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Assessing Lipid Coating of the Human Oral Cavity after Ingestion of Fatty Foods

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The coating on the oral surface plays a significant role in mouthfeel perception, which is an important attribute governing perception of reduced-fat or low-fat food products. The aim of this work was to develop efficient methods to quantify the coating of the lipids covering the oral surface after ingestion of fatty foods. Three different approaches were assessed to investigate the in-mouth behavior of fluid foods in a subject. A first approach determined the mass of lipids retained in the oral cavity by rinsing it out. A second approach evaluated the thickness of the lipid coating on the tongue by fluorescence measurement using a dye incorporated in oil. Finally, a third approach measured local lipid thickness by adsorption of the lipids on a filter paper. All applied methods showed results in the same range. The thickness of the lipid coating was on average between 5 and 32 μm. The total lipid recoveries obtained by controlled rinses from the oral cavity were approximately 96 and 50% for single filter paper. A fast, simple, and quantitative method was developed to measure the thickness of the lipid coating on an oral surface after ingestion of fatty foods. This work presents the potential of the method to investigate in-mouth distribution and residues of lipids and establishes new avenues to study in-mouth behavior of food components and its influence on the sensory perception.

KEYWORDS: Lipids; in-mouth; food residue; fluorescence; texture

INTRODUCTION

Dietary choices are strongly influenced by the perception of food taste, smell, and texture, which depend strongly on the lipid content in foods (*1*). Removal of lipids from food significantly changes characteristic aroma and texture properties of many foods and affects their perception. Many studies have shown that texture is an important parameter, but not the only one, by which lipid content is judged (*2*). Texture perception takes place during the dynamic process of food breakdown in the mouth and is affected by oral processes, such as motility, saliva, and temperature (*3, 4*). Recent studies have underscored the importance of an interaction between the oral environment and the food structure, which might drive substantially the resulting sensory perception. Weenen et al. (*5*) showed that texture attributes of semisolid foods can be grouped into six categories. One of the six categories is particularly related to the sensory functionality of oil. It includes attributes such as fatty, creamy, and coating. Another study performed by de Wijk et al. (*6*) suggested that the rough-creamy sensory dimension is primarily related to fat content. They proposed lubrication (friction) and flavor release as possible mechanisms by which lipids can affect sensory attributes. Creaminess and fatty aftertaste are among the sensory attributes that are the most typical for emulsified foods (*5–7*). All of these findings identify lipids as one of the key drivers for the sensory perception of food.

The release of flavor from the mouth coating can be modulated with designed emulsions. These emulsions may directly interact with the oral mucosa (*8*) and are suspected to deposit more oil onto the mouth surfaces. This in turn could lead to higher flavor availability. Another study showed that the coalescence of the emulsion occurs during the movement between two surfaces, which was done in conditions comparable to rubbing the tongue against the palate (*9*).

Lipid mouth coating is defined as residual lipids from food that stick onto the oral surface, and it is believed to contribute to several sensory attributes. However, a mechanism of formation of the lipid mouth coating is not known. There is almost no information on its composition and structure or on possible interactions that contribute to its formation. It is postulated that fat lubricates the movement of the food bolus surface along the oral tissue, which decreases perceived dryness and roughness and increases perceived fattiness and creaminess (*10*). Hence, fat-related attributes along the rough-creamy dimension primarily reflect surface properties and are driven by the amount of

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fat. Those attributes can be differentiated in preswallowing (mouthfeel) and postswallowing (afterfeel) sensations. The latter seems to be investigated more easily, because it should be possible to isolate and analyze the stimulus on oral mucosa, responsible for the afterfeel. Recently, a method to study mouth coatings by measuring the turbidity of oral water rinses was reported (*10*). The turbidity of oral rinses was correlated with sensory attributes such as creamy, fatty, sticky, and airy, for a series of dairy desserts varying in fat content between 0 and 15%. However, this study did not reveal the composition of the mouth coating. Another study (*11*) evaluated semiquantitatively the mouth coating by taking and evaluating swabs from the tongue with ATR FT-IR spectroscopy. Very recently, Adams et al. (*12*) visualized in vivo food residues in the mouth. They showed that pure oils are emulsified with saliva during oral processing.

The aim of this work was to develop a simple, fast, and reliable method to obtain quantitative information on the lipid coating after ingestion. In this study we have compared three different approaches to quantify low amounts of lipids remaining on the oral surface. The lipids that are in contact with the tongue and the palate are substantially responsible for the sensory impact of a product. Therefore, a direct measure of undisrupted lipid coating provides valuable information and contributes to the understanding of the behavior of the food components in the mouth and their influence on the perception.

MATERIALS AND METHODS

Materials. Samples used for evaluation of the lipid mouth coating were medium-chain triglycerides (MCT) Delios V by Cognis (Monheim, Germany) and curcumine 95% as a natural extract from Naturex (Avignon, France). Bottled water Vittel from Nestlé (Vittel, France) was used for rinses. Materials for lipid extraction were chloroform and methanol, both of reagent grade from Merck (Darmstadt, Germany).

Oral Processing Protocol. One subject was used in this study to evaluate the methods. The subject was not allowed to brush her teeth or to eat or drink anything besides water during the 0.5 h preceding the test. Prior to ingestion of the sample, the subject had to rinse his mouth with water and swallow. Then various samples were given at room temperature and were masticated for 30 s and subsequently spat out twice (processing time of about 5 s). Then the mouth coating with lipids was either collected or directly measured with three different methods called, respectively, the "mouth rinse" method, the "fluorescent probe" method, and the "filter papers" method.

Quantification of Lipid Mouth Coating by the Mouth Rinse Method. The mouth rinse method was based on determination of lipids in the mouth coating by rinsing them out of the oral cavity and extracting and quantifying them by measuring the mass. Two amounts of MCT (0.5 and 5 mL) were masticated following the oral processing protocol described above. Then the mouth was rinsed with 5 mL of warm water at 45 °C for 60 s, which was then spit out into a glass tube that was used to quantify the lipids. During the rinsing the panelist was not allowed to swallow. Lipid extraction was performed with a mixture of chloroform/methanol 2:1 by volume (*13*). The samples were shaken by turning the closed test tubes up and down for 30 s, then shaken for 30 s with a vortex, and finally centrifuged for 10 min at 20 °C and 2000 rpm to obtain a clear separation of the two phases. The lower phase was transferred with Pasteur pipet into preweighed tubes. The solvent was evaporated under nitrogen flow at 40 °C until a constant mass was reached. All measures were done in triplicate.

Evaluation of Curcumine as a Marker of the Lipid Mouth Coating. Curcumine, an oil-soluble fluorescent dye, was added to oil samples. After ingestion of samples, residual lipids were directly measured by fluorescence on the oral surface. Because curcumine is not commonly used as a marker, we had to confirm its efficacy and show that its concentration in oil should stay the same during the oral processing. We evaluated the amount of lipids in the mouth coating collected with the mouth rinse method. The amount of lipids was quantified respectively by weight as a control and by fluorescence of curcumine in oil.

Various amounts of pure MCT or MCT with 65 ppm of curcumine extract were masticated following the previously described procedure. Lipids were extracted as described for the mouth rinse method. On the one hand, the lipids in the mouth coating of pure MCT were quantified by weight. On the other hand, the quantity of lipids in MCT samples with curcumine was measured by fluorescence at 440 nm excitation and 515 nm emission wavelengths at room temperature (Perkin-Elmer LS50B). Calibration to link the fluorescent intensity of the samples to the amount of the lipids was done as follows: Various volumes (0–150 μL) of MCT containing 65 ppm of curcumine were added to 6 mL of water. Afterward, the lipids were extracted in the same way as from the samples, and the fluorescence intensity of a lower phase was measured. At the end the results of both determinations were compared. A second extraction confirmed that curcumine is not partially soluble in the water phase. Curcumine has shown persistent stability.

Determination of the Thickness of the Lipid Mouth Coating by the Fluorescent Probe Method. Respectively, 0.5 and 5 mL of MCT, containing 65 ppm of curcumine, were masticated as described above. Immediately thereafter the samples were spat out, and the tongue was moved back and forth against the palate to spread the lipids in the oral cavity. The thickness of the mouth coating was directly measured on the tongue by fluorescence. 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 measurements on solids. 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.

Fluorescent intensity was linked to the thickness of the lipid coating and was done as follows: Various volumes (0.2, 0.3, 0.4, 0.5, 0.6, and 0.8 mL) of MCT with 65 ppm of curcumine were spread with a pipet on a plastic Petri dish with a diameter of 8.5 cm at room temperature. The Petri dish was carefully shaken by hand, until oil was evenly spread. Measurements were taken from 16 predefined points. The average intensity of these 16 points was used for a calibration curve. Fluorescence intensity of curcumine linearly decreases with temperature. The mean thickness of the lipid coating was calculated by dividing the volume of MCT spread by the surface of the Petri dish (56.7 cm^2) and multiplied by a factor 10^4 to express thickness in micrometers. The calibration curve was adjusted (reduced by 12.3%) to compensate for the fluorescent difference due to the difference in temperature between the stretched out tongue (32 °C) and the laboratory (23 °C).

Determination of the Thickness of the Lipid Mouth Coating by the Filter Papers Method. Lipids were absorbed onto the filter paper by pressing a filter paper to the tongue surface, and the lipids were extracted from filter papers and quantified by fluorescence. A Hybond P membrane from Amersham Bioscience (Freiburg, Germany) was cut into square pieces of 4 cm^2; 650 ppm of curcumine extract was added to the MCT just before the test. At least three repetitions were done with 0.5 and 5 mL of MCT intake volume. Samples were masticated in the mouth for 30 s and subsequently spat out twice (processing time of about 5 s). The filter papers were then placed onto the tongue and removed after 15 s. To check the recovery of lipids from the tongue with filter papers, four filter papers were consequently placed on the tongue. Each filter paper had a surface of 4 cm^2 with a surrounding band to cover 9 cm^2 in total. Only the inner square of 4 cm^2 was used for lipid evaluation. The filter papers were then transferred into glass tubes to quantify the content of lipids. Prior to lipid extraction, 1 mL of water was added to the samples. Lipids were extracted with a mixture of chloroform/methanol as described above, and the fluorescence was measured in the lower phase using the calibration curve.

RESULTS

Quantification of Lipid Mouth Coating by the Mouth Rinse Method. To validate this method, three points needed to be addressed. First, we needed to know what the recovery of the extraction method was; second, we needed to determine if it was possible to recover all of the lipids from the mouth

coating; and third, we chose a protocol for the collection of the mouth coating.

Recovery of the Lipids with the Extraction Method. Recovery of the extraction method was checked by mixing various amounts of MCT (10 mg–1 g) with 1 mL of saliva for 30 s on a vortex in four replicates. The detection limit was below 10 mg, and the recovery was >95% for all amounts of MCT with a coefficient of variation of 2.5%.

Quantitative Recovery of Lipid Mouth Coating. During food ingestion lipids are deposited on the oral surface. For quantitative determination of mouth coating, all lipids deposited on the oral surface needed to be washed out with the mouth rinse. We checked in preliminary trials how many rinses of 5 mL of warm water were necessary by establishing a mass balance. Already in the first 5 mL rinse almost the whole mouth coating, independent on the oil intake, was quantitatively recovered, 96 ± 2%, for oil intake ranging from 30 mg to 1 g.

Protocol for Collecting Lipid Mouth Coating. We found that 5 mL of warm water was enough to collect all lipids from the mouth coating. However, several other parameters, such as the oral processing time, number of spit outs, and saliva secretion could influence the retention of lipids on oral surfaces. Experiments were done at three various oral processing times (5, 30, and 60 s). There was only some slight variation of recoverd lipids observed between the various oral processing times. An oral processing time of 30 s was chosen to maintain free movement of the tongue and at the same time ensure overall distribution of the lipids. The influence of number of spit outs was done for higher (20 mL) and lower (0.5 mL) intake volumes. With higher intake volumes there was a high decrease of lipid mouth coating from the first (650 mg) to the second spit out (220 mg), which was not observed with lower intake amounts. This might be due to some bulk retention of lipids at higher volumes, simply because it is harder to spit all out at once when the higher volume is ingested. The influence of saliva secretion was not investigated, but the subject was instructed to swallow just before ingestion of the sample to minimize the effect of residual saliva in mouth.

Quantification of Lipid Mouth Coating. Lipids were quantified in the mouth coating after ingestion of two samples of MCT (0.5 and 5 mL), each in triplicate and repeated on another day. The coefficient of variation for each mouth coating measurement by the mouth rinse method is maximum at 17%. The day-to-day variations were, respectively, 102 ± 14 and 82 ± 12 mg on days 1 and 2 for 0.5 mL of MCT intake and 138 ± 26 and 147 ± 19 mg on days 1 and 2 for 5 mL of MCT intake.

Determination of the Thickness of the Lipid Mouth Coating by the Fluorescent Probe Method. *Evaluation of Curcumine as a Lipid Marker.* Curcumine was chosen as a fluorescence marker because it is readily available as a plant extract, is well soluble in oil, is not toxic, and is used as a food colorant. Curcumine is stable over time and does not show any fluorescence quenching. In addition, excitation and emission wavelengths of curcumine do not interfere with absorption and fluorescence wavelengths of proteins, which are present in oral surface. We checked that curcumine is soluble only in lipids and does not distribute between aqueous and lipid phases. The mouth coating was collected and extracted as described for the mouth rinse method. Lipids were quantified either by sample weight or by fluorescence of the sample, which was very well correlated with a linear curve [$Y = 0.9934X$; $R^2 = 0.9748$; Y axis represents lipid mouth coating by fluorescence (mg); X axis represents lipid mouth coating by weight (mg)]. Prior to this, a calibration curve was obtained to link the amount of oil with curcumine fluorescence intensity. Curcumine concentration was constant in oil; therefore, a calibration curve of fluorescence intensity as a function of amount of oil was obtained ($Y = 9.1657X$, $R^2 = 0.9863$). These findings confirmed the use of curcumine as a suitable marker for in vivo evaluation of the mouth coating.

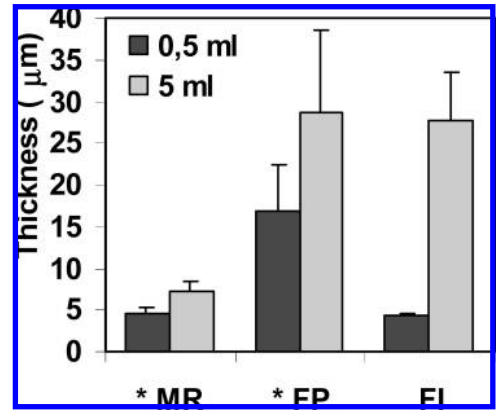

Figure 1. Comparison between the approaches to quantify the thickness of the lipids on oral surface for two different oil intakes: MR, mouth rinse method; FP, fluorescence probe method; FI, filter paper method. The values are represented as means of at least triplicates, * that were performed twice on two different days.

Determination of the Thickness of the Lipid Mouth Coating. The fluorescence of curcumine on the tongue surface was measured by fluorescent probe designed for measuring fluorescent on surfaces. In this setting the distance between the sample and detector was constant and enabled quantitative measurements. Measured fluorescence intensity of probe needs to be related to the thickness of lipid layer. Therefore, plastic Petri dishes were used for calibration with various volumes of lipids to obtain different thicknesses. The drawback of this model is the fact that it is difficult to homogeneously spread smaller volumes (≤0.2 mL) over a large (56.7 cm^2) surface. The wetting capacity of the liquids toward the Petri dish is very important. However, the effect can be overcome by increasing the number of measurements and by setting the position of measurements on a Petri dish. The fluorescence of the curcumine is dependent on the temperature. Therefore, we had to take into account the difference of intensity because the calibration was done at 23 °C on a Petri dish and mouth coating was measured on a stretched out tongue at 32 °C. Therefore, the calibration curve obtained on a Petri dish was reduced by 12.3% and resulted in the following equation: $Y = 5.1309X$; $R^2 = 0.9895$; Y axis represents fluorescence intensity (au), X axis represents thickness of lipids (μm). Measuring on an oral surface in vivo was quite challenging. After the subject had spit out the bolus, the tongue was moved once forth and back against the palate prior to fluorescence measurement. However, even small movements or contractions of the tongue changed the fluorescence intensity. Therefore, an averaged time of the measurement was set to 0.5 s to obtain an average of 40 measurements for each position and potentially minimize the effect of the tongue movements. The tongue surface was scanned manually just after spitting. The measurements were done on two positions of the tongue, always in the same order. As the results show (**Figure 1**), different thicknesses of lipid mouth coating were obtained on the tongue dependent on the amount of oil that was ingested. Lipids were quantified in the mouth coating after ingestion of two MCT intakes (0.5 and 5 mL), each in at least triplicate and repeated on another day. The coefficient of variation for each mouth coating measurement by the fluorescent probe method is

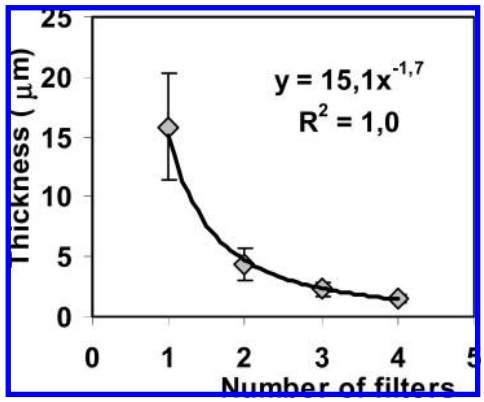

Figure 2. Thickness of lipids obtained by each filter paper consequently deposited on the same tongue area. Already with the first four filter papers, we estimate a total thickness of 24.0 μm. If we assume using more filter papers following the trendline curve, the total thickness reached with 100 filter papers is 30.5 μm.

maximum of 20%. The day-to-day variations were, respectively, 25.5 ± 8.3 and 8.26 ± 2.9 μm for days 1 and 2 for 0.5 mL of MCT intake and 32.0 ± 12.2 and 25.3 ± 7.5 μm for days 1 and 2 for 5 mL of MCT.

Determination of the Thickness of the Lipid Mouth Coating by the Filter Paper Method. To confirm the results from the fluorescent probe method, the filter paper method was developed. The same protocol was followed as for the fluorescent probe method. The surface of the filter papers is constant. However, slight movements of the tongue muscles might enlarge the tongue surface in contact with filter papers. Therefore, more (or fewer) lipids may stick to the paper and contribute to the variation of the results. However, to minimize this effect, we have applied filter papers on the tongue with a surrounding band, which was not used for quantification. We have observed that the filter papers do not recover all of the lipids from the tongue surface after one coating (**Figure 2**).

Calculation of Mean Thickness by the Mouth Rinse Method. The mouth rinse and fluorescent probe methods cannot directly be compared. Previous study (*14*) evaluated the mean total surface area of the mouth, which was found to be 215 ± 13 cm^2. The teeth, keratinized epithelium, and nonkeratinized epithelium, respectively, contribute to about 20, 50, and 30% of the total surface area. For our comparison we took 100% of the overall surface. The volume of lipids on the tongue was calculated from the weight and specific weight of MCT. Mean thickness was simply obtained by dividing the volume by the surface. The results obtained by the filter paper and fluorescent probe methods can be directly compared, because both measurements are related to the amount of oil for a given surface and allow direct calculation of the thickness (**Figure 1**).

DISCUSSION

The results from this study demonstrate that the coating of lipids, remaining on the oral surface after swallowing, can be measured quantitatively. For this purpose we have evaluated three different approaches to measure lipid coating on the oral surface.

The mouth rinse method and the fluorescent probe method are both found to be suitable to determine the amount of lipids remaining on the oral surface after swallowing. Both methods are affected by experimental parameters, mainly by the oral processing protocol. The experimental protocol needs to be adapted to the particular question. The mouth rinse method can easily be performed in any chemical laboratory as no specific equipment is needed. However, the method is time-consuming compared to the fluorescent probe method. The methods quantitatively determine the amount of lipids that remain on the oral surface after swallowing. Nevertheless, when this kind of experiment is performed, the variability should not be underestimated. The critical point of the mouth rinse method is the rinse. Warm water is sufficient to collect the lipid coating, probably due to the soap properties of saliva (*15*). This is in coherence with another study (*11*) that has reported much shorter persistence of lipids in the mouth than expected. The mouth rinse method allowed us to quantitatively recover lipids that coat the oral surface. The fluorescent probe method is much more convenient. It does not depend on recovering mouth rinses, it is done much more quickly, and the amounts of lipids can be determined very locally at specific spots. The lipids could be analyzed by fluorescence only in the presence of a marker. For this purpose we needed a relevant marker, which can be traced during processing and ingestion (*16*). We selected curcumine among edible biomarkers and among chlorophylls and tocopherols. Curcumine was found to be suitable for the purpose; it is stable, and its signal is sufficient for tracing purposes ($Y = 0.9934X$; $R^2 = 0.9748$). With the fluorescent probe method the thickness of lipids deposited on a specific oral area can be measured. In a previous study Adams et al. (*12*) showed that lipids deposited on the oral surface are in contact with saliva and do form droplet dispersion. Referring to this finding, it is interesting to note that oil dispersion does not interfere with the fluorescent measurements. This was confirmed by comparing the thicknesses of lipid dispersions determined by fluorescent probe measurements and by filter papers. Both methods have given very similar results.

When the mouth rinse method is compared with the fluorescent probe method, smaller calculated values for thickness were observed with the former method. The difference originates from the localization of the lipids. The fluorescent probe method measured lipids only on the tongue, whereas the mouth rinse method took an average of all oral surfaces. The lips, palate, and dorsal area of the tongue are actively involved in the texture perception of liquid or semisolid foods. For this reason, we could expect the deposition of lipids on these surfaces to be thicker. Our measurements were not done on the same day, and a small day-to-day variation might be expected.

The thickness of the lipid coating was found to be between 4 and 30 μm for the filter paper method and between 17 and 29 μm for the fluorescent probe method. Both methods measured the lipid mouth coating at a similar area of the tongue. The smaller thickness was observed for the filter paper method at an intake volume of 0.5 mL of oil. This might be a result of poor recovery of lipids form the oral surface. So far, no suitable method is available for measuring the lipid coating in the oral cavity. However, one study estimated an average thickness of the salivary film in the mouth to be between 70 and 100 μm (*14*).

We may envisage further applications of the fluorescent probe method to study other food molecules that could coat the oral cavity. Appropriate selection of fluorescent labels could enable simultaneous investigation of food residual components, such as lipids, proteins, and carbohydrates. This technique offers not only quantitative information but also information on its spatial distribution on the oral surface. This technique has also the potential to measure the rate at which food compounds are cleared from the mouth.

In this study we have demonstrated that by increasing the oil intake from 0.5 to 5 mL, the residual amount of lipids is

increased (**Figure 1**). The same trend is described in sensory studies, where the fatty mouthfeel is increased with the fat content (*6, 17*). Hence, we might hypothesize that the amount of lipids deposited on the oral surface is related to the mouthfeel perception. Our approach has the potential to study in-mouth distribution and retention of lipids after ingestion. The coating on the palate and tongue plays a significant role in a mouthfeel perception, which is a critical attribute influencing the perception of reduced-fat or low-fat food products (*2*). Direct measurements of undisrupted residues on the oral surface provide information that contributes to our understanding of food material movements during oral processing. Therefore, our work opens up new avenues to investigate the behavior of food components during oral processing and its influence on the perception.

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