INTRODUCTION

Diacylglycerides (DAGs) are one of the minor components in vegetable oils but play an important role in the physical properties of the products where they are present [1]. Several chromatographic techniques are available to determine DAGs, including GC [2] and HPLC [3] procedures. To prevent breakdown of DAGs caused by high temperature during GC analysis, these procedures require a pretreatment (i.e., silylation) with hazardous chemicals. For several oils an additional step has to be included to avoid co-elution of short chain triacylglycerides (TAGs) and DAGs. The procedures based upon this GC methodology are accurate and correct; nevertheless they are long and time consuming. HPLC is usually carried out at ambient temperature, so protection of the hydroxyl group is not necessary. Although excellent separation of the different lipid classes can be achieved using a gradient, one is restrained to the use of an evaporative light scattering detector (ELSD). However, it’s response is nonlinear and depends on the fatty acid composition of the DAGs. Furthermore, the runtime is longer due to the conditioning of the column after each run. To overcome the limitations of the ELSD, a fast isocratic straight phase HPLC method with refracto-index (RI) detection was developed which can be easily implemented in QC laboratories. The GC method was used as reference method during this study.

OBJECTIVE

To set up an isocratic HPLC procedure for the determination of DAGs, which can be used for routine analysis in a QC laboratory.

METHODOLOGY OF CURRENT PROCEDURES

Straight phase HPLC with gradient separation: A small amount of sample is weighed and dissolved. An internal standard is added after which the sample is analyzed on a HPLC system. Separation on a silica column is accomplished by means of a gradient. Detection takes place using an ELSD.

GC method: An internal standard is added to the sample prior to separation on a basic alumina oxide SPE column. The TAGs and DAGs are eluted and both fractions are silylated using BSTFA and pyridine. The trimethylsilyl (TMS) derivatives are analyzed on a non-polar capillary GC column. The Flame ionization detector (FID) is used for detection.

METHOD DEVELOPMENT

To employ the RI detector, an isocratic HPLC separation of the DAGs had to be developed. Due to the non-polar nature of the sample, a mixture of hexane and toluene was chosen as solvent. The elution of the DAGs was controlled by the amount of ethyl acetate that was added as modifier. Formic acid was added to control the pH and retention time of the free fatty acids. All these solvents were also used in the gradient analysis. Quantification was done using an external calibration curve which was set up by injecting different volumes of a stock solution that consisted of a mixture of four different DAGs. To improve the reproducibility of the procedure the added amount of solvent to dissolve the sample was weighed using an analytical balance.

HPLC CONDITIONS

The conditions for the isocratic HPLC procedure were the following:

- Sample concentration: 50 mg/ml (HPLC solvent)
- Column: Acentis Express HLLC (100x2.1 mm; 2.7 µm)
- Solvent: hexane/toluene/ethyl acetate (82/15/3 v/v %) with 0.15 % formic acid
- Flow: 0.6 ml/min
- Injection volume: 10 µl
- Equipment: Shimadzu DGU-20A, LC-20AD, SIL-20AC, CTO-20AC and RID-10A

RESULTS AND DISCUSSION

Once method development was finalized, five different oils were treated on a silica column to remove all DAGs. The purified TAGs were spiked with a known amount of DAGs to obtain a range of samples with different levels of DAGs, exceeding the normal range found in these particular oils. These samples were analyzed with all three methods. A typical chromatogram of a Crude Palm Oil using the isocratic HPLC procedure is given in Figure 1. The runtime was 20 min.

The results of the HPLC analysis with RI detection clearly show that the RI detection is in good agreement with the reference method (GC-FID), as shown in Figure 2. The typical, non-linear behavior of the ELSD detector leads to a concentration dependent variance.

CONCLUSIONS

A new isocratic HPLC procedure was developed that is suitable for daily routine use in QC laboratories for the analysis of DAGs. The results are in good agreement with the GC reference method. During validation the following performance characteristics were found: measuring range: 0-10 %, reproducibility: \( x=5.43 \% \), \( s=0.42 \% \). Because there is hardly any pretreatment, the time needed for work-up is limited and operators are not exposed to hazardous chemicals needed for silylation, with benefits for health and the environment. The use of a calibration curve and RI detection improved both accuracy and trueness compared to the gradient HPLC method with ELSD.

REFERENCES

2. AOCS Cd 11b-91 Determination of mono- and diglycerides by capillary gas chromatography
3. AOCS Cd 11d-96(09) Mono- and Diglycerides by HPLC-ELSD

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