# Lipase Alcoholysis Of Triglycerides To Produce "Tallodiesel" As A Transport Fuel

S Booth, A.J. Long, M.H. Lucas, A. Catalina, BLC Leather Technology Centre

G Williams, Biocatalysts Limited

## Background

The "Tallodiesel" project was a research programme carried out to test the efficacy of enzyme induced biodiesel production from tallow as a potential for the next generation of transport fuel use. The aim was primarily to test the technical innovation, subsequently to assess the economic potential and explore opportunities for application to market.

Biodiesel is an alternative to petroleum-based diesel fuel made from renewable resources such as vegetable oils or animal fats. Chemically it comprises a mix of mono-alkyl esters of long chain fatty acids. A lipid transesterification production process is normally used to convert the base oil to the desired esters and remove free fatty acids. The biggest source of feedstock for biodiesel production is oil from crops or other similar cultivatable material. Plants utilize photosynthesis to convert solar energy into chemical energy. It is this chemical energy that biodiesel stores and is released when it is burned. Therefore plants can offer a sustainable oil source for biodiesel production.

Animal fats similarly contain chemical energy that is released when burned. However they are limited in supply and it would certainly not be efficient to raise animals simply for their fat. Producing biodiesel with animal fat that would have otherwise been discarded i.e. from the tanning industry, could, however, replace a small percentage of petroleum diesel usage and provide an environmentally benign disposal route for this material. Unfortunately the chemical transesterification of tallow is restricted by the high content of free fatty acids. Chemical esterification of FFA liberates water, which may cause hydrolysis and saponification of the fat feedstock, leading to the production of soaps. This will negatively affect the yield of the reaction and the recovery of the biodiesel product.

The UK Rendering Association estimates that over 200,000 tonnes of tallow are derived from animal carcass rendering in the UK domestic market annually. This is in comparison to some 60 million tonnes of tallow/fats globally. This material is either disposed of or sold as a low-grade raw material for industrial application.

This research provided technological proof for the biochemical conversion of low-grade tallow into "tallodiesel" by enzyme mediated alcoholytic transesterification of fats and free fatty acids to alkyl esters. The associated techno-economic evaluation supports the potential roll out of this technology by demonstrating that the process provides a method for an economically beneficial conversion.

**Tallow as a raw material**

In the initial stages of the research the aim was to characterise and formulate the stoichiometry of tallow and emulsified fat sources from a selection of tanning industry production facilities. Although the fats are recovered at the early stages of the leather process the production methods contain variations and the raw material sources are not necessarily common, the biggest difference being between the species of animals processed.

Tallow samples were acquired from four tanneries, three tanneries being processors of bovine material, the fourth tannery a processor of ovine material. The iodine values were as expected for saturated animal fats although the ovine tallow was very low compared to reported values. The saponification values were also similar to reported values. The unsaponifiable matter values of two tanneries were also close to reported values. However, the values for the tallow from the other two tanneries were considerably higher than reported. As expected, the free fatty acid content of all the tallows was high (Table 1).

Table 1 Chemical analysis of industrial tallow samples

| **Tallow source** | **Iodine Value** | **Saponification Value** | **Unsaponifiable material (% m/m)** | **Total free fatty acids (%)** |
|---|---|---|---|---|
| **("W")** | 45.7 | 250.9 | 3.0 | 6.6 |
| **("X")** | 46.1 | 133.0 | 1.1 | 2.1 |
| **("Y")** | 35.1 | 162.6 | 1.8 | 2.8 |
| **("Z")** | 2.6 | 202.6 | 3.8 | 2.3 |

**Enzyme suitability**

There are many potential enzyme materials that could have the potential for fat breakdown but their performance under a number of different reaction conditions is not necessarily suitable. It was therefore necessary to screen enzyme sources, determine the enzyme kinetics and optimise the efficiency of conversion of tallow, thereby determining the suitability of the enzymes as agents for tallodiesel production.

Nine enzymes were investigated for the synthesis of ethyl esters from the sample tallows. TLC analysis of the reactions suggested that most of the enzymes produced esters and that the concentration of triglyceride present (by visual assessment of the triglyceride spot on the TLC plate) was inversely proportional to the concentration of esters formed. The presence and identity of the esters was confirmed by GC-MS. No esters were detected in the treated ovine tallow, suggesting that the activity of the enzymes had been inhibited.

The detected compounds were esters of myristic acid, palmitic acid, 9-palmitic acid, stearic acid and oleic acid, the fatty acids typically associated with bovine tallow (Table 2). Several of the enzymes showed very high apparent conversion efficiencies. The distribution of esters when compared to reported figures for FAME of beef tallow, suggests that the conversion was incomplete. However, it is clear from the data and the observed reduction in solids in the reaction mixtures, that significant conversion of the tallow to esters had occurred.

Table 2 Quantification and distribution of ethyl esters detected by GC-MS following lipase treatment of ("X") tallow. Values calculated from methyl ester standards.

| | **Mass (g) / % distribution of ethyl esters detected in lipase treated samples** | | | | | |
|---|---|---|---|---|---|---|
| **Enzyme** | Ethyl myristate | Ethyl 9-palmitate | Ethyl palmitate | Ethyl oleate | Ethyl stearate | % ethyl ester yield |
| **D** | 0.45 / 5.83 | 0.89 / 11.72 | 3.92 / 51.21 | 1.25 / 16.24 | 1.14 / 15.00 | 38.25 |
| **F** | 0.32 / 5.73 | 0.85 / 15.31 | 2.43 / 44.06 | 1.28 / 23.13 | 0.65 / 11.77 | 27.65 |
| **H** | 0.55 / 4.81 | 1.27 / 11.01 | 3.58 / 31.27 | 2.21 / 19.30 | 3.85 / 33.61 | 57.30 |
| **J** | 0.29 / 5.88 | 0.59 / 11.69 | 2.22 / 43.93 | 1.12 / 22.12 | 0.83 / 16.38 | 25.25 |
| **L** | 0.78 / 4.64 | 1.55 / 9.10 | 4.69 / 27.65 | 2.87 / 16.95 | 7.06 / 41.66 | 84.75 |
| **N** | 0.75 / 4.67 | 1.48 / 9.13 | 4.92 / 30.26 | 2.85 / 17.59 | 6.23 / 38.35 | 81.15 |
| **P** | 0.22 / 4.04 | 0.67 / 12.74 | 1.90 / 36.29 | 0.93 / 17.82 | 1.52 / 29.11 | 26.2 |
| **R** | 0.27 / 5.52 | 0.83 / 17.78 | 1.81 / 38.47 | 0.89 / 18.89 | 0.91 / 19.34 | 23.55 |
| **T** | 0.05 / 4.97 | 0.25 / 24.48 | 0.34 / 33.43 | 0.24 / 23.84 | 0.13 / 13.28 | 5.05 |

Of the nine enzymes tested in this work, only certain types were seen to effectively convert the tallow into ethyl esters under the experimental conditions evaluated. However, under laboratory conditions, using an enzyme offer equivalent to £0.15 per kg of tallow, conversion efficiencies of over 80% were achieved (80 % tallodiesel 8% glycerol, 12% fatty matter residue).

**Fuel Characteristics**

Having characterised the tallow and identified a number of suitable enzymes the subsequent phase explored the chemical and physical properties of the produced fuel to determine its acceptability for application in conventional engines or combined heat and power plants (Table 3).

Table 3 Testing of tallodiesel (laboratory scale process)

| Test | Method | Units | Specification | | Results |
|---|---|---|---|---|---|
| | | | Minimum | Maximum | |
| **Density @ 15°C** | EN ISO 12185 | $kg/m^3$ | 860 | 900 | 870.6 |
| **Viscosity @ 40°C** | EN ISO 3104 | $mm^2/s$ | 3.5 | 5.0 | 5.54 |
| **Flash Point** | EN ISO 3675 | °C | 120 | | 44 |
| **Sulphur Content** | EN ISO 20865 | mg/kg | | 10 | 73 |
| **Carbon Residue** | IP 13 | %m/m | | 0.3 | 0.41 |
| **Cetane Number** | EN ISO 5165 | | 51 | | 50.2 |
| **Total Contamination** | EN 12662 | mg/kg | | 24 | 13 |
| **Acid Value** | EN 14104 | mg KOH/g | | 5 | 10 |
| **CFPP** | BS EN 116 | °C | | | 5 |

The results for the tallodiesel in the initial stages of the research indicated critical aspects of the process that need to be addressed. A flash point of the product that is very low suggests that the tallow has not fully converted and that there is some residual alcohol left in the fuel. In the initial analysis the sulphur content was also high indicating the need to obtain the tallow from green fleshings or the need to incorporate a sulphur removal stage in the production process.

**Mechanics of mixing**

It is apparent that due to interface conditions between the enzyme, the alcohol and the tallow that the mechanics of the reaction, i.e. mixing in the reaction vessel, is of crucial importance. The research indicated that the chemistry works. However, simply scaling up the masses of the reactants is not enough to ensure that the reaction will proceed in bulk. In order to progress the technology to a commercially viable operation it is clear that reactor vessel design is a crucial determinant of feasibility. Effective reactor design may also be a possible means of reducing the amount of enzyme required and hence the cost of conversion. The length of time for conversion is a function of the interface conditions between the reactants. Correct reactor design to optimise the mechanics of mixing indicates potential for significant reduction in reaction times.

**Techno-economic evaluation**

Given the already high cost of transport fuel, tallodiesel is unlikely to be considered as an option unless the costs of production are at least comparable to other fuels. Biodiesel is already favoured with an attractive taxation rate in comparison to conventional petroleum based fuels; however enzymes are traditionally expensive materials that contribute a major portion of the costs of production. The potential costs of biodiesel production in the UK were evaluated. This is an essential aspect of the project, illustrating that a technically feasible approach could be progressed to an economic reality. Fuel taxation rates in the UK will be dissimilar from those in other parts of the world and these must therefore be taken into account when evaluating the economic viability of the process in different markets.

Biodiesel production becomes increasingly economically interesting as the alternative disposal costs rise or as fuel taxation policies promote biofuels. The various disposal routes for animal fleshings in the UK were investigated and the production of biodiesel was found to be of potential economic interest. Landfilling the waste incurs cost. Composting, (where possible), also results in incurred costs but these are lower than for landfill due to the avoidance of landfill tax. By contrast even a medium sized tannery may be able to produce biodiesel from tallow in a profitable manner and larger tanneries should certainly be capable of it.

Although it was anticipated that the residues would only be a minor proportion of the products of the reaction, these require consideration. Exploration of the quantity, character and opportunities for any residue produced was undertaken to identify potential disposal options as these may prove an additional source of income. This potential income steam was discounted in the techno-economic evaluation

**Conclusion**

The project research indicated that enzyme mediated alcoholysis of tallow is a potentially viable route for the production of biodiesel, however it has yet to be undertaken commercially. There is a good indication that the method would provide an ideal route for the disposal of animal by-product and that the added value would result in a no-cost option that has every potential for resulting in profit, provided that correct economies of scale apply.