

Interactions in interesterified palm and palm kernel oils mixtures. II – Microscopy and Differential Scanning Calorimetry

By Renato Grimaldi^{1*}, Lireny Aparecida Guaraldo Gonçalves¹, Luiz Antonio Gioielli² and Ilka S. Simões²

¹Laboratório de Óleos e Gorduras – Faculdade de Engenharia de Alimentos (FEA) – Universidade Estadual de Campinas (UNICAMP), Caixa Postal 6091, CEP 13081-970, Campinas, SP, Brazil

²Faculdade de Ciências Farmacêuticas – Universidade de São Paulo (USP)

RESUMEN

Interacciones entre mezclas de aceites de palma y palmiste interesterificados. II – Microscopía y Calorimetría Diferencial de Barrido (DSC).

Fueron interesterificados en el laboratorio mezclas de aceite de palma (PO) y aceite de palmiste (PKO) en diferentes proporciones (100/0, 80/20, 60/40, 50/50, 40/60, 20/80 y 0/100) bajo condiciones predeterminadas (0.4% metóxido de sodio, 20 minutos, 100°C). Las catorce muestras fueron caracterizadas antes y después de la interesterificación por Microscopía de Luz Polarizada y por Calorimetría Diferencial de Barrido (DSC). Los resultados mostraron el efecto de varios factores sobre la forma y anchura de los cristales. El área media de los cristales revela el aumento de tamaño de los mismos cuando aumenta la proporción de PKO, con valores que varían entre $2.7 \times 10^3 \mu\text{m}^2$ para PO y $1.8 \times 10^6 \mu\text{m}^2$ para PKO. Después de la interesterificación, la anchura de los cristales fue menor para las formulaciones de PO/PKO de 100/0, 80/20, 60/40 y 20/80, y fue mayor en las otras. La forma polimórfica β' se observó en la muestra de aceite de palma puro. Los resultados mostrados por las curvas de fusión, presentan valores de "onset" desde -19.6°C para los picos correspondientes a los triglicéridos más insaturados, hasta 20.7°C para los más saturados. Los valores mayores de entalpía de fusión de los picos más saturados del aceite de palma, es 38.7 J.g^{-1} antes y 48.4 J.g^{-1} después de la interesterificación, mostrando un grupo saturado más estable. De forma global, la interesterificación causa un incremento en el gradiente de cristalización y mejora la compatibilidad en las fracciones de PO/PKO.

PALABRAS-CLAVE: Aceite de palma interesterificado - Aceite de palmiste interesterificado - Calorimetría diferencial de barrido - Microscopía de luz polarizada - Polimorfismo.

SUMMARY

Interactions in interesterified palm and palm kernel oils mixtures. II – Microscopy and Differential Scanning Calorimetry.

Palm oil (PO) and palm kernel oil (PKO) compositions (100/0, 80/20, 60/40, 50/50, 40/60, 20/80 and 0/100) were interesterified in laboratory scale under predetermined conditions (0.4% sodium metoxide, 20 minutes, 100°C). The fourteen samples, before and after interesterification, were characterized by Polarized Light Microscopy and Differential Scanning Calorimetry (DSC). Results showed the effect of various factors on the form and width of crystals. The mean area of crystals revealed the increase of crystals when PKO was added, with values varying from $2.7 \times 10^3 \mu\text{m}^2$ to PO and $1.8 \times 10^6 \mu\text{m}^2$ to PKO. After interesterification, the

crystal widths were lower at PO/PKO 100/0, 80/20, 60/40, 20/80 fractions and were higher to others. The beta-prime polymorphic form was observed in the pure palm oil sample. The results showed in melting curves, onset values from -19.6°C to more unsaturated peaks until 20.7°C to more saturated ones. The higher values to more saturated peak in a melting curve to palm oil, 38.7 J.g^{-1} before and 48.4 J.g^{-1} after interesterification, showed a more stable saturated group. In a general way, the interesterification promoted an increase of crystallization rate and a better compatibility between PO/PKO fractions.

KEY-WORDS: Differential scanning calorimetry - Interesterified palm kernel oil - Interesterified palm oil - Polarized light microscopy - Polymorphism.

1. INTRODUCTION

The utilization of chemical interesterification in fats and oils mixtures is very important and versatile, which allows the combination of different fats and oils properties, with final use in margarines and shortenings. The raw materials react in a catalyst presence at mean temperature of 100°C , that promoting a random distribution of fatty acids bound to triacylglycerides, leading to the formation of new compounds with different physical properties (Allen, 1996).

Several instrumental techniques are used to monitoring the interesterification reaction, that include differential scanning calorimetry as an important tool to investigate transition of crystalline forms during fats melting (Zeitoun *et al.*, 1993). When the fat is heated, it can exhibit multiple melting phases, such as each crystallization step represents a transition phase from a less stable polymorphic form to a more stable one (Schmidt *et al.*, 1996). The peak temperature transition can be an important indicator of crystal polymorphic form, since the more stable form has a higher melting point (Zeitoun *et al.*, 1993).

Nassu (1994) studied a thermal behavior of deodorized Brazilian oils like soybean, cottonseed, palm, palm kernel and coconut, besides hydrogenated, fractioned and interesterified oils. In other study, Grimaldi (1998) characterized eight

samples of hydrogenated fats, used in margarine productions.

Melting termograms, through differential scanning calorimetry supply valorous information on how the products melt in the mouth during mastication. The partial area values, identified under the melting peak (endothermic), is equivalent to the remaining solids percent at the selected temperature and this value is higher than the values determined through nuclear magnetic resonance (Ali and Dimick, 1994).

Cebula and Smith (1991) utilized DSC in the investigation of influence of progressive changes on the formulation of cocoa butter substitutes and compounds with saturated triacylglycerides and other polar compounds, normally present in bakery fats, through calorimetric curves. DSC was considered as a sensible technique to shortening characterization, permitting a good control in comparing with similar thermal behavior and that present melting and crystallization at similar conditions. Changes in a thermal profile are attributed to differences in the triacylglycerides compositions of products.

Deman *et al.* (1989) used DSC to peak temperature, enthalpy and crystallization temperature verification. In another study, Deman *et al.* (1991) many shortenings were characterized getting different melting curves.

Another technique utilized in a crystallization study is polarized light microscopy, that can distinguish between liquid and solid phases, because the crystals are anisotropic whereas liquid fat is isotropic (Rousseau *et al.*, 1996).

Exams of randomized lard (chemically interesterified) with polarized light microscopy showed that its composition presented small spherulites rather than large ones in the native lard (Rousseau *et al.*, 1996).

Hurtová *et al.* (1996) accomplished random interesterification of sunflower and canola oils mixtures with fully hydrogenated oils. In these reactions, sodium hydroxide and sodium methoxide were used as catalysts. The evaluations were made with polarized light microscopy, where the crystalline structure was observed. The composition photos showed small crystal clusters, characteristics for beta prime form. The crystallization of original samples was very fast, promoting clusters with higher crystals, characteristics of beta form.

Rousseau *et al.* (1996) used the polarized light microscopy in the visualization of butter and canola oils mixtures before and after interesterification. The addition of canola oil promoted a gradual aggregation of crystalline structure. The micro photos of butter oil before the reaction showed a dense network of spherulites, with measure varying from 10-25 μm in size. After the reaction, a crystal network was composed of spherulites of varied densities,

measuring around 15 μm , with a lacy network of small crystals.

The objective of this work was to make an investigation of interesterified palm and palm kernel oils fractions and their characterization through differential scanning calorimetry and polarized light microscopy. Another objective was the visualized of eutectic systems between binary mixtures of palm and palm kernel oils.

2. EXPERIMENTAL

2.1. Material

Refined palm oil (PO) and palm kernel oil (PKO), supplied by Companhia Refinadora da Amazônia (CRA), Brazil.

PO/PKO mixtures (%) – 100/0, 80/20, 60/40, 50/50, 40/60, 20/80, 0/100 before and after interesterification reaction in the following conditions: 0.4% MeONa, 100°C, 20 minutes.

Sodium methoxide, supplied by BASF S.A.

2.2. Methods

Microscopy analysis of crystals

Slide preparation– the samples were melted at 60-70°C to complete melting of crystals. The laminas to microscopy were put on the heating plate (Thermal Microscope Stage, TS-4 series, PHYSITEMP INSTRUMENTS INC.) adjusted at 50°C, fitted with a bath (Thermo Bath, model TB-85, Shimadzu Corporation, Japan) during 5 minutes to temperature stabilization. The samples were put on the laminas with the aid of capillary tubes (1 drop \cong 12mg) and dispersed with the cover. The crystallization was carried at 25 and 35°C during 48 hours.

Crystal visualization– with polarized light microscopy (Olympus System Microscope, model BX 50, Olympus America Inc.). Amplification of 40X, 100X and 200X, in accord to width of crystals. The visualization was done at constant temperature.

Thermal Analysis (DSC)– Method AOCS Cj 1-94 (1997).

Equipment: DSC-7 Thermal Analysis Perkin Elmer

Analysis conditions:

Weight sample: ~10mg

Melting curves: 10min (80°C), 80°C to -40°C (10° C/min); 30min at -40°C; 1min (-40°C), -40°C to 80°C (5° C/min).

3. RESULTS AND DISCUSSION

The laminas, conditioned according to methods section, were measured and the crystal data are

Table I
Statistical data to crystal parameters of PO/PKO mixtures at 25°C and 35°C before and after chemical interesterification

Fraction	Mean Area (μm^2)		Mean Diameter (μm)	
	25°C	35°C	25°C	35°C
PO/PKO 100/0 b	2.7×10^3	5.8×10^4	58.0	247.6
PO/PKO 100/0 a	119.8	861.7	14.5	31.9
PO/PKO 80/20 b	2.9×10^3	7.5×10^4	59.6	316.5
PO/PKO 80/20 a	684.7	1.3×10^4	29.1	110.4
PO/PKO 60/40 b	5.9×10^3	1.0×10^5	83.6	360.6
PO/PKO 60/40 a	310.2	3.7×10^4	21.5	214.1
PO/PKO 50/50 b	4.9×10^3	3.9×10^4	75.9	220.5
PO/PKO 50/50 a	2.4×10^4	—	173.4	—
PO/PKO 40/60 b	5.8×10^3	1.3×10^4	84.6	122.1
PO/PKO 40/60 a	2.8×10^4	—	177.0	—
PO/PKO 20/80 b	2.4×10^5	—	494.4	—
PO/PKO 20/80 a	4.6×10^5	—	420.2	—
PO/PKO 0/100 b	1.8×10^6	—	1.9×10^3	—
PO/PKO 0/100 a	9.8×10^5	—	981.7	—

b - before interesterification; a- after interesterification.
 Mean of standard deviations observed at each temperature - $\pm 10\%$.

showed on Table I. The parameters available were mean area and diameter.

The reading temperature was based on previous experience. In some cases at 35°C, the samples were completely melted. The interesterification promoted a decrease of crystal sizes of 100/0, 80/20 and 60/40 PO/PKO fractions, while that to other samples, the interesterification promoted an increase of crystal sizes, with exception of PKO, where the mean area was from 1.8×10^6 to $9.8 \times 10^5 \mu\text{m}^2$.

Many factors influence lipid crystallization, like tempering method, interactions between components and crystallization time. The eutectic effect, visualized at PO/PKO fractions is another parameter that affects the crystallization rate.

The evaluation parameters, calculated through obtained termograms, were similar to those cited by Nassu (1994). The Table II shows melting enthalpy values of PO/PKO compositions before and after interesterification.

The melting termograms and images at 25°C can be visualize in the next figures.

The interesterification of PO/PKO 100/0 fraction excluded the area below base line in the melting curve, related to an increase of crystallization rate. The process avoided the simultaneous melting and crystallization, observed through the portion below the base line and visualized at 100/0 fraction before interesterification (Nassu, 1994).

This behavior of simultaneous crystallization and melting was studied by Cebula and Smith (1991). At this work, the effect of different crystallization and melting rates in pure triacilglyceride termograms

Table II
Melting enthalpies (J.g^{-1}) at PO/PKO mixtures before and after chemical interesterification

Fraction	Peak 1	Peak 2	Peak 3
PO/PKO 100/0 b	42.2	—	38.7
PO/PKO 100/0 a	16.2	10.5	48.4
PO/PKO 80/20 b	22.0	56.6	—
PO/PKO 80/20 a	7.3	80.8	—
PO/PKO 60/40 b	4.2	80.9	—
PO/PKO 60/40 a	1.1	90.7	—
PO/PKO 50/50 b	2.0	84.8	—
PO/PKO 50/50 a	2.7	79.5	—
PO/PKO 40/60 b	3.1	98.0	—
PO/PKO 40/60 a	2.6	68.6	—
PO/PKO 20/80 b	1.8	98.5	—
PO/PKO 20/80 a	1.9	82.4	—
PO/PKO 0/100 b	—	112.5	—
PO/PKO 0/100 a	—	96.4	—

b - before interesterification; a- after interesterification.

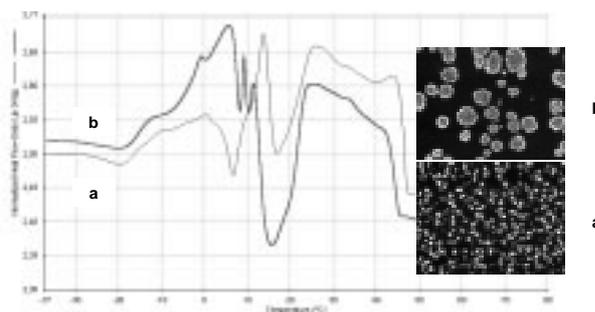


Figure 1
 Melting curves and images of PO-100 before (b) and after (a) chemical interesterification.

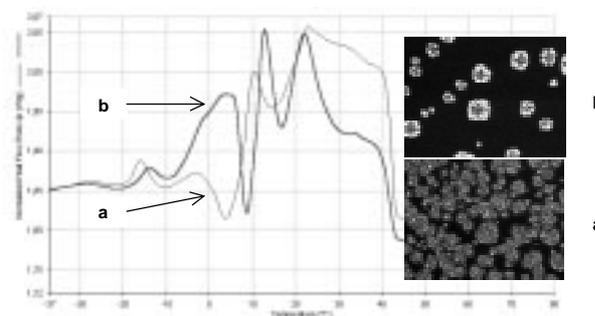


Figure 2
 Melting curves and images of PO/PKO 80/20 before (b) and after (a) chemical interesterification.

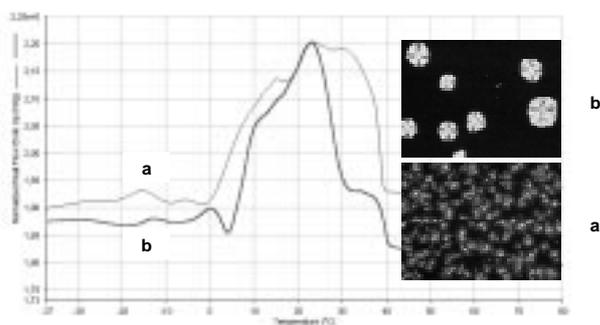


Figure 3
Melting curves and images of PO/PKO 60/40 before (b) and after (a) chemical interesterification.

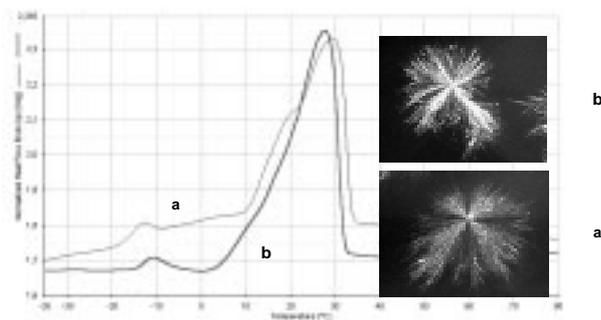


Figure 6
Melting curves and images of PO/PKO 20/80 before (b) and after (a) chemical interesterification.

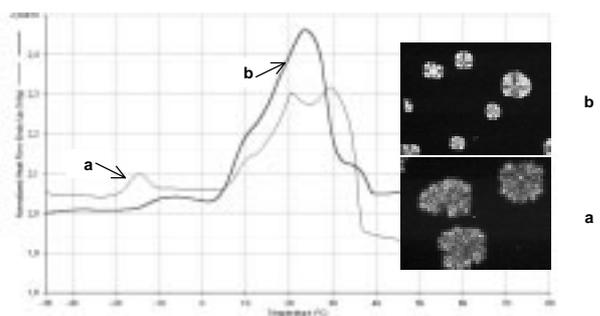


Figure 4
Melting curves and images of PO/PKO 50/50 before (b) and after (a) chemical interesterification.

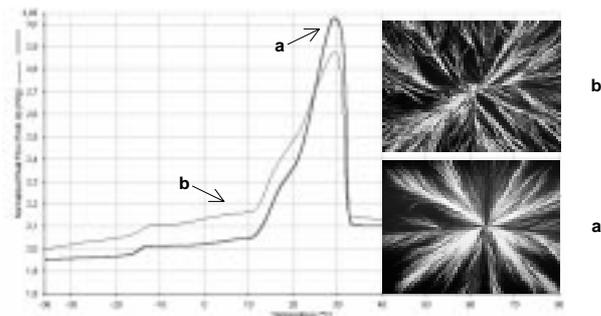


Figure 7
Melting curves and images of PKO-100 before (b) and after (a) chemical interesterification.

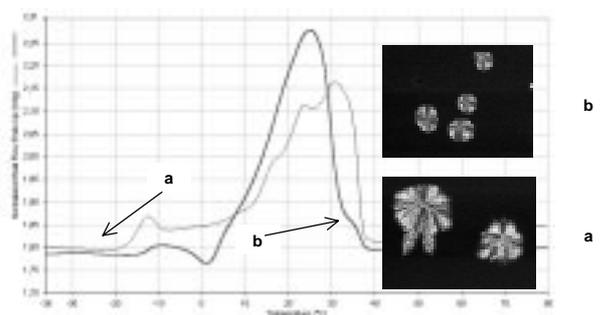


Figure 5
Melting curves and images of PO/PKO 40/60 before (b) and after (a) chemical interesterification.

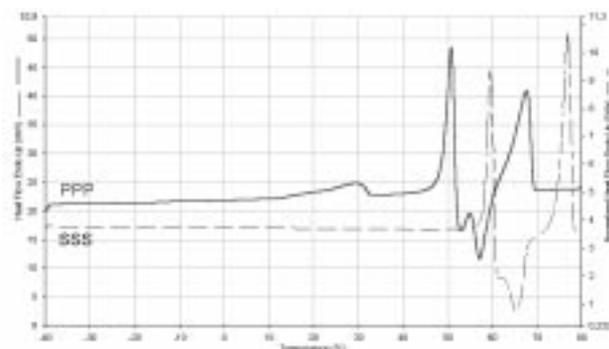


Figure 8
Melting curves of pure triacylglycerides (PPP and SSS).

(POS and POP) was studied. After fast POP crystallization, different heating rates showed many polymorphic transitions to the same original sample.

The PO/PKO 80/20 showed many peaks in the melting curves before interesterification, with similar characteristics after reaction, and small differences in more unsaturated region (0°C). The complexity of

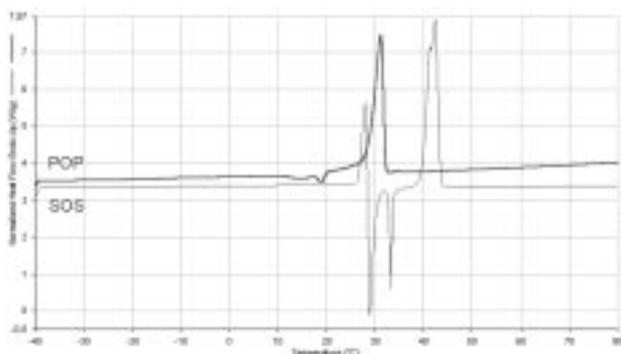


Figure 9

Melting curves of pure triacylglycerides (POP and SOS).

termograms can suggest a high incompatibility between triacylglycerides of the samples. Other melting curves presented similar behavior, with only a large melting peak.

The palm kernel oil presented a single melting peak, due to a high concentration of trisaturated triacylglycerides. The interesterification reaction to palm kernel oil modified the crystalline morphology, with more structured crystals. Berger and Ong (1985) cited that after interesterification, the applicability of palm kernel oil was improved, due to its better plasticity, characteristics of beta prime crystals.

Kaiserberger (1989) accomplished the thermal characterization of many oils and fats and concluded that the melting range and curve profile are dependent of many combined effects like triacylglycerides composition, polymorphism and thermal history. The Figures 8 and 9 show the melting curve to pure triacylglycerides.

The mathematic model, calculated by multiple regression for two components mixtures, according to Hare (1974) is the following:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_{1,2} X_1 \cdot X_2$$

Where Y is the estimated mean area or mean diameter of crystals. X_1 is palm oil proportion, X_2 is palm kernel oil proportion. $X_2 + X_1 = 100$. The R^2 is used to estimate the adjustment of the mathematical model for the to obtained data (Table III).

A decrease of absolute values of $\beta_{1,2}$, after the interesterification proved the better compatibility of palm and palm kernel oils.

4. CONCLUSION

Palm and palm kernel fractions showed characteristics of eutectic system, mainly at 80/20

Table III
Interactions between PO/PKO mixtures before and after chemical interesterification. Mean area and diameter data

Mean area (μm^2)	Coefficients			R^2
	β_1	β_2	$\beta_{1,2}$	
b	1.64×10^5	1.57×10^6	-3.79×10^6	0.89317
a	5.13×10^4	9.55×10^5	-1.93×10^6	0.98114
Mean diameter (μm)	β_1	β_2	$\beta_{1,2}$	R^2
b	204.93	1740.20	-3814.80	0.93881
a	49.82	930.50	-1536.86	0.98354

b - before interesterification; a- after interesterification

and 50/50 PO/PKO fractions. The chemical interesterification at 100°C and 20 minutes with 0,4% of sodium methoxide minimized the incompatibility between the fractions and improved the plasticity.

Crystallization profile of interesterified samples showed a better compatibility between the oils, with a sharp and well-defined peaks. The mathematic model using the diameter and area data of crystals, proved the decrease of eutectic system after chemical interesterification.

ACKNOWLEDGEMENTS

The authors would like to express many thanks to FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo by the financial support and to CRA (Companhia Refinadora da Amazônia) by the supplier of palm and palm kernel oils.

REFERENCES

- Allen, D.A. (1996). Interesterification – a vital tool for the future? *Lipid Techn.* 11-18.
- Al., A.R.M. and Dimick, P.S. (1994). Thermal analysis of palm mid-fraction, cocoa butter and milk fat blends by differential scanning calorimetry. *J. Am. Oil Chem. Soc.* **71**, 299-302.
- AOCS (1997). Official Methods and Recommended Practices of the American Oil Chemist's Society. Champaign, v.1-2.
- Berger, K.G. and Ong, S.H. (1985). The industrial uses of palm oil and coconut oils. *Oléagineux*, **40**, 613-621.
- Cebula, D.J. and Smith, K.S. (1991). Differential scanning of confectionery fats. Pure triglycerides: effects of cooling and heating rate variation. *J. Am. Oil Chem. Soc.* **68**, 591-595.
- Deman, L., Man, J.M. and Blackman, B. (1989). Physical and textural characteristics of some shortenings and margarines. *J. Am. Oil Chem. Soc.* **66**, 128-132.
- Deman, L.; Man, J.M.; Blackman, B. (1991). Physical and textural characteristics of some North American shortenings. *J. Am. Oil Chem. Soc.* **68**, 63-69.

- Grimaldi, R., Nassu, R.T., Gonçalves, L.A.G. and Moreira, R.N.C. (1998). Characterization of hydrogenated fats for margarine manufacturing purposes. *Grasas Aceites*, **49**, 1-8.
- Hare, L.B. (1974). Mixture designs applied to food formulation. *Food Techn.* **28**, 50-62.
- Hurtová, S., Schmidt, S., Zemanovic, J., Simon, P. and Sekretar, S. (1996). Random interesterification of fat blends with alkal. catalysts. *Fett/Lipid*, **98**, 60-65.
- Kaisersberger, E. (1989). DSC investigations of thermal characterization of edible fats and oils. *Thermoch. Acta*, **151**, 83-90.
- Nassu, R.T. (1994). Estudo do comportamento térmico de óleos e gorduras por calorimetria de varredura diferencial (DSC). - Tese (Mestrado), Depto de Tecnologia de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Campinas, SP, Brasil.
- Rousseau, D., Forestière, K., Hill, A.R. and Marangoni, A.G. (1996). Restructuring butterfat through blending and chemical interesterification. 1. Melting behavior and triacylglycerol modifications. *J. Am. Oil Chem. Soc.* **73**, 963-972.
- Schmidt, S., Hurtová, S., Zemanovic, J., Sekretar, S., Simon, P. and Ainsworth, P. (1996). Preparation of modified fats from vegetable oil and fully hydrogenated vegetable oil by randomization with alkal. catalysts. *Food Chem.* **55**, 343-348.
- Zeitoun, M.A.M., Neff, W.E., List, G.R. and Mounts, T.L. (1993). Physical properties of interesterified fat blends. *J. Am. Oil Chem. Soc.* **70**, 467-471.

Recibido: Septiembre 2000
Aceptado: Mayo 2001