Characterization of Used Cooking Oils by High Performance Liquid Chromatography and Corona Charged Aerosol Detection

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Overview

Purpose: To develop analytical methods to characterize used cooking oils by HPLC.

Methods: High-pressure liquid chromatography (HPLC) methods using the Thermo Scientific™ Dionex™ Corona™ ultra RS™ Charged Aerosol Detector, ultraviolet photodiode array detector (DAD), fluorescence detector (FLD), and the Thermo Scientific™ MSQ Plus™ mass spectrometer (MS) were developed and are detailed.

Results: A variety of cooking oils, five used oils ("gutter oils") and two fresh oils were analyzed, and their results are presented. The universal lipids method provided the fastest and most differentiating results to distinguish different oil qualities, and the HPLC-FLD-MS method provided information on aldehyde content of the samples.

Introduction

Cooking oils come from a variety of sources, including olive, rapeseed, peanut, grapeseed, mustard, corn, and many others. Cooking oil must be monitored for quality and contamination. When cooking oil is heated, it can undergo many chemical changes including oxidation of unsaturated fatty acids, triglyceride decomposition, and the formation of potentially cytotoxic oxidation products such as 4-hydroxy-trans-2-nonenal (HNE) and other aldehydes that are purported to be associated with Parkinson's, Alzheimer's, Huntington's, atherosclerosis, liver diseases, and stroke. Rancidity during long term storage can also occur and is associated with the content of polyunsaturated fatty acid content. Although these issues make used oil unfit for use in the kitchen, and unhealthy for human consumption, it can still act as a useful resource as a raw material for biofuels production. As there is a significant price difference between high quality cooking oils and lower quality biofuel raw materials, the possibility exists for unscrupulous people to filter and decolorize used cooking oils and sell them as high quality cooking oils. Such treated used oils are referred to as gutter oils (GO).

To provide some means of distinguishing fresh oil from gutter oil, we developed four HPLC methods. Three of these methods used the Corona ultra RS charged aerosol detector to determine different lipid components and ratios that were present in the samples. A fourth method used sample derivatization, fluorescence and mass spectrometry to determine aldehyde content.

One method, based on a universal lipids analytical method, separates the majority of analytes by reversed phase (RP-HPLC) with focus on triacylglycerides (TAG) and their composition, diacylglycerides, and minor components. A second RP-HPLC method quantifies the free fatty acids resulting from base hydrolysis of the samples, with detectable changes in fatty acid composition. A third method, using normal phase (NP-HPLC), separates the samples by lipid class, including TAGs, free fatty acids, and DAGs–a faster and simpler result than the first method. The charged aerosol detector is ideal for these analyses based on its relatively uniform response factor for non-volatile analytes, and its high sensitivity. The detector operates by nebulizing analytes after they elute from the column, and placing a charge on the resulting analyte particles. Peak area is proportional to the amount of analyte (mass) entering the detector. Typical limits of quantitation are in the single-digit nanograms on column, and replicate injection precision values are two percent relative standard deviation on peak area.

A fourth method, for aldehyde determination using HPLC with fluorescence (FLD) and mass spectrometry of derivatized oil samples, is also described with associated results. Identifications of aldehyde degradant components were made by mass spectrometry.

Methods

Liquid Chromatography System: Reversed Phase

HPLC System:	UltiMate 3000 DGP-3600RS pump, WPS-3000RS autosampler, and TCC-3000RS column oven

Liquid Chromatography System: Normal Phase

HPLC System:	UltiMate 3000 LPG-3400SD pump, WPS-3000RS autosampler, and TCC-3000RS column oven

RP-HPLC-Corona ultra RS Universal Lipids Method

Sample Preparation: 100 µL (or equivalent mass) of sample dissolved in 900 µL methanol /chloroform (1:1)
HPLC Column: Thermo Scientific™ Accucore™ C8, 2.6 µm 4.6 × 150 mm HPLC
Column Temp.: 40 °C
Mobile Phase A: Methanol/water/acetic acid (650:350:4)
Mobile Phase B: Acetonitrile
Mobile Phase C: Isopropyl alcohol
Detector: Corona ultra RS
Detector Filter: 3
Nebulizer Temperature: 20 °C
Sample Temperature: 20 °C
Flow Rates: Elution Pump: 0.8 mL/min, Inv. Grad. Pump: 1.2 mL/min
Injection Volume: 5 µL
Flow Gradients:

Elution

Time (min)	%A	%B	%C
-5.0	100	0	0
0.0	100	0	0
1.0	100	0	0
2.0	50	50	0
15.0	45	55	0
23.0	0	100	0
30.0	0	100	0
60.0	0	35	65

Inverse

Time (min)	%A	%B	%C
-5.0	0.0	66.7	33.3
0.0	0.0	66.7	33.3
1.7	0.0	66.7	33.3
2.7	33.3	33.3	33.3
15.7	40.0	26.7	33.3
23.7	66.7	0.0	33.3
30.7	66.7	0.0	33.3
60.7	66.7	33.3	0.0

RP HPLC-Corona ultra RS Method for Omega Lipids/Free Fatty Acids

Sample Preparation: Oil samples (50 µL aliquot) were dissolved/dispersed in 5 mL isopropanol/water (3:2) and 1 mL of 5 M KOH. All samples were heated in an 80 °C water bath for 1 h with occasional stirring. After samples were cooled, a 500 µL aliquot was removed and 25 µL of formic acid was added to neutralize the sample.

HPLC Column: Thermo Scientific™ Acclaim™ C30, 3 µm, 3.0 × 250 mm
Column Temp: 30 °C
Mobile Phase A: Methanol/MP B/acetic acid (900:100:3.6)
Mobile Phase B: Acetone/acetonitrile/tetrahydrofuran/acetic acid (675:225:100:4)
Detector: Corona ultra RS
Detector Filter: 3
Nebulizer Temperature: 10 °C
Flow Rate: Elution Pump: 1.0 mL/min, Inv. Grad. Pump: 1.0 mL/min
Injection Volumes: 5 µL
Flow Gradients:

Elution

Time (min)	%A	%B
0.0	100	0
1.0	40	60
13.0	30	70
22.0	5	95
24.0	5	95
29.0	100	0

Inverse

Time (min)	%A	%B
0.0	5	95
1.0	5	95
2.0	65	35
14.0	75	25
23.0	100	0
25.0	100	0
25.0	5	95
29.0	5	95

NP-HPLC-Corona ultra RS Method for Acylglycerols and Free Fatty Acids

Sample Preparation: ~40 µL/mL of oil was dissolved in iso-octane/isopropanol (95:5)
HPLC Column: Glass-lined, titanium fritted, non-endcapped CN, 3 µm, 4.0 × 150 mm, at 40 °C
Mobile Phase A: Iso-octane
Mobile Phase B: Methyl-t-butyl ether, 0.4% acetic acid
Mobile Phase C: iso-Octane/n-butyl acetate/methanol/acetic acid (500:167:333:4)
Detector: Corona ultra RS charged aerosol detector
PowerFunction: 2.0
Detector Filter: 5
Nebulizer Temp.: 15 °C
Sample Temperature: 15 °C
Flow Rate: 1.0–1.2 mL/min
Injection Volumes: 1–5 µL

Flow Gradient:

Time (min)	Flow Rate (mL/min)	%A	%B	%C
0.0	1.0	100	0	0
0.0	1.0	100	0	0
2.0	1.0	95	5	0
6.0	1.0	93	7	0
12.0	1.0	60	40	0
13.0	1.2	40	0	60
13.5	1.2	20	0	80
13.7	1.2	20	80	0
15.0	1.2	40	60	0
16.0	1.2	100	0	0

Aldehydes Analyzed by Reversed-Phase HPLC with FLD and MS Detection

Sample preparation: A 500 µL aliquot of oil sample solution (10 mg/mL in tetrahydrofuran), was mixed with 1000 µL of Hantzsch reagent in a 1.5 mL centrifuge tube. The solution was heated at 75 °C for 1 hour. Samples were centrifuged (10,000 g for 3 mins), and the supernatant was transferred to an HPLC vial.

The Hantzsch reagent was prepared as follows: 15 mL of denatured alcohol and 1 mL of water was mixed prior to the addition of 2 g of ammonium fromate. Once dissolved, a 1 mL volume of formic acid and 50 mg of 1,3-cyclohexanedione was added. The solution was brought to a 20 mL volume through the addition of denatured alcohol.

HPLC Column:	Acclaim 120 C18, 3 µm, 3.0 × 150 mm at 50 °C
Mobile Phase A:	Water
Mobile Phase B:	n-propanol
Detector 1:	UltiMate 3000 FLD-3400RS Fluorescence detector
Wavelengths:	Excitation: 388 nm Emission: 455 nm
Sensitivity:	2, Data Collection: 5 Hz
Detector 2:	MSQ Plus mass spectrometer
Probe Temperature:	400 °C
Ionization:	+mode ESI, Cone potential: +75 V
Dwell Times:	1.00 s for SIM, 4.00 s for FSM
Flow Rate:	0.5 mL/min, flow split (1:1) between FLD and MS
Injection Volumes:	1 µL
Flow Gradient:	

Time (min)	%A	%B
-5.0	98	2
0.0	98	2
2.0	98	2
45.0	65	35
52.0	5	95
62.0	5	95
65.0	98	2

Data Analysis
All HPLC chromatograms were obtained and compiled using Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, 7.1 SR 1.

Results

RP-HPLC-Corona ultra RS detector Universal Lipids Method

Qualitative comparisons were made between gutter oil samples and fresh soybean oil, and the chromatogram overlays are shown in Figure 1. Relative to the fresh oil, a few differences between gutter oil and soybean oil were found: fatty acid (measured by oleic acid peak area), monoglyceride, and DAG amounts increased, while TAGs decreased; and there was a change in the triglyceride distribution to heavier triglycerides, as indicated by the relative amounts of equivalent carbon number triglycerides (ECN). ECN is calculated using C-2n, where C is the number of carbon atoms and n is the number of double bonds in the alkyl chains. Other unidentified analyte peaks were also noted in the used oils. Levels of analytes that showed significant differences between soy bean oils and GOs are highlighted in red in Table 1. For comparison purposes the values for the oleic acid, DAG, and TAG component areas are relative to total peak area in the chromatogram, and the ECN amounts are relative to the total TAG peak area.

From a qualitative observation, the gutter oils GO4 and GO5 were of the least quality, being a cloudy, rancid gel. This is reflected in the high free oleic acid content, low triglycerides, and highest ECN 52:ECN 40 ratios.

FIGURE 1. HPLC-Corona detector chromatogram of unused soybean oil and five gutter oils, normalized to the ECN 46 peak.

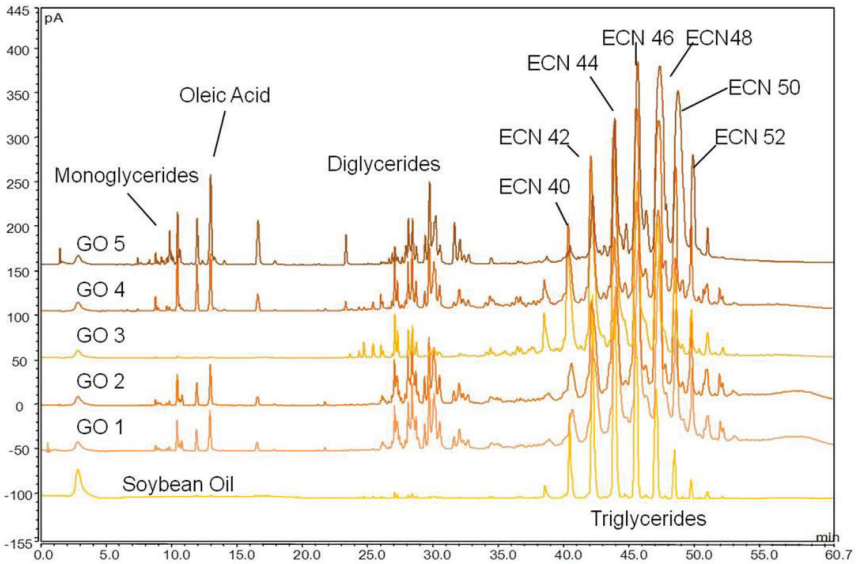


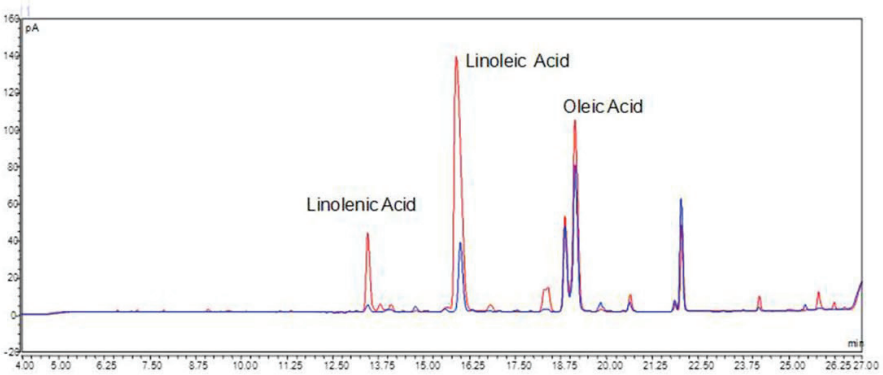
TABLE 1. Relative peak area analysis of two unused soybean samples and five gutter oil samples.

Oil	Oleic Acid (Area-%)	DAG (Area-%)	TAG (Area-%)	ECN 40 (Area-%)	ECN 48 (Area-%)	ECN 52 (Area-%)
Soybean-1	0.02	3.37	96.12	9.23	14.29	2.06
Soybean-2	0.03	1.10	98.77	7.66	13.96	1.39
GO1	1.21	8.70	80.99	5.62	24.04	3.72
GO2	0.93	14.35	82.77	4.12	22.81	4.29
GO3	0.02	4.50	91.82	11.06	13.96	2.33
GO4	1.46	11.76	79.01	6.38	23.75	6.42
GO5	2.47	11.76	79.40	1.50	27.90	6.90

RP HPLC-Corona ultra RS detector method for Omega Lipids/Free Fatty Acids

Samples were hydrolyzed and analyzed to investigate any differences in the free fatty acid profiles of the different oil samples. In Figure 2, HPLC-Corona detector chromatogram overlays of soybean oil (#1) and a gutter oil (GO #5), showing changes in the fatty acid profile of the two oils as indicated by the arrows. MS analysis (-ve, ESI, -55V) revealed saturated and unsaturated fatty acids found in soybean oils. Agreeing with the results found in the Universal method, there were decreases in linoleic and linolenic acids compared to oleic acid with gutter oil compared to the soybean oil.

FIGURE 2. HPLC-Corona detector chromatogram of fatty acids in unused soybean oil (red) and a gutter oil (#5) (blue).



NP-HPLC-Corona ultra RS detector method for Acylglycerols and Free Fatty Acids

Oil samples were prepared and analyzed by normal phase chromatography, which separates by the polar/hydrophilic moieties on the analytes. The free fatty acids were separated from the triglycerides, and the gutter oils were found to contain more free fatty acids and less triglycerides than the fresh soybean oil. The results of two oils are summarized in Table 2. Like the universal lipids method, increases in fatty acid and diglyceride content relative to the triglycerides was evident in the gutter oil samples.

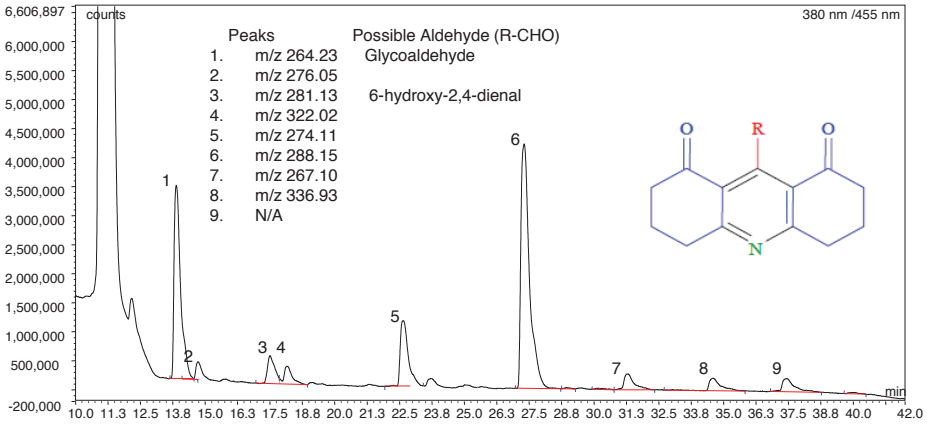
TABLE 2. Relative peak area analysis for gutter oil #2 and soybean oil #2 by normal phase chromatography

Oil	Free Fatty Acid (Area-%)	DAG (Area-%)	TAG (Area-%)	Other (Area-%)
Soybean-2	0.0	0.04	99.4	0.56
GO2	0.3	6.8	91.5	2.6

Aldehydes Analyzed by Reversed-Phase HPLC and FLD-MS

Aldehydes in the samples were derivatized to their fluorescent acridine forms by the Hantzsch synthesis. Aldehyde derivatives were measured using HPLC-FLD; MS was used to obtain a molecular weight and possible identity of each aldehyde derivative. A chromatogram of GO4, showing a number of aldehyde derivatives is presented in Figure 3. Molecular weights (m/z) of the acridine derivatives are also given. Aldehyde peaks were identified by fluorescence and presence of a sodium adduct. All of the peaks shown in this figure are significantly larger or altogether new, compared to those found in unused soybean oil, indicating that aldehydes are produced upon heating. No 4-HNE was found in the samples, possibly due to its reactivity and the age of the samples undergoing analysis.

FIGURE 3. HPLC-FLD-MS analysis of acridine-derivatized aldehydes found in GO4.



Conclusions

- Four separate HPLC methods were used to characterize gutter oil compared to unused soybean oil controls.
- Different “qualities” of gutter oil were also evident, with GO3 consistently closest to unused soybean oil and GO5 being the worst.
- The universal lipids method still provided some distinguishing characteristic of GO3, seen as an increased amount of DAG content.
- Of the four methods developed and investigated for this study, the universal lipids method provided the most consistent determination between used and fresh cooking oils.
- The second method of choice was the aldehyde method, using HPLC-FLD-MS of derivatized aldehydes.
- The normal phase method would best serve as a fast screening tool for detection of samples with DAG and free fatty acids present.

References

1. Zarkovic, N. 4-Hydroxynonenal as a bioactive marker of pathophysiological processes, *Molecular Aspects of Medicine*, **2003**, 24, 281–291.

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