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Solid Fat Content Estimation by Differential Scanning Calorimetry: Prior Treatment and Proposed Correction

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Abstract The objective of this work was to develop a corrected method for solid fat content estimation by differential scanning calorimetry (DSC), as important differences are usually observed between the results given by DSC and pulsed nuclear magnetic resonance (NMR). Cold storage after full melting of fats was necessary to avoid the appearance of exothermic peaks in the modulated temperature DSC thermograms, in order to make an appropriate estimation of melting energy. Different fats were analyzed by NMR and DSC, obtaining considerably higher solid fat content values with the latter, uncorrected method. These differences were attributed to the fact that consumed energy per unit of melted mass tends to increase with the increase of the melting temperature of each fraction of the fats. A linear correlation between melting enthalpy and melting point of different triglycerides was used to estimate the energy per unit of mass consumed at each temperature. From these data, an estimated transformation of melting energy into melted mass was performed and new solid fat content values were calculated. The results obtained from this correction were much closer to the measurements made by NMR, in comparison to the uncorrected DSC method.

Keywords Differential scanning calorimetry · Pulsed nuclear magnetic resonance · Solid fat content · Melting enthalpy · Comparison of techniques

Introduction

Solid fat content (SFC), as an expression of the solid–liquid ratio in fats, is an important parameter for characterization and quality control of fats. One of the most common methods for SFC determination is pulsed nuclear magnetic resonance (NMR), which has proved itself to be a valid technique [1, 2]. However, this method requires individual measurements at each required temperature, limiting the information that can be obtained.

Differential scanning calorimetry (DSC) has been proposed as an alternative method for SFC determination since it has the advantage of obtaining a thermogram including the whole temperature range from one unique measurement [1, 2]. The calculation of SFC by DSC can be performed from the partial integration of the thermograms obtained [3, 4]. Additional information can be obtained by modulated temperature DSC, giving the reversible and non-reversible components of the total heat flow [5, 6]. However, differences have been found between results obtained by NMR and DSC, generally higher SFC values being observed with the latter method [1, 7, 8]. These differences have been attributed to different factors such as tempering methods and weight of samples [9]. Other authors attributed the differences between these methods to a technical question, as NMR data are calculated on the base of the relative numbers of protons present in the triglycerides in the solid and liquid phases, while DSC values are obtained on the basis of the melting enthalpies of these triglycerides [8]. Moreover,

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the NMR and DSC methods are essentially different, as the former is static while the latter is dynamic.

Deroanne [10] took into account the differences between the melting enthalpies of different fractions of fats in order to correct the SFC values obtained by DSC. However, that method has the disadvantage of requiring fractionation of the fat in order to obtain the melting enthalpies of each fraction, and important differences were still observed between SFC values given by that method and NMR [1]. The present work proposes an alternative correction from the consideration of the energy/mass ratio that varies with the melting point of the triglycerides.

The objective of this work was to analyze the effect of prior treatment of the fat on the DSC thermograms and to propose a corrected method for SFC estimation by DSC from the transformation of melting energy into melted mass. In this way, new results were obtained and compared to the values obtained by NMR.

Experimental Procedures

Materials

The different fats were provided by CALSA (Lanús, Argentina). The following fats were used: refined bovine fat (RBF); olein of bovine fat (OBF); stearin of bovine fat (SBF); partially hydrogenated soybean oil with melting points of 25 °C (S25), 36 °C (S36), and 42 °C (S42); low trans vegetable fat obtained by interesterification (LTF). The fatty acid composition of the samples, determined by gas chromatography, is shown in Table 1. A commercial sunflower oil was also used to complete the curve of melting enthalpy versus major melting peak temperature.

Prior Treatment of Fats

Fats were stored at 4 °C for at least 1 month before being treated and analyzed. In order to homogenize the fat, three

different treatments were performed on RBF and LTF samples: (1) full melting at 60 °C and immediate DSC measurement; (2) partial melting at 40 °C and immediate DSC measurement; (3) full melting at 60 °C and storage at 4 °C for 1 day before DSC measurement.

Modulated Temperature Differential Scanning Calorimetry (DSC)

The thermal behavior of the different fats, enclosed in sealed aluminium pans, was determined using an MDSC Q-200 (TA Instruments; New Castle, DE). Samples were cooled to −90 °C at 10 °C/min (with an isotherm at that temperature during 10 min) and heated to 80 °C at 5 °C/min. Measurements were performed with a modulation amplitude of ±1 °C every 60 s. All measurements were performed in duplicate. From the thermograms obtained, total melting enthalpy (ΔH), major melting peak temperature, and absolute melting energy (E) were measured. ΔH is expressed as energy per unit of mass (in J/g), while E corresponds to the energy (in J) consumed by the sample mass; the module of E , thus, depends on the quantity of mass. The measurement of ΔH and E was performed after drawing a straight baseline from the beginning to the end of fat melting (Fig. 1a). SFC calculation by the uncorrected DSC method was made by the partial integration of the thermograms at different temperatures, according to the following equation:

$$\text{SFC}_E = \frac{\int_{T_0}^{T_f} E dT}{\int_{T_0}^{T_f} E dT} \times 100 \quad (1)$$

where E is the melting energy as a function of the temperature (T), T_0 is the initial temperature of melting and T_f is the final temperature of melting.

Pulsed Nuclear Magnetic Resonance (NMR)

SFC of RBF and LTF samples was determined using a pulsed NMR spectrometer (Minispec-mq20, Bruker; Germany). Fats were treated according to the IUPAC [11] and AOCS [12] methods. Both methods include full melting of the fat and then cooling to 0 °C before bringing the samples to the measurement temperature. In the IUPAC method, the cooling is performed directly to 0 °C (60 min), while in the AOCS method, the cooling is more gradual because it is performed at different shorter stages with intermediate temperatures. The determination of SFC was performed at different temperatures: 0, 10, 21, 27, 33, 38 and 45 °C. All measurements were performed in quintuplicate.

Table 1 Fatty acid composition of different fats

Sample	Fatty acid types (%)			
	Saturated	Monounsaturated	Polyunsaturated	Trans
RBF	53.06	39.19	2.65	4.99
OBF	47.46	43.80	3.18	5.48
SBF	66.71	26.43	1.69	5.09
S25	20.12	36.44	23.45	19.97
S36	20.29	36.55	0.84	41.87
S42	28.15	29.46	0.45	41.91
LTF	48.86	12.83	37.49	0.83

Results and Discussion

Effect of Prior Treatment on the Thermal Behavior of Fats

Figure 1 shows the thermograms of modulated temperature DSC for two fats (RBF and LTF) subjected to three

different treatments. In these profiles, the total heat flow and its reversible and non-reversible components can be observed, whose endothermic peaks (below the baseline) correspond to the melting of fat. In the cases of full melting and immediate DSC measurement, the presence of exothermic peaks (above the baseline) were observed in the non-reversible component (Fig. 1a, b). This phenomenon

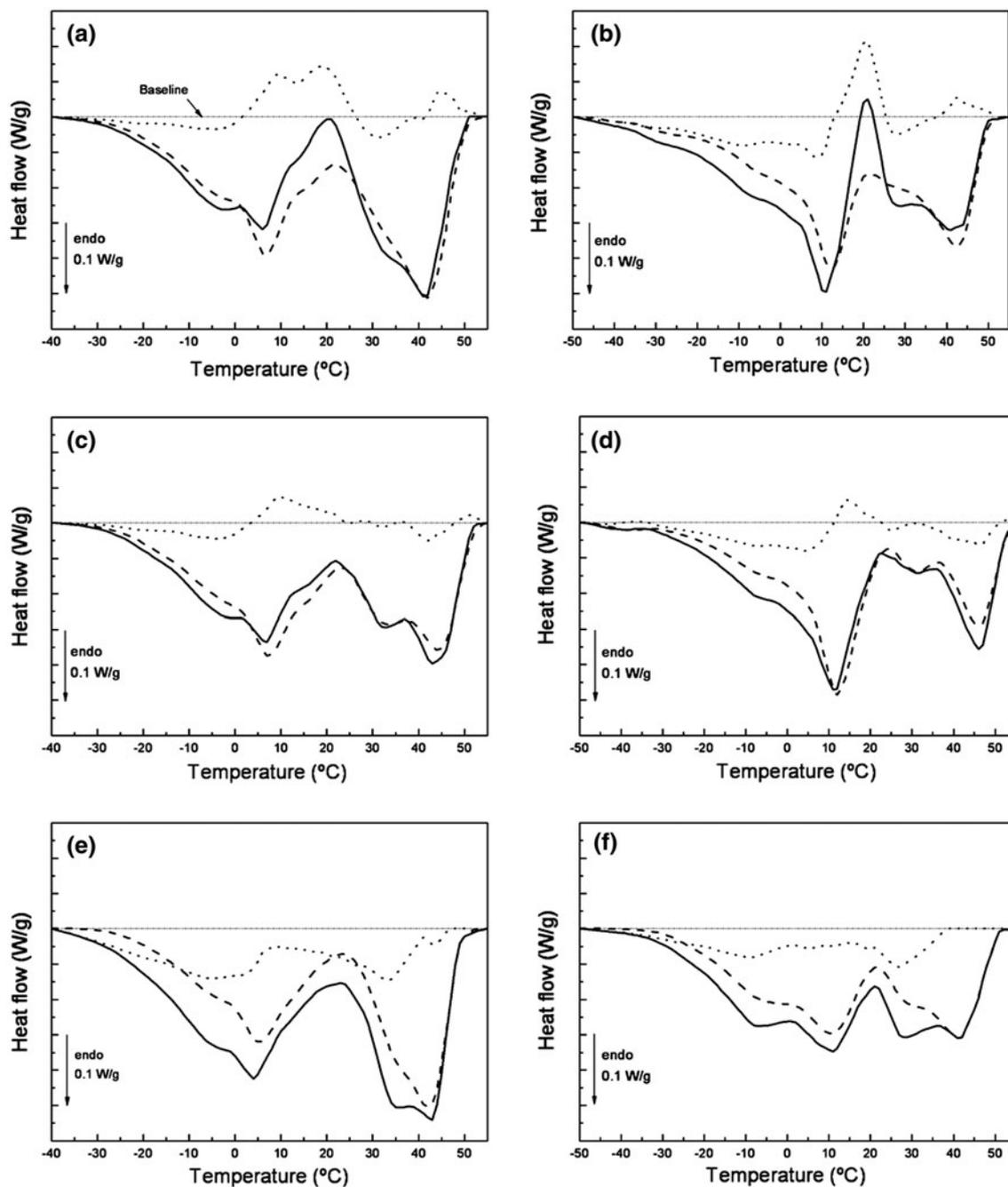


Fig. 1 DSC melting thermograms of fats, showing total heat flow (solid line), reversible heat flow (dashed line), and non-reversible heat flow (dotted line) as a function of temperature. **a** RBF, immediately after full melting; **b** LTF, immediately after full melting; **c** RBF,

immediately after partial melting; **d** LTF, immediately after partial melting; **e** RBF, with full melting +1 day at 4 °C; **f** LTF, with full melting +1 day at 4 °C

led to smaller absolute values of total heat flow than the reversible component where those exothermic peaks exist, indicating that melting energy information is lost in the total heat flow. This behavior can be attributed to the formation of metastable polymorphs (e.g. α -form crystals) due to a relatively fast cooling of the fully melted samples, which would not give them enough time to form more stable crystals ($\alpha \rightarrow \beta' \rightarrow \beta$). The exothermic peaks observed during heating were a consequence of the recrystallization of metastable crystals towards more stable polymorphs after the melting of those crystals [13]. This effect was even more pronounced when the measurements were made under the conditions recommended by AOCS for the determination of melting properties of fats, where full melting and cooling of samples are performed in the DSC [14] (data not shown). With regard to immediate measurements performed after partial melting, exothermic peaks were also observed in the non-reversible component but to a much lower extent (Fig. 1c, d). As the higher melting point crystals formed after long storage at 4 °C were not melted, they maintained their stable structure so that a lower quantity of metastable crystals would have been formed after fast cooling. Moreover, thermograms obtained after partial melting also showed a richer fraction of crystals at the highest temperature peak, which could be attributed to the growth of existing crystals that act as nuclei, favoring the formation of high melting point crystals during the cooling stage in DSC. On the other hand, the exothermic peaks were absent in samples that were fully melted and stored at 4 °C for 1 day (Fig. 1e, f). The prior cold storage of fats favors the formation of more stable polymorphs given sufficient time [13], explaining the last result. Even though no exothermic peaks remained after storage at 4 °C for 1 h (data not shown), longer cold storage time is recommended to favor the formation of the most stable polymorphs. The presence of exothermic peaks is an obstacle for the calculation of consumed melting energy, because the crystallization observed in the non-reversible component occurs at the same time as the melting observed in the reversible component. Because ΔH must be calculated from the total heat flow, the presence of exothermic peaks does not allow the correct calculation of melting energy because it masks part of the information. Thus, it is convenient to use the thermograms obtained after cold storage of the fat, so that ΔH can be used as an appropriate estimation of melting energy, for example, for SFC calculation.

Transformation of Melting Energy into Melted Mass

The thermograms obtained by DSC can be used for the approximate SFC calculation by their partial integration at different temperatures. Other authors used this method for

SFC estimation and obtained higher values than those corresponding to NMR method [1, 7, 8]. These differences could be attributed to the different melting enthalpies of each fraction of the fat, as it was previously suggested by Deroanne [10]. Thermograms obtained for different fats show a higher area below the baseline at higher temperature of the major melting peak, corresponding to a higher ΔH (Fig. 2a, b). The increase of ΔH (corresponding to the entire melting curve) with increasing major melting peak temperature can be observed in Fig. 2c. This is an expected result because ΔH of triglycerides increases with the increasing length of the hydrocarbon chains and decreases with an increasing number of double bonds [13]. This variability of ΔH indicates that the lower melting point fractions of fats consume less energy per unit of mass than the higher melting point fractions; this could be a main factor for the differences usually observed between SFC calculated by DSC and NMR. Thus, it seems to be necessary to add a correction to the DSC method by the transformation of energy percentages into mass percentages, where data corresponding to pure triglycerides should be used.

Figure 3 shows a linear correlation between the ΔH and the melting point of common triglycerides in their most stable polymorph, using data from different bibliographic sources [15–17]. Although some triglycerides are relatively far from the obtained line, most of them fit quite well in the correlation. The use of data, corresponding to pure triglycerides, responds to the need of uniform criteria, as it would be impossible to obtain a correlation between ΔH and melting temperature that can be adjusted to all type of fat blends. Moreover, pure triglycerides present a defined thin melting peak in DSC thermograms, allowing a more appropriate correlation between ΔH and melting temperature (e.g. total ΔH values in Fig. 2c are lower than in Fig. 3 at the same temperature because of the lower ΔH of the lower melting point fractions of the fats). Because the energy consumed per unit of mass tends to increase with the melting point of the triglycerides, the equation obtained from the linear correlation can be used to estimate the quantity of melted mass at each temperature. This calculation was made on the basis of the following equations:

$$\Delta H = 98.13 + 1.62 \times T \quad (2)$$

$$m = \frac{E}{\Delta H} \quad (3)$$

where ΔH is the melting enthalpy in J/g as a function of the melting temperature in °C (T), and m is the melted mass in g corresponding to the absolute energy in J (E) consumed at a defined temperature range around T ; the ideal temperature range would tend to zero, but a range of 1 °C gives sufficient accuracy. Equation 2 corresponds to the

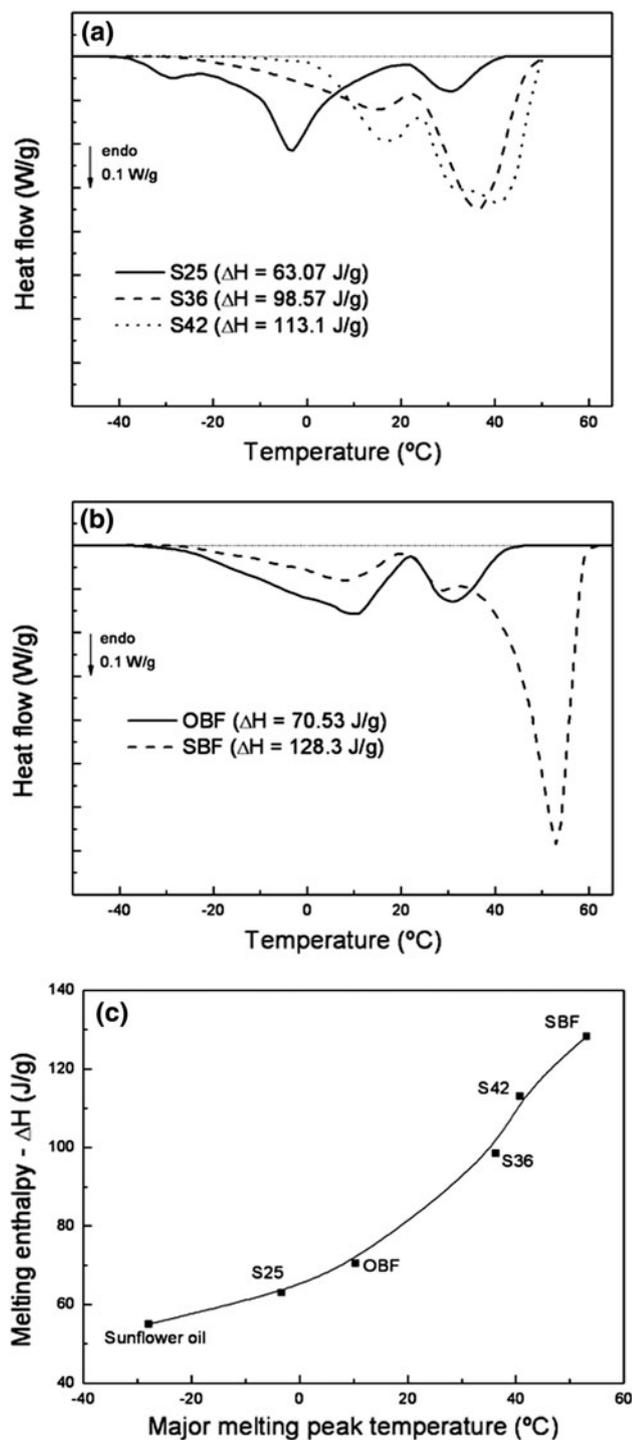


Fig. 2 **a** DSC melting thermograms (total heat flow) of partially hydrogenated soybean oils with different melting points. **b** DSC melting thermograms (total heat flow) of different fractions of bovine fat. **c** Total melting enthalpy (ΔH) versus major melting peak temperature obtained by DSC for different fats

line obtained in Fig. 3. Because the calculations are based on data corresponding to the most stable polymorph of triglycerides, it is important to subject the fats to a

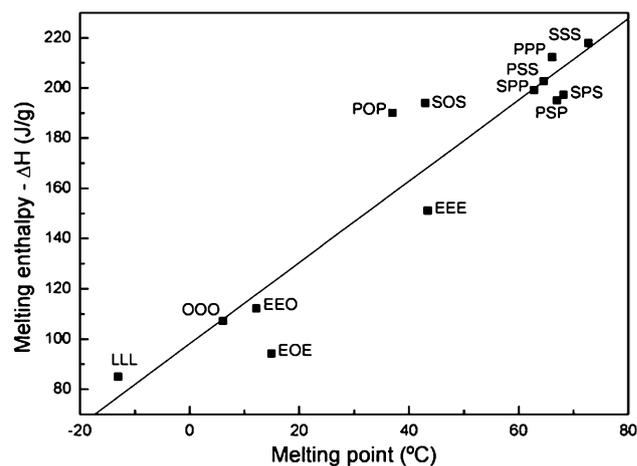


Fig. 3 Linear correlation between total melting enthalpy (ΔH) and melting point of different triglycerides in their most stable polymorph (generally β). $R = 0.94$. *P* palmitic acid, *S* stearic acid, *E* elaidic acid, *O* oleic acid, *L* linoleic acid. Data were obtained from different sources [15–17]

sufficient cold storage time. The estimation of mass seems to be quite accurate because the total mass calculated by this method (by the sum of all masses obtained at each temperature) agreed with the actual weighed mass in the DSC pan to within 5 %.

Figure 4 shows the percentages of consumed energy and melted mass of two different fats, for temperature ranges of 1 °C. The consumed energy was obtained directly from the DSC thermograms and the melted mass was calculated according to Eqs. 2 and 3. It was observed that the melted mass percentage was higher than the consumed energy percentage at lower temperatures, while the contrary occurred at higher temperatures. Thus, the differences between the DSC and NMR methods for SFC calculation would be in part attributed to a variation of consumed energy/melted mass ratio with temperature. In this way, mass profiles can be obtained in replacement of energy profiles, which can be used for a better estimation of SFC.

Calculation of Solid Fat Content by the Corrected DSC Method

As melted mass (m) can be expressed as a function of the temperature (T), SFC can be calculated from the following equation:

$$\text{SFC}_m = \frac{\int_{T_0}^{T_f} m dT}{\int_{T_0}^{T_f} m dT} \times 100 \quad (4)$$

where T_0 is the initial temperature of melting and T_f is the final temperature of melting.

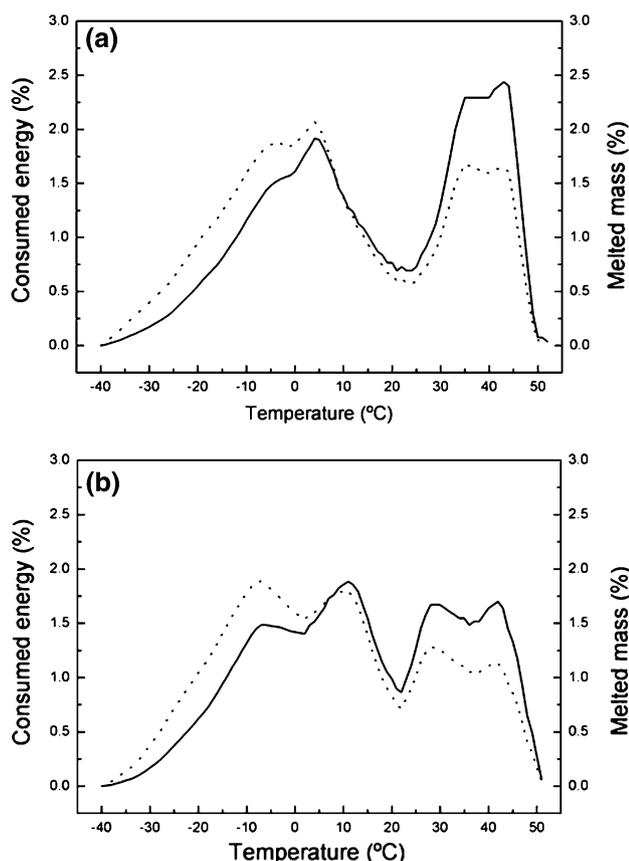


Fig. 4 Percentages of consumed energy (*solid line*) and melted mass (*dotted line*) as a function of temperature, calculated from DSC data. **a** RBF, with full melting +1 day at 4 °C; **b** LTF, with full melting +1 day at 4 °C

SFC calculations were performed for two different fats by four methods (Fig. 5): uncorrected DSC; corrected DSC; NMR by IUPAC [11]; and NMR by AOCS [12]. In the case of the uncorrected DSC, the SFC calculation was made according to Eq. 1, while the results for corrected DSC

correspond to Eq. 4. For both samples, it was observed that the SFC values obtained by uncorrected DSC were much higher than those obtained by both NMR methods at most temperatures. This is in agreement with the results observed by other authors [1, 7, 8]. On the other hand, the SFC calculated by corrected DSC as explained in this work gave results that were much closer to those obtained by NMR. For the RBF sample (Fig. 5a), the value determined at 0 °C by corrected DSC was in agreement with the one obtained with NMR according to IUPAC. However, from 10 °C to the highest temperature, the corrected DSC values were very similar to those obtained with NMR by AOCS, and up from 27 °C the three methods were in agreement. In the case of the LTF sample (Fig. 5b), the SFC calculated by corrected DSC at the lowest temperatures were higher than those obtained by both NMR methods, but they were still much closer than the ones obtained by uncorrected DSC. In this fat, at 21 °C the corrected DSC value was similar to NMR according to IUPAC, and as for the RBF sample, the three methods gave similar results above 27 °C. Although some differences were observed between the corrected DSC method and NMR methods at the lowest temperatures, it should be considered that the NMR methods also showed differences between them at those temperatures, because of their different heat treatments [18]. Moreover, it should be mentioned that the DSC method demands full crystallization of the fat while samples are cooled just to 0 °C for NMR measurements, so the differences in tempering methods may still play an important role. Furthermore, there will always be a fundamental difference between both methods: DSC is dynamic and NMR is static. Thus, the objective was not to obtain a fitting between both methods, but to develop a correction that leads to more comparable results. Nevertheless, the conversion of consumed energy into melted mass allowed to obtain SFC values that were much closer to those given by NMR methods.

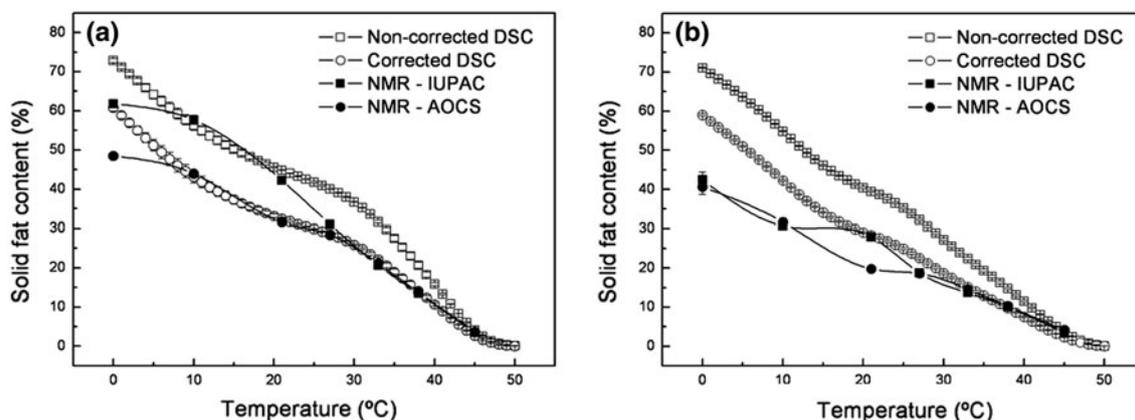


Fig. 5 Solid fat content (SFC) as a function of temperature determined by different methods. **a** RBF, with full melting +1 day at 4 °C; **b** LTF, with full melting +1 day at 4 °C. SFC values determined by

DSC and NMR are means of two and five replicates, respectively, and *error bars* indicate SD

Conclusions

The studies performed in this work show that fat should be subjected to cold storage after melting in order to avoid the formation of exothermic peaks and obtain an appropriate estimation of melting energy by DSC. This work also demonstrates that it is necessary to make a correction of the DSC method for SFC estimation, where an approximate transformation of energy into mass must be made. Although some differences between the corrected DSC method and determinations by NMR at low temperatures were observed, which could be attributed to the differences in tempering methods, at higher temperatures the results were in agreement. Even though the proposed correction may not be perfect, the results show that the transformation of energy DSC profiles into mass profiles leads to SFC values that are more comparable to those obtained by NMR. In this way, DSC could be used as a valid alternative tool instead of NMR for SFC estimation, giving its characteristic advantages such as obtaining values over the whole temperature range from one unique measurement.

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