Differential Scanning Calorimetry Index for Estimating Level of Saturation in Transesterified Wax Esters[1]

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ABSTRACT: Differential scanning calorimetry (DSC) thermograms of fatty esters can give valuable information on melting characteristics and heats-of-fusion enthalpy (ΔH). A series of jojoba liquid wax esters was constructed by transesterifying native jojoba oil with 5–50% completely hydrogenated jojoba wax esters. This series, when subjected to a standardized DSC tempering method with heating/cooling cycles, exhibited an excellent correlation for level of saturation based on area changes in endothermic ΔH. Endothermic events were recorded for native (ΔH_A) and completely hydrogenated (ΔH_C) jojoba wax esters. A third endotherm, ΔH_B, was observed when they were transesterified. Based on a multiple regression program, the best fit ($R^2 = 0.98$) using ΔH data was: % saturation = $16.847 - 0.162\ (\Delta H_A) + 0.209\ (\Delta H_B) + 0.600\ (\Delta H_C)$. Standard errors for predictions were approximately 1.045 at 0% saturation, 0.770 at 25% saturation, and 1.158 at 50% saturation. Endothermic events A, B, and C each represent the respective diunsaturated, monounsaturated, and saturated contents of wax esters in the transesterified blends. This was verified by measuring the dropping points for both the native and completely hydrogenated wax esters. These findings provide an index which can predict the degree of saturation in transesterified wax ester blends and serves as a research tool in process and product developments.

JAOCS 73, 271–273 (1996).

KEY WORDS: Differential scanning calorimetry, index of saturation, jojoba wax, transesterified wax esters.

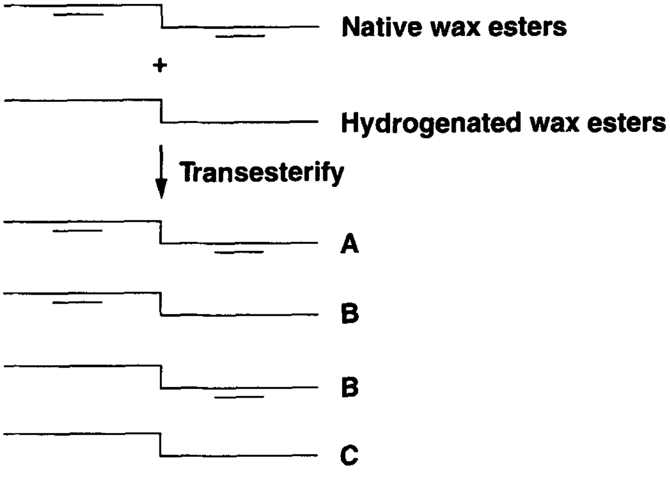
Differential scanning calorimetry (DSC) has been used not only to develop a profiling technique to characterize tropical oils, such as palm, palm kernel, and coconut (1), but also to identify other vegetable oils and their detection in baked food products (2). DSC melting curves have proven useful for monitoring polymorphic changes in mixtures of confectionery fats (3), for evaluating the effects of liquid fats on the melting points and polymorphic behavior of cocoa butter (4), for detecting adulterants such as animal fats in butter (5), and also for monitoring physical interactions of ternary blends of fats (6).

Application of statistical and mathematical techniques to DSC melting curve data can be used to devise a solid-fat index (7,8). Dyszel and co-workers used these techniques to characterize the country of origin of pistachio nuts (9), macadamia nuts (10), and peanuts (11). The primary objective of our research was to devise a DSC index to estimate the amount of saturation present in transesterified blends of jojoba wax esters based on heats-of-fusion enthalpies (ΔH). Native jojoba liquid wax, our starting material, is an esterified mixture of predominantly monoenoic fatty acids (12–14) with monoenoic alcohols to form diunsaturated esters.

EXPERIMENTAL PROCEDURES

Materials. The calibration (provided by JDA) sets consisted of native jojoba wax esters chemically transesterified with proportionate blends of completely hydrogenated jojoba wax esters to give a series consisting of 5, 10, 15, 20, 30, 40, and 50% saturation. These transesterified blends contained diunsaturated, monounsaturated, and completely saturated esters labeled A, B, and C, respectively (see Scheme 1).

Analyses. Four replicates of each calibration set were analyzed by the American Oil Chemists' Society (AOCS) recommended DSC procedure Cj 1-94 (15), which consisted of



SCHEME 1

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[1]Presented at the 1995 AOCS Meeting, May 7–11, 1995, San Antonio, Texas.

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a series of heating and cooling cycles. Samples were each heated from room temperature to 80°C and held for 10 min to destroy crystal memory, cooled at 10°C/min to –40°C and held for 30 min to allow for polymorphic changes and finally heated at 5°C/min to 80°C to define melting profile. Analyses were performed with a Perkin-Elmer DSC 7 equipped with a glove box, Intercooler II (liquid nitrogen cooling unit), and TAS 7 software package (Perkin-Elmer Corp., Norwalk, CT). Transesterified wax ester samples of about 2.5 mg, rather than the recommended 7.0 mg, were each weighed in nonvolatile aluminum pans to 1 µg accuracy on a Cahn 29 Automatic Electrobalance (Cahn Instruments Inc., Cerritos, CA). The sample pans were crimped but not hermetically sealed. An empty crimped aluminum pan was used as a reference. A dual standardization with *n*-decane and indium was used to calibrate the instrument. Data from the final heating scan from –40 to 80°C, heated at 5.0°C/min, was used to devise the mathematical index. The ΔH was calculated by fitting a straight line from peak onset to peak conclusion with area recorded as joules per gram (J/g). Dropping point of the native and completely hydrogenated wax esters was determined by AOCS recommended procedure Cc 18-80 (15) using a Mettler FP90 Central Processor with a FP83HT dropping point cell.

Relationships between ΔH and % saturation of wax esters were measured by linear regressions in the General Linear Models procedure of the Statistical Analysis Systems software package (SAS/STAT Guide for Personal Computers, Version 6 ed; SAS Institute, Cary, NC). Regression results were examined and compared by *P*-values, R^2, and visual evaluation of residuals. A calibration model was constructed from this data using multilinear regression algorithms to fit the original data.

RESULTS AND DISCUSSION

DSC thermograms of native jojoba liquid wax esters gave one endothermic event with peak onset of 4.358°C, peak maximum of 11.818°C, and ΔH_A of 123.564 J/g; whereas thermograms of completely hydrogenated jojoba wax esters had a peak onset of 66.575°C, peak maximum of 69.066°C, and ΔH_C of 218.269 J/g. The designation "A" of ΔH represents the diunsaturated form, and "C" represents the saturated form of the starting materials (see Scheme 1). Native wax esters, primarily the diunsaturated form, gave a dropping point of 13.5°C which was 1.7°C beyond the peak maximum observed by DSC. On the other hand, the completely saturated wax esters had a dropping point of 72.3°C as compared to the DSC peak maximum of 69.1°C. As the dropping points are in close agreement with the DSC peak maxima, the "A" endothermic event must represent the diunsaturated form and the "C" event represents the completely saturated form. Figures 1 and 2 show DSC thermograms of transesterified blended wax esters. Transesterified wax ester blends at 5 and 10% saturation gave two endothermic events labeled A for the event at approximately 5°C, and B (see definition of B in Scheme 1) for the event at approximately 20°C. At 15% saturation a third endotherm is first observed at about 45°C and becomes more prominent as % saturation increased to 50%. Based on our findings with the dropping points, endotherm B must represent the monounsaturated forms in the transesterified blends. As % saturation increases, ΔH_A decreases and ΔH_B and ΔH_C increase. As shown in Figure 3, the amounts of diunsaturated and saturated forms in the transesterified blends follow a straight line function while that for monounsaturated forms follow a quadratic function. Because of the scatter of points for ΔH_A, its correlation coefficient $R^2 = 0.85$ is poor in comparison with the correlation coefficients of 0.98 and 0.95 for ΔH_B and ΔH_C, respectively. With the application of multilinear regression analysis to the data sets for the calibration model, an algorithm was derived where % saturation = $16.487 - 0.162\ (\Delta H_A) + 0.209\ (\Delta H_B) + 0.600\ (\Delta H_C)$ with $R^2 = 0.98$. Fitting this derived model back to the original data (Fig. 4) yielded a standard error for predictions of 1.045 at 0% saturation, 0.770 at 25% saturation, and 1.158 at 50% sat-

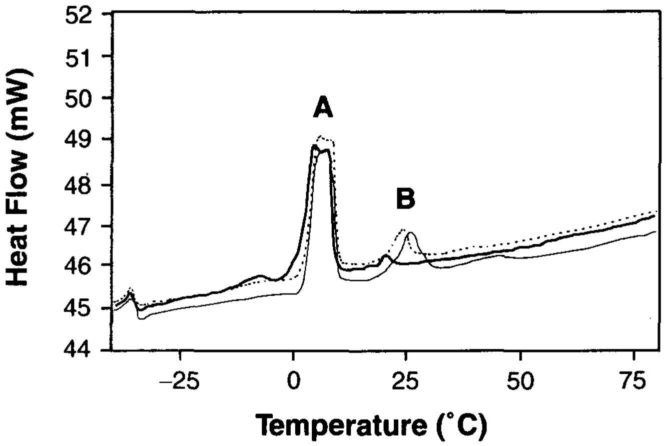

FIG. 1. Differential scanning calorimetry thermograms of transesterified wax esters: —, 5% saturation; ----, 10% saturation; —, 15% saturation where A is the diunsaturated and B the monounsaturated species.

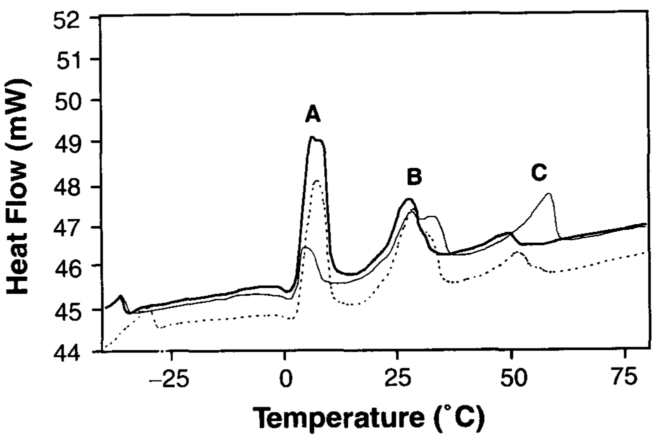

FIG. 2. Differential scanning calorimetry thermograms of transesterified wax esters: —, 20% saturation; ----, 30% saturation; —, 50% saturation where A is the diunsatured, B the monounsaturated, and C the saturated species.

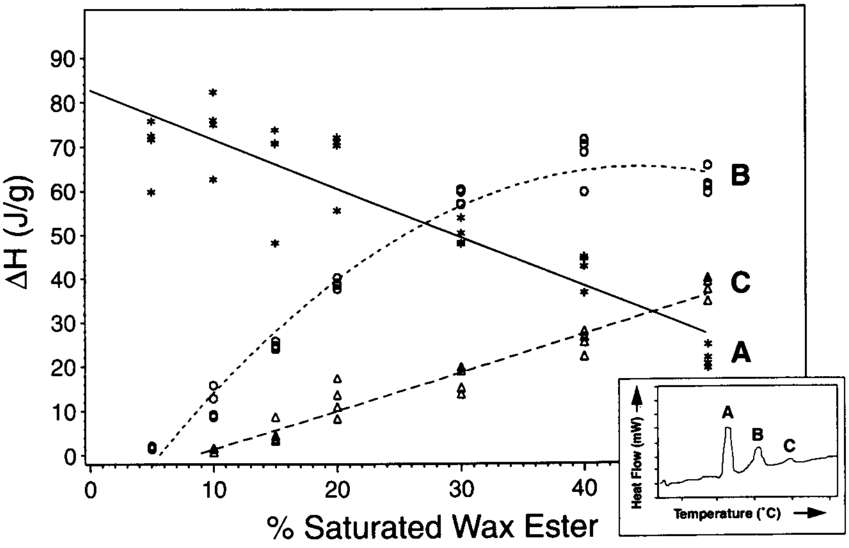

FIG. 3. Relationship of level of saturation designated as % saturation in transesterified wax esters with fusion enthalpy (ΔH). Diunsaturated species: $\Delta H_A = 88.36 - 1.278$ (% saturation), $R^2 = 0.85$; monounsaturated species: $\Delta H_B = -20.23 + 3.873$ (% saturation) $- 0.0439$ (% saturation)^2, $R^2 = 0.98$; saturated species: $\Delta H_C = -7.28 + 0.864$ (% saturation), $R^2 = 0.95$. Endotherms A, B, and C in insert represent the diunsaturated, monounsaturated, and saturated species, respectively.

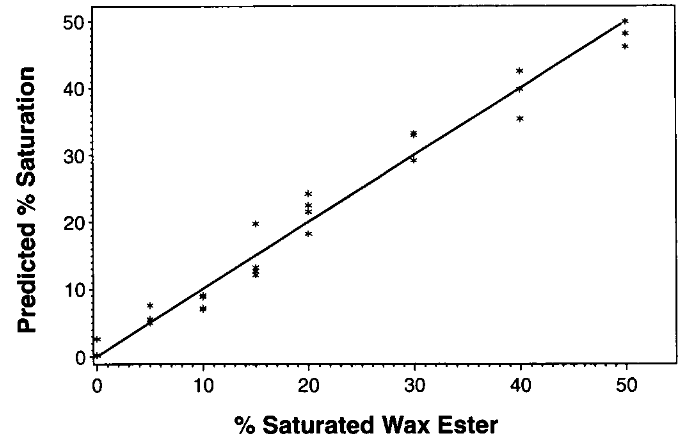

FIG. 4. Actual data vs. predictions based on fusion enthalpy (ΔH) where the level of saturation designated as % saturation = $16.487 - 0.162\ (\Delta H_A) + 0.209\ (\Delta H_B) + 0.600\ (\Delta H_C)$, $R^2 = 0.98$. ΔH_A, ΔH_B, and ΔH_C represent the diunsaturated, monounsaturated, and saturated species, respectively.

uration. Based on this algorithm derived from ΔH of the diunsaturated, monounsaturated, and saturated forms of wax ester blends, we derived an index that precisely predicts the degree of saturation in these blends. This index can be used as a research tool in process and product development.

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[Received March 30, 1995; accepted October 19, 1995]