Steryl Glycosides and Acylated Steryl Glycosides in Vegetable Oils and Fatty Acid Methyl Esters

Effects on the Filterability of Biodiesel

Study on the SG and ASG Contents of Vegetable Oils and FAME

Research on the Correlation between Filterability of Biodiesel and SG and ASG Contents

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This study was carried out in cooperation with AGQM member companies from January 2009 until June 2010

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Table of Content

Abbreviations ......................................................................................................................... 3
Abstract .................................................................................................................................. 4
Introduction ............................................................................................................................ 5
Project Definition ................................................................................................................... 6
Execution of the Project ......................................................................................................... 7
Test Methods .......................................................................................................................... 7
Evaluation of the Data ............................................................................................................ 8
Assessment of the Results .................................................................................................... 19
Summary .............................................................................................................................. 19

Abbreviations

ASG Acylated Steryl Glycosides
CFPP Cold Filter Plugging Point
FBT Filter Blocking Tendency
SG Steryl Glycosides
CST Cold Soak Test
csFBT cold soak Filter Blocking Tendency
OVID Verband der ölsaatenverarbeitenden Industrie in Deutschland e.V.
Abstract

When Diesel is fuelled, steryl glycosides (SG) and acylated steryl glycosides (ASG) are one of the causes – apart from e.g. microbial contamination – for sporadically observed filter plugging. Those compounds are natural parts of vegetable oils and – especially in palm and soybean oils – their concentration can be up to some hundred mg/kg. The objective of a study\(^1\) – jointly carried out by AGQM, OVID and the ASA (American Soybean Association – International Marketing) – was the inspection of typical oil mill processes with regard to any reducing of the SG and ASG contents of rapeseed and soybean oils.

The influence of the Biodiesel production process on the contents of SG and ASG had not been investigated before. Based on the SG and ASG contents of the vegetable oils used and the Biodiesel produced therewith, it is thus the aim of this report to obtain indications whether and to which extent any reduction can be achieved during the production process by means of a suitable process management. Additional tests on the filterability of the Biodiesel samples are meant to show whether a correlation between filterability and the contents of SG and ASG can be identified.

The examination of the vegetable oils used shows that the SG and ASG contents range between 5 mg/kg (limit of quantification of the test method) and 350 mg/kg, with a tendency to higher levels for ASG. The ASG contained in the oil are mostly transformed into SG during the transesterification process, so that Biodiesel made from conventional base catalysed processes usually shows a higher SG contents than the vegetable oil used for its production.

Therefore, it is not possible to give a statement concerning the removal of ASG during the production process; due to the transformation into SG the proportional ratio of the two components is intensely shifted. Since on the other hand in total the contents found in Biodiesel (\(\leq 20\) mg/kg) is lower than that in the oils, obviously many Biodiesel production processes are apt to reduce the SG contents today already.

The determination of the filterability (Filter Blocking Tendency, FBT) shows that there is no indication for a clear correlation between filterability and the SG and ASG contents. It tends to be more difficult to filter samples with higher SG and ASG contents than samples with low contents; however, in some cases a reverse behaviour can also be observed; i.e. despite low SG and ASG contents there are high FBT values and/or good filtration properties at high contents. Considering those results it seems that the FBT test is not suitable to safely assess the filterability; implementing the so-called ‘Cold Soak’ (cooling of the sample to 4.5°C for 16 h before filtration) does not reveal any additional information either.

Based on the report at hand, a recommendation cannot be derived for a limit value of the SG and ASG contents which could on the one hand be obtained by technically justifiable effort and on the other hand safeguard that filtration problems with Biodiesel can be safely detected and/or ruled out.

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\(^1\) J. Haupt, G. Brankatschk, T. Wilharm, “Steryl Glucoside Content in Vegetable Oils as a Risk for the Production of Biodiesel”, [www.agqm.de](http://www.agqm.de)
Introduction
In recent years there have been sporadic observations that when Biodiesel-blended Diesel fuel (B5/B7) is fuelled at filling stations plugging of fuel filters and deposits of solid substances in storage tanks may occur. One possible cause – apart from microbial contamination for example – may be steryl glycosides (SG) and acylated steryl glycosides (ASG) which are a natural part of vegetable oils. Especially in the cases of palm and soybean oils they can be contained in concentrations of up to some hundred mg/kg. The content of SG and ASG is not restricted by DIN EN 14214; therefore, there is hardly any data as to which concentration of these compounds are normally contained in Biodiesel since standardised analysis does not exist so far. The feedstock ‘vegetable oil’ is hardly examined in that respect either.

In order to ensure the unlimited usability of Biodiesel, information concerning production process steps and feedstock properties, which influence the SG and ASG contents, is essential for Biodiesel producers. Therefore, a study\(^1\) jointly carried out by AGQM and OVID and funded by ASA (American Soybean Association – International Marketing), assessed which production steps in oil mills lead to decreasing SG and ASG contents. Rapeseed and soybean oil samples – taken at various points of the production process – were analysed in a laboratory and converted into Biodiesel, the SG and ASG contents of which were then also examined. It turned out that the transesterification leads to a decrease of SG and ASG; however, when handling highly contaminated oils the effect is too small to guarantee sufficient quality. Therefore, when dealing with oils with high SG and ASG contents, the authors recommend the introduction of an additional refining step in the Biodiesel production to achieve a decrease of SG and ASG.

During the base catalysed transesterification, being the standard process for the production of Biodiesel, ASG are at least partly converted into SG. Thus it is possible that a Biodiesel may contain more steryl glycosides than the vegetable oil used for its production. In order to obtain a reliable comparison between the qualities of the vegetable oil and the Biodiesel, both the acylated as well as the non-acylated form in feedstock and product must be determined.

In contrast to the acylated form, steryl glycosides are hardly soluble in FAME and only crystallise slowly out of the product. The process can be accelerated by cooling; for that the forming crystals can serve as nuclei for highly melting components, e.g. saturated monoglycerides.

The purpose of fuel filters is to remove solid particles from the fuel to avert detrimental influences on the injection system. The kind and size of the particles have an influence on the plugging of a filter. Large particles remain in the filter but they do not seal the filter pores immediately; however, if the particles are of a size corresponding to the diameter of the filter pores, even small amounts can already block the filter and significantly reduce the filterability.

The possibility to predict the suitability of fuels by the determination of the filterability is an important concern of the automotive industry. Of all currently implemented test methods to determine the filterability, IP 387 mod. (FBT Filter Blocking Tendency) and ASTM D 7501 (Cold Soak Test) are the ones most frequently used for the testing of Biodiesel. Of those two FBT is favoured in Europe, whereupon cold treatment (‘Cold Soak’) prior to measurement is

\(^1\) J. Haupt, G. Brankatschk, T. Wilharm, “Steryl Glycoside Content in Vegetable Oils as a Risk for the Production of Biodiesel”, www.agqm.de
considered as well. The US Biodiesel standard D 6751 stipulates a limit value for CST, whilst in Europe equivalent specifications have not been established so far.

During the course of this study the filterability according to IP 387 was examined. The thermal pre-treatment of the sample (Cold Soak FBT) was integrated in order to find out whether increased validity of the test method can be achieved and additional information gained. A change in the filterability by means of cooling can bring about conclusions with regard to a future improving of this Biodiesel property by thermal treatment:

- Any post-cooling deterioration of the FBT indicates very small, visually not perceivable particles plugging the filter pores
- A reduced FBT value indicates the “freezing” of interfering components and/or the formation of ‘large’ particles

Noticing a distinct tendency would be a reason to carry out further investigation for the optimisation of the crystallising conditions.

Another aspect which was to be assessed with this study is a possible correlation between the SG and ASG contents and the FBT and/or Cold Soak FBT. Such a correlation may lead to a recommendation for a limit value for the SG and ASG contents; considering the analytical deficits of all known filtration tests (poor precision, time-consuming) it would be desirable to be able to judge the filtration behaviour of fuels via the determination of the problem components. However, prior to any final fixing of limit values, the correlation between measuring data and practical behaviour of Biodiesel used in B100 and B7 fuels must still be examined.

**Project Definition**

Among the many different causes for filter plugging, the influence of SG and ASG on the filterability of Biodiesel was to be examined first because it was assumed to be one of the main causes. The following questions were to be clarified by this study:

- Does the Biodiesel production process influence the SG/ASG contents of the final product?
- Is there a correlation between the SG/ASG contents of Biodiesel and its filterability?
- Is it sensible to integrate the ‘cold soak’ into the FBT test method?
- Is it possible to give a recommendation for a limit value on the basis of the data at hand?

For that purpose, a best possible comprehensive overview of the SG and ASG contents of the vegetable oils used for Biodiesel production and the Biodiesel produced thereof was to be gained first. Additionally, data was collected concerning the current situation of the filterability of Biodiesel in Germany. For that it was advantageous to carry out the data gathering along with AGQM’s sampling at the Biodiesel production plants because that way a multitude of additional parameters is now available, if necessary, without having to carry out new tests. An additional advantage is the fact that data comes from a wide-spread spectrum of producers and production processes.
Execution of the Project

In 2009 and the first half of 2010 additional analyses – apart from inspecting the Biodiesel according to DIN EN 14214 – were carried out in line with AGQM’s sampling at the production plants. As far as possible, samples of the vegetable oils used were taken. A total of 153 samples of vegetable oil and 203 Biodiesel samples were inspected. Below, the campaigns are referred to as 1-09 to 6-09 and 1-10 to 3-10.

All samples were tested for their SG and ASG contents; there was an additional filterability test (FBT B and Cold Soak FBT) of the 120 Biodiesel samples from campaigns 5-09 to 3-10, in order to assess a possible correlation between filterability and SG/ASG contents. The samples came from a large number of German Biodiesel producers, an Austrian producer and a trader from the circle of AGQM members. That way, a wide overview of the Biodiesel quality produced in Germany is achieved.

The data gathering was started with the determination to which extent SG and ASG was contained in the oils used and which contents of SG and ASG were to be found in the Biodiesel produced thereof. There were no differentiated individual assessments of the rapeseed or soybean oils. In Germany winter quality is almost exclusively produced with rapeseed oil, so any influence of other vegetable oils on the Biodiesel quality can only be found in transition or summer qualities.

Despite the fact that so far there are no assured findings whether filtration tests reflect the actual behaviour of fuels in a vehicle, they are considered an indication for the suitability of fuels. For this study the filterability (FBT) of Biodiesel was determined according to IP 387 method B; that counts for untreated samples and in cases of pre-cooling (cold soak). It was the objective of the test to find out whether a correlation between the SG and/or ASG contents and the FBT value could be found and whether integrating the cooling step is a sensible addition to the test method.

Test Methods

HPTLC-Method for the Determination of SG and ASG

**Method:** High Performance Thin Layer Chromatography (HPTLC), automated method

**Preparation of Samples:** Pyridine is added to the sample in order to dilute unsolved parts of SG and ASG. Afterwards the sample is diluted with n-heptane / MTBE 2:1 (v/v).

**Solid Phase Extraction:** the sample is filtered through a filter cartridge. The sample matrix is separated as filtrate; SG and ASG remain in the filter. The filter is washed with n-heptane/MTBE. Afterwards SG and ASG are eluted with acetone and methanol. The solvents are evaporated; the residue is dissolved with THF.

**HPTLC:** For each SG and ASG standards of three different concentrations are applied to the TLC plate. The sample to be analysed is applied as well. The TLC plate is developed using a

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2 For reasons of anonymity every producer or trader received an identification number. Not every producer or trader was sampled during every sampling session which explains the data gaps.
mixture of DIBK/glacial acetic acid/water at a ratio of 90:25:3 (v/v). Afterwards the TLC plate is dried at 200 mbar and 120 – 150°C for at least 20 min.

**Development:** The TLC plate is immersed into a solution of Copper (II) Acetate and afterwards heated to 90°C for approx. 5 min. SG and ASG become visible as grey-blue bands. The TLC plate is read by a TLC scanner at 620 nm as soon as possible after the development because the colour intensity fades with time.

For SG and ASG the limit of quantification for this method is 5 mg/kg.

**Filtration Test**

In this study the determination of the Biodiesel filterability was carried out with the FBT method (IP 387). Method B was used as basic method in its standard version and also using a previous cooling step. With both methods a defined volume (300 ml) is filtered until the build-up of a determined counter pressure (105 kPa). The test is terminated if the following criteria are fulfilled: the filtration volume of 300 ml is reached at a counter pressure of < 105 kPa or the pressure rises to 105 kPa with a filtrate of less than 300 ml. The FBT value which is considered assessment criterion is calculated according to the following formula:

\[ FBT = \sqrt{1 + \left( \frac{P}{105} \right)^2} \]

where:
- \( P \) is the maximum pressure measured when filtering 300 ml (abort criterion 300 ml of filtrate reached).
- \( V \) is the maximum volume filtered until a pressure of 105 kPa is reached (abort criterion 105 kPa reached).

If values exceeded 1.41 not all of the volume could be filtered. At present in Europe or the USA there is no limit value according to which a sample is rated good or bad; however, in Australia there is a FBT limit value of 2.0 for Diesel fuels. Still, there is no definite and dependable connection to the actual behaviour of the Diesel fuels.

The following evaluation implements a random differentiation between samples with FBT values of up to and more than 2.00. All samples comply with the seasonal requirements concerning the CFPP.

For the determination of the Cold Soak FBT, before filtration the samples are cooled at 4.5°C for 16 h. Afterwards they are brought to 20°C without any additional heating. Significant differences between the FBT and Cold Soak FBT values indicate a change of the sample influenced by temperature; the precision of the method – which even decreases with rising values for FBT – must be considered though when interpreting the measuring data.

**Evaluation of the Data**

The study assessed the data obtained during the course of AGQM’s routine checks at Biodiesel production plants. Due to the varying sources of procurement it was not possible to determine the origins of each used feedstock; therefore, the evaluation can only give a survey of the vegetable oils customary on the German market without any consideration of the differing production processes in the oil mills.
In order to allow for a graphical evaluation the SG and ASG contents below the limit of quantification (5 mg/kg) were assigned the value 5 mg/kg. Thus the value ‘5 mg/kg’ represents SG and/or ASG levels of $\leq 5$ mg/kg.

All collected data was considered in the overall assessment and evaluated with regard to the SG and ASG contents in vegetable oils, the SG and ASG contents in Biodiesel and the Biodiesel filterability. An individual evaluation per producer will only be done for substantial data sets.

**Vegetable Oils – Evaluation of the SG and ASG Contents**

Figures 1 and 2 show the SG content of vegetable oils used in campaigns 1-09 to 3-10. The maximum value amounts to 215 mg/kg. An allocation of the measuring data according to the time of year is not identifiable.

**Figure 1**: SG content of vegetable oil, campaigns 1-09 to 3-10, producers 1 - 18
Figures 3 and 4 show the ASG content of vegetable oils used in campaigns 1-09 to 3-10 for the Biodiesel production. The maximum value amounts to 324 mg/kg.

**Figure 2:** SG content of vegetable oil, campaigns 1-09 to 3-10, producers 19 - 35

**Figure 3:** ASG content of vegetable oil, campaigns 1-09 to 3-10, producers 1 - 18
Figure 4: ASG content of vegetable oil, campaigns 1-09 to 3-10, producers 19 - 35

Figure 5 illustrates the allocation of the SG and ASG contents of the vegetable oils. 93 per cent - the biggest proportion of the samples by far - contain a maximum of 100 mg/kg of SG and/or ASG.
In individual cases oils of more than 200 mg SG and/or ASG are used. It proves right that the ASG share in vegetable oil is mostly higher than that of SG. The determination of the SG content alone is therefore not explicit enough to monitor the vegetable oils used.

**Biodiesel - Assessment SG und ASG Contents**

The following figures show the SG contents (6 and 7) and the ASG contents (8 and 9) of Biodiesel of campaigns 1-09 to 3-10. Whilst some producers show relatively stable values during production, the contents of others fluctuate considerably. However, in total the values are very low, with smaller concentrations of ASG than of SG.

![Figure 6: SG content in Biodiesel, campaigns 1-09 to 3-10, producers 1 to 18](image-url)
Figure 7: SG content in Biodiesel, campaigns 1-09 to 3-10, producers 19 to 35

Figure 8: ASG content in Biodiesel, campaigns 1-09 to 3-10, producers 1 to 18
A seasonal influence on the contents of SG and ASG in Biodiesel cannot be identified. However, so far there is still too little data to allow for the interpretation of the fluctuation of the SG and ASG contents between campaigns; maybe long-term monitoring could reveal the reasons.

Figure 10 shows the allocation of the SG and ASG contents. In 86% of the samples the SG levels were 20 mg/kg or less, in 13% of the samples tested an SG level between 20 and 50 mg/kg at the most was found. Only 1% of the samples had a level above 50 mg/kg. The ASG content of 67% of the samples ranged up to 5 mg/kg, 96% up to 20 mg/kg. The maximum ASG value amounts to 33 mg/kg.
Biodiesel – Filterability

The evaluation comprises the samples of campaigns 5-09, 6-09 and 1-10 to 3-10. For the evaluation of the data in this case samples with an FBT and csFBT value of ≤ 2.00 were denoted ‘well filterable’, samples with an FBT and csFBT value of > 2.00 were denoted ‘poorly filterable’. The denomination was effected at haphazard.

The following figures 11 (campaigns 5-09 and 6-09) and 12 (1-10 to 3-10) show the results of the filtration tests.
The graphs reveal that the values for FBT and csFBT comply within the precision of the method. Evidently no additional information can be obtained by the thermal pre-treatment of the Cold Soak Method. This is also confirmed by the allocation of the FBT and csFBT measuring data (figure 13): 54 % of the samples show an FBT value of 2 at the most for both methods; 70 % of the samples range up to 3 for both FBT and csFBT.
In figure 14 the FBT values are plotted against the SG content. The data points do not indicate a clear correlation between filterability and SG contents. Though there is a tendency for poor filterability in combination with high SC contents there is also a number of data sets which do not support this tendency: there are observations of combinations of low SG contents with poor filterability and also of high SG levels with good FBT results. This is an indication that the SG content is not the only cause for problematic filterability.
In figure 15 the FBT values are plotted against the ASG content. Here it is even more clearly noticeable that no correlation between ASG content and filterability can be derived. In particular, even an ASG level of 5 mg/kg is no guarantee for good filtration properties of the sample.
Evidently SG and ASG have a clear influence on the filterability of FAME but they are not the only and/or triggering factor for filtration problems. It is likely for other components to overlay or aggravate the effects which are caused by SG and ASC; this has already been demonstrated by other tests. Additional tests in this field are imperative.

**Assessment of the Results**

**Correlation between Filterability and Steryl Glycoside Content**

Table 1 illustrates the frequency allocation of poor and good filtration depending on the SG and ASG contents:

<table>
<thead>
<tr>
<th></th>
<th>FBT and Cold Soak FBT ≤ 2,00 (well filterable)</th>
<th>FBT or Cold Soak FBT &gt; 2,00 (poorly filterable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SG and ASG ≤ 10 mg/kg</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>SG or ASG &gt; 10 mg/kg</td>
<td>31</td>
<td>47</td>
</tr>
</tbody>
</table>

Tab. 1: Filterability and SG/ASG contents in Biodiesel

The data clearly shows the already mentioned tendency for poor filterability with higher SG contents but they also document that many products show a deviant behaviour. Standard analytics according to the requirements of DIN EN 14214 do not indicate why poorly filterable samples with an SG and/or ASG content of less than 10 mg/kg show this noticeable behaviour. Properties not included in the test scope are presumably responsible for this effect.

There are two explanations for the good filtration properties of samples with SG and/or ASG contents of 10 mg/kg: either the crystals are so small that they are not held back by the filter or it is due to hindered crystallisation. Crystal nuclei, variations in temperature, moving the sample etc. can cause delayed crystallisation of SG and ASG at a later point in time and thus worsen the Biodiesel filterability with time. This is an effect which can in fact be observed in practice.

**Recommendation for Limit Value**

From the data at hand a recommendation for an SG and/or ASG limit value cannot be derived. The current data does not reveal a clear and exploitable correlation between the steryl glycoside contents and the filterability; both SG and ASG contents, neither individual nor added, can be put in correlation to the filtration behaviour. However, without such a correlation no statement can be made with regard to whether the stipulation of a limit value for steryl glycosides could bring about the guarantee for the unproblematic use of a fuel.

**Summary**

**Scope of the Test**

In addition to the requirements of DIN EN 14214 and as part of AGQM’s sampling at the production plants, 203 Biodiesel and 153 vegetable oil samples were tested for their contents of steryl glycosides (SG) and acylated steryl glycosides (ASG). The samples were gathered and analysed during nine campaigns (1-09 to 3-10) from January 2009 until June 2010. The
samples of campaigns 5-09 to 3-10 were additionally tested for their filterability according to FBT and Cold Soak FBT (csFBT).

A large number of AGQM member Biodiesel producers as well as one AGQM trading company joined in the project. They represent a production capacity of about 3.8 Mio tons/a (Germany’s total: 4.9 Mio tons/a); thus the Biodiesel qualities used for this study are a representative reflection of the situation in Germany.

Quality of the Vegetable Oils Tested

The vegetable oils used during 2009 and the first half of 2010 showed significant variations in their SG and ASG contents. In most cases the levels for SG were above those for SG, with more than 90 % of all samples tested containing no more than 100 mg/kg SG and/or ASG. This shows that in most cases oils with low SG and ASG contents are used already.

Quality of the Biodiesel Tested

The SG and ASG contents of the Biodiesel samples tested in this study in the years 2009 and 2010 were relatively low; the contents only amounted to more than 30 mg/kg in just 4 % (SG) respectively 2 % (ASG) of the 203 samples. Many samples fell below the ASG limit of quantification of 5 mg/kg in Biodiesel with the SG contents mainly ranging up to 20 mg/kg. Just five of all samples tested (2%) contained more than 40 mg/kg SG. On the one hand this is achieved by using just slightly contaminated vegetable oils; on the other hand it is obviously possible to achieve a significant decrease of the SG and ASG contents by using suitable production management. However, when assessing the measuring data the ASG are converted to SG during the transesterification process, so that a quantitative evaluation of the reduction during the process is only possible restrictedly. In case vegetable oils with low SG and/or ASG contents are on hand already a significant reduction induced by the Biodiesel production process can no longer be observed.

Correlation between Filterability and SG Content

The evaluation of the results of the filtration tests reveals no correlation between the SG and ASG contents and the values for FBT and Cold Soak FBT (csFBT). On the one hand there are samples which show poor filterability despite low SG and ASG contents; on the other hand the filterability of samples with significantly higher SG and ASG contents was better than that of others with comparably little contents. Although there is a tendency, that in many cases lower SG and ASG contents lead to better filterability, a relatively large number of samples shows a deviate behaviour. Apparently the SG and/or ASG contents are not the only influencing factors to cause a deterioration of the filterability of FAME.

Further Action

In most Biodiesel plants the feedstock cannot be unmistakably allocated to the Biodiesel batch tested due to logistic circumstances and production management. In this context, another project - already underway – to achieve the reliable allocation of the feedstock to the
final product, is meant to reveal additional findings as to the influence of the production process on the presence of SG and ASG. Additionally the test for the filterability of Biodiesel-Diesel blends (B7) is planned with the objective to examine changes in the filtration properties due to the blending of fuels. In previous test sequences reference indicating some unexpected behaviour of blend fuels could be found.

The evaluation of various parameters – determined during AGQM’s production plant sampling – is meant to reveal whether the contents of saturated monoglycerides – maybe in combination with the SG and ASG contents – correlates with the filterability.