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Monitoring Lipase-Catalyzed Interesterification for Bulky Fat Modification with FT-IR/NIR Spectroscopy

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This work demonstrates the application of FT-IR and FT-NIR spectroscopy to monitor the enzymatic interesterification process for bulky fat modification. The reaction was conducted between palm stearin and coconut oil (70:30, w/w) with the catalysis of Lipozyme TL IM at 70 °C in a batch reactor. The blends and interesterified fat samples in liquid form were measured by attenuated total reflectance based FT-IR (spectra region, 1516−781 cm^{-1}) and transmission mode based FT-NIR (spectra region, 5369−4752 cm^{-1}) with the temperature of both controlled at 70 °C. The samples in solid form were also measured by reflectance-based FT-NIR (spectra regions, 7037−6039 and 5995−5612 cm^{-1}) at room temperature. Calibrations of FT-IR and FT-NIR for conversion degrees (evaluated by triglyceride profile), solid fat contents (SFC), and dropping points of interesterified products were carried out by using partial least-squares regression. High correlations ($r > 0.96$) were obtained from cross validations of the data estimated by FT-IR, FT-NIR, and the above-mentioned conventional analytical methods, except for correlations ($r = 0.90-0.95$) between FT-IR and SFC profiles. Overall, FT-NIR spectroscopy coupled with transmission mode measured at 70 °C had the highest correlations, which also had the closest conditions to the sampled products in the process, indicating a great potential for implementation as an on-line control for monitoring the enzymatic interesterification process.

KEYWORDS: Lipase-catalyzed interesterification; margarine fats; FT-IR; FT-NIR; interesterification degree; solid fat content; dropping point

INTRODUCTION

Bulky plastic fats modified by enzymatic interesterification reactions have received increasing interests in both academia and industries (*1*−*4*). Previously, these types of fats, which are in great demand, had been largely modified by hydrogenation, fractionation, and chemical interesterification for the applications in margarine and shortening production. Enzymatic interesterification has the following inherent advantages compared to chemical interesterification: (1) it offers a clean process; (2) it is a simple and easy-to-control process; (3) more natural fats are produced if *sn*-1,3-specific lipase is used; (4) it is an environmentally friendly production. Furthermore, using Lipozyme TL IM, an immobilized *Thermomyces lanuginosa* lipase, as the biocatalyst for interesterification might offer a cost-efficient alternative to the conventional chemical interesterification (*4*).

For enzymatic interesterification the primary issues are the stability of enzyme and the precautions to keep constant reaction conversion for the production process. Monitoring the real-time conversion degree during enzymatic interesterification is necessary for successful in-process control of bulky fat modification. At present, conventional monitoring methods for such production are based on both chemical and physical changes, such as triglycerides (TAGs) and solid fat content (SFC) profiles (*4*, *5*). Almost all of these methods are tedious, time-consuming, sample-destructive, and inappropriate for on-line control. Therefore, a fast, reliable, and efficient method is expected to monitor the enzymatic interesterification process.

Successful applications of Fourier transform infrared (FT-IR) and near-infrared (FT-NIR) spectroscopy in the field of edible oils and fats analysis (*7*−*19*) imply the potential utilities of spectroscopic techniques to monitor the enzymatic interesterification process for bulky fats modification. FT-IR and FT-NIR spectroscopy combined with chemometrics for data treatment have been proved to be fast, simple, sample-nondestructive, and efficient tools for both qualitative and quantitative analysis of various food products. They are potentially useful for on-line/at-line monitoring (*6*). In the oils and fats filed, FT-IR and FT-NIR have been applied as alternatives for the quantitative determination of peroxide value (*7*−*10*), iodine value (*11*−*13*), saponification number (*12*), free fatty acids (*14*−*16*), and *cis*- and *trans*-fatty acids content (*17*, *18*). van de Voort et al. (*19*) reported that IR regions of ester linkage carbonyl band (1750−1740 cm^{-1}) and portions of the oil fingerprint region (1550−1050 cm^{-1}), which represented fatty acid compositions and

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distributions, contributed to the successful calibration of FT-IR method with conventional dilatometry method for the determination of solid fat index (SFI) profiles of hydrogenated soybean oils. Rodrigues et al. (*20*) also reported the feasibility of NIR spectroscopy to quantify the SFC of different fat blends using NMR data for calibration. In the study, high correlations were obtained between the NIR estimated values and NMR analyzed values for SFC at 10, 20, 30, and 35 °C.

Enzymatic interesterification for bulky fat production involves the exchange of acyl groups among triglycerides. Theoretically, there is no change of fatty acid compositions during the reaction. This means that the reaction leads only to the redistributions of fatty acids on glycerol backbones. The redistributions might cause the change of molecular vibrations within or between the lipid structure, such as methyl ($-CH_3$), methylene $)-CH_2-$), methyne ($-CH$), ethylenic systems ($-CH{=}CH_2$, $-CH{=}CH-$, and $C{=}CH_2$), and ester linkage ($C-O-C$). Additionally, diglycerides (DAGs) and free fatty acids (FFA), produced as byproducts during the enzymatic interesterification (*3*, *4*), probably have effects on spectral analysis. It was found that IR and NIR spectra have corresponding changes with the appearances of DAGs and FFA in fat samples. This is because both of them contain the $-OH$ group, which has a distinct absorption in IR and NIR spectra (*14*−*16*, *21*).

One objective of this study was to compare the feasibility of FT-IR and FT-NIR spectroscopy for monitoring enzymatic interesterification process. On the other hand, the proper condition for monitoring was selected. A blend (palm stearin/coconut oil, 70:30) and 12 interesterified products produced from two batch reactions were used for the investigation. Samples at liquid state (at 70 °C) were examined by both attenuated total reflectance (ATR)-based FT-IR and transmission-based FT-NIR. Samples at solid state (at room temperature) were also analyzed by FT-NIR spectroscopy coupled with reflectance mode. Correlations between FT-IR or FT-NIR spectroscopy and conventional analysis methods for quantitative determination of conversion degree (based on changes of TAG profiles), SFC profiles, and dropping point (DP) were examined.

MATERIALS AND METHODS

Materials. The refined, bleached, and deodorized palm stearin and coconut oils were supplied by Karlshamns AB (Karlshamn, Sweden). The compositional characteristics of the oils and blend were given in a previous paper (*3*). Lipozyme TL IM, a silica-granulated *T. lanuginosa* lipase, was supplied by Novozymes A/S (Bagsvaerd, Denmark). The water contents of Lipozyme TL IM and the oil blend were 6.0 and 0.06%, respectively. The monoacid TAG standards (18:0, 24:0, 30:0, 36:0, 42:0, 48:0, 52:0, 54:3, 54:6, and 54:9, where the first number represents the total carbon number of the acyl groups and the second number represents the total number of double bonds) for HPLC analysis were from Sigma Inc. (St. Louis, MO) with the purity of >97%. All other chemicals and reagents for the analysis were of analytical or chromatographic grades.

Interesterification (Ester−Ester Exchange) in 600 g Batch Reactor. About 600 g of blend (palm stearin/coconut oil, 70:30) was used for the reaction. Details for the reduction of byproducts through the washing procedure before reaction were the same as described before by Zhang et al. (*22*). The reaction was conducted at 70 °C with 4% lipase dosage and stirring rate of 300 rpm. Samples were withdrawn during the reaction from the sampling valve and filtered by syringe membrane filters. The final product was directly filtered through an in situ filter.

Reference Analysis of Conversion Degree, SFC Profiles, and DP. Conversion degree (eq 1) was defined as the change of peak ratio from the HPLC analysis of triglyceride profiles during the reaction

$$\text{conversion degree (\%)} = 100 \times (P_{11}/P_{16}) \quad (1)$$

where P_{11} is the area percentage of peak 11 (ECN44) and P_{16} is the area percentage of peak 16 (ECN48) from the HPLC-analyzed TAG profiles. The detailed method has been previously described by Zhang et al. (*3*). SFC was measured with a Minispec mq 20 NMR analyzer (Bruker, Germany) according to AOCS official method Cd 16b-93 (1993). DP was measured by Mettler Toldo (FP83HT) according to AOCS official method Cc 18-80 (1999).

FT-IR Spectroscopy. The instrument was a Bomem FT-IR/NIR spectrometer (FTLA 2000-154, Bomem Inc., Québec, Canada) equipped with a dual SiC−quartz−halogen source module and an extended range DTGS detector capable of scanning the spectral range of 12000−500 cm^{-1}. The spectrometer was controlled by an IBM Pentium 4 PC running under Windows XP-based Bomem-Grams/AI software (Galactic Industries Co., Salem, NH). IR spectra were collected over the range of 4000−400 cm^{-1} based on an attenuated total reflectance (ATR) sample handling accessory. The analysis was performed at 4 cm^{-1} resolution and 128 scans per spectrum, and the temperature was controlled at 70 °C during the analysis. Every fat sample had three replicates.

FT-NIR Spectroscopy Analysis of Liquid Fat Samples at 70 °C. A Bomem FT-IR/NIR spectrometer (FTLA 2000-154, Bomem Inc.) was used for collecting NIR spectra. The sample-handling accessory for NIR analysis was a temperature-controllable multivial-holding block capable of accepting 8-mm (o.d., path length) transparent glass vials with a volume of ∼1 mL. The fat samples (∼0.8 mL in each glass vial) were first melted at 70 °C before analysis and then scanned over the range of 12000−4000 cm^{-1} with temperature controlled at 70 °C. Every fat sample determination had three replicates. All samples and background spectra were taken at 16 cm^{-1} resolution and 128 co-added scans per spectrum. Air background spectra were collected every hour during the analysis.

FT-NIR Spectroscopy Analysis of Solid Fat Samples at Room Temperature. The analysis was applied on a Spectrum one NTS FT-NIR spectrometer (Perkin-Elmer, Inc., Wellesley, MA) coupled with a near-infrared reflectance accessory (NIRA). Before the analysis, samples were melted at 70 °C, and 1 mL of sample was transferred into a 1.5-mL glass vial (o.d., 11 mm). Every sample determination had three replicates. The thickness of fat samples was ∼10 mm to guarantee that light could not penetrate the sample. The melted samples were kept at room temperature for 10 min before scan to ensure that all samples were analyzed under similar conditions and then scanned over the range of 14000−4000 cm^{-1}. All samples and background spectra were taken at 16 cm^{-1} resolution and 75 co-added scans per spectrum.

Calibration and Validation. Raw FT-IR and FT-NIR spectra data were transferred to a multivariate data analysis software, Unscrambler 8.05 (CAMO Process A/S, Oslo, Norway), for quantitative analysis. FT-IR and FT-NIR spectroscopy are secondary methods of analysis (*9*, *23*); the development of quantitative analysis methods requires calibration with a set of standards (reference value) of known composition, prepared gravimetrically or analyzed by primary chemical methods. In this study, the conversional analysis results were used as the reference values (Y variants) and spectra data were used as the X variants for the calibrations. For FT-IR or FT-NIR spectroscopy to determine a complex system, such as oils and fats, the simple quantitative analysis method is not sufficient; therefore, more sophisticated multivariate analysis techniques are required. Partial least-squares (PLS) regression is a statistical approach to quantitative analysis and has been largely applied in mid-IR and NIR spectroscopy. Calibrations were developed with PLS regression for the quantitative analysis. Each calibration was assessed by using the leave-one-out cross-validation procedure and optimized in terms of the appropriate number of factors by minimizing the root-mean-square error of prediction. The analysis results from the traditional analysis were input into the program as the references for investigating the correlation between IR/NIR spectra and the reference methods.

The performance of calibration models were assessed by means of the correlation coefficient r, which expresses the linear relationship between reference values and predicted values, and the root mean square error [RMSE(Y)] (eq 2), which is described below as the square root of the residual Y-variance divided by the scaling factor

$$\mathrm{RMSE}(Y) = \sqrt{\frac{\sum_{i=1}^{n}(Y_{\mathrm{pred}} - Y_{\mathrm{ref}})^2}{n}} \quad (2)$$

where Y_{pred} is the Y values predicted from the calibration model, Y_{ref} is the reference Y values, and n is the scaling factor. If Y_{pred} in eq 2 is based on the calibration samples, the resulting RMSE(Y) is called RMSEC(Y) and it shows the modeling error. RMSEP(Y), which is based on the test set validation, is used for estimating the prediction error.

The optimal number of PLS factors was evaluated by comparing correlation coefficients (r) and root mean square error [RMSE(Y)]. The use of an optimal number of PLS factors in the calibration minimized the RMSEP.

RESULTS

Lipase-Catalyzed Interesterification. Figure 1 shows the change of conversion degree at different reaction times within 24 h of reaction. The big changes occurred in the first 4 h, and then the reaction gradually reached the equilibrium stage. At the same time, the changes of chemical properties also reflected the changes of physical properties with the same tendency, such as SFC and DP (**Table 1**). The average standard deviations of reference analysis methods are also given in **Table 1**, which show the good reproducibility of the reference methods and indicates that the reference methods are reliable and can be used for the calibration of FT-IR and FT-NIR determinations in this study.

FT-IR Analysis. The interesterified products produced during the enzymatic interesterification were examined by ATR-based FT-IR spectroscopy with temperature controlled at 70 °C. **Figure 2A** illustrates the multiplicative scatter correction (MSC) preprocessed IR spectra of the fat samples in the region 1516−781 cm^{-1}. This IR region mainly represents the oil fingerprint absorption region, including C−O−C vibration in esters and C−H bending vibrations; meanwhile, it also represents the C−H out-of-plane bending vibrations. It was the significant region for the determination of SFI (*19*).

By using this region for our determinations, the calibration statistics derived from the PLS model are presented in **Table 2**. Factors of 4−8 were required to account for the variation in conversion degree, DP, and SFC measured at 10, 20, 30, 35, and 40 °C. Root-mean-square errors of calibration and prediction, that is, RMSEC and RMSEP, were acceptable for detecting the process of interesterification. In general, a good linear relationship was demonstrated between the FT-IR predictions obtained from PLS models and the conventional analysis data (figures not shown).

FT-NIR Spectroscopy Analysis of Liquid Fat Samples at 70 °C. NIR radiation contains an amount of energy that gives rise to absorption bands that correspond mainly to overtones and combinations of fundamental vibrations. The most sensitive bands are those derived from the O−H, C−H, and N−H vibrations (*23*). In this study, it was found that the NIR region 5369−4752 cm^{-1} appears to be highly significant for DP, SFC profiles, and conversion degree. **Figure 2B** shows the raw NIR spectra of the interesterified products in the region 5369−4752 cm^{-1}. This broad region mainly represents the absorption bands of the second overtones of C═O stretching vibrations (*24*) and O−H combination vibration. The C═O vibration was mainly from ester (RCOOR) groups, the absorptions of which were assumed to change with changes in fatty acids distributions. O−H band absorption was probably from free fatty acids (FFA) and diglycerides (DAGs), which were produced as intermediates and byproducts during the reaction. Blanco et al. (*21*) demonstrated that the mixture of glycerides (mono-, di-, and triglycerides) has an absorption in the NIR region 5000−4545 cm^{-1} due to the absorption of combination bands for the O−H bond in the mono- and diglycerides. Additionally, combination bands for water could also appear in this region due to trace amounts of water in the reaction system.

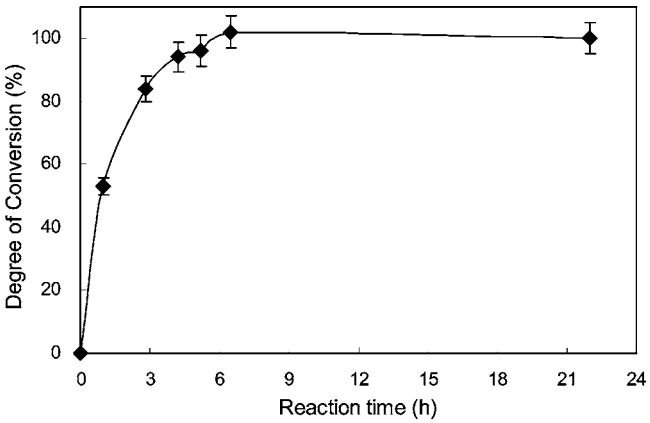

Figure 1. Effect of operation time on conversion degree for Lipozyme TL IM-catalyzed interesterification between palm stearin and coconut oil (70:30, w/w) in a batch reaction. Reaction conditions: temperature, 70 °C; stirring, 300 rpm; lipase dosage, 4 wt %; no additional water.

Table 1. Values Determined by Conventional Methods of Conversion Degree, Solid Fat Content (SFC) at 10, 20, 30, 35, and 40 °C, and Dropping Point (DP)[a]

reaction time (h)	conversion degree (%)	DP (°C)	SFC (%)				
			10 °C	20 °C	30 °C	35 °C	40 °C
0.0	0.00	59.60	51.49	48.70	45.16	42.97	39.79
1.0	53.12	55.16	48.61	46.06	36.18	31.59	26.99
2.8	84.00	51.38	41.14	38.99	25.88	21.35	16.71
4.2	94.14	49.82	40.05	33.66	22.29	17.75	13.31
5.2	96.13	49.36	39.95	32.96	21.48	16.92	12.55
6.5	102.00	48.88	40.18	31.94	20.72	16.17	11.73
22.0	100.00	47.90	40.13	29.67	19.90	15.32	10.93

[a] The standard deviations for conversion degree were calculated on the basis of duplicate experiments with three determinations in the analysis step for each sample (six data groups). The absolute values for each sample point were below 0.36. The standard deviations for dropping point (DP) and solid fat content (SFC) were also calculated on the basis of duplicate experiments with three determinations in the analysis step for each sample (six data groups). The absolute values for each sample point were below 0.27 and 0.20, respectively.

The calibration statistics are listed in **Table 2**. Few factors (n = 4−5) were required to account for the variation in conversion degree, DP, and SFC measured at 10, 20, 30, 35, and 40 °C. RMSEC and RMSEP values derived from PLS models for conversion degree, DP, and SFC profiles were acceptable for control of the interesterification process. Meanwhile, both calibration and prediction errors were smaller than the values obtained from IR spectra.

FT-NIR Spectroscopy Analysis of Solid Fat Samples at Room Temperature. The NIR region 7143−6849 cm^{-1} has been reported (*21*) to correspond to the first overtone for the O−H bond on mono- and diglyceride in a mixture of glycerides. Meanwhile, absorptions in the region 5882−5556 cm^{-1} have been found corresponding to the first overtone of C−H bonds in fatty acids (*21*). In this study it was found that NIR regions 7037−6039 and 5995−5612 cm^{-1} gave highly significant correlations with DP, SFC profiles, and conversion degree. **Figure 2C** illustrates the second derivative and MSC prepro-

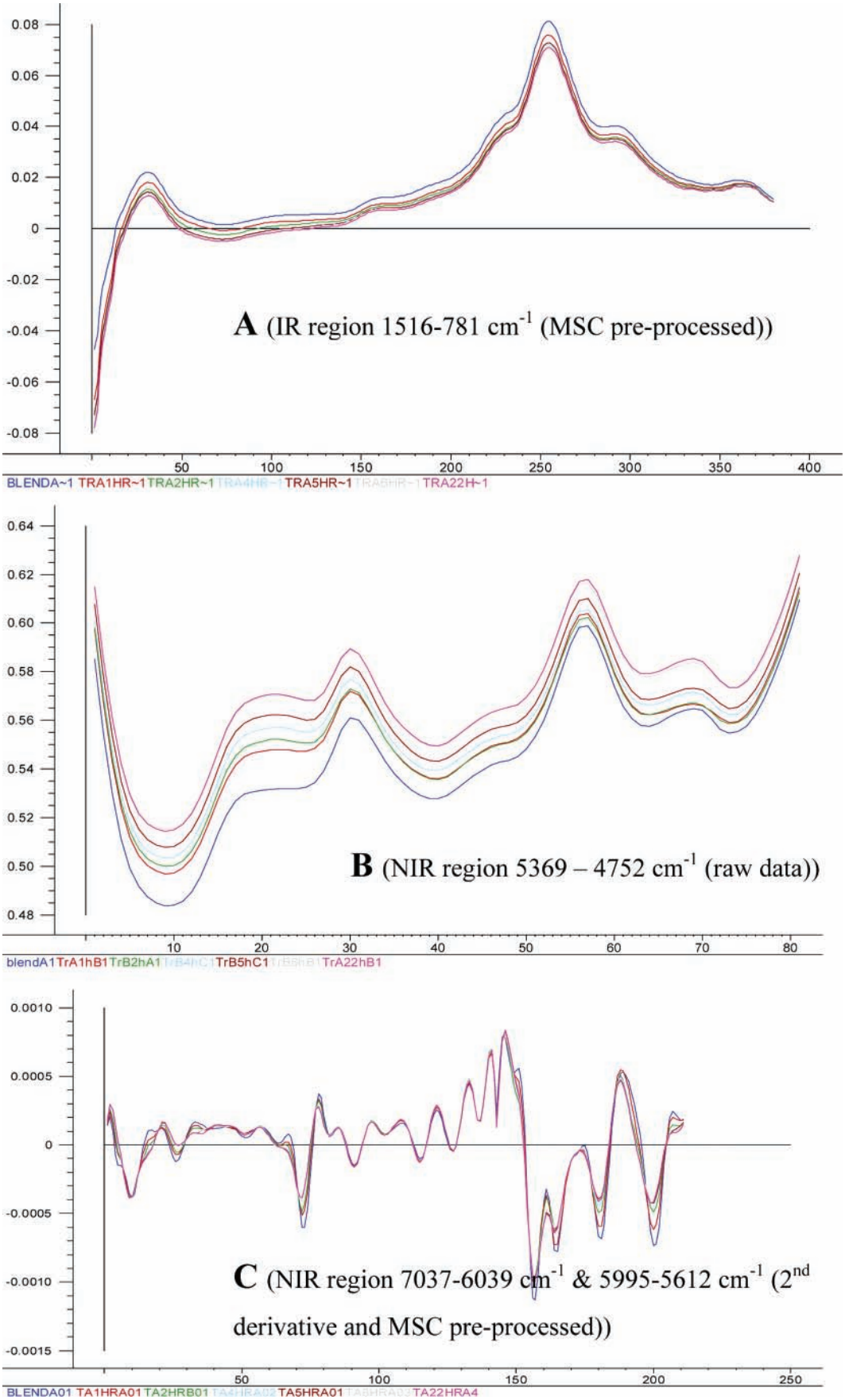

Figure 2. Spectra region used for calibrations: (**A**) MSC preprocessed IR region 1516–781 cm^{-1}; (**B**) transmission-based NIR region 5369–4752 cm^{-1} (raw data); (**C**) reflectance-based NIR regions 1421–1656 and 1668–1782 nm (second-derivative preprocessed).

cessed NIR spectra of interesterified products in the regions 7037−6039 and 5995−5612 cm^{-1}.

Statistical results obtained for the PLS calibrations are shown in **Table 2**. Only one or two factors were required to account

Table 2. Calibration Results for Conversion Degree, SFC at 10, 20, 30, 35, and 40 °C, and DP from Partial Least-Squares (PLS) Calibrations

		r	RMSEC	RMSEP	PLS factor
conversion degree[a]	IR	0.975	5.320	8.153	8
	NIR (70 °C)	0.981	5.593	7.228	4
	NIR (26 °C)	0.993	3.367	4.290	2
DP[b]	IR	0.960	0.708	1.162	8
	NIR (70 °C)	0.987	0.513	0.655	4
	NIR (26 °C)	0.993	0.395	0.484	2
SFC at 10 °C[c]	IR	0.914	1.271	1.910	8
	NIR (70 °C)	0.948	1.197	1.467	4
	NIR (26 °C)	0.983	0.650	0.848	2
SFC at 20 °C[c]	IR	0.903	1.815	3.055	4
	NIR (70 °C)	0.963	1.096	1.827	5
	NIR (26 °C)	0.981	0.943	1.339	2
SFC at 30 °C[c]	IR	0.947	1.977	2.991	8
	NIR (70 °C)	0.980	1.451	1.863	4
	NIR (26 °C)	0.994	0.831	1.000	2
SFC at 35 °C[c]	IR	0.953	1.965	2.977	7
	NIR (70 °C)	0.983	1.445	1.833	4
	NIR (26 °C)	0.991	1.233	1.310	2
SFC at 40 °C[c]	IR	0.946	1.759	2.945	8
	NIR (70 °C)	0.983	1.489	1.915	4
	NIR (26 °C)	0.991	1.270	1.369	1

[a] Variation range of conversion degree is 0–100% during reaction. [b] Variation range of dropping point is 60–47 °C during reaction. [c] Variation range of SFC (at 20 °C) is 48–30% during reaction.

for the variation in conversion degree, DP, and SFC data at 10, 20, 30, 35, and 40 °C, respectively. RMSEC and RMSEP for conversion degree, DP, and SFC profiles were much lower than the errors obtained for calibrations of IR spectra. This indicates that this method has better reproducibility for determining the conversion degree, DP, and SFC profiles of interesterified products than IR spectroscopy.

DISCUSSION

The results presented above clearly indicate that both FT-IR and FT-NIR spectroscopy have good correlations with conventional analysis methods for the determination of conversion degree, DP, and SFC profiles of the interesterified fat products. **Figure 3**, as one typical illustration, presents the "leave one out" cross-validation plot obtained from the calibration models for the conversion degree, SFC at 20 °C, and DP, respectively. These plots illustrate good linear relationship between the FT-NIR predictions obtained from PLS models and the conventional analysis data. The calibrations of conversion degree, DP, and SFC at 10, 20, 30, 35, and 40 °C were developed on the basis of the same FT-IR or FT-NIR spectral regions. By way of explanation, during the enzymatic interesterification, the changes of both chemical and physical properties of the interesterified products are determined by the changes of the molecular structures and compositions of the fat, in particular by the changes of fatty acid distributions in the glycerol backbone. Such information was extracted from the FT-IR or FT-NIR spectrum through the use of sophisticated multivariate analysis techniques, that is, PLS regression in this study, which allowed the subtle spectral variations to be correlated to changes in the chemical and physical properties, as measured by conventional methods. This indicates that FT-IR and FT-NIR spectroscopy can be used for monitoring the changes of both chemical and physical properties of bulky plastic fats during the enzymatic interesterification, so as to be able to monitor the reaction progress. This is a breakthrough for the possible on-line control of the enzymatic modification process because FT-IR and FT-NIR methods are evidently faster and easier than the conventional analysis methods. For example, with FT-IR and FT-NIR spectroscopy, the measurement of SFC in off-line operation was possible in only 2−5 min. It will be even faster with a particular on-line connection or design. One limitation of the FT-IR and FT-NIR approach for monitoring the enzymatic interesterification process is the estimation errors, which could be high compared to those of the conventional methods. For process control in industrial operations, it is important to closely follow unexpected happenings that could dramatically change the reaction progress, such as serious enzyme poisoning, heating system break down, and leakage. In such cases, a rough but instant automatic monitoring will help. Certain errors are most likely acceptable for these applications. However, the precision of the prediction can be improved by repeated improvement of the calibration models through time-to-time additional reference samples in a specific production process and product. The models can be also improved with more reference samples at different reaction stages with more possible reaction situations, which may happen in real production processes for the specific case.

To make a comparison of calibration results obtained from different FT-IR and FT-NIR protocols, FT-NIR had higher correlations with conventional analysis methods and FT-NIR-derived calibrations had lower errors for calibration and prediction. Furthermore, FT-NIR is faster than FT-IR spectroscopy. In this study, each sample took ∼5 min for FT-IR analysis, whereas FT-NIR analysis required only <2 min. This indicates that FT-NIR is more suitable for the purpose. Whereas calibration results for FT-NIR analysis of interesterified products in liquid form at 70 °C and in solid form at room temperature were very close, FT-NIR analysis of the interesterified products in solid form exhibited higher correlations with conventional methods. This could be caused by noises (*23*). Most noises were removed by preprocessing in FT-NIR analysis of the interesterified fat products in solid form at room temperature, whereas FT-NIR analysis of liquid interesterified products at 70 °C used raw spectral data as the calibration set. In this study it was found that preprocessing did not improve the calibration in FT-NIR analysis of liquid interesterified products. In general, the FT-NIR analysis method with liquid interesterified fat products at 70 °C is more suitable for monitoring the reaction because the industrial process of lipase-catalyzed interesterification is generally controlled at ∼70 °C.

The aim of this study was to assess and compare FT-IR and FT-NIR spectroscopy methods for the possibility of monitoring enzymatic interesterification. Thus, one pivotal consideration is the feasibility of these approaches in the application of real-time, that is, on-line/at-line, monitoring. On-line/at-line monitoring by FT-NIR spectroscopy has become widespread in recent years because FT-NIR presents a possibility for rapid, easy-to-handle, and cost-effective monitoring of different reactions. Knothe (*25*, *26*) reported the successful applications of fiber-optic FT-NIR spectroscopy for rapid monitoring of transesterification in biodiesel production. His research claimed the feasibility of FT-NIR in monitoring a real-time production of fatty materials. A fiber-optic probe can be also used in this study for on-line monitoring. The similar protocol can be used with transmission-based FT-NIR spectroscopy at the process temperature. For on-line monitoring using reflectance-based FT-

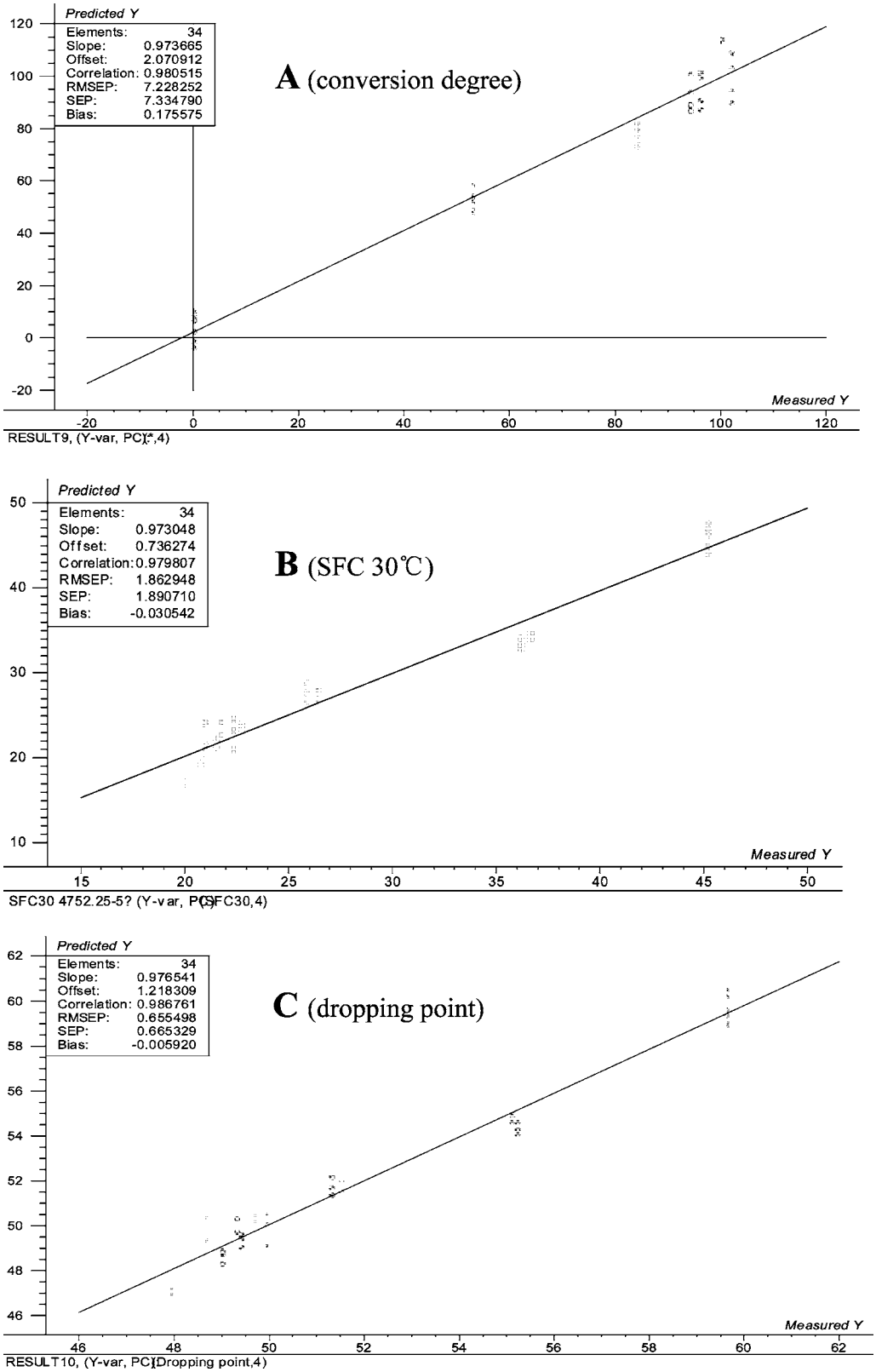

Figure 3. Cross-validation plots of transmission-based FT-NIR predicted values versus reference determined methods for determinations of (**A**) conversion degree, (**B**) solid fat content (SFC), and (**C**) dropping point (DP), as derived from the partial least-squares (PLS).

NIR with solid samples, a particular design of the sampling and sample preparation should be made in order to make it possible.

Application of FT-IR spectroscopy for on-line monitoring is more difficult because sample handling is special. Accurate FT-IR measurement usually requires samples being extraordinarily homogeneous and the amount of sample (e.g., path length) meeting the limit of intensity because FT-IR absorption has much higher intensity compared to FT-NIR absorption (*9*, *23*). However, Dubé et al. (*27*) used ATR-based FTIR spectroscopy for monitoring biodiesel production, indicating the possibility of ATR-FTIR in quantitative on-line monitoring.

The conclusion of this study is that both FT-NIR and FT-IR spectroscopy methods could possibly be used as fast analysis

methods for quantitative determination of the conversion degree (defined by changes of TAG profiles), SFC profiles, and DP of bulky fats during the enzymatic interesterification process. In particular, FT-NIR spectroscopy had better correlations with conventional analysis methods and it is also quicker and simpler than FT-IR spectroscopy in practical uses. With such feasibility, the calibration can be further improved with various sampling situations in a practical processing operation. On-line setup as well as the tolerance of the off-line determinations to the dynamic processing conditions will of course be the further challenges ahead for the real uses in an industrial plant.

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