J Am Oil Chem Soc (2007) 84:803–807
DOI 10.1007/s11746-007-1113-x

ORIGINAL PAPER

Separation and Quantification of Vegetable Oil Based Polyols by High Performance Liquid Chromatography with Evaporative Light Scattering Detection

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Received: 2 May 2007 / Revised: 10 July 2007 / Accepted: 12 July 2007 / Published online: 15 August 2007

Abstract Polyols with terminal primary alcohol functionalities were obtained from canola oil via an ozonolysis and hydrogenation process. A high performance liquid chromatography (HPLC) method with evaporative light scattering detection (ELSD) was developed for separating and quantifying the polyol products. Linear calibration curves were obtained for the mono-ol, diol and triol components with correlations (r^2) above 0.98. According to the standard curve, the content of mono-ol, diol and triol can be obtained from their HPLC-ELSD chromatograms.

Keywords Polyol · Canola oil · High performance liquid chromatography · Evaporative light scattering detection · Calibration curve

Introduction

Vegetable oil is an abundant renewable source that can be used for the manufacture of polyurethanes (PUs) so long as it comprises double bonds in its structure, which can be converted to alcohol functionalities to make polyols, the monomers for polyurethane production. There are many ways to synthesize polyols from various vegetable oils. Some research groups have sought to introduce alcohol functionality by hydroformylation of double bonds [1] followed by hydrogenation or epoxidation then ring opening [1, 2]. The above mentioned methodologies have produced TAG polyols with hydroxyl functionality situated in the middle of the fatty acid chains. For the synthesis of polyols with terminal hydroxyl groups from vegetable oils, the process of ozonolysis is generally adopted [3, 4]. In our research group, a process of ozonolysis followed by hydrogenation (also demonstrated to be feasible at an industrial scale) has been successfully utilized with canola oil to make polyols with terminal hydroxyl groups [5]. These polyols are capable of producing high-quality PU foams and elastomers [6, 7].

Gel permeation chromatography (GPC) and FTIR methods were used in Petrovic's work to characterize terminal hydroxyl polyols [3]; FTIR and ^{13}C-NMR were adopted in Tran's research for characterization of similar structures [4]. Neither of these analytical methods allowed for quantification of the components of the polyols. Currently, there is no literature published on the separation and quantification of different components in polyol products from vegetable oils. In this paper, we report a methodology for the separation and quantification of different components in polyols, including saturated triacylglycerols (TAGs), mono-ols, diols and a triol, utilizing high performance liquid chromatography (HPLC) and an evaporative light scattering detector (ELSD).

Evaporative light scattering detection for HPLC has been widely used for the separation and quantification of lipid classes [8–16] using stationary phases of silica [17], diol [18], cyanopropyl [19], or bonded polyvinyl alcohol [20]. The use of ELSD has some advantages in the area of lipid analysis: (1) it is not sensitive to the solvent flow rate and ambient temperature [21]; (2) it eliminates the need for lipid derivatization; and (3) it allows for the use of solvents that are usually not suitable for UV detection such as chloroform, which has a similar absorbance region to the lipids [22].

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Experimental

Materials

One hundred percent pure canola oil was supplied by Canbra Foods Limited, Lethbridge, AB, Canada. Raney nickel 2800 (slurry in water) catalyst was purchased from Sigma-Aldrich Co., USA. The ozone generator (Azcozon Model RMV16-16) was purchased from Azco Industries Ltd., Canada. The hydrogenation vessel (2 L) was purchased from Parr Instrument Co., USA. The Wiped-Blade Molecular Distillation apparatus (Model VKL 70/ICL–04) was purchased from Incon Processing, IL, USA.

Methods

Synthesis and Characterization of Polyols from Canola Oil

Canola oil (100 g) was dissolved in ethyl acetate (400 mL) and ozonized at room temperature for 1 h (or 30, 40, 50 and 60 min in different batches). An oxygen flow rate of 5 L/min was passed through the ozone generator allowing for an ozone concentration of 62 g/cm^3. The ozonolysis crude product was then hydrogenated for 3 h at 135 °C and 100 psi using Raney Ni (10% weight of ozonolysis crude product) as a catalyst. Ethyl acetate was removed by rotary evaporation and wiped blade molecular distillation was used to remove low molecular weight by-products to obtain polyols products.

HPLC System

The HPLC system consisted of a dual Milton Roy pump with a 20 μL auto-injector. A 250 × 4 mm Betasil Diol-100 (5 μm particle size) column was used (purchased from Thermo Hypersil-Keytone) and maintained at 50 °C with a Biorad column heater. The detector was an Alltech ELSD 2000 evaporative light scattering system maintained at 100 °C with a gain setting of 16 (on the 16 unit scale) and a nitrogen flow rate of 3 L min^{-1}. The mobile phase comprised of two solvents, *A* and *B*. *A* was 100% heptane and *B* was 50% heptane with 50% isopropyl alcohol (IPA). A run consisted of a linear gradient of 100% *A* to 70% *A* and 30% *B* over 30 min with a flow rate of 2 mL min^{-1}.

HPLC Standard Curves

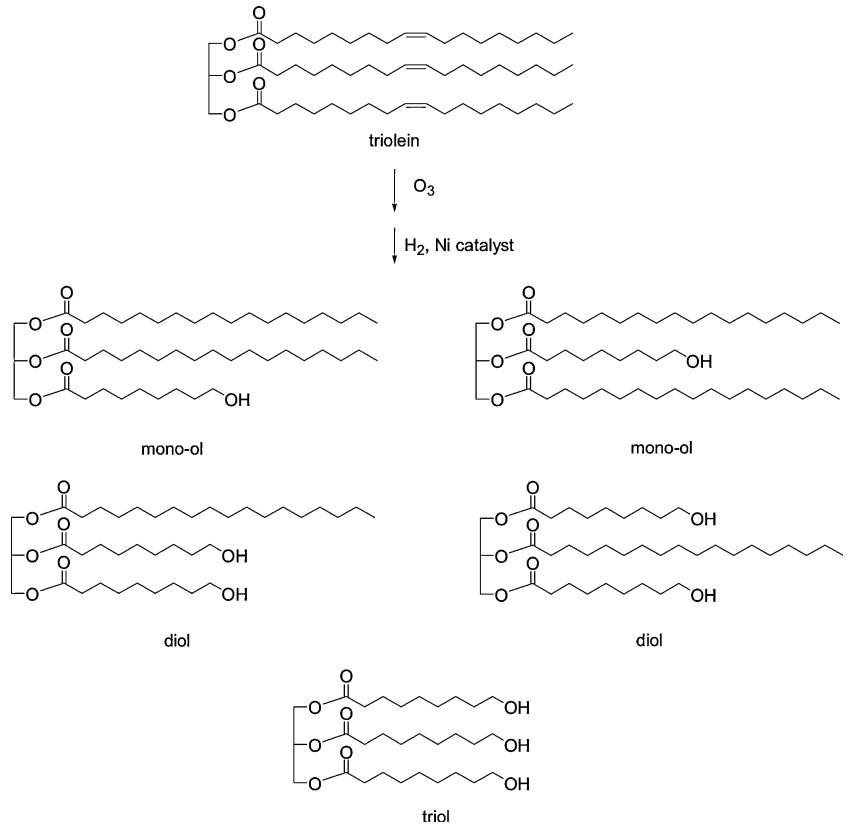
Pure mono-ol, diol and triol standards were obtained by isolating polyol products by flash silica chromatography. The purification process, chromatographic data and compound characterization are reported in detail in a previous publication [5]. Dichloromethane (DCM) solutions of known concentrations of each standard were injected separately. Each concentration of the standard solution was injected three times and the average peak area was plotted against the concentration to obtain the standard curve. Correlation (r^2) was determined for all curves by linear regression analysis.

Results and Discussion

Polyols were obtained by ozonolysis of canola oil in ethyl acetate followed by hydrogenation. Canola vegetable oil contains more than 90% of unsaturated fatty acids [23], including oleic acid, linoleic acid, and linolenic acid, all of which have a double bond at carbon nine that can be cleaved by reductive ozonolysis to provide terminal hydroxyl polyols. The polyol products obtained are a mixture of saturated TAGs along with mono-ols, diols and triols. The presence of saturated alkyl chains in the TAG products is due to two main reasons: (1) in addition to unsaturated fatty acids being present in canola oil, there are also small amounts of saturated fatty acids present (mainly palmitic and stearic acid); (2) ozone may not have reacted with all of the double bonds and hydrogenation of the unreacted double bonds produces TAGs containing saturated alkyl chains. Using triolein as an example, Scheme 1 illustrates the mechanism of polyol production from unsaturated TAGs and the structures of these products.

In our study, an HPLC-ELSD method was developed to separate and quantify the components in the polyol mixture. A diol-based column and gradient elution system provided good separation of saturated TAGs, mono-ols, diols and triols within a relatively short run time. Figure 1 shows a typical HPLC chromatogram of polyol products (after ozonolysis for 1 h) in canola oil. As four main peaks were observed by HPLC using the diol-based column, it was logical to assign them according to the polarities of those components in the polyols. Beginning with the first peak, which has the shortest retention time, the peaks were assigned to the saturated TAGs (the least polar components), followed by TAGs with a single alcohol group (mono-ols) then TAGs with two alcohol groups (diols) and finally TAGs with three alcohol groups (triol), the most polar component. The peak of mono-ols (seen clearly in the insert of Fig. 1) was very small due to the relatively small amount of mono-ol in the polyol product. A small peak was always observed at 24.5 min. This could be due to a transesterification product also containing three hydroxyl groups, which would have similar polarity to the main triol product. The transesterification mechanism is shown in Scheme 2. Due to the high temperatures used during

Scheme 1 Production of polyols from triolein



distillation to remove unwanted byproducts and also high pressures used in the hydrogenation steps, transesterification could have occurred between the triol and the alcohol by-product.

Purified mono-ol, diol and triol samples were analyzed individually on the same HPLC system and the results shown in Fig. 2. The retention times of the mono-ol, diol and triol components were 7.3, 14.9 and 25.3 min, respectively, which correlates to the previous HPLC chromatogram shown in Fig. 1. Expansion of the mono-ol and diol peaks of the HPLC revealed some peak splitting as shown in Fig. 2. This is due to different hydroxyl and alkyl group substitutions in the *sn*-1(3) or *sn*-2 positions of the mono-ols and diols i.e., mono-ol and diol isomers. The retention times and peak resolution for the mono-ol, diol and triol components were highly reproducible.

Calibration curves for the mono-ol, diol and triol are shown in Fig. 3. The peak area increased linearly with increasing standard concentration within a certain range: mono-ols, 193–650 μg mL^{-1} diols, 288–1370 μg mL^{-1}, and triols 268–671 μg mL^{-1}. When the standard concentration was below this range, the response deteriorated; above this range, the peaks reached the upper detection limit. The r^2 for a linear curve fitting for the mono-ol and diol was above 0.99, but the r^2 for the triol was 0.98. To quantify the polyol products from one reaction batch, the batch was either concentrated or diluted to obtain the concentration of each component within the range. Peak area of each component was obtained from the

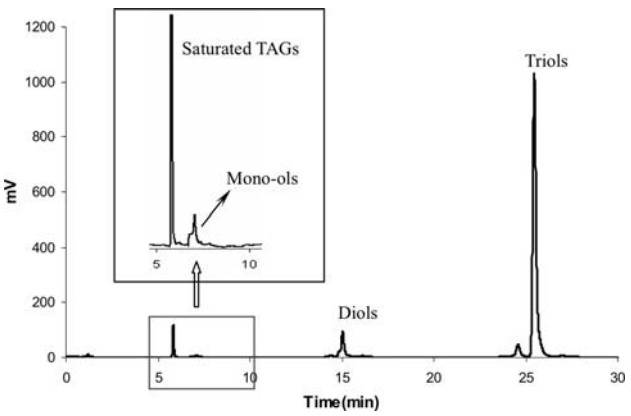
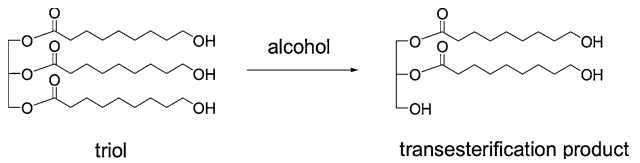


Fig. 1 HPLC of polyol products from canola oil



Scheme 2 Transesterification between triol and the other alcohol

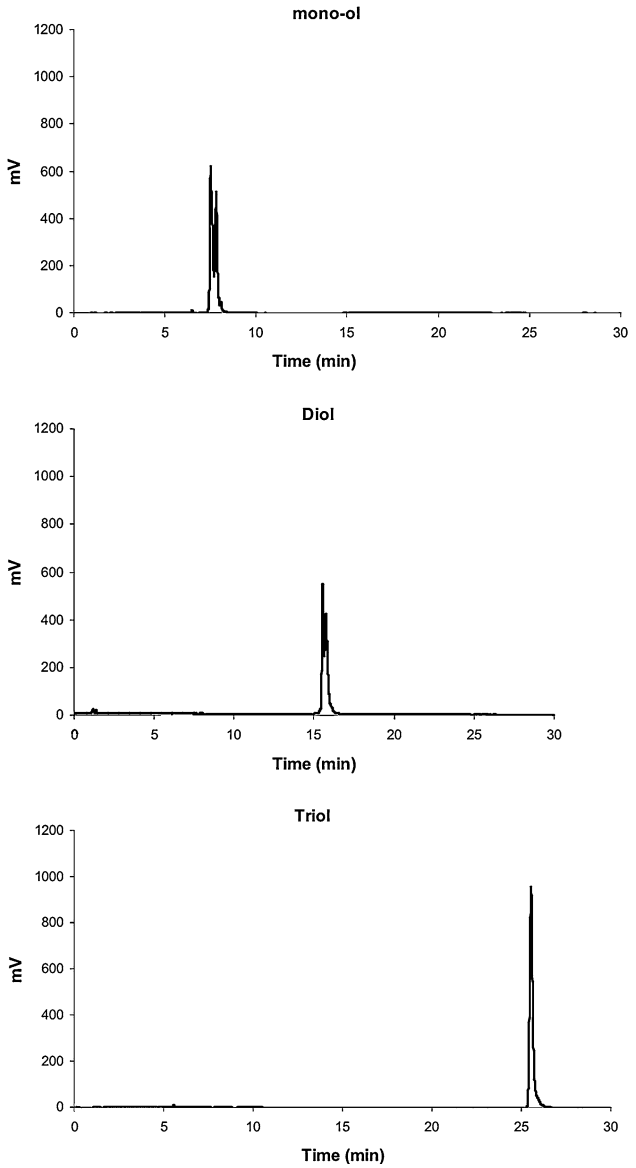


Fig. 2 HPLC of pure mono-ol, diol and triol

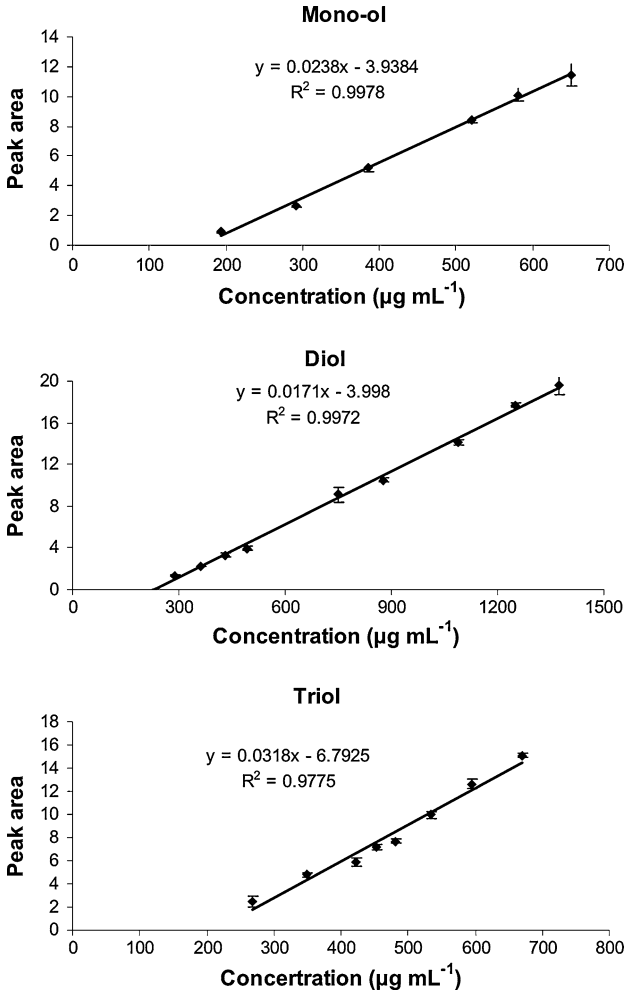


Fig. 3 Calibration curves for mono-ol, diol and triol products

chromatogram and according to the calibration curve equation, the concentration of each component was calculated. After comparison with the total sample concentration, the content of each component was obtained. Table 1 shows the content of mono-ol, diol and triol in the polyol products. It can be seen that with increasing ozonolysis time, the content of mono-ol decreased while that of triol increased. The reason is that over time more double bonds can react with ozone and following hydrogenation, more hydroxyl groups are obtained.

It was also found that the detector response was different for mono-ols, diols and triols therefore, in order to accurately quantify the components of the polyol mixture, individual calibration curves for each component was necessary. Unlike UV-detection, ELSD response is independent of the chain length and degree of unsaturation of the fatty acid chain [13, 21], therefore the calibration curves reported

Table 1 Content of mono-ol, diol and triol in the polyol products from varying ozonolysis time

Ozonolysis time (min)		30	40	50	60
Content (%)	Mono-ol	20.29 ± 1.05	14.11 ± 0.72	8.39 ± 0.04	4.72 ± 0.03
	Diol	45.78 ± 0.84	46.77 ± 1.30	34.52 ± 1.08	26.00 ± 0.48
	Triol	16.79 ± 0.23	30.17 ± 0.84	50.54 ± 0.32	60.18 ± 1.16

in this paper are also applicable to other polyols with similar terminal primary alcohol functionalities.

Acknowledgments We thank the Alberta Crop Industry Development Fund (ACIDF), the Alberta Agricultural Research Institute (AARI), AVAC Ltd., Natural Sciences and Engineering Research Council (NSERC), the Agriculture and Food Council (AFC), the Alberta Canola Producers Commission (ACPC), Bunge Corp. granting agencies for funding portions of this study. The authors acknowledge the technical contribution of Mr. Ereddad Kharraz and the editing efforts of Dr. Hayley Wan and Dr. Laziz Bouzidi.

References

1. Lyon CK, Garrett VH, Frankel EN (1974) Rigid urethane foams from hydroxymethylated castor oil, safflower oil, oleic safflower oil, and polyol esters of castor acids. J Am Oil Chem Soc 51:331–334
2. Hu YH, Gao Y, Wang DN, Hu CP, Zu S, Vanoverloop L, Randall D (2002) Rigid polyurethane foam prepared from a rape seed oil based polyol. J Appl Polym Sci 84:591–597
3. Petrovic ZS, Zhang W, Javni I (2005) Structure and properties of polyurethanes prepared from triglyceride polyols by ozonolysis. Biomacromolecules 6:713–719
4. Tran P, Graiver D, Narayan R (2005) Ozone-mediated polyol synthesis from soybean oil. J Am Oil Chem Soc 82:653–659
5. Narine SS, Yue J, Kong X (2007) Production of polyols from canola oil and their chemical identification and physical properties. J Am Oil Chem Soc 84:173–179
6. Narine SS, Kong X, Bouzidi L, Sporns P (2007) Physical properties of polyurethanes produced from polyols from seed oils: I. Elastomers. J Am Oil Chem Soc 84:55–63
7. Narine SS, Kong X, Bouzidi L, Sporns P (2007) Physical properties of polyurethanes produced from polyols from seed oils: II. Foams. J Am Oil Chem Soc 84:65–72
8. Beermann C, Green A, Mobius M, Schmitt JJ, Boehm G (2003) Lipid class separation by HPLC combined with GC FA analysis: comparison of seed lipid compositions from different *Brassica napus* L. varieties. J Am Oil Chem Soc 80:747–753
9. Stith BJ, Hall J, Ayres P, Waggoner L, Moore JD, Shaw WA (2000) Quantification of major classes of Xenopus phospholipids by high performance liquid chromatography with evaporative light scattering detection. J Lipid Res 41:1448–1454
10. Grizard G, Sion B, Bauchart D, Boucher D (2000) Separation and quantification of cholesterol and major phospholipid classes in human semen by high-performance liquid chromatography and light-scattering detection. J Chromatogr B 740:101–107
11. Neff WE, List GR, Byrdwell WC (1999) Quantitative composition of high palmitic and stearic acid soybean oil triacylglycerols by reversed phase high performance liquid chromatography: utilization of evaporative light scattering and flame ionization detectors. J Liq Chromatogr Relat Technol 22:1649–1662
12. Homan R, Anderson MK (1998) Rapid separation and quantitation of combined neutral and polar lipid classes by high-performance liquid chromatography and evaporative light-scattering mass detection. J Chromatogr B 708:21–26
13. Silversand C, Haux C (1997) Improved high-performance liquid chromatographic method for the separation and quantification of lipid classes: application to fish lipids. J Chromatogr B 703:7–14
14. Breton L, Serkiz B, Volland JP, Lepagnol J (1989) A new rapid method for phospholipid separation by high-performance liquid-chromatography with light-scattering detection. J Chromatogr B Biomed Appl 497:243–249
15. Christie WW (1986) Separation of lipid classes by high-performance liquid-chromatography with the mass detector. J Chromatogr 361:396–399
16. Christie WW (1985) Rapid separation and quantification of lipid classes by high-performance liquid-chromatography and mass (light-scattering) detection. J Lipid Res 26:507–512
17. Murphy EJ, Rosenberger TA, Horrocks LA (1996) Separation of neutral lipids by high-performance liquid chromatography: Quantification by ultraviolet, light scattering and fluorescence detection. J Chromatogr B Biomed Appl 685:9–14
18. Perona JS, Ruiz-Gutierrez V (2004) Quantification of major lipid classes in human triacylglycerol-rich lipoproteins by high-performance liquid chromatography with evaporative light-scattering detection. J Sep Sci 27:653–659
19. Elhamdy AH, Christie WW (1993) Separation of nonpolar lipids by high-performance liquid-chromatography on a cyanopropyl column. HRC J High Resolut Chromatogr 16:55–57
20. Christie WW, Urwin RA (1995) Separation of lipid classes from plant-tissues by high-performance liquid-chromatography on chemically bonded stationary phases. HRC J High Resolut Chromatogr 18:97–100
21. Christie WW (1992) Advances in lipid methodology-one. the Oily Press, Ayr
22. Becart J, Chevalier C, Biesse J (1990) Quantitative-analysis of phospholipids by HPLC with a light-scattering evaporating detector—application to raw-materials for cosmetic use. HRC J High Resolut Chromatogr 13:126–129
23. Firestone D (1999) Physical and chemical characteristics of oils, fats, and waxes. AOCS Press, Champaign