

INVESTIGACIÓN

Solid fat content determination: Comparison between pNMR and DSC techniques

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RESUMEN

Determinación del contenido de grasa sólida: Comparación entre las técnicas pNMR y DSC.

La Calorimetría Diferencial de Barrido (DSC) ha sido una de las técnicas más populares de análisis térmico durante las dos últimas décadas. El presente trabajo muestra la utilización de DSC en la determinación del contenido de grasa sólida en las grasas. Los valores del contenido de grasa sólida fueron calculados por medio de áreas parciales de las curvas de calentamiento de DSC. Comparando con los valores de Resonancia Magnética Nuclear Pulsante (pRMN), los valores de DSC fueron generalmente más altos que los de pRMN. Las diferencias entre muestras y las técnicas entre sí parecen ser las responsables por presentar esta relación. La calorimetría diferencial de barrido es una técnica rápida para la determinación del contenido de grasa sólida, temperaturas y entalpías de las transiciones de fase, además de proporcionar una curva característica de cada muestra.

PALABRAS-CLAVE: Calorimetría Diferencial de Barrido — Control de calidad — Grasa sólida (contenido) — Resonancia Magnética Nuclear pulsante — Técnicas (comparación).

SUMMARY

Solid fat content determination: Comparison between pNMR and DSC techniques.

Differential Scanning Calorimetry (DSC) has been one of the most popular thermal analysis techniques during the last two decades. The present work shows the utilization of DSC on the determination of solid fat content in fats. Solid fat content values were calculated through the partial areas of DSC heating curves. In a comparison with pulse NMR values, DSC values were generally higher than the pNMR ones. Differences between samples and the techniques themselves seems to be responsible for this fact. DSC is a fast technique on solid fat content, characteristic temperatures and phase transitions enthalpies determinations besides giving a characteristic curve of each sample.

KEY-WORDS: Differential Scanning Calorimetry — Pulsed Nuclear Magnetic Resonance — Quality control — Solid fat (content) — Techniques (comparison).

1. INTRODUCTION

Solid-liquid ratio in fats, expressed as Solid Fat Content (SFC), is a routine analysis for the quality control and in monitoring processes such as fractionation, hydrogenation and tempering (Raemy & Lambelet, 1991). Dilatometry, the widest and oldest method used is time-consuming and not accurate. On the contrary, pNMR (pulsed Nuclear Magnetic Resonance) and DSC (Differential Scanning

Calorimetry) are easier and faster techniques, which are considered very practical and useful for determining solid-liquid ratio in fats. Pulsed NMR spectroscopy has been investigated by various authors and it has proved to give accurate and reliable results provided that certain conditions, like sample tempering are fulfilled (Lambelet, 1983). At the application temperature, solid-liquid ratio in fats can be determined from the DSC melting curves by partial integration (Kaisersberger, 1989). The partial areas values are obtained according to the equation (Raemy & Lambelet, 1991):

$$SFI(T) = \frac{\int_{T_0}^T H(T)dT}{\int_{T_0}^{T_1} H(T)dT}$$

where T_0 is the onset temperature of melting and T_1 is the end temperature of melting.

DSC offers a flexible means for tempering the fat at different temperature regimes prior the determination, as well as a thermal fingerprint of the fat. The latter is of importance in distinguishing between two fats with identical SFC values (Biliaderis, 1983).

DSC and dilatometry techniques were first compared by Miller et al. (1969). Bentz & Breidenbach (1969) and Walker & Bosin (1971) also reported studies comparing DSC, NMR and dilatometry. Differences observed between the techniques were attributed to the tempering methods and weights of samples (Walker & Bosin, 1971). Lambelet (1983 a,b) also found different values for DSC and NMR techniques which was related to the difference of the chemical composition of the fats. Deroanne (1977) used DSC to find solid fat content of palm oil and a procedure which corrected values obtained by this technique was proposed, in order to reduce differences between DSC and NMR methods. Bentz & Breidenbach (1969) had already suggested considering DSC as a new technique for oils and fats analysis. Recently DSC melting properties of fats and oils was proposed as a new method by the American Oil Chemists' Society (AOCS).

In this study the solid-liquid ratio values were determined by Differential Scanning Calorimetry (DSC) and pulsed Nuclear Magnetic Resonance (pNMR). The results obtained by these two techniques were compared and discussed.

2. EXPERIMENTAL

Material

12 different types of oils and fats were used: palm oil, palm olein, palm stearin, babassu oil and hydrogenated soybean, cottonseed oil, babassu oil, palm kernel oil and palm kernel stearin. The samples were gently supplied by Gessy Lever Ltda. (Valinhos, SP) and Refinadora de Oleos Brasil (São Caetano do Sul, SP).

Characterization of samples

Samples were characterized by the following analysis: iodine value [AOCS method Cd 1b-87 (1988)], softening point [AOCS method Cc 3-25 (1988)] and fatty acid composition through analysis of methyl esters by gas-liquid chromatography. The instrument used was a Perkin Elmer Sigma 3B gas-liquid chromatograph with ionization detector and the results were obtained by a Perkin Elmer integrator LCI - 100. The methyl esters were obtained according to Hartman & Lago (1973).

Solid Fat Content by pNMR

The instrument used was a Bruker Minispec PC120 NMR spectrometer. NMR tubes were heated at 60°C for 10 minutes prior to immersion at 0°C for 60 minutes. The

solid fat content was then measured between successive tempering procedures for 30 minutes at 10°, 20°, 30°, 35°, 37.5° and 40°C. This treatment corresponds to the IUPAC method 2.150 (1987).

Differential Scanning Calorimetry

The instrument used was a Perkin Elmer DSC7 Differential Scanning Calorimeter. Nitrogen was the carrier gas and calibration was done with indium, after checking the operation with distilled, deionized water. 10-15mg of sample were enclosed in hermetically sealed aluminum pans and run against air (empty pan) as reference. For DSC experiments, two tempering methods were tested: a) DSC₀ method: heating the sample to 80°C for 5 minutes to destroy any previous crystalline structure. Cooling rapidly to 60°C and holding for 5 minutes before crystallizing at 0° for 10 minutes. From 60 to 0°C, the cooling rate was 200°C/min. This temperature procedure was similar to IUPAC method 2.150 (1987) and it was done for comparison to pNMR results. b) DSC₁ method: heating the sample to 80°C for 5 minutes, cooling at 10°C/min to -50°C, holding at this temperature for 5 minutes to crystallize the sample. After one of these tempering methods, heating curves were recorded from initial temperature to 80°C at a heating rate of 10°C/min. The resulting DSC data was collected and processed by the PE TADS System using the Partial Areas Program.

Table I
Fatty acid composition of vegetable oils

Sample	C 8:0	C 10:0	C 12:0	C 14:0	C 16:0	C 16:1	C 18:0	C 18:1	C 18:2	C 18:3	C 20:0
SON	-	-	-	0.12	11.41	0.11	9.51	22.31	2.05	0.24	0.18
SOB	-	-	-	0.12	11.16	0.11	13.04	49.21(a)	4.84 (b)	0.25	0.44
AGH	-	-	-	0.84	23.31	0.74	3.36	35.02	0.91	0.01	0.25
AGI	5.34	4.25	35.89	13.30	11.06	-	23.67	36.28 (a)	2.66 (b)	10.11 (b)	0.86 (c)
PMD	-	-	0.46	0.82	38.23	-	5.32	59.39	1.12	-	-
PME	-	-	-	0.80	49.83	-	6.59	10.11 (b)	2.71	-	-
PMO	-	-	-	0.63	35.43	-	5.08	42.47	12.19	0.10	0.39
PMH	-	-	-	0.68	40.22	-	56.30	32.62	9.28	0.50	0.37
PKR	4.35	3.57	48.71	16.34	8.15	-	2.34	44.53	13.35	0.59	0.37
PKE	2.62	3.02	54.85	21.06	8.35	-	2.47	2.07	0.19	0.01	-
BBD	6.01	5.11	43.87	16.30	9.04	-	3.51	0.52 (b)	2.22	-	-
BBH	5.90	5.07	42.74	15.54	9.08	-	15.69	14.30	0.94	-	-
								2.02	1.12	-	-
								2.85 (a)			

SON-hydrogenated soybean oil type "N"; SOB-hydrogenated soybean oil type "B"; AGH-hydrogenated cottonseed oil; AGI-interesterified cottonseed oil; PMD-deodorized palm oil; PME-palm stearin; PMO-palm olein; PMH-hydrogenated palm oil; PKR-refined palm kernel oil; PKE-palm kernel stearin; BBD-deodorized babassu oil; BBH-hydrogenated babassu oil.

C8:0 - Caprylic acid
C10:0 - Capric acid
C12:0 - Lauric acid
C14:0 - Myristic acid
C16:0 - Palmitic acid
C16:1 - Palmitoleic acid
C18:0 - Stearic acid
C18:1 - Oleic acid
C18:2 - Linoleic acid
C18:3 - Linolenic acid
C20:0 - Arachidic acid

(a) Unidentified: probably trans isomer and/or positional of C18:1. (b) Unidentified: probably trans isomer and/or positional of C18:2. (c) Unidentified

Table II.
Iodine value and softening point of vegetable oils

SAMPLE	IODINE VALUE (g I ₂ /100 g sample)	SOFTENING POINT (°C)
SON	76	32.0
SOB	69	37.3
AGH	73	32.7
AGI	9	36.4
PMD	60	36.2
PME	43	49.8
PMO	65	20.1
PMH	3	58.4
PKR	16	28.4
PKE	7	31.7
BBD	17	24.4
BBH	7	35.5

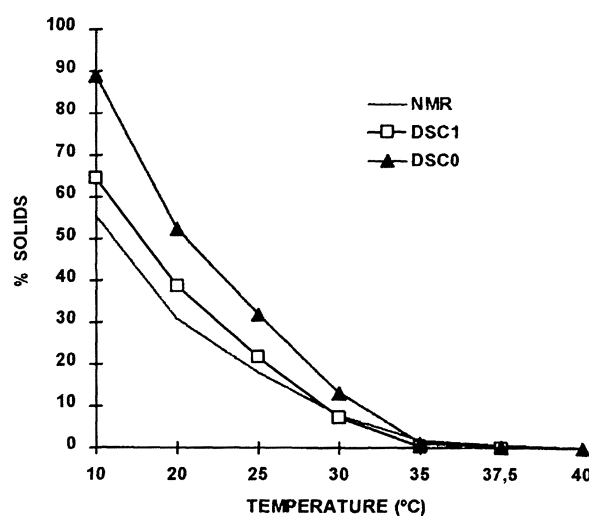
SON-hydrogenated soybean oil type "N"; SOB-hydrogenated soybean oil type "B"; AGH-hydrogenated cottonseed oil; AGI-interesterified cottonseed oil; PMD-deodorized palm oil; PME-palm stearin; PMO-palm olein; PMH-hydrogenated palm oil; PKR-refined palm kernel oil; PKE-palm kernel stearin; BBD-deodorized babassu oil; BBH-hydrogenated babassu oil.

3. RESULTS AND DISCUSSION

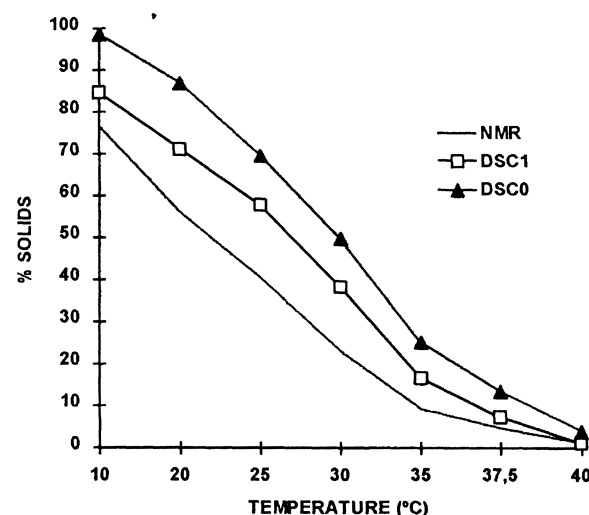
The results of the characterization of the samples are showed in table I and II. Fatty acid composition showed great variability of the samples (table I). Iodine value varied from 3 to 76 g I₂/g sample and softening point, from 20.1 to 58.4°C (table II).

For all samples, except palm oil and palm stearin, solid-liquid ratio values obtained by pNMR method were lower than the DSC ones. Solids-temperature curves for all samples are shown in figures 1 to 6. Differences between the two methods are higher at lower temperatures, becoming almost the same at higher temperatures when solid fat content diminishes. These results agree with those obtained by Lambelet (1983 a, b). Lower pNMR values than DSC ones might be explained from a theoretical point of view: NMR data are calculated on the base of the relative amount of protons present in the triglycerides in the solid and liquid phases, while DSC values are obtained on the base of the melting enthalpies of these triglycerides. The variation in heat of fusion is always greater than the corresponding variation in the relative amount of protons. It therefore results that lower NMR than DSC values are predicted throughout the fusion of the fat. However, when the fat is completely solid, this theory assumes that both methods give identical results, and this was not experimentally observed by Lambelet (1983 a, b). High DSC values may also come from attributing a 100% solid fat content value at the beginning of the measurement, without knowing anything about the sample state before this point. For the temperature procedure DSC₁, all of the DSC measurements had started at -50°C, being assumed that all samples under investigation are completely solid at this temperature. NMR values may be too low if an amorphous phase is present in the sample, which is compared by the

NMR apparatus to a liquid phase. The presence of an amorphous phase can be shown by another technique such as X-ray diffraction (Lambelet, 1983). On the other side, Bentz & Breidenbach (1969) and Walker & Bosin (1971) found higher values for the NMR method if comparing to DSC, which was attributed to chemical differences between the samples, lack of tempering for the NMR procedure and samples weight.

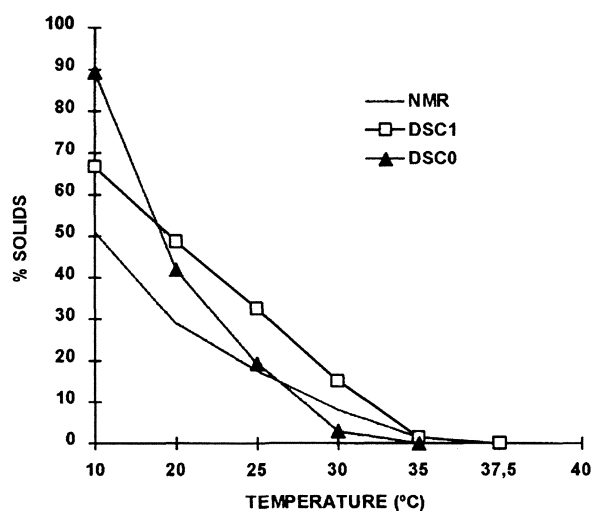


(a)

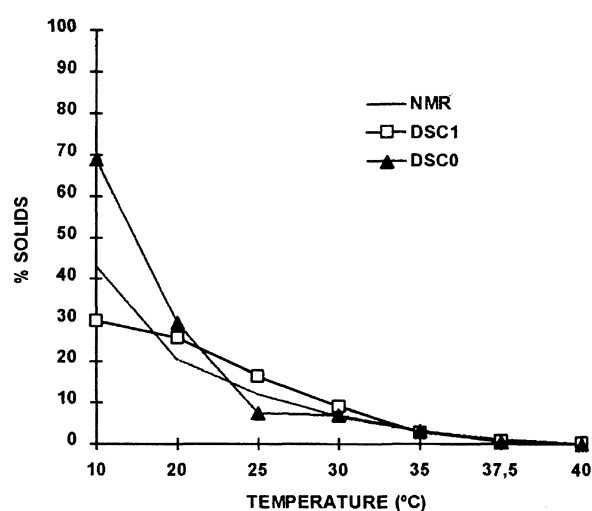


(b)

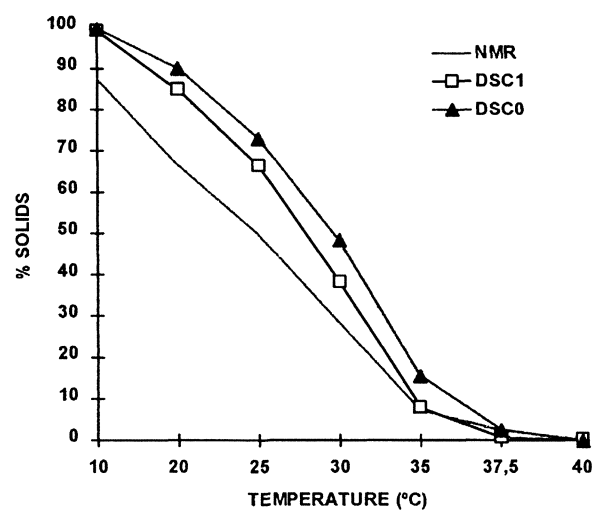
Fig. 1.
Solids-temperature curves for DSC and pNMR methods for hydrogenated soybean oil samples (a) "N" hydrogenated soybean oil; (b) "B" hydrogenated soybean oil.



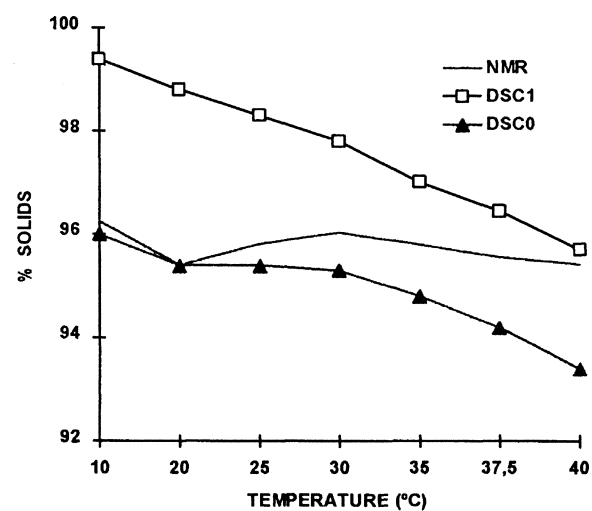
(a)



(a)



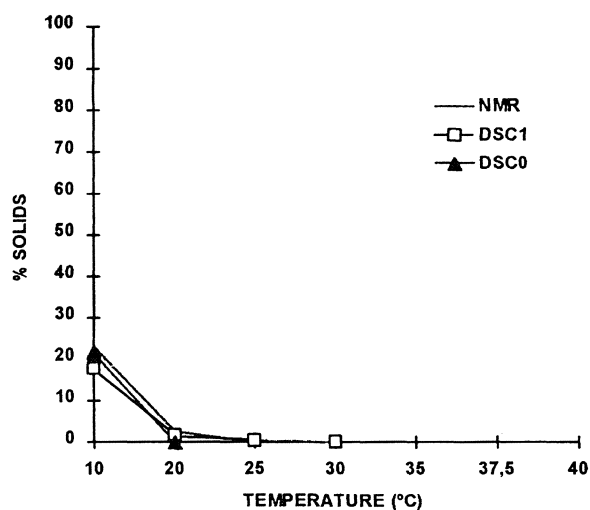
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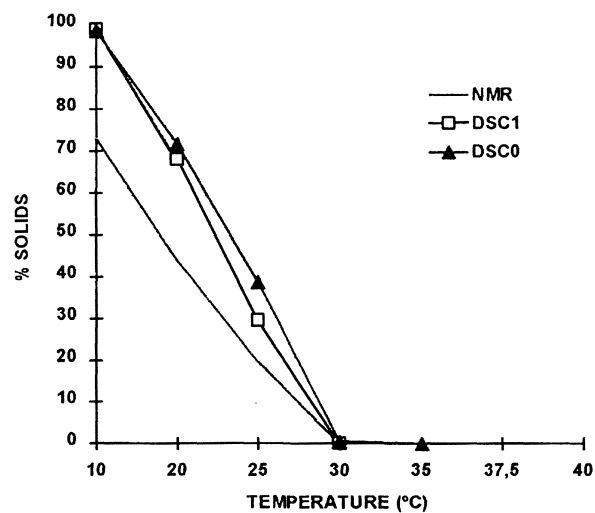
(b)

Fig.2.
Solids-temperature curves for DSC and pNMR methods for cottonseed oil samples: (a) hydrogenated cottonseed oil and (b) interesterified cottonseed oil.

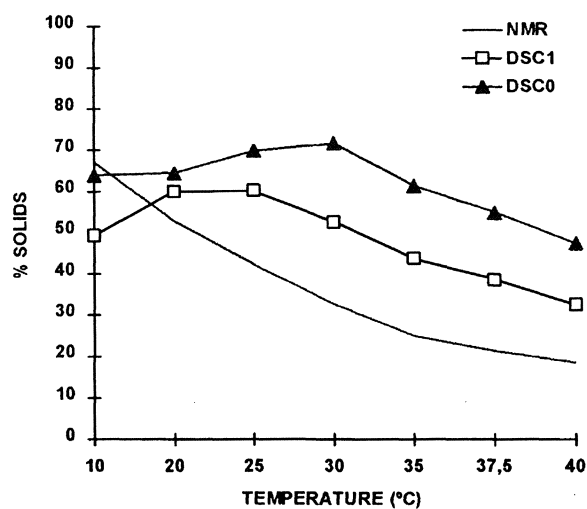
Fig.3.
Solids-temperature curves for pNMR and DSC methods for palm oil samples: (a) deodorized palm oil; (b) hydrogenated palm oil.



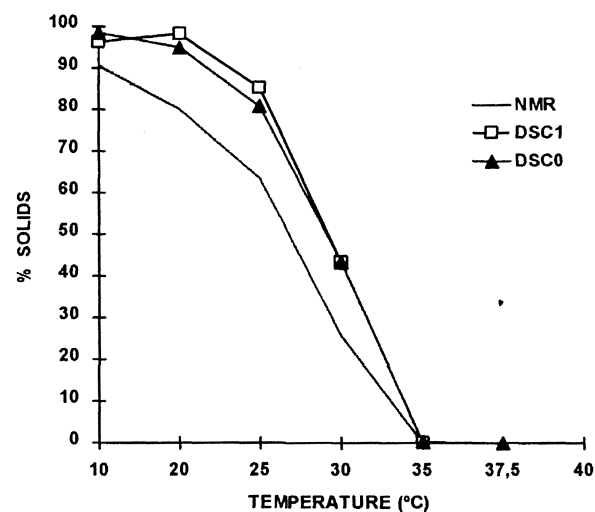
(a)



(a)



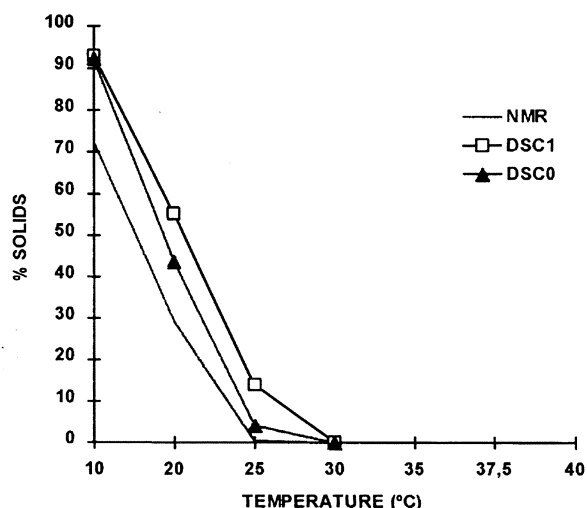
(b)



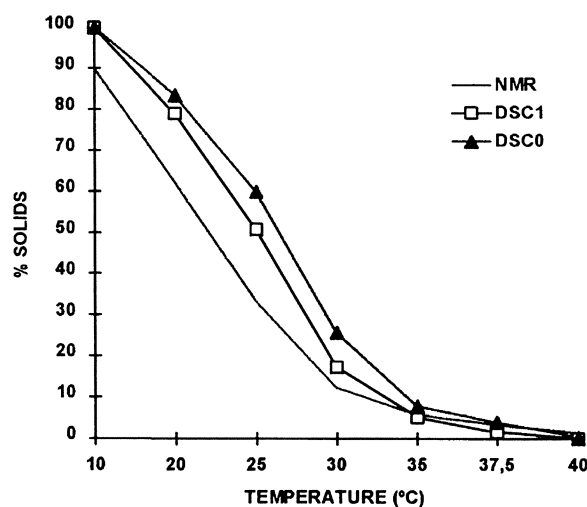
(b)

Fig.4.
Solids-temperature curves for pNMR and DSC methods for palm oil samples: (a) palm olein; (b) palm stearin.

Fig.5.
Solids-temperature curves for pNMR and DSC methods for palm kernel oil samples: (a) palm kernel oil; (b) palm kernel stearin.



(a)



(b)

Fig.6.

Solids-temperature curves for pNMR and DSC methods for babassu oil samples: (a) deodorized babassu oil and (b) hydrogenated babassu oil.

Values obtained from DSC two tempering procedures showed that DSC₀ method had higher values than DSC₁. At DSC₀ method, the rapid cooling rate does not allow the complete solidification of samples, mainly of soybean, cottonseed and palm oils due their fatty acid composition. The supposition that partial areas at 0°C was 100% was not true, leading to higher values of solid fat content if comparing to DSC₁ method, where the cooling rate was slower and the melting curve was recorded from -50°C. From this point to 0°C, almost all samples began to melt.

This fact explains why the solid fat content values of DSC₁ method calculated from partial areas are lower at 0°C than that obtained by DSC₀.

Some samples, such as hydrogenated cottonseed oil and deodorized babassu oil showed the opposite behaviour, when higher DSC₁ values were observed. It may be due sampling, where small pieces of sample were taken from solid fats without melting, being not representative. These fats might show some different crystalline forms or even crystallize in such a way that they show a different behaviour when melting, leading to these results.

Palm stearin showed a completely different behaviour if compared to the other samples. DSC heating curve of this sample shows a valley amongst the peaks between the 20 and 25°C, which dropped below the baseline. When partial areas values were calculated the exothermic peak representing a crystallization is considered an addition to the SFC values, increasing them. The same phenomenon happens with palm oil and hydrogenated palm oil, but this effect was so small that it did not affect the results.

Both DSC tempering methods solid fat content values showed higher values than the pNMR ones. Correlation values between pNMR and the two DSC tempering methods are shown in table III. A good correlation was found for almost all samples, excepting the already known cases of palm oil, palm stearin and hydrogenated palm oil. Non-linear relationships were also found by Lambelet (1983a) for milk fat samples which have in common with palm oil and palm stearin the presence of two independent fractions. Hydrogenated palm oil showed a low correlation value because solid fat content values varied in a narrow temperature range (see figure 3), although it is not necessarily source of discrepancy between methods. It has to be considered that this sample is a very unusual one, with a high softening point and a very low iodine value.

Table III
Correlation values between pNMR and DSC methods

Sample	Correlation values	
	NMR vs. DSC ₀	NMR vs. DSC ₁
SON	0.9985	0.9982
SOB	0.9781	0.9845
AGH	0.9918	0.9789
AGI	0.9835	0.9941
PMD	0.9872	0.9225
PME	0.5296	0.6655
PMO	0.9946	0.9991
PMH	0.6837	0.5548
PKR	0.9869	0.9931
PKE	0.9863	0.9790
BBD	0.9985	0.9905
BBH	0.9740	0.9893

NMR-Nuclear Magnetic Resonance; DSC₁-Differential Scanning Calorimetry/start temperature - 50°C; DSC₀-Differential Scanning Calorimetry/start temperature 0°C

SON-hydrogenated soybean oil type "N"; SOB-hydrogenated soybean oil type "B"; AGH-hydrogenated cottonseed oil; AGI-interesterified cottonseed oil; PMD-deodorized palm oil; PME-palm stearin; PMO-palm olein; PMH-hydrogenated palm oil; PKR-refined palm kernel oil; PKE-palm kernel stearin; BBD-deodorized babassu oil; BBH-hydrogenated babassu oil.

4. CONCLUSIONS

Results show that DSC can be considered an alternative technique on solid fat content determination, besides giving a fingerprint of each sample and its thermal parameters such as onset temperatures and phase transition enthalpies. Sampling and tempering methods have to be considered as important factors when obtaining DSC curves.

ACKNOWLEDGEMENTS

The authors acknowledge the valuable collaboration of Prof. Dr. Pedro Luís Onofrio Volpe (IQ - UNICAMP) and the financial support given by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brasília, Brasil) and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, São Paulo, Brasil).

REFERENCES

- Bentz, A. P. & Breidenbach, B. G. (1969). -"Evaluation of the differential scanning calorimetric method for fat solids".- J. Am. Oil Chem. Soc. **46**, 60-63.
- Biliaderis, C. G. (1983).- "Differential scanning calorimetry in food research - a review".- Food Chem. **10**, 239-265.
- Deroanne, C. (1977).- "L'analyse calorimétrique différentielle, son intérêt pratique, pour le fractionnement de l'huile de palme et la détermination de la teneur en phase solide".- Lebensm. Wiss. Tech. **10**, 251-255.
- Hartman, L. & Lago, R. C. (1973).- "Rapid determination of fatty acid methyl esters from lipids".- Lab. Practice **22**, 475-476, 494.
- IUPAC (International Union of Pure and Applied Chemistry). (1987).- "Standard Methods for the Analysis of Oils, Fats and Derivatives".- 7th. ed.- Blackwell Scientific Publications - Oxford.
- Kaisersberger, E. (1989).- "DSC investigations of the thermal characterization of edible fats and oils".- Thermochim. Acta **151**, 83-90.
- Lambelet, P. (1983a).- "Comparison of N.M.R. and D.S.C. methods for determining solid content of fats. Application to milk fat and its fractions".- Lebensm. Wiss. Tech. **16**, 90-95.
- Lambelet, P. (1983b).- "Comparison of N.M.R. and D.S.C. methods for determining solid content of fats. Application to cocoa butter and its admixtures with milkfat".- Lebensm. Wiss. Tech. **16**, 200-202.
- Miller, W. J.; Koester, W. H.; Freeberg, F. E. (1969).- "The measurement of fatty solids by differential scanning calorimetry".- J. Am. Oil Chem. Soc. **46**, 341-343.
- Raemy, A. & Lambelet, P. (1991).- "Thermal behaviour of foods".- Thermochim. Acta **193**, 417-439.
- Walker, R. C. & Bosin, W. A. (1971).- "Comparison of SFI, DSC and NMR methods for determining solid-liquid ratios in fats".- J. Am. Oil Chem. Soc. **48**, 50-53.

Recibido: Marzo 1995

Aceptado: Octubre 1995