PRODUCTION OF WAX ESTERS IN *CRAMBE*

Outputs from the EPOBIO project - November 2006
Prepared by Dr Anders S Carlsson
Flagship leaders: Prof Sten Stymne, Dr John Dyer

Full Report: ISBN 10: 1-872691-03-X ISBN 13: 978-1-872691-03-9
Download from: http://www.epobio.net/publications.htm

EXECUTIVE SUMMARY

EPOBIO is an international project to realise the economic potential of plant-derived raw materials by designing new generations of bio-based products that will reach the marketplace 10-15 years from now. At a Workshop held in Wageningen in May 2006 a wide range of experts considered the Flagship theme of plant oils and identified a lubricant product developed in the non-food oilcrop *Crambe abyssinica* as the first target for EPOBIO to consider. This report sets out the conclusion of a detailed literature review and an analysis of environmental impacts and the economic case. It also takes account of inputs from international scientists and industrialists.

Bio-renewables, such as plant-derived oils, are a sustainable means of providing the essential products needed by society. In this context, plant oils are already major agricultural commodities with around 20% by value used for non-food applications. Two plant-derived fatty acids, erucic and lauric acid, have been competing with petroleum alternatives for many years. Historically, cost has been the major bottleneck limiting the development of new plant-derived oils. But, in the context of the escalating cost of crude oil and also the increasing concerns about both finite supply and security of supply, there is an emerging strategic need to develop additional renewable products from plant oils.

This report shows that the production of wax esters for the manufacture of lubricants, from the non-food oilcrop *Crambe abyssinica* can become viable in Europe. Viability would be further enhanced in scenarios where the hulls and meal remaining after oil extraction are used to produce heat and/or electricity for use in the production process. In addition to the economic benefit of using the co-products for bioenergy, this alternative to the use of fossil-derived energy would have the advantage of reducing carbon dioxide emissions and making a contribution to renewable energy targets. The reduced environmental impact of this renewable product demonstrates sustainable production.
Crambe is a low input crop when compared to many other oilcrops that could be cultivated in Europe. This offers potential to reduce the use and hence environmental burden of fertilisers and water. *Crambe* has been chosen as the candidate crop platform for industrial production of wax esters because its oil is not suitable for use in food applications. This is an essential requirement since the manufacture of wax esters in *Crambe* can only be achieved through genetic modification of the plant. The report recommends a gene discovery programme to identify the relevant enzymes for production of the relevant wax esters in high yields in parallel to optimisation of a routinely applicable plant transformation system for *Crambe* and an agronomy programme to achieve a robust, mainstream agricultural crop.

There is existing intellectual property (IP) in the area of wax ester production in oilcrops – this is typical of many applications involving the use of plant biotechnology. Analyses of the existing patent landscape and the opportunity for commercial development of wax esters in *Crambe* will be essential tasks needed to underpin future research and development of this application.

The implications for the use of a genetically modified plant, the impact of current GMO regulations in Europe and the associated substantial regulatory compliance costs have to be considered. Small and medium sized enterprises are unlikely to be able to bear the costs associated with these issues and so future exploitation is likely to be undertaken only by multinationals. Taken together, these constraints have the potential to limit development in Europe and lead to a continuing dependence on imported fossil oil and a continuing loss of competitive advantage to other countries and regions where the cultivation of genetically modified crops is not constrained.

The risks associated with the use of a genetically modified crop can be mitigated in a number of ways. First, the use of a crop which cannot be used for food or feed is important. This is considered essential from a regulatory perspective, given that the infrastructure in agriculture cannot ensure 'fail-safe' separation of different varieties/traits in the same crop species. However, the use of a non-food crop can have negative consequences since oilcrops such as *Crambe* have not been optimised for mainstream agriculture and their oil yield needs to be improved. Second, risks can be further mitigated by the choice of a crop for which inter-species crosses with the closest-related species give sterile offspring. It is not anticipated that *Crambe* will be able to cross easily with its related species. A third means of risk mitigation is the adoption of the same identity preservation practices for the cultivation of non-food GM crops as those already in place for the cultivation of GM foodcrops.

The Common Agricultural Policy (CAP) has decoupled subsidy from production and brought a new emphasis on market forces. *Crambe* for oil production could be grown on maincrop land and also on set-aside land, for as long as that cultivation option is retained in the CAP. The development of a new crop with clear market potential could help underpin commercially focussed farming in the future. Developments such as this will help to create and sustain employment both in the farming sector and in rural areas. In addition, the optimisation of processing an oilcrop for wax ester production will link into opportunities for integrated and zero waste, rural biorefineries further delivering economic benefit in rural areas.

Beyond Europe there is significant potential to develop alternative sources of wax esters. The report notes that current jojoba varieties have scope for improvement in collaboration with developing countries. Collaboration with the US on alternative crops such as soybean could benefit from information exchanged by research programmes on European oilcrops such as *Crambe*.

CELL WALL SACCHARIFICATION

Outputs from the EPOBIO project - November 2006

Prepared by Dr Ralf Möller

Flagship leaders: Prof Markus Pauly, Prof Sarah Hake

Full Report: ISBN 10: 1-872691-04-8   ISBN 13: 978-1-872691-04-6

Download from: http://www.epobio.net/publications.htm

EXECUTIVE SUMMARY

EPOBIO is an international project to realise the economic potential of plant-derived raw materials by designing new generations of bio-based products that will reach the market place 10-15 years from now. At a Workshop held in Wageningen in May 2006 a wide range of experts considered the Flagship theme of plant cell walls in relation to biorefining. They identified the need to improve the efficiency with which lignocellulosic plant cell walls, the most abundant renewable resource on earth, can be converted into sugars and other useful bioproducts through biorefining, as the first target for EPOBIO to consider. This report sets out the conclusion of a detailed literature review and also takes account of inputs from international scientists and industrialists.

Biorefining is the production of chemicals, materials, fibres, products, fuels or power from agricultural/forestry raw materials. First generation biorefineries use simple feedstocks such as sugar, starch or vegetable oil, but second and third generation biorefineries are already in development and will use biomass feedstocks that largely consist of lignocellulose cell walls from plant-based feedstocks. The biorefinery is already recognised to have a key role to play in the production of renewable fuels including bioethanol and biodiesel. Significantly, future generations of biorefineries will be integrated, zero-waste systems producing many bioproducts and materials from a diverse range of feedstocks.

Cost-effective, efficient conversion of plant cell walls into their components is key to realising the full potential of the biomass lignocellulose feedstock. Plant cell walls have evolved to resist breakdown, whether from mechanical or chemical forces or from microbial attack. This resistance to breakdown is a massive bottleneck for the development of second generation biorefineries. Understanding the complexity of plant cell walls and ways in which sugars can be more efficiently released from the walls (saccharification) were considered to be a major priority for EPOBIO.

From a policy and regulatory perspective, the development of efficient and cost-effective biorefineries is important for a number of reasons. Biorefineries can make a positive contribution to the delivery of international targets and governmental commitments for reductions in greenhouse gas emissions whilst also addressing energy supply issues. Innovation directed to the development of new generations of more efficient biorefineries will deliver a major improvement in the level of the greenhouse gas emission reductions achieved. Biorefineries are a key strategy of the Knowledge-Based Bio-Economy (KBBE), delivering renewable and sustainable products able to compete with existing fossil-derived products.

The production of biofuels in biorefineries and reducing dependence on fossil reserves are driven by a number of strategic imperatives including the price, finite nature and security of supply of fossil oil. Other drivers include the detrimental environmental impact of fossil-derived fuels and mineral oils compared with the renewable and sustainable nature of plant-derived alternatives. There are also important regulatory drivers such as the indicative target in the EU of 5.75% biofuels by 2010, a target that is under review with further proposals likely. In the US, policy initiatives include the Energy Action Plan, mandating an increase in the use of bioethanol and biodiesel, and the Advanced Energy Initiative, promoting the development of practical and competitive methods for the production of bioethanol from lignocellulose.

There is also increasing concern about the environmental impact of the expansion of oil palm, soybean and sugar cane cultivation for biofuels leading to deforestation in Indonesia, Malaysia and Brazil. The future development of second generation lignocellulosic biorefineries in Europe and the US affords the potential to track and evidence environmental impacts and benefits, increase biofuel production in those regions whilst, in parallel, addressing environmental concerns about the use of imported material.

In the context of a Common Agricultural Policy that has cut the link between subsidy and production and brought a new focus on the market, biorefineries will provide an additional outlet for the agriculture sector, especially in the newer Member States. Structural funds could readily be used to support biorefinery investment in those countries as well as in less prosperous Objective 1 regions of the EU. New income opportunities are linked to the potential for diversification in agriculture. New commercial markets will not only help the viability of farming but will also encourage sustainability and develop the wider rural economy and infrastructure.

Biorefineries are also highly relevant to policies that aim to support developing countries. Biorefineries in developing countries could readily deliver social and economic benefits through the production of biofuels and energy for local use integrated with bioproducts for export. Clear technical standards would need to be set to ensure the market and supply chains develop on a sound commercial basis.

An important aim of biorefining is to maximise the value derived from the biomass feedstock. The harsh chemical and physical treatments currently used in biorefineries involve a significant energy use and can often lead to a loss of value in bioproducts. New processes that protect the by-products and enhance their value will support wealth creation and add further value to agricultural outputs. For efficient biorefining the component parts of the biomass must be released in a way that protects their value. To minimise input costs biorefineries will also need to be able to use a wide range of feedstocks.

The composition and molecular organisation of plant cell walls vary between feedstocks and are responsive to environmental change. The report identifies that there is a need to develop molecular and analytical tools to characterise the diverse range of biomass feedstocks and, in parallel, design novel high throughput assays for their digestibility. Research into cell wall pre-treatment is also needed. The use of cellulases is fundamental to efficient biorefining and there is a need to further optimise cellulases. Also, novel hydrolases need to be identified to improve breakdown of the complex and highly resistant plant cell walls.

The scale of the work needed is both significant and international requiring multidisciplinary collaboration. A single integrated project spanning the diverse research areas would ensure continuous feedback and a full exchange of know-how and materials.

ALTERNATIVE SOURCES OF NATURAL RUBBER

Outputs from the EPOBIO project - November 2006

Prepared by Dr Jan van Beilen

Flagship leaders: Prof Yves Poirier, Dr Bill Orts

Full Report: ISBN 10: 1-872691-08-0 ISBN 13: 978-1-872691-08-4

Download from: http://www.epobio.net/publications.htm

EXECUTIVE SUMMARY

EPOBIO is an international project to realise the economic potential of plant-derived raw materials by designing new generations of bio-based products that will reach the market place 10-15 years from now. At a Workshop held in Wageningen in May 2006 a wide range of experts considered the Flagship theme of biopolymers and identified a detailed assessment of the potential for new sources of rubber production as the first target for EPOBIO to consider. This report sets out the conclusion of a detailed literature review and an analysis of environmental impacts and the economic case. It also takes account of inputs from international scientists and industrialists.

Natural rubber is a unique biopolymer of strategic importance that, in many of its most significant applications, cannot be replaced by synthetic alternatives. The raw material is supplied either as latex or in dry rubber form. This study has identified three reasons why new alternative feedstock supplies should be developed:

- Increasing evidence of allergic reaction to the proteins in natural rubber obtained from the rubber tree *Hevea brasiliensis* and an immediate need to develop natural rubber sources that do not cause such allergic responses.
- A disease risk to existing supplies of raw material, from *Hevea brasiliensis* that could potentially decimate current production.
- Predicted shortages of supply of natural rubber.

Hevea rubber in latex applications, which include around 40,000 household items, is responsible for moderate to severe allergic reactions. The incidence of those reactions has increased dramatically in the last 15 years and it is now accepted that 1-6% of the general population suffer from latex allergies. Some studies have shown that up to 17% of healthcare workers are at risk of reactions. In the US, the American Society for Testing and Materials has developed a new standard (ASTM 1076-06) for Category 4 natural rubber latex in response to the allergy issue. Previous standards measured physical performance rather than protein content and applied only to *Hevea*-based raw materials. The new standard offers an opportunity for manufacturers looking to develop protein-free high performance latex from other sources. It provides a new level of materials safety for medical product manufacturers and will mean that employers will be able to address these health issues by using alternative products.

The EPOBIO analysis shows that the shrub guayule has greatest potential as an alternative source of rubber that would also meet the protein content requirements of the new ASTM standard. There is a need to develop improved extraction and processing technologies and take forward crop improvement. Two new economic opportunities arise, in the cultivation of the crop and in industrial production of guayule-based rubber products.

The guayule shrub is well suited to the semi-arid areas of Southern Europe and, in the context of the reformed and market-focussed Common Agricultural Policy would offer an alternative production choice in areas such as those currently dominated by cotton production. The EU uses 8% of world production of natural rubber latex, and 14.5 % of world production of natural (dry) rubber. It can be assumed that future guayule lines will produce a yield of 10% natural rubber and since plant biomass is typically 10 tons per hectare per year, natural rubber would be produced from guayule at a rate of 1 tonne per hectare per year. Significantly, current EU demand could be met from 1,205,000 hectares of land. This is equivalent to 9% of arable land in Spain, or 1.5% of arable land in the EU.

Guayule has the additional benefit of being a low input crop with the potential to reduce environmental impact and contribute to sustainable development. Although current varieties could be grown immediately, the species is relatively unimproved and there is potential to improve rubber yield and quality, water use and other agronomic issues. There is therefore a new commercial opportunity for farmers in the short term, as well as the potential to create and sustain employment both in the farming sector and in rural areas in the longer term. This will help maintain and develop the rural infrastructure.
Currently, the existing processing technology and industrial expertise for the delivery of guayule latex and guayule dry rubber is based solely in the US. There is an urgent need for Europe to develop production and processing capability in order to address the potential new market and to avoid the loss of competitive position. We anticipate the development of a new extraction and processing industry using existing guayule varieties will take place in parallel to agronomic improvement of the crop and expansion of its cultivation.

As knowledge of the potential to develop biorefineries grows, there would be opportunity to incorporate the production of guayule latex into integrated, zero waste biorefinery systems. This could provide new income opportunities and further support for rural areas. The current opportunity to develop processing technology in the EU and to improve crops for the EU would also help the economic sustainability of both the agriculture sector and a wider industry.

At the strategic level there is a risk to the existing supply of raw material from *Hevea brasiliensis*. South American Leaf Blight has all but ended *Hevea* rubber production in South America and would have a similar devastating effect if it spread to Asia. Risk mitigation through the early development of guayule production, processing and crop improvement gives Europe an opportunity to establish a platform from which to build quickly in the event of a failure of supply.

The disease risk alone may not give sufficient justification for the development of new, alternative supply and processing capability in Europe. But it should be noted that demand for *Hevea* rubber is expected to exceed supply by 25% by 2020. One reason for this is the replacement of rubber trees with palm trees in order to meet the increasing demand for oil for the manufacture of biofuels, driven by increasing regulatory targets in Europe and the US. Meeting the demand for rubber has strategic importance given its essential use in products such as aeroplane tyres, personal protection products in medical applications, dental equipment and emergency equipment such as intravenous tubing.

This report also identifies a need to examine further sources of supply beyond guayule, for example, from Russian dandelion. This is important in risk mitigation since multiple supply chains are preferable to one alternative source of supply. This brings with it an opportunity to develop crops that could be cultivated beyond the semi-arid regions of Europe and the US. The report recommends that the molecular-based research needed to develop potential alternative sources of supply in the longer term should be put in place.

The expansion of guayule cultivation and processing could readily take place in conjunction with developing countries. The crop is well suited to the climates of many developing countries and cultivation and processing in those geographic regions would also be driven by the shortage of supply issues and growing markets. Collaborative work involving the EU and developing countries should be investigated as a priority.