



# Development and validation of a simple and reliable alternative method for process monitoring and final product quality control during fatty acid ethyl esters production

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## ABSTRACT

As the production of biofuels increase, there is an urgent need to easily analytically control their production at the plant level as well as to assess the quality of the final products. Especially method capable of determining fatty acid ethyl ester content of 96.5% is crucial for utilization in praxis. In this work, a refractive index method with required sensitivity was developed and validated by means of a standard reference gas chromatography method. Validation with a considerable set of real unique samples obtained at pilot scale was performed for both purposes – process monitoring at high conversions and final product quality control. The results confirmed a favourable degree of accuracy with a relative deviation lower than 3.5% from the reference value given by the gas chromatography. Moreover, application of the method for quality control of fresh and long-term stored samples revealed that the deterioration of final products during storage can be detected. The developed refractive index method is thus suitable for the simple and rapid evaluation of the quality of produced fatty acid ethyl esters and for analytical monitoring of their production process.

## 1. Introduction

Due to the ever-growing population and industrialization, the demand for energy is continuously increasing. Currently, one of the alternative sources of energy is biodiesel. Biodiesel is considered, because of its physicochemical properties, as a renewable and environmentally friendly alternative fuel for diesel engines - either pure, or in a mixture with conventional diesel fuel [1–4]. Chemically, biodiesel is defined as a mixture of monoalkyl esters of saturated and unsaturated long-chain fatty acids. Biodiesel is obtained via transesterification reactions of vegetable oils and animal fats, where the triglycerides react with short-chain alcohol, usually methanol or ethanol, in the presence of a catalyst. Simultaneously, glycerol is produced as a byproduct [5,6]. The transesterification reaction is dependent on the selected process parameters, namely type of alcohol, alcohol/oil molar ratio, reaction time, reaction temperature, type and amount of catalyst. In addition, feedstock quality and composition (especially content of free fatty acids and water) plays significant role [7,8].

The most commonly used alcohol in transesterification reactions is methanol due to its low production cost, high reactivity with fatty acid and easier separation of biodiesel and glycerol [2,9,10]. However, use of

ethanol seems to be a better alternative because it can be produced from biological sources and residues, is less toxic, improves the cloud point and oxidation stability of the biodiesel [11] and emits less carbon monoxide and nitrogen oxides than in case of biodiesel production with methanol [12–15].

Product analysis is a very important part of biodiesel production. Due to the large variability of raw materials, standardization was introduced. The final products must meet specific standards for biodiesel on the market. Several analytical procedures have been developed to assess the quality of biodiesel [16–23]. Gas chromatography (GC), the most common method for the determination of the composition of biodiesel, is prescribed by national and international standards across the world [5,16,17,20,24]. This chromatographic method and its techniques represent a complete analysis of total alkyl esters, glycerides (mono-, di- and tri-) and free glycerol. We have developed a GC method for the simultaneous determination of main reaction components during biodiesel production, which can be used to monitor the composition of the reaction mixture in transesterification kinetics studies [25]. Another widespread chromatographic method is high-performance liquid chromatography (HPLC). The HPLC does not require derivatization of samples for analysis and is less time consuming, compared to GC. Various

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detection methods were studied using HPLC to monitor the production of biodiesel [26–31]. Furthermore, spectroscopic methods were used by many researchers for the evaluation and monitoring of the biodiesel production [32,33]. The first reported spectroscopic method used for the assessment of methyl esters was <sup>1</sup>H Nuclear magnetic resonance (NMR) [34]. The reaction kinetics of the transesterification was also studied by <sup>13</sup>C NMR [35]. Other authors followed up on these findings and studied the use of NMR in off-line and real-time monitoring of the biodiesel production reaction [36–41]. The transesterification reaction can be on-line monitored by near-infrared spectroscopy (NIR) using a fiber-optic probe. The NIR shows good correlation with NMR analysis; however, these methods are less sensitive than GC for quantification of minor components [42–46]. Analytical methods using Fourier transform infrared spectroscopy (FTIR) have been developed to monitor the biodiesel content in the reaction mixture during transesterification reaction [47–50]. Izida et al. used fluorescence spectroscopy for the on-line in situ monitoring of the same reaction [51] and transesterification reaction modelling [52]. Ghesti and co-workers investigated the potential application of Raman spectroscopy to monitor and quantify ethyl esters [53,54].

As the production and use of biodiesel increase, there is an urgent need to easily and continuously monitor the conversion of glycerides to biodiesel at the plant level as well as to assess the quality of final products. However, above mentioned methods are often too sophisticated for the industrial implementation, since they require specialized sample preparation (e.g. time-consuming and sometimes complex derivatization), expensive equipment and qualified personnel which presents limitation especially for smaller industrial production units. Therefore, it is convenient to develop a simple, fast, reliable, and accurate analytical method that do not require complicated and expensive equipment.

A potentially simple and fast methods could be associated with physical properties. Since the viscosity difference between triglycerides and corresponding methyl esters resulting from transesterification is approximately one order of magnitude [55], this physical parameter was widely investigated for the purpose of transesterification reaction monitoring. It was shown that viscosities of refined methyl esters are in good agreement with the GC analysis [55–57]. A simple and relatively inexpensive monitoring method was developed based on measurements of the deviation of the density of the reaction medium during the transesterification reaction [55,57,58]. Non-traditional physical properties such as impedance [59] and speed of sound [60] were also studied. Wenlei et al. find out that the conversion of oils to esters could be determined by applying a simple linear correlation with hydroxyl content of the reaction mixture or refractive index (RI) of the product, since the components of the reaction mixture, i.e. glycerin, monoglycerides (MG), diglycerides (DG), triglycerides (TG), and methyl esters have different hydroxyl contents and RI; these results were in good agreement with reaction conversion determined by <sup>1</sup>H NMR spectroscopy measurements [61]. Ghanei et al. proved that RI and specific gravity of refined fatty acid methyl esters (FAME) had high enough precision for predicting of the extent of the reaction [56]. The use of RI measurements for monitoring of a transesterification reaction was further investigated [57,60,62] and this method is considered as a simple, rapid, inexpensive and suitable for process control [62]. In addition, the possibility of on-line monitoring of transesterification reaction by RI measurements was studied [60,63–65] and it was shown that for a reliable assessment of reaction conversion it is necessary either to separate the reaction phases or refine the reaction mixture [60,64,65], or preferably use RI measurement in an off-line manner [60].

Most of the discussed methods based on physical properties measurements were aimed at monitoring of the overall course of the reaction. However, ester content in biodiesel should be higher than 96.5 % wt. According to international standards (e.g. Ref. [21]) specifying the biofuel quality; consequently, a method appropriate for industrial purposes of final process control should be reliable and precise enough in

order to distinguish even small differences in ester content during the termination phase of the reaction. Furthermore, such method would be appropriate even for final biofuel quality assessment. To the best of our knowledge, no RI method achieving the required potential has been reported yet. In addition, most of the works studying RI measurements investigated the methanolysis reaction and ethanolysis reaction was reported only in paper [62].

In our work, the performance of RI measurements at high conversions of ethanolysis reaction was investigated and the method was validated by means of a reference method based on gas chromatography. In addition, we have tested different methods of reaction mixture refining and compared their impact on the RI measurements reliability. The validated method was used at a pilot scale for the purposes of reaction monitoring, reaction endpoint verification and the quality assessment of final product – fatty acid ethyl esters (FAEE).

## 2. Materials and methods

### 2.1. Materials

Pure rapeseed oil for laboratory scale experiments was purchased from a local grocery store, food grade rapeseed oil used in pilot-scale experiments was supplied by company Fabio PRODUKT. Ethanol (99.8%) was obtained from BC-Chemservis s. r.o. Analytical grade sodium hydroxide (99.7%), adipic acid and *n*-butanol were supplied by the IPL Company, sulfuric acid (>95%) by Sigma–Aldrich and refined Glycerol from PENTA s. r.o.

### 2.2. Analytical methods

#### 2.2.1. Sample preparation for RI measurement

Three different sample preparation methods were examined:

- Approximately 5 ml of the reaction mixture was withdrawn and analysed without any further modification
- Approximately 5 ml of the withdrawn reaction mixture was carefully shaken with 5 ml of 0.05 M sulfuric acid aqueous solution in the test tube, to stop the reaction immediately. The mixture was centrifuged for 15 min at 6000 rpm; after that, the upper layer of the sample was taken and analysed.
- The sample preparation was the same as described in B), but after centrifugation the upper layer of the sample was vigorously shaken with glycerol at ratio 1:1 (v/v) and centrifuged for 15 min at 6000 rpm. The sample thus prepared, with described additional glycerol extraction, was subjected to analysis of refractive index measurements.

#### 2.2.2. RI measurement

The refractive index was measured by Exacta Optech RMI Abbe Refractometer mod. RMI. The measurement accuracy was of  $10^{-4}$  at 40 °C – thermostatically controlled by Thermo Haake P5 Circulating Water Bath.

#### 2.2.3. Sample preparation for GC measurements

Reaction monitoring: Approximately 2 ml of the reaction mixture were withdrawn and mixed with a *n*-butanolic solution of adipic acid (0.1 mol/L, 3 ml) in a vial and vigorously shaken to stop the reaction immediately.

Final product quality control & RI calibration for reaction monitoring: Samples for GC measurements were prepared according to method C) described in 2.2.1.

The subsequent sample preparation for GC analysis was according to Ref. [25] in both cases.

#### 2.2.4. Gas chromatography measurement

The composition of the biodiesel reaction mixture, (i.e. content of

FAEE, glycerol, TG, DG and MG), was determined by gas chromatography (Master GC Fast Gas Chromatograph, DANI Instruments S. p.A.) according to the method described in Ref. [25]. Each sample was at least twice injected into the GC chromatograph.

### 2.3. Transesterification reaction

#### 2.3.1. Laboratory-scale experiments

The pure rapeseed oil was subjected to transesterification reaction with ethanol at different reaction conditions. 250 g of rapeseed oil were weighed into glass reactor equipped with a thermometer, reflux condenser and stirrer. The part of ethanol was added, and the reaction mixture was heated to the required reaction temperature. Then the desired amount of catalyst solution in ethanol (amount required for dissolution of NaOH) was added. Addition of catalyst is taken as time zero of the reaction. The reaction conditions were following: the molar ratio of oil to ethanol 1:6, NaOH concentration of 0.5–1.0 % wt. (relative to the initial weight of oil), as a catalyst. The reaction mixture was stirred intensively (2000 rpm) for 2 h under reflux at different reaction temperatures ranging from 40 to 60 °C. Samples of the reaction mixture were withdrawn periodically and processed as described above.

#### 2.3.2. Pilot-scale experiments

The results obtained in the laboratory scale were implemented into the pilot-scale. The reaction conditions were set depending on the experiments performed at the laboratory scale. 65 kg of rapeseed oil (food grade, as specified in section 2.1.) was subjected to a transesterification reaction with ethanol (molar ratio of fat to ethanol 1:6) and NaOH (0.5 % wt., relative to the initial weight of oil) as a catalyst. Sampling and their further processing for GC and RI analysis were as described above. The reaction mixture was stirred intensively for 2 h under reflux at a reaction temperature of 50 °C. The mixture was then transferred to the glass gravitational separator. After 30 min of separation, the bottom layer was removed and excess ethanol was distilled off from the upper layer – crude FAEE. The distillation residue was then washed with 30 % wt. of water, separated by means of gravitational separation (the bottom water layer was removed), refined with glycerol in a similar manner (washed with 10 % wt. of glycerol) and finally separated again by means of gravitational separation (the bottom glycerol layer was removed) to obtain the final refined product.

### 2.4. Analytical data evaluation

Conversion of the transesterification reaction in reaction time  $\tau$  was calculated from GC glyceride analysis and initial reaction mixture composition ( $\tau = 0$ ) by means of the following formula:

$$\text{Conversion} [\%](\tau) = 100 - \frac{TG[\%](\tau) + DG[\%](\tau) + MG[\%](\tau)}{TG[\%](0) + DG[\%](0) + MG[\%](0)} 100$$

## 3. Results and discussion

### 3.1. Method development and optimization

Successful characterization of TG ethanolysis reaction mixture composition relies on the difference in the value of refractive indexes between input feedstock (TG,  $n_D = 1.46575$  in case of rapeseed oil) and final products (FAEE,  $n_D = 1.44725$  in case of ethyl esters prepared from the rapeseed oil). Despite said difference is in this case slight, it allows distinguishing the FAEE from the initial TG. Nevertheless, the sensitivity and reliability of the measurement are dependent on sample preparation, as was pointed out in the literature [60,64,65]. Consequently, we have tested three methods of sample preparation, starting with the measurement of raw sample of the reaction mixture (method A) and ending with sample measured after refining by means of double extraction (method C). Fig. 1 and Table 1 document that sample

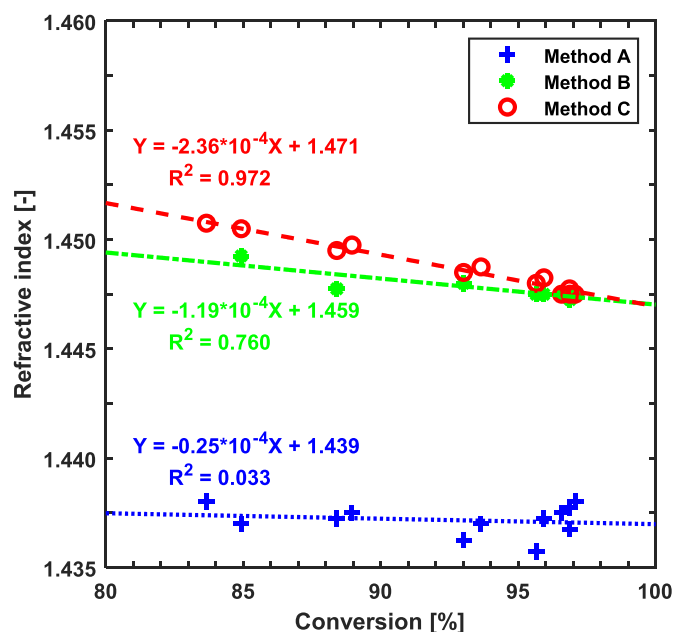


Fig. 1. Comparison of three methods of sample preparation: dependence of refractive index on reaction conversion determined by GC.

Table 1

Regression analysis of the methods of sample preparation.

Method	R - Pearson correlation coefficient	R <sup>2</sup> - Coefficient of determination	p-value	
			Intercept	Slope
A	-0.181	0.033	<0.05	0.57
B	-0.872	0.760	<0.05	<0.05
C	-0.986	0.972	<0.05	<0.05

preparation significantly influences the measurement sensitivity and precision. Differences in RI between samples prepared by the most simple method A were insignificant in the conversion range of 84–97% ( $R^2 = 0.03$ ) in contrast to method C, which achieved the highest sensitivity (slope of  $-2.36 \times 10^{-4}$  % of conversion) and reliability in terms of  $R^2$  reaching a value of 0.97. As a result, method C was chosen as optimal and used in further work. Although method C is more laborious compared to methods A and B, it is still simple and straightforward for the purposes of routine analysis and process control. Let us note that the purpose of the additional sample refining with glycerol in method C was to extract water and other polar compounds present in the sample after previous washing with 0.05 M aqueous solution of sulfuric acid.

During the course of transesterification reaction, TG are converted to FAEE and several reaction intermediates are present in the reaction mixture. Said changes in reaction mixture composition are reflected in changes of its refractive index. Precisely, changes in composition of the reaction mixture ester phase were recorded by RI in samples prepared by method C. In the next step, we investigated the correlation of RI data with concentration of basic reaction components and reaction conversion in the corresponding reaction mixture samples. Table 2 summarizes gained results. As can be seen, the highest correlation coefficient was

Table 2

Pearson correlation coefficient of selected parameters with RI data.

Parameter	R - Pearson correlation coefficient
TG	0.942
DG	0.986
MG	0.727
FAEE	-0.999
Conversion	-0.998

obtained in case of FAEE content ( $-0.999$ ) and reaction conversion ( $-0.998$ ), which are therefore the most suitable parameters for method calibration. This result was expected as the FAEE present major compound in the final product and conversion expresses extent of the reaction. Surprisingly, our data indicate an unexpectedly good correlation with DG concentration ( $0.986$ ), the reaction intermediate. This result is striking especially in comparison with correlation to TG ( $0.942$ ), main compound of the feedstock.

### 3.2. Method calibration and validation

Conversion was chosen as an appropriate parameter for the purpose of ethanolsis reaction monitoring and reaction endpoint verification. Firstly, the method was calibrated with the help of standards prepared from reaction mixture samples taken at different times. Composition of the calibration standards was determined by the standard reference method – GC. As a result, the calibration standards reflected the composition of a real reaction system with presence of MG and DG in contrast to common approach – preparation of calibration standards from mixtures of TG and FAEE [62]. As can be seen in Fig. 2, linearity of the calibration chart was good with a high value of coefficient of determination. Let us note that obtained result is in agreement with published data – similar absolute values of calibration curve were obtained in the work of Santos et al. [62] despite the authors worked with different type of vegetable oil (soybean oil).

In order to perform robust validation of the suggested method, a set of kinetic measurements carried out at different reaction conditions was realized. Samples of reaction mixtures were analysed by the GC reference method and the developed RI method. In total, we have used 40 unique samples for method validation. Validation parity plot (Fig. 2) reveals that in the majority of the cases, the difference between RI method and reference GC method was lower than 3.5%, even in the area of main interest, i.e. high conversions of transesterification reaction (range of 85–100%) where most of the validation samples were present. Said difference corresponds to the expectable experimental error even in the case of the reference GC method [25]. In addition, no significant dependence of measurement precision on the reaction conversion was observed and the difference threshold of 3.5% holds for the whole investigated range of concentrations.

Calibration based on FAEE content was chosen for the purpose of final product quality determination and control. Especially in this case, the method must be sensitive enough to distinguish whether the samples

do fulfil the common limit of ester content  $\geq 96.5$  % wt. We have used data from the same calibration samples as in the previous case of conversion calibration and added randomly chosen data from the final product quality assessment obtained by means of the reference GC method (totally 27 samples were used for the calibration). Fig. 3 showing calibration curve in the range of interest (FAEE 90–99 % wt.) reveals higher scatter of the data which was expected due to the narrow measurement range and corresponding high demands on method sensitivity. Overall, the linearity was acceptable with the favourable value of coefficient of determination ( $0.94$ ).

The method was further validated by means of 50 unique validation samples of final products obtained mostly at pilot scale. Parity plot comparing results of RI analysis with reference GC method (Fig. 3) documents that the difference between both methods was in most cases again lower than 3.5%. More precisely, 95% of validation samples achieved relative deviation lower than 3.5% from the reference value given by the GC method, (see distribution of deviation in Fig. 4). These results shall be evaluated in the view of expectable error of the standard method EN 14 103 for ester content evaluation – the standard itself [23] reports reproducibility limit around 3 % wt. of the absolute value and similar interlaboratory comparison [66] came to the same difference in the range of 3 % wt. between reported results of an identical sample. In our case, we compare different methods and the validation was performed on a series of unique samples. As a result, the expectable GC method error, which is present in validation samples, must be taken into account. In conclusion, validation confirmed good sensitivity and resolution of RI method for the purposes of final product quality control.

### 3.3. Reaction monitoring and endpoint verification at pilot scale

Validated method was used for monitoring of the course of reaction at a pilot scale. The reaction conversion was assessed by both methods – RI and reference GC. As can be seen in Fig. 5, both methods are in agreement, which was nevertheless stated in the RI method validation. The RI method was able to follow the course of the reaction and is therefore suitable for basic kinetics measurements performed e.g. on site at industrial scale. It can also be used for the purposes of reaction endpoint verification because it is possible to distinguish even relatively small increase of conversion equal to approximately 2%. For example, in the pilot-scale experiment, the conversion was in 105 min equal to 98% according to the RI method with no measurable progress in 150 min – see Fig. 5. Said conclusion was confirmed by GC measurement (increase

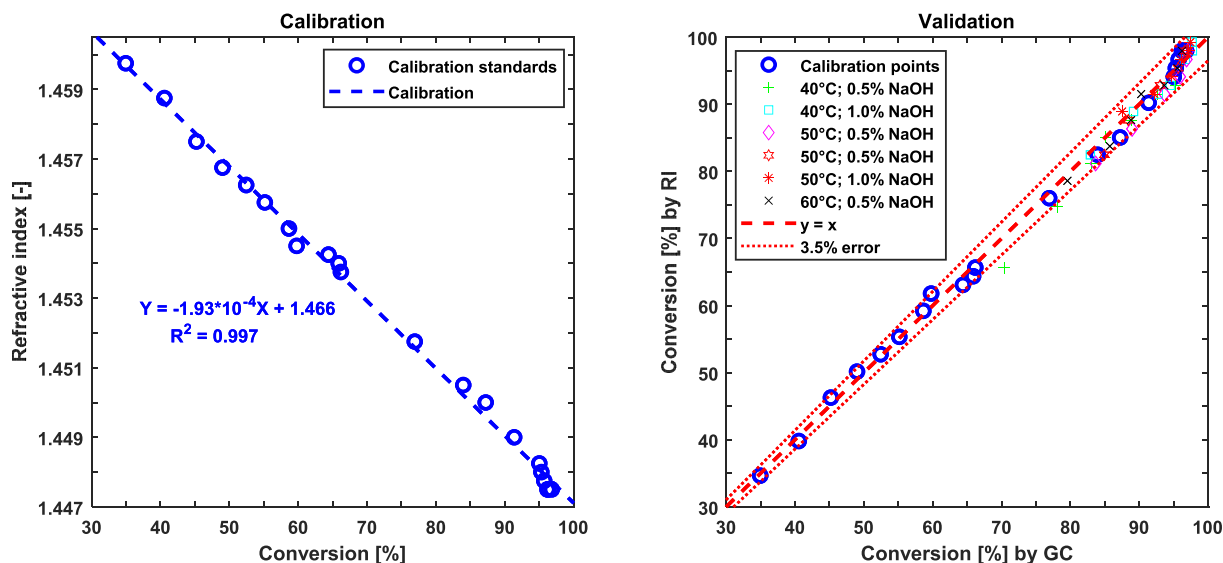


Fig. 2. Calibration chart for reaction monitoring by means of reaction conversion determination and Validation of reaction monitoring – parity plot of conversion obtained by means of RI vs. reference method based on GC; dotted lines show prediction interval.

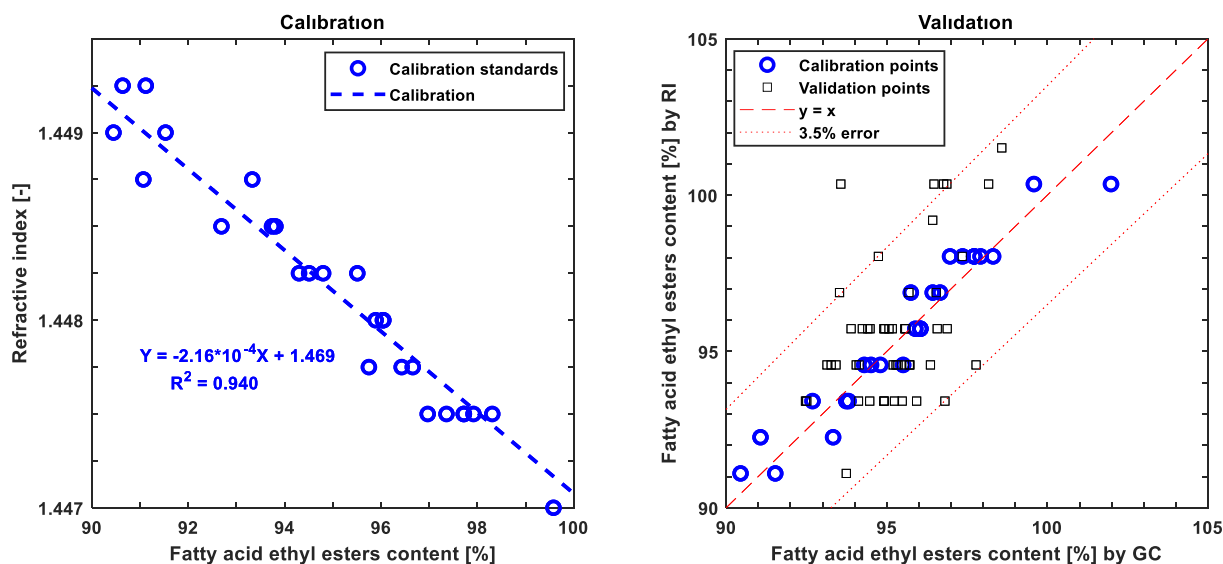


Fig. 3. Calibration chart for product quality control by means of FAEE content determination and Validation of final product quality control – parity plot of FAEE content obtained by means of RI vs. reference method based on GC; dotted lines show prediction interval.

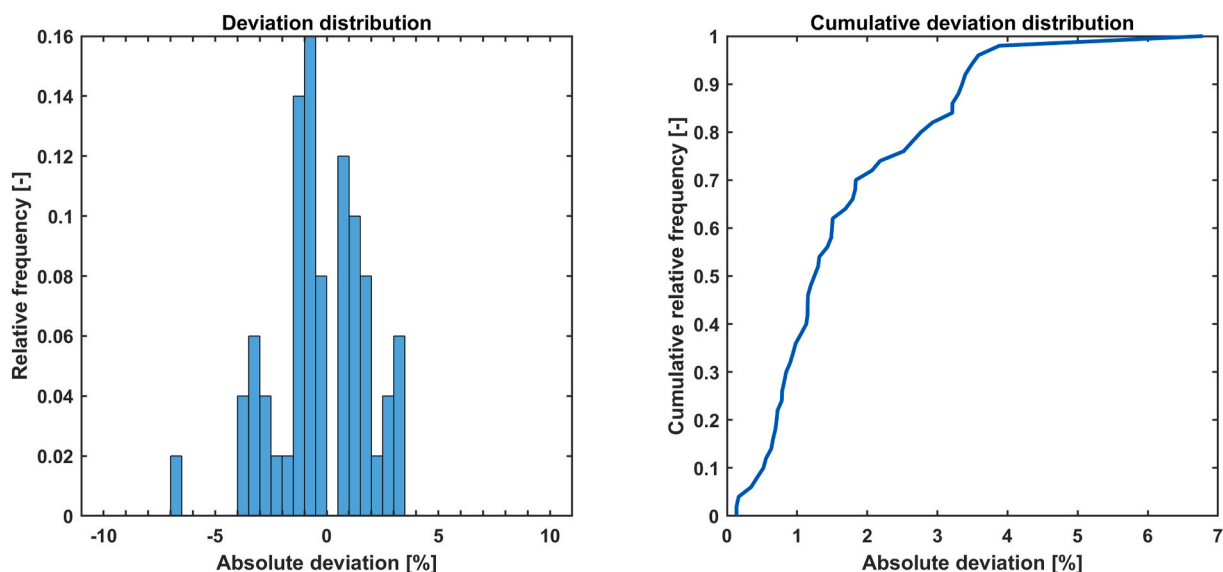


Fig. 4. Distribution of deviation between developed RI method and reference GC method.

in conversion between 105 and 150 min was 0.3% according to GC data). Based on these results, reaction time of 105 min is sufficient because there is no significant progress in reaction conversion with increased reaction time. Furthermore, the method can be applied in similar manner at prescribed time interval to check that the extent of reaction achieved required value and thus serve as method of routine process control.

### 3.4. Determination of final product quality at a pilot scale

Validated method was further used for quality assessment of the final products prepared at a pilot scale. Since the pilot unit was operated batchwise, the quality of final FAEE obtained in each batch was monitored. Fig. 6 shows results of FAEE quality monitoring in case of 11 batches. As can be seen, FAEE content – final product quality indicator, was in most batches higher than the threshold value of 96.5 % wt. In two batches the FAEE content was lower than this threshold and consequently these batches did not meet the quality criteria. There was close

agreement between the quality control performed by means of the RI method and reference GC method in all discussed cases. As a result, the RI method proved to be suitable for final product quality monitoring purposes.

In addition, we performed quality control analysis also for final product samples which were stored for long time (around 2.5 years) – see green data points in Fig. 6. Despite FAEE content of all samples was higher than 96.5 % wt. in the time of their manufacture, the RI method showed that after long-term storage their quality does not meet the threshold value. Said conclusion was further confirmed by the results of GC analysis. Control measurement of peroxide value (batches no. 14–16) revealed that samples were affected by oxidation (all peroxide values > 120 meq/kg). Therefore, the RI method was able to capture deterioration of final products also during their long-term storage and can be used even for this purpose.

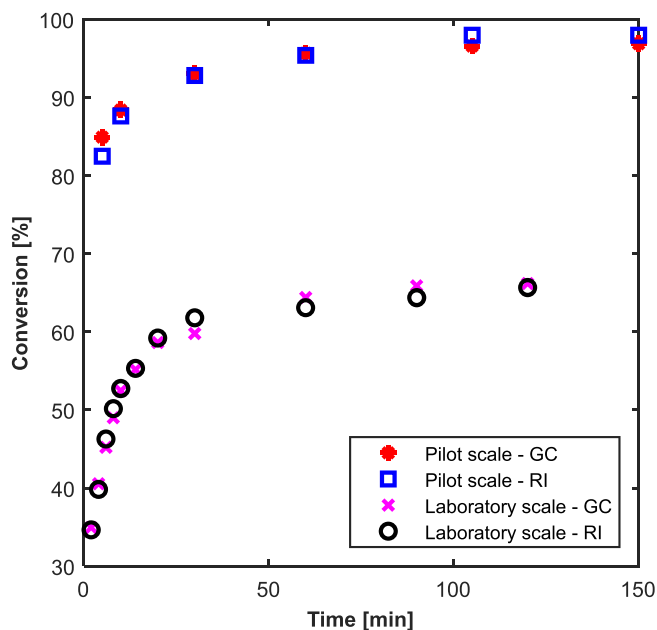


Fig. 5. Monitoring of the reaction conversion - Pilot scale experiment (50 °C, 0.5% NaOH) and Laboratory scale experiment (40 °C, 0.1% NaOH).

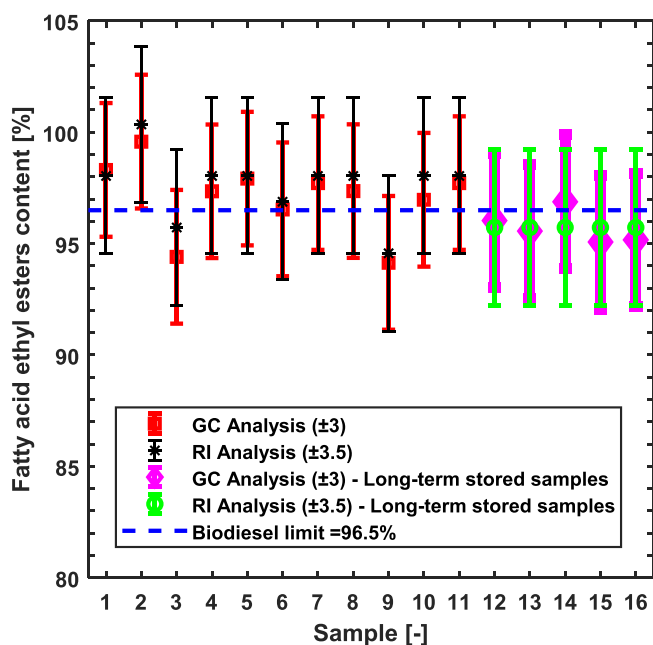


Fig. 6. Final product quality measurement for batches prepared at a pilot scale. Batches in green indicate long-term stored samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

### 3.5. Effect of presence of free fatty acids

Free fatty acids (FFA) are usually present in lipids used for biofuel production and consequently impact of their presence in the sample on the reliability of the RI method should be addressed. FFA interfere with the most common alkali catalysed transesterification (FFA react with catalyst which results in soap formation) [67]. Therefore, vegetable oils or animal fats used for biofuel production by this reaction are usually refined and contain low amount of FFA. FFA content is further limited in final biofuels, e.g. European standard for biodiesel quality [21]

prescribes maximal acid value of 0.5 mg KOH/g ( $\sim 0.25$  % wt. FFA). We have investigated the effect of FFA presence on the results of the RI method and found out that if FFA content is lower than 1 % wt. ( $\sim 2$  mg KOH/g), FFA do not interfere with the analysis. In addition, in case of FFA content approximately 5 % wt. ( $\sim 10$  mg KOH/g), the error caused by the FFA presence was within the error of the RI method (3.5%). In other words, suggested RI method is suitable for samples with low FFA content, which represents the majority of industrial samples since alkali catalysed transesterification is the most abundant method. The FFA content in final biofuels is limited by regulation and it should be routinely checked by rapid and inexpensive acid value determination. On the other hand, the RI method is not suitable for monitoring of esterification/transesterification of lipids with high FFA content.

## 4. Conclusions

The results of this study showed that the refractive index measurements are sensitive and reliable enough to monitor the course of rapeseed oil ethanolysis even in the area of high conversions (85–100%) and to determine the final product quality (FAEE content  $\geq 96.5$  % wt.). The key factor assuring the method sensitivity is sample preparation performed by means of double extraction. Rigorous calibration of the method was performed, with the refined samples of a real reaction mixture containing MG and DG. The validation based on 40 unique samples taken in 6 different reactions revealed that in the majority of the cases the difference in reaction conversion determination between the RI method and the reference GC method was lower than 3.5 %. Therefore, the method proved suitable for basic kinetics measurements performed e.g. on site at industrial scale and for routine reaction extent/endpoint verification.

The FAEE content calibration proved to be an optimal parameter for the purpose of final product quality determination. Calibration curve in the range of interest (FAEE 90–99 % wt.) had expected higher scatter of the data due to the narrow measurement range. However, the linearity was acceptable with a favourable value of coefficient of determination (0.94). The method was further robustly validated by means of 50 unique samples of final products. 95 % of validation samples reached a relative deviation lower than 3.5 % from the reference values of FAEE content given by the GC method. The developed method was used for quality control analysis of final products prepared at pilot scale, both fresh and long-term stored. The RI method was able to prove the threshold value of FAEE content of 96.5 % wt. and to reveal the deterioration of final products during long-term storage. In general, the method is suitable for samples with low FFA content (preferably  $< 1$  % wt.).

In conclusion, the developed simple RI method proved sensitive enough to replace usual GC measurement not only for reaction monitoring but also for final product quality control. Utilization of GC is then advisable predominantly in case of samples which are at the threshold values to confirm results of RI analysis. Such an approach is beneficial, especially in the case of smaller production units, as RI method is inexpensive and simple in comparison with the standard GC method.

## Credit author statement

**Jakub Husár:** Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization; **Jiří Pecha:** Conceptualization, Investigation, Methodology, Writing - Review & Editing, Supervision; **Lubomír Šánek:** Methodology, Validation, Investigation, Resources, Writing - Original Draft.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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