

Effect of Fat Nature and Aroma Compound Hydrophobicity on Flavor Release from Complex Food Emulsions

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Complex food emulsions containing either hydrogenated palm kernel oil (vegetable fat) or anhydrous milk fat (animal fat) were flavored by using different aroma compounds. The fats differed by their fatty acid and triacylglycerol compositions and by their melting behavior, while the aroma compounds (ethyl butanoate, ethyl hexanoate, methyl hexanoate, mesifurane, linalool, diacetyl, *cis*-3-hexen-1-ol, and γ -octalactone) differed by their hydrophobicity. Application of differential scanning calorimetry to fat samples in bulk and emulsified forms indicated differences in the ratio of solid-to-liquid between temperatures ranging from 10 to 35 °C. Solid-phase microextraction coupled with GC–MS analysis indicated that flavor release from food emulsions containing animal or vegetable fat differed depending on both the fat nature and flavor compound hydrophobicity. The release of diacetyl was higher for emulsions containing animal fat, whereas the release of esters was higher for emulsions containing vegetable fat. The release of *cis*-3-hexenol, linalool, γ -octalactone, and mesifurane (2,5-dimethyl-4-methoxy-(2*H*)-furan-3-one) was very similar for the two fatty systems. The above results were discussed not only in terms of aroma compound hydrophobicity, but also in terms of structural properties of the emulsions as affected by the lipid source.

KEYWORDS: Emulsion; fat nature; liquid-to-solid ratio; flavor release; temperature; aroma compound hydrophobicity

INTRODUCTION

The overall flavor impression is built upon integration of the temporal flavor profiles as the volatile compounds stimulate the olfactory cells during eating. There is thus a need to fully understand flavor release, taking into account their interactions with macroingredients, and the physical breakdown of the food matrix when consuming a solid food (1).

During consumption, viscoelastic food products can undergo a complete phase change from semisolid to liquid. The changes in their physical states, due to changes in temperature and dissolution with saliva, would be accompanied by changes in flavor release patterns. Due to its solvent properties, fat concentration strongly influences the release of flavor compounds and their perception during consumption of the food product, this effect being dependent on the physicochemical characteristics of the aromas, for example, stale and cherry flavors were released faster and perceived at higher intensities as fat content decreased (2). In their study investigating the effect of sugar and fat on sensory perception of frozen food products, Guinard et al. (3) showed that increased fat or sugar caused a better acceptability of the products by the consumers. However manufacturers try to substitute milk fat with fat replacers in

order to create products that meet the demands of health-conscious consumers. When milk fat was replaced by whey protein fat replacers, significant differences in sensory attributes resulted (4). However different types of fats are commonly used in the industry. There is, until now, little information on the effect of fat composition on flavor release and perception.

Fatty acid composition and arrangement in lipids affect their melting behavior and contribute to the overall structural and sensory properties of colloidal food emulsion systems. In dispersed systems, the resistance to fat droplet destabilization is due to structural properties of dispersed fat globules and the composition of the dispersing and interfacial phases (5, 6). Furthermore, the partition of the volatile aroma compounds corresponds to the distribution between the oil, aqueous, and gas phases. Most of the aroma compounds, being hydrophobic, have a great affinity for the lipids and thus a lower vapor pressure and higher sensory threshold in oil than in water (7, 8).

The physical state of the lipid also affects aroma compound partitioning. The proportion of solid-to-liquid state of the oil phase depends on the nature of the lipids and the temperature of the medium. By comparing anhydrous milk fats of different melting points, no significant difference in flavor release was observed when all the fats were in a liquid state, whereas below the fats melting points, the volatility of ethyl hexanoate was highly dependent on the percentage of fat in the liquid state (9,

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10). Maier (11) showed that retention of volatile compounds by triacylglycerides (TAG) in a solid state increases with increasing temperature and decreases for TAG in the liquid state with increasing temperature. Small variations in temperature around the melting point of the lipids can affect the proportion of solid-to-liquid state. This modification can change the vapor-condensed phase partition coefficient. The sorption of volatile compounds on fatty foods in the liquid state is more important than in the solid state due to better solubility. Indeed, an increase in temperature increases the liquid-to-solid ratio, which results in stronger retention of the volatile compounds, but as soon as the volatile compound is totally soluble in the liquid fat, the phenomenon is totally explained by the van't Hoff law (12).

The volatility of flavor compounds in oil depends on chain length and degree of unsaturation of the fatty acids contained in the triglycerides. The volatility of ethyl hexanoate in vegetable oils differing by their percentages of oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) decreased when the level of unsaturation increased (9). The oil/water partition coefficients determined for ethyl butanoate and 2,5-dimethylpyrazine were lower in emulsions with tributyrine (C4) than in those with trioleine (C18:1), which was mainly explained by the differences in fatty acid chain length (13).

On the other hand, studies focused on physical stability of food emulsions containing various milk proteins indicated that total or partial replacement of skimmed milk powder (SMP) by a whey protein isolate (WPI) led to a higher level of fat droplet agglomeration (5, 14) and changes in the evolution of fat droplet crystallinity under cooling experiments (14).

In the present study we investigated the effects of the nature of fat (from animal or vegetable source), used to prepare complex food emulsions, on the flavor release of aroma compounds differing by their hydrophobicity, in parallel with the characterization of parameters of physical stability of the corresponding model systems.

EXPERIMENTAL PROCEDURES

Characterization of Fats. The composition in diacylglycerols and triacylglycerols was determined by thin-layer chromatography with FID detection (Iatroscan MK5), after dilution of liquid fat in chloroform-methanol (2/1). The composition of fatty acids was determined by gas-chromatography analysis of methyl esters prepared by addition of NaOH-methanol on a mixture of fat with hexane. A fused-silica capillary column DB-Wax, 50 m, 0.32 mm i.d., 1 μ m film thickness (J&W Scientific) was employed. The carrier gas was helium (35 cm³s⁻¹). The GC oven heating was started at 50 °C and then increased to 220 °C at a rate of 5 °C·min⁻¹. The injector was maintained at 250 °C and the FID detector at 250 °C. Identification of methyl esters was done by comparison with pure standard compounds injected in the same conditions.

Emulsion Preparation. The emulsions were prepared using a pilot plant, as previously described (14). The emulsions consisted (all in weight proportions) of 3% whey protein, 5.3% lactose, 0.8% mineral ions from milk permeate ultrafiltrate, 14% sucrose, 3% glucose syrup (dextrose equivalent 40), and a mixture of mono- and diglycerides (0.2%) and stabilizers locust bean gum, guar gum, and carrageenan (0.3%). We used two fats at the same weight proportion (9%). One fat was a partially hydrogenated palm kernel oil (PKO), and the other was an anhydrous milk fat (AMF). These fats were provided by Aarhus Oliefabrik A/S and Lactalis Industrie, respectively. Their fatty acid and triacylglycerol composition is given in Table 1.

The emulsion systems were flavored with eight aroma compounds (ethyl butanoate, ethyl hexanoate, mesifurane (2,5-dimethyl-4-methoxy-(2H)-furan-3-one), methyl hexanoate, *cis*-3-hexenol, diacetyl, linalool, and γ -octalactone) which have different hydrophobicities (Table 2). A mixture of these compounds in propylene glycol was obtained from flavor suppliers (Food Ingredients Specialities, (FIS), York, England).

Table 1. Diacylglycerol (DAG), Triacylglycerol (TAG), and Fatty Acid Percent Composition of Anhydrous Milk Fat (AMF) and Palm Kernel Oil (PKO) Used To Prepare Food Emulsion Model Systems (Iatroscan for DAG and TAG and gas-chromatography analysis of methyl esters for fatty acid composition)

fat	PKO	AMF	fat	PKO	AMF
DAG	4.8	1.7	C18:0	3.0	10.4
TAG	95.2	98.3	C18:1	22.8	27.2
C12:0	32.1	3.7	C18:2	4.4	2.9
C14:0	10.6	11.8	C18:3		0.7
C16:0	22.3	30.9	C20:1		0.76

Table 2. Aroma Compounds: Odor Threshold, Odor Description, log *P* Value, and Concentration in the Emulsion

aroma compound	odor threshold, mg·kg ⁻¹ in water (lit. cited)	odor description	log <i>P</i> (15)	concn (mg·kg ⁻¹)
ethyl butanoate	0.001 (16)	fruity	1.72	10.00
ethyl hexanoate	0.0003 (17)	fruity	2.80	2.80
mesifurane	0.025 (16)	caramel	1.40	2.80
methyl hexanoate	0.087 (17)	fruity	2.26	2.00
<i>cis</i> -3-hexen-1-ol	0.5 (17)	green	1.61	1.60
diacetyl	0.003 (17)	buttery	-2.26	0.56
linalool	0.005 (18)	fruity	2.91	0.40
γ -octalactone		coconut, peach	2.34	0.24

They appear by concentration order in the blend in Table 2. Final concentrations of flavor compounds in emulsions varied between 0.16 and 10 ppm.

Melting Behavior of Fats by Differential Scanning Calorimetry (DSC). Differential scanning calorimetry (DSC, Perkin-Elmer 7, Software Pyris) was used to study the melting behavior of bulk and emulsified fat samples, as previously described (19). The apparatus was calibrated for total heat of reaction using pure samples of indium ($\Delta H = 28.45 \text{ J}\cdot\text{g}^{-1}$) and for onset transition temperature using indium and lauric and stearic acids ($T_{\text{onset}} = 156.6, 41, \text{ and } 72 \text{ }^\circ\text{C}$, respectively). The samples (around 15 mg) were put in an aluminum pan at room temperature, with an empty pan used as a reference pan. In the first experiments the samples were heated from -30 (bulk fat) or -10 (food emulsions) to $50 \text{ }^\circ\text{C}$, at $5 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$, and the heat of melting ΔH_0 was calculated from the area between the peak transitions and a straight baseline drawn from the initial to the final heat flow deviation. In the second experiments samples that were stored at $-30 \text{ }^\circ\text{C}$ were immediately put in the DSC cells and held for 30 min at various temperatures ranging from 5 to $25 \text{ }^\circ\text{C}$ and then heated to $50 \text{ }^\circ\text{C}$, at $5 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$. The heat of melting reaction $\Delta H(T)$, determined from annealed emulsions, was postulated to be due to remaining fat solids at temperature T and used to calculate the percentage (%) of liquid at temperatures ranging from 10 to $30 \text{ }^\circ\text{C}$ following the equation

$$\% \text{ liquid } (T) = 100[\Delta H_0 - \Delta H(T)]/\Delta H_0$$

Characterization of Emulsions. Structural parameters such as fat globule size distribution and amount of adsorbed proteins in the emulsions were determined as previously described (14) by light scattering (Mastersizer apparatus, MS 1000, Malvern Instruments, Orsay-France) and Kjeldahl method, respectively. Light scattering measurements and protein determination were done from three and two independent samplings, respectively. After centrifugation (15 000g for 30 min) of the food emulsion samples, the aqueous phases were separated from the cream layers and analyzed for their protein content using the Kjeldahl method (14).

Solid-Phase Microextraction (SPME) Coupled with GC-MS (SIM Mode) Analysis. Emulsion samples (5 g) were placed in 20 mL vials and allowed to equilibrate 1 h at different temperatures (10, 15, 20, and $25 \text{ }^\circ\text{C}$). A SPME fiber, PDMS/DVB, polydimethyl siloxane/divinylbenzene, 65 μ m (Supelco Park, Bellefonte, PA), was used for volatile compound sampling using the methodology already described (20).

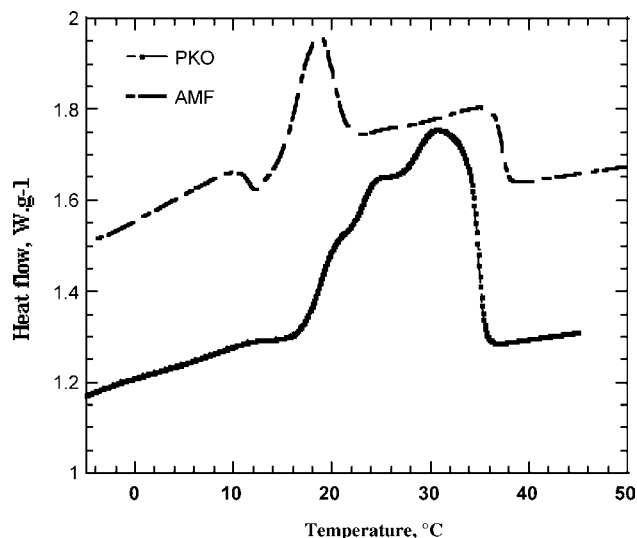


Figure 1. DSC heating curves obtained from vegetable (PKO) and animal (AMF) fat samples.

Volatile compounds were desorbed by inserting the fiber into the GC injector, set at 250 °C for 10 min, 1 min for desorption (purge off) and 9 min for cleaning (purge on).

All SPME operations were automated using a MPS2 MultiPurpose Sampler (Gerstel, Applications, Brielle, The Netherlands).

GC-MS Analysis. A HP 6890-GC equipped with a split/splitless injector coupled with a mass-selective detector 5970 (Hewlett-Packard, Palo Alto, CA) was used. A fused-silica capillary column DB-Wax, 50 m, 0.32 mm i.d., 1 μm film thickness (J&W Scientific), was employed. The carrier gas was helium (35 $\text{cm}^3\cdot\text{s}^{-1}$).

The GC oven heating was started at 50 °C and then increased to 220 °C at a rate of 5 $^\circ\text{C}\cdot\text{min}^{-1}$. The total analysis time was 39 min. The injector was always maintained at 250 °C.

The mass spectrometer was operated in a mass range from 29 to 300 at a scan rate of 1.89 $\text{s}\cdot\text{scan}^{-1}$. The quantification was realized by SIM mode (selective-ion monitoring). The selected and specific ions were 43 for diacetyl, 85 for γ -octalactone, 82 for *cis*-3-hexenol, 87 for methyl hexanoate, 88 for ethyl butanoate, 93 for linalool, 101 for ethyl hexanoate, and 142 for mesifurane.

Statistical Analysis. A two-way analysis of variance was done, testing the following factors: fat, temperature, and fat-temperature interaction. Sample means were calculated (on three experiments) and compared using Newman-Keul's test at the 5% level.

RESULTS

Melting Behavior of Bulk and Emulsified Fats. Examples of melting curves observed by DSC from lipid samples used for emulsion preparation are shown in **Figure 1**. These two curves indicated that AMF and PKO samples, which have different TAG, DAG, and fatty acid composition (**Table 1**), have different melting behaviors. The temperatures corresponding to the peak maximum are different: they are located at ca. 18 °C for anhydrous milk fat and 30 °C for palm kernel oil. However, the temperature of melting completion is very similar for the two fat samples. The trend of the ratio of liquid-to-solid content in lipid samples and in corresponding food emulsions, as a function of melting temperature, is shown in **Figure 2a** and **b**. It indicated formation of different liquid proportion upon heating at temperatures ranging from 15 to 30 °C, but both fats were to 100% in the liquid state at 35 °C. Due to differences in fatty acids and DAG/TAG composition (**Table 1**), the proportion of liquid was higher for the animal fat (AMF) than for the vegetable one (PKO), whatever the holding temperature, ranging from 10 to 30 °C. Furthermore, the curves in **Figure 2a** indicated that 50% liquid was formed at ca. 18 °C for the animal fat instead

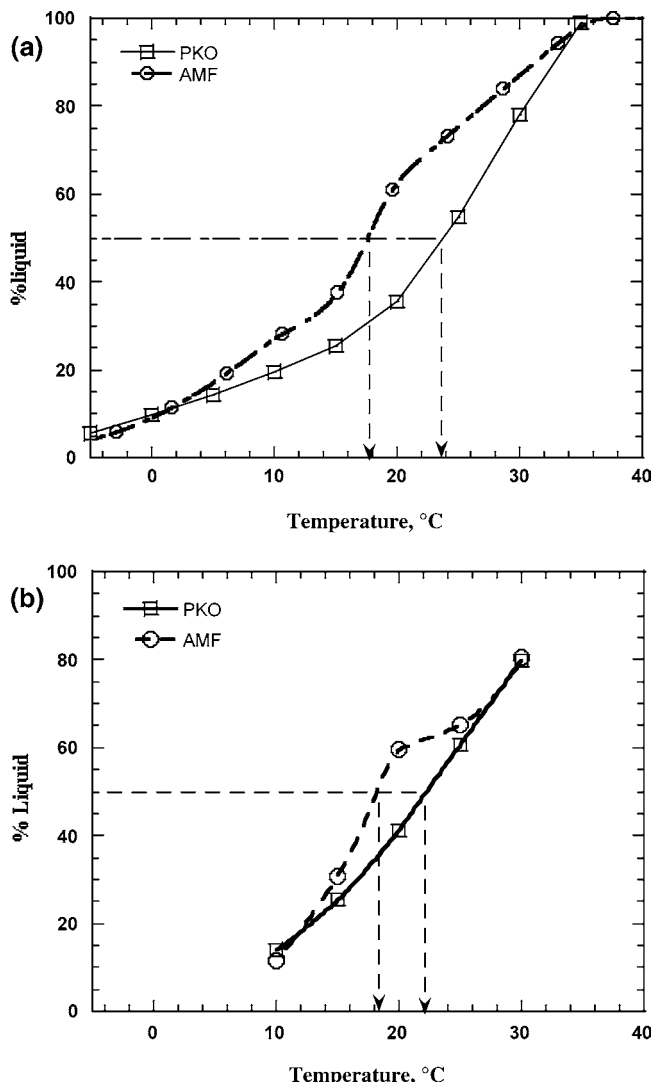


Figure 2. (a) Evolution of liquid proportion in bulk animal fat (AMF) and bulk vegetable fat (PKO) as determined by differential scanning calorimetry (DSC). (b) Evolution of liquid proportion in food emulsion model systems containing either anhydrous milk fat (AMF) or palm kernel oil (PKO).

of 23 °C for the vegetable fat. At 25 °C, the highest temperature used for solid-phase microextraction analysis, the proportion of liquid for AMF had a lower value (65%) in the emulsified than in the bulk sample (75%), whereas this difference was slightly lower for PKO.

Structural Properties of Food Emulsion Models. The fat globule size distributions observed from the two food emulsions, after dispersion either in distilled water or in SDS (sodium dodecyl sulfate) solution (**Figure 3a** and **b**), indicated a bimodal shape, as found for other food milk emulsions where caseins were replaced by whey proteins (14). Values of their corresponding median droplet size ($D_{0.5}$) are reported in **Figure 4a**. When light scattering measurements were made after dilution in distilled water, the $D_{0.5}$ value was higher for the emulsion prepared with animal fat (4.37 μm) than with vegetable fat (2.16 μm). When samples of the two food emulsions were dispersed in SDS solutions, the globule size distributions remained bimodal (**Figure 3b**) but shifted to a lower size value ($D_{0.5} = 1.1 \mu\text{m}$), indicating the dissociative action of SDS molecules.

The proportion of protein adsorbed (P_{ads}) at the oil-water interface relative to protein concentration (P_0) used in emulsions was calculated as follows: $P_{\text{ads}} = (P_0 - P_{\text{aqueous}})/P_0$. The results reported in **Figure 4b** indicated that the proportion of proteins

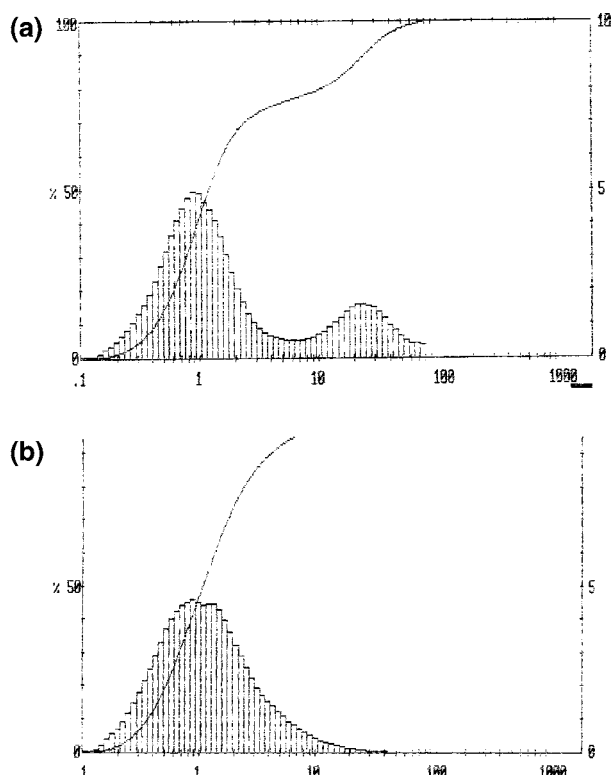


Figure 3. (a) Fat globule size distribution of emulsion realized with vegetal fat (PKO). (b) Fat globule size distribution of emulsion realized with vegetal fat (PKO) after SDS addition.

in the cream layers was higher for the emulsions prepared with the vegetable fat (28%) than with the animal one (17%). Thus, changing the lipid source in food emulsion may be followed by changes in structural properties.

Flavor Release Analysis by Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry (SPME-GC-MS). Solid-phase microextraction (SPME) coupled with GC-MS (SIM mode) was used to quantify aroma release in the vapor phase (Figures 5–8). The polydimethyl siloxane divinylbenzene (PDMS/DVB) coating fiber was chosen because its sensitivity was adapted to the eight aroma compounds (21). The experiments were done at 10, 15, 20, and 25 °C in triplicate.

Results are presented as peak areas. The repeatability of the experiments is good (most of the variation coefficients are lower than 5%). Table 3 shows that the effect of temperature is higher than the effect of fat. Some fat-temperature interactions occur but with a greater probability than both single effects. Looking at the Figures 5–8 we observed that the effect of fat on flavor release depends on the flavor compound. The effect of temperature on volatility is predominant to the effect of the physical state of the fat on the retention of volatile compounds, as already observed by Meynier et al., who found a linear relation between the logarithm of the partition coefficient and the inverse of temperature (22). The release of hydrophobic compounds such as ethyl hexanoate ($\log P = 2.80$), methyl hexanoate ($\log P = 2.26$), and linalool ($\log P = 2.91$) was greater in emulsion with the vegetable fat (PKO) than in emulsion containing the animal fat (AMF). We observed the opposite result for the release of hydrophilic compound such as diacetyl ($\log P = -2.26$). The flavor release of *cis*-3-hexen-1-ol ($\log P = 1.61$) and mesifurane ($\log P = 1.4$), compounds of intermediate hydrophobicity, did not vary significantly as a function of the nature of fat, whereas that of ethyl butanoate ($\log P = 1.72$) varied following the effect observed for the other esters. No significant effect was observed

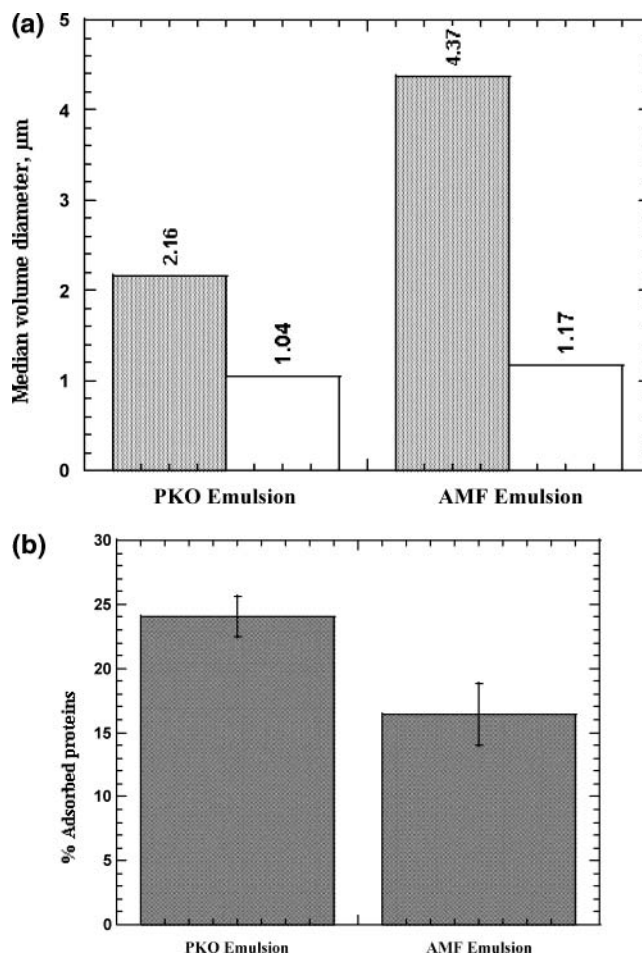


Figure 4. (a) Values of median droplet size, as determined by light scattering after dispersion in distilled water (D0.5 H₂O) and in SDS solution (D0.5 SDS), for emulsions containing vegetable fat (PKO) or animal fat (AMF). (b) Values of proportion of adsorbed proteins (%Pcream) at the fat droplet surface in emulsions containing vegetable fat (PKO) or animal fat (AMF).

Table 3. Two-Way Analysis of Variance of Flavor Release from the Two Emulsions (AMF, PKO), for the Eight Aroma Compounds

	fat effect		temperature effect		fat-temperature interaction	
	F(1,16)	p	F(3,16)	p	F(3,16)	p
ethyl butanoate	89.9	<0.0001	131	<0.0001	4.6	0.017
ethyl hexanoate	460	<0.0001	887	<0.0001	2.1	ns
methyl hexanoate	322	<0.0001	45	<0.0001	3.5	0.04
<i>cis</i> -3-hexenol	3.6	ns	2143	<0.0001	22.5	<0.0001
diacetyl	58	<0.0001	71	<0.0001	12.6	0.0002
mesifurane	18.3	0.0006	731	<0.0001	9.6	0.0008
linalool	155	<0.0001	1161	<0.0001	20	0.0002
γ -octalactone	0.03	ns	966	<0.0001	3	ns

for γ -octalactone, even if this compound was considered as hydrophobic ($\log P = 2.34$).

DISCUSSIONS AND CONCLUSIONS

The two fats differed by their chemical composition and melting behavior. They led to food emulsions characterized by different structural parameters such as mean droplet size and amount of adsorbed proteins at the oil-water interface and a different trend in flavor release.

The release of the chosen flavor compounds as a function of the two fats experimented in the present study differed according

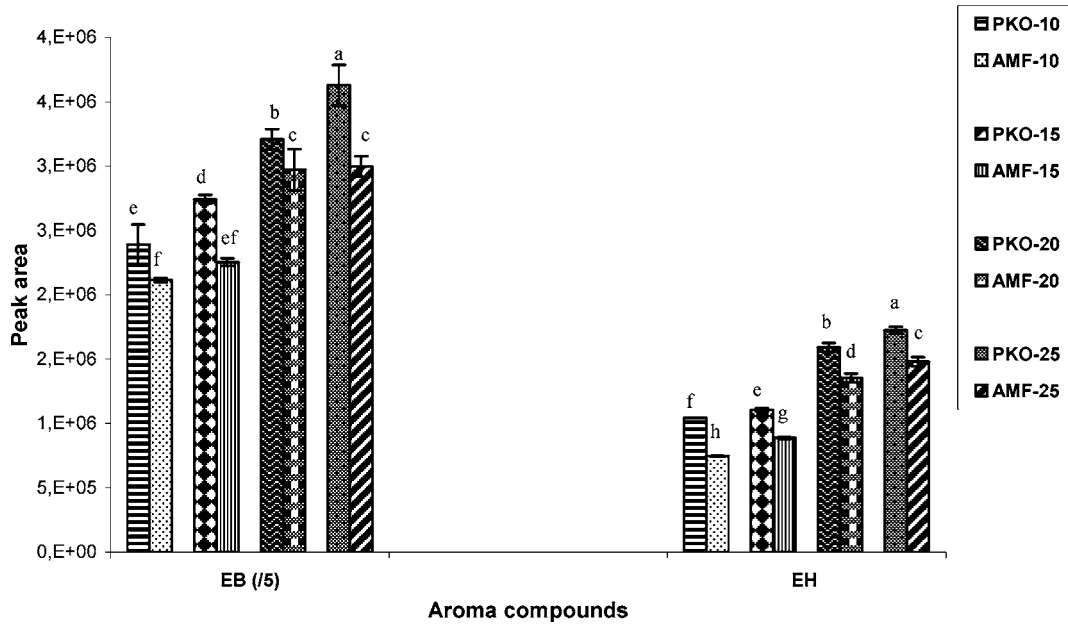


Figure 5. Flavor release of Ethyl Butanoate (EB) and Ethyl Hexanoate (EH) as influenced by temperature (10, 15, 20, and 25 °C) and emulsions (PKO: palm kernel oil; AMF: anhydrous milk fat). Means with the same letters are not significantly different at the 5% level.

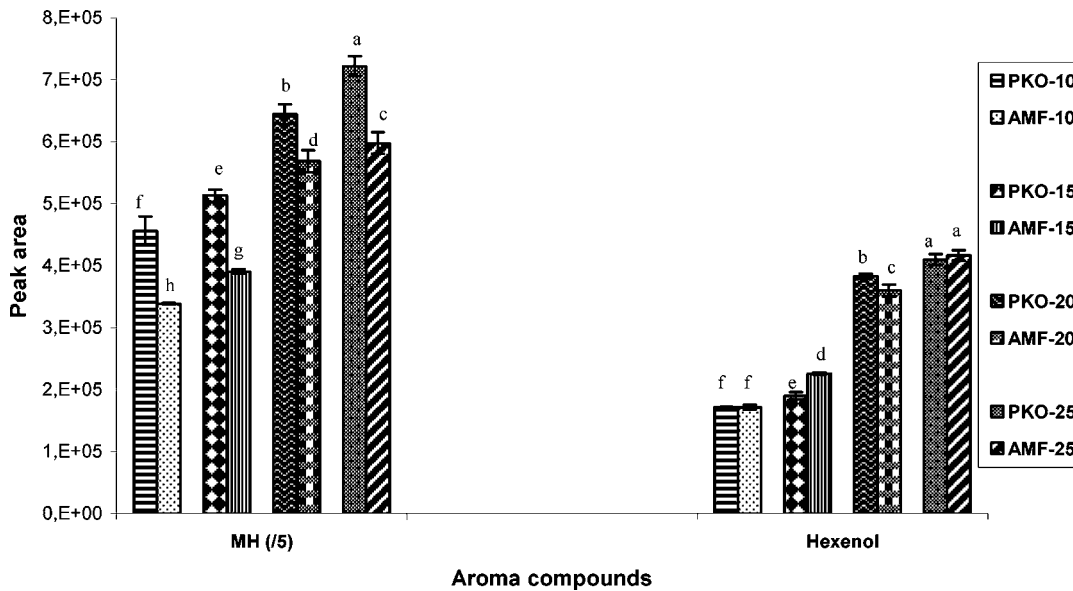


Figure 6. Flavor release of Methyl Hexanoate (MH) and Hexenol as influenced by temperature (10, 15, 20, and 25 °C) and emulsions (PKO: palm kernel oil; AMF: anhydrous milk fat). Means with the same letters are not significantly different at the 5% level.

to their hydrophobicity and chemical class, whereas Roberts et al. (10) observed the same behavior for all compounds studied, which are different from those tested in the present study.

Diacetyl, more soluble in water than in fat (23), was released to a greater extent from food emulsion containing AMF than PKO, except at a temperature of 25 °C, the temperature for which the proportion of liquid is higher than 60% in the two emulsions. This fat effect could be partly explained by the higher triacylglycerol content of AMF, in combination with its higher proportion of liquid as a function of temperature. However, in a model cheese, diacetyl volatility was not affected by the physical state of fat (from tributyrin to anhydrous milk fat), whereas in the same cheese the volatility of diallyl sulfide decreased by 20% in the presence of tributyrin compared to anhydrous milk fat (24). Notice that food emulsions containing AMF have a greater droplet size than those with PKO, which could explain the greater release of diacetyl. Results obtained on salad dressings showed that the most hydrophilic compounds

such as diacetyl were better released from emulsions with a larger droplet size (from 21 to 80 μm) (25). However, in model emulsions no influence of droplet size (7–15 μm) was observed by the same authors on diacetyl, whereas a decrease in the release of diacetyl was observed by other authors by increasing the droplet size from 0.65 to 1.1 μm (26). Other structural parameters than droplet size may also influence flavor release, together with the presence of other constituents such as emulsifiers, which may interact with aroma compounds.

cis-3-Hexen-1-ol and mesifurane, compounds with intermediate hydrophobicity, were not influenced by the nature of the fat. These results are in agreement with those obtained by Miettinen et al. (27), who did not observe significant differences in flavor release of *cis*-3-hexenol from food emulsions containing either 9% animal fat or 9% vegetable fat.

Among the most hydrophobic aroma compounds, independent of temperature, esters were more released from food emulsion containing PKO, which contained a higher proportion of DAG

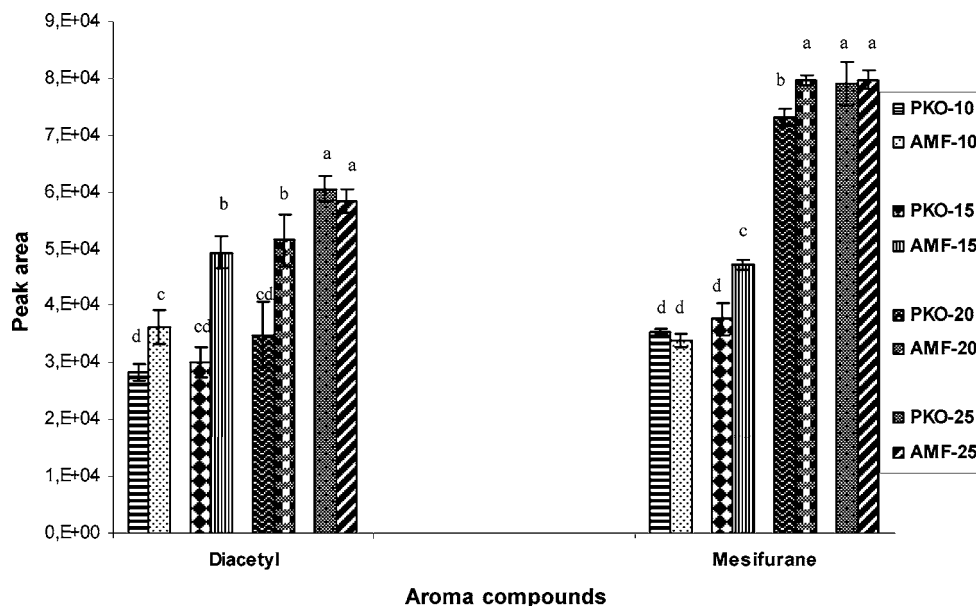


Figure 7. Flavor release of Diacetyl and Mesifurane as influenced by temperature (10, 15, 20, and 25 °C) and emulsions (PKO: palm kernel oil; AMF: anhydrous milk fat). Means with the same letters are not significantly different at the 5% level.

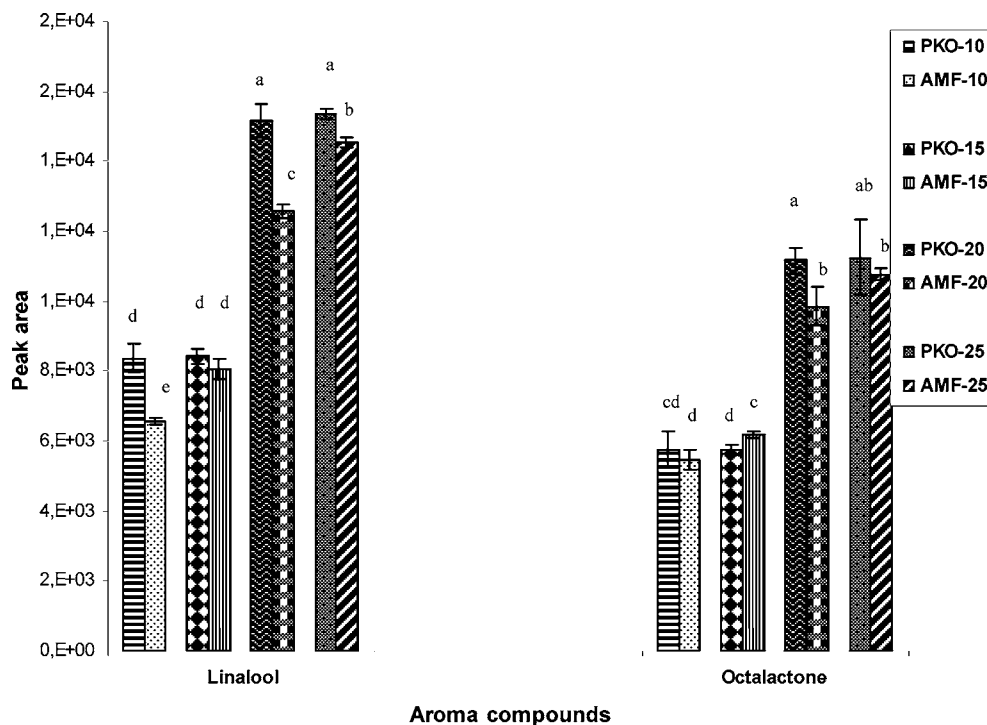


Figure 8. Flavor release of Linalool and Octalactone as influenced by temperature (10, 15, 20, and 25 °C) and emulsions (PKO: palm kernel oil; AMF: anhydrous milk fat). Means with the same letters are not significantly different at the 5% level.

than AMF and thus had a lower liquid proportion upon heating, as evaluated by DSC. This result is in agreement with previous data (28) showing that the release of ethyl hexanoate increased by increasing the percentage of solid-to-liquid fat, due to a lower solubility. In their study on frozen food systems differing by the nature of fat, Miettinen et al. (27) did not find any difference in the release of ethyl butanoate and ethyl hexanoate. However, in salad dressings (25) an increase in release of hydrophobic compounds was observed by decreasing the droplet size, results which were confirmed in a model emulsion made with the same interfacial protein concentration (29). However, esters are known to interact with milk proteins (20). Food products made with AMF contain a lower proportion of adsorbed proteins and thus a higher proportion of soluble proteins, which are susceptible

to retain the esters. This can explain the lower release of esters from AMF food products. No significant difference was observed for γ -octalactone at the different temperatures. This means that hydrophobicity, as estimated by the log P value, cannot be the only parameter used to explain the differences observed in flavor release between the two types of fats studied. This observation is confirmed by the behavior of ethyl butanoate, which is closer to that of the other esters than to that of the flavor compounds of the same hydrophobicity (*cis*-3-hexenol and mesifurane).

In previous studies (6, 14, 19) we showed that replacing skimmed milk powder by whey proteins resulted in changes in fat droplet crystallinity, emulsion stability, and protein composition of surface layers around fat droplets. In the present study,

we showed that changing the nature of the fat also has effects not only on characteristic structural parameters of fat droplets in food emulsion model systems but on flavor release. The level of fat droplet agglomeration was higher for AMF-based food emulsions in comparison with PKO-based food emulsions. In addition, flavor compounds appeared to be more or less released depending on both the proportion of liquid-to-solid content of fat and aroma compound hydrophobicity and chemical class.

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