Distribution and Quantification of Oil Uptake in French Fries Utilizing a Radiolabeled ^{14}C Palmitic Acid

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A novel method utilizing ^{14}C palmitic acid for image analysis and liquid scintillation for quantifying oil uptake of French fries undergoing deep-fat frying is described. Radiolabeled computerized image analysis of the fried product furnished comprehensive visual information on the distribution and localization of oil uptake which was limited to the crust. A significant high linear correlation ($R^2 = 0.977$; $P < 0.001$) was found between oil uptake measured by liquid scintillation and differential scanning calorimetry, and between liquid scintillation and radiolabeled imaging ($R^2 = 0.861$; $P < 0.001$). The high specificity and sensitivity of the method allows quantifying uptake of very low oil concentrations ranging from several milligrams. Oil quantification via liquid scintillation was more sensitive than image analysis and requires up to 3 order of magnitude lower concentration of the radiolabeled marker. The low concentration and inherent stability of the radiolabeled chemical make this technique uniquely suitable for studying oil uptake mechanism during deep-fat frying of foodstuffs.

Keywords: *Deep-fat frying; radiolabeled; liquid scintillation; image analysis; oil uptake.*

INTRODUCTION

Fats and oils have a unique appeal offering special properties that improve the overall palatability of foods. Frying is a complex and important operation in the industrial or institutional preparation of foods (Varela, 1988). It involves transfer of heat from the surrounding oil to the interior of a food product. In addition, the mass transfer is characterized by the movement of water as vapor from the product into the frying medium and subsequent oil absorption by the product (Saguy and Pinthus, 1995). Several chemical and physical changes also occur during frying, including starch gelatinization, protein denaturation, and crust formation.

Quantification of oil uptake is important for studying the mechanism of oil uptake and its specific sites and to generate concentration profiles for mass transfer studies (Baumann and Esher, 1995). Determination of oil uptake is commonly carried out by solvent extraction in a Soxhlet or a similar apparatus (Pinthus et al., 1992; Kozempel et al., 1991; Lamberg et al., 1990). Solvent extraction methods require large samples (ca. 2−10 g) that depend on the amount of oil, size reduction, and predrying. Recently, gas chromatography was implemented for fast lipids quantification (Marx and Stender, 1997). A novel approach utilizing a DSC method to determine the amount of frying oil in small samples (<100 mg) circumvents the need for solvent extraction (Aguilera and Gloria, 1997).

A number of studies have been conducted to show the uptake of oil during the frying process. Oil uptake of deep-fat fried food products was localized in the outer crust surface (Gamble et al., 1987; Farkas et al., 1992; Keller et al., 1986; Pinthus et al., 1995; Varela, 1977). Low-magnification light microscopy studied with an oil soluble dye revealed that oil intrusion during frying was accumulated in an oil layer of approximately 1 mm (Keller et al., 1986, Lamberg et al., 1990). Differential scanning calorimetry data showed that in French fries, the crust contained up to 6 times more oil than the core (Aguilera and Gloria, 1997). In an alginate restructured potato product fried for 1 and 5 min, only ca. 35−38 and 60−85% of oil uptake was localized at the crust, respectively. These values are expected to be higher for longer frying duration (Pinthus et al., 1995). Magnetic resonance imaging (MRI) has been also used as a noninvasive analytical tool for determining the oil and water concentration gradients within a porous material, showing similar oil concentration at the crust surface (Farkas et al., 1992).

Utilization of radiolabeled ^{14}C isotopes for studies in the food industry was first suggested for ethanol (Faltings, 1952) and has been applied since in numerous applications (e.g., diffusion of glucose on surfaces of vegetable slices, Duckworth and Smith, 1962; fermented spirits, McWeeny and Bates, 1980; vinegar, Krueger and Krueger, 1986; caffeine, Allen, 1961; protein fat and lactose, Noble et al., 1981). The ability to utilize liquid scintillation counting and imaging for quantitative and qualitative measurements could provide several distinct advantages over conventional methods (Noble et al., 1981).

Whole body autoradiography techniques have been available for many years for studying the biological fate of chemicals (Ulberg and Larrson, 1981; Wilson and Hall, 1981). This technique that is most frequently employed with radiolabeled chemicals provides a visual analysis of tissue distribution. Recent developments and capabilities of image analysis techniques explain its increased use for toxicology studies (Wilson and Hall, 1991). However, its application to quantify changes in food processing has not been reported. The objective of this study was to demonstrate a novel application of ^{14}C palmitic acid for macroquantitative radiolabel imaging of oil absorption geometry and for microqualitative

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measurements of oil uptake during deep-fat frying of French fries.

MATERIALS AND METHODS

Radiolabeled Palmitic Acid. *1-*^{14}*C*-palmitic acid (cat. No. 12195, ICN Pharmaceuticals, Inc., Irvine, CA) dissolved in ether with an initial total concentration of (250 μCi/mL), a specific activity of 53 mCi/mmol, and 99% purity was used. The ^{14}C-radiolabeled fatty acid (1.67 mL), was transferred to a 10 mL volumetric vial, and commercial sunflower oil (Coop, Switzerland) was used to fill the volume. The ether was evaporated under a nitrogen stream, and the remaining oil was further dissolved in 240 mL of Sunflower oil utilized for deep-fat frying.

Deep-Fat Frying. Frying was performed in a 500 mL glass beaker placed over an electrically heated plate. The oil was heated to 180 °C for 2 h prior to frying (Pinthus et al., 1992). French fries were hand cut fresh from locally obtained Bintje potatoes and cut to shape (0.8 × 0.8 × 10 cm). Samples (6.5 ± 0.4 g) were fried individually for 5 min. After frying, the samples were left to cool on a paper towel at ambient temperature. The French fries were then weighed and placed in a freezer (−30 °C) for further analysis.

Radiolabel Imaging. The frozen French fries were sliced by a special knife prepared by inserting 20 razor blades spaced at 0.25 cm apart mounted on an aluminum frame, and yielded thin slabs of equal 0.25 cm thickness. The thin slabs were placed on an imaging plate (10 pieces in each column) and were left overnight for counting on an Instant Imager (Electronic Autoradiography System, Packard, Meriden, CT). The computerized Imager was calibrated using a ^{14}C-labeled test source grid provided by Packard.

Scintillation Counting. Weighed samples were cut into small fine pieces with a scalpel and transferred to a scintillation vial (20 mL). One milliliter of Soluene 350 (Packard, Zurich, Switzerland) was added, and the samples were left overnight in a shaking bath (40 °C and 150 rpm). Twenty milliliters of scintillation cocktail fluid (Ultima Gold, Packard) was added, and the radioactivity was determined in a liquid scintillation counter (LKB Model 1219, Turku, Finland). Samples were counted for 5 min and data were converted to disintegrations per minute (dpm) using a quenching coefficient and an established quenching curve. Initial slice weight of each individual sample was used to account for sample weight, yielding dpm/g.

DSC Runs. A differential scanning calorimeter (DSC 820, Mettler Instrument AG, Volkestwil, Switzerland) was utilized according to a previous method (Aguilera and Gloria, 1997). Duplicate samples (40−60 mg) were placed in aluminum pans (100 μL) and their DSC was recorded within the temperature range of 10 to −80 °C at a rate of 1 °C/min using an empty pan as reference. Oil in the fried sample was calculated by dividing the integrated area under the exothermal peak corresponding to the oil by the crystallization enthalpy of the frying oil (determined on TAS810 Mettler datastation). As the enthalpy of the oil changed with frying duration and conditions, appropriate oil samples were used to determine the appropriate values corresponding to oil during the frying (Gloria and Aguilera, 1997).

Water Content. After the DSC runs, the lids of the aluminum pans were perforated (three small holes), placed in an oven at 105 °C overnight, cooled in a desiccator with P_2O_5, and weighed. Water content was derived from the weight loss.

Oil Absorption. The derived water content was used for the calculation of oil concentration on a dry basis excluding oil (%, DB).

Determination of Accuracy and Sensitivity. Liquid scintillation counting method was correlated to control oil samples prepared by weighing known amounts of oil directly into the counting vials and following the procedure outlined above. Samples for the scintillation were prepared following two different approaches: identical samples were used first for the DSC measurement, or samples were divided and analyzed in parallel. Radiolabeled image analysis data were also correlated with liquid scintillation counting measurements.

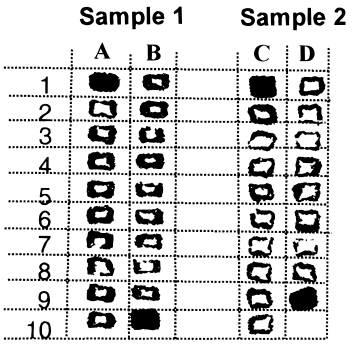

Figure 1. Typical radiolabeled image view of two deep-fat fried French fries samples.

Thin Layer Chromatography (TLC). To verify the status of the *1-*^{14}*C*-palmitic acid after the deep-fat frying process, the radiolabeled material was dissolved in toluene (A. P. Merck, Darmstadt, Germany) yielding a stock solution of 200 μCi/mL. Five microliters of the stock solution was dissolved in 1 mL of either toluene or fresh sunflower oil, yielding a final concentration of 1 μCi/mL similar to the concentration containing the radiolabeled palmitic acid used in the frying experiments. Five microliters was applied to a silica gel (F254 Merck, Darmstadt, Germany) and was developed for 30 min at ambient temperature in hexane (Merck, Darmstadt, Germany). Additionally, 1 μL samples were developed applying a different mobile phase polarity (5% methanol in dichloromethane; Merck, Darmstadt, Germany). The plates were scanned using the image analyzer described above.

Statistical Analyses. Microsoft Excel 95 linear regression was used.

RESULTS AND DISCUSSION

Typical radioactivity digitized images of ^{14}C-labeled French fries fried in oil containing [1-^{14}C]palmitic acid at 1.02 nCi/mg oil for 5 min are depicted in Figure 1. The image (samples 1 and 2) includes for each sample up to 20 slices of 0.25 cm thickness and shows the absorption of radioactive palmitic acid. The dark area represents a higher concentration of absorbed oil. From the visual information on the distribution of the oil, it is apparent that except for the first and last slices (placed with their end upside toward the imager), oil was concentrated at the crust, while no penetration toward the center was observed. The method allows visual and computerized analysis of very small sections which is not normally feasible with conventional methods. Similar oil intrusion depth of ca. 1 mm was reported for potato using microscopical techniques (Keller et al., 1986; Lamberg et al., 1990). However, our imaging data seem not to support values derived by utilizing a DSC method for determining oil concentration at the core of the product. This discrepancy could be possibly explained by the large variability in the determination of oil concentration at the core (Aguilera and Gloria, 1997), or by differences related to potato variety and porosity. Each individual slice was further analyzed using a built-in software program for the quantification of the radioactive concentration and correlated to liquid scintillation counting.

Specificity, sensitivity, and accuracy are important characteristics of an analytical tool. The specificity of the liquid scintillation counting method depends on the radiolabeled material (Figure 2), showing a linear relationship ($R^2 = 0.996$; $P < 0.001$) in the range zero to 25 mg of oil. Moreover, extending the range of added control oil up to 430 mg showed a similar linear

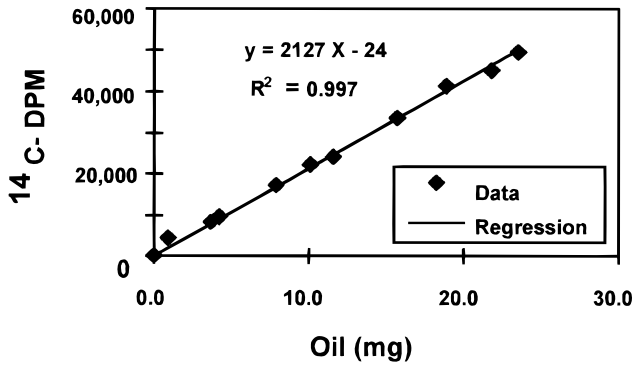

Figure 2. Calibration curve for liquid scintillation and oil (n = 11).

relationship where the slope of the line changed insignificantly (from 2127 to 2153, respectively). In both cases, the intercept was not zero, but within the background level of 20−30 dpm, hence indicating that liquid scintillation counting is linear and directly proportional to ^{14}C. The number of disintegrations per minute (dpm) ranged from 30 to 55 000 for 0−25 mg of oil, thus indicating that the level ^{14}C utilized could be significantly lowered. The samples were recounted several times within a period of a week, yielding a coefficient of variation (standard deviation/average) below 0.3%, except for the zero oil, where the dpm was within the background noise level (20−30 dpm). The 95% confidence interval of the slope was ±24.2 corresponding to ca. ±0.2% at the average range of 25 000 dpm. This clearly indicates the high precision of the method.

The liquid scintillation calibration data also suggest that if the sample weight to be used for the determination is higher, the amount of the radiolabeled material required to be added to the frying oil could be decreased. For most practical purposes, in foods undergoing deep-fat frying, the amount of ^{14}C could be reduced by 1−3 orders of magnitude for liquid scintillation counting, depending on the sample size used for the analysis and the amount of oil absorbed. It is also worth noting that according to safety standards (Decree no. 814.501 of the Swiss Federal Counsel), the maximum dose for inhalation or ingestion is 5.6×10^{-10} Sv/Bq and a maximum exposure (skin, hand, foot) is 150 mSv per year. These data indicate that for routine oil uptake determinations, the method could be utilized with minimal health hazards. However, for radiolabeled imaging, the level of ^{14}C should be maintained higher as its efficiency is significantly lower compared with liquid scintillation.

Fried oil at the conditions of the test showed an exothermic peak of crystallization at −62.3 °C and enthalpy of 18.0 J/g, indicating a shift from −56.3 °C and 31.5 J/g obtained for fresh oil. These changes are expected due to deterioration occurring in the oil with frying time (Gloria and Aguilera, 1997). Comparison between the amount of oil absorbed during deep-fat frying determined by liquid scintillation counting and DSC (Figure 3) shows a good correlation ($R^2 = 0.977$; $P < 0.001$). Oil absorption ranged from 10 to 23% (DB) and is typical for French fries operation (Kozempel et al., 1991). The insignificant discrepancy observed between the two methods is expected, as only half of the samples were identical. It could also reflect differences in accuracy of the two methods utilized.

Comparison between imaging and liquid scintillation data (Figure 4) showed that, probably due to the shield provided by the potato slice, radiolabeled imaging of ^{14}C was less accurate. Typical sections should range from 10 to 100 μm, with 20−50 μm sections being most commonly used (Wilson and Hall, 1991). This thickness range requires a microtome and could be applied if required. The range utilized was significantly higher, thus furnishing an explanation for the need to increase the concentration of the radiolabeled chemical and the somewhat lower accuracy observed. Nevertheless, a linear relationship was obtained ($R^2 = 0.861$; $P < 0.001$), indicating that depending on the precision required, qualitative and quantitative information could be collected with the radiolabeled imaging. This visual and comprehensive information is most valuable for studies investigating the mechanism of oil uptake and for experimental frying, where porosity, crevices, and imperfect food surfaces are expected.

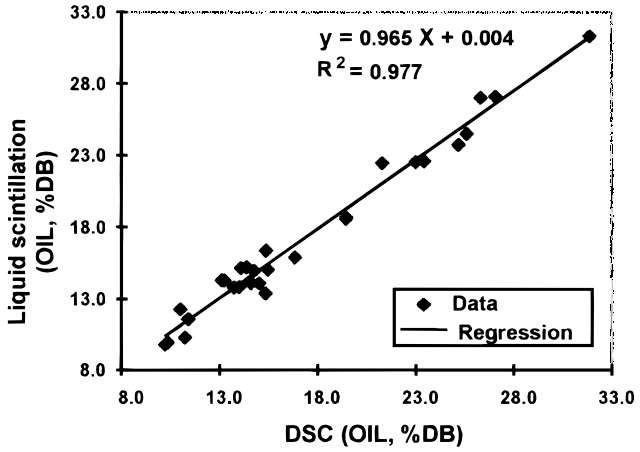

Figure 3. Comparison between oil determination in French fries by liquid scintillation and DSC method (n = 27).

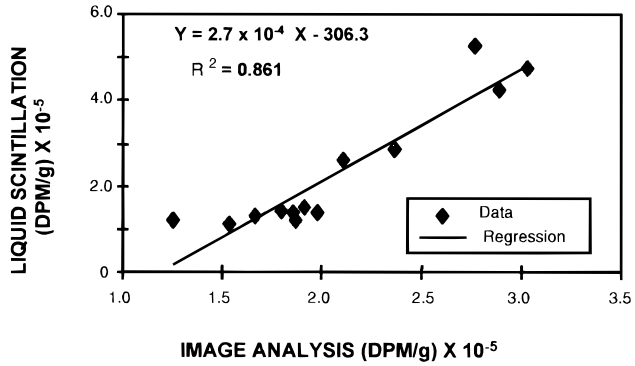

Figure 4. Comparison between liquid scintillation and image analysis of oil in French fries (n = 13).

To quantify the oil absorption in a single French fry, 20 thin slices from one French fry were analyzed by both imaging and liquid scintillation (Figure 5). The data shows that the two ends absorbed the highest quantity of oil. This high oil absorption is expected, as the surface area exposed during frying is larger. However, there are other parts of the French fry that absorb higher concentration of oil as could be explained merely by the surface exposed. This elevated concentration is probably due to local microstructural differences which could be crevices and imperfect surface caused during the cutting.

Stability of the radiolabeled [1-^{14}C]palmitic acid was tested upon the completion of the frying using TLC technique. No apparent deterioration was observed due to the typical temperature and heat exposure during deep-fat frying (Figure 6). The larger spots observed on the TLC plate (Figure 6A) for both fried and fresh

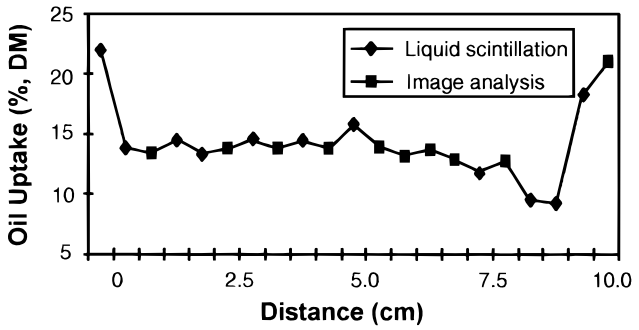

Figure 5. Typical distribution of oil uptake in a French fry ($n = 21$).

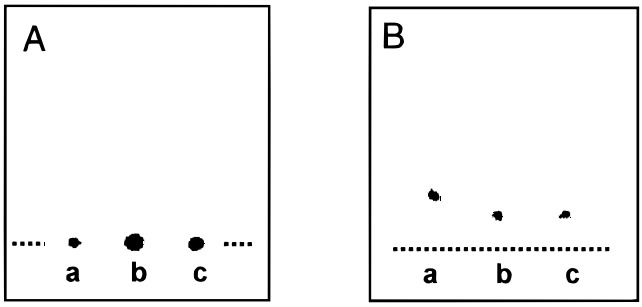

Figure 6. Radiolabeled image view of a silica gel TLC plate separation of [1-^{14}C]palmitic acid dissolved in (a) toluene, (b) deep-fat fried sunflower oil (6 h, 180 °C), and (c) fresh sunflower oil), eluted in hexane (5 μL, A), and 5% methanol in dichloromethane (1 μL, B).

oil are probably due to the higher viscosity of the oil samples. When the polarity of the mobile phase was altered (Figure 6B), both the fresh and fried oil samples showed an identical distance traveled lower than the [1-^{14}C]palmitic acid counterpart ($R_F = 0.11$, 0.11, and 0.22, respectively). This difference is probably due to altered mobility, affected by the accompanying extraneous materials from the oil. It also had an impact on the concentration of the radiolabeled compound quantified with the image analysis. However, as both the spiked fresh and fried oil samples had similar activities, it was concluded that [1-^{14}C]palmitic acid could be considered practically stable under typical deep-fat frying conditions.

In conclusion, utilization of a radiolabeled material for quantifying oil uptake during deep-fat frying of a food product proved highly accurate. It offers imaging capabilities identifying the exact sites of oil penetration and uptake. It also furnishes a unique capacity for the determination of very low oil concentrations in food samples. The method is straightforward and could be applied for routine and fast analyzes of a large number of samples.

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