

Research Paper

Temperature-dependent solubility of wax compounds in ethanol

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The ability of ethanol to dissolve wax compounds, as an alternative to traditional lipid solvents, was investigated for the recovery of cuticular lipids from biomass. The solubilities of fatty esters with carbon chain lengths from 40 to 54 were measured in ethanol over a temperature range of 30–80°C. The greatest increase in solubility was observed between 40° and 60°C for the long chain waxes that are characteristic of flax cuticle lipids. The solubility of a 52-carbon wax increased by a factor of four over this temperature range. The Van't Hoff equation was used to estimate enthalpy of solution values. Ethanol was an effective lipid solvent at these modestly elevated temperatures and offers an economical method to recover lipid co-products from biomass prior to conversion to bioethanol.

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1 Introduction

The conversion of biomass or waste cellulosic materials to ethanol *via* chemical or biochemical technologies is an active area of research that promises to supplement the current supply of biofuels without consuming commodity crops such as corn and soybean. The large amount of lignocellulose waste generated from agricultural and forestry harvesting operations represents an alternative feedstock for conversion to ethanol by saccharification and fermentation processes. Additional cellulosic material will become available through the cultivation of energy crops such as switchgrass in the Central United States and bermudagrass in the Southern United States [1]. All of these biomass sources contain minor amounts of natural products that could be recovered as valuable co-products. It is known that the separation of some bioactive compounds from the cellulose fraction improves the conversion efficiency and yield of bioethanol. For example, the phenolic acid components of lignin show an inhibitory effect on enzyme activity

and suppress the microbial production of ethanol in the fermentation process [2].

The waxes that coat the outer layer of plant tissues, the cuticular lipids, represent one class of compounds of particular interest as co-products. These lipid compounds are concentrated on the surface of the plant cuticle and provide moisture control and serve as a protective barrier for the plant. If the waxes could be economically recovered from biomass prior to conversion it would provide an additional marketable co-product. Extraction with the traditional lipid solvents, *e.g.*, hexane, methylene chloride, and petroleum ether, pose significant risks to human and environmental health. The use of compressed gases such as carbon dioxide or propane can be effective to extract lipid compounds but require high-pressure vessels and specialized equipment. In contrast to the traditional nonpolar lipid solvents are such polar solvents as ethanol that are not usually considered effective for lipids. However, ethanol at 50°C was shown to be an effective solvent for the extraction of corn oil [3, 4]. Additionally, the composition of the ethanol extracts contained higher levels of beneficial phytochemical compounds than hexane extracts, *e.g.*, tocopherols and phytosterols [5]. Ethanol has been used advantageously as a co-solvent to modify the polarity of pressurized carbon dioxide or water for the extraction of natural products [6, 7]. More recently the application of hot ethanol to recover waxes from flax fiber processing waste containing cuticle tissue was also demonstrated [8]. These results motivated the current investigation of the temperature-dependent solubility

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Abbreviations: C_s , solution concentration (mol/kg); dT , temperature differential; ΔH , solution enthalpy (J/mol); \ln , natural logarithm; R , gas constant (J/mol K); T , temperature (K)

of fatty esters in ethanol and the possibility of use in biomass-to-ethanol conversion.

The use of hot ethanol for the extraction of lipids from biomass and its integration with a biomass-to-ethanol conversion process offers technical benefits. The availability of ethanol that is produced at these facilities provides a local source of solvent and the appropriate process equipment. The temperature-dependent solubility of lipids in ethanol allows for a simple separation scheme to fractionate the lipids and recycle the ethanol. The defatted biomass would be more readily wetted by aqueous media in downstream operations after removal of lipid components due to surface effects.

This study examined the temperature-dependent solubility of long-chain waxes as model compounds found in biomass, and reports on the application of the Van't Hoff equation to interpret the solubility of the waxes in ethanol. The application of the temperature-dependent solubility of these compounds in ethanol represents an effective separation scheme to recover lipids from biomass prior to conversion to bioethanol.

2 Materials and methods

2.1 Solubility study

The solubility of long-chain waxes in ethanol was determined as a function of temperature by placing 50 mg of a standard wax compound into a 2-mL glass vial with a 5 mm \times 2 mm magnetic stir bar and filling the vial with anhydrous reagent grade ethanol (Aaper, Shelbyville, KY). Wax standards obtained from Nu-Chek Prep (Elysian, MN, USA) included stearyl behenate and behenyl stearate. Higher molecular weight wax compounds previously isolated and purified from flax included triacontanyl hexadecanate, dotriacontanyl hexadecanate, dotriacontanyl octadecanate, tetratriacontanyl octadecanate, and hexatriacontanyl octadecanate [9]. The glass vial was fitted with a septum through which a syringe could draw a 20- μ L sample. The vial was placed in a dry block heater (Pierce Co., Rockford, IL, USA) and heated to 80°C with slow stirring. The temperature of the block was monitored by a thermometer placed into a vial of glycerin located in the block. The temperature was allowed to equilibrate for 30 min before a sample was taken. The temperature was reduced in 5°C decrements to 30°C with samples taken at each temperature level. Each sample was immediately transferred to 0.2 mL spectroscopic grade cyclohexane (Sigma-Aldrich, St. Louis, MO, USA) placed into a 2-mL glass vial containing a low-volume insert and analyzed by gas chromatography (GC). Experiments were performed in triplicate.

2.2 Analysis

GC analysis of fatty compounds was performed on the Agilent 6890 gas chromatograph fitted with a DB5-HT column

(15 m \times 0.250 mm \times 0.1 μ m). Helium carrier gas was used at a total flow rate of 10 mL/min with an inlet temperature of 280°C and a detector temperature of 400°C. Injections were made in splitless mode with 1- μ L volumes. The oven temperature program started at 120°C for 1 min, increased to 380°C at 10°C/min, and was held at 380°C for 5 min. Calibration curves were prepared from standard solutions at concentrations of 0.01, 0.05, 0.1, 1.0, and 2.0 mg/mL of the wax compounds in cyclohexane. All analyses were performed in duplicate.

3 Results and discussion

The temperature-dependent solubility of the long-chain wax compounds associated with plant cuticle tissue was investigated in a series of experiments using hot ethanol. While ethanol is a relatively polar solvent, it can solubilize lipids at elevated temperatures and may provide an alternative to conventional lipid solvents. Solutions of the individual wax compounds were incubated for 30 min at each temperature in hot anhydrous ethanol. Samples were removed by syringe and analyzed by GC to determine solubility as a function of temperature. The saturated condition was evident by the presence of insoluble material that increased with decreasing temperature over the course of the experiment. Results obtained with wax compounds of 46- to 54-carbon chain length are presented in Tab. 1. Average values ($n=3$) for the solubilities of each wax are reported with the associated standard error (SE). These wax compounds comprised a saturated fatty alcohol of between 30- and 36-carbon chain length esterified to either palmitic (C16:0) or stearic acids (C18:0). The longer chain length waxes, *e.g.*, 50, 52, and 54 carbons, exhibited a large increase in solubility between 40° and 60°C. For example, the solubility of the 52-carbon wax increased from 0.049 mg/mL at 40°C to 0.191 mg/mL at 60°C. In contrast, the shorter chain-length waxes of 46 and 48 carbons did not show a significant change in solubility over this temperature range. The role of structure on solubility was explored with a pair of esters that both have a total chain length of 40 carbons but differ in the respective alcohol and acid components, *e.g.*, stearyl behenate and behenyl stearate. Solubilities were determined for this pair of esters over the same temperature range. ANOVA was used to test for significant differences between the solubility values of the two esters. Both esters showed the trend of increasing solubility in ethanol with increasing temperature; however, results of the F test indicated that no significant differences existed between the solubility of stearyl behenate and behenyl stearate in ethanol at the temperatures tested (for $\alpha = 0.05$).

The Van't Hoff equation (Eq. 1) was applied to the temperature-solubility data to estimate the enthalpy of solution for these waxes. Plots of the natural log of the concentration of the ester, C_s (mol/kg), *versus* the reciprocal of the absolute temperature, $1/T$ (K) were made. The slope of such a plot is

Table 1. Solubility of wax esters in ethanol with increasing temperatures and carbon chain length.

Carbon chain length	Solubility in mg/mL (SE) ^{a)}				
	40°C	45°C	50°C	55°C	60°C
46	0.039 (0.003)	0.041 (0.002)	0.041 (0.004)	0.041 (0.001)	0.042 (0.002)
48	0.045 (0.002)	0.047 (0.002)	0.051 (0.004)	0.052 (0.003)	0.051 (0.002)
50	0.048 (0.001)	0.059 (0.003)	0.076 (0.002)	0.105 (0.004)	0.11 (0.005)
52	0.049 (0.003)	0.055 (0.004)	0.083 (0.006)	0.162 (0.003)	0.191 (0.013)
54	0.043 (0.002)	0.048 (0.003)	0.055 (0.003)	0.093 (0.006)	0.145 (0.010)

a) SE, standard error.

proportional to the enthalpy of solution, ΔH (J/mol). Values of the slope were obtained from the regression equations and are presented in Tab. 2 for the longer chain waxes of 50, 52, and 54 carbons that displayed temperature-dependent behavior. The shorter chain wax esters were not included in these calculations since they did not exhibit a strong change of solubility with temperature. The application of the Van't Hoff equation assumes that the enthalpy is not a function of temperature. Eq. (1) is obtained from the integration of Eq. (2) with ΔH treated as a constant. This assumption is reasonable over a small temperature interval but would not generally be true.

$$\ln(C_s) = -\frac{\Delta H}{RT} \quad (1)$$

$$\int d \ln(C_s) = \int \frac{\Delta H dT}{RT^2} \quad (2)$$

The enthalpy values obtained from the slopes are reported with the associated standard error (SE) and correlation coefficient (r^2) values. The non-linearity in the raw data was attributed to the temperature dependence of the enthalpy term. This is reflected in the values of the correlation coefficients. As noted above, if the temperature dependence of the enthalpy were known, then the appropriate expression could be substituted into Eq. (2) and formally integrated. Alternative approaches to estimate the temperature dependence of solubility phenomena include the use of equations of state combined with partial or excess molar properties [10, 11]. These methods are powerful and require complete sets of experimental data for development and validation. Simpler techniques that describe solubility in terms of partition coefficients show promise for mixtures, although temperature variations are not fully explained [12].

Table 2. Heats of solubility calculated for wax esters (from linear portion of Van't Hoff plot).

Carbon chain	ΔH [KJ/mol]	SE	r^2
50	−38.684	1.887	0.972503
52	−67.839	3.991	0.960594
54	−54.936	5.305	0.900422

The observed temperature-dependent solubility of these waxes in ethanol is important for two reasons. The increase in solubility at elevated temperature permits more lipid material to be removed from the biomass substrate and transported away from the solids with the bulk solution. The decrease in solubility that occurs at reduced temperatures allows the lipid compounds to be separated from the solvent in a subsequent product recovery step. By controlling the temperature reduction in this step a fractionation of the extracted lipids would be achieved allowing separation by chain length with recycle of the ethanol [6].

4 Conclusion

The solubility of wax compounds in ethanol was shown to increase with increasing temperature over the range of 40–60°C. This behavior was proposed as the basis for an economical separation method to recover lipid compounds from biomass used as feedstock for biomass-to-ethanol conversion processes. Such lipid extracts represent an additional co-product that can improve the overall process economics. Additionally, the crude extract can be fractionated by decreasing the temperature to precipitate the waxes and thereby achieve product separation by carbon chain length. Implementation of this method with bioethanol production provides the benefits of an integrated process.

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Conflict of interest statement

The author has declared no conflict of interest.

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