International Journal of Food Science +Technology

Institute of Food Science +Technology ifst

International Journal of Food Science and Technology 2020, **55**, 1265–1271

Original article

In situ monitoring of the biocatalysed partial hydrolysis of cocoa butter and palm oil fraction

Anne Stöbener,[1] Mario Fabuel Ortega,[1] Christoph J. Bolten,[2] Edwin Ananta,[3] Shantha Nalur[4] & Andreas Liese[1]*

1 Institute of Technical Biocatalysis, Hamburg University of Technology (TUHH), 21073 Hamburg, Germany
2 Department of Biology, Institute of Material Sciences, Nestlé Research, Société des Produits Nestlé SA, CH-1000, Lausanne 26, Switzerland
3 Science & Technology Department, Nestlé Development Center Singapore, 29 Quality Road, 618802 Singapore, Singapore
4 Science & Technology Department, Nestlé Product Technology Center, Bakersfield CA 93313, USA

(Received 6 June 2019; Accepted in revised form 29 August 2019)

Summary Partial glycerides are widely used ingredients in confectionery products that can be produced from natural fats. In a biocatalytic partial hydrolysis of cocoa butter and palm oil fractions, a product mixture containing 1.5% monoglycerides and 5.5% diglycerides intended for the use in confectionery products was created. This study is a proof of principle that shows the feasibility of monitoring the biocatalytic partial hydrolysis of these two natural fats *in situ* with ATR-FTIR spectroscopy. An economic approach was utilised for calibration since partial glyceride calibration standards are costly and poorly available. The released compounds were quantified by means of chemometric modelling, and the model was validated with gas chromatography. Resulting root mean square errors were in the low per cent range. Additionally, the results indicate that distinction of the released free fatty acids is possible with ATR-FTIR spectroscopy.

Keywords Biocatalysis, Fourier-transform mid-infrared spectroscopy, *in situ* monitoring, natural fat, partial hydrolysis.

Introduction

The demand for natural food products has strongly increased. Ingredients perceived as artificial or chemical are replaced by natural alternatives, and processes are replaced by sustainable alternatives. In the food industry, natural fats like palm oil and its fractions are building blocks that affect the functional and sensory properties of foods. They add richness to a variety of products and carry the flavour of fat-soluble ingredients (Dunkley, 1982; Drewnowski, 1992). Partial glycerides, especially monoglycerides of fats, are widely used as emulsifiers (Kralova & Sjoblom, 2009). They are traditionally produced by glycerolysis of fats with alkaline or acidic catalysts (Sonntag, 1982). In contrast to this chemically catalysed reaction route, biocatalysis allows hydrolysis to be performed at mild reaction conditions. For example, palm oil has been hydrolysed by *C. rugosa* at 37 °C (Khor *et al.*, 1986). Yet, in this process, hexane was used as a solvent, which is approved in the EU as extraction solvent, but has to be removed from the product to guarantee a maximum residue limit of 1 mg kg^{-1} (DIRECTIVE 2009/32/EC). Solvent-free biocatalytic approaches can combine the avoidance of hazardous chemicals with the advantage of milder reaction conditions than the corresponding chemical reactions, resulting in sustainable processes (Liese *et al.*, 2006). Monoglyceride formation in such a process is limited by phase behaviour and can be influenced by surface-active additives (Baum *et al.*, 2016). Another biocatalytic synthesis route is the partial hydrolysis of a natural fat, where the triglycerides are not completely hydrolysed to glycerol and free fatty acids, but the process is stopped at the stage of conversion where the product has the aimed composition. An advantage of this approach is the avoidance of ingredients perceived as artificial/chemical by consumers since only natural fat and water are needed. Effective control and end point determination of this bioprocess is only possible if the concentrations of all compounds are known. Yet, in the biocatalysed partial hydrolysis of a natural fat, a complex mixture of partial glycerides is generated. Quantification is possible with gas chromatography (GC) or high-pressure liquid chromatography (HPLC) (Marcato & Cecchin, 1996; Isidorov *et al.*, 2007). However, these chromatographic

*Correspondent: E-mail: Liese@tuhh.de

doi:10.1111/ijfs.14366

methods are laborious and time-consuming. Additionally, they do not deliver an online signal for process control. Sampling for chromatography from multiphase systems as present in solvent-free hydrolysis and glycerolysis of fats is an additional challenge and accompanied by high errors.

A promising alternative to chromatographic methods is Fourier-transform mid-infrared (FTIR) spectroscopy because the infrared spectra of mono-, di- and triglycerides can be distinguished (O'Connor *et al.*, 1955). FTIR spectroscopy has become a valuable technique in the analysis of fats and oils. For example, the free fatty acid content of fats and oils can be quantified using the carbonyl absorbance of the carboxylic acid group at 1711 cm^{-1} (Mahesar *et al.*, 2014). In combination with Attenuated Total Reflectance (ATR) probes (Minnich *et al.*, 2007), providing an IR penetration depth of only a few micrometres, FTIR spectroscopy can be used to determine the concentrations of all components in the continuous phase of a water-in-oil emulsion *in situ* (Müller *et al.*, 2010). *In situ* ATR-FTIR spectroscopy has been successfully used to monitor the concentrations of mono-, di- and triglycerides in the solvent-free esterification of lauric acid and glycerol (Mueller *et al.*, 2011). The full hydrolysis of castor oil has been monitored with ATR-FTIR spectroscopy (Khaskheli *et al.*, 2015). Yet, in this application the technique was employed offline after centrifugation of the final product. Due to the necessary excess water contents, in full hydrolysis reactions often oil-in-water emulsions are formed. Online ATR-FTIR analysis of the dispersed phase is possible via μ-membrane-based phase separation in a circulation loop (Kaufhold *et al.*, 2014).

The aim of this study was the *in situ* monitoring of a partial hydrolysis of two natural fats with ATR-FTIR spectroscopy. Cocoa butter was chosen as a high-value fat with a complex mixture of triglycerides, while a palm stearin was a widely used low-cost material comprising a simpler triglyceride combination. In contrast to the previously cited literature, the reaction was monitored inline in the upper phase of a mixer/settler reactor. The partial hydrolysis was performed with the goals of reaching high monoglyceride content, as well as intending to use the hydrolysis products as intermediates in food manufacturing. Hence, no solvents or further additives were used in the hydrolysis. Chemometric modelling of the FTIR spectra for quantification was evaluated for the two natural fats.

Materials and methods

Standards, natural fats and biocatalyst

The following substances were used as calibration standards in gas chromatography: the free fatty acids palmitic (Carl Roth GmbH & Co. KG, ≥98% Karlsruhe, Germany), oleic (Panreac AppliChem GmBH, pure Ph. Eur., pharma grade, Darmstadt, Germany) and stearic acid (Carl Roth GmbH & Co. KG, ≥98%), the monoglyceride 3-palmitoyl-sn-glycerol (Sigma-Aldrich GmbH, ≥99%, Munich, Germany), the diglycerides 1,2-dipalmitoyl-sn-glycerol (Avanti Polar Lipids Inc., Alabaster, AL, USA, ≥98%) and glyceryl 1,3-distereate (Sigma-Aldrich GmbH, ≥99%). For triglycerides, a reference material (triglycerides in cocoa butter IRMM® certified Reference Material; Sigma–Aldrich GmbH) was used. Tricaprin solution was used as internal standard (ASTM® D6584 Tricaprin Solution 8000 μg mL^{-1} in pyridine, analytical standard; Restek, Bad Homburg, Germany). The silylating reagent MSTFA (n-methyl-n-trimethylsilyl-trifluoracetamide; Macherey-Nagel, Düren, Germany) was used for derivatisation.

The fats used in this study were cocoa butter (Cargill GmbH, Hamburg, Germany), and palm oil fraction (Loders Croklaan, Wormerveer, the Netherlands). Both fats were refined, bleached and deodorised. The main components of the cocoa butter were the triglycerides palmitin-olein-palmitin (POSt, 33%), stearin-olein-stearin (StOSt, 26%) and palmitin-olein-palmitin (POP, 16%). The oleic acid at the sn-2 position is responsible for the cocoa butter specific melting behaviour. The stearin fraction of palm oil was used in this study. The main component was tripalmitin (PPP, 65%). The triglyceride POP and its regioisomers PPO were also present. Triglycerides containing stearic acid (i.e. POSt) were present in very low concentrations.

The biocatalysts used were food-grade Lipozyme 435 (Novozymes, Bagsværd, Denmark) and CalB FG (c-LEcta, Leipzig, Germany), both *Candida antarctica* lipase B, physically adsorbed on a hydrophobic methacrylate carrier (average diameter 400 μm).

Gas chromatography

For gas chromatography, 10 mg of fat was dissolved in 600 μL pyridine/chloroform (1:1 vol). 60 μL of the solution, 20 μL tricaprin solution as internal standard and 20 μL MSTFA were incubated at 80 °C for 45 min. GC analysis was performed with a GC 5890 Series II (Hewlett-Packard, Böblingen, Germany), on an UltiMetal Vf5ht (15 m × 0.25 mm × 0.1 μm; Agilent Technologies Deutschland GmbH, Böblingen, Germany) column, starting at 90 °C for 2 min and heating to 380 °C at a heating rate of 15 °C min^{-1}, with a total runtime of 31 min. FID detection was employed. Typical retention times were 9–10 min for free fatty acids (FFAs), 12–13 min for monoacylglycerides (MAGs), 16.4 min for the internal standard, 18–18.5 min for diacylglycerides (DAGs), and 21–23 min for triacylglycerides (TAGs). Peak areas of TAGs,

DAGs and MAGs were summed up for analysis, while peak areas of free fatty acids FFAs were analysed individually and in sum. All GC analyses were performed in duplicates. Typically, standard deviations of 5% were observed in these duplicates. These high deviations resulted from two factors: only a split inlet was available in the gas chromatograph, which increases discrimination of high boiling compounds (Grob, 1979), and traces of the dispersed phase that may be present in the drawn offline sample.

Spectroscopy and multivariate analysis

ATR-FTIR spectra were measured with a Vertex 70 spectrometer (Bruker Optik GmbH, Ettlingen, Germany), equipped with a silver halide fibre diamond ATR probe (IN350-T) and a liquid nitrogen-cooled mercury–cadmium–telluride (MCT) detector. Spectra were recorded with 256 scans per spectrum from 4000 to 600 cm^{-1} using the OPUS 7.0 software package (Bruker Optik GmbH). The spectra were pre-processed by baseline correction (concave rubber band, 64 points, 10 iterations; OPUS software package), cutting (2000–600 cm^{-1}), normalisation to the CH_2 vibration at 1462 cm^{-1}, and mean centring (The Unscrambler X, Version 10.5; Camo Software, Oslo, Norway). Quantification of the compounds from the FTIR spectra was done with multivariate analysis. For this purpose, the partial least squares (PLS) algorithm was chosen. The algorithm was established in 1979 for chemometric analysis (Gerlach *et al*., 1979). It has similarities to principal component analysis (PCA) and is a widely used multivariate method (Wold *et al*., 2001). The procedure for spectra quantification is as follows. For calibration, a data set consisting of spectra and the respective concentrations of all compounds (determined by GC) in the mixture is needed. The concentrations are correlated to the spectra, and regression coefficients are yielded that can be applied to determine concentrations from further FTIR spectra. Validation of the model is typically performed by comparing concentrations determined from FTIR data to the respective concentrations determined with the reference method.

The multivariate calibration and regression in this study were performed with The Unscrambler X. A total number of fifty two training samples (thirty one cocoa butter, twenty one palm oil fraction, IR spectra and corresponding concentration data from GC) were available for calibration. The non-iterative partial least squares algorithm was applied to calculate a chemometric model. A total number of sixteen test samples (thirteen cocoa butter, three palm oil fraction) were available for validation.

The quality and predictive power of the model are typically evaluated by root mean square errors (RMSE) (Brereton, 2003):

$$\mathrm{RMSE} = \sqrt{\sum_{i=1}^{n} (\hat{x}_i - x_i)^2}/n$$

where $\hat{x}_i$ is the amount of the respective compound (in weight per cent) determined offline, x_i is the amount determined by the chemometric model and n is the number of data points. The root mean square error of calibration (RMSEC) relates the predicted value for every calibration sample to its reference value and thereby characterises the quality of the fit. For calculating the root mean square error of cross-validation (RMSECV), data points are successively left out of the regression and then predicted by the resulting model. Thereby, an estimation of the predictive power of the model is given, and possible overfitting can be indicated. Again similarly to PCA, the PLS model is calculated in several ranks called PLS factors. With an increasing number of PLS factors, the RMSEC usually decreases, while the RMSECV first decreases, and then increases again. The increase of the RMSECV with the PLS factor is an indication of overfitting. A minimum RMSECV is a criterion for the selection of PLS factors. The root mean square error of prediction (RMSEP) is calculated from samples not included in the calibration data set and hence is a measure for the predictive ability of the model in external validation.

Experimental setup and reaction conditions

The *in situ* FTIR spectroscopy was investigated in a mixer/settler reactor (MSR, $V = 1000$ ml) agitated by a tangential turbine (100 r.p.m.). The setup is visualised in Fig. 1a. The turbine is placed at the interface between aqueous and oil phase and agitates the reaction mixture without flow in axial direction. Hence, a reaction zone forms in the middle of the reactor while an aqueous phase in the lower part and an oil phase in the upper part persist. The reaction temperature was 70 °C for all FTIR measurements.

The fat was melted completely, and water was added before the FTIR probe was introduced into the MSR from the top to ensure immersion in the upper oil phase. The reaction was started by adding the immobilised enzyme to the stirred reaction mixture. Samples for GC were withdrawn in intervals of 30 and 60 min by a syringe, and suspended immobilised enzyme was separated by a polyamide net (width: 200 μm).

Results and discussion

Hydrolysis reaction and FTIR spectra

The hydrolysis reaction was carried out with the aim of releasing a high amount of partial glycerides, especially monoglycerides. We found that a temperature of

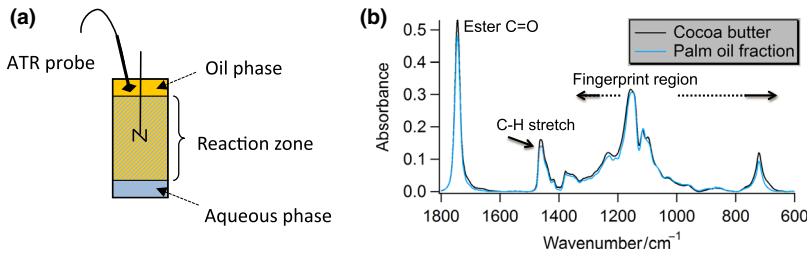

Figure 1 (a) Schematic setup of the mixer/settler reactor showing the positioning of the ATR probe. (b) FTIR spectra of the employed natural fats before hydrolysis. [Colour figure can be viewed at wileyonlinelibrary.com]

70 °C, high enzyme concentrations and low amounts of water favour the formation of monoglycerides. Both enzymes applied in this study did not significantly differ in terms of product composition. A mixture of 99% (wt/wt) fat and 1% (wt/wt) water and an enzyme load of 3% resulted in the hydrolysis of 33%(wt/wt) of the triglycerides after a reaction time of 8 h. The product mixture contained approximately 5.5% (wt/wt) diglycerides and 1.5% (wt/wt) monoglycerides. To achieve higher monoglyceride contents, water could be replaced by glycerol (glycerolysis) and additives could be applied (Baum *et al.*, 2016). Nonetheless, this was not the scope of this study.

Typical FTIR spectra of the two fats before hydrolysis are shown in Fig. 1b. Both unhydrolysed fats show an intense vibration at 1750 cm^{-1}. This is the C=O vibration of the esterified acid carbonyl group. In the course of the hydrolysis, this vibration's amplitude will decrease and a vibrational band at 1720 cm^{-1} will emerge, which is the C=O vibration of the free fatty acid carbonyl group.

Calibration of the chemometric models

The natural fats employed as starting materials comprise triglycerides of the three different FFAs as main components: Oleic (O), palmitic (P) and stearic (St) acid. The cocoa butter contains three main triglycerides with oleic acid in sn-2 position, and palmitic and/or stearic acid in sn-1 and sn-3 positions (POP, POSt, StOSt). The major triglyceride in the palm oil fraction is tripalmitin, and minor amounts of other triglycerides (mainly POP, PPO, very low concentrations of triglycerides containing stearic acid) are present. Their hydrolysis leads to a complex mixture of regioisomers, the number of isomers being further enhanced by non-enzyme catalysed acyl migration (Boswinkel *et al.*, 1996). From a purely combinatorial point of view, fifteen distinguishable diacylglycerides can be formed from the three major triacylglycerides. The calibration of a chemometric model with mixtures prepared from pure components is not possible at reasonable cost for a natural fat containing a high number of regioisomers of saturated and unsaturated fatty acids. The same applies to chromatographic calibration. In the latter, partial glycerides were analysed as equivalents of one available component. For chemometric calibration, at specific time points in a running reaction, samples were taken for gas chromatographic analysis to correlate concentrations with the IR spectra with the matching timestamp. In the case of cocoa butter, a total of thirty one offline samples measured by GC were used for PLS regression. For the palm oil fraction, twenty one GC offline samples were included for calibration. In sum, five models were calculated. The first models for cocoa butter and palm oil fraction were calculated with all analytes as sums without distinguishing FFAs (four analytes of interest: TGs, DGs, MGs and FFAs). The models were then calculated again where the FFAs were distinguished (six analytes of interest: TGs, DGs, MGs, palmitic, oleic and stearic acid). Finally, a combined model with the data from cocoa butter and palm oil was calculated. In Table 1, the PLS parameters of calibration and cross-validation are presented for the different components (FFAs, MAGs, DAGs, and TAGs). The number of PLS factors for each component was chosen so, that the root mean square error of cross-validation was minimum, while the predicted vs. reference plot of the respective compound was close to a unity line. If deviations between PLS factors were not significant, a lower number of PLS factors was preferred to avoid overfitting.

The calibration of the chemometric models yielded coefficients of determination close to unity for the chosen number of factors. Root mean square errors of calibration and cross-validation were generally low. This shows a satisfactory fit and is prerequisite for validation of the models.

The regression coefficients of a PLS model can give a plausibility test of the model. In the following section, regression coefficients of the triglycerides are discussed to exemplify this(data not shown). In all calculated models, regression coefficients for the triglycerides are dominated by a strongly positive value at 1750 cm^{-1}, corresponding to the C=O vibration of the esterified acid carbonyl group. Further positive values are within the fingerprint region. These cannot be

Table 1 PLS calibration parameters for cocoa butter, palm oil fraction and a combined model from both fats

	FFA	P/O/St	MG	DG	TG
Cocoa butter (31 training samples)					
Factors	3	2/3/1	4	7	3
R^2 cal	0.96	0.95/0.96/0.95	0.91	0.98	0.97
RMSEC	0.91	0.23/0.37/0.38	0.11	0.25	1.76
RMSECV	1.03	0.26/0.43/0.42	0.13	0.49	2.06
RMSEP	0.74	0.17/0.19/0.34	0.26	0.68	1.51
Palm oil fraction (21 training samples)					
Factors	3	4/4/4	5	7	2
R^2 cal	0.99	0.99/0.98/0.92	0.98	0.99	0.95
RMSEC	0.37	0.29/0.08/0.05	0.05	0.21	2.07
RMSECV	0.81	0.59/0.17/0.06	0.07	0.56	2.80
RMSEP	0.52	0.54/0.04/0.03	0.12	0.23	5.38
Combined model (52 training samples)					
Factors	4	6/6/6	3	9	5
R^2 cal	0.94	0.95/0.93/0.91	0.88	0.98	0.96
RMSEC	1.08	0.69/0.55/0.61	0.13	0.27	2.08
RMSECV	1.19	0.83/0.66/0.73	0.14	0.51	2.90
RMSEP	0.47	0.94/0.38/0.70	0.24	0.74	3.27

assigned unambiguously to a specific vibration, but do have further impact on the regression. High absorbance in these spectral regions will be interpreted as high concentrations of TG. In contrast, at 1720 cm^{-1}, corresponding to the C=O vibration of the FFA carboxyl group, a negative regression coefficient is observed. Hence, a high absorbance in this region will correlate with low concentrations of TGs.

Validation and application

In order to validate the method, the PLS models were used for concentration prediction in the course of a further hydrolysis reaction of the respective fat. The root mean square errors of prediction are featured in Table 1. The partial hydrolysis concentration prediction of cocoa butter is presented in Fig. 2 and shows a good agreement between online and offline data for the triglycerides, with an RMSEP of 1.5%-w/w and a maximum relative deviation of 5%. The free fatty acids are predicted reliably, except for concentrations approaching zero in the first hour of the hydrolysis reaction. Partial glycerides were predicted with slightly lower R^2 compared to the other compounds. Especially diglyceride prediction required higher PLS factors than the other compounds and deviated from the reference for longer prediction times. One possible explanation for this could be the large number of regioisomers due to the process of acyl migration, resulting in up to ten peaks in the chromatogram, not all of which were baseline separated. These peaks were analysed as a sum parameter. A different explanation could be the partial glyceride concentrations which stayed low throughout the hycdrolysis reaction. The results for the palm oil fraction are comparable to the cocoa butter results (Table 1). In analogy to cocoa butter, a larger PLS factor is required for diglycerides. Yet, probably due to the smaller number of calibration samples, the triglyceride and palmitic acid RMSEs of cross-validation and prediction are larger than for the cocoa butter samples. Concentration prediction for a further hydrolysis reaction is possible as presented in Fig. 2. The results show that for both model fat systems, FTIR spectroscopy in combination with chemometric modelling is a suitable method to monitor concentrations during hydrolysis.

To evaluate FFA distinguishing with FTIR spectroscopy, the models for both fats were calculated a second time, and each FFA was implemented as a single response. As can be noticed in Table 1, when summing up the RMSEs of P, O and St, the FFA value is approximately yielded. Changes in the other components RMSEs are negligible. Hence, the additional complexity of six predictors instead of four does not increase model uncertainty. The *in situ* monitoring of singular FFAs during the partial hydrolysis is shown in Fig. 3. This validation data set shows a reasonable agreement between predicted and reference data, indicating that FFAs can be distinguished with FTIR spectroscopy. This is especially interesting, since the FFA composition has strong influence on the sensory properties, and the natural fat may release FFAs that decrease the quality of the hydrolysis product.

The results shown in Fig. 3 are in accordance with the fatty acid composition of the TGs in the starting materials. While for cocoa butter, all three free fatty acids are observed in the resulting product mixture, from the palm oil fraction mostly palmitic acid is released with smaller amounts of oleic acid.

Finally, a combined model for both fats was calculated with a total number of fifty two training samples. Due to the different fatty acid composition of the two natural fats, combining the data sets corresponds to an increase in the calibration range of the PLS model. The RMSEs of the combined model are significantly larger than the RMSEs for the single fat models. Consequently, the RMSEPs in Table 1 show that the prediction of the concentration of the compounds is less reliable. Free fatty acids are overestimated, and diglycerides are underestimated by the combined model. Nonetheless, the reaction course can be followed by the combined model. Concentrations predicted for triglycerides are mostly within the uncertainty limits of the offline samples. This shows that a combined chemometric model for fat hydrolysis is possible for fats with a profile of FFAs as different as cocoa butter and palm oil fraction. Predictions for the currently over- and underestimated compounds could be improved by increasing the number of calibration

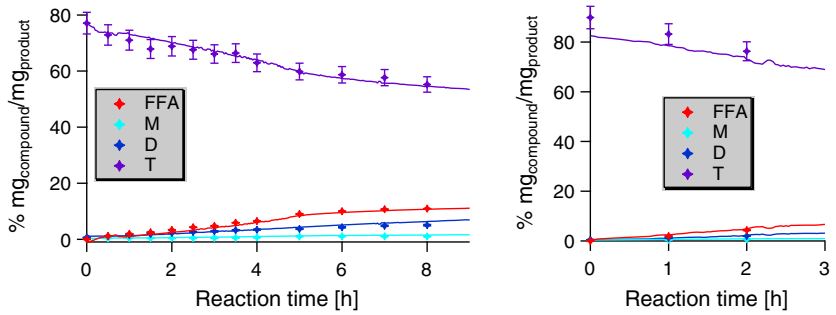

Figure 2 Comparison of *in situ* FTIR (lines) and offline data (diamonds) during the partial hydrolysis of cocoa butter (left) and palm oil fraction (right); mixer/settler reactor, 100 rpm; 70 °C; reactant mixture: 99% fat, 1% water; biocatalyst: CalB FG (cocoa butter), Lipozyme 435 (palm oil fraction); enzyme load: 3% referring to mass of the fat. [Colour figure can be viewed at wileyonlinelibrary.com]

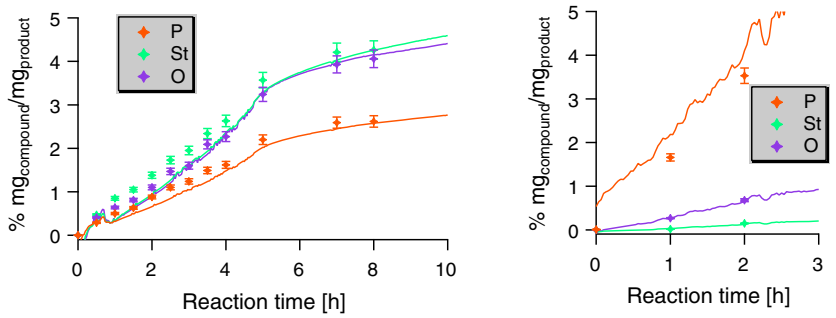

Figure 3 Comparison of FFA prediction between *in situ* FTIR (lines) and offline data (diamonds) during the partial hydrolysis of cocoa butter (left) and palm oil fraction (right); mixer/settler reactor, 100 rpm; 70 °C; reactant mixture: 99% fat, 1% water; biocatalyst: CalB FG (cocoa butter), Lipozyme 435 (palm oil fraction); enzyme load: 3% referring to mass of the fat. [Colour figure can be viewed at wileyonlinelibrary.com]

samples. A possible, though costly way to improve the concentration prediction, would be the use of pure components and their mixtures to increase the range of the calibration data set. Individual analysis of the fatty acid composition of all compounds could furthermore improve the model. Nonetheless, the results indicate that a hierarchical modelling approach using submodels for each fat is an effective and economic way to achieve concentration prediction of partial fat hydrolysis.

Conclusion

The partial hydrolysis of two natural fats was monitored *in situ* with FTIR spectroscopy. The reaction led to a product containing 1.5% monoglycerides and 5.5% diglycerides intended for confectionery applications. FTIR spectra were measured in the upper oil phase of a mixer/settler reactor. Using PLS chemometric modelling, summarised quantification of the formed compounds (FFA, MG, DG) and residual triglycerides was possible with low errors. Furthermore, the FFAs released during hydrolysis can be quantified individually with FTIR spectroscopy. While it is possible to analyse the hydrolysis with one combined model for both natural fats, the results show that an approach with single models for each fat is more accurate and economical. It is shown that calibration can be performed with partial glycerides of exemplary fatty acids. This is of special importance since calibration standards of all compounds present in the product mixture would be costly and difficult due to the poor commercial availability. The approach chosen in this study can be utilised in research and production to monitor natural fat partial hydrolysis and also glycerolysis or alcoholysis reactions in real time, allowing to stop conversion at the optimal product composition.

Acknowledgments

We thank Uta Naefken for performing part of the hydrolysis reactions.

Data availability statement

Research data are not shared.

Ethical guidelines

Ethics approval was not required for this research.

Conflict of interest

There is no conflict of interest.

References

Baum, S., Ritter, E., Smirnova, I., Schilling, M. & Liese, A. (2016). The role of phase behavior in the enzyme catalyzed synthesis of glycerol monolaurate. *RSC Advances*, **6**, 32422–32429.

Boswinkel, G., Derksen, J.T.P., van't Riet, K. & Cuperus, F.P. (1996). Kinetics of acyl migration in monoglycerides and dependence on acyl chainlength. *Journal of the American Oil Chemists Society*, **73**, 707–711.

Brereton, R.G. (2003). *Chemometrics: Data Analysis for the Laboratory and Chemical Plant*. Chichester, UK: John Wiley & Sons Ltd.

Directive 2009/32/EC of the European Parliament and of the Council of 23 April 2009 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients; accessible online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02009L0032-20100916; accessed 28.01.2019.

Drewnowski, A. (1992). Sensory properties of fats and fat replacements. *Nutrition Reviews*, **50**, 17–20.

Dunkley, W. (1982). Reducing fat in milk and dairy products by processing. *Journal of Dairy Science*, **65**, 454–458.

Gerlach, R.W., Kowalski, B.R. & Wold, H. (1979). Partial least squares path modeling with latent variables. *Analytica Chimica Acta*, **112**, 417–421.

Grob, K. (1979). Evaluation of injection techniques for triglycerides in capillary gas chromatography. *Journal of Chromatography*, **178**, 357–392.

Isidorov, V.A., Rusak, M., Szczepaniak, L. & Witkowski, S. (2007). Gas chromatographic retention indices of trimethylsilyl derivatives of mono- and diglycerides on capillary columns with non-polar stationary phases. *Journal of Chromatography A*, **1166**, 207–211.

Kaufhold, D., Fagaschewski, J., Sellin, D., Strompen, S., Liese, A. & Hilterhaus, L. (2014). Novel μ-membrane module for online determination of the free fatty acid content in the dispersed phase of oil-in-water emulsions. *Analytical and Bioanalytical Chemistry*, **406**, 3157–3166.

Khaskheli, A.A., Talpur, F.N., Ashraf, M.A., Cebeci, A., Jawaid, S. & Afridi, H.I. (2015). Monitoring the Rhizopus oryzae lipase catalyzed hydrolysis of castor oil by ATR-FTIR spectroscopy. *Journal of Molecular Catalysis B: Enzymatic*, **113**, 56–61.

Khor, H.T., Tan, N.H. & Chua, C.L. (1986). Lipase-catalyzed hydrolysis of palm oil. *Journal of the American Oil Chemists Society*, **63**, 538–540.

Kralova, I. & Sjoblom, J. (2009). Surfactants used in food industry: a review. *Journal of Dispersion Science and Technology*, **30**, 1363–1383.

Liese, A., Seelbach, K. & Wandrey, C. (2006) *Industrial Biotransformations*, 2nd edn. Weinheim, Germany: Wiley_VCH.

Mahesar, S.A., Sherazi, S.T.H., Khaskheli, A.R., Kandhro, A.A. & Uddin, S. (2014). Analytical approaches for the assessment of free fatty acids in oils and fats. *Analytical Methods*, **6**, 4956–4963.

Marcato, B. & Cecchin, G. (1996). Analysis of mixtures containing free fatty acids and mono-, di- and triglycerides by high-performance liquid chromatography coupled with evaporative light-scattering detection. *Journal of Chromatography A*, **730**, 83–90.

Minnich, C., Buskens, P., Steffens, H. *et al.* (2007). Highly flexible fiber-optic ATR-IR probe for inline reaction monitoring. *Organic Process Research & Development*, **11**, 94–97.

Mueller, J., Baum, S., Hilterhaus, L., Eckstein, M., Thum, O. & Liese, A. (2011). Simultaneous determination of mono-, di-, and triglycerides in multiphase systems by online fourier transform infrared spectroscopy. *Analytical Chemistry*, **83**, 9321–9327.

Müller, J., Neumann, M., Scholl, P. *et al.* (2010). Online monitoring of biotransformations in high viscous multiphase systems by means of FT-IR and chemometrics. *Analytical Chemistry*, **82**, 6008–6014.

O'Connor, R., DuPre, E. & Feuge, R. (1955). The infrared spectra of mono-, di-, and triglycerides. *Journal of the American Oil Chemists' Society*, **32**, 88–93.

Sonntag, N.O.V. (1982). Glycerolysis of fats and methyl esters - Status, review and critique. *Journal of the American Oil Chemists Society*, **59**, 795A–802A.

Wold, S., Sjöström, M. & Eriksson, L. (2001). PLS-regression: a basic tool of chemometrics. *Chemometrics and Intelligent Laboratory Systems*, **58**, 109–130.