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Acceptability and Digestibility of Emulsions in a Rat Model: Effects of Solid Fat Content and Lipid Type

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Abstract The present study sought to determine if alterations in the chemical nature and form of fat in food would reduce digestibility while maintaining acceptability in rats. Oil-in-water emulsions ($d < 1$ μm) were prepared with either liquid palm oil, solid hydrogenated palm oil or solid docosane, all stabilized with sodium caseinate. The emulsions were incorporated into a fat-free rodent feed, and each offered over 5 days to separate cohorts of 12 male Sprague–Dawley rats housed in metabolic cages. The feed formulated with solid hydrogenated palm oil was significantly less acceptable than the feeds containing either liquid palm oil or docosane (feed intake 4.9, 26.6 and 32.1 g/animal/day respectively). The proportion of the fat retained (i.e. absorbed) was significantly less in the animals consuming the feed formulated with solid docosane than in the animals consuming either the liquid or solid palm oil (retention 8.7, 99.6, and 97.2%, respectively). The appearance of the feces from the rats fed docosane was different from the rats fed the triacylglycerol samples and thermal analysis revealed many of the solid alkane droplets had not coalesced during passage through the rat's digestive system. These results indicate that indigestible fats can be packaged into food in a manner that does not compromise the acceptability of the product, and does not produce any apparent intestinal distress.

Keywords Fats and oils · Fat substitutes · Emulsions/colloids · Fat crystallization

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Introduction

Cardiovascular disease accounted for approximately 29% of all deaths worldwide in 2004, and reduction in the consumption of high-fat foods is recommended in order to reduce cardiovascular disease burden [1]. Yet in spite of repeated health messages to reduce fat in the diet, per capita apparent fat intake continues to increase. Worldwide, fat intake increased from 71.7 g per capita in the late 1990s, to 78 g in 2001–2003, with intakes in the developed nations being much higher. In North and South America, for instance, intakes increased from 116.9 g in the late 1990s, to 128 g per capita, in 2001–2003 [2]; intakes in Europe were a few grams lower, but also increased across the same time period. Despite known health risks, it is extremely difficult for people to eat less fat.

There have been considerable efforts to reformulate foods to have less energy from fat yet provide comparable sensory qualities by replacing the naturally occurring triacylglycerols with fat substitutes and fat replacers. For example Olestra is a chemically synthesized fatty acid ester of sucrose that has similar physical properties to a triacylglycerol oil yet cannot be digested [3]. Clinical trials with Olestra have not shown adverse gastrointestinal side effects [4, 5]. However, Olestra is expensive and anecdotal reports of gastric discomfort have limited its general acceptance.

Alternatively, naturally occurring solid triacylglycerols appear to be absorbed to a lesser extent than liquid oils. For example, Apgar et al. [6] showed that cocoa butter was less digestible than corn oil by Sprague–Dawley rats (59–72 vs. 93–97%) and Kaplan and Greenwood [7] showed that hydrogenation lowered the bioavailability of soybean oil in rats from 97 to 31%. While crystalline fat cannot be readily substituted for liquid oil as a bulk lipid in food due to the difference in texture, the rheology of stable oil-in-water emulsions is not affected markedly by droplet crystallization. Therefore, it may be possible to incorporate highly hydrogenated oils into food as a reduced energy food emulsion. However the effect of crystallinity on the digestibility of emulsified lipids is not well understood.

The process of emulsion breakdown begins in the mouth where salivary mucins and shear between the tongue and palate cause extensive flocculation and coalescence [8]. In the stomach there is some enzymatic hydrolysis of the surface proteins which may lead to coalescence as well as possible flocculation induced by the change in pH [9]. In the small intestine bile salts can displace any remaining emulsifier from the droplet surface and the process of fat droplets hydrolysis, transport and absorption begins [10]. The structure of the emulsion can affect the digestibility of the lipid [11]. For example, Mun et al. [12] showed that changing the emulsifier changed the in vitro reactivity of lipase with emulsified triacylglycerols. Interestingly, in a similar in vitro model Bonnaire et al. [13] showed that the rate of digestion of a solid triacylglycerol emulsion was slower than a comparable liquid triacylglycerol emulsion. However, in vitro measurements of digestion must be treated with caution as the in vivo processes are inevitably more complex. While emulsion structure and lipid crystallinity may limit the digestibility of fats, it is unclear what happens to the undigested material in vivo. Certainly it is well known that indigestible liquid oils are excreted in the feces and can cause discomfort. However, even if the interfacial layers are digested, there is no mechanism for solid fat particles to coalesce and they may be excreted as individual colloidal particles dispersed in the feces with possibly less associated discomfort.

The goal of this work was to investigate the effect of the chemical nature of a fat (alkane vs. triglyceride) and the form of the fat (solid vs. liquid) on acceptability and digestibility in rats. Further we sought to investigate the form of the fat in the feces. The fats selected were palm oil and hydrogenated palm oil as examples of liquid and solid triacylglycerols (digestible fats) and docosane as a solid indigestible fat. Others have shown that rats will readily consume digestible and non-digestible fats and fat-like substances [14–17]. Nutritive fats and non-nutritive greasy substances are acceptable to rats [e.g. 14] and are equally preferred initially, but preference for the nutritive item develops with longer-term exposure [16–18]. Among nutritive fats and oils, solids are generally preferred, and are more acceptable, than are liquids [19, 20]; however, equal acceptability also has been reported [20]. To our knowledge, fat particle size was not controlled in these previous studies, and excretion/retention was not assessed.

In our initial experimental design we planned to compare docosane with liquid indigestible oil (i.e., mineral oil). However preliminary studies showed that while the animals would consume mineral oil emulsions over a 48 h period, their fur coats were completely covered with oil as a result of their grooming the anal area and spreading the oil over their bodies. We therefore discontinued this part of the study over concerns of thermoregulation and nutrition, and the preliminary findings are only discussed qualitatively.

Materials and Methods

Preparation of Emulsions

The aqueous phase of the emulsion was prepared by mixing 1.85% (w/w) bovine sodium caseinate (Sigma Chemical Company, St. Louis, MO), 0.37% (w/w) sodium saccharin (Sigma Chemical Company, St. Louis, MO) and 1.2% (w/w) sugar-free soluble fiber supplement (Benefiber, Novartis Consumer Health, Inc., Parippany, NJ) in distilled water before storing overnight to allow complete hydration. The lipid ingredients were palm oil (liquid triacylglycerol, LTAG) (Sans Tans 25), hydrogenated palm oil (solid triacylglycerol, STAG) (27 Stearine); both donated by Loders Croklaan North America LLC., Channahon, IL), and docosane (solid alkane, SA) (Sigma–Aldrich, Inc., St. Louis, MO). The lipids were heated (50, 80, and 80 °C, respectively) to ensure they were liquid prior to homogenization. Aliquots (34.14%, w/w) were then coarsely mixed with aqueous phase at the same temperature using a Polytron mixer (15 s, maximum speed, Brinkmann Instruments Inc., Westbury, NY) and then passed through a two-stage valve homogenizer (Panda, Niro Soavi, Italy) set to 200 bar for the first stage; 20 bar for the second stage. Emulsions were recycled through the homogenizer until a particle size under 1 μm was reached. Particle size distributions of the emulsions were measured using a laser diffraction particle analyzer (LA-920, Horiba, Irvine, CA). Samples were diluted with distilled water to approximately 0.001% fat in the measuring chamber to avoid multiple scattering effects during measurements. The scattering pattern was used by the internal software of the instrument to calculate the particle size of the droplets using a relative refractive index of 1.09.

Aliquots of the emulsion were mixed with 18% (w/w) of powdered fat free rodent diet (AIN-93G-MODIFIED, Bio-Serv, Frenchtown NJ; calories as protein: 18.50%; fat:

0.0%; carbohydrate: 68.59%; 3.50 kcal/g) with a hand mixer until all the powder became hydrated and well distributed in the emulsion to form the emulsion feed. The resulting emulsion feed was 28% fat, 0.2% saccharin, 1.0% casein sodium salt, 18% fat free chow and 0.65% of a sugar-free fiber supplement and 52.15% water. The fat free chow and soluble fiber were added to increase the volume of feces produced while the saccharin was added to increase the palatability of the feed. Fresh samples were prepared the day before the beginning of each trial and stored at 4 °C until required for use when they were allowed 1 h to warm to room temperature before being offered to the animals.

Animals

Male Sprague–Dawley (Harlan, Indianapolis, IN) rats, 80 days of age at the start of the study, were used as subjects. Animals were individually housed in hanging stainless steel wire cages in a temperature- and humidity-controlled environment placed on a 12:12 light:dark cycle. Three groups of 12 rats were received every 2 weeks for the evaluation of the three emulsion feeds (total number of rats = 36).

Upon arrival the rats were maintained for 4 days on a nutritionally complete commercially available pelleted rodent diet (Laboratory Rodent Diet 5001, PMI Feeds, Richmond IN; percent of calories as protein: 28.05%, fat: 12.14%, carbohydrate: 59.81%; 3.3 kcal/g), which was placed in hanging metal food hoppers at the front of the cage. On the fifth day, animals were transferred to hanging stainless steel metabolic cages that allowed for the separation of powdered chow, urine and feces. From days 5 through 9 the rats were maintained on the powdered fat free rodent diet placed in a stainless steel cup located in an enclosed alley that protruded beyond the back of the cage to prevent the contamination of fecal and urine samples. Fecal samples were collected from each animal on days 3–5 of the powdered chow regimen (days 7–9 of the study) and frozen (−80 °C) until required for analysis.

On days 10–14 of the rats were maintained on one of three emulsion feeds (STAG, LTAG and SA). All feeds were allowed to come to room temperature and were placed in the metabolic cages 15 min prior to the start of the dark period. Daily feed intake was measured and fecal samples were collected from each animal from days 3 to 5 of the fat/fat free chow emulsion regimen (days 12–14 of the study) 3 h after the start of the light cycle. Pooled fecal samples were frozen (−80 °C) until required for analysis.

It was observed in a pilot study that each emulsion would partly dry out during the overnight period. To calculate the amount of dehydration, two "blank" samples of each emulsion were filled to the same amount in a similar cup as was presented to the rats and placed on a table in the animal colony. Each sample was weighed at the beginning and end of the presentation period and the average loss in water weight due to evaporation was subtracted from the weights of the amounts consumed by each of the rats.

All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Tap water was freely available to the animals at all stages.

Fecal Fat Analysis

Frozen fecal samples were pulverized and homogenized using a laboratory blender (Warning Commercial, Torrington, CT). They were then dried at 70 °C for about 72 h in a vacuum oven (Model 5830, National Appliance, Co., Portland, OR) connected to an Air Cadet vacuum/pressure station (Model 7059-40, Cole-Palmer Instrument Co., Chicago, IL) until a constant weight was obtained. Fat was quantified by duplicate, after extraction with petroleum ether (60–80 °C) into pre-weighed dried aluminum cups, using a Soxtec Extraction Unit (HT 1043 Tecator, Prairie, MN). After extraction, the aluminum cups containing the fat were dried and weighed again. The fat content was obtained from the difference in cup weight and was expressed as a percentage of dry mass.

Differential Scanning Calorimetry

Calorimetric measurements were carried out using a VP-DSC micro-calorimeter (Microcal, Northampton, MA). Freshly prepared emulsions (without the addition of other feed ingredients) and thawed fecal samples were diluted with ultrapure water to a fat content <0.2 wt%. Aliquots (513.1 μL) were loaded into the instrument and heated to 80 °C at a rate of 55 °C h^{-1}, maintained for 10 min then cooled to 5 °C at 55 °C h^{-1} held for 10 min and reheated to 80 °C at the same rate. All samples were run against a reference cell filled with water.

Statistical Analysis

Data analysis was carried out using the PROC GLM procedure of the Statistical Analysis Software (SAS) (version 9.1, SAS Institute, Inc., Cary, NC). Differences were assessed using the Tukey's Studentized Range (HSD) post-hoc test and considered significant when $p < 0.05$.

Results and Discussion

Fecal samples from rats fed fat free powder (i.e., pooled samples from days 7 to 9) contained no detectable fat (Table 1). Rat feces typically contain very little fat even

Table 1 Total dry feces and fat percentage in feces for rats fed fat free chow and 28% fat emulsion feeds

Diet	Total dry feces (g)	Fat in dry feces (%)
Fat free chow ($n = 36$)	4.34 ± 0.82^{B}	ND
Palm oil (LTAG, $n = 12$)	1.78 ± 0.18^{B}	6.63 ± 0.31^{C}
Hydrogenated palm oil (STAG, $n = 12$)	0.64 ± 0.07^{B}	16.84 ± 1.45^{B}
Docosane (SA, $n = 12$)	32.42 ± 3.47^{A}	79.08 ± 0.99^{A}

Values are means ± standard error and corresponds to the pooled feces samples of the last 3 days of the 5-day feeding period. Values in the same column with different superscript letters are significantly different ($p < 0.05$)

ND not detectable by performing fat ether extraction

when the animals are fed a normal diet [21] and clearly the fat-free chow served to reduce this to a baseline of effectively zero.

All of the emulsion diets were acceptable to the rats (Table 2) and their intake produced sufficient feces for analysis (Table 1). However the LTAG and SA were much more acceptable than the STAG. In addition, even though the amount of feed consumed was not statistically different between the LTAG and SA diets, energy intake was significantly lower when the SA diet was consumed (t test, $p < 0.0001$), due to the reduced energy density of the diet. There is no obvious pattern relating the properties of the oil to acceptability (i.e., solid vs. liquid, alkane vs. triacylglycerol) and at present we have no explanation for the differences in acceptability observed. Others have reported differences in the acceptability of solid and liquid, digestible and indigestible oils in rats as described in the introduction. Our results with the LTAG and SA diets are consistent with other reports showing acceptability of diets containing either palm oil [e.g., 22, 23], or non-digestible oil-like substances [14–17], respectively.

In contrast to the LTAG and SA diets, intake of the STAG diet was quite low. Hydrogenated palm oil has rarely been used in rat feeding studies. In several studies it was used to assess effects of fatty acid deficiencies [24–26]. However, the present study was not long enough for deficiencies to develop [27], thus the low intakes were not likely due to the lack of n-3 and n-6 fatty acids. In addition, if a lack of essential fatty acids were the cause of the reduced intake, then the SA diet should have had the same effect. Instead, SA intakes were comparable to LTAG. In a study in which fatty acid deficiency was not present, the presence of 4% hydrogenated palm oil did not result in reduced intake [15]. The present study indicates that at a higher concentration, hydrogenated palm oil is not acceptable to rats, although the reason for that remains unclear.

When the animals consumed the fat emulsion diets, fat was found in all fecal samples (i.e., pooled fecal samples from days 12 to 14) at a concentration depending on the lipid type (SA $\gg$ STAG $>$ LTAG; Table 1). The proportion of fat not excreted and hence absorbed by the rats was calculated from the differences between daily fat intake and fat in feces (Table 1). While almost all of the triacylglycerol oils were absorbed, almost none of the SA was absorbed.

It is not surprising that almost all of the alkane consumed was excreted in the feces as mammals lack the enzymes to digest such oils. Palm oil, like most triacylglycerols, is readily digestible (e.g., Manorama and Rukmini [28] reported fat absorption of 94% for palm oil) and it appears neither emulsification nor hydrogenation provided protection to the fat in the present work. This is contrary to the findings of Kaplan and Greenwood [7] who showed that extensive hydrogenation can reduce the digestibility of fats in rats (e.g., the digestibility coefficients were 30.9% for hydrogenated soybean oil and 97.0% for partially hydrogenated vegetable oil). Similarly, when Granlund et al. [29] generated a very high melting point fat by totally hydrogenating fish oil, rats excreted greater quantities of this lipid in the feces than of soybean oil in a control diet. It seems likely that the higher digestibility of the hydrogenated palm oil used in this work is due to the fact that the sample was not fully hydrogenated and contained a fraction of liquid oil or alternatively the fat in the present work was emulsified and therefore more accessible to the digestive enzymes. We hypothesize that as the lipase begins to act on the liquid oil fraction the reaction products act as a solvent to dissolve the solid fraction and facilitate its subsequent breakdown. Other workers have shown that the digestibility of typical food lipids does not depend on

Table 2 Feed intake and fat retention data for rats fed the 28% fat emulsion feeds

Fat emulsions	Feed intake (g/day/rat)	Fat retention[#] (g/day/rat)	Retention (%)
Palm oil (LTAG, $n = 12$)	26.65 ± 1.63^{A}	7.58 ± 0.46^{A}	99.60 ± 0.02^{A}
Hydrogenated palm oil (STAG, $n = 12$)	4.93 ± 0.59^{B}	1.37 ± 0.16^{B}	97.21 ± 0.34^{A}
Docosane (SA, $n = 12$)	32.13 ± 2.91^{A}	0.59 ± 0.16^{B}	8.72 ± 3.36^{B}

Values are means ± standard errors. Values in the same column with different superscript letters are significantly different ($p < 0.05$)

[#] Because the "retention" data was not normally distributed, the statistical analysis was also performed with log-transformed values and the same differences were found

crystallinity. For example Rumpler et al. [30] demonstrated that the energy available from beef tallow and corn oil is similar in humans; while beef tallow is solid it has a liquid oil fraction [31].

The appearance of the feces was characteristic of the feed consumed (Fig. 1). LTAG and STAG emulsion diets led to the production of feces darker than those from the fat-free diet while the SA emulsion led to the production of whiter, harder and more brittle pellets due to their high fat content. There were no obvious signs of distress in the animals consuming any of the test diets. This is in contrast to preliminary studies conducted with similar rats and a similar diet formulated with mineral oil. Although the mineral oil diet was acceptable to the rats, its consumption led to the almost immediate excretion of large volumes of liquid oily feces. This diet was discontinued due to concerns of thermoregulation and nutrition but the contrast with the SA feed is evident.

Although a significant fraction of the fat ingested as an emulsion is excreted in the feces it is not clear to what extent the emulsion structure survives the digestion process. The LA emulsions used in the preliminary work were completely disrupted during gastric transit, but even if the interfacial protein was digested in the SA emulsion there is no obvious mechanism for coalescence in the absence of liquid oil. The fecal pellets contained too much insoluble material for any residual droplets to be sized by light scattering and it was not possible to detect the fine particles by optical microscopy. However it is possible to differentiate emulsified from bulk fats from differences in their

Fig. 1 Feces collected from rats fed **a** fat free chow; **b** LTAG/fat free chow emulsion; **c** STAG/fat free chow emulsion and **d** SA/fat free chow emulsion (pooled samples of the last 3 days of feeding period)

crystallization behavior [32]. Bulk lipids typically crystallize a few degrees below their bulk melting point while the same fat in a fine emulsion droplet can be supercooled by 10–20 °C before it crystallizes.

The positions of the melting transitions in the heating thermograms of fecal and emulsion samples were similar, although the peaks from the feces were smaller due to their lower fat content (Fig. 2). The final melting points were similar to the melting points of the corresponding bulk fats (STAG: 36 °C and SA: 36–39 °C). Melting behavior is characteristic of the fat present and its polymorphic form but not significantly characteristic of the structure of any emulsion present [33]. Consequently we can conclude that the fat in the feces is of a similar chemical composition (i.e., not contaminated by other fatty substance excreted in the rat's intestine) and the crystals were in the same polymorphic form.

The onset of crystallization in the STAG emulsions occurred at about 27 °C below the melting point which is characteristic of crystal nucleation in fine dispersions. However, the same STAG in the feces of rats fed these emulsions crystallized only 7 °C below the melting point which is much more characteristic of crystallization in a bulk fat. The onset of crystallization in the SA emulsions occurred about 25 °C below the melting point of the fat which is once again characteristic of crystal nucleation in a fine dispersions. The crystallization thermogram of the feces was more complex, with one portion of the fat crystallizing at the same temperature as the emulsified docosane and another portion at a slightly higher temperature. However, none of the docosane in the fecal samples had crystallization behavior corresponding to bulk docosane ($\sim$37 °C).

It appears that the STAG emulsion is broken during the processes of digestion and the undigested lipid merges to form larger droplets prior to excretion. On the other hand, a good portion of the dietary SA emulsion is excreted as intact droplets. This may be because although the STAG is solid at body temperature it is a complex mixture of components with a small liquid phase that can facilitate partial coalescence.

In summary, rats readily accepted feeds prepared from emulsions of a solid alkane and liquid palm oil triglycerides but less readily from solid palm oil triacylglycerides. Only a small fraction of the triacylglycerides was detected in the feces and this was apparently composed of larger fat aggregates and not the original droplets. The solid triacylglyceride was absorbed to a similar extent as the liquid. Thus, the incorporation of solid palm oil droplets into the food reduced acceptability without providing meaningful protection from fat retention. Whether this would be the case with other triacylglycerols bears further investigation.

In contrast to the results obtained with the solid triacylglyceride, acceptability was high and retention was low

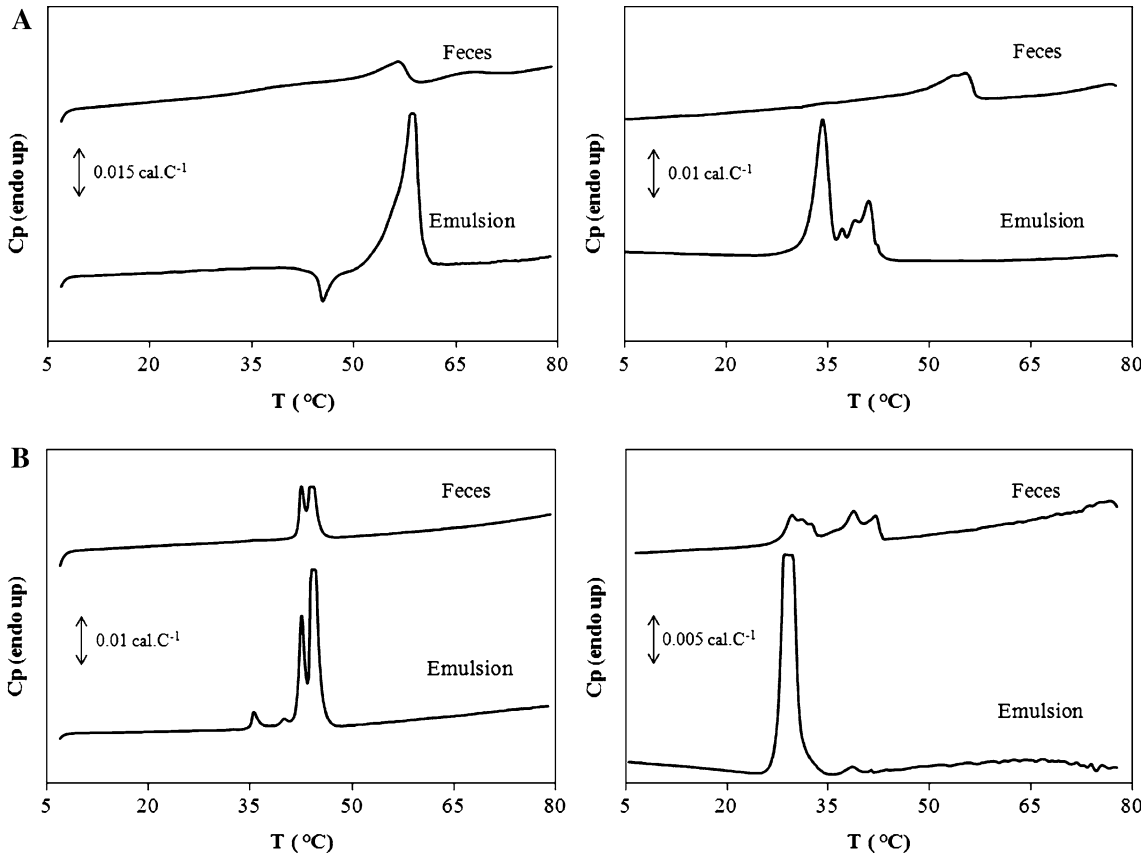

Fig. 2 Heating (*left*) and cooling (*right*) thermograms of STAG (**a**) and SA (**b**) emulsions and their corresponding feces samples

when the solid alkane was incorporated into the food. Such an approach may provide a useful way to reduce the fat content of food, while maintaining palatability. In addition, almost all of the alkanes were excreted in the feces; the solid alkanes apparently as intact droplets. Any indigestible lipid would probably absorb fat-soluble vitamins and carry them out of the body; therefore, some supplementation would be necessary to allow safe long-term intake.

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