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Continuous Hydrolysis of Cuphea Seed Oil in Subcritical Water

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Abstract Cuphea seed oil (CSO) is a source of decanoic acid which is useful in the preparation of estolide lubricants among other applications. Decanoic acid and other free fatty acids (FFA) can be hydrolyzed from CSO using a catalyst like KOH, followed by neutralization with HCl and extraction with hexane. This procedure, however, uses caustic materials, hazardous solvents and generates waste salt streams. This study investigated the use of water without catalysts to hydrolyze CSO in a continuous flow tubular reactor. Parameters such as the interaction of pressure and temperature, temperature, water to cuphea oil fatty acid residue (H_2O:COFAR) molar ratio, and flow rate were examined. The lowest conversions of CSO to FFA were at the lowest temperature (i.e., 300 °C) and the hydrolysis was ca. 90% at 350 °C and 13.8 MPa and ca. 80% at 365 °C and 13.8 MPa. Hydrolysis increased with pressure and leveled off at 13.8 MPa. Hydrolysis increased with temperature and leveled off at ca. 330 °C. The optimal H_2O:COFAR molar ratio was found to be 6:1. Conversion rates were inversely proportional to flow rate with 95% conversion at the lowest flow rate (i.e., 0.25 mL/min) corresponding to the longest residence time (i.e., ca. 45.2 min). These results demonstrate a continuous subcritical water process for hydrolyzing CSO to FFA that is effective, requires no catalysts and does not generate a waste salt stream.

Keywords Cuphea seed oil · Flow reactor · Free fatty acid · Hydrolysis · Subcritical water

Introduction

Cuphea seed oil (CSO) has been investigated as a source of medium chain fatty acids (i.e., C8–C14) for use in chemical manufacturing, including detergents, shampoos, lubricants and wood preservatives [1–5]. Decanoic acid (C10) is one of the most abundant fatty acids (FA) in CSO (i.e., ca. 66–82%) [6, 7] and it has found use as a wood preservative [8], termiticide [9], in dental compositions [10], fabric softeners [11], and cosmetics [12, 13]. However, one of the most promising uses for decanoic acid is as estolide biobased lubricants [6].

Saturated/capped estolides which use saturated FAs to terminate the oligomerization process have been developed which have physical properties that could help eliminate common problems associated with the use of vegetable oils as functional fluids [14–16]. By varying the capping material (i.e., the terminal FA) on the estolide oligomer, the crystal lattice structure of the material can be disrupted as it approaches its pour point, leading to estolides with excellent low-temperature properties, pour points of −36 °C, and cloud points of −41 °C without an additive package [17]. Estolides utilizing C-10 decanoic acid as the terminal FA have especially useful properties as lubricants

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name USDA implies no approval of the product to the exclusion of others that may also be suitable.

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based on the physical properties exhibited by these C-10 materials [6].

In order to synthesize these saturated estolides, the crude CSO must first be refined, bleached, deodorized (RBD) and then hydrolyzed to form the saturated free fatty acids (FFA). The hydrolysis of CSO has been accomplished using catalysts such as 2.0 M KOH and subsequently neutralized with 1-M HCl and extracted with hexane [6]. Although this method is effective, it uses caustic materials, hazardous solvents and leads to waste salt streams upon neutralization of the reaction mixture.

Holliday et al. [18] demonstrated that hydrolysis of vegetable oils can be rapidly achieved in a closed reactor (i.e., batch) without the need for a catalyst. They describe the use of subcritical water (i.e., below its critical temperature of 374 °C) at high densities (i.e., ca. 0.7 g/mL) as an effective means of hydrolyzing soybean oil, linseed oil, and coconut oils to FFA using hydrolytic reaction conditions ranging in temperatures of 260–280 °C. The hydrolysis occurs rapidly, usually within 15–20 min, yielding 97% or better conversion. Although both Holliday et al. [18] and King et al. [19] observed some geometric isomerization from the natural all *cis* form to *trans* isomers of the linolenic acids at reaction temperatures as low as 250 °C, CSO contains ca. 94% saturated fatty acids and very little unsaturated fatty acids [20], so the potential for isomerization of *cis* to *trans* is not a problem for CSO as it was for the critical water hydrolysis of soybean oil.

King et al. [19] extended their hydrolysis concept using a flow reaction system similar to that used in conventional fat splitting. The hydrolysis was conducted in an open tube without internal mixing elements or catalytic agents, at volumetric proportions where the water content in the reactor exceeded that of the vegetable oil. Optimal results were obtained at water to oil ratios between 2.5:1 and 5:1 (v/v). Lower water/oil ratios produced incomplete hydrolysis, while higher ratios produced no improved conversion. The hydrolysis of soybean oil (97% or better conversion) occurred readily between 10 and 15 min of residence time at 330–340 °C in the flow system. Under the above conditions, reaction times <10 min resulted in decreased conversion of vegetable oil to FFA. Yoshida et al. [21] used subcritical water hydrolysis (300 °C, 8.4 MPa for 5 min) in a sealed rector to obtain fatty acids from fish meat. Krammer and Vogel [22] discuss the subcritical water hydrolysis of esters as well as the underlying reaction mechanisms. Tavakoli and Yoshida [23] reported a maximum hydrolysis of squid waste triglycerides at 240 °C and 40 min. Minami and Saka [24] describe the use of subcritical water hydrolysis (320 °C, 20 MPa for ca. 10 min) of rapeseed oil prior to methyl esterification for biodiesel production.

The objective of this study was to apply critical fluid technology to the subcritical water hydrolysis of cuphea seed oil to produce FFA for subsequent use in the synthesis of products such as estolides. We investigated a very wide range of experimental conditions to determine the general conditions required to effectively hydrolyze CSO. Some experiments included treatments with very low densities of water (i.e., steam) and potentially low rates of hydrolysis to determine the optimal conditions for the hydrolysis of CSO. Parameters such as interaction of pressure and temperature, temperature, water to cuphea oil molar ratio, and flow rate were examined to determine the optimal operating conditions.

Materials and Methods

Cuphea Seed Oil

Oil from the cuphea germplasm line PSR 23 (*Cuphea viscosissima* × *C. lanceolata*) was obtained from cuphea seeds harvested from USDA plots in Morris, MN, USA and Peoria, IL, USA. Clean, whole cuphea seeds (9.7% moisture content) were milled using a pair of smooth rolls (model SP900-12 Roller Mill, Roskamp Champion, Waterloo, IA, USA) with the gap between the rollers set to 0.25 mm. The milled seeds were immediately loaded into a preheated seed cooker (Laboratory Seed Cooker/Conditioner model 324, French Oil Mill Machinery Co., Piqua, OH, USA). The seed temperature was monitored to not exceed 93 °C during cooking. Cooking time was 75 min, and the final moisture content of the cooked milled seed was 5.9% (dry based). The cooked milled seeds were screw pressed using a heavy duty laboratory screw press (model L 250, French Oil Mill Machinery Co., Piqua, OH, USA). The filtered crude oil was acid degummed to remove the phosphatides. Free fatty acids were removed by neutralizing the degummed oil with 4.15 N (20°Be′) NaOH, and the resulting soapstock was separated by centrifugation. The refined oil was bleached using 4.5% bleaching clay (Tonsill 167 FF, Sud-Chemie Adsorbents, Inc., Meigs, GA, USA) and 2% activated carbon (Darco KB, Norit Americas, Inc., Marshall, TX, USA). The bleached oil was deodorized for 2 h at 260 °C with sparge steam at 1–2% w/w per hour to yield refined bleached deodorized (RBD) CSO. Gardner color measurements were made using a Lovibond 3-Field Comparator from Tintometer Ltd Salisbury, England) using AOCS Method Td 1a-64 [25].

Subcritical Water Apparatus

The hydrolysis apparatus allowed oil and water to be pressurized, mixed, heated, cooled, and depressurized.

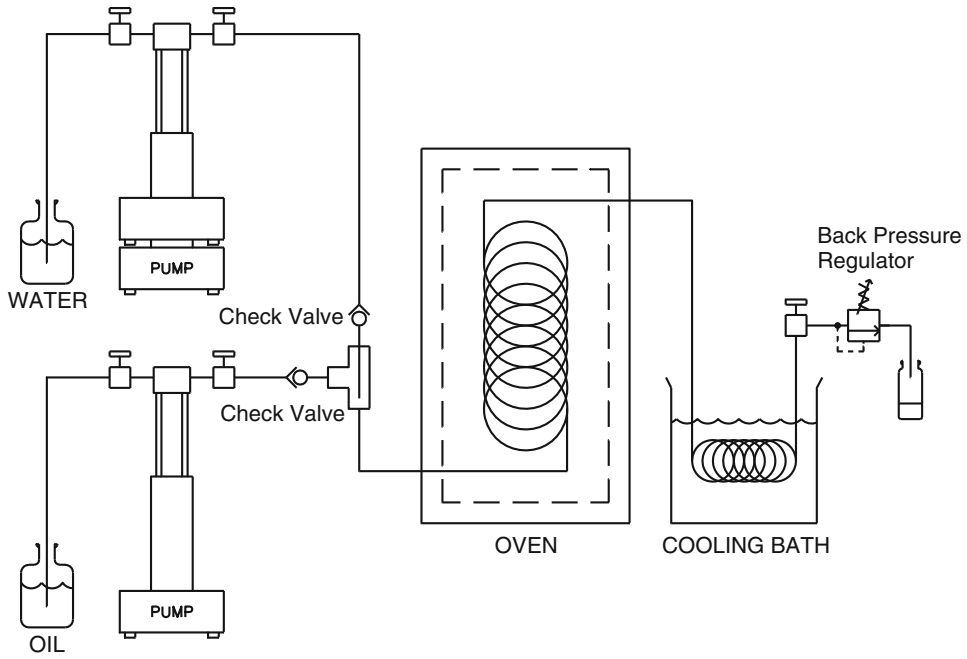

Fig. 1 Schematic of subcritical hydrolysis apparatus

A schematic drawing of the apparatus is shown in Fig. 1. Two syringe pumps (Models 100DX, ISCO, Inc., Lincoln, NE, USA) provided co-current feed flows of water and oil through 3.18 mm OD stainless steel tubing at the desired flow rates and ratios. Check valves (Part Numbers SWO2200, Autoclave Engineers, Inc., Erie, PA, USA) were used in each line to prevent back-flows. A lab-built mixer was fashioned using stainless steel reducing unions and a tee tube fitting (Swagelok Company, Solon, OH, USA) such that water sprayed through a 1.59 mm OD tube into the oil as it flowed by (Fig. 1). This mixture then entered the oven coil, which was 617 cm of 3.18 mm OD, 1.52 mm ID, Hastelloy tubing (internal volume 11.3 mL). The temperature of the oven (Series 3710A, Applied Test Systems, Inc., Butler, PA, USA) was controlled with a lab-built temperature control box. The hydrolyzed oil and water mixture was cooled as it exited the oven through a coil of stainless steel tubing submerged in a container of water. Pressure was maintained throughout the system using an adjustable back pressure regulator (Model Number 26-1722-44, Tescom Corporation, Elk River, MN, USA). System pressure was monitored using a pressure module (Model 700P31, Fluke Corporation, Everett, WA, USA). The depressurized hydrolyzed oil and water mixture was collected in glass vials at the output port of the back pressure regulator at a flow rate that was the sum of the two feed pumps settings.

General Operation Procedure

Start-up of the system included turning on the oven and setting the back pressure regulator. Water flow at 2 mL/min was commenced until the oven temperature and system pressure reached their set points. Water flow was then stopped briefly so that the water and oil flows called for in the experiment could be set and commenced. Since the hydrolysis reactor coil with a volume of 11.3 mL was filled with just water at this point, the first 20 mL collected at the back pressure regulator was discarded. After that, two samples per experimental treatment were collected at ca. 5 min intervals and each treatment was replicated two to four times. Shutdown involved turning off the oven, stopping the oil flow, and flushing the system with water at 13.8 MPa and a flow rate of 2 mL/m for 25 min as the oven cooled.

Interaction of Pressure and Temperature

The interaction of pressure and temperature on the hydrolysis of cuphea oil was studied at all combinations of three temperatures (i.e., 300, 350 and 365 °C) with 11 pressures (i.e., 0.4, 0.9, 1.7, 3.4, 6.9, 10.3, 13.8, 17.2, 20.7, 24.1, 27.6 MPa). The total flow rate was 2 mL/min and the water to cuphea oil fatty acid residue (H_2O:COFAR) molar ratio was 3.3:1. Each triglyceride molecule was considered to have three fatty acid residues. The entire experiment (i.e., all pressure temperature combinations) was replicated twice with two samples taken per replication.

Effect of Temperature

To better refine the optimal temperature for hydrolysis of the CSO, the effect of temperature was tested at seven different temperatures (i.e., 300, 310, 320, 330, 340, 350,

and 360 °C). All tests were at 13.8 MPa with a total flow rate of 2 mL/min and a H_2O:COFAR molar ratio of 3.3:1. There were four replications performed of each temperature and two samples drawn at each replication.

Effect of Water:Cuphea Oil Fatty Acid Molar Ratio

The effect of water to cuphea oil fatty acid residue (H_2O:COFAR) molar ratio was tested at 350 °C, 13.8 MPa and a total flow rate of 2 mL/min. The H_2O:COFAR molar ratios tested included: 1:1, 2:1, 3:1, 4:1, 6:1, 8:1, 12:1, 16:1, 24:1, 32:1, 48:1, 64:1 and 96:1. There were four replications of each ratio and two samples drawn at each replication.

Effect of Flow Rate

The effect of total flow rate (i.e., oil plus water) was studied and covered a 16-fold range. The total flow rates tested were 0.25, 0.5, 1.0, 2.0 and 4.0 mL/min with corresponding residence times of 45.2, 22.6, 11.3, 5.7 and 2.8 min, respectively. All tests were at 350 °C, 13.8 MPa and a H_2O:COFAR molar ratio of 6:1. There were two replications performed of each flow rate and two samples drawn at each replication.

Chemical Standards

Samples of FFA (i.e., decanoic, dodecanoic, tetradecanoic, hexadecanoic and octadecanoic), Monoacylglycerides (i.e., monocaprin, monolaurin, monomyristin, monopalmitin, and monostearin), Diacylglycerides (i.e., dicaprin, dilaurin, dimyristin, dipalmitin, and distearin) were purchased from Nu-Chek Prep, Elsian, MN, USA. These standards as well as the CSO sample were used to identify the peaks in the treatment samples.

Compositional Analyses

The compositions of the product mixtures were determined by off-line supercritical fluid chromatography (SFC). The oil portion of each sample vial was prepared for analysis by first separating the water from the oil. A small amount (ca. 0.5 grams) of sodium sulfate was added to the vial, which was then heated to 60 °C. The warm vial was centrifuged (IEC Clinical Centrifuge, International Equipment Company, Needham, MA, USA) at 2,550 rpm for 10 min. Two drops of the separated oil were added to a 2-mL glass vial, followed by the addition of 6 drops of diethyl ether and 1 mL of hexane.

The SFC analyses were conducted with a Series 4000 SFC (Selerity Technologies, Inc., Salt Lake City, UT, USA) equipped with a flame ionization detector (FID) held at 350 °C. SFC/supercritical fluid extraction-grade carbon dioxide (Airgas Inc., Radnor, PA, USA) was used as the carrier fluid. A SB-Methyl-100 capillary column (10 m by 50 μm i.d., 0.25 μm film thickness) (Selerity Technologies, Inc., Salt Lake City, UT, USA) was used with a program of 100 °C isothermal, 10.1 MPa hold for 5 min, and a ramp of 1.5 MPa/min to 31.4 MPa. A solution containing ca. 5 mg/mL was injected into the SFC (500 nL loop) and the relative amounts of free fatty acids (FFA), monoacylglycerides (MAG), diacylglycerides (DAG) and triacylglycerides (TAG) were determined from the FID area percentages. A single SFC analysis was performed on each sample.

Statistical Analyses

Analyses of variance (ANOVA) were conducted on composition (i.e., FFA, MAG, DAG, and TAG) percentage data using Statistix 7 software (Analytical Software, Tallahassee, FL, USA). Main effects were tested using *F* tests and means were compared using Bonferroni's comparison of means.

For the interaction of pressure and temperature data, mathematical equations that described the data were determined using TableCurveTM 2D curve-fitting software (Systat Software, Inc., Richmond, CA, USA). Weighted regression sigmoidal curves were obtained for each of the three temperatures (i.e., 300, 350, and 365 °C) of FFA percentage as a function of pressure. Standard weights of 1/variance were used for weighting mean values of FFA percentage for replicate pressure values. Confidence interval bands at the 99% level were calculated and FFA percentages of interest were compared using non-overlapping of the confidence intervals to denote significant differences.

Results and Discussion

Interaction of Pressure and Temperature

The effects of the interaction of pressure and temperature on the hydrolysis of cuphea oil are shown in Fig. 2. The ANOVA indicated that there were highly significant main effects of both pressure ($F_{10,33} = 3{,}715$, $P < 0.0001$) and temperature ($F_{2,33} = 1{,}098$, $P < 0.0001$) as well as the pressure by temperature interaction ($F_{20,33} = 58.1$, $P < 0.0001$) on the conversion of CSO to FFA. The mean square error was 2.0 with 33 degrees of freedom (*df*). The equation models for all three temperatures were highly significant ($P < 0.000001$) and all regression parameters for all equations were significant at $P < 0.05$ except for the intercept value of 0.486 for the 300 °C temperature

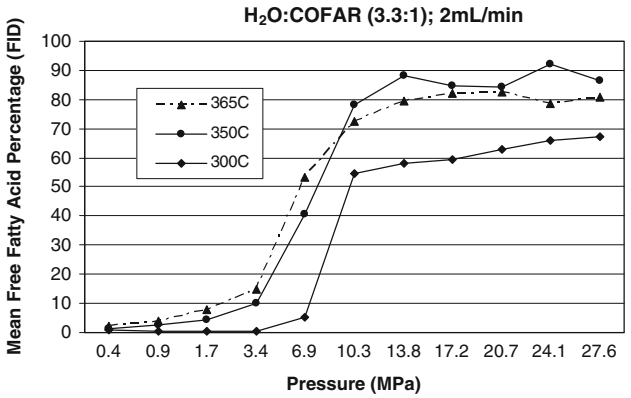

Fig. 2 Interaction of pressure and temperature on subcritical hydrolysis of cuphea seed oil

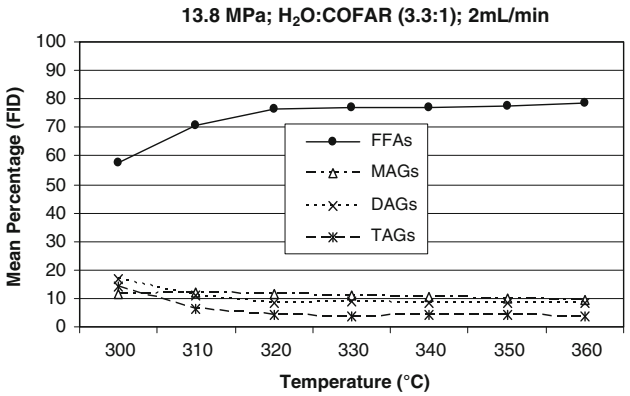

Fig. 3 Effect of temperature on subcritical hydrolysis of cuphea seed oil

equation, which was not significantly different from zero. The equations where Y = FFA percentage and X = pressure in MPa for 300, 350 and 365 °C, are: $Y = 0.486 + 65.12/(1 + \exp(-(X - 9.032)/0.835))$ $R^2 = 0.999$; $Y = 87.948/(1 + \exp(-(X - 7.111)/1.77))$ $R^2 = 0.9998$; and $Y = -4.384 + 86.239/(1 + \exp(-(X - 5.732)/2.193))$ $R^2 = 0.9998$, respectively.

As expected, the lowest conversions were at the lowest temperature tested (i.e., 300 °C) and at this temperature, hydrolysis never exceeded 70%. In addition, hydrolysis increased with pressure and this was especially evident between the pressures of 3.4 and 10.3 MPa. At 13.8 MPa, the hydrolysis of CSO was significantly higher at 350 °C (i.e., ca. 88%) than at 365 °C (i.e., ca. 80%). However, the hydrolysis of CSO did not increase significantly with pressures higher than 13.8 MPa.

Interestingly, even under conditions where the density of the water was very low (i.e., 350 °C, 13.8 MPa, $d = 0.07$ g/mL), the hydrolysis was very high (i.e., ca. 88%) compared to conditions with a much higher density of water (i.e., 300 °C, 13.8 MPa, $d = 0.72$) where the hydrolysis was only ca. 58%. Because the water and oil were mixed outside the oven and then entered the oven together, the temperature of the mixture increased as it moved through the reaction coil. This transition in temperature would cause a change in the density of the water and possibly its phase as well. It is possible that the hydrolysis occurred at some point during this transition.

Effect of Temperature

The effect of temperature when the pressure was 13.8 MPa is shown in Fig. 3. The ANOVAs indicated that there were highly significant main effects of temperature on the percentage of all components analyzed including FFA ($F_{6,18} = 427.4$, $P < 0.0001$); MAG ($F_{6,18} = 18.0$, $P < 0.0001$); DAG ($F_{6,18} = 152.5$, $P < 0.0001$); and TAG ($F_{6,18} = 158.8$, $P < 0.0001$). The mean square error was 0.6 (18 *df*). The conversion of the cuphea seed oil triglyceride to the hydrolyzed free fatty acids increased with temperature and leveled off at about 330 °C. At 300 and 310 °C, the percentage FFA was only ca. 60 and 70%, respectively. At temperatures higher than 330 °C there was no further increase in FFA conversion.

Effect of Water:Cuphea Oil Fatty Acid Molar Ratio

The results of the water to cuphea oil fatty acid residue molar ratio experiments are shown in Fig. 4. The ANOVA indicated that the H_2O:COFAR molar ratio had a highly significant effect on the percentage of all components analyzed including FFA ($F_{12,36} = 85.1$, $P < 0.0001$); MAG ($F_{12,36} = 82.0$, $P < 0.0001$); DAG ($F_{12,36} = 47.5$, $P < 0.0001$); and TAG ($F_{12,36} = 527.3$, $P < 0.0001$). The mean square error was 4.8 (36 *df*). It was expected that low H_2O:COFAR molar ratios would result in incomplete hydrolysis and that conversion would increase with H_2O:COFAR molar ratios until at some level the hydrolysis

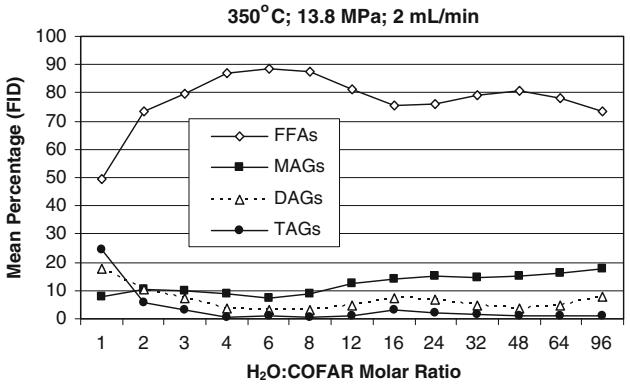

Fig. 4 Effect of water to cuphea oil fatty acid residue molar ratio on subcritical hydrolysis of cuphea seed oil

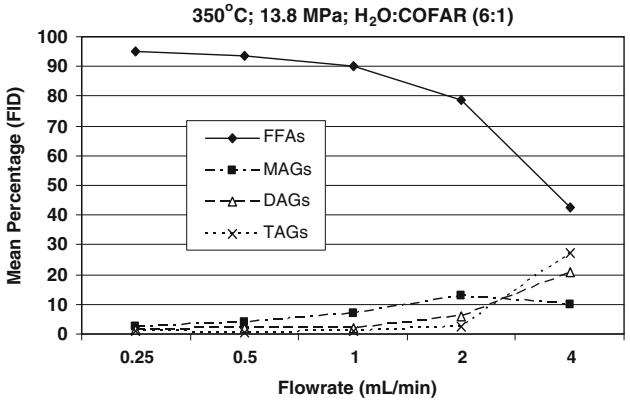

Fig. 5 Effect of total flow rate on subcritical hydrolysis of cuphea seed oil

would not increase any further with higher ratios as reported by King et al. [19]. This, however, was not the case at least for CSO. At the 1:1 molar ratio, conversion was only ca. 50% and it increased with the molar ratio up to ca. 90% at the 6:1 molar ratio. At higher molar ratios (i.e., 8:1 and higher) however, the conversion dropped and was only between 70 and 80% for molar ratios of 16:1 or higher. At these higher molar ratios, the lower conversion to FFA is accompanied by an increase in MAG. These MAG may be acting as a surfactant and forming micelles around the CSO and inhibiting the complete hydrolysis of the CSO within the micelle.

Effect of Flow Rate

The effect of total flow rate through the reaction chamber is shown in Fig. 5. The ANOVA indicated that the flow rate had a highly significant effect on the percentage of all components analyzed including FFA ($F_{4,4} = 551.7, P < 0.0001$); MAG ($F_{4,4} = 71.6$, $P = 0.0006$); DAG ($F_{4,4} = 2{,}615.7$, $P < 0.0001$); and TAG ($F_{4,4} = 216.3$, $P = 0.0001$). The mean square error was 1.7 (4 *df*). Conversions of 90–95% FFA were observed at the lowest flow rates (i.e., 0.5 and 0.25 mL/min, respectively) and longest residence times (i.e., 22.6 and 45.2 min, respectively). There was a decrease in the conversion of the CSO to FFA as the flow rate increased. This decrease was especially sharp between flow rates of 2 and 4 mL/min (i.e., residence times of 5.7 and 2.8 min, respectively) where only ca. 80 and 40% conversions were achieved, respectively. This is probably a result of the fast flow rates not allowing sufficient residence time in the reactor for the hydrolysis to occur. King et al. [19] reported similar findings.

Conclusions

Our results demonstrated that CSO can be effectively hydrolyzed in a continuous flow subcritical water reactor to give yields of ca. 90% conversion to FFA using a temperature of 350 °C, a pressure of 13.8 MPa, a H_2O:COFAR molar ratio of 6:1 and a flow rate of 1 mL/min.

The colors of the FFA samples generated were generally light brown and these FFA would require some sort of purification such as distillation before they could be used in subsequent syntheses. Although these colored compounds have not been identified, they are thought to be polymerized and/or oxidized by-products. Lubricant manufacturers and consumers prefer light colored products (i.e., those with low Gardner values) and this applies to estolides as well [20]. Because the color of decanoic estolides is a function of the color of the decanoic acid used in their synthesis, the decanoic acid used in estolide syntheses must be as light as possible. However, because commercial decanoic acid is not as refined as other mid-chain fatty acids, removal of color bodies is necessary. Kugelrohr-distillation [6], short-path distillation [26] and centrifugal molecular distillation [20] have all been used to purify FFA prior to incorporation into estolides. Sorbents could also be used to purify the FA.

In addition, the RBD CSO used in these hydrolysis studies had a starting Gardner color value of 3 and this undoubtedly contributed to the dark color of the hydrolyzed FFA. If a lighter CSO had been used as the starting material, a lighter FFA product may have been obtained. Recently, it has been shown that CSO obtained by supercritical carbon dioxide (SC-CO_2) has a lower Gardner color (i.e., ca. 2) than the RBD CSO used in this research [27]. It is likely that upon hydrolysis, the SC-CO_2-derived CSO may give a lighter FFA product than did the RBD CSO.

These results demonstrate the use of a continuous-flow conversion process employing subcritical water as an alternative hydrolysis method which is effective and devoid of catalyst residues for the processing of triglyceride-based fats and oils to their component fatty acid constituents.

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