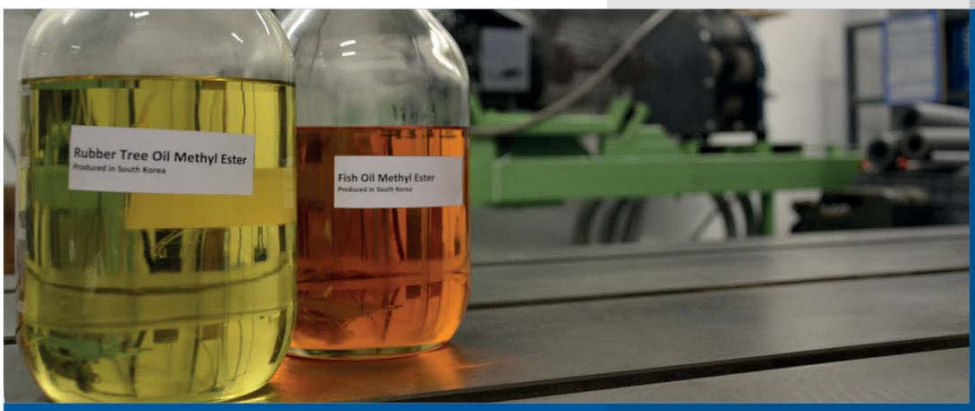
FUELS JOINT RESEARCH GROUP



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Biofuels from non-food feed-stocks

Preparation, Fuel Properties and Engine Emissions

Herausgeber: Jürgen Krahl, Axel Munack, Peter Eilts, Jürgen Bünger

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Promofuel Promotion of Advanced Biofuels

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Promotion of Advanced Biofuels:

Preparation, Fuel Properties and Engine Emissions

Final Report

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0. Introduction

"PROMOFUEL" is a research project for the development of advanced biodiesel fuels, whereby the consortium consists of three experienced partners in the field of biofuels. The project is focusing on new non-food feedstocks for biodiesel production, which until today are mainly excluded from the traditional biodiesel production because of the chemical properties of these feedstocks. Most of the non-food feedstocks for biodiesel production like microbial oils, Jatropha, Camelina etc. contain fatty acids with high unsaturation, leading to low oxidation and storage stability. The project will bring new understanding of the relation between chemical composition and fuel behavior. Two biodiesel test fuels were prepared in larger scale out of rubber seed oil and fish oil. These oils are chosen as representatives of non-food feedstocks or microbial oils. With the test fuels engine and emission tests were carried out and the mutagenicity of the emissions are tested in order to get a better understanding of the influence of the chemical structure on the toxicity of particulate emissions. Laboratory tests have been carried out with partial hydrogenation in order to enhance the oxidative stability and to increase the shelf life. The results of the project are disseminated in a final workshop and will be the basis of further development and research on advanced biofuels between Europe and Korea.

The two main objectives of "PROMOFUEL" are developing scientific facts for the suitability of new non-food feedstocks for traditional biodiesel production and to get better knowledge on the influence of the type of feedstock on environmental and health effects of engine emissions.

The goal is to enhance the feedstock variability for traditional biodiesel plants, where huge investments have been done within the last years. The production of fatty acid methyl esters (FAME biodiesel) today is still the most economical way for the production of alternative diesel fuel, the technology is well established and there is already a huge infrastructure of biodiesel plants existing worldwide. However, due to the lack of sufficient sustainable feedstocks there is a huge overcapacity of biodiesel production units especially in Europe.
Expectations for new technologies for alternative diesel fuels like biomass to liquid (BtL) could not been fulfilled until now because of serious technological and logistical problems. Hydrotreated vegetable oils are already produced in industrial scale they could be used for aviation fuel or as admixture for diesel fuel, leading to even higher blends as B10. However, these technologies need high investment and also running costs because of more sophisticated technology. Furthermore, until now only food grade vegetable oils can be applied for this technology, leading to higher production costs than traditional biodiesel, so the future is rather uncertain. The strategy of the project is to establish criteria for the use of new non-food feedstocks, which normally contain higher amounts of higher unsaturated fatty acids like linoleic and linolenic acid as well as polyunsaturated fatty acids. Most of new

potential seeds like Jatropha, Camelina or other potential crops contain higher amounts of linoleic and linolenic acid, which limits the use of these feedstocks in higher extent because of regulations in internal specifications. Also oil from microalgae or other micro-organisms, which are supposed to be the most promising feedstocks for biofuels in the future, contain fatty acids with high unsaturation, even fatty acids with 5 or more double bonds. Within this project two representatives of these feedstocks are selected for basic investigation, rubber seed oil and fish oil. Rubber seed oil stands for oil with higher amounts of linoleic and linolenic acid, whereas fish oil can be seen as model substance for micro algae oil, because micro algae are the main feed for fish. The main objections for these feedstocks for biodiesel production are the poor oxidation and storage stability resulting from high unsaturation. To overcome these problems within this project it should be tested, what maximum amount of unsaturated products could be blended with mineral diesel in order to meet EN 590. Furthermore partial hydrogenation of the double bonds of these high unsaturated feedstocks should be carried out in order to get products, which are more stable but still have very good cold temperature behavior. Such a strategy is far more economic than total hydrogenation, which additionally needs a step of hydro isomerization which only can be done in specified plants.

However, the technical suitability of an advanced fuel cannot be the only parameter of judgment: Availability of feedstocks as well as sustainability including emissions and especially health effects must be taken into account, too.

Therefore, the other main objective of “PROMOFUEL” was to monitor the state of the art knowledge on emissions and health effects relative to different biodiesel feedstocks and engine conditions.

Limited knowledge is available on the influence of chemical structure of the different biodiesel feedstocks on the engine and emission behavior. Especially health effects of soot emissions coming from diesel engines are big concern in the overall emission of combustion engines. Mutagenicity tests of emissions with pure vegetable oils, pure biodiesel and blends with diesel fuels have shown that there is a strong difference between the different fuels and blends. Pure vegetable oils seem to have higher mutagenicity than biodiesel and petro diesel, blends of biodiesel with diesel fuel have significant lower mutagenicity, but surprisingly emissions of B20 have higher mutagenic potential than other blends.

Possible reaction products from diesel fuel/biodiesel blends are in the focus of interest. It is well known in literature that emissions especially from B20 (20 % blend of biodiesel in fossil diesel) can lead to a maximum of mutagenicity.

Within this project different biodiesel samples from new feedstocks like rubber seed oil and fish oil were prepared and B20 blends have been used in engine tests. The influence of chemical composition and emissions was studied and the mutagenic potential of the particulate matter is measured and compared with the results of the other feedstocks. So a relation between chemical composition and grade of mutagenic potential of engine emissions should be found.

In the long term, the consortium of KORANET partners wants to understand the interactions between fuels components and their influences on the emissions and especially the health effects. In the future, fuels with high biogenic content shall be designed with regard to the technical, environmental and health-related aspects. So “PROMOFUEL” should develop scientific facts for the promotion of new non-food feedstocks for biodiesel production, on the one side in testing the possibility of finding the optimum blend of higher unsaturated feedstocks or in chemical modification, on the other side finding out the influence of chemical composition on the health effects of emissions.

1. Non-conventional biodiesel sample preparation

1.1. Introduction

In Korea, biodiesel is getting more important because it is recognized as the promising tool to mitigate CO_2 in transport sector. Since the annual diesel consumption in Korea is two times higher than gasoline, implementation of biodiesel has been started since 2006. With strong support from Korean government, the biodiesel production capacity has increased rapidly. Current production capacity of biodiesel in Korea is about 700,000kL/year but the actual production is only 400,000kL/year due to the shortage of feasible feedstocks since most feedstocks consumed in Korea are the imported palm, soybean oils and the waste cooking oil collected in land. The waste cooking oils are also derived from palm and soybean oils. Non-conventional feedstocks like rubber seed oil, fish oil and algal oils are emerging as the promising feedstocks for making biodiesel because they are inedible and may be cheaper than the conventional feedstocks. Another possible feedstock is winter rapeseed which may be cultivated in paddy fields in Korea during winter. Since all those feedstocks considered in Korea are containing high unsaturation fatty acids, evaluation of the biodiesels made from the non-conventional feedstocks as motor fuel will be very important.

The cooperation between Europe and South East Asia and especially Korea in the field of biofuels and biodiesel is very important because South East Asia is the major producer for vegetable oils with long tradition in oleochemical utilization of especially palm oil, but on the other side there is a big demand for feedstocks for biodiesel production. So it is very important to find and develop new non-food feedstocks, so Korea might have a high potential for the production of microbial oils like oil from micro algae. So this project could be the basis for the development of future intensive research in this area.

The objective of the work is the preparation of 20L of fatty acid methyl esters(FAMEs) out of rubber seed oil and fish oil for Partner 1 (University of Graz) and Partner 2 (Coburg University) to do the works, the partial hydrogenation of the FAMEs and the engine tests and emissions. For preparation of non-conventional biodiesel samples, we also established the reaction conditions for conversions of rubber seed and fish oils into FAMEs and analyzed the compositions of the FAMES and their fuel properties

1.2. Materials and Methods

1.2.1. Materials

About 200kg of rubber seeds were purchased from the local company in Thailand. The oil is mechanically extracted from the seeds after dehulling and roasting in Korea. Rubber seed oil extracted was used for preparation of rubber seed fatty acid methyl ester (FAME).
Salmon oil was purchased from a local supplier in Germany and used for making salmon oil FAME.

1.2.2. Pretreatment and Transesterification of Rubber Seed Oil

For the esterification of free fatty acids contained in rubber seed oil, the mixture of oil, methanol and sulfuric acid was mixed in 50L reactor at 65^{o}C for 3 hours (figure 1). The pretreated oil was used for the transesterification. Rubber seed oil was again mixed with methanol and potassium hydroxide at 65^{o}C for 40 minutes.

Figure 1: Reactor for conversion of rubber seed oil into FAME.

1.2.3. Analytical Methods

EN ISO 661 (animal and vegetable fats and oils e preparation of test sample) was used to measure the free fatty acid content containing oil and biodiesel. Acid value and free fatty acid content were calculated using the equations given in the literature [1].
Fatty acid methyl ester contents were analyzed based on EN standard 14103 using an Agilent 6890 capillary gas chromatograph (Agilent technologies) equipped with a Agilent-INNOWAX column (30 m* 0.32 mm* 0.5 mm). Methyl heptadecanoate (Fluka) as an internal standard material was used for quantification of FAME, which was calculated by the equations given in the literature [2].

1.3. Results

1.3.1. Rubber Seed Oil FAME Preparation

Properties of the extracted rubber seed oil are summarized in Table 1. Since the acid value of rubber seed oil is about 36, a two stage process (esterification of free fatty acids into FAME by acid catalysts and transesterification into FAME by alkali catalyst) is adopted for converting the rubber seed and salmon oils into their FAMEs [3].
The acid value of the rubber seed oil during the esterification is shown in figure 2 As shown in the figure, the acid value was dropped to 1 after the pretreatment.

Table 1: Properties of oil extracted from dehulled rubber seeds.

Property	Rubber seed oil
Density, g/ml	0.868
Viscosity, cP @ 20^{o}C	114.5
Kinematic viscosity, cSt @ 40^{o}C	35.3
Heating value, cal/g	9468.35
Total contamination, %	1.71
Water content, %	0.1404
Acid value, mg KOH/g	35.91
Unsaponifiables, %	0.84

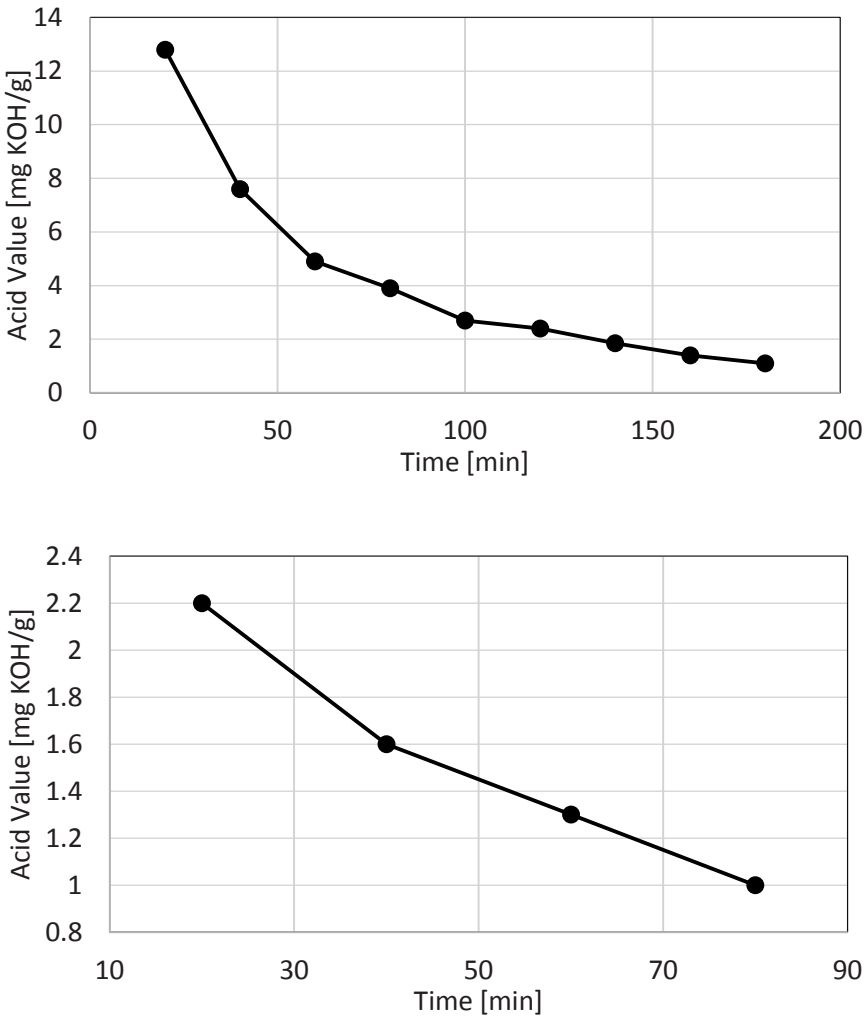

Figure 2: Acid value profile of rubber seed oil with respect to time. Reaction conditions: Methanol/oil 25.3% w/w, H2SO4/oil 0.81% w/w, 65oC, 200 rpm.

The esterified rubber seed oil was again transesterified to convert the residual triglycerides into FAME with KOH as a catalyst. The FAME profile is shown in figure 3.

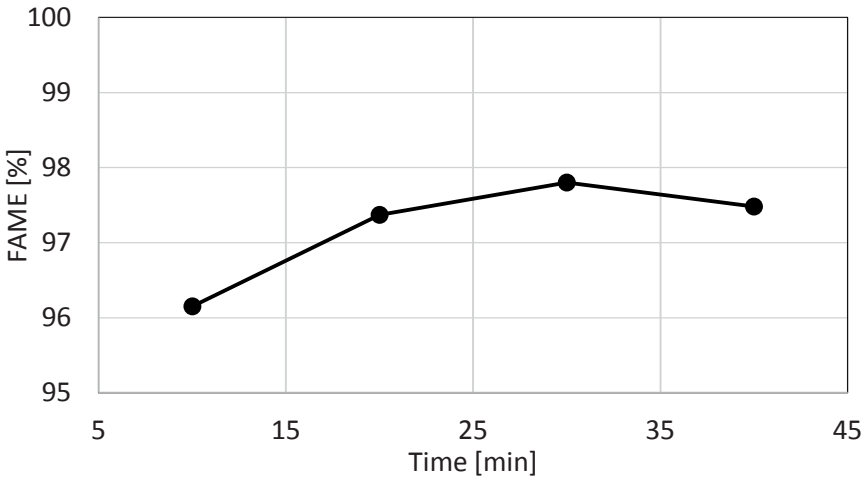

Figure 3: Rubber seed oil FAME profile with respect to time. Reaction conditions: Methanol/oil 33% w/w, KOH/oil 0.9% w/w, 65^{o}C, 200rpm.

1.3.2. Purification of Rubber Seed oil FAME

After transesterification, FAME needs to be separated from the reaction products. So the FAME was separated by sedimentation. The methanol in FAME was also removed by vacuum evaporation. After methanol removal, washing was applied to remove the traces of catalysts and other soluble impurities with distilled water. Rotary evaporator, operating at 90^{o}C and 20mbar, was used to remove water for 30 minutes. Finally FAME was distilled to get high purity product. Properties of the purified rubber seed FAME are summarized in Table 2.

Table 2. Properties of rubber seed FAME[1]

Parameter	Values
FAME content, wt. %	98.6
Acid value, mg KOH/g	0.78
Water content, vol %	0
Total glycerin, wt. %	0.01
Mono glycerides	0.002
Di Glycerides	0.001
Tri Glycerides	0.001
Free glycerol	0.008

[1]: Properties of FAME was determined by the analysis protocol specified in EN14214.

Composition of rubber seed oil FAME was also determined by the methods described in the literature [2]. Table 3 shows the composition of rubber seed oil FAME. As summarized the Table 3, rubber seed oil FAME has much higher contents of linolenic acid (C18:3) that should lead to very poor oxidation stability of FAME.

Table 3: Fatty acid composition of rubber seed, soya and rapeseed oils.

Fatty acids	Rubber seed*	Soya [4]	Rapeseed [4]
Myristic acid, C14:0	0.10	-	-
Palmitic acid, C16:0	8.07	11-12	3-5
Palmitoleic acid, C16:1	0.24	-	-
Stearic acid, C18:0	9.21	3-5	1-2
Oleic acid, C18:1	22.51	23-29	55-65
Linoleic acid, C18:2	33.17	52-56	20-26
Linolenic acid, C18:3	26.19	6-8	8-10
Arachidic acid, C20:0	0.00	-	1-2
Behenic acid, C22:0	0.40	-	0.5
Erucic acid, C22:1	0.00	-	-
Not identified	0.00	-	-
Total	100.00	100.00	

*: This work.

1.3.3. Preparation of Salmon Oil FAME

Since the oil has high polyunsaturated fatty acid (PUFA) contents, the oil is used as the model compounds of the algal oils. Like rubber seed oil, the Salmon oil has high free fatty acid contents (18.6 of acid value), so two stage conversion process has been used to make FAME from Salmon oil. Identical reaction conditions, applied for making FAME from rubber seed oil, were employed for converting salmon oil into FAME. For the esterification of free fatty acids in Salmon oil, the required reaction time is shorter than those for rubber seed oil. As shown in figure 4, the acid value of Salmon oil is decreased to lower than 2 after 120 minutes.

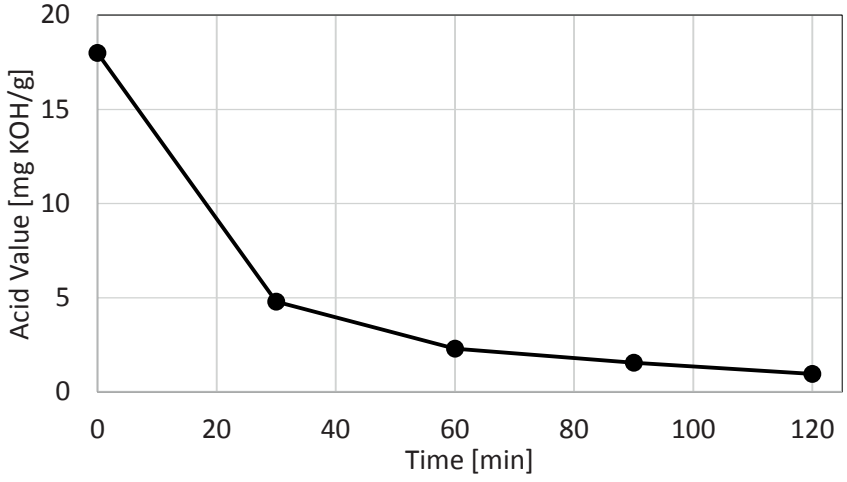

Figure 4. Acid value profile of Salmon oil with respect to time. Reaction conditions: Methanol/oil 25.3% w/w, H_2SO_4/oil 0.81% w/w, 65°C, 200 rpm.

1.3.4. Analysis of Salmon Oil FAME

Pretreated Salmon oil by esterification was again transesterified to get FAME with alkali catalysts. The reaction conditions are also same with those applied for rubber seed oil. Same purification protocol was also used for purification of Salmon oil FAME.
The FAME has following properties (Table 4).

Table 4: Properties of Salmon oil FAME[1]

Parameter	Values
FAME content, wt. %	98.4
Acid value, mg KOH/g	0.22
Water content, vol %	0.034
Total glycerin, wt. %	0.05
Mono glycerides	0.13
Di Glycerides	0.06
Tri Glycerides	< 0.01
Free glycerol	< 0.01

[1]: Properties of FAME was determined by the protocol specified in EN14214.

The PUFA content of salmon oil FAME having more than 4 double bonds is 5.4 % between that of fresh water algae and that of marine algae. Although the PUFA content of Salmon oil is some lower than that of marine algae, it may represent the characteristics of algal oil FAME. So the salmon oil FAME was supplied to Partner 1 and 2 for performing the works on partial hydrogenation and engine tests.

Table 5: Fatty acid compositions of Salmon and algal oils.

Fatty acid	Salmon oil	C. vulgaris[5] (Freshwater algae)	Marine algae	
			Nannochloropsis[6]	Isochrysis galbana[7]
Myrisic acid, C14:0	6.1	0.3	3.2	23.1
Myristoleic acid, C14:1	0.1	-	3.2	-
Palmitic acid, C16:0	11.2	26.1	9.2	14.0
Palmitoleic acid, C16:1	4.3	-	19.8	3.0
Stearic acid, C18:0	1.5	7.7	2.1	1.1
Oleic acid, C18:1	19.8	22.3	3.6	14.0
Linoleic acid, C18:2	6.0	21.0	1.1	5.0
Linolenic acid, C18:3	33.9	7.1	9.1	7.0
Arachidic acid, C20:0	0.2	-	-	-
Gadoleic acid, C20:1	4.4	-		-
Behenic acid, C22:0	0.1	-	-	-
PUFA (> 4 double bonds)	**5.4**	**-**	**26.5**	**29,0**
Not identified	7.1	14.0	10.4	3.8
Total	100	100	100	100

1.4. References

[1] AOCS Official Method Cd 3d-63. Acid value. Official method and recommended practices of the AOCS. AOCS Champaign. 5th ed., Illinois; 2003.

[2] British standard method. Fat and oil derivatives Fatty Acid Methyl Esters (FAME) determination of ester and linolenic acidmethylester contents. BS EN14103; 2003

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2. Characterization and Evaluation of the Biodiesel Samples

2.1. Scope

Partner 1 (Graz University) analyzed the biodiesel samples according to the European biodiesel specification EN 14214. Out of the fatty acid distribution it will be calculated in what amount these samples could be blended with other biodiesel samples in order to meet the limits for the content of unsaturated fatty acids. Tests with different synthetic antioxidants will be carried out in order to select the most effective one for enhancement of oxidative stability.

2.2. Preface

Each 5l of different biodiesel samples were sent by Korean partners in order to evaluate their quality. Biodiesel was prepared from rubber seed oil (RSO) and waste fish oil (WFO) by corresponding esterification and transesterification steps (see 1.2). For further purification, the samples were distilled. In order to evaluate now the final composition, these samples were fully analyzed according to European fuel specifications (EN14214).

2.3. Materials and Methods

Analysis was carried out according the standardized methods given by EN 14214 [1]. All chemicals, solvents and equipment used, followed the given normative methods of EN14214. Antioxidants, used for stability tests were purchased from following suppliers: Vulkanox BKF (2,2'-methylene-bis-(4-methyl-6-*tert*-butylphenol)), Vulkanox ZKF (2,2'-methylene-bis-(4-methyl-6-cyclohexylphenol)) donated by Bayer AG (Leverkusen, Germany); 2,5-Di-*tert*-butyl-hydroquinone (DTBHQ)and 2,5-*tert*-butyl-hydroquinone (TBHQ) was provided by AECI-Aroma and Fine Chemicals (Richards Bay, South Africa); 2,6-di-tert-butyl-4-methyl-phenol (butylated hydroxytoluene, BHT) and pyrogallol (PY) from Sigma-Aldrich Handels GmbH, Vienna, Austria and of minimum 98% purity.

2.4. Results

2.4.1. Rubber Seed Oil Methyl Esters (RSOME)

Table 6: Analysis Data of RSOME

Parameter	Unit	Test method	Limits EN 14214	Result
Ester content	%(m/m)	EN 14103	≥ 96.5	96.72
Density at 15°C	kg/m³	EN ISO 3675	860 – 900	887.4
Viscosity at 40°C	mm²/s	EN ISO 3104	3.50 – 5.00	4.27
Flash point	°C	EN ISO 3679	≥ 101	159
Sulfur content	mg/kg	EN ISO 20846	≤ 10.0	1.4
Carbon residue (CCR 10%)	%(m/m)	EN ISO 10370	≤ 0.30	0.19
Sulfated ash content	%(m/m)	ISO 3986	≤ 0.02	0.01
Water content	mg/kg	EN ISO 12937	≤ 500	125
Copper strip corrosion (3h at 50°C)	Rating	EN ISO 2160	Class 1	1
Oxidation stability, 110°C	Hours	EN 14112	≥ 6.0	**0.47**
Acid value	mgKOH/g	EN 14104	≤ 0.50	**1.18**
Iodine value	g/100 g	EN 14111	≤ 120	118.71
Linolenic acid methyl ester	%(m/m)	EN 14103	≤ 12.0	**14.8**
Polyunsaturated (≥4 double bonds) methyl ester	%(m/m)		≤ 1	<0.1
Methanol content	%(m/m)	EN 14110	≤ 0.20	<0.01
Monoglyceride content	%(m/m)	EN 14105	≤ 0.80	<0.01
Diglyceride content	%(m/m)	EN 14105	≤ 0.20	<0.01
Triglyceride content	%(m/m)	EN 14105	≤ 0.20	<0.01
Free glycerol	%(m/m)	EN 14105	≤ 0.02	0.025
Total glycerol	%(m/m)	EN 14105	≤ 0.25	0.025
Group I metals (Na+K)	mg/kg	EN 14538	≤ 5.0	<0.2 / <0.2
Group II metals (Ca+Mg)	mg/kg	EN 14538	≤ 5.0	<0.2 / <0.2
Phosphorus content	mg/kg	EN 14107	≤ 4.0	<0.2
CFPP	°C	EN 116		3
Fatty Acid Distribution:		A.O.C.S. Ce 1-62		
Lauric acid (C12:0)	%(A)			0.04
Myristic acid (C14:0)	%(A)			0.15
Myristoleic (C14:1)	%(A)			0.05
Palmitic acid (C16:0)	%(A)			9.22
Palmitoleic acid (C16:1)	%(A)			0.2
Stearic acid (C18:0)	%(A)			8.15
Oleic acid (C18:1)	%(A)			24.79
Linoleic acid (C18:2)	%(A)			35.11
Linolenic acid (C18:3)	%(A)			14.06
Arachidic acid (C20:0)	%(A)			0.33
Gadoleic (C20:1)	%(A)			0.15
Behenic acid (C22:0)	%(A)			0.12
Unidentified	%(A)			7.63

2.4.2. Squid Oil Methyl Esters (SOME)

Table 7: Analysis Data of SOME

Parameter	Unit	Test method	Limits EN 14214	Result
Ester content	%(m/m)	EN 14103	≥ 96.5	**92.76**
Density at 15°C	kg/m³	EN ISO 3675	860 – 900	878.9
Viscosity at 40°C	mm²/s	EN ISO 3104	3.50 – 5.00	4.79
Flash point	°C	EN ISO 3679	≥ 101	157
Sulfur content	mg/kg	EN ISO 20846	≤ 10.0	6.1
Carbon residue (CCR 10%)	%(m/m)	EN ISO 10370	≤ 0.30	0.29
Sulfated ash content	%(m/m)	ISO 3986	≤ 0.02	0.006
Water content	mg/kg	EN ISO 12937	≤ 500	177
Copper strip corrosion (3h at 50°C)	Rating	EN ISO 2160	Class 1	1
Oxidation stability, 110°C	Hours	EN 14112	≥ 6.0	**0.78**
Acid value	mgKOH/g	EN 14104	≤ 0.50	**1.71**
Iodine value	g/100 g	EN 14111	≤ 120	42.8
Linolenic acid methyl ester	%(m/m)	EN 14103	≤ 12.0	0.25
Polyunsaturated (≥4 double bonds) methyl ester	%(m/m)		≤ 1	<0.1
Methanol content	%(m/m)	EN 14110	≤ 0.20	<0.01
Monoglyceride content	%(m/m)	EN 14105	≤ 0.80	<0.01
Diglyceride content	%(m/m)	EN 14105	≤ 0.20	<0.01
Triglyceride content	%(m/m)	EN 14105	≤ 0.20	<0.01
Free glycerol	%(m/m)	EN 14105	≤ 0.02	0.009
Total glycerol	%(m/m)	EN 14105	≤ 0.25	0.009
Group I metals (Na+K)	mg/kg	EN 14538	≤ 5.0	<0.2 / <0.2
Group II metals (Ca+Mg)	mg/kg	EN 14538	≤ 5.0	<0.2 / <0.2
Phosphorus content	mg/kg	EN 14107	≤ 4.0	<0.2
CFPP	°C	EN 116		7
Fatty Acid Distribution:		A.O.C.S. Ce 1-62		
Lauric acid (C12:0)	%(A)			0.35
Myristic acid (C14:0)	%(A)			5.44
Myristoleic (C14:1)	%(A)			0.07
Palmitic acid (C16:0)	%(A)			37.12
Palmitoleic acid (C16:1)	%(A)			1.48
Stearic acid (C18:0)	%(A)			0.22
Oleic acid (C18:1)	%(A)			9.12
Linoleic acid (C18:2)	%(A)			37.7
Linolenic acid (C18:3)	%(A)			4.66
Arachidic acid (C20:0)	%(A)			0.19
Gadoleic (C20:1)	%(A)			0.21
Behenic acid (C22:0)	%(A)			0.24
Eicosadienoic acid (C20:2)	%(A)			0.09
Docosahexaenoic acid (C20:5)	%(A)			0.05
Unidentified	%(A)			3.06

2.4.3. Fish Oil Methyl Esters (FOME)

Table 8: Analysis Data of FOME

Parameter	Unit	Test method	Limits EN 14214	Result
Ester content	%(m/m)	EN 14103	≥ 96.5	98.4
Density at 15°C	kg/m³	EN ISO 3675	860 – 900	885.3
Viscosity at 40°C	mm²/s	EN ISO 3104	3.50 – 5.00	4.41
Flash point	°C	EN ISO 3679	≥ 101	172
Sulfur content	mg/kg	EN ISO 20846	≤ 10.0	11
Carbon residue (CCR 10%)	%(m/m)	EN ISO 10370	≤ 0.30	**0.69**
Sulfated ash content	%(m/m)	ISO 3986	≤ 0.02	0.01
Water content	mg/kg	EN ISO 12937	≤ 500	339
Total contamination	mg/kg	EN 12662	≤ 24	**25**
Oxidation stability, 110°C	Hours	EN 14112	≥ 6.0	**0.42**
Acid value	mgKOH/g	EN 14104	≤ 0.50	0.17
Iodine value	g/100 g	EN 14111	≤ 120	117.07
Linolenic acid methyl ester	%(m/m)	EN 14103	≤ 12.0	0.32
Polyunsaturated (≥4 double bonds) methyl ester	%(m/m)		≤ 1	**12.64**
Methanol content	%(m/m)	EN 14110	≤ 0.20	<0.01
Monoglyceride content	%(m/m)	EN 14105	≤ 0.80	0.13
Diglyceride content	%(m/m)	EN 14105	≤ 0.20	0.06
Triglyceride content	%(m/m)	EN 14105	≤ 0.20	<0.01
Free glycerol	%(m/m)	EN 14105	≤ 0.02	<0.01
Total glycerol	%(m/m)	EN 14105	≤ 0.25	0.05
Group I metals (Na+K)	mg/kg	EN 14538	≤ 5.0	**11** / 1.0
Group II metals (Ca+Mg)	mg/kg	EN 14538	≤ 5.0	0.7 / 1.1
Phosphorus content	mg/kg	EN 14107	≤ 4.0	1.0
CFPP	°C	EN 116		-7
Fatty Acid Distribution:		A.O.C.S. Ce 1-62		
Myristic acid (C14:0)	%(m/m)			3.07
Myristoleic acid (C14:1)	%(m/m)			0.12
Palmitic acid (C16:0)	%(m/m)			6.22
Palmitoleic acid (C16:1)	%(m/m)			2.80
Stearic acid (C18:0)	%(m/m)			1.35
Oleic acid (C18:1)	%(m/m)			17.06
Linoleic acid (C18:2)	%(m/m)			5.09
Linolenic acid (C18:3)	%(m/m)			34.73
Arachidic acid (C20:0)	%(m/m)			0.20
Gadoleic acid (C20:1)	%(m/m)			5.26
Not identified	%(m/m)			13.45

2.4.4. Distilled Fish Oil Methyl Esters (DFOME)

Table 9: Analysis Data of DFOME

Parameter	Unit	Test method	Limits EN 14214	Result
Ester content	%(m/m)	EN 14103	≥ 96.5	99.8
Density at 15°C	kg/m³	EN ISO 3675	860 – 900	882.1
Viscosity at 40°C	mm²/s	EN ISO 3104	3.50 – 5.00	4.26
Flash point	°C	EN ISO 3679	≥ 101	156
Sulfur content	mg/kg	EN ISO 20846	≤ 10.0	4
Carbon residue (CCR 10%)	%(m/m)	EN ISO 10370	≤ 0.30	**0.62**
Sulfated ash content	%(m/m)	ISO 3986	≤ 0.02	0.01
Water content	mg/kg	EN ISO 12937	≤ 500	384
Total contamination	mg/kg	EN 12662	≤ 24	1
Oxidation stability, 110°C	Hours	EN 14112	≥ 6.0	**0.47**
Acid value	mgKOH/g	EN 14104	≤ 0.50	0.18
Iodine value	g/100 g	EN 14111	≤ 120	85.97
Linolenic acid methyl ester	%(m/m)	EN 14103	≤ 12.0	0.50
Polyunsaturated (≥4 double bonds) methyl ester	%(m/m)		≤ 1	5.41
Methanol content	%(m/m)	EN 14110	≤ 0.20	0.02
Monoglyceride content	%(m/m)	EN 14105	≤ 0.80	0.03
Diglyceride content	%(m/m)	EN 14105	≤ 0.20	<0.01
Triglyceride content	%(m/m)	EN 14105	≤ 0.20	<0.01
Free glycerol	%(m/m)	EN 14105	≤ 0.02	0.01
Total glycerol	%(m/m)	EN 14105	≤ 0.25	0.03
Group I metals (Na+K)	mg/kg	EN 14538	≤ 5.0	0.9 / <0.2
Group II metals (Ca+Mg)	mg/kg	EN 14538	≤ 5.0	0.5 / 0.9
Phosphorus content	mg/kg	EN 14107	≤ 4.0	0.9
CFPP	°C	EN 116		-1
Fatty Acid Distribution:		A.O.C.S. Ce 1-62		
Lauric acid (C12:0)	%(m/m)			0.10
Myristic acid (C14:0)	%(m/m)			5.05
Myristoleic acid (C14:1)	%(m/m)			0.05
Palmitic acid (C16:0)	%(m/m)			11.23
Palmitoleic acid (C16:1)	%(m/m)			4.31
Stearic acid (C18:0)	%(m/m)			1.53
Oleic acid (C18:1)	%(m/m)			19.83
Linoleic acid (C18:2)	%(m/m)			5.96
Linolenic acid (C18:3)	%(m/m)			34.85
Arachidic acid (C20:0)	%(m/m)			0.17
Gadoleic acid (C20:1)	%(m/m)			4.37
Behenic acid (C22:0)	%(m/m)			0.12
Not identified	%(m/m)			7.07

2.4.5. Discussion

RSOME:

The sample showed the typical fatty acid distribution, known for rubber seed oil. Noticeable is the high amount of higher (more than 1 double bond) unsaturated fatty acids. Especially the content of linolenic acid (C 18:3) with 14.8%m/m is above the given specification limit. Linked to the high amount of unsaturated fatty acid esters in the sample is of course the very low oxidation stability with 0.47h. For practical applications, such biodiesel has to be stabilized by use of appropriate antioxidants. Furthermore, the high acid value reflects, that an additional washing or even purification step will be necessary. High acidity is directly linked to corrosion effects on engine parts.

SOME:

Originally, squid oil was selected by the Korean partners as representative feedstock with on the one hand nonfood application (waste) and on the other hand with an expected higher content of polyunsaturated fatty acids. However, as the analysis results showed, the content of polyunsaturated (≥4 double bonds) fatty acids is insufficiently low. Therefore, fish oil from salmon was selected. Detailed information on the composition is given enclosed. Anyway, squid oil methyl esters are analyzed according the given specifications and besides low polyunsaturated ester content (which in fact is within specs limits) also other parameters exceed the limits. With 92.76 %m/m, the ester content is significantly below the minimum requirement. As the other parameters describing the transesterification behavior are within the given limits, it seems that some other byproducts like unsaponifiable species are responsible for this low ester content. A further purification step is therefore recommended. Linked to the high amount of unsaturated esters (C18:2, C18:3) is the poor oxidation stability with 0.78h. Stabilization by antioxidants is therefore necessary. Dramatically high is also the acid value. Insufficient purification (washing) or transesterification might be the reason for that. Here, an additional purification step is also necessary to decrease the acid content. Another aspect to be considered is that due to a high amount of especially palmitic acid in the oil, the low temperature behavior is very poor. With a CFPP of +7°C for the neat biodiesel winter operability is not ensured without blending with fossil diesel.

FOME distilled and undistilled:

Methyl esters prepared from fish oil (salmon oil) have been chosen as source for polyunsaturated rich material. The originally planned utilization of algal oil failed due to lack of oil world-wide. As the analysis of the final esters showed, higher amounts of especially C20:5 and C22:6 fatty acids can be found. However, during distillation the amount decreases from 12.64% m/m to 5.41%m/m. Such polyunsaturated species are highly affected by temperature in combination with oxygen contact and degradation is the consequence. This is

also underlined by a very low value for oxidation stability. Therefore, it is unambiguous to stabilize such material by appropriate antioxidants. The evaluation of the efficiency of different antioxidants is given in (2.6.). Related to the high amount of unsaturated species in the product, also the value for carbon residue is increased. This relation has been investigated intensively and a correlation between degree of unsaturation and carbon residue increase has been found [2,3]. As carbon residue is not further a quality parameter of biodiesel since the last adoption of the method, it is from our perspective still important to point out, that carbon residue values in context to polyunsaturated rich material should be re-evaluated.
What was really astonishing when analyzing the fish oil biodiesel, is the relatively low value for Iodine number. As the fatty acid profile showed, significant amounts of palmitic acid can be detected. This is quite unusual for fish oil. But due to lack on further information on the feedstock it can be hardly evaluated and discussed. Anyway, during distillation the Iodine numbers drop down further as consequence of the decrease (alteration) of the polyunsaturated fatty acid esters.

2.5. Blending Behavior

As the fatty acid distribution of the individual feedstocks respectively biodiesel produced showed significant high amounts of unsaturation, blending experiments with biodiesel and fossil diesel were not carried out. However, these experiments were carried out with the hydrogenated biofuel as given in chapter 3.3.

2.6. Stability Improvement

In order to guarantee a certain stability of the biodiesel produced, it was necessary to test several specific antioxidants. The stability improvement is necessary of course to fulfill quality requirements, but also to avoid negative influences of degradation products on engine performance and exhaust emission tests. As the selected feedstocks respectively produced methyl esters are very rich in unsaturated and polyunsaturated fatty acids it is quite harder to improve their stability to a certain extent. “Classic” antioxidants used for biodiesel prepared from vegetable oils or animal fats are not as effective in case of higher unsaturation [4]. Therefore, antioxidants known for higher effectiveness are tested [5]. The oxidation stabilities of the different biodiesel produced within this project are listed in table 10. As can be seen, all of them are far beyond the required limits, consequence of their fatty acid distribution.

Table 10: Stability of Neat Samples

Sample	Oxidation Stability [h]
RSOME	0.47
FOME	0.42
DFOME	0.47

Stability improvement experiments were carried out under classical Rancimat conditions as given by EN1214. Several additives are tested in different concentrations as listed in table 11.

Table 11: Influence of Additives on IP

Additiv [mg/kg]	RSOME IP [h]	FOME IP [h]	DFOME IP [h]
Vulkanox BKF			
1000	4.44	5.09	4.80
500	2.11	2.71	1.97
250	1.34	1.78	1.08
100	0.90	0.65	0.60
0	0.47	0.42	0.47
Vulkanox ZKF			
1000	3.22	4.18	4.11
500	1.97	2.77	2.01
250	1.21	1.61	1.67
100	0.88	0.91	0.85
0	0.47	0.42	0.47
DTBHQ			
1000	**8.66**	**8.03**	**6.91**
500	**7.06**	**6.43**	3.67
250	5.73	5.20	2.18
100	3.80	2.54	1.64
0	0.47	0.42	0.47
TBHQ			
1000	**8.14**	**7.28**	**7.12**
500	5.38	3.55	4.92
250	2.77	2.84	3.07
100	2.01	1.22	1.53
0	0.47	0.42	0.47
PY			
1000	**7.25**	**6.03**	5.88
500	**6.68**	4.67	4.31
250	4.28	2.91	3.70
100	3.71	1.25	1.56
0	0.47	0.42	0.47
BHT			
1000	2.06	2.88	1.98
500	1.27	1.74	1.33
250	0.79	1.21	0.74
100	0.60	0.68	0.52
0	0.47	0.42	0.47

As can be seen, only slight and mostly insufficient improvements on stability can be archived. To fulfill quality requirements, relatively high concentrations are necessary. Evaluating the individual antioxidants tested on the different methyl esters, only DTBHQ and TBHQ at 1000 mg/kg are suitable. It was therefore recommended to use these antioxidants for stabilization of the biodiesel used in the project.

2.7. References

[1] European Committee for Standardization: EN 14214 (2013); Liquid petroleum products - Fatty acid methyl esters (FAME) for use in diesel engines and heating applications - Requirements and test methods.

[2] Mittelbach, M.; Enzelsberger, H.; Transesterification of Heated Rapeseed Oil for Extending Diesel Fuel. *J. Am. Oil Chem. Soc.*, (1999),**76**(5), 545-550.

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[5] Schober, S.; Mittelbach, M.; The Impact of Antioxidants on Biodiesel Oxidation Stability. *Eur. J. Lipid Sci. Technol.* (2004), **106**, 382-389.

3. Partial Hydrogenation of Biodiesel Samples in Lab-scale

3.1. Scope

Fish oil methyl esters are partially hydrogenated by partner 1 (Graz University) in order to reduce the amount of polyunsaturated fatty acids and to meet the EN 14214 specifications. These reactions are carried out in laboratory scale at very mild conditions (room temperature, ambient pressure), so the reaction will be stopped at the stage of mono unsaturation. These products on the one side should have sufficient stability, on the other hand also good cold temperature behavior.

3.2. Materials and Methods

3.2.1. Feedstock

Fatty acid methyl esters were prepared from fish oil (VF Cuxhaven, Germany) by transesterification with methanol according to an internal method. This Feedstock was tested on its properties according to well-established methods given in EN 14214, and was partially hydrogenated without further pretreatment.

3.2.2. Partial Hydrogenation

Partial hydrogenation of the prepared fish oil methyl esters was carried out in a 500 mL batch reactor (Parr Inst., USA). For each experiment 50 g of fish oil methyl esters (180 mmol) were diluted with technical grade n-hexane (Roth, Austria) to an overall volume of 200 mL and 0.5 g of palladium on activated carbon was added (10 % Pd basis Aldrich, USA). This reaction mixture was processed at a constant hydrogen pressure of 10 bars at room temperature (20-28°C) while stirring was kept constant at 300 rpm. The gas was consecutively supplied from a high pressure gas burette (Parr Inst., USA) which acted as a gas reservoir. To achieve partial hydrogenation the molar amount of hydrogen was limited; therefore the reaction was stopped by depressurizing the system after defined amounts of hydrogen were consumed. This was measured via the pressure drop within the gas reservoir at a constant temperature. Before further analysis the solvent was removed on a rotary evaporator (Heidolph, Germany). Products with a nominal degree of saturation of 100, 75, 66, 50, 33 and 25 % were obtained.

3.2.3. Product Analysis

All obtained products were tested on, in the present context, critical fuel properties. These properties were the oxidation stability, Iodine value, cold flow properties as well as the distillation range [1-6], and were compared to requirements according to EN 14214. Furthermore, the same tests were carried out on blends of the product with a nominal degree of saturation of 50 % with fossil diesel fuel. Blending rates were 5, 10, 20 and 30 % v/v. These results were compared to EN 590.
The product composition was determined by gas chromatography (Agilent 7890 A GC equipped with a flame ionization detector) with an Agilent J&W HP-INNOWax capillary column (30m x 0,250 mm x 0,15 um polyethylene glycol). 1 µL of a sample solution (3 % m/m) with 1 mg/mL of C23:0 as an internal standard was split injected (split ratio 30:1, injector temperature 240°C). Separation was achieved by a temperature program starting at a temperature of 150°C, which was raised by 5°C/min to 240°C, and finally held for 15 min. The carrier-gas flow (He) was 0.70 mL/min.

3.3. Results and Discussion

Fatty acid methyl esters derived from fish oil (FOME) have excellent cold flow properties because of its high amount of highly unsaturated compounds. This circumstance on the other hand leads to a very poor oxidation stability which makes it not fully unsuitable for the use as biodiesel. According to EN 14214 the maximally allowed amount of polyunsaturated compounds is 1 % m/m. To reduce the polyunsaturated methyl ester content of about 14 % m/m in the feedstock used to fit within the specification's requirement and therefore increase the oxidation stability while maintaining satisfying cold flow properties, partial hydrogenation was carried out. This could be achieved in any case in less than 15 min, although almost full hydrogenation (P100) took several hours. The experimental results and properties of the products as well as the feedstock are summed up in Table 12.

Table 12: Product and Feedstock Properties and Results

Product	Hydrogenation		≥4 db	Iodine Value	Oxidation stability 110°C	Cold flow properties		
	nH_2 [mmol]	t [min]	[% m/m]	[g/100g]	[h]	CP [°C]	PP [°C]	CFPP [°C]
FOME	Feedstock		14.39	118	0.17	-3	-3	-7
P25[a]	70.6	5.7	3,52	86	0.56	3	-6	-11
P33	88.5	3.5	1,01	75	1.01	1	-6	-9
P50	133.4	13.8	n.d.[b]	59	4.62	7	3	0
P66	179.8	12.3	n.d.	45	5.40	15	15	18
P75	191.4	14.3	n.d.	41	18.66	18	18	11
P100	273.3	348.2	n.d.	n.d.	>70	solid		
Requirements according to EN14214			max. 1	max. 120	min. 8	n.s.[c]		

[a] Product X, where X is the nominal degree of saturation

[b] not detectable

[c] not specified

Obtained partially hydrogenated products are classified according to their degree of saturation defined by the molar amount of hydrogen consumed during the reaction in relation to the fully hydrogenated product. The approach to control the excess of hydrogenation by hydrogen supply rather than time was chosen to eliminate temperature influences on the reproducibility. These occurred mainly by pressurization and dissolution of the gas in the medium. Increases in temperature occurred despite water-cooling, measured temperatures ranged from 20 to 28°C. As figure 5 confirms this deviation had significant influence on the reaction time. However the data is consistent concerning hydrogen consumes, that way the degree of saturation could excellently be controlled.

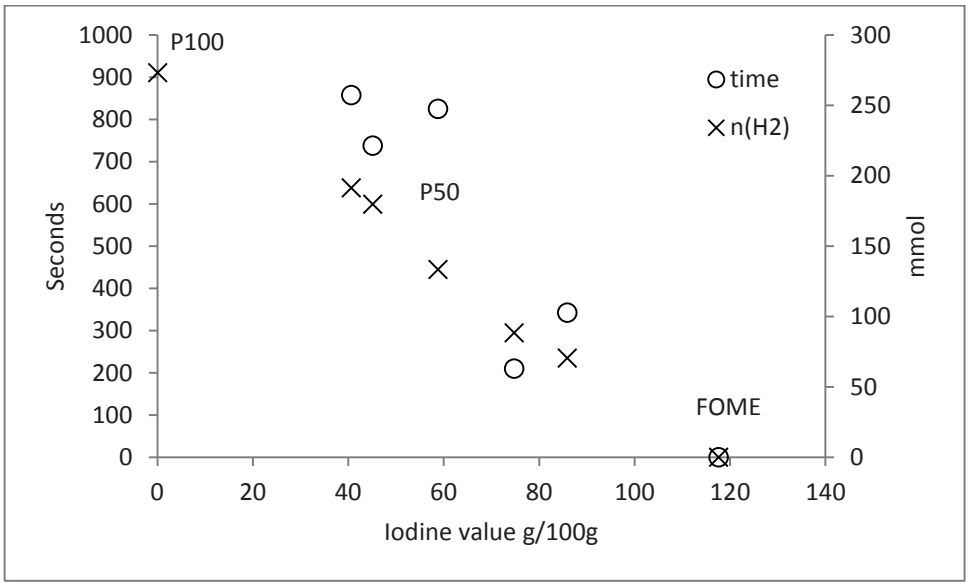

Figure 5: Dependency of Iodine Value on Molar Hydrogen Amount and Time

Figure 6 shows an overlay of the chromatograms of three of the products and the feedstock.

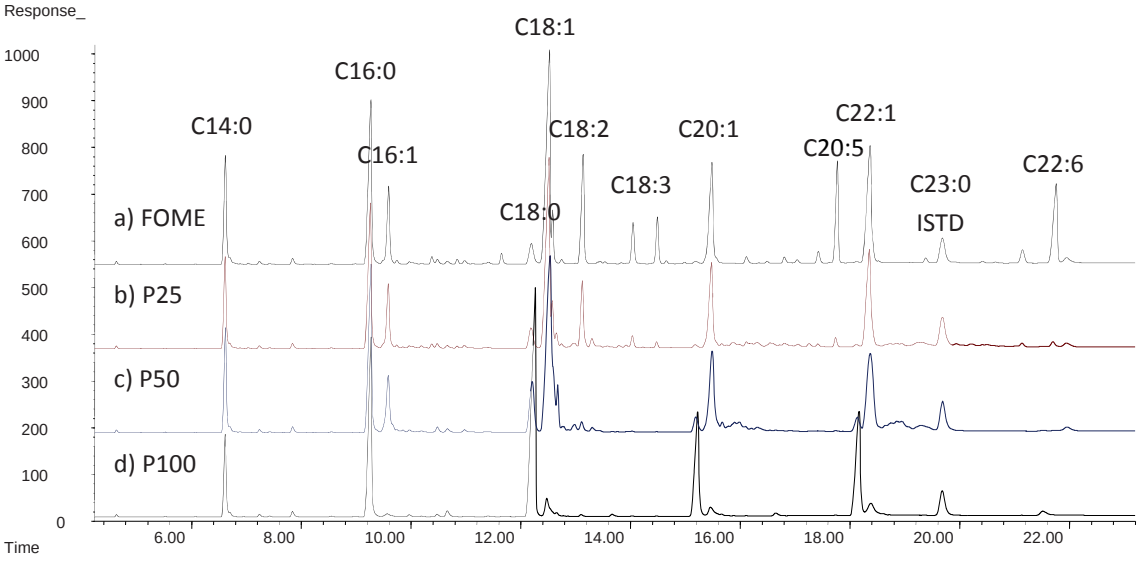

Figure 6: Feedstock Chromatograms

As the percentage composition (figure 7) confirms, polyunsaturated esters are quickly hydrogenated to less unsaturated compounds as they are nearly vanished in P33 (about 1 %).

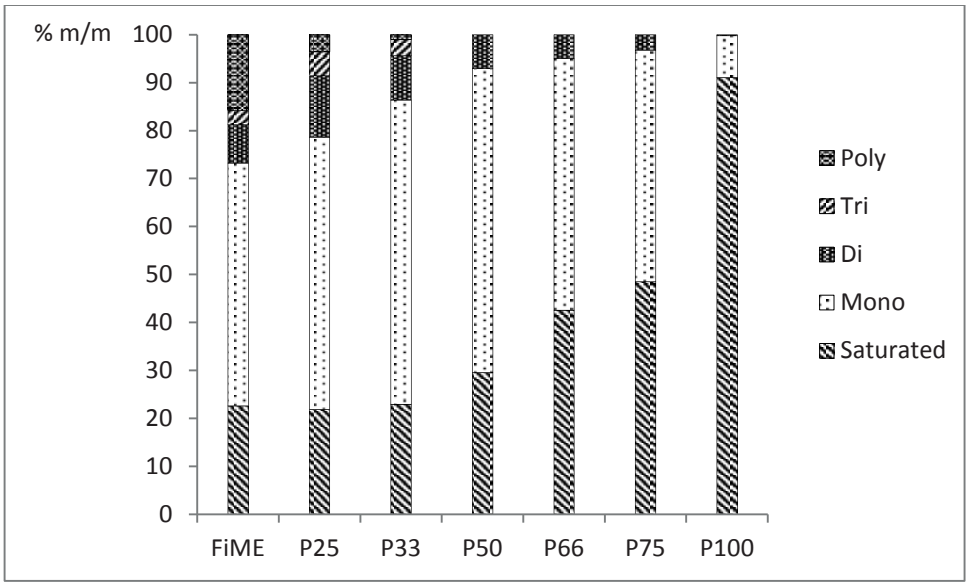

Figure 7: Percentage Compositions of Obtained products and Feedstock

Until P50 the level of saturated compounds remains almost at a constant level. This product is virtually free from poly and tri-saturated esters and has a very high content of monounsaturated esters of about 63 % while saturated esters are only comprised in nearly the same excess as in the feedstock. Compared to the products with a higher degree of saturation also the cold flow properties remained in an acceptable range. Because of these advantages this product was further taken as a component for blending with fossil diesel fuel. At the moment a maximum of 7 % v/v of FAME is allowed to be contained in fossil diesel fuel according to EN 590, which is the European standard for fossil diesel fuel. Hence, FAME addition significantly influences the oxidation stability blends with more than 2 % v/v, need to pass the oxidation stability test according to EN 15751. To do so the blend has to be stable for more than 20 h. This was the case for all blends tested even in higher blending ratios up 30 % v/v. Also the requirements as winter diesel for temperate climatic zones are fulfilled (Tab. 13).

Table 13: Blending Ratios vs. Cold Flow Behavior

Blending Rate [% v/v]	Iodine Value [g/100g]	Oxidation stability 110°C [h]	Cold flow properties [°C]		
			CP	PP	CFPP
5	5.51	>70	-6	-25	-27
10	9.49	>70	-6	-24	-25
20	15.63	38.00	-5	-18	-20
30	21.76	37.52	3	-15	-17

Selected boiling profiles are given in figure 8.

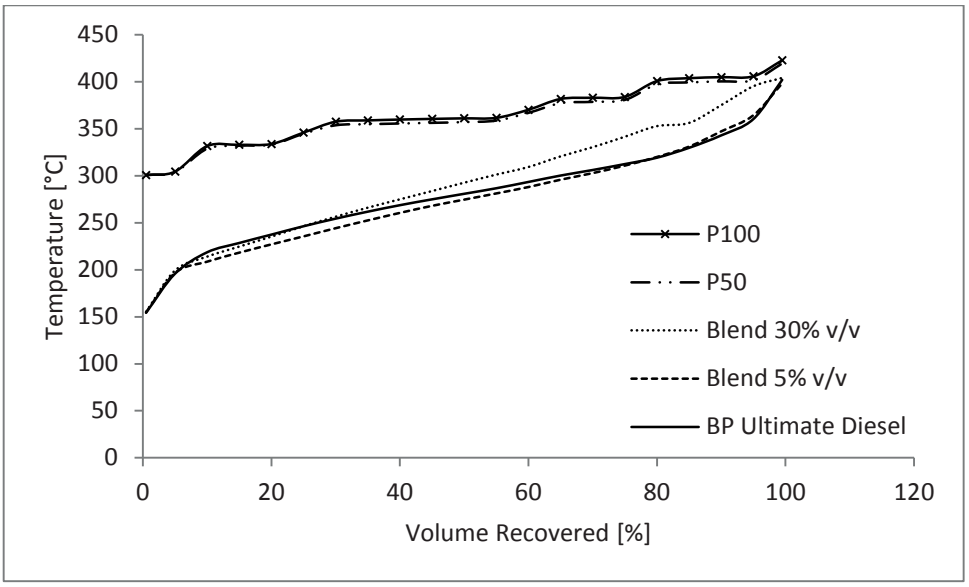

Figure 8: Boiling Profiles

All Blends fully meet the specified requirements given within EN 590 in term of boiling behavior. Neat products were only plotted for comparison reasons but do not need to fulfill any requirements according to EN 14214.

3.4. Conclusion

Polyunsaturated fatty acids occur in high amounts in fish oil therefore the oxidation stability is less suitable for fuel applications. By a time-efficient partial hydrogenation over common Pd/C at room temperature, these undesirable compounds could be selectively removed from biodiesel derived from fish oil. The product composition could be controlled by the hydrogen supply, that way it was possible to hydrogenate selectively towards monounsaturated compounds, while keeping saturated compounds at almost the level on which they occurred in the feedstock, hence the cold flow properties remained in a suitable range. Blends of so obtained products with fossil diesel fuel were tested on crucial fuel properties. All blends fulfill the requirements by EN590 even in higher blending rations then allowed therein and therefore represent excellent blending components for fossil diesel fuel.

3.5. References

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[2] European Committee for Standardization (CEN). EN 14111:2001. Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of Iodine Value; CEN: Brussels, Belgium, 2001.

[3] European Committee for Standardization (CEN). EN 116:97. Diesel and Domestic Heating Fuels - Determination of Cold Filter Plugging Point; CEN: Brussels, Belgium, 1997.

[4] ASTM International. ASTM D2500-11. Standard Test Method for Cloud Point of Petroleum Products; ASTM International: West Conshohocken, PA, 2011.

[5] ASTM International. ASTM D97-12. Standard Test Method for Pour Point of Petroleum Products; ASTM International: West Conshohocken, PA, 2012.

[6] International Organization for Standardization (ISO). ISO 3924:2010. Petroleum Products; Determination of Boiling Range Distribution. Gas Chromatography Method; ISO: Geneva, Switzerland, 2010.

4. Emissions of Rubber Tree Oil Methyl Ester and Fish Oil Methyl Ester

4.1. The Environmental Relevance of Diesel Engine Emissions

These individual exhaust gas components are briefly described in terms of their formation and effects on humans and the environment.

4.1.1. Regulated Exhaust Gas Components

Hydrocarbons (HC)

The combustion of organic materials ideally takes place according to the following gross reaction equation under the creation of carbon dioxide and water

$$C_n H_m + \left(n + \frac{m}{4} \right) O_2 \rightarrow n\, CO_2 + \frac{m}{2} H_2O \,.$$

In real combustion processes, this ideal conversion of fuel to water and carbon dioxide is not achieved. Depending on the engine and its operating conditions (load level, engine temperature, etc.) partially oxidized compounds can develop or unburned fuels can be emitted. The sum of these components is called hydrocarbons, and is measured as a lumped parameter.

Through the multiplicity of substances belonging to this class of substances, no simple statement on the direct impact on humans is possible. Hydrocarbon emissions can sometimes be of low toxicological relevance (i.e., alkanes or alkenes), but they can also be carcinogenic (i.e., benzene). Furthermore, the hydrocarbons are of significance in atmospheric chemistry since some of these substances contribute to the development of summer smog. A correlation between a total hydrocarbon emission value and the resulting health and environmental damage cannot be undertaken due to the complex composition of this group of substances.

Carbon Monoxide (CO)

Carbon monoxide is also the result of the incomplete combustion of fuels. In addition to these engine processes, household and industrial combustion processes, the oxidation of methane in the troposphere as well as the decomposition of chlorophyll can be named as sources of

emissions. CO is constantly oxidized to CO_2 in the atmosphere or eliminated by soil bacteria. The average residence time in the troposphere is less than half a year. Through the high conversion of CO in the atmosphere, the main danger is less at the global level than at the local level, and particularly in closed rooms. Carbon monoxide is a colorless and odorless gas and links 250 times more strongly to hemoglobin than oxygen. With increased carbon monoxide concentrations in inhaled air, this suppression of oxygen leads to suffocation symptoms through to death. Acute poisoning occurs beyond 2,000 ppm, sub-acute at just 500 ppm CO (Marquardt and Schäfer, 1994).
Overall, the carbon monoxide emissions occurring through engine combustion, in consideration of the other environmental pollution caused by vehicle traffic, are insignificant. Only 7.5% of CO emissions originated in 2002 from diesel vehicles (2.5 % from passenger cars and 5 % from heavy duty vehicles, VDA 2004). In future, the emissions will decrease. Furthermore, the major part of traffic CO emissions originates from spark ignition engines (VDA, 2008).

Nitrogen Oxides (NOx)

The nitrogen oxides nitrogen monoxide (NO) and nitrogen dioxide (NO_2) are in contrast to CO and HC by-products of complete combustion. Nitrogen monoxide results as so-called “thermic NO” in the oxygen rich parts of the flame, or in the subsequent reaction zone (Baumbach, 1993). The reaction mechanism is called Zeldovich reaction (Fernando et al., 2006; Warnatz et al., 2001) in accordance with:

$$O_2 \rightarrow 2O\cdot$$
$$O\cdot + N_2 \rightarrow NO + N\cdot$$
$$N\cdot + O_2 \rightarrow NO + O\cdot$$
$$N\cdot + \cdot OH \rightarrow NO + H\cdot$$

The reaction begins at about 1300 °C as a consequence of the start of oxygen dissociation. In the engine a noteworthy NO level can first be seen beyond about 1900 °C (Mattes et al., 1999). In addition the nitrogen oxide known as “prompt NO” results from the reaction of HC radicals with air nitrogen and intermediary developed hydrocyanic acid. The latter plays only a minor quantitative role. Overall more than 90 percent of the total nitrogen oxide emissions are nitrogen monoxide. Characteristic of NO is its effort to react with oxygen – and particularly quickly with ozone – to form NO_2.
Pure nitrogen monoxide does not irritate the lungs, but if no conversion to NO_2 occurs, it develops methemoglobin after resorption via the respiratory tract. NO is an endogenous modulator of the blood vessel tone and thus a well-studied substance in terms of physiology and metabolism (Lenz et al., 1993).

Nitrogen dioxide is a gas with a piercing odor and red-brown color. It irritates the lungs and mucosa at a very low concentration. As a free radical, NO_2 is basically in a position to abstract hydrogen from fatty acids and thus to cause lipid peroxidation. This peroxidation ultimately leads to a loss of function in biological membranes. The destruction of membranes is the higher-ranking toxicity principle, while the lipid peroxidation presents the initial reaction. Living cells counteract this process with protective and reparative mechanisms so that it first occurs by extremely high concentrations of NO_2 which are hardly attainable in the free atmosphere. In the presence of water, NO_2 disproportions to nitrous acid and nitric acid. The nitrous acid (HNO_2) or its salts can react with secondary amines to mutagenic nitrous amines. Nitrite in the blood system can oxidize hemoglobin to methemoglobin through which the capacity to transport oxygen can be lost. NO_2 works in the same way, probably through the nitric acid (HNO_3) which develops as a cellular poison in the respiratory tract. Exposure to 9 mg/m³ NO_2 causes reduced fluidity of plasma membranes. Changes in the fluidity of membranes affect a range of fundamental cellular functions such as transmembrane transport, certain enzyme activities and receptor-ligand interactions. An activation of anti-oxidative enzymes and lipid peroxidation were observed after exposure to about 0.7 mg/m³ NO_2 (Marquardt and Schaefer, 1994). Nitrogen dioxide is a substance for which evidence of a carcinogenic effect can be found in in vitro and animal studies.
Of additional significance is the ability of NO_2 to serve as a preliminary substance for photo oxidant development, particularly for ozone. The leaching of nitrous oxide from the atmosphere takes place through the formation of nitrous acid or nitric acid and their subsequent wet deposition as so-called acid rain.
The worldwide road traffic causes 17.5 % of anthropogenic nitrogen oxide emissions (Mollenhauer, 2002). In the year 2002, in Germany diesel passenger cars caused 9 % and heavy duty vehicles 53 % of NO_x emissions of all traffic emissions (VDA, 2004).

Particulate Matter (PM)

In contrast to the concept “diesel engine emissions,” no general definition for diesel particles exists. According to the definition of the Environmental Protection Agency (EPA) in the U.S.A., particles shall be understood in the following as all substances present in diluted exhaust in solid or liquid form at a temperature of under 51.7 °C (meaning 125 °F) and which can be deposited on a filter (Code of Federal Regulations). The exhaust gas sample temperature is limited to ensure that all organic compounds with higher boiling points that could be of concern for health reasons and which could be adsorbed on carbon particulate matter are documented by the analysis. The temperature reduction of the exhaust gas samples is achieved by mixing the exhaust gas with air in a dilution system. In this manner the exit of the exhaust gas into the environment is simulated.

The emitted particle mass consists of a multiplicity of organic and inorganic substances. The main constituents of the organic substances are unburned or only partially burned fuel and lubrication oil. The inorganic substances include soot (carbon), sulfates, water and metallic compounds. Shavings and rust particles originating directly from the engine or the exhaust gas system, as well as derivates of organo-metallic fuel and lubricant additives, are included in the metallic compounds. The percentage of these substances in the total particle mass depends on many parameters. In addition to constructive parameters such as form of the combustion chamber and design of the injection system, the point of operation, or rather the overall load configuration, the fuel and lubricant quality as well as the wear of the engine show also an influence here (Wachter and Cartellieri, 1987; Bosch, 2007).

The development of particles is initiated by the soot development in the combustion chamber. Soot develops if the fuel enters an area with high temperature and low oxygen supply. The reactions leading to soot or particle formation are only incompletely clarified in a quantitative manner. The most probable hypothesis is the Acetylene Theory (Klingenberg et al., 1992). According to this theory, crack and dehydration reactions occur at the beginning of the soot development, which lead to the decomposition of long-chain fuel molecules into short-chain unsaturated hydrocarbons like acetylene. Through accumulation, with further hydrogen split off, acetylene develops cyclic and polycyclic aromatic hydrocarbons (PAH). Further addition and dehydration reactions lead to an increase in the carbon portion of the molecules, until ultimately the first particles develop with a diameter of more than 0.001 µm, from which the so-called primary particles coagulate with a diameter of 0.01 to 0.08 µm. The form in which the particles leave the combustion chamber is very diverse. Both small primary particles as well as larger agglomerates that are composed of primary particles and partly form some spatially-branched chains and spherical clusters have been observed (Lipkea and Johnson, 1978; Amann and Siegla, 1982; Jing et al., 1996).
In the consideration of particle development it is always differentiated between the processes in the combustion chamber, those in the exhaust tract, and those in the mixing of exhaust gas with ambient air, i.e. at the exit into the atmosphere.
For the particle development in the combustion chamber, the soot oxidation that starts practically simultaneously to the soot formation is of great significance. The speed of the soot formation, initiated by crack reactions as well as oxidation reactions, is strongly dependent on pressure, temperature and air ratio. At high temperatures and low levels of oxygen (small air ratio) the speed of the crack reactions is higher than that of the oxidation reactions, which leads to an increased development of soot. At high temperatures and large air ratios, in contrast, the oxidation speed is higher so that a subsequent soot combustion is possible (Meurer, 1966; Hühn, 1970; Houben and Lepperhoff, 1990; Glassman, 1996; Burtscher, 2004).
A non-homogeneous mixed preparation of fuel and air in the combustion chamber always causes particle development in diesel engines. These can thus be considered products of incomplete combustion, which can be reduced through higher temperatures, but that at the

cost of higher NO_x emissions. These inescapable conflicts are at the moment considered the greatest problem of direct injection diesel engines and are called the “diesel dilemma” or “NO_x / PM trade-off” (Bosch, 2007; Flynn et al., 1999). Publications show that even modern, direct injection Otto engines tend to show analogue behavior (Lake et al., 1999). To solve this problem, three different methods are used simultaneously: fine tuning of the engine and its injection system, the exhaust gas treatment, and the optimization of fuels. The three methods mentioned should be adjusted to each other in order to reduce the particle and nitrogen oxide emissions at the same time.

In environmental studies it was observed that short increases in particle concentration in the air led to an increase in the number of patients with respiratory and circulatory illnesses brought to hospitals or who die (Dockery and Pope, 1994; Samet et al., 1995; Katsouyanni et al., 1997; Samet et al., 2000; Wichmann et al., 2000; Li et al., 2008; Stöger und Schulz, 2004). Long term studies showed that in urban areas with high particle air pollution featured more chronic respiratory and circulatory diseases than in non-rural areas (Dockery et al., 1993; Pope et al., 1995). A study published in 1999 found a link between a risk of lung cancer with particles but also with ozone and sulfur dioxide (Abbey et al., 1999). Overall the available data show that particle emissions could be linked to negative health impacts, above all for sensitive persons (children, older people and the sick). An estimate of the global health risks with consistently increasing particle air pollution resulted worldwide in 8 million additional deaths for the years 2000 to 2020 (Working Group on Public Health and Fossil Fuel Combustion, 1997).

In other studies, the particle size distribution of the emissions is considered since fine (< 2.5 µm) and ultra-fine (< 0.1 µm) particles are more strongly linked to health risks than the particle sizes already used for evaluation (< 10 µm) (Seaton et al., 1995; Peters et al., 1997; Schwartz und Neas, 2000; Oberdörster, 2001; Frampton et al., 2004).

4.1.2. Non-Regulated Exhaust Gas Components

Mutagenicity of the Soluble Organic Fraction of the Particles

For a health related assessment of different diesel fuels it is necessary not only to determine the exhaust gas emissions from diesel engines but also perform toxicological studies.

Combustion exhaust gases contain a multiplicity of different toxic elements that have not yet be conclusively studied. In the group of PAH-related compounds, far more than 150 individual substances have been identified, of which the larger portion shows a mutagenic or carcinogenic effect. Since chemical analytical proof of all substances in exhaust gas is not technically or financially feasible, and provides no statement on their toxic potency, a

laboratory test method was searched for in order to document the genetic impacts of individual substances or mixtures of substances, such as, for example, combustion residues.
In 1973, Ames and his colleagues published the experimental instructions for an in vitro test system which showed the mutagenic characteristics of a broad spectrum of substances through a re-mutation of genetically manipulated *Salmonella typhimurium* strains. The study of bacterial mutations has in the meantime gained a permanent place as a scientific method for estimating the gene-toxicological and carcinogenic effects of working substances and chemical pollution of the environment (OECD Guideline 471). Between 80 and 90 percent of the carcinogenic substances have also been shown to be mutagenic (Maron and Ames, 1983).
In 1978, the ability of DEE to cause genetic damage was first shown in an Ames Test (Salmonella Microsome Test) by Huisingh et al. (1978), and was then confirmed in further extensive studies (Clark and Vigil, 1980; Claxton and Barnes, 1981; Siak et al., 1981; Belisario et al., 1984).

Aldehydes and Ketones

Aldehydes and ketones result from incomplete fuel combustion in an engine. Complete oxidation of hydrocarbons to carbon dioxide and water is disrupted by the creation of aldehydes and ketones. Thus compounds remain that are only partially oxidized. The obvious relationship observed for Otto fuels between the aromatic content of the fuel and the carbonyl emissions is not measurable for diesel fuels (DF). Thus despite extensive studies, no certain information on the aldehyde creation in diesel engine emissions (DEE) is available (Prescher et al., 1997). Aldehydes and ketones generally have a penetrating, mucous-irritating odour. The best known representative of this class is formaldehyde, the negative impacts of which are well known on human health (Informationsschrift Umweltpolitik, 1992). Formaldehyde and acetaldehyde are, together with 1,3 butadiene, benzene and particle organic materials among the most relevant toxic air pollutants according to the US Clean Air Act (Gorse et al., 1991). In 1998, these so-called “air toxins” were expanded to include DEE.
Acrolein, which has proven to be characteristic in some engines running on rapeseed oil methyl ester (RME) (Wurst et al., 1990; Krahl, 1993; Schäfer et al., 1998), is also considered a potential carcinogen (Zhaohui et al., 2006; Office of Health and Environmental Assessment, 1990).
In the following, the effect of formaldehyde as a main component of aldehyde emissions of diesel engines will be explained more closely.
At room temperature, formaldehyde is a colorless gas with a penetrating odor. Under atmospheric conditions, including sunlight, formaldehyde is converted to, among other things, CO_2. In the presence of NO_2, it has a half-life period of 35 minutes, without NO_2, of 50 minutes. Formaldehyde is known longest for its use in medicine and science as a disinfectant, sterilizer and preservative. Today it is mainly used in plastic production as an adhesive and as a binding substance in wood processing and in the production of paper, textiles and paint. The main

formaldehyde emissions are from motor vehicles (especially without catalysts) and the sites where formaldehyde is produced and used as well as heating systems (gas, oil, coal and wood), heating plants, refineries and airplanes. The formaldehyde concentrations in the ambient air are between 0.1 µg/m^3 (clean air areas, maritime regions) und 160 µg/m^3 (urban centres).

The perception level of the penetrating odor of formaldehyde is, depending on the test person, within the range of 0.06 to 0.22 mg/m^3. Known effects of formaldehyde exposure are mucous irritations in the eyes and in the upper respiratory system. The effect threshold for irritations of the eyes is at 0.06 and for the respiratory system at 0.12 mg/m^3. Asthmatics, but also healthy persons, can react to formaldehyde with respiratory problems. Here, concentrations are above those which already result in eye irritations. Due to the sensitising effects, a sub-acute exposure at the workplace as well as in private interior areas can lead to asthmatic-type reactions. Long term exposure to formaldehyde in interior areas can lead to memory annoyance, concentration difficulties and sleeping problems (Marquardt und Schäfer, 1994).

The frequency of nasal and nasopharynx tumours in workers is attributed to the direct inhalation contact with formaldehyde. This frequency shows a direct dependence on exposure. The suspicion that formaldehyde also leads to leukaemia and tumours in the central nervous system has not been proven. In contrast to animals, for which the carcinogenicity has been classified as sufficiently secure, the carcinogenicity of formaldehyde in humans has not been sufficiently attested. According to the former MAK List (2004), within the limits of a MAK value of 0.37 mg/m³ no mentionable contribution to cancer risk is expected. This is in accordance with Category 4 of the cancer-causing substances. Acetaldehyde, 2-propenal, and 2-butenal are, in contrast, considered to belong to Category 3B of the cancer causing substances and are believed to cause cancer (MAK List, 2004). The International Agency for Research on Cancer (IARC) has until now found no aldehydes or ketones in diesel engine emissions classified as cancerous to humans. Thus, one can in no case speak of a certain cancerous effect in humans by the aldehydes and ketones in diesel engine exhausts (Bünger et al., 2000).

The direct health damaging effects of formaldehyde are, however, not the sole reason for the environmental relevance of the carbonyl compounds emitted in motor exhaust fumes. These nonetheless show only short half-life periods in the atmosphere (Lofti et al., 1990). In addition it is significant that aldehydes and ketones have a great potential for building photo oxidants in the presence of NO_x (Carter and Bufalini, 1991; Carter, 2007).

The cytotoxicity of DEE is caused above all by aldehydes (National Research Council, 1982; WHO, 1996) and becomes evident through irritations of the mucous membranes in the eyes and upper respiratory system (Ulfvarson et al., 1987; Scheepers and Bos, 1992).

To measure these toxic effects, cell cultures of human and animal origin can be exposed against the DEE extracts. The amount of cell damage can then be determined with special dyes (neutral red) (Borenfreund and Puerner, 1984).

PAH from Diesel Engine Combustion

Generally, compounds comprised of two or more condensed aromatic rings of carbon atoms are called polycyclic aromatic hydrocarbons. According to the currently valid version of the Diesel Fuel Norm DIN EN 590, up to 11 % (w/w) of polycyclic aromatic hydrocarbons may be present in fuels. Thus a possibility exists from the outset that PAH exhaust comes from the exhaust system of the diesel engine with non-combusted fuel. Principally PAHs always develop in combination with the incomplete combustion of organic materials. Consequently, there are numerous PAH sources in addition to diesel engines. Included here are also processes like the roasting of coffee or the smoking of a cigarette (Marquardt and Schäfer, 1994; Schauer et al., 2003). The main cause of PAHs are, in addition to the transportation sector, above all industrial facilities in which mineral oil processing takes place, steel works, paper plants, but also small combustion facilities as found in residential homes. The German Environmental Agency estimates the emissions of Benzo[a]pyrene, a very useful routing substance for PAHs, for the year 1994 at 14 t, whereby the contribution of the small furnace facilities is estimated to comprise 66 % (Umweltbundesamt, 2001). Studies by Larsen and Baker (2003) in the air surrounding Baltimore, determined the portion of various PAH emittants related to the gas and particle phase. There they calculated the contribution of diesel and Otto engines to each be 16 to 26 %, the carbon combustion to 28 to 36 %, the oil combustion from 15 to 23 %, and the wood burning or other combustion processes from 23 to 35 %. Glaser et al. (2005) conducted PAH studies on soil close to and distant from roads for motor vehicles. There they found an average PAH load of between 1 and 10 µg/kg in areas far from vehicles, and two decimal powers higher near to particularly busy roads.
In the following, the creation process and the characteristics of PAHs originating from diesel engine combustion are explained in more detail.

Formation Process of PAHs

The development of polycyclic aromatic hydrocarbons is linked very closely to diesel particles. Ultimately PAH occur at least as an interim synthesis step in the models of Bockhorn (1994) and Amann and Siegla (1982). During the development of diesel particles, a portion of the available PAHs escapes in the gas phase and leaves the exhaust stream through later condensation on the particles. In the figure 9, Klingenberg et al. (1992) show a largely accepted reaction scheme for the development of PAHs, which takes acetylene as starting substance.

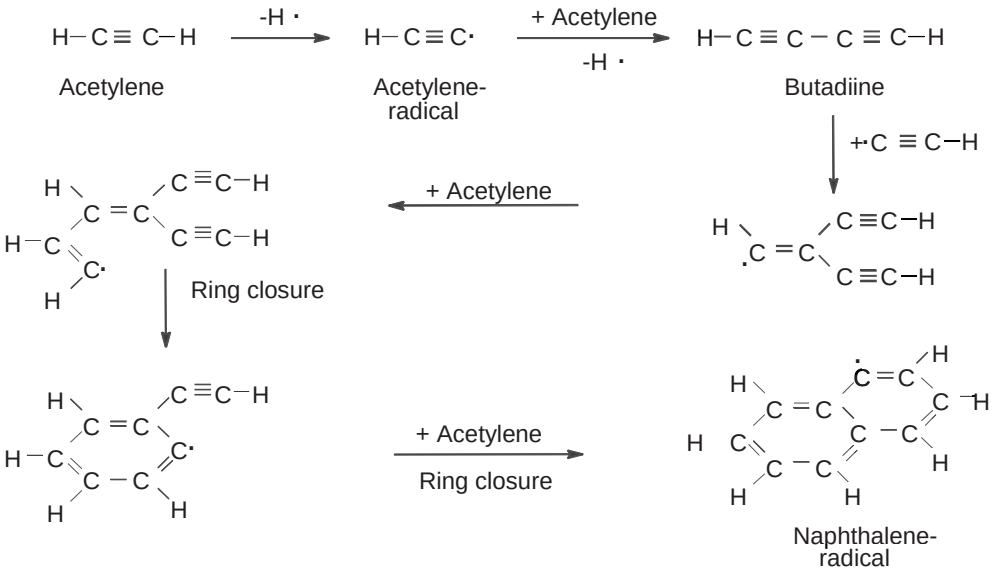


Figure 9: PAH development mechanism starting from acetylene (Klingenberg et al., 1992)

After acetylene becomes an acetylene radical through hydrogen abstraction, butadiene is created that, with other acetylene radicals or acetylene molecules, leads to cyclic and polycyclic products. These products are the precursor of particle creation through coagulation. In the combustion heat, further surface growth occurs and then simultaneous graphitisation. Since the PAH development is a precursor to particle development, favourable combustion zones with a high fuel portion, high temperatures and low oxygen levels favour the creation of polycyclic aromatic hydrocarbon compounds, too.

As further important reaction mechanism is a method via condensation reactions of previously existing aromatic systems (Amann and Siegla, 1982). Unfortunately the exact processes have not been completely described with absolute certainty (Warnatz et al., 2001). The condensation mechanism, in view of the diesel engine combustion, leads to the simple assumption that the reduction in the aromatic content in fuel leads to a reduction in the PAH emissions in exhaust (Mi et al., 2000). In contrast, according to research results by Westerholm et al. (1988) more than half of the emitted PAHs are completely re-created during the combustion process.

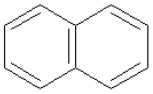
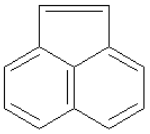
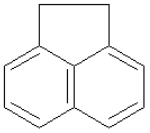
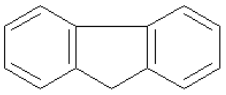
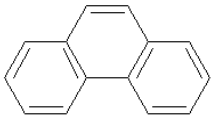
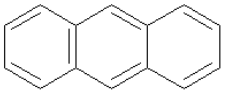
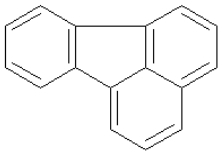
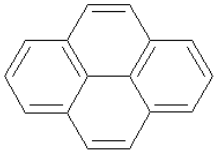
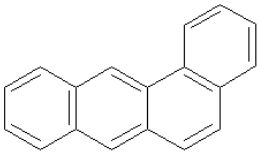
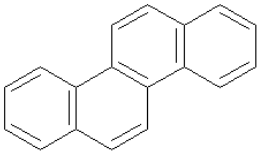
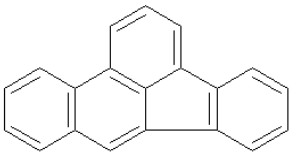
Characteristics of PAHs

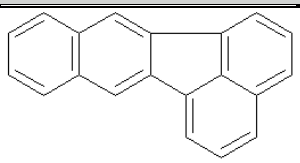
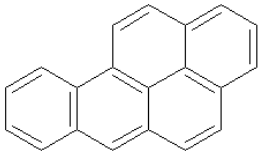
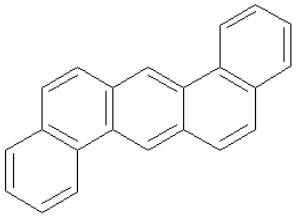
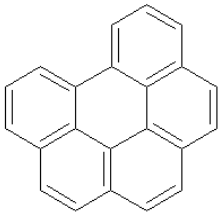
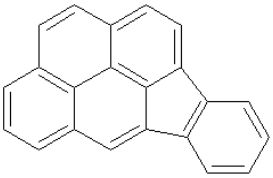
An enormous range of very different PAH molecules exists. In the air, more than 500 PAH types were found (Marquardt und Schäfer, 1994). Schauer et al. (2003) reported more than 100 particle-bonded PAHs and more than 150 PAHs bonded to cigarette smoke. Related to DEE, PAHs occur in both the gas and the particle phases. The selected sample conditions largely determine which PAHs take a gas form, and which are adsorbed at the particle phase of the

diesel particles. Thus for example in exhaust studies with low pressure impactors only up to 10 % of the total emissions of the three ring compounds like phenanthren or antracene could be detected on the impactor foils, while the other 90 % remained in the gas phase (von Borstel, 1993). Within the framework of the same study, von Borstel (1993) found retrieval rates of between 30 and 50 % for the four ring molecules like fluroanthene, pyrene, ben[a]anthracene and chrysene. Only the larger five ring molecules could be separated up to the missing rest of an average of 10 % quantitatively on the impactor foils. In the literature, above all PAHs beyond a size of four rings or rather 16 hydrogen atoms are regarded as particle-bonded PAH (Kweon, 2003; Larsen and Baker, 2003; Schauer et al., 2003; Zielinska et al., 2004).

In pure form and at room temperature, the compounds are often found as colourless crystalline bodies. Detailed physical information on the best known representative of this group can be found in Fiedler et al. (1997). Due to the enormous number of PAHs, for the practical chemical analysis, for example in the creation of the drinking water regulations in Germany or for American environmental regulations (EPA) certain routing substances were found and a targeted representative selection was made. For the analysis of exhaust, the 16 PAHs in the following table 14 reflect the widely accepted analytes that go back to determinations by the EPA. In the framework of this report, the diesel particles were analysed for these substances. In the table, in addition to the names, the sum formulas, the structural formulas and the boiling points are also shown.

Table 14: Overview of the 16 EPA-PAHs according to the EPA 610 method

Name	Abbreviation	Molecular formula	Chemical structure	Boiling point [°C]
Naphthalene	Nap	$C_{10}H_8$		218
Acenaphthylene	-	$C_{12}H_8$		280
Acenaphthene	Ace	$C_{12}H_{10}$		278
Fluorene	Flu	$C_{13}H_{10}$		295
Phenanthrene	Phe	$C_{14}H_{10}$		340
Anthracene	Ant	$C_{14}H_{10}$		342
Fluoranthene	Fla	$C_{16}H_{10}$		375
Pyrene	Pyr	$C_{16}H_{10}$		387
Benz[a]anthracene	BaA	$C_{18}H_{12}$		438
Chrysene	Chr	$C_{18}H_{12}$		488
Benzo[b]fluoranthene	BbFla	$C_{20}H_{12}$		481

Name	Abbreviation	Molecular formula	Chemical structure	Boiling point [°C]
Benzo[k]fluoranthene	BkFla	$C_{20}H_{12}$		480
Benzo[a]pyrene	BaPyr	$C_{20}H_{c2}$		496
Dibenz[a,h]anthracene	DBAnt	$C_{22}H_{14}$		524
Benzo[ghi]perylene	BPer	$C_{22}H_{12}$		543
Indeno[1,2,3-cd]pyrene	IPyr	$C_{22}H_{12}$		522

Due to the low volatility of the PAHs, their distribution is linked to the presence of particles such as dust, soot or pollen. This also means that their distribution primarily takes place through the air. From an occupational medicine point of view, the main intake of polycyclic aromatic hydrocarbons is also via the respiratory system. Dependent on the size of the inhaled dust or particles, the PAHs adsorbed there enter into the bronchioles, where up to 70 % can be carried into the blood (GESTIS Materials Database, 2004). The skin resorption of PAHs is only little known; related to the digestive tract, mean respiration rates of under 50 % have been found. Oral intake occurs above all via smoked or grilled foods and leafy greens like salad, spinach and cabbage (Larsen and Baker, 2003; Marquardt and Schäfer, 1994).

The acute toxicity of the PAHs is mostly considered to be small, and also the chronic toxicity, which can be seen in the respiratory system, the skin or the liver, doesn't play an important role. Solid knowledge is available here through animal studies. This is not true for results on cancerous effects. The most well-known example is certainly smoking as a cause of lung cancer. Numerous epidemiological studies with chimney sweeps at the beginning of the 20th

century quickly made the cancerous effects of soot and ashes obvious and at the same time named the polycyclic aromatic hydrocarbons as the cause (Marquardt and Schäfer, 1994). Additionally after exposure to PAHs genetic changes become apparent, too. Genotoxicity tests carried out for the main three, four and five ring systems provided weak to clear proof of significant mutagenic potential (GESTIS materials database, 2004). PAHs are the most frequent and well known genotoxic or rather carcinogenic chemical compounds in the atmosphere (Savela et al., 2003).

One part of the primary PAHs also reacts with parts of the air and exhaust to substituted PAHs, for example like Nitro PAH, Oxy PAH, Alkyl PAH, Thio PAH (IARC, 1989; Scheepers and Bos 1992; Winer and Busby, 1995). A well-studied example for the increase of mutagenic potential of DEE through particle-associated PAH in the air is the development of Nitro PAH (Atkinson and Arey, 1994).

PAHs found in nature, or also during the analytical processes in an engine test bench, are subject to very different conditions which can contribute to degradation or artefact development. In particular gases like oxygen, ozone, nitrogen oxides and sulfur oxide or also the presence of light or the impact of high temperatures lead to a degradation of PAHs (Krahl, 1993; DIN ISO 11338-2, 2003). However, it is assumed that the half-life period of PAHs in soil or rather in water sediments can comprise months, years or even decades (Marquardt and Schäfer, 1994; Jacob et al., 2006).

The analytical characterisation of PAHs necessarily differs on the basis of the type of sample. In this context the sample preparation plays an especially important role. In the case of PAH emissions caused by diesel engines both gaseous as well as particle-tied PAH emissions occur. Numerous research groups take the particle mass filters, which are loaded with the well-defined diluted exhaust, as the basis for their studies, in order to ascertain the condensation of a large portion of highly volatile PAHs on the filter (Postulka and Lies, 1981; Kraft et al., 1982; von Borstel, 1997; Durbin et al., 2000). Alternatively the sampling of undiluted exhaust is also used often (Kraft et al., 1982; Krahl, 1993; von Borstel, 1997; Claußen and Wollmann, 2003; Herbst, 2004).

Normally liquid and gas chromatographic measurement equipment is used for the analytical proof. Fluorescence detectors (FLD) and mass spectrometers (MS) are frequently used for detection. Gratz et al. conducted a ring study in 2000 with a high performance liquid chromatography (HPLC) with downstream FLD and, in comparison, a gas chromatography (GC) in combination with MS and found that both methods provide adequate resolution and sensitivity for the quantification of traces of PAH in complex environmental matrices. In the framework of this report a HPLC FLD method was applied, even though acenaphthylene could not be considered in the results, due to the lack of fluorescence characteristics.

4.2. Materials and Methods

4.2.1. Engine and Engine Testing Conditions

The studies were carried out on an emissions test facility at the Thünen-Institute of Agricultural Technology in Braunschweig. A Farymann engine 18W was used (Table 15).

Table 15: Technical data of the test engine Farymann 18W

Stroke of cylinder	55 mm
Bore of cylinder	82 mm
Number of cylinders	1
Stroke volume	290 cm^3
Normal rate of revolutions	3000 min^{-1}
Rated power	5.2 kW
Maximum torque	15.3 Nm
Exhaust gas standard	Tier 4

The crankshaft of the test engine is coupled with a controllable eddy-current brake MT12 (WEKA Company) which determines the engine load and thus enables to adjust the speed with the throttle control. In the framework of this project the load points were in accordance with the 5-mode cycle (Welschof, 1982). In figure 10 the preset torque and revolution rates, related to the maximal load or to the normal rate of revolutions, are presented graphically. . In table 16 the exact values of the five modes are listed. The engine runs in each mode at least 60 seconds before sampling regulated and non-regulated emissions. The weighting is, in each case, denoted above the individual point. To realize the weighting factors, the sampling time was adapted to the factors in consideration with the exhaust gas volume.

Table 16: Speed and torque during the tested modes

Operation Point	Speed [min^{-1}]	Torque [Nm]
A	2840	12.9
B	2650	7.0
C	1850	5.1
D	3000	1.9
E	1050	0

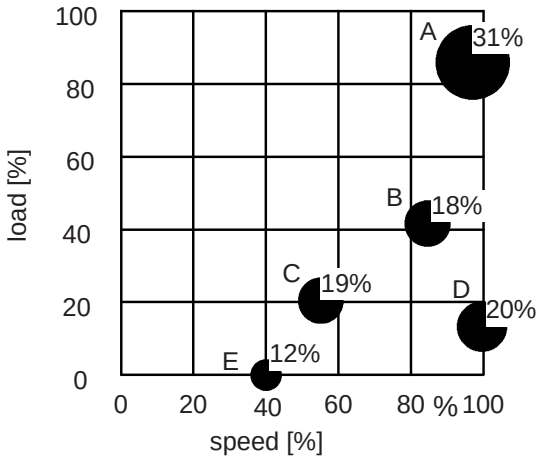


Figure 10: 5-mode test cycle

4.2.2. Fuels

In total, six fuels were studied. They can be found in the following table with the appropriate abbreviations.

Table 17: Studied fuels

Fuel appellation	Fuel
DF	Fossil diesel fuel
RME	Rape seed oil methyl ester
FOME	Fish oil methyl ester
RSOME	Rubber tree oil methyl ester
FOME20	20% blend of FOME in DF
RSOME20	20% blend of RSOME in DF

DF and RME were used as reference fuels. Comparisons of these two fuels were carried out since almost 20 years at different models of the Farymann engine. Thus, engine failure could be easily detected. The biodiesel RME with analysis was provided by Prokon Bio-Ölwerk Magdeburg, the DF with analysis from Haltermann GmbH in Hamburg, Germany. Fuel analyses are listed in Table 18 and 19.
FOME and RSOME were produced within the scope of this project.

Table 18: Fuel analyses for DF as well as limits acc. to DIN EN 590:2013

Property		Limits		
		Min.	Max.	DF
Density (15 °C)	[g/mL]	0.820	0.845	0.8344
Kin. Viscosity (40 °C)	[mm²/s]	2.000	4.500	2.876
Cetane Number	[-]	51.0		53.5
Flash Point	[°C]	55		85
CFPP	[°C]		0 / -20	-21
HFRR	[µm]		460	205
Water Content	[mg/kg]		200	35
Oxidation Stability	[g/m³]		25	< 0.001
Acid Number	[mg KOH/g]		0.02	< 0.01
Sulfur Content	[mg/kg]		10*	1.8
Carbon Residue	[% wt]		0.30	< 0.1
Ash Content	[% wt]		0.010	< 0.001
Hydrogen Content	[% wt]			13.59
Carbon Content	[% wt]			86.41
Monoaromatics	[% wt]			20.4
Diaromatics	[% wt]			4.8
Triaromatics	[% wt]			< 0.1
Polyaromatics	[% wt]		8.0	4.8
Total Aromatics	[% wt]			25.2
FAME Content	[% vol]		7	0***
Calorific Value MJ/kg	[% wt]			43.171

* according to 10. BImSchV (2009), ** according to DIN 51628, *** none added
n.d.: not determined

Table 19: Fuel analysis for RME and limits according to EN 14214

Property		Limits		
		Min.	Max.	RME
Density (15 °C)	[g/mL]	0.875	0.900	0.883
Kin. Viscosity (40 °C)	[mm²/s]	3.50	5.00	
Cetane Number	[-]	51		> 51
Flash Point	[°C]	120		>101
CFPP	[°C]		0	-15
Water Content	[mg/kg]		500	180
Oxidation Stability	[h]	6.0		> 6
Acid Number	[mg KOH/g]		0.50	0.19
Sulfur Content	[mg/kg]		10	5.9
Carbon Residue	[% wt]		0.30	< 0.3
Ash Content	[% wt]		0.02	< 0.02
Iodine Number	[g Iod/100g]		120	<120
Ester Content	[% wt]	96.5		>96.5
Linolenic acid methyl ester	[% wt]		12	9.8
Monoglycerides	[% wt]		0.80	0.49
Diglycerides	[% wt]		0.20	0.14
Triglycerides	[% wt]		0.20	0.07
Methanol	[% wt]		0.20	<0.02
Free Glycerol	[% wt]		0.020	< 0.01

4.2.3. Analytical Methods for Regulated Exhaust Gas Emissions

The regulated exhaust gas components carbon monoxide, hydrocarbons and nitrogen oxides were determined with a commercial gas analyzer and sampled each second. A mean was determined from the values sampled in the last minute of an operating point.

Hydrocarbons

A gas analyzer from the Ratfisch Company (RS 55-T) was used to determine the hydrocarbons. This measurement instrument works with a flame ionization detector (FID). The test gas is led into a helium hydrogen flame which burns in an electrical field. The hydrocarbons contained in the test gas are ionized through the flame and thus lead to a change in the electrical field, through which the HC content is calculated. The hot and previously filtered exhaust gas is led

to the HC analyzer through a pipe, heated to 190 °C and controlled by a thermostat. The purpose of the heated gas path is to prevent a premature condensation of the hydrocarbons with a high boiling point.

Carbon Monoxide

The CO gas analyzer BA-5000 (Bühler Technologies) works with non-dispersed infrared light (NDIR process). Here the test gas flow (filtered and cooled below its dew-point) is divided into two equal flows each flowing through a cuvette. One of the cuvettes is irradiated with an infrared light, the wavelength of which is tuned to the characteristic absorption of carbon monoxide. Thus this partial flow is heated and it leads in a canal connecting both cuvettes to a compensatory flow which is measured through a micro flow sensor and can be calibrated as a measure for the content of the component CO.

Nitrogen Oxides

The nitrogen oxides are analyzed with a chemical luminescence detector (CLD) from the EcoPhysics Co. (CLD 700 EL ht). In the oxidation from NO to NO_2, about 10 percent of the NO_2-molecules reach an electronically stimulated condition, from which they immediately, and under the emanation of photons, return to a non -stimulated condition (luminescence). These photons are identified and are a measure of the NO content. To determine the total content of NO_x ($NO + NO_2 = NO_x$), a branch current of the hot and filtered sample is first led through a converter in which NO_2 is reduced to NO. The nitrogen dioxide content is calculated as the difference in the measured values of NO_x and NO.

Particulate Matter

Particulate matter of each test cycle was collected from the undiluted exhaust part stream onto one glass fiber filter coated with PTFE (Teflon) (T60 A20, Pallflex Products Corp., Putnam, CT, U.S.A.). Sampling proceeded in each mode in consideration of their weighting factors with a constant sampling flow of 25 L/min. According to VDI-Guideline 3872 part 1 the exhaust gas phase was cooled under 50 °C using an intensive cooler (Schott, Germany) and condensates were collected separately. Further condensed compounds were desorbed from the cooler with 100 mL methanol and added to the condensates. Every fuel was tested five times with two identical sampling constructions, resulting in 10 particle filters to determine particulate matter for each fuel. The filters were conditioned (22° C, rel. humidity 45 %), weighed before and after sampling to determine the sampled particulate matter. After determination of the particulate matter the filters were stored together with the condensates at -18° C to determine PAH emissions and mutagenicity of the exhaust gas.

4.2.4. Analysis Methods for Non-regulated Exhaust Gas Emissions

Mutagenicity of the Soluble Organic Fraction of the Particles

Sampling and processing of samples:
For determination of mutagenicity, filters and condensates were taken from sampling procedure of the particulate matter.
The extraction takes place in a Soxhlet apparatus with 150 mL dichloromethane, since the greatest mutagenic activity can be gained with this solution (Siak et al., 1981). The length of extraction is 12 hours and amounts to 50 to 60 extraction cycles. The extracts as well as the condensates were reduced by rotary evaporation (Heidolph, Kehlheim, Germany) and dried under a stream of nitrogen. They were re-dissolved in 4 mL DMSO immediately before use.

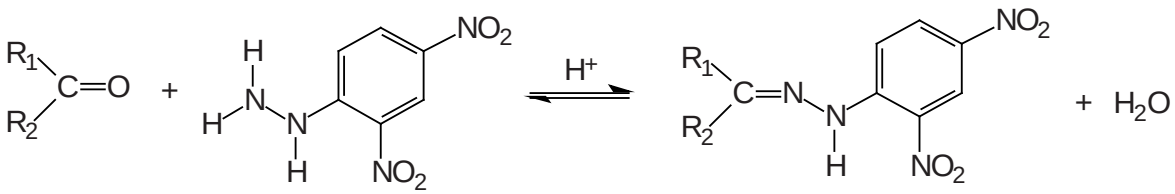
Mutagenicity assay:
Ames et al. (1975) developed the *Salmonella typhimurium*/mammalian microsome assay that detects mutagenic properties of single compounds as well as of complex mixtures by reverse mutation of a series of *Salmonella typhimurium* tester strains, bearing mutations in the histidine operon. Depending on the tester strain different types of mutations can be detected. In this study tester strains TA98 and TA100 were used, detecting mutagens that cause frameshift mutations and base-pair substitutions. These strains were shown to be most sensitive to mutagens of organic extracts of diesel engine particles (DEP) (Clark and Vigil 1980, Claxton 1983). This study employed the revised standard test protocol (Maron and Ames 1983).
Extracts and condensates were tested in the following log 2 dilutions: 1.0, 0.5, 0.25, 0.125. Each concentration was tested both with and without 4 % S9 Mix. Every extract and condensate was at least tested in triplicate. Plates were incubated at 37° C for 48 h in the dark, and revertant colonies on the plates were counted using an electronically supported colony counting system (Cardinal, Perceptive Instruments, Haverhill, Great Britain). The bacterial background lawn was regularly checked by microscopy, as high doses of the extracts proved toxic to the tester strains, resulting in a thinning out of the background.
In order to consider enzyme-caused changes in mutagenicity, the Tests were performed with and without metabolic activation by microsomal mixed-function oxidase systems (S9 fraction). Preparation of the liver S9 fraction from male Wistar rats was carried out as described by Maron and Ames (1983). Phenobarbital and β-naphthoflavone (5,6-benzoflavone) were used for induction of liver enzymes. These substances were proven to be safe and adequate substitutes for Arochlor 1254 (Matsushima et al. 1976). The mutagens methyl

methanesulfonate (10 µg/mL in distilled water), 2-aminofluorene (100 µg/mL in DMSO), and 3-nitrobenzanthrone (1000 pg/mL in DMSO), were used as positive controls.

Acceptance criteria and statistics:
Mutagenic response is classified positive if a reproducible, dose-dependent increase of the number of revertant colonies is observed (Krewski et al. 1992, Mortelmans and Zeiger 2000). Revertant numbers of the positive results (means ± standard deviations) are estimated from the initial linear part of the dose-response curves by a linear regression model. Differences between the tested fuels are calculated for significance using Student´s t-test for independent variables, two-sided.

Carbonyls

The concentrations of carbonyls were determined with DNPH cartridges. These cartridges contain 2,4-dinitrophenylhydrazine (DNPH) coated silica gel. Aldehydes and ketones react on the surface of the silica gel with DNPH according to following reaction:



R_1 and R_2 = H or hydrocarbon chain

The sampling takes place out of the raw exhaust gas. The sample tube is heated to 80 °C to avoid condensation. DNPH and the reaction products, hydrazones, react with nitrogen dioxide. To minimize this reaction, potassium iodine cartridges are placed before the DNPH. This potassium iodine reacts with nitrogen dioxide without disturbing the carbonyle flow.
The sampling flow is set to 0.5 L/min. The sampling time at the end of each mode was set according to the exhaust gas volume flow and the weighting factor.

After sampling the hydrazones and excess DNPH were flushed with acetonitrile out of the cartridges into a 2 mL volumetric flask. This solution was analyzed by HPLC with UV detection (370 nm)

Polycyclic Aromatic Hydrocarbons (PAH)

For determination of PAH emissions, filters and condensates were taken from sampling procedure of the particulate matter.
The filters were extracted using an extractor fexIKA 50 (IKA, Germany) for four hours with toluene (GC ultra-gradient Grade, Roth, Germany) as extracting solvent. The extracts were reduced by rotary evaporation and dried under a stream of nitrogen while solving the extracts in acetonitrile (ultra- gradient grade, Roth, Germany). During the sample preparation the samples were never evaporated to dryness to avoid losses of the PAH. Finally, they were filled into 2 mL volumetric flasks with acetonitrile for further use.
The condensates were extracted three times with 10 to 30 mL of toluene:dichloromethane (1:1, Pestilyse, Roth, Germany) for 5 minutes in an ultrasonic bath. The eluents were merged, and subsequently treated as the particulate extracts.
The extracted PAH were separated and quantified using a HPLC with fluorescence detection. The HPLC system is listed in Table 20. After accumulation of the PAH on a pre-column (ChromSpher Pi, Varian, Germany) using the effect of donor acceptor complex chromatography (DACC), the PAH were dissolved by acetonitrile/water gradient, and separated by an analytical column.

Table 20: Parameters of the HPLC

System	VWR Hitachi Elite LaChrom
Auto sampler	Hitachi L-2200, volume: 0.1 mL
Pump	Hitachi L-2130, flow rate: 1.5 mL/min
Oven	Hitachi L-2350, temperature: 24 °C
Fluorescence detector	Hitachi L-2480, cell volume: 12 µL
DACC	ChromSpher Pi, 20.0 mm · 3.0 mm, Varian, Germany
Analytical column	Supelcosil LC-PAH, 25 cm · 4.6 mm, particle size 5 µm, Supelco
Eluent	Acetonitrile/water (HPLC grade)

Calibration of the HPLC system was done using a 16 PAH standard (LGC Promochem, Germany) in different dilution levels. The quantification of the PAH was performed by an internal standard (para-quaterphenyl, Fluka, Germany) with was added at the beginning of the extraction process.
The analyzed PAH are listed below:

Table 21: 16 PAH according to EPA method 610

Name	Number of rings	Abbreviation
Naphthalene	2	Nap
Acenaphthylene	3	non fluorescent
Acenaphthene	3	Ace
Fluorene	3	Flu
Phenanthrene	3	Phe
Anthracene	3	Ant
Fluoranthene	4	Fla
Pyrene	4	Pyr
Benz[a]anthracene	4	BaA
Chrysene	4	Chr
Benzo[b]fluoranthene	5	BbFla
Benzo[k]fluoranthene	5	BkFla
Benzo[a]pyrene	5	BaPyr
Dibenz[a,h]anthracen	5	DBAnt
Benzo[ghi]perylene	6	BPer
Indeno[1,2,3-cd]pyrene	6	IPyr

4.3. Results

For all evaluations a minimum of three measurements and a maximum of 6 measurements were included, whereby the average was created from all individual results.

4.3.1. Results for the Regulated Exhaust Gas Components

Hydrocarbon Emissions

Using fish oil methyl ester (FOME) or rubber tree oil methyl ester (RSOME) the emissions of hydrocarbons increased to 170% of those from fossil diesel fuel. This increase of the highly unsaturated methyl ester is clearly higher than the increase from RME. The B20 blend have smaller increase in HC emissions, as expected, if a linear dependency between the high of emissions and the percentage of methyl esters is assumed.

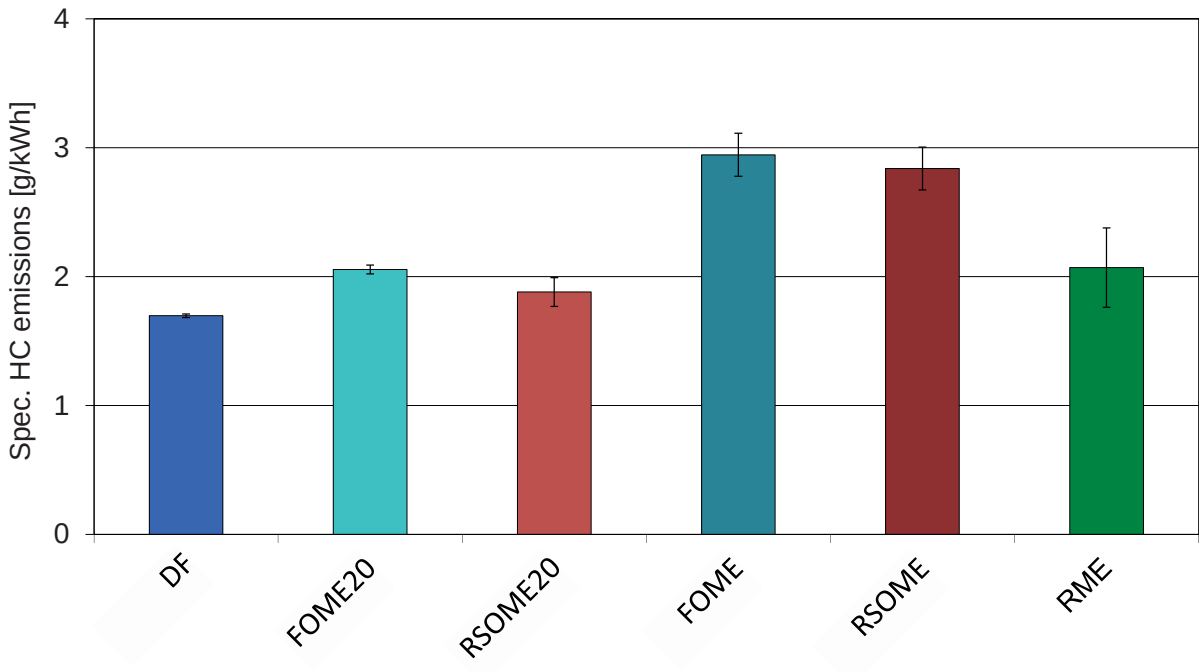


Figure 11: Specific HC emissions (5-mode test, Farymann 18W)

Carbon Monoxide Emissions

The carbon monoxide emissions show the same trend as the HC emissions (figure 12). The highly unsaturated methyl ester shows the highest emissions, whereas RME has only a slight increase in CO emissions compared to DF.

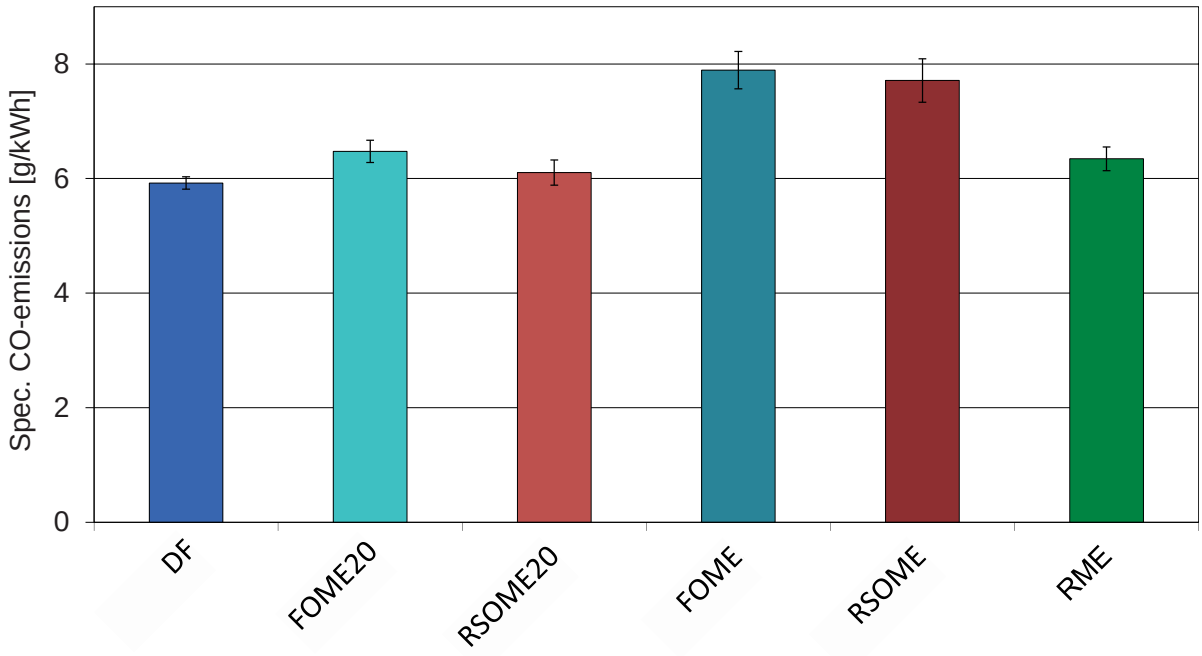


Figure 12: Specific CO emissions (5-mode test, Farymann 18W)

Nitrogen Oxides Emissions

The nitrogen oxides emissions (NOx) differ only slightly. Against the normal trend of most diesel engine, this engine shows a small decrease of 5 % using RME instead of DF. The emissions of FOME and RSOME are in the same level as RME. The emissions of the B20 blends are also comparable to the emissions of the pure methyl esters. Here a nonlinear must be assumed.

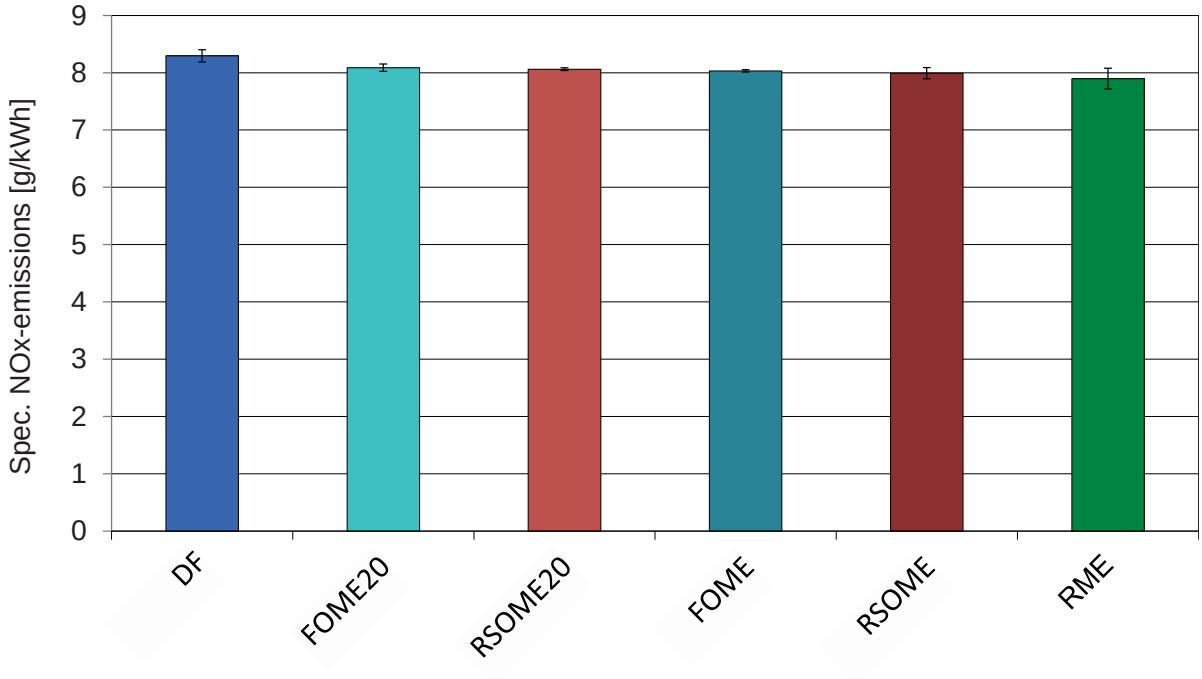


Figure 13: Specific NO_x emissions (5-mode test, Farymann 18W)

Particulate Matter Emissions

The particles were sampled in apparatus based on the VDI-guideline 3872 part 1 from the raw exhaust gas. The sampled particles were also used for determination of the mutagenicity and the PAH emissions. Therefore, two sampling lines were used. In figure 14 the both line are presented separately. Except for the B20 blend of FOME both lines shows comparable results. In all, the methyl esters had factor 2 higher particle emissions compared to fossil diesel fuel. This trend is known for this type of engine. The increase of emissions is due to unburned fuel, as previously studies had shown. The emissions of the B20 blend are within a linear trend between the raw fuels.

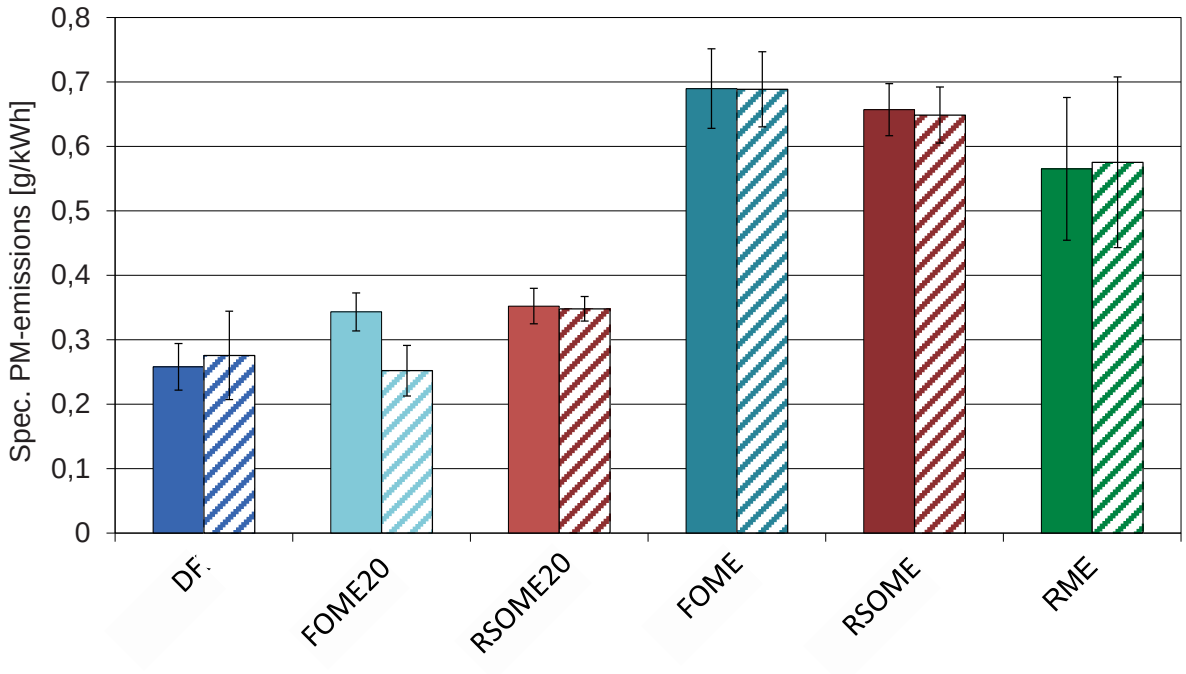


Figure 14: Particulate emission (5-mode test, Farymann 18W)

4.3.2. Results of the Non-regulated Exhaust Gas Components

Carbonyl Emissions

Most of the carbonyl emissions (Figure 15) consist of formaldehyde, acetaldehyde and acrolein. Acetone couldn't be determined due to high background levels. 2-butanone and n-butyraldehyde were determined together because of equal retention times. For m-tolualdehyde only small amounts could be detected. Slightly divergent retention times for this analyte could refer to other isomers of tolualdehyde.
In general, the carbonyl emissions shows the same trend as the HC emissions. The methyl esters have higher emissions than fossil diesel fuel. The emissions of B20 blend are in the same range as the emissions of fossil diesel fuel. In detail, the emissions of formaldehyde are lower with the B20 blends, therefore the B20 blend emits more acrolein.

PAH Emissions

PAH were sampled both, particle-bound on filters and in the condensate. Figures 16 and 17 show the separated results for the particle extract and the condensate. The lightweight PAH were mainly found in the condensate and the higher PAH were found in the filter fraction. Also the lightweight PAH were found in die diesel consisting fuel (neat diesel fuel and the blends) In total of all PAH RME had the highest emission, whereas fossil diesel fuel emitted the lowest amount of PAH. RSOME is almost at the level of DF; FOME shows about 30 % higher emission then RSOME. The blend emits at the same level as RSOME.

The high emissions of Biodiesel may be by reason of the high emission of particulates. In all, these results doesn't fit to older results and aren't coherent. Therefore, further experiments has to be done, too check the results.

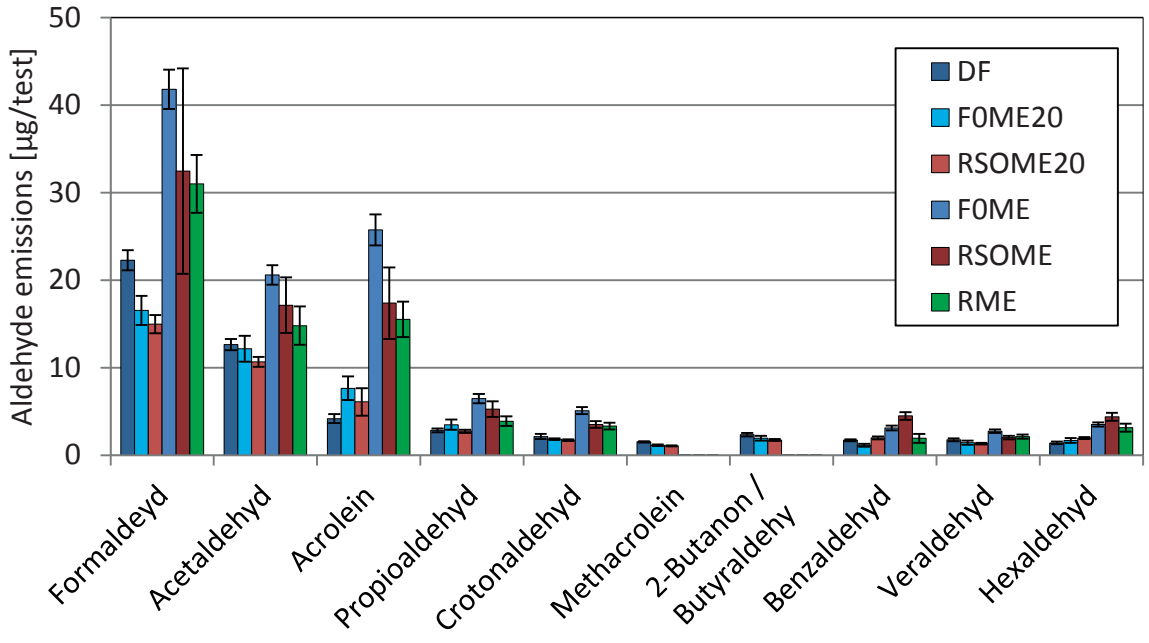


Figure 15: Specific carbonyl emissions (5-mode test, Farymann 18W)

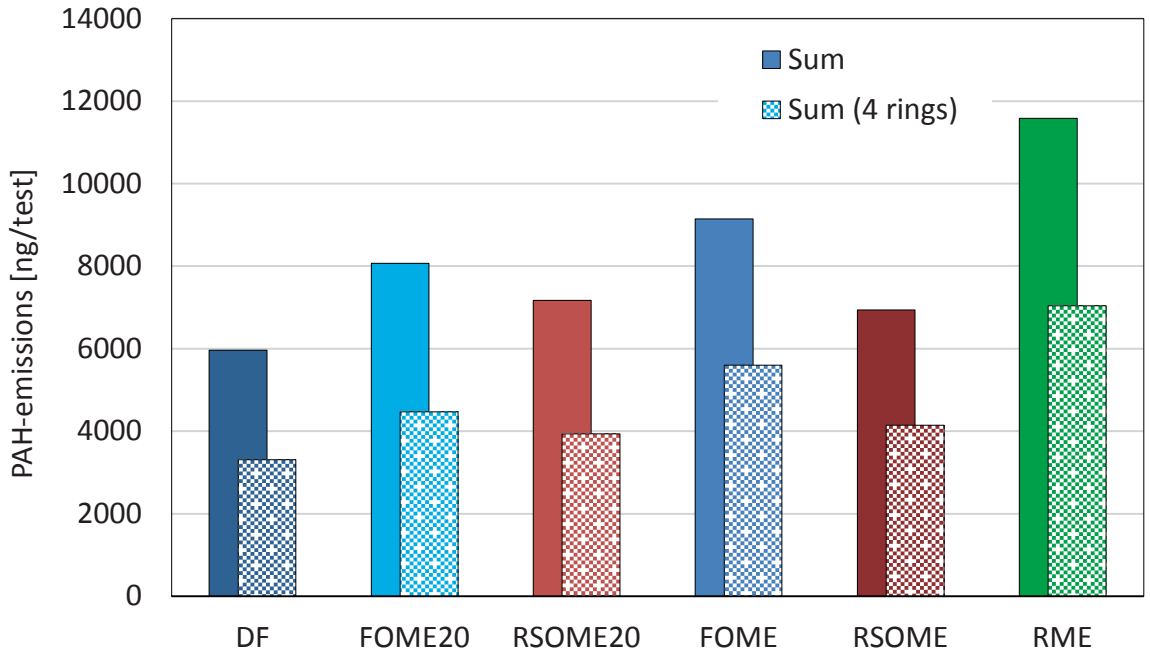


Figure 16: PAH in particulate sampled during the during the 5-mode test (Farymann 18W)

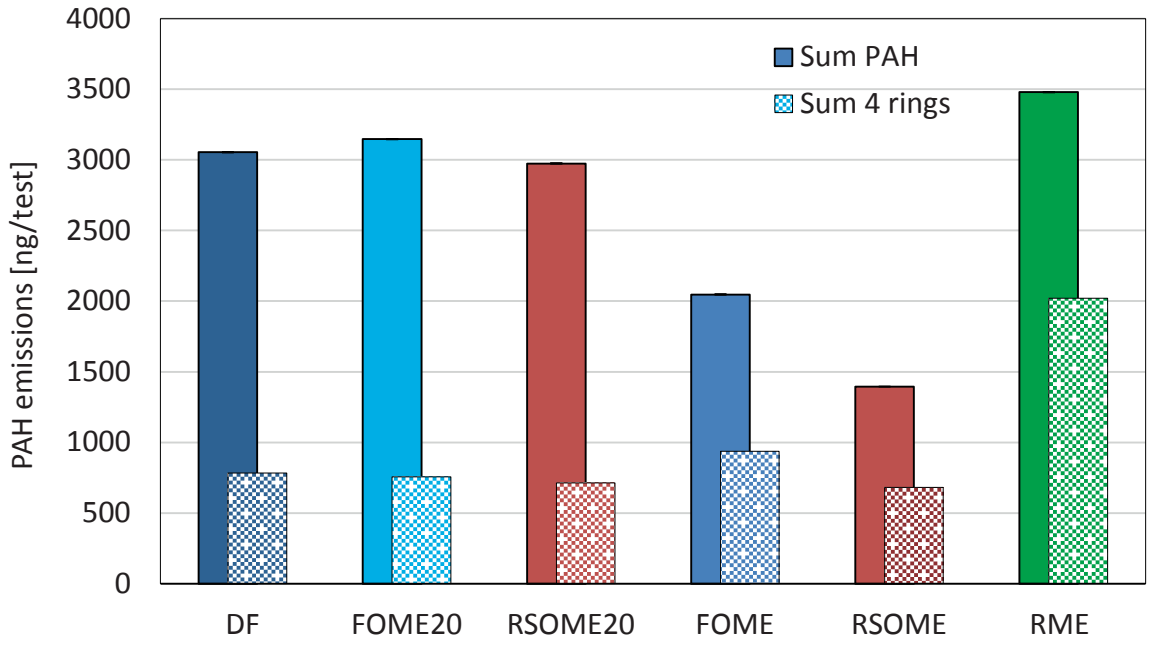


Figure 17: PAH in condensate sampled during the 5-mode test (Farymann 18W)

Mutagenicity of the Organically Soluble Particle Fraction

The extraction of the particle filters was performed at the University of Bochum. In both test strain TA98 and TA100, the mutagenicity of DF was significantly the highest of all tested fuels. About seven eighth of the mutagenicity was found in the particulate fraction. The condensate contributes only a minor amount. In the test strain TA98 RME and FOME shows the same mutagenicity whereas RSOME had a significant lower mutagenicity. In the test strain TA100 RSOME and FOME shows a lower mutagenicity then RME. The mutagenicity of the blends are more in the range of the methyl esters and not as expected in the range of the fossil diesel fuel.

In all fuels the direct (-S9) mutagenicity is higher than the indirect (+S9) after metabolic activation of extracts by rat liver enzymes. This speaks for the theory that the largest part of the mutagenicity was caused by substituted PAH (for example, Nitro-PAH). These are mostly direct mutagens while the native PAH require a metabolic activation through the formation of epoxides.

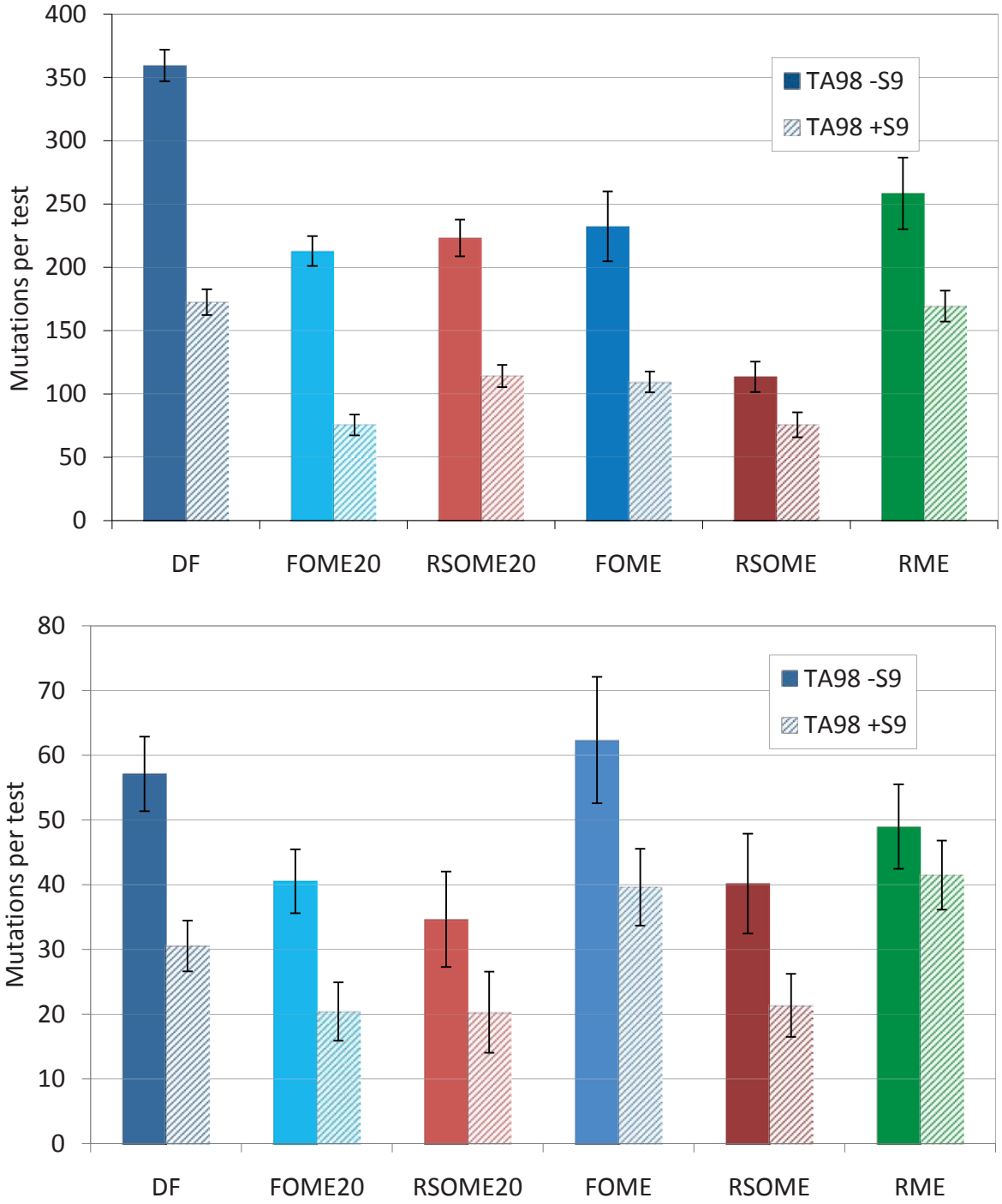


Figure 18: Mutagenicity of PM extracts (above) and condensates (below) in strain TA98 (5-mode test, Farymann 18W)

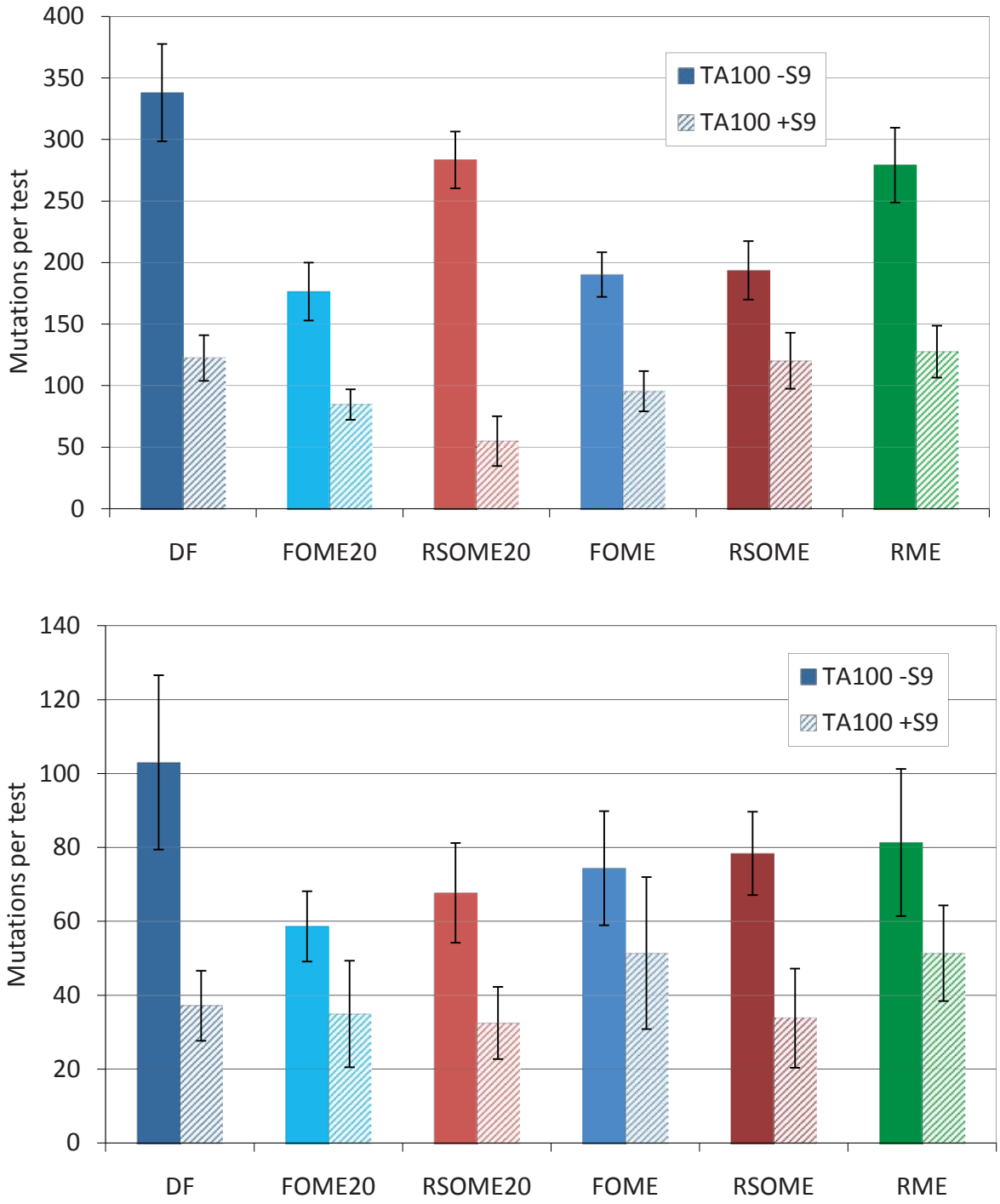


Figure 19: Mutagenicity of PM extracts (above) and condensates (below) in strain TA100 (5-mode test, Farymann 18W)

4.4. Conclusion

Rubber tree oil methyl ester (RSOME) and fish oil methyl ester (FOME) were tested in a one-cylinder engine in respect to their regulated and non-regulated emissions. The esters were used as neat fuel an in a 20 % blend with fossil diesel fuel. As reference fossil diesel fuel (DF) and rapeseed oil methyl ester (RME) were tested as well.

The regulated emissions and the emissions of the aldehydes followed the known trend for RME compared to DF. The emissions of hydrocarbons, carbon monoxide, particulate and aldehydes increased while the emission of nitrogen oxides decreased slightly. Compared to RME the highly unsaturated methyl esters shows always higher emissions, whereat the emissions of FOME seemed to be slightly higher than those of RSOME.

The measurement PAH emissions and the mutagenic effect gave no clear trend. The data isn't always reasonable. Therefore, the fuels should be tested in further test series. Nevertheless it could be stated, that the highly unsaturated methyl esters do not increase PAH emissions and mutagenicity significantly.

4.5. Literatur

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