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Project title: Reducing Food Processing Waste

Instrument: Specific Targeted Research or Innovation Project (STREP)
Thematic priority: 5 - Food Quality and Safety

FINAL SCIENCE REPORT

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EXECUTIVE SUMMARY

The large quantities of food processing co-products produced within the EU globally contain potentially marketable components, such as proteins, polysaccharides and phytochemicals. The economic recovery and up-grade of these components from co-products to provide value-added food-grade commodities has been a long term problem within the EU. The aspiration is generally compromised by their microbiological instability and heterogeneous composition. However, co-product exploitation and waste minimization is vital to sustainable development and to address environmental concerns about waste disposal.
The overall aim of Repro has been to develop advanced and integrated (bio)processing methods to enable total transformation of food-derived plant-based co-products into higher value food and feed products. The underlying drivers have been to achieve this objective whilst avoiding microbiological hazards, ensuring that the co-product processing stream has inbuilt traceability and that the final products comply with existing food regulations and are acceptable to the consumer; (environmentally and socioeconomically). The co-products selected as raw materials were Brewers' spent grain from barley and vegetable trimmings, in particular from brassica vegetables. The Repro consortium comprised 13 partners, selected for their recognised expertise and relevance to the achievement of the project objectives *(farm-to-fork*).

Generic Process Development
REPRO collaborative research was used to develop (enzyme-based) biotools for the controlled deconstruction of co-products. Selected commercially-sourced enzymes had their activity profiles determined to standardise assay procedures and dosing protocols. Recombinant enzymes were also considered, including a cutinase produced within Repro. Biotools were integrated with advanced processing technologies to produce novel and innovative products. Microbial modelling led to the development of protocols which successfully stabilised co-products, minimised microbial hazards during processing and extended the shelf life of the final products.

Three generic processes for plant co-product valorisation were developed:
- Extrusion processing with and without the involvement of bio-processing
- Enzyme-aided fractionation of spent grain to provide routes for protein and functional saccharide products;
- Enzymatic liquefaction or enzyme-aided fractionation of vegetable trimmings to isolate pectins and phytochemicals.

The developmental research explored how the manipulation of the activity profile of an enzyme cocktail can strongly affect the chemistry and macromolecular properties of isolated products; an important consideration when correlating enzyme activity requirements for the selective solubilisation or modification of the cell wall matrix.
Two scenarios were selected from the generic process development to be mathematically modelled using chemical engineering principles. Each model was evaluated for socioeconomic and environmental acceptability *(farm-to-fork).*

Scenario 1: *Production of snacks by extrusion of BSG* evaluated snacks based on whole spent grain and chick-pea flour. Summarised results, reflecting a REPRO-developed "traffic light" code for process acceptability, showed:

Energy:	Highly Acceptable (best-case scenario)
Microbiological Safety:	Safe products
LCA:	Environmentally more acceptable than production of feed or disposal in landfill
Legislative Compliance:	Novel Foods Legislation has to be addressed for spent grain use as an ingredient in products created
Consumer acceptability:	Acceptable; (with reservations)

Cost/benefit analysis: Acceptable; (could be improved by ingredient replacement manipulation)

Scenario 2: *Liquefaction of Vegetable Trimmings* involved the extraction of phytochemical-rich juice and the enzymatic release of specialised pectins showed:

Energy: Energy efficiency is crucial; (Acceptable only in best-case scenario)
Microbiological Safety: Safe products
LCA: Environmentally more acceptable than production of feed or disposal in landfill but enzyme production: energy costs important
Legislative Compliance: Acceptable; due to use of approved methods and familiar food–grade ingredients
Consumer acceptability: Acceptable (with reservations)
Cost/benefit analysis: May be acceptable but some scenarios (processing costs and energy efficiency) are seen as expensive

Overall, the exploitation of spent grain by incorporation into snack products and vegetable trimmings by liquefaction and fractionation are highly positive, with the caveat that compliance with current novel foods legislation is required.

The third bio-process developed; *The Partial Liquefaction of Spent Grain* was not as comprehensively modelled and evaluated. The process showed that economically important components, including peptides, water-soluble antioxidants, and fibre-rich residues could be produced, but a cursory evaluation highlighted the requirement for large quantities of enzymes. This presents a significant energy and environmental burden, which will require further targeted research and development to find high activity and robust enzymes, which will have a bearing on the future for the exploitation of ligno-cellulosic residues through biotechnological approaches, e.g. for bioalcohol production.
The Targeted Exploitation Programme within Repro demonstrated the successful incorporation and evaluation, through feed trials, of spent grain as a protein source in fish feed. This has led to the filing of a patent. Furthermore, the potential to exploit novel fibre products, based on cereal residues, and rheologically-active pectins, has been demonstrated and evaluated in emulsion-based dairy food systems.

In conclusion, REPRO has demonstrated that biotechnology, in conjunction with modern processing systems, has the potential to deliver achievable co-product exploitation. Underpinning this approach is the demonstrated ability to liberate valuable constituents from readily available low-value by-products for commercial and sustainable use. This is perceived as becoming increasingly important and relevant to the industrial exploitation of nutrient-rich co-products in the food chain and to help alleviate building pressures on the economics of a sustainable world food supply. Repro has delivered a user-friendly and cost-effective way to identify and evaluate a range of important risk criteria and the use of a predictive simulation tool facilitates the analysis of costs, benefits and risks associated with exploitation of food-grade processing waste streams (both generic and local).. The introduction of virtual processing scenarios has also shown their advantage to focus where information gaps exist. Hence areas requiring further research can be readily identified.
It is to be recommended that predictive simulation models form an important module in the development of advanced and integrated bio-processing methods and for these models to be adopted by industry, to enable total transformation of plant-based co-products into higher value environmentally friendly and consumer-acceptable products. This should benefit the economic recovery and up-grade of co-products to provide value-added commodities, a long term problem within the EU.

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1 Introduction

1.1 Background

Very large quantities of agro-industrial (food processing) waste co-products are produced in the EU and globally every year. These include in excess of **3.4 million tonnes of spent grain** from the brewing industry, and over **1 million tonnes of vegetable trimmings** from the vegetable processing industry (Eurostat data indicates total vegetable sector waste is in excess of 10 million tonnes; the AWARENET project has indicated that in 2004, the EU agrifood chain activities accounted for 222 million tonnes of waste). These plant-derived waste co-products are known to contain significant amounts of potentially valuable components which remain unexploited waste in the current processes.

Whilst a key underlying driver for upgrading food-chain waste is **environmental concern**, with increasing pressures on food supply to an increasing world population and the search for alternatives to fossil fuels then the successful exploitation of co-products is becoming politically and economically more critical. Waste streams contain valuable nutrients that can be introduced into the food chain through processing and their ligno-cellulosic components can be exploited through biofuel production. Co-product exploitation and waste minimization is vital to *sustainable development*, a concept which originated from the 1992 Rio conference. International commitments on sustainability are now underpinned by demanding *legislative controls* to minimize the impact of waste on the environment and human health. **In the EC, the importance of reducing waste** has been reflected in the stringent legislation, particularly the EC Council Directive 1999/31/EC" (26 April, 1999) to *reduce biodegradable waste disposed to landfill* (35% of 1995 levels by 2016) and the Hazardous Waste Directive (91/689/EEC) *including cereal and vegetable food processing wastes.*

The Euro-legislation is implemented via appropriate national regulations (such as the UK **Landfill Tax**). Across EU member states, the amount of organic waste sent to landfill **will be severely limited**. The impact of the legislation on food processors is being amplified by restrictions on feeding many co-products to animals, and the reduction in animal husbandry across Europe as a result of public concerns' such as dietary well being and safety issues such as BSE.

Hence, despite their valuable constituents, agri-waste co-products have remained a substantial negative cost for the food industry. A sustainable future for many food processors requires that these co-products are exploited so as to prevent them from becoming waste. In an economic climate of rising food prices and forecast food shortages it is foreseen that there will be an increasing political pressure to exploit co-products.

1.2 Previous research and State of the Art

The many **potentially exploitable components** in such wastes include: nutrients and micronutrients (protein, dietary fibre, prebiotics, antioxidants and other bioactive polyphenolics), rheological agents (hydrocolloids, gelling agents, films and coatings), texturised residues, flavours and colourants (juices). These could be utilised in *high-value products (e.g. cosmetic, pharmaceutical, nutraceutical)*, as well as in contributing to *medium value food and feed ingredients*. As a result, there have been numerous attempts, including EC-funded research, to increase the utilisation of waste through the commercial extraction of such components. **However, few waste valorization processes have been developed, and there has been little impact on the overall levels of waste**. This is mainly due to the following factors:

1. Many wastes suffer from **biological, particularly microbiological instability**, resulting in **hazardous, noxious wastes**. Risk Assessments of food waste streams are **rare**, but have been applied to the risk of microbiological contamination and transmission of chemical

residues when fed to animals or composted for application to agricultural land. There is a requirement for the identification of Critical Control Points (CCPs) in any process of co-product exploitation in order to ensure the *safety of workers in developmental research as well as the safety and stability of co-products* for further use in the food or other industries. In addition, there is a requirement for waste streams and co-products derived from them to be stabilised in order to *prevent chemical, enzymatic and microbiological deterioration* during storage, transport and further processing. Again, there is very little research on these aspects. However, food-processing methodology can be applied (such as freezing, chilling, acidification, drying, solubilising in ethanol) as appropriate for the purposes for which the waste streams might be used. The integration of such stabilisation protocols with the CCPs could then eliminate microbiological and chemical hazards. Finally, there is a paucity of knowledge concerning the traceability of many co-products. Meeting these is a prerequisite for creating a sound basis for international exploitation of otherwise unstable plant-based food processing wastes.

2. Most projects have been limited in scope and have not had the scientific and technological know-how to address the immense **complexity and heterogeneity** of biological waste materials; conventional processes are "too crude". There is an increasing awareness that precision enzyme biotools have a role to play in co-product exploitation. Individual enzymes or enzyme mixtures have previously been employed to extract singular components/fractions from agro-food waste and plants in general, **but not as a means to facilitate the deconstruction and upgrading of the whole waste.** For example, the enzymatic processing of proteinaceous waste streams has previously been investigated by many research groups, in several settings and with various starting materials. However, there are relatively few reports on *fully controlled fork-to-farm approaches* for producing plant-waste-derived products (e.g hydrolysates), with potential bioactivity or functionality**.**

3. Economically viable up-grading requires **whole-waste utilisation** and therefore a very high degree of **multidisciplinarity** in the R&D process. Furthermore, the food industry, which is **commodity**-based with **low profit** margins, has not had sufficient investment confidence to exploit the many research findings to develop new processing regimes; this is especially true of SMEs with their lack of scale economies, and attenuates the potential for innovation. However, the national legislation to implement EC regulations on waste disposal and resulting costs are now within the economic and accountancy-based time scale of interest of many companies and many are highly supportive of a serious attempt to address these problems. What is needed is a demonstration that well managed and integrated research can not only develop potential processes to exploit co-products, but can demonstrate that such novel approaches can address the concerns of the different stakeholders. These include economic, environmental, legal and technological issues which all impact on consumer and societal acceptability.

1.3 Aim of REPRO

The overall aim of this study has been to **develop advanced methods to enable total transformation of food-processing-derived plant-based organic waste co-products into high added value food and feed products**.

This has been achieved by focusing the multidisciplinary expertise of a Pan-European team of participants on **4 key scientific and technical objectives:**

1) To develop approaches and procedures as means to obtain ***traceable, microbiologically safe and stable*** co-products for partners during project and protocols for implementation after project;
2) To develop ***precision enzyme-based bioprocesses*** to deconstruct and tailor co-product components*;*
3) To ***integrate the enzyme-based bioprocesses with advanced physical processes***, thereby developing ***hybrid bio-processing systems***;

4) To minimise market risk by:
   (a) ensuring socioeconomic and environmental ***acceptability*** of new processes and products by the ***consumer, retailer, and regulator*** *(fork-to-farm concept);*
   (b) developing sustainable dissemination and exploitation routes via stakeholder interaction platforms, and an international, web-based dissemination platform to link co-product producers, users and process R&D experts.

REPRO has developed *integrated methods* by targeting two high-volume waste co-products which are representative of a wide range of waste streams: **Spent grain** (barley residues from the brewing industry) and **vegetable trimmings** (such as leaf, stem and pod tissues). These co-product wastes are rich in plant biopolymers, phytochemicals, nutrients and micronutrients. Within REPRO applied biochemical, enzymatic and physical processing tools have been developed to achieve the most *cost-effective and safe methods* for obtaining new added-value products. Methods developed for spent grain will be applicable to cereal co-products generally whilst those developed for vegetable trimmings will be transferable to all fruit and vegetable co-products to be exploited in different industrial sectors.

In addition to industry-wide dissemination, **two main specific targets** have been considered for exploitation: **fish feed development**, for which new sources of protein and omega 3 fatty acids will be sought (this seeks to address recent fears of toxicant levels in farmed fish which result from the use of sea-fish-based fish feed) and the impact of extracted components on the stability of food and **model emulsion systems**.

These objectives have **ensured** that methodologies and products / processes derived thereof are fully evaluated in relation to the consumer interests (**"Fork-To-Farm"** approach) and through integration of research with a pragmatic dissemination approach with SME and industrial communication have enabled REPRO to contribute towards creating a competitive and dynamic European knowledge-based economy.

1.4 Approach – WP design and function / Structure of the project

The structure of REPRO is shown in Figs. 1.4.1 & 1.4.2.

Figure 1.4.1: technical scope of REPRO

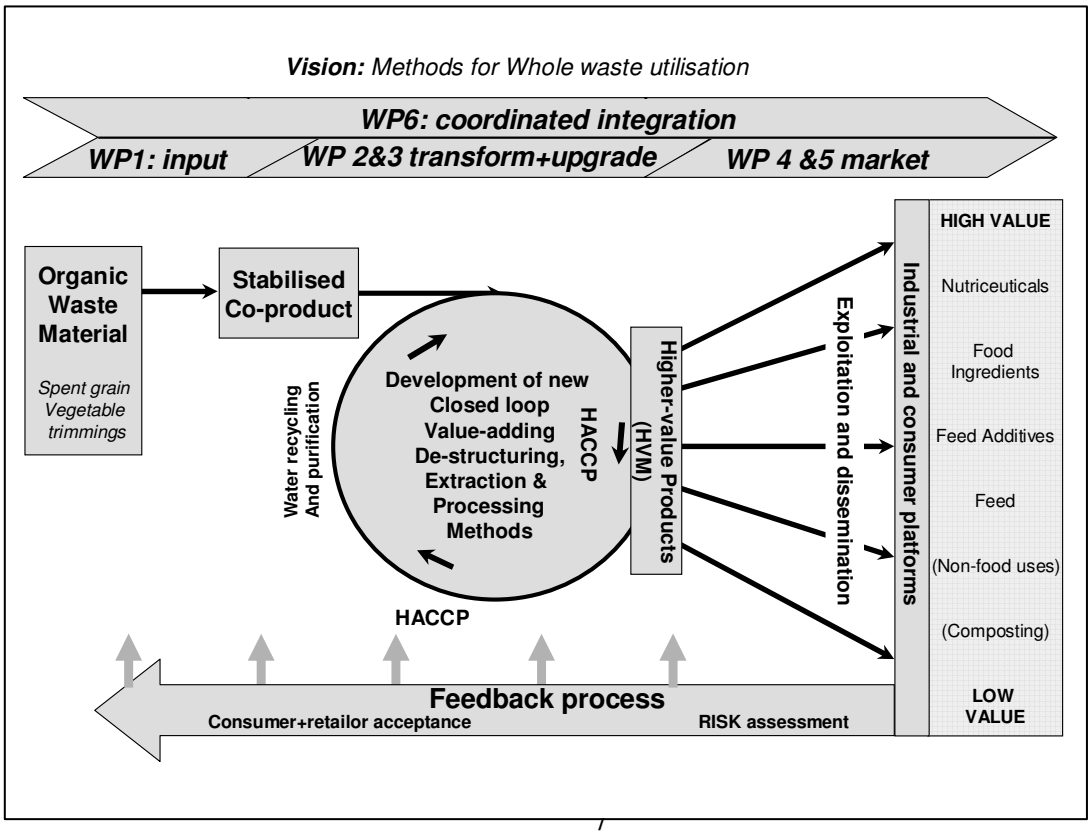
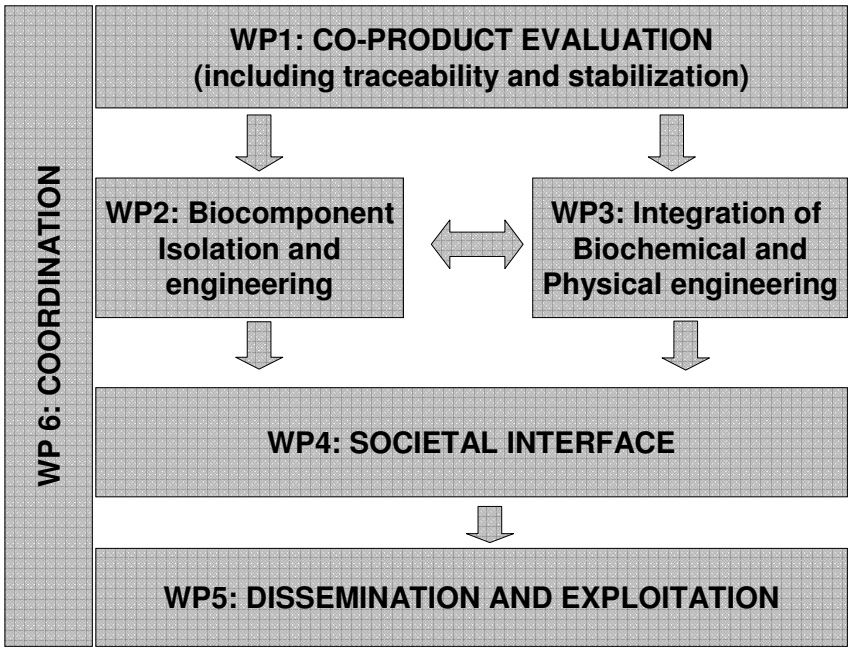


Figure 1.4.2: Organisation and Integration of Activities within REPRO



Summary of Work Package activities:

Development of approaches and procedures for microbiological safety, stability and co-product traceability (***Work Package 1***)***:***

Microbiological and Chemical Risk Assessment: To subject waste streams to a Risk Assessment for chemical and microbiological hazards. This has involved the processes of *Hazard identification, Hazard characterization, Exposure assessment and Risk characterization* following the guidelines of the Codex Alimentarius Commission. Such approaches are commonplace for foods and Risk Assessments are an integral part of **Good Manufacturing Practice** in the food industry.
Stabilisation of co-products: in conjunction with the development HACCP, to evaluate approaches for stabilising co-products;
Traceability and composition of co-products: To evaluate selected co-products and record on a bespoke database.

*Development of precision enzyme-based bioprocesses to deconstruct and tailor co-product components (**Work Package 2):***

To develop *intelligent, enzyme-based toolboxes to precisely manipulate the raw materials for improved product isolation, and tailored techniques for pre- and post-processing of biopolymers for added value functionalities*. A **major thrust of REPRO** has been the integration of these precision biotools with advanced processing methods.

While chemical extraction of plant cell wall hydrocolloids may lead to a high yield of recovery: enzymatic extraction provides a more precise and environmentally-friendly approach, allowing further manipulation of both the residue and the extractable products into such products as sweeteners and/or stabilisers, antimicrobial agents, natural antioxidants, prebiotics or food flavourings and colorants. The complimentarity of **enzymologists** (both protein and polysaccharide

specialists together with **cell wall chemists** in the consortium has facilitated a systematic approach for **tailored manipulation of isolated polymers and residues in conjunction with the extraction of cellular contents including phytochemicals and proteins**. This project thus includes a strong **biotechnology** component (enzymes, enzyme development) and this is important for Europe (which is a large enzyme producer). Crucially, "basic" biotechnology is combined with "applied" biotechnology, and it is precisely this combination what the biotechnology industry needs to make progress.

*The integration the bioprocesses with advanced physical processes, thereby developing hybrid processing systems incorporating closed-loop water-recycling activities (**Work Package 3) :***

To consider a range of appropriate technologies (including those listed below) for integrating biotools (WP2) into processing systems. Where appropriate, to develop the *technologies for the upgrading of food processing co-products*.
To develop engineering models to describe the most promising processing lines.
To use outputs from the models to develop a method for simultaneous assessment of quality, environmental impact and process economy in order to evaluate optimal process conditions under given constraints.
Techologies considered: the following technologies that have *already proven their technical applicability* in fields such as food, chemical and pharmaceutical processing will be employed in the fractionation of biotool-treated co-products, and in the development of novel hybrid processes:

Extrusion-extraction. to combine *efficient extrusion-extraction* process with enzymatic treatment in order to open up the structure of the material to be extracted, or to convert the raw material into valuable components (e.g. peptides, oligosaccharides). Also, the application of extrusion technology as a single processing system for use in the upgrading of waste co-products (effectively exploiting extrusion as a texturizing tool).

Supercritical CO_2 extraction. This is a proven technology for several industrial applications (e.g. decafeination, drug extraction from plants), though not yet used extensively for extraction of value-added components from food processing by-products. This *safe and clean technology* will be used in conjunction with enzymatic pre-treatments for extraction of small molecules (e.g. secondary metabolites, phytochemicals).

Membrane filtration. This is widely used for *water purification* and in the chemical, food processing and pharmaceutical industries. Membrane filtration can be applied in many relevant areas including: product pre-concentration, clarification, sterile filtration and fractionation.

*Ensuring acceptability of new processes and products by the consumer, retailer, and regulator (**Work Package 4**):*

Socio-Economic Risk Assessment: An integral part of REPRO is to *understand the cost-benefits of waste revalorisation processes*. Such economic Risk Assessment helps to ensure that **only those co-products for which there is likely to be an economic market will be produced from the waste streams**. This has been complemented by assessment of specific exploitation routes, involving organisations with expertise in production of feed ingredients. Equally important is the social aspects of producing new food materials and ingredients from substrates that might otherwise be regarded as waste. The BSE crisis highlighted the importance of consumer and retailer acceptance of any food products. Therefore, a **"Farm-to Fork"** principal has been applied in REPRO, such that end user perception of using such co-products in the food chain will be assessed fully.

*Development of sustainable dissemination and exploitation routes **(Work Package 5)**:*
Prior to REPRO there was no centralised information and technology platform that food processors and related waste producers could use to address their excess co-products. Instead, they must rely on *fragmented and incomplete* information from national research laboratories and independent consultants. REPRO has sought to address this by producing **stakeholder**

interaction platforms to enable pragmatic dissemination of REPRO outputs to all interested stakeholders.

Coordination ***(Work Package 6):***
To coordinate the project and ensure that objectives are met.

1.5 Expertise contracted

The consortium (Table 1.5.1) comprises a highly experienced group of **world-class food research establishments and SMEs from the EU and candidate countries**, all of which have considerable experience in attempting to add value to food processing co-products through national and EC funded projects, many of which have been coordinated by REPRO partners.

Table 1.5.1. The Repro Consortium

Role *	Participant No.	Name	Short name	Country	Expertise
CO	**1**	Institute of Food Research	**IFR**	GB	Coordination of REPRO; Microbiology and safety (stabilisation and storage of co-products); enzymology; chemical characterisation of cell wall components
CR	**2**	Agrotechnology and Food Innovations BV	**A&F**	NL	Process development and chemical engineering
CR	**3**	Netherlands Organisation for Applied Scientific Research	**TNO**	NL	Purification of high-value components; evaluation of legislative compliance of new processes
CR	**4**	Institut National de la Recherche Agronomique	**INRA**	FR	Enzymology with reference to vegetable trimmings exploitation and pectin extraction
CR	**5**	VTT Technical Research Centre of Finland	**VTT**	FI	Recombinant technology; development of biotools for cereal residue exploitation
CR	**6**	SIK – Institutet foer livsmedel och bioteknik	**SIK**	SE	Process modelling (mathematical; microbiological); evaluation of environmental impact by LCA
CR	**7**	Agricultural University of Norway	**AUN**	NO	Biotool development (proteases); oversea fish feed development
CR	**8**	Manchester Metropolitan University	**MMU**	GB	Extrusion and snack manufacture
CR	**9**	Fundacion GAIKER	**GAIKER**	ES	Knowledge transfer (website, platform management, newsletter publication); separation technology and expertise.
CR	**10**	Gaziantep University	**Gantep**	TR	Extrusion (in conjunction with P8)
CR	**11**	CSIR Food, Biological and Chemical	**CSIR**	ZA	Development of fish feed (cereal residues) and related products.

		Technologies			
CR	**12**	Wageningen University	**WU**	NL	Socio-economic evaluation of new processes; exploitation of biotools in cell wall degradation.
CR	**13**	Latvian State Institute of Wood Chemistry	**LIWO**	LV	Evaluation of extracted components in food and model emulsion systems.

2. Project Execution

2.1 Introduction

Project execution requires the fulfilment of the Contractual Deliverables associated with Repro (Annex1). These have been organised in 6 Work Packages within the project (see Figure 1.4.2). Technological developments have been pursued using established and innovative methodology. The output from technology development is its integration and evaluation in selected process scenarios, including Life Cycle Analysis, consumer acceptance and regulatory requirements, as described below.

2.2 Technology development

2.2.1 Raw Material Quality: Traceability and stabilisation technologies

2.2.1.1 Traceability of raw material

The traceability of raw materials and methods for their stabilisation (D 1.1, D1.6) were documented as Standard Operating Procedures (SOPs). The SOPs are maintained on the Repro website (www.repro-food.net), under Material reference and stabilisation. The SOPs were developed to cover the available pre-delivery history of supplied materials and to document in a standard format the subsequent stabilisation, processing and sample analysis. This included a standard sample identification; e.g. SGP01001 = spent grain, partner 1, sample delivery number 1 and VTP04001 = vegetable trimmings, partner 4 sample delivery number 1. Stabilisation history includes a microbiological assessment and the preparation of alcohol insoluble residues (AIR). A prototype database (MS Office Access) was developed, to facilitate policing sample traceability during the project. Problems with database transportability between partners limited its use in the project. With hindsight it would be appropriate to have database technology web-based and in place from the beginning of a project.

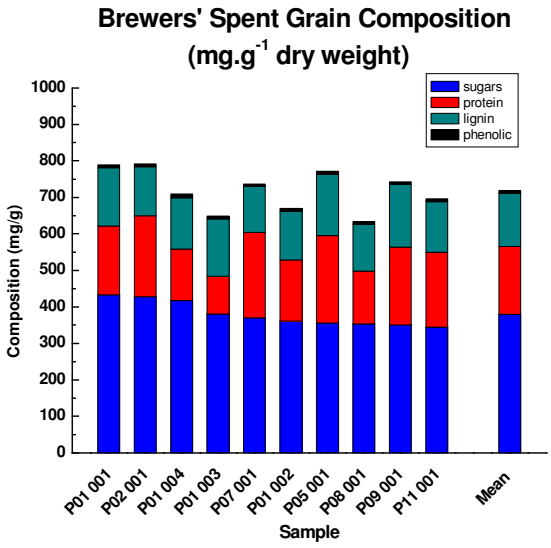
Evaluation of BSG from a range of suppliers

Analyses of supplied BSG for chemical composition (Figure *2.2.1.1*) and microbial ecology (Table *2.2.1.1*) showed that different sources were relatively consistent in composition: ~40% polysaccharide; 20% protein; 15% lignin (D 1.8). Differences in polysaccharide content between breweries could be ascribed to differences in residual starch / limit dextrins in samples (2.5 – 10% sample weight). Protein content also varied between breweries, with BSG from lager producers having the higher protein content. Aerobic thermophiles were the predominant microbes present and in the fresh BSG were within non-hazard guidelines (D 1.2). The screening of BSG samples showed that BSG is a relatively uniform feedstock, with a persistent microflora but a suitable substrate for exploitation provided microbial proliferation can be controlled. This work will be submitted for publication.

Table 2.2.1.1. Representative Microbiological colonisation of brewers' spent grain

Media /Micro-organisms	**Log_{10} micro-organisms/g brewers' spent grain**					
	P01 003	P02 001	P05 001	P08 001	P09 001	P11 001
PCA 20°C Aerobic Mesophiles	2.9 – 5.33	3.59 – 4.43	3.0 – 4.7	3.1 – 3.6	5.9 – 6.12	2.6 – 3.2
PCA 55°C Aerobic Thermophiles	5.33 – 6.6	6.0 – 7.0	2.25 –4.0	2.0 – 2.3	3.96 – 5.3	2.6 – 3.2

Figure 2.2.1.1



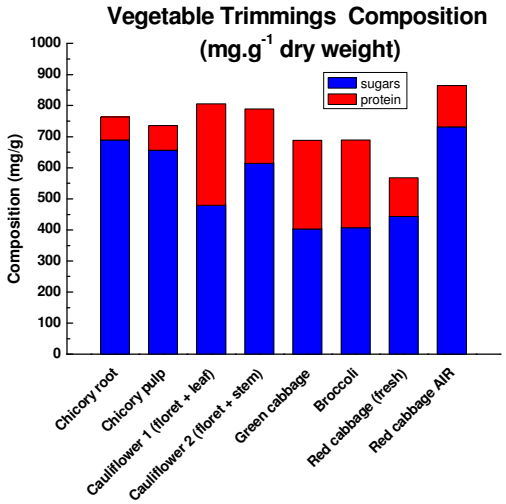
Evaluation of Vegetable Trimmings (VT) from a range of suppliers

Analyses of supplied VT for chemical composition (Figure *2.2.1.2*) and microbial ecology (Table *2.2.1.2*) showed protein and polysaccharides were the major components (D1.8) and pectins are a major component of the polysaccharides. Only traces of lignin or cell wall-bound phenolic acids are present in VT, though VT do provide a potentially rich source of phytochemicals, eg anthocyanins in red cabbage. Composition of VT is dependant on vegetable type and anatomical origin of the VT. Composition is more variable than for BSG, which may affect the exploitation of VT as a chemical feedstock. The apparent quantity of pectin varied from 28% for cauliflower to 44% for chicory roots. Aerobic mesophiles were the predominant microbes present in VT and overall microbial colonisation was much higher than for BSG (D1.2), which again may compromise the successful exploitation of VT unless appropriate stabilisation techniques can be implemented.

Table 2.2.1.2. Microbiological characterisation of Vegetable Trimmings

Media / Micro-organisms	**VTP04 001**	**VTP09 001**	**VTP08 001**	**VTP09 002**	**VTP03 001**
	Chicory		Broccoli and Cauliflower		Red cabbage 1
PCA 20°C Aerobic Mesophiles	6.88 – 7.68	7.66 – 8.01	6.16 – 7	3.86 – 6.18	6.72 – 7.07
PCA 55°C Aerobic Thermophiles	<2.0	2.55 – 3.98	<2.0	<2.0	<2.0

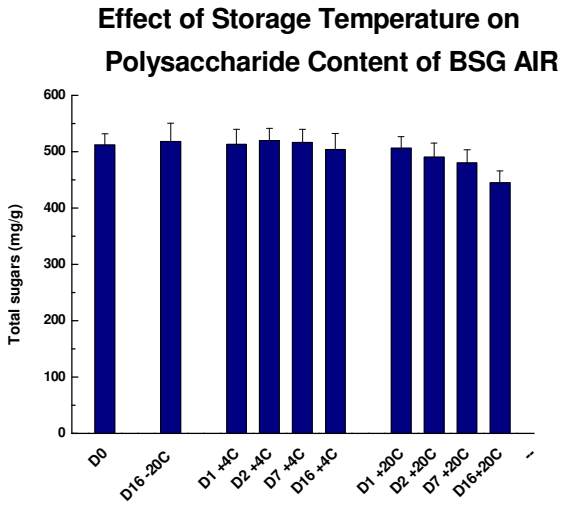
Figure 2.2.1.2



2.2.1.2 Impact of storage on raw material quality

Changes in raw material quality and microbial ecology for BSG and VT were monitored under controlled storage regimens. When frozen or dried there was negligible change in material composition during 16 days storage (D1.7). Storage at room temperature (20^0C) resulted in a loss of polysaccharide, especially residual starch in BSG (Figure 2.2.1.1) and a corresponding increase in microbial colonisation (D1.7; D1.4). This information has been crucial to the development and validation of stabilisation protocols (D1.3 +1.9), identifying critical control points for supply, stabilization and storage of co-products and microbial hazard modelling for novel processes (D3.6). Storage of VT an ambient temperature resulted in rapid microbial spoilage. Two full research papers will be published from the storage work.

Figure 2.2.1.1



2.2.1.3 Traceability and stabilisation technologies - Output.

Screening of BSG and VT for composition and microbial colonisation of fresh material and during controlled storage has identified the importance of stabilisation technologies for the successful processing and exploitation of these raw materials. This has important implications for selecting appropriate techniques to maintain material quality but ensure microbial inactivation during enzymatic and physical processing treatments of BSG and VT (WP2 and WP3).

2.2.1.4 Stabilisation technologies

Various stabilisation methods were investigated for stabilizing brewers' spent grain and vegetable trimmings. Table 2.2.1 summarises these stabilisation technologies and their qualities with respect to the application are given in global terms. This table is based on experiments (reported in periodic partner reports) and general knowledge.

Table 2.2.1. Overview of technologies for stabilisation of vegetable trimmings and BSG.

Technology	Microbial stability	Product quality	Energy use	Energy use (kJ/kg water)	Availability of equipment	Investment costs	Economy
Air (or nitrogen) drying	Good, depending on water activity	Good at low temperatures, medium at higher temperatures	High	5000	Commercially	Low	Good
Drying in superheated steam	Good and product sterilised / pasteurised	Good, no oxidation	Medium, low	3000 / 1500 with heat recovery	Limited / under development	Medium	Medium, good with high product quality
Drying with an adsorbent	Good, depending on water activity	Good, low temperatures possible	Medium	3500	Limited / under development	Medium	Medium
Freeze drying	Good, depending on water activity	Very high	High	Over 10,000	Commercially	High	Low (good if high product quality is wanted)
N_2-freezing	Good	Good, depending of freezing speed en storage. No/small changes compared to fresh	High	Depending on storage time	Commercially	Medium / high	Depending on further processing
Freezing	Good	Good, depending of freezing speed en storage. No/small changes compared to fresh	Medium	Depending on storage time	Commercially	Medium	Depending on further processing
Preservation by addition of acid	Good	Good, other product for further processing	Low / absent	-	Commercially	Low	Depending on further processing
Fermentation by inoculation with sauerkraut juice	Good	Good, other product for further processing	Low / absent	-	Commercially	Low	Depending on further processing

Extrusion as stabilisation technology

Extrusion processing is a high temperature short time (HTST) continuous process with the ability to process high volumes of material at high pressures and high shear rates. The combination of these process characteristics was thought to provide a means of both drying and reducing the microbial load of the BSG in one unit operation. Experimental trials were carried out using a twin-screw extruder as reported previously in the 6 and 9 month periodic reports (MMU). Initial trials highlighted the difficulty in feeding the wet BSG into the screws of the extruder, limiting the feed rates and screw speeds that could be utilised. Reductions in the moisture content were minimal on the first pass through the extruder although significant reductions were achieved after several passes (see Figure 2.2.1).

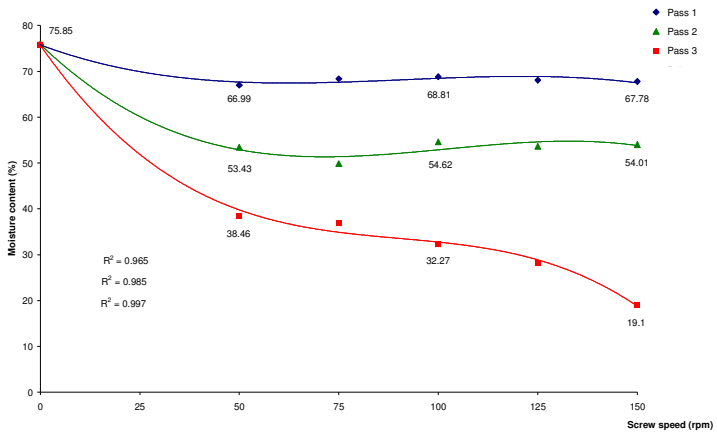


Fig. 2.2.1. Reduction in moisture content of BSG after successive passes thought the extruder at varying screw speeds (0 screw speed represents BSG moisture prior to extrusion)

Microbial loads on the un-extruded BSG were found to be low, of the order of 10^3 cfu/g. A reduction in the microbial load to below 10^2 cfu/g was achieved through extrusion. It was, however, found that on a single pass though the extruder the heat shock could stimulate germination of spores and thus multiple passes through the extruder would be required to ensure microbial decontamination. From the studies undertaken it was concluded that extrusion could be used to stabilise BSG however there were no significant advantages over more conventional drying techniques which may require less capital expenditure.

2.2.2 Enzyme tools

2.2.2.1. Development of cutinase

Cutinases and suberinases are polyesterases, which are able to degrade or partially depolymerize cutin and suberin. Cutinases (EC 3.1.1.74) are frequently produced by phytopathogenic fungi and pollen since they are involved in the disruption of the cuticular barrier of the host plant during the initial stage of fungal infection (Kolattukudy, 1984; Longhi and Cambillau, 1999). The aim in the REPRO project was to screen and produce cutinase originating from a non-pathogenic microbial strain.

Cutinase activity was screened from a variety of different microbes originating from VTT culture collection. *Humicola grisea* cutinase was first cloned and characterized. The cloned *Humicola* cutinase was observed to be too close with the sequence of *Humicola insolens* patented by Novozyme and thus attempts to screen for other strains were carried out. *Coprinus cinereus* and *Trichoderma reesei* were previously found to be able to produce so-called polyesterases having

activity on various polyesters, such as cutin. Cutinase genes from *C. cinereus* and *T. reesei* found with homology searches were cloned and expressed in *T. reesei* under the strongly inducible promoter of the major cellulase gene *cbh1*. The best transformants were selected and out of those the cutinase from *C. cinereus* 09668 seemed to be most promising candidate and it was produced in 20L fermenter cultivation yielding 10 g of purified protein. *Trichoderma reesei* cutinase was also produced in 20 L fermenter.

The novel cutinases were biochemically characterized (Table 2.2.2). CcCUT showed one band in SDS-PAGE with a molecular mass of 22 kDa, which is close to the values (20–25 kDa) reported previously for known cutinases. According to SDS-PAGE, TrCUT has somewhat higher molecular mass (28 kDa). Substrate specifity of the cutinases was investigated using *p*-nitrophenols esterified with fatty acids with varying chain length. CcCUT and TrCUT had higher activity on shorter (C2 – C10) than on longer (C16 and C18) fatty acids. Surprisingly, the activities on *p*-NP acetate (C2) and propionate (C3) were observed to be clearly higher than on *p*-NPB (C4). The C4/C16 ratio of CcCUT and TrCUT was 1.8 and 3.1, respectively. Typically, cutinases have high activity on C2 - C8 fatty acids and the ratio C4/C16 ratio is between 1-4. CcCUT was rather stable at temperatures up to 50°C but residual activity decreased sharply at 60°C. The pH optimum of CcCUT was around 7-8, whereas TrCUT was shown to have two clearly different pH optima (around 4 and 8). Thus TrCUT is suitable for treatments in more acidic range.

Table 2.2.2. Biochemical properties of *Coprinus cinereus* cutinase 09668 (CcCUT) and *Trichoderma reesei* cutinase (TrCUT).

Property		**CcCUT**	**TrCUT**
Molecular weight, kDa (SDS-PAGE)		22 (20.8[a])	28 (25.9 [a])
Length of mature protein (aa)		181	231
Thermostability (pH 5)	T½ 50°C	>20 h (70%)	>20 h (80%)
	T½ 55°C	3 h	n.d.
	T½ 60°C	< 1h	1.5 h
pH stability (20h)	50°C	6-9	4–7
	23°C	4-9	n.d.
pH optimum (with *p*-NPB)		7-8	4 and 8
Activity (nkat mg^{-1})	Lipase	234	88
	CE	0	n.d.

a) theoretical Mw, n.d. not determined

Role of cutinase in hydrolysis of cutin was evaluated using isolated apple cutin as substrate with and without Triton X-100 addition. Both cutinases were able to hydrolyse apple cutin. However, CcCUT seemed to be more efficient in used conditions. When isolated apple cutin was treated with extensive cutinase dosage about 10% of the cutin was solubilized as mono- and oligomers and addition of Triton X-100 did not enhance the hydrolysis level. When 0.1% of hydrophobin II was added into the treatment the hydrolysis levels were increased to 16%. The hydrolysis could also be verified by microscopical analyses.

Table 2.2.3. Treatment of apple cutin with TrCUT1 and CcCUT1 preparations.

Enzyme	Dosage nkat/g	Hydrolysis degree
Ref	0	0.51
TrCUT1	1000	1.21
CcCUT1	1000	2.61
	10 000	10.08
	10 000 + HFBII	7.7
	100 000 + HFBII	16.0

* % of substrate, calculated as stearic acid (284.5 g/mol)

The results on cutinase discovery have been collected to a scientific manuscript: Kontkanen H, Westerholm-Parvinen A, Saloheimo M, Bailey M, Rättö M, Marzia M, Nakari-Setälä T, Buchert J. Novel polyesterase hydrolysing cutin and suberin from *Coprinus cinereus*. To be submitted

2.2.2.2. Development of other enzyme systems

Recombinant feruloyl esterases (E.C. 3.1.1.73) from *Aspergillus niger* (AnFaeA), *Neurospora crassa* (NcFae-1) and *Talaromyces stipitatus* (TsFaeC) were expressed in *Pichia pastoris*, obtaining protein at 10-30 mg quantities. These esterases were used in formulating a minimal enzyme mixture for the solubilisation of BSG.

2.2.2.3. Understanding of activity profiles commercial enzymes

Several commercial enzymes were selected for REPRO projects from different suppliers and their activity profiles were determined, as shown in Table 2.2.3 and D2.1. Activity assay procedures were collated from different partners and standardized dosing protocols were used in the measurements. Based on the activity profiles and hydrolysis experiments the enzymes for the REPRO processes were selected (Table 2.2.4).

Table 2.2.4. Summary of commercial hydrolytic enzymes used in REPRO processes
* activity profile carried out.

Substrate	Enzyme type	used for	Enzyme names	Result
BSG	carbohydrate degrading non-proteolytic enzyme mixture	selective solubilisation of carbohydrates	Depol 740L*, Depol 686, Laminex BG, Spezyme, Econase CE*, Celluclast	Solubilization degree highest with Depol 740, Econase CE selected as no FAE activity present
BSG	proteases with no carbohydrate degrading activity	selective solubilisation of protein	Promod 184, Promod 298, Promod 24, Promod 439, Promod 439, Papain, Neutrase, Alcalase, Bromelain,	Alcalase and the essentially equivalent Promod439 most efficient, Alcalase selected
Red cabbage liquefaction	multienzyme-mixture, pectinolytic activities major components	as complete solubilisation as possible	Rapidase C600, Rapidase liq+, Maxazyme	Rapidase liq+ selected
Chicory root and cauliflower	non-pectinolytic mixture, protease + cellulase	selective solubilisation of non-pectin components	Promod 298L*, 648L*, 184P*, 24L*, Cellulyve *, Neutrase*	Protease 24L + Cellulyve selected

2.2.3 Matrix deconstruction by enzymes aiming either partial or total solubilisation

2.2.3.1. Brewers' spent grain

A number of different approaches were tried to obtain maximum solubilisation of BSG by systematic release of carbohydrates and proteins. Commercial enzymes, predominantly food-approved, screened for relevant activities (Table 2.2.4) (D2.1) were used to release primarily carbohydrates from spent grain (section 2.2.3.1.1) or protein (section 2.2.3.1.2). Results from these studies formed the basis of an integrated hydrolysis model (D2.2, D3.1 and D3.4) (section 2.2.3.1.3.) designed to isolate feruloylated oligosaccharides and peptides from BSG.

2.2.3.1.1. Enzymatic solubilisation of carbohydrates from BSG

Different non-proteolytic enzyme mixtures were compared for their efficiency to solubilise carbohydrates from BSG without concomitant protein solubilisation. The activity profiles of the enzymes were assessed, both as a single point value and as a function of pH. Many of these carbohydrase mixtures were found to contain lower levels of proteases, an activity not present on the manufacturers' data sheets. The treatment times varied from 5 hours to 8 days and temperatures were 40 or 50ºC. The enzyme dosages were adjusted based either on HEC activity (endoglucanase) or xylanase activity. Solubilisation yield after 20 hours treatment with Econase and Econase+Depol was 15% and 20%, respectively. When the treatment was performed for 8 days, the solubilisation yield increased to 20 and 28%, which corresponded 45 and 60% solubilised carbohydrates. From the microbiological contamination point of view these conditions were found to be unacceptable and therefore further treatments were performed for 5 hours at 50°C. In these conditions the hydrolysis yields were in the range of 13-14% (Table 2.2.5). The solubilised fraction composed of about 40% of cellulose derived sugars and about 60% of xylan derived sugars. Depending on the FAE activity present in the enzyme preparation the arabinoxylo-oligosaccharides were either substituted with ferulic acid (Econase) or FA was liberated in the hydrolysis as free FA (Depol 740). Enzyme catalysed solubilization of protein during the hydrolyses was found to be neglible in most cases, maximally amounting to about 3% of the total.

Table 2.2.5. Solubilisation yield (%) expressed as solubilised monosaccharides from the original dry mass under different treatment conditions. Enzyme dosage was 5000nkat/g based on either endoglucanase or xylanase activity.

Enzyme	40ºC/20hours	50ºC/5hours	50ºC/20hours	50ºC/8days
Econase CE	16	13	14	21
Spezyme	17	13.5		
Depol 740		14		
Depol 686		13		
Econase+Depol 740			20	28

The ability of different Depols (740, 686 and 670) to solubilise dry matter and N were further studied either alone or in mixed reactions with Alcalase (running conditions; pH 6.8, 4hours). In all cases Depol740 gave best yields in terms of DM solubilisation (around 2-3% better solubilisation of DM than Depol686 and Depol670 which were at the same level). For Depol740 the degree of DM solubilisation was 22%.

The *Trichoderma* preparation (Depol 686) was most efficient at 50°C in solubilising spent grain at low pH (pH 3-6) while the *Humicola* preparation (Depol 740) was the best performer over the whole range. Profiling of key glycoside hydrolase, esterase and protease activities across this pH range demonstrated that solubilisation of spent grain by *Trichoderma* enzymes corresponded to the range of maximum activities. This was not the case with the *Humicola* enzymes, where maximum solubilisation of the substrate occurred at pH 9.1, at which pH the determined activities were low. Protease activity in Depol 740 over the higher pH range did correspond to high solubilisation, but inhibition of this activity resulted in only a 5% decrease in solubilisation. This suggests an unidentified factor produced by *Humicola* improved the enzyme-catalyzed solubilisation of lignocellulosic material.

The enzymatic solubilization of BSG at pH 6.0 was found to require the cooperative activity of a protease, a xylanase and cellulose-degrading enzymes. Addition of α-arabinofuranosidases and/or feruloyl esterases to this combination did not significantly increase overall solubilization. While co-addition of the three enzymes solubilised 30% of the biomass in 3 hours at 50°C, the same amount as a fungal multi-enzyme preparation, 10% more was removed by sequential treatment of the material with removal of hydrolysis products between each step, irrespective on the order of

addition. Additional activities and possibly other non-catalytic proteins not available within the project, coupled with improved knowledge of recalcitrant structures in lignin-containing materials and the use of high pH, could possibly improve the extent of hydrolysis.

2.2.3.1.2. Enzymatic solubilisation of protein from BSG

The impact of different proteases on enzymatic solubilisation of BSG was investigated by UMB using commercial proteases such as alcalase, papain, neutrase, protamex, bromelain and an in-house hyperstable engineered protease, boilysin. Treatment with proteases alone resulted in up to 77% solubilisation of the BSG proteins. Pretreatment with carbohydrases had small effects on protein solubilisation, but did, under some conditions increase the protein solubilisation yield from 77% to slightly above 80% (Fig. 2.2.2). Alcalase (from Novozymes) and an equivalent sample called ProMod439 (from Biocatalysts), applied at 60°C, were each clearly the most efficient enzymes in terms of protein solubilisation. These optimal results were obtained using pH 8.0 and high Alcalase dosages. At almost 20 times lower enzyme dosages protein solubilisation under otherwise identical conditions was still considerable (64%).

The peptides produced by Alcalase had lower average molecular weight than peptides produced by the less effective enzymes. Gel filtration experiments showed that almost all soluble products had mass below 1000 Da, with an average in order of 500 Da (tetra-hexapeptides). Amino acid composition analyses showed that Alcalase treatment solubilises proline and glutamine (constituents of barley hordein) slightly more efficiently than the other amino acids in BSG. These results are presented in a manuscript which UMB has submitted to J. Agric and Food Chem.

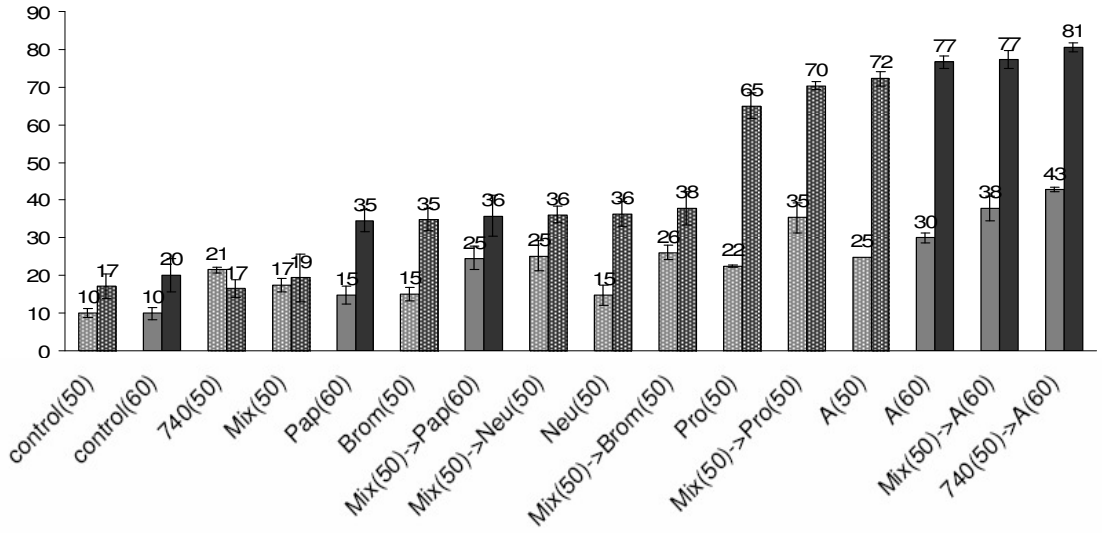


Fig.2.2.2. Solubilisation of BSG (%) with different enzymes. Hydrolysis was carried out at pH 6.8 for 18h. “740” and “Mix” indicate that the material was pre-treated by Depol740 or a mix of carbohydrases. The figure shows solubilisation of % dry matter (lighter columns, left in each pair) and % nitrogen (darker columns, right in each pair). Dotted columns represent reactions performed at 50ºC. Abbreviations; 50 or 60, hydrolysis at 50ºC or 60ºC, respectively; Pap, Papain; Neu, Neutrase; A, Alcalase; Brom, Bromelain; control, no enzymes added.

IFR (P1) also showed that treatment of BSG with the Promod proteases released almost all of the non-cellulosic glucose containing material, which was shown by WU to be composed of low molecular mass and primarily monomeric sugars. This suggests that this material probably derives from incomplete degradation of starch and β-glucan.

2.2.3.1.3. Development of two-stage process for BSG solubilisation

The REPRO project identified four different process models for BSG valorisation and out The process presented in Fig. 2.2.3 was selected after an extensive lab-scale process optimisation procedure, (D2.2), using the proteases Alcalase and ProMod439 and the carbohydrase preparations Depol740 (with FAE activity) and Econase (new name Rohament CL). Hydrolysis protocols were optimised with respect to temperature (affecting both microbial contamination and enzyme speed), pH, enzyme dose, order of enzyme and processing time. The order proteases and carbohydrase did not greatly influence solubilisation. Also, similar solubilisation yields were obtained in one-step (both enzymes acting simultaneously) and two-step reactions. Depol740 was more effective in solubilising dry matter than Econase. The extra solubilisation yield obtained with Depol740 is probably due to the FAE activity present. Based on this extensive lab-scale process development work, and considering the production goal (FAX+peptides+residue), the final process selected for upscaling was two-step process, with (FAE-free) Econase first (> FAX), following by Alcalase treatment for peptide release. These results are described in a joint UMB-VTT –IFR publication.

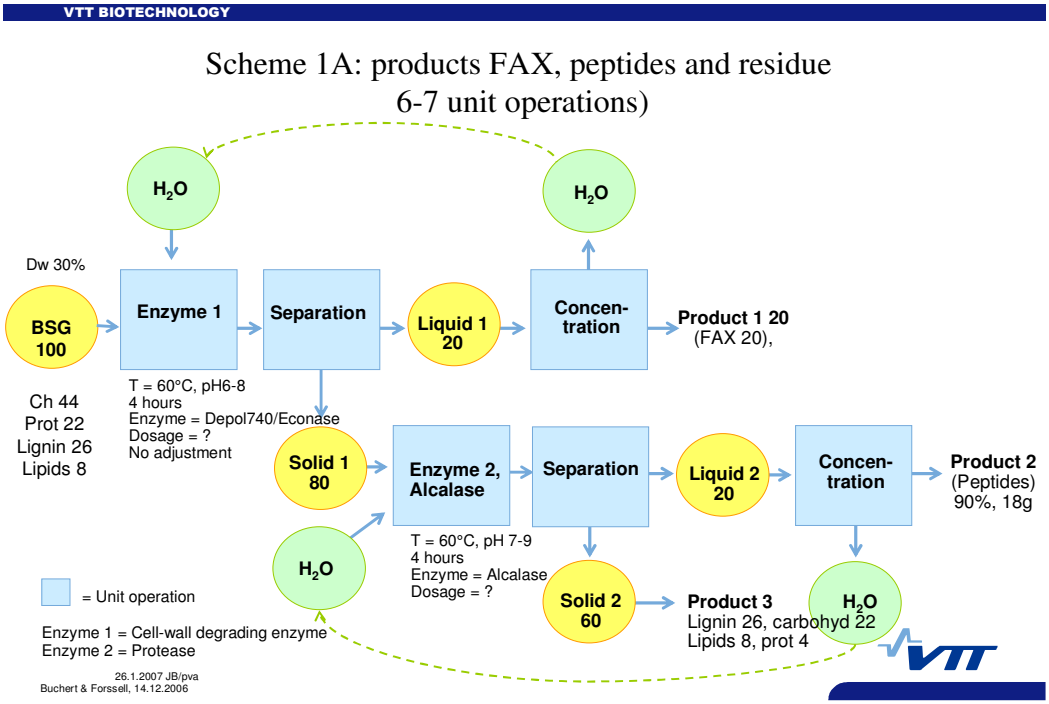


Fig. 2.2.5. Two stage BSG solubilisation process

A subsequent comparative study of hydrolysis of BSG samples from different European Breweries (collected and stabilised in REPRO), aimed at verifying whether the processes described above are generally applicable to European BSG streams, is in progress at UMB and will be concluded for publication.

2.2.3.2. Vegetable trimmings – Extraction of Pectins

Both chemical and enzymatic methods were applied to extract pectins from AIR prepared of chicory roots and cauliflower by-products (D2.3). In the enzymatic stage the enzymes were selected to be devoid of pectinolytic activity with high protease and cellulase activities. Polymeric pectins were solubilised by using a proper combination of enzymes (proteases and cellulases). According to the results of optimization tests performed using different enzymatic combinations

(protease + cellulase) (for more details see 1st, 2nd and 3rd annual progress reports), 2 enzymatic couples, namely Neutrase + Cellulyve and Promod 24L + Cellulyve were demonstrated to be the most efficient for pectin extraction. Laboratory scale extraction of pectins from vegetable trimmings with selected enzymes was performed according to the scheme presented below (Fig. 2.2.4). Modified Hairy Region was also isolated from the treatment.

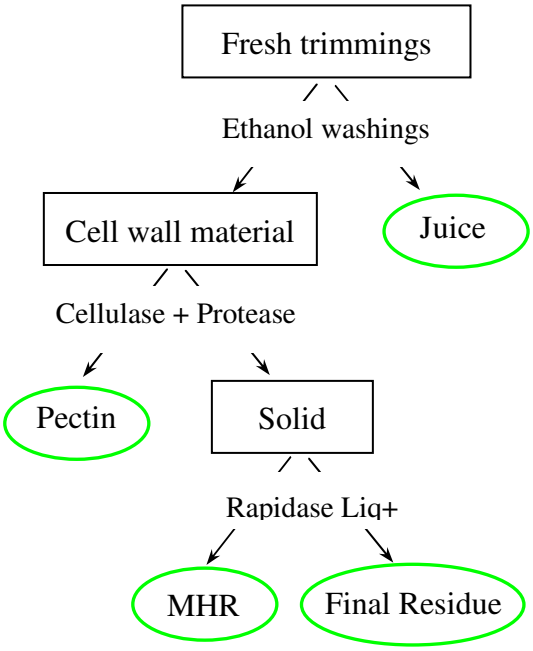


Fig. 2.2.4. Isolation scheme for pectin and HMR isolation from vegetable trimmings

In the case of chicory and red cabbage trimmings, extractions were performed either at 40°C for 16h or at 50°C for 4h. The yields of pectins extracted at 40°C for 16h were higher compared to those obtained at 50°C for 4h. Although sugar composition, DM and DA of pectins extracted in these two different conditions were very similar, their molar masses were considerably different. Indeed, extraction at 50°C for 4h allows for the extraction of high molar mass pectins, which demonstrates that pectins were better preserved in these conditions (for more details, see deliverable D2.4 and 3rd periodic annual progress report). The residues recovered after extraction of native pectins were removed for Rapidase Liq+ treatment in order to produce pectic oligosaccharides, so-called Modified Hairy Regions (MHR).

The association of protease and cellulase allows the extraction of native pectins from all vegetable trimmings. The addition of the PME to the enzymatic mixture allows to extract low methoxy pectins. Moreover, the amount of the residue recovered after pectin extraction can be considerably decreased by the treatment with Rapidase Liq+.

2.2.3.3. Total solubilisation process for red cabbage process

In this research red cabbage trimmings were used in a liquefaction process, where the different options of a possible process were investigated. Red cabbage trimmings have the advantage that, next to pectin, also colorants and glucosinolates can be isolated allowing us to demonstrate that the process developed is rather flexible in handling different types of valuable compounds yielding ingredients for the food industry. The different parts of the trimmings were investigated for their levels in polysaccharides, proteins and anthocyanins and it was found that the compositions did not make it necessary to split the trimmings in separate streams which would have made the pilot scale process much more complicated. Not only the starting material was treated with enzymes

directly, also the residual wastes after pectin extraction (either using chemical or enzymatic extraction) were used. We found that the commercial enzyme preparation Rapidase Liq+ was quite effective enzyme preparations to obtain total liquefaction, since more material with a high molecular mass was degraded. Obviously, such an enzyme treatment will degrade part of the pectin molecules and could be used only when the pectins have been extracted already or will not be extracted at all. In laboratory scale extractions possible end-products and process options were identified and screened.

The results will be collected to a manuscript: Schroot, J, Zykwinksa A, Panouille M, Hinz S, Borge G.I.A., Bonnin E and Schols H.A. (2008) Recovery of valuable compounds from red cabbage trimmings.JAFC, to be submitted.

2.2.3.4. Plant cell wall architecture

The plant cell wall is a complex, multifunctional extracellular organelle which plays an essential role in nearly every aspect of plant physiology, from cell division, tissue extension and support through to ripening and senescence (Brett and Waldron, 1996). The cell wall also provides the basis for exploitation, from structuring agents and fuels through to nutritional and bioactive functionality (Waldron et al., 2003). Primary cell walls are a rich source of polysaccharides, among which pectins, hemicelluloses and cellulose, and proteins. These different cell wall components are organized in interpenetrated networks (Carpita and Gibeaut, 1993).

2.2.3.5.1. Influence of cereal wall architecture on enzyme accessibility

BSG contains flowering glume material, rachilla and lodicule remains, and parts of the embryonic axis, in addition to bran layers, and partially degraded fragments of the endosperm (Figure 2.2.5). In comparison to wheat bran, this makes the composition of BSG physically more heterogeneous and chemically more complex.

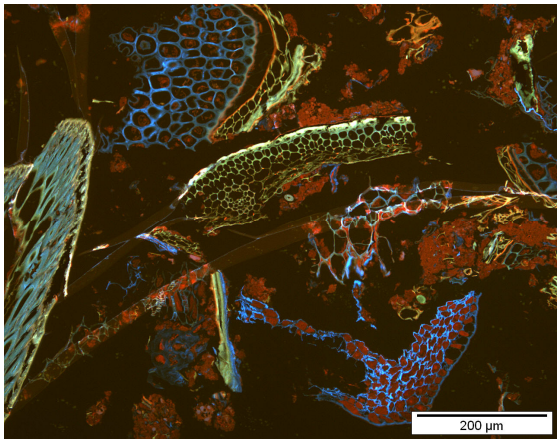


Figure 2.2.5. Confocal micrograph of BSG. The samples are stained with Calcofluor and Acid fuchsin which make cell-wall blue and protein red. Lignified cell-walls appear yellow.

BSG comprises approximately 80% barley cell wall material, which is partly lignified and rich in arabinoxylan polysaccharides and simple phenolic compounds such as ferulic acid (Hernanz *et al.*, 2001; Jay et al, 2007). The remaining 20% is mainly protein. From REPRO, we have found that the non-starch components in BSG gathered from breweries across the EU do not significantly vary, the main difference being the levels of storage polymers, starch and protein, while the apparent lignin content of BSG measured as Klason lignin (14.5%) is higher than previously reported in the literature (4%). The extent of enzymatic solubilisation of BSG was found to depend on whether the substrate was 'wet', i.e. straight from the brewer's mash tun under microbially stable conditions, or had been dried. Dried BSG was the better substrate for the enzymes present in the commercial preparations. Up to 30% of the spent grain biomass was solubilised with multi-enzyme

preparations containing xylanase, cellulase and protease as the main activities. The major limiting factor in obtaining total liquefaction of BSG was the presence of lignin entwined with the carbohydrates. Pretreatment techniques designed to open up the cell wall matrix of BSG, such as extrusion and stream explosion, did not aid BSG liquefaction under the conditions explored in this project, although limited success was found using chemical pre-treatments (Partner 12 2nd Year report). The improved solubilisation with sequential addition of enzymes must therefore either remove inhibitors of enzyme activities or allow enzymes to attack only the insoluble matrix rather than the preferred solubles. A chemical delignification was performed at the end of the project and further work is required to see if the lignin-free material can be substantially broken down by the enzyme cocktails used in REPRO or other activities are still required for the liquefaction of lignocellulosic raw materials such as BSG.

2.2.3.5.2 Influence of dicotyledonous wall architecture on enzyme accessibility

In comparison to BSG, the enzymatic liquefaction of vegetable trimmings went almost to completion. When red cabbage was solubilized by Rapidase liq+, the pectic polysaccharides were broken down to uronic acids and MHR, suggesting that the enzymes present in Rapidase Liq+ contain activities to break down the homogalacturonan segments and part of the neutral sugar side-chains, but not the arabinogalactans and arabinan comprising the side-chains in the rhamnogalacturonans. Pre-treatment of the red cabbage through fine milling rather than steam blanching was shown to be important for enzyme accessibility and subsequent digestion.

Pectins present in primary cell walls of chicory roots and cauliflower were isolated by first enzymatically deconstructing the cellulose/hemicelluloses and protein networks, respectively. The use of cellulases and proteases led to pectin extraction, suggesting that pectins in the tissues examined during REPRO could form close interaction either with the cellulose/hemicellulose and/or protein networks (Panouillé et al, 2006). Combination of protease and cellulase activities allowed the extraction native pectins without modifying chemical structure, contrary to the standard chemical extraction method used during industrial pectin processing. It appears that the same enzymatic digestion process applied to different vegetable trimmings results in the isolation of different amounts of pectins. Moreover, extracted pectins differed by their chemical composition (including sugar composition, degrees of methylation and acetylation) and molar masses. These findings emphasize the important heterogeneity of cell walls, their elementary components and their organisation. Indeed, the presence of at least two pectic populations was hypothesized for pectins isolated from chicory roots: (*i*) one pectin population with long side chains and (*ii*) one pectin population with short side chains. The presence of these two pectic populations can be explained by different entanglement of pectins with other cell wall components. It is thought that pectins with long side chains are more integrated within cell walls due to high entanglement and/or association with cellulose/hemicellulose network and thus, they are more difficult to extract. In contrary, pectins with short side chains may play a role within the matrix and are loosely integrated with cell walls, and thus, they are easier to isolate (Zykwinska et al, in preparation). Compositional analysis of the enzyme-extracted pectins shows that they contain low amount of associated proteins. This protein fraction may constitute further evidence that pectins and proteins are covalently bound in cell walls of dicotyledonous plants.

2.2.4 Extrusion

2.2.4.1. Extrusion of BSG

BSG was incorporated into ready-to-eat expanded chick pea and tarhana snacks and its effects on the textural and functional properties of extrudates have been studied (D3.2). Dried and milled BSG at levels of 10–30% was added to the formulation mix containing wheat flour, corn starch, yogurt powder, onion powder, tomato powder, yeast, dill, mint, paprika and salt. A commercial

sample of BSG was supplied by a local brewery (Manchester, UK). The results obtained from the analysis of the extrudates are discussed in terms of the interaction between the ingredients and effects of processing conditions. The samples were processed in a twin-screw extruder with a combination of parameters including constant feeding rate of 25 kg/h, process temperatures 80–120^{o}C and screw speeds of 150–350 rpm. Pressure, torque and material temperature during extrusion were recorded. The extrudate properties of nutritional and textural characteristics were measured. Image technique investigations provided useful information on internal structure of the extruded products, total cell area, and their contribution to the appearance and texture. It was found that addition of BSG significantly increased protein content, phytic acid and bulk density, decreased sectional expansion index, individual area and total area of the cells. The higher level of BSG resulted in cells with thicker walls with a rougher surface (see 12 month MMU report for chick –pea work and 24 month MMU report for tarhana work).

2.2.4.2. Extrusion of Vegetable Trimmings

The incorporation of cauliflower trimmings into ready-to-eat expanded products and their effect on the textural and functional properties of extrudates have been studied. Dried and milled cauliflower at levels of 5-20% was added to the formulation mix: wheat flour, corn starch, oat flour, egg whites, milk powder, onion powder, tomato powder, carrot powder, dill and mint, paprika and salt. The results obtained from the analysis of the extrudates are discussed in terms of the effect of cauliflower co-products on nutritional and textural characteristics, and the effects of processing conditions. The samples were processed in a twin-screw extruder with a combination of parameters including: solid feed rate of 20-25 kg/h, water feed adjusted to 9-11%, screw speed of 250-350 rpm and process temperatures 80 – 120^{0}C. Pressure, torque and material temperature during extrusion were recorded. It was found that addition of cauliflower significantly increased the dietary fibre and levels of protein in the raw ingredients. Extrusion cooking significantly increased the level of phenolic compounds and antioxidants but significantly decreased protein in vitro digestibility and fibre content in the extruded products. The expansion indices, total cell area of the products, wall thickness all showed negative correlation with the level of cauliflower. Sensory tests indicated that cauliflower could be incorporated into ready-to-eat expanded products up to the level of 10% (see 36 month MMU report).

2.2.4.3. Combination of extrusion and bio-processing

Work carried out in fulfilling D3.1 (as reported in the periodic reports) has shown that Brewers Spent Grain (BSG) can be utilised as a fibre source in extruded snack products. The maximum level of BSG addition during this process has been found to be around 25-30%. Above this level the sensory properties, in particular the level of expansion and degree of hardness, of the product become unacceptable. In pre-treating the BSG with selected enzymes it was hoped that the structure of arabinoxylan and protein would be opened up resulting in increased expansion and reduced hardness.

Initial trials carried out using two commercially available enzyme preparations (one cellulase and one xylanase), to pre-treat the BSG, suggested that treatment with xylanase would improve the level of expansion in the extruded products. A more comprehensive trial was subsequently carried out using three enzyme preparations to act on different components of the BSG, and is documented in the 24 and 36 month periodic reports. The results of the trial were complex and showed all of the enzyme treatments to have effects on the physical properties of the final extrudates, most notably reducing the break strength (Figure 2.2.6) which was perceived as a desirable softening of the product.

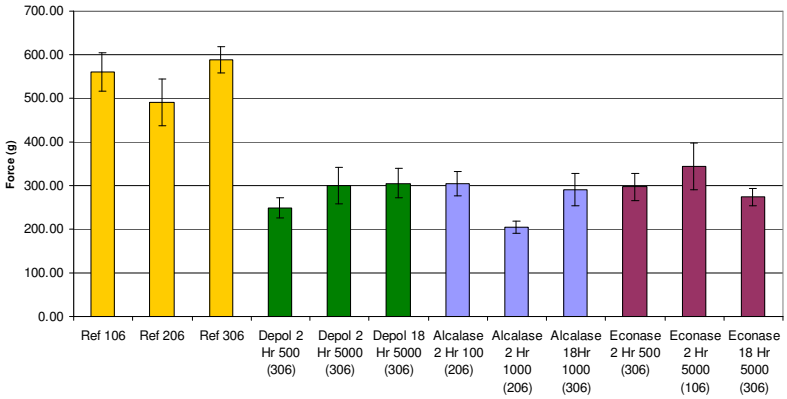


Figure 2.2.6. Effect of enzyme treated BSG on extrudate break strength (Ref 106, 206 and 306 are untreated control samples)

Examination of the untreated and treated BSG samples using scanning electron microscopy (SEM) showed the complex structure and evidence of the effects of the enzyme treatment (Figure 2.2.7). Increases in the digestion times and enzyme dosage did not always result in more pronounced changes in the extrudate properties suggesting that the complex structure of the BSG may limit the action of single enzyme treatments and in order to improve extrusion behaviour significantly multiple enzyme treatments may be required.

The conclusions of the studies undertaken were that enzyme processing of the BSG can significantly improve the extrusion behaviour of BSG although, changes may also be detrimental and thus a fuller understanding of the changes brought about by the enzymes is required in order to tailor the properties of the BSG to the requirements of the extrusion process.

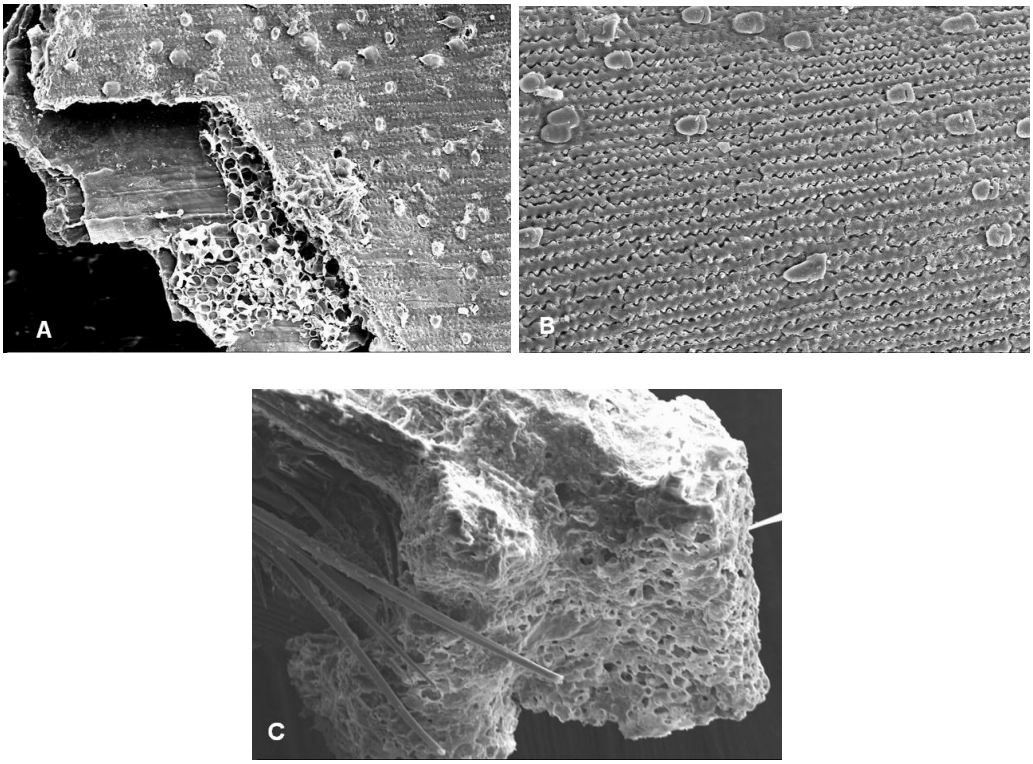


Figure 2.2.7. SEM images of untreated and enzyme treated BSG (A: Untreated BSG x500 magnification, B: Depol treated BSG after 2 hrs x1000 magnification, C: Depol treated BSG after 18 hrs x500 Magnification)

2.2.5. Fermentation of BSG to produce fungal oil containing EPA (C20:5n3)

Different fermentation technologies were investigated by CSIR to produce ω-3 enriched material to be incorporated into fish feed (Deliverable 5.6) to (partially) replace fish oil in the fish diet (Fig. 2.2.8). The fungus used for fermentation has GRAS status. The fungal oil produced by this *Mortierella* fungi is patented (US patent no 6,749,849, 2004) for use in baby formula and is regarded as safer than fish oil, which could contain mercury, organochlorine pesticides and PCB's (Jacobs *et al*, 2004).

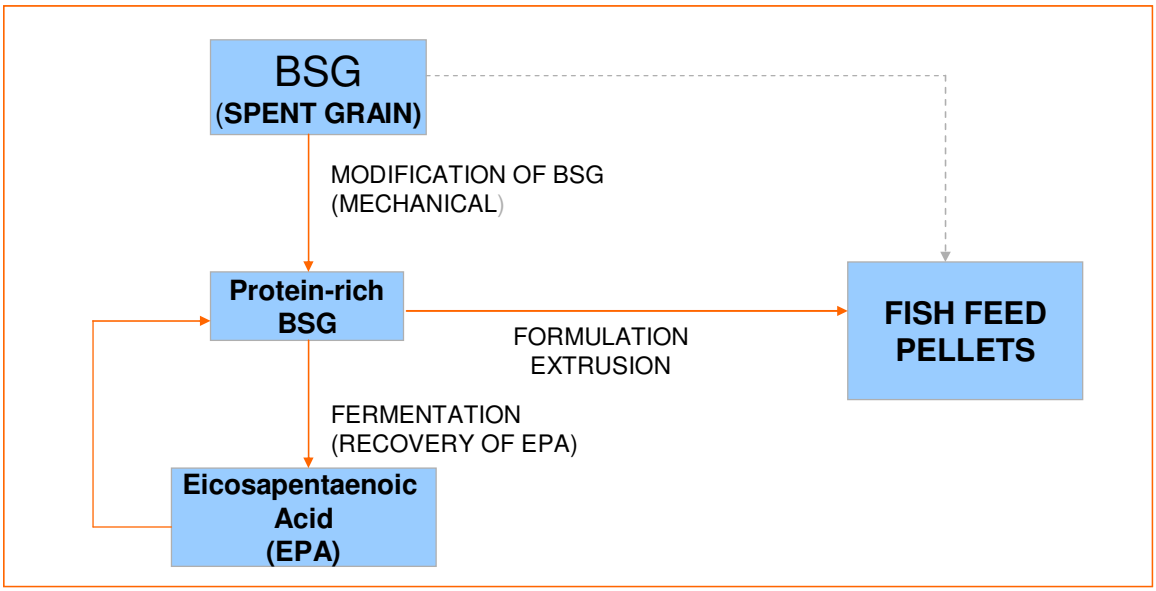


Figure 2.2.8. Flow diagram of the production of EPA-enriched fish feed

The following 3 types of fermentation were investigated. i.e. solid state fermentation (SSF), submerged culture and fluidised bed technology. The production of EPA using solid state fermentation (SSF) of *Mortierella spp.* on BSG was investigated and the highest yield achieved was 3.9 mg EPA/g BSG (with the addition of 10% linseed oil, containing the ω-3 EPA precursor). Submerged fermentation with a synthetic medium (not containing BSG) in shake flasks resulted in higher EPA production than solid state fermentation of BSG. Submerged fermentation using modified protein-rich BSG (obtained from a CSIR patented process, see section 2.4.4) to replace the nitrogen source was then investigated. This application does not utilise large volumes of spent grain waste product, but could provide a cost effective alternative for the production of EPA and other valuable HUFA. The highest yield obtained was 2.1 mg EPA per g BSG added to the medium. Fluidised bed system was investigated in small scale with BSG suspended on membrane filters in fluid filled Petri dishes. Growth of fungi in the BSG yielded 2.3 mg EPA per g of BSG, the highest yield (without linseed oil addition) obtained in any of the fermentation processes. Fermentation technology for the production of EPA-enriched fish feed has potential economical and environmental benefits (see 2.4.4).

2.2.6 Process and product descriptions on selected processes

2.2.6.1. Pilot-scale BSG process

The selected process for BSG solubilization was scaled up using fresh BSG obtained from a local brewery and using their pilot scale facilities. The target products were defined to be a feruloylated arabinoxylo-oligosaccharide fraction, henceforth termed 'FAX', and a peptide fraction. FAX is intended to be used as prebiotic oligosaccharide, peptide fraction could be used as food ingredient

as e.g. bioactive peptide. In the process significant amounts of unhydrolyzed residues are formed which could be used as thickening agent, as a low calorie filling or as antioxidant.

36 kg of wet and fresh BSG was treated first with Econase at 60ºC for 4 hours, from which the soluble fraction was removed and after adjusting pH to 8.5 treatment of solids were continued with Alcalase at 60ºC for another 4 hours, and finally the unsolubles were separated from the liquids. Thus three product streams were obtained; the first solubles (FAX), the second solubles (peptides) and the unsolubles (residue). Process parameters and mass balances are presented in Fig. 2.2.9.

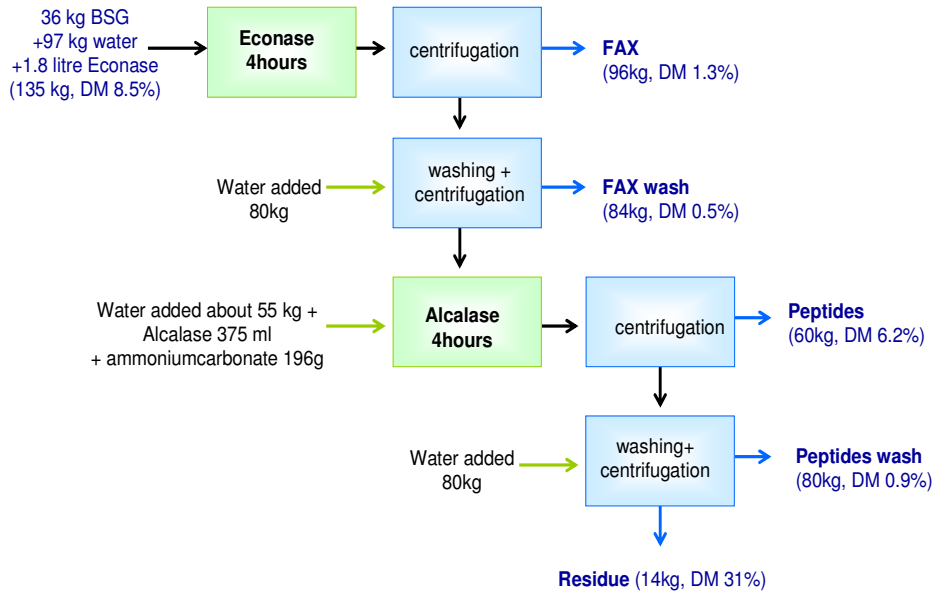


Fig. 2.2.9. Flow diagram of the BSG process in pilot scale

The separation processes of the fractions were also investigated in pilot scale (TNO). In this process different product fractions are produced: desweetened FAX, sugar syrup, peptides and dewatered biomass. For the removal of monosacharides from the FAX fraction an adsorptive separation process is foreseen. The appropriate technology for this separation method is Simulated Moving Bed (SMB) chromatography. A full scale design of this SMB process was made by model calculations. From Table 2.2.6 it can be concluded that the maximum removal of monosaccharides is 92%. The minimum loss of oligo- and polysaccharides is respectively 30% and 45%, resulting in a maximum yield (di-, oligo- and polysaccharides) of 69%. The chosen adsorbent is suitable for the removal of monosaccharides from the FAX fraction, however with this adsorbent a considerable loss of di-, oligo- and polysaccharides (> 31%) is inevitable.

Table 2.2.6 Performance SMB for various SMB sizes.

	Feed [kg/hr]	Product [% of feed]		
Volume SMB [m^3]		4	40	400
Mono	155	52	26	7
Di	85	56	77	93
Oligo	198	57	69	70
Poly	162	55	55	55
% mono's removed	-	48	74	92
Yield [%]	-	56	66	69
Dilution [-]	-	1.9	1.7	1.6
Productivity [$kg/m^3/hr$]	-	3.8	0.45	0.05

The dried FAX fraction was white powder, the dried peptide fraction was dark brown powder and the residue resembled much the original spent grain. 50-60% of the total dry mass was solubilised based on mass balance calculation. More than 80% of the original proteins were released in the process. About 26% from the original carbohydrates were solubilised into the FAX fraction, and the protein content of the peptide fraction was about 55%. In spite of the fact, that fresh BSG was used the degree of solubilisation was very much the same as obtained in lab scale experiments with dried and milled BSG. This confirmed the earlier observation that milling did not significantly affect the degree of solubilisation of BSG. Both FAX and residue fractions showed rather high antioxidative capacity analysed as DPPH radical scavenging activity indicating that they contain high quantity of oxygen active phenolic-OH groups (Table 2.2.7). The lignin characteristics of the lignin fraction were also characterized by LIWC.

Table 2.2.7. Product characteristics. ND= not determined

Product	**Chemical composition**			**Antioxidativity**
	carbohydrates, %	protein/peptides, %	lignin, %	
FAX	84	10	nd	++
Peptide	7	55	nd	?
Lignin residue	70	5	21	++

The protein fraction was also analyzed with respect to it molar mass. The molar mass of the peptides was found to be in the range of 0.1-5 kDa. The result is similar to that obtained in small scale. The second wash water was also found to contain a considerable amount of peptides (13% of the total amount of solubilised peptides) and thus is potentially valuable.

2.2.6.2 Red cabbage process

The red cabbage process was also tested in pilot scale using using Neutrase, Cellulyve, and Rapidase Liq+ as enzymes (Fig. 2.2.10). The yields and physicochemical properties of the pectins obtained by both chemical (pectin A) or enzymatical (pectin II) processes were rather similar to the pectins extracted on lab scale (section 2.2.3.2) The MHR had typical characteristics as found before although slight difference in arabinose and galactose levels were observed (Table 2.2.8). MHR is expected to be suitable as a soluble soy polysaccharide fraction having rather similar structures has been described to have good food stabilizing properties (Nakamura et al., 2001). Oligouronides could be useful as immunomodulating or anti-bacterial binding agent within the colon of humans (Stahl et al, 2003).

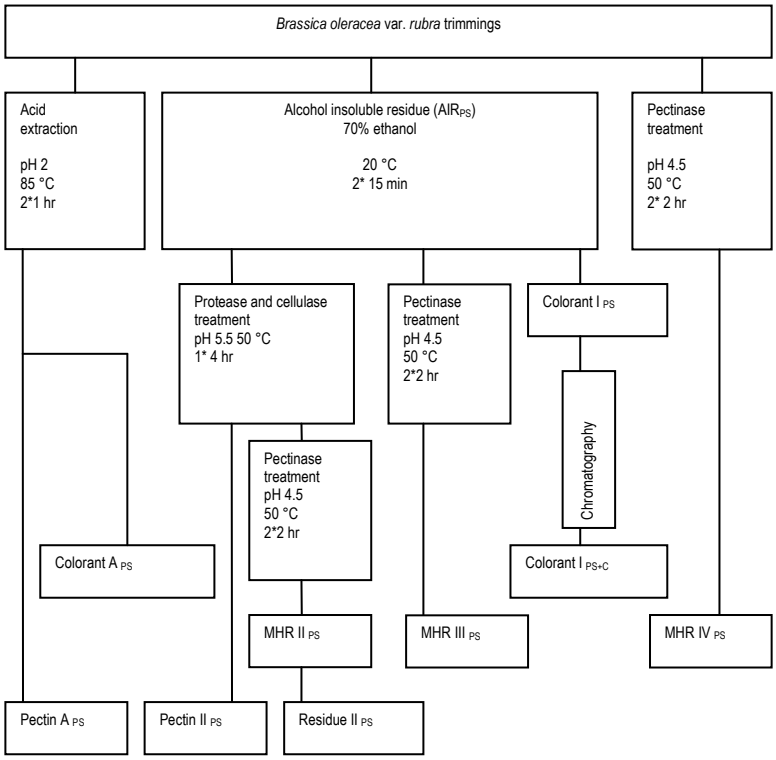


Fig. 2.2.10. Red cabbage process

The present study shows that red cabbage trimmings can be converted into valuable products with an industrial perspective. The insoluble carbohydrate rich fraction can be used as such or it can be treated with green labelled enzymes in order to solubilise either pectin or modified hairy regions (MHR). The soluble colorant rich fraction can be used as a food ingredient after removal of the cabbage flavour via an adsorptive chromatographic separation with 90% recovery.

Table 2.2.8 . Sugar, protein, ash and anthocyanin contents and weight-average molar masses (kDa) of red cabbage trimmings, AIR, pectins, modified hairy region (MHR) and colorant obtained at pilot scale.

Sample	Yield [%]	[mol%]								Total sugars	Protein	Ash	Antho-cyanin	Total	MW
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	GalA	$mg.g^{-1}$	$mg.g^{-1}$	$mg.g^{-1}$	$mg.g^{-1}$	$mg.g^{-1}$	kDa
Trimmings	100	1	0	5	3	5	4	63	19	357	118	74	7.7	643	ND
AIR $_{PS}$	37.8	2	1	12	5	3	6	38	34	459	133	74	0.4	898	ND
Pectin A $_{PS}$	6.1	3	0	7	2	1	10	13	64	415	154	147	0.3	716	20,100, 500
Color. A $_{PS}$	51.5	1	0	6	1	4	4	56	27	364	170	110	2.3	693	ND
Pectin II $_{PS}$	8.3	2	0	8	2	1	6	15	65	167	41	353	<0.1	869	20, 100, 500
MHR II $_{PS}$	22.0	2	0	10	3	3	4	57	21	328	108	217	<0.1	739	5, 10
Residue II $_{PS}$	6.8	1	1	6	7	5	4	49	28	401	206	78	<0.1	838	ND
MHR III $_{PS}$	24.7	3	1	14	4	3	6	32	37	321	80	192	<0.1	781	5, 20, 50
MHR IV $_{PS}$	3.2	10	1	10	5	6	16	13	39	299	149	41	14	695	5, 20, 50
Color. I $_{PS}$	62.2	1	0	1	0	8	2	82	7	524	109	73	4.5	747	ND
Color. I $_{PS+C}$	2.9	0	0	1	1	1	1	76	21	301	54	5	125	562	ND

ND, not determined; Color., colorant
* Yield is expressed in % of dry trimmings
PS – pilot scale, PS+C – pilot scale after chromatographic treatment

2.2.6.3. Technical / functional characteristics of the products

2.2.6.3.1. BSG products

The chemical composition of BSG shows that the presence of ferulate offer considerable scope to modify the cross-linking of feruloylated polysaccharides and hence the mechanical properties of these residues. In solution ferulic acid (FA) can be readily polymerised by horse radish peroxidise, but when esterified to a polysaccharide the profile of diferulates becomes restricted. This situation also exists *in muro* and suggests structural constraints may limit the availability of FA for cross-linking. At relatively low polysaccharide concentration, (~3%), steric restrictions were apparent in gels prepared using isolated polysaccharides. Mechanical properties such as swelling also appear fixed at these relatively low polysaccharide concentration(s). This limits the potential to modify mechanical properties *in muro* through oxido-reductase activity.

The impact of the isolated BSG fractions on colonic bacteria was measured. A fermentation experiment was performed using fecal inocula. To see differences between the 'untreated' original BSG and econase/ alcalase treated BSG, the original BSG was also fermented in the same way as the econase/ alcalase residue of BSG. The fermentation of the original BSG and econase/ alcalase residue of BSG was followed during 144 hours by analysis of the optical density at 600 nm, pH, HPSEC, total sugar content, HPAEC, and SCFA and lactate analysis. The faecal bacteria were unable to use the residual carbohydrates of the lignin fraction. It is assumed that the residue of BSG after an enzyme treatment with econase and alcalase consist of a complex of carbohydrates, protein, and cutin covered with a lignin 'layer'. Thus, the lignin fraction could be used as inert bulking agent increasing the dietary fibre content of the product. The relatively high antioxidativity of the fraction implies its simultaneous suitability as technological antioxidant.

2.2.6.3.2. Vegetable products

The functional properties of the isolated pectins were analyzed (Table 2.2.9). Native pectins extracted with protease and cellulase were characterized by DM>50. Therefore, their gelling properties were studied in the presence of sucrose at acidic pH. When a PME was added to protease and cellulase mixture, the DM of pectins was decreased (<<50), which allows to study gelling properties of Low Methoxy pectins in the presence of calcium. Pectins with high DA and protein content (chicory, cauliflower pectins) were tested for their emulsifying properties in the presence of rapeseed oil (for more details, see the 3rd periodic annual progress report). It was observed that native pectins formed weak gels in the presence of sucrose at low pH. Demethylated pectins (or low-methoxy pectins) were therefore able to gel in the presence of calcium at pH 6.

Table 2.2.9. Summary of the functional properties of pectins

Pectin	Functional properties
Chicory[a]	Emulsifying/stabilising
Chicory[b]	Weak gel/sucrose (Extra Slow Set)
Chicory[c]	Weak gel/calcium (Ultra Rapid Set)
Citrus[b]	Strong gel/sucrose (Medium Rapid Set)
Citrus[c]	Strong gel/calcium (Rapid Set)
Cauliflower[b]	Emulsifying/stabilising

a: Neutrase+Cellulyve 40°C 16h
b: 24L+Cellulyve 50°C 4h
c: 24L+Cellulyve+PME 50°C 4h

Pectins (isolated from red cabbage trimmings by acid hydrolysis) were also used as emulsifiers for obtaining of oil-in water emulsions (70:30 by volume). It was demonstrated, that the emulsions viscosity and stability is strongly pectin concentration dependant (D5.5). At pectin concentration ≥8% in the water phase emulsions were stable during several weeks in weakly acidic (pH 3,5-4,0) media. Highly viscous (butter-like) and storage-stable emulsions were obtained using water-soluble pectin fractions. The emulsions were not influenced by presence of electrolyte (NaCl) at concentrations 0-2% in the water phase. Thus, red cabbage pectins can be used as comparatively effective emulsifiers for oil-water systems. The red cabbage pectins were also used as thickener for milk products. Milk fermentation process by yogurt culture (*Streptococcus thermophilus and Lactobacillus delbrueckii subsp.Bulgaricus)* was not affected by pectin addition but some casein sedimentation was observed at distinct stages of the process. Due to the low viscosity of the red cabbage pectins the used pectin concentrations were higher than citrus pectins. Red cabbage residue, obtained after enzymatic treatment of trimmings, possessed high water and yogurt whey retention value (180-190%) and, introduced in yogurt in quantity till 8%, did not induced casein sedimentation increase the product viscosity and can be used as thickener for the sour milk products.

One of the applications of low Mw pectin fragments could be the use as prebiotics to stimulate a healthy colon microflora in human. Fermentation was carried out using fecal inoculum. Fermentation was monitored by bacterial growth, utilisation of oligo- and polysaccharide material and the production of short chain fatty acids. The results show clearly that low Mw pectic material isolated from red cabbage is not readily fermented by colonic bacteria. The oligomeric fraction obtained as filtrate after ultrafiltration of the liquefied material, however, is readily fermentable. However, in addition to oligogalacturonic acid(s), this fraction contained a large proportion of

glucose, which will not reach the colon and will be taken up in the small intestine. The results also showed that a high amount of butyric acid was formed during fermentation of this fraction, but there was no increase of butyric acid after glucose was completely degraded. Mainly lactic acid was formed after glucose degradation. All together these results suggest that the oligomer fraction might be used as a prebiotic after glucose removal. However, the presence of any positive health effect of this fraction is not clear at this point, since no butyric acid seems to be formed.

2.2.7 Discussion

REPRO project developed three different processes for plant by-product valorisation, i.e. an enzyme-aided fractionation of BSG, total enzymatic liquefaction of red cabbage or enzyme-aided fractionation procedure for different vegetable trimmings to isolate pectins or MHR. The chemistry and macromolecular properties of the isolated products was strongly affected by the activity profile of the used enzyme mixture. Valuable information on correlation of plant cell wall chemistry to the needed enzymes for hydrolysis of the matrix was obtained in REPRO project.

Up to 30% of the spent grain biomass was solubilised with multi-enzyme preparations containing xylanase, cellulase and protease as the main activities. The significant part of the carbohydrates remained in the unhydrolyzed fraction. More research is still needed to get insight to the chemical composition as well as location of these carbohydrates in the hydrolysis residue. Protease treatments can digest the majority of endosperm protein present in BSG. All fractions obtained from BSG, i.e. the FAX, peptide and also unhydrolyzed lignin enriched fraction have potential use as food ingredients. BSG was also found to be suitable fermentation subtsrate for fatty acid production as well as to be incorporated into different snack recipes to increase their dietary fibre content.

Red cabbage trimmings can be converted into valuable products with an industrial perspective. The insoluble carbohydrate rich fraction can be used as such or it can be treated with green labelled enzymes in order to solubilise either pectin or modified hairy regions (MHR). The soluble colorant rich fraction can be used as a food ingredient after removal of the cabbage flavour via an adsorptive chromatographic separation with 90% recovery.

In addition to the process development, novel enzyme tools were identified and the role of these enzymes in biomass valorisation remains to be studied in future projects.

2.3 General Methods

Methods for use within Repro were generally adopted from existing partner use and expertise. Emphasis was placed on the documentation of methods as SOP so that traceability of results was guaranteed and also that the methodology was readily transferable to other operators, either within the consortium or associated with the stakeholder platform activities.

2.3.1 Procedures for microbiological safety, stability and co-product traceability (WP1):

2.3.1.1 Documented Standard Operating Procedures (SOP) – Traceability SOP were prepared for source and history of waste materials (D1.1). The SOP documentation, maintained on the Repro website, was completed on delivery of each sample. Each material used was supplied as currently available from commercial sources of BSG and VT. BSG samples were obtained from large scale breweries and micro-breweries and included producers of lager-type beer and ale-type beer. Vegetable Trimmings (VT) obtained were from commercial sources of vegetable processors, as food-grade trimmings.

2.3.1.2 Documented Standard Operating Procedures (SOP) – Composition Analysis and Microbial Ecology

On delivery, samples of material were assessed for microbiological risk, using selective media to enumerate resident microorganisms (D1.2), and requirements to maintain microbial stability during storage and processing (D1.4 & D1.5). Samples were also stabilised as alcohol insoluble residues (AIR) for chemical characterisation (D1.6), as detailed in the respective SOP and maintained on the website.

SOP for quantifying constituent chemical components were prepared for:

- Klason Lignin
- Quantification of soluble phenolic acids by colorimetry
- Quantification of constituent sugar sugars in polysaccharides by acid hydrolysis and GC
- Uronic acid content by colorimetry
- Starch quantification by enzyme digestion and colorimetric determination of glucose
- Mixed-linkage β-glucan quantification by enzyme digestion and colorimetric determination of glucose
- Determination of degree of methylation and acetylation of polysaccharides by titration
- Quantification of constituent cell wall bound phenolic acids by HPLC
- Quantification of constituent flavonol glycosides from vegetables by colorimetry
- Protein by kjeldhal digestion and determination as Total Nitrogen x 6.25

Methods used for analysis are routine procedures for screening samples such as BSG and VT and thus can be considered to provide the baseline traceability reference for present and future sample structure and composition characterisation. The traceability baseline also provides the reference against which to evaluate the consequences of subsequent sample treatment activities (D1.8).

2.3.2 Development of precision enzyme-based bioprocesses to deconstruct and tailor co-product components (WP2):

2.3.2.1 Enzyme source

Commercial enzymes chosen for evaluation (D 2.1) were sourced based on them being predominantly approved for food use by the manufacturers. Although Alcalase, the protease preparation used in the processing steps of BSG, is not food approved, it is used in REPRO for proof-of-concept studies to identify the type of protease activity and mode of action required.

Fungal cutinases were cloned and expressed in *Trichoderma reesei* using standard molecular biology protocols. The enzymes were produced in a laboratory-scale fermenter, and purified by immobilized metal affinity chromatography. Recombinant esterases, already available in the

consortium at the beginning of the project, were obtained by expressing fungal genes encoding the required feruloyl esterase under control of the methanol promoter in the yeast *Pichia pastoris*. These recombinant enzymes were for research purposes only and not meant for inclusion in food-grade materials.

2.3.2.2 Enzyme activity profiling

Activities of multi-enzyme mixtures and purified enzymes were determined through specific assaying against a model substrate, following previously published assay conditions for each activity. For example, xylanase activity was assayed against 1% (w/v) wheat arabinoxylan (Megazyme) or Birchwood xylan; endoglucanase activity was assayed against Whatman filter paper; □-glucosidase activity assayed on p-nitrophenyl-□-glucopyranoside; polygalacturonase ("pectinase") activity assayed against orange peel polygalactuonic acid; rhamnogalacturonan hydrolase was assayed against sugar beet rhamnogalacturonan; protease activity was assayed against haemoglobin, casein or dye-linked casein; pectin methyl esterase assayed against lime pectin; feruloyl esterase activity assayed against methyl ferulate (Apin Chemicals); and cutinase assayed against apple cutin. For examining activities for processing spent grain and leafy tissues as in Deliverable 2.2, activities were determined under the experimental pH and temperature conditions, and added to the reaction mix. A four hour incubation period at 60^{o}C was chosen to ensure microbiological stability and downstream food processability rather than the addition of chemical anti-microbial agents which may not meet legislative approval.

2.3.2.3 Enzyme activity optimization

The behaviour of the selected mono- and multi-component enzymes in the digestion of spent grain and leafy tissues, with respect to temperature, pH and time of incubation, were followed by altering one variable at a time and measuring the effect. This data provided the optimum conditions used in the liquefaction studies in Deliverable 2.2, proteolytic modification studies in Deliverable 2.3 and 2.4, and preparation of low methoxy pectins from high methoxy pectins in Deliverable 2.4.

2.3.3 Integration of bioprocesses with advanced physical processes, thereby developing hybrid processing systems incorporating closed-loop water-recycling activities (WP3):

2.3.3.1 Stabilisation technology (D3.3)

For stabilisation of the REPRO-products the following technologies have been considered as a separate unit operation:

- Air drying (or nitrogen)
- Drying in superheated steam
- Drying with an adsorbent
- Freeze drying
- N_2-freezing
- Freezing

In addition since some valuable components in VT are lost during processing as a result of enzymatic processes, which are activated during cutting and pulping the feed material VT stabilization of the products started early in the processing.
The following stabilisation techniques have been tested.

- Addition of lactic acid
- Addition of acetic acid
- Fermentation by inoculation of sauerkraut juice
- Fermentation by inoculation of sauerkraut juice and NaCl

2.3.3.2 Extrusion –chickpea snacks (D 3.2)

To evaluate the effects of both the extrusion variables and the addition of the BSG on the final snack extrudate characteristics, the design chosen was a five factor (screw speed, moisture content, BSG level, throughput and barrel temperature) 5 level (100-300 rpm, 10-20 %, 10-30 %,

10-35Kg hr-1, 80-140ºC) full central composite design consisting of 50 experimental runs in total. This design was selected after carrying out a trial experiment to determine the range of processing conditions suitable for the development of chickpea snacks The extruder used for the experiments was a co-rotating, twin-screw extruder (Werner Pfleiderer) with a screws diameter of 37.4 mm and two circular dies with 4 mm diameter. The screw profile has self wiping elements except for a 60 mm section consisting of short reverse and forwarding elements. The L/D ratio was 27:1. The extruder was equipped with rospen twin-screw volumetric feeder and Watson – Marlow 505 DI pump. Once the extrusion response parameters, screw speed, die temperature and
die pressure were constant, extrudates were cut with a sharp knife (approx. 40 cm long) as they emerged from the die and left to cool at room temperature for about 30 min, packaged in plastic bags and stored at room temperature until analysed. Extrudates were analysed for bulk density, lateral expansion, hardness, moisture, fat, protein, PIVD, WAI, WSI, phytic acid, total antioxidant capacity, total phenolic compounds and fibre. For consumer acceptability, the physical characteristics of bulk density, sectional expansion index, hardness and colour were the parameters considered of most importance.

2.3.3.3 Hazard analysis (HACCP) (D3.6)

When developing new products and processes it is very important to assess the hazards in an early phase and to prepare HACCP (Hazard Analysis Critical Control Points) for controlling those hazards. What hazards may be present? Which of the identified hazards require control? Which control measures are effective and to what extent?
The microbiological assessments gave preliminary quantitative descriptions, to identify steps where contamination, reduction and growth of possible pathogens could occur. As the processes were finalized and more product and process information were available, predictive microbiology was used to calculate the actual number of bacteria in the products along the production line (exposure assessment). Critical control points and important parameters were identified.
In order to take into account variations in product and process parameters the Monte Carlo simulation technique was also demonstrated on the BSG snack and one of the red cabbage processes. This made it possible to simulate the number of bacteria, not only as worst case scenarios or mean values, but also to get information of the probability distributions, relevant to identify extreme values likely to cause food safety problems. Spread sheet models were constructed in MS Excel and the add-in software @risk was used to perform the Monte Carlo simulations.

2.3.3.4 Model development

For the process lines developed in (D3.4) models for simultaneous assessment of quality, environmental and economy were developed, in order to evaluate optimal process conditions under given constraints (D3.5). The working procedure is iterative, involving persons having different key competences. The approach gives the possibility to simultaneously evaluate the effect of changes in the process with respect to process efficiency, environmental impact and microbiological risks.
An “integration working methodology” was first developed in collaboration with the partners involved, to integrate the engineering/bioengineering aspects with microbiological risk assessment, environmental analysis and legal and consumer aspects and a general risk inventory. A general simulation framework for modelling was developed to evaluate different production scenarios. The most suitable process conditions, in combination with environmental impact, process economy and microbiological safety were thus highlighted for selected process alternatives.
The general simulation tool is developed as an integrated part of the developed working procedure. The output from the process simulation is used as inputs to the microbiology and environmental sub-models, i.e. the model is process driven.
From a selection of the alternative pilot scale, two process lines were then selected for complete evaluation aiming at exploring different closed-loop scenarios. The selected processes were:

- extrusion of BSG to produce a chick pea snack (D3.2)

- valorisation of red cabbage for producing a phytochemical rich juice dietary fibre and/or a pectin rich fraction and/or a MHR rich fraction (D3.1)

Based on the similarities of the valorisation of red cabbage trimming, only the energy consumption for each process step was evaluated for a third process:

- liquefaction of BSG (D3.1)

2.3.4 Ensuring acceptability of new processes and products by the consumer, retailer, and regulator (WP4):

2.3.4.1 Regulatory assessment

A generic approach is used to assess the regulatory acceptance of processes and products involved in the upgrading of co-products from the food industry and to provide an overview of the hazards and risks of the products and processes involved by means of a risk inventory for the 3 model products (D3.1). Regulatory assessment is made against prevailing EU legislation for input substances.

The approach provides insight in the regulatory position of the starting material and the assessment of regulatory acceptance processes to be gained. Based on the regulatory position and the risk inventory a set of risk management options to meet the regulatory requirements can be drawn. This is based on a traffic lights model to illustrate particular scenarios for permitted substances in products/

2.3.4.2 Socio-economic assessment

The socio-economic assessment (D4.2) is divided into four clusters of work:

1. Literature review & Interviews with retailers
2. Delphi study
3. Focus Group discussion
4. Cost-Benefit analysis

The literature review and interviews identifies central themes that make up consumers' views of potential risks and benefits attached to new food processing technologies and the introduction of novel (modified or functional) food products. A short literature review was made that deals with concepts around waste and dirt, in order to investigate what possible elements can be found here, that could form contributing success or failure factors in relation to the nature of the raw material (waste) used to develop novel foods. Interviews were held with retailers and the outcomes used to narrow down the field of further research.

On the basis of the literature review and the interviews:

- A first and second round Delphi study was developed
- Focus Groups were run
- Triangulation ensured results capture all relevant consumer attributes and perceptions.

The Delphi Study explores the ideas of the consumers and the retailers and includes a literature review, development of a questionnaire, selection of experts, approaching experts, description of the data, data analysis, and data evaluation (D4.2). It is used to narrow down the research of consumer preferences.

Consumer focus groups assess the ideas of the consumers and the retailers more in-depth. This includes literature review, development of a format, selection of focus group members, managing the focus groups, description of the results, analysis of the results, and evaluation of the results. In the focus groups ethnographic methods are used to understand consumer representations of salient issues associated with innovative production processes.

2.3.4.3 Life Cycle Analysis (LCA)

The performance of an LCA (D4.3) is divided into four main parts: Goal and scope definition, Inventory analysis, Impact assessment and Interpretation of results. In the goal and scope

definition, the system to be studied and the purpose of the study is defined. System boundaries are chosen, preferably reflecting the boundary between the natural and the technical system under study, that is, normally starting with extraction of raw materials and ending with some sort of waste treatment. The inventory analysis consists of gathering of data about the resource use, energy consumption, emissions and products resulting from each activity in the production chain. All in- and outflows are then calculated on the basis of a unit of the product called the functional unit. The choice of this unit should represent the function of the product. From some activities, more than one product may be the outcome. In such cases, the total environmental impact is often divided between the main product and by-products, a procedure known as allocation in LCA methodology. Allocation is based on the most relevant relationship between the main product and by- products in each case, e.g. mass, energy content or economic value. Another approach is to include the by-products in the system and separately assess another production system for this product, which can then be subtracted from the original system in order to obtain results for the main product. This latter approach is called system expansion and is recommended by ISO.

2.3.5 Development of sustainable dissemination and exploitation routes (WP5):

Dissemination activities were organised as publications, website operation, project reporting and development of a stakeholder platform

2.3.5.1 Publications (D5.1)

Standard operating procedures (SoP) were developed for the preparation, circulation and approval of publication manuscripts by the Repro consortium. Submitted / published manuscripts were maintained on the Repro website (2.3.5.2)

2.3.5.2 Web site (D5.2)

A dedicated REPRO project Web site is: www.repro-food.net, was established. Within the website the Home page gives directions to general information about the project and the Repro consortium. The home is page has unrestricted public access. The website also contains a password protected restricted area for access by the REPRO consortium partners and acts as a virtual project office, enabling on-line project management.

2.3.5.3 Project reporting (D5.2)

Periodic project dissemination is provided by a REPRO Newsletter, published every 6 months.

2.3.5.4 Creation of stakeholder platform (D5.3 and D5.4)

Companies from different fields, research centres, consultancies, consumers and civil services are invited to register in a global platform to promote information exchange amongst the participants. The main objective of the platform is to establish a participative forum to reach a global overview on food wastes and by products management options and identify potential actions in this field.

2.3.6 Coordination Activities (WP6)

Project Management / Co-ordination activities involved the establishment of effective liaison between the science partners, administrative officials and finance officials associated within the Repro consortium and with the EU Commission.

Existing protocols within the Consortium were adopted for:

- Scheduled production of contractual deliverables – financial statements / information for audits (D6.1)
- The collation of regular financial statements from partners (D6.2)
- Arrangement of all coordination meetings and interim meetings (D6.3)
- Contractual deliverables on time– scientific reports and milestones (D6.4)
- Ensure delivery of scientific reports (D6.5)
- Monitoring, evaluation and managing scientific activity via Management structure (D6.6)
- Promote equal opportunities (D6.7)

2.4 Evaluation of selected process scenarios

2.4.1 Specific Scenario: BSG Extrusion

Background

Specific problem with BSG

BSG is difficult to exploit, mainly due to the following:

- Possible microbial problems due to high moisture content
- BSG needs to be cooled quickly or dried immediately after collection from the brewery
- Quick transport between brewery and processing plant is required
- Lack of materials in BSG to support expansion of extruded products.

Rationale for the process development (Reasons for using extrusion)

Extrusion is an interesting and promising process, because:

- It is a short time high temperature process and uses high volumes of material which will include BSG
- Possibility to obtain expanded snack with good sensory qualities by selecting appropriate ingredients
- Economical process
- It does not create additional by-products.

Rationale for choosing various unit processes within the whole process:

The rest of the processes in the process line are needed due to the following:

- Extrusion demands dry feed, hence pressing/drying is needed
- Needs to be dried and milled to uniform particle size for consistent feed rate
- Mixing required to obtain homogeneous raw material to allow for uniform feed rate and uniform distribution of ingredients in extrudate.

Extrusion

Extrusion processing is a high temperature short time (HTST) continuous process with the ability to process high volumes of material at high pressures and high shear rates. The combination of these process characteristics was thought to provide a means of both drying and reducing the microbial load of the BSG in one unit operation. Experimental trials were carried out using a twin-screw extruder as reported previously in the 6 and 9 month periodic reports. Initial trials highlighted the difficulty in feeding the wet BSG into the screws of the extruder, limiting the feed rates and screw speeds that could be utilised. Reductions in the moisture content were minimal on the first pass through the extruder although significant reductions were achieved after several passes

Specific method developments

A number of ready to eat snack products have been developed, with added Brewer's Spent Grain (BSG) using extrusion cooking. All snacks were made using the same process flow. The flow diagram for the standard production of the snack is given in Figure 1.

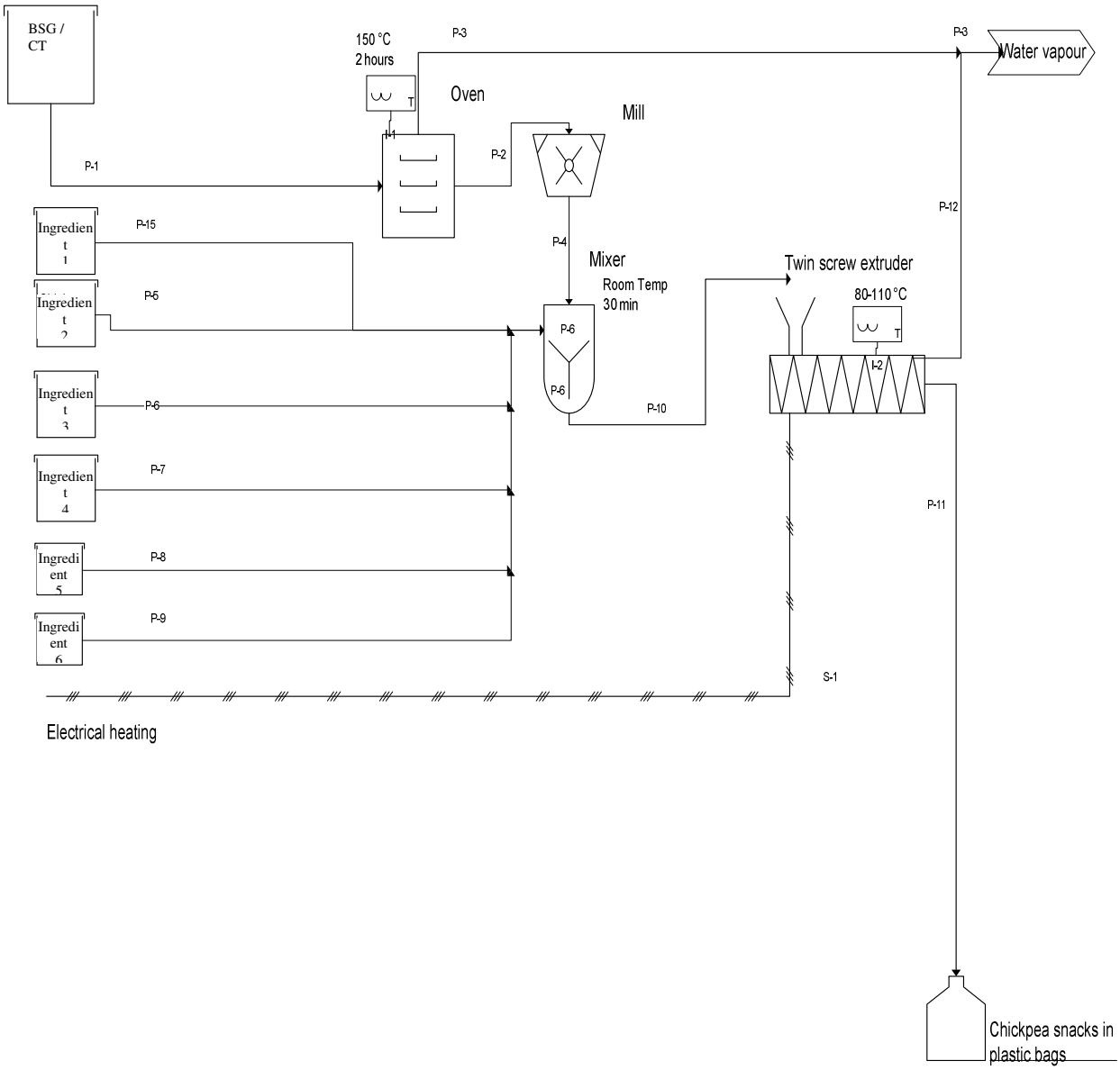


Figure 2.4.1.1. Flow diagram for snack production

Modelling

A process model was created (D3.5) based on the pilot scale process described in Figure 2.4.1.1 (= pilot process scheme) .The pilot trail was evaluated by the model as well as a potentially best case assuming that the production of the BSG takes place at the site of a brewery using excess heat for the drying, which implies that we can omit the transport, the chill step and the environmental impact of the drying step.

The production of snack using one ton of BSG was simulated. The BSG was assumed to have a dry matter content of 20%. A formulation based on 21 % BSG and 9% rice flour on, 30% Chickpea flour and 20% maize flower as main ingredients was assumed. In addition carrot powder (5%), tomato powder (5%) onion powder (2%), Oregano (1,5%) and Basil (1,5%), salt (0,5%), yeast (0,5%), Gum Arabic (2%) and oil were added. From one ton of BSG 926 kg snacks were produced. The transportation distance from the brewery to the factory was assumed to be 50 km. A Western European energy system was assumed. It was further assumed that 10% of the process waste went to landfill, 30% to incineration and 40% was used as compost and 20% as feed. The

cost for handling losses of product during production was assumed to be negligible and was thus set to zero. All input data are given in D3.5.

Ingredients: The ingredients dominate the environmental impact (Figure 2.4.1.2) due to that some of the ingredients chosen have a relatively very high impact e.g. the rice flour which is a major ingredient contributes 5 times more to the GWP than maize flour. Tomato powder and onion powder are other (minor) ingredients which have a high environmental impact. Gum Arabic dominates by far the costs of resources needed to upgrade BSG to a snack (Figure 2.4.1.3). The costs for the energy was small, being 0,2 Euro/kg snacks produced.

Processing: The most energy requiring process step in the pilot scale is the drying step (Figure 2.4.1.4), Simulations showed that it was possible to decrease the energy consumption from 2.1 kWh/kg produced snack to 1 kWh per kg produced snack assuming and decrease from 20% dry mater content to 40% dry matter content of the feed for a process run under the same conditions as the pilot trail. The energy consumption in the pilot scale case was1,9 MWh and 0,2 MWh for the best case scenario. As seen in Figure 2.4.1.4.

RESULTS

Energy used in the process

The simulation results fro energy consumption, divided in the different unit operations are presented in Figure below. The left chart shows the initial setup of the process, and the left shows the improved set up, where the initial simulation results were used to streamline the process line. The improvement potential was very large, as shown be the differences between the charts.
It is obvious that some processes accounts for the lions share of the total, namely

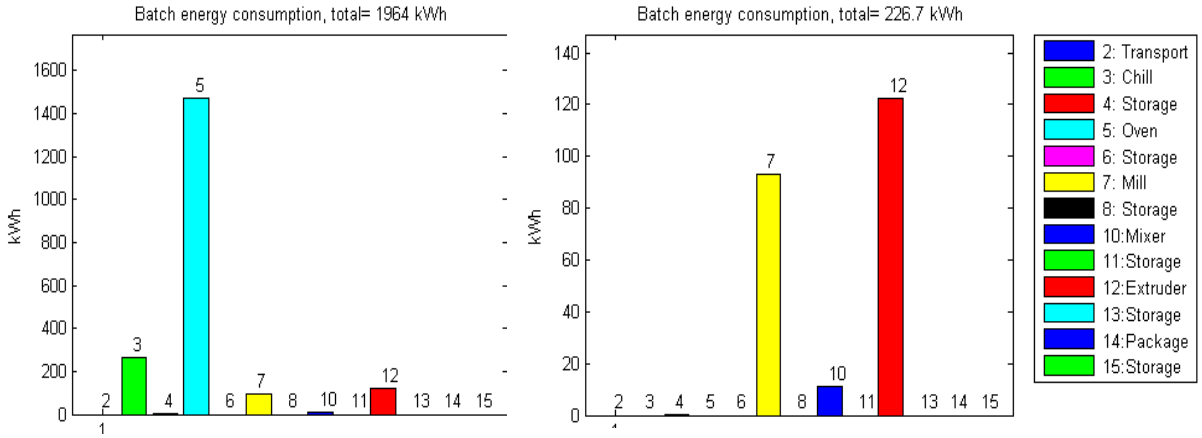


Figure 2.4.1.2. Energy used for the "best case" scenario for the different steps in the process line

Environmental impact and direct costs for the processing line

Below the simulated direct environmental impact and direct costs are presented.

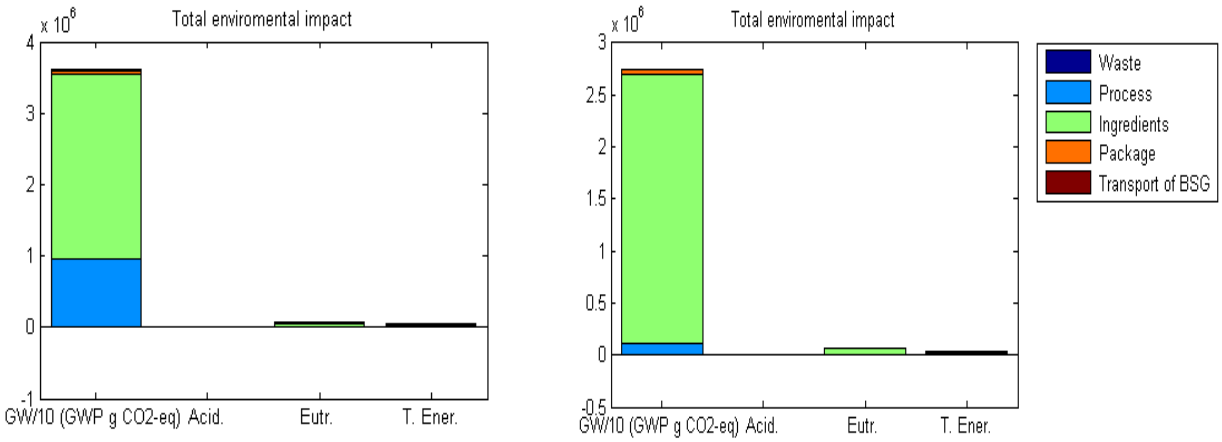


Figure 2.4.1.3. Direct environmental impact from the process line

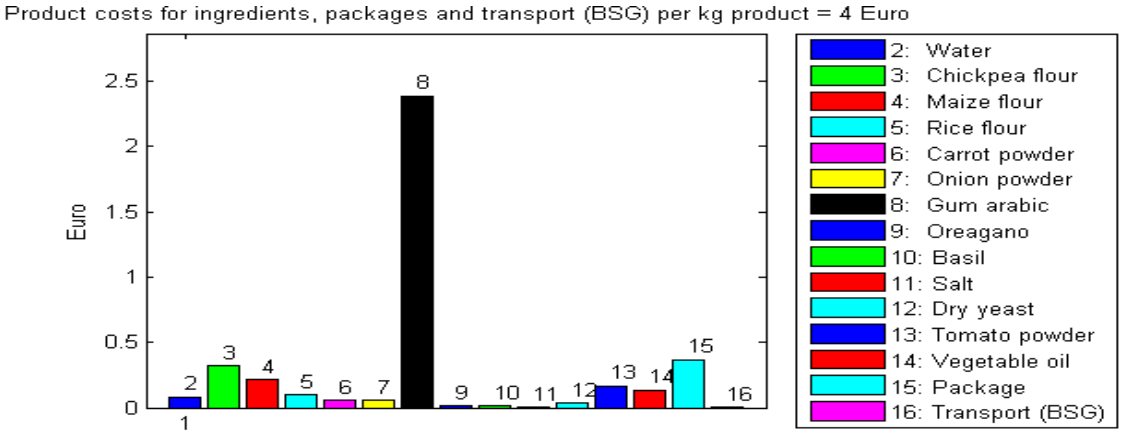


Figure 2.4.1.4. Costs of ingredients and transport per kg BSG

Microbiology

The identified pathogens for the brewers spent grain (BSG) snack process were:

- *Salmonella spp*
- Spore forming bacteria (*Bacillus cereus*)

From the qualitative and quantitative assessments of the BSG snack process, the following parameters and steps which require control were identified. The microbiological acceptance is summarised in Figure Y.

- The drying of BSG (time and temperature) will effectively reduce potential pathogens, both spores (*B.cereus)* and vegetative *Salmonella*. The dry BSG is also stable (a_w < 0,9) so no growth occurs during an intermediate storage
- The quality of the added dry ingredients during mixing are important (prevalence ingredients, minimize potential pathogens)
- There is a thermal reduction in the extruder (time and temperature) for vegetative bacteria but there is no reduction of spores (*B. cereus*)
- The drying of the final product will prevent further growth of bacteria (a_w < 0,9), in fact it will be a non-thermal inactivation of *Salmonella* during storage.

Environmental assessment by Life Cycle Assessment (LCA)

Description of the studied scenarios

After discussions with the different partners it was decided that the process developed should be assumed to be located in the UK. This means that data for surrounding systems and energy production and waste management was UK data. The process and the modelling work are described in more detail in D 3.6 and associated reports for WP3.

Since the three scenarios in the LCA ("what to do with the BSG"-question) deliver different products ("Landfill": no products, "Feed": animal nutrition, and "Snack": snacks) the results must be adjusted to make the scenarios comparable. We have chosen to perform a so called "multiple functional unit approach". This means that all scenarios should provide the same products, and if all products can not be produced from BSG, alternative means of production must be used, and the environmental impact and resource use for these alternative, or complementary, processes is added to the scenarios total. The extrusion process was assumed to be the same for all snack production. In short, BSG replaced rice flour, other ingredients were the same. A compilation of the products delivered from the systems and what complementary production used is presented in Table 2.4.1.1 below.

Table 2.4.1.1. Amounts of all products produced in the scenarios (kg), treating 1 ton of BSG

Scenario	BSG snack produced (kg)	Alternative snack produced (kg)	BSG as feed (kg)	Alternative feed (kg)
Snack	926	0	0	220 [a]
Feed	0	926	1000	0
Landfill	0	926	0	220 [a]

[a] Nutritionally equivalent to 1000 ton of wet BSG

Results

The environmental impact for different ways of using BSG is compared. It can be considered the question the brewery or an authority might ask. Generally the results show that it is beneficial for the environment to use BSG as snack ingredient instead of using it for feed and always better than disposal by landfilling. The reason is the fact that the "BSG comes for free" as compared to the alternative ingredients, and it is environmentally more efficient to produce the feed directly than to use the BSG as feed and produce alternative ingredients (rice meal).

In Figure 2.4.1. the results for contribution to global warming is presented. The BSG snack is the preferred option, and landfilling the worst. The reason is that in the "Feed scenario", alternative productions of ingredients results in more global warming gasses than the alternative feed production in "Snack scenario". The "Landfill scenario" is charged with both alternative ingredients and alternative feed production, and in addition the landfilled BSG cause emissions of the greenhouse gas methane.

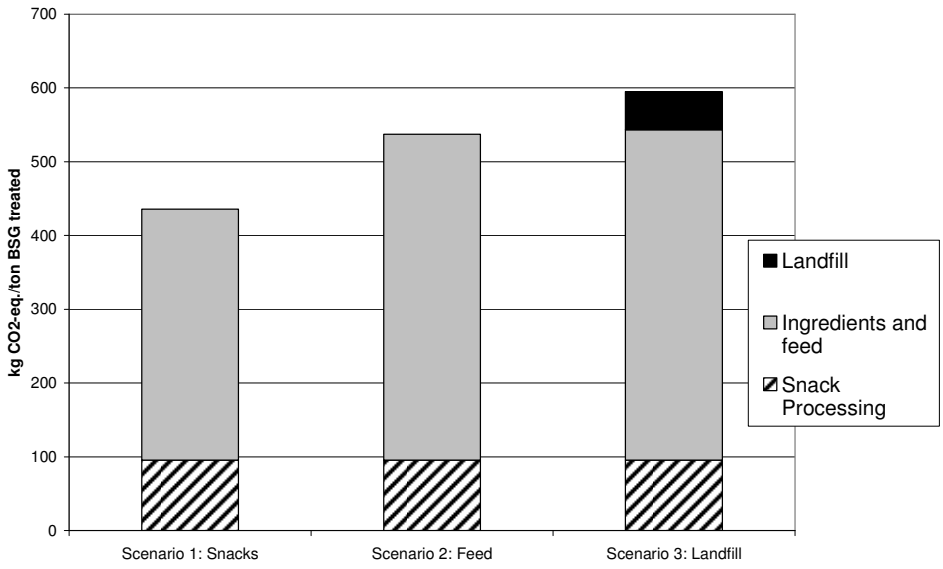


Figure 2.4.1.5 Results for global warming potential, for the comparison of different ways of using/disposing BSG

The emissions of potentially eutrofying substances are presented in Figure ***2.4.1.***2.4.1.6. For this effect category the feed use scores best and landfilling worst. The reason is that the ingredients replaced by BSG do not cause as much Eutrophication as the feed production needed in the snack scenario.

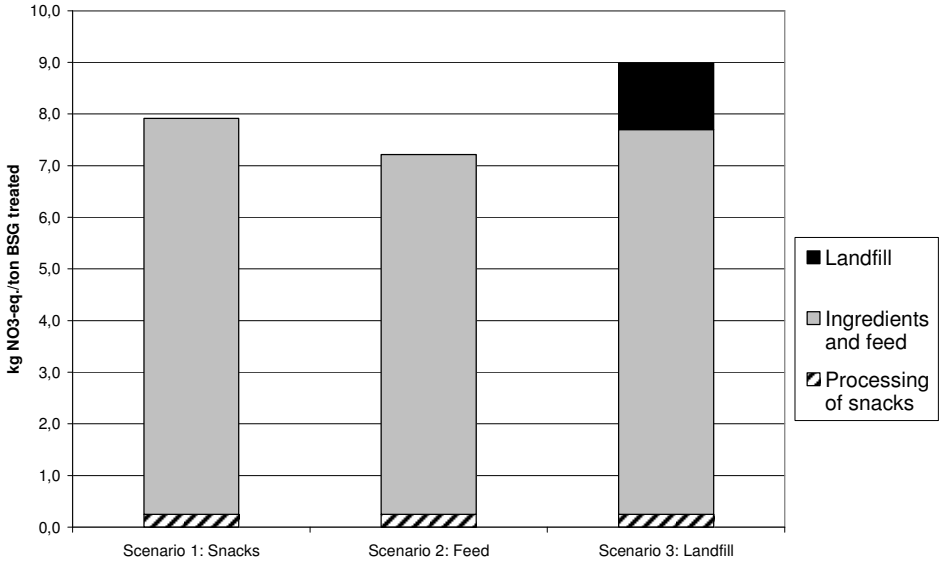


Figure 2.4.1.6. Results for Eutrophication potential for the comparison of different ways of using/disposing BSG

The use of primary energy carriers ("Fuels") and emission of acidifying substances were also quantified, but the differences between scenarios were small.

Discussion and Conclusions

The most important conclusion for extrusion processes is that the choice of ingredients is important. This is explained by the fact that the process itself uses relatively little energy. It is also important what alternative production of feed that is assumed, ideally several alternatives should

be tested to create a more solid ground for conclusions and create a broader understanding of the system.

Legislative issues (D4.1)

Regarding the chickpea snack based on brewer's spent grain and the dissolution of brewer's spent grain using enzymes, results show that the following substances are qualified as novel food, input material brewer's spent grain, and output substances the chickpea snack based on brewer's spent grain, desweetened FAX, peptides and enriched lignin. In case output substances peptides and enriched lignin are used for feed purposes a risk assessment needs to be done. Regarding the use of enzymes, legislation is not harmonized within the EU, therefore national regulations apply and these need to be checked when enzymes are used.

Substances qualified as novel food may not be put on the EU market at this moment. Means for repair is to submit a novel food dossier according to Regulation EC No 258/97. The decision to submit a novel food dossier is up to stakeholders.

Further risk management options to meet regulatory requirements can be summarized as follows:

Food business operators should consider and treat co-products from food industry which feed back into the food production chain as food. This means that all relevant legislation should be applied and control systems put in place.

Member States & EU Government should consider and treat co-products from food industry which feed back into the food production chain as food. This means that current food legislation should apply. In case edible parts of products are defined, e.g. for setting maximum residue limits, a reconsideration of the 'edible parts' may be required. Furthermore monitoring programs regarding e.g. contaminants and pesticides can be developed by national enforcement.

In summary, not all products developed can be put on the market immediately. Permission according Regulation (EC) No 258/97 is required for a number of substances. This procedure may take up to five years.

Cost-Benefit analysis

We compared the BSG chickpea snack with a chickpea snack without the dried BSG, which instead contains a higher amount of rice flour. The cost for handling losses of product during production was assumed to be negligible and was thus set to zero. We assume that the costs for labour, equipment, management, capital, and buildings are the same in both cases. Only the costs for ingredients and the process costs of both kinds of snacks have been compared. We cannot calculate the benefits. The price of the BSG chickpea snack is unknown because there is no product yet for sale in the market. Also prices may vary when we take into account or not the health effect of the dietary fibre in the BSG chickpea snack. The reference scenario contains 21% BSG on dry matter bases (initial load: 100 kg wet BSG, yield: 92,6 kg snacks).

The main results are summarised in the list below:

- The cost processing 1 kg dried BSG (4,8 kg wet BSG) is 18.3 Euro. The extra costs are gum Arabic, €11, and energy costs €0.77.
- A major difference between rice flour and BSG flour is that wet BSG needs to be chilled and dried, in addition of milling. Cost for energy consumption of per kg dry BSG €0.77. Of this amount €0.65 is from the oven, and € 0.12 is from the chilling.
- The transport distances, based on simulations of 50 and 1000 km, are not critical concerning costs.

Consumer acceptability

The relations value and volume on acceptability.

High Volume	Normal volume	Low volume
X		

High volume reduction is the priority for reprocessing in the opinion of consumers and reprocessing BSG **does** answer this criteria completely.

High value	Normal value	Low value
	X	

As it is hard to imagine products, value is neutral, but related to the demand that BSG ingredients should make food products cheaper.

Quality of used compound

Fresh/unprocessed	By-product	Slurry/waste/sump
		X

BSG is hard to identify with. Associations with bad smell and an unhygienic mass are made. It scores low compared to other by products, such as trimmings and rejected fresh supply.

The influence of required communication on consumer trust

Normal Communication	Extra Communication	High need extra Communication
X		

Participants indicate not to be overly interested in detailed information. General information is considered enough. Transparency is thought important and an information campaign is said to raise more questions than it is seen as reassuring. Information should be available to interested consumers.

The influence of the need for quality and safety control measures on consumer trust

Normal HACCP	Low need extra HACCP	High need extra HACCP
	X	

Established techniques need no extra HACCP, but innovative technologies need dedicated HACCP. As BSG carries some degree of distrust, demands are made for safety assurance prior to the reprocessing itself. Trust is mainly put in marketing interest of the producer and the regulations of the food authority. A voluntary upscaling of HACCP would raise more question than offer assurance concerning the safety and quality.

Overall acceptability for BSG

	X	

BSG reprocessing is acceptable under conditions.
Brewers Spent Grain ends low on the scale of suitability for reprocessing as compared to other by-products, such as trimmings and rejected vegetable supplies. BSG has associations with bad smell, dirtyness and unhygienic, and is not something consumers easily identify with. It is harder to imagine possible products. As it is assumed that BSG under new regulations would not be permitted as animal feed anymore, food quality and safety for human consumption are brought forward as areas of concern.
Extracting fiber from BSG is thought to deliver a significant volume reduction, which is appreciated by consumers. Assurances are sought around the lead time and storage conditions before reprocessing, to control optimum quality of the by-product. Also it is indicated that reprocessing is required and should be such that the product is freed of all possible contaminants.
Consumers have no real objections against ingredients made from BSG, but there is a general feeling that one wouldn't want to know about their origin. Detailed information is hardly

appreciated, but should be available to the ones looking for it. It is also felt that acceptance would be easier if the ingredients are used in existing already familiar food products. Notions like better use of resources – environmentally friendly- are seen as supportive for acceptation. Reprocessed BSG is perceived as second grade and consumers are thus not prepared to pay a premium for them. Additional attributes, such as the healthiness would be welcomed as a positive side effect and could also contribute to acceptability.

Conclusions and recommendations

- Compared to the case of the BSG chickpea snack, next to the other ingredients, 21% more rice flour has to be bought. The total assessment for the case of the chick pea snack is in the yellow zone, because we don't know the benefits yet.
- The BSG in the case of the BSG chickpea snack is almost for free (green zone). Compared to the case of the chickpea snack, the costs for gum Arabic and for the drying step pull the total assessment for the case of the BSG chickpea snack to the red zone. It is worth noting that it is a minor ingredient gum Arabic that dominates the production costs. To reduce the overall production costs alternatives to gum Arabic may be considered.
- The drying step is an expensive step. Costs can be decreased by pressing the BSG before processing and possibly by drying the product at the brewery using other energy sources e.g. excess steam. If a cheap alternative for gum Arabic can be found, the total assessment of the BSG chickpea snacks will be in the green zone.
- The transport has a small influence on cost.

Summary Results

BSG pilot scale	Oven	Chill Mill Extruder	Transport Storage Mixer
BSG best case scenario		Mill Extruder	Transport Storage Mixer

Summary of results for Energy consumprion

Input			BSG
Process		potential pathogens from added ingredients limited reduction in the extruder	effective reduction during drying of BSG
Output			no further growth in the final dry snack product

Summary of the microbiological acceptance of the BSG snack process.

BSG best case scenario	Eutrophication	Acidification Energy use	Global warming

			potential

Summary of results for Life Cycle assessment (LCA)

Chickpea snack with rice flour		Total cost assessment Costs Rice Flour (€1.16) Costs other ingredients and process costs (€ 6.53)	
BSG chickpea snack	Total cost assessment Cost of gum Arabic (€11) Cost of extra energy (€0.77)	Costs other ingredients and process costs (6.53)	BSG (free)

Summary of the Cost-Benefit acceptance of the BSG snack processes.

REPRO SCORECARD SUMMARISING EVALUATIONS OF BSG – SNACK EXTRUSION PROCESS

PROCESSING SCHEME: ACCEPTABILITY CRITERIA AND RATINGS		
CRITERION No	**DESCRIPTION**	**RATING**
1	ENERGY UTILISATION	
2	MICROBIOLOGICAL SAFETY	
3	LIFE CYCLE ASSESSMENT	
4	LEGISLATIVE COMPLIANCE	
5	CONSUMER ACCEPTABILITY	
6	COST-BENEFIT ANALYSIS	
	OVERALL ASSESSMENT	

2.4.2 *Specific Scenarios: Red cabbage trimmings*

Introduction

In excess of **1 million tonnes of vegetable trimmings** from the vegetable processing industry (Eurostat data indicates total vegetable sector waste is in excess of 10 million tonnes). These plant-derived waste co-products are known to contain significant amounts of valuable components which remain unexploited waste in the current processes.

Vegetable trimmings (from the vegetable processing industry) represent "soft" (i.e. non-lignified) plant food wastes.

They contain tissues representative of most tissue types, from stem tissues through to leaf material. Hence, by focusing on these, it is envisaged that methods developed during the project will be applicable to up-grading nearly all other plant-based co-products. Hence, **this focused project will deliver *generic solutions***. Although various by-products from agricultural crops could be used for the isolation of pectic materials, red cabbage trimmings have the advantage that in addition to pectic polysaccharides, they are also rich in colorants and glucosinolates. Isolation of these would enable the project to demonstrate that the process developed is rather flexible in handling different types of valuable compounds yielding ingredients for the food industry.

Specific problem with RC trimmings

RC is difficult to exploit, mainly due to the following:

- Possible microbial problems due to high moisture content
- RC and other VT needs to be chilled rapidly or dried immediately after production to limit microbial proliferation
- Quick transport between producer and processing plant is required

Red cabbage trimmings *(Section 2.2.6.2*) were found to contain around 45% (dry matter) of carbohydrates. It can be estimated that this is predominantly polysaccharide fraction and is represented by pectins, hemicelluloses and cellulose in a 5:1:4 ratio. Trimmings also contain proteins, ash, anthocyanins and glucosinolates; respectively 19%, 12%, 1% and 1.% Both lab scale and pilot plant scale studies showed that red cabbage trimmings can be converted into valuable products with an industrial perspective. In laboratory scale extractions, possible end-products and process options were identified and screened. Pilot scale experiments added value since process conditions used reflected industrially relevant microbiological and chemical criteria. The ethanol insoluble carbohydrate rich fraction can be used as such or it can be treated with green labelled enzymes into either pectin or modified hairy regions (MHR). The soluble colorant rich fraction can be used as a food ingredient after removal of the cabbage flavour via an adsorptive chromatographic separation with 90% recovery. The final residue recovered after pilot scale Rapidase Liq+ treatment represents only 6.8% of dry trimmings used for experiments. The residue contains only small amounts of pectins, as 11% of the GalA initially present in dry trimmings was quantified, respectively at pilot scale. The gelling and / or thickening and / or prebiotic properties of the pectin and MHR obtained from Brassica rubra trimmings should point out what the economic perspective is of these products. References: Nakamura el al. (2001), Schols, H.A., and Voragen, A.G.J. (1994), Schroot, J et. al. (2008)

Process line development

A process scheme for a full utilization of the red cabbage trimmings for production of a phytochemical rich juice, pectin and then further processing the residue to a MHR-rich fraction was developed (Figure 2.4.2.2). By alcohol extraction as shown in Figure 2.4.2.1 the vegetable trimmings can be separated into a phytochemical rich juice (after alcohol recovery) and a polysaccharide rich fraction called cell wall material (CWM). The latter can be modified using enzymes to obtain specifically tailored carbohydrates. Possibly the CWM can also be used as such, as a fibre rich water binding ingredient since it can hold 25 times its own weight in water.

Modelling

Based on the process schemes given in Figures 2.4.2.1and 2.4.2.2, a simulation model (D3.5) for integrated evaluation of different process scenarios was developed. The scale chosen for the simulations was a daily production based on 10 tonnes of red cabbage trimmings. The production scale is based on the assumption that Dutch industry will produce 3300 tonnes red cabbage trimmings a year, which corresponds to 15% of the total production. It was further assumed that the transport of the trimmings to the factory was short, an average distance of 50 km was assumed.

For each process step the costs, use of resources (raw material, energy chemicals), microbiological status, and level of valuable compounds (anthocyanins, glucosinolates, uronic acid and neutral sugars), costs and environmental impact was followed. Seven scenarios were evaluated (D3.5) aiming towards successive improvements and a closed loop approach for all process streams.

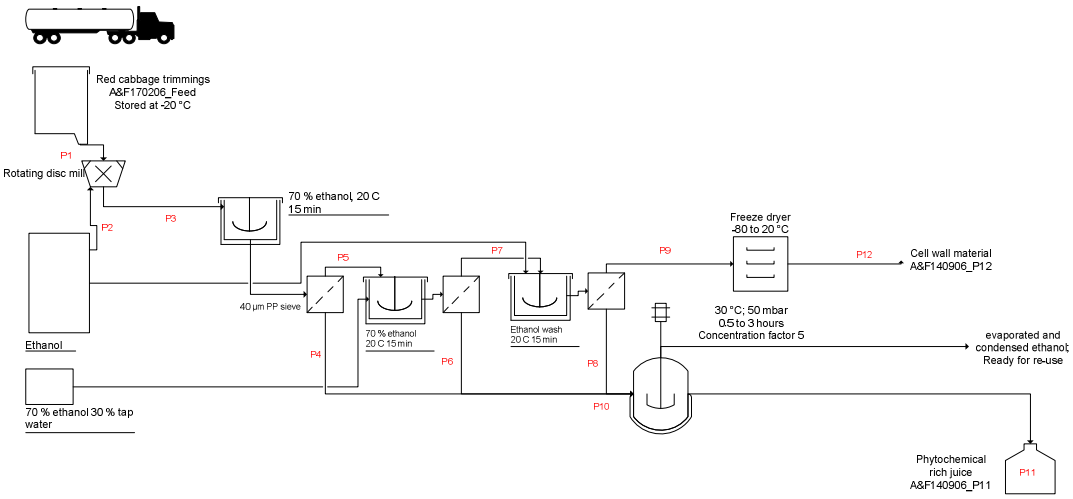


Figure 2.2.4.1. Flow diagram extraction of red cabbage trimmings using alcohol.

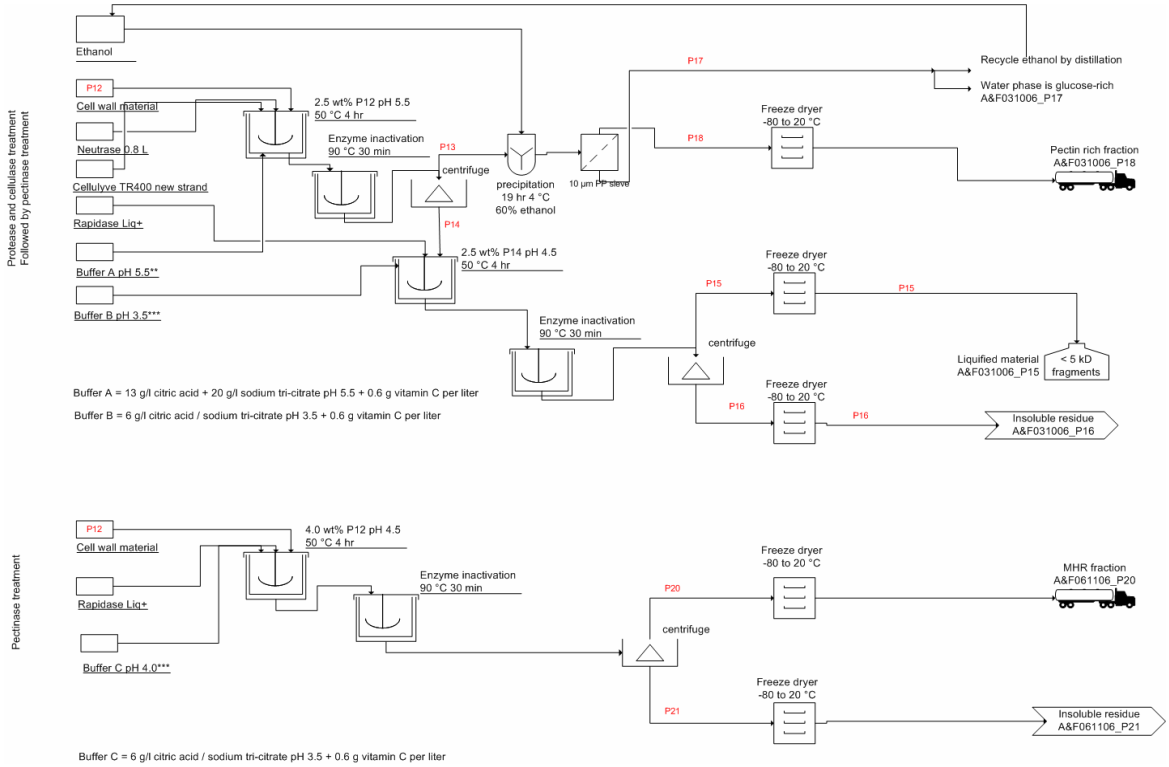
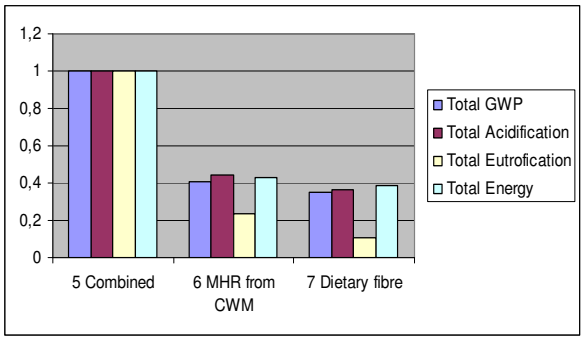


Figure 2.4.2.2. Flow diagrams liquefaction of cell wall material using a cellulase and a protease followed by a pectinase (top) and liquefaction of CWM using only a pectinase (bottom).

Scenario 1: Pilot scale trail with an addition of a distillation step for retrieving the alcohol from the phytochemical rich juice (Referens scenario)
Scenario 2: 100% re-circulation of alcohol in all process steps
Scenario 3: Conventional drying instead of freeze drying. The heat of evaporation is used as an estimate for the energy consumption
Scenario 4: Natural gas is used in all steps involving heating
Scenario 5: Scenario 2-4 are combined to one scenario (Combined scenario)
Scenario 6: As scenario 5 but only MHR is produced besides the phytochemical rich juice
Scenario 7: As scenario 5 but only dietary fibres are produced besides the phytochemical rich juice

The evaluation of the pilot scale scenario (Scenario 1) identifies three critical steps being the distillation process for recovering alcohol and the two freeze drying steps. These process steps are associated with large energy use and the freeze drying steps are also associated with losses of alcohol, which adds to environmental impact as well as costs. The simulations show that by combining the changes suggested in scenario 2-4, both costs (Figure 2.4.2.3) and environmental impact could be reduced with a factor of five or more. From Scenario 5-7 it can be concluded that the product mix plays an important role for the impact of the process itself (Figure 2.4.2.4). Figure 2.4.2.5 shows the process energy requirements for the different scenarios and Figure 2.4.2.6 shows the energy usage for each unit operation for scenario 5, where both pectin and MHR are produced from the cell wall material in a highly improved manner. Additional improvements aiming at a closed loop production is re-circulation of the buffer by e.g. using a dense ultra filtration membrane to decrease the amount of buffers needed and at the same time lower the salt contents in the products. Moreover, a concentration step will as well decrease the water contents before drying thus give considerable energy savings (D3.5). If the production line is placed in connection to a food factory excess of heat may be used, which will improve the process economy and decrease the environmental impact further.

Figure 2.4.2.3 Simulated costs/ton for scenario 1- 5 and in detail for scenario 5.



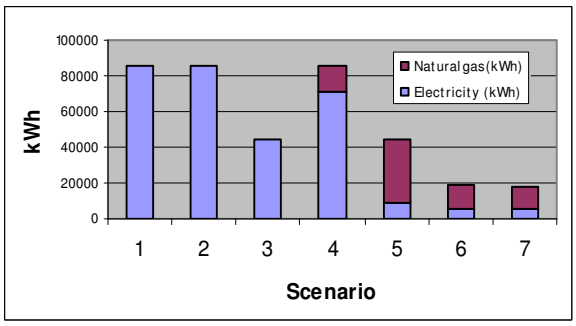


Figure 2.4.2.4 Relative environmental impact scenario. 5-7.

Figure 2.4.2.5 Process energy consumption for

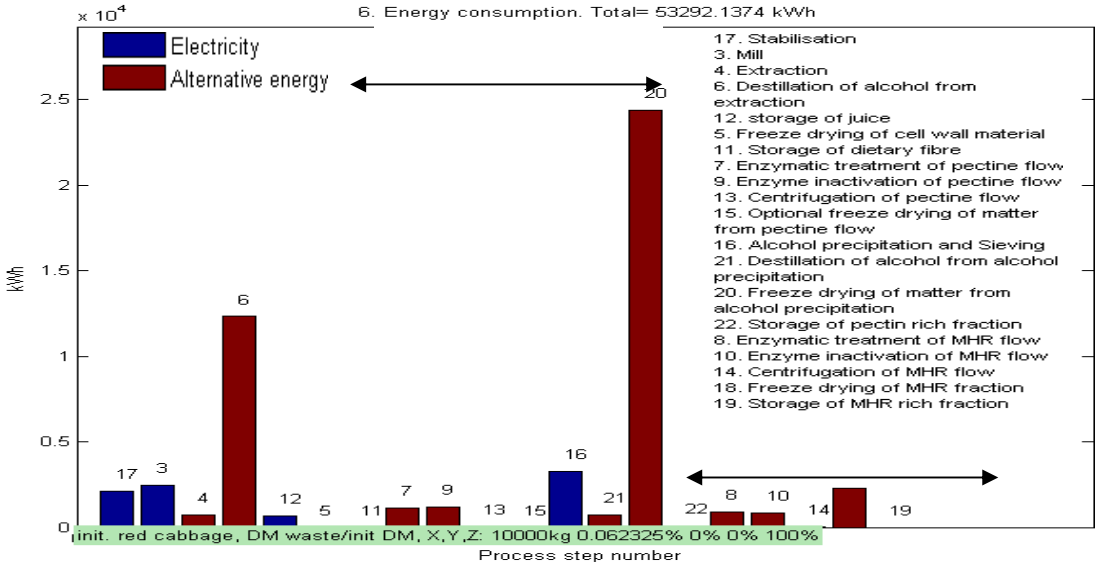


Figure 2.4.2.6 Energy consumption for each process step in the combined scenario (scenario 5). Alternative energy refers to natural gas and conventional drying is used in the "freeze drying" step

Evaluation of energy consumption

Taking into consideration that the pectin fraction can be concentrated up to ten times before drying the energy consumption for producing two product mixes were evaluated in a relative manner; where red means a potential critical process step.

Products: Phytochemical rich juice, Pectin, MHR	• Evaporation/ Destillation	• Alcohol precipitation (stirring) • Stabilisation • Milling • Drying Pectin * • Drying MHR*	• Extraction • Storage • Enzymatic treatment of Pectin* • Enzymatic treatment of MHR*
Products: Phytochemical rich juice, MHR	• Evaporation/ Destillation	• Stabilisation • Milling • Drying of MHR*	• Extraction • Storage • Enzymatic treatment of MHR*

**Excess heat may be used if available*

Figure 2.4.2.7: Evaluation of energy consumption for two selected best production scenarios.

Evaluation of Microbiological Risks (D3.6)

The identified possible pathogens for the red cabbage processes were:

- *Listeria monocytogenes*
- *E.coli O157*
- *Salmonella spp*
- Spore forming bacteria (*Bacillus cereus*)

From the qualitative and quantitative assessments of the red cabbage processes, the following parameters and steps which require control were identified. The microbiological acceptance is summarised in Figure 2.4.2.7

- The quality of the raw materials (prevalence of spore forming bacteria, *B. cereus*) are important because the reduction of *B.cereus* is limited in the red cabbage processes (this can be considered pre requisities or Good Hygienic Practice (GHP) more than a critical control point)
- The non-thermal reduction during several ethanol steps (time and ethanol concentration) will effectively reduce vegetative bacteria (*Salmonella, Listeria, E.coli O157*)
- The thermal reduction during enzymatic treatment and enzymatic deactivation (time and temperature) will effectively reduce vegetative bacteria (*Salmonella, Listeria, E.coli O157*) and *B.cereus* to some extent in the pectin and MHR processes
- The drying of the final product (pectin and MHR) will prevent further growth of bacteria (a_w <0,9)
- The freezing storage of the phytochemical rich juice will prevent further growth of bacteria (temperature).

Input		• Red cabbage trimmings	
Process		• Limited reduction of spores (*B.cereus)*	• Effective reduction of veg. bacteria
Output			• No further growth in the dry pectin and MHR • No further growth in the phytochemical rich juice (freezing storage)

Figure 2.4.2.7: Summary of the microbiological acceptance of the red cabbage processes.

Environmental assessment (T4.3)

The modeling of the scenarios provided information on the relative environmental impact of the scenarios. In order to evaluate the environmental impact in a systems perspective, i.e. compare the developed processes with alternative means of taking care of the trimmings, a comparative LCA was performed. The two most promising alternatives from the modeling was chosen and alternative means of taking care of the trimmings were identified based on statistics from the Netherlands, being use as feed or composting. Here only the results for maximized MHR production are presented since it demonstrates the most environmentally benign scenario. The study is reported in detail in D4.3. We used a so called “systems expansion approach”, which means that the three scenarios (RCT Liquefaction, feed, compost) all should deliver the same products, be it from RCT or conventional production. This meant that the Liquefaction scenario was charged with emissions for producing feed and compost, the feed scenario with emissions for producing MHR and compost and the compost scenario with emissions for producing MHR and feed. By this approach the scenarios become comparable.

A summary of the results is presented in Figure 2.4.2.8 and 2.4.2.9. The former presents the result for global warming potential and the latter the eutrophication. Also results for acidification and energy use were generated. The reason for this choice is that global warming potential is an extremely important parameter, and eutrophication was the only impact were liquefaction did not turn out to be superior, and works as an example of how these results can be used to direct improvements measures.

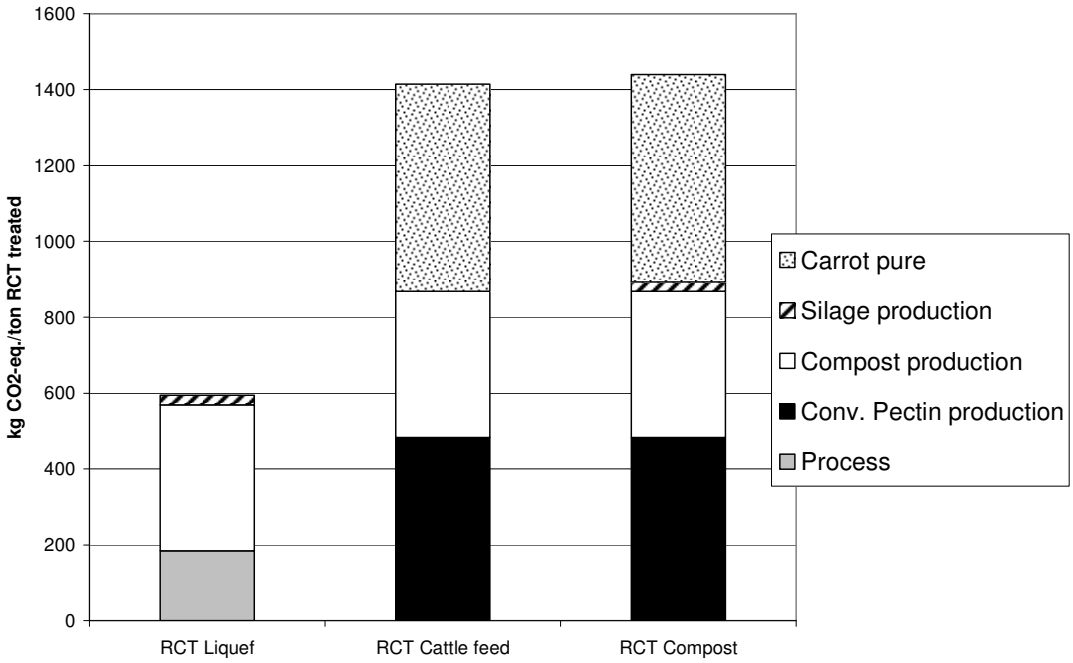


Figure 2.4.2.8. Potential global warming effect for liquefaction of Red cabbage trimmings with maximum MHR production, compared to alternative routes

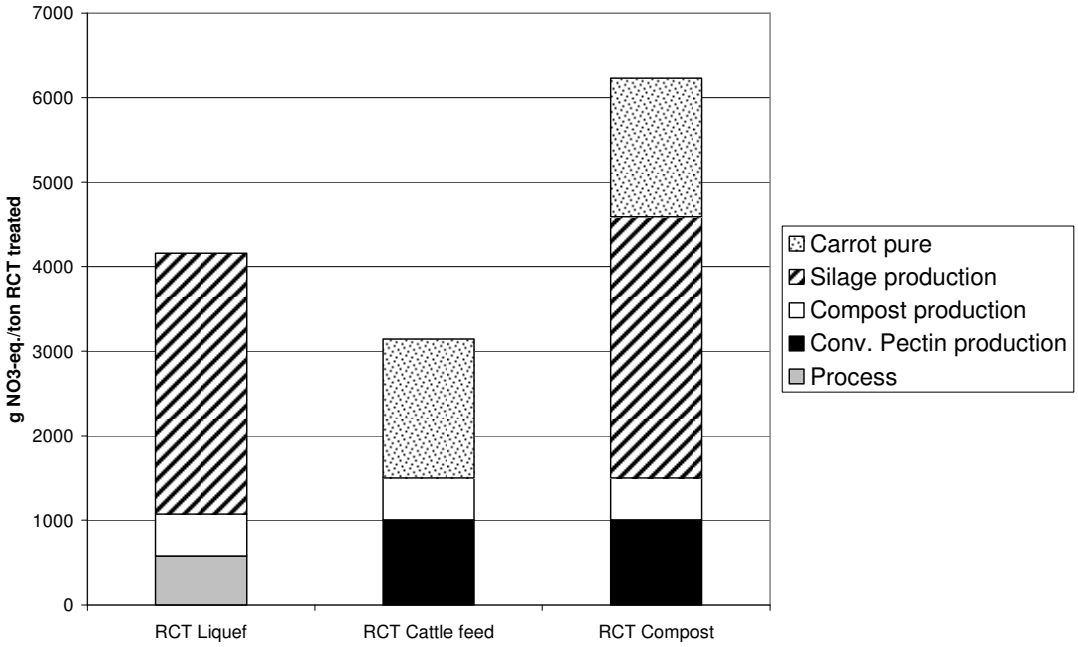


Figure 2.4.2.9. Potential Eutrophication for liquefaction of Red cabbage trimmings with maximum MHR production, compared to alternative routes.

The conclusions are summarised in Figure 2.4.2.10, where also the results for a liquefaction process maximising pectin (not shown above) is included.

Global warming potential			MHR-max Pectin-max
Energy use			MHR-max Pectin-max
Acidification		Pectin-max	MHR-max
Eutrophication	Pectin-max	MHR-max	

Regulatory acceptance

Figure 2.4.2.11 shows the regulatory acceptance of the substances involved in the process 'liquefaction of red cabbage trimmings'.

Input			**•Red Cabbage Trimmings**
Additions		**Enzymes: •Neutrase •Cellulyve (conditions apply)**	**•ethanol •isopropanol •citric acid •tri sodium citrate •ascorbic acid, •sodium hydroxide**
Output		**•NF: vegetable juice containing a.o. glucosynolates, colors •NF: modified hairy regions**	**•Colorant •Pectins •Dietary fibre**

Figure 2.4.2.11 Regulatory acceptance of substances in the process 'liquefaction of red cabbage trimmings'

The output substances *vegetable juice containing a.o. glucosinolates, colours* and the *modified hairy regions* are qualified as novel food and may not be put on the EU market at this moment. The decision to submit a novel food dossier is up to stakeholders.

Regarding the use of enzymes national regulations apply and these need to be checked when enzymes are used.

Further risk management options to meet regulatory requirements now and in the future can be summarized as follows. Food business operators and Member States & EU Government should consider and treat the used co-products from food industry as food. Current food legislation shall apply accordingly.

Consumer acceptance

SLIDERS

The relations value and volume on acceptability

High Volume	Normal volume	Low volume
		X

High volume reduction is the priority for reprocessing in the opinion of consumers. It is questioned how much waste is left after extraction.

High value	Normal value	Low value
		X

The value of the red colorant is mainly seen as economic and in that sense not found interesting for consumers.

Quality of used compound

Fresh/unprocessed	By-product	Slurry/waste/sump
X		

Trimmings are an easily to identify with by-product that have no direct associations with waste.

The influence of required communication on consumer trust

Normal Communication	Extra Communication	High need extra Communication
X		

Participants indicate not to be overly interested in detailed information. General information is considered enough. Transparency is thought important and an information campaign is said to raise more questions than it is seen as reassuring. Information should be available to interested consumers.

The influence of the need for quality and safety control measures on consumer trust.

Normal HACCP	Low need extra HACCP	High need extra HACCP
X		

Consumers have no clear opinion, but do formulate contextual demands. Established techniques need no extra HACCP, but innovative technologies need dedicated HACCP. As red cabbage trimmings are easily accepted as resource, there is less need felt for extra measures. Trust is mainly put in marketing interest of the producer and the regulations of the food authority. A voluntary up-scaling of HACCP would raise more question than offer assurance concerning the safety and quality.

OUTCOME FOR RED CABBAGE

			X	

Red cabbage colorant extraction does not answer consumer expectations.

Disposal of easy to identify with by-products, such as red cabbage trimmings is seen as wasteful. The trimmings are easily accepted as a resource for further processing. Therefore, there are hardly concerns about food safety. General standards are accepted as sufficient on the assumption that regulations and producers' interests are sufficient tools to assure quality and safety. Reprocessing red colorant from Red Cabbage however, is thought to have only a marginal impact on waste volume reduction. It is found that reprocessing should primarily serve the goal of waste volume reduction and not economic interests. Consumers question how much waste is left after reprocessing. Consumers further question whether other options can reduce the waste volume

further. Also, even high quality ingredients are perceived as second grade when produced from residue flows (or "waste") and consumers are thus not prepared to pay a premium for them. Additional attributes, such as the healthiness of the red colorant, are not sought after specifically, but are welcomed as a positive side effect and could contribute to acceptability. The main goal however, a sustainable environment, remains the most important. There is only a moderate desire to be informed about the reprocessing of residues into ingredients. It is accepted that we do not know much about most food products. Notions like better use of resources – environmentally friendly- are seen as supportive for acceptation. Detailed information about the nature of the used residues and the reprocessing technique are hardly appreciated, but should be available to the ones looking for it.

Comparison cost versus benefits

The cost versus benefit was evaluated for the production pectins and MHR (scenario 5) and for the production of only MHR (scenario 6).

- Costs per day (10 tonnes) are: resources (enzymes, buffers, and water), electricity, natural gas, and transport.
- Benefits per day (10 tonnes) are: natural colorant (€50 kg), MHR (€7 kg), pectin (€7 kg), and fibre (€1 kg).
- In both cases (with or without pectin) the costs per day for labour, capital, management, buildings, and equipment are left out of the calculations. For example, it is unknown if it is possible to incorporate the cases into an existing production facility. For this reason our recommendations are only based on the process costs.

Conclusions and recommendations

- In the best case Pectin path with MHR the benefits minus the process costs is a loss of €1914. Without even taking into account the other costs we can conclude that the total cost/benefit assessment for this case is in the red zone (see figure 2.4.2.12).
- In the best case MHR alternative the benefits (€1942) minus the process costs (€ 3856) is a gain of €491, but there will also be other costs. For this reason the total cost/benefit assessment is placed in the yellow zone (see figure 2.4.2.12). After the inclusion of the other daily costs, the total cost/benefit assessment will also be in the red zone.
- Energy efficient drying including pre-concentration will substantially contribute to cut costs and lower the environmental impact of the process.
- Alternative energy resources to electricity should be used as far as possible
- For the optimised process (reference scenario) the costs of enzymes and energy are dominant.

Best case Pectine path with MHR	• Total cost /benfit assessment • €1914 loss • (€3856 cost)		• (€1942 benefit)
Best case MHR alternative	• (€1381 cost)	• total cost/benefit assessment	• (€1872 benefit) • €491 gain

Figure 2.4.2.12: Summary of the Cost-Benefit acceptance of the red cabbage processes.

Discussion

The results obtained clearly show that the enzymatic treatment applied to red cabbage trimmings significantly reduces the amount of by-product from the vegetable trimmings industry yielding

interesting food grade ingredients. The potential of legislative acceptability of the developed process or parts thereof is high, since reagents, unit operations and products are basically accepted for human food production. However, MHR is also novel Food and cannot be put on the market until approval of EU after submission of a novel food dossier, which is up to stakeholders.

The environmental for the liquefaction process compared to the present route of disposal showed that there are environmental gains by introducing liquefaction. The most promising alternative was the maximised production of MHR. The reasons for the general results was basically that the red cabbage trimmings “come for free”, i.e. no emissions has occurred during the production of the raw material which is the case for the conventional (or present) production of MHR and vegetable juice (which is the largest product volume). The sensitive part for the liquefaction process is energy use, which was demonstrated for the pectin maximised process, where the liquefaction showed higher environmental impact than to use the trimmings as feed for one environmental impact category and similar for another. Hence, in order to optimise this process energy efficiency is key. Generally, data gathering was difficult for the assessment of conventional production, so the results must be interpreted with care, and should be looked at as indications rather than detailed results. The methods developed within the project have showed to be valuable in capturing the important issue of environmental impact of novel processes and products.

According to the cost benefit analysis the production of MHR together with the colorant is to prefer. The designed process should be looked upon as a worked out example. Costs and energy demand will be highly dependent on the situation at the actual process site, though the analysis clearly shows that there is a potential for a safe and sustainable closed loop recovery of leafy vegetable trimmings..

References.

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Schols, H.A., and Voragen, A.G.J. (1994). Occurence of pectic hairy regions in various plant cell wall materials and their degradability by rhamnogalacturonase. Carbohydrate Research 256, 83-95.

Schroot, J, Zykwinksa A, Panouille M, Hinz S, Borge G.I.A., Bonnin E and Schols H.A. (2008) Recovery of valuable compounds from red cabbage trimmings.JAFC, to be submitted.

REPRO SCORECARD SUMMARISING EVALUATIONS OF VT LIQUEFACTION PROCESS

PROCESSING SCHEME: ACCEPTABILITY CRITERIA AND RATINGS		
CRITERION No	**DESCRIPTION**	**RATING**
1	ENERGY UTILISATION	
2	MICROBIOLOGICAL SAFETY	
3	LIFE CYCLE ASSESSMENT	
4	LEGISLATIVE COMPLIANCE	
5	CONSUMER ACCEPTABILITY	
6	COST-BENEFIT ANALYSIS	
	OVERALL ASSESSMENT	

2.4.3 Specific Scenario: BSG liquefaction

Background

Compared to the two integrated processes described earlier (Section 2.4.1: BSG extrusion and Section 2.4.2: Red cabbage trimmings liquefaction), the BSG liquefaction is generally less developed. This is mainly because of the complexity in disassembling the lignified tissues. The experiments are only on pilot scale for the enzymatic treatment, and the data for the physical parts of the process are assumptions based on experiences. Information and data on the products are also lacking, as alternative ways of production and possible use of products. Moreover, no detailed modelling for scenario evaluation has been undertaken, so the possibilities to adjust and optimise the process remain limited. This means the evaluations must be considered as indicators and recommendations for further development work rather than final results. Nevertheless we think it is valuable to present the integrated process, since it exemplifies much of the core aims within REPRO, and also is an example of an interesting process for further development. BSG contains several components for exploitation. Residual starch and protein have potential for incorporation into other food or feed processing systems. This leaves the large quantity of lignocellulose which presents the greatest challenge for exploitation. Hence REPRO has focused on the enzymatic extraction of (a) the protein component (which has a commodity market value) and (b) the partial liquefaction of the residual cell wall polysaccharide to create added valued, soluble oligosaccharides with anti-oxidant capacity. The process of enzymatic liquefaction (D2.2) is described in section 2.2.6.1, but the process scheme is presented in Figure *2.4.3.1*. Basically the process uses enzymes to solubilise BSG but leaves an insoluble residue. Thus two phases, one liquid and one solid result, although the ultimate objective is to maximise the solubilisation. The different streams are separated using physical methods and dewatered to yield marketable products. The three major products identified from the liquid phase are:

1. FAX (Feruloylated arabinoxylan components – as oligomers and polymers), with potential antioxidant applications in foods / nutraceuticals and forseen as the most valuable product.

2. Glucose syrup (a commodity, which could be an alternative or replacement in sugar or starch derived products)

3. Peptides (amino acid and peptide-enriched component), the value of which fraction requires more detailed evaluation.

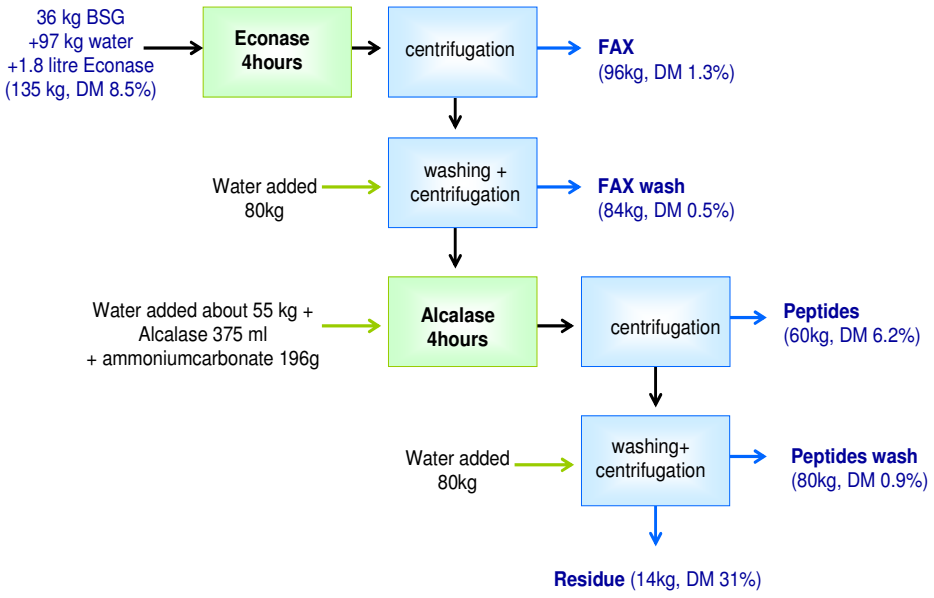

Figure 2.4.3.1. Principal description of the process

A more detailed large-scale processing scheme (D3.4) is shown in Figure 2.4.3.2.

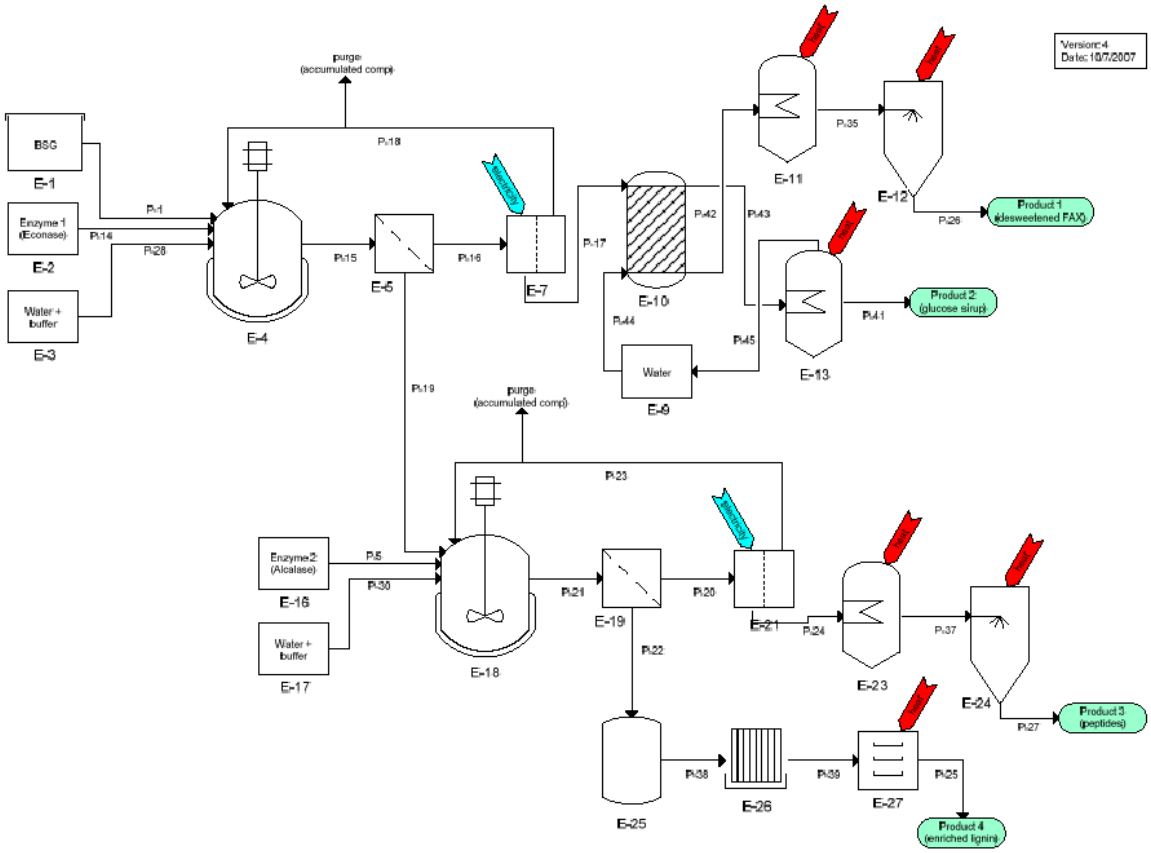

Figure 2.4.3.2 Process flow sheet for BSG valorisation process

Environmental assessment with LCA

Since detailed data on any important product were unavailable we cannot do a full “multiple functional unit approach” as was done in the other two case scenarios. This decision is in accordance to the methodology developed within REPRO. Conclusively, the total environmental impact for the process is quantified, and thereafter these impacts are divided by the total value for the products produced.

One very important note for this study is the uncertainty for the data for enzyme production, initially it was not considered as important since the amounts of enzymes routinely used are low, so even with weak data quality the conclusions drawn are solid; even a ten-fold increase or decrease in environmental impact per kg enzyme does not affect the results. However, the BSG liquefaction process requires significantly increased volumes of enzymes; hence the data quality is crucial. Due to the process development in the latter stage of the project and the proprietary nature of LCA data for enzyme production, the opportunity to obtain improved data for analysis during the lifespan of Repro was limited. However, it was decided to carry out the LCA, using a sensitivity analysis to cover the range of possible outcomes resulting from different data for enzyme production.

In the basic study we use the data presented above, which is an average of five types of enzymes, and as sensitivity analysis we use data for the lowest data, a factor 10 lower than the average. This is not ideal and should be considered as a “proof of principle” of the assessment method rather than an evaluation of actual detailed experimental results.

The scenarios analysed were:

1. Using BSG as feed, calculated on environmental impact (=transport) divided with the value for feed.

2. Using BSG for liquefaction. This includes the environmental impact for processing and also the avoided emissions from excess heat. We need data on BSG combustion, which can be based on the chemical composition of the solid phase combined with the efficiency for the boiler. Excess heat is assumed to replace fuel oil (D4.3).

Results

As described in the Methods section (Section2.3.4.3) the results are presented as environmental impact per Euro value created. In
Table ***2.4.3.1*** the results for the base case (average data for enzyme production) is presented. The result for GWP is very negative for the liquefaction process, as a consequence of the high energy use for enzyme production, and the fact that the enzymes used are produced in Denmark, where the electricity is to a large extent fossil fuel based.
The result for acidification is more positive for the liquefaction process which is a benefit of the use of the remaining solid residue. This is assumed to be a replacement fuel for oil in a district heating plant, whereas the heat used in the process in produced from natural gas. This was assumed to a very reasonable solution for a practical implementation of the process. The perceived outcome is that the avoided emissions from the oil are larger than the emissions from the natural gas. In short, one MJ produced from oil emits more than one MJ produced from natural gas.

The results for Eutrophication are considered more detrimental for the liquefaction process, which is explained by the large emissions from enzyme production. The same can be said for energy use.

Table 2.4.3.1. Environmental impact and energy use per Euro value created for the two alternative uses of BSG

	GWP (g CO2-eq./Euro	Acidification (g SO2-eq./Euro)	Eutrophication (g NO3-eq./Euro)	Use of primary energy carriers (MJ/Euro)
BSG Liquefaction	2630	-9	12	8
BSG as feed	420	3	4	7

To test the sensitivity for data for enzyme production the lowest values found for enzyme production was used. The results from this sensitivity analysis are presented in
Table ***2.4.3.2***. It is apparent (and not surprising) that the liquefaction process comes out better. But still the global warming potential is higher for liquefaction, which warrants attention when considering the development of this process.

Table 2.4.3.2. Environmental impact and energy use per Euro value created for the two alternative uses of BSG, using lower emission factors for enzymes

	GWP (g CO2-eq./Euro	Acidification (g SO2-eq./Euro)	Eutrophication (g NO3-eq./Euro)	Primary energy carriers (MJ/Euro)
BSG Liquefaction	477	-16	-1	-6
BSG as feed	420	3	4	7

Consumer acceptance

No direct assessment for this specific process was performed, but the results for the BSG extrusion can be transferred to the process as well as the raw material (see above)

Discussion and Conclusions

The LCA study suffers from the fact that no data on alternative production of FAX was available. This made the multiple functional unit approach impossible. The alternative approach, to use the economic value is often a good one, but in this case it was found to be extremely sensitive to the value that could be assumed for the feed, since it was so close to zero, and variable. Even very small differences gave large changes in the environmental impact. Moreover, the prices assumed for the products were also less accurate. Conclusively this shows the importance to plan studies of this kind early, and to give time and resources for complementary inventories of alternative production and sensitivity analyses. Moreover, the process is more or less energy self sufficient, besides the energy used for enzyme production. The fact that the lignin part is a very useful solid biofuel means the process is a "full waste solution", as opposed to many other up-grading processes where new side-streams often occur, often large volumes, low value and difficult to manage in an economic and environmentally sound way.

The *most important improvement option* is to decrease the dosage of enzymes required, alternatively find enzymes that are cheap and has a low energy demand for their production. THERE IS A PAUCITY OF INFORMATION ON THIS ISSUE. The second most important issue is to evaluate the exploitation potential of the products, both the FAX and also the peptides. Such evaluation is currently not possible due to the lack of a market for equivalent products.

REPRO SCORECARD (ESTIMATED) SUMMARISING EVALUATIONS OF BSG LIQUEFACTION PROCESS

PROCESSING SCHEME: ACCEPTABILITY CRITERIA AND RATINGS		
CRITERION No	**DESCRIPTION**	**RATING**
1	ENERGY UTILISATION	
2	MICROBIOLOGICAL SAFETY	
3	LIFE CYCLE ASSESSMENT	
4	LEGISLATIVE COMPLIANCE	
5	CONSUMER ACCEPTABILITY	
6	COST-BENEFIT ANALYSIS	
	OVERALL ASSESSMENT	

2.4.4 Specific Scenario: Production of fish feed from BSG

2.4.4.1 Introduction

Two target species of marine finfish were used for feeding trials in REPRO namely: *Rhabdosarghus globiceps* (Stumpnose, which is a sea bream or marine sparid), and *Argyrosomus inodorus*, described as a member of the mulloway group of fish and also known as Silver Cob. Both these species are target species for aquaculture in South Africa and were chosen in collaboration with the South African Department of Environmental Affairs and Tourism. Both species are also popular restaurant and export species threatened with overfishing in the wild.

The raw material chosen for inclusion in fish feeds was a brewers spent grain stream obtained from a South African Brewery and distributed by a feed manufacturing company. The company provides wet and dried brewers spent grain to the South African feed industry and they are interested in adding value to their products for inclusion in fish feeds.

Many marine finfish, which are mostly carnivorous by nature, utilise carbohydrates very poorly (Koshio, 2002; Krogdahl et al., 2004 and Fountoulaki et al., 2005). Typical examples include Mediterranean sea bream (*Sparus Aurata*, also defined as belonging to a general group of fish named “sea breams”) which performed poorly when feeds contained high levels of glucose were given (Koshio, 2002), and salmon which developed digestibility problems when precooked maize starch was included in their diets (Krogdahl et al., 2004). These authors reported indications of liver damage or liver stress in these cases. According to the Fish Database, sea bream and salmon are strict carnivores (www.fishbase.org). The fish database describes Silver Cob as a Piscivore, indicating that it only eats other live fish. There is some confusion regarding the feeding habits of Stumpnose, as a member of the marine sparids it is believed to be carnivorous, yet the fish database describes it as having omnivorous feeding habits too. If it is omnivorous, it indicates a possible higher tolerance for feeds from plant origin which will make the use of even more BSG in feeds for Stumpnose possible.

The use of brewers spent grain as a feed ingredient can partly obviate the problem of carbohydrate intolerance in marine finfish because the malting and extraction processes during beer production remove most of the starch (or better described as the fermentable carbohydrates). If the process is optimised, starch levels can be reduced to very low levels with typical residual starch levels in BSG being reported as varying between 5 and 26% depending on the brewing process (Musatto et al., 2005) as opposed to, 63 - 65% starch in the barley grain (MacGregor & Fincher, 1993).

In the REPRO project, BSG was tested as a possible ingredient in formulations for fish feeds, both as is, and after separation of the BSG into two fractions namely a high protein and a high fibre fraction. In both cases, BSG was dried immediately after receiving from the brewery or after the separation step, in order to ensure microbiological stability. Carnivorous fish typically need 50% or more protein in their diets, which necessitated the development of an affordable separation method to produce a high protein BSG stream. A PCT patent application for the separation process was filed and published (PCT application No. PCT/IB2007/052769), evaluation results are pending.

2.4.4.2 Brewers spent grain fractionation

The patented fractionation process is summarised in Figure 2.4.4.1. The process consists of a one-step wet separation technique where wet brewers spent grain directly from the brewery is separated using a small amount of water. The two fractions are separated through a screen, and dried.

High protein
BSG

High fibre
BSG

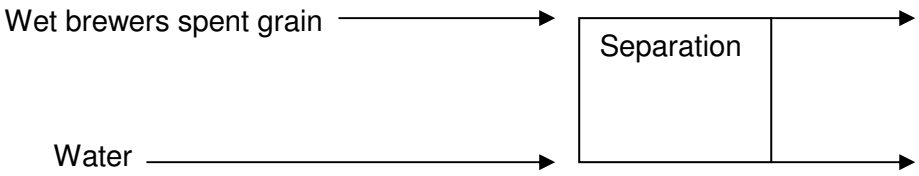

Figure 2.4.4.1. Abbreviated schematic representation of the BSG brushing separation process.

In Table 2.4.4.1 the comparative compositions of dried unmodified BSG and dried high protein BSG are shown. It shows a doubling of the protein content (Dumas method for nitrogen using a factor of 6.25), a small increase in the lipid fraction and a three-fold decrease in the crude fibre content for the high protein fraction. Reduction of fibre is crucial because fish as a single stomach animal cannot digest it and generally it causes bulky faeces, and water pollution, as well as interfering with nutrient absorption in the gut. The dietary fibre has also been reduced, and it consists mainly of arabinoxylans. The true effect of these non-starch polysaccharides in fish digestion is unknown, it may have prebiotic effects, but no data in the literature exist for the species tested.

In Table 2.4.4.2 the proximate composition of the high protein streams of the three processing runs for producing the separated BSG using the patented process is given. Standard deviations were small indicating excellent repeatability of the separation process. Variations in proximate composition will therefore be strongly linked to the composition of the ingoing BSG, which may vary slightly depending on the beer brewing process (for example Laager vs. Stout beers using different barley cultivars). It can therefore be concluded that the separation process will produce a high protein stream with an average protein content of at least 40%. Losses in the process were negligible because of the design of the machine allowing all ingoing BSG to be captured again in the two outgoing fractions. In Figure 2.4.4.2 the two fractions are shown after separation and drying. The high protein fraction was used as is in fish feed formulations.

Table 2.4.4.1. Comparison between composition of dried BSG raw material and the high protein BSG stream (g/100g sample, dry base)

Nutrient	Analytical method	CSIR dried BSG protein concentrate (%)	South African BSG stream (% dry weight)
Protein	Protein (Dumas), AACC 46-30,1999	41.2	21.7
Ash	Ash, In house method	4.1	3.3
Fat	Fat (Soxhlet), AACC 30-25, 1999	12.85	9.0
Carbohydrates	Carbohydrates – by difference	36.34	49.2
Crude Fibre	Fibre (Crude), AACC 32-10, 1999	5.52	16.8

Table 2.4.4.2 Proximate composition of dried high protein BSG produced from three BSG separation runs (g/100g sample)

Analysis	Protein stream 1	Protein stream 2	Protein stream 3	Mean	*Std. Deviation*
Moisture	3.5	4.2	4.2	3.97	*0.40*
Protein	40.26	39.76	39.69	39.90	*0.31*
Ash	4.11	4.6	4.68	4.46	*0.31*
Fat	13.8	13.2	13.5	13.50	*0.30*
Crude fibre	6	5.7	5.9	5.87	*0.15*
Dietary fibre	32.3	32.5	32	32.27	*0.25*
Energy (kJ)	1712	1685	1687	1694.67	*15.04*

Figure 2.4.4.2 Dried high protein BSG (left) and dried high fibre BSG (right).

The amino acid requirements of marine finfish is a complex field and it is an expensive exercise to determine. In order to save time, many researchers base the amino acid requirements of feeds based on the amino acid composition of whole minced fish of the target species. However, Halver, 1978, conducted an experiment using Chinook Salmon where amino acids were tested individually against a reference diet, and the maximum growth rate was determined for each amino acid level. By doing so, they were able to determine the true dietary amino acid requirement of Chinook Salmon. In Figure 2.4.4.3, a comparative amino acid profile is given of the Chinook Salmon amino acid requirements, the amino acid profile of fish meal used as a control in a feed experiment, as well as the amino acid profile of egg protein which is often used as a protein standard in feeding. The profiles of selected cereals typically used in feed formulations as well as the profile of the BSG used in REPRO are also shown. Only the profiles of the ten essential amino acids are given here. Of particular importance is the large difference in amino acids such as lysine and arginine between the profile of fish meal and the profiles of the true requirements. Lysine is typically deficient in cereals when compared to that of fish meal and eggs, which is clear in this comparison. The other amino acids that are deficient are methionine and arginine. However, when compared with the fish meal and the egg amino acid profiles, the true requirement seems to be less stringent, and the BSG is only slightly deficient in arginine, lysine and methionine for maximum growth, and the cost of supplementing the BSG with only these quantities will therefore be significantly less. No such comparative studies have ever done for the Stumpnose and the Cob tested in REPRO. This fell outside the scope and the budget of the project, but it has been identified as a serious gap in the literature, and for future work to focus on species specific feeds, such systematic studies of true requirements will be necessary to replace a general assumption approach of analysing the whole fish.

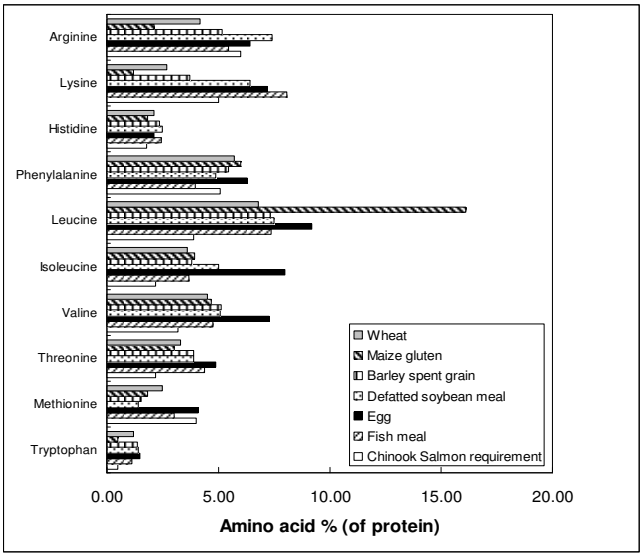

Figure 2.4.4.3. Amino acid profiles of reference protein sources and plant proteins as compared to the true protein requirement of Chinook Salmon (a carnivorous finfish). Barley spent grain analysed by CSIR Biosciences, South Africa, and other data obtained from Halver, 1978, Pereira & Oliva-Teles, 2003, and O'Keefe, 2007.

In Figure 2.4.4.4 the amino acid profiles using 18 amino acids of the high protein BSG stream are compared to the amino acid profile of the unmodified BSG and a typical fish meal control feed (full feed pellet) manufactured for the REPRO work by the CSIR. The amino acid profile did not change after BSG separation except for a reduction in the glutamine content, but the standard deviation of the glutamine determination was high. As glutamine is not an essential amino acid, the reduction is not important. Compared to the fish meal control pellets, the BSG streams were deficient in arginine and lysine, which is typical of cereals.

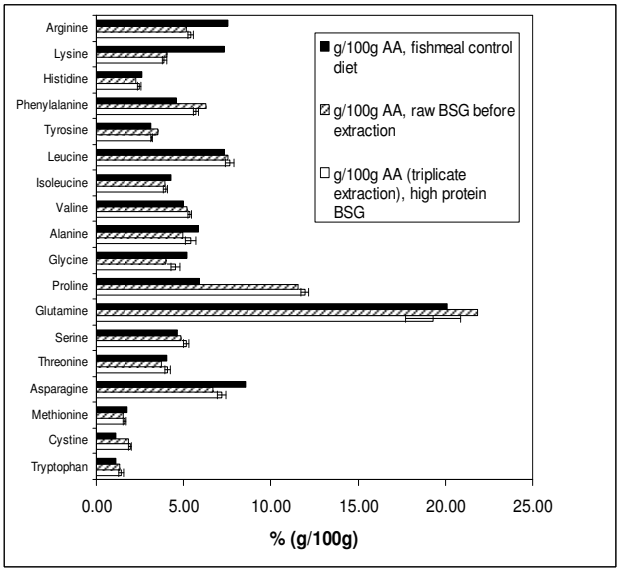

Figure 2.4.4.4. Amino acid profile of high protein BSG produced in South Africa (data obtained from REPRO project), BSG obtained from Alvoer (Pty). Ltd.

The fish species tested required 50% protein in the diets, and therefore the feeds were supplemented with a small amount of fishmeal. In Figure 5 the amino acid profiles of the final fish meal/BSG protein feed are given, compared to a fish meal control feed. The final mixed BSG feed

was deficient in lysine when compared to full fish meal, but based on literature data for true requirements that could be found, it was decided not to fortify the feed with lysine.

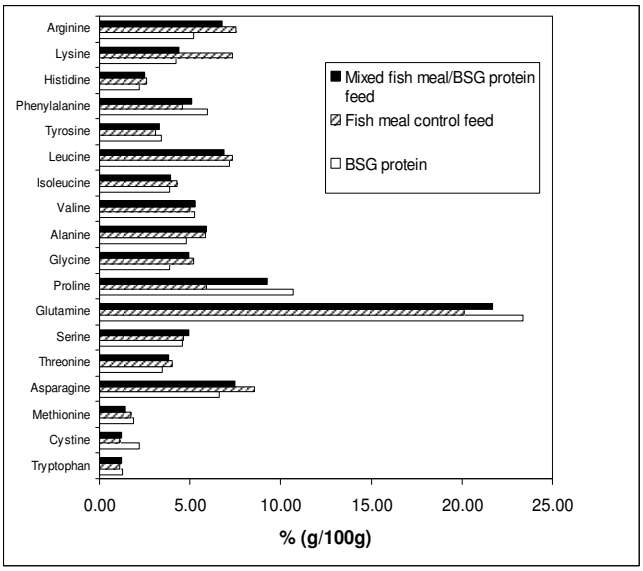

Figure 2.4.4.5. The effect of BSG inclusion on amino acid profiles of fish feeds.

The lysine level is relatively low in most plant protein sources, and in whole barley this level amounts to around 3.5% of the total protein (Kent, 1983). However, lysine is not evenly distributed in the grain. The highest percentage occurs in the proteins found in the germ (6.3% lysine as a percentage of total protein), while lysine levels in the storage proteins in the endosperm are low (0.2 to 1.6%) (Shewry, 1993). Forster and Ogata, 1998, reported that dietary requirements for lysine for sea bream were between 3.6 and 4.4% of dietary protein, thus indicating that the protein that can be obtained from BSG is suitable for feed inclusion provided that the lysine is available. Lysine availability can be negatively influenced by high extrusion temperatures when the feed pellets are produced if residual reducing sugars due to incomplete malting are present. Lysine reacts readily with reducing sugars as a result of Maillard browning, rendering the lysine unavailable for digestion. Therefore, if the barley protein can be removed from the fibrous portion to produce a protein concentrate (e.g. as partly is the case in BSG), the protein-enriched material has more potential as a fish feed ingredient to replace fish meal.

Extruder settings used for fish pellet production are given in Table 2.4.4.3. The objective was to ensure gelatinisation of starchy components in the formulation used as binders, while preventing harsh conditions which may lead to lysine damage. Product temperature was low and no puffing of the pellets occurred. Pellets were dried afterwards at 50°C and are shown in Figure 2.4.4.6.

Table 2.4.4.3. Extruder settings for fish pellet production

Description	Value
Heater 1 (front) ℃	110
Heater 2 (back) ℃	80
Screw speed (rpm)	150
Feed rate (machine setting)	3.2
Feed rate (kg/hr)	4.1
Water dose (machine setting)	10
Water dose (pulse setting)	35
Product temperature (℃)	83
Pressure (Bar)	14
Die size (mm)	4
Raw material feeder: must eliminate bridge formation	

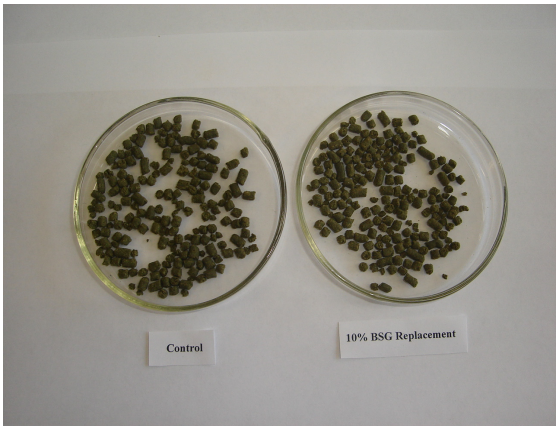

Figure 2.4.4.6. Examples of fish feed pellets

Proximate composition of feeds showed that feeds were balanced in terms of protein content, fat content and carbohydrates (Table 2.4.4.4).

Table 2.4.4.4. Proximate composition of fish feed pellets used for the Stumpnose and the Silver Cob feeding trials

Nutrient	Control pellets (with fish meal)	With 10% fungal material (of formulation)	With 46% of fish meal replaced with high protein BSG
Protein	47.7	47.1	47.2
Omega 3	3.2	2.6	1.6
Total fat	13.1	13.1	13.3
Carbohydrates	30.4	31.4	30.0
Ash	8.8	7.96	6.6
Crude fibre	0	0	2.8

In Tables 2.4.4.5 and 2.4.4.6 the feed performance results are shown for the two fish species tested. Feed results are described in terms of various standard formulas and these are:
SGR – Specific Growth Rate - % biomass increase per day per fish (average)

FCR – Feed conversion Ratio – mass eaten per mass gained per fish for the total period
WG (%) - % weight gained (wet fish weight) measured for a specific period of feeding.

In both experiments, the BSG containing feed produced better results in terms of weight gained and feed conversion ratios when compared to the control feed and the fungal biomass mix with fishmeal.

Table 2.4.4.5. The effect of formulation on *Rhabdosarghus globiceps* (Stumpnose) juveniles as measured by % Weight Gain (WG), Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR). Measure 2 was after one month (4 weeks) and measure 3 after two months (8 weeks) of feeding.

	Measure 2			Measure 3		
Formulation	**WG %**	**SGR**	**FCR**	**WG %**	**SGR**	**FCR**
Control	35.9	1.5	2.5	20.6	1.1	4.2
Improved BSG replacement	31.0	1.3	2.2	24.2	1.1	1.9
Control + Fungal material	31.3	1.3	2.2	20.4	1.0	3.0

Table 2.4.4.6. The effect of formulation on *Argyrosomus inodorus (*Silver Cob) juveniles as measured by Weight Gain % (WG), Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR). Measure 1 was after 6 weeks, measure 2 is after 10 weeks, measure 3 after 13 weeks and measure 4 after 17 weeks of feeding.

	Measure 1			Measure 2			Measure 3			Measure 4		
Formulation	**WG %**	**SGR**	**FCR**	**WG %**	**SGR**	**FCR**	**WG %**	**SGR**	**FCR**	**WG%**	**SGR**	**FCR**
Control	30.4	0.89	1.17	30	0.93	1.09	12	0.6	1.6	24	0.7	1.3
Improved BSG replacement	9.5	0.3	1.87	42	1.26	0.81	16	0.8	0.9	31	0.9	0.9
Control + Fungal material	21	0.64	1.14	29	0.91	1.09	16	0.76	1.2	Feed Depleted		

Fungal material was limited due to scale-up limitations and the feed was depleted after measure 3 (Table 2.4.4.6). BSG containing pellets resulted in slightly higher consumption volumes, which can be ascribed to a slightly higher crude and dietary fibre content, and also possibly due differences in pellet textures as the BSG tended to soften the texture making it more palatable to fish. Other conclusions from Table 6 were: (1) No toxicity effects were observed for the high-protein BSG; (2) Inclusion of the High-protein BSG compared well with a fishmeal-only diet, (3) The inclusion of the fungal material also did not show toxicity effects. Also, the Feed Conversion Ratios were excellent and compared well for those of Atlantic Salmon (Nordgarden et al, 2003). Due to limited fish stock available, these results are preliminary and need to be repeated on a larger scale which fell outside the scope of the REPRO project in terms of allocated funds. These results are based on 'single group' testing and whilst growth rates are indicative of the feed availability, insufficient data is available at present to do statistical analysis.

Silver Cob only eats other live fish in the wild (www.fishbase.org) and the positive results showing the tolerance to high inclusion levels of BSG (46% of fishmeal) and the inclusion of fungal biomass provides a sound basis for future experimental work in this field. This work has shown that partial replacement of the feeds with plant-based (BSG-derived) protein material is possible and feasible.

It has also shown that the Silver Cob fish readily adapts to eating pellets as opposed to live feed, providing that they are weaned properly at a young age. The fish seemed to learn and adapt to the

feeding protocol, usually becoming responsive to the feeding method i.e. if feed floats, the fish will learn to eat from the surface to a certain extent in spite of being a mid-water feeder in the wild. Behavioural aspects of fish reared in captivity for the target species are not known and it is possible that experimental results may be positively influenced by implementing species specific adaptation procedures, this work relates to fisheries management for a specific species which needs further investigation. Typical fish adaptation times for this project consisted of a three week period in the specific tank system with the specific feed to be tested, before the actual experiment was recorded.

2.4.4.3. Conclusions

Typical amino acid profiles available in the literature for sparid feeds are based on profiles for fish meal. That might not be the ideal comparison as it has been shown for salmon that true amino acid requirement differs markedly from the amino acid profile of fish meal. Those true requirements have only been done with very few fish species due to the high cost and time constraints of such trials. However, it is a necessity and research gap that must be addressed for true quantitative assessment on the inclusion of plant-based proteins in fish feed in any future work. The biggest problem is arginine and lysine deficiency in the BSG (typical of cereals), however, these deficiencies are not large. In general, the fish adapted excellently to the inclusion of the high protein BSG and preliminary feed results were very positive, laying down the basis for future research work.

At this stage, no work has been done on the effect of anti-nutritional factors that could arise from BSG and the presence of non-starch polysaccharides such as arabinoxylan. Amino acid fortification can be done, and further work will be necessary to evaluate novel fractionation processes to reduce fibre and increase protein content of the BSG fraction.

2.4.4.4 References

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2.5 Overall Conclusions and Recommendations

Conclusions

The aim of REPRO has been to develop new and acceptable methods to upgrade large, poorly-addressed waste co-products [vegetable trimmings and spent grain], to higher-value materials for food production and to utilize whole waste. This has necessitated an integrated approach, bringing together the expertise and activities of a range of researchers, from life-scientists and chemical engineers through to experts in consumer, legislative and environmental criteria. Only by bringing together these different actors can an objective and overarching assessment of new processing systems be achieved.

The first objective in REPRO was to develop approaches and procedures as means to obtain *traceable, microbiologically safe and stable* co-products.

REPRO has evaluated vegetable trimmings (VT) and cereal waste streams (Brewers' spent grain BSG), and has assessed their suitability, traceability, safety and means to ensure microbiological safety via identification of HACCP.

REPRO has demonstrated that in the modern food chain, it is entirely feasible to expect, and record traceability of co-products for bulk waste streams, and to exploit current technologies to maintain food-grade quality characteristics.

The ability to stabilise and exploit food-grade waste streams is becoming increasingly relevant for the utilization of constituent nutrients in the food chain as global food supplies become limited and existing supply sources need to be efficiently exploited.

The second objective in REPRO was to develop *precision enzyme-based bioprocesses* to deconstruct and tailor co-product components.

REPRO has successfully developed several enzyme-based bioprocesses to disassemble and tailor BSG and VT and their components, involving the development of novel enzyme cocktails, and the provision of a new recombinant cutinase.

In the case of vegetable trimmings, REPRO has demonstrated that it is possible to either

(i) completely liquefy such waste streams, providing the basis for selected fractionation or
(ii) extract intracellular components using appropriate solvent technology and then more selectively (enzymatically) disassemble the plant cell wall in order to obtain potentially exploitable hydrocolloids.

In the case of partially lignified (cereal-derived) co-products, then only partial liquefaction is feasible. However, the REPRO research has demonstrated that it is possible to produce bulk quantities of high value components, and that all components, even the insoluble residues, may be exploited.

The third objective in REPRO was to *integrate the enzyme-based bioprocesses with advanced physical processes*, thereby developing *hybrid bio-processing systems*.

REPRO has successfully demonstrated the feasibility of developing unit processes from the physical and enzyme based bioprocesses (above) and then incorporating these into full process scenarios.

Three main processing lines (with numerous permutations) have been established. These are

(i) direct exploitation of co-products using extrusion technology for the production of snacks
(ii) liquefaction and exploitation of vegetable trimmings
(iii) partial liquefaction and exploitation of cereal co-products.

In addition, REPRO has demonstrated that these (complex) processes can be depicted as chemical engineering models which can then be subjected to scenario analysis and evaluated for a range of risk criteria.

The fourth REPRO objective was to minimise market risk of such new processes by ensuring

(i) socioeconomic and environmental *acceptability* of new processes and products by the *consumer, retailer, and regulator (fork-to-farm concept);*

(ii) continued dissemination of the new knowledge during and after the project.

REPRO has successfully evaluated several of the main processing scenarios for market risk in relation to their economic and environmental acceptability. This approach has included full life-cycle assessment, evaluation of regulatory acceptance and in-depth consumer assessment of selected processes. The results for the fully assessed processes (extrusion of snacks and liquefaction of vegetable trimmings) have demonstrated that the new REPRO processes are more environmentally friendly than disposing of by landfill, or through the production of animal feed. Although only partially assessed, the REPRO research on partial disassembly of cereal residues has indicated that the high levels of enzymes required may be environmentally less sound. Further work is required in this area, but the conclusions will have a bearing on the exploitation of lignocellulose for bioalcohol production – an area of increasing interest.

Regarding the continual dissemination of REPRO outputs, in addition to the routine (and extensive) activities of individual partners in promoting their research activities, the REPRO outputs will be promoted by continuation of the current REPRO website, and the forthcoming Special Interest Groups (a UK national activity, and an EFFoST- related international activity) which will focus on sustainability of the food chain. In addition, the Total Food series of conferences will continue to promote these outputs, and the next Total Food 2009 meeting will include a REPRO session.

As part of a targeted exploitation programme within REPRO, the potential role of exploiting novel fibre and rheologically-active products has been evaluated in emulsion-based food systems. Materials evaluated include recalcitrant residues from cereal products in dairy products, particularly in relation to particle size and impact of complex mixtures, therefore addressing problems of sedimentation of larger particles. In addition, the potential for exploiting cereal co-products (BSG) as a protein source in fish feed has been successfully evaluated and demonstrated. The research has led to the filing of a patent on mechanical separation of protein from residual grain material. Fish feeding trials using feed supplemented with BSG-derived protein successfully carried out.

The REPRO project has focused on developing approaches for whole waste exploitation, and through the evaluation of societal impact has highlighted many of the key risks that must be mitigated in order for entrepreneurial actors to exploit modern science and technology in order to exploit co-products and waste streams. To that end, REPRO has developed a risk scorecard which provides a clear means to describe the complex information related to the exploitation of a co-product.

A colour-coded “Risk Scorecard” which describes the relevant risk criteria associated with the exploitation of a co-product has been developed.

Recommendations

Most food chain wastes contain prospective high value components. REPRO has demonstrated that biotechnology, in conjunction with modern processing systems, has the potential to underpin approaches to liberate these components for commercial and sustainable use. However, the effective development and exploitation of this technology is expensive. The food industry is mature, with a low rate of growth. Hence, there is very limited opportunity for investment in this type of R&D. Until recently there was a perceived limited opportunity for investment in Repro-type research by the food industry but with a changing scenario for global food suoply and the very real conflict between food and fuel production, then Repro-associated activities will become increasingly relevant to the future development of the food industry.

REPRO has verified through experimental approaches that acceptable exploitation of waste streams will be greatly enhanced by the identification and exploitation of the highest value components within a risk-evaluated whole-waste exploitation approach. Furthermore, REPRO has been able to identify and evaluate a range of important risk criteria which need to be addressed if

waste co-products are to be exploited. However, this is not feasible for every co-product, and poor / incorrect perceptions of such risks are likely to reduce investment prospects. This is further exacerbated by the relatively low rewards to the processor.

Industry needs a more user-friendly and cost-effective way to obtain such information. We have concluded that what is needed is a predictive simulation tool to enable the costs, benefits and risks associated with exploitation of waste streams (both generic and local) as a source of multiple-value outputs, to be estimated.

This might be achieved by developing a software-based modelling system which enables the user to

(i) Evaluate the components of co-products streams from appropriate composition databases;
(ii) Create virtual processing scenarios based on defined unit processes to liberate potentially valuable components whilst ensuring whole co-product exploitation, and
(iii) Assess societal criteria at the press of a button in order to evaluate a co-product for potential exploitation routes and markets.

The REPRO participants, in conjunction with 10 other possible partners are currently exploring future opportunities within Framework 7.

APPENDIX 1. REPRO DELIVERABLES

Deliverable	Title
WP1; Procedures for microbiological safety, stability and traceability	
D 1.1	Documented Standard Operating Procedures for source and history of Waste materials at each time point.
D 1.2	Microbiological risk assessment of waste materials
D 1.3 + 1.9	Developed protocols for the use and selection of basic technologies for providing input into WP 2, 3; validated protocols for the use and selection of basic technologies for storage and stability
D 1.4	Risk assessment of co-products, as a function of storage time and stabilization variables
D 1.5	Documented critical control points for supply, stabilization and storage of co-products for each source i) to partners; ii) post-project
D 1.6	Standard Operating Procedures for plant content analysis for Spent Grain and Process Trimmings
D 1.7	Defined waste materials by composition and structure as a function of storage time
D 1.8	Defined co-products by composition for providing input into WP 2, 3.
WP2 Development of precision enzyme-based bioprocesses	
D 2.1	Novel enzymes and their mode of action determined
D 2.2	Total liquefaction processes for spent grain and process trimmings
D 2.3	Method for partial modification of waste matrix for enhanced extractability of hydrocolloids (Parts 1 and 2) **Part 1**: Preparation of material and extraction of pectic polysaccharides **Part 2**: Modification of BG by proteolysis
D 2.4	Methods for controlled modification of pectin chemistry (degree of pectin methyl esterification, hairyness, etc.), xylan chemistry and protein chemistry in lab scale.
WP3; Integration of bioprocesses with advanced physical processes	
D 3.1	Detailed description of developed processes converting cereal derived and leaf based organic by-products into added – value product(s)
D 3.2	Detailed description of developed processes converting cereal derived and leaf based organic by-products into a texturised added – value product(s)

D 3.3 Detailed description of developed process for mild drying with significant preservation of yield of bioactive compounds

D 3.4 Detailed description of engineering models and validated results.

D 3.5 Detailed description of the scenarios evaluated and the results of the evaluations

D 3.6 A microbiological hazard analysis for the new processes developed

WP4; Ensuring acceptability of new processes and products

D 4.1 Upgrading of co-products from food industry – technology assessment report on the acceptability of processes and products and their legal aspects

D 4.2 A socio-economic Assessment report on the economic feasibility and efficiency of the processes and products.

D 4.3 Life Cycle Assessment report on environmental improvements for some of the proposed new technologies for leafy vegetables, and on environmental improvements for some of the proposed new technologies for brewers spent grain.

WP5 Development of sustainable dissemination and exploitation routes

D 5.1+ annex Plan for Technical and Scientific Publications

D 5.2 Establish web site and provide regular newsletter and information on other REPRO publications (scientific and technical) to more than 500 interested parties.

D 5.3 Working plans for creation and operation of platforms

D 5.4 Creation and operation of platforms

D 5.5 Evaluation of Extracted Components on Rheology of Emulsions

D 5.6 Evaluation of Fish Feed sector exploitation opportunities

WP6 Coordination Activities

D 6.1 Contractual deliverables on time – financial statements and information for any audits

D 6.2 Collation of regular financial statements from partners

D 6.3 Arrange all coordination meetings and interim meetings

D 6.4 Contractual deliverables on time– scientific reports and milestones

D 6.5 Ensure delivery of scientific reports

D 6.6 Monitoring, evaluation and managing scientific activity via Management structure

D 6.7 Promote equal opportunities