

Lipid Deposition on the Tongue after Oral Processing of Medium-Chain Triglycerides and Impact on the Perception of Mouthfeel

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Lipids between the tongue and palate strongly contribute to the sensory impact of a product. Mouthfeel is a sensory attribute responsible for distinguishing reduced fat from full fat food products. The aim of this work was to quantify the distribution, deposition, and retention of lipids on the tongue and palate upon oral processing and relate this to texture. The thickness of lipid deposition was measured in mouth by fluorescence. A trained panel evaluated the perceived intensity of samples to describe lipid Mouthfeel. The thickness of lipid deposition on the tongue shows spatial variation (10–100 μm) and stopped increasing after intakes higher than 8 mL of medium-chain triglycerides (MCTs). After 2 min, only 25% of the lipid deposition was retained on oral surfaces. A difference in the thickness of lipid deposition of 25 μm resulted in significantly different perception. This work describes the in-mouth behavior of food lipids and its influence on texture perception.

KEYWORDS: Lipids; tongue; in mouth; clearance; fluorescence; Mouthfeel; texture; perception

INTRODUCTION

Modern consumers ask for less fat in their foods without reducing its pleasantness (1). The food industries are under considerable pressure to find the best way to reduce fat while maintaining the perception in their traditional and novel products. However, fat plays a variety of roles in foods, and therefore, it is perceived by different mechanisms (2). In recent years, several studies have tried to understand the mechanisms that drive fat perception. At the beginning, it was believed that fat is perceived only as a food texture. However, we know now that lipids also influence the aroma profile, the taste, and the perceived temperature of food.

Texture perception is complex and opens many research avenues (3). In semi-solids and liquid foods, it is related to product viscosity (4). Nevertheless, correlating texture perception to viscosity is not straightforward, because many foods are not Newtonian solutions. The viscosity of non-Newtonian fluids changes depending upon the shear force applied to the product. Researchers have started to evaluate the shear forces that occur in mouth. In addition, viscosity also changes because of the degradation of thickeners by salivary amylase (5). Lubrication

is another factor related to texture, and it has been largely investigated in the recent years. It is strongly influenced by product properties, such as viscosity, as well by the environment, such as temperature, force, wettability, and roughness of the surface (6–9). Knowing all of these parameters should allow for the prediction of the lubricating properties of the product in mouth.

Lipids are a good solvent for many aromas. The aroma profile changes with modified lipid content (10). Many studies have explored this area, and it has been shown that, besides the product matrix and aroma properties, also the oral environment influences the aroma release (11). Some studies have hypothesized that lipids are sensed, because of a different heat-transfer capacity of lipids (12) or because they contribute to a taste stimuli by free fatty acids (13, 14). For a better understanding of possible mechanisms and their interactions, it is important to study the behavior of lipids in mouth. Knowing how lipids interact, are spread, and are retained on the oral surface should bring valuable insight in understanding the differences in perception of aroma, texture, or even taste.

Therefore, novel *in vivo* instrumental methods that reflect perceived oral texture are very helpful (15). Recently, Adams et al. (16) visualized *in vivo* food residues in mouth. They showed that pure oils are emulsified with saliva during oral processing. Another study (17) correlated the turbidity of oral water rinses with sensory attributes, such as creamy, fatty, sticky, and airy, for a series of dairy desserts with fat contents varying between 0 and 15%. However, this study did not address the

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compositional changes of lipid deposition and did not provide any quantitative data on the distribution and retention of food compounds on the oral surfaces. Another study (18) described lipid deposition semiquantitatively by taking and evaluating swabs from the tongue with attenuated total reflection Fourier transform infrared (ATR FTIR) spectroscopy. The follow-up study (19) showed no difference in deposition on oral surfaces; however, clearance of lipids was much faster compared to the clearance of proteins or carbohydrates from the oral surfaces. Electrostatic interactions of saliva or mucus with food components is a possible mechanism that drives the deposition of compounds on oral surfaces (20, 21).

Lipids that are in contact with the tongue and palate could strongly influence the sensory impact of a product. Therefore, the direct measurement of undisrupted residue provides valuable information and contributes to the understanding of the behavior of food components in mouth and their influence on perception. The aim of this work was to investigate the influence of oil volume, after being processed in mouth, on the deposition of lipids on the tongue and palate. The focus of the study was also on the spatial variation of the thickness of the lipid deposition and its retention over time. We observed a correlation between the lipids deposited on the oral surface and the perception of Mouthfeel. For our study, we have used medium-chain triglycerides (MCTs), because it is tasteless and constituted mainly from two fatty acids.

MATERIALS AND METHODS

The design of our study was to investigate the lipid deposition on oral surfaces and its impact on the perception of Mouthfeel. We investigated factors that influence lipid deposition, such as MCT intake volume, time after spitting out, and the position at the tongue surface. A sensory study was performed to evaluate the impact of the differences measured as a lipid deposition on a mouthfeel perception.

Materials. Samples used were MCTs Delios V by Cognis (Monheim, Germany) and curcumin 95% as a natural extract from Naturex (Avignon, France). Bottled Vittel water by Nestlé (Vittel, France) was used for rinses. Plastic Pasteur pipettes and Falcon tubes by Becton Dickinson Labware (Le Pont de Claix, France) were used to deliver samples to subjects.

Oral Processing Protocol. Subjects performed the test at 9 a.m. in the morning. Prior to oral processing of each sample, the subjects rinsed their mouth with water. Various amounts of MCT were given at room temperature. Samples were freely moved around the mouth for 30 s and subsequently spat out twice (processing time of about 5 s). After spitting out, the subjects moved the tongue back and forth against the palate. Then, either thickness of lipid deposition or perceived intensity was measured (Figure 1).

Determination of the Thickness of Lipid Deposition on the Tongue and Palate. Six subjects (three men and three women, with a mean age of 27 years) performed the test at 9 a.m. in the morning. Various amounts of MCTs (0.5–16 mL), containing 65 ppm curcumin, were manipulated as described previously. Then, the intensity of lipid deposited on the oral surface was respectively measured, immediately

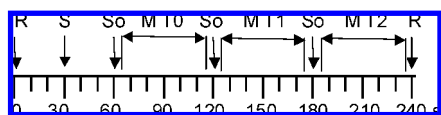


Figure 1. Timeline of each sample manipulation. Subjects rinsed (R) the mouth prior to the test. The stimuli (S) was delivered and moved freely around the mouth. After 30 s, subjects spat out (So) the stimuli. Immediately after the expectoration (T0), the measurement (M) was performed. After 1 min from the first expectoration (T1), the subjects spat out again and the second measurement was performed. After 2 min from the first expectoration (T2), the subjects spat out again and the last measurement (M) was performed, followed by rinsing (R).

after spitting out (T0), after 1 min (T1), and after 2 min (T2) (Figure 1). A total of 12 points of measurement were evenly distributed on the dorsal surface of the tongue (Figure 2). Five measurements were taken in the lateral area, including one on the tip of the tongue. Additional measurements were taken in the central part of the tongue, which was separated into front and back areas. Besides measurements on the tongue, one point was measured also in the middle of the palate. Fluorescence was measured with a Cary Eclipse from Varian (Victoria, Australia) coupled with a fluorescence remote read fiber-optic probe fitted with a tip for measurement of the solid surface (22). Measurements were performed at an excitation wavelength of 440 nm and an emission wavelength of 515 nm, with an average measuring time of 0.5 s at 32 °C. Tests were done in triplicates. The background of an oral surface was subtracted from the measured intensity. The fluorescent intensity was translated into the thickness using various amounts of MCTs spread on a Petri dish.

The raw thickness data were first log-transformed because they were heteroscedastic (standard deviation was dependent upon the mean). Data from each subject were then normalized (all measurements were transformed into *z* scores) to remove subject variability and to only keep variability because of the amount of MCTs. A three-way analysis of variance (ANOVA) was finally performed on the normalized log-transformed data to estimate the impact on the thickness of lipid deposition. MCT intake volume, position of measurement on the tongue, and time after spitting out the sample were chosen as factors for ANOVA. Fisher's least significant difference (LSD) was selected as the multiple comparison procedure. A 95% confidence level was applied for all tests.

Mouthfeel Perception. The sensory panel consisted of nine subjects (women, with a mean age of 45 years), who were previously trained on using the defined sensory attributes (Table 1) for evaluating in mouth sensations when consuming of semi-liquid food products. They evaluated samples on a 10-point scale with anchors (1, not at all; 10, very) for three attributes describing lipid deposition ("lubricating film", "fatty film", and "sticky film"). Samples were evaluated immediately after spitting out or 1 min after spitting out. Tests were performed in duplicate. The sensory attributes were evaluated only after spitting out and not during oral processing. Consequently, the attention of the panelists was drawn toward the perception of the actual deposition of lipids on the oral surface and not to the primary perception of oil volume put in mouth. However, we can not totally exclude that primary perception affects final perception.

The subjects were seated in sensory booths with controlled temperature and ventilation. A red light was used to minimize the visual input. To avoid possible olfactory cues, the subjects wore nose clips throughout testing. The subjects received all five samples during a session of 1 h, which was replicated 3 days later. The sample presentation was balanced among panelists according to a Latin square design. The samples were coded with 3-digit codes and were served in Falcon tubes. Three samples of pure MCTs (0.5, 1, and 2 mL) were delivered directly into the mouths of panelists with a pipet, to avoid visual clues of different volumes. Subjects were instructed to follow the previously described mastication protocol. The three attributes were evaluated immediately after first spitting out and again after 1 min (Figure 1). Subjects were instructed to thoroughly rinse their mouths with water between samples. During the sessions, panelists could drink and rinse with water ad lib. Scoring was made through a computer on an unstructured linear scale anchored on each end with the labels "none" (value of 0.0) and "very" (value of 10.0) presented according to the test design by the FIZZ software (Biosystems, Couternon, France). An ANOVA was performed on the raw sensory data to estimate the impact of the MCT volume intake and time on the thickness of lipid deposition. A Duncan pair comparison test was selected as the comparison procedure. A 95% confidence level was applied for all tests. MCT intake volume and time after spitting out were chosen as factors for ANOVA.

RESULTS

Thickness of Lipid Deposition. The thickness of lipid deposition increased with increasing MCT volumes up to 8 mL (Figure 3). The thickness of the lipid deposition increased

Table 1. Description of Sensory Attributes and Protocol for Evaluation

sensory attribute	description (from “none” to “very”)	evaluation protocol
“lubricating film”	sensation of a thin deposition film covering the mouth; it is essentially perceived on the lips and the palate/tongue and makes them slide with ease on one another	after spitting out the product: slide your tongue on the palate and on the lips and then slide your lips
“fatty film”	sensation close to the feeling of having a layer of fat or oil covering the mouth	after spitting out the product: slide the tongue on the palate and lips and the lips on one another
“sticky film”	describes the force needed to unstick the tongue from the palate; sensation of a product adhering between the tongue and the palate; could also be perceived between the lips	after spitting out the product: put your tongue onto the palate and unstick it; do the same with the lips

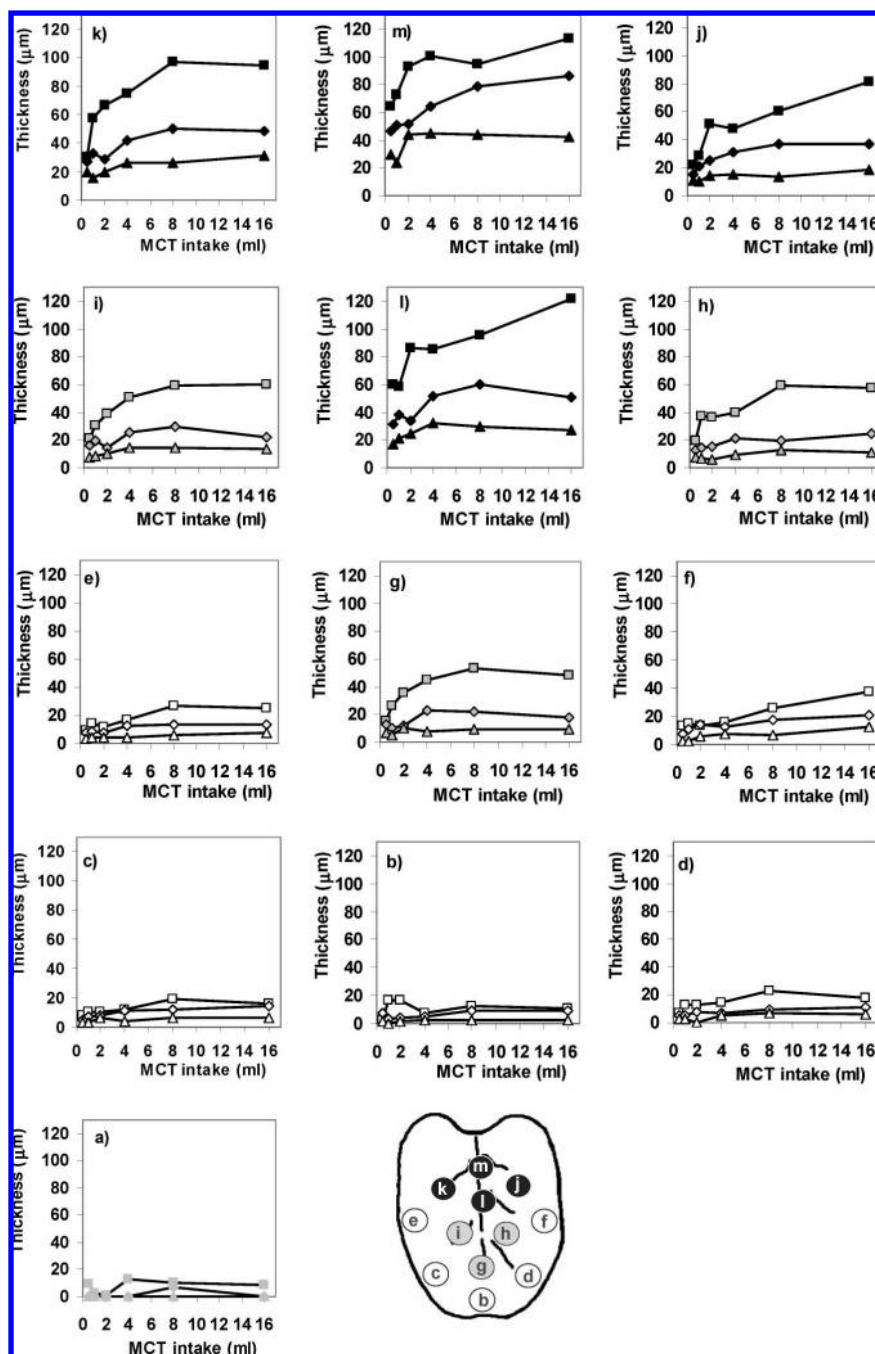


Figure 2. Influence of MCT intake volume on the thickness of lipid deposition at each position (a, palate; b–m, tongue) by the oral surface, depending upon the time after spit out [square, immediately after (T0); diamond, 1 min after (T1); triangle, 2 min after (T2)]. The line to connect points is only used for the visualization of the thickness, depending upon MCT intake at different times after expectoration. Each point is a mean of six panelists performed in triplicates. Different colors represent different areas of the tongue (black, back; gray, front; white, lateral).

very rapidly at low volumes and much slower at higher volumes. Already, 1 mL of MCT intake reached more than

half of the thickness for the volume of MCT intake at 8 mL. Furthermore, an increase from 8 to 16 mL of the volumes of

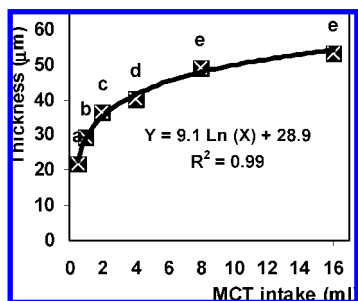


Figure 3. Regression of the thickness of lipid deposition on the oral surface, dependent upon the MCT intake volume. Each point is a mean of all oral positions for six panelists performed in triplicates. Different letters represent significant difference.

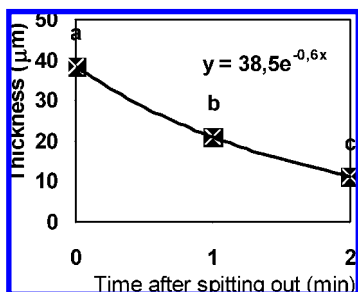


Figure 4. Regression of the thickness of lipid deposition on the oral surface, dependent upon the time after spit out. Each point is a mean of all oral positions at all MCT intake volumes for six panelists performed in triplicates. Different letters represent significant difference.

MCT did not significantly change the thickness, suggesting that the oral surface possibly reached a saturation level for lipid deposition.

Spatial Variation of Lipid Deposition. Distribution of lipids on the tongue and palate was not uniform (Figure 2). The thickness of lipid deposition on the palate was much lower than that on the tongue, which was always below 10 μm . The thickness of lipids on the tongue varied significantly depending upon the place of measurement. The measurement positions on the tongue were chosen in a such way that covered most of the tongue. There was less lipid deposition on the lateral area of the tongue than on the central area (Figure 2). Even in the central area, differences were observed between the front and back of the tongue. Positions in a front area had significantly lower mouth coating than the positions at the back of the tongue (Figure 2). Increase in thickness was also observed between the positions in lateral areas, whereas the tip of the tongue had significantly less lipid deposition compared to the back.

All positions on the tongue showed an increase in thickness of the lipid deposition, with an increasing volume of MCTs ingested. When the volume exceeded 8 mL, no significant increase of thickness could be observed (Figure 3).

Retention of Lipids on Oral Surfaces. Lipids were only weakly retained on oral surfaces. In fact, the clearance of lipid deposition followed an exponential decrease. The thickness was reduced by almost half after 1 min, and slightly more than a quarter of the thickness was left after 2 min (Figure 4).

The difference in thickness of the lipid deposition between the different volumes of MCT intake decreased with time after spitting out. After 2 min, the thickness of lipids was reduced to almost the same level, independent of the volume of MCT intake.

The lipid deposition shows spatial variation on the tongue. This spatial variation was observed also during clearance of the lipids (Figure 2). From our results, it seems that the rate of clearance is similar for all positions on the tongue.

Table 2. Logarithmic Regression ($Y = A \ln(X) + B$) of the Thickness of the Lipid Deposition Depending upon the Volume of MCT Intake at T_0^a

position	A	B	R^2
b	0.8	10.4	0.04
c	2.8	9.7	0.74
d	3.7	10.8	0.77
e	5.0	12.0	0.84
f	6.4	13.6	0.73
lateral	3.7	11.3	0.83
g	10.4	26.4	0.88
h	10.7	30.4	0.88
i	12.1	30.9	0.97
front	11.1	29.2	0.95
j	16.1	31.8	0.93
k	18.5	50.9	0.92
l	17.3	66.6	0.90
m	13.2	76.0	0.98
back	16.3	56.3	0.97
mean	9.1	28.9	0.99

^a Regressions are made for all positions on the tongue and their means [b–m, positions on the tongue (Figure 2)].

Regression of the Thickness of Lipid Deposition on Oral Surfaces. Our results showed that the deposition of lipids on oral surfaces followed a logarithmic curve as a function of the volume of MCT intake (Figure 3). The distribution of lipids on the tongue shows spatial variation. Different areas of the tongue also showed different saturation curves (Table 2). The fitting parameters of the logarithmic function increased with the tendency of the position for higher lipid deposition. Positions on the same area of the tongue had similar constants (Table 2). The retention of lipids on oral surfaces was weak and could be described by an exponential decrease (Figure 4). These two functions are the regression of the mean thickness for the lipid deposition, dependent upon the intake volume of pure MCTs from immediately after to 2 min after spitting out (eq 1).

$$\text{thickness } (\mu\text{m}) = (9.1 * \ln V + 28.9) * e^{-0.6 * t} \quad (1)$$

This equation estimates the thickness of lipid deposition as a function of the intake volume of pure MCT (Figure 3) at any time after spitting out (Figure 4) [V is the volume of pure MCT intake (mL) and t is the time after spitting out (min)]. For this equation, we have used mean values of all oral positions for six panelists performed in triplicates to obtain fitting parameters 9.1 and 28.9. For a fitting parameter -0.6 , we used a mean of all oral positions and all MCT intake volumes for six panelists performed in triplicates.

Mouthfeel Perception. Subjects rated three sensory attributes for all of the samples according to perceived intensity. Greater volumes of pure MCT intake increased perceived intensity for the “fatty film” attribute (Figure 8) and for the “lubricating film” attribute (Figure 7) immediately after the first spitting out. A significant increase in perceived intensity for “fatty film” and “lubricating film” was observed between samples of 0.5 and 5 mL of pure MCT. There was no significant difference observed between all of the volumes of MCT for the “sticky film” attribute.

The clearance of lipids from the oral cavity was observed 1 min after the first spitting out, and it decreased ratings to a much lower perceived intensity for all attributes. Differences between the samples decreased, but they were still significantly different between 0.5 and 5 mL samples for “lubricating film” and “fatty film”.

Link between Perceived Intensity and Thickness of Lipid Deposition. The attributes “fatty film” and “lubricating film” best described the relationship with measured thickness of lipid

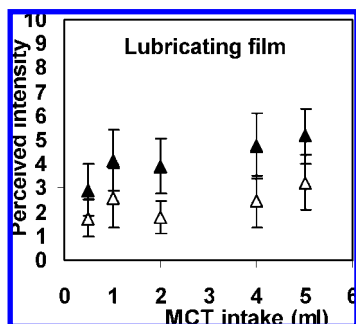


Figure 5. Perceived intensity of “lubricating film” as a function of the volume of MCT intake and depending upon the time after spitting out at T0 (▲) and T1 (△).

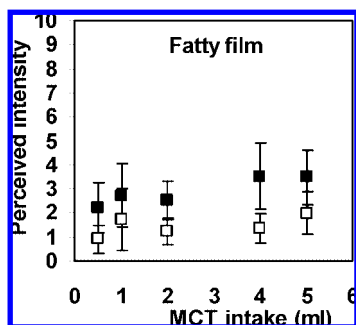


Figure 6. Perceived intensity of “fatty film” as a function of the volume of MCT intake and depending upon the time after spitting out at T0 (■) and T1 (□).

deposition (**Figures 7 and 8**). A significant difference was perceived between the highest and lowest MCT volumes. This was correlated with the thickness of lipid deposition that was also the most different between the highest and lowest MCT volume. Even though the increase in the MCT volume did not statistically significantly change the perception, it showed a tendency to increase intensity of perception. However, there was a significant increase in the thickness of lipid deposition for each MCT intake volume until saturation was reached.

An approximately 50% decrease was observed in the thickness of lipid deposition after 1 min. The same decrease was observed with the perceived intensity of the sensory attributes (**Figures 5 and 6**), which were related to the thickness of lipid deposition and therefore also decreased to around 50% after 1 min (**Figure 7 and 8**).

The distribution of lipids on the tongue and palate shows spatial variation. The thickness of lipids on the tongue varied significantly depending upon the location of measurement. Three main areas of the dorsal surface of the tongue were assigned on the basis of differences in thickness of lipid deposition: lateral, front, and back. All areas of the tongue possibly contributed to the perception of the sensory attributes.

DISCUSSION

In our study, we have used MCTs as a sample to show some trends of lipid behavior on oral surfaces after expectoration. Only future work will show how far the differences of oil in physicochemical properties are influencing this behavior. The volume of MCT influences the amount of lipids that adheres to the oral surfaces and is directly related to the perception. This finding is in agreement with all previous studies that describe an increase in perception ratings because of higher fat content (*1, 6, 10*). Interestingly, the amount of lipids staying

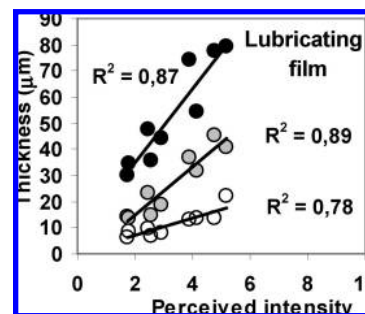


Figure 7. Correlations of perceived intensity of “lubricating film” with the mean thickness of the lipid deposition of each area on the tongue (R^2 , correlation coefficient; white circle, lateral; gray circle, front; black circle, back). The thickness of the lipid deposition for 5 mL of an intake volume of MCT was measured on a different day as for other volumes.

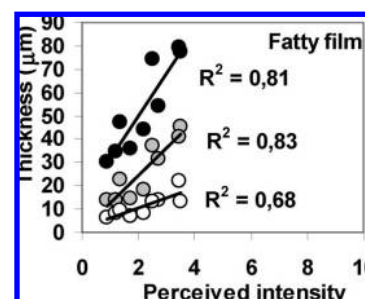


Figure 8. Correlations of perceived intensity of “fatty film” with the mean thickness of the lipid deposition of each area on the tongue (R^2 , correlation coefficient; white circle, lateral; gray circle, front; black circle, back). The thickness of the lipid deposition for 5 mL of an intake volume of MCT was measured on a different day as for other volumes.

on the oral surface increased logarithmically. Meaning that after a certain volume of oil, lipid deposition was no longer significantly increased. Few studies also showed with an isoviscous emulsion system that, after a certain percent of fat, the perception of Mouthfeel does not increase anymore (*9, 23*). Pure oil is a good model to study the behavior of lipids in mouth toward oral surfaces. However, lipid behavior in mouth might be impacted by the food structure, such as emulsion droplets.

Another question is what kind of adhering forces might be involved in the deposition of lipids on oral surfaces. Therefore, we evaluated the spatial variation and retention of lipids on oral surfaces. We found that the thickness of lipid deposition on the palate was much lower than on the tongue. A previous study did not show selective retention of oil in the oral cavity nor in different subjects (*19*). This might be due to their oral sampling method using swabs. The latter method has a strong limitation in quantitative evaluation. Another study shows the importance of surface wettability for lubrication of food products. The palate with its smooth surface has a much more hydrophilic surface compared to a rough tongue surface (*8*). Therefore, this could explain the tendency of lipids to adhere to the tongue surface rather than to the palate. Moreover, the thickness of lipids on the tongue significantly varied, depended upon the area of the tongue. Lateral areas of the tongue showed lower lipid thickness than central areas. The central area of the tongue has larger papillae, resulting in more hydrophobic surfaces than lateral areas of the tongue (*24*). This speaks in favor of the importance of surface wettability for lipids to adhere to the surface. However, in our study, we observed spatial variation of the lipid deposition. The origin of these differences might be the microstructure of a deposition on the tongue, stronger rubbing

of the lateral area of the tongue against the palate and teeth, or higher exposure of lateral areas to saliva flow.

In agreement with other studies, we showed a fast clearance of lipids from the oral surfaces (19). The clearance of lipids could be described as an exponential decrease, which was observed also in a previous study for the clearance of sucrose (25). The effect of saliva secretion during oral processing on actual deposition and clearance of the lipids on the tongue and palate is not known. We found that lipids were washed at similar rates, independent of the amount of oil ingested and the position in the oral cavity. This is in agreement with a previous study, which states that oil is cleared from all oral surfaces at comparable rates (19).

In our study, we have showed that the intake volume of oil influences lipid deposition on oral surfaces. The lipid deposition has shown spatial variation. A total of 75% of lipid deposition has been cleared out from the oral surface just after 2 min. However, one could criticize the interpretation of the fluorescence intensity at the tongue to the thickness of lipid deposition, because we did not take into account the exact surface of the tongue. More investigation is needed to understand the structure of the lipid deposition. The height of papillae is much higher than the thickness of lipid deposition that we have measured. A saliva-covered tongue surface is quite hydrophilic, and even after the mucous layer is removed, a distinct contact angle remains (7). There are several ways how oils could be dispersed in the mouth into an emulsion (26). Therefore, it is not evident that the remaining lipids are present in form of a spread layer. More likely, unless the amount of lipids is very high, adherent lipids would be present as patches at the tongue surface. Another possibility is that the adherent lipids are present in the form of emulsion droplets in a mucus layer, such as Adams et al. indicate (16). One could also argue that a significant part of the oil will reside in the clefts between the papilla and, therefore, might not be measured with our method. Observed spatial variation of the thickness could be due to different heights of papillae on the tongue or the difference in forces acting while the tongue is moved against the palate. Future research should be made in a direction to better understand the microstructure of the food depositions on the oral surface.

Sensory attributes were linked with the deposition and retention of lipids on the tongue and palate. "Fatty film" and "lubricating film" were correlated with the thickness of lipid deposition. After 1 min, the clearance of lipids from the oral cavity was observed and sensory intensity ratings were decreased to a much lower perceived intensity for all attributes. The thickness of lipids deposited on the tongue related well to sensory attributes. Our study has shown that we require optimal thickness of lipid deposition to generate a "lubricating film". According to a recently published study (27), a difference of 25 μm in thickness is detected between the tongue and palate. This relates well to our findings, in which the mean thickness of lipids remaining on the tongue was around 25 μm and, therefore, perceived significantly different as well.

We conclude that lipid deposition on oral surfaces, especially on the tongue, is related to sensory perception. The thickness of lipid deposition on the tongue shows spatial variation and increased with an increasing amount of oil until saturation was reached. The lipids were, however, only weakly retained on the oral surfaces.

ABBREVIATIONS USED

MCT, medium-chain triglyceride; ANOVA, analysis of variance; LSD, least significant difference; T0, immediately after spitting out; T1, 1 min after spitting out; T2, 2 min after spitting out.

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