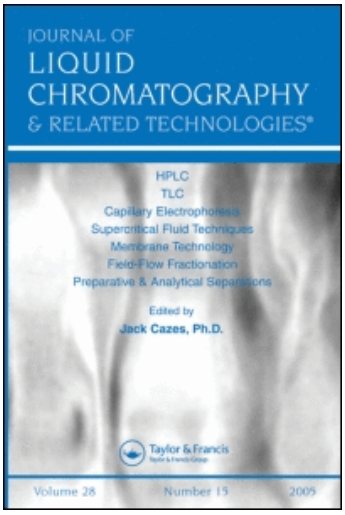

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

ANALYSIS OF CORN OIL IN ETHANOL MISCELLA BY GEL PERMEATION CHROMATOGRAPHY

Jason Kwiatkowski; D. Darnoko[a]; Munir Cheryan[b]
[a] Department of Food Science and Human Nutrition, Agricultural Bioprocess Laboratory, University of Illinois, Urbana, IL, U.S.A. [b] Department of Agricultural Engineering, Department of Food Science and Human Nutrition, Agricultural Bioprocess Laboratory, University of Illinois, Urbana, IL, U.S.A.

Online publication date: 06 May 2002

To cite this Article Kwiatkowski, Jason , Darnoko, D. and Cheryan, Munir(2002) 'ANALYSIS OF CORN OIL IN ETHANOL MISCELLA BY GEL PERMEATION CHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 25: 9, 1379 — 1386
To link to this Article: DOI: 10.1081/JLC-120004753
URL: http://dx.doi.org/10.1081/JLC-120004753

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

J. LIQ. CHROM. & REL. TECHNOL., 25(9), 1379–1386 (2002)

ANALYSIS OF CORN OIL IN ETHANOL MISCELLA BY GEL PERMEATION CHROMATOGRAPHY

Jason Kwiatkowski,[1] D. Darnoko,[2] and Munir Cheryan[1,2,*]

[1]Department of Agricultural Engineering, and
[2]Department of Food Science and Human Nutrition,
Agricultural Bioprocess Laboratory, University of Illinois,
1302 W. Pennsylvania Ave., Urbana, IL 61801, USA

ABSTRACT

A method for the analysis of corn oil and free fatty acids in ethanol miscella was developed using gel permeation chromatography. Two Phenogel columns were connected in series to a refractive index detector and tetrahydrofuran was used as the mobile phase. Sample preparation involved only dilution with the mobile phase and filtration. Individual free fatty acids eluted as one peak. Reproducibility of the method was good with a relative standard deviation of 0.94–3.17%.

Key Words: Corn; Corn oil; High performance liquid chromatography; Free fatty acids; Ethanol

*Corresponding author. E-mail: mcheryan@uiuc.edu

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

INTRODUCTION

Hexane is the most common solvent for vegetable oil extraction from oilseeds. Due to several potential safety and toxicity problems with hexane, studies are being conducted with alternate solvents such as ethanol. However, ethanol also extracts non-oil components such as proteins, carbohydrates, and pigments, and the concentration of oil is usually very low in the extract (0.5–4%). Thus, conventional Soxhlet or gravimetric methods of oil analysis are difficult and may give non-reproducible results when used for analysis of oil in ethanol miscella. High-performance size-exclusion chromatography (HPSEC) has been used for the separation and quantification of glycerides, fatty acids, and their esters.[1–4] There are currently no reports on its use for monitoring the extraction of corn oil with ethanol. This work reports on the analysis of corn oil and free fatty acids in ethanol miscella using HPSEC.

EXPERIMENTAL

Materials

Reference standards such as oleic acid, linoleic acid, and palmitic acid of >99% purity were purchased from Nu-Chek-Prep, Inc. (Elysian, MN) and Sigma Chemical Co. (St. Louis, MO). Refined corn oil was purchased from a local grocery store. Crude extracts of corn oil were prepared by extraction of ground corn with absolute ethanol at a solvent-to-solids ratio of 4 mL ethanol/g corn, 50°C and extraction time of 30 minutes. The slurry was filtered through Whatman No. 1 filter paper.[5] The clear filtrate was subjected to HPLC analysis.

Instrumentation

The HPLC system consisted of a ThermoSeparation Products P1000 isocratic pump with a ThermoSeparation Products RefractoMonitor Model IV refractive index detector and a Hewlett Packard 3395 integrator (Fremont, CA). The columns were two 300×7.6 mm Phenogel columns of 5 μm and 50 A pore size (Phenomenex, Torrance, CA), connected in series and protected by a 50×7.6 mm guard column of the same packing material. The mobile phase was HPLC grade tetrahydrofuran (THF) at a flow rate of 1 mL/min at room temperature and the sample injection size was 10 μm.

Sample Preparation

If the sample was clean and contained mostly oil (e.g., when standards were being analyzed or the corn was extracted with absolute ethanol), little or no sample preparation was needed. The oil-ethanol sample was diluted with THF in a 1 : 1 ratio and filtered through a 0.2 μm syringe filter before injection.

However, if extractions were performed with lower ethanol concentrations or other polar solvents, protein and other non-oil components could also be extracted. These components could cause a blockage of the HPLC columns. Such samples were treated with hexane to extract the triglycerides and other hexane-soluble components. The hexane treatment, typically, was the addition of 2 mL of hexane to 2 mL of the sample and mixing for 5 minutes at ambient temperature. After standing for 10 minutes to allow separation of the hexane and ethanol phases, the lighter hexane phase that contained the oil was removed, while the heavier ethanol phase was washed again with 2 mL of hexane. This washing procedure was repeated 3–5 times, depending on the residual oil in the sample. The individual hexane fractions were combined, diluted with THF in a 1 : 1 ratio, and filtered through a 0.2 μm syringe filter before injection.

RESULTS AND DISCUSSION

Elution Characteristics

Corn oil is composed mainly of triglycerides with palmitic, oleic, and linoleic acid comprising a majority of the side chains. Figure 1 shows a

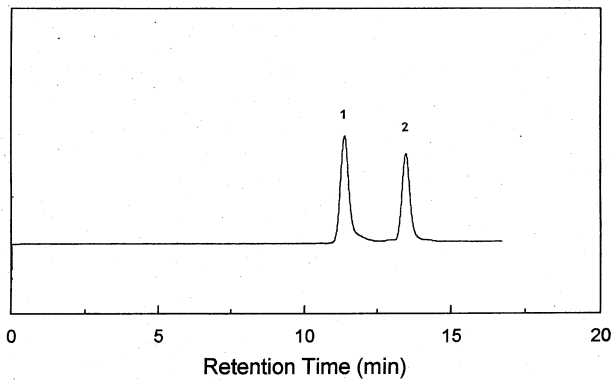

Figure 1. Gel permeation chromatogram for standard compounds. 1 = corn oil, 2 = free fatty acids (palmitic + oleic + linoleic).

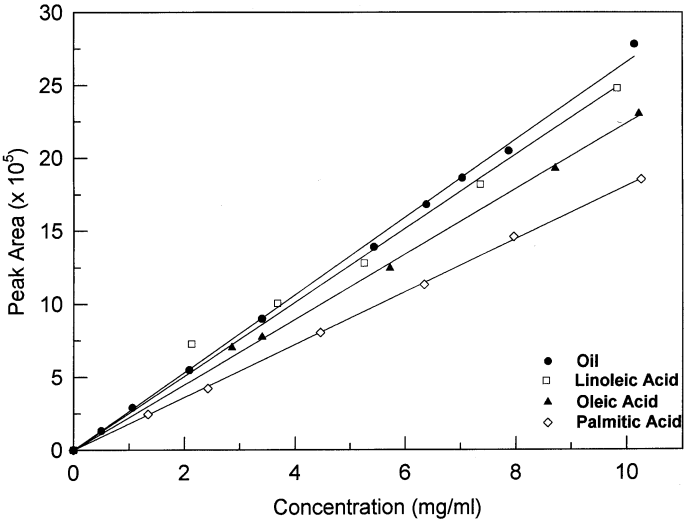

Figure 2. Calibration curves for corn oil and free fatty acids.

chromatogram of a standard containing pure corn oil and free fatty acids (FFA). There is a distinct separation of the corn oil peak, which has a retention time of 11.38 minutes under these analytical conditions, and the free fatty acids, which elute together as one peak at 13.44 minutes.

Figure 2 shows the calibration curve for corn oil and the individual free fatty acids made with reference compounds of refined corn oil, palmitic acid, oleic acid, and linoleic acid. Standard solutions were injected three times each into the HPLC and the corresponding peak areas were plotted against concentration. Table 1 shows the statistical analysis of the calibration curves. The standard solutions have good linearity within the concentration range examined, as shown by the high correlation coefficients.

Table 1. Standard Curves of Corn Oil and Fatty Acids*

Compound	Retention Time (min)	A	R^2
Corn oil	11.378	2.6594	0.998
Palmitic acid	13.443	1.8079	0.999
Oleic acid	13.443	2.2356	0.998
Linoleic acid	13.443	2.5296	0.988

*Concentration (g/L) = Peak Area $\times 10^{-5}$/A.

Table 2. Reproducibility of Analysis for Standard Corn Oil and Fatty Acids

Repetition	Sample 1 (Oil, g/L)	Sample 2 (Oil, g/L)	Sample 3 (Oil, g/L)	Sample 4 (FFA, g/L)
1	1.2081	4.0289	1.6913	0.7565
2	1.1195	4.0790	1.6986	0.7598
3	1.1403	4.1284	1.7252	0.7717
4	1.1205	4.0764	1.7243	0.7713
5	1.1407	4.0080	1.6983	0.7596
Average	1.1458	4.0641	1.7075	0.7638
Standard deviation (%)	0.0363	0.0472	0.0160	0.0072
Relative std. dev. (%)	3.1683	1.1604	0.9376	0.9376

The reproducibility of the method was studied by multiple injections of three different samples of pure corn oil and one sample of commercial free fatty acids. As shown in Table 2, the relative standard deviation was 0.016–0.047% for oil and 0.0072% for free fatty acids.

Ethanol Extracts of Oil in Corn

Figure 3 shows a chromatogram of an ethanol extract of whole ground corn. Two major peaks are visible: the oil peak at a concentration of 5.6 g/L oil and the

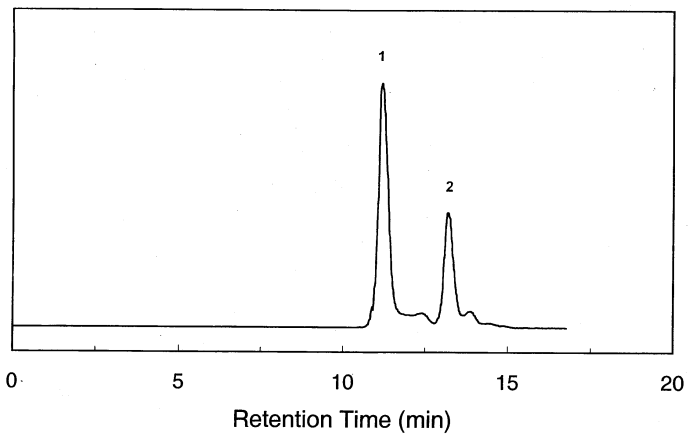

Figure 3. Gel permeation chromatogram of an extract of whole ground corn extracted with absolute ethanol. 1 = corn oil, 2 = non-oil components.

Table 3. Variation of HPLC Parameters for 10 Injections*

Parameter	Average	Standard Deviation	Relative Standard Deviation (%)
Retention time (min)	10.657	0.0029	0.0272
Peak area	1,606,497	15,491	0.9643
Oil content (g/L)	5.587	0.1097	1.9635

*Sample was a batch extract of whole ground corn extracted with absolute ethanol.

peak for free fatty acids. Table 3 shows the variability of HPLC parameters during analysis of the ethanol extracts. The relative standard deviations were 0.0272% for the retention time of corn oil and 1.96% for the oil concentration.

Pretreatment of Samples

If the ethanol extractant contains water, it will decrease the proportion of oil being extracted from corn, while increasing the amount of non-oil components in the extract.[5] If the samples contain a significant quantity of non-oil components, the columns could get blocked. Pretreating the samples with hexane was an effective way of overcoming this problem. The optimum number of hexane washes was determined, as shown in Table 4. For corn extracted with 90% ethanol, there was no detectable oil after the third stage of washing. For the 70%

Table 4. Optimum Number of Washes with Hexane*

	Concentration of Oil in Extract (g/L)	
Sample	90% Ethanol	70% Ethanol
Extract wash 1	0.276	0.002
Extract wash 2	0.045	0.002
Extract wash 3	0.002	0.000
Extract wash 4	0.000	0.000
Extract wash 5	0.000	–
Combined extracts	0.131	0.002
Calibration factor (A)	2.464	2.460

*The sample was an extract of whole ground corn which had been extracted with either 90% ethanol or 70% ethanol. It was then treated with hexane as described in the text.

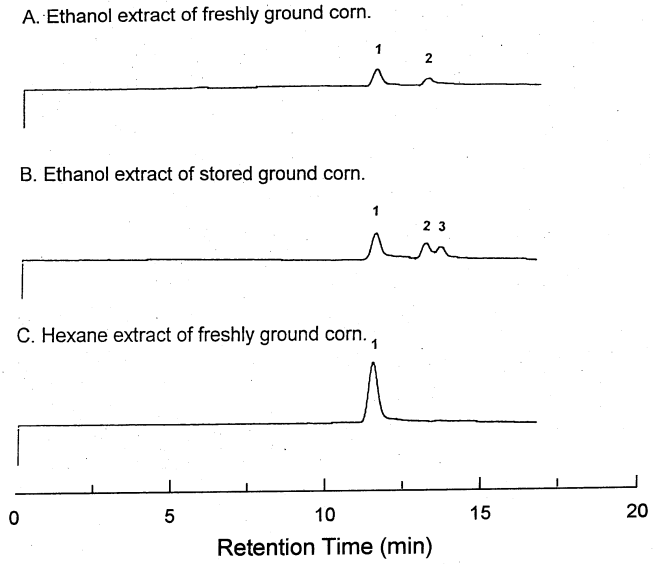

Figure 4. Gel permeation chromatogram of extracts of whole ground corn. A: Freshly ground corn extracted with absolute ethanol. B: Ground corn stored for several weeks extracted with absolute ethanol. C: Freshly ground corn extracted with hexane. 1 = corn oil, 2 = non-oil components, 3 = free fatty acids.

ethanol extracts, oil was barely detectable in the first and second stage of washing. Thus, hexane washing was done 3 times to ensure that a quantitative recovery of the oil was obtained. Table 4 also shows the calibration factor (A) for the oil in the hexane-treated samples.

Figure 4 shows a series of chromatograms obtained with various extracts of whole ground corn. Chromatogram A shows an ethanol extract of freshly ground corn. There are two peaks, one for oil and the other for non-oil components. Chromatogram B shows a chromatogram obtained with ground corn that had been stored for several weeks and then extracted with absolute ethanol. A peak for free fatty acids is also visible, probably due to the partial hydrolysis of triglycerides by lipase. Chromatogram C is a hexane extract of fresh ground corn, which shows only one peak for the triglycerides and none for other non-oil components.

CONCLUSIONS

Compared to the Soxhlet method or other gravimetric methods for determining oil content, a major advantage of gel permeation chromatography is

its short analysis time. Since there is no interaction between sample and the polymeric gel material in the column, a much smaller liquid sample may be used. The ethanol extracts require minimal sample preparation and the total analysis time is significantly reduced. Triglycerides and free fatty acids are separated completely, although different fatty acids gave similar retention times.

ACKNOWLEDGMENTS

This study was partly supported by the Illinois Department of Commerce and Community Affairs, the Illinois Corn Marketing Board, and the USDA National Research Initiative Competitive Grants Program (Award No. 97-35504-4296).

REFERENCES

1. Christopoulou, C.N.; Perkins, E.G. J. Am. Oil. Chem. Soc. **1986**, *63*, 679–684.
2. Fillieres, R.; Benjelloun-MLayah, B.; Delmas, M. J. Am. Oil. Chem. Soc. **1995**, *72*, 427–432.
3. Trathnigg, B.; Mittelbach, M. J. Liq. Chromatog. **1990**, *13*, 95–105.
4. Darnoko, D.; Cheryan, M.; Perkins, E.G. J. Liq. Chromatog. & Rel. Technol. **2000**, *23*, 2327–2335.
5. Kwiatkowski, J. M.S. Thesis, University of Illinois at Urbana-Champaign (2001).

Received December 25, 2001
Accepted January 20, 2002
Manuscript 5718