BIOScopes

Improvements needed for the biodiesel standard EN 14214

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Final report for Lot 1 of the Bioscopes project

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BIOScopes:
Biodiesel Improvement On Standards, Coordination of Producers and Ethanol Studies

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The European BIOScopes project is carried out by Ecofys for the European Commission's Directorate-General Energy and Transport, in cooperation with AGQM, EBB, NEN, University of Graz, Atrax, ITERG, Abengoa, SSOG, ASG, ADM, Ecotraffic, BAFF, LACCO, UNGDA, LBB, O2Diesel, and VTT.

BIOScopes is concerned with the improvement of the EN 14214 biodiesel quality standard, and the increased use of bioethanol in the diesel market.

The following reports have been published:

- Lot 1: Improvements needed for the biodiesel standard EN 14214
- Lot 2: Heavy-duty ethanol engines
- Lot 3a: Fatty acid ethyl esters (FAEE)
- Lot 3b: Ethanol-diesel blends

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Abbreviations

CEN	European Committee on Standardization
CEN/CMC	CEN Central Management Centre
CEN/TR	CEN Technical Report, an informative (non-normative) product of CEN
CFPP	Cold Filter Plugging Point, item of the (bio)diesel quality standard
CP	Cloud Point, item of the biodiesel quality standard
EN 14213	European quality Standard for FAME for heating oil
EN 14214	European quality Standard for automotive FAME
EN 590	European quality Standard for automotive diesel
FAEE	Fatty Acid Ethyl Ester, biodiesel from esterification of fatty acids with ethanol
FAME	Fatty Acid Methyl Ester, biodiesel from esterification of fatty acids with methanol
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
ICP	Ion Coupled Plasma, type of spectroscopic method
JWG	Joint Working Group
PM	Particle Matter, emission of small particles, dangerous for health
prEN	preliminary CEN Standard or enquiry text
RME	Rapeseed Methyl Ester, common biodiesel from rapeseed
TF FAME	CEN/TC 19 taskforce concerned to prepare the FAME quality specification
UAP	Unique Acceptance Procedure, a single formal ballot procedure to accept a draft Standard in CEN
WG 24	Working Group under CEN that has the scope to draft the diesel and biodiesel quality standards for automotive application, also CEN/TC 19/WG 24

Executive summary

Background and actions performed

When the first European biodiesel specifications had been developed in the beginning of this century, CEN, the European Standardization Committee, had to build on the experience of that time. In the end it appeared to be that some test methods to determine the product quality were inaccurate when applied to blends in diesel or were even non-existing. The objective of the project was to update the test methods by standardizing the (existing) field experience and if necessary develop new methods. The data of accuracy (precision) needed substantial improvement. Moreover, new test improvements for the determination of the storage stability needed to be examined for their feasibility. This was linked to the choice for the only available reliable predictor at that time: the Iodine Value.

The project thus included literature research on stability based on a paper on the state of the art of biodiesel production, quality and use, as well as interlaboratory studies on test methods and standards' preparation work for acceptance of the improvements in CEN. A total of six parameters and their characterization methods as referred in the biodiesel specification EN 14214:2004, were the basis of the project's work:

1. Ester content, (EN 14103:2003);
2. Diglyceride content (EN 14105:2003);
3. Triglyceride content (EN 14105:2003);
4. Free glycerol content (EN 14106:2003);
5. Alkaline content (prEN 14538:2002);
6. Polyunsaturated esters content (no standard available).

Next, work has been done on oxidation stability and FAME detection testing as these were needed to complete the revision of EN 14214.

The project team of course had to work closely with existing CEN groups. All work was directly fed into the joint-working group (JWG) between CEN/TC 19 and CEN/TC 307, which had been established around the start of the project to advise on biodiesel test method improvements. Next, regular reporting to and participation in the groups that had to decide upon the revision of the diesel and biodiesel specifications was done by the project partners in person.

Next, the team, when discussing and assessing test method improvements invited the cooperation of CEN experts. In addition, participating laboratories for the interlaboratory study where sought all over Europe. In the end, the team assisted in drafting the text for the (revised) standards and short reports to support certain decisions in CEN on the next steps for the test methods.

Iodine Value

Concerns on possible engine problems and failures have often been attributed to high Iodine Value feedstock. But due to Iodine Value limitation in the European Biodiesel Specifications the use of potential feedstock is discriminated so far. The critical evaluation of Iodine Value versus feedstock, stability, engine experience, fuel quality and world-wide standardization concerns led to following conclusions: The Iodine Value on its own is not sufficiently good enough for describing stability concerns. The stability of biodiesel is a result of position and content of double bonds in the different fatty acid methyl esters of biodiesel as well as the content of antioxidants and the production technology used. Therefore oxidation stability is the most important parameter in the context of possible problems in engine parts.

Together with oxidation stability, the content of linolenic acid methyl esters and polyunsaturated esters are sufficiently enough to exclude the use of higher portions of very “instable” and higher unsaturated feedstock (e.g. linseed oil and fish oil). Engine problems with blends or neat biodiesel are predominantly caused by bad oxidation stability and not by high Iodine Values. An appropriate high oxidation stability of biodiesel which can be reached by adequate feedstock or added antioxidants, is sufficient enough for the quality of the fuel.

The conclusion from this project is that a further limitation of Iodine Value at 120 g I_2/100 g does not seem to be necessary. The increase of Iodine Value up to 140 should be anyway compensated by an appropriate adjustment of the value for oxidation stability. Based on comprehensive existing experience especially with soybean oil methyl esters, concerns on problems caused by the use of higher Iodine Value biodiesel can be disproved. To guarantee further high quality biodiesel especially in context with stability, parameters like linolenic- and poly-unsaturated ester content as well as adjusted oxidation stability are currently much more meaningful. Further investigations, however, are necessary to study the influence of biodiesel with high Iodine Value on engine oil dilution and stability of engine oil.

Test methods and standards

The optimal test method for ester content has not been found. Many improvements have been tested, also on the basis of an alternative fats and oils test (EN ISO 5508). Both optimization of the internal standard as modification of the sample preparation have been tested. An alternative by HPLC-SEC has been assessed, but it was decided to turn back to the gas chromatograph. Finally, a good repeatability for the updated GC test method has been developed. While the results for linolenic acid methyl ester were judged to be “good to excellent”, the preliminary reproducibility results for ester content were somewhat disappointing and also difficult to interpret. In the end, the statistical evaluation of the Round Robin test showed an unacceptable precision for a minimum limit of 96.5%. Possibly, this may be related to the inexperience with the test. So it is advisable to work out the GC test into a standard (revision of EN 14103) and redefine the precision in one or two years time. Next, one may decide to also define a HPLC test, determine its precision and use this as the referee test method in cases of dispute, but offer the GC for day-to-day testing.

The method for di- and tri-glycerides has been successfully updated by elimination of a calibration step and improvement of the internal calibration standards. One essential change to the test method is the addition of one dedicated standard for each of the three “family members” (mono-, di-, triglycerides). Control of the GC columns had to be improved, too. Next, stability of the reference glycerides appeared to be a problem, delaying progress in the work as such. The team has forwarded the resulting text to CEN for further work, becoming a revision of EN 14105. For di-glycerides the renewed precision is acceptable for the limit as wished for in EN 14214, whereas for tri-glycerides more experience seems to be needed in a couple of years time.

The study has further revealed that for free glycerol no improvements could be made to EN 14105, but that the generally used methodology gives sufficient information to comfort the industries need. It is thought that the test method's precision will improve due to more experience in the field.

The test method for alkali content (EN 14538) has proven by the team to be applicable and has been accepted for incorporation in the pending revision of EN 14214.

The method for polyunsaturated esters has been developed and almost finalised. These esters are defined as having 4 or more double bonds. Determination is done by gas chromatography/FID detection using an internal calibration with C 23:0 methyl ester. The theoretical detector correction factors relative to C 23:0 internal standard for different poly-unsaturated ester types are applied to the analytical data for optimum accuracy. The test has been accepted as a new work item under CEN/TC 19. The study has delivered good precision data via a first internal Round Robin test. It showed application possibilities to contents as low as 0.2 %, with the option to accomplish about 0.1 % after further optimization of the test method. Unfortunately the statistical results of a larger RR were not available for this report. The standard text has been forwarded to CEN/CMC and is to be balloted as prEN 15779:2008.

The existing test for determination of the contents of FAME in middle distillates like diesel and heating oil is based on infrared spectroscopy. It works quite well, however, the precision was not determined in the concentration range below 1.7 %. Additionally, the mineral oil industry was complaining about poor precision of the method in general, not allowing to blend as close to the usual blend level of 5 %. A modification of the method was undertaken. The major change is measuring without sample dilution in the low concentration range (a Method “A” and “B” have been introduced) and the change of the solvent. This revision will now be worked upon by CEN.

The oxidation stability test has been greatly improved. The text has been drafted and forwarded to CEN to allow EN 15751 to become a Standard when the revised EN 14214 will hopefully be published at the end of 2008. The test has its limitations towards diesel containing FAME levels at less than 2%.

A two-step revision of EN 14214 has been accepted, with the first aim to include the test method results concerning alkaline metals, free glycerol and oxidation stability. Next, CEN has been advised

to incorporate the revised or new methods on glycerides and esters in the biodiesel specification in the near future. On relaxation or deletion of the Iodine Value requirement a discussion is still pending.

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1 General Introduction

1.1 Introduction to the work

Fatty Acid Methyl Ester (FAME) is a liquid biofuel that has a history of automotive use. Car manufacturers have both good and bad experiences with this product, because of the difference in quality of the FAME (derived from different biological sources, having different cold operability, lubricity, ignition and stability qualities and with the changes of containing other types of or different levels of contaminants), which affects the technical performance of car engines. Satisfactory standard specifications concerning fuels are necessary for the reduction of exhaust emissions from engines. In addition the fuel must be fit for purpose i.e. maintain it's quality throughout the supply chain from producer to end-user and enable car engines to perform properly under varying climatic conditions. All these qualities have been extensively investigated within European standardization and specifying bodies (such as CEN).

From 1997 to 2003 a co-operative study of CEN Technical Committee 307 on "Oilseeds, animal and vegetable fats and oils" and CEN Technical Committee 19 on "Petroleum products, lubricants and related products" has been executed in which a dozen tests have been developed. These test methods had to identify specific parameters of FAME. With those a producer or a blender is able to identify the suitability of FAME for blending in European diesel according to the EU Directive (EN 590). Two separate FAME specifications have been drafted and published in 2004:

- EN 14213 Heating fuels — Fatty acid methyl esters (FAME) — Requirements and test methods;
- EN 14214 Automotive fuels — Fatty acid methyl esters (FAME) for diesel engines — Requirements and test methods.

Both specifications were designed for neat biodiesel fuel and for blending up to 5% in regular diesel fuel.

When the study was concluded, four methods – when applied to FAME blended in diesel – appeared to have a precision that did not meet the requirement for setting a reasonable limit, following the internationally accepted standard EN ISO 4259, section 8.2 ('For a single limit, the specified limit shall be not less than twice the reproducibility R'). This so-called "2R-requirement" is essential for comparison of laboratory tests. Not only will this prevent extensive discussions between two trading parties, but it will also secure the diesel quality in the end.

At the time of development of the first biodiesel specifications not much field experience was available. The majority was on rapeseed methyl ester (RME) which seemed to work well. Many requirements were thus related to this feedstock and test methods could only be judged for application

and precision on RME (see also 2.2). Now that the supply of feedstock becomes diverse, the test methods need to be re-checked for a wider range of esters.

Since its publication, EN 14214 has become a market specification. This has also resulted in its use for non-specified (and non-regulated) applications, such as higher levels of blending in diesel, just because the quality is the one most available on the market. These purposes do not fall within the scope of the work. Other quality standards have been published in the rest of the world, including some added test methods. Also these are not dealt with in this study.

1.2 Objectives

The objective of the present project is to update the test methods by standardizing the (existing) field experience and if necessary develop new methods. It is the aim to optimize the existing test procedures in that way that the data of accuracy (precision) will be improved substantially. Moreover, new test improvements for the determination of the storage stability needed to be examined for their feasibility. Principles for the determination of important characteristic features of quality of mixtures are to elaborate. For the parameters “Fatty acids with more than 3 double bonds“ and “storage stability” there are no standardized test procedures up to now. These methods have to be elaborated almost from scratch. The aim is to deliver (revised) CEN Standards and a report on the comparison of the test methods.

Secondly, a working paper on the state of the art of biodiesel production, quality and use as well as standardization, based on literature review and experiences in practice, is developed. This report lays out inter-relationship between ester origin, Iodine Value, biodiesel composition and stability. The aim was to identify preliminary answers to questions such as:

- Can the Iodine Value be increased and under what conditions?
- Can we increase the blend percentage?
- Do we need different specifications/requirements for FAME for blends and FAME for biodiesel at 100% level?

Final answers on the above questions can of course only be given to the full satisfaction of the engine manufacturers and biodiesel producers after engine field tests, but for that the timeframe of this project is too limited. The working paper will therefore only make sure that during the inter-laboratory testing and drafting of the CEN Standards no things are overlooked.

1.3 Milestones and deliverables

There were four milestones foreseen, all having several deliverables. (The work package leaders are indicated in between brackets).

M1 Internal study on the state of the art of the various biodiesel origins and fuel uses (Mr. Mittelbach):

a) Small status report on actions and questions for the group;
b) Literature survey;

c) Final report on state-of-the-art;
d) Advice on additional necessary testing (in cooperation with AGQM);
e) Advices on how to implement the results into the EN 14214 specification (in cooperation with AGQM and NEN);
f) Identification of further work on engine or field tests to assess inter-relations of stability testing, Iodine Value and actual engine work (in cooperation with AGQM and NEN);

M2 Inter-laboratory study and technical report to CEN on updated test methods (Mr. Fischer):
a) Draft test methods;
b) Lab studies;
c) Round robin set-up;
d) Sample preparation and distribution;
e) Statistical evaluation report;
f) Updated test method drafts;
g) Draft text for CEN Technical report;

M3 Publication of the first draft (revised) standard(s), the so-called prEN's (Mr. Costenoble/Mrs. Lacoste of Iterg):
a) Draft test methods in CEN-format;
b) New work item proposals for (revision of) test methods and CEN/TR to TC 19 / TC 307;
c) prEN texts of test methods sent to CEN/CMC;
d) CEN/TC 19 decision to publish CEN/TR;
e) prEN enquiry results known and tabled;
f) Decision on next steps for enquiry results;

M4 CEN/TC 19 resolution to revise the actual existing CEN (bio)diesel specifications (Mr. Costenoble):
a) CEN/TR text in CEN format to JWG and TC 19;
b) CEN/TC 19 decision to revise EN 14214 and EN 590;
c) CEN/TR published.

1.4 Project partners

This project concerns Lot 1 of the BIOScopes project.

University of Graz

The Institute of Chemistry of the University of Graz (shortly called Uni Graz), an Austrian research institute, with large experience in application and shortcomings of the existing standards.

Arbeitsgemeinschaft Qualitätsmanagement Biodiesel e.V.

The Association of Quality Management Biodiesel (AGQM) having extensive experience in the coordination of research and development projects and an internationally recognised partner for questions concerning the Quality Assurance of Biodiesel.

Nederlands Normalisatie-instituut

NEN, the Dutch Standardization institute, since 1981 the holder of the secretariat of CEN/TC 19, the CEN Technical Committee dealing with EN 14214, EN 14213 (heating fuel) and EN 590.

European Biodiesel Board

The European Biodiesel Board, EBB, the biodiesel producers' representative organization for Europe.

2 Study

2.1 Division of work

The project is focussed around three work packages, which indicates the division of work amongst the Lot 1 partners:

- 1a: FAME test method revision and assessment – led by AGQM, Mr. J. Fischer
- 1b: standardization and dissemination of the FAME test results – led by NEN, Mr. O. Costenoble
- 1c: working paper on Iodine Value and stability in relation to different feed stocks and blend percentages of FAME – led by Uni Graz, Mr. M. Mittelbach

To accomplish the work for sub-package 1b, NEN has sub-contracted tasks to ITERG (Institut des Corps Gras) in France, which had chaired and coordinated the original drafting of the fats and oil test methods within CEN/TC 307 under the EC Mandate for FAME.

2.2 Scope

The baseline was the examination of a total of six parameters and their actual characterization methods as referred in the biodiesel specification EN 14214:2004:

1. Ester content, (EN 14103:2003 under CEN/TC 307);
2. Diglyceride content (EN 14105:2003 under CEN/TC 307);
3. Triglyceride content (EN 14105:2003 under CEN/TC 307);
4. Free glycerol content (EN 14106:2003 under CEN/TC 307);
5. Alkaline content (prEN 14538:2002 under CEN/TC 19);
6. Polyunsaturated esters content (no standard available).

Test methods for ester content and alkaline metals were tested twice (two ring tests for each method) during the period of the Mandate M/245 (1997-2003) without reaching the precision required. The conclusion was that existing methods have reached their limits and other technical work may be needed.

To expand the metal content investigation not only Group I (Na and K), but also Group II (Ca and Mg) metals have been identified by the car manufacturers as a matter of concern. This has lead to the introduction of a new test method, in addition to the existing EN 14108 and EN 14109. This method was only at draft stage (prEN 14538) when EN 14214 had been published, but during the BIOScopes project it has been slightly updated and introduced in the FAME specification. The method is based on German experience, but has not gone through a major inter-laboratory study. Introducing a second, completely different test method with the need for additional equipment is not only confusing, but also cost ineffective for the laboratories. A challenge related to metals is the measurement of phosphorus

content, which is now done according to a separate standard, but might be included in the test method of this project.

Improvement is also needed for diglycerides, triglyceride and free glycerol content determination. The determination method for glycerides (EN 14105) namely seems to have worse precision in the real world than indicated in the CEN Standard. But the partners think it is possible to improve the method so it can fulfil the biodiesel specification. It may also be used to replace EN 14106, according to its actual scope only for free glycerol content determination, a more straight forward GC method. An improved determination, which gives the benefit of doing only one test, is the goal of the project. At the time of drafting EN 14214, both test methods were used in the labs and neither could be discriminated for precision.

The specification on polyunsaturated ester content was introduced in order to limit at 1 % the content of polyunsaturated FAME with more than 3 double bounds. At the time of the Mandate no test method was available for such a complicated determination in term of identification and quantification (1 % of FAME with more than 3 double bounds), so technical work is needed before any standardisation step.

Three items referring to behaviour of biodiesel submitted to oxidative conditions have been defined by CEN/TC 19 depending on the uses:

- Oxidation stability, where the parameter is the influence of oxygen and ambient air, for both automotive diesel and heating oil applications;
- Thermal stability, where the parameter is influence of temperature in absence of oxygen, for both automotive diesel and heating oil applications;
- Storage stability, where the influence of time and storage conditions is essential, for only heating oil applications.

At the end of the Mandate M/245, one CEN/TC 307 test method based on Rancimat test (EN 14112) has been chosen for FAME oxidation stability.

However, the stability characteristics for different biodiesel were not known at that time. To overcome the problem CEN/TC 19 choose to require a certain Iodine Value as an indication for the stability. This requirement was based on existing field experience, which at that time was merely based on rapeseed methyl ester as basis for biodiesel. But the biological origin of the source cannot be a requirement within a fuel specification that is based on quality to drive an engine. Therefore, the purpose of this BIOScopes Lot 1 project is also to correlate past experience with results of the new test methods on all kind of biodiesels. This means first of all that different sources will be incorporated in the laboratory tests to check the suitability of the reviewed test methods for different types of biodiesel.

2.3 General execution

Lot 1 has had two meeting on 1 February 2006 and 18 July 2006. After the last meeting each work package leader has called up package meetings when necessary. Only coordination meetings between the overall co-ordinator and the Lot co-ordinator took place since.

External groups

The relation with other, external groups has been done as follows:

- The CEN/TC 19 - CEN/TC 307 JWG on test methods has been used as the focal point for the work of Lot 1a. The BIOScopes partners are all nominated experts in this group and have acted as the main driving forces behind the actual work. The work package leader, being the JWG convenor, has always reported of the progress to both CEN/TC 19/WG 24 and to CEN/TC 19/WG 24/TF FAME (see under 2). The latter, having more frequent meetings and the most debates on progress of EN 14214 revisions has been used as steering group as indicated in the original proposal;
- Reporting to CEN/TC 19/WG 24, responsible for decisions on revision of the (bio)diesel fuel specifications, Mr. Fischer has reported on two occasions. Any feedback, especially when this WG had been discussing the EC Mandates, and indications towards needed timeframes has been fed back into Lot 1a;
- ITERG has taken care of reporting to CEN/TC 307 and in its working group on fats & oil testing, which are both responsible for several of the actual test methods. Also NEN has given input and posed questions regarding the revision of the test methods (see also §2.5);
- To the CEN/TC 19 plenary meeting an official report has been sent at the beginning of March 2007. This included the request for a short term (updating of test methods) and a long term (broadening of the feedstock) revision of EN 14214.

2.4 Lot 1a: FAME test method revision and assessment

The AGQM representative leading this part of the work has been Dr. J. Fischer. He is also chairman of the joint-working group on FAME testing under CEN/TC 19 and CEN/TC 307.

This so-called FAME Taskforce was set up in 2004 to further elaborate on the EN 14214 test methods. It consists of several organizations that have already been engaged in the original Mandate work on FAME. The work of the FAME TF will be incorporated within the BIOScopes Lot 1 project, as will be arranged by the envisaged AGQM representative. This concerns discussion on test methods, set-up of a inter-laboratory test and discussion on the results.

Within the TF five laboratories will do the basic discussion and testing on the test methods: Institut Des Corps Gras - ITERG (FR), ASG Analytik (DE), Stazione Sperimentale Oli e Grassi (SSOG, IT), Uni Graz (AT) and ADM Oelmuehle (DE). AGQM will set-up the inter-laboratory test, based on the methods generated in the FAME TF. In the actual testing the labs of all organizations will participate, next to already identified labs, such as BP (DE), Novaol (FR), Bunge Europe (HU), EHN Combustibles Renovables (ES), Shell Hamburg (DE), ITS Sunbury Technology Centre (UK) and Total (FR).

The planning for this work was as follows:

1. Draft test methods and initial quality checks;
2. Lab studies;
3. Round robin set-up;

4. Sample preparation and distribution;
5. Statistical evaluation;
6. Update the draft test method;
7. Draft text for CEN Technical Report (CEN/TR).

2.5 Lot 1b: Standardization and dissemination of the FAME test results

The foreseen steps within this standardization work are the following:

1. Draft test methods in CEN-format;
2. New work item proposals for (revision of) test methods and CEN/TR to CEN/TC 19 (fuels and related products) and CEN/TC 307 (fats and oils);
3. prEN texts of test methods sent to CEN/CMC;
4. prEN enquiry results known and tabled;
5. Decision on next steps for enquiry results.

NEN coordinated the work with ITERG. Part of it is the official adoption of the revision of the test methods in CEN/TC 307. ITERG has, as an active participant in the JWG, taken care of the drafting of the test methods text for all the lab studies and thus preparing the texts for CEN/TC 307. NEN, as CEN/TC 19 Secretariat, has the contacts with CEN and CEN/TC 307 to request further handling of the texts.

The first issue under this part of the project of course, was to bring in line the initiatives under BIOScopes and those already under way in CEN. Mainly that concerned the acceptance that the most important test method issues were addressed. BIOScopes partners needed to take part in the work and the envisages Round Robin work of Lot 1a (see §2.4) needed to be done. That was accepted as part of the work of the JWG.

Next to the actual test methods investigation, NEN had to initiate the work items concerning the new or revised test methods on the programme of work of CEN/TC 19 and CEN/TC 307. The official start of the standards drafting had to be accepted by the 30 Member States under CEN. The assistance of ITERG was called upon to prepare the draft texts and later to update those on comments coming from experts in WG 24.

2.6 Lot 1c: Working paper on Iodine Value and stability of FAME

The literature study work has followed the following steps:

1. Literature survey;
2. Report on state-of-the-art, especially on Iodine Value relations, including advices on how to implement the results into EN 14214, the automotive biodiesel specification.

All work has been done by the University of Graz. First report has been finalized at the end of 2006. A summary has been presented to and discussed in the CEN/TC 19/WG 24/TF FAME. Thus to support

the drafting of the specification towards oxidation stability and Iodine Value the report has been updated with new literature in 2007.

3 Results

3.1 Lot 1a: FAME test method revision and assessment

Ester content

As mentioned before, EN 14103 was regarded as unworkable from the beginning. The original method determines methyl ester and linolenic acid methyl ester contents via gas chromatography. Some improvements were tried within ring tests by one of the partners, AGQM, but they failed completely. Then the group looked at the methodology used in EN 14105 for glycerides. The GC methods were revised by the BIOScopes group and several modifications were tested in small lab studies. All of them failed.

ITERG further took up the task to optimize EN 14103. A survey study using 4 samples in 5 labs showed, that using a combination of two internal standards in conjunction with the use of theoretical response factors for the Flame Ionization Detector (FID) the gave the smallest dispersion in the results. However even these results did not allow full compliance to the "2R" rule in all cases. In view of these results from intensive and comprehensive testing and variation of all relevant parameters, it looks like there is not much probability left to accomplish the intended precision improvement.

It was therefore proposed to mark this test method as not really suitable for the determination of ester content. The method is still needed for determination of linolenic acid esters. As one alternative, the use of an HPLC/SEC (size exclusion chromatography) method was discussed, where molecules are separated by molecular size. With the probable advent of new FAME sources like animal fats, it was also recognised that all test methods using a separation mechanism will need to be checked if they can cope with the various new FAME compositions like shorter chains from coconut oil or C17 from animal fats.

Another activity to determine ester content by FTIR was undertaken within the BIOScopes group. Such an alternative method has potential for the determination of the ester content, but detects every type of C=O band and is not able to differentiate between e.g. partial glycerides, fatty acids and methyl esters. Therefore, it would be necessary to combine the measurement with a calculation step (ester content is the IR result minus contents of free fatty acid and glycerides).

The following results were obtained after the studies and the following modifications for the revision of the EN 14103 are proposed:

- To use C19-FAME as internal standard instead of C17-FAME in order to allow the analysis of animal fat biodiesel;

- To modify the sample preparation by weighting 250 mg of internal standard (methyl nonadecanoate (C19:0))and 250 mg of biodiesel sample and to dilute in 5 ml solvent (iso-octane);
- To determine the water content by Karl-Fischer of the internal standard at the reception of each lot;
- To slightly adjust the gas chromatograph and the column used (the formerly required Carbowax 20M not being available to all laboratories);
- To prefer provided standards of high purity and low water content, if possible (i.e. by Nu Chek Prep).

Finally, the conclusion of the work on the determination of the ester content is that the attempts to improve the precision of the method failed. The group was able to identify some improvements in handling and execution of the measurement which can be implemented in a revision of the standard method.

The full report on the results is given in Annex B.

Di- and triglyceride content

EN 14105 is also a gas chromatography method but after a transformation step (silylation). Modifications of the method, the sample preparation and the internal standards for calibration have been attempted and studied. Several experimental modifications for improvement of EN 14105, especially with respect to free glycerol and glycerides were undertaken. It has been found that calibration time intervals may have an influence, so better control for checking or recalibration is necessary.

One of the major problems is the calibration of the triglycerides. To improve the calibration and the ability to measure also short chain glycerides, two different triglycerides were chosen as reference substances. This enables the operator to check the efficiency of the GC column and of the apparatus. It was observed that, during the use the column shows a tendency to increase its retention activity towards triglycerides, so that the calibration coefficients increase, leading to a lower triglyceride evaluation.

Next, the use of a second control peak, i.e. TG C57 as second internal standard ("IS2") has been studied. Solubility issues for "IS2" can be resolved by changing the solvent from pyridine to THF. The feasibility for this solvent change has already been established. Preliminary RR exercises to test these modifications were executed.

Using this technique the control peak allows for each run to check the efficiency of the column, in terms of triglyceride irreversible absorption. If the obtained values are out of the control parameters, GC maintenance and a new calibration must be done. However, one of the main problems was to get a stable solution of reference substance, which now seems to be possible using a different solvent.

After several interlaboratory tests the method modification seems to be suitable and easy to carry out. The short term stability of modified standard solution has been evaluated in a positive way. The possible peaks overlap in the MG C19 monoglyceride can be easily solved or neglected. The obtained results are comparable with the ones coming from EN 14105:2003. The suggested procedure shows some advantages, as compared to the actual standard:

- Elimination of calibration process for MG, DG, TG, while it remains necessary for free glycerol evaluation;
- Solution of the problem related to GC capillary column degradation, as no calibration factors are used. In other words the internal standard for each family has the same response factor of each component belonging at the same family. As a better control and to avoid poor TG response a criteria of column acceptance based on relative response between MG, DG and TG standard will be introduced in the text;
- Precision of the determination of diglycerides could be improved significantly. Using the revised version of EN 14105 the “2R” rule can be fulfilled, being at least a partial and important success of the work;
- In terms of tri-glycerides the wished for limit can still not be specified in EN 14214 when applying the "2R" rule to the RR results.

The full report on the results is given in Annex B.

Free glycerol

The determination of free glycerol by GC was considered as a work item for the BIOSCOPES project as there is one major problem: the peak of free glycerol is overlapped by traces of Diesel fuel, and therefore the quantification is impossible in those samples. However, by now there is no solution for this problem.

Since this is not a major problem for the standardisation, and since the methods (EN 14105 and EN 14106) are suitable for use the group decided to focus on the improvement of the more relevant GC test methods.

Alkali and metals content

Soon after the start of BIOScopes it was concluded that EN 14538 (now updated to measure both Group I and Group II alkali metals) fulfils the needs. No further activities in this field were initiated by BIOScopes.

Polyunsaturated fatty acid esters

Since this concerned development of a new standard test method, a literature study has been undertaken by the University of Graz. There are several methods available to measure polyunsaturated fatty acids (PUFA), however there are no available precision data. The sensitivity of the chosen method has to be very high, since we have to measure concentrations below one percent in total, having in mind that there exists a variety of PUFAs in different ratios. The group decided to continue work on a method on the basis of AOCS (Ce 1b-89), which is very similar to EN 14103 and is a gas chromatographic method, too. It was investigated for suitable determination of esters with 4 to 5

double bonds, being the ones most present. While there is quite a number of possible isomers, many of them not being very prominent in market relevant samples like fish oil, it was proposed and accepted to confine the test method to a controlled set of the most often occurring identified and commercially available "key components".

A lab study for polyunsaturated ester methods has been further developed on the basis of work at Uni Graz. This was then further investigated via a mini round robin test in order to gain a survey of the achievable precision data. This showed that such a test method may be applicable to contents as low as 0.2 %, with the option to accomplish about 0.1 % after further optimization of the test method. A Round Robin test inside BIOScopes has been done, in which no real interference with other "saturated" fatty acid have been observed.

Some sample problems in the first exercise made the project team to arrange for a second Round Robin test with 20 participants. This RR was executed in February - March 2008. Unfortunately the statistical results were not available for this report.

FAME content in middle distillates

The determination of the contents of FAME in middle distillates like diesel and heating oil is getting more important since a lot of European countries have started blending FAME to these products. The existing test method EN 14078, based on infrared spectroscopy was working quite well, however, the precision was not determined in the concentration range below 1.7%. Additionally, the mineral oil industry was complaining about poor precision of the method in general, not allowing to blend as close to the usual blend level of 5% v/v as they desired.

A modification of the method was undertaken. The work was carried out together with a group of experts from the German mineral oil industry. The major change is measuring without sample dilution in the low concentration range (Method “A” and “B”) and the change of the solvent.

Additionally the suitability for esters of different feedstock was tested. The method did not show any problems in detecting and identifying different FAMEs. Suitability of the revised EN 14078 for low levels (< 0.1 % *v/v*) of FAME content could be demonstrated, and the overall precision of the method could be improved. The work at a whole has been carried out by the BIOScopes group in cooperation with the DIN FAM group on infrared spectroscopy.

3.2 Lot 1b: Standardization and dissemination of the FAME test results

General administration

The JWG on "Sampling and analysis of fats and oils and related products for fuel applications" between CEN/TC 19 and CEN/TC 307 has effectively been initiated in June 2006. The first meeting was on 11 September 2006 and during the project time it has had 4 meetings in total. Its scope was defined as: "To standardize sampling, test and determination methods, including establishment of

precision data, relevant to fats and oils derived ester type products to be used as, or blended into, fuels and bio-fuels for transport and stationary engines. Primarily attention is methodology related to fatty-acid methyl esters (FAME). Quality assessment of related fuel products, such as fatty acid ethyl esters (FAEE), is also incorporated. Pure fats and oils, also for fuel applications, are excluded from the work. Test methods' applicability shall be assessed for both the 100% biofuel, as-well-as for fuel blends thereof". Originally, pure vegetable oils were within the scope, but the JWG requested exclusion, which NEN had to arrange for through a ballot within both TC's.

NEN assisted the JWG with its internal procedures and work plan. Constant exchange of information between the JWG, WG 24 and the TF FAME was personalized by NEN and AGQM representatives.

Publication of the first draft (revised) standard(s)

Based on indications of the JWG and decisions in the TF FAME, NEN has initiated the following revisions:

- EN 14103:2003 under CEN/TC 307 at the end of 2006, agreed upon by resolution 9/2006;
- EN 14105:2003 under CEN/TC 307 at the end of 2006, agreed upon by resolution 9/2006;
- EN 14078:2003 under CEN/TC 19 in May 2007, agreed upon by resolution 56/2007.

EN 14538:2006 did not need to be revised (see §3.1).

Revision of EN 14112 (oxidation stability) was requested to, but rejected by CEN/TC 307 as the updates were only aimed at biodiesel (FAME) for fuel applications. Hence, CEN/TC 19 had to create a „new“ method for fuel purposes, which was accepted as a new work item by resolution 54/2007 in September 2007. The text of this so-called revised Rancimat has been sent to CEN for translation and initiation of the enquiry commenting ballot, under the number prEN 15751 (planned to be finalised on 10 April 2008).

As most of the studies were still under development nearing the end of the project (see §3.1), which means the majority of preparations on the text had to be been done quickly. The updates of EN 14103 and EN 14105 have been made by ITERG. The infra-red method update has been translated into English as the majority of work was done in Germany.

The PUFA method has been written from scratch on the basis of an existing method from AOCS. It has been accepted as a CEN/TC 19 method during a ballot at the end of 2007. Next, the text has been finalised and send to CEN for translation and further processing. The ballot on prEN 15779 has been initiated on 24 March 2008, making thus the text publicly available before the end of the project. Due to some problems with the samples in the final RR, the statistical evaluation and correct precision determination could not be finalised within the project time. Hence, the complete text was not available at the end of the project.

Revision of actual (bio)diesel specifications

The planning for this work was as follows

1. CEN/TR text on test methods study in CEN format to JWG and CEN/TC 19;

2. CEN/TC 19 decision to revise EN 14214 and EN 590 (automotive diesel fuel specification);
3. CEN/TR published.

At the end of the BIOScopes project not much more than a sketch of the CEN/TR is available due to the delays in the work of Lot 1a. It has been decided to await full finalization of the JWG work programme (which also contains work on phosphorus and density) and then produce a full report under CEN/TC 19 and CEN/TC 307.

The actual decision to revise the two fuel specifications was a point of discussion and decision at the CEN/TC 19 meeting week in May 2007. The discussion was somewhat distracted by the fact that a new Mandate by the EC for 10% FAME in diesel had been given. So much of the efforts needed to go into checking how that could be accomplished in that area. Effectively, CEN/TC 19 accepted to revise EN 14214 through a one ballot procedure at short notice. The target of the effective initiation of this so-called Unique Acceptance Procedure (UAP) ballot is April 2008, allowing publication of the revised EN 14214 before 2009.

Concerning the BIOScopes work, this UAP revision includes the requirement to use the revised Rancimat (EN 15751) and the ICP for alkaline metals (EN 14538). The revision is to make blending of FAME up to 10% in diesel fuel possible. The elements that will be included in this intermediate revision are not all those studied under BIOSCopes, because first all test method standards need to be published in their revised form. Conclusions of the project on esters, glycerides and PUFA (prEN 15779) have not yet been fully discussed in CEN/TC 19 within the timeframe of the BIOScopes project. The work on FAME detection (EN 14078) has been accepted, but the revision of the test method needs to be finalized first.

The discussion on revising EN 590 to allow the new EN 14214 FAME at 10% (B10) has for the moment been directed into a similar two-step process. A start will be made in 2008 with a UAP ballot on 7% FAME allowance on the basis of the first revision text of EN 14214, followed by a later B10 revision. The 7% has been chosen for reasons of given engine manufacturers warranties and the need to meet the 5.75 energy content percentage aspirations as laid down by the EC for 2010 and by some Member States for an earlier year. This means that the updated EN 14214 will be incorporated in the first B7 revision, but at the earliest in a 2009 publication of EN 590.

Apart from the above mentioned UAP revision, CEN/TC 19/WG 24 has accepted in its November 2007 meeting to have a next full revision once all methods and their correlation with engine functioning will be known. A more comprehensive revision of EN 14214 is thus foreseen. Discussion on an eventual change of the actual requirement (limit) for PUFA is pending, but for the first UAP revision it will remain as is. Next, the revised test methods for ester content (set as a limit for fatty acid methyl ester content), di- and tri-glycerides content need to be incorporated in the newly revised EN 14214.

Also on Iodine Value versus oxidation stability both the TF FAME and WG 24 have not yet drawn final conclusions. No consensus could be found until now on:

- Oxidation stability: Discussion on limit change to 8h for the revised EN 14112. Market data suggest that greater stability is required. Alternative methods and requirements have been proposed:
  - Use of EN ISO 12205 (the regular diesel stability test) at a higher temperature of 115°C combined with a so-called "ΔTAN" acid value detection test;
  - The "PetrOxy"

  These tests methods will be evaluated and compared with EN 15751. In 2008 a complete inter-laboratory test with all three oxidation stability determination methods will be organised;
- Iodine Value: Diverging views remain on how to treat it (even with the information from the BIOScopes project): keep as is (120), raise moderately (130), raise considerably (140) or delete and rely on oxidation stability limit. With the little experience with PUFA testing, recent field data on diesel fuel stability in Germany and recent problems with stereo-glycosols made many experts beware for too quick conclusions and changes to what is known to work.

It is expected that the above will be concluded parallel to the decisions on the full 10% FAME in diesel specification. This should follow directly after the 2009 publication of EN 14214, maybe in a 1 to 1½ year's period thereafter.

3.3 Lot 1c: Working paper on Iodine Value and stability of FAME

After evaluation of the existing data material (literature, reports, etc.) some aspects can be pre-summarized:

- Oxidation stability is the most important parameter in the context of possible problems with engine parts;
- A clear dependence between Iodine Value and oxidation stability can not be proven explicitly;
- From the current status, Iodine Value is not the parameter which sufficiently describes potential degradation (stability) problems. Linolenic acid ester content and oxidation stability will be much more significant;
- Engine and long term tests with higher Iodine Value biodiesel (up to Iodine Value 140) did no show any negative results which can be attributed to the Iodine Value. Most problems reported, are generated by bad fuel quality. However, engine problems occur if Iodine Value is significantly higher than 140 (>160). On the other hand, the question is if such biodiesel as neat product is a topic, because such fuel will fail anyway in several other quality parameters (CFPP, Ox.Stab, Linolenic ester content, and others);
- Countries which have Iodine Value excluded in their specifications did not report any problems which might be coming from Iodine Value;
- Influence of Iodine Value on other biodiesel quality parameters: no direct correlation between Iodine Value and other biodiesel parameters can be pointed out.

A detailed report is given in Annex A.

At this moment the following possible scenarios for EN 14214 have been studied and proposed to CEN/TC 19 working groups: if retained, and unchanged, the Iodine Value gives insufficient additional

information on the feedstock and the biodiesel quality (especially stability). Next, raising Iodine Value e.g. up to 140 may expect from engine experiences no significant negative effects. In fact, neat biodiesel with Iodine Value 140 will not meet EN 14214 in other parameters. Especially oxidation stability will be much more difficult to improve. Important is that the final product meets all parameters of EN 14214 (irrespective of the Iodine Value). The last option is abandonment of Iodine Value, which gives a similar scenario as described for higher Iodine Value. Furthermore, blends of very high Iodine Value oils with very low Iodine Value oils can be identified by other parameters.

However, all three scenarios are possible without impact on changing biodiesel quality. In other words, the biodiesel have to meet the EN 14214 specification limits in any case. Iodine Value alone does not guarantee quality. Currently the limits for Iodine Value exclude other feed stocks. On the other hand blending high Iodine Value oils with low ones up to total Iodine Value of 120 should also not be the solution to evade this problem. Further investigations, however, are necessary to study the influence of biodiesel with high Iodine Value on engine oil dilutions and stability of engine oil. In this field a lack of experience can be observed.

Advice on additional necessary testing (improved determination methods of oxidation stability is needed) has been forwarded to and investigated the partners in Lot 1b.

4 Results and conclusions

The BIOScopes project encountered many delays in the test methods evaluation, due to both administration (for instance late payments for the subcontractors) and technical drawbacks encountered. This has also delayed the work on drafting the test methods and preparing formal decisions on actual revision of EN 14214 and EN 590. The effective multi-laboratory Round Robin took place from November 2007 to February 2008. This means that the envisaged CEN/TR on the FAME test methods precision studies cannot be a deliverable of the BIOScopes project. However, its future publication under CEN/TC 19 is envisaged.

In total, 4 meetings of the JWG took place over the period of time of the BIOScopes projects and the concluding meeting has been planned for April 2008, with a final acceptance discussion planned for the CEN/TC 19/WG 24 meeting in May 2008.

Effectively, the work in Lot 1a and Lot 1c has been finalised under the BIOScopes project and Lot 1b supplied the basic texts as far as possible due to the technical drawbacks and discussions around the other Lots in the relevant CEN/TC's:

- Improvements of the test method for ester content could not be achieved. A good repeatability for the updated GC test method has been developed. While the results for linolenic acid methyl ester were judged to be "good to excellent", the preliminary reproducibility results for ester content were somewhat disappointing and also difficult to interpret. In the end the latest statistical evaluation of the Round Robin test showed an unacceptable precision for a minimum limit of 96.5% or higher. Possibly, this may be related to the inexperience with the test. Some improvement on the practice of the method were found. It is advisable to work out the test into a standard (revision of EN 14103) and redefine the precision in one or two years time. The draft text has been forwarded to the CEN/TC 307 for further work. The planned enquiry has not been delivered in time before the end of the project, due to the earlier indicated delays and problems. The limit in EN 14214 has been redefined as "fatty acid methyl ester content", as it is the full product without all contaminants, ethanol and water. The minimum limit has been debated but not been changed so far;
- The method for di-glycerides has been successfully updated by elimination of a calibration step and improvement of the internal calibration standards. For tri-glycerides insufficient precision has been opbserved for the actual limit in EN 14214. The text has been forwarded to the CEN/TC 307 for further work and will become a revision of EN 14105. Also here, the envisaged enquiry text has not become effective within the project timeframe;
- The study has revealed that for free glycerol no improvements could be made, but that the generally used methodology gives sufficient information to comfort the industries need;

- The test method for alkali content (EN 14538) has proven to be applicable and has been accepted for incorporation in the pending revision of EN 14214;
- The method for polyunsaturated (≥ 4 double bonds) esters has been developed and accepted as a new work item under CEN/TC 19. The study has delivered good precision data via a first internal Round Robin test. The standard text has been forwarded to CEN/CMC and is to be balloted as prEN 15779:2008. Effective publication is to be expected after the revised EN 14214 text, as the full statistical evaluation could only be done after the end of the project;
- The oxidation stability test has been greatly improved. It has been accepted as a new test method under CEN/TC 19 making it specific for fuel purposes. The text has been drafted and forwarded to CEN for a shortened enquiry ballot, which lasts until April 2008. This time frame should allow the final EN 15751 Standard to be available when the revised EN 14214 will be published at the end of 2008. The test has its limitations towards diesel containing FAME levels at less than 2%. For these cases on alternative or parallel test and requirement need to be discussed in WG 24;
- The test for determination of the contents of FAME in middle distillates has been improved. The major change is measuring without sample dilution in the low concentration range (A method "A" and "B" have been introduced) and the change of the solvent. Based on the revised method a round robin test has been executed and has shown acceptable precision data for the determination of FAME content in a wide range of concentrations. This revision will now be worked upon by CEN;
- A full study on Iodine Value versus oxidation stability and other requirements has been finalised, updated and presented to the relevant CEN groups. In parallel to the presentation of this report, the Lot 1c deliverable will be made public to CEN (WG 24 and TF FAME);
- A two-step revision of EN 14214 has been accepted, with the first aim to include the test method results concerning alkaline metals, free glycerol and oxidation stability. Next, CEN/TC 19/WG 24 has been advised to incorporate all other above indicated revised or new methods in the biodiesel specification in the near future. On relaxation or deletion of the Iodine Value requirement a discussion is still pending.

Although at the end of the study no final CEN publication can be offered, the Lot partners, looking at the many technical issues they had to take care of, conclude that they have delivered reasonable results. The use of it of course is only to be decided upon by industry and other stakeholders at the CEN/TC 19 table.

5 Recommendations

As some of the delays were not only due to technical reasons, but also because of financing questions in relation to funds, the Lot 1 partners would advise to have single financing work. This type of laboratory and pre-standardization work should be separated from other more research and literature study work. Especially if the time scales are different the attention at the start of the project is divided amongst too many issues. Lab work needs investments from the beginning, whereas researchers may have less pressing financing needs for their hours of work.

Of course, the partners advise the relevant CEN Technical Committees to accept and publish the resulting test methods for content of glycerides, polyunsaturated fatty acids and FAME, plus the renewed oxidation stability determination. Next, these standards need to be incorporated in future revisions of EN 14214 and EN 14213.

The work to develop an alternative method for the determination of ester content in FAME looks now promising. In the end, the statistical evaluation of the Round Robin test showed an unacceptable precision for a minimum limit of 96.5% ester content for the test method that resulted from our work. It is advisable for CEN/TC 307 to work out the draft test into a standard (revision of EN 14103) and redefine the precision in one or two years time. Next, CEN may decide to separately standardize the HPLC-SEC test, determine its precision and use this as the referee test method in cases of dispute, but offer the GC for day-to-day testing. Another point of discussion is if the ester content shall be limited or only reported to check for eventual fraud or process problems.

For tri-glycerides more experience with the updated procedure needs to be awaited before the wished limit can be required following the "2R" rule. The precision date will have to be evaluated statistically based on a large scale round robin test, which is also valid for the ester content.

With regards to Iodine Value an increase up to 140 g Iodine/100 g linked with an additional adjustment of oxidation stability up to 8h by EN 15751 (to be published) is recommended. Such an adjustment together with the limitations of linolenic acid as well as polyunsaturated fatty acids (a suitable method for the determination is now available) should be sufficient to open the range of feedstock for biodiesel production on the one side and to guarantee long-term engine performance.

Based on comprehensive existing experience especially with soybean oil methyl esters, concerns on problems caused by the use of higher Iodine Value biodiesel can be disproved. Further investigation, however, are necessary to study the influence of biodiesel with high Iodine Value on engine oil dilution and stability of engine oil. In this field a lack of experience can be observed.

The Lot 1 partners prefer their lab and RR work on test methods to be published in a CEN Technical Report (CEN/TR) summarizing all the work done and still going on in the JWG to update EN 14214. Officially, such has not yet been decided in CEN/TC 19.

Annex A Improvement of analytical methods

BIOScopes

Biofuels initiative: New Applications

TREN/D2-44/2005

Improvement on Standards, Co-ordination of Producers & Ethanol Studies

Final Report on BIOSCOPES Lot 1 Task a

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1 Introduction

The objective of the BIOSCOPES project (Lot 1 Task a) is to improve the test methods by standardizing the (existing) field experience and if necessary develop new methods. It is the aim to develop further the existing test procedures that way that the data of accuracy will be improved clearly. Moreover, new attempts for determination of the storage stability are to examine for its feasibility. Principles for the determination of important characteristic features of quality of mixtures are to elaborate. For the parameters “Fatty acids with more than 3 double bonds“ and “storage stability“ there are no standardized test procedures up to now. These methods have to be elaborated anew. The results will be (revised) CEN Standards and a report on the comparison of the test methods.

A total of six parameters and their actual characterization methods should be examined:

1. ester content, (EN 14103 under CEN/TC 307)
2. diglyceride content (EN 14105 under CEN/TC 307),
3. Triglyceride content (EN 14105 under CEN/TC 307),
4. Free glycerol content (EN 14105/EN 14106 under CEN/TC 307),
5. FAME content in middle distillate (EN 14 078 under CEN/TC 307) and
5. Polyunsaturated esters content.

At the start of the project it was also part of the work program to work on the ICP method for the determination of earth alkaline metals (Ca, Mg), EN 14538, with the aim to include the alkaline metals (Na+K). However, CEN TC 19 WG 27, together with a German expert group had started the work in the meantime and finished the necessary modification of EN 14538 right after the start of BIOScopes, so that no further work on this parameter needed to be executed.

The test methods for ester content was tested twice (two ring tests) during the Mandate M/245 without reaching the precision required so existing methods have reached their limits and other technical work may be needed. Some improvements were tried within ring tests by one of the partners, AGQM, but they failed completely.

Improvement is also needed for diglycerides, triglyceride and free glycerol content determination. EN 14105, which is used for the determination of glycerides seems to have worse precision in the real world than indicated in the standards. But the partners think it is possible to improve the method so it can fulfil the biodiesel specification. It may also be used to replace EN 14106 for free glycerol content determination. However, as the work on glycerides was extremely time consuming the group agreed on giving the work on free glycerol a low priority and to set the focus on the remaining tasks. Since the determination of free glycerol in the presence of diesel fuel and the resulting interference is not leading to field problems this was not regarded as a major problem.

The specification on polyunsaturated ester content was introduced in order to limit at 1 % the content of polyunsaturated FAME with more than 3 double bounds. At the time of the Mandate no test method was available for such a complicated determination in term of identification and quantification (1 % of FAME with 4 or more than 4 double bounds), so technical work is needed before any standardisation step.

This report of the working group for Lot 1a of the BIOSCOPES project includes the relevant results obtained from experimental work on the existent methods and proposals for alternative methods which have been received and evaluated by March 31st, 2008.

Responsible for the individual work items are:

Ester content (EN 14103 or substitute method) :	ITERG: application and completion of a method based on SSOG proposal
Contents of glycerol and glycerides (EN 14105) :	SSOG
FAME content in middle distillates using IR method (EN 14078) :	ASG
Content of poly-unsaturated esters (PUFA) :	University of Graz

We did not succeed in reaching all of our aims but by far most of the work could be finished successfully.

I want to thank all partners of our group for their outstanding work and expertise which assured the success of this project.
I also have to thank Ortwin Costenoble for his support as leader of lot 1.

Dr. Juergen Fischer

Workpackage leader

2 Ester Content (EN 14103 or Substitute Method)

The determination of the ester content is one of the most critical methods included in EN 14214. Since all modifications proposed and tested so far have failed, the experts of Lot 1a commonly feel that there is no chance to improve this method, and that the goal of this project should be to find a suitable and precise replacement for EN 14103.

Two methods have been proposed, and the results derived so far are described in this chapter.

2.1 Optimisation of the determination of ester content – previous experience

In order to develop an alternative method for EN 14103 standard for the determination of fatty acid methyl ester purity, trials were conducted by ITERG using size-exclusion liquid chromatography (gel permeation).

2.1.1 Method and reagent

Method

Ester content is determined after dilution of the sample in tetrahydrofurane (THF) at a concentration of 2 mg/ml, by injection of 20µl of the solution into a size-exclusion high performance liquid chromatograph using two serial columns of polystyrene-divinylbenzene (PLGEL), 300 mm of length, 7,5 mm of diameter, 100 Å of porosity and 5 µm of particle size. Elution is carried out with tetrahydrofurane with a flow rate of 0.8 ml/min. The detection is done following the difference of refractive index compared to the solvent. The calibration is carried out with solutions of methyl ester of oleic acid in THF, with a range of concentration from 0.5 to 10 mg/ml.

Samples

Six samples coming from a former ring test organised by AGQM in 2004 were used to evaluate the developed method. Results obtained by ITERG using the EN 14103 were used as reference values.

These values are the followings:

Samples	1	2	3	4	5	6
CPG ester content (%)	95.7	93.6	98.3	98.1	96.2	100.1

2.1.2 Choice of the chromatographic conditions

Different solvents were tested in order to understand the risk of co-elution between the components that may be present in the samples: glycerol (GLY), free fatty acids (AGL), sterols, monoglycerides (MG), diglycerides (DG), triglycerides (TG).

Free fatty acids and sterols are the components that present the higher risk of co-elution (figure 1). Relative retention times of these components, taking the methyl ester peak as the reference peak, are in the range 0.95 to 1.05. Surprisingly, their retention times depend on the nature of the elution solvent.

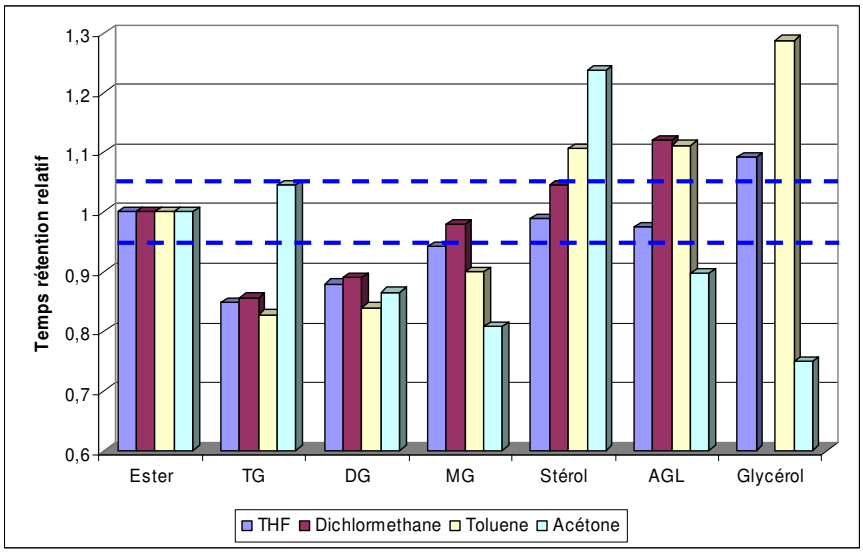

Fig. 1: Influence of the elution solvent choice on the relative retention time of the components using size-exclusion HPLC (GPC)

So the order of elution is totally modified when THF is replaced by acetone, due to a low solubility of triglycerides in acetone:

THF :	TG< DG < MG < AGL < STEROL < ESTER < GLY
Acetone :	GLY < MG < DG < AGL < ESTER < TG < STEROL

Although some of these solvents, as toluene, may present a real advantage for the separation of methyl esters from sterols and free fatty acids, we have decided to choose the Tetrahydrofurane for solubility difficulty identified when working with either acetone, dichloromethane or toluene.

An example of chromatogram obtained for a methyl ester spiked with sterols is given in figure 2. It is clearly seen in these elution conditions, that it is impossible to get a satisfying resolution between methyl esters, sterols and free fatty acids.

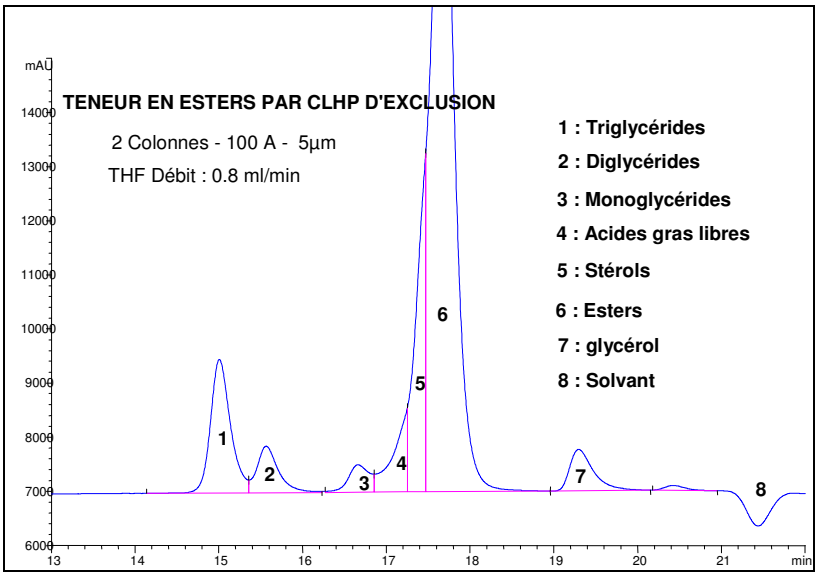

Fig. 2: Size-exclusion HPLC (GPC) chromatogram of a biodiesel sample diluted in THF and spiked with sterols

Relative retention times are comparable to the ones that were determined some years ago using three columns of 100 Å (table 1).

Tab. 1: Methyl ester content - size-exclusion HPLC (GPC) analysis of reference products

Reference products	Molecular Weight	3 columns (previous trials)		2 columns (2005)	
		Retention time (min)	Relative retention time	Retention time (min)	Relative retention time
Polymers of triglycerides	> 1800	17.4	0.68	nd	
Dimers of triglycerides	1772	20.2	0.79	nd	
Triolein	885	21.9	0.85	15.0	0.85
Diolein	621	22.7	0.88	15.5	0.88
Sterol ester	678	23.1	0.90	nd	
Dimers of methyl esters	594	23.3	0.91	nd	
Monoolein	356	24.3	0.95	16.6	0.94
Sterol	414	25.1	0.98 (ß-sitosterol)	17.5	0.99 (cholesterol)
Oleic acid	282	25.2	0.98	17.2	0.97
Methyl-esters	***296***	***25.7***	***1.00***	***17.7***	***1.00***
Glycerol	92	27.8	1.08	19.3	1.09

nd : not determined

2.1.3 Choice of the external standard

Using different standard for the calibration of the ester content determination using size-exclusion HPLC (GPC), it appears that the detector response is dependent on the chemical structure of the standard (figure 3). The saturated fatty acid methyl esters (C16 and C17) are less detected than the mono-unsaturated fatty acid methyl esters (C18:1), and mono-unsaturated fatty acid methyl esters are less detected compared to triglycerides (C18:1 TG). So it seems important to adapt the choice of the external standard to the nature of the methyl esters to be analysed.

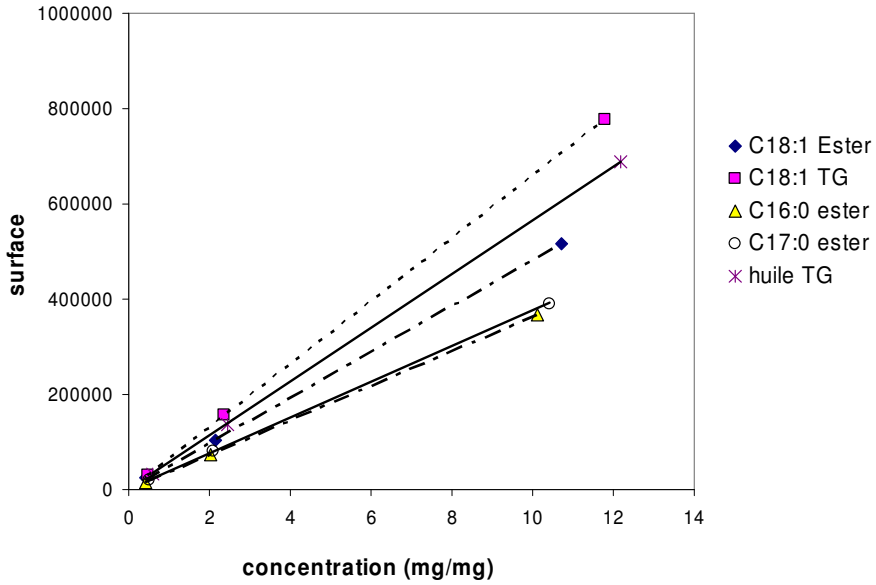

Fig. 3: Ester content determination by size-exclusion HPLC (GPC) analysis - Effect of the structure of the external standard on the refractometric detector response

In the following trials, results got with a calibration either with methyl heptadecanoate or methyl oleate were compared (figure 4).

Using methyl heptadecanoate leads to an overestimation of the ester content for the 6 samples tested, compared to their ester content obtained with GC method (EN 14103). **So, methyl oleate was chosen as the external standard for the calibration of the ester content determination by size-exclusion HPLC (GPC).**

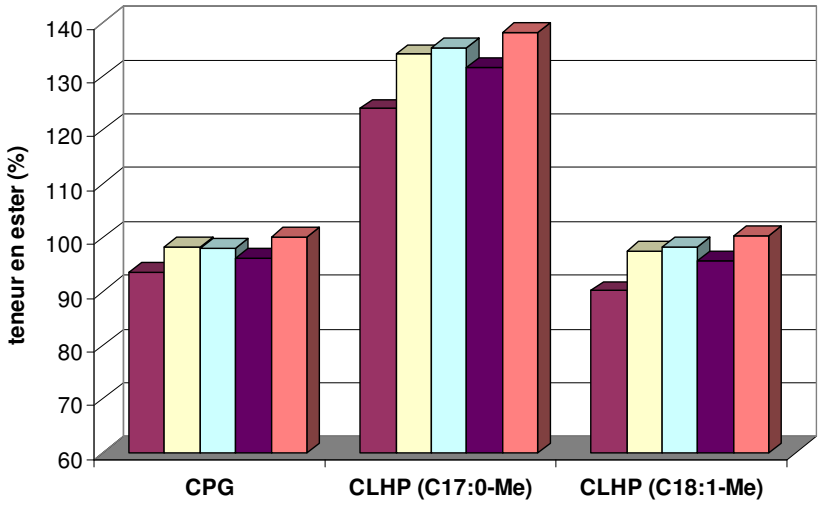

Fig. 4: Effect of the choice of the external standard on the determination of ester content determination by size-exclusion HPLC (GPC) for 6 samples of known content (dilution at 2 mg/ml in THF - 2 columns of PLGEL 100 Å) - Comparison with results obtained using the GC method (EN14103 standard)

2.1.4 Calibration method

First, calibration solutions of methyl oleate within the concentration range of 0.1 - 10 mg/ml were used (figure 5 - case A). Concentrations determined by size-exclusion HPLC (GPC) for 4 validation samples were very similar to the reference GC values. On the other hand,

samples 1 and 2 present underestimated ester content with size-exclusion HPLC method, respectively of 9 % to 4 %.

When the calibration is done spiking the samples with methyl oleate at different concentrations, at 0.5 and 1 mg/ml, results got for ester content determination are not improved (figure 5 -case B).

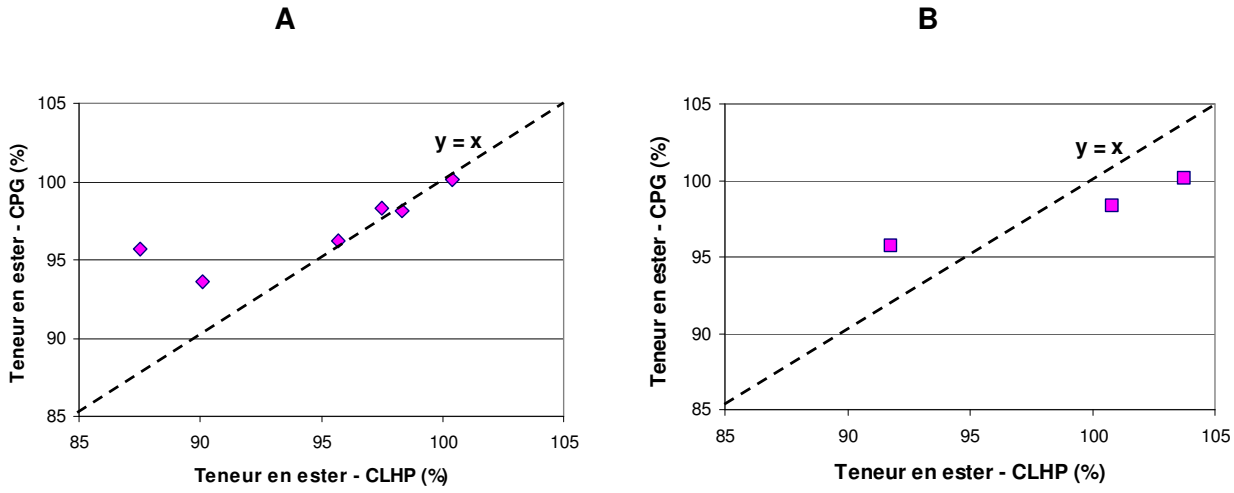

Fig. 5: Influence of the calibration mode on the ester content determined using the size-exclusion HPLC method compared to the results obtained with the GC method (EN 14103) for different biodiesel samples - A) external calibration - B) calibration with methyl oleate spiking

2.1.5 Conclusion

The ester content determination by size-exclusion HPLC (GPC) in the chromatographic conditions chosen, with 2 columns of PLGEL of 100 Å porosity and tetrahydrofuran elution, allows approaching the results obtained with the reference method EN 14103.

However, free fatty acids and sterols presence in noteworthy quantity may induce an overestimation of the ester content due to co-elution difficult to overcome.

These results show the necessity to work on the improvement of this HPLC method by trying other elution solvents (mixture of THF and toluene?) and by adjusting the external standard nature to the fatty acid composition of the biodiesel samples to be analysed.

Determination simultaneously of free fatty acid content, ester content by GC method (EN 14103) and by HPLC method for a representative number of samples, would allow assessing the accuracy of the size-exclusion HPLC method.

2.2 Optimisation of the determination of ester content by using modified EN 14105

The scope of ITERG pre-trials was to assess the feasibility of the method chosen during the first meeting of BISOCOPES- Lot 1 (27.03.06) and which protocol was written by P. Bondioli (SSOG). This method is based on the analytical conditions used in the glycerides content analysis described in the EN 14105 standard.

This document is enclosed at this report as Annex 1.

2.2.1 Samples used for ITERG trials

Samples received from University of Graz (April 2006):

- Biodiesel sample from soy oil
- Biodiesel sample from palm oil
- Biodiesel sample from used frying oil

Samples from ITERG:

- Biodiesel sample from rapeseed oil

Samples received from ADM (May 2006):

- Biodiesel samples from coconut oil and animal fat

2.2.2 Analytical conditions (SSOG method)

Column DB5 HT (10 m – 0.32 mm – 0.1 µm)

Oven temp = 50 °C hold for 1 min, programmed at 7 °C/min up to 240 °C, programmed at 15 °C/min up to 360 °C, final temperature hold for 7 min

Detector temperature = 370 °C

Remark: all the trials conducted by ITERG in April 2006 were done using a 15 m long DB5 column, due to a late arrival of the new purchased column.

2.2.3 Effect of silylation on monoglyceride elution

The injection with or without silylation is very instructive when looking at the elution of monoglyceride in figure 6:

- non silylated mono-olein is eluted before the FAME C24:1 and C24:0 (red chromatogram),
- silylated mono-olein is eluted after the FAME C24:1 and C24:0 (blue chromatogram).

The method to be developed must be simple but it must avoid the risk of confusion between the peaks to be integrated.

It seems better to keep the silylation step that will "push" the mono-olein peak away from the FAME elution zone that should be defined as ***"integration of peaks from C6-FAME up to C24-FAME"***.

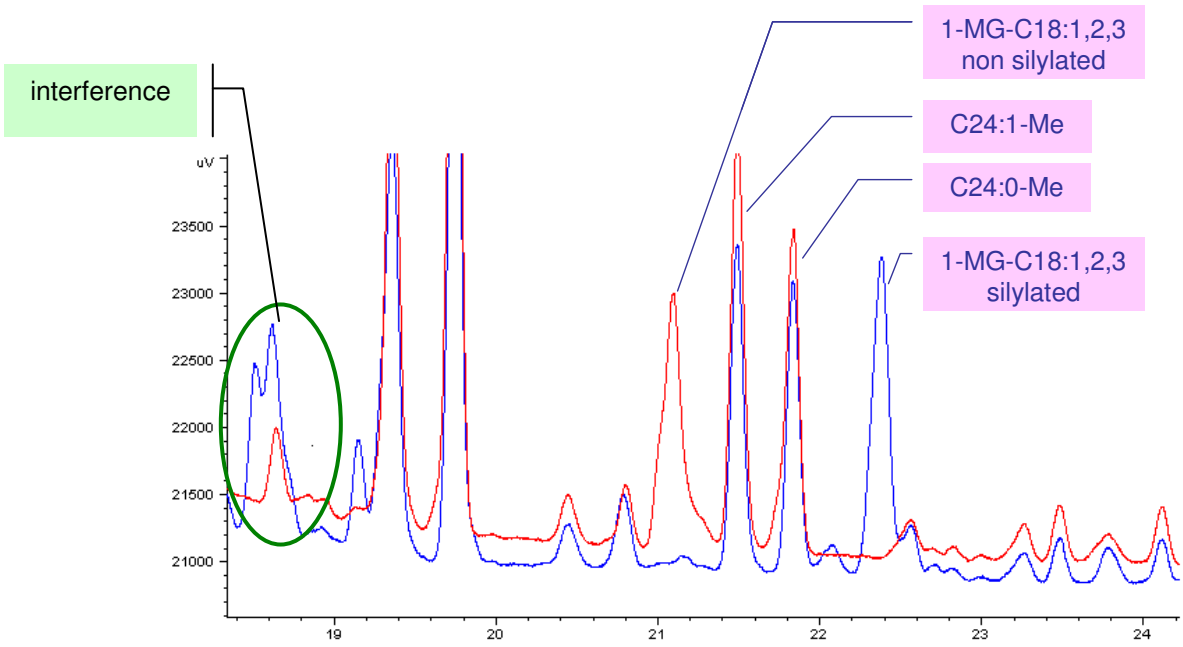

Fig. 6: Effect of silylation on monoglyceride elution for rapeseed Biodiesel (in red : rapeseed oil methyl ester non silylated - in blue : rapeseed oil methyl ester silylated)

Remark: Looking at the chromatogram obtained for the silylated rapeseed oil methyl ester, non-identified peak appears just before the elution of the C22:1 and C22 fatty acid methyl esters (green circle - blue chromatogram). This peak may have a noticeable impact on the calculation of the ester content if all the peaks eluted from C6-FAME up to C24-FAME are taking into account in the calculation, as it was the case in the previous EN 14103 method (see § 2.6. Impact of the selection of the peaks to be integrated on the ester content determination)

2.2.4 Addition of internal standard in biodiesel

The addition of C15-FAME may cover a small peak of “natural” C15-FAME present in the rapeseed biodiesel sample (figure 7).

Addition of C19-FAME in rapeseed biodiesel may also cover a small peak of “natural” C19-FAME present in the rapeseed biodiesel sample (figure 7). Compared to the addition of C15-FAME, the elution zone for C19-FAME is more risky than due to the number of small peaks eluting in the area.

The chromatograms obtained for biodiesel from soy oil, palm oil and used frying oil do also present a small peak of “natural” C15-FAME. Peak of “natural” C19-FAME” is also found in biodiesel from soy oil and used frying oil (figures 8 to 10).

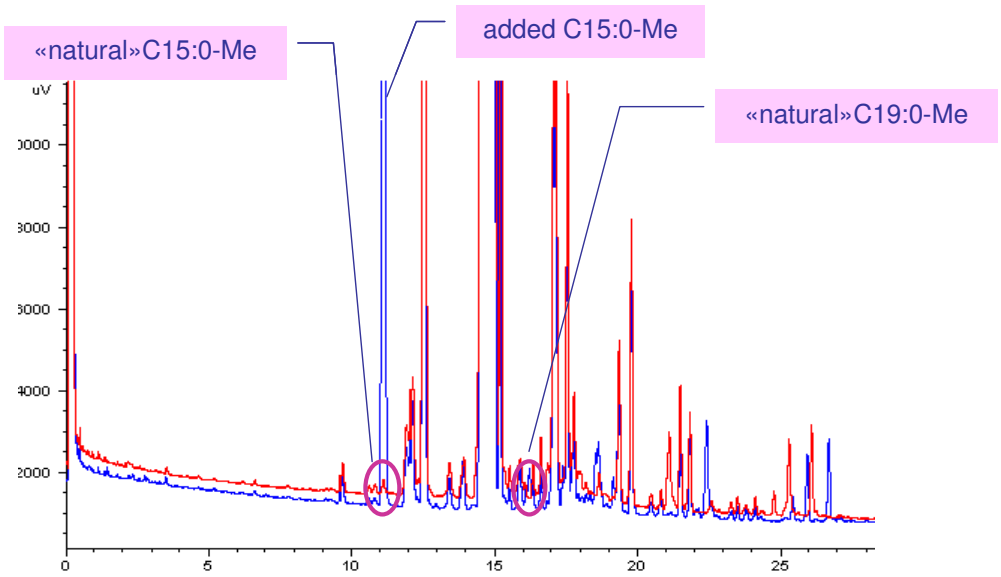

Fig. 7: Effect of the addition of C15:0-FAME elution for rapeseed Biodiesel (in red : rapeseed oil methyl ester non silylated - in blue : rapeseed oil methyl ester silylated + internal standard C15:0-FAME)

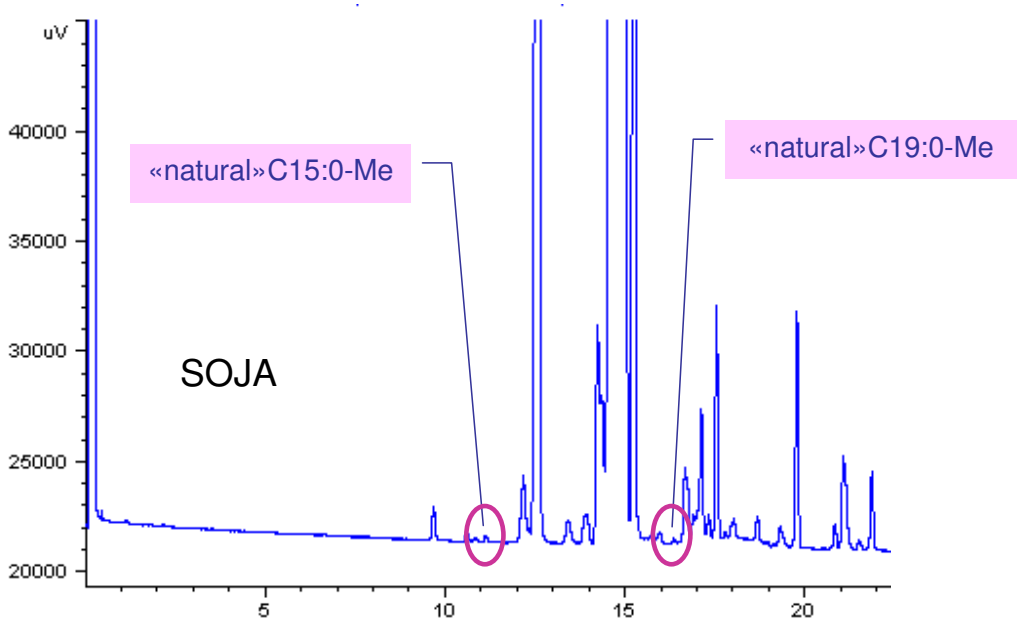

Fig. 8: FAME elution zone for biodiesel from soy oil (in blue : soy oil methyl ester non silylated without internal standard)

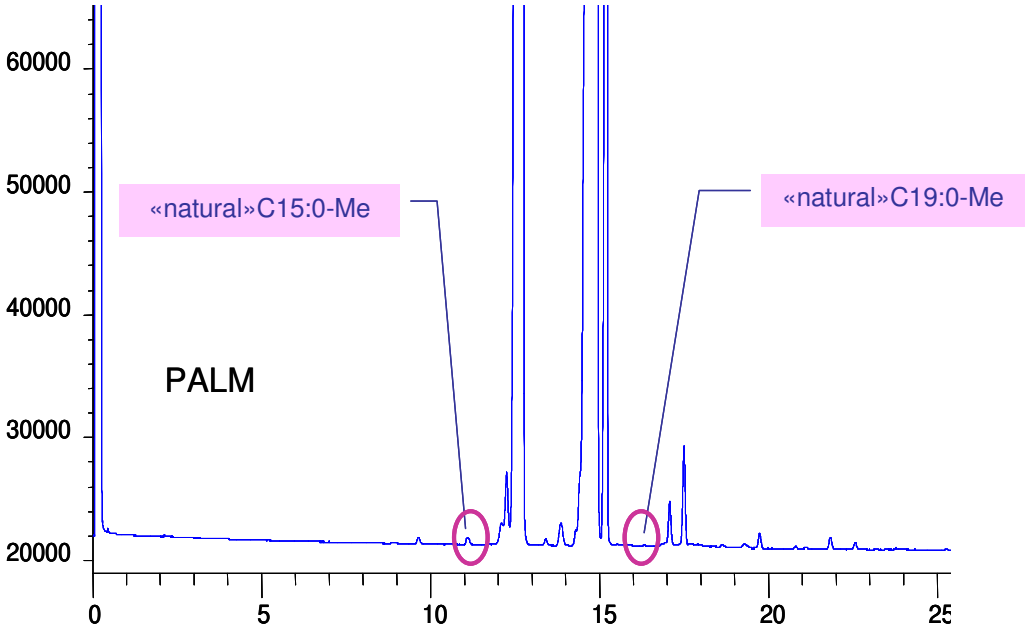

Fig. 9: FAME elution zone for biodiesel from palm oil
(in blue : palm oil methyl ester non silylated without internal standard)

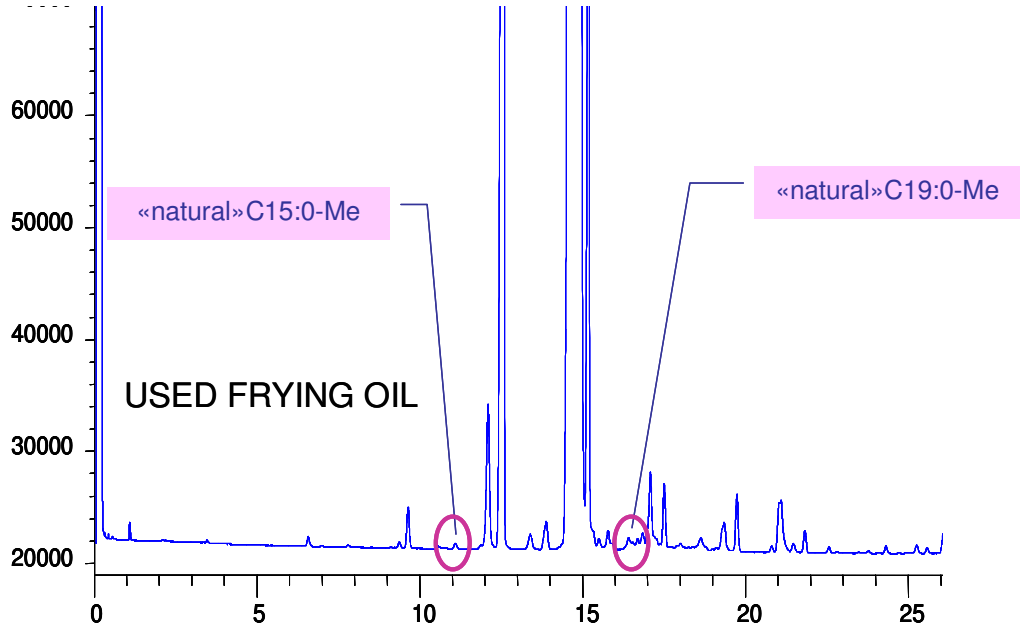

Fig. 10: FAME elution zone for biodiesel from palm oil
(in blue : used frying oil methyl ester non silylated without internal standard)

In conclusion, it seems better to choose the C15-FAME as an internal standard, even it we know that we will "mask" a peak of this FAME naturally present in very low quantities in the samples tested: palm oil FAME, soy oil FAME, rapeseed oil FAME, used frying oil FAME.

2.2.5 Calculation of the response factor of the FAME according to their chain length in our analytical conditions

The calculation was first done using the SSOG proposed method (revised method) with a 15 m long DB5 HT column (table 2)

Tab. 2: Calculation of the response factor (RF) using either C15-FAME or C19-FAME

FAME	Mass (mg)	Area	Normalised area	RF C15	RF C19
C6	53.9	169757	3149	1.52	1.66
C8	75.6	272507	3605	1.33	1.45
C14	51.8	235698	4550	1.05	1.15
C15	63.8	305124	4783	1.00	1.09
C16	62.6	309123	4938	0.97	1.06
C18	54.4	271390	4989	0.96	1.05
C19	60.9	318853	5236	0.96	1.00
C20	47.0	248692	5291	0.91	0.99
C22	46.3	248578	5369	0.89	0.97

From C6-FAME to C22-FAME we observe a large variation of the response factor using the internal standard C15-FAME, from 1.52 to 0.89 that may impact on the global FAME content for some special fat like tropical oils. **For most classical vegetable oils, the variation of the response factor is less important, from 1.05 for C14-FAME to 0.96 for C18-FAME.**

Figure 11 presents the relationship between the length of the carbon chain of the FAME and the relative response factor using C19-FAME.

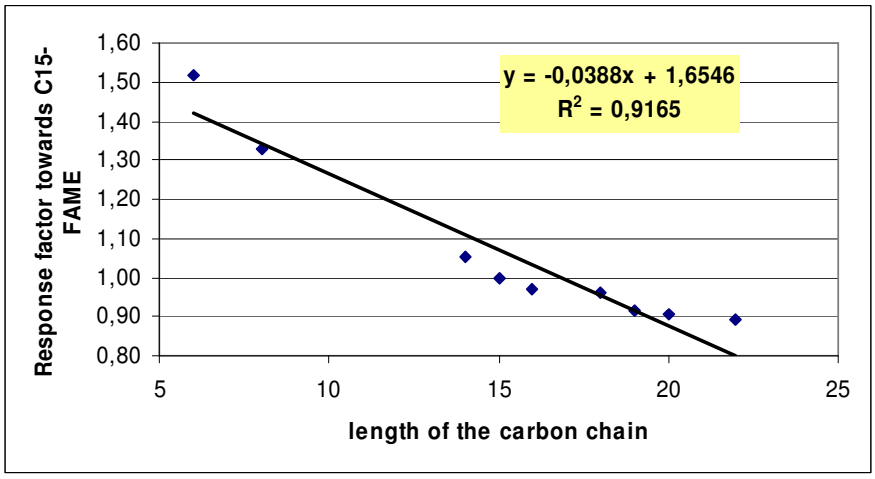

Fig. 11: Relationship between response factor and chain length of FAMEs

Although the first 5 FAMEs present a rather good correlation (figure 12), the general correlation between the response factor and the carbon chain length, from C6-FAME to C24-FAME, is not so good (R^2 = 0.916).

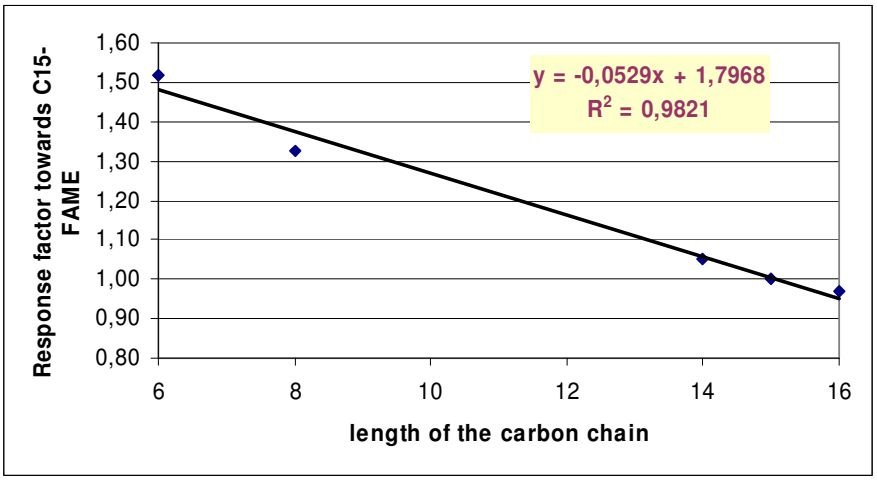

Fig. 12: Relationship between response factor and chain length of FAMEs from C6-FAME to C16-FAME

This calculation will be revisited before the end of May with the just purchased reference products covering the entire range of FAME proposed in the revised method: C6 -C8 - C10 - C12 - C14 - C16 - C18 - C20 - C22 - C24.

2.2.6 Impact of the selection of the peaks to be integrated on the ester content determination

This work was planned in order to see if the integration of all the peaks eluted from C6-FAME up to C24-FAME (former EN 14103) lead to a real different result from the one obtained when integrating[1] only the identified FAME peaks.

Different Biodiesel samples were analysed, after silylation, in presence of the internal standard C15-FAME, according to the SSOG procedure, using a 10 m long DB5 column.

Tab. 3: Impact of the selection of the peaks to be integrated on the ester content determination for different biodiesel samples

Biodiesel samples	Ester content (%) all peak integrated	Ester content (%) selected peak integrated
Rapeseed oil	102.7	99.5
Coconut oil	92.6	91.6
Palm oil	103.4	102.8
Used frying oil	100.6	99.3
Soy oil	102.9	101.4
Animal fat	not calculated	98.6

The difference between the two sets of data is between 0.6% and 3%. This difference corresponds to the possible interfering peaks of minor diglycerides.

In order to reduce the overestimation of the ester content, it is important to integrate only the identified FAME.

[1] Remark: the response factors were not used in this calculation as the reference products necessary were not arrived.

2.2.7 Utilisation of the response factor on the ester content determination

2.2.7.1 Calculation of the response factor of the FAME according to their chain length in our analytical conditions

The calculation was first done using the SSOG proposed method (revised method) with a 15 m long DB5 HT column (table 4).

Tab. 4: Calculation of the response factor (RF) using either C15-FAME or C19-FAME

FAME	Mass (mg)	Area	Normalised area	RF C15	RF C19
C6	53.9	169757	3149	1.52	1.66
C8	75.6	272507	3605	1.33	1.45
C14	51.8	235698	4550	1.05	1.15
C15	63.8	305124	4783	1.00	1.09
C16	62.6	309123	4938	0.97	1.06
C18	54.4	271390	4989	0.96	1.05
C19	60.9	318853	5236	0.91	1.00
C20	47.0	248692	5291	0.90	0.99
C22	46.3	248578	5369	0.89	0.98

This calculation was revisited at the end of May with the just purchased reference products covering the entire range of FAME proposed in the revised method: C6 -C8 - C10 - C12 - C14 - C16 - C18 - C20 - C22 - C24 and with a new 10 m long DB5 HT column (table 5).

Tab. 5: Calculation of the response factor (RF) using C15-FAME (31 may 2006)

FAME	RF 1	RF 2	Mean value	RSD	CV %
C6	nd	nd	nd	nd	nd
C8	1.351	1.354	**1.352**	0.0020	0.15%
C10	1.218	1.222	**1.220**	0.0030	0.25%
C12	1.086	1.086	**1.086**	0.0001	0.01%
C14	1.021	1.020	**1.021**	0.0011	0.11%
C15	1.000	1.000	**1.000**	0.0000	0.00%
C16	0.971	0.969	**0.970**	0.0015	0.15%
C18	0.926	0.925	**0.926**	0.0007	0.07%
C20	0.910	0.910	**0.910**	0.0001	0.02%
C22	0.897	0.898	**0.897**	0.0009	0.10%
C24	0.868	0.867	**0.868**	0.0003	0.04%

The response factors are very stable when the mixture is injected either on a 10m-long DB5 column or a 15m-long DB5 column.

2.2.7.2 Impact of the utilisation of the response factor on the ester content determination

This work was done at the end of May 2006 (table 6). The ester content results obtained using the response factors are lower than the ones calculated without the use of the response factors excepted for the biodiesel based on coconut oil, mainly due to the presence of short length fatty acids.

Tab. 6: Impact of the utilisation of the response factors on the ester content determination for different biodiesel samples

Biodiesel samples	Ester content (%) selected peak integrated without using response factors	Ester content (%) selected peak integrated using response factors
Rapeseed oil	99.5	92.3
Coconut oil	91.6	**97.6**
Palm oil	102.8	**97.2**
Used frying oil	99.3	92.6
Soy oil	101.4	94.4
Animal Fat	98.6	92.7

Compared to the specification fixed by EN 12214, only two samples are complying with the 96.5 % limit and the biodiesel samples from rapeseed, used frying oil, soy and animal fat have ester content values under 96.5%. These results seems underestimated and they should be compared with the ones obtained with the EN 14103 standardised method.

2.2.8 Results

The results obtained up to now show that:

- **it is better to analyse the FAME silylated rather than non silylated, in the chromatographic conditions given by SSOG,**
- **C15-FAME should be prefer to C19-FAME even if small traces of natural C15-FAME do exist in some biodiesel samples,**
- **it is preferable to integrate only the identified FAME peaks than to integrate all the peaks as it was done in the previous EN 14103 method, due to some non identified peaks present between C20-FAME and C22:1-FAME.**

2.3 FURTHER OPTIMISATION

2.3.1 OPTIMISATION OF ESTER CONTENT DETERMINATION EN 14103 - GC method

It was decided to verify that the existing EN 14103 was suitable for the determination of ester content for new FAME compositions like shorter chains from coconut oil or C17 from animal fats. Instead of C17-FAME as internal standard, C19-FAME was chosen in order to let the possibility to quantify C17-FAME coming from the biodiesel.

2.3.1.1 Samples used for ITERG trials

- biodiesel sample from palm oil (ring test organized by ASG on EN 14103)

- blend of biodiesel from rapeseed oil and coconut oil 90:10 (ring test organized by ASG on EN 14103)
- biodiesel sample from coconut oil 1 (ring test organized by ASG on EN 14103)
- biodiesel sample from coconut oil 2 (ADM)
- biodiesel sample from animal fat (ITERG)
- biodiesel sample from used frying oil (IFC, Graz).

2.3.1.2 Analytical conditions

2.3.1.2.1 Method 1: EN 14103 with C19-FAME as internal standard

Preparation of the sample

Methyl nonadecanoate of known purity, Nu Chek Prep, reference N-19-M

Methyl nonadecanoate 10 mg/ml solution in isooctane: accurately weigh approximately 500 mg of methyl heptadecanoate in a 50 ml volumetric flask and make up to mark with isooctane.

Accurately weigh approximately 250 mg of sample in a 10 ml vial, and then add 5 ml of methyl nonadecanoate solution using a pipette.

GC conditions

Column used: DBWAX (30 m - 0.25 mm - 0.25 µm)

Oven temp = 60°C hold for 2 min, programmed at 10°C/min up to 200°C, programmed at 5°C/min up to 240°C, final temperature hold for 7 min

Hydrogen pressure = 70 kPa

Split flow = 10 ml/min

The integration shall be carried out as from the methyl hexanoate (C_6) peak up to that of the methyl nervonate ($C_{24:1}$) taking all the peaks into consideration, including the minor ones.

2.3.1.2.2 Method 2: EN 14103 with modification of the sample preparation

According to the proposal of Paolo Bondioli and Sandro Sgro, we have decided to increase the amount of the added internal standard, C19-FAME.

Abstract of Paolo Bondioli message of July 17th 2007

Sandro Sgro observed that simply increasing the amount of IS Me C17 to be added at sample from the original 50 mg up to 250 mg, repeatability and internal reproducibility greatly increase. We did some test and we can confirm this remark. In the standard operating condition a so huge amount of added IS does not provoke FID saturation.

Preparation of the sample

Methyl nonadecanoate of known purity, Nu Chek Prep, reference N-19-M

Accurately weigh approximately in a 10 ml vial, 250 mg of sample and 250 mg of methyl nonadecanoate, then add 5 ml of isooctane using a pipette.

Vortex the solution for 1 minute.

GC conditions

Column used: DBWAX (30 m - 0.25 mm - 0.25 µm)

Oven temp = 60°C hold for 2 min, programmed at 10°C/min up to 200°C, programmed at 5°C/min up to 240°C, final temperature hold for 7 min

Hydrogen pressure = 70 KPa

Split flow = 10 ml/min

The integration shall be carried out as from the methyl hexanoate (C_6) peak up to that of the methyl nervonate ($C_{24:1}$) taking all the peaks into consideration, including the minor ones.

2.3.1.3 Assessment of the repeatability of GC method 1: EN 14103 with C19-FAME as internal standard

Five samples were analyzed in quadruplicate in order to assess the repeatability of the EN 14103 with a minor modification of the internal standard used, C17-FAME instead of C19-FAME. The suitability of the method was also evaluated for new FAME compositions like shorter chains from coconut oil or C17 from animal fats.

Tab. 7 : Assessment of the repeatability of the determination of ester content (% m/m) with GC method 1: EN 14103 with C19-FAME as internal standard - 4 replicate per sample

	22/06/2007	22/06/2007	18/07/2007	18/07/2007	MEAN	SD	RSD	2 * SD
COCO (1)	100,3	95,2	104,0	99,3	99,7	3,61	3,62%	7,23
PALM	95,9	97,0	99,7	99,2	97,9	1,8	1,80%	3,53
RAP/COCO 90:10	97,5	97,1	99,0	98,2	98,0	0,84	0,86%	1,68
ANIMAL FAT	95,9	93,7	95,7	96,2	95,4	1,14	1,20%	2,29
COCO (2)	101,1	94,6	99,4	99,5	98,6	2,81	2,85%	5,63
							moy	4,07

The repeatability was evaluated calculating the “uncertainty” that was chosen as equal to 2 times the standard deviation.

Table 7 and Figure 13 show that the widest variation of the results is obtained for coconut biodiesel samples. The mean value for the repeatability for the 5 samples studied is 4,1 % (m/m) with method 1.

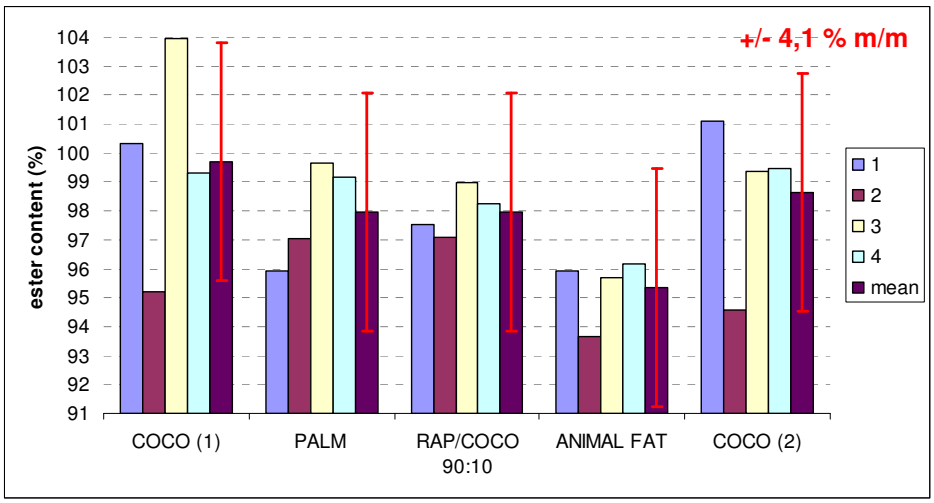

Figure 13: Assessment of the repeatability of the determination of ester content (% m/m) with GC method 1: EN 14103 with C19-FAME as internal standard - 4 replicate per sample

2.3.1.4 Assessment of the repeatability of GC method 2: EN 14103 with modification of the sample preparation

Five samples were analyzed in triplicate in order to assess the repeatability of the EN 14103 with two modifications:

- Modification of the internal standard used, C17-FAME instead of C19-FAME,

- Modification of the sample preparation, weighting an equal amount of sample and internal standard.

The suitability of the method was also evaluated for new FAME compositions like shorter chains from coconut oil or C17 from animal fats.

Tab. 8: Assessment of the repeatability of the determination of ester content (% m/m) with GC method 2: EN 14103 with modification of the sample preparation - 3 replicate per sample, 2 injections

	27/07/2007	27/07/2007	27/07/2007	27/07/2007	27/07/2007	27/07/2007	MEAN	ET	CV	2 * ET
PALM	97,84	98,35	98,94	97,95	98,00	97,33	98,1	0,54	0,55%	1,08
RAP/COCO 90:10	97,23	97,07	97,05	97,07	96,79	96,91	97,0	0,47	0,16%	0,95
ANIMAL FAT	96,25	96,00	95,83	96,82	97,04	96,56	96,4	0,47	0,49%	0,95
COCO (2)	97,77	95,17	95,35	95,80	95,78	95,87	96,0	0,93	0,97%	1,86
UFO	90,82	90,98	90,88	90,65	90,45	90,82	90,8	0,19	0,21%	0,38
									moy	1,04

The repeatability was evaluated calculating the “uncertainty” that was chosen as equal to 2 times the standard deviation.

Table 8 and Figure 14 show that the variation of the results is decreased with method 2 compared to those obtained with method 1. The mean value for the repeatability for the 5 samples studied is 1,0 % (m/m) with method 2.

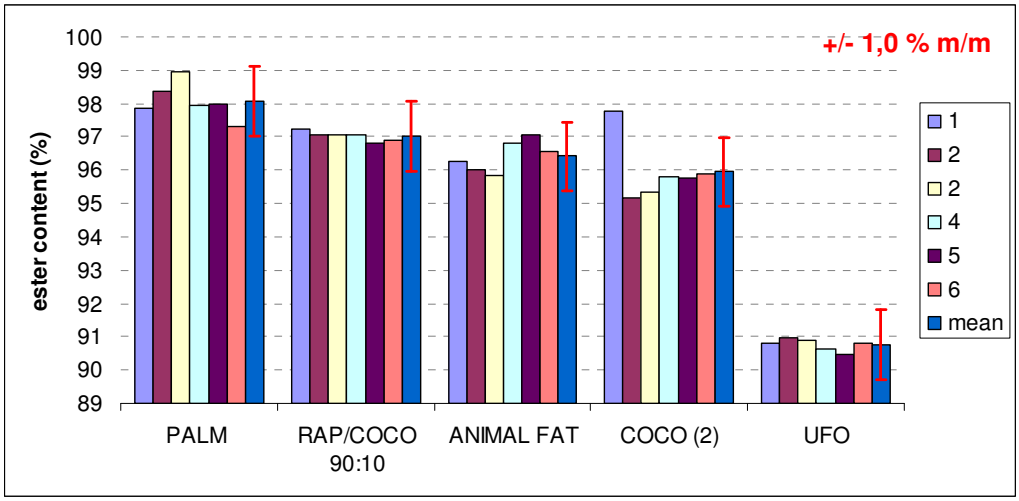

Fig 14: Assessment of the repeatability of the determination of ester content (% m/m) with GC method 2: EN 14103 with modification of the sample preparation - 3 replicate per sample, 2 injections

2.3.1.5 Modification of EN 14103

By increasing the content of the internal standard added to the biodiesel sample, the precision of ester content determination by GC was increased in the executed pre-tests. The repeatability calculated as equal to 2 times the standard deviation was between 0,4 % and 1,9 % m/m when 250 mg of C19-FAME is used as internal standard, whereas the repeatability was between 1,7 % and 7,2 % when only 50 mg of C19-FAME is used in a solution.

As a result of these investigations, the following modifications for EN 14103 were proposed:

- to use C19-FAME as internal standard instead of C17-FAME in order to allow the analysis of animal fat biodiesel,
- to modify the sample preparation by weighting 250 mg of internal standard and 250 mg of biodiesel sample and to dilute in 5 ml solvent,
- to determine the water content by Karl-Fischer of the internal standard at the reception of each lot,
- to prefer standards provided by Nu Chek Prep[2] for their purity and low content of water, when it is possible.

2.3.1.6 Round Robin Test using the modified EN 14103 for the Determination of Ester Content and Linolenic Methyl Ester Content

Finally, in January 2008 a round robin test was executed with the modified version of EN 14103. Six different samples were tested by 17 participants in different labs in Austria, France, Germany, Italy and Spain. The following methyl esters were tested:

- Rapeseed oil ME (2)
- Soybean oil ME
- Palm oil ME
- Rapeseed oil ME / Soybean oil ME
- Soybean oil ME / Coconut oil ME

The ring test was organised by ASG, the results were collected by ITERG. The statistical evaluation was executed by ITERG, additional calculation will be done by DIN/FAM. Unfortunately it was impossible to include these results in this report.

Figure 15 shows the raw data obtained from the measurement of the samples. Some of the labs reported results constantly above or below the mean values; however, due to the wide spread of the values only a few results could be eliminated as outliers (Table 9).

[2] http://www.nu-chekprep.com/

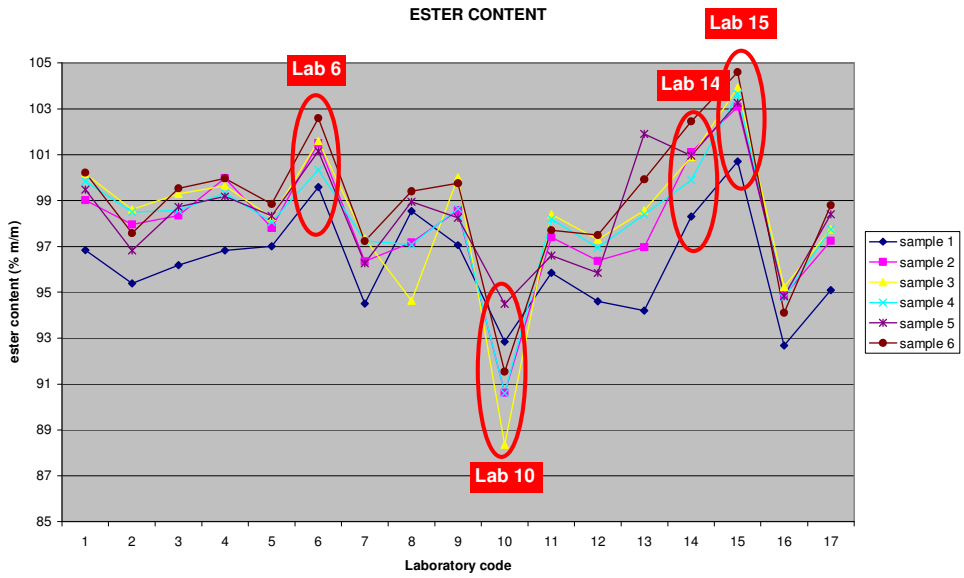

Fig 15: Raw data obtained from the Round Robin Test on ester content with the modified EN 14103

Table 9: Statistical evaluation of the round robin test on ester content and linolenic methyl ester content

EN 14103 Ester content	1	2	3	4	5	6	
Number of participating laboratories (P)	17	17	17	17	17	17	
Number of laboratories retained after eliminating outliers (p)	16	16	14	15	15	16	
Number of test results in all labs (n)	32	32	28	30	30	32	
Mean (m)	96,46	98,36	99,16	98,57	98,21	99,39	
Repeatability standard deviation (sr)	0,37	0,30	0,34	0,35	0,19	0,36	
Repeatability relative standard deviation (RSDr)	0,4	0,3	0,3	0,4	0,2	0,4	
Repeatability limit (r)	1,04	0,84	0,96	0,99	0,53	1,02	0,90
Reproducibility standard deviation (sR)	2,12	2,16	2,17	2,00	2,45	2,48	
Reproducibility relative standard deviation (RSDR)	2,2	2,2	2,2	2,0	2,5	2,5	
Reproducibility limit (R)	5,94	6,04	6,07	5,61	6,87	6,94	6,24

EN 14103 Linolenic FAME content	1	2	3	4	5	6	
Number of participating laboratories (P)	17	17	17	17	17	16	
Number of laboratories retained after eliminating outliers (p)	16	16	16	15	14	13	
Number of test results in all labs (n)	32	32	32	30	28	26	
Mean (m)	8,82	8,20	6,26	6,79	7,47	0,24	
Repeatability standard deviation (sr)	0,10	0,05	0,11	0,07	0,02	0,18	
Repeatability relative standard deviation (RSDr)	1,1	0,6	1,7	1,0	0,2	74,5	
Repeatability limit (r)	0,27	0,14	0,30	0,20	0,05	0,49	0,19
Reproducibility standard deviation (sR)	0,34	0,33	0,31	0,32	0,30	0,16	

Reproducibility relative standard deviation (RSDR)	3,8	4,0	5,0	4,8	4,1	69,8	
Reproducibility limit (R)	0,94	0,92	0,87	0,91	0,85	0,46	0,90

The plot of the reproducibility and the repeatability against the mean values (Fig. 16a, 16b) show that for the ester content the mean value of the precision data is acceptable.

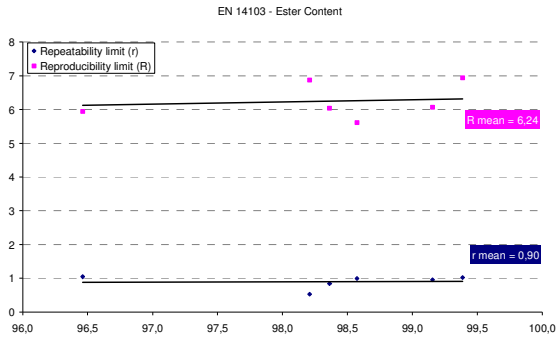

Figure 16a: Ester content, plot of precision values against mean values

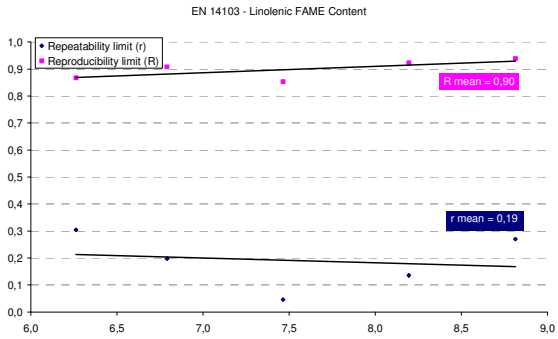

Figure 16b: Linolenic acid methyl ester content, plot of precision values against mean values

Compared to the precision data of the original method EN 14103, generally spoken the results of the ring test show no improvement. Table 10 compares the precision data of the method in use with the revised version.

Table 10: Comparison of precision data

Ester content

	EN 14103 (original)	EN 14103 (modified)
Repeatability	1,6 % (m/m)	0,90 % (m/m)
Reproducibility	3,1 % (m/m)	6,24 % (m/m)

Linolenic Acid Methyl Ester Content

	EN 14103 (original)	EN 14103 (modified)
Repeatability	0,1 % (m/m)	0,19 % (m/m)
Reproducibility	0,311xL+0,02 % (m/m)	0,90 % (m/m)

Unfortunately there is no clear result. While for the determination of the ester content the repeatability could be improved significantly proving that the handling of the procedure is enhanced, the reproducibility is far beyond the precision which is required for a standard test method.

The statistical evaluation of the linolenic acid methyl ester determination gives exactly the opposite result: the repeatability is worse than in EN 14103, while the reproducibility is considerably better.

During the pre-tests the water content of the standard substances was identified as a cause for the bad precision; however, due to the fact that only a few participants of the ring test determined the water content this presumption could neither be confirmed nor disproved yet.

2.3.1.7 Conclusion on the determination of ester by GC methods

After several attempts to improve the precision of the existing method (EN 14103) and to develop new methods we have to state finally that we found some partial results indicating potential opportunities to reach the target; however, the overall result is that all attempts failed. Neither changing the existing method for ester content nor modifying EN 14105 showed the desired effect, though there is still a little hope for EN 14103.

2.3.2 OPTIMISATION OF ESTER CONTENT DETERMINATION EN 14103 - HPLC-SEC method

2.3.2.1 Samples used for ITERG trials

- Biodiesel sample from palm oil (ring test organized by ASG on EN 14103)
- Blend of biodiesel from rapeseed oil and coconut oil 90:10 (ring test organized by ASG on EN 14103)
- Biodiesel sample from coconut oil 1 (ring test organized by ASG on EN 14103)
- Biodiesel sample from coconut oil 2 (ADM)
- Biodiesel sample from animal fat (ITERG)
- Biodiesel sample from used frying oil (IFC, Graz)
- Biodiesel sample from rapeseed oil (ITERG).

2.3.2.2 Analytical conditions

HPLC-SEC Method

Ester content is determined after dilution of the sample in tetrahydrofuran (THF) at a concentration of 2 mg/ml or 5 mg/ml, by injection of 20 µl of the solution into a size-exclusion high performance liquid chromatograph using 2 or 3 serial columns of polystyrene-divinylbenzene (PLGEL), 300 mm of length, 7,5 mm of diameter, 100 Å of porosity and 5 µm of particle size. The columns are heated at 30°C. Elution is carried out with tetrahydrofuran with a flow rate of 1 ml/min. The detection is done following the difference of refractive index compared to the solvent.

External calibration

The calibration is carried out with reference solutions of methyl ester in THF, at a concentration of 2 mg/ml. The calibration factor for the Cx-FAME is calculated as:

$$\mathbf{CF_{Cx} = Peak\ area/(Cx\text{-}FAME\ concentration)}$$

According to the fatty acid methyl ester composition of the biodiesel samples, various pure FAME standards were bought (Nu Check Prep) and studied: C12-FAME, C16-FAME, C18-FAME, C18:1-FAME.

Quantification 1: external calibration

The ester content was calculated using only one FAME reference according to the following formula:

$$\text{Ester content (\% m/m)} = \frac{\Sigma \text{ Peak area}}{CF_{Cx} \times \text{Sample concentration}}$$

For the analyzed biodiesel samples, all the peaks eluted between C12-FAME and C18:1-FAME were considered for the ester content determination.

Quantification 2: internal normalization

It is assumed that the whole of the components are represented on the chromatogram, so that the total of the areas under the peaks represents 100 % of the constituents.

It is also assumed that all the components have the same response factor towards refractometric detector.

For the analyzed biodiesel samples, all the peaks eluted between C12-FAME and C18:1-FAME were considered for the ester content determination.

2.3.2.3 Calibration factors obtained using various FAME standards

According to the chain length and the insaturation, the calibration factor is different (table 9).

Tab. 9: HPLC-SEC method for ester content determination - Calibration factors for various FAME standards - 3 serial PLGEL columns - dilution to 2 mg/ml - 2 injections

Standard	Retention time (min)	Relative RT/ C18 :1	Calibration Factor CF_{Cx}
C8:0	22,5	1,11	not done
C12:0	21,4	1,06	23533
C14:0	20,9	1,03	not done
C16:0	20,4	1,01	30020
C18:0	20,0	0,99	32971
C18:1	20,2	1,00	40845
C18:2	20,4	1,01	48375

The longer the chain of the FAME is, the higher the signal is. The insaturation increases also the refractometric signal.

The retention time depends also on the chain length and for some biodiesel samples, ester content may represent several HPLC peaks when the chain length of the FAME is not homogeneous (Annex 1).

2.3.2.4 Quantification of ester content for some biodiesel samples in HPLC-SEC using an external calibration

Using the calibration factors calculated previously (§ 2.3), some biodiesel samples were analyzed. The results were compared to the ester content determined by GC previously (§ 1.3).

The effect of the standard used for the external calibration is tremendous (table 10 and figure 15).

Tab. 10: HPLC-SEC method for ester content determination - Effect of the calibration on the ester content - 3 serial PLGEL columns - dilution to 2 mg/ml - 2 or 3 replicate per sample

	ESTER CONTENT CALCULATED USING THE EXTERNAL CALIBRATION WITH STANDARD FAME :					
SAMPLES	Ester content (% m/m)	C12	C16	C18	C18:1	C18:2
COCO (1)	trial 1	101,8	79,8	72,6	58,6	49,5
	trial 2	108,0	84,7	77,1	62,2	52,6
	mean	104,9	82,2	74,9	60,4	51,0
	2 * SD	8,9	6,9	6,3	5,1	4,3
	RSD (%)	4,22%				
PALM	trial 1	155,2	121,6	110,8	89,4	75,5
	trial 2	159,4	125,0	113,8	91,9	77,6
	mean	157,3	123,3	112,3	90,6	76,5
	2 * SD	6,0	4,7	4,3	3,5	2,9
	RSD (%)	1,92%				
RAP/COCO 90:10	trial 1	178,7	140,1	127,6	103,0	86,9
	trial 2	180,8	141,7	129,0	104,1	87,9
	mean	179,7	140,9	128,3	103,6	87,4
	2 * SD	2,9	2,3	2,1	1,7	1,4
	RSD (%)	0,81%				
COCO (2)	trial 1	100,8	79,0	72,0	58,1	49,0
	trial 2	104,0	81,5	74,2	59,9	50,6
	trial 3	104,9	82,2	74,8	60,4	51,0
	mean	103,2	80,9	73,7	59,5	50,2
	2 * SD	1,2	1,0	0,9	0,7	0,6
	RSD (%)	2,06%				
ANIMAL FAT	trial 1	161,7	126,8	115,4	93,2	78,7
	trial 2	162,0	127,0	115,6	93,3	78,8
	trial 3	161,6	126,7	115,4	93,1	78,6
	mean	161,8	126,8	115,5	93,2	78,7
	2 * SD	0,5	0,4	0,4	0,3	0,2
	RSD (%)	0,12%				
RAP	trial 1	180,7	141,6	129,0	104,1	87,9
	trial 2	176,7	138,5	126,1	101,8	86,0
	mean	178,7	140,1	127,5	103,0	86,9
	2 * SD	5,6	4,4	4,0	3,2	2,7
	RSD (%)	1,58%				

For the rapeseed/coco 90:10 biodiesel sample, the ester content varies from 87 % to 180 %, whether the external calibration is carried out with a C18:2-FAME or a C12:0-FAME solution.

It is a confirmation that we need to adjust the choice of the standard used for the calibration according to the composition of the FAME contained in the biodiesel sample.

Figure 5, comparing the ester content measured by HPLC-SEC and GC method 1, facilitates the choice of the calibrating FAME.

For ester content determination in coco biodiesel, C12-FAME is recommended.

C18:1-FAME is recommended as calibrating solution for the quantification of ester content in palm, rapeseed/coco blend, and animal fat biodiesel samples.

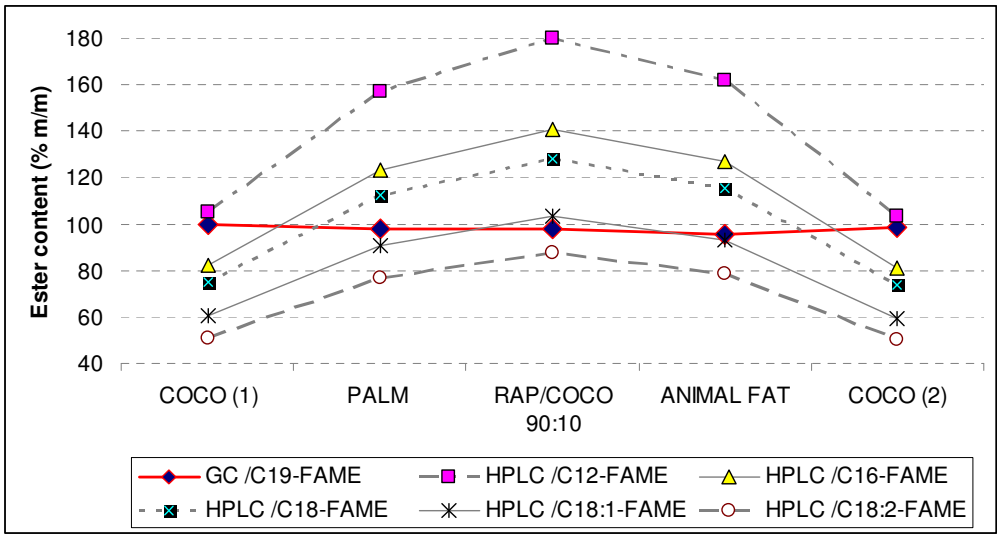

Fig. 15: HPLC-SEC method for ester content determination - Effect of the calibration on the ester content - 3 serial PLGEL columns - dilution to 2 mg/ml - 2 or 3 replicate per sample

The repeatability was evaluated calculating the “uncertainty” that was chosen as equal to 2 times the standard deviation. The mean value for the repeatability for the 6 samples studied is 3,0 % (m/m) with the external calibration.

Taking into account the repeatability, which is rather high, it seems that results obtained by GC or by HPLC-SEC are comparable (figure 16).

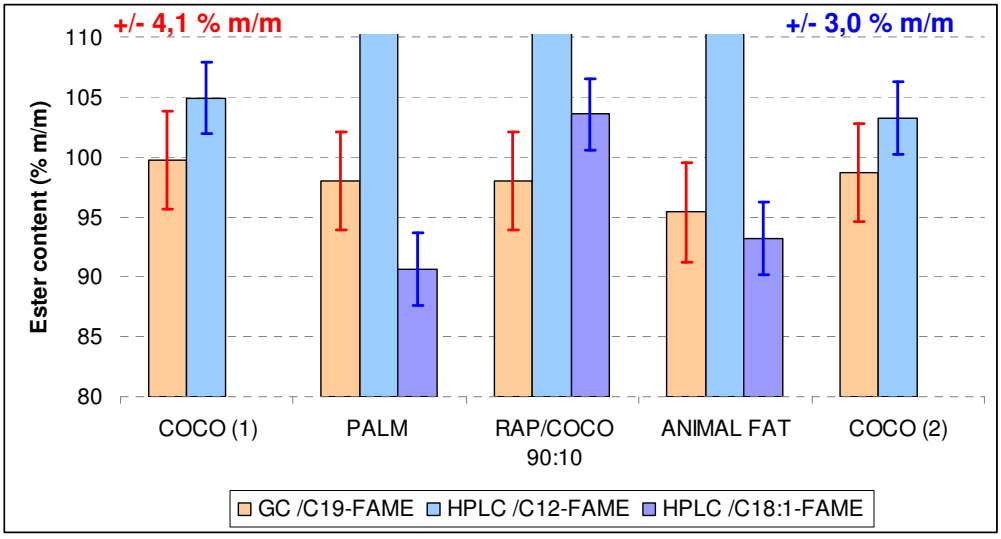

Fig. 16: HPLC-SEC method for ester content - Comparison of the results obtained with the calibration with C12-FAME and C18:1-FAME to the GC results - 3 serial PLGEL columns - dilution to 2 mg/ml - 2 or 3 replicate per sample

However, the repeatability is not improved compared to the current EN 14103 method.

So we decided to let down external calibration and to assess internal standardization as we can easily assume that all the components present in the biodiesel samples are eluted on the column.

2.3.2.5 *Quantification of ester content for some biodiesel samples in HPLC-SEC using internal normalization*

Two biodiesel samples were analyzed in quadruplicate in order to assess the repeatability of the HPLC-SEC method using internal normalization. These samples had been previously analyzed by GC, as a reference method. Table 11 and figure 17 present these results.

Tab. 11: HPLC-SEC method for ester content - Quantification with internal normalization – 2 serial PLGEL columns - dilution to 5 mg/ml - 4 replicate per sample, 2 injections

Sample	Trial	inj 1	inj 2	mean	SD	2 * SD	RSD
UFO	1	90,59	91,52	91,25	0,37	0,74	0,41%
	2	91,05	91,41				
	3	90,96	91,33				
	4	91,78	91,40				
COCO (2)	5	97,40	97,44	97,48	0,05	0,10	0,05%
	6	97,44	97,49				
	7	97,49	97,47				
	8	97,53	97,54				

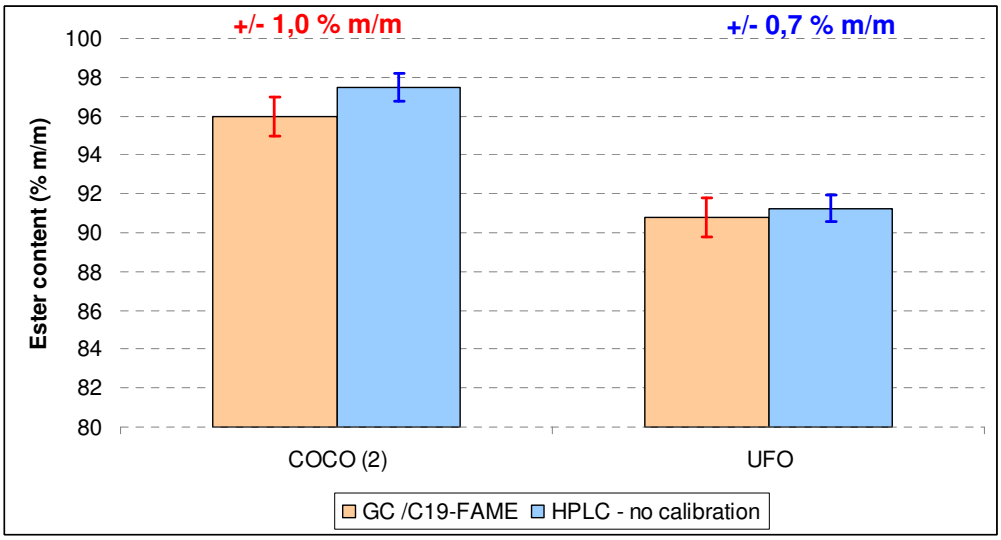

Fig. 17: HPLC-SEC method for ester content - Comparison of the results obtained with internal normalization to the GC results - 2 serial PLGEL columns - dilution to 5 mg/ml - 4 replicate per sample, 2 injections

Repeatability, calculated as equal to 2 times the standard deviation, is very low for both samples, under 0,8 % (m/m). But, this precision should be confirmed with more samples, as only 2 samples were analyzed.

Comparison of the HPLC-SEC results to the GC results for both samples is correct (figure 17).

So it seems that determination of ester content with HPLC-SEC and internal normalization gives similar results with GC determination, with a good repeatability.

2.3.2.6 Conclusion on ester content determination by HPLC-SEC method

We have confirmed that an external calibration does not lead to repeatable results for the determination of ester content by HPLC-SEC, and that internal normalization is much more precise.

The repeatability calculated as equal to 2 times the standard deviation is lower than 0,8 % when internal normalization is used, whereas the repeatability is between 0,2 % and 8,9 % when external calibration is used.

If the work on HPLC-SEC with internal normalization is to be pursued, the following step should consists on analyzing at least 10 other samples to verify its concordance with GC results and its repeatability.

If the results obtained here are confirmed, we could say that HPLC-SEC and GC revised method are enough precise to be proposed to the European standardization.

2.3.3 Special Annex: HPLC-SEC Chromatograms

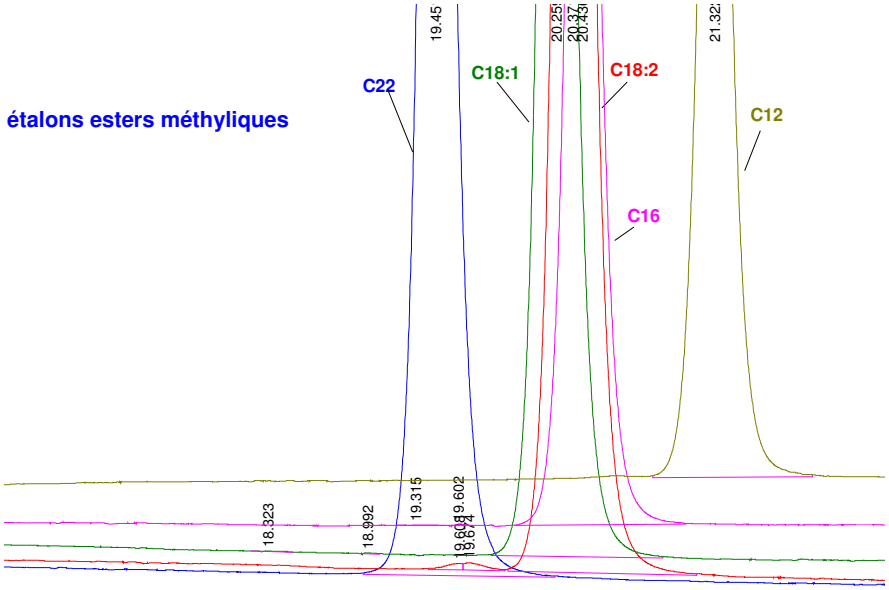

Fig 18: HPLC-SEC method for ester content determination - Chromatogram of FAME standard solutions at 2 mg/ml - 3 serial PLGEL columns

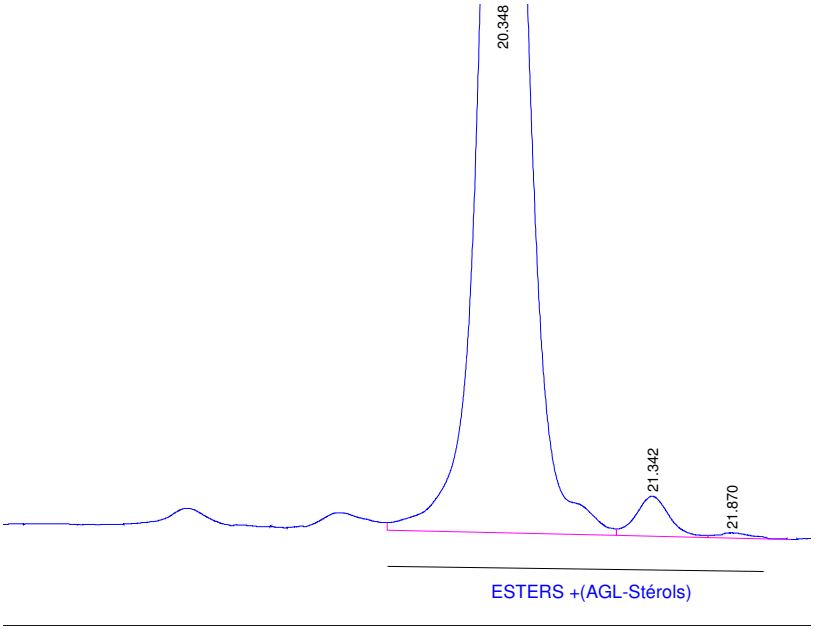

Fig 19: HPLC-SEC method for ester content determination - Chromatogram of a "rapeseed +coco" biodiesel sample diluted at 2 mg/ml - 3 serial PLGEL columns

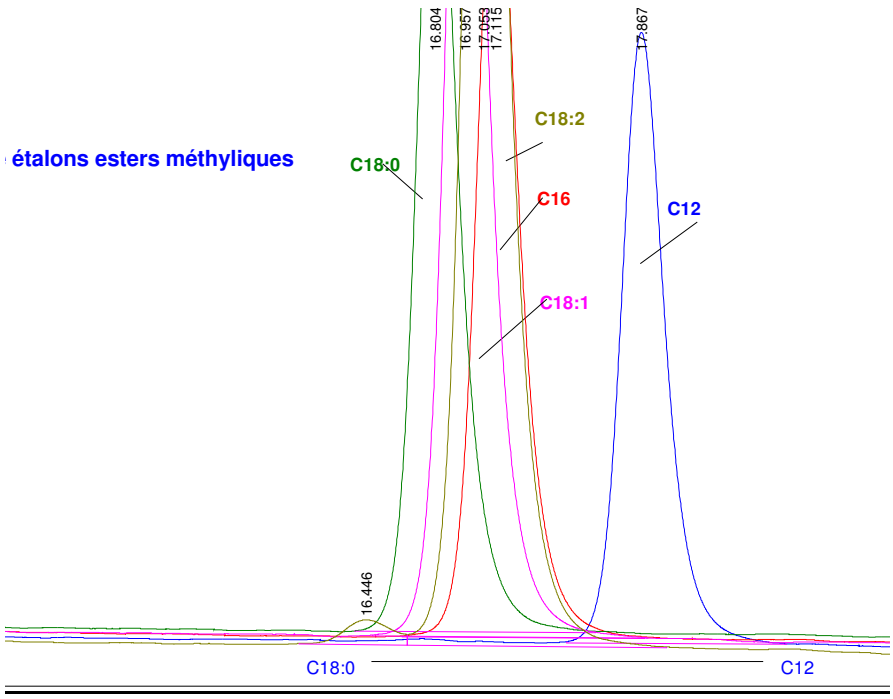

Fig 20: HPLC-SEC method for ester content determination - Chromatogram of FAME standard solutions at 2 mg/ml - 2 serial PLGEL columns

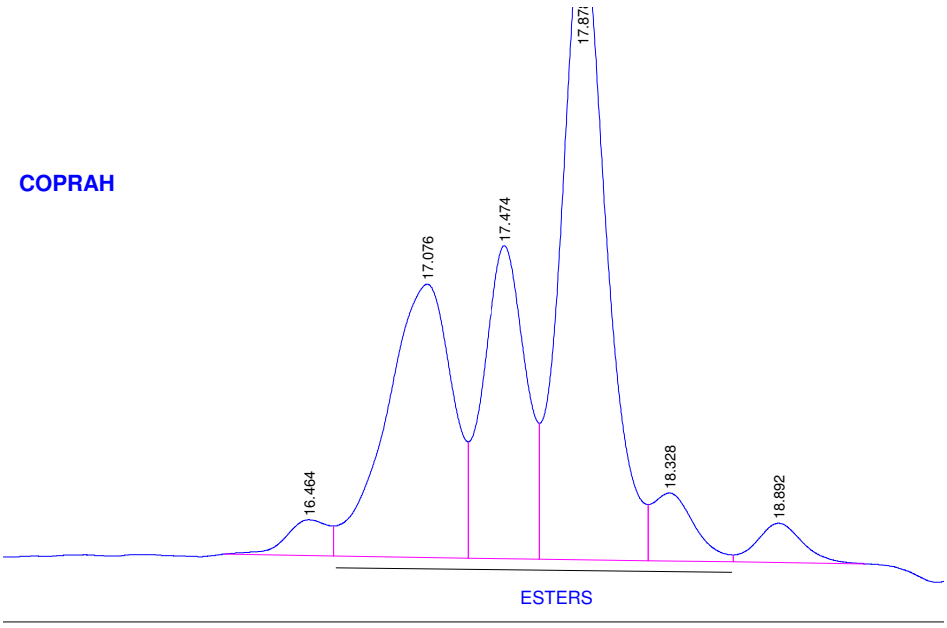

Fig. 21: HPLC-SEC method for ester content determination - Chromatogram of a coco biodiesel sample diluted at 5 mg/ml - 2 serial PLGEL columns

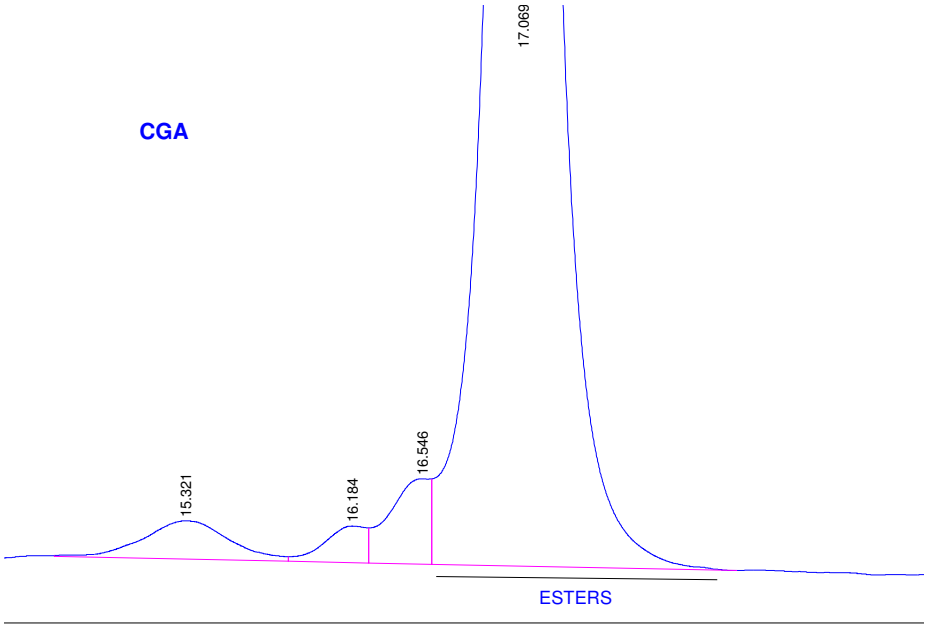

Fig. 22: HPLC-SEC method for ester content determination - Chromatogram of an animal fat biodiesel sample diluted at 5 mg/ml - 2 serial PLGEL columns

3 Content of Glycerol and Glycerides (EN 14105)

3.1 FOREWORD

In this report the activity carried out within the BIOSCOPES project by the SSOG team is reported.

During the period January –March 2006 a preparatory activity, concerning of set-up of the analytical system, bibliography review and some preliminary test has been carried out. This activity is not discussed here in detail.

- On the contrary the activity planned after the Berlin meeting of day March ,27th is reported in detail and organised in the following sections:
- Preparation of draft for a modified EN 14103 method;
- Preparation of draft for a modified EN 14105 method;
- Experimental activity to verify the practical suitability of the modifications proposed for EN 14105.

3.2 DRAFT OF MODIFIED EN 14105

Some days after the Berlin meeting, the draft of the modified EN 14105 was delivered to the other partners for their consideration. In this case only few minor modifications were necessary and as a consequence the document does not consist of a complete procedure but only of the suggested modifications and improvements, along with the necessary indication for a proper location into the original text. After the experimental activity carried out in SSOG Institute some amendments and some additions were necessary, leading to the preparation of Version 1, enclosed at this report as Annex 2. This is the first dissemination of this document.

3.3 EXPERIMENTAL ACTIVITY ON MODIFIED EN 14105

The experimental activity agreed during the Berlin meeting and here reported consists on the introduction of a control peak in triglyceride zone, which is eluted after the peaks naturally present in Biodiesel samples to introduce an objective criterion to continuously evaluate the behaviour of GC column in terms of triglyceride irreversible absorption.

It is well known that the GC column used for this method continuously degrades during use, leading to a variation of the response factor IS2/triglyceride. For this reason after calibration this response factor tends to decrease, leading to the practical low estimation of triglyceride content. Also diglycerides are affected by this drawback, even if at a lesser extent. The time distance between calibration and analysis in different labs was evaluated by the expert group as the main reason of lack in reproducibility of this EN 14105 method.

In practical terms an additional reference compound was added to the sample and in order to avoid an increase of the working time and the additional mistakes due to a third standard solution addition, a joint solution IS2 + TG C57 was prepared. The preparation of this joint solution poses some additional problems, due to poor solubility of TG C57 in pyridine. To partly solve this problem a more diluted solution must be prepared (20 ml instead of 10 ml of final volume, containing approx. 80 mg of IS2 and 50 mg of TG C57). The solution, when stored in refrigerator, shows a precipitate of TG C57 that can be re-dissolved by a gentle heating immediately before use. This warning was inserted in Version 1 of draft.

The first test carried out had the meaning to evaluate possible interferences between the peaks of a Biodiesel sample and the one of the new reference compound. We can exclude this problem also in consideration of what illustrated in figure 23.

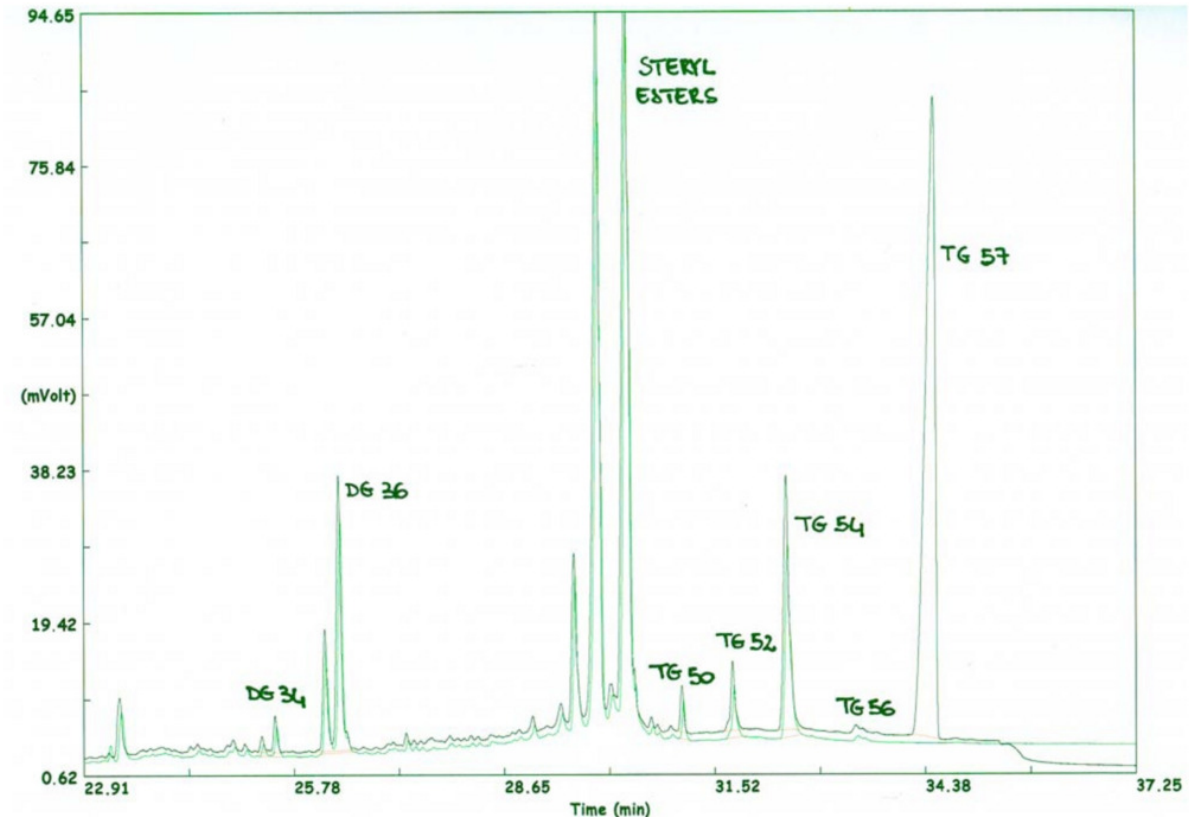

Fig. 23: Overlay chromatogram of a Biodiesel sample analysed with or without C57 addition

A second test was carried out by analysing three different Biodiesel samples with or without TG 57 addition. The results are summarised in table 12.

Tab. 12: Comparison of results obtained during the analysis of three different Biodiesel samples with and without TG C57 addition (as % m/m)

Parameter	**Sample 1**		**Sample 2**		**Sample 3**	
	EN 14105	modified	EN 14105	modified	EN 14105	modified
Free glycerol	n.d.	n.d.	n.d.	n.d.	0.008	0.007
Monoglycerides	0.66	0.70	0.64	0.68	0.62	0.65
Diglycerides	0.14	0.16	0.15	0.17	0.13	0.14
Triglycerides	n.d.	n.d	n.d.	n.d.	0.11	0.15
Tri with TG C57	--	0.03	--	0.03	--	0.14

From the evaluation of these preliminary data we can observe that the obtained results are very similar, and discrepancies fall into the limit of reproducibility values (samples were analysed in two different times without and with TG C57 addition). A particular case is represented by triglycerides concentration in Sample 1 and 2. The original standard does not allow to report the presence of triglycerides, even if there is an evident trace signal on the GC path, because of the negative b value for calibration equation (b = - 0.0457), leading to a negative concentration for triglycerides, obviously not reported.

The second test we carried out was a reproducibility check, carried out by repeating the analysis of sample 3 during 8 different replicates, carried out during the same days, by repeating 8 times sampling, sample derivatisation and GC analysis.

The results are reported in table 13.

Tab. 13: Repeatability test
Results obtained by 8 replicates on the same sample (as % m/m).

	RIF 1	**RIF 2**	**RIF 3**	**RIF 4**	**RIF 5**	**RIF 6**	**RIF 7**	**RIF 8**
GLY	0.007	0.007	0.007	0.008	0.007	0.008	0.008	0.008
MONO	0.649	0.654	0.640	0.651	0.641	0.649	0.649	0.649
DI	0.135	0.134	0.132	0.136	0.133	0.135	0.131	0.134
TRI	0.131	0.133	0.136	0.139	0.134	0.141	0.128	0.140
TRI (C57)	0.149	0.150	0.144	0.136	0.144	0.146	0.141	0.144
RF	1.17	1.15	1.11	1.02	1.10	1.10	1.11	1.10

Finally in table 14 the repeatability values calculated from EN 14105:2003 standard, for the performance of the analyst during repeatability evaluation for given method and the values obtained by the same analyst during the test shown in table 9 are reported.

Tab. 14: Comparison of repeatability values

	EN 14105:2003 Standard	**EN 14105:2003** Laura Della Bella	**Modified EN 14105** Laura Della Bella
Free Glycerol	0.002	0.002	0.002
Monoglycerides	0.081	0.093	0.016
Diglycerides	0.012	0.016	0.006
Triglycerides	0.025	0.005	0.015
Tri through TG C57			0.015
Response Factor			0.147

From the analysis of reported data we can come to the conclusion that the suggested modification does not have any apparent impact on the feasibility and on the repeatability of standard method.

On the contrary a strong impact on reproducibility must be foreseen because the suggested modification might allow to constantly monitor the *health status* of each GC column, allowing to detect the need for extra maintenance in addition to the laboratory maintenance programme.

3.4 OPTIMISATION OF GLYCERIDES CONTENT DETERMINATION EN 14105[3]

The scope of ITERG pre-trials was to assess the interference between diglycerides and dimers of esters using the EN 14105 method which protocol was improved by P. Bondioli (SSOG).

So we added to a sample of Biodiesel from used frying oil, some dipalmitine (DG with C32) and distearine (DG with C36) in order to get a chromatogram that would be added in the text of the method. It was also planned to add DG with C36, but the standard was not available rapidly.

3.4.1 Analytical conditions

Column DB5 HT (10 m – 0.32 mm – 0.1 µm)

Oven temp = 50 °C hold for 1 min, programmed at 7 °C/min up to 240 °C, programmed at 15 °C/min up to 360 °C, final temperature hold for 7 min

Detector temperature = 370 °C

3.4.2 Results

Using the required column and the required condition of temperature of the oven, **we pointed out a co-elution between C32-diglycerides and dimers of FAME (figure 24).**

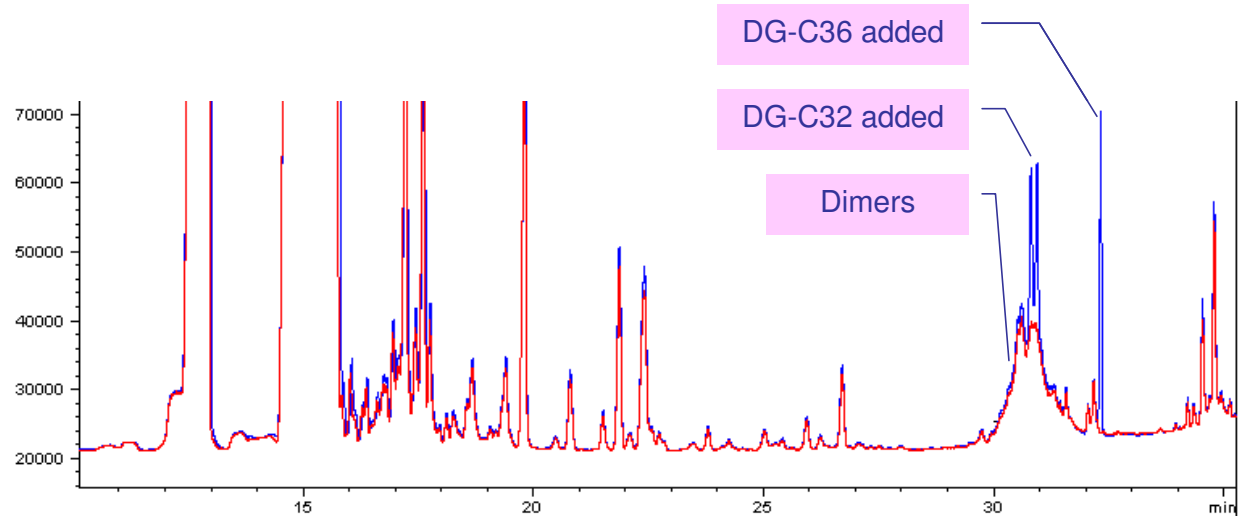

Fig. 24: Effect of the addition of DG-C32 and DG-C36 in Biodiesel from used frying oil

We have decided to run the same analysis using a less polar column (DB1 HT - 15 m – 0.32 mm – 0.1 µm) and we observed the same phenomena.

[3] This part describes the ITERG activities on this topic.

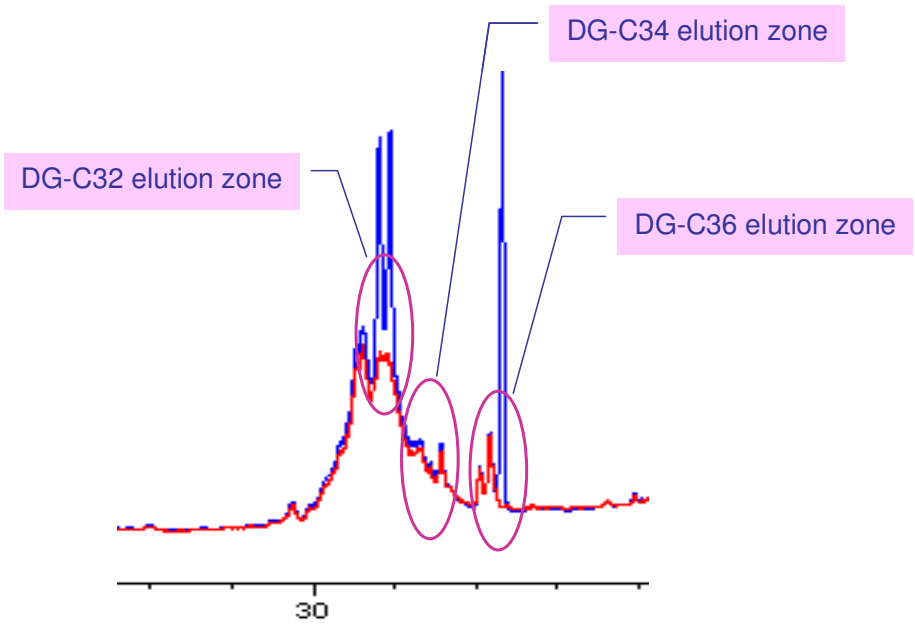

Fig. 25: Effect of the addition of DG-C32 and DG-C36 in Biodiesel from used frying oil - Zoom of the elution area of diglycerides

Unless palm oil is the major oil of the Biodiesel from used frying oil, the content of diglycerides is not very much underestimated, considering only the peaks in the area of DG-C36.

We must take into consideration, however, that the utilisation of palm oil for frying is increasing, due to the decision of lowering trans-fatty acid in fried products that enable companies to use partially hydrogenated fats.

Dimers of methyl esters interfere with diglycerides, and the diglyceride content of Biodiesel may be underestimated if used frying palm oil is included. It is really necessary to add some chromatograms of this area for this type of product and maybe to work on the temperature program of the oven.

3.5 SAMPLE CHROMATOGRAMS

The following sample chromatograms are to be included in revised EN 14105:

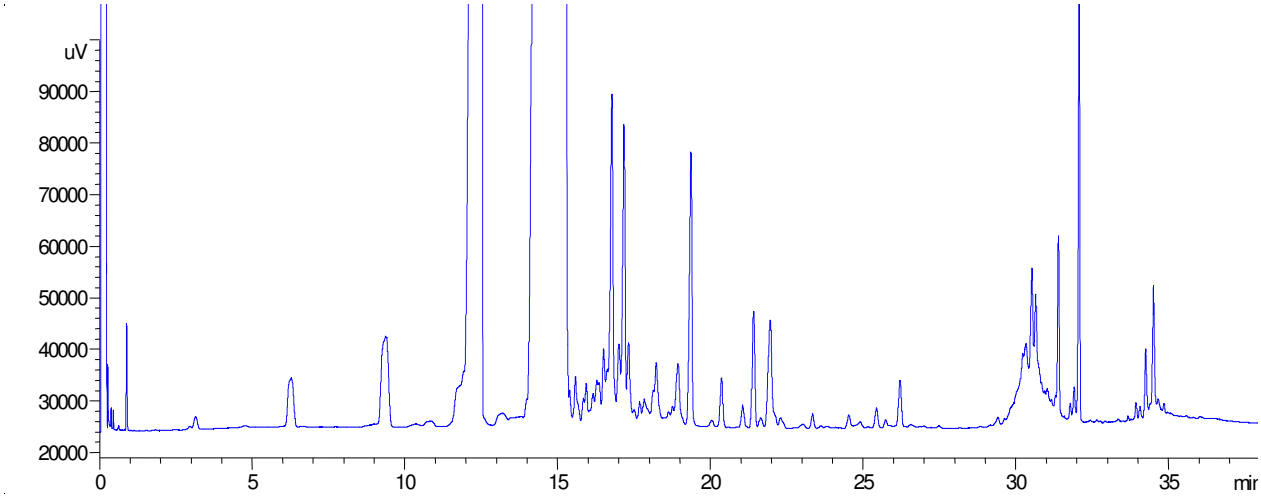

Fig. 26: Chromatogram of a sample of Biodiesel from used frying oil naturally rich in dimers and spiked with diglycerides with 32, 34 and 36 atoms of carbon

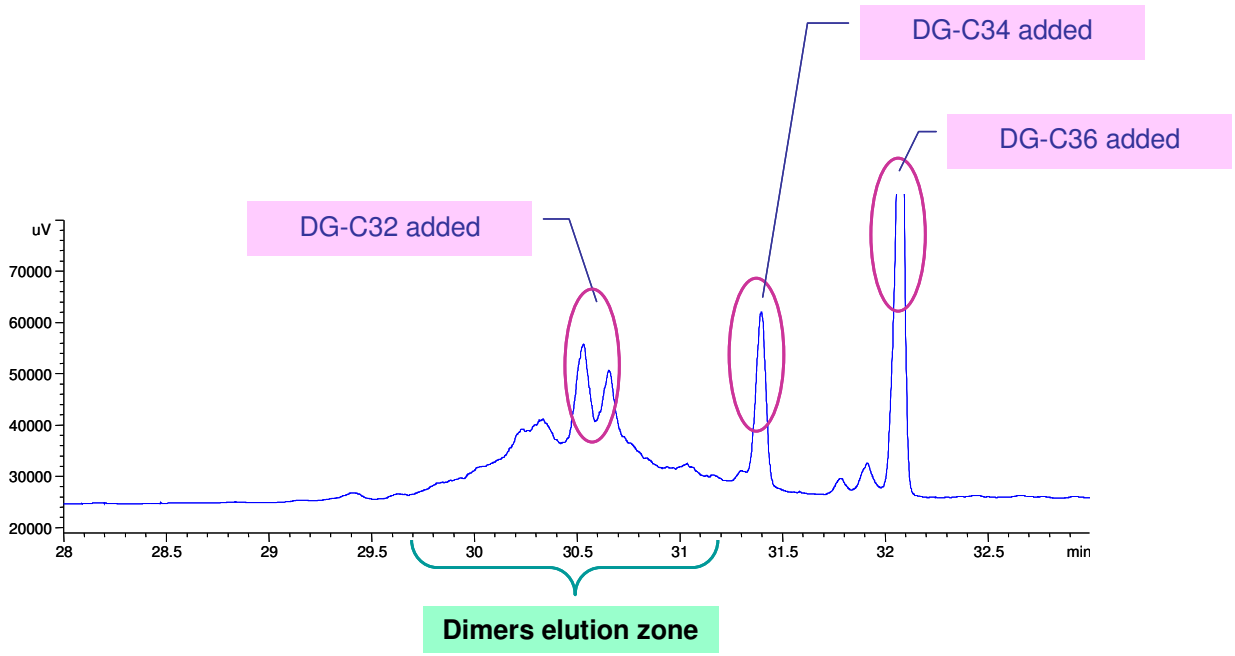

Fig. 27: Chromatogram of a sample of Biodiesel from used frying oil naturally rich in dimers and spiked with diglycerides with 32, 34 and 36 atoms of carbon - zoom of the diglycerides elution zone

3.6 FURTHER OPTIMISATION (RESULTS OBTAINED AFTER MODIFICATION OF IS2 AND USE FOR MODIFIED EN 14105:2003 STANDARD)

Following what anticipated and discussed during the last CEN meeting held in Vienna on May, 14 our lab did some preliminary work in order to investigate about the feasibility of using a modified IS2 composition, allowing to avoid the preliminary calibration process.

The idea was to carry out the measurement directly, using the internal standardisation and avoiding the use of response factors. To do so an internal standard for each family (mono-, di- and triglycerides) must be used and obviously the internal standard peaks do not have to interfere with sample peaks.

After discussion in Vienna the two preliminary steps were agreed. These steps are listed below:

1. Evaluation of modified IS2 solution in terms of solubility of new ingredients, cold stability, possibility to restore the solution at ambient temperature after a period in the refrigerator at 4°C, chemical stability of mono- and diglycerides, in terms of isomerisation mainly;
2. *Conditio sine qua non* was that the modified method should give the same results as the old one.

3.6.1 Evaluation of modified IS2 solution

A modified IS2 solution was prepared by dissolving in tetrahydrofurane 50 mg of C19 monoglyceride, 50 mg of C38 diglyceride and 50 mg of C57 triglyceride in a calibrated 20 ml flask. All products are readily soluble in solvent by gentle stirring, without heating. The so obtained solution was kept at ambient temperature for 24 hours: no precipitation was observed.

After that a 48 h storage in a refrigerator at 4°C was done. The flask showed a precipitate that spontaneously re-dissolves when restored at ambient temperature (as in the case of modified IS2 containing TG C30 and TG C57 in use for the last mini RRT).

The GC path shown in figure 28 was obtained injecting the standard solution after TMS derivatisation after the 48 hours storage at 4°C.

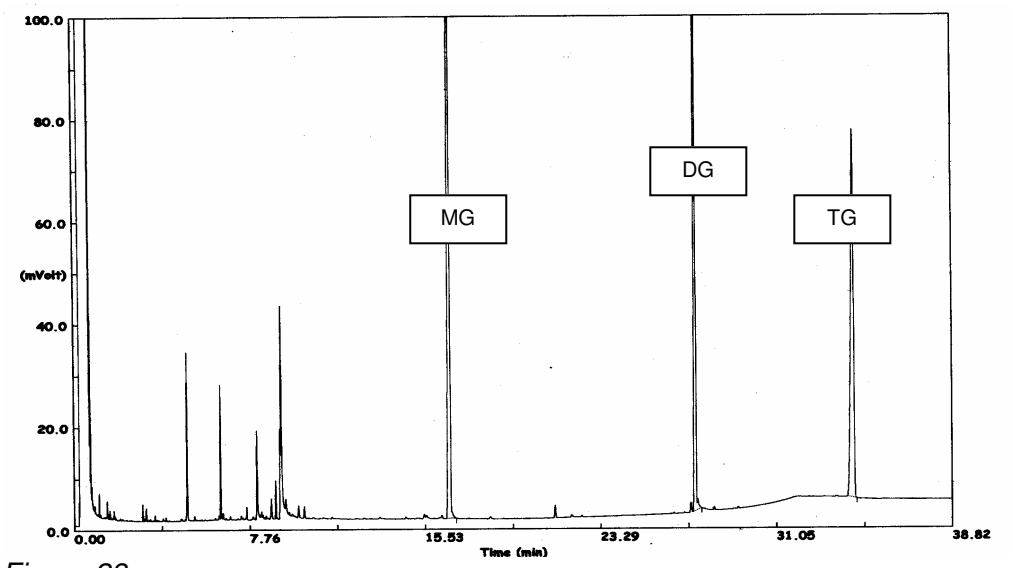

Figure 28

We can observe that the three peaks MG, DG and TG, corresponding at the three added products are very clean and do not show any isomerism evidence.

The methylesters eluted in the first part of diagram belong to a contamination of the analytical system. In any case no signal attributable to C19 free fatty acid TMD derivative is present on the graph.

After four months of storage the GC path of the standard solution shows the following diagram (figure 29)

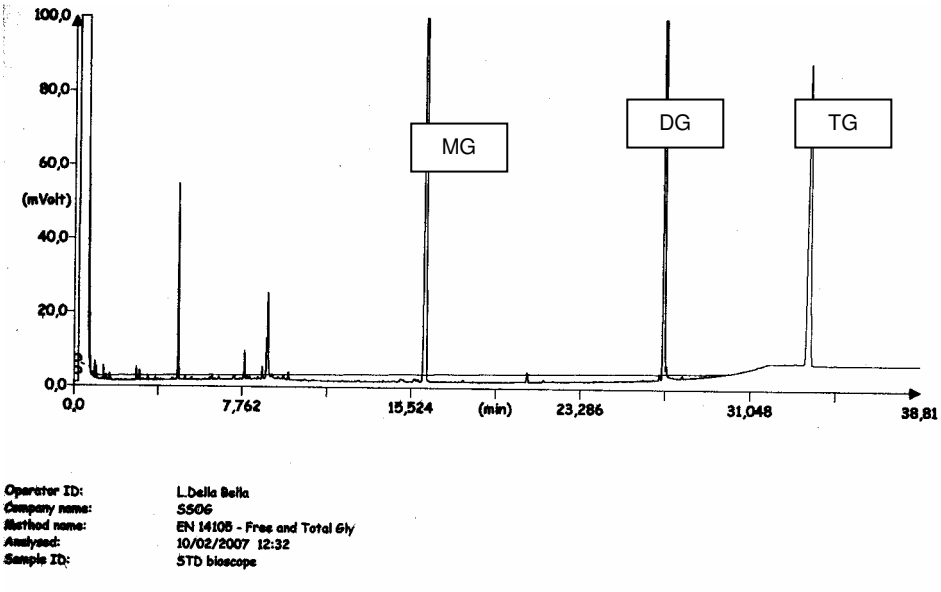

Figure 29

No changes in Internal Standard constituents can be detected, so we can conclude that the solution can be regarded as stable for almost four months, if stored in a refrigerator at 4 °C.

3.6.2 Samples evaluation

For this preliminary samples evaluation the two biodiesel sample (A and B) distributed for the mini RRT were used. The obtained results (in duplicate) are compared in the following tables 15 and 16 with the ones obtained during said RRT (mean values of 4 labs).

Tab. 15: Sample evaluation (A)

Sample A	**Test 1**	**Test 2**	**Avg value of mini RRT**
Monoglycerides	0,643	0,654	0,673
Diglycerides	0,155	0,163	0,193
Triglycerides	0,194	0,205	0,223

Tab. 16: Sample evaluation (B)

Sample B	**Test 1**	**Test 2**	**Avg value of mini RRT**
Monoglycerides	0,675	0,669	0,656
Diglycerides	0,139	0,139	0,156
Triglycerides	0,077	0,076	0,085

The results do not look so bad also in consideration of the dispersion we obtained during the mini RRT. The only observed possible interference is around the C19 monoglyceride, where two peaks (one before and one after) were observed. Separation could be critical but the proportion between peaks and standard might allow to minimise this risk. Also the adoption of a 15 metres column, as required by French representatives, might improve this separation. The two peaks are already present in the samples (check your chromatograms obtained during the RRT) and it should be very interesting to know something about their identity. In the following figures 30 and 31 the chromatograms obtained for sample A and B are reported.

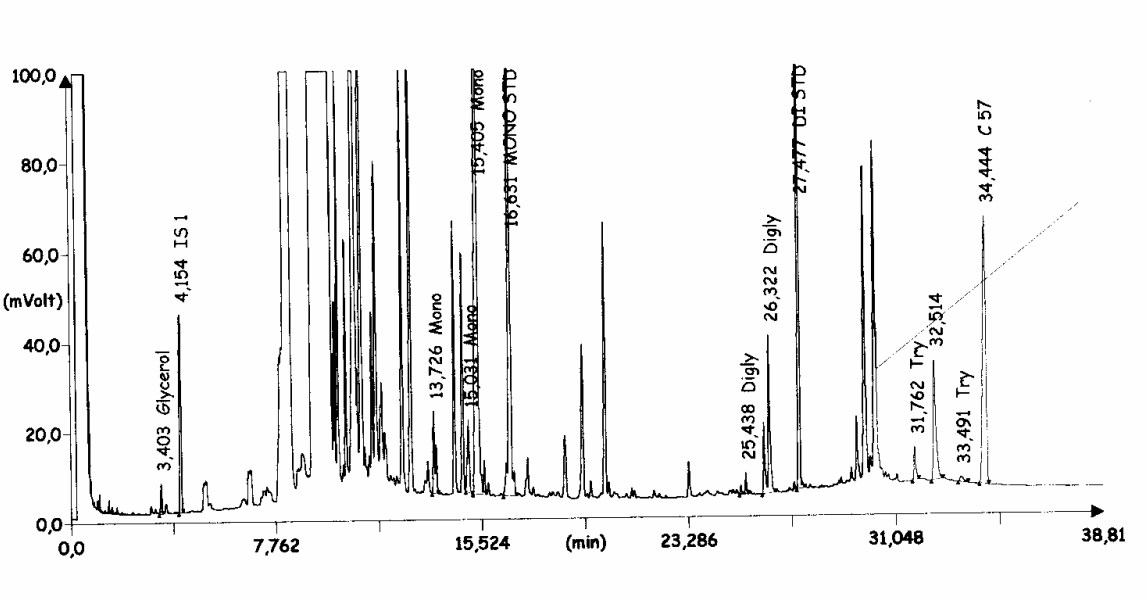

Figure 30 – Sample A

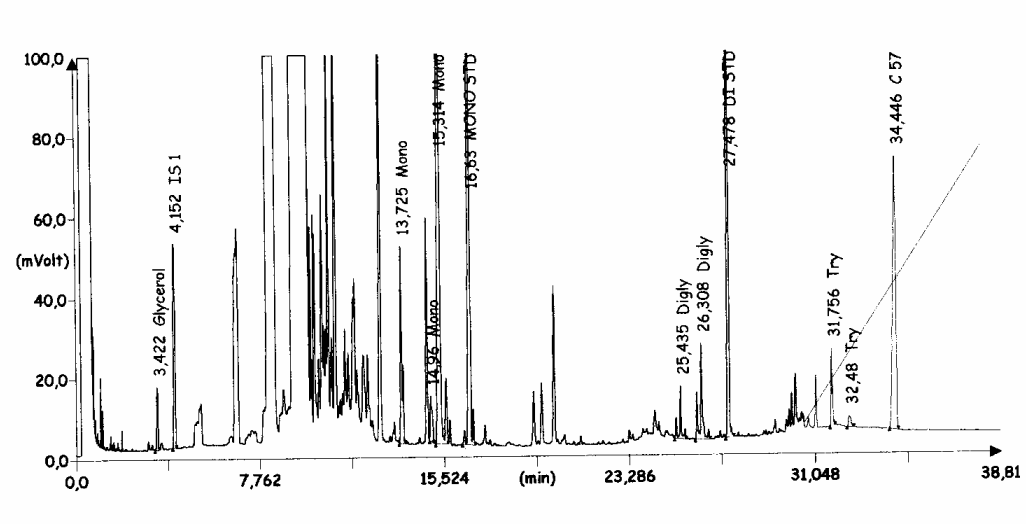

Figure 31 – Sample B

The figures 32, 33 and 34 represent a zoomed picture of the standard path and of the previous shown diagrams, respectively.

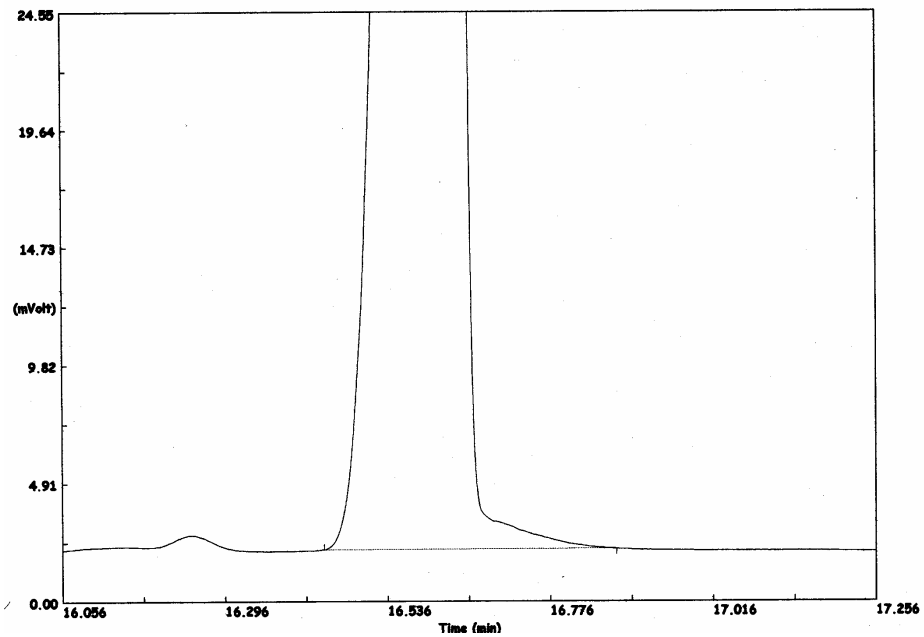

Figure 32 – Zoom of MG C19 standard

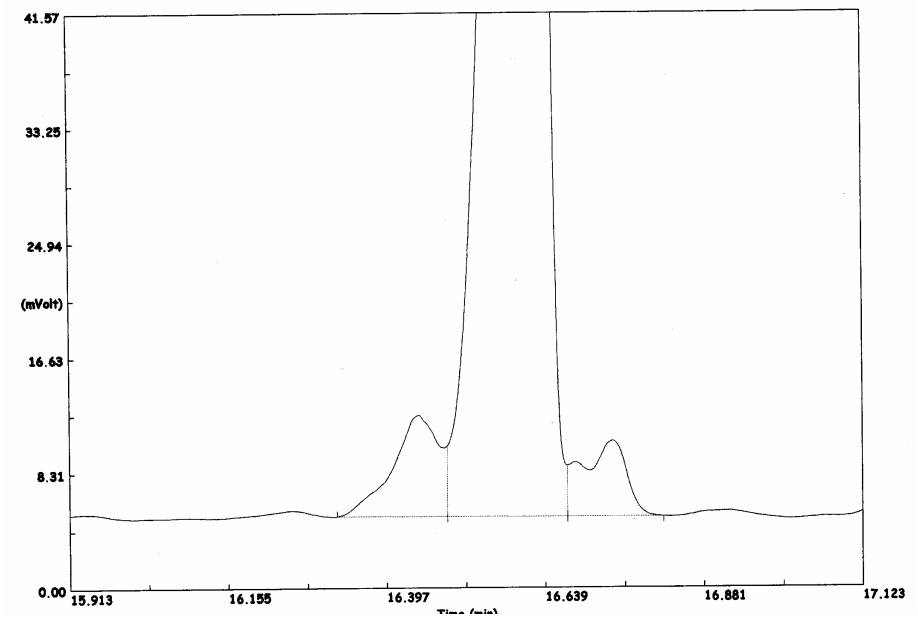

Figure 33 – Zoom of MG C19 in sample A

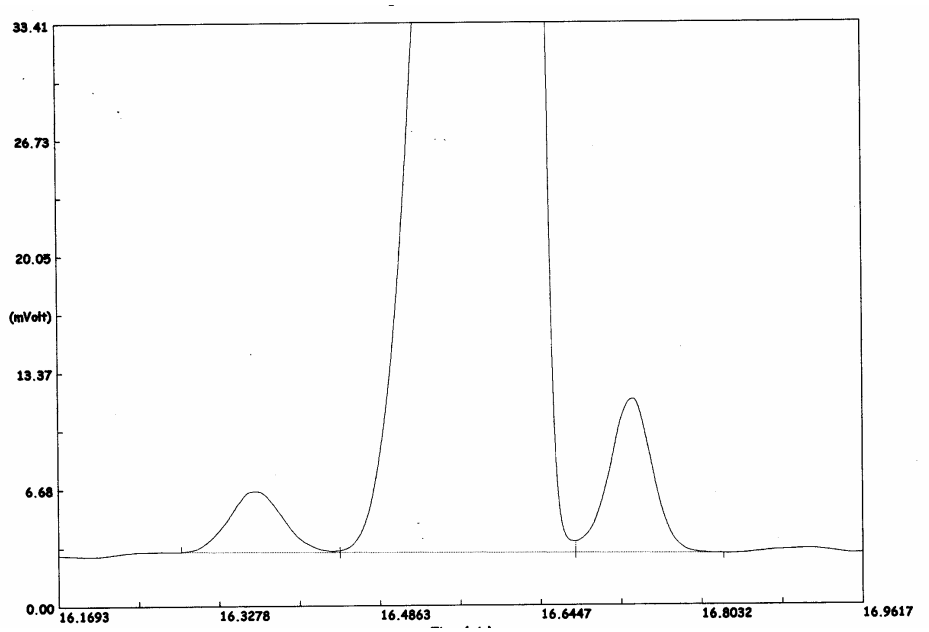

Figure 34 – Zoom of MG C19 in sample B

3.6.3 FOLLOW UP OF THE PRELIMINARY TESTS

After this first test we decided to verify using other samples the suitability of the suggested procedure. Four biodiesel samples distributed in occasion of one mini ring test for EN 14103 modified method were available:

- Sample 1, from coconut;
- Sample 2, from palm;
- Sample 3, from soy/rape;
- Sample 4 from coconut/rape.

Of course the biodiesel sample 1, produced from coconut oil was not suitable for analytical control, because of the presence of MG, DG and TG with low M.W. that could overlap with other interesting peaks belonging to other families. The last sample, notwithstanding the same problem, was used, thanks to the low content on coconut biodiesel.

In every case we must remember that the suggested modified method does not allow in any case the analysis of biodiesel coming from palm kernel and coconut oil.

In the following tables the results obtained in duplicate using the modified procedure are compared with the ones obtained using the standard EN 14105:2003.

Tab. 17 Comparison of proposed and standard procedure

Sample 2	New method Test 1	New method Test 2	EN 14105:2003 Test 1	EN 14105:2003 Test 2
Free glycerol	0,018	0,018	0,015	0,015
Monoglycerides	0,257	0,259	0,248	0,256
Diglycerides	0,057	0,057	0,045	0,048
Triglycerides	0,092	0,094	0,089	0,093

Sample 3	New method Test 1	New method Test 2	EN 14105:2003 Test 1	EN 14105:2003 Test 2
Free glycerol	0,004	0,004	0,005	0,005
Monoglycerides	0,662	0,655	0,637	0,640
Diglycerides	0,137	0,138	0,129	0,127
Triglycerides	0,031	0,031	0,017	0,017

Sample 4	New method Test 1	New method Test 2	EN 14105:2003 Test 1	EN 14105:2003 Test 2
Free glycerol	0,272	0,272	0,213	0,217
Monoglycerides	0,617	0,618	0,560	0,565
Diglycerides	0,110	0,111	0,102	0,104
Triglycerides	0,016	0,016	0,010	0,010

Evaluating the obtained results we can observe some systematic differences, for triglycerides in Samples 3 and 4 and for monoglycerides in Sample 4. Nevertheless, for a better evaluation of what here stated we must remember that these parameters in EN 14214:2003 standard for biodiesel are reported using two decimal digits and in every case the observed differences widely fall within the repeatability interval for EN 14105 standard.

3.6.4 CONCLUSIONS

After these tests the method modification seems to be suitable and easy to carry out.

The short term stability of modified standard solution IS 2 was been evaluated in a positive way after more than four month of storage at 4°C.

The possible peaks overlap in the MG C19 monoglyceride can be easily solved or neglected.

The obtained results are comparable with the ones coming from EN 14105:2003.

The suggested procedure shows some advantages, is compared with the actual standard:

- elimination of calibration process for MG, DG, TG, while it remains necessary for free glycerol evaluation;
- solution of the problem related to GC capillary column degradation, as no calibration factors are used. In other words the IS for each family has the same response factor of each component belonging at the same family. As a better control and to avoid poor TG response a criteria of column acceptance based on relative response between MG, DG and TG standard will be introduced in the text.

3.6.5 ORGANISATION OF A ROUND ROBIN TEST WITHIN CEN TC19/TC307 JWG

After the evaluation of the positive obtained results the partners agreed to launch a RRT within the CEN Committee, involving approximately 20 laboratories.

On day January, 11th ASG Analytik, partner in this project distributed six different biodiesel samples, along with 300 mg of each reference standard substances (glyceryl monononadecanoin, glyceryl dinonadecanoin, glyceryl trinonadecanoin) and the test procedure for the modified method, as reported in Annex 1.

The dead line for results presentation to SSOG was set on day February, 15th. Owing to the low number of received results after the above mentioned dead line it was agreed to wait for additional two weeks, in order to receive a greater number of results. At the end of February, when the session was definitely closed, 16 laboratories provided their contribution. After results organisation in .xls files data were transferred to ITERG who will take care of statistical evaluation.

3.6.6 ROUND ROBIN RESULTS

Finally, in January 2008 a round robin test was executed with the modified version of EN 14105. Six different samples were tested by 16 participants in different labs in Austria, France, Germany, Italy and Spain. Methyl esters of varying sources and of varying glyceride content were examined

The ring test was organised by ASG, the results were collected by SSOG. The first statistical evaluation was executed by ITERG, additional calculation will be done by DIN/FAM. Unfortunately it was impossible to include these results in this report, so the given precision data have to be regarded as preliminary.

The following table 18 show the reproducibilities and the repeatabilities of the modified test method for each component. In figure 35 to 37 the reproducibility is plotted against the mean value of the round robin test for each sample. Table 19 and 20 compares the newly evaluated precision equations with the ones given in EN 14105.

Tab. 18 Reproducibility and repeatability of single components

Monoglycerides						
Mean value [% m/m]	0,4265	0,4338	0,6155	0,7612	0,3031	0,8101
Reproducibility	0,1480	0,1683	0,1779	0,0752	0,0718	0,1634
Repeatability	0,0371	0,0356	0,0618	0,0752	0,0222	0,0442
Diglycerides						
Mean value [% m/m]	0,1420	0,1597	0,1724	0,2994	0,1846	0,0808
Reproducibility	0,0594	0,0524	0,0549	0,0946	0,052	0,0428
Repeatability	0,0173	0,0169	0,0200	0,0519	0,0215	0,0116
Triglycerides						
Mean value [% m/m]	0,1726	0,0534	0,0725	0,1548	0,3188	0,0719
Reproducibility	0,1402	0,0566	0,0641	0,1349	0,121	0,0509
Repeatability	0,0204	0,0160	0,0148	0,0198	0,0319	0,0114
Free Glycerol						
Mean value [% m/m]	0,0074	0,006	0,0134	0,0008	0,0031	0,0121
Reproducibility	0,0071	0,0065	0,0095	0,0072	0,0037	0,009
Repeatability	0,0014	0,0010	0,0023	0,0022	0,0007	0,0018
Total Glycerol						
Mean value [% m/m]	0,1561	0,1458	0,2027	0,2629	0,1393	0,2383
Reproducibility	0,0365	0,0415	0,0476	0,0638	0,0283	0,0436
Repeatability	0,0093	0,0147	0,0217	0,0373	0,0049	0,0138

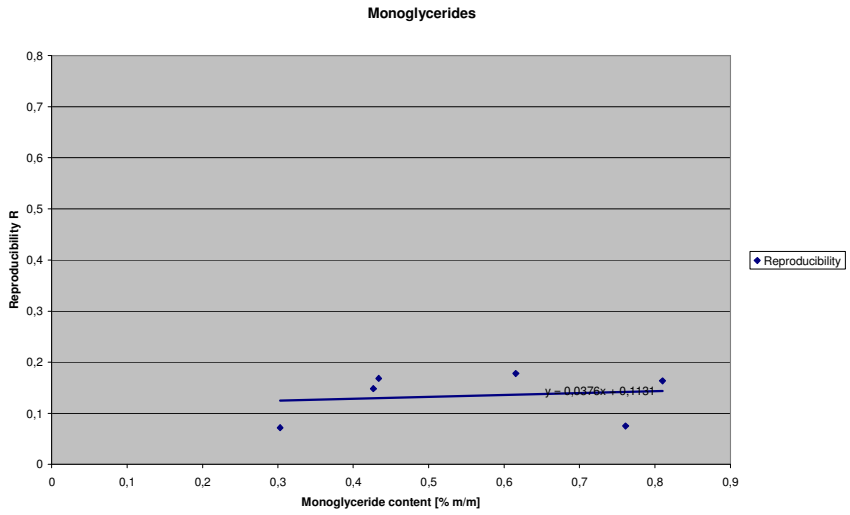

Fig. 35: Monoglycerides: reproducibility plotted versus monoglyceride content

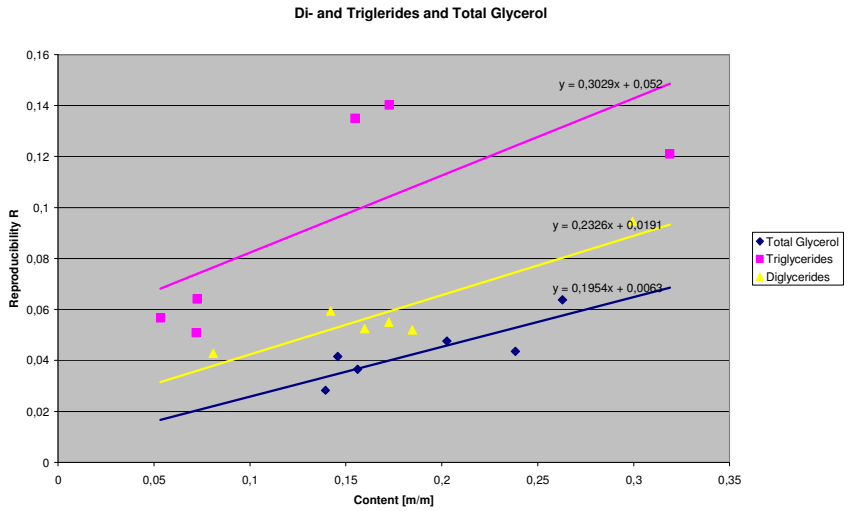

Fig. 36: Di-, Triglyceride and total glycerol content: reproducibility plotted versus content

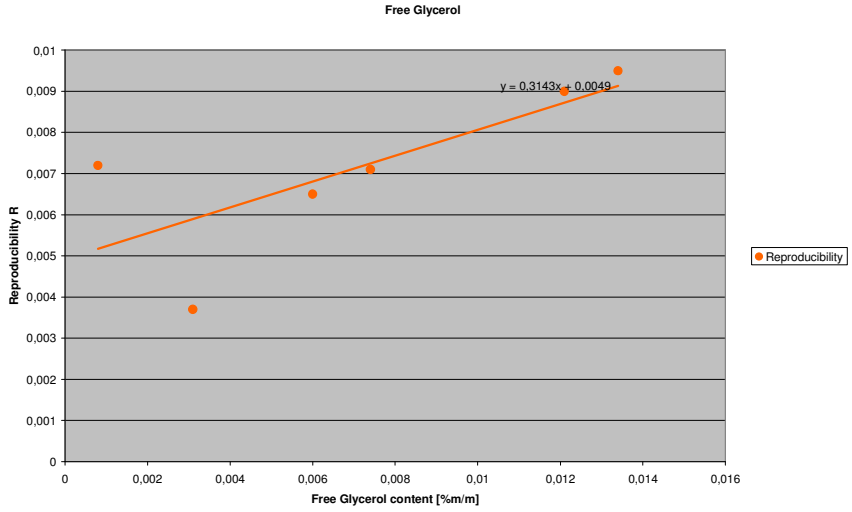

Fig. 37: Free glycerol: reproducibility plotted versus free glycerol content

The graphs show that the number of samples analysed in the ring test could have been larger. The line of best fit, in particular for triglycerides is hard to draw. Unfortunately we were limited to real world samples as it is too difficult to prepare defined samples with these components.

However, the precision equations are of sufficient quality. Even for triglycerides we were able to achieve at least the borderline of the “2R” rule. Table 18 and 19 give a comparison of the precision equations of the original and the modified method.

Table 19: comparison of EN 14105 repeatability versus modified method

Repeatability	**EN 14105**	**Revised EN 14105**
Monoglycerides	r = 0,119x + 0,004	r = -0,0248x + 0,0329
Diglycerides	r = 0,060x + 0,004	r = 0,1921x - 0,0101
Triglycerides	r = 0,1565x + 0,004	r = 0,0686x + 0,0094
Free Glycerol	r = 0,0538x + 0,0014	r = 0,048x + 0,0012
Total Glycerol	r = 0,0687x + 0,004	r = 0,1752x - 0,0165

Table 20: comparison of EN 14105 reproducibility versus modified method

Reproducibility	EN 14105	Revised EN 14105
Monoglycerides	R = 0,124X + 0,133	R = 0,1708X + 0,0612
Diglycerides	R = 0,192X + 0,025	R = 0,2326X + 0,0191
Triglycerides	R = 0,2099X + 0,0641	R = 0,3029X + 0,052
Free Glycerol	R = 0,5983X + 0,003	R = 0,3143X + 0,0049
Total Glycerol	R = 0,4472X - 0,01	R = 0,1954X - 0,0063

Conclusions

The graphs show that in some cases it is not easy to place a line through the measuring points. Since real samples had to be used in the round robin tests, there are gaps in the measurement range which was focused on. A higher number of samples with an optimised spread of FAME contents would have led to better results; however, that could not be realised under the given conditions.

The statistical assessment at hand must still be considered preliminary. Still, all in all a positive effect of the modifications of the test method can be noticed. If only the figures of the original and the latest precision equations are compared, for some components the situation seems to have even deteriorated or in other words, no improvement seems to have been achieved. But if you consider that the precision data given in the standard could not be achieved in practice or in the round robin tests, then it is a poor comparison. The revised EN 14105 allows for an improved precision of almost all parameters compared to the practice and thus leads to the fact that at least the “2R-Rule” for diglycerides can clearly be met. At least marginally the rule is met for triglycerides also which is a significant improvement.

Even though this parameter was not the focus of the work, improved precision data for the determination of free glycerol could also be achieved.

The handling of this method as well as its assessment is simplified greatly by the application of internal standards. Furthermore, it is possible to check the performance of the columns by adding standard substances because calibration factors are not used any longer.

At the present state of affairs the revised EN 14105 is an improvement compared to the original method. The revision should be integrated into the standardisation process at CEN TC 307.

4 FAME Content in Middle Distillates Using IR Method (EN 14078)

4.1 Introduction

Middle distillates blended with fatty acid methyl esters (FAME) are becoming more and more popular in the European Union (EU). For example, in Germany almost all diesel fuels according to EN 590 are blended with approximately 5% (V/V) FAME. Thus it is necessary to have an analytical method for the determination of FAME content in diesel fuel which is able to fulfil the requirements of authorities and industry concerning the precision range. Since 2004 the standard EN 14078, an infrared spectroscopic method, exists in the EU. Unfortunately it shows poor accuracy in lower FAME concentration ranges. Consequently it was necessary to improve the method.

A working group within the German Institute for Standardization (DIN) suggested to replace cyclohexane as the required solvent with FAME free diesel fuel respectively heating oil and to avoid dilution via using different IR cells for different FAME concentrations. The modified procedure was tested successfully in Germany. To verify the suitability of the method, precision data need to be evaluated based on a large scale round robin test.

4.2 Preliminary Investigations

Before the round robin test was organised, it was necessary to test the modified EN 14078 with a wider variety of biodiesel feedstocks and common diesel fuels of different origin. Three different middle distillates and four different biodiesels were chosen to investigate possible influences on the measurement method.

First of all the three different middle distillates were analysed as blank and blended samples with 5% (V/V) rape seed oil methyl ester (RME). Figure 4-1 shows the resulting spectra.

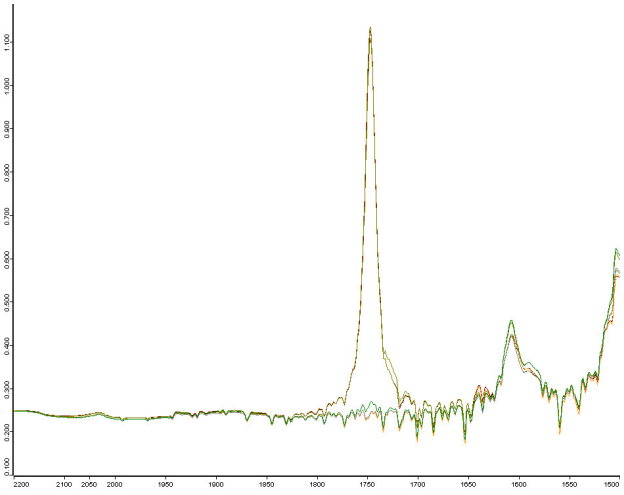

Fig. 4-1: IR spectra of three different middle distillates blended with 0% and 5% (V/V) rape seed oil methyl ester

It can be seen that the peak for the ester group appears around a wave number of 1748 cm^{-1} and that it is not influenced by different diesel fuels. Beside that the three middle distillates without RME show now response in this wave number area. This was the first important finding.

In a second series of investigations biodiesel from different feedstocks were blended with one diesel fuel in successive concentrations between 1 and 10% (V/V). Figure 4-2 shows the results for these blends.

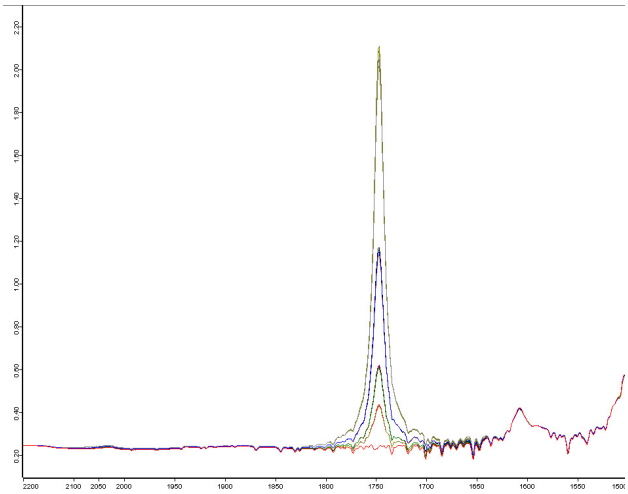

Fig. 4-2: IR spectra of one middle distillate blended with FAME from different feedstocks (palm, rape, soy, used fat) in concentrations of 1,2,5 and 10% (V/V)

These results lead to the second important finding that even different FAME feedstocks don't influence the IR signal and that no peak shift occurs. Therefore it was possible to prepare a calibration curve which is shown in figure 4-3.

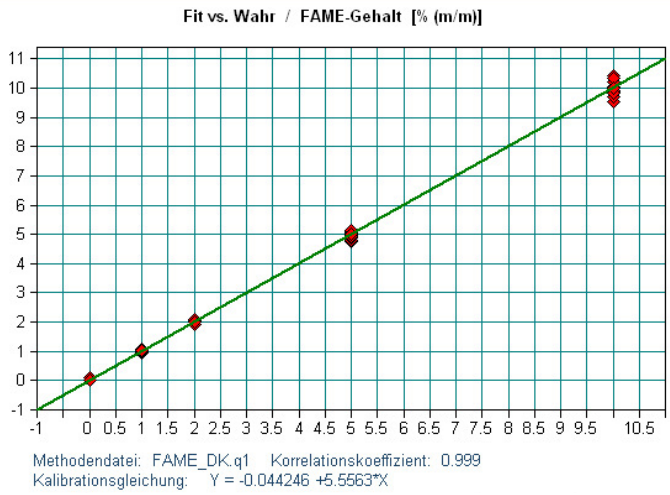

Fig. 4-3: Calibration curve for different middle distillates blended with FAME from different feedstocks in concentrations of 1,2,5 and 10% (V/V)

Table 4-1 summarises the analysed test mixtures for the provided calibration.

Table 4-1: Overview of the measured middle distillate FAME blends

		FAME Concentration in % (V/V)				
Middle Distillate	FAME	0	1	2	5	10
DK1	PME	X	X	X	X	X
	RME		X	X	X	X
	SME		X	X	X	X
	UFME		X	X	X	X
DK2	PME	X	X	X	X	X
	RME		X	X	X	X
	SME		X	X	X	X
	UFME		X	X	X	X
DK3	PME	x	X	X	X	X
	RME		X	X	X	X
	SME		X	X	X	X
	UFME		X	X	X	X

PME: Palm Oil Methyl Ester RME: Rape Seed Oil Methyl Ester

SME: Soybean Oil Methyl Ester UFME: Used Fat Methyl Ester

The determined calibration curve underlines the capability of the modified EN 14078 to detect a broad range of concentrations of FAME in diesel fuel without any disturbance because of different diesel fuels or biodiesels.

4.3 Round Robin Test

After finishing the preliminary investigations the round robin test could be prepared to evaluate precision data. Ten samples of diesel fuel with different FAME contents between 0,5 and 20% (V/V) (approx. 4 to 180 g/L) and three samples of heating oil with different FAME contents between 0,05 and 0,2% (V/V) (approx. 0,5 to 2,0 g/L) were set up. 20 participants took part and received their test samples at the end of April 2007 as well as the exact description how to proceed. Meanwhile the round robin test is evaluated. Fig. 4-4 and 4-5 show the results for diesel fuel and heating oil.

RR Number 00152
RR Title FAME content in Diesel Fuel prEN 14078 - (IR) new method
RR Date 2007-06
Test result FAME content
Unit g FAME / Litre
No of Samples 10

Sample	DK 1		DK 2		DK 3		DK 4		DK 5	
Lab										
1	8,52	8,49	17,34	17,01	25,85	25,79	42,93	42,78	51,23	51,16
2	8,53	8,51	17,17	17,17	25,83	25,89	42,91	42,86	51,37	51,37
3	8,46	8,47	17,22	17,17	25,82	25,89	42,15	42,12	*H 46,42*	*46,04*
4	9,02	9,03	17,76	17,75	26,79	26,77	43,86	43,97	52,17	52,51
5	9,10	8,91	17,95	17,90	26,76	26,66	43,95	43,85	52,08	52,04
6	8,64	8,66	17,54	17,37	26,11	25,98	43,31	43,25	51,45	51,34
7	9,19	9,07	17,92	17,88	26,54	26,62	44,48	45,12	52,60	52,92
8	8,57	8,53	17,06	17,14	25,55	25,76	42,69	43,03	50,87	51,09
9	8,42	8,34	16,94	16,78	25,52	25,38	42,98	42,97	54,65	54,40
10	8,61	8,58	17,41	17,35	26,24	26,06	43,70	43,54	52,41	52,07
11	9,14	9,09	17,08	17,06	25,27	25,20	42,04	41,69	*H 34,16*	*34,07*
12	8,85	8,85	17,20	17,15	25,45	25,40	42,30	42,25	50,25	50,10
14	8,77	8,71	17,43	17,44	26,12	26,10	43,40	43,19	51,21	51,06
15	9,68	9,53	18,35	18,50	25,60	25,87	42,56	42,58	49,88	49,88
16	8,61	8,57	17,30	17,21	25,75	25,84	43,57	43,25	52,21	51,89
17	8,60	8,56	17,11	17,18	25,72	25,62	43,91	44,91	*C 48,08*	*51,04*
18	8,56	8,82	17,26	17,29	*C 26,69*	*25,71*	42,97	43,03	51,22	51,38
19	8,55	8,45	16,94	16,75	25,43	25,39	42,03	42,48	50,08	49,81
Labs / valid Labs		(18) 18		(18) 18		(18) 17		(18) 18		(18) 18
Mean		8,752		17,363		25,898		43,128		51,556
Repeatability, r		0,212		0,249		0,251		0,712		0,463
Reproducibility, R		0,943		1,213		1,365		2,323		3,626

Lab	DK 6		DK 7		DK 8		DK 9		DK 10	
1	60,30	60,58	77,18	76,89	86,30	86,23	128,36	128,22	171,88	171,92
2	59,93	59,93	77,05	77,05	85,61	85,36	125,84	125,84	167,87	167,87
3	58,35	58,96	75,42	76,17	86,16	86,71	124,84	125,33	171,23	171,87
4	*H 63,97*	*63,99*	81,06	81,09	90,21	90,61	129,59	129,65	171,59	172,11
5	60,70	60,99	77,17	76,79	88,08	88,18	129,49	129,01	174,25	174,73
6	59,91	60,18	78,67	77,77	87,83	86,79	126,75	127,31	169,46	168,53
7	61,35	61,29	79,83	79,43	89,20	89,66	132,87	132,75	180,44	180,55
8	59,53	59,74	*C 74,85*	*77,06*	85,50	85,71	124,78	125,20	167,85	168,70
9	60,34	60,79	77,01	76,26	89,54	88,30	125,68	125,94	169,88	167,89
10	60,63	60,22	77,72	78,03	86,59	87,11	128,38	128,18	169,56	166,83
11	*H 39,37*	*39,27*	*H 50,76*	*50,57*	*H 56,35*	*56,21*	*H 102,22*	*102,28*	*H 136,28*	*135,97*
12	58,75	58,75	75,80	75,75	84,40	84,45	123,10	123,00	164,50	164,70
14	59,67	59,16	80,25	80,08	*C 80,08*	*88,81*	130,17	129,77	172,84	171,85
15	58,84	58,68	75,60	75,44	84,12	84,08	120,16	120,16	163,60	163,40
16	59,96	59,59	77,95	77,14	84,89	84,89	127,39	126,71	168,52	170,57
17	*C 62,79*	*64,36*	80,76	80,83	87,77	85,74	125,42	124,57	169,58	168,64
18	59,70	60,23	77,32	77,57	85,69	85,96	125,05	126,00	168,70	165,32
19	58,13	58,21	73,78	74,28	80,06	82,90	*C 124,64*	*122,18*	165,18	163,11
Labs / valid Labs		(18) 15		(18) 16		(18) 15		(18) 16		(18) 17
Mean		59,779		77,556		86,354		126,733		169,571
Repeatability, r		0,723		0,988		2,116		0,973		3,059
Reproducibility, R		2,745		6,017		6,761		9,094		12,480

Remarks:
1 Lab no. 13 was eliminated because it did not supply duplicate results
2 results are displayed only to two decimal places to fit on one page, but excel sheet with 3 digits can be made available
3 Outliers are marked (C = Cochran, H = Hawkins)

Fig. 4-4: Interlaboratory comparison results for the modified EN 14078 method with diesel fuel (Sample 1 to Sample 10)

Round Robin No.	00154
Round Robin Title	FAME in HEL - new procedure prEN 14078 - (IR)
Date of RR	2007-06
Test method	Straight forward MID - IR
Test result	FAME content
Unit	g FAME / Litre
Sample description:	HEL (= domestic Heating oil Extra Light)

C = Cochran
H = Hawkins

Sample Name		HEL 1			HEL 2			HEL 3	
Lab No		M1	M2		M1	M2		M1	M2
1	+	0,613	0,605	+	0,995	0,995	+	1,844	1,848
2	+	0,681	0,681	+	1,057	1,049	+	1,910	1,910
3	+	0,666	0,640	+	1,056	1,023	+	1,898	1,842
4	***H***	***0,960***	***0,960***	***H***	***1,350***	***1,350***	***H***	***2,210***	***2,220***
5	***H***	***1,246***	***1,293***	***H***	***1,533***	***1,533***	***HC***	***3,543***	***2,202***
6	+	0,662	0,678	+	1,034	1,046	+	1,978	1,923
7	+	0,713	0,713	+	1,041	1,052	+	1,892	1,898
8	+	0,769	0,811	+	1,105	1,105	+	1,986	1,986
9	+	0,637	0,637	+	1,040	1,040	+	1,901	1,899
10	+	0,664	0,665	+	1,055	1,058	+	1,908	1,902
11	+	0,638	0,636	+	1,036	1,029	+	1,891	1,887
12	+	0,700	0,750	+	1,100	1,100	+	1,950	1,950
14	+	0,623	0,624	+	1,018	1,027	+	1,861	1,859
15	***H***	***0,098***	***0,135***	***H***	***0,604***	***0,645***	+	1,718	1,779
16	+	0,654	0,644	+	1,041	1,051	+	1,905	1,915
17	+	0,600	0,610	+	1,180	1,180	+	2,020	2,010
18	+	*0,784*	*0,784*	+	*1,048*	*1,048*	+	*1,895*	*1,897*
19	+	*0,784*	*0,784*	+	*1,048*	*1,048*	+	*1,895*	*1,897*
(Number) / Valid Labs		(18) 15			(18) 15			(18) 16	
Sample Means		0,682			1,057			1,902	
Repeatability r		0,041			0,022			0,054	
Reproducibility R		0,193			0,132			0,184	

Remarks

1 Labs 4 and 5 were identified as outliers for all three samples
2 Lab 13 was not included because no duplicates were reported
3 FAME contents in examined heating oil were from about 0,7 to 2 g FAME / Liter, equivalent to 0,08 .. 0,2 %(V/V)

Fig. 4-5: Interlaboratory comparison results for the modified EN 14078 method with heating oil (HEL 1 to HEL 3)

The results for laboratory 13 are not reported because no duplicates were provided. Both figures show good results for repeatability and reproducibility. Thus the precision footprint for both ranges could be calculated. Fig. 4-6 and 4-7 show the precision data.

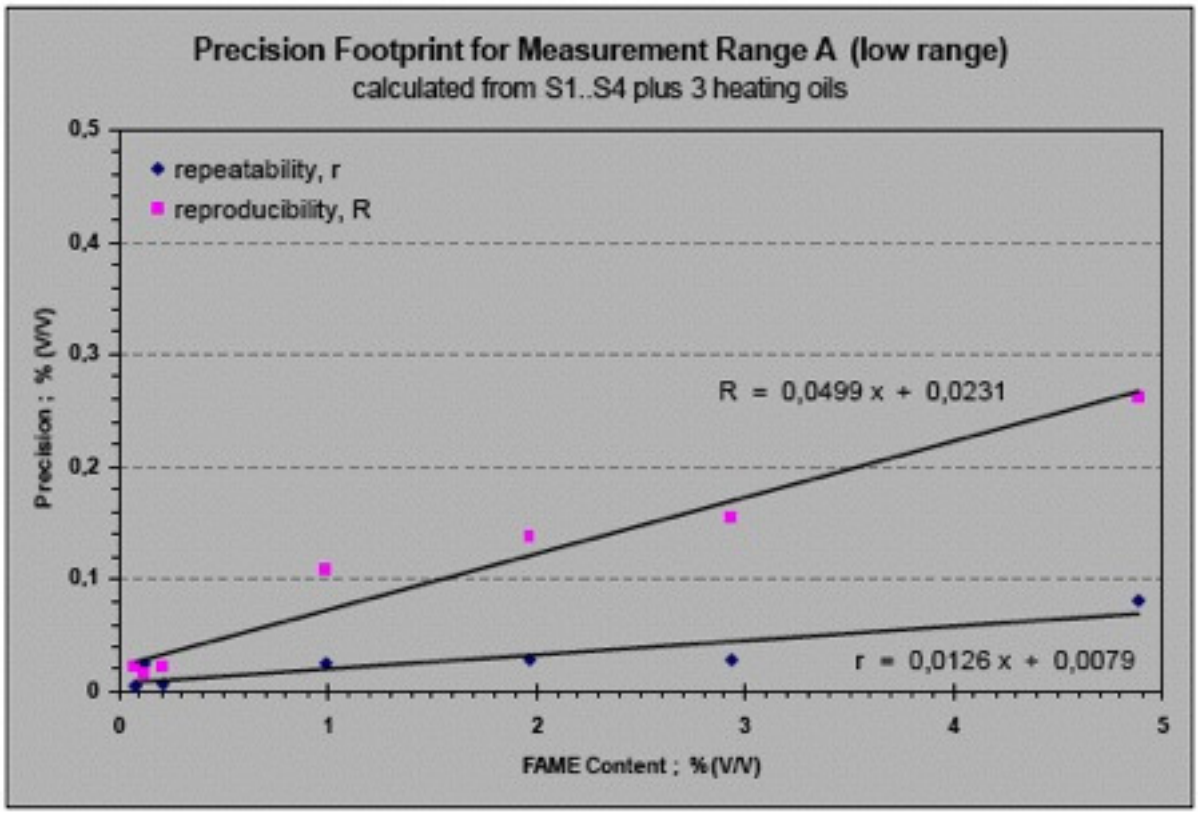

Figure 4-6: Precision data for the measurement range 0,05 to 3,00 %(V/V)

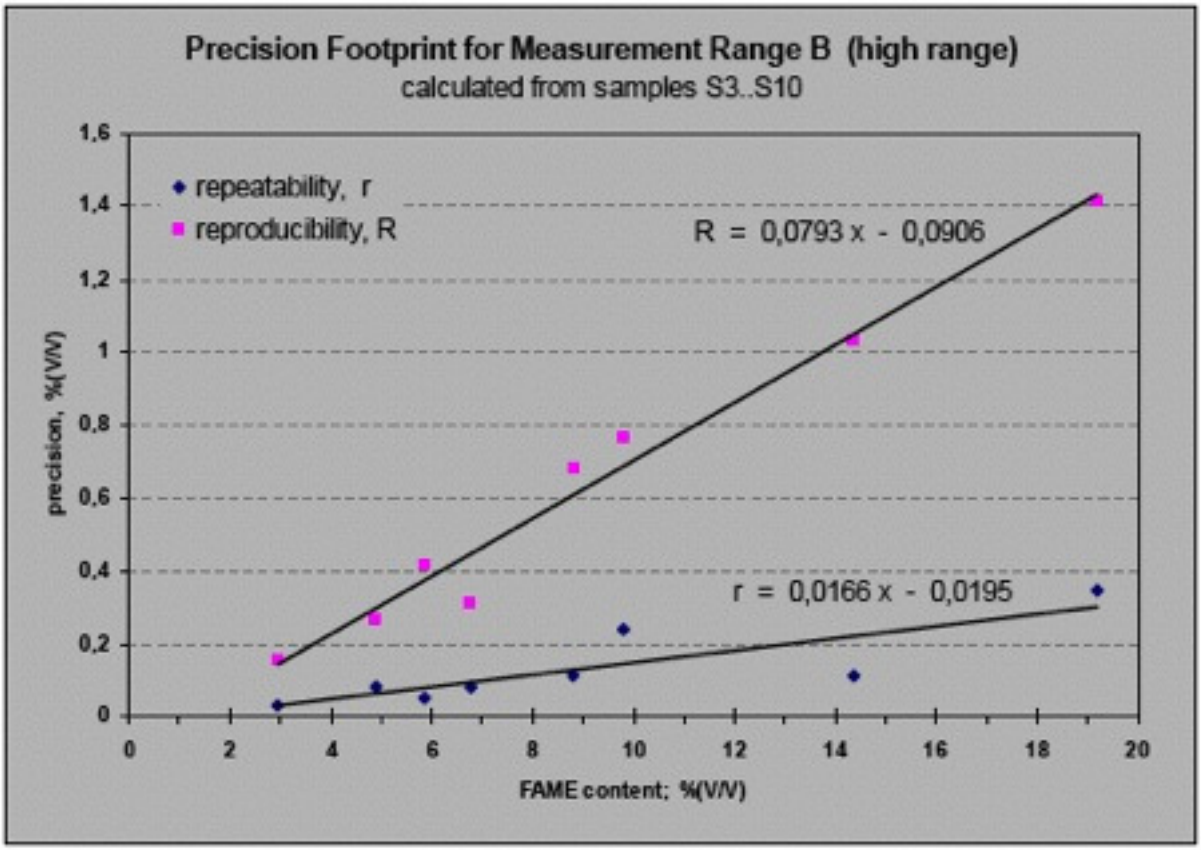

Figure 4-7: Precision data for the measurement range 3,0 to 20,0 %(V/V)

It could be seen that it was necessary to determine two measurement ranges because of different dilution and IR cell requirements. Therefore it is recommended to use two decimal places for the range 0,05 to 3,00 %(V/V) instead of one decimal place for the range between 3,0 to 20,0 %(V/V). Possible limit values should be set analogically.

The results of the round robin test lead to the implementation of a new national standard in Germany E DIN 51527-1. Sooner or later the national standard will merge in a modified version of EN 14078. It was necessary to establish E DIN 51527-1 because a new official standard was needed and it was impossible to formulate a modified EN 14078 within the available time-frame.

5 Content of Polyunsaturated Esters (PUFA)

For polyunsaturated fatty acid methyl ester (PUFA), the European Biodiesel standard EN 14214 is giving a limit of max. 1% m/m. However, so far there exists no standardised method to measure this parameter.

In the following the results of a literature study, with the purpose of identifying potential test methods for PUFA, are listed. The most promising candidate is described and will be discussed and tested.

5.1 SCOPE

The purpose of the report is to describe a possible procedure for the determination of the polyunsaturated fatty acid methyl ester content of FAME. It gives an overview on the current status of research, carried out in order to finally establish a draft standard for future purposes. Not all experiments have been finished yet but first recommendations can be done.

5.2 EVALUATION OF A SUITABLE METHOD – LITERATURE RECHERCHÉ

In fact many methods seem to be suitable as basis for the development of a method for the determination of PUFA. However, due to the current status of existing methods given by EN 14214 and due to the equipment actually available in the different laboratories and Biodiesel plants it should be the target to select a method capable from the instrumental side, for them. For this reason, a gas chromatographic method should be the choice. Other methods described in literature (see references) mostly include HPLC methods coupled with e.g. MS detection. Such methods are widely being used in medical sector and would lead to additionally investment costs due to expensive equipment for FAME analysing institutions. However, a comprehensive literature citation is given at the end of this document.

5.3 BASICS OF A METHOD

5.3.1 Normative References

This document / draft is based on A.O.C.S Official Method Ce 1b-89. Modifications of the method are given in detail in the corresponding paragraphs. If it would be necessary to determine PUFA directly out of marine oils, the sample preparation should be according to A.O.C.S. Official Method Ce 2-66.

5.3.2 Principle

Determination of the percentage of PUFA present in FAME by gas chromatography/FID detection using internal calibration with C 23:0 ethyl ester. Theoretical detector correction factors relative to C 23:0 (IS) for 20:5 n-3 (0.99) and 22:6 n-3 (0.97) should be applied to the analytical data for optimum accuracy.

5.3.3 Apparatus

- Capable gas chromatograph with capillary injection system (preferable split mode at a split ratio of 1:100) and a flame ionization FID detector.
- Injection port 250°C, detector 250°C
- Oven temperature profile – Initial temperature 170°C, initial hold time, 0 min; Program rate 10°C/min up to 200°C; 2°C/min up to 225°C final temperature; final hold time 10 min.
- Capillary column: should be of flexible fused silica; 30 meters length, 0.25µm film thickness and 0,20 – 0,32 mm ID. The liquid phase must be bonded Carbowax or an

equivalent polyglycol e.g. HP –INNOWax, SUPELCOWAX 10, Carbowax, CB-WAX, DB-23 or equivalent types.
- Carrier gas, hydrogen or helium, 99.99% purity or better. Additional gas depending on instrumentation (compressed air, oxygen) but have to be of high purity, also oxygen scrubber could be mandatory with Wax-type columns.
- Data analysis according the corresponding instrumentation software.

5.3.4 Reagents

- C 23:0 ethyl ester, purity min. 99.5%
- Iso-Octane, min. analytical grade

If additionally sample preparation of marine oils is necessary the corresponding reagents are amended as follows:

- Solution of sodium hydroxide, reagent grade, 0,5N in methanol
- Methyl alcohol, reagent grade
- BF_3, 12% in methanol
- Saturated solution of sodium chloride, reagent grade, in water
- Sodium sulphate, anhydrous, reagent grade
- Petroleum ether, redistilled, b.p. 30 – 60°C

5.3.5 Additional Equipment

- Screw-cap vials 1.5 ml with leak-tight Teflon lined caps
- Screw-cap or crimp cap vials 10 -20 ml
- 1, 2 and 5 ml volumetric pipettes
- Pasteur type pipettes
- Volumetric flask, 25 ml
- Analytical balance, ± 0.0001 g

For marine oil samples:

- 50 ml flat bottom boiling flasks or Erlenmeyer flasks
- Water cooled condenser
- Heating facility
- Boiling chips, free of fat
- Separator funnels, 250 ml

5.3.6 Procedure

a) Accurately weigh (± 0.1 mg) approximately 25 mg of C 23:0 ethyl ester internal standard (IS) into a 25 ml volumetric flask and fill up to mark with iso-octane.
b) Accurately weigh (± 0.1 mg) approximately 30 mg of FAME into a (10 ml) tube
c) Pipette 2 ml aliquot of the IS into the tube, cap and mix thoroughly.
d) Transfer 1 ml of the above solution into a screw-cap vial
e) Inject 1 µl under appropriate gas chromatographic conditions (see Apparatus).

Note 1: The above given procedure reflects the currant status of the method development. Changes, especially in connection with sample and IS amount and concentration are under evaluation at the moment. New Biodiesel from fish oil as well as different fish oils are being expected these days. These samples give information about the actual quality (PUFA distribution) of fish oil and FAME of fish oil on the market. They will be used for further validation of this method.

5.3.7 Calculation

The PUFA content C, expressed as percentage (wt-%), can be calculated according eq. 1:

$$C = \frac{\sum A_{PUFA} \times CF_{PUFA}}{A_{IS}} \times \frac{C_{IS} \times V_{IS}}{W} \times 100 \qquad \text{(Eq. 1)}$$

where

A_{PUFA}	are the peak areas of the polyunsaturated fatty acid methyl esters (see note 2)
CF_{PUFA}	are the theoretical correction factors of the corresponding PUFA
A_{IS}	is the peak area of the internal standard C 23:0
C_{IS}	is the concentration in mg/ml of the internal standard
V_{IS}	is the volume (ml) of the internal standard solution being used
W	is the weight (mg) of the sample

Results should be expressed to one decimal place. (This might change when the limit of quantification has been evaluated)

Note 2: In fact, the strongest contribution of the PUFA found in marine oil samples comes from EPA or DHA. But experiments had shown that additionally (depending on the fish oil source) significant amounts of C 18:4, C 20:3, C 20:4, C 22:4, C 22:5 and other PUFA isomers can be identified in such samples. Especially for the determination of neat fish oil FAME, these compounds have to be taken into account together with their corresponding correction factors. All above mentioned other PUFA have been identified so far and can be given as reference chromatogram in the method. However, fish oil FAME will not be often analysed in its neat form. First experiments of B5 fish oil FAME blends with “normal” Biodiesel showed, that only EPA, DHA and possibly C 20:4 and C 22:5 can be identified as main PUFA compounds in this mixture. At the moment the reasonable minimum level for this compound will be evaluated. Therefore the prior mentioned expected new fish oil and fish oil FAME samples will give more information, but these experiments are currently in progress. Moreover, these experiments will help to (re)establish reasonable limits for PUFA in the specifications.

5.4 NEXT STEPS TO ESTABLISH A METHOD

Three different biodiesel from different fish oils have been prepared in lab scale. These fish oil are being used as blending components for the RR tests.

In this context also the selected PUFA’s which will be determined by the method have been purchased as reference substances. The following PUFA’s, representing the predominant ones in marine oils and corresponding esters are: C 20:4, C 20:5, C 22:5, and C 22:6.

On internal standard the decision to use C23:0 methyl ester has been verified. Up to now no problems with overlapping signals with this IS could be observed.

IS amount has been set on 5mg/ml which is suitable for pure FAME from fish oil with high concentration of PUFA and also for blends with PUFA concentrations of 0.1% m/m.

Current status: A suitable method for the determination of PUFA methyl esters in biodiesel has been developed (see enclosed). The optimum IS standard amount has been evaluated

and verified by several internal test measurements. Concerning PUFA concentration the determination limit of 0.1% m/m for each compound (C 20:4, C 20:5, C 22:5, and C 22:6) can be noted. The linearity is given up to 5% m/m whereby the question how exact the result should be given, at PUFA concentrations far above the specification limit, has to be discussed.

5.5 FINAL STEPS TO ESTABLISH THE METHOD

The results of the performed internal RR test showed good statistic results concerning repeatability and reproducibility (Tab. 1).

Sample	1	2	3	4
No of participating laboratories	4	4	4	4
Mean value % (m/m)	**0.79**	**2.75**	**0.41**	**1.26**
Repeatability standard deviation, % (m/m)	0.002	0.003	0.001	0.002
Reproducibility standard deviation, % (m/m)	0.005	0.005	0.007	0.008
Repeatability limit, r, % (m/m)	0.006	0.008	0.003	0.006
Reproducibility limit, R, % (m/m)	0.014	0.015	0.020	0.022

Tab. 1: Statistical Results of Internal RR

In this context and to guarantee linear behaviour (area versus concentration) of the PUFA to be measured tests with single PUFA have been performed in concentration ranges between 0.1 – 2.5%. The results showed linear behaviour ($R^2 > 0.997$) for all four characterized PUFA (C 20:4, C 20:5, C 22:5, and C 22:6).

Final evaluation of the comments and observations made on the internal RR a pre-Draft method for CEN have been set up (see Annex 1).

IS amount has been set on 1mg/ml which is suitable for total PUFA concentrations between 0.1 – 3 % m/m. It has been found that for high PUFA concentrations (>10 % m/m) *e.g.* neat FAME from fish oil, the proposed method is not suitable and if applicable has to be adjusted.

15 European laboratories are participating in the final RR which could not be finished before the editorial deadline of this report. The receivable results and statistical evaluation will become part of the standard method subsequently given but unfortunately anyway after project end. In total 5 different fish oil methyl esters have been prepared and selected for this RR. The mentioned fish oils were selected from different sources (fish types) which represent the predominant varieties and to give information on the expectable oil characteristics in this context. Oils were: Salmon oil (crude), tuna oil (crude), and herring oil from Norwegian producers and cod liver oil (crude) and mackerel oil from Island. Samples (oil as well esters) were stored at -20°C until use. Additionally the methyl esters were additivated with 1000 mg/kg (BHT) to prevent oxidative degradation especially of the poly-unsaturated esters.

5.6 REFERENCES FOR THE METHOD BASICS

To find a promising base to develop a test method 154 literature citations have been evaluated:

Adlof,R.O. Application of silver ion chromatography to the separation of conjugated linoleic acid isomers. In: Advances in Conjugated Linoleic Acid Research, Volume 2., pp. 37-55 (edited by J.L. Sebedio, W.W. Christie & R.O. Adlof, AOCS Press, Champaign, IL.) (2003).

Adlof,R.O., Menzel,A. and Dorovska-Taran,V. Analysis of conjugated linoleic acid-enriched triacylglycerol mixtures by isocratic silver-ion high-performance liquid chromatography. J. Chromatogr. A, 953, 293-297 (2002).

Adlof,R. and Lamm,T. Fractionation of cis- and trans-oleic, linoleic, and conjugated linoleic fatty acid methyl esters by silver ion high-performance liquid chromatography. J. Chromatogr. A, 799, 329-332 (1998).

Adlof,R.O. The Lindlar-catalyzed reduction of methyl santalbate: A facile preparation of methyl 9-cis,11-trans-octadecadienoate-9,10-d(2). J. Am. Oil Chem. Soc., 76, 301-304 (1999).

Adlof,R.O. Separation of conjugated linoleic acid-containing mono-, di-and triacylglycerols by silver-ion high-performance liquid chromatography. Eur. J. Lipid Sci. Technol., 103, 614-617 (2001).

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Annex B Literature survey

BIOSCOPES

Biofuels Initiative: New Applications
Biodiesel: Improvement On Standards,
Coordination Of Producers & Ethanol Studies

Reference: TREN/D2-44/2005

LOT 1c Literature Survey

Draft
Final Report

Prepared for
European Commission
Directorate-General Energy and Transport

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1. Aim:

Within a literature study the state of the art of biodiesel production and use will be outlined. Production capacities and actual production of biodiesel in different countries worldwide will be estimated. The state of the specifications in different countries will be discussed with special focus on the differences in parameters and limits. Also an overview of the different use of biodiesel, either 100 % or blends with mineral diesel, will be given.

Special focus will be put on other oil sources than rape seed oil, especially the use of high linoleic oils with higher iodine values or the use of oils and fats with higher content of saturated fatty acids like animal fat or palm oil. It will be discussed, which limits will be out of specifications. A relationship between oil source, iodine value, and oxidation stability will be shown.

Engine experience and fleet tests with biodiesel from other sources than rape seed oil will be discussed.

The aim of the literature study is to identify possibilities to increase the quantities of biodiesel by using alternative feedstocks, to identify the need for further research and engine tests and the need for adaptations of the existing specifications EN 14214 and EN 590.

2. Introduction:

The world-wide biofuel industry and therein especially the biodiesel production have reached a tremendous annual growth during the last couple of years. This growth is not only limited on increasing production capacities but includes also economic, technological, logistic and distributive aspects and measures. Main responsible for these developments are legislative actions for the implementation of biofuels as fossil fuel substitutes as stated *e.g.* by the European Fuels Directive (Directive 2003/30/EC), the regulation of national and international quality standards and quality monitoring and of course measures for the reduction of pollutant emissions as required by the Kyoto target. The global expansion of biodiesel usage can be clearly underlined by a map reflecting the world-wide status of biodiesel initiatives as given in figure 1.

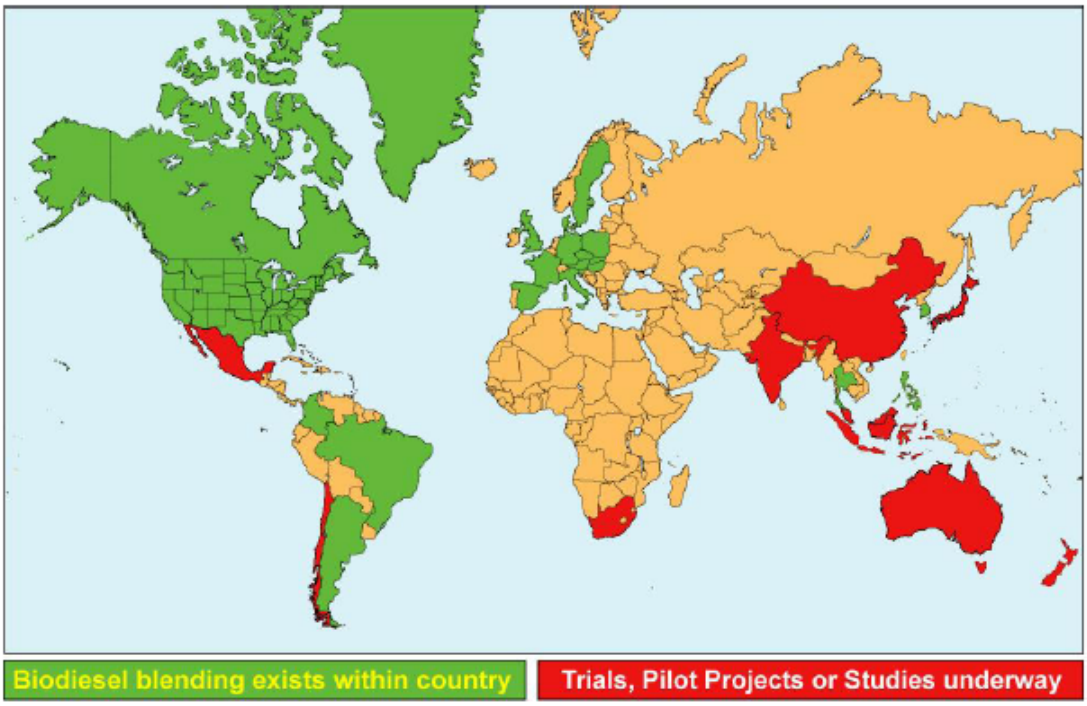


Fig. 1: Global View on Biodiesel Usage 2006

Source: IFQC Global Biofuels Center, August 2006.

In fact, if the global biodiesel production is classified into production amounts and feedstock consumption, the European situation is by far dominating the market (see 3.2). This can be pointed out furthermore by itemization of the main feedstocks for biodiesel production as shown in figure 2.

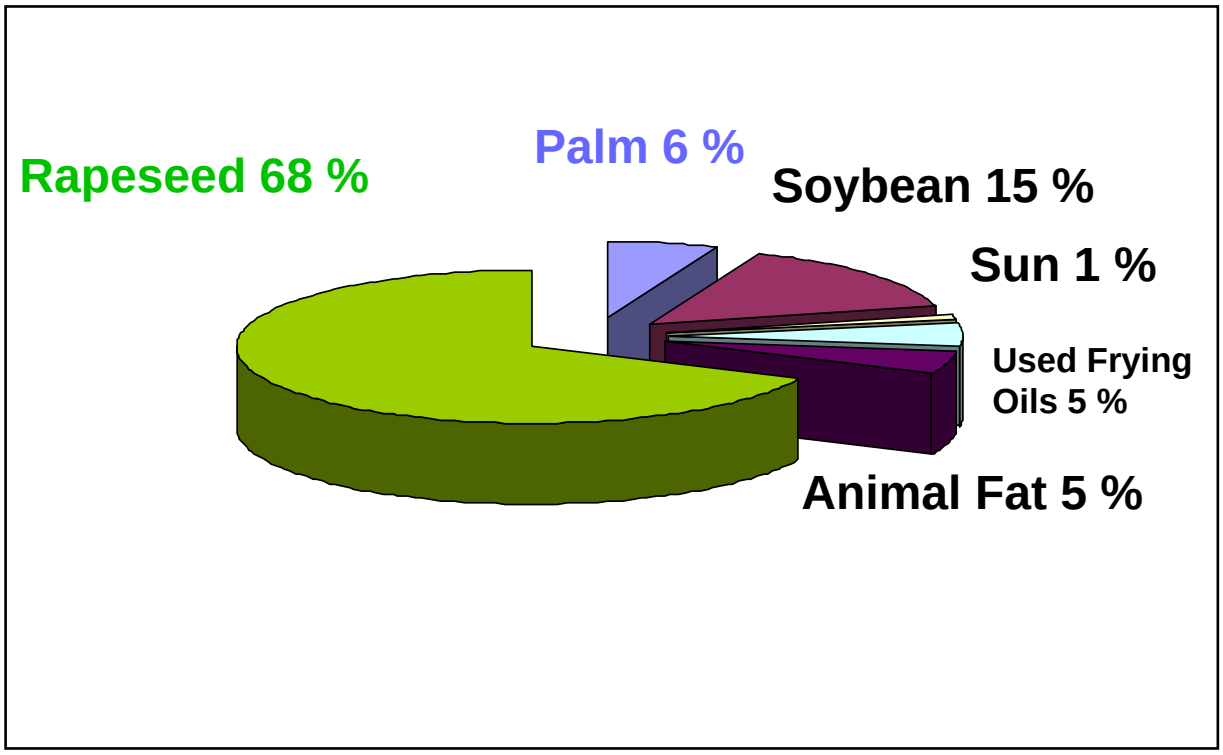


Fig. 2: Global Raw Material Sources for Today's Biodiesel Production, 2006

Meanwhile, this positive trend on establishing alternative fuel sources has become some problematic aspects especially focusing on the European situation. Due to the enormous demand for vegetable oils and fluctuating prices on the world market, the need for alternative "oil-sources" becomes more and more urgent. As a first result, new biodiesel production technologies which facilitate the use of *e.g*. waste cooking oils or even waste animal fats have been developed and already adopted in different plants. To ensure a uniform quality of the product independently of the raw material used for the production, the European Commission mandated CEN – the European Committee for Standardization – to develop standard specifications concerning minimum requirements and testing methods for biodiesel used as fuel for diesel engines and for heating purposes which finally have been adopted 2003 known as

EN 14214 standard for FAME. This quality requirement is setting a high level for biodiesel quality within the EU and has been used as guideline for many countries which are establishing their own national specific biofuel standards. Unfortunately and perhaps unintended, EN 14214 indirectly also regulates the choice of feedstock for biodiesel production. Especially the iodine value limited by EN 14214 at 120gI_2/100g prevent producers of using soybean oil as the worlds most produced vegetable oil available at much lower prices than compared to rapeseed oil. Of course the limit of iodine value has been set at this level as a result of intensive studies and experiences on this topic. Especially car manufacturers insisted on iodine value as parameter because of apprehensions concerning engine problems as a result of degradation products which are formed intensified at feedstocks (respectively biodiesel) with higher iodine value. So objections concerning influence of varying stability of biodiesel with different iodine values in terms of possible engine problems resulted in the current valid limitation of iodine value within EN 14214. But on the other hand further parameters describing directly or indirectly stability concerns of biodiesel are included in EN 14214 (oxidation stability, linolenic acid methyl ester content, poly-unsaturated methyl ester content). These parameters far better describe degradation tendencies of biodiesel and the question is now if the iodine value has become obsolete and might be reconsidered in terms of limit and necessity. So therefore the upcoming discussion and evaluation of different aspects concerning iodine value should give an indication about pros and cons of iodine value with special focus on biodiesel quality, emission criteria and engine experience. Finally, different scenarios will be described which evaluate the impact of an increased limit for iodine value as well as an exclusion of iodine value on biodiesel quality. Furthermore it will be pointed out if the current biodiesel specification contains enough parameter describing stability behavior of biodiesel and if iodine value from this point of view can be therefore changed or even replaced.

3. Biodiesel Production World-Wide:

3.1. Production Technology

There are different possibilities to classify the different biodiesel production technologies. It can be distinguished according to the type of catalyst between homogenously or heterogeneously catalyzed processes; or distinguished according to the reaction conditions between low and high temperature and pressure reactions; or between continuous or batch operation. On the other hand it is also possible to classify according to the type of feedstock. The so-called single feedstock technologies are using half or fully refined vegetable oils like rape-seed, soy-bean, sunflower etc. With these technologies the content of free fatty acids in the feedstock should be very low, so the formation of soaps is limited. Normally alkaline catalysts like sodium methoxide or potassium hydroxide are used, and the soaps formed as side products during the reaction are either removed by water washing steps or recycled by (post-)esterification with acid catalysts after glycerol phase conditioning. With this technology also a small amount of other feedstocks like recycled frying oil or higher acidic palm oil can be blended to the refined vegetable oils.

The so-called multi-feedstock technologies are additionally capable of processing feedstock with higher amounts of free fatty acids. Here a so-called pre-esterification of the free fatty acids is necessary, or during a high pressure and temperature process all fatty material is directly converted in FAME in one step. These processes are capable to process any type of feedstock, including acid oils, animal fats, high acidic palm oil or even fatty acids. The reaction conditions can easily be adapted to the change of feedstock.

Though a differentiation of these two technologies often is not very easy, especially with newer developments of technology, the terms single- and multi-feedstock technologies are broadly used in the biodiesel terminology, and therefore will be used as followed.

3.1.1. Single Feedstock Technologies

The biggest biodiesel production units with capacities of more than 100.000 t/y mainly use fully refined vegetable oils with low content of water and free fatty acids. In that case they use a solution of sodium methoxide or sodium hydroxide in methanol in order to get very low formation of soaps. After a continuous transesterification process, which mostly is conducted in 2 steps at moderately elevated temperature and ambient pressure, the glycerol layers, which are formed at the bottom layer of the reaction mixture, are separated and the raw methyl ester phase is further purified, mainly by different water washing steps. The final product is dried and can be used directly, without distillation, as biodiesel. Most of these biodiesel plants are combined with an oil seed crushing and refining facility, so it is possible to use highly refined oils as raw material. Also these big facilities mainly have an own glycerol purification technology including distillation of raw glycerol into pharmaceutical grade glycerol. Today the biggest biodiesel production plants mainly are not adapted to use oils with high content of free fatty acids like palm oil or waste oils.

In most single feedstock production plants the glycerol phase is further processed in order to get pharmaceutical grade glycerol. Excess methanol is removed by distillation and the glycerol is distilled under high vacuum and treated with charcoal. The yields for these single feedstock technologies are almost 100 %, because side reactions like saponification are kept to a minimum due to low water and free fatty acid content in the starting material.

3.1.2. Multi Feedstock Technologies

The so-called multi feedstock technologies are capable of processing all kinds of various feedstocks, including vegetable oils with higher content of free fatty acids like unrefined oils or palm oil but also waste oils or animal fats. The main difference to single feedstock technologies is the use of additional reaction steps, like pre-esterification of free fatty acids. So in a first step free fatty acids are pre-esterified

with the use of acidic catalysts, followed by one or two alkaline catalyzed transesterification steps. The raw fatty acid methyl esters are purified by water washing steps and additionally can be further refined by vacuum distillation. The main advantage of this technology is the fact that the yield of conversion of fatty acid material into fatty acid methyl esters is almost 100 %. The highest yield can be obtained, when remaining soaps in the glycerol layer are recycled by acidification of the glycerol and separation of free fatty acids, which can be reintroduced into the pre-esterification step or first step of transesterification[1]. Another approach for converting high acidic oils into fatty acid methyl esters is the conversion of fatty acids into glycerides, followed by traditional transesterification[2].

3.1.3. Small Scale Production Units

Especially in countries, where the biodiesel development just begins the production capacity of plants is mostly lower than 5.000 t/a, and using different feedstock and different production technologies. Mostly these plants have not been built by big biodiesel technology companies, but the technology has been developed by individual groups and organizations based on own experience and development. The glycerol layer mostly is used directly without any purification e.g. as substrate for biogas plants, or will be purified to be sold as raw glycerol. The catalyst for transesterification is mainly potassium hydroxide, because it leads to the highest conversion rates. Several of these production plants are organized as co-operatives, using vegetable oils produced locally, and also the biodiesel will be used by the members directly. Most of the very small production units don't have their own facilities for quality control, so the quality of the product might vary and it is not guaranteed to meet EN 14214.

3.1.4. Large Scale Production Units

In contrast to small scale production units, the currant trend in countries where biodiesel is already established (especially in Europe) the tendency of biodiesel plant sizes > 100.000 t/a is definitely observable. But also countries which have large feedstock amounts available (*e.g.* China, Indonesia, Brazil, USA) are planning to build large biodiesel plants with capacities up to 350.000 t/a (see table 2). Such large scale production units additionally have also special requirements on the location where they are built. Main aspect, due the large amount of feedstock delivery and product dispatch are the access to adequate transportation possibilities *e.g.* harbor, railway, navigable rivers, and motor-ways.

3.2. Production Capacities

The world-wide biodiesel production capacities and amounts increased dramatically during the last couple of years. Due to the high demand of biodiesel in order to fulfil the European Fuels Directive the number of large scale biodiesel production plants increased significantly. Although the total production amount increased, several EU Member States will have to enforce there activities on biodiesel production. Table 1 gives an overview on the currant biodiesel production amounts in Europe. Furthermore, other countries have also established several biodiesel production facilities and are planning to expand their capacities dramatically as demonstrated in table 2.

Country	2003	2004	2005	2006
Austria	50	100	125	134
Belgium			55	85
Cyprus			2	2
Czech Republic			188	203
Denmark	41	44	81	81
Estonia			10	20
Finland			0	0
France	500	502	532	775
Germany	1025	1088	1903	2681
Greece			35	75
Hungary			0	12
Ireland			0	1
Italy	420	419	827	857
Latvia			5	8
Lithuania			10	10
Luxemburg			0	0
Malta			2	3
Poland			100	150
Portugal			6	146
Slovakia			89	89
Slovenia			17	17
Spain		70	100	224
Sweden	8	8	12	52
UK	5	15	129	445
NL			0	0
TOTAL	2049	2246	4228	6070
EU-10			423	514

Tab.1: Production of Biodiesel in the EU (1000t)

Source: European Biodiesel Board[3].

Biodiesel Production Technologies 2006

Suppliers and Capacities

Status 7/06

No	Location AT	Year	Capacity tpy	Location BDI	Year	Capacity tpy	Location CD PROCESS system Connemann-ADM	Year	Capacity tpy	Location DesmetBallestra	Year	Capacity tpy	Location LURGI	Year	Capacity tpy	Location + others worldwide	Year	Type	Capacity tpy
1	Asperhofen	**91**	**200**	Mureck (A)	**93**	**9.000**	Leer	**91**	300	Livorno II (I)	05	**100.000**	Marl	01	**100.000**	Compiégne(F)	**93**	IFP	**20.000**
2	Ochsenfurt	00	**65.000**	Bruck (A)	**93**	**20.000**	Leer	**93**	7000	Thessaloniki(GR)	05	**20.000**	Malchin	02	**40.000**	Livorno (I)	**93**	Montedis	**80.000**
3	Schwarzheide	03	**100.000**	Oloumuc (CZ)	98	**30.000**	Leer	**95**	**120.000**	Torres Novas (E)	05	**40.000**	Marl	05	**100.000**	Rouen (F)	96	Henkel	**150.000**
4	Brunsbüttel	05	**150.000**	Kentucky(US)	03	**5.000**	Bratislava (SK)	98	**50.000**	Delaware (US)	06	**20.000**	Pamplona	01	**50.000**	Bitterfeld	99	sim. CD	**150.000**
5	Neuss	03	**150.000**	Malchin	00	**12.000**	Magdeburg I	00	**75.000**	Romania	06	100.000	Rostock	06	150.000	Schwedt	05	sim. CD	**200.000**
6	Jihlava (CZ)	98	**35.000**	Barcelona(E)	03	**6.000**	Hamburg I	01	**200.000**	Poland	06	50.000	Frankfurt	06	200.000	Wittenberge	99	xxx	**65.000**
7	Reus (E)	03	**50.000**	Niederpölln	01	**50.000**	Hamburg II	05	**300.000**	Brazil	06	100.000	Mannheim	06	100.000	Borken	01	xxx	**35.000**
8	Ecodasa?	05	**25.000**	Arnoldstein	04	**25.000**	Lisboa (P)	05	**100.000**	Tracopol (P)	06	66.000	Lülsdorf	06	125.000	Sêtes (F)	06	IFP	**160.000**
9	Kyritz	05	**30.000**	Motherwell	05	**50.000**	Sternberg	06	**150.000**	Arcor (E)	07	100.000	Cargill (US)	06	100.000	Karlshamn(SE)	06	NBT	**100.000**
10	Trzebinia (PL)	05	**100.000**	Australia	06	50.000	Lubmin	06	60.000	Brazil	07	50000	Schrobenhs	06	100.000	Verdun (F)	95	xxx	**100.000**
11	Santander (E)	06	150.000	Vienna (A)	06	95.000	Ocana (E)	06	100.000	Wroclaw (PL)	06	150.000	Neubrandbg	06	40.000	Zistersdf (A)	03	Energea	**30.000**
12	Chikago (US)	05	130.000	Huesca (E)	06	25.000	Mainz	06	275.000	PasirGudang MAL	06	100.000	Darwin (AUS)	06	150.000	Teesside (UK)	06	Energea	**250.000**
13	Sunoil (NL)	06	60.000	Litauen	06	100.000	Velva (USA)	06	250.000	Immingham (UK)	06	100.000	Piesteritz	06	200.000	Ancona (I)	95	xxx	**120.000**
14	Hungary	06	10.000	Lünen	06	18.000	Falkenhagen	06	100.000	Singapore	07	100.000	Cuenca (E)	06	35.000	Bari (I)	95	xxx	**50.000**
15	Saar	06	100.000	Barcelona II	06	25.000	Latur (IN)	06	15.000	Bulgaria	07	100.000	USA	05	**15.000**	Emmelev(DK)	02	xxx	**50.000**
16	Thailand	07	200.000	Sevilla (E)	07	50.000	Magdeburg II	06	200.000	Selangor (MAL)	07	100.000	USA	06	100.000				
17	Eberswalde	07	250.000	Almeria (E)	07	6.000	Ulsan (K)	06	100.000	Linares (E)	07	100.000	Ertvelde (B)	07	95.000				
18	Magdeburg	07	200.000	Lünen 2 (D)	07	50.000	VR China	06	250.000	Brazil	07	100.000	Serbia	07	100.000				
19	Madison (USA)	07	150.000	Arnoldstein 2 (A)	07	25.000	Seoul (K)	06	35.000	Torres Novas II (P)	07	40.000	Enns (A)	07	100.000	**+ estimate:**			
20	Marbach (D)	07	150.000	Ventspils (LT)	07	100.000	Porto (P)	06	100.000	Owensboro (USA)	07	150.000	Rotterdam	07	200.000	40-50 further plants in EU,			
21	Torun (PL)	07	150.000	Portugal	07	25.000	Valencia (E)	06	75.000	Brazil	07	100.000	Rotterdam	07	200.000	capac. 2-20.000 tpy diverse			**400.000**
22	Krakau (PL)	07	150.000	Dänemark	07	50.000	Czechovice (PL)	07	100.000	Malaysia	07	100.000	?		100.000	50-60 plants in US,worldwide			**350.000**
23	?		200.000	?		200.000	Malaysia	07	330.000	Guymon (USA)	07	100.000	?		200.000	+50 plants under constr.			400.000
24							Clairpool (USA)	07	350.000	Seoul (KR)	07	200.000	?		200.000				
27							Bioestry (PL)	07	60.000	Malaysia	07	100.000							
28							Malaysia	07	200.000	Immingham II (UK)	07	100.000							
29							Camanche (USA)	07	200.000	?		100.000							
30							Brasilien	07	250.000	?		200.000				xxx = own technology			
31							Indonesia	07	250.000							grey background = operating plants			
32				**CD PROCESS**		offered by:	Indonesia	07	350.000										
33				**GEA-Westfalia**		**LIPICO**	Indonesia	07	350.000										
34				**MAN-Ferrostaal**		**Buss-SMS**	Bratislava II (SK)	07	100.000										
				CIMBRIA-SKET															
				TecnicasReunidas															
				CROWN															
35				**BRATNEY**		see left:	Licensees for CD PROCESS system Connemann-ADM												
		Sum	**2.605.200**			**1026.0.00**			**5.102.300**			**2.686.000**			**2.800.000**				**2.710.00**
	Total Sum = 16.929.500			**tpy Biodiesel Worldwide 07**												c: connemann-ADM 8/06			

Tab. 2: Estimated Production Capacities 2007

Source: ABI

4. Feedstocks:

The global demand for oils/fats, both for food and non-food purposes, is anticipated to expand significantly: during 2006/07 (table 2), world consumption is expected to increase by almost 6 million tonnes or 4 percent. An important factor is the fast growing use of oils/fats as fuels and as feedstock for biodiesel production. Such utilization is expected to expand further in the European Union and the United States, while production is starting in various other countries, including Argentina, Brazil, Canada, Indonesia, Malaysia and the Philippines. The key oils commonly used are soybean and rapeseed oil, but palm and coconut oil as well as animal fats are also being used. Private sector investment into the development of biodiesel industries continues to be strong, irrespective of the uncertain development of mineral oil prices and of the possibility that plants may not be running at full capacity. Government incentives and other public support measures, together with existing or prospected mandatory blending requirements explain this trend. According to private sources, global utilization of oils/fats as biofuels should exceed 10 percent of total consumption in 2006/07. As to total consumption of oils/fats, the anticipated reduction of rapeseed, groundnut and sunflower oil supplies should increase the dependence on soy and palm oil in 2006/07. Together these two oils should account for half of total consumption. Traditionally, the bulk of the expansion in global demand of vegetable oils occurs in the developing world. However, in the last two years, considerable growth has also occurred among developed nations due to biofuel production in these countries, a trend that is expected to continue in 2006/07. Among developing countries, demand expansion is expected to be led by Asia. Particularly noteworthy are China, where population and GDP growth drives food and non-food consumption, and Malaysia, with rising production of palm oil for use as fuel and biodiesel feedstock[4].

	2004/05	2005/06 estim.	2006/07 forecast
		million tons	
Soybeans	216.1	218.7	224.3
Cottonseed	44.6	42.3	43.4
Rapeseed	45.9	48.8	46.5
Groundnuts (unshelled)	34.7	35.4	33.8
Sunflower	25.4	30	29.6
Palm kernels	8.9	9.5	9.6
Copra	5.2	5.2	5.4
Total	**380.8**	**389.9**	**392.6**

Tab. 2: World Production of Major Oilseeds

Source FAO[4].

A more detailed information on the production of 17 oils and fats since 2003/2004 (including a forecast for 2006/2007) is given in table 4.

	2003/2004	2004/2005	2005/2006	2006/2007	increase
World total	**130.3**	**138.4**	**145.8**	**152.9**	**22.6**
Soybean	30.9	32.9	34.8	36.6	5.7
Palm	29.9	33.3	35.2	37.6	7.7
Rape/Canola	14.4	15.7	17.7	18.6	4.4
Sunflower	9.6	9.4	10.5	10.8	1.2
Cottonseed	4.2	5.0	4.9	5.0	0.8
Peanut	4.8	4.5	4.6	4.5	-0.3
Corn	2.0	2.1	2.2	2.3	0.3
Olive	3.2	3.0	2.7	3.1	-0.1
Palm-kernel	3.5	3.9	4.1	4.3	0.8
Coconut	3.1	3.2	3.3	3.3	0.2
Butter	6.4	6.6	6.8	7.0	0.6
Lard	7.3	7.5	7.7	7.9	0.6
Tallow	8.1	8.3	8.4	8.5	0.4

Other commodities not included in this table are fish oil (~1.0 MT), sesame (~0.8 MT), linseed (~0.7 MT), and castor (~0.5 MT). MT; million metric tons.

Tab. 4: Production of 17 Oils and Fats

Source: INFORM adapted from *Oil World Annual 2006*[5].

Additional data including interesting predictions for the period 2005/06 to 2015/16 have been evaluated by the Malaysian Palm Oil Board (MPOB) and by the Food and Agriculture Policy Research Institute (FAPRI) as given in table 5.

	MOPT (MT)				FAPRI (MT)			
	1995	2005	10-year increase		2005/06	2015/16	10-year increase	
			MT	%			MT	%
Soybean	20.4	33.3	12.9	63	34.1	44.9	10.8	32
Palm	15.2	33.3	18.1	119	34.3	50.4	16.1	47
Rapeseed	11.0	16.0	5.0	46	16.2	19.9	3.7	23.0
Sunflower	8.6	9.7	1.1	13	10.3	12.2	1.9	18
Palm-kernel	1.9	3.9	2.0	101	4.2	5.8	1.6	40
Sub total	**57.1**	**96.2**	**39.1**	**68**	**99.1**	**133.2**	**34.1**	**34**
Peanut	4.4	4.5	0.1	2	4.8	5.3	0.5	12
Coconut	3.3	3.1	-0.2	-6				
Cottonseed	3.9	5.0	1.1	29				
Corn	1.9	2.1	0.2	13				
Olive	1.9	2.9	1.0	54				
Castor	0.5	0.5	0	12				
Sesame	0.6	0.8	0.2	40				
Linseed	0.7	0.6	-0.1	-13				
Vegetable oils (13)	**74.3**	**115.7**	**41.4**	**56**				
Butter	5.7	6.5	0.8	15				
Lard	5.7	7.5	1.8	33				
Tallow	7.5	8.2	0.7	9				
Fish	1.3	1.1	-0.2	-18				
Animal Fats (4)	**20.2**	**23.3**	**3.1**	**16**				
World total (17)	**94.4**	**139**	**44.5**	**47**				

MT; million metric tons

Tab. 5: Production (MT) of Oils and Fats in the Period 1995 to 2005
with Predictions for the Period 2005/06 to 2015/16
Source: INFORM[5].

The above mentioned increased demand for oils for biodiesel production especially in the EU can be underlined by production statistics as given in table 1 and 2. In this context, the dependency of European biodiesel producers on rapeseed-oil as traditional feedstock can be pointed out in table 6. More than 50% of the available rapeseed oil is being used for biofuel production in 2006 and the proportion will be increased according forecasts for 2007 and beyond.

October/September

	2001/02	2002/03	2003/04	2004/05	2005/06*
Total	4.000	4.140	4.370	5.270	6.260
- Food Sector	2.880	2.690	2.600	2.670	2.600
- Non Food Sector (a)	1.120	1.450	1.770	2.600	3.660
thereof RME-biodiesel (b)	1.120	1.450	1.730	2.440	3.320
- Direct Use (c)	-	-	40	160	340

*estimates

(a) mainly biodiesel

(b) rape methyl ester

(c) direct use of refined rape oil as fuel

Tab. 5: EU-25 Rapeseed Oil Utilization (1000t)

Source Report from the Commission to the Council[6].

Increasing feedstock demand connected with increasing rapeseed-oil prices on the world market induced producers to search for (cheaper) alternatives. The current development on biofuel industry enforced the need of opening the feedstock market also for other suitable vegetable oil sources in order to fulfill biofuel quotas given by the European Fuels Directive. However, the current European biodiesel specification limits the use of feedstock. Blending of rapeseed-oil with other oils is becoming more and more important in biodiesel production taking into consideration that the quality of the final product has to meet the given specifications. Concerning other possible feedstock sources especially the iodine value limitation is the most restricting parameter. High iodine value feedstocks are often discussed of having poor stability and therefore higher engine damaging potential. On the other hand feedstocks with

low iodine values (= higher degree of saturation) fail in its low temperature behavior. Unfortunately most of these mentioned oils and fats would be available on the world market in larger amounts at (compared to rapeseed-oil) much reasonable prices. An overview on iodine values of biodiesel produced from other potential feedstocks is given in table 7.

Higher Unsaturated:

Biodiesel	Iodine No.
Soybean	117-143
Sunflower	110-143
Safflower	126-152
Linseed	168-204
Corn	103-140
Camelina	-180
Fish-oil	-200

Higher Saturated:

Biodiesel	Iodine No.
Tallow	35-48
Palm	35-61
Palm kernel	12-18
Coconut	6-12
Babassu	10-18
Lard	30-40
Palm stearine	35-40

Tab. 7: Iodine Number of Biodiesel from Other Feedstocks

Other possible raw materials with iodine values between 90 and 120 gI_2/100g could be *e.g.* cotton-seed oil, castor oil, poppy-seed oil, Jatropha, olive oil, sesame oil and peanut oil. Specific and characteristic differences in quality of biodiesel from all the mentioned feedstocks will be discussed in the corresponding chapter [see 6.2.].

5. Iodine Value and Standardization:

Since the world-wide first biodiesel standard for rapeseed oil methyl esters (RME) has been established in 1991 in Austria[7] it took three years until the iodine value as a parameter describing stability behavior of biofuels has been included in the German specifications for vegetable oil methyl esters[8]. The limit of iodine value in the DIN V 51606 specifications for vegetable oil methyl esters was set at that time at ≤ 115 gI_2/100g in accordance on the typical iodine value of rapeseed oil. In the following years, different European countries established their own national standards on biodiesel which mostly also contain a parameter for iodine value but with varying

limits. Sweden has defined a limit of ≤ 125 gI_2/100g in the SS 15 54 36 standard in 1996[9]; Austria set a limit of ≤ 120 gI_2/100g in the ON C 1191 in 1997 for fatty acid methyl ester[10]; France implemented two biofuel specifications in 1997 with two different limits for iodine value. The limit for ≤5% blends in fossil diesel fuel is defined with ≤ 115 gI_2/100g[11] and the limit for ≤5% blends in domestic heating fuel with ≤ 135 gI_2/100g[12]. In 2001, Italy implemented a minimum requirement concerning iodine value in their standards for biofuel application as automotive fuel or for heating limited with ≤ 120 gI_2/100g[13,14]. The different country specific biofuel standards, after reevaluation of parameters and limits, were integrated in the European biodiesel specifications for fatty acid methyl esters EN 14214 and EN 14213 in July 2003[15,16]. The limits for iodine value were set at ≤ 120 gI_2/100g for FAME used as automotive fuel and ≤ 130 gI_2/100g for FAME used for heating purposes. Concerning the quality parameters, all member countries of the EC have to fulfill the given requirements except for low temperature behavior where each member state can define a country specific limit depending on the climatic conditions. In fact, concerning iodine value Spain has defined a different national limitation. Spain's renewable fuels decree (1700/2003) sets the technical standards for biofuels, including a deviation from the EC directive in Article 7, Paragraph 3 regarding maximum iodine content (140 vs. 120 in the EU directive), which, as a result facilitates the use of soybean and sunflower oil in the production of biodiesel. This decree is valid only for B100 use of biodiesel whenever biodiesel is used as blending component the limit of ≤120gI_2/100g is still valid.

Outside the EU the situation concerning iodine value limitations is different. Iodine value is excluded or not yet defined in numerous national biodiesel standards. Up to now iodine value is excluded in the ASTM specifications[17], the Australian biodiesel specifications[18], the Korean specifications, and the Canadian specifications (CAN/CGSB-3.520). The Canadian B1 – B5 standard requires that the biodiesel component blended into the fuel meets either ASTM D6751 or EN 14214 which is (among other parameters) an inconsistence concerning iodine value. A specification for blends with B6 to B20 in Canada is being developed. However many other countries have not definitely defined limits for iodine value *e.g.* New Zealand (NZS 7500:2005 Automotive biodiesel - Specification for manufacture and blending) and

Brazil (ANP 255 “take note”)[19]. On the other hand an iodine value limit ≤120gI_2/100g is included in the provisional South African biodiesel standard which is based mainly on EN 14214 as well as in the proposed Japanese biodiesel specifications (JIS). Indonesia set a limit on iodine value of ≤115gI_2/100g (SNI 04-7182-2006) and Argentina of ≤150gI_2/100g[19].

However, world-wide upcoming specifications on biodiesel introduced so far or even under development, are mainly based on ASTM D6751 or EN 14214 or a “mixture” thereof. Therefore many countries are interested on the further development of the mentioned standards especially focussing on changes in iodine value which limits the feedstock for production as well as restricts biofuel trade.

6. Iodine Value and EN 14214 Parameters

6.1. General Considerations

The iodine value is a measure of total unsaturation within a mixture of fatty materials, regardless of the relative shares of mono-, di-, tri-, and polyunsaturated compounds. It is expressed in grams of iodine which react with 100 g of the respective sample. Iodine value currently is limited to ≤ 120 gI_2/100g in the European biodiesel fuel specification EN 14214 respectively limited to ≤ 130 gI_2/100g in the European biodiesel for heating specification EN 14213. A more detailed comparison of iodine value limits within the different world-wide biofuels specifications is given in the corresponding chapter [see 5].

Engine manufacturers have long argued that fuels with high iodine value tend to polymerize and form deposits on engine nozzles, piston rings and piston ring grooves, when they are heated[20]. Highly unsaturated compounds have also been linked with decreased oxidation stability, causing the formation of various degradation products, which can negatively affect engine operability [see also 7]. Moreover, unsaturated esters introduced into the engine oil are suspected of forming

high-molecular compounds, which may reduce the lubricating quality[21]. Iodine value has found to correlate with viscosity and Cetane number, which both decrease with increasing degree of unsaturation. The limit of 120 gI_2/100g in the European biodiesel fuel specification excludes several promising oil sources such as soybean- or sunflower oil from serving as raw materials for biodiesel production, unless the resulting esters are blended with suitable biodiesel types or fossil diesel fuel. However, the results of various engine tests indicate that polymerization reactions occur to a significantly extent only in fatty acid esters containing three or more double bonds[22,23,244] which merely constitute a small share of the fatty acid patterns of soybean- or sunflower seed oil. The presence of these compounds even in small amounts has disproportionately strong effects on oxidative stability as well[25]. It has been argued that this is due to the fact that bis-allylic positions within ester molecules are particularly prone to oxidative attack. So therefore linolenic acid methyl ester (with double bonds at Δ9, Δ12 and Δ15, containing two bis-allylic positions at C-11 and C-14), shows dramatically decreased oxidation stability as compared to saturated and also mono- and di-unsaturated esters of the same chain length. Therefore, it might make sense to limit the content of highly unsaturated compounds within biodiesel fuels rather than the total degree of unsaturation as it is expressed by iodine value[22,26]. Following this line of argumentation, it was suggested replacing iodine value in biodiesel standards by parameters such as “allylic position equivalents” and “bis-allylic position equivalents”, which would far better correlate with the actual oxidative stability of a given sample[27].
In this context, genetic engineering or conventional plant breeding might be the key to a more favorable fatty acid composition for seed oils displaying high iodine values. So engine and emission tests have successfully been conduced on high oleic acid sunflower methyl ester fuels. Enriched in oleic acid, and with only a negligible content of linoleic acid, these fuels show iodine values well below the limit of EN 14214[28].

Two procedures for the determination of iodine value are provided by EN 14214. On the one hand, a titrimetric method using Wijs reagent is suggested (EN 14111). Here the dissolved sample is reacted with a defined amount of Wijs solution (containing iodine monochloride in glacial acetic acid) to facilitate the addition of the halogen to

all carbon-carbon double bonds contained. As a next step the mixture is allowed to react with potassium iodide solution. ICl which was not consumed in the first step now oxidizes an equivalent amount of KI to I_2. The liberated elemental iodine is titrated with sodium thiosulfate. After an analogous analysis of a blank solution, iodine value can be calculated from the obtained results.

Alternatively, iodine value can also be calculated from the relative methyl ester contents as determined by capillary gas chromatography according to EN 14103. This method is admitted as a viable alternative to the titrimetric procedure in an annex of EN 14214. Here iodine value is obtained by adding up the respective contributions of each unsaturated ester contained in the sample, multiplying the methyl ester mass percents with conversion factors characteristic of each compound (see table 8). This method is a further development of the AOCS official method Cd 1c-85 for the determination of iodine value in edible oils[29].

Methyl Ester	Conversion Factor	Methyl Ester	Conversion Factor
C 12:1	1.1953	C 20:4	3.1874
C 14:1	1.0558	C 20:5	4.0096
C 16:1	0.9454	C 22:1	0.7198
C 18:1	0.8560	C 22:2	1.4479
C 18:2	1.7237	C 22:3	2.1844
C 18:3	2.6034	C 22:4	2.9295
C 18:4	3.4954	C 22:5	3.6822
C 20:1	0.7820	C 22:6	3.7076
C 20:3	2.3755	C 24:1	0.6785

Tab.8: Conversion Factors for Methyl Esters

6.2. Influence of Iodine Value on EN 14214 Quality Parameters

In order to give an idea on the direct or indirect influence of feedstocks with iodine values significantly different to the typical range of rapeseed oil, a comparison between the effects of such feedstocks on EN 14214 quality parameters will be given. These results will be the basis together with engine experience and fleet test

observations, for the description and evaluation of possible scenarios how limits for iodine value can be handled in upcoming adopted European biodiesel quality standards.

6.2.1. Density:

The influence of feedstocks with low or high iodine values on the density of the produced biodiesel is only very small. Of course slight differences (increase, decrease) can be observed but for the given limits of EN 14214 no problems will be expected.

6.2.2. Viscosity:

Similar to density observations, no significant influences on viscosity are expected. The iodine value of the feedstock is not directly responsible for differences in viscosity. But it should be pointed out that some feedstocks, especially castor oil, due to the uncommon fatty acid distribution (in case of castor oil the high content of ricinoleic acid) can lead to biodiesel viscosities above the specification limit.

6.2.3. Flash Point:

Influences of iodine value on flash point are not expected because the flash points of the different saturated and unsaturated fatty acid methyl esters are very similar and far above the given limit. Influences on flash point are mainly caused by the technology used for biodiesel production which means that a complete removal of methanol must be guaranteed.

6.2.4. Sulfur Content:

Sulfur content can be influenced significantly by the feedstock. But the compounds affecting sulfur content must not be correlated to the iodine value of the feedstock. Influencing factors are *e.g.* content of glucosinolates (grade of refining of the vegetable oil, seed variety), amino acids and proteins. Especially feedstock coming from animal fat tends to have higher sulfur contents than compared to vegetable oils.

6.2.5. Carbon Residue:

This parameter can not be evaluated all-embracing because especially for high iodine value feedstocks the data material is insufficient. But some conclusions can be given. Feedstocks, respectively biodiesel with iodine values up to 120 gI_2/100g, did not have any significant influence on carbon residue. Excluded in this context are feedstocks coming from waste and recycled material (recycled frying oils, and waste animal fats). Biodiesel from such feedstock can have carbon residue values above the given limit but this effect is not caused by iodine value itself but from the content of polymerized triglycerides in the feedstock[73]. Such polymers are mostly formed during the several heating cycles which the oil undergoes and remain partially as corresponding polymeric fatty acid methyl esters in biodiesel. Biodiesel with iodine values up to 160 gI_2/100g did not show any significant increases in carbon residue compared to “normal” biodiesel. Problematic is the situation on biodiesel with very high iodine values. However vegetable oils with iodine values in the region of up to 200 gI_2/100g did not lead to significant changes in carbon residue of the corresponding methyl esters. On the contrary biodiesel derived from marine (fish) oils which have a high content of poly-unsaturated fatty acids in the feedstock tend to have carbon residue values outside the given specification limit. These observations have not been investigated sufficiently enough but the content of poly-unsaturated acids has obviously a dramatic influence on carbon residue. So therefore a clear statement on the influence of high iodine values on carbon residue can not be given because plant oils on the one hand did not show any significant effect whereas on the other hand marine fats and oils and waste materials can have an impact.

6.2.6. Cetane Number:

This parameter is influenced by the chain length of the fatty acid ester as well as by the level of unsaturation. In summary, the results are that Cetane number is lower with increasing unsaturation and higher with increasing chain length, i.e., uninterrupted CH_2 moieties. So therefore iodine value directly influences Cetane

number of the biodiesel leading to values below the given specification limit in case of biodiesel with high iodine values[30,32,32].

6.2.7. Sulfated Ash Content:

Ash content is not influenced by the iodine value of the feedstock itself but from the content of inorganic residues during biodiesel processing. So therefore this value is only affected by technological problems.

6.2.8. Water Content:

Water content depends on the quality (purity) of the feedstock as well as on the technology used for biodiesel purification. Iodine value has no influence. However it should be mentioned that water content of biodiesel is also a result of the hygroscopic nature of fatty acid methyl esters. If there is a possible influence of unsaturation on hygroscopic behavior has not been investigated so far.

6.2.9. Total Contamination:

Total contamination is not affected by iodine value but rather by the feedstock quality and process technology.

6.2.10. Copper Strip Corrosion:

This parameter is difficult to be evaluated due to the lack of experimental data. An effect of iodine value is absolutely possible because iodine value influences the stability of the ester and therefore also might influence this kind of determination but for more detailed information additional tests should be carried out.

6.2.11. Oxidation Stability:

Oxidation stability of biodiesel is definitely the mostly discussed parameter in connection with iodine value. The background is that iodine value has been predominately introduced as parameter describing the degradation tendency of a

fatty acid methyl ester. So iodine value can be classified as a parameter giving an idea on the stability of biodiesel. In fact this (iodine) value can not be taken as a definite indicator how long a sample is stable but could give an indication. Therefore, the parameter oxidation stability (given in [hours] has been introduced, which better describes the rate of degradation under forced aging conditions of a fatty acid methyl ester sample. Oxidation stability is therefore a parameter (besides linolenic acid methyl ester content) which far better describes the degradation tendency and therefore the stability of biodiesel. On that reason also in the U.S. the importance of a stability describing parameter has been identified and therefore the parameter oxidation stability (minimum requirement 3 hours) has recently been introduced into the ASTM biodiesel specifications The important question is if there is a correlation between iodine value and oxidation stability which could help to predict the stability when feedstocks others than rapeseed-oil are being used for production. If feedstocks are purchased iodine values are more often known than oxidation stability (whereby actually both parameters should be given in the certificate of analysis if oil quality has to be evaluated). The current opinion is that oxidation stability is correlated with iodine value because the higher the content of poly-unsaturated fatty acids (fatty acids with 3 or more double bonds) and therefore the iodine value the lower should be the measured oxidation stability. In fact, higher iodine values are correlating with lower oxidation stability but that does not mean that lower iodine values automatically should lead to higher oxidation. The reason is that the stability of biodiesel is a combination of several factors which interact. First of all, the stability is influenced by the content of (poly-) unsaturated fatty acid esters. The double bonds of the ester chain are very attractive for oxygen attack leading to peroxides and hydroperoxides. During further degradation processes these products form smaller molecules like aldehydes, ketons, and short chain organic acids which are responsible for problems within the engines. On the other hand double bonds are able to react thermally induced with other fatty acid ester chains forming polymers often classified as sediments gums and residues which lead to filter blocking and injector failures. However, these mentioned behaviors are going hand in hand with increasing iodine values. But on the other hand, the oxidation stability of fats and oils and the corresponding methyl esters is influenced also by the content of natural

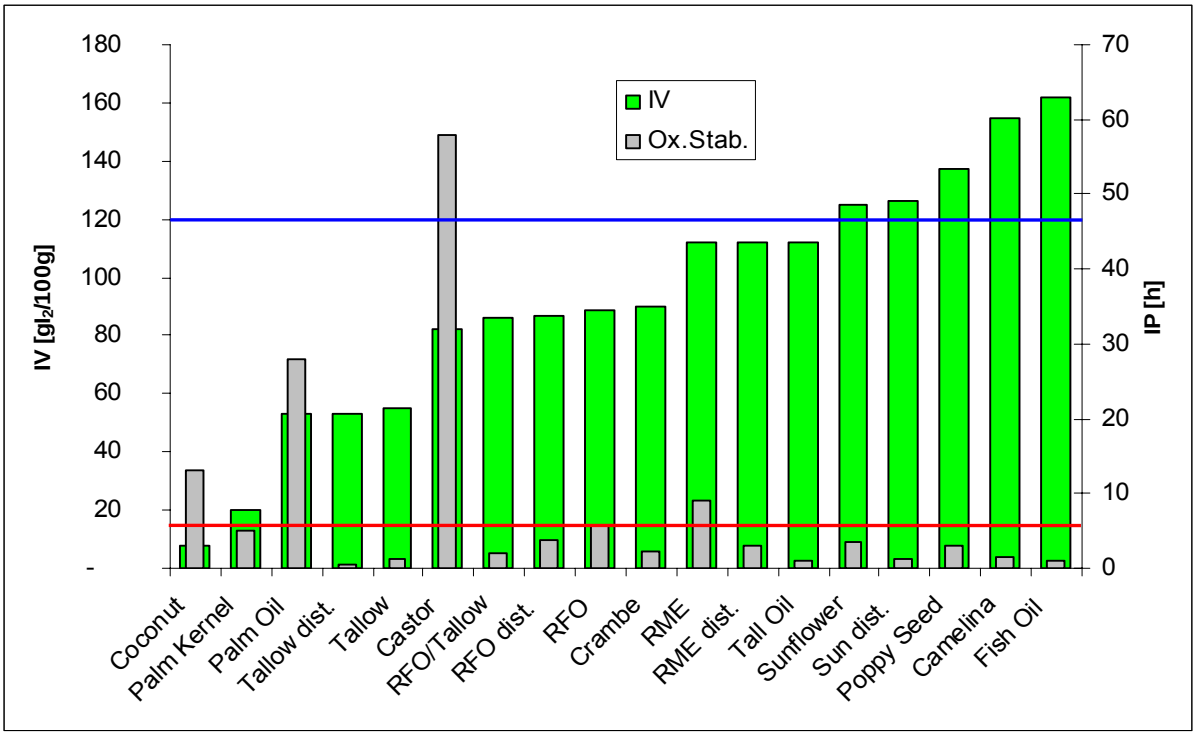
protecting compounds (antioxidants) as well as by the production technology used. Every vegetable oil or fat has a specific content of different mostly phenolic type antioxidants (tocopherols, carotenoids,..) which naturally protect against oxidative degradation. As an example the content of natural antioxidants in different biodiesel samples is given in table 9.

Sample	α-Tocopherol [mg/kg]	γ-Tocopherol [mg/kg]	Carotenoids [mg/kg][c]
RU[a]	263	320	70
RD	98	142	n.d.
SU	264	12	n.d.
SD	84	nd.	n.d.
TU	n.d.	n.d.	n.d.
TD	n.d.	n.d.	n.d.
UU	80	77	n.d.
UD	trace[b]	32	n.d.

[a]Abbreviations: RU, Rapeseed-ME undistilled; RD, Rapeseed-ME distilled; SU, Sunflower-ME undistilled; SD, Sunflower-ME distilled; TU, Tallow-ME undistilled; TD, Tallow-ME distilled; UU, Used Frying Oil-ME undistilled; UD, Uused Frying Oil-ME distilled; n.d., not detectable.
[b]Bellow 10mg/kg.
[c]Carotenoids were calculated by using carotene as reference.

Tab. 9: Content of Natural Antioxidants
Source BIOSTAB Project Results[33].

Especially two important objects can be pointed out. The content of natural antioxidants is strongly dependent on the feedstock used. Especially animal fats, used cooking oils, and also coconut fat (not mentioned in table 8) have very low levels of natural antioxidants. This should lead to lower oxidation stabilities although the iodine value is very low. On the other hand, highly purified biodiesel, *e.g.* done by distillation after transesterification in order to remove contaminants and by-products, is also characterized by lower antioxidant levels, which is in fact a negative side effect of the purification. For this reason such products should also have lower oxidation stabilities independently on the iodine value of the feedstock used. In fact these predictions can be underlined by a comparison of iodine value versus measured oxidation stabilities of different biodiesel samples as given in figure 3.



red line = stability limit, blue line = IV limit of EN14214

Fig. 3: Iodine Value versus Oxidation Stability of Biodiesel

Biodiesel with iodine values >120gI_2/100g without any exception had oxidation stabilities far below the given limit of 6 hours. But on the other hand, also biodiesel with low iodine values can have measured oxidation stabilities below this limit. Especially feedstocks which contain almost no natural antioxidants (*e.g.* tallow) or distilled products have very low oxidation stabilities.

Due to the limitation of oxidation stability of ≥ 6h induction period at 110°C given by EN 14214 it is necessary to additivate biodiesel with appropriate antioxidants in order to increase the stability. A wide range of antioxidants have been tested and evaluated especially during the BIOSTAB project and some of these products meanwhile are commercially used for biodiesel[33,74,75]. However, these additives only influence the stability and do not change anything on the fatty acid distribution respectively the iodine value of a biodiesel sample. Therefore, measuring iodine value of an additivated sample does not give any information on its stability. The only argument that can be underlined is, that biodiesel with high iodine values are much more difficult to additivate (higher amounts, more specific antioxidants needed) in an

appropriate way. The specification limit for oxidation stability can hardly be reached. On the other hand low iodine value feedstocks and biodiesel are can be stabilized more easily even with much lower amounts of antioxidants added.
Summarizing it can be said that iodine value itself is not the most suitable parameter describing the stability of biodiesel. However, the question is if oxidation stability and especially the method used for the determination and linolenic acid methyl ester content and polyunsaturated fatty acid esters are sufficient.

6.2.12. Acid Value:

Acid values can not be correlated with iodine value of the biofuel. This value is mainly influence by the content of free fatty acids in the ester and therefore affected by feedstock quality and processing technology used.

6.2.13. Linolenic Acid Methyl Ester:

It is of course evident that this parameter is mainly influenced by the iodine value of the feedstock respectively biodiesel. Feedstocks with higher content of linolenic acid are *e.g.* camelina oil (38%), linseed oil (45-70%), soybean oil (4-10%), high erucic rapeseed oil (7-12%),and walnut oil (9-15%). But it should be pointed out that high iodine value must not lead to high contents of linolenic acid methyl ester and a statement in this connection is not valid. The content depends on the fatty acid distribution of the feedstock. So therefore it is more correct to argue that biodiesel with high content of linolenic acid methyl ester therefore has also high iodine values. However, linolenic acid methyl ester content is an important parameter for exclusion of instable feedstocks.

6.2.14. Polyunsaturated Methyl Esters:

In this context the same argumentation than mentioned at linolenic acid ester content is valid. High content of polyunsaturated esters predominately found in biodiesel from marine oils, lead to high numbers for iodine value. High iodine value is not automatically an indicator for high content of poly-unsaturated fatty acid methyl

esters. In this context it should be also mentioned that polyunsaturated methyl ester content in another very important parameter for predicting stability.

6.2.15. Methanol Content:

Methanol content is not affected by iodine value but mainly by process technology (methanol recovery).

6.2.16. Glycerides (mono-, di-, tri-) and Glycerol

Considerations on the influence of iodine value on glyceride and glycerol content have to be done from two different aspects. The first aspect is regarding to the glyceride and glycerol content of the biodiesel itself. Basically, these parameters are influenced only by process technology which means how complete the transesterification of the oil has been performed. Therefore, a correlation between iodine value and glyceride/glycerol content cannot be found. On the other hand the aspect of suitability of the method for the determination of these parameters on biodiesel from different feedstocks should be kept in mind. Iodine value reflects also the heterogeneity of the fatty acid distribution of the oils and fats. Feedstocks with low iodine values are mostly characterized by high content of saturated and even uncommon fatty acids. As an example coconut or palm kernel oil have high contents of lauric-, myristic-, and other shorter chain fatty acids. For such products it can be clearly stated that the determination method EN 14105 is not suitable for evaluation of glyceride/glycerol content (but these observations are part of BIOSCOPES Lot 1a and will be discussed there). Summarizing it can be said that especially if low iodine value feedstocks (palm, palm kernel, coconut) which have high potential of being used in future as cheap biodiesel feedstock, will have to lead to an re-evaluation or adoption of EN 14105 method.

6.2.17. Ester Content:

For the ester content of a biodiesel sample the same statement as discussed on glyceride/glycerol method is valid. No influence of iodine value on ester content is

expected but the method for the determination has to be adopted which is also part of BIOSCOPES Lot 1a.

6.2.18. Group I Metals:

The content of group I metals is not influenced by the iodine value of the feedstock.

6.2.19. Group II Metals:

The content of group I metals is not influenced by the iodine value of the feedstock.

6.2.20. Phosphorus Content:

The content of group I metals is not influenced by the iodine value of the feedstock.

6.2.21. Low Temperature Behavior – CFPP:

Low temperature behavior of biodiesel is defined mainly by the content of saturated fatty acid esters in the biofuel. So therefore the iodine value can be correlated with CFPP values. However, this is true only for decreasing iodine value. The lower the iodine value and therefore the higher the amount of saturated fatty acid esters the higher is the resulting CFPP. Typical examples are biodiesel produced from animal fats, coconut fat, or palm oil with CFPP values mostly > +5°C. On the opposite, increasing iodine value (increased content of unsaturated fatty acid esters) should lead to decreasing CFPP values. But in fact such forecasts can not be verified in practice. As an example: rapeseed oil methyl esters normally have CFPP values between -10 to -15°C and an iodine value in the range of 115gI_2/100g. Soybean oil or sunflower oil methyl esters with iodine values in the range of 130gI_2/100g showed CFPP values between -6 and -2°C depending on the feedstock. Biodiesel from camelina or linseed oil with iodine values between 170 and 190 gI_2/100g showed CFPP values of -5 and -9°C respectively. For low temperature behavior the overall fatty acid distribution of the sample, especially saturated versus unsaturated fatty acid ester, is responsible as demonstrated in figure 4. A forecast only on iodine value data is not suitable. Additionally, as mentioned on oxidation stability, CFPP of

additivated (winterized) biodiesel can not be identified or predicted only having iodine value as data.

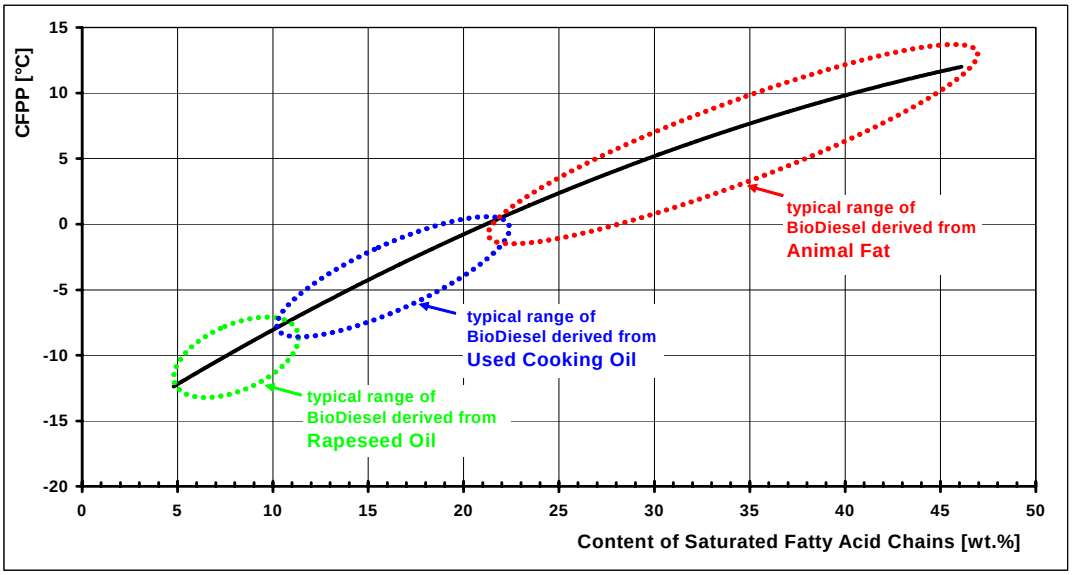


Fig.4: Degree of Saturation versus CFPP of biodiesel

Source: Biodiesel International AG, Internal Report

7. Engine Experience and Emission Characteristics

As mentioned above, iodine value has an influence on other biodiesel quality parameters. This will be additionally discussed in chapter 6. However, besides the impact on fuel quality, one of the most important aspects when discussing iodine value limits and regulations is to investigate and compare the real world behavior of fatty acid methyl esters with different iodine values when used in vehicles and engines. Additionally, emission characteristics of such fuels have to be evaluated. For this reason the enclosed evaluation of engine experiences and emission characteristics is based on published data material with special focus on experience with high iodine value fuels. It should be noted that most of the evaluated literature is coming from the U.S. because soybean oil is the predominant feedstock there for

biodiesel production. Therefore, most data available is concerning investigations on soy based biofuels whereby also some other high iodine value fuels have been investigated in few studies mainly done by European research groups. The enclosed literature overview is reflecting findings and observations on the different topics and will be executively summarized at the end of this chapter.

C. Strong, C. Erickson and D. Shukla (2004) reviewed, amongst others, engine performance characteristics as well as emissions and air quality impacts of neat biodiesel from soybean oil as biodiesel blends[34]. Key findings of this review were:

- In general, engine performance has not appeared to suffer significantly because of the introduction of biodiesel. There may, however, be some peak power loss and some increase in fuel consumption.

- Recent studies have shown no significant wear concerns with biodiesel, especially when biodiesel is blended with good quality petroleum diesel. Material compatibility with seals and gaskets may be a concern on B100 or in older engines.

- One engine concern arises when an engine alternates between different fuel types. Conventional diesel leaves deposits in engines that biodiesel, as a solvent, will clean out. This can mean additional costs for replacing fuel filters initially, but these additional costs are not sustained over time. Moreover, this is less of an issue if a low biodiesel blend (B20 or less) is used or if biodiesel is used as an additive (B2).

- Cold weather product storage for low (less than B20) biodiesel blends should not be a problem. Biodiesel blends are already used on a widespread basis in several cold weather locations, including Yellowstone National Park, Glacier National Park, Grand Teton National Park and Malmstrom Air Force Base. Moreover, biodiesel has been approved by the EPA as a fuel additive (B2 or less). At least one public filling station in Montana blends biodiesel into its conventional diesel.

- Numerous emissions studies have been conducted, and ably summarized by EPA (see subsequent to this). Most tests have been completed with B20 biodiesel blends. Biodiesel blends show emissions benefits for SO_2, CO, CO_2, HC and PM. Biodiesel blends show increased NO_x emissions, which may be partly or fully mitigated by changing engine timing.

The mentioned report[35] of EPA therefore will be discussed in this context more detailed. The EPA (U.S. Environmental Protection Agency) conducted a review of studies comparing the emissions of heavy-duty highway engines using diesel No. 2 with similar vehicles using biodiesel (B100) or biodiesel blend fuels. The report focused on studies examining heavy-duty highway engines because of the lack of studies on that topic using Federal Test Procedure (FTP) testing. Statistical correlations were developed for highway engines, and these were compared to those estimated through heavy-duty engine studies. The report concluded the following: "For PM and HC, the vehicle data appears to produce emission benefits that are smaller than those predicted by the [statistical] correlations. For NO_x the vehicle data appears on average to produce emission reductions whereas the [statistical] correlations predict emission increases. For CO, the vehicle data appears to produce larger emission benefits than the [statistical] correlation predictions. Based on this comparison, they do not believe that the vehicle data can be used to represent the emission effects of biodiesel on heavy-duty diesel engines." Vehicles carrying a greater load will produce more vehicle emissions; however, there are no indications that the emissions impacts of biodiesel change as vehicle load increases. They conducted a regression analysis estimating the relative emissions change as a function of the percent of biodiesel used in the fuel blend. Furthermore analyses for biodiesel based on the percent blend of biodiesel using different types of feedstock (soy, rapeseed and animal) have been performed. This study documented the percentage for each type of biodiesel used (soy, rapeseed or animal-based) and its respective change, relative to diesel No. 2, of four types of emissions – NO_x, PM, HC and CO. As this review encompassed nearly 40 studies, it is believed that it provides a comprehensive picture of the differences in emissions between biodiesel and

diesel. Table 10 summarizes the change in levels of toxic emissions between diesel No. 2 and a soy-based B20 blend – a commonly used biodiesel blend.

Source Effect	Percent Change in Emissions
NO_x	2.0%
PM	-10.1%
HC	-21.1%
CO	-11.0%

Tab. 10: Source Impacts on Using Soy-Based B20 Compared to Average Diesel Source[35].

The following source impacts, for emission reductions due to soy-based biodiesel compared to diesel No. 2, are ranked from greatest to lowest: HC, CO, PM. These comparative benefits are chiefly related to biodiesel's high oxygen and low sulfur content[36]. Conversely soy-based biodiesel NO_x emissions increased slightly as compared to diesel No. 2. The report also examined whether there were statistically significant differences in emissions levels between biodiesel and diesel. They expressed the results of this analysis in terms of p-values. A p-value greater than 0.05 illustrates that there is no statistically significant difference at a 95 percent confidence interval. The p-values in Table 11 show that emissions levels are significantly different between biodiesel and diesel No. 2 for all four pollutants. They also show that there is a significant difference between biodiesel types for NOx, PM and CO emissions. There was no significant difference in HC emissions between biodiesel types (animal fat, rapeseed and soy).

	NO_x		PM		HC		CO	
Do Source Effects…	Test	P-value	Test	P-value	Test	P-value	Test	P-value
Change with % biodiesel?	Yes	0.0001	Yes	0.0001	Yes	0.0001	Yes	0.0003
Animal x % biodiesel	Yes	0.0001	Yes	0.0001	Yes	0.5525	Yes	0.0001
Rape x % biodiesel	Yes	0.0311	*No*	*0.6316*	*No*	*0.9162*	Yes	0.0164
Soy x % biodiesel	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*

Tab. 11: P-Values for Biodiesel Source Effects

Source [35].

NO_x

As seen in table 10, soy-based B20 emits 2.0 percent more NO_x than diesel No. 2. According to the EPA study, the biodiesel types are significantly different from one another. As seen in figure 5, each type of biodiesel showed higher emissions of NO_x than diesel No. 2. Soy-based biodiesel showed the most significant increase in NO_x emissions, animal-based biodiesel the smallest, and rapeseed showed an increase between the two.

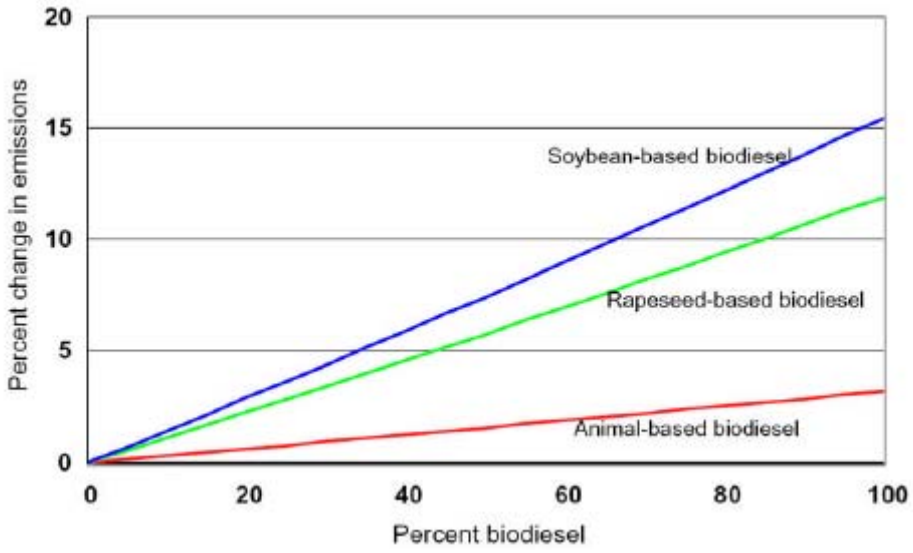


Fig. 5: Biodiesel Source Effects of NO_x

Source [35].

The increase in NO_x is partially rooted in biodiesel's higher cetane number. K. Walker from the Scottish Agricultural College reports that higher NO_x emissions result primarily from the shorter ignition delay time of biodiesel[37]. According to staff from the Montana Department of Environmental Quality, recent studies suggest that NO_x emissions are related to engine technology and injection pressure: slow and medium speed (medium-pressure) diesel engines show no or little increase in NO_x while engines with high injector pressure (~300 psi) show slight rises in NO_x[38]. The piston of the engine moves due to advancement of a hot flame front that results from the ignition of the air-fuel mixture. The typical air-to- fuel mixture for a diesel engine is 7 parts of air to 10 parts of fuel. As air contains 80 percent nitrogen, most of the air-fuel mixture is nitrogen. NO_x is created when an oxygen/nitrogen mixture is subjected to high temperatures and pressures. At the start of combustion, the combustion chamber on a diesel engine is filled with air. The oxygen and nitrogen mixture is under high pressure and is fairly hot. If there is a delay in the ignition timing, a large amount of accumulated fuel suddenly ignites, creating a very hot flame front, and probably creating a large amount of NO_x[39].
This problem can be overcome by changing the engine timing. Steve Howell of the Society of Automotive Engineers reported that research indicates retarding engine timing to lengthen ignition time can mitigate increases in NO_x emissions from biodiesel[40]. This statement is echoed by Dr. Walker: "Adjustment of injection timing and engine operating temperature will result in these levels (of nitrogen oxides with biodiesel) being reduced below mineral diesel levels."[39].

Although the mean engine timing has the potential of matching the cetane number, there still lies the problem of biodiesel's cetane rating varying more than diesel. According to the EPA study, biodiesel has a "widely varying natural cetane number" relative to diesel[35]. Both conventional diesel and biodiesel can be blended to achieve a certain cetane number. Therefore, regardless of engine timing modification, biodiesel is statistically expected to emit more NO_x than diesel due to its greater cetane level variance. It's important to note that cetane numbers in association with engine timing are a significant factor in NO_x emissions, but not the only factor. The National Biodiesel Board notes that, because of biodiesel's lack of sulphur, a variety

of NO_x control technologies may be applied that would not be applicable with conventional diesel[41]. Examples of these technologies include NO_x adsorbers and lean NO_x catalysts, which have demonstrated the potential to control greater than 50 percent of diesel engine NO_x emissions. The Manufacturers of Emission Controls Association (MECA) adds the lower sulphur fuel can lead to adoption of control technologies to impact HC and PM emissions as well, such as commercially available PM and HC filters that use a NO_x catalyst to help destroy diesel particulate emissions. MECA adds that lower sulphur fuel "further enhances the performance of other PM and HC control technologies, such as oxidation catalysts and catalyzed diesel particulate filters, which can operate on current diesel fuels."[42].

There are other strategies that may be used to reduce NO_x in biodiesel blends. According to staff from the Montana Department of Environmental Quality, chassis dynamometer testing conducted at lower temperatures (~35° F) showed that less NO_x was produced at lower operating temperatures. Therefore, the actual NO_x impact of biodiesel in Montana may be less than indicated by the EPA analysis[38]. Lowering the aromatic content in the base fuel, or using diesel No. 1 (kerosene) as a base fuel, can both be effective. Cetane enhancers di- tert-butyl peroxide (DTBP) and ethyl-hexyl nitrate (EHN) may also be helpful[43]. Another option is to blend biodiesel using different feedstocks. It has been reported that iodine values in animal-based biodiesel are lower than those for soy- or rapeseed-based biodiesel, so it has been suggested by Thornton that blending high iodine value fuels and low iodine value fuels could help mitigate the increase in NO_x[44]. Exhaust gas recirculation has also been shown to reduce NO_x of B20 by 10 percent, in comparison with the results obtained through altering injection pressure and optimizing engine timing[45].

PM

According to the EPA study, B20 soy-based biodiesel produces 10.1 percent lower PM emissions than diesel No. 2[35]. Further, the percentage of PM being emitted decreases as the percentage of biodiesel increases, as seen in figure 6. It should be noted that there was no significant difference between soybean and rapeseed-based

biodiesel PM emission, but both exhibited a greater PM emission than that of animal based biodiesel.

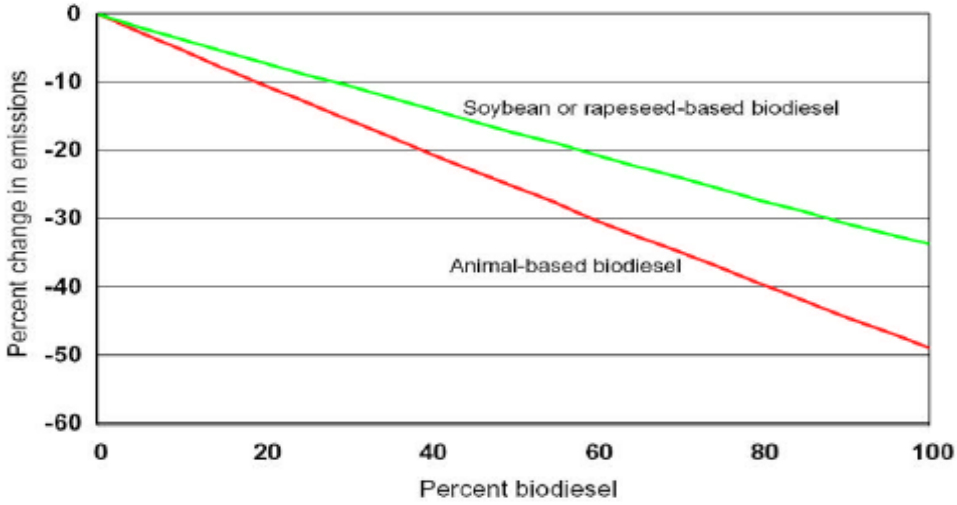


Fig. 6: Biodiesel Source Effects of PM

Source [35].

There is a trade-off in diesel engine technology between producing lower PM emissions (as seen in figure 6), while creating more NO_x emissions (as seen in figure 5. The trade-off of PM and NO_x emissions might be a problem. First, Alfuso found that rapeseed methyl ester reduced smoke levels in direct injected diesel engines[46]. Figure 5, 6, and figure 7 show that soy, rapeseed and animal-based biodiesel blends have similar emissions attributes to that of the rapeseed methyl ester used in Alfuso's experiment; it is likely that that there will also be a reduction in smoke. More specifically, it is possible that the trade-off of PM and NO_x for soy, rapeseed and animal-based biodiesel blends also tend to produce less smog.

HC

Biodiesel has noteworthy benefits in reducing HC emissions relative to diesel No. 2. As seen in table 10, soy-based B20 emits 21.1 percent fewer HC than diesel No. 2. The EPA report shows that there is no significant difference in the percent reduction

of HC emissions between different feedstock types (rapeseed, soy and animal-based)[35].

CO

As shown in table 10, B20 (soy-based) biodiesel produces 11.0 percent less CO than No. 2 diesel fuel. This trend is similar to biodiesel base types (soy, rapeseed and animal), as seen in figure 7. The EPA report indicates that CO emission reductions vary according to the feedstock used. An analysis by the University of Idaho shows that CO and other emissions vary by iodine value, which represents the amount of unsaturated carbon bonds. High oleic vegetable oils have the lowest CO emissions, lower PM emissions, relatively little impact on NO_x emissions, and are the most stable[38]. As seen in figure 7, the following biodiesel bases are in order of decreasing percent reduction of CO per percent biodiesel: animal, rapeseed then soybean-based biodiesel.

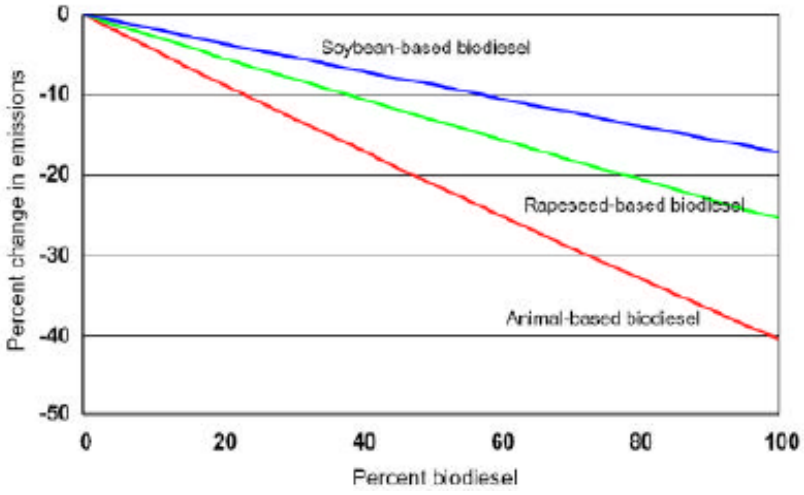


Fig. 7: Biodiesel Source Effects of CO

Source [35].

CO_2

Regarding carbon dioxide, the EPA report was not able to identify a clear difference between biodiesel and diesel. It noted that carbon dioxide benefits are attributed to biodiesel because of its nature as a renewable resource; however, the report did not

quantify those benefits[35]. A Department of Energy fact sheet on biodiesel did seek to quantify these benefits. It noted that plants that are used to make biodiesel draw CO_2 from the atmosphere and recycle it back as the plants decompose. Because of the renewable nature of biodiesel, DOE estimated that biodiesel from soybean oil produces 78 percent less CO_2 than diesel[47,48].

Concerning NO_x and PM emissions of soybean oil methyl esters and blends numerous studies and articles have been published. Some of them are incorporated in the studies mentioned above[34,35] and the others will be given enclosed. Some findings can be interpreted as similar to that already discussed but in terms of overall literature research on this topic should be nevertheless cited and summarized.

Graboski et al. (1999) investigated the effect of biodiesel composition on NOx and PM emissions from a DDC series 60 engine[49]. As an important part of this study, data on emissions based on the EPA heavy-duty transient cycle have been measured to demonstrate the sensitivity of engine emissions to biodiesel ester composition.

- In total, 27 neat biodiesels and 3 B-20 blends (with EPA certification diesel) were tested (see table 12). Seven fuels prepared from various natural feedstocks were obtained from IGT, and three of these were also tested as B-20 blends. Twenty fuels were prepared at the Colorado School of Mines, primarily from nearly pure (or technical grade) fatty acids. Nevertheless, many of these fuels were not as pure as originally intended because of high levels of impurities in the feedstocks. These fuels covered a very wide range of realistic feedstocks as well as chemical properties such as fatty acid chain length and number of double bonds in the fatty acid chain. Fuels were analyzed for a wide range of properties including water and sediment, free and total glycerine, iodine value, peroxide value, acid number, cetane number, density, kinematic viscosity, gross heating value, and carbon, hydrogen, and oxygen content. The specific fatty acid esters present in the fuels were also determined by GC/MS analysis. Regulated pollutant emissions, along with

certain non-regulated pollutants, were measured on a 1991 DDC Series 60 engine via the heavy-duty transient test (40 CFR Part 86 Subpart N). Emissions from biodiesel fuels were compared with emissions for EPA certification diesel. This allows comparison with a conventional diesel, and most importantly, a measure of engine drift. Volatile organic fraction, PM sulfates, and emissions of certain aldehydes were also measured for many of the fuels. Samples for PAH and biological activity analysis were collected.

Biofuel	Iodine No.	Biofuel	Iodine No.
Methyl Laurate	0.3	1:2 Methyl Stearate: Methyl Linseed	116
Methyl Palmitate	0.5	Oxidized Methyl Soy	131
Methyl Stearate	0.5	Oxidized Ethyl Soy	118
Ethyl Stearate	1	High Acid No Methyl Oleate	84
Methyl Oleate	90	High Glyceride Ethyl Soy	117
Ethyl Oleate	79	Methyl Soy	n.d
Methyl Linoleate	151	Edible Methyl Tallow	n.d
Ethyl Linoleate	140	Inedible Methyl Tallow	n.d
Methyl Linolenate	165	Methyl Canola	n.d
Ethyl Linseed	157	Methyl Lard	n.d
Methyl Soy (Soygold)	121	Methyl LFFAG	n.d
Methyl Hydrogenated Soy	6	Methyl HFFAG	n.d
Ethyl Soy	122	B20 Inedible Methyl Tallow	n.d
Ethyl Hydrogenated Soy	6	B20 Methyl Soy	n.d
2:1 Methyl Stearate: Methyl Linseed	66	B20 Methyl LFFAG	n.d

* n.d.; not determined

Tab. 12: List of Biodiesel Fuels Tested[49]

(LFFAG=low free fatty acid grease, HFFAG=high free fatty acid grease).

- All fuels tested met water and sediment specifications as well as free and total glycerine specifications (with the exception of one fuel produced with high glycerine value). Not all fuels met the acid number specification, in particular fuels with a high stearate content were not liquid at or near room temperature making acid removal difficult. Other properties are generally in good agreement with literature values.

- Certification fuel runs exhibited a mean NO_x emission level of 4.59 g/bhp-h (+/- 0.3%) with no significant drift over the 4 month test program. PM emissions averaged 0.261 g/bhp-h (+/-2.7%). A small downward drift in PM emissions was evident (11% over the course of the project). Thus, no correction for drift

was applied to the NO_x emissions data but a correction was applied to the PM data.

- Testing of the biodiesels produced from natural sources indicated that PM emissions were dependent on the oxygen content of the fuel only. All fuels reduced PM relative to certification diesel. NO_x emissions varied considerably with biodiesel feedstock but all increased NO_x relative to certification diesel. The most highly unsaturated fuels (canola and soy) produced the highest NO_x emissions. But based fuel economy was the same for all biodiesels and certification fuel.

- For the fuels prepared from chemically pure esters, all reduced PM relative to certification diesel but the PM reduction was not proportional to oxygen content in all cases. Almost all of these fuels increased NO_x relative to certification fuel. The exceptions were methyl palmitate, methyl laurate, ethyl stearate, and ethyl ester of hydrogenated soybean oil. No consistent difference in emissions was observed between ethyl and methyl esters of the same feedstock. High peroxide value (1800), acid number, and glycerine content had no effect on regulated pollutant emissions in this short term study. However, these out-of-spec fuel properties may cause fouling in longer term studies.

- Regression analysis of the results indicated that emissions could be correlated by one parameter, either density of cetane number (which were highly correlated with each other). PM reduction was proportional to oxygen content for biodiesels with a cetane number of greater than about 45 (density greater than 0.89). For fuels with cetane number less than 45 PM reduction was less. NO_x emissions were also well correlated with biodiesel density or Cetane number. These results suggest that neat biodiesels with cetane numbers greater than about 60 may produce NO_X emissions equal to or less than certification fuel. The impact of molecular structure is implicit in either the density or cetane number. More saturated esters have higher cetane numbers

and lower densities than less saturated esters. Thus, the lower the iodine value, the lower the NO_X emission. Data collected also demonstrate the effect of chain length. The density of shorter chain length saturated esters is greater than longer chain saturated esters and the NO_X emission is also greater. However, methyl laurate, with cetane number 61.2 and density 0.873, is NO_X neutral compared to certification fuel.

- The results presented here are engine specific. Other engines and calibrations will probably give similar results but the impact of the NO_X/PM trade-off for diesel engines will change the overall results but probably not the trends.

- The NO_X behaviour of biodiesel blends is complex. Insufficient physical property data are available to characterize the effect of blending at this time, although provisionally for 25 blends a linear combination of NO_x emissions seems appropriate. The effect of oxygen on particulate matter is well characterized by considering only the oxygen content of the blend. This conclusion appears to be robust.

Chang and van Gerpen (1997) studied the fuel properties and engine performance of biodiesel prepared from modified feedstocks[50]. Within this project, biodiesel fuels were prepared from feedstocks with modified compositions including the methyl esters of a low palmitic soybean oil, a partially transesterified soybean oil (BIOGREEN), a synthetic blend of saturated esters, and a commonly used methyl soyate. These esters were blended with No. 2 diesel fuel in 20% and 50% concentrations. The blended fuels were then tested in a diesel engine (John Deere model 4276T four-cylinder, four stroke, turbocharged diesel engine) to investigate the effect of biodiesel composition on performance, combustion characteristics, and emissions. The results obtained can be summarized as follows:

- Methyl esters of soybean oil have higher cetane numbers than No. 2 diesel fuel, and they can be used as cetane improvers. The esters with higher amounts of saturated compounds (lower iodine value) had higher cetane numbers than the unsaturated esters (higher iodine value). The neat esters

contain about 11% oxygen by weight and can be used as an oxygenated additive for diesel fuel. The energy content of the esters is approximately 12% lower than of No. 2 diesel fuel.

- The fuel injection timing was advanced slightly when the ester blends were used. The maximum advance was one degree of crankangle when 20% BIOGREEN was used.
- The engine performance of the ester blends was similar to that of No. 2 diesel fuel with nearly the same thermal efficiency, slightly higher fuel consumption, and lower power output.
- All ester blends showed the same combustion stages as the diesel fuel but a shorter ignition delay. They also showed a lower premixed burning rate and fraction, higher bulk gas temperature, and faster diffusion combustion rate than No. 2 diesel fuel. The maximum changes of combustion characteristics were provided by the 50% blend of synthetic fuel of methyl palmitate and methyl stearate (OPT). the partially transesterified soybean oil had a slightly longer ignition delay, 0.25 degrees of crankangle longer than the baseline diesel, and a slower combustion rate during the diffusion stage.
- All fuel blends lowered the CO, HC, particulate, and soot emissions. The 50% blend with only methyl palmitate and methyl stearate had the largest effect an the reduction of CO, HC, particulate, and soot emissions compared with diesel fuel, 24%, 15%, 32% and 44% respectively. Of the 20% blends, this mixture of methyl palmitate and methyl stearate also gave the largest reduction of CO, HC, particulate, and soot emissions. The blends with the saturated esters produced the lowest increase of SOF (soluble organic fraction) in the particulates. The NO_x emissions of the fuel blends were higher than for diesel fuel. The blends with 50% of the synthetic fuel increased NO_x emissions by 5% which was the smallest increase among the 50% blends. The 20% synthetic fuel blend did not show a significant increase of NO_x emissions.
- The saturated esters showed higher cetane number, greater reduction of CO, HC, and particulates, and less NO_x emissions increase than the unsaturated esters.

Another study evaluated the impact of biodiesel source material and chemical structure on emissions of criteria pollutants from a heavy-duty engine[51]. Biodiesel produced from a variety of real-world feedstocks as well as pure (technical grade) fatty acid methyl and ethyl esters were examined for emissions performance in a heavy-duty truck engine. The objective was to understand the impact of biodiesel's chemical structure, specifically fatty acid chain length and number of double bonds, on emissions of NO_x and particulate matter (PM). A group of seven biodiesel samples produced from real-world feedstocks and 14 produced from pure fatty acids were tested in a heavy-duty truck engine using the U.S. heavy-duty federal test procedure (transient test). It was found that the molecular structure of biodiesel can have a substantial impact on emissions. The properties of density, cetane number, and iodine value were found to be highly correlated with one another. For neat biodiesels, PM emissions were essentially constant at about 0.07 g/bhp-h for all biodiesels as long as density was less than 0.89 g/cm^3 or cetane number was greater than about 45. NO_x emissions increased with increasing fuel density or decreasing fuel cetane number. Increasing the number of double bonds, quantified as iodine value, correlated with increasing emissions of NO_x. Thus the increased NO_x observed for some fuels cannot be explained by the NO_x/PM trade-off and is therefore not driven by the thermal NO formation. For fully saturated fatty acid chains the NO_x emissions increased with decreasing chain length for tests using 18, 16, and 12 carbon chain molecules. Additionally, there was no significant difference in NO_x or PM emissions for the methyl and ethyl esters of identical fatty acids.

Knothe (2005) reviewed the dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters[52]. He mentioned that since the source of biodiesel varies with the location and other sources such as recycled oils are gaining interest, it is important to possess data on how the various fatty acid profiles of the different sources can influence biodiesel fuel properties. The evaluation showed that the fuel properties of biodiesel are strongly influenced by the properties of the individual fatty acid esters in biodiesel. Both moieties, the fatty acid and the alcohol, can have considerable influence on the fuel properties such as cetane number with relation to

combustion and exhaust emissions, cold flow, oxidative stability, viscosity, and lubricity. Generally, cetane number, heat of combustion, melting point, and viscosity of neat fatty compounds increase with increasing chain length and decrease with increasing unsaturation. It therefore appears reasonable to enrich (a) certain fatty ester(s) with desirable properties in the fuel in order to improve the properties of the whole fuel. It may be possible in the future to improve the properties of biodiesel by means of genetic engineering of the parent oils, which could eventually lead to a fuel enriched with (a) certain fatty acid(s), possible oleic acid that exhibits a combination of improved fuel properties.

In this context it is important to consider that among the influences of fuel composition on the quality the iodine value of the biofuel has by far the most influence on other quality parameters as well as on emission characteristics, especially NO_x. Therefore several studies have been made in order to investigate this influence as partially mentioned above, and to search for strategies to eliminate such effects. Szybist et al. (2005) evaluated formulation strategies to eliminate the biodiesel NO_x effect[53]. They explored the efficiency of (1) reducing the iodine value of soy-derived biodiesel fuels through increasing the methyl oleate (methyl ester of oleic acid) content and (2) addition of cetane improvers, as strategies to combat the biodiesel NO_x effect: the increase in NO_x emissions observed in most studies of biodiesel and biodiesel blends[34,35,40,43-56]. This is accomplished by spiking a conventional soy-derived biodiesel fuel with methyl oleate or with cetane improver. The impact on bulk modulus of compressibility, fuel injection timing, cetane number, combustion, and emissions were examined. The conventional B20 blend produced a NO_x increase of 3-5% relative to petroleum diesel, depending on injection timing. However, by using a B20 blend where the biodiesel portion contained 76% methyl oleate, the biodiesel NO_x effect was eliminated and a NO_x neutral blend was produced. The bulk modulus of petroleum diesel was measured to be 2% lower than B20, yielding a shift in fuel injection timing of 0.1-0.3 crank angle. The bulk modulus of the high methyl oleate B20 blend was measured to be 0.5% lower than B20, not enough to have a measurable impact on fuel injection timing. Increasing the methyl oleate portion of the biodiesel to 76% also had the effect of increasing the cetane

number from 48.2 for conventional B20 to 50.4, but this effect is small compared to the increase to 53.5 archived by adding 1000 ppm of 2-ethylhexyl nitrate to B20. For the particular engine tested, NO_x emissions were found to be insensitive to ignition delay, maximum cylinder temperature, and maximum rate of heat release. The dominant effect on NO_x emissions was the timing of the combustion process, initiated by the start of injection, and propagated through the timing of maximum heat release and maximum temperature.

McCormick et al. (1997) studied the effect of several oxygenates on regulated emissions from heavy-duty diesel engines[57]. Oxygenates produce a significant reduction in emissions of particulate matter (PM-10) from diesel engines but in most cases also causes the nitrogen oxide emissions to increase. In their work, several oxygenates having a wide range of properties were blended with No. 2 diesel at the 1 and 2wt% oxygen level. Emissions were measured using the hot start portion of the U.S. Heavy-Duty transient Test (40 CFR, part 86, Subpart N) in both a 2-stroke and a 4-stroke engine. It was found that at this oxygen level PM reductions on the order of 10-15% were obtained regardless of oxygenate chemical structure. The oxygenates differently affected the integrated NO_x emissions. Methyl esters of soybean oil increased NO_x by 2-3%, decanoic acid had no effect, and octanol may have slightly decreased NO_x. Examination of real time NO_x concentrations data for octanol and soy esters indicates that both oxygenates increase NO_x during portions of the cycle where the engine is generating high torque at low speed with a little or no turbo boost. For octanol, there is a compensating reduction in NO_x at high speed of load. Several hypotheses regarding the effect of oxygenates on diesel NO_x emissions are furthermore discussed.

In another study, fuel additive and blending approaches to reduce NO_x emissions from biodiesel were evaluated by McCormick et al.[58]. Blending of 20% biodiesel with petroleum diesel is well known to cause a significant reduction in PM emissions but also can cause NO_x emissions to increase by 1 to 3 percent. This study has examined a number of approaches for NO_x reduction for 20% biodiesel/petroleum diesel blends (B20). These approaches included blending with a nominally 10%

aromatic diesel, zero aromatic Fischer-Tropsch (FT) diesel, and use of fuel additives. Biodiesel produced from soybean oil and from yellow grease was examined. Testing was conduced in a 1991 DDC Series 60 truck engine using the U.S. heavy-duty FTP. Emissions of NO_x, PM, CO, and THC are reported. Relative to certification diesel the B20 fuels exhibited 20% lower PM emissions but 3.3 and 1% higher NO_x emissions for soy and yellow grease based blends, respectively. The 10% aromatic fuel exhibited 12% lower PM and 6% lower NO_x. FT diesel had the lowest emissions with a 33% reduction in PM and 16% lower NO_x. For B20 (soy + cert fuel), lowering of the base fuel aromatic content from 31.9 to 7.5% lowered NO_x by 6.5%. Linear interpolation between data points for B20 produced using cert fuel and produced using 10% aromatic fuel suggests that, if all other factors are equal, a base fuel having 25.8 aromatics should provide a NO_x neutral B20 (relative to certification diesel having 31.9% aromatic content). Note that the cetane numbers of the certification diesel and the 10% aromatic diesel are essentially the same. Blending of FT diesel may produce a NO_x neutral fuel at biodiesel content as high as 55%. The cetane enhancers di-tert-butyl peroxide and 2-ethyl-hexyl nitrate are both effective at reducing NO_x from B20 blends in this engine, while retaining the PM emission benefits of biodiesel. Ferrocene was also examined but demonstrated no emission benefits.

Regulated emissions from biodiesel tested in heavy-duty engines meeting 2004 emission standards were investigated by McCormick et al.[59]. Biodiesel produced from soybean oil, canola oil, yellow grease, and beef tallow was tested in two heavy-duty engines. The biodiesels were tested neat and as 20% by volume blends with a 15 ppm sulphur petroleum-derived diesel fuel. The test engines were the following: 2002 Cummins ISB and 2003 DDC Series 60. Both engines met the 2004 U.S. emission standard of 2.5 g/bhp-h NO_x + HC (3.35 g/kW-h) and utilized exhaust gas recirculation (EGR). All emission tests employed the heavy-duty transient procedure as specified in the U.S. Code of Federal Regulations. Reduction in PM emissions and increase in NO_x emissions were observed for all biodiesels in all engines, confirming observations made in older engines. On average PM was reduced by 25% and NO_x increased by 3% for the two engines tested for a variety of B20 blends.

These changes are slightly larger in magnitude, but in the same range as observed in older engines. The cetane improver 2-ethyl-hexyl nitrate was shown to have no measurable effect on NO_x emissions from B20 in these engines, in contrast to observations reported for older engines. The effect of intake air humidity on NO_x emissions from the Cummins ISB was quantified. The CFR NO_x/humidity correction factor was shown to be valid for an engine equipped with EGR, operating at 1700 m above sea level, and operating on conventional or biodiesel.

The effect of biodiesel fuel composition on diesel combustion and emissions were analyzed by Schmidt and Van Gerpen (1996)[60]. The first part of their study investigated three possible reasons why an ester of soybean oil can reduce exhaust emissions. The impact of fuel's oxygen content, long chain hydrocarbons and cetane improver were evaluated. The results indicate that:

- The cetane number of ester/diesel fuel blends was higher for long chain length esters. Saturated esters increased the cetane number more than unsaturated esters.
- The solid portion of the particulate decreased by 33% and oxides of nitrogen emissions increased by 25% as the oxygen content of the intake air was increased from 20.5% to 22.0%.
- Biodiesel's particulate reducing effect was attributed to a combination of its oxygen content and its displacement of aromatic and shorter-chain hydrocarbons with long-chain esters.

The second part of this study involved evaluating the emissions and performance characteristics of the fatty esters found in soybean-based biodiesel. The results indicated that.

- Particulate emissions were significantly reduced when the diesel engine was fuelled with blends of methyl palmitate with diesel fuel. The 50% blend of methyl palmitate gave the largest particulate reduction of 30%.
- The soluble portion of the particulate decreased as ester unsaturation increased.
- The changes in NO_x emissions were small when biodiesel/diesel fuel blends were used. NO_x emissions for all of the 50% fatty ester blends with diesel fuel,

except methyl palmitate and isopropyl stearate, increased between 0 and 5%. The 50% blends of methyl palmitate and isopropyl stearate with No.2 diesel fuel reduced NO_x emissions 2 to 3% compared with diesel fuel alone. None of these changes were statistically significant at the 90% confidence level.

- All of the esters demonstrated a significant reduction in hydrocarbon emissions.
- Brake specific fuel consumption increased for all of the fatty esters, but thermal efficiency of the engine did not change when esters are used.

Reyes and Sepúlveda (2006) investigated PM-10 emissions and power of a diesel engine fuelled with crude and refined biodiesel from salmon oil[61]. They assessed the power response and level of particulate emissions for blends of diesel – crude biodiesel and diesel – refined biodiesel. Crude and refined biodiesel were prepared from salmon oil with a high content of free fatty acids, throughout a process of acid esterification followed by alkaline transesterification. Blends of diesel – crude biodiesel and diesel – refined biodiesel were tested in a diesel engine (5959 cm^3, six cylinder, four stroke, water cooled diesel engine Mercedez Benz model OM-366) to measure simultaneously the dynamometric response and the particulate material (PM-10) emission performance. The results indicate a maximum power loss of about 3.5% and also nearly 50% of PM-10 reduction with respect to diesel when a 100% refined biodiesel was used. For blends with less content of either crude or refined biodiesel, the observed power losses are lower but at the same time lower reduction in PM-10 emissions are attained.

Haas et al. (2001) investigated the engine performance of biodiesel fuel prepared from soybean soapstock[62]. Emissions data for both the neat fuel and a 20 vol% blend in low-sulphur petroleum diesel were collected according to the Environmental Protection Agency heavy-duty transient cycle protocol using a DDC Series 60 engine on an engine test stand. The emissions profile of biodiesel from soapstock was quit similar to that of biodiesel produced from refined soy oil. Compared with petroleum diesel fuel, emissions of total hydrocarbons, particulates, and carbon monoxide were reduced 55%, 53%, and 48% respectively, with neat soapstock biodiesel. Total NO_x

increased 9%. Operation on a 20 vol% blend of soapstock biodiesel in petroleum diesel gave reductions of 27.7%, 19.7%, and 2.4%, respectively, in total hydrocarbons, particulate matter, and carbon monoxide, relative to petroleum diesel. Nitrogen oxide emissions increased 1.3%. In the context of engine emissions, these data suggest the suitability of the methyl ester of soy soapstock as a diesel fuel.

Besides exhaust emission behaviour of biodiesel with higher iodine value a second and probably more important aspect is the evaluation of engine performance if higher iodine value biodiesel is being used. Again, most projects and studies on this specific topic have been conducted in the U.S. using mostly soybean oil methyl ester as fuel. Evaluating literature data it should be pointed out that especially at the beginning of performance tests in the mid 90's several problems on long endurance tests occurred as reviewed by Graboski and McCormick[63]. But pre-summarizing these results it should be mentioned that the findings can not clearly be compared with tests performed later on. Because in this "early years" of biodiesel investigation varying biodiesel qualities have been used for these tests so that the conclusions can not be interpreted as a result or effect of high iodine value fuel which should be the goal of this study. So therefore the results discussed enclosed are given chronologically leading finally to the more comparable results obtained more recently.

The National Biodiesel Board (NBB) funded a 1000-hr durability test using a DDC 6V-92TA, electronic controlled engine and a 20% methyl soy-ester blend[64]. Approximately 10000 gallons of the B-20 blend were consumed during the test. At about 700 hr the following conditions were observed: low fuel return pressure, low horsepower, low fuel consumption, and high crankcase blowby. It was observed that fuel returning to the day tank was black and significant deposits on air box covers, piston components, and injectors were observed. The fuel lines, fuel filters, and fuel transfer pump were replaced during a brief shutdown at 700 hr. After a longer shutdown at 750 hr, it was found that engine parameters had returned to normal. During the final 250 hr, performance deteriorated in a pattern similar to the first 750 hr. After engine tear down, substantial deposits were found on many engine

components. The source of these deposits appeared to be the lube oil. Cavitation erosion of the injector needle valves had caused injectors to deteriorate to the point that almost no fuel atomization was occurring. Deteriorating fuel pump seals were proposed to have introduced microscopic air bubbles into the fuel causing the cavitation erosion. Elevated soot and wear metals were observed in the lubricant. Broken fire and compression rings were also found on several cylinders. Softening of fuel system seals was observed.

A second NBB funded durability study was performed using a 1987 Cummins N14 engine and B-20 soy methyl ester[65]. The test was intended to be of 1000-hr duration but was terminated at 650 hr due to failure of the engine PT pump, a part of the fuel system. Failure was caused by build up in the pump of a residue composed of fatty acid esters, free fatty acids, and acid salts. This same residue has plugged a fuel filter and the PT pump earlier in the durability test. Fuel injectors were in good condition at the end of the test. Oil analysis revealed no significant degradation. It was proposed that the operational problems experienced during this test were caused by instability toward oxidation of the B-20 fuel. Notable, both the neat soy diesel and No.2 diesel used to make the B-20 used in this study were found to be stable.

Additional studies provide some confirmation of the problems noted above. Lucas (1995) reported similar fuel filter and pump problems to those described above in his study of soy methyl ester B-20 formulated with JP-8 (jet fuel)[66]. Blackburn (1983) reported 50% oil viscosity loss in 50 hr using ethyl soyate[67]. Siekman et al. (1982) investigated mixtures of several methyl esters (from soybean oil, iodine value 128, and babassu oil, iodine value 17) and lubricating oil in their engine and laboratory tests[68]. Results have shown that the amount of double bonds introduced is significant in increasing viscosity and reducing total base number. GC analysis of extracted ester shows degradation mainly in fatty acids with conjugated double bonds. Bench tests and mixing-driving tests led to similar results.

The first study which really focuses on iodine value versus engine performance has been published by Prankl and Wörgetter (1995)[69]. Long term tests have been carried out using a 1-cylinder engine. Five test fuels were used with an iodine value from 100 to 180 $gI_2/100g$. The engine ran over 250 hours with each test fuel. The engine oil

was pre-mixed with 10% of the test fuel. During the run, oil samples were taken and the content of fatty acid methyl ester was analyzed. A high increase of the oil viscosity could be observed with all test fuels independently from the iodine value. Because of this high increase, the engine oil had to be changed at the half interval. The content of fatty acid methyl esters decreased from 10% at start to below 2% at the end of each test run. No significant differences in cleanness and formation of deposits on cylinder, combustion chamber, valves and injectors could be observed. Increasing deposits with increasing iodine value were found at piston rings on the 2nd ring groove.

Further research on technical performance of vegetable oil methyl esters with a high iodine value have been carried out in addition within an ALTENER project by Prankl et al. as summarized below[70]. In Phase I long-term tests were carried out with a single-cylinder engine on the test bench. Rapeseed oil-, sunflower oil-, and camelina oil methyl esters were used as test fuels with varying iodine values (RME iodine value 107, SME iodine value 132, and CME iodine value 150). After 256 hours with each fuel the engine oil was analyzed and the engine parts were inspected. An increase of the viscosity, total base number, n-pentane- and toluene insolubles could be found with an increasing iodine value. A correlation between the increase of viscosity and the engine oil temperature could be found. The inspection of the engine parts showed no differences of carbon deposits on the cylinder and injectors. Small differences were observed in deposits of the bottom of the 2nd ring groove and on the area between 1st and 2nd piston ring. These results encouraged to start a practice test (Phase II) with pure camelina oil methyl ester (iodine value 150). During Phase II (1998) a fleet test with camelina oil methyl ester was carried out. 9 vehicles (5 tractors and 4 passenger cars) as well as one stationary engine were operated with CME during 1 to 3 oil-changing intervals. The running time with CME ranged between 160 and 730 hr with tractors and between 10.000 and 46.000 km with passenger cars. Before and after the test a performance test of the tractors was carried out at the test bench. After the test the condition of the engines was documented. The examinations at the test bench hardly showed any alterations in the performance data of the tractors. Engine performance and efficiency tended to be higher after the test. The measurement of the smoke emissions led to 50% lower

values with CME compared to diesel fuel. At the end of the test, though, the results were slightly higher than at the beginning. The analysis of the engine oil showed that in 4 tractors the oil was considerably diluted with fuel. Thus, the viscosity and the total base number decreased. The soot content was low in all oil samples. However, the samples displayed a high amount of total contamination, which largely consisted of ageing products of the fuel. In the final examination it was determined that the engines were in a satisfying and clean condition considering their running time. No remarkable changes were observed concerning deposits in the cylinder head, the valves, the exhaust port, the piston top land, the cylinder liner, and the piston surface. Minor deposits were found in the inlet port and in the inlet valves. Trumpet-like deposits surrounding the nozzle holes were found in the direct injection engines. The groove base of the piston rings was practically free of deposits. During the entire fleet test hardly any problems occurred. During the project the operation of the fleet was documented in log-books by the vehicle operators. The fuel filters of various vehicles had to be replaced. In one case sealing parts of the injection pump had to be replaced.

Investigations on the impact of biodiesel on fuel system component durability have been reported by Terry (2005)[71]. The main conclusions of the findings can be summarizes as followed:

- The highly oxidised B100 biodiesel and biodiesel blends prepared for this study have significantly different physical and chemical characteristics to non-oxidised biodiesel and biodiesel blends. The oxidised biodiesel have been prepared by heating of the samples up to 57°C under constant sparging of air at a flow rate of 4 l/min for 29 days. The B20 test blend containing highly oxidised biodiesel may have been more highly oxidised than is likely to occur in the real world.
- Fuel filter blocking and fuel separation was observed during testing of the highly oxidised B20 test fuel in this study. Products of oxidation in the test fuel and decomposition reactions occurring under the conditions of test probably accelerated fuel separation in the fuel blend. Fuels likely to present this composition cannot be recommended for use for commercial automotive fuels.

- Phase separation and filter blockage did not occur during testing of B5 and B20 blends prepared from biodiesel which had been less extensively oxidised and which contained lower water and sediment contents. The tests indicate the behaviour of oxidised fuels under conditions of test are not dependant on the concentration of oxidised component and may be due to the extent of oxidation of the biodiesel component.
- B5 fuel prepared from oxidised biodiesel did not cause abnormal wear in either the injector or pump wear tests conducted in this study. Fuel filter blocking and fuel separation was not encountered during testing of this fuel.
- The results produced from injector wear tests indicate that the lubricity of the test fuels are adequate for the protection of diesel injector components running under similar conditions. The injector component wear test on the highly oxidised B20 blend failed to reach completion due to fuel filter blockage. It should be noted that the test method used for this study was a novel 500 hour test procedure. Commercial decisions around lubricity quality should not be based on a single test.
- The ratings produced from pump lubricity tests indicate that all test fuels are within the range normally expected for commercially available automotive diesel fuel running under the test conditions selected for this 500 hour test procedure. The rotary pump wear test on the highly oxidised B20 blend failed due reach completion due to fuel filter blockage. It should be noted that commercial decisions around lubricity quality should not be based on the results of single test.
- None of the candidate test fuel blends tested showed any adverse effects on the wear ratings of the common rail fuel pumps using a novel 500 hour test procedure. The test results indicate that the lubricity of the test fuels is adequate for the protection of common rail pumps running under similar conditions. It should be noted that commercial decisions concerning lubricity should be based on more than one test.
- Material compatibility testing of candidate elastomers has shown that fluorocarbon elastomers of medium to high fluorine content are most compatible with the test fuels under the specified conditions at concentrations

of 20% or below. The results show that other candidate materials tested exhibited good resistance to changes in physical properties but exceeded the typically acceptable levels of degradation in one or more tests. These materials may be less compatible with biodiesel blends under certain applications.

Most recently Prankl et al. published the final report on the LOCAL AND INOVATIVE BIODIESEL project[72]. The objective was to contribute to the biofuels market share of 5.75% by 2010 as indicated by the Biofuels Directive. Starting with a list of 24 different raw materials that were considered to be suitable for the production of biodiesel, 25 samples could actually be purchased and tested in the lab. They were furthermore used for emission tests. Five out of these 25 were additionally objected to engine endurance tests. Emissions of a single cylinder engine were measured with diesel fuel and 23 fatty acid methyl esters with iodine values between 12 and 189 gI_2/100g. A clear dependence could be found between iodine number and NO_x emission. Weak dependency was found between carbon monoxide while hydrocarbons are independent from the iodine value. Endurance tests were carried out with seven different test fuels to find influences of the fuel on the long term performance. A single cylinder engine was operated 256 hours at varying load and speed with diesel fuel, rapeseed oil methyl ester, animal fat methyl ester, coconut oil methyl ester, and soybean oil methyl ester, a blend of 30% jet fuel and RME and jatropha oil methyl ester. Engine performance (power, consumption) was determined at the beginning and the end of each test. A significant loss of power caused by decreased fuel injection volume and efficiency after 256 hr was observed with rapeseed oil-, animal fat-, and coconut oil methyl ester. Engine oil viscosity decreased with all fuels; with RME and animal fat methyl ester viscosity dropped most while the other fuels have shown a slight increase towards the end of each test. Engine oil dilution with fuel was less than 1% in all cases. No significant differences were found in wear, contamination or additive content. Engine oil dispersancy was decreasing mainly with RME but also with animal fat, soybean oil, and jatropha methyl esters. The engine parts were inspected carefully by the engine manufacturer. No remarkable differences were found in coke deposits on piston, piston ring grooves

and gap clearance of piston rings. Neither significant difference nor unusual wear marks were visible at cylinder liners. Most differences were found at the injectors: carbon built-ups at the sprayholes were higher with all biodiesel fuels than with fossil diesel. Extreme cratering was found with coconut oil methyl ester and with jet fuel-RME blend. The determination of the nozzle flow rate has shown a decrease with all fuels, highest change was found with RME and animal fat methyl ester. The spray formation was influenced by external coke deposits; an influence of carbon deposits inside the holes is supposed, but not verified yet. All in all, differences between the investigated “innovative” biodiesel were considerably low, an ideal fatty acid profile could not be found. Low temperature flow properties are most important in countries with cold winters. For those, fatty acids double bonds are unavoidable. The NO_x emissions are significantly influenced by the proportion of double bonds, saturated fatty acid esters result in low NO_x emissions; for the evaluation the NO_x-HC trade-off must be considered: favourable NO_x emissions are accompanied by increased hydrocarbon emissions. Fuel quality has been proved again as crucial factor for sustainable market development. Especially for B100 the quality on the market will decide, an ambitious quality monitoring system is indispensable.

8. Summary of Engine Experience and Emission Performance:

In order to recapitulate the above mentioned huge amount of data concerning emission behaviour and engine experience of biodiesel with higher iodine value, the main features, findings and observations can be summarized as follows:

- Overall, the different studies showed that the use of high iodine value biodiesel (with special emphasis on soybean oil methyl ester) lead to comparable results found at “conventional” biodiesel produced *e.g.* from rapeseed oil. Comparable means, similar behaviour in fuel consumption, engine performance, load transmission and even in emission characteristics but with some differences.

- Emission characteristics of high iodine value biodiesel basically underlined the beneficial effects of all other types of biodiesel but with one major exemption. The NO_x emissions, which generally tend to be higher compared to conventional diesel if biodiesel is used, increased significantly with increasing iodine value of the fuel. This can be pointed out most emphatically by a correlation as given in figure 8.

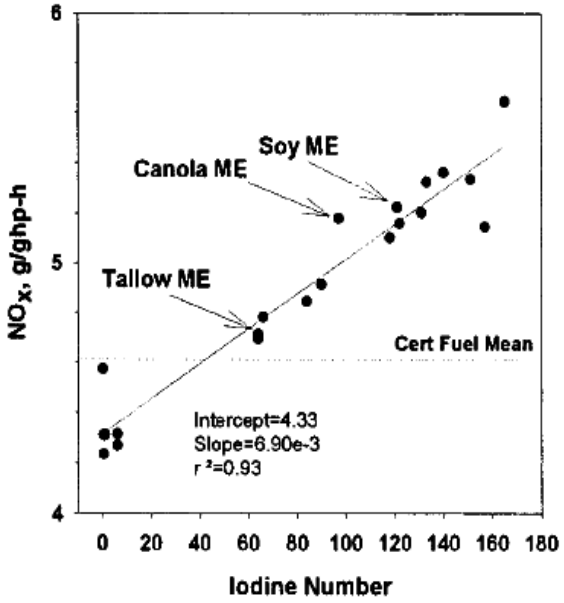


Fig. 8 Dependency between Iodine Value and NO_x Emission
Source: [51].

- Other emissions, like CO, HC, and particulate matter are partially slightly different than compared to rapeseed oil methyl ester or even higher saturated fatty acid methyl esters but generally far better than compared to conventional diesel fuel.
- Engine performance did not show dramatic effects which clearly can be attributed on the higher iodine value. Biodiesel with iodine values up to 150 gI_2/100g have been used without any main differences in engine performance. Higher iodine value biodiesel have not been investigated sufficiently enough until now.

- Biodiesel blends up to B20 have been investigated without any significant differences in engine performance than compared to conventional diesel fuel.

9. Conclusion:

Within this study, many aspects concerning experience with biodiesel with higher iodine values have been evaluated. The background why iodine value within EN 14214 is currently under discussion is that due to the tremendous increase of biodiesel production within the EU the need of feedstocks also dramatically increased. Due to significant differences in feedstock prices, more and more biodiesel producers tend to use feedstock blends others than rapeseed oil as raw material for their production. In order to ensure that a possible opening of the feedstock market in context with cheap oil sources like soybean oil will not lead to consequences with current biodiesel quality as well as possible up-coming problems in engines this study critically reflects all these mentioned facts. Summarizing the following conclusions can be made:

- Due to the iodine value limitation the current European biodiesel specification EN14214 discriminates several potential feedstocks for biodiesel production. Feedstocks like soybean oil, sunflower oil, Jatropha oil, or cottonseed oil cannot be used as 100%.
- It has been feared that the higher (thermal, storage, and oxidation) instability of oils with higher contents on polyunsaturated fatty acids will lead to engine problems and failures.
- Based on the above mentioned reasons, the parameter for oxidation stability with an minimum limit of 6 hours at 110°C has been introduced in the EN14214 biodiesel specifications.
- Oxidation stability is the most important parameter in the context of possible problems in engine parts.
- The iodine value on its own is not sufficiently good enough for describing stability concerns. The stability of biodiesel is a result of position and content of double bonds in the different fatty acid methyl esters of biodiesel as well as the content of antioxidants and the production technology used.

- Together with oxidation stability, the content of linolenic acid methyl esters and polyunsaturated esters are sufficiently enough to exclude the use of higher portions of very “instable” and higher unsaturated feedstocks (*e.g.* linseed oil and fish oil).
- Engine problems with blends or neat biodiesel are predominantly caused by bad oxidation stability and not by high iodine values.
- An appropriate high oxidation stability of biodiesel which can be reached by adequate feedstocks or added antioxidants, is sufficient enough for the quality of the fuel. A further limitation of iodine value does not seem to be necessary.
- In order to guarantee a high stability in blends with fossil diesel, new methods for the determination of oxidation stability are currently under investigation.
- The withdrawal of iodine value could be compensated by an appropriate adjustment of the value for oxidation stability

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