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The General Applicability of in Situ Transesterification for the Production of Fatty Acid Esters from a Variety of Feedstocks

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Abstract We previously described a method for fatty acid methyl ester (FAME) production wherein acylglycerol transesterification was achieved by reacting flaked full fat soybeans with alkaline methanol to create a product that met ASTM specifications for biodiesel. In the present work we explore the general applicability of this approach, termed in situ transesterification, to feedstocks other than soybeans. Materials investigated were distillers dried grains with solubles (DDGS), which is a co-product of the production of ethanol from corn, and meat and bone meal (MBM), a product of animal rendering. For both feedstocks, reaction conditions giving maximum lipid transesterification were predicted by statistical experimental design and response surface regression analysis, and then verified experimentally. Successful transesterification was achieved at ambient pressure and 35 °C. For DDGS, partial drying markedly reduced the methanol requirement to achieve a high degree (91.1% of maximum theoretical) of transesterification. Elevated reaction temperatures (to 55 °C was explored) caused little or no shortening of the time to completion. Protein was not removed from the DDGS during this treatment. For MBM, drying was not required to achieve a high degree (93.3%) of transesterification. The remaining meal retained approximately 90% of the protein originally present. Coupled with the previous work with soybeans, the data

presented here indicate that in situ transesterification is generally applicable to lipid-bearing materials, which could substantially increase the supply of biodiesel.

Keywords Biodiesel · Distillers dried grains · Fats and oils utilization · Fatty acid ester · In situ transesterification · Meat and bone meal · Transesterification

Introduction

The production and use of fatty acid esters, particularly methyl esters (FAME), as a fuel for compression ignition (diesel) engines is a rapidly growing technology that has penetrated the transportation fuels sector in much of Europe and is in the process of doing so in the United States and other countries. Compared to petroleum-derived diesel fuel, the use of biodiesel offers advantages of reduced engine emissions of pollutants, domestic sourcing, and renewability.

It is estimated that the United States produced and consumed approximately 30 million gal of biodiesel in 2004 [1]. European consumption exceeded this value by at least tenfold. Biodiesel production is growing rapidly with 2006 US production estimated to be 245 million gal and expected to exceed 700 million gal in 2015 [2]. A majority of biodiesel is produced from edible vegetable oils, soybean oil in the United States, and presently accounts for approximately 10% of annual US soybean oil production if all was made from that feedstock. There is concern that at anticipated future production levels the use of edible oils for fuel will compete significantly with food uses. This would result in undesirable increases in both food and biodiesel costs, a particularly damaging occurrence in the

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case of biodiesel, which even at current prices, has difficulty competing with petroleum fuel on an economic basis. Feedstock expense is the major contributor to the cost of biodiesel production [3].

Alkali-catalyzed transesterification of fats and oils in the presence of a simple monohydroxy alcohol, usually methanol, is the predominant technology used for FAME synthesis. For vegetable oils this requires the prior recovery of the lipid by pressing or solvent extraction, and the partial purification of this oil. We have previously described an alternate method of FAME synthesis from soybean oil, termed in situ transesterification, which does not require the extraction and purification of the oil prior to FAME synthesis. Rather, thinly flaked soybeans are themselves the feedstock for this process, and alkali-catalyzed transesterification takes place directly within the flakes during incubation in alkaline alcohol [4]. This process operates at high efficiency, and under optimum reaction conditions achieves maximum theoretical conversion of the acylglycerols of the feedstock into FAME. Furthermore, the resulting ester preparation meets the ASTM specifications for biodiesel [5].

The desirability of ensuring future supplies of biodiesel feedstocks, while at the same time minimizing economic pressures on high quality lipids, has led to the development of methods to use low quality lipids, such as waste greases and soapstock for biodiesel production. Other low-value lipid sources exist, but have received little or no attention in this regard. One of these is Distillers Dried Grains with Solubles (DDGS), a co-product of the fermentation of corn to produce ethanol via the dry grind process. Another is Meat and Bone Meal (MBM), a product of the animal rendering industry that is composed of the inedible portions of animals slaughtered in meat production. Both are produced in substantial quantities, have low economic value, and contain 5–10% lipid, which could be converted to FAME. These materials are not suitable for the recovery of their lipid by hexane extraction or yield preparations of poor quality. In the present work we report the results of an investigation of the ability of in situ transesterification to convert the lipids in DDGS and MBM to FAME.

Experimental Procedures

Chemicals

DDGS was the product of an industrial corn-to-ethanol fermentation plant, and was provided by Dr. Vijay Singh, Department of Agricultural and Biological Engineering, University of Illinois, Urbana, IL, USA. It consisted of solid particles with a size range such that 88 wt% passed

through a 20-mesh screen and 24% passed through a 50-mesh screen. Oven drying (77 °C) the DDGS to constant weight indicated 8.7 wt% moisture. Extraction with hexane for 4.5 h in a Soxhlet apparatus followed by HPLC analysis (below) of the resulting oil indicated a 8.8 wt% triacylglycerol (TAG) content.

MBM, obtained from a commercial rendering facility, was provided by Dr. Rafael Garcia of our facility. It consisted of particles with a size range such that 74% of the mass passed through a 20-mesh screen, and only 2% passed through a 50-mesh screen. The moisture content, determined by oven drying as described above, was 2.0 wt%. Soxhlet extraction with hexane followed by HPLC of the resulting oil indicated 9.1 wt% triacylglycerol content.

DDGS and MBM were stored at 4 °C.

As required, feedstocks were incubated in a convection oven (77 °C) until the desired moisture levels were attained.

Soygold brand biodiesel, produced from refined soybean oil, and used as a chromatography standard was the product of Ag Environmental Products, Lenexa, KS, USA. Soybean oil TAG and FFA for use as chromatography standards were obtained from Sigma-Aldrich. Palmitic, stearic, oleic, linoleic, and linolenic acids, mixed in amounts proportional to their mass abundance in soybean oil [6], served as the FFA standard. A mixture of FAME, whose composition reflected the fatty acid content of soy oil (RM-1), was the product of Matreya, Inc. (Pleasant Gap, PA, USA). Organic solvents were B&J Brand™ High Purity Grade (Burdick & Jackson, Inc., Muskegon, MI, USA). Other chemicals were Analytical Reagent grade quality or better.

Conduct and Optimization of in situ Transesterification

The lipid-bearing material (5.00 g for MBM, and an amount equal to 5.00 g of dry matter in the case of DDGS, for which multiple moisture levels of substrate were examined) was mixed with methanol in which sodium hydroxide had been previously dissolved ("alkaline alcohol") in screw-capped bottles of a capacity at least five times the reaction volume. These were mixed by orbital shaking at a speed sufficient to keep the solids well suspended. Reactions were conducted in heated chambers at the desired reaction temperatures.

Central Composite Response Surface design methods [7] were employed to investigate coordinately the effects and interactions of the amount of alkaline methanol, its NaOH concentration, and reaction time on the yields of FAME, FFA and unreacted TAGs in the liquid phase. Preliminary studies (data not shown) were conducted to focus the statistically designed work in the region of variable space giving greatest FAME production.

For the final optimization of the reaction conditions for 5-g reactions of DDGS, the amounts of alkaline methanol tested were 12, 14, 18, 22, and 24 mL; NaOH concentrations were 0.20, 0.25, 0.35, 0.45, and 0.50 N; and reaction times were 0.5, 1.5, 2.5, 3.5, and 4.5 h. This series of variables was tested at DDGS moisture levels of 2.62, 6.86, and 8.7 wt%. Each series involved 20 reactions at various combinations of these levels.

For optimization of the reaction conditions for transesterification of 5 g of MBM, the amounts of alkaline methanol tested were 5.0, 6.67, 10.0, 13.3, and 15.0 mL, NaOH concentrations were 0.1, 0.133, 0.20, 0.27, and 0.30 N; and reaction times were 0.5, 1.0, 2.0, 3.0, and 3.5 h. Each series involved 20 reactions.

Following reaction, bottles were allowed to sit for 15 min at room temperature to allow the solids to settle. The liquid phase was removed, the solids were washed twice by resuspending in 10 mL methanol, the washes were pooled with the reaction liquid, and the mixture was analyzed by HPLC to determine the contents of FAME, TAG, diacylglycerols (DAG), monoacylglycerols (MAG) and free fatty acids (FFA). (Preliminary experiments demonstrated that two washes were sufficient to remove all FAME from the solids.)

For DDGS and MBM, best-fit equations correlating the amounts of FAME, FFA and TAG produced with the reactant compositions were constructed using SAS/STAT software [8]. Numerical analysis of these equations and examination of the corresponding three-dimensional (3D) surfaces allowed identification of the conditions predicted to give maximum FAME yield with minimum contamination by FFA and TAG.

To conduct time-course studies, multiple replicate reactions were conducted, each consisting of 5 g substrate and the amounts of alcohol and NaOH predicted to give maximum reaction. At selected times, a pair of tubes was processed as described in "Analytical Methods", to determine the FAME content. The results of duplicate determinations are presented; individual values differed from the average by between 2 and 15% in these determinations.

Analytical Methods

Acylglycerols (AG), FAME, and FFA in the products of in situ transesterification were determined by HPLC on a diol-modified silica column (LiChrosorb 5 Diol, Varian, Walnut Creek, CA, USA), which was developed isocratically with a mixture of hexane and acetic acid (99.9:0.1 vol%) at a flow rate of 0.5 mL/min. Peaks were detected by evaporative light scattering and quantitated by reference to standard curves constructed with known pure compounds.

Identification of the fatty acids in the FAME product was achieved by GC, with flame ionization detection. The column was a Hewlett–Packard Innowax fused-silica capillary, 30 × 0.53 mm (internal diameter) run as previously described [9]. FAME assignments were made by comparison with known FAME.

Nitrogen contents (wt%) were determined using a LECO FP2000 Nitrogen Analyzer (LECO Corpn., St Joseph, MI, USA). To estimate protein content, nitrogen values were multiplied by 6.25 for DDGS, the value applicable to corn [10], and 6.45 for MBM (R. Garcia, personal communication).

Results and Discussion

In Situ Transesterification of DDGS

Reactions were conducted at 35 °C since reaction rates were unacceptably low at lower temperatures. Higher temperatures were not explored in detail since complete transesterification occurred in less than two hours at 35 °C. Previous work with flaked soybeans demonstrated that in situ transesterification occurred, and that the methanol required for the reaction was greatly reduced, when moisture was completely removed from the flakes prior to reaction [5]. In contrast, with fully dry DDGS the degree of transesterification never exceeded 25% of the theoretical maximum. In an effort to achieve higher FAME yields, the effect of substrate moisture level on the reaction was investigated.

Table 1 lists the reaction conditions, predicted by Central Composite Design experiments conducted at each selected feedstock moisture level, for maximum transesterification of the endogenous acylglycerols in DDGS into FAME. These experiments also identified conditions that would yield only partial conversion of the AG in DDGS into FAME. The latter data are not discussed here. However, if one wished to convert only a portion of the lipids in

Table 1 The effect of moisture content on the reaction conditions predicted to yield optimum FAME production during the in situ transesterification of 5 g (dry weight) of DDGS at 35 °C

Moisture (wt%)	Methanol (mL)	NaOH (N)	Time (h)	FAME yield (% max. theoretical)
2.6	14.0	0.40	1.2	100
2.6	18.0	0.30	0.83	98.7
6.9	22.0	0.35	1.5	99.5
6.9	24.0	0.20	1.8	100
8.7	18.0	0.45	4.2	99.5
8.7	20.0	0.25	0.5	99.7

a feedstock to FAME, leaving some of them unreacted, e.g., to enhance the nutritional energy value of the residuum for use as an animal feed, it is clear that conditions to achieve this could be identified.

We sought reaction conditions giving optimum FAME production using substrate with as near its natural moisture level as was possible. This would reduce feedstock drying costs. However, in the case of full moisture (8.7%) DDGS a relatively large amount of methanol, 20 mL, was required to achieve near-quantitative FAME yield from 5 g of DDGS (Table 1). This is undesirable, due to the high cost that would be subsequently expended in recovering unreacted methanol by distillation. Increasing the NaOH concentration in the methanol phase from 0.25 to 0.4 N slightly reduced the methanol requirement, to 18 mL (Table 1). However, the cost of the additional alkali would probably negate any cost reduction due to this small decrease in methanol use. In addition, with the latter conditions the reaction time to achieve high levels of transesterification increased approximately eightfold, to 4.2 h.

The removal of 20% of the moisture from DDGS (to 6.9%) did not reduce the methanol requirements of the reaction (Table 1). In fact, at this moisture level optimal transesterification required at least 22 mL of methanol per 5 g of substrate.

With more complete drying, however, a reduction in methanol requirement for high level transesterification was observed (Table 1). With DDGS at 2.6% moisture, which corresponds to removal of 70% of the substrate moisture, the methanol requirement was reduced to 18 mL of 0.3 N NaOH per 5 g DDGS at a reaction time of 0.83 h (50 min). By increasing the NaOH concentration to 0.4 N the amount of methanol required for high degrees of transesterification was further reduced to 14 mL (Table 1) with 70 min reaction time.

Thus, high degrees of transesterification with minimal methanol usage were predicted to occur at moisture levels approximately 30% of that typically found in DDGS. At that moisture level (2.62 wt%) the relationship between FAME yield in 35 °C reactions containing a 5-g dry wt equivalent of DDGS and other reaction variables, as predicted from the Central Composite Design data, is described by Eq. 1:

$$\text{FAME} = 1110 - 2.33\,V + 1180\,N - 0.974\,T - 63.6\,VN - 0.0158\,VT + 1.19\,NT + 0.963\,V^2 + 131\,N^2 + 0.00158\,T^2 \quad (1)$$

where FAME is the predicted FAME yield (mg), V methanol volume (mL), N normality of NaOH, and T reaction time (h).

The R^2 value for the fit of this equation to the experimental data was 0.84. This indicates that the reliability of the model is reasonably high. We have typically observed R^2 values in excess of 0.92 for similar equations describing the in situ transesterification of flaked soybeans [4]. With DDGS, however, we have repeatedly observed poorer fit between predicted and observed degrees of esterification. Duplicate reactions often show greater variability in results than we have experienced with other feedstocks. For DDGS, the standard deviation of the mean of six replicate reactions conducted at the center-point conditions of the statistically designed experiment was 16% of the value of the mean. The cause of this variation is not yet known.

Figure 1 presents the 3D surface of FAME yields predicted by Eq. 1 at 1.2 h (70 min) of reaction as a function of the amount of methanol and the concentration of NaOH in that methanol. Maximum theoretical FAME yield in this system is 397 mg. The surface in Fig. 1 generally exceeds this value. This suggested that shorter reaction times might achieve full transesterification. We found that this was not the case for 30 min reactions. We did not explore reaction times between 30 and 70 min, since there would be little value in exercising such control over reaction length on an industrial scale. The surface also suggested high FAME production with volumes of methanol below 12 mL. However, this area was outside the region explored experimentally, and thus Eq. 1 lacked predictive reliability at low volumes. Experiments subsequently conducted using small methanol volumes (6–10 mL) gave FAME yields below 35% of theoretical maximum, probably
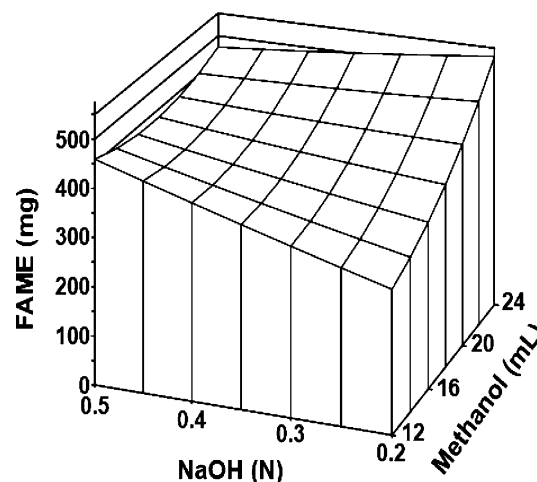

Fig. 1 Predicted FAME production (from Eq. 1) at 70 min of reaction time as a function of methanol volume and NaOH concentration during the in situ transesterification of 5 g of DDGS, 2.62 wt% moisture, at 35 °C. Maximum theoretical FAME production: 397 mg

because the DDGS substrate was not thoroughly wetted by the liquid phase.

To validate the predictions of Eq. 1 and Fig. 1, in situ transesterification reactions were conducted under the optimal conditions for the transesterification of 2.62 wt% moisture DDGS with 14 and 18 mL of methanol. FAME production was 91.1 ± 1.7% and 90.1 ± 0.1% of maximum theoretical for these conditions, respectively. This is acceptably close to the full transesterification predicted by the experimental model. Reactions of slightly longer duration have consistently yielded theoretical maximum transesterification. Under optimum conditions for FAME production, the production of FFA and AG was negligible, with these species constituting only 0.3–0.4 wt% of the lipid-based mass in the product.

Since preliminary experiments indicated that a high degree of transesterification was achieved in a relatively short time at a low temperature (35 °C), the effects of higher temperatures on the reaction were not explored in
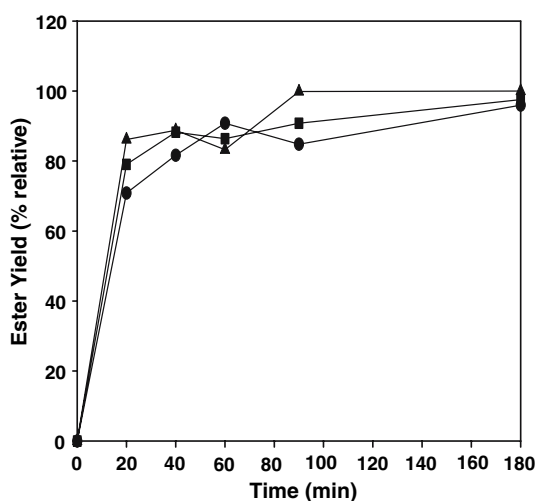

Fig. 2 Time course of FAME production over time as a function of reaction temperature in reactions containing 5 g of DDGS (2.3 wt% moisture), and 14 mL of methanol containing 0.4 N NaOH. Reaction temperatures: *filled circles*: 35 °C, *filled squares*: 45 °C, *filled triangles*: 55 °C. Data presented are the averages of duplicate determinations. Most individual determinations differed from the averages by 10% or less

the statistically designed experimentation. It was reasoned that if this method were applied in industry, the lowest temperature giving an acceptable degree of completion in a relatively short reaction would be used to save energy. To briefly explore the effect of temperature, replicate time-course reactions were run at 35, 45, and 55 °C. The results (Fig. 2) suggest that an elevation in reaction temperature caused, at best, only a modest acceleration of reaction rate.

The fatty acid composition of the FAME fraction produced from DDGS by in situ transesterification at optimal conditions (14 mL methanol/0.4 N NaOH) was comparable to that typically seen for corn oil (Table 2). Thus, as reported for soybeans [5], in situ transesterification does not exhibit fatty acid selectivity in the transesterification event.

As received, the DDGS contained 28.0 ± 0.2 wt% protein. Following in situ transesterification under optimal reaction conditions (14 mL liquid phase reaction) the protein content was 32.4 + 0.2 wt%. An increase in protein content would be expected if little or no protein was solubilized during transesterification, since lipid removal by the reaction increases the relative proportion of protein. Given an AG content of 8.8% in DDGS, and a transesterification efficiency of 91%, one calculates that the loss of lipid should increase the protein content to 30.4% if no protein were lost. The actual measured protein content was greater than this following transesterification suggesting that all or nearly all of the protein was retained while a small amount (approximately 6% of meal mass) of non-proteinaceous, non-lipid material was removed from the meal during transesterification.

Conditions for the optimal transesterification of dry flaked soybeans involved amounts of methanol comparable to those identified here optimal for DDGS, but alkali concentrations of only 0.1 N NaOH, not 0.3–0.4 N as for DDGS [5]. The pH of a mixture of 5 g of DDGS in 15 mL water was 4.2, whereas the pH of a comparable mixture of flaked soybeans was 6.4. Some, or all, of the greater alkali requirement for effective transesterification of DDGS was thus probably due to the inherent acidity of this substrate. Modifications in the technology of preparing DDGS that reduced its acidity could lower the alkali requirement for effective in situ transesterification. Of course, an analysis

Table 2 Fatty acid composition (wt%), determined by GC, of methyl esters produced by in situ transesterification of DDGS and MBM

Source material	16:0	18:0	18:1	18:2	18:3
DDGS	12.9	1.6	28.5	55.5	1.4
Corn oil [14]	9.2–11.8	1.1–1.7	19.5–30.4	53.0–65.3	1.2–2.1
MBM	25.2	19.7	35.6	1.9	0.3
Beef tallow [15]	20.9–28.9	7.0–26.5	30.4–48.0	0.6–1.8	0.3–0.7

Typical fatty acid compositions of the triacylglycerols of corn oil and beef tallow are provided for reference

of the cost of such modifications, and their impact on overall process economics, would be required.

In Situ Transesterification of MBM

Reactions were conducted at 35 °C since high levels of transesterification were achieved in relatively short time at this temperature, while reaction rates were unacceptably low at lower temperatures.

During production MBM is dried rather thoroughly, typically to a final moisture content of 2–4 wt%. Therefore, it was first investigated in an "as received" state rather than investing further energy in complete drying. Equation 2 presents the predictive equation, derived via Central Composite Design experimentation, for FAME production from 5 g MBM at 35 °C:

$$
\begin{aligned}
FAME = {} & -1080 + 170\,V + 4740\,N + 31.1\,T \\
& - 282\,VN + 2.67\,VT - 118\,NT - 5.18\,V^2 \\
& - 3380\,N^2 - 3.68\,T^2
\end{aligned}
\tag{2}
$$

where FAME is the predicted FAME yield (mg), V methanol volume (mL), N normality of NaOH; and T reaction time (h). The R^2 value for the fit of this equation to the experimental data was 0.87, indicating a reasonably good fit between actual and predicted results. The reproducibility of the reaction for MBM was much better than that for DDGS (above); the standard deviation of the mean of the FAME content of six replicate reactions conducted at the center-point reaction conditions of the statistically designed experiment differed from the mean by only 1.3% of the mean.

Table 3 presents selected conditions predicted to yield high levels of FAME, derived from Eq. 2. High efficiencies of transesterification were predicted for reactions containing 12–15 mL of methanol per 5 g of MBM, which is similar to the case of DDGS (Table 1). For MBM the alkali requirement was approximately one-half that seen with DDGS.

Table 3 Selected conditions predicted by Eq. 2 to give maximum FAME production during the in situ transesterification of 5 g of MBM at 35 °C

Methanol (mL)	NaOH (N)	Time (h)	FAME yield (% max. theoretical)
15.0	0.10	3.0	100
14.0	0.10	2.5	99
13.0	0.10	2.5	97
12.0	0.10	3.0	96
12.0	0.18	2.0	92

As with DDGS, we desired to identify a set of reaction conditions that were predicted to give a high level of transesterification at minimum alcohol levels. As shown in Table 3, this occurred in reactions consisting of 5.0 g MBM and 12.0 mL of methanol containing 0.2 N NaOH. Table 3 also indicates that optimal conditions can be identified for achieving high degrees of transesterification in reactions using less alkali, or longer reaction times. Also, a variety of conditions could be identified that were consistent with partial conversion of the lipid in MBM to FAME (data not shown). Since high degrees of transesterification were achieved with feedstock with its endogenous moisture levels, reactions using dried MBM were not explored.

Figure 3 displays the 3D surface predicted by Eq. 2 for FAME production from MBM as a function of methanol volume and NaOH concentration during 2.0 h of reaction at 35 °C. This reaction duration was judged to be reasonable for use in an industrial setting. Analysis of Eq. 2 and Fig. 2 indicated that the minimum methanol volume needed to achieve maximum FAME production in a 2.0 h reaction was 12.0 mL at NaOH concentration of 0.20 N. FAME yield under those conditions was predicted to be 418 mg, 91.7% of theoretical maximum. Confirmatory reactions run at these settings gave a yield of 425.5 ± 10 mg (93.3% max. theoretical yield). Triacylglycerols and FFA were undetectable by HPLC, and thus constituted <0.4% of the mass of the FAME in these samples.

As with DDGS, the time-course of FAME production was determined at 35, 45, and 55 °C to explore the effect of
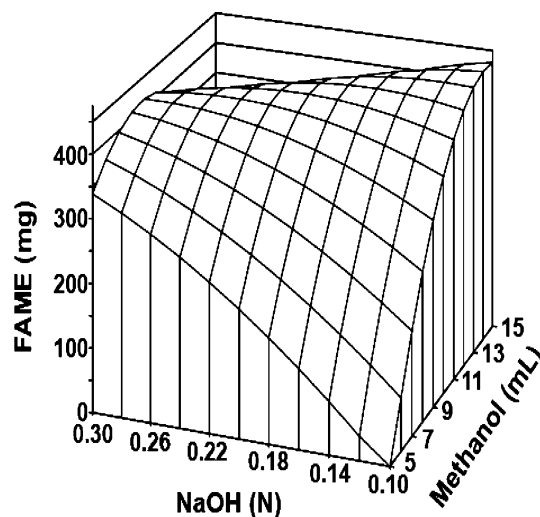

Fig. 3 Predicted FAME production (from Eq. 2) at 2.0 h of reaction as a function of methanol volume and NaOH concentration during the in situ transesterification of 5 g of MBM at 2.62 wt% moisture. Reaction temperature: 35 °C. Maximum theoretical FAME production: 456 mg
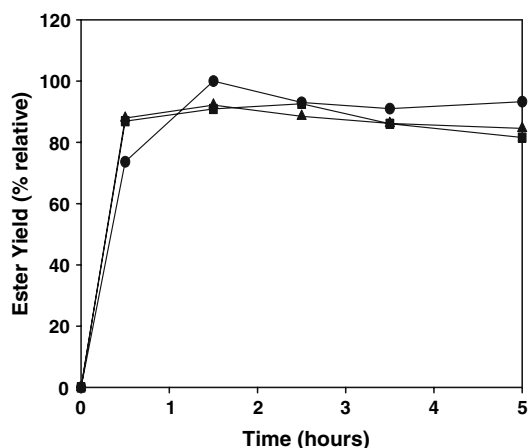
Fig. 4 Time course of FAME production as a function of reaction temperature in reactions containing 5 g of MBM (2.3 wt% moisture), and 1 mL of methanol containing 0.2 N NaOH. Reaction temperatures: *filled circles*: 35 °C, *filled squares*: 45 °C, *filled triangles*: 55 °C. Data presented are the averages of duplicate determinations. Most individual determinations differed from the average by 10% or less

temperature on the reaction. The reactions contained 5 g MBM and 12 mL of 0.2 N NaOH in methanol. The results (Fig. 4) indicated little if any increase in reaction rate or yield at elevated temperatures.

The fatty acid content of the FAME produced from MBM by in situ transesterification under optimum conditions was typical of that observed for beef tallow (Table 2). As seen with DDGS, this indicated that in situ transesterification did not exhibit fatty acid selectivity.

The protein content of MBM as received was 47.5 ± 1.8 wt%. Following in situ transesterification under optimum conditions identified above, it was 47.1 ± 1.1 wt%. Accounting for the loss of mass due to lipid removal during transesterification, this indicated that approximately 10% of the protein in MBM was removed during transesterification. This is relevant to the possible use of the post-transesterification meal in animal nutrition, and should not amount to a detrimental reduction in protein content. The question of nutritional availability of the protein in materials subjected to in situ transesterification remains to be investigated.

Perspective

The conditions defined here as optima were chosen to maximize FAME production while minimizing the use of alcohol and conducting the reactions at moderate temperatures. Other optima could be found, and could involve only partial FAME yields, higher temperatures, shorter reaction times, etc., as desired by the practitioner.

In producing FAME from isolated oils it is important to achieve degrees of transesterification in excess of 98%, in part to reduce contamination of the product by unreacted acylglycerols. Such high conversions are not essential to successful FAME production for fuel use by in situ transesterification, since unreacted TAG do not appear in the ester product. We have consistently found unreacted TAG to remain in the meal. When reaction conditions were chosen to maximize FAME production, residual TAG levels in the post-reaction solid phase were correspondingly low.

Both DDGS and MBM contain free fatty acids, and we have detected FFA at levels of 7–10% of total hexane extractable material in DDGS. Free fatty acids combine with sodium ions to form fatty acid salts (soaps), and thereby reduce the availability of catalyst to conduct transesterification. Evidently, however, FFA levels were insufficient in the materials studied to significantly retard transesterification. As with conventional production of biodiesel from feedstocks containing FFA, the greatest impact of FFA on the conduct of in situ transesterification may be to necessitate, for each batch of feedstock, determination of the amount of catalyst required to neutralize the FFA and any other acids in the material, and to attain sufficient catalyst concentration to achieve transesterification of the glycerides in the feedstock. In general, since DDGS and MBM are industrial products generated at multiple independent operations, one expects variation in their compositions across space and time. The data presented indicate the general applicability of the direct transesterification of these materials, but the optimum conditions for FAME production may vary on a batch-to-batch basis for these feedstocks.

In previous studies with soybeans [4] we have shown that of the glycerol released by transesterification, >90% was found in the liquid phase of the reaction mixture. In that work the glycerol separated from the methyl ester phase upon removal of the methanol. It is likely that released glycerol has a similar fate in the case of the substrates investigated here.

In situ transesterification has now been demonstrated to be an effective method for FAME production from soy flakes [4], DDGS and MBM, three very distinct lipid substrates. Additionally, we have observed that the method achieves FAME synthesis from corn germ and canola (data not shown). It seems reasonable to conclude that this approach is valid for the production of FAME from virtually any lipid-bearing material. This could considerably expand the supply of biodiesel, without impacting edible oil prices. US production of DDGS in 2004 was 7.3 million tons [11]. Assuming a typical oil content of 8.8 wt%, as typically found, this would be sufficient to produce 176 million gal of biodiesel, more than twice current US

annual production. Similarly, the 3.3 billion pounds of MBM produced in 2003 [12] would produce 40 million gallons of biodiesel if all were subjected to in situ transesterification. Sources such as these, and others amenable to in situ transesterification, appear able to greatly increase supplies of biodiesel. Before the potential of this method for FAME synthesis can be realized, the suitability as an animal feed of the meal exiting the process must be determined, since meal sale is a vital component of oil and of biodiesel production economics. We have conducted some studies of this type with soybean meal subjected to in situ transesterification. The results indicated that residual methanol was readily removed from the meal, which then performed well as a component of aquaculture diets for trout [13]. Further studies on the feedstocks investigated here are anticipated.

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