Critical Fluids as Process Environment for Adding Value and Functionality to Sunflower Oil; A Model System for Biorefining

Baig M. N.[1,3,5], Alenezi R.[1], Leeke G. A.[1], Santos R. C. D.[1], Zetzl C.[2], King J. W.[3], Pioch D.[4] and Bowra S.*

1. Department of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK,

2. Department of Thermal and Separation Processes, Technical University Hamburg Harburg, 21073, Germany,

3. Department of Chemical Engineering, Bell, University of Arkansas, Fayetteville, AR 72701, USA,

4. Génie des Procédés d'Elaboration des Bioproduits, CIRAD Dpt Amis , Montpellier, Cedex 5, France

5. Department of Research and Development, Phytatec (UK) Ltd, Plas Gogerddan, Aberystwyth SY23 3EB,

Email: steve.bowra@phytatec.com, Fax:+44 1978 823241

Abstract

The European Technology Platform for Sustainable Chemistry in its Strategic Research Agenda of 2006 developed three visionary project ideas. One stated vision was integrated biorefining, which was defined as a process that would enable the production of platform chemicals, material and biofuels from biomass and ideally in a sustainable manner. Critical fluids offer environmental advantages over chemical solvents, while providing enhanced separation, and chemical selectivity[1]. Therefore the objective is to establish the use of critical fluids for the recovery of products from biomass and combine this with the transformation of selected molecules to add value. This overall objective was achieved by selecting sunflower oil as a model substrate. As the first step, sub-critical water (SCW) mediated hydrolysis of sunflower vegetable oil (triacylglycerides) creating free fatty acid and glycerol was studied in a continuous flow reactor[2]. In the second step of the process, FFA's were bio-catalytically transformed to fatty acid esters using lipase within super fluid environment. A factorial and a central composite design have been used to evaluate the influence of operating conditions on the hydrolysis as well as enzymatic esterification process material balance[3]. The response surface equation was optimized to identify the optimum process conditions for maximum FFA yield. The process conditions predicted to yield 100% esters were: pressure X1, 200 bar; temperature X2, 60ºC; FFA to ethanol ratio X3, 2.0; enzyme concentration X4, 9.70 (wt%) and residence time X5, 60 min. Experiments conducted under these conditions gave a yield of esters of 94.3%, close to the predicted result. In summary, this study presents optimised process conditions for continuous flow SCW hydrolysis of sunflower oil and the subsequent transformation of fatty acid to ethyl ester and also discusses the process advantages of coupling the two steps to one continuous flow unit.

Mis en forme : Couleur de police : Automatique

Supprimé : ¶

Introduction

A bio-based economy is the stated vision for the 21st century. Underpinning this goal is increased utilisation of biomass to create industrial feed stocks thereby loosening the current dependence on fossil fuel. Integrated biorefining is a broad all encompassing term which describes the ambition of achieving full utilisation of primary agricultural biomass or organic waste and creating platform chemical, biofuels and materials. To support this goal requires bio-processing technology development, which in turn evokes the selection of solvents. Critical fluids are recognised as environmentally 'green'. Water is potentially the most benign alternative to organic solvents. Sub-critical water is hot water maintained anyway between 100 °C and 347 °C and below 221 bar its critical point. Sub critical water has successfully been used as a solvent for extraction of range of compounds, which find application in health care, cosmetic and pharmaceutical industries [4]. However, sub critical water can also be used to hydrolyse natural occurring polymers e.g. triacylglycerides and hemicellulose[2,5], although in batch conditions especially decomposition of monomers can occur unless the operating conditions are not controlled. Supercritical carbon dioxide is an equally benign solvent and has been investigated for its suitability to support biocatalysis. Enzymes as industrial biocatalysts offer significant environmental and economic benefits over conventional chemical methods of manufacture and processing, thus the combination of SC CO_2 and enzymes offer significant potential with regards to developing environmentally responsible bio-processing. In fact the benefits of using SC CO_2 to support biocatalysis are often in the product quality and overall reduction of waste rather than the enzyme kinetics and/or biochemistry. However despite the existing body of work on lipase performance in SC CO_2 there only a few studies which undertake the evaluation of the impact of multiple variables relevant to process development. To address this issue and at that same time limit the number of empirical experiments response surface methodology (RSM) which enables the evaluation of the effects of multiple parameters, alone or in combination, on response variables and also predicts their behaviour under a given set of conditions have been adopted[6] and successfully validated.

In the light of the above the objective of this research is to demonstrate the utility of critical fluids to support hydrolysis of vegetable oil and enzyme mediated transformation of fatty acids, a product of the hydrolysis. To aid this objective a five factorial response surface model was designed for the continuous flow lipase mediated ethanolysis and validated. The result will be presented in the light of existing knowledge to address the impact of each parameter.

Supprimé : is

Experimental

Materials

Refined Sunflower oil was used as a model substrate for the sub-critical water hydrolysis experiments. (Sainsbury, UK). The esterification experiments were conducted with 89% pure Oleic acid (Sigma Aldrich) and an immobilised Lipase, Lipozyme TL IM®) (Novozyme) from *Thermomyces lanuginosus* with specific activity of 250 IUN/g. The reagents used were ethyl alcohol and sodium hydroxide from Fisher Scientific.

Methods

Hydrolysis of sunflower oil-continuous flow process

A continuous flow tubular reactor was designed and constructed to enable sub-critical water hydrolysis of sunflower oil. Experiments were conducted at pressures between 100 to 200 bar and temperatures of 250 to 390 ºC. Two water to oil ratio were used i.e. 50:50 and 80:20 v/v.

Enzymatic esterification of FFA - continuous flow Process

Continuous enzymatic esterification was carried out in a modified tank reactor loaded with enzymes through which oleic acid, ethanol and SC-CO_2 were pumped

Determination of FFA and fatty acid ethyl esters content

The degree of vegetable oil hydrolysis was determined by measuring the increase in acidity using the standard AOC protocol and a photometric technique (MicroChem II analyser). A decrease in acidity was used to calculate the % of fatty alcohol formation using the same protocols.

Experimental Design

Response surface methodology (RSM) based on calibrating full quadratic models around the central composite design (CCRD) was adopted to develop a five factorial experimental design to optimise the reaction conditions for lipase mediate ethanolysis of oleic acid. Similar statistical techniques were adopted to design the sub-critical water hydrolysis experiments but are not shown. The experimental parameters pressure of the reaction X1, bar; temperature of the reaction X2, ºC; FFA to ethanol ratio, X3, enzyme concentration, X4 as wt. percentage of the fatty acids and X5, residence time, in min; were selected as the variables to maximize the response (conversion of FFA to esters). The experimental design generated using the RSM suggested 26 experiments with five variables at five levels. A second order polynomial equation was developed to study the effects of variables on the esters yield. The equation indicates the effect of variables in terms of linear, quadratic and cross-product terms.

$$Y = A\circ + \sum_{i=1}^{N} A_i X_i + \sum_{i=1}^{N} A_{ii} X_i^2 + \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} A_{ij} X_i X_j \qquad Eq.1$$

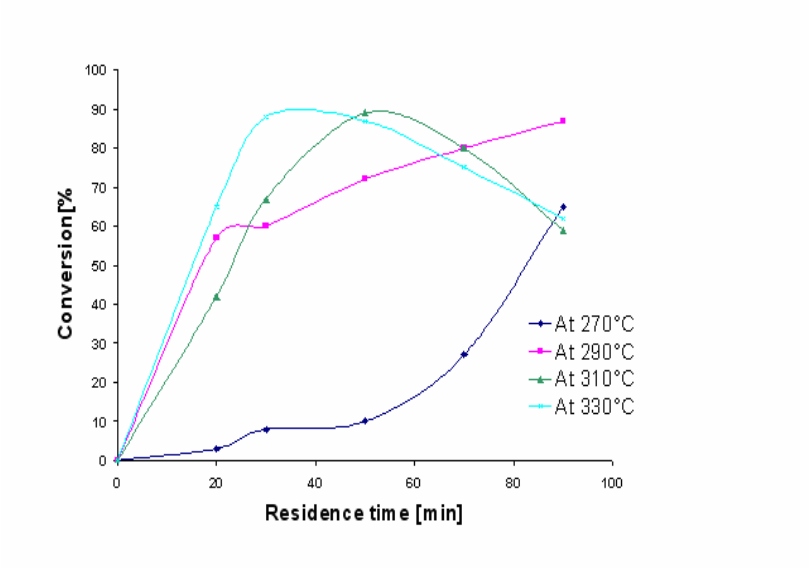
where Y is the yield of esters (%), X_i the variable, A_0 the constant term, A_i the coefficient of the linear terms, A_{ii} the coefficient of the quadratic terms, A_{ij} the coefficient of the cross-product terms and N the number of variables. The coefficients of the equation were determined by using Design Expert 7.0 software. The analysis of variance (ANOVA) and the lack-of-fit for the final predictive equation were also done using the same software.

A graphical representation of the above equation in the form of contour plots was used to describe the individual and cumulative effect of the test variables on the response. (Not shown)

Results and discussion

The preliminary results of the sub-critical water mediated hydrolysis of refined sunflower oil are illustrated in figure 1.

Fig 1 Percentage conversion of triglyceride to FFA in a continuous flow hydrolysis process



A more complete analysis will be presented by R .Alenezi *et al* in these proceeding. It is notable that at 270 ^{0}C the rate of hydrolysis appears to be significantly slower compared to 290 ^{0}C, 310 ^{0}C and 330 ^{0}C, as adjudged by the time taken to reach 50% hydrolysis, which at 270 ^{0}C is 80 mins compared to 15-20 mins for the other treatments. While at 290 ^{0}C the initial rate is high after 90 min the plateau as not been reached, where as for the 310 ^{0}C and 330 ^{0}C the maximum of approximately 90% are achieved after 45 min and 25 min respectively, thereafter the acidity drops indicating thermal transformation. Interestingly, the finally level of residual acidity converge for both 310 ^{0}C and 330 ^{0}C. The results demonstrate that sub-critical water can facilitate the hydrolysis of oil but with extended residency time at elevated temperature appears to result in decomposition. The conventional Colgate-Emery process of steam splitting of oils involves temperatures of 240-260 ^{0}C and high pressures. However the resulting products are often unstable and require re-distillation to remove impurities and products of degradation. In addition to this, highly unsaturated heat sensitive oils cannot be used in the Colgate-Emery process without prior hydrogenation. In the light of the sub-critical water results further analysis is currently underway to assess what effect the treatment has on fatty acid molecules.

Table 1 illustrates the range of variables employed to conduct the actual set of experiments undertaken and the percentage conversion of esters obtained.

Exp No.	Pressure [bar] X1	Temp [deg C] X2	Ethanol Conc. [volume factor of FFA] X3	Enzyme Conc. [wt%] X4	Time [min] X5	Yield (based on Model) [%]	Yield(based on Exp) [%]
1	150	50	1.50	7.50	10	21.0	17.0
2	100	40	1.00	5.00	20	12.0	15.0
3	200	60	2.00	5.00	20	17.5	19.0
4	200	40	2.00	10.00	20	39.5	41.0
5	200	60	1.00	10.00	20	43.5	45.0
6	100	60	2.00	10.00	20	40.5	42.0
7	150	50	1.50	3.70	40	43.9	40.0
8	150	35	1.50	7.50	40	45.0	41.0
9	150	50	2.25	7.50	40	46.0	42.0
10	150	50	1.50	7.50	40	56.0	60.0
11	225	50	1.50	7.50	40	71.0	67.0
12	150	50	1.50	7.50	40	56.0	61.0
13	150	50	1.50	7.50	40	56.0	60.0
14	150	50	1.50	7.50	40	56.0	61.0
15	75	50	1.50	7.50	40	43.0	39.0
16	150	50	0.75	7.50	40	47.0	43.0
17	150	50	1.50	7.50	40	56.0	60.0
18	150	65	1.50	7.50	40	49.0	45.0
19	150	50	1.50	11.20	40	82.0	78.0
20	200	40	2.00	5.00	60	63.5	65.0
21	200	60	1.00	5.00	60	65.5	67.0
22	100	60	2.00	5.00	60	58.5	60.0
23	200	40	1.00	10.00	60	88.5	90.0
24	100	40	2.00	10.00	60	83.5	85.0
25	100	60	1.00	10.00	60	85.5	87.0
26	150	50	1.50	7.50	70	88.0	84.0
27[a]	**200**	**60**	**2.00**	**9.70**	**60**	**100.0**	**94.3**

[a] *Validation experiment at optimal conditions*

Table 1: Experimental design showing level of variables along with experimental and predicted yield

The predicted response values deviated from experimental data within the range of 5% which is considered an acceptable technical error. The Model F-value of 9.14 implies the model is significant and given the emperical results the predective strength of RSM has again been reinforced.

The results of the continuous lipase mediated esterification of free fatty acids with ethanol indicates that at a pressure of around 200 bar the maximum ethyl ester yield was obtained. In the present study, increased pressure, theoretically, would enhance the solubility of reactants in SC-CO_2 and hence the yield of fatty acid ethyl esters (FAEE). However results show that the yield of FAEE did not correlate with an increase in pressure between 100 and 200 bar, Shishikura et al[9] observed similar effect of pressure on yield of FAEF and offered two explanations, 1) a decreased partition rate of substrates in the liquid phase and 2) increased reaction volume. With increased pressure, the solubility of the substrates in the SC-CO_2 phase increased markedly and decreased in the enzyme phase, in which the catalysis takes place. This causes dilution of the substrate and results in the retardation of esterification.

Supprimé : no

Temperature significantly affected enzyme catalysis in SC-CO_2. Results show that, the maximum ester yield was obtained at 60 °C with other variables at their optimum values; this is in line with the temperature optimum of the lipase used which is from *Thermomyces lanuginosus* a thermal tolerant microrganism. The yield of esters decreased at temperatures below and above this value. Temperature is widely understood to influence the three dimensional conformation and hence the stability of the enzyme. On the other hand, temperature also affects the partitioning of substrates between the SC-CO_2 phase and enzyme phase. At higher temperatures the decrease in the yield could be due to the reduced availability of the alcohol substrate (ethanol) as reported by Ramamurthi et al[10]. however high concentration of ethanol has also been reported to reduce enzyme activity[8].

Supprimé : .

Supprimé : H

Supprimé : activity[6]

Results illustrate that enzyme concentration has a significant impact on yield of FAEE. The effect of enzyme concentration on the yield of FAEE is in agreement with the established results i.e., increased concentration of enzyme gives increased yield of the FAEE in the range (5–15%) studied. The saturation effect of enzymes on the yield of FAEE is not observed within the experimental range studied.

A higher concentration of enzyme is required to achieve a greater yield at lower pressures. At higher pressures, 60–70% of maximum FAEE yield can be obtained with lower enzyme concentration, but maximum yield cannot be achieved. The incubation time and enzyme concentration show a synergistic effect resulting in a maximum level. This means higher enzyme concentrations and longer incubation times are necessary to achieve the maximum yield of FAEE.

Water content is one of the important factors to be considered during the enzymatic reaction, especially, esterification reactions using lipases, as these are disturbed by water generated through the reaction. The generated water could shift the reaction equilibrium in favour of hydrolysis and thus lower the reaction rate. However, the esterification reaction proceeded unaffected even without adding a moisture removing aid such as a molecular sieve in the presence of SC CO_2, this is in line with previous reports.

Conclusion

The rate of the hydrolysis was enhanced by increasing temperature, pressure and residence time. The highest yield (up to 90%) was obtained under the conditions - 35 minutes residence time, 80:20 v/v water to oil ratio, 200 bar and 330 °C.

Optimization of the ethyl esterification of FFA of oleic acid by CCRD design shows that, all the five reaction variables have effects on the yield of the FAEE individually and in association with other reaction parameters under SC-CO_2. The mutual effect of temperature and ethanol concentration on the yield of FAEE is highly pronounced. The predicted model fits well with the experimental results. The water generated as a product of the esterification reaction, dissolves in the SC-CO_2 and thereby its inhibitory effect nullified.

Supprimé :

The pressure, 200 bar; temperature, 60 ºC; FFA to ethanol ratio of 2; enzyme concentration, 10% (w/w); and incubation time, 60 min were found to be the optimum conditions to achieve the maximum yield of FAEE.

The results show promise for the integration of a continuous flow hydrolysis and an esterification process into a one step continuous procedure for ester production.

References

[1] Hutcheson, K.W., Foster, N.R., ACS Symposium Series 608, American Chemical Society: Washington, DC, 1995.

[2] Holliday, R. L., King, J. W., List, G. R., Industrial and Engineering Chemistry Research, Vol. 36, 1997, p. 932-935

[3] D.C. Montogomery, Design and Analysis of Experiments, John Wiley and Sons, New York, 1991, p. 542–547

[4] Ayalal, R. S., Luque de Castro, M. D., Food Chemistry,Vol. 75 , 2001, p. 109-113

[5] Patil, T.A., Butala, D.N., Raghunathan, T.S., Shankar, H. S., Reaction Kinetics. Industrial and Engineering Chemistry Research, Vol. 27, 1998, p. 727-735

[6] Nagesha, G. K, Manohar B and Udaya Sankar K, The Journal of Supercritical Fluids Vol. (missing), 2004, p. 137-145

[7] Wong, W.C., Basri, M., Razak, C.A.N., Salleh, A.B., J. Am. Oil Chem. Soc., Vol. 77, 2000, p. 85–88

[8] Marty, A., Dossat, V., Condoret J.S., Biotechnol. Bioeng., Vol. 56, 1997, p. 232–237

[9] Shishikura, A, Fujimoto, K, Suzuki, T, Arai, K,Improved lipasecatalyzed incorporation of long-chain fatty acids into medium-chain triglycerides assisted by supercritical carbon dioxide extraction, J.Am. Oil Chem. Soc. Vol.71, 1994, p. 961–967.

[10] Ramamurthi, S, Bhirud, P.R, McCurdy A.R, Enzymatic methylation of canola oil deodorizer distillate, J. Am. Oil Chem. Soc. Vol. 68, 1991, p. 970–975.

Supprimé : [6] Knez Z., Habulin M., Krmelj, J. Supercritical Fluids, Vol. 14, 1998, p. 17–27.¶

Supprimé : 7

Supprimé : 8

Supprimé : 9

Supprimé : A.

Supprimé : K.

Supprimé : T.

Supprimé : K.

Supprimé : 10

Supprimé : S.

Supprimé : P.R.

Supprimé : A.R