New Technologies to Determine Solid Fat Content On-line

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ABSTRACT: The determination of solid fat content (SFC) is an important analytical procedure in the food industry. The most common way to determine SFC is by low-resolution pulsed NMR (p-NMR). Although this technique is very sensitive and easy to use, it has the disadvantage that it cannot be used for on-line measurements. The present work compares new technologies to determine SFC on-line. On-line ultrasonic spectroscopy and NMR-MOUSE (NMR mobile universal surface explorer) techniques were compared with off-line p-NMR measurements and there was a good correlation between the values obtained. Ultrasonic measurements accurately described the SFC variation, whereas NMR-MOUSE determinations need to be improved to some extent owing to a strong temperature and motion dependence. These two techniques can be used as on-line methodologies to determine SFC during the crystallization of fats.

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KEY WORDS: Fat crystallization, NMR-MOUSE, on-line measurements, p-NMR, solid fat content, ultrasonics.

Analytical techniques based on measurements of the interaction of ultrasonic waves with food materials have been shown to be particularly suitable for studying the properties of fatty foods. Instrumental measurements can reduce the dependence on time-consuming chemical and sensory analysis. The measurement should provide some information about the food (e.g., temperature, composition, structure, concentration) that will be useful in controlling final product quality. The response time is crucial, so whereas laboratory tests on finished products are valuable, measurements made on-line of the freshly made or in-process foods are better. The determination of solid fat content (SFC) by low-resolution pulsed NMR (p-NMR) is an internationally accepted procedure and an important analysis for producers and users of fats and oils. Although this technique has been widely used by researchers and industrial professionals because it is very sensitive and accurate, it cannot be applied on-line. This fact has encouraged scientists to develop new techniques that can be used to measure SFC values on-line. Recent work has proven that ultrasonic technology can fulfill this promise (1,2). Ultrasound techniques have been used in numerous areas, such as medicine, oceanography, and materials science. Several authors have described the application of ultrasonic waves to the determination of SFC in fat systems (3–9). Ultrasonic technology has advantages over many other techniques because it can be applied to systems that are optically opaque, concentrated, and electrically nonconducting. In addition, ultrasonic measurements are rapid and precise, are nondestructive and noninvasive, can be fully automated, are nonhazardous, and are particularly suitable for on-line measurements.

The NMR-MOUSE (NMR mobile universal surface explorer) is a new NMR instrument that allows *in situ* measurements. In this instrument (NMR-MOUSE), the magnetic field is applied to the sample from one side (single-sided NMR). The magnetic field penetrating the object is inhomogeneous, and only a limited number of the wide variety of NMR experiments can be performed. Nevertheless, the information acquired using the NMR-MOUSE is well suited for the quality control of soft materials such as elastomers, food, creams, biological tissue, and wet porous material (10,11). The advantage of the NMR-MOUSE device is its ability to analyze the sample just by locating the sensor on the sample surface. There is no need to cut the sample into pieces to load a sample tube. As different measurement depths can be chosen, samples also can be characterized through packaging material (12). In food science research, this new technique has already been used to measure the water/oil contents in food emulsions (13).

The objective of this work was to compare SFC determinations using the traditional off-line p-NMR technique with both the ultrasonic and NMR-MOUSE technology.

MATERIALS AND METHODS

Crystallization experiments at 30°C, SFC determinations, and ultrasonic experiments were carried out with two fat samples (1). Interesterified hydrogenated palm oil was used as sample A and partially hydrogenated palm oil and palm stearin as sample B. Both samples were diluted with 50% of canola oil.

NMR-MOUSE. The minispec MOUSE device (NMR-MOUSE), Mobile Universal Surface Explorer (Bruker Optics Inc., Milton, Ontario, Canada) is a handheld NMR instrument for relaxation measurements in the near-surface volume of unrestricted size samples. It contains two permanent magnets mounted with antiparallel polarization on a yoke. In the gap between the magnets, a radio frequency tank circuit is positioned, which generates the radiofrequency field ($\boldsymbol{B}_1$) necessary for the observation of an NMR signal. Details of the construction and use of the NMR-MOUSE were described by Eidmann *et al.* (14) and Guthausen *et al.* (15). Specifically, in this study, a spin

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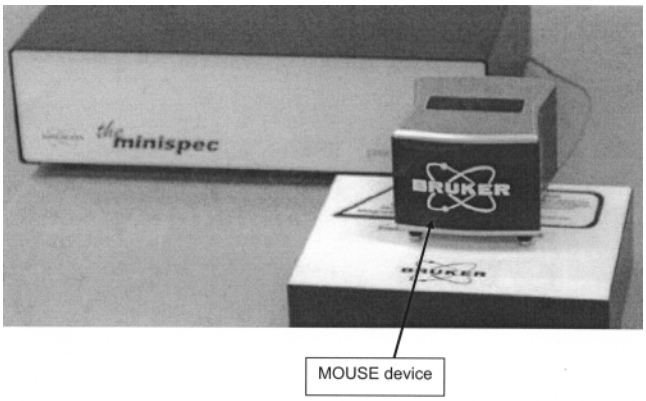

FIG. 1. Mouse device used to measure the solid fat content (SFC) on-line by means of NMR-mobile universal surface explorer (NMR-MOUSE).

echo pulse sequence was used: a 90° excitation pulse, followed by a 0.1 ms evolution delay, then a 180° refocusing pulse, followed by a refocusing delay. The signal intensity was sampled at the echo (approximately at 0.2 ms) over a period of 0.01 ms. For the 90° pulse, the transmitter attenuation was set to 12 dB and the pulse length was 6.67 μs. For the 180° pulse, the transmitter attenuation was set to 6 dB and the pulse length was 6.67 μs. The approximate depth of penetration of the resonator was 3 mm, and the NMR frequency was 14.8 MHz. The signal was averaged using 256 scans, and the recycle delay between scans was 0.1 s. The receiver gain was set to 115 dB.

The MOUSE device shown in Figure 1 was placed at the bottom of the crystallization cell, which was made of 2-mm glass to allow the electromagnetic waves to reach the sample (Fig. 2). Measurements with the NMR-MOUSE technology were performed with and without agitation. To simplify the nomenclature, we will refer to the traditional low-resolution p-NMR as mq20 and to the single sided p-NMR as NMR-MOUSE.

Crystallization cell. Figure 2 shows the crystallization cell especially designed to study on-line the crystallization process of fat systems. This cell was designed to reproduce an on-line measurement similar to the one that is taking place in a plant pipe. In the lateral view of the cell (Fig. 2a), we can see the windows where the transducers for the ultrasonic measurements are placed. We can also note the NMR-MOUSE device in the bottom of the cell and the impeller together with the thermocouple. Figure 2b is an upper view of the crystallization cell again showing the transducer windows and the NMR-MOUSE device. A picture of the crystallization cell appears in Reference 1.

Calibration curve for NMR-MOUSE technology. To study the NMR-MOUSE device response to different SFC values, we constructed a calibration curve with interesterified hydrogenated palm oil (sample A). Sample A was added to canola oil in different proportions (100, 80, 50, 20, and 0% addition of sample A to canola oil) to obtain blends with different SFC values. Sample A and its dilutions were heated to 120°C and held at this temperature for 30 min. After the samples had completely melted, they were placed in a petri dish and crystallized at room temperature (22°C) for 24 h. After this period, the petri dishes were placed on top of the MOUSE device in order to perform the measurement (Fig. 1). The melted samples were also placed in NMR tubes to perform the traditional p-NMR measurement using a Bruker mq20 pulser NMR after crystallization at 22°C for 24 h.

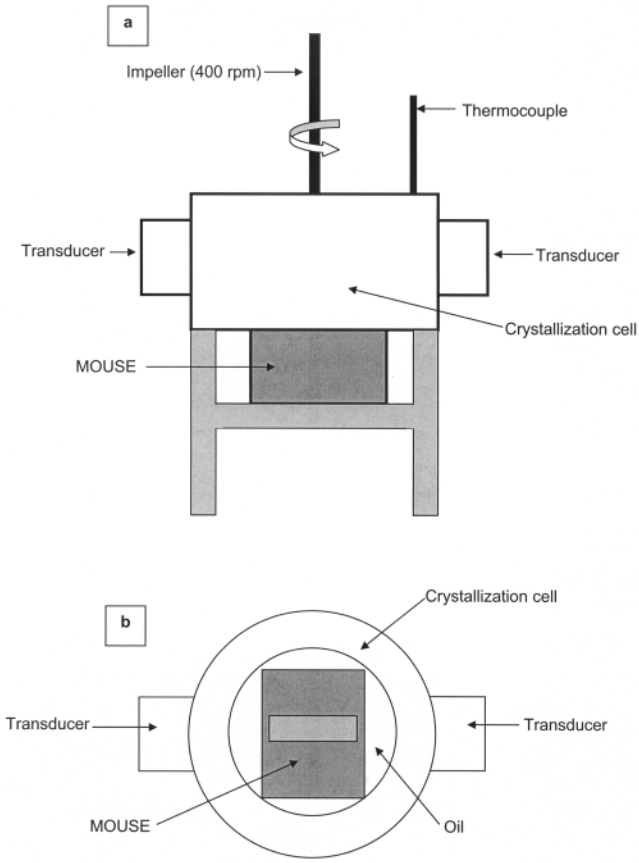

FIG. 2. Experimental design showing the crystallization cell to measure SFC on-line by means of ultrasonics and the NMR-MOUSE device. (a) Lateral view, (b) upper view. For abbreviations see Figure 1.

ANOVA test. Significant differences obtained with the three methods were analyzed by means of a one-way ANOVA test ($P < 0.05$).

RESULTS AND DISCUSSION

Calibration curve for NMR-MOUSE. The SFC was measured for sample A and its dilutions (100, 80, 50, 20, and 0% of sample A in canola oil) using the p-NMR technique (mq20). These values were converted into liquid percentages since the NMR-MOUSE measures the proportion of liquid in the sample. NMR-MOUSE measurements were made on the samples crystallized statically at 22°C in petri dish plates as described in the Materials and Methods section. The NMR-MOUSE reading was plotted against the liquid content determined from SFC measurements using the mq20. A linear correlation between these measurements was found (Fig. 3a) with an r^2 of 0.989.

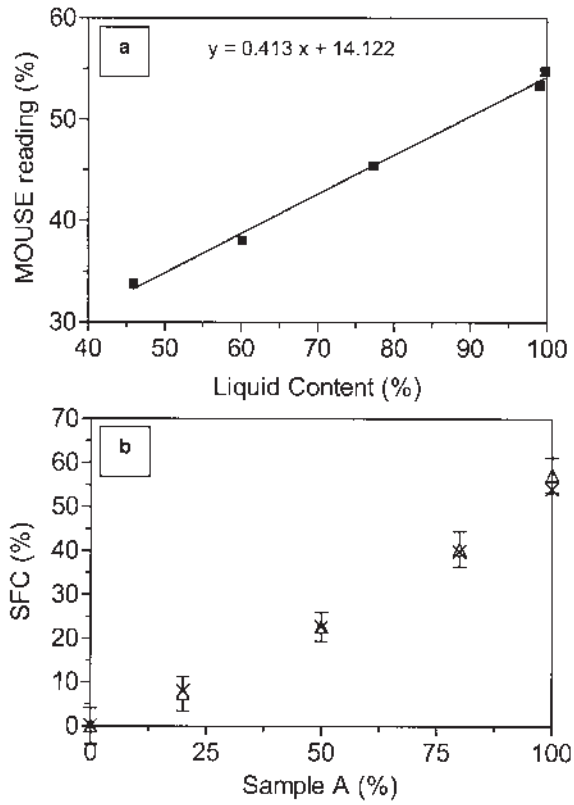

FIG. 3. (a) Calibration curve for the NMR-MOUSE technology showing the linear relationship between the NMR-MOUSE reading and the conventional pulsed NMR (p-NMR) technology, mq20 (shown as liquid percentage). (b) Comparison of SFC values of different dilutions of sample A measured by traditional p-NMR, mq20, and NMR-MOUSE (x and △, respectively). For abbreviations see Figure 1.

These results suggest that there is a good correlation between the values obtained by the NMR-MOUSE and the mq20 when samples are crystallized and measured under static conditions. Considering the inhomogeneity of the magnetic field generated by the NMR-MOUSE, the effect of the molecular orientation of fat on the NMR-MOUSE measurement was studied. Therefore, NMR-MOUSE measurements were performed with the petri dishes placed at different positions (rotating the petri dishes by 45°). Five measurements were made for each petri dish position for all dilutions. No significant differences ($P < 0.05$) were found for the measurements performed in the different positions for all dilutions. SFC values obtained by means of mq20 and the NMR-MOUSE technology for sample A and its dilutions are plotted in Figure 3b. The NMR-MOUSE data reported in this figure represent the average of the values obtained for all positions on the petri dish. It can be seen from this figure that data obtained using both techniques were not significantly different.

SFC measurements using the NMR-MOUSE. Several researchers have shown that the NMR-MOUSE can be used to measure the fat content of packaged dairy products (12,13). However, none of them had explored the use of this new technology to measure the variation of SFC on-line during the crystallization of a fat product. Therefore, as described in the Materials and Methods section, two samples (A and B) diluted with canola oil (50:50) and canola oil itself were crystallized at 30°C with 400 rpm of agitation. Two different kinds of experiments were carried out to study whether motion during the dynamic crystallization affected the NMR-MOUSE measurement. In one experiment, agitation was stopped momentarily during the measurement (approximately 1 min), and then restarted to let the crystallization continue under shear, until the next measurement was performed. In the second experiment, the measurement was performed without stopping the agitation in the cell. When the SFC evolution was measured with and without agitation for both samples (A and B diluted with 50% of canola oil), significant differences were found between these values and the ones obtained using the mq20 method (Fig. 4). When NMR-MOUSE determinations were made on canola oil with and without agitation, a significant effect of temperature on the measured signal was observed. Even though canola oil did not crystallize under the experimental conditions used, a signal was detected by the NMR-MOUSE device (Fig. 4e). To eliminate the temperature effect on the NMR-MOUSE reading, a correction was made to the sample readings by subtracting the canola oil measurements from the sample ones. The corrected data are also shown in Figure 4. We can see that for the static experiments (Figs. 4a and 4c) the corrected values were closer to the ones determined by the mq20 method. When measurements were made under agitation (without stopping the impeller), even the corrected values obtained by NMR-MOUSE determinations were significantly different from the ones obtained by means of the mq20 technique. Figures 4b and 4d show some negative SFC values determined by the NMR-MOUSE. These negative values do not have any physical significance and thus they can be attributed to the motion of the material during the measurement. Therefore, measurements made under static conditions are the ones that better describe the SFC evolution.

SFC determination by means of ultrasonics. SFC was measured on-line by means of an ultrasonic technology developed previously in our laboratory (1,2). Figure 5 shows the comparison between the SFC obtained by the mq20, NMR-MOUSE, and ultrasonic technology for both samples (A and B) diluted 50% with canola oil and crystallized at 30°C. As expected, SFC (measured by means of the mq20) increased as crystallization took place for both samples until a plateau that depended on the specific crystallization behavior of each sample was reached. Sample A diluted with 50% of canola oil had an m.p. of 46.1°C, and this sample reached its SFC plateau ~20 min after reaching the crystallization temperature. The final SFC was ~15%. Sample B diluted with 50% of canola oil had a m.p. of 41.2°C and reached an SFC plateau of ~10% after 50 min. The higher the m.p., the higher the degree of supercooling of the sample at a constant crystallization temperature. Therefore, the final SFC obtained was higher and this SFC level was generally achieved sooner than for low supercoolings.

NMR-MOUSE data reported in Figure 5 are the corrected values obtained for the static condition since these values were the ones that better correlated with the mq20 technique. SFC

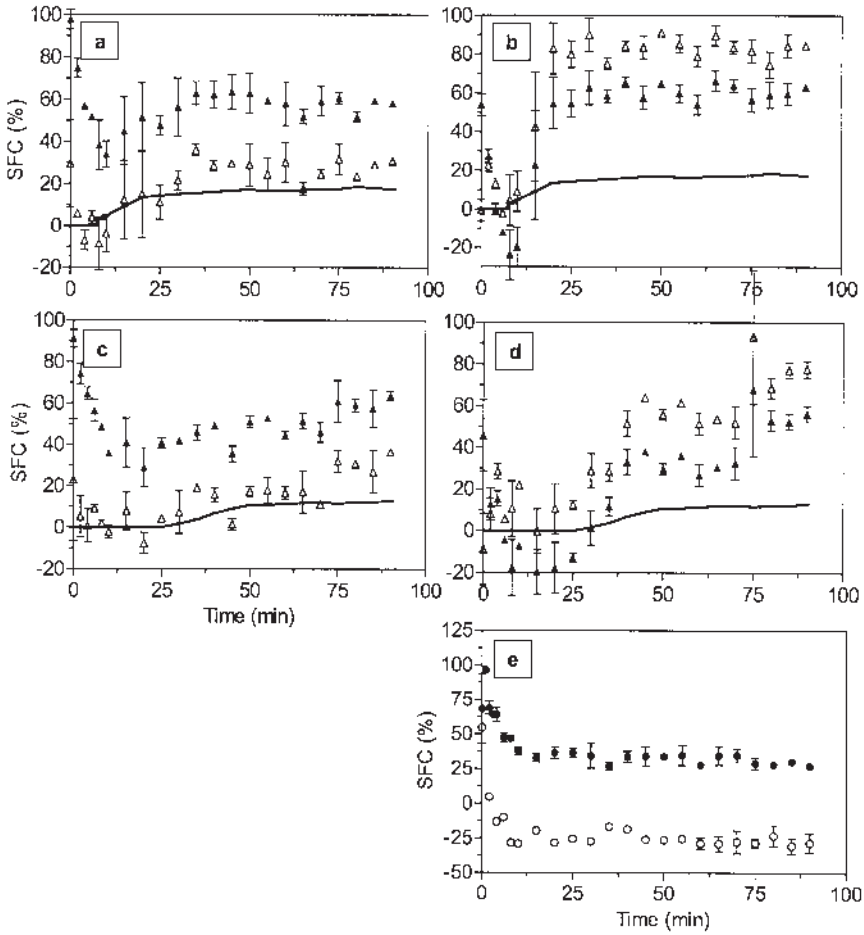

FIG. 4. Comparison of SFC determination using NMR-MOUSE technology and traditional p-NMR technique. Sample A (50% dilution with canola oil): (a) static and (b) with agitation. Sample B (50% dilution with canola oil): (c) static and (d) with agitation. Each figure shows the NMR-MOUSE data (▲), the corrected NMR-MOUSE data (△), and the traditional p-NMR data, mq20 (line with no symbols). (e) Canola oil measured with NMR-MOUSE in static condition (●) and with agitation (○). For abbreviations see Figures 1 and 3.

values measured by means of NMR-MOUSE increased during the crystallization process, reaching a plateau at ~37 min. The final SFC obtained was ~25% for sample A diluted with 50% of canola oil. The same general behavior was observed for sample B. Therefore, NMR-MOUSE technology showed a good correlation with the mq20 values, especially at intermediate SFC values. The error in the determination seems to be more significant at the beginning of the crystallization process (due to temperature fluctuations) and at long crystallization times after the SFC plateau is reached. All the techniques used in this study are strongly dependent on temperature since they measure relaxation properties of the material. But in the case of the mq20, the magnet is always at 40°C and therefore the measurements are independent of temperature variations that may occur in the crystallization cell.

Ultrasonic data reported in Figure 5 were measured with 550 kHz transducers. No significant differences were found between the 550 kHz and 1 MHz transducers (1); therefore, only the 550 kHz results are reported here. SFC measured by ultrasonics accurately described the SFC variation during the crystallization process. Sometime after the SFC plateau was reached, ultrasonic measurements could not be performed owing to the high attenuation of the signal. This attenuation depends on crystal size and the final SFC, which depends indirectly on the crystallization temperature and the chemical nature of the sample that is being crystallized (1,2).

A statistical analysis was made (paired *t*-test) of both samples that showed that the values obtained from determinations made with the mq20 and the ultrasonic technology were not significantly different ($r = 0.9908$ and 0.9909 for samples A and B, respectively, $P < 0.05$). On the other hand, when the same statistical analysis was performed between the measurements made with the mq20 and the NMR-MOUSE, significant differences were found ($r = 0.5954$ and 0.5648 for samples A and B, respectively, $P < 0.05$).

Although both ultrasonics and NMR-MOUSE can be used to determine SFC on-line, more accurate results are obtained with ultrasonics. The fact that NMR-MOUSE measurements

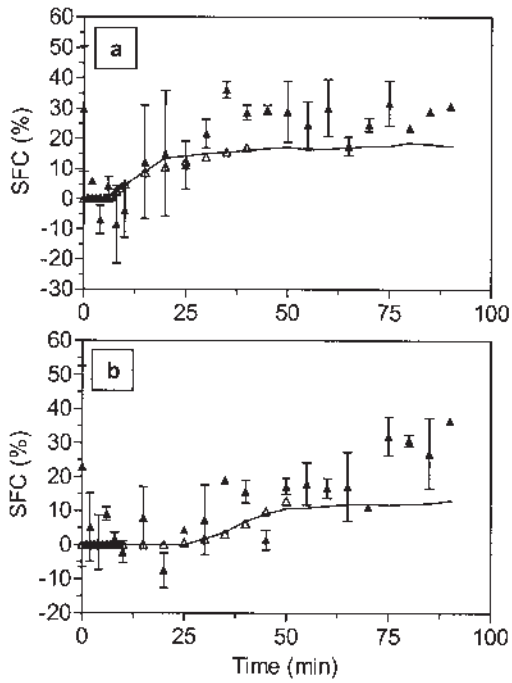

FIG. 5. Comparison between SFC values measured with traditional p-NMR, mq20 (line with no symbols), ultrasonics (△), and NMR-MOUSE (▲) for sample A (a) and B (b), diluted with 50% of canola oil crystallized at 30°C. For abbreviations see Figures 1 and 3.

have to be carried out under static conditions is a serious limitation for on-line measurements but, as described by other authors, it can be used as a quality control tool since very reliable data are obtained for, e.g., packaged margarines and creams (15). Therefore, owing not only to sample motion dependence but also to temperature variation, further investigations need to be carried out to apply the NMR-MOUSE technology in on-line measurements. Ultrasonic technology, on the other hand, proved to be an accurate technique to measure SFC on-line since no corrections needed to be made. Previous data (1) showed that this technique can be used even for high SFC values and that measurements do not depend on sample motion. Transducers can be placed in the pipe walls where the fat is being crystallized, and therefore the crystallization process can be followed in real time.

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