two model studies by Wiet et al. (1993) and Metcalf and Vickers (2002), the results of model systems do not always correspond with real food products.

Carunchia Whetstine et al. (2006) demonstrated what happened to flavor compounds when fat was removed from aged full fat Cheddar cheese. Two full fat cheeses, aged 9 and 39 months, were evaluated. The fat was removed from the cheeses by a novel process which removed 50% of the fat content. Flavor volatiles and sensory properties of the full fat cheeses and the same cheeses with the fat removed were evaluated. Descriptive sensory evaluation and direct solvent extraction with solvent-assisted flavor evaporation (SAFE), and gas chromatography/olfactometry with aroma extract dilution analysis (GC/O) and (AEDA), and GC/mass spectrometry (GC/MS) were used as methods of analysis. The reduced fat cheeses were very similar in flavor profile to the full-fat reformed cheeses, with slightly lower milk fat/lactone and rosy/floral flavor. The extracted cheese fat had very little flavor exhibiting low intensities of fruity and catty flavors. The results suggested that compounds responsible for catty, fruity and sulfur flavors stayed or partitioned partially in the fat fraction and were detected in the removed cheese fats. The lack of other compounds such as milk fat/lactone and cooked/milky flavor and basic tastes in the removed fat as well as the similarity of the fat reduced cheeses to the full fat cheeses suggested that most flavor-contributing compounds were retained in the aqueous phase. Interestingly, milk fat flavor was not found in the removed cheese fat even though its chemical source, lactones, were associated with milk fat. The sensory threshold for gamma octalactone and d-decalactone was 10 times higher in oil than water which may explain why it was not detected by sensory analysis in the cheese fat. Based on previous studies, it is likely that the sensory thresholds for flavor compounds in the reduced-fat cheese were lower than in the cheese fat or full-fat

cheese since reduced fat cheese has a higher water to fat ratio as compared with full fat cheeses or cheese fat. Even if some volatile compounds were removed with the cheese fat, the decrease in sensory threshold of certain compounds might still result in a similar sensory flavor profile to full fat products. Most flavor compounds are hydrophobic, thus, higher water to fat ratio leads to lower thresholds. This experiment showed that as the fat phase was removed from aged cheese, the aqueous phase retained the majority of non-polar flavor compounds. The cheese fat itself had very little flavor and few flavor compounds.

Aroma extract dilution analysis (AEDA) was used to characterize aroma-active compounds in cheeses and cheese fat. AEDA is a semi-quantitative technique used with gas chromatography olfactometry (GC-O) and does not necessarily suggest the concentration of the compounds. Thirty three aroma-active compounds with \log_3 flavor dilution (FD) factors >1 were identified. Compounds with \log_3 FDs differing by more than or equal to 2 suggested possible concentration differences. The Log_3 FD in full and reduced fat cheeses was similar for most compounds. There were differences in esters (fruity flavor) between 39 month cheeses and cheese fat, with higher concentrations of ethyl butanoate and ethyl hexanoate in cheese fats. This indicated that esters were removed with the fat and were not retained in the liquid-aqueous phase which corresponded with sensory results. The Log_3 FD value for phenylacetaldehyde was highest in the 9 month full-fat cheese but was lower in the reduced-fat cheese and cheese fat. This indicated that the partitioning of phenylacetaldehyde in reduced-fat cheese and cheese fat was similar. Acetic acid had low Log_3 FD value in cheese fats compared to full and reduced fat cheeses whereas the results for butanoic and hexanoic

acid displayed no clear trends. As a more polar compound, acetic acid was more likely to partition in the aqueous phase and be present at higher concentrations in cheeses than cheese fat (Carunchia Whetstine et al., 2006).

Francis et al. (2005) studied the effects of fat on milk flavor, aftertaste, and volatile compounds as a function of serving temperature. A randomized complete block design was used with 2 fat contents, 2 temperatures (4C, 15C) and 3 replications. Descriptive analysis was conducted using 5 trained panelists, volatile compounds were analyzed using solid-phase microextraction-gas chromatography (SPME-GC) on the same day as sensory evaluation and volatile compounds extracted were analyzed by gas chromatography-mass spectrometry (GC-MS). Using descriptive analysis, serving temperature did not seem to have a significant effect on sensory flavor and texture attributes of milk. Milk fat levels, however, contributed to some texture, flavor, and attribute differences. Nonfat milk was described as more chalky, less viscous, and less fat feel than whole milk texture. The flavor of non fat milk was also described to have less sweet and sweet aromatic, flat, and sour aromatic than whole milk samples. This coincided with Metcalf and Vickers (2002) who reported that fat enhanced sweet taste. The absence of milk fat affected the maximum aftertaste intensities in dairy milk. The cooked aftertaste attribute for the nonfat milk remained at high intensity at 15 s and 90 s as compared to cooked aftertaste of whole milk. This study found that fat content affected 8 sensory descriptors: fat feel, fat aftertaste at 15 s, fat aftertaste at 90 s, fatty mouthfilm at 15 s, fatty mouthfilm at 90 s, sweet aromatics, overall sweet aftertaste at 15 s, and overall sweet aftertaste at 90 s. Pearson correlation coefficients

between headspace compounds and 8 sensory descriptors were positively correlated. This study demonstrated that fat content significantly influenced the sensory aftertaste attributes of milk.

1.2e Volatile Compounds in Dairy Fat

In a study by Francis et al. (2005), the volatile compound concentrations of nonfat and whole milk were analyzed using SPME gas chromatography-mass spectrometry (GC-MS). They identified and quantified 7 compounds that are important to milk out of more than 100 peaks. The compounds associated with fresh flavor in nonfat and whole milks included benzaldehyde, ethyl caproate, heptanal, 2-heptanone, hexanal, nonanal, and pentanal. All the compounds except for hexanal were found at greater concentrations in whole milk as compared to nonfat milk which suggested that fat levels affected the flavors and odors contributed from these compounds to dairy products.

Chin and Rosenberg (1997) identified different volatile compounds formed in reduced fat and full fat cheeses at different ripening conditions. SPME-GC was used for headspace volatile analysis. Fat affected the formation of volatile free fatty acids and other volatile compounds in Cheddar cheese. At 10 days, full fat cheeses were significantly higher in acetoin, butanoic acid, octanoic acid, d-decanolactone and d-dodecanolactone compared to reduced fat cheeses whereas reduced fat cheeses were higher in ethanol and acetic acid. Thus, removing fat removed compounds that contributed to flavor of Cheddar cheese. Principle component analysis (PCA) of volatile compounds showed clear separation between full-fat and reduced fat cheeses after 6 months. Results suggested that full fat samples were

characterized by butanoic and hexanoic acids, acetoin and d-decanolactone. Acetoin is related to diacetyl which contributes to buttery notes in Cheddar cheeses. Reduced fat samples were characterized by high levels of decanoic and dodecanoic acids, and ethanol. The results suggested that fat levels influenced volatile compound formation leading to distinction in flavor of Cheddar cheese.

Carunchia Whetstone et al. (2006) identified various potent odorant compounds in aged cheeses using AEDA. In 39 month old full fat and reduced fat cheeses, the potent compounds which had either low thresholds or appeared in high concentration were identified. Compounds that appeared to be in higher concentration or had low threshold (higher Log_3 FD factors) in 39 month full-fat cheeses compared to reduced fat cheeses were butanoic acid (rancid cheese odor), 2-methyl-3-furanthiol (cooked/brothy), dimethyl trisulfide (cabbage/sulfur), 1-octen-3-one (metallic/mushroom), ethyl hexanoate (fruity), octanal (citrus), (E,E)-2,4-octadienal (fatty/green), furaneol, (E)-2-nonenal (fatty/old books), and 4-methyl octanoic acid (waxy/animal). Compounds that appeared to be in higher concentration or have low thresholds (higher Log_3 FD factors) in 39 month reduced fat cheeses compared to full-fat cheeses were acetic acid (vinegar), diacetyl (buttery), 2-methyl thiophene (plastic bottle), propyl butyrate (fruity), 2-phenethanol (sweet/floral) and gamma-octalactone (coconut). In removed fat, compounds that had high Log_3 FD factors compared to the full- and reduced-fat cheese were 2-acetylthiazole (nutty) and hexanoic acid (sweaty). This study suggested that removing fat altered the flavor compounds in the end products leading to different flavors perceived in the end products.

1.3 Effects of Fat on Food Texture and Model Systems

1.3a Effects of Fat on Texture of Dairy Products

Two early experiments conducted by Mela (1988) investigated the factors that caused the perception of fat in fluid dairy products. Untrained panelists were asked to rate fat content, creaminess, pleasantness of milk, whole milk, half-and-half, a mixture of half-and-half and heavy cream, and heavy cream. Three conditions: normal, reduced visual stimulation, and reduced visual and olfactory stimulation were carried out. Visual and olfactory cues were eliminated by masking samples with a black background, testing under low intensity red light, and using nose clips. Creaminess and fat content were positively correlated ($p < 0.01$) under all conditions conveying that perception of fat content and creaminess was derived from similar cues. The results of the first experiment suggested that creamy texture influenced the perception of 'fat content' (Mela, 1988).

Similar results on creaminess in association with fat content were found by deWijk et al. (2004). They studied texture of vanilla custard desserts using sensory and instrumental measurements and found 2 sensory dimensions, one contained 'melting' to 'thick' and another contains 'rough' to 'creamy-soft' attributes for commercial vanilla custard desserts. In a subsequent experiment, they used models of vanilla custard desserts with altered thickness and fat contents (0%-4.5%). Most custards with different fat contents was located along the rough-creamy/soft dimension suggesting that fat impacted these attributes. Samples with high fat were located at the creamy/ soft and fatty (coating) sensation side and samples with low-fat and soy based custard were located at the rough side (dry and afterfeel

sensations). The presence of fat influenced mouth-coating and afterfeel attributes such as creaminess, fattiness and roughness. Fat also increased most flavor attributes but decreased odor, chemical and sickly flavor and sour taste. Fat was not shown to affect thickness and melting mouthfeel attributes. The mechanism of fat may be due to the degree of physical friction between oral tissues and the food (deWijk et al., 2004) and astringency (Janhoj et al., 2006).

The findings that fat contributed to creaminess lead to further investigation on food texture. Mela (1988) conducted a second experiment to examine the impact of viscosity on the texture of fluid dairy products. The viscosity of distinct fat content dairy products was adjusted by the addition of a pregelatinized cold water swelling modified cornstarch to be approximately equal to the level of heavy cream. By enhancing viscosity of lower fat dairy products, the perceived fat content was increased (Mela, 1988). This study suggested that viscosity was one of the physical properties affecting the texture and perceived fat content of food. Prindiville et al. (1999) found a corresponding result on the effect of fat on food texture. They studied the effect of milk fat on the sensory properties of chocolate ice cream by altering fat levels in ice cream. Lower fat ice cream was less viscous and appeared to be more foamy and more separated compared to higher fat ice cream. Lower fat ice creams were perceived as less creamy, less smooth, faster melting, more chalky and less firm in mouthfeel.

To better understand the role of fat on texture of the food, perception of texture attributes and microstructure of dairy products were investigated (Bryant et al., 1995; Drake

et al. 1996, 1998; Irigoyen et al., 2002; Marshall et al., 1990; Prindiville et al., 2000).

Prindiville et al. (2000) studied the effect of fat replacers on sensory properties in non-fat and 2.5% milk fat chocolate ice cream. They found that a low milk fat score of assigned by trained panelists influenced the intensity of cocoa and mouthfeel. Ice cream containing milk fat melted the slowest which suggested that milk fat stabilized the emulsion through clumping of the fat globule at air cell walls. Milk fat provided high mouth-coating characteristics and a creamy texture compared to non-fat ice cream with fat replacers (Prindiville et al., 2000).

Drake et al. (1996, 1998) observed the microstructure of Cheddar cheese to compare the effects of different fat mimetics on fat reduction. Full fat cheese contained 36.6% moisture where as reduced fat (13.5%) cheese contained a significantly higher percentage of moisture (45.7%) (Drake et al., 1996). Firmness of cheeses was compared using a cone penetrometer on a TA.XT2 Texture analyzer and they found that the low-fat cheese was significantly firmer than the full-fat cheese. This result was similar to sensory scores. The sensory scores from trained judges (n=12) indicated that full fat cheeses had less firmness, crumbliness and rubberiness compared to low fat cheeses. The scanning electron micrographs of full-fat and low-fat cheeses were different. The full-fat cheeses contained more fat globules (observed as smooth black holes) and a smooth and homogeneous protein matrix as compared to low-fat cheese (Drake et al., 1996, 1998).

Fat reduction increased hardness and springiness while decreasing the adhesiveness and cohesiveness of Cheddar cheese (Bryant et al., 1995; Mistry and Anderson, 1993;

Irigoyet et al., 2002; Gwartney et al., 2002). Bryant et al. (1995) studied the microstructure of Cheddar cheese to understand the physico-chemistry behind fat reduction. The microstructure of cheese containing lower fat (27 %) had a more compact protein matrix and less milk fat globules dispersed within the network compared to the higher fat cheeses (32 and 32%) which they hypothesized contributed to the altered texture perception of the lower fat cheeses. Mistry and Anderson (1993) also found similar results when they evaluated different variety cheeses (Cheddar, Mozzarella, processed, and Swiss cheeses) with different fat levels. For all cheeses, milk fat reduction was compensated by increased moisture, total protein, and ash. In full-fat Cheddar cheese, the microstructure showed a smooth-surfaced protein matrix with large number of fat globules evenly distributed within the matrix. In reduced-fat Cheddar cheese, there were fewer fat globules dispersed within the protein matrix. The fat globule sizes were more uniform compared to full-fat Cheddar cheeses and appeared to be flat (not layered). The protein matrix became more dense and not as open, thus, the cheese became more firm and rubbery. The protein matrix of Mozzarella cheese was similar to 33% low fat Cheddar cheese. The size of fat globules was more uniform and smaller than those in Cheddar cheese. In full-fat processed cheese, the fat globules were uniform in size and evenly distributed which was characteristic of emulsified cheeses. They also consisted of a flat protein matrix, unlike 33% low fat processed cheese where the protein matrix appeared rough (Mistry and Anderson, 1993).

The effect of fat reduction on cheese texture and physical properties was studied using modified descriptive analysis and torsional fracture methods (Gwartney et al., 2002).

Sensory texture profiling by trained panelists found reduced-fat cheeses (14-18 %) to have higher springiness, hardness, fracturability, waxiness, chewiness and fracture stress scores than full fat cheeses. The normal fat cheeses (28-32 %) were higher in cohesiveness, stickiness, smoothness, and meltability. PCA results showed a clear distinction between full- and reduced- fat samples. Reduced-fat cheeses loaded on the negative component of PC1 which included waxiness, fraturability, chewiness, hardness and springiness. Full-fat cheeses loaded on the positive component which was described by stickiness, cohesiveness, meltability and smoothness similar to what was found by Bryant et al. (1995). Yates and Drake (2007) studied texture properties of Gouda cheese using descriptive analysis of texture and confirmed that these specific attributes were also impacted by fat reduction. Reduced fat content Gouda cheeses displayed a springier texture with higher recovery rates, decreased adhesiveness, cohesiveness and degree of breakdown (Yates and Drake, 2007).

Cheese is a visco-elastic material that has a mixture of fluid- and solid-like properties and can be characterized by applying stress and strains to the cheese (Foegeding and Drake, 2007). Gwartney et al. (2002) used torsional fracture analysis to determine the fracture properties of cheeses. Torsion fracture measures the point from the initial formation up to the point of fracture which is best related to sensory first-bite attributes. Fracture stress determines the cheese hardness which could describe sensory chewiness, smoothness, and meltability (Foegeding and Drake, 2007; Gwartney et al., 2002). Fracture stress values were lower for the full-fat samples than reduced fat products. Correlating the sensory and torsion measurement, fracture stress results were positively correlated with attributes springiness,

hardness, and cheesiness and negatively correlated with smoothness and meltability.

Fracture strain was positively correlated with springiness and negatively correlated with stickiness and oily mouthfeel (Gwartney et al., 2002).

Rheology and sensory analysis were used to investigate the properties that relate to the perceived cheese texture in young cheeses namely Mozzarella and Monterey Jacks (Brown et al., 2003). Rheological analysis, small strain oscillatory, creep and large strain analysis, were used to measure texture properties instrumentally. The human textural perception measurement was measured by descriptive sensory analysis. There were correlations observed between instrumental and sensory data. Perceived firmness was positively correlated to fracture modulus, complex modulus, and storage modulus which measured the ratios of force to deformation increase with firmness. Hand firmness and first-bite firmness were positively correlated to complex modulus and storage modulus. Fracture modulus was positively correlated to hand firmness, degree of breakdown, and chewdown adhesiveness but negatively correlated with hand springiness and hand rate of recovery (Brown et al., 2003).

Kucukoner and Haque (2006) studied the impact of fat on the physicochemical properties of Cheddar cheese using trichloroacetic acid precipitation (TCA 12%) for protein concentration and an Instron testing machine for textural analysis. Their results on texture analysis with stress and strain values in full-fat (32%) and reduced-fat (6%) cheeses agreed with the findings of Gwartney et al. (2002). This could be partly due to the soluble protein content which was found to impact the physical properties of Cheddar cheese at different fat

levels and ripening stages. The results suggested that fat influenced the protein and moisture content in Cheddar cheese. Low-fat cheeses contained higher moisture. Concurrent with Bryant et al. (1995) and Mistry and Anderson (1993), they found low-fat cheeses to contain higher protein. The total soluble nitrogen, which reflected the content of total soluble proteins, peptides and amino acids, was higher in low-fat cheeses throughout the ripening stages from 0 to 6 months. Higher peptide concentrations can also contribute to bitterness in low-fat cheeses (Kucokoner and Haque, 2006).

1.3b Roles of Fat on Creaminess

Creaminess is the term often related by consumers to describe the flavor and/or texture properties of full fat foods. To investigate the fundamental properties of creaminess, Elmore et al. (1999) used preference mapping techniques to define this terminology. Trained panel consistency terms such as thickness, slow melt rate, denseness, mouth coating, visual airiness and smoothness were related to the consumer term creaminess (Elmore et al., 1999). They also found that puddings with higher fat content were perceived to be related to dairy flavor. Samples with high fat were also perceived as opaque and yellow. The mean hedonic scores for creamy texture indicated that samples containing more fat were preferred. This finding coincided with findings from cheese studies (Pagliarini, 1997; Hamilton et al., 2000; Jack et al., 1992) and a yogurt study by Richardson et al. (2000). The characteristics of yogurt preferred by consumers included creaminess, firm mouthfeel, acetaldehyde, and smoothness. Consumers disliked powdery, milk flavor, and bitter yogurts. Richardson et al. (2000) noted that consumers perceived products to be creamiest when they were higher in

fat, exhibited dairy flavors and textures of characteristic of creaminess. There was also a positive correlation between the creaminess perceived by consumers and product liking by consumers (Richardson et al., 2000).

Janhoj et al. (2006) studied low-fat yogurt creaminess and found many underlying descriptors that were associated with creaminess. Sensory evaluation was measured with descriptive analysis using 12 trained panelists. Products with 3.5% fat had high smoothness. A second sensory dimension was related to creaminess. High creaminess was correlated with butter flavor, cream flavor, sweet taste, and fatty after mouthfeel. Products that had high creaminess were the ones with skim milk powder and had 4.8 or 5.4 % w/w microparticulated milk protein. High creaminess was associated with percentage of fat in milk solids non fat (MSNF). High intensity of dry after mouthfeel, astringency and sour taste were negatively correlated to creaminess. Products that fell under those categories were mainly the lowest fat level (0.3 and 0%) products. Texture related descriptors and some others such as creamy smell, butter flavor, cream flavor and fatty after mouthfeel were also important when describing creaminess (Janhoj et al., 2006).

More recent studies relating fat level to rheological properties were conducted (Janhoj et al., 2007; Jellema et al., 2005; and Ma et al., 1996). Janhoj et al. (2007) studied the sensory and rheological characterization of acidified milk drinks using 17 acidified milk drinks with varying formulation and fat contents (3-8 % milk solid non fat). Sensory evaluation was conducted by descriptive analysis and the rheological properties were measured using a Bohlin-CVOR controlled stress rheometer. The flow curves from obtained

results were fitted using non-linear regression to the Power law model. They found that the term creaminess in these beverages was comprised of several descriptors. Descriptors related to texture such as viscosity (visual and oral) and resistance were related to creaminess. Flavor descriptors such as cream flavor, buttermilk aroma and buttermilk flavor were also related. Transparency, sweet, boiled milk aroma and boiled milk flavor were negatively correlated to creaminess. The relationship of smoothness and creaminess were also identified (Janhoj et al., 2007). Creaminess was rated higher in all high fat samples which may be due to increased dairy flavors such as buttermilk and cream flavor intensities and decreases in flavors negatively correlated to high fat i.e. boiled milk flavor. Also, the characteristic of creaminess may exceed smoothness at higher levels of viscosity so that samples with low smoothness can still be perceived as creamy (Janhoj et al., 2007). Rheological results suggested that samples with the same formulation but varying in fat contents (2 and 8.5 %) had different viscosity and flow.

In relating creamy mouthfeel in custards to rheological properties, 3 different groups of rheological properties were identified (Jellema et al., 2005). Jellema et al. (2005) used 4 series of vanilla custard samples prepared using different types of modified starch or carboxy methylcellulose (CMC) (Jellema et al., 2005). All custards contained 6 % (w/w) sugar and 0.1 % (w/w) vanilla flavoring. Milk fat contents ranged between 0.5-5 %. Sensory properties were investigated using quantitative descriptive sensory analysis (QDA). Characteristics such as mouthfeel and afterfeel attributes were emphasized. Odor attributes, flavor/taste attributes, mouthfeel attributes and aftertaste/feel attributes were rated. As for

rheological measurements, 2 types of small-deformation measurements (dynamic stress and frequency sweep) and 2 types of large-deformation measurement (a flow curve and steady shear rate measurement) were performed. A PLS regression model was built and a model of the attribute creamy mouthfeel was plotted. Similar to Janhoj et al. (2007), they found that the studied parameter, creamy mouthfeel, had multidimensional attributes. Thick, airy, sticky, and fatty had positive contributions to the model whereas rough mouthfeel, heterogeneous and melting had negative contributions, similar to what was demonstrated by deWijk et al. (2003).

Three groups of products that represented different rheological properties were identified (Jellema et al., 2005). Group 1 described properties at rest where there was small deformation without break-up of the structure of the product when force was applied. Group 2 described structure break-up, start of flow when stress or shear rate was applied. Group 3 described the flow of the products after mechanical breakdown of the structure. Viscoelastic materials incorporated aspects of fluid-(viscous) and solid-(elastic) like behaviors (Foegeding et al., 2003; Foegeding and Drake, 2007). From rheological data, group 1 possessed linear viscoelastic range where as there was little effect on the custards when stress and strains were applied. Group 2 indicated where the viscoelastic behavior of the custard changed from elastic to predominantly viscous. The rheological parameters that described the flow of group 2 just after structure breakdown were measured at 33 and 100 Pa. Half of the samples fell between group 2 and 3. Custards with higher creaminess rating were related to high initial stiffness, low stress or strain, structure break down, and relatively easy flow. Creamy,

airy and fatty mouthfeel attributes were negatively related to group 2 (structure break-up) and described using rheological variables. Thick and sticky mouthfeel were related to group 3, the flow behavior. Thus, in order to assess thickness and stickiness, the product needed to be deformed in the mouth. Melting was negatively related to thickness as well as the creamy, airy and fatty mouthfeel. Creamy mouthfeel was positively related to thick, airy, sticky and fatty and was negatively related to rough mouthfeel, heterogeneous and melting. Rough mouthfeel was hard to relate to any rheological variables (Jellema et al., 2005).

Friction was another factor that influenced creaminess (deWijk et al., 2005). The work of deWijk et al. (2005) on creaminess found that texture attributes were summarized by 2 sensory dimensions: 'rough-creamy' and 'melting-thick'. They found that the rough-creamy dimension was correlated to fat content and coefficient of friction. Custards with higher fat content had lower friction and were perceived as creamier and less rough. As fat content increased from 5-20 %, the friction of starch-based and starch/CMC custard products decreased. The same trend prevailed in different fat levels of vanilla custard dessert with canola oil with no CMC added. The inverse correlation of fat and friction was further investigated in these desserts. Fat and frictions were found to be affected by the number of fat droplets and size of the droplets. Smaller droplets reduced friction. This may be due to the droplets being less deformable than the larger ones resulting in smaller surface area, hence, a reduction in friction. Friction increased with fat droplet size and particle size in mayonnaise (above 4 μm , $r=0.96$). Increases in friction were positively correlated to roughness, prickling, dry-mealiness, and heterogeneity and inversely related to creaminess,

fattiness, stickiness, slipperiness and smoothness sensation. They concluded that fat lowered friction especially at low levels in foods (between 0-5 %) to 10 %. For foods with very high fat content such as mayonnaises and dressings, friction became less related to perceived roughness but was still related to some fat-based attributes i.e. fatty after-feel (deWijk et al., 2004).

Creaminess is likely different things in different foods. Kilcast and Clegg (2002) studied creaminess and its relationship with food particle size and shape. Creamy texture and oil droplet size depended on fat content. Creamy texture was defined by ‘overall creamy texture perceived in the cream range’ with increases with increasing drop size for each fat content with higher creamy texture scores. In one of the experiments, model particle systems with 5 different types of solid particles were assessed including 3 calcium carbonate types of particles and 2 white fused aluminum types of particles with different size distributions. The particle sizes were dispersed into a 0.3 % solution of xanthan gum. Particle concentration was a major influence on perceived textural characteristics . Samples with smaller particles were more creamy than the control and vice-versa. Creaminess decreased with an increase in particle concentration. Creaminess also decreased with increasing viscosity. In their second experiment, 9 samples were produced with 3 formulations with different fat contents (10 or 20 %), and either presence or absence of particle sizes. Samples with the highest fat content of 20 % and homogenized to the least extent had the highest overall creaminess as perceived by 14 trained panelists. Overall creaminess was defined by ‘a personal assessment of the overall creaminess (flavor and

texture) of the sample'. With the same extent of homogenization, samples with 20 % fat scored higher than those with 10 % fat. Added flavor was shown to have little or no influence on overall creaminess.

1.4 Fat Substitutes

The removal of fat affects the texture and flavor release from foods, therefore, industries seek fat substitutes in order to imitate full-fat products. When replacing fat, either carbohydrate or protein or both may be used as the substitute (Schmidt et al., 1993; Plug and Haring, 1993). Carbohydrate based fat replacers consist of different types of polysaccharides and sugar polyalcohols (Plug and Haring, 1993). Most carbohydrate fat replacers are highly polar and consist of dipole-dipole interactions and hydrogen bonds. In some starches, the hydroxyl group is present outside the coil of the helical starch structure forming lipophilic regions where lipophilic flavors are bound. For example, cyclodextrin addition to nonfat milks and yogurts resulted in similar flavor-release profiles to products containing higher fat content (Reineccius et al., 2004). This shows that carbohydrates have the potential to bind flavor and may be utilized in reduced-fat products.

Protein- based fat replacers were shown to have better interaction with flavor and to provide more similar characteristics to fat containing products compared to carbohydrate-based fat replacers (Schmidt et al., 1993; Schirle-Keller et al., 1994; Liou and Gruen, 2007). The amino acid sequence of a protein determines its chemical and physical characteristics. Disulfide bridges and hydrogen bonds affect binding properties and solubility of proteins (Plug and Haring, 1993). Liou and Gruen (2007) studied the effect of fat level on 5 sensory

properties of strawberry flavor compounds with or without fat replacers: Simplese (protein-based), Litesse (carbohydrate-based) and Litesse/Simplese mixes using descriptive analysis in 5 ice-cream bases containing 10 or 4 % fat. They found both fat replacers to successfully imitate the texture characteristics of a 10 % control ice cream. Texture attributes including smoothness, creaminess and mouth coating were highest in the control samples, but there were no significant differences in those texture attributes in 4% milk fat ice-cream with fat replacers from the 10 % milkfat ice cream control. In coldness intensity, Litesse was not different from the 10 % milkfat ice cream control which could partly be due to having a high water-holding capacity. Litesse was also better for inhibiting icy crystal growth compared to Simplese. Sensory results showed that all fat replacers slightly increased the intensity of sweet aftertaste in 4 % milkfat ice cream. However, the milky flavor in 10 % milkfat ice cream remained the highest.

In a study of the effect of fat level on sensory perception using a descriptive test of five flavor chemicals in ice cream, Liao and Gruen (2007) evaluated 5 flavor compounds with different functional groups: *cis*-3-hexen-1-ol (grassy flavor), ethyl-3-methyl-3-phenylglycidate (candy flavor), α -ionone (violet flavor), γ -undecalactone (peach flavor) and Furaneol (cooked sugar flavor) (Liao and Gruen, 2007). The compounds interacted with fat replacers depending on their polarity and thus influenced the intensity of flavor perceived. *cis*-3-hexen-1-ol and Furaneol compounds were hydrophilic whereas ethyl-3-methyl-3-phenylglycidate, α -ionone, and γ -undecalactone compounds were hydrophobic. Lipophilic compounds were less noticeable at higher fat contents due to the binding of lipophilic flavor

compounds to fat suppressing flavor release (Liuo and Grunn, 2007). Fat replacers impacted furaneol, α -ionone and γ -undecalactone flavors. Ice cream containing Litesse had higher intensities of violet and peach flavors than ice cream containing Simplese, while ice cream with Simplese had higher intensities of Furaneol. Being a protein-based fat replacer, Simplese interacted more with lipophilic flavor compounds (ketones, aldehydes) due to its possession of hydrophobic binding sites. Thus, perceived sensory intensities were decreased with increased binding of the volatile compound (Liou and Grun, 2007; Schirle-Keller et al., 1994). No instrumental analysis to evaluate released headspace concentration was used. Overall, the sensory results showed that the flavor profile of the ice cream with protein-based Simplese was more similar to the 10% full fat ice-cream than the ice cream with carbohydrate-based Litesse.

Schirle-Keller et al. (1994) studied the influence of fat content and fat replacers (protein-based and carbohydrate-based) in a model system on vapor pressure of different functional group flavor compounds using gas chromatography. As fat-solubility increased, the relative vapor pressure of all flavor compounds decreased in oil-containing systems (Schirle-Keller et al., 1994). Fat dissolves many flavor components and results in a reduction of vapor pressure (Buttery et al., 1971;1973). Protein-based fat replacers (Simplese-100 and 300) were found to exhibit similar properties to oil-containing systems showing a decrease in relative flavor pressure when aldehydes were added. However, the carbohydrate-based fat replacer (Splendid and Stellar) had no effect. These results suggested that protein-based fat replacers were better at mimicking oil-containing systems compared to

carbohydrate-based replacers regarding their affect on volatilities of compounds. In this study, reactions with ketones were not significant (Schirle-Keller et al., 1994).

Concurrent to the 2 previous studies using protein (Simplese) and carbohydrate-based (Maltodextrin) fat replacers, Schmidt et al. (1993) also found ice cream with Simplese to be more similar to the full-fat control in terms of rheological properties (Schmidt et al., 1993). They compared the 2 fat replacers in batches of ice milks with 2-5 % milk fat to 4.8 % conventional ice milk (control) on the rheological, freezing and melting characteristics. They found that the Simplese had the same viscosity and consistency index to the control. During whipping, Maltodextrin did not incorporate air as much as the control or Simplese. Simplese, therefore, was better at mimicking the properties of the 4.8 % fat control than Maltodextrin.

Another study was conducted on the effect of 2 types of whey protein-based fat replacers, Dairy Lo and Simplese, and 2.5 % milk fat, and cocoa butter on sensory properties of low-fat and non-fat chocolate ice cream (Prindiville et al., 2000). The differences between the 2 fat-replacers were that Simplese was made of round microparticulates of whey proteins from 0.1 to 3.0 micrometers (The NutraSweet Kelco Company, 1994) whereas Dairy Lo was a thermally denatured whey protein concentrate (Anonymous, 1994). Ice creams with Dairy Lo appeared to be more viscous, darker in color in chocolate ice cream, and displayed higher chocolate aroma intensities compared to ice creams with Simplese (Prindiville et al., 2000). Ice cream with Dairy Lo and a control low fat ice cream had similar thickness and mouth coating effects which were greater than ice

cream containing Simplese. Ice creams with both fat replacers and cocoa butter had higher cocoa flavor intensity compared to a control low fat ice cream. There were no significant differences in consumer acceptability between samples although trained panelists documented differences. According to the trained panel, after storage duration for 6 weeks, ice creams with Simplese were creamier such that they had the smoothness and body associated with milk fat compared to Dairy Lo. Ice creams with both fat replacers were icier than ice cream containing milk fat.

1.5 Consumer Attitudes

The consumer acceptance of dairy products does not only include sensory properties, but the information on the product is crucial (Grunert et al., 2000; Kahkonen et al., 1999). Consumers consider health benefits, therefore, public communication is important for products involving healthy images. Reduced fat food products, however, have poor sensory quality images amongst consumers (Hamilton et al., 2000; Richardson et al., 1999; Folkenberg et al., 2003b; Kahkonen et al., 1997). Consumers prefer full-fat dairy products and other products. However, when presented with the fat level information in cheese products, the preference for reduced-fat cheese increased despite initial lower scores on sensory properties (Grunert et al., 2000; Kahkonen et al., 1999). These results demonstrate that health benefits affected consumer preference but there is certainly a need to improve the sensory properties of reduced fat dairy products.

Consumer demographics and education are another factor influencing their purchase and perception of reduced-fat products (Richardson et al., 1999; Kahkonen et al., 1999;

Hamilton et al., 2000). Consumer cluster studies showed that mostly females and young adults preferred low fat dairy products (Richardson et al., 1999; Kahkonen et al., 1999). Females associate dieting and weight loss to the consumption of reduced fat foods while males believe physical activity is the key to weight loss (Hamilton et al., 2000). Education increases health awareness of consumers (Kahkonen et al., 1999; Margetts et al., 1997).

Fat levels contribute greatly to consumer liking of dairy products. In a study of Gouda cheese, Yates and Drake (2007) showed that the consumer overall liking scores increased with increasing fat content (up to 24.6 % fat). 24.6 % fat cheese had the best liking score and was described as smooth and creamy. Going beyond that fat level, cheeses that contained higher fat (32 %) received a decreased texture liking score (Yates and Drake, 2007). The texture and flavor liking contributed greatly to overall liking scores. One reduced fat (15 % fat) Gouda cheese, however, received a higher liking score than a 16.1 % fat cheese and this difference was attributed to the flavor of the cheese. This study indicated that improving the flavor of reduced fat cheese improved the liking scores. (Yates and Drake, 2007).

In studies of sensory properties of Cheddar cheese, full fat products were preferred over the reduced fat cheese (Hamilton et al., 2000; Jack et al., 1992). Consumers described reduced fat cheese as slightly rubbery and “horrible tasting” where as full fat cheeses were described as creamier, fuller flavored, tastier, and smoother textured (Hamilton et al., 2000). Jack et al. (1992) found reduced-fat cheeses were firmer, more rubbery and drier than full fat cheeses while full-fat cheeses were described as moist, soft and smooth (Jack et al., 1992).

Maturity of the cheese also influenced consumer perceptions. More mature cheeses were described as crumbly, dry and hard whereas less mature cheeses were described as rubbery and soft. Low fat cheeses were described using immature cheeses terminologies, suggesting flavor and texture development were delayed.

In accordance with Gouda and Cheddar cheese studies, a study of sensory profiles and preferences of Mozzarella cheese indicated that the loadings of smooth paste, creamy and juicy characteristics separated the low-fat and standard-fat products (Pagliarini, 1997). The majority of consumers preferred full-fat products as they discriminated products using flavor attributes such as juicy, acid, salty, yogurt odor, sweet and milky characteristics. There was a small group of consumers that preferred low-fat products to all others. Interestingly, there was one reduced fat product profiled that was similar to the full-fat products. This conveys that with appropriate production technologies, reduced fat cheeses with sensory properties similar to full fat standard cheese can be made.

1.6 Conclusion

Reduced fat food products are perceived to have poor sensory quality image amongst consumers. Thus, many efforts are put into improving the sensory characteristics of reduced fat foods.

In dairy products, fat contributes greatly to the flavor and texture. For the flavor to be perceived, a sufficient concentration of flavor compounds in the vapor phase or aqueous phase must be perceived. The rate of release of vapor compounds depends on the partition coefficient of the compounds, molecular interactions between flavor components, the

compound's disposition in the food, the components of the food, the viscosity of the food matrix, and the particle size of the food components. Changes in fat content in the food matrix, therefore, alter the rate of flavor release.

Fat contributes to creamy flavor, mouthfeel, sweet taste, sweet odor, and buttery odor (Koeferli et al., 1995; Folkenberg and Martens, 2003a) and affects basic tastes as it increases sweet, salty, sour and umami tastes, and suppresses bitterness (Metcalf and Vickers, 2002). In dairy products, fat contributes to specific flavors and odors of dairy products such that acetoin, butanoic acid, octanoic acid, d-decalactone and d-dodecalactone were higher in full-fat cheeses compared to reduced fat cheeses (Chin and Rosenberg, 1997). The removal of fat alters the flavor compounds in the end products leading to different flavors perceived in the end products (Carunchia Whestine et al., 2006).

Food texture attributes such as creaminess, smoothness, roughness and mouthfeels are affected by fat content. The mechanism of how fat affects the texture may be due to the degree of physical friction between oral tissues and the food and astringency (deWijk et al., 2004; Janhoj et al., 2006). In cheese, the microstructure is different with different levels of fat and in turn contributes to changes in cheese texture (Drake et al., 1996, 1998). Reduction of fat content leads to a springier texture with high recovery rates, decreased adhesiveness, cohesiveness and degree of breakdown in cheese (Yates and Drake, 2007).

As fat plays many important roles in sensory quality of food products, industries seek for fat substitutes to imitate full-fat products. Protein-based fat replacers were shown to have better interaction with flavor to provide similar characteristics to fat containing products

compared to carbohydrate-based fat replacers. This function of protein was attributed to the amino acid sequences which determined its physico-chemical characteristics and allowed various protein interactions to different flavor compounds (Schmidt et al., 1993; Liou and Gruen, 2007).

1.7 Objectives

Reduced fat dairy products with improved sensory properties are in high demand. Studies have demonstrated that altering flavor of the cheese can lead to better consumer acceptance (Yates and Drake, 2007; Pagliarini et al., 1997). Many efforts have been made to study how fat levels affect the behavior of flavor compounds and have been reviewed earlier. Many studies have been conducted on instrumentally measuring the effects of fat levels on the equilibrium headspace concentration, flavor compound partition coefficient, and volatile release of flavor compounds (Fabre et al., 2006; Roberts et al., 2003; Guyot et al., 1996, Bakker and Mala 1996). Sensory evaluation such as time intensity, odor intensity and flavor release were also of interest (Roberts et al., 2003; Bakker and Mela, 1996; Plug and Haring, 1993).

There have been no studies on the roles of fat and pH on the sensory detection threshold of different compounds and the impact of the matrix-compound partition coefficient, viscosity, and particle size and distribution on sensory threshold values. Detection threshold is defined by the American Society for Testing and Material (E 679-91, 1997) as “the lowest concentration of a substance in a medium relating to the lowest physical intensity at which a stimulus is detected as determined by best estimate criteria’. Sensory

threshold values are crucial for product developers, especially when the original form of a food product is altered, i.e. change in fat level or acidified. The purpose of this study was to investigate the threshold value of 3 prominent flavor compounds in dairy products which varying physicochemical properties: diacetyl (buttery), delta-decalactone (coconut) and furaneol (burnt sugar) and their behavior at different fat levels and neutral and acidified pH (5.5).

The detailed aims are:

- 1) To determine the best estimate threshold (BET) for the 3 compounds at varying fat levels (0, 10, 20, and 100 %) at neutral pH (+/-0.1) and acidified pH 5.5 (+/-0.1).
- 2) The parameters that affect the BET values will be investigated:
 - a) Partition coefficient of the compounds in different matrixes will be measured by determining the compound-matrix headspace-liquid partition coefficient.
 - b) The viscosity at 25 C of the matrix will be measured
 - c) The particle size and distribution of the matrix will be measured

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CHAPTER 2

The Roles of Fat and pH on the Detection Thresholds and Partition Coefficients of Three Compounds: Diacetyl, Delta-Decalactone, and Furaneol, in Water, Oil, and Emulsions

ABSTRACT

The effect of fat and pH on the best estimate threshold (BET) of 3 prominent dairy product flavor compounds with varying physicochemical properties: diacetyl, delta-decalactone, and furaneol in water, oil and oil-in-water model emulsions (at 10 and 20 % fat at neutral and acidified pH 5.5) were investigated. The headspace-matrix partition coefficients ($K_{HS/matrix}$) of each compound in the different matrixes were established. The rheology and particle size of the emulsions used in this study were also investigated. The particle size and the viscosity of the emulsions did not affect the BET or the partition coefficients. Reducing fat from 20 to 0 % did not affect the BET value or partition coefficient of diacetyl ($P>0.05$). Increased fat content increased the BET value and decreased the partition coefficient ($P<0.05$) of the most lipophilic compound in the study, delta-decalactone ($P<0.05$). Fat content did not affect the BET of furaneol ($P>0.05$), but did have an effect on the partition coefficient. At pH 7, addition of fat decreased the partition coefficient of furaneol whereas at pH 5.5, addition of fat increased the partition coefficient. Adjustment of pH from 7.0 to 5.5 did not impact the BET values of delta-decalactone, but did affect the partition coefficients of furaneol at all fat levels and impacted diacetyl at 0 % fat. The partition coefficient results generally agreed with the BET values on the effect of fat and pH, although the partition coefficient test was more sensitive to the differences in the matrix composition than a threshold test.

Key words: Detection threshold, BET, partition coefficients, fat, pH, diacetyl, delta-decalactone, furaneol

Introduction

The main goal in the food industry is to produce food products that are accepted and purchased by consumers. Fat usually contributes to an increase in overall liking of food products (Richardson, 2000; Folkenberg et al., 2003). However, excessive consumption of products high in fat contributes to diseases such as coronary heart disease, stroke, hypertension, diabetes, obesity and cancer (Sinclair and O'Dea, 1990; Hu and Willette, 2002). Thus, achieving healthy products that are low in fat, but appealing in flavor and texture, are challenging to product developers.

Flavor perception is a complex process. Selective binding of flavor compounds to specific receptor proteins in the olfactory epithelium is required for olfactory sensory perception to occur (Kinsella, 1998). To obtain a sensory response, a sufficient concentration of flavor compounds in the vapor phase must be achieved. In modifying foods for fat reduction or altering the pH of food products, the release of aroma compounds is often affected (Bakker and Mala, 1996; Guyot et al., 1996; Fabre et al., 2006; Roberts et al., 2003). Understanding the physicochemical parameters which affect flavor release and perception such as equilibrium headspace partition coefficient and mass transport are the foundation in developing food products with lowered fat content with improved flavor profiles.

Many studies have evaluated how fat concentrations affect the headspace concentration, flavor compound partition coefficients ($K_{HS/matrix}$), and volatile release of flavor compounds (Fabre et al., 2006; Bakker and Mala 1996, Bayarri et al., 2006). Partition values describe the distribution of a volatile compound between two, well-defined phases in a

system at equilibrium (Taylor, 2002). Partition coefficient of the headspace and the matrix ($k_{hs/matrix}$), therefore, represents the concentration of a flavor compound in the headspace over the concentration of a flavor compound in the matrix at equilibrium: $k_{hs/matrix} = C_{hs}/C_{matrix}$. Similarly, the partition coefficient of the liquid phase, i.e. oil-water partition coefficient, can be described as $K_{o/w}$, and is known as the hydrophobicity of a compound or Log P. Log P is derived from the logarithm of the n-octanol/water partition coefficient with hydrophilic molecules having a negative log P and hydrophobic molecules having a positive log P (Taylor, 2002).

Some studies have related instrumental results to sensory measurements to investigate the impact of fat content on time intensity, odor intensity and flavor release of both real foods and model emulsions (Roberts et al., 2003; Guyot et al., 1996). Roberts et al. (2003) compared nosespace, headspace and sensory intensity ratings to evaluate the effect of fat on flavor release of compounds in water and in milk with three different fat contents. The release of diacetyl was not affected by changes in milk fat levels (skim milk, 2.7 % fat milk and 3.8 % fat milk). Benzaldehyde was slightly affected by fat content. Its concentration in the nosespace significantly decreased in whole milk compared to water. Changes in fat content had the largest effect on the percent (concentration) of compound released in the nosespace for the most lipophilic compound in the study, beta-damascenone (Log P=2.79). Sensory orthonasal intensity correlated well with the instrumental measurement of nosespace. By sensory analysis, lipophilic compounds also displayed the lowest intensities at the highest milk fat concentration (3.8 %) and the highest intensities in skim milk.

Guyot et al. (1996) investigated the effect of fat and pH on sensory odor intensities and headspace partition coefficients in model emulsions. Sensory and instrumental results were also complementary. Compounds with ionizable groups (butyric acid) were impacted by pH (Guyot et al., 1996). Aroma and release of a hydrophobic compound (delta decalactone) were increased in aqueous solution while opposite effects were documented for polar compounds (butyric acid, diacetyl). They reported that the odor intensity of delta-decalactone decreased as fat content increased at both pH's studied (4.5 and 5.2). In contrast, the odor intensity of diacetyl was not affected when the emulsion fat content was increased up to 83 % w/w fat.

Limited studies have addressed sensory detection thresholds as they relate to fat content. Van Aardt et al. (2001) reported retronasal detection thresholds of acetaldehyde in whole, lowfat, nonfat, and chocolate flavored milk, and spring water during storage in amber glass containers. There were no significant differences in the threshold values at the 3 different fat levels in milk. Compared with the 3 white milks with different fat levels, chocolate flavored milk had higher threshold values and water had lower threshold values. These differences were attributed to the flavor masking properties of the milk and chocolate milk compared to water rather than fat or matrix component effects. Adhikari et al. (2006) investigated retronasal thresholds of three flavor compounds (diacetyl, hexanal, and delta-decalactone) in skim milk. They reported interactions between the compounds. The presence of one other compound either suppressed or increased the threshold value of the other compound. No instrumental studies were conducted in this study and the impact of fat

content was not addressed.

Other physicochemical parameters that affect flavor release are interfacial mass transfer and diffusion (Taylor, 2002). The penetration theory of interfacial mass transfer developed by Harrison et al. (1997) is often used to model flavor release from liquid emulsions in dynamic systems. The mass transfer coefficient relates to the solute diffusion coefficient in the penetration theory and was predicted by the Stokes-Einstein equation to be inversely proportional to the square root of viscosity (Baines and Morris, 1987; Wilke and Change, 1955). Terta et al. (2006) studied the effect of viscosity on the flavor release rate and air/liquid partition coefficient of limonene in polysaccharide solutions. They demonstrated that increased viscosity reduced the partition coefficient of a non-polar compound limonene (Log P=4.57). In contrast, a slightly hydrophilic compound studied, trans-2-hexanal (Log P=1.58), was not impacted by viscosity (Terta et al., 2006). Increasing the viscosity by increasing the polysaccharide concentration decreased the release rate of both compounds. They concluded the viscosity mainly affected the release rate and had a smaller impact on partition coefficient (Terta et al., 2006). Contradicting results were reported by Charles et al. (2000) who studied the effect of droplet size and viscosity on flavor release from salad dressings. They found that large droplet size emulsions contributed to lower viscosity and in turn increased the release of hydrophilic compounds. They attributed the effect to less resistance to mass transfer in less viscous emulsion systems. For hydrophobic compounds, the rate of release increased with smaller droplet size (increased viscosity) (Charles et al., 2000). McNulty and Kerel (1993) explained that the release of

hydrophobic compounds involved the transfer from oil to water and then from water to air. Smaller droplet size increased the surface area of the oil droplets, and thus, enhanced the rate of transfer of the hydrophobic compounds from oil to water. The release of hydrophilic compounds was, therefore, higher in emulsions with larger droplet sizes (decreased viscosity).

The droplet size may also indirectly affect flavor distribution in emulsions due to changes in the total distribution of emulsifier between adsorbed and nonadsorbed states (McClement, 2002). Large droplet size requires less emulsifier as compared to smaller droplet size. In emulsions with constant emulsifying agent, the fraction of the adsorbed emulsifying agent to the droplet increased when the droplet size decreased. Thus, if an emulsifier can bind or solubilize flavor molecules, and the adsorbed state is different from the unadsorbed state, the flavor distribution will be different with changes in droplet size and would impact flavor release and partition coefficient (McClement, 2002). Emulsifying agents such as proteins and polysaccharides may influence flavor release and partition coefficients (Seuvre et al., 2000; Guidchard et al., 2000; Giroux et al., 2007; Juteau et al., 2004).

To our knowledge, there have been no studies on the roles of fat and pH on the sensory detection threshold of different compounds in matrices with altered fat levels and the impact of the compound-matrix partition coefficient, emulsion rheological characteristics and particle size on sensory threshold values. The objective of this study was to investigate the threshold values of 3 prominent dairy product flavor compounds with varying physicochemical properties: diacetyl (buttery) ($\log P = -2.0$), delta-decalactone (coconut)

(Log P= 3.4) and furaneol (burnt sugar) (Log P=1.4) and their behavior in water, oil or oil-in-water model emulsions at 10 and 20% fat and at acidified (pH=5.5) and neutral pH (pH=7.0). The partition coefficients of the compounds, which could potentially cause differences in threshold values, were measured as well as the rheology and the particle size of the emulsions used in the study.

Materials and Methods

Chemicals

Diacetyl (2,3-butanedione, 97 %, CAS number: 431-03-8) and furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone, 99 %, CAS number: 3658-77-3) were obtained from Aldrich Chemical Co. (St. Louis, MO). Delta-decalactone (97 %, CAS number: 705-86-2) was obtained from Alfa Aesar (Ward Hill, MA).

Treatments

Chemicals were added to five matrices at two different pH levels. The matrices included deionized water, vegetable (soybean) oil (Wesson brand, ConAgra Foods, Inc, Omaha, NE), emulsions prepared with 10 or 20 % v/v vegetable oil in water with 1 % w/v fresh curd calcium caseinate (Erie Foods International Inc., Erie, IL), and 1 % w/v Fresh curd calcium caseinate (0 % vegetable oil) in water (Cas control). The three emulsion matrices (10 and 20 % fat and Cas control) were evaluated at neutral pH (7.0 +/-0.1) and pH 5.5 (+/-0.1) acidified with lactic acid (Fischer Scientific, Pittsburgh, PA). All emulsions were

manufactured in duplicate and measurements subsequently evaluated on the experimental replications.

Emulsion Preparation

Emulsions were prepared according to the methods of Meynier et al. (2005) with minor alterations. Briefly, calcium caseinate (Cas) (1 % w/v) was dissolved in deionized water. The Cas solution was heated to 50 C in a scraped surface steam kettle (Groen, Trout Valley, IL) for 30 min. Vegetable oil was heated to 50 C. A coarse dispersion (10 or 20% fat v/v) was prepared by blending the Cas solution at high speed in a KB7207 Krups blender (Krups, Shelton, CT) for 1 min and slowly adding hot oil. The coarse dispersion was fed immediately into a homogenizer (Panda 2K, Niro Inc., Columbia, MD) at a pressure of 800 bar (80 bar second stage, 720 bar first stage). The Cas 0 % fat control was prepared by blending the 1 % Cas solution for 1 min followed by homogenization at the same pressure. The emulsion was chilled to 5 C in the refrigerator overnight. Lactic acid (Fischer Scientific, Pittsburgh, PA) was used to adjust the pH of the 5C emulsions and Cas control to pH 5.5 for the acidified treatments (0 % fat Cas control, 10 and 20 % fat emulsions) using a Mettler-Toledo Seven Easy pH meter (Mettler-Toledo Inc., Columbus, OH). Volatile compounds were added to propylene glycol (J.T.Baker, Phillipsburg, NJ) to make stock solutions. An aliquot of stock solution was added after the emulsions were made or to water or oil at 25 C.

Rheology

Rheological properties of emulsions at 25 C were determined in duplicate using a Stress Tech controlled stress Rheometer (ATS Rheosystems, Bordentown, NJ/Rheologica

Instruments AB, Lund, Sweden) with a CC 25 Couette assembly. Samples (15 ml) were pre-sheared for 30 s at 30 s^{-1} . The viscosity was measured as shear rate was increased from $50 - 300 \text{ s}^{-1}$ and then decreased from 300 to 50 s^{-1} to check for hysteresis. The measurement interval was 10 sec. Shear rate and shear stress were fitted to a power law model to obtain K (consistency coefficient) and n (flow behavior index).

Particle size

Particle size distribution of the fat emulsions was measured using a Mastersizer 2000 with a Hydro 2000-S liquid sample dispersion unit (pump speed 2250 rpm) with software version 5.40 (Malvern Instruments, Westborough, MA). A combination of a red (633 nm) and blue laser (466 nm) were used. The sample material (fat) refractive index was set at 1.458 and an absorption value of 0.00001. The blue light refractive index for fat was set at 1.460 with an absorption value of 0.00001. The dispersant (water) refractive index was set at 1.33. The density of the particulate material was set at 0.902 g/cm^3 . The general purpose predictive model type was used, with the particle shape set to spherical. Size range of particles to be detected was 0.020 to 2000 μm . The obscuration limits were set from 7 to 9% to achieve a consistent amount of sample loading and to minimize the risk of multiple light scattering. The emulsion and dispersant temperatures were between 22 to 24 C. Background and sample measurement time was 5 seconds and 5000 snaps. There were 3 measurement cycles with no delay between measurements. The average of the 3 cycles was reported. The majority of samples had residual values for the statistical model that were between 0.4 to 1 %, with occasional samples having residuals between 1 and 2 %.

Flavor-Protein binding

To test if protein had an impact on the binding of flavor compounds in the emulsions, static headspace analysis (see static headspace analysis method section) was conducted for each compound in 1, 2, and 3 % (w/v) protein in deionized water. The protein solutions were prepared with the same method used for preparation of the 1 % caseinate (Cas) control. All three compounds were added to 10 ml of protein solution in 40 ml vials. The same procedures applied for headspace partition coefficient determination (described later) were used. The final concentrations in the vials were: diacetyl=10 ppm; delta-decalactone=100 ppm; and furaneol=1000 ppm. The concentrations were chosen based on the concentrations that provided detectable signals in preliminary studies. A high furaneol concentration relative to the other two compounds was required, likely due to its thermal degradation under normal GC conditions (Rouseff et al., 1998). The GC oven temperature program used was that for headspace partition coefficient determination of delta-decalactone (total run time of 18.67 min). The MS was set in single ion monitoring (SIM) detection mode to quantify diacetyl (ion 86, 0-5 min), furaneol (ion 128, 5-10 min), and delta-decalactone (ion 99, 10-18.67 min). The areas under the curves were compared to each other to determine if increasing protein concentration affected the volatile headspace concentration. All injections were done in duplicate.

Threshold Determination

The best estimate orthonasal threshold (BET) of each compound/matrix combination (8 threshold tests for each compound: oil, water, two different pHs for Cas

control and the two emulsions) was determined using the ASTM ascending forced choice method of limits procedure E679-79 (ASTM, 1997). All compounds were dissolved in propylene glycol (J.T.Baker, Phillipsburg, NJ) on the day of the test. Stock solutions (Table 1) in the appropriate matrix (water, oil, protein solution, or emulsion) were serially diluted (factor of 3), and 15 ml of each was poured into clean, labeled 56 ml plastic soufflé cups and lidded. Blank samples of the appropriate matrix were prepared with the same amount of propylene glycol in each series. The incubation time in the cups was 2-3 h. Preliminary studies confirmed that this was a sufficient time to achieve equilibrium for each compound.

Panelists (n = 25) were given these concentrations in a series with two appropriate blanks (deodorized water, oil, 1 % protein or emulsion). Seven ascending series were tested each time. Series were presented in ascending concentration, and each series was presented in a randomized order and evaluated by panelists using the ASTM method with the sure/not sure modification detailed by Lawless et al. (2000). Subjects were instructed prior to testing and participated in at least three practice orthonasal threshold tests prior to participating in this study. Subjects were told to open each soufflé cup from the side and to briefly sniff the headspace of each cup in the series without completely removing the cup lid. Subjects rested 1 min between each set of three and were also instructed to sniff their sleeve to assist clearing their nasal passageways between cups. Subjects were asked to choose the one item from the three that they thought different, and to give a certainty judgment (sure/not sure). The individual best estimate threshold was taken as the geometric mean of the last concentration with an incorrect response and the first concentration with a correct response except for the

following sequence: if the subject indicated a “not sure” response for the correct choice, that concentration was increased by a factor of 1.41, to adjust for the possibility of a chance correct response (Lawless et al., 2000). Threshold testing was conducted in duplicate for each compound on different days. Group thresholds were taken as the geometric mean of the individual best estimate thresholds.

Static Headspace Analysis

The equilibrium headspace/matrix partition coefficient of each emulsion or protein solution at both pH's, oil, or water was measured by static headspace sampling (Jung and Ebeler, 2003; Nahon et al., 2000). Ten g of liquid matrix (1 % protein solution, emulsion, oil, or water) were added to a 40 ml amber screw top vial (28 x 98 mm, Supelco, Bellefonte, PA) with a PTFE/Silicone Septum (Supelco, Bellefonte, PA). An aliquot of stock solution made up with propylene glycol (concentration: diacetyl = 2380 ppm, delta-decalactone = 11600 ppm, furaneol = 12500 ppm) was added to achieve a certain concentration for each compound/matrix combination (Table 2). All vials were thoroughly mixed by vortexing for 1 min.

The vials were then sonicated for 30 min at 25 C and incubated at 40 C for 30 min. After the equilibration time, a sample (Table 2) of the headspace was directly injected onto an HP 6890 gas chromatograph equipped with a 5973 inert mass spectrophotometer (GC-MS) (Agilent Technologies, Santa Clara, CA) using a 1 ml gastight syringe (Hamilton, Bonaduz, Switzerland) in splitless mode (splitless injection sleeve, Supelco, Bellefonte, PA). The GC was equipped with a ZB-5ms column (0.25 mm x 30 m x 0.25 μ m film thickness)

(Phenomenex Zebron, Torrance, CA). The MS was initially set in scan mode: Diacetyl—low mass of 40 and high mass of 90; delta-decalactone and furaneol—low mass of 35 and high mass of 250, with a run time of 30 min to determine the retention time for each compound. Later, single ion monitoring (SIM) mode was used to detect the ions of interest, i.e. diacetyl = ion 86, delta-decalactone = ion 99, and furaneol = ion 128. All injections were conducted in duplicate.

The injector was maintained at 250 C at a pressure of 7.08 psi for all compounds. The oven temperature profile was different for each compound. For diacetyl, the initial temperature was 40 C for 1 min, followed by ramping to 100 C at 10 C/min, and then to 200 C at 30 C/min. The total run time was 10.33 min. For delta-decalactone, the initial temperature was held at 40 C for 1 min, ramped to 200 C at 10 C/min, and then to 250 C at 30 C/min. The total run time was 18.67 min. For furaneol, the initial temperature was held at 40 C for 1 min, ramped to 100 C at 10 C/minute, then further to 250 at 30 C/minute. The total run time was 12 min.

The area under the peak acquired from headspace injection was an unknown value. To convert the unknown area to the amount of volatile compound concentration partitioned in the headspace, a standard curve for each compound was generated. For standard curves, stock solutions were prepared by adding compounds to methanol (EMD, San Diego, CA) (delta-decalactone and furaneol) or diethyl ether (diacetyl). Ten ml of solvent was added to a 20 ml screw thread headspace vial (Microliter Analytical Supplies, Inc., Suwanee, GA). An aliquot of stock solution was added to the vial. One μ l of the solution containing a known

concentration of each compound was directly injected onto the GC (10 µl- microliter #701, Hamilton Co., Reno, NV) and analyzed using the same conditions as described previously with headspace injection. All injections were done in duplicate.

The partition coefficient of the compound in the headspace and the matrix, $K_{HS/matrix}$, was calculated using equation 1:

$$(1) \quad K_{HS/matrix} = C_{HS}/C_{matrix}$$

where C_{HS} was the concentration of the volatile compound in the headspace and C_{matrix} was the concentration of the compound in the matrix. At equilibrium, the concentration of compound in solution was calculated from the initial volatile concentration in the liquid, C'_{matrix} , the volume of the headspace, V_{HS} , and the volume of the solution, V_{matrix} as the volatile compound in the liquid distributed between the air phase and the matrix. The compound concentration in solution was calculated using equation 2:

$$(2) \quad C_{matrix} = C'_{matrix} - C_{HS} (V_{HS}/V_{matrix})$$

(Jung and Ebeler, 2003).

By dividing the headspace concentration (C_{HS}) by the matrix concentration (C_{matrix}), the partition coefficient ($K_{HS/matrix}$) was obtained.

Statistical analysis

Threshold test

As is commonly done when estimating BET values, the threshold values for each individual were log transformed and then the geometric mean was computed. The antilog then produced the group estimate of the BET. The delta method (Sen and Singer, 1993) was

used to compute the standard error of the group BET estimates. The estimated group BET values were approximately normally distributed as a result of the Central Limit Theorem. Hence, pairwise comparisons of the group estimates of the BET values were formed using $Z = (BET_2 - BET_1) / (\sqrt{SE_1^2 + SE_2^2})$ as a test statistic that was standard normal under the null hypothesis of common BET values. This pairwise comparison approach was adopted to reduce the type II error rate which, if too large in an initial study like this, may result in failing to find differences that can be subsequently investigated more thoroughly.

Partition Coefficient Test

Statistical analysis was conducted using SAS software version 9.1 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was used to test significance at 0.05 significance level. Tukey's Honestly Significant Difference (HSD) test was used to determine differences among treatments of interest.

Viscosity, protein-flavor interaction, and particle size

Statistical analysis was conducted using XLStat (Addinsoft, New York, NY). Analysis of variance (ANOVA) was used to test significance at 0.05 significance level. Tukey's Honestly Significant Difference (HSD) test was used to determine differences among treatments.

Results and Discussion

Emulsion Characterization

All emulsions at 10 and 20% fat displayed reversible, shear-thinning characteristics over the shear rate range tested, 50 – 300 s⁻¹ in the presence of propylene glycol. The

viscosity of emulsions and the 1% Cas control increased with an increase in fat content (Figure 1). The apparent viscosity at the shear rate of 50s^{-1} was significantly greater at 20 % fat compared to 10 % fat and the 1% Cas control ($P<0.05$). This result was expected as an increase in the fat fraction essentially means increased disperse phase in the same emulsion volume. The viscosity was not different at pH 7 than pH 5.5 at the same fat levels ($P>0.05$). Hunt and Dalgleigh (1995) noted that characteristics of emulsions at pH values close to the pI of the protein had two competing effects: 1) flocculation of emulsion droplets and 2) the formation of a gel with the whey protein in solution (Hunt and Dalgleigh 1995). Flocculation of emulsion droplets and larger droplet size occurred at pH 5.5 which was closer to the pI of casein (pI=4.6) compared to neutral pH, and resulted in a decrease in viscosity but the decrease was not statistically significant ($P>0.05$).

The shear stress-shear rate plot of all fat level emulsions (10, 20 and 0% Cas control), with and without propylene glycol, was fitted with a Power-law model:

$$\sigma = k\dot{\gamma}^n$$

Where: σ = shear stress, $\dot{\gamma}$ = shear rate, k =consistency coefficient, n =flow behavior index.

Propylene glycol did not impact the K or the n values ($P>0.05$) in the emulsions (results not shown). Moreover, addition of fat had no significant effect on K (at pH 7: 0, 10 and 20 % fat were 0.0047, 0.0034 and 0.0043, respectively; at pH 5.5, 0, 10 and 20 % fat were 0.0022, 0.0033, and 0.0039, respectively) or n (at pH 7: 0, 10, and 20 % were 0.75, 0.84, and 0.87, respectively; at pH 5.5: 0, 10 and 20 % fat were 0.89, 0.84, 0.88, respectively) ($P>0.05$).

This means that the droplet interactions and emulsion structure were not significantly

different from each other and that the emulsions with varying fat had similar flow behavior.

Viscosity does affect flavor release in dynamic systems (Taylor, 2002; Harrison et al., 1997). In a static system, however, viscosity is not a major concern and partition coefficient is often a more sufficient tool used to study the behavior of flavor compounds (Terta et al., 2006; Juteau et al., 2004). Thus, viscosity and rheology of the emulsions in the current study will not be discussed further.

b) Particle size analysis

Droplet size may indirectly affect flavor distribution in emulsions due to changes in the total distribution of emulsifiers between adsorbed and nonadsorbed states (McClement, 2002). Large droplet size requires less emulsifier due to less surface area to cover. In our experiment, pH did not have an effect on the droplet size (mean $d(4,3) = 0.57 \mu\text{m}$ and $0.61 \mu\text{m}$, for 10 % fat at pH 7 and 5.5, respectively; $d(4,3) = 1.17 \mu\text{m}$ and $1.99 \mu\text{m}$, for 20 % fat pH 7 and 5.5 respectively) ($P > 0.05$). However, increasing the fat content (from 10 to 20 %) increased the droplet size by about 50 % ($d(4,3) = 0.59 \mu\text{m}$ and $d(4,3) = 1.58 \mu\text{m}$ respectively) ($p < 0.0001$). The vapor pressure of a compound contained within an emulsion droplet could be impacted by the droplet size. Greater vapor pressure within a droplet increases with lower droplet size. However, this effect would be unlikely to be significant in food emulsions as it would only impact small droplets ($d < 0.1 \mu\text{m}$) (McClement, 2002).

Flavor-Protein Interaction

In emulsions with constant emulsifying agent content, the fraction of emulsifying

agent adsorbed to the droplet interfaces increased as the droplet size decreased due to more surface area (McClement, 2002). That means if an emulsifier can bind or solubilize flavor molecules, and the adsorbed binding state is different from the unadsorbed binding state, the flavor distribution will be different with changes in droplet size (McClement, 2002). In this experiment, the same amount of emulsifying agent was used with all the treatments (1 % Cas). Due to the constant emulsifying agent used, 10 % fat had more protein adsorbed on the droplet interface compared to 20 % fat emulsion. If interfacial proteins bind to the compounds tested, a greater extent of binding may occur with 10 % fat emulsion than 20 % fat. This effect potentially alters the partitioning of the compounds in the headspace and BET results and was thus investigated in the current study.

Static headspace analysis confirmed that proteins interacted with diacetyl and furaneol at pH 7 but not at pH 5.5 ($P < 0.05$) (Table 3). Proteins did not interact with delta-decalactone at either pH ($P > 0.05$). Diacetyl headspace concentration decreased as protein in solution increased from 1 to 3 %. However, furaneol headspace concentration increased as protein in solution increased from 1 to 3 % at pH 7.0. There were no significant differences in protein binding at pH 5.5 for all compounds. Protein binding to odorant compounds occurs when the binding sites are available and not engaged in protein-protein interactions (Cheftel et al., 1985). At acidified pH, proteins are more aggregated compared to neutral pH, and this could lead to less binding sites available to bind to the flavor compounds. Thus, at neutral or alkali pH, casein binds more carbonyl, alcohol and ester volatile compounds than at acidic pH (Lindsay, 1985). This was consistent with the results observed in this study.

Even though there were some significant differences in the binding of flavor compounds to proteins, the influence of oil concentration in the emulsion was shown to have much greater effects in emulsions with 0.1 to 3.2 % whey proteins and 1 to 8 % oil (Giroux et al., 2007). Therefore, although proteins affected the headspace concentration of some compounds, the contribution of fat would be much more than the protein effect on the BET and partition coefficients in the current study.

Threshold Determination

Olfactory threshold is very hard to measure as it varies from trial to trial and moment to moment (Lawless and Heymann, 1998). Many external factors that can affect olfactory threshold include: the purity of the stimuli and the background aroma of the control stimuli. The internal factors include the subject's physical state, attentiveness and motivations, and how much experience they have (Lawless and Heymann, 1998). Even within the same session and the same individual, variability in response is observed (Lawless et al., 1995). Thus, a threshold test can be considered as 'levels of detection that occur 50 % of the time' (Lawless and Heymann, 1998).

Effect of Fat on BET Values

Diacetyl

1.1 Effect of Fat on Detection Threshold of Diacetyl in Water vs. Vegetable Oil

Diacetyl is a compound that contributes a buttery odor in dairy products (Guyot et al., 1996; Bakker and Mala, 1996). It is a hydrophilic compound with a hydrophobicity value

(Log P) of -2.0. The threshold values of diacetyl in different matrices are shown in Table 4. The best estimate threshold value (BET) of diacetyl in water was significantly lower than the BET in oil ($P < 0.05$) (Table 5a). These threshold values were consistent with values of 5 ppb and 50 ppb in water and oil, respectively, reported by Druaux and Voilley (1997). Some previous studies did not report any difference between orthonasal water and sunflower oil threshold values for diacetyl (Van Gemert, 2003), and these differences could be due to different methods and dispersed phase (sunflower oil) used (Lawless and Heymann, 1998).

Our diacetyl BET values while in agreement with previous threshold reports, however, contradict with the sensory odor intensity of diacetyl found by Guyot et al. (1996). They found diacetyl to have higher odor intensity in 99 % paraffin oil as compared to 0% fat which means a greater amount of diacetyl was detected at higher fat content. This would imply that the BET value of diacetyl in oil was lower than in water. Being a hydrophilic compound, diacetyl has some solubility in water, which results in less volatile compound in the headspace and thus a required higher concentration to be perceived in the headspace (Landy et al., 1996; Guyot et al., 1996). The differences between our study and that of Guyot et al. (1996) may be due to the background odor of the vegetable oil which prevented panelists from detecting diacetyl in vegetable oil at lower concentrations (Lawless and Heymann, 1998). Also, the paraffin oil used in by Guyot et al. (1996) study is a purified liquid saturated hydrocarbon acquired from petroleum (Chayen et al., 1994). It is different from the composition of the vegetable oil which is unsaturated. Relkin et al. (2004) found that diacetyl release was greater in animal fat than vegetable fat. The differences in fat

composition in our study compared to Guyot et al. (1996) may also be the source of the threshold differences.

1.2 Effect of Fat on Detection Threshold of Diacetyl in Oil-in-Water Emulsions

At neutral pH and pH 5.5, the addition of 1 % calcium caseinate did not significantly affect the BET values (Table 5a). At pH 5.5, addition of 10 or 20 % fat did not impact BET values of diacetyl. This finding coincides with results of Bennett et al. (1964), who did not find a significant difference in the retronasal threshold of diacetyl in skim milk or cream. Roberts et al. (2003) also did not find any significant differences in sensory nosespace of diacetyl in water, skim milk (milk protein), and fat content of the milk (0, 2.7, and 3.8 % fat) at neutral pH. Guyot et al. (1996) also found no difference in the sensory odor intensity and headspace partition coefficients of diacetyl at pH 5.2 in 0, 15 and 45 % fat emulsions. These experiments attributed their findings to the low hydrophobicity of diacetyl. However, at pH 7, the BET value of diacetyl in 10 % fat was significantly lower than 0 and 20 % fat. The pattern was similar to that of the pH 5.5 such that 10 and 20 % was lower than 0 % fat, although at pH 5.5, the result was not significant. Thus, the significance found statistically may be due to the variability in the population tested that contributed to smaller standard error.

Delta-Decalactone

2.1 Effect of Fat on Detection Threshold of Delta-Decalactone in Water vs. Vegetable Oil

Delta-decalactone is a compound with a high hydrophobicity value (Log P = 3.4) and

contributes a coconut aroma (Guyot et al., 1996). There was a significant difference between the BET value in water (66.0 ppb) and vegetable oil (1550.0 ppb) ($P < 0.05$) (Table 5a). The threshold in water was in the same range as that reported by van Gemert (2003) of 100 ppb. The increased threshold pattern of the compound in water vs. oil was also consistent with previous studies (van Gemert, 2003; Engel et al., 1998). Moreover, a similar pattern was found with taste thresholds of delta-decalactone in water and butter oil, 140 ppb and 1400 ppb (Siek et al., 1969). The markedly increased BET value of delta-decalactone in vegetable oil was due to the great solubility of this hydrophobic compound in the hydrophobic oil matrix. Hydrophobic compounds have a great affinity for lipids, and thus have lower vapor pressure in lipids and increased sensory thresholds in oil (Bakker et al., 1996; Druaux and Voilley, 1997)

2.2 Effect of Fat on Detection Threshold of Delta-Decalactone in Oil-in-Water Emulsion

There were no significant differences among the BET values for delta-decalactone with the addition of 1 % Cas control at either pH ($p > 0.05$) (Table 5a). At pH 7, the BET value at 10 % fat was 550 ppb, which was significantly higher than the 1% Cas control (44 ppb). The 20 % fat emulsion also had an increased BET value compared to the 1% Cas control but the value was not significant. The threshold for 20 % fat was expected to be significant. However, with threshold tests, there are many sources of variability. At pH 5.5, addition of fat at both 10 and 20 % increased the BET value ($P < 0.05$).

Furaneol

3.1 Effect of Fat on Detection Threshold of Furaneol in Water vs. Vegetable Oil

Furaneol is composed of a polar furanone ring with short hydrophobic side chains (Log P=1.4) (Relkin et al., 2004). The BET values in water (22.3 ppb) and oil (27.4 ppb) were not significantly different from each other (Table 5a). Our BET value in water is consistent with results reported by Larsen and Poll (1992) who found the detection threshold to be 1-10 ppb. Preininger and Grosch (1994) reported the odor threshold of furaneol in sunflower oil to be 25-50 ppb which also agreed with the value found in this study. A wide range of BET values have been published for furaneol from 1-10 ppb to 1700 ppb (van Gemert, 2003). This may be due to different testing methods implied with each test or the instability of the compound as it is sensitive to oxidation and unstable at all pH's (Roscher et al., 1997).

3.2 Effect of Fat on Detection Threshold of Furaneol in Oil-in-Water Emulsion

The BET values of furaneol in 1 % Cas was not significantly different from the BET of furaneol in water at both pH's ($P>0.05$) (Table 5a). Moreover, there were no differences in BET value observed as fat levels were increased from 0 to 10 to 20 % (v/v). These results suggest that reducing fat from 20 to 0 % fat do not have a large impact on the detection threshold of furaneol.

Effect of pH on BET values

Diacetyl

There were no significant pH differences in BET values for diacetyl at all fat levels

(Table 5b). These results contradict Bennett et al. (1964) who found the threshold value of diacetyl to increase as pH decreased. Bennett et al. (1964) studied the impact of pH and cream on retronasal threshold of diacetyl compound using a paired comparisons method. In skim milk, the retronasal threshold value was 10, 5, 50, and 200 ppb at pH 6.8, 6.0, 5.5, and 5.0 respectively.

Delta-Decalactone

There was no effect of pH on the BET value of delta-decalactone (Table 5b). The pH range tested in our study may not be sufficient to find a pH effect on delta-decalactone. Kearney et al. (1993) reported that the pKa of the carboxyl group on the delta-hydroxy decanoic acid form was 4.46. Below that pKa, a large proportion of the compound is protonated and forms a lactone ring whereas above the pKa, a higher amount of the compound will be in the hydroxyacid form (Kearney et al., 1993). If thresholds were determined below the pKa, 4.46, differences in BET values may be observed.

Furaneol

There were no pH differences in BET values for furaneol at any fat level tested (Table 5b). These results contradict the results reported by Buttery et al. (1995). Buttery et al. (1995) demonstrated that furaneol had lower odor thresholds in water at pH 4.5 compared to pH 7. However, there were differences in the threshold methods used compared to the current study. Buttery et al. (1995) did not follow an ASTM method and used 2 sample presentation (1 sample and 1 blank) rather than 3 sample presentation (1 sample and 2 blank samples). pH may affect furaneol due to its instability at all pH's, especially pH less than 3.5

and greater than 5 (Roscher et al., 1997). Shu et al. (1985) found that the amount of the opened chain of the furan ring of furaneol occurred more readily at lower pH than higher pH which is likely due to acid breakdown of the furan ring, thus, leading to lower concentrations of the furan ring structure at lower pH. This could possibly explain lower detection threshold at lower pH found by Buttery et al. (1995) as other aroma active volatile products such as acetaldehyde, hydroxyacetone, 1-hydroxy-butanone, 3-hydroxy-2-butanone, and 2,3-butanedione are formed.

Partition Coefficients

Effect of Fat on Partition Coefficient

Diacetyl

1.1 Effect of Fat on Partition Coefficient of Diacetyl in Water vs. Vegetable oil

There was a significant difference in the static headspace diacetyl concentration between diacetyl in oil and water (Table 6) (Table 7a) ($P < 0.05$). Diacetyl in oil had a higher air/matrix partition coefficient ($K_{HS/matrix}$) of 4.80×10^{-5} compared to water, 2.60×10^{-5} . Our results agreed with previous published values: Salvador et al. (1994) also found diacetyl in sunflower oil to have higher $K_{HS/matrix}$ (6.3×10^{-4}) compared to water (4.5×10^{-4}). A higher $K_{HS/matrix}$ value means more volatile compounds distributed in the headspace when the system is at equilibrium. Since diacetyl is a hydrophilic compound, we expected higher partition coefficients in oil compared to water.

1.2 Effect of Fat on Partition Coefficient of Diacetyl in Oil-in-Water Emulsions

At neutral pH, there was a significant difference in the partition coefficient of diacetyl in the 1% calcium caseinate control compared to water (Table 6) (Table 7a). Fare et al. (1998) found that diacetyl reacted with the primary amino groups of protein, for instance, the terminal amino groups and mainly the E-lysyl residues. They suggested that there were 2 groups of binding sites presented on sodium caseinate for diacetyl. Strong bonding occurred via covalent bonding between polar amino group and the carbonyl group on the diacetyl (Schiff bases formation between carbonyl groups and the E- or alpha- amino group) whereas weak bonding occurred via hydrogen bonding (Fare et al., 1998; Cheftel et al., 1985). At pH 7, the amino acids with a carboxyl group (aspartic and glutamic acids) are ionized and not reactive to the ketone groups and methyl groups of diacetyl (Fare et al., 1998). Fare et al. (1998) confirmed that addition of diacetyl to glycosylated sodium caseinate (7.5 % w/v) resulted in an absence of diacetyl bound to glycosylated sodium caseinate. In the presence of typical (nonglycosylated) calcium caseinate in this study, lower $K_{HS/matrix}$ values suggested some interaction between diacetyl and calcium caseinate. Hansen and Booker (1996) also demonstrated flavor interactions with casein and whey protein with benzaldehyde, d-limonene, and vanillin (at 0 to 0.5 % w/v proteins in water). However, whey proteins exhibited greater binding of flavor compounds than casein as they unfolded with heating and tended to bind a greater amount of flavor compounds.

There were no significant differences in the partition coefficient of diacetyl with altered fat content (0, 10 and 20 %) at pH 7. It was expected that 20 % fat would have a

higher $K_{HS/matrix}$ value as our study had found diacetyl in oil to have a greater $K_{HS/matrix}$ value than diacetyl in water. However, 20 % fat was an emulsified system with 3 phases: oil, water, and an oil and water interface. Hydrophilic compounds are partitioned in the water phase, thus, less affected by increases in oil fraction (Druaux and Voilley, 1997). Our results agreed with Dubois et al. (1996) who found up to 30 % change in fat content did not affect the volatility of diacetyl in model cheese at pH 4.9.

At pH 5.5, unlike neutral pH, there was no effect of protein binding observed in the headspace concentrations when comparing the 1 % calcium caseinate control to water (Tables 6, 7b). Protein is more aggregated at pH closer to pI, therefore, less protein binding sites, i.e. on amino groups, were available to bind to the carbonyl group of diacetyl. Also, amino acids are in a more protonated form, thus, more of the hydrogen is available for hydrogen bonding at acidified pH. Thus, no effect of protein binding was shown in the headspace. There was also no significant effect of fat on the flavor compound at both fat levels. Our results agreed with Guyot et al. (1996) who found no significant differences on the partition coefficient of diacetyl from 0 to 49 % fat content at pH 5.2 although the values increased with increasing fat content.

Delta-Decalactone

2.1 Effect of Fat on Partition Coefficient of Delta-Decalactone in Water vs. Vegetable Oil

There was a significant effect of fat on the partition coefficient of delta-decalactone in water compared to oil (Tables 6, 7a) ($P < 0.05$). The partition coefficient of oil was significantly lower than water. The observed result was expected due to the high

hydrophobicity value of delta-decalactone (Log P=3.4). The liquid/liquid partition coefficient of delta-decalactone showed that this compound was mainly dissolved in the hydrophobic organic phase (Guyot et al., 1996). Roberts et al. (2003) also found highly lipophilic compounds to have a significant decrease in headspace concentration with increasing fat content.

2.2 Effect of Fat on Partition Coefficient of Delta-Decalactone in Oil-in-Water Emulsion

At both pH's, there were no significant difference in partition coefficient with addition of casein to water ($P > 0.05$). This result agrees with the flavor-protein interaction experiment, where no protein binding to the compound was observed. There were significant differences observed with addition of 10 and 20 % oil, with lower $K_{HS/matrix}$ as compared to 1 % Cas control at both pH's. Guyot et al. (1996) found the partition coefficient of delta-decalactone in water was $1.03E^{-4}$ at pH 5.2. However, in their study, this volatile was not detected in the headspace when the fat content increased. This result agrees with our study, as greater amounts of delta-decalactone adsorbed in a matrix with higher fat lead to less (or undetectable) concentration in the headspace. Again, the affinity of hydrophobic compounds to a hydrophobic matrix contributes to these results.

Furaneol

3.1 Effect of Fat on Partition Coefficients of Furaneol in Water vs. Vegetable Oil

There was no significant difference in the partition coefficients of furaneol (Log P=1.4) in water as compared to vegetable oil (Tables 6, 7a). Since furaneol has a hydrophilic furanone ring with a hydrophobic side chain (Milo and Reineccius, 1997), it would be least

impacted by fat content as compared to highly hydrophilic or highly hydrophobic compounds.

3.2 Effect of Fat on Partition Coefficient of Furaneol in Oil-in-Water Emulsions

There was an effect of protein at neutral pH on the partition coefficient of furaneol as compared to water, with 1 % Cas control having a greater partition coefficient. The same trend was found in the flavor-protein interaction experiments. The partition coefficients of furaneol in emulsions with 10 and 20 % vegetable oil were significantly smaller than the partition coefficient in the 1% Cas control (Table 6, 7a). This could be due to the protein effect, where unadsorbed proteins drive furaneol to the headspace. At pH 7, the protein solution contained more net negatively charges and could repulsed hydrophilic ring of furaneol, and thus could lead to greater furaneol in the Cas control headspace. At pH 5.5, the net charge was lower compared to neutral pH, thus, there was no significant difference in the partition coefficient in water compared to 1 % Cas control at pH 5.5. At pH 5.5, the emulsions with 10 and 20 % vegetable oil had partition coefficients greater than the 1 % Cas control. Lower partition coefficients in 0 % fat (Cas control) mean more compound concentrated in the liquid phase (than in the headspace).

Effect of pH on partition coefficients

Diacetyl

Table 7b shows the effect of pH on the partition coefficient of diacetyl, delta-decalactone and furaneol. There was a pH effect on partition coefficient of the 1 % Cas control, with pH 7 having a lower partition coefficient compared to pH 5.5. Thus, at pH 7,

less compound was partitioned in the headspace at equilibrium. This result agrees with our protein binding experiment and other studies that demonstrated that proteins do bind to diacetyl at neutral pH (Fare et al. 1998; Hansen and Booker, 1996).

Delta-Decalactone

There were no pH effects on the partition coefficients at all fat levels ($p < 0.05$) for delta-decalactone (Table 7b). Guyot et al. (1996) found no effect of pH in the range they studied (4.2 and 5.2) on partition coefficients of delta-decalactone, although the vapor/liquid partition coefficient was slightly higher at pH 5.2 than 4.5. They explained that at that pH range, the structure of delta-decalactone was in the same delta-hydroxy decanoic acid form (Guyot et al., 1996). The pKa of the carboxyl group on the delta-hydroxy decanoic acid form is 4.46 (Kearney et al., 1993). Below that pKa, a large proportion of the compound is protonated and forms a lactone ring whereas above the pKa, a higher amount of the compound will be in the hydroxyacid form (Kearney et al., 1993). The pH range of the current study may not be wide enough to perceive the effect of pH on delta-decalactone as both are above its pKa.

Furaneol

pH impacted the partition coefficient of furaneol (Table 7b). In the 1 % Cas control, the partition coefficient was greater at neutral pH compared to pH 5.5. However, at 10 and 20 % vegetable fat, the opposite prevailed, higher partition coefficients were observed at pH 5.5 than pH neutral. Again, unadsorbed proteins do affect furaneol at pH 7. This may lead to greater furaneol in the headspace at pH 7 as compared to pH 5.5.

Relating Sensory Detection Threshold to Partition Coefficient of the Compounds

Partition coefficients agreed with BET values for the hydrophobic compound, delta-decalactone, when investigating the effect of fat. The aroma intensity, headspace concentration, and sensory intensity rating of hydrophobic compounds tend to be more affected by fat compared to hydrophilic compounds (Bayarri et al., 2006; Robert et al., 2003). Robert et al. (2003) found the percent (concentration) of beta-damascenone, the most hydrophobic compound in their study, released in the nosespace to be more affected by fat than less hydrophobic compounds studied. Greater $K_{hs/matrix}$ values for delta-decalactone in water than in oil coincided with smaller BET values of delta-decalactone in water as compared to oil. Also, a similar trend prevailed as we compared 0, 10 and 20 % fat at pH 7 and pH 5.5, where fat seemed to have an effect by solubilizing the compound and resulted in higher BET values and lower partition coefficients. There were no effect of pH on the BET and partition coefficient of delta-decalactone.

For hydrophilic and slightly hydrophilic compounds, diacetyl and furaneol respectively, partition coefficients, for the most part, agreed with BET values. There were no fat effects on the BET and the partition coefficients of diacetyl in 1 % Cas, 10 and 20 % fat emulsions. There was also no flavor-protein interaction observed in both tests at pH 5.5. However, at pH 7, there was a protein binding effect demonstrated in the headspace partition coefficient, but the effect was not significant in the threshold test. Moreover, volatile compounds partitioned greater over oil, however, the BET of diacetyl was greater in oil compared to water rather than lower which would be expected based on the partition

coefficient. For furaneol, there was no effect of fat or protein on the detection threshold, although, there were some effects on the partition coefficients. The pH effects on BET values were not large enough to be significant. However, the partition coefficient showed pH effects on diacetyl at 0 % (1 % Cas control) and on furaneol at all fat levels.

Partitioning of compounds caused differences in threshold values in the static system used in this study. Viscosity and the particle size had no effect on thresholds. To study the impact of fat or pH on model emulsions, the partition coefficient was more consistent than the threshold test as it depends purely on the composition of the matrix (Harrison et al., 1997; Martuscelli et al., 2008), and threshold tests can be variable due to variability of human subjects. However, sensory perception is necessary for studying the impact of fat and pH on human sensory detection. As instruments and humans function differently, the ability to detect some compounds may also differ. For instance, furaneol had the smallest partition coefficient compared to diacetyl and delta-decalactone in general, but its BET values was not greater than that of delta-decalactone. Clearly, changes detected instrumentally do not necessarily mean that a change in sensory detection will occur.

Fat and pH ranges evaluated in this study did not impact the BET values of furaneol. For diacetyl, changes in the detection threshold remained insignificant up to 20 % fat at both pH's. Eliminating fat (1 % Cas) only affected the detection threshold of delta-decalactone, a hydrophobic compound. This suggests that hydrophobic compounds are mostly affected by the reduction of fat which agrees with Robert et al. (2003) who found lipophilic compounds to have the lowest intensities in milk containing 3.8 % fat compared to skim milk. Changes

in pH from 7.0 to 5.5 did not affect the BET results of any of the three compounds at the fat levels evaluated.

Fat usually contributes to overall acceptability in food products (Richardson, 2000; Folkenberg et al., 2003). Adjusting fat in a product may interfere with the consumer's acceptability in a negative way. Results from this study allow product developers to understand the impact of fat and pH on detection thresholds of different flavor compounds in order to adjust the flavor profile of a product accordingly. For instance, the concentration of diacetyl affects the aroma and flavor of cottage cheese, with 1.0 ppm diacetyl receiving the highest ratings in full fat cottage cheese (Antinone et al., 1994). From our study, reducing fat from 20 to 0 % did not affect the detection threshold of diacetyl at neutral or acidified pH (5.5). This suggests that in a reduced fat or fat free cottage cheese, it is likely that diacetyl concentrations do not need to be adjusted in order to achieve the level that is acceptable to consumers. Our study results demonstrate that fat has a greater impact on delta-decalactone as significant differences were observed when fat content was reduced. Delta-decalactone contributes to coconut/milk fat flavor in many fat-rich foods (Guyot et al., 1996; Singh et al. 2003). Lactones are fat-derived aroma compounds (Alewijn et al., 2006), thus, lowering fat decreases lactone formation in the final product. However, the BET value for delta-decalactone was lower at 0 % fat compared to 20 % fat or 100 % oil, thus, addition of small amounts of delta-decalactone should be sufficient in order to achieve products with similar intensity to the original form as there is less fat to solubilize the compound. Furaneol is one of the key odorants in cheese (Milo and Reineccius, 1997) and is derived from amino acid

and carbonyl compounds (Griffith and Hammond, 1988). When fat in cheese is reduced, furaneol does not need to be altered as our study found that fat content did not impact the detection threshold of furaneol at both neutral and pH 5.5 (cheese).

Taken as a whole, our study with three distinct volatile compounds suggests that changes in matrix (fat content and pH) may affect partition coefficient and sensory threshold. These changes may contribute to perceived sensory changes. Other sensory effects from altering food matrices may be more complex and involve interactions with other flavor and matrix components.

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Table 1: Concentration of stock solutions for diacetyl, delta-decalactone, and furaneol for threshold tests

Compounds	Stock 1 (ppm)	Stock 2 (ppm)
Diacetyl	2376.00	5.94
Delta-Decalactone	2313.00	5.78
Furaneol	13612.00	170.16

*Stocks in parts per million

Table 2: Diacetyl, delta-decalactone, and furaneol concentrations and volumes of headspace sampled

Matrix	Diacetyl		Delta-Decalacatone		Furaneol	
	Total conc. (ppm)	Headspace volume (μl)	Total conc. (ppm)	Headspace volume (μl)	Total conc. (ppm)	Headspace Volume (μl)
Water	10	300	100	500	2000	500
Oil	10	300	5000	500	2000	500
0% 5.5	10	300	100	500	3000	300
0% 7.0	10	300	100	500	3000	300
10 %5.5	10	300	500	500	3000	300
10 % 7.0	10	300	500	500	3000	300
20%5.5	10	300	500	500	3000	300
20% 7.0	10	300	500	500	3000	300

*0% (5.5 or 7) = 0% Fat (1 % calcium caseinate) at pH 5.5 or pH 7, 10 % (5.5 and 7) = Emulsion containing 10 % fat at pH 5.5 or pH 7, 20 % (5.5 or 7) = Emulsion containing 20 % fat at pH 5.5 or pH 7. Total concentration (in parts per million), Headspace volume (in microliters)

Table 3. Peak areas (area counts) of diacetyl, delta-decalactone and furaneol in 1, 2, and 3% protein (w/v) solutions at pH 7.0 and pH 5.5.

pH	Compound	1%Cas	2%Cas	3%Cas
7.0	Diacetyl	174000 ^a	138000 ^{a,b}	137000 ^b
7.0	Delta-Decalactone	12800 ^a	13600 ^a	14200 ^a
7.0	Furaneol	21600 ^b	26500 ^a	36600 ^a
5.5	Diacetyl	384000 ^a	409000 ^a	405000 ^a
5.5	Delta-Decalactone	21800 ^a	18300 ^a	11600 ^a
5.5	Furaneol	59900 ^a	95600 ^a	46300 ^a

*1, 2, and 3% Cas: 1, 2 and 3% (w/v) calcium caseinate in water

^{a,b}Means in a row not followed by a common letter are statistically different (P<0.05)

Table 4. Best Estimate Threshold (BET) Results of Diacetyl, Delta-Decalactone, and Furaneol in Different Matrices

Matrix	Diacetyl		Delta-Decalactone		Furaneol	
	BET (PPB)	S.E.	BET (PPB)	S.E.	BET (PPB)	S.E.
Water	6.0	2.21	66.0	15.2	22.3	6.99
0% Fat pH 7.0	40.8	17.6	43.5	17.4	90.8	41.0
0% Fat pH 5.5	44.9	20.1	35.8	12.0	46.4	15.8
10% Fat pH 7.0	5.6	1.66	546	176	56.4	25.2
10% Fat pH 5.5	9.2	2.62	294	103	67.8	23.4
20% Fat pH 7.0	21.8	7.43	113	42.8	66.9	20.3
20% Fat pH 5.5	8.6	2.90	329	123	134	60.0
Oil	99.5	38.0	1550	365	27.4	9.71

*0% Fat (pH 5.5 or 7) = 0% fat 1% calcium caseinate in water at pH 5.5 or pH 7, 10 % Fat (pH 5.5 and 7) = Emulsion containing 10 % (v/v) fat at pH 5.5 or pH 7, 20% Fat (pH 5.5 or 7) = Emulsion containing 20% (v/v) fat at pH 5.5 or pH 7. BET= Best Estimate Threshold values (PPB=in parts per billion), S.E.= Standard Errors of BET values

Table 5a. Paired Comparisons Demonstrating the Effect of Fat on the BET of Diacetyl, Delta-Decalactone and Furaneol

Matrices	Diacetyl	δ -Decalactone	Furaneol
Water vs. Oil	Sig. ¹	Sig	N.S.
Water vs. 0% pH 7	N.S.	N.S.	N.S.
0% pH 7 vs. 10% pH 7	Sig.	Sig.	N.S.
10% pH 7 vs. 20% pH 7	Sig.	Sig.	N.S.
0% pH 7 vs. 20% pH 7	N.S.	N.S.	N.S.
Water vs. 0% pH 5.5	N.S.	N.S.	N.S.
0% pH 5.5 vs. 10% pH 5.5	N.S.	Sig.	N.S.
10% pH 5.5 vs. 20% pH 5.5	N.S.	N.S.	N.S.
0% pH 5.5 vs. 20% pH 5.5	N.S.	Sig.	N.S.

*0% (pH 5.5 or 7) = 0% fat 1% calcium caseinate in water at pH 5.5 or pH 7, 10 % (pH 5.5 and 7) = Emulsion containing 10 % (v/v) fat at pH 5.5 or pH 7, 20% (pH 5.5 or 7) = Emulsion containing 20% (v/v) fat at pH 5.5 or pH 7.

¹Sig.= Significant (P<0.05), N.S.= Not significant (P>0.05)

Table 5b. Paired Comparisons Demonstrating the Effect of pH on BET of Diacetyl, Delta-Decalactone and Furaneol

Matrices	Diacetyl	δ-Decalactone	Furaneol
0% pH 7 vs. 0% pH 5.5	N.S. ¹	N.S.	N.S.
10% pH 7 vs. 10% pH 5.5	N.S.	N.S.	N.S.
20% pH 7 vs. 20% pH 5.5	N.S.	N.S.	N.S.

*0% (pH 5.5 or 7) = 0% fat 1% calcium caseinate in water at pH 5.5 or pH 7, 10 % (pH 5.5 and 7) = Emulsion containing 10 % (v/v) fat at pH 5.5 or pH 7, 20% (pH 5.5 or 7) = Emulsion containing 20% (v/v) fat at pH 5.5 or pH 7.

¹Sig.= Significant (P<0.05), N.S.= Not significant (P>0.05)

Table 6. Headspace Partition Coefficient ($K_{HS/matrix} E^5$) Results of Diacetyl, Delta-Decalactone, and Furaneol in Different Matrices

Matrices	Diacetyl		δ -Decalactone		Furaneol	
	$K_{HS/matrix} E^5$	Stdev.	$K_{HS/matrix} E^5$	Stdev.	$K_{HS/matrix} E^5$	Stdev.
Water	2.60	0.28	1.70	0.73	0.031	0.0073
0% Cas pH 7.0	1.50	0.33	2.20	0.57	0.082	0.038
0% Cas pH 5.5	2.70	0.45	1.60	0.28	0.022	0.0056
10% Fat pH 7.0	1.50	0.21	0.23	0.053	0.025	0.0076
10% Fat pH 5.5	1.60	0.60	0.26	0.091	0.090	0.0097
20% Fat pH 7.0	1.70	0.21	0.15	0.028	0.029	0.011
20% Fat pH 5.5	1.60	0.41	0.10	0.046	0.072	0.029
Oil	4.80	1.47	0.01	0.0019	0.060	0.014

*0% Fat (pH 5.5 or 7) = 0% fat 1% calcium caseinate in water at pH 5.5 or pH 7.0, 10 % Fat (pH 5.5 and 7) = Emulsion containing 10 % (v/v) fat at pH 5.5 or pH 7, 20% Fat (pH 5.5 or 7) = Emulsion containing 20% (v/v) fat at pH 5.5 or pH 7, $K_{HS/matrix} E^5$ = Partition Coefficients E^5 , Stdev= Standard Deviation

Table 7a. Paired Comparisons Demonstrating the Effect of Fat on Partition Coefficients of Diacetyl, Delta-Decalactone and Furaneol

Matrices	Diacetyl	δ-Decalactone	Furaneol
Water vs. Oil	Sig. ¹	Sig	N.S.
Water vs. 0% pH 7	Sig.	N.S.	Sig.
0% pH 7 vs. 10% pH 7	N.S.	Sig.	Sig.
10% pH 7 vs. 20% pH 7	N.S.	N.S.	N.S.
0% pH 7 vs. 20% pH 7	N.S.	Sig.	Sig.
Water vs. 0% pH 5.5	N.S.	N.S.	N.S.
0% pH 5.5 vs. 10% pH 5.5	N.S.	Sig.	Sig.
10% pH 5.5 vs. 20% pH 5.5	N.S.	N.S.	N.S.
0% pH 5.5 vs. 20% pH 5.5	N.S.	Sig.	Sig.

*0% (pH 5.5 or 7) = 0% fat 1% calcium caseinate in water at pH 5.5 or pH 7, 10 % (pH 5.5 and 7) = Emulsion containing 10 % (v/v) fat at pH 5.5 or pH 7, 20% (pH 5.5 or 7) = Emulsion containing 20% (v/v) fat at pH 5.5 or pH 7.

¹Sig.= Significant (P<0.05), N.S.= Not significant (P>0.05)

Table 7b. Paired Comparisons Demonstrating the Effect of pH on Partition Coefficients of Diacetyl, Delta-Decalactone and Furaneol

Matrices	Diacetyl	δ-Decalactone	Furaneol
0% pH 7 vs. 0% pH 5.5	Sig. ¹	N.S.	Sig.
10% pH 7 vs. 10% pH 5.5	N.S.	N.S.	Sig.
20% pH 7 vs. 20% pH 5.5	N.S.	N.S.	Sig.

*0% (pH 5.5 or 7) = 0% fat 1% calcium caseinate in water at pH 5.5 or pH 7, 10 % (pH 5.5 and 7) = Emulsion containing 10 % (v/v) fat at pH 5.5 or pH 7, 20% (pH 5.5 or 7) = Emulsion containing 20% (v/v) fat at pH 5.5 or pH 7.

¹Sig.= Significant (P<0.05), N.S.= Not significant (P>0.05)

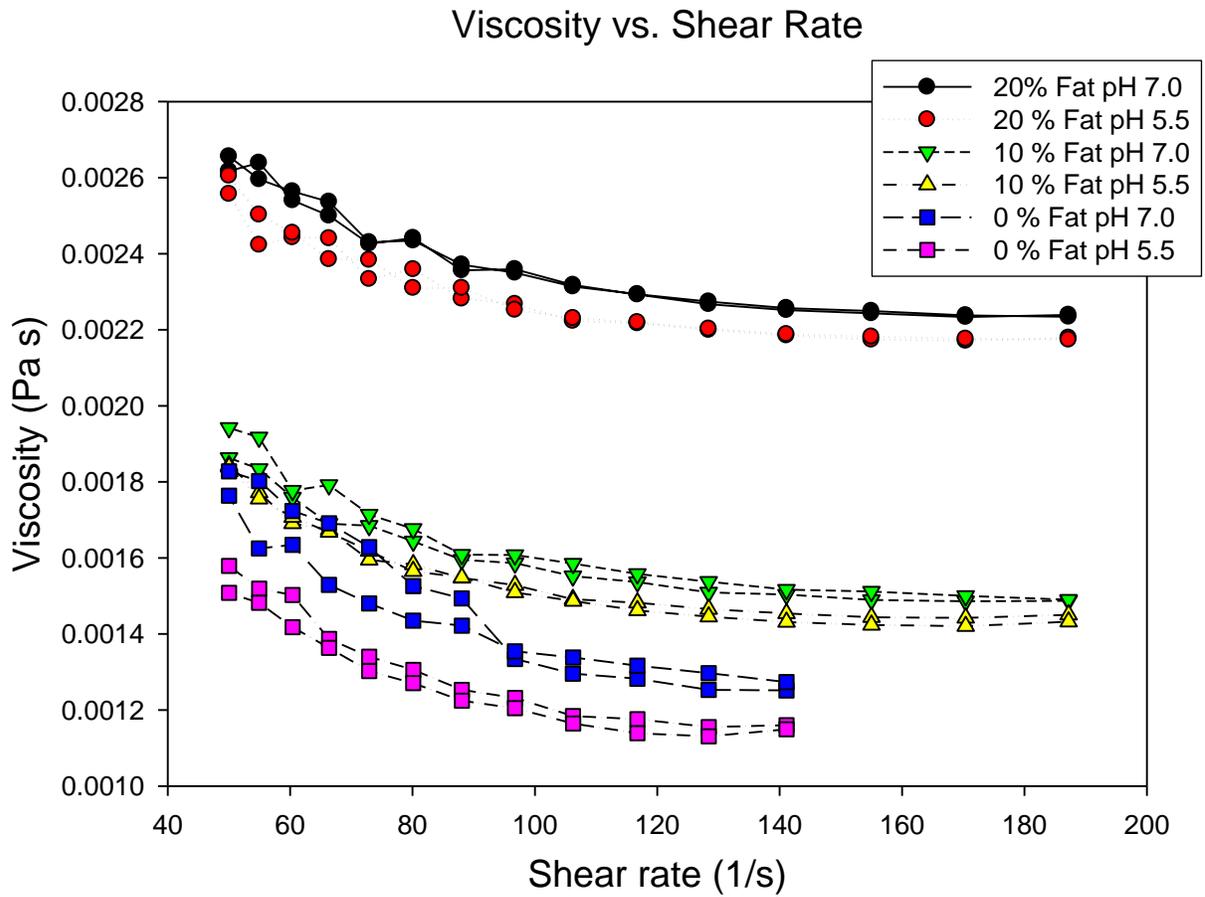


Figure 1. The viscosity vs. shear rate for 1% Cas control (0% fat), 10 and 20% fat emulsions at pH 5.5 and pH 7