Total Food

Exploiting co-products - minimizing waste

Edited by Keith Waldron, Craig Faulds and Andrew Smith

FORWARD

The "waste" issue has become topical for the European Food Industry due to legislative pressures. The EC landfill directive has led to an increase in costs and a reduction in landfill opportunities in many States. This is of considerable economic consequence: every year, Europe produces over 220 million tonnes of food waste. With increasing disposal costs, alternative uses of co-products are increasingly being sought. Many co-products have the potential to provide new and natural sources of neutraceuticals and functional ingredients. Indeed, there are numerous research activities and many scientific publications providing the basis for extracting value from waste streams. However, the difficulties of exploiting co-products require more than scientific and technical know-how. They also require an understanding of social values and economic limitations, from market potential to risks associated with diversification. Nevertheless, successes like those highlighted in the whey industry demonstrate that appropriate use of science and technology can turn waste streams into highly valuable income streams.

The purpose of Total Food 2004 has been to provide an international forum to enable a wide range of interest groups including food processors, research scientists, consumer scientists and non Governmental organizations to meet and exchange knowledge about co-product exploitation. Topics have ranged from an environmental overview of food chain activities and consumer acceptance through to the development of pharmaceuticals and biohydrogen production. Focused workshops have enabled delegates to table questions to a global expertise base from over 22 countries and identify emerging problems and potential solutions. As a record of the knowledge communicated, this Proceedings Volume contains the formal publication of the Plenary lectures, short presentations and posters.

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Keith Waldron, Craig Faulds and Andrew Smith

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Institute of Food Research

PROCEEDINGS

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Key Drivers

Perspectives on Environmental Externalities and Side-Effects in the Food Chain

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The Real Costs of Food

When we buy or bake our daily bread, do we ever wonder about how much it really costs? We like it when our food is cheap, and complain when prices rise. Indeed, riots over food prices date back at least to Roman times. Governments have long since intervened to keep food cheap in the shops, and tell us that policies designed to do exactly this are succeeding. In most industrialised countries, the proportion of the average household budget spent on food has been declining in recent decades. Food is getting cheaper relative to other goods, and many believe that this must benefit everyone as we all need to eat food. But we have come to believe a damaging myth. Food is not cheap. It only appears cheap in the shop because we are not encouraged to think of the hidden costs of damage caused to the environment and human health by certain systems of agricultural production. Thus we actually pay three times for our food. Once at the till in the shop, a second time through taxes that are used to subsidise farmers or support agricultural development, and a third time to clean up the environmental and health side-effects. Food looks cheap because we count these costs elsewhere in society. As economists put it, the real costs are not internalised in prices.

This is not to say that prices in the shop should rise, as this would penalise the poor over the wealthy. Using taxes to raise money to support agricultural development is also potentially progressive, as the rich pay proportionally more in taxes, and the poor, who spend proportionally more of their budget on food, benefit if prices stay low. But this idea of fairness falters when set against the massive distortions brought about by modern agricultural systems that additionally impose large environmental and health costs throughout economies. Other people and institutions pay these costs, and this is both unfair and inefficient. If we were able to add up the real costs of producing food, we would find that modern industrialised systems of production perform poorly in comparison with sustainable systems. This is because we permit cost-shifting - the costs of ill-health, lost biodiversity and water pollution are transferred away from farmers, and so not paid by those producing the food nor included in the price of the products sold. Until recently, though, we have lacked the methods to put a price on these side-effects.

Agriculture's Multifunctionality

We should all now be asking: what is farming for? Clearly, in the first instance, to produce food, and we have become very good at it. Modern agriculture is a great success, but only if our measures of efficiency are narrow. Agriculture is unique as an economic sector. It does more than just produce food, fibre, oil and timber. It has a profound impact on many aspects of local, national and global economies and ecosystems. These impacts can be either positive or negative. The negative ones are worrying. Pesticides and nutrients leaching from farms have to be removed from drinking water, and these costs are paid by water consumers, not by the polluters. The polluters, therefore, benefit by not paying to clean up the mess they have created, and have no incentive to change behaviour. What also makes agriculture unique is that it affects the very assets on which it relies for success. Agricultural systems at all levels rely for their success on the value of services flowing from the total stock of assets that they control, and five types of asset, natural, social, human, physical and financial capital, are now recognised as being important (Costanza *et al*., 1997; Pretty and Ward, 2001; Pretty, 2002; Uphoff, 2002; Pretty, 2003).

Natural capital produces nature's goods and services, and comprises food, both farmed and harvested or caught from the wild, wood and fibre; water supply and regulation; treatment, assimilation and decomposition of wastes; nutrient cycling and fixation; soil formation; biological control of pests; climate regulation; wildlife habitats; storm protection and flood control; carbon sequestration; pollination; and recreation and leisure. *Social capital* yields a flow of mutually beneficial collective action, contributing to the cohesiveness of people in their societies. The social assets comprising social capital include norms, values and attitudes that predispose people to cooperate; relations of trust, reciprocity and obligations; and common rules and sanctions mutually-agreed or handed-down. These are connected and structured in networks and groups.

Human capital is the total capability residing in individuals, based on their stock of knowledge skills, health and nutrition. It is enhanced by access to services that provide these, such as schools, medical services, and adult training. People's productivity is increased by their capacity to interact with productive technologies and with other people. Leadership and organisational skills are particularly important in making other resources more valuable. *Physical capital* is the store of human-made material resources, and comprises buildings, such as housing and factories, market infrastructure, irrigation works, roads and bridges, tools and tractors, communications, and energy and transportation systems, that make labour more productive. *Financial capital* is more of an accounting concept, as it serves as a facilitating role rather than as a source of productivity in and of itself. It represents accumulated claims on goods and services, built up through financial systems that gather savings and issue credit, such as pensions, remittances, welfare payments, grants and subsidies.

As agricultural systems shape the very assets on which they rely for inputs, a vital feedback loop occurs from outcomes to inputs. Thus sustainable agricultural systems

tend to have a positive effect on natural, social and human capital, whilst unsustainable ones feed back to deplete these assets, leaving less for future generations. For example, an agricultural system that erodes soil whilst producing food externalises costs that others must bear. But one that sequesters carbon in soils through organic matter accumulation helps to mediate climate change. Similarly, a diverse agricultural system that enhances on-farm wildlife for pest control contributes to wider stocks of biodiversity, whilst simplified modernised systems that eliminate wildlife do not. Agricultural systems that offer labour-absorption opportunities, through resource improvements or value-added activities, can boost economies and help to reverse rural-to-urban migration patterns.

Agriculture is, therefore, fundamentally multifunctional. It jointly produces many unique non-food functions that cannot be produced by other economic sectors so efficiently. Clearly, a key policy challenge, for both industrialised and developing countries, is to find ways to maintain and enhance food production. But the key question is: can this be done whilst seeking both to improve the positive side-effects and to eliminate the negative ones? It will not be easy, as past agricultural development has tended to ignore both the multifunctionality of agriculture and the pervasive external costs (Pretty, 2002; Pretty *et al*., 2003; Stephens *et al*., 2003).

This leads us to a simple and clear definition for sustainable agriculture. It is farming that makes the best use of nature's goods and services whilst not damaging the environment. It does this by integrating natural processes such as nutrient cycling, nitrogen fixation, soil regeneration and natural enemies of pests into food production processes. It also minimises the use of non-renewable inputs that damage the environment or harm the health of farmers and consumers. It makes better use of the knowledge and skills of farmers, so improving their self-reliance, and it makes productive use of people's capacities to work together to solve common management problems. Through this, sustainable agriculture also contributes to a range of public goods, such as clean water, wildlife, carbon sequestration in soils, flood protection and landscape quality.

Putting Monetary Values on Externalities

Most economic activities affect the environment, either through the use of natural resources as an input or by using the `clean' environment as a sink for pollution. The costs of using the environment in this way are called externalities. As they are side effects of the economic activity, they are external to markets, and so their costs are not part of the prices paid by producers or consumers. When such externalities are not included in prices, they distort the market by encouraging activities that are costly to society even if the private benefits are substantial. The types of externalities encountered in the agricultural sector have several features. Their costs are often neglected, and often occur with a time lag. They often damage groups whose interests are not represented, and the identity of the producer of the externality is not always known (Baumol and Oates, 1988; EEA, 1998).

In practice, there is little agreed data on the economic cost of agricultural externalities. This is partly because the costs are highly dispersed and affect many sectors of economies. It is also necessary to know about the value of nature's goods and services, and what happens when these largely unmarketed goods are lost. As the current system of economic accounting grossly underestimates the current and future value of natural capital, this makes the task even more difficult. It is relatively easy, for example, to count the treatment costs following pollution, but much more difficult to value, for example, skylarks singing on a summer's day, and the costs incurred when they are lost.

Several studies have recently put a cost on the negative externalities of agriculture in China, Germany, Netherlands, the Philippines, UK and the USA (for summary, see Pretty, 2004). When it is possible to make the calculations, our understanding of what is the best or most efficient form of agriculture can change rapidly. In the Philippines, researchers from the International Rice Research Institute found that modern rice cultivation was costly to human health. They investigated the health status of rice farmers exposed to pesticides, and estimated the monetary costs of significantly increased incidence of eye, skin, lung and neurological disorders. By incorporating these into the economics of pest control, they found that modern, high-pesticide systems suffer twice, as with nine pesticide sprays per season they returned less per hectare than the integrated pest management strategies, and cost the most in terms of ill-health. Any expected positive production benefits of applying pesticides were overwhelmed by the health costs. Rice production using natural control methods has multifunctionality in contributing positively both to human health as well as sustaining food production (Rola and Pingali, 1993).

At the University of Essex, we developed a new framework to study the negative externalities of UK agriculture. This used seven cost categories to assess negative environmental and health costs - damage to water, air, soil and biodiversity, and damage to human health by pesticides, micro-organisms and disease agents. The analysis of damage and monitoring costs counted only external costs, as private costs borne by farmers themselves, such as from increased pest or weed resistance from pesticide overuse, are not included. We conservatively estimated that the external costs of UK agriculture, almost all of which is modernised and industrialised, to be at least £1.5 billion each year in the 1990s. Another study by Hartridge and Pearce (2001) has also put the annual costs of modern agriculture in excess of £1 billion (2001). These are costs imposed on the rest of society, and effectively a hidden subsidy to the polluters (Pretty *et al.*, 2000, 2001). The annual costs arise from damage to the atmosphere (£316 million), to water (£231 million), to biodiversity and landscapes (£126 million), to soils (£96 million) and to human health (£777 million). Using a similar framework of analysis, the external costs in the USA amount to nearly £13 billion per year (Pretty *et al.*, 2001).

Putting a Monetary Value on Agricultural Landscapes

Landscapes are culturally valuable, and the aesthetic value we gain from them owes

much to their emergence from agricultural practices. They are, of course, almost impossible to value in monetary terms. However, many proxies can be used, including how much governments are willing to pay farmers to produce certain habitats or landscapes, how often the public visits the countryside, and how much they spend when they get there. In the UK, several studies of agri-environmental policies have sought to put a value on positive environmental and landscape outcomes (Willis *et al*., 1993; Hanley *et al*., 1998). These schemes have attempted to restore some of the habitat and other positive countryside attributes that were lost during intensification as well as protect those attributes not yet lost.

UK agri-environmental schemes have been designed to deliver benefits in several forms, including biodiversity, landscape patterns, water quality, archaeological sites, and enhanced access. Benefits may accrue to those in the immediate area of a scheme, to visitors from outside the area, and to the public at large. The annual per household benefits, using a variety of valuation methods such as contingent valuation, choice experiments and contingent ranking, vary from £2-30 for most Environmentally Sensitive Areas, rising to £140 for the Norfolk Broads and £380 for Scottish machair grasslands. If we take the range of annual benefits per household to be ten to thirty pounds, and assume that this is representative of the average households' preferences for all landscapes produced by agriculture, then this suggests national benefits of the order of £200-600 million. Expressed on a per hectare basis, this suggests annual benefits of £20-60 per hectare of arable and pasture land in the UK.

On the one hand, these are likely to be overestimates, assuming agri-environment schemes have already targeted certain landscapes because of their higher value. On the other hand, they could be substantial underestimates, as they omit to value such benefits as pathogen-free foods, uneroded soils, emission-free agriculture, and biodiversity-producing systems, as well as focusing on the outcomes of a scheme rather than the whole landscape. There are too few studies yet to corroborate these data.

Actual visits made to the countryside are another proxy measure of how much we value landscapes. Each year in the UK, day and overnight visitors make some 433 million visit-days to the countryside and another 118 million to the seaside (Pretty *et al*., 2003). The average spend per day or night varies from nearly £17 for UK day visitors, to £33 for UK visitors, and just over £58 for overseas overnight visitors. This indicates that the 551 million visit-days to the countryside and seaside result in spending of £14 billion per year. This is three and a half times greater than the annual public subsidy of farming, and indicates just how much the landscape is valued.

Could Better Policies Help?

These external costs and benefits of agriculture raise important policy questions. In particular, should farmers receive public support for the public benefits they produce in addition to food? Should those that pollute have to pay for restoring the

environment and human health? These two principles are called `the provider gets' and `the polluter pays', and they are important to both industrialised and developing countries. Three categories of policy instruments are available: advisory and institutional measures, regulatory and legal measures, and economic instruments. In practice, effective pollution control and supply of desired public goods requires a mix of all three approaches, together with integration across sectors.

Advisory and institutional measures have long formed the backbone of policies to internalise costs and so prevent agricultural pollution. These rely on the voluntary actions of farmers, and are favoured by policy makers because they are cheap and adaptable. Advice is commonly given in the form of codes of good agricultural practice, such as recommended rates of application of pesticides and fertilizer, or measures for soil erosion control. Most governments still employ extension agents to work with farmers on technology development and transfer. A variety of institutional mechanisms can also help to increase social capital and the uptake of more sustainable practices, including encouraging farmers to work together in study groups, investing in extension and advisory services to encourage greater interaction between farmers and extensionists, and encouraging new partnerships between farmers and other rural stakeholders, as regular exchanges and reciprocity increase trust and confidence, and lubricate co-operation.

Regulatory and legal measures are also used to internalise external costs. This can be done either by setting emissions standards for the discharge of a pollutant, or by establishing quality standards for the environment receiving the pollutant. Polluters who exceed standards are then subject to penalties. There are many types of standards, such as operating standards to protect workers, production standards to limit levels of contaminants of residues in foods, emissions standards to limit releases or discharges, such as of silage effluents, and environmental quality standards for undesirable pollutants in vulnerable environments, such as pesticides in water. But the problem with such regulations is that most agricultural pollutants are diffuse, or non-point, in nature. It is impossible for inspectors to ensure compliance on hundreds of thousands of farms in the way that they can with a small number of factories. Regulations are also used to eliminate certain practices, and include bans on spraying of pesticides close to rivers and on straw-burning in the UK, and the mandatory requirement to complete full nutrient accounts for farms, such as in the Netherlands and Switzerland. A final use for regulations is the designation and legal protection of certain habitats and species, which are set at national or international levels.

Economic instruments can be used either to ensure that the polluter bears the costs of the pollution damage and the abatement costs incurred in controlling the pollution. They can also be used to reward good behaviour. A variety of economic instruments are available for achieving internalisation, including environmental taxes and charges, tradable permits, and targeted use of public subsidies and incentives. Environmental taxes seek to shift the burden of taxation away from economic `goods', such as labour, towards environmental `bads', such as waste and pollution. Clearly the market prices

for agricultural inputs do not currently reflect the full costs of their use. Environmental taxes or pollution payments, however, seek to internalise some of these costs, so encouraging individuals and businesses to use them more efficiently. Such taxes offer the opportunity of a `double dividend' by cutting environmental damage, particularly from non-point sources of pollution, whilst promoting welfare. However, many opponents still believe that environmental taxes stifle economic growth.

The alternative to penalising farmers through taxation is to encourage them to adopt non-polluting technologies and practices. This can be done by offering direct subsidies for adoption of sustainable technologies, and by removing perverse subsidies that currently encourage polluting activities. An important policy principle suggests that it is more efficient to promote practices that do not damage the environment rather than spending on cleaning up after a problem has been created. Many governments provide some direct or indirect public support to their domestic agricultural and rural sectors. Increasingly, payments are being shifted away from being production-linked, such as through price support or direct payments, to being retargeted to support sustainable practices. Generally, though, only small amounts of total budgets have been put aside for environmental improvements though such policies as the US Conservation Reserve Programme, the European Union's agri-environmental and rural development programmes, and the Australian Landcare programme. Many now believe that all public support for farming should be entirely linked to the provision of public environmental and social goods.

The substantial external costs of modern agriculture, and the known external benefits of sustainable agricultural systems, pose great challenges for policy makers. A range of policy reforms could do much to internalise some of these costs and benefits in prices. In practice, as no single solution is likely to suffice, the key issue rests on how policy makers choose an appropriate mix of solutions, how these are integrated, and how farmers, consumers and other stakeholders are involved in the process of reform itself. Attention will therefore need to be paid to the social and institutional processes that both encourage farmers to work and learn together, and result in integrated cross-sectoral partnerships. Policy integration is vital, yet most policies seeking to link agriculture with more environmentally-sensitive management are still highly fragmented.

The problem is that environmental policies have tended only to green the edges of farming. An essentially modernist agriculture remains much as it ever was, but is now tinged green. Non-crop habitats have been improved, perhaps some hedges, woodlands and wetlands. But the food is largely produced in the conventional manner. The bigger challenge is to find ways of substantially greening the middle of farming - in the field rather than around the edges. A thriving and sustainable agricultural sector requires both integrated action by farmers and communities, and integrated action by policy makers and planners. This implies both horizontal integration with better linkages between sectors, and vertical integration with better linkages from the micro to macro level. Most policy initiatives are still piecemeal, affecting only a small part of an individual farmer's practices, and so not necessarily leading to substantial shifts towards sustainability.

Concluding Comments

In this paper, I have adopted a fairly narrow economic perspective to set out some of the real costs of modern agricultural and food systems. The side-effects, or externalities, of food production systems are substantial, yet these do not appear in the price of food. The costs of lost biodiversity, water pollution, soil degradation, and ill-health in humans are shifted elsewhere in economies and, because they are difficult to identify and measure, they are easily lost. Allocating monetary values to these externalities is only one part of the picture, as these methods are inevitably inexact, but they do illustrate the size of the problem. The term multifunctional, when applied to agriculture, implies a system that does more than just produce food. Agriculture shapes landscapes, water quality, biodiversity and carbon stocks in soils. All of these are important public goods, and represent new income opportunities for farmers. But progress is slow, as policy reforms have lagged behind. There is a need for radical integration of policies to support transitions towards agricultural systems that minimise their external costs and maximise their positive side-effects.

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Waste Legislation and its Impact on the Food Industry

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Introduction

The Waste Management Problem in the Food industry

The food and drink industry is one of the largest industry sectors in Europe and is of prime importance to the European economy. The production of waste in this sector is a very large and increasing problem. The total amount of food waste (and by-products) produced in the EU has been estimated at approximately 222 million tonnes per year[1] .

Although the food industry does not produce as much hazardous waste as other sectors, it generates a high volume of biodegradable waste. When it decomposes, biodegradable waste produces methane and toxic materials that pose an environmental risk if not managed effectively. Furthermore, food processing activities are associated with the discard of large quantities of effluent and residues with a high BOD and COD content. The organic content of these effluents and residues results in a high treatment cost.

Waste prevention, minimisation and valorisation are widely recognised as more desirable solutions for waste management than "end of pipe" treatment. It is not surprising that legislation both at the European and national level is a key driving force in promoting the use of these solutions. The development of technology to secure economic solutions to the food waste problem must, however, take place within the regulatory framework laid down to protect the environment and the public.

This paper will consider the waste management strategies in place at the European and National level. It will then deal with the development of waste legislation within the EU. The main regulations affecting the food industry will then be addressed in more detail. The paper will conclude by looking at the problems that have arisen at the Community level with respect to the definition of waste. The paper draws on the work undertaken by ADAS and other partners during the AWARENET Project[2] under the Growth Programme of the EU 56th Framework.

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[1] AWARENET Handbook

1. Waste Management Strategies in the EU and UK:

It is estimated that approximately 3 billion tonnes of waste are generated each year in Europe[3] ; industrial waste, which includes food manufacturing and processing waste, is 15% of this figure. A Community Strategy for Waste Management[4] was therefore published in 1989 and amended in 1996 to deal with this problem. The Waste Strategy[5] as amended sets out the legal principles that will form the framework of European Waste Policy. These legal principles include:

- "The prevention principle: waste production should be limited as far as possible
- "The polluter pays principle: the cost of dealing with waste should be met by the person that produced it
- "The precautionary principle: problems associated with waste should be anticipated
- "The proximity principle: waste should be dealt with as close as feasible to its place of production

Three areas of importance are also identified within the strategy, notably:

- "A waste management hierarchy: priority must be given to waste prevention, recovery, optimisation and minimisation of disposal
- "Producer responsibility: producers must take back end of life products
- "Control of waste shipments: rules are set for imports and exports of waste within EU countries.

The Sixth Environment Action Programme (6th EAP) of the European Union, "Environment 2010: Our future, Our choice", also identifies natural resources and waste as an environmental priority area to be tackled for improvements. The programme states that waste prevention should be viewed as an integrated product policy approach and recognises that further measures are needed to encourage recycling and recovery of waste. It also introduces several Thematic Strategies, one of which is waste prevention and recycling. In 2003, the Commission adopted Communication (2003) 301 towards the Thematic Strategy on the prevention and recycling of waste. This Communication includes an assessment of the European waste policy in relation to prevention and recycling with a view to identifying means to further develop waste management policy in line with the waste management hierarchy set out in the Community's waste strategy described above.

The UK has also adopted a waste strategy called the "Waste Strategy 2000 for England and Wales". It describes the Government's vision for managing waste and resources better. It sets challenging targets for industry, for example requiring the reduction of industrial waste landfilled to 85% of 1998 levels. With landfill tax already

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[2]AWARENET Project, Final Report and Handbook published in February 2004 and available from Gaiker (Spain)
[3]EEA Europe's Environment: the third assessment
[4]EC Communication (89) 339 Final on the overview of the Community Strategy for Waste Management
[5]EC Communication COM(96)339

at a high level and due to increase by £1 per tonne each year, there is a real incentive to find alternative uses for the waste produced.

2. Development of Waste Legislation

2.1 Development of Waste Legislation in the EU

The introduction of the Waste Framework Directive (WFD) 75/442/EEC set the foundation for Community policy on waste management. This Directive was amended by Directive 91/156/EEC to incorporate the guidelines set out in the Community Strategy for Waste Management 1989. The main provisions of the amended Directive are to define "waste", to set out the principles of the waste management hierarchy and to enforce the principles of proximity and self-sufficiency. It also requires Member States to establish waste management plans.

The introduction of the WFD led to the subsequent development of additional waste regulatory regimes. Some of these are particularly important to the food sector and have severe implications to this industry. The most significant regulatory regimes are the Integrated Pollution Prevention and Control Directive, the Landfill Directive, the Animal By-Products Regulation and the Waste Incineration Directive. These will be described in more detail in section 3 and 4 below.

2.2 Implementation of waste legislation in the UK

There are two main types of legislation made by the European Union, notably Directives and Regulations. Both Directives and Regulations must be transposed into Member States national law but the means of implementation differ. Most legislation is in the form of Directives, which set common objectives and deadlines for member States to implement these through the enforcement of national legislation. Regulations do not need to be transposed into domestic law as they have direct applicability. For the implementation of Directives, the UK Government employs a consultation process (usually 12 weeks duration) to ensure stakeholders have an opportunity to comment on what is being proposed. In addition, the Code of Practice on Consultation, recently published, commits the Government to carry out a Regulatory Impact Assessment to determine the risks, benefits and costs of implementation. Reference to the Defra (and EU) Websites can quickly identify proposed legislation in this process and provides an insight into Government policy and plans for waste management.

The WFD transposition into English and Welsh law was staggered and started with the Environmental Protection Act 1990, part II. Subsequent regulations were then introduced, such as the Duty of Care Regulations 1992 and the Waste Managing Licensing Regulations. The Environment Act 1995 came into force to more closely reflect the definition of waste given in the Waste Framework Directive. More recent regulations have also been established in response to the new Directives at the EU level. These will again be considered in section 3 and 4.

3. Main waste legislation affecting the food industry:

The AWARENET study revealed that there are four current pieces of legislation and one impending Directive that are particularly important in regulating waste from the food sector. These are the Integrated Pollution Prevention and Control Directive (IPPC), The Landfill Directive, the Animal By-Products Regulation, the Waste Incineration Directive and the Draft Biowaste Directive. These regulations will be considered in turn below.

3.1. Council Directive 96/61/EC concerning integrated pollution prevention and control

This Directive sets out measures to prevent, reduce and eliminate pollution at source. It is designed to prevent or minimise air, water and soil pollution emission from industrial installations with a view of achieving a "high level of environmental protection". The polluter pays principle is an important theme within the Directive.

Member States must issue operating permits for certain large food installations. The permits must contain conditions based on Best Available Techniques (BAT). The provisions of the Directive cover the following food related activities:

- "Slaughterhouses with carcass production capacity greater than 50 tonnes/day
- "Treatment and Processing intended for the production of food products from:
    - a) Raw animal materials other than milk (production capacity 75 tonnes/day)
    - b) Vegetable raw material (production capacity 300 tonnes/day). Where the finished product is a combination of animal and vegetable waste, if the animal products are incidental or less than 10%, it is treated as vegetable waste.
- "Treatment and processing of milk (quantity of milk received greater than 200 tonnes/day)
- "Installations for the disposal and recycling of animal carcasses and animal waste with a treatment capacity over 10 tonnes/day
- "Installations for intensive rearing of poultry or pigs with more than
    - a) 40,000 places for poultry
    - b) 2,000 places for production pigs (over 30kg)
    - c) 750 places for sows

The Directive applies to new installations with effect from October 1999. Existing installations will gradually become subject to the Directive according to a timescale for permit application. For the treatment of raw meat and for the disposal and recycling of animal carcasses and animal waste, the timescale for permit application is between 1 June and 31 August 2004. For the treatment of vegetables and milk, the window for

permit application is between 1 January and 31 March 2005. For intensive farming activities, the window of application is between 1 November 2006 and 31 January 2007.

The Pollution Prevention and Control (PPC) Regulations 2000 transposed the IPPC Directive into UK law.

3.2. Council Directive 1999/31/EC on the landfill of waste:

This Directive sets challenging national targets for the reduction of biodegradable municipal waste going to landfill. The aim is to encourage prevention, recycling and recovery of waste in preference to landfill use. The use of recovered materials and energy must also be promoted.

Strict conditions apply to landfill sites and their operators. Sites are to be classified into one of three categories, namely inert, hazardous and non-hazardous. Certain hazardous and other wastes, including liquids, are prohibited from landfills. Co-disposal in landfills of hazardous and non-hazardous waste must end by July 2004. All wastes must be pre-treated before they can be accepted on landfill sites. It will be the responsibility of waste producers to pay for the pre-treatment of their waste before landfilling.

All landfill operators must apply for a permit under the PPC Regulations by 31 March 2007. This will enable all landfill sites to come under a single regime. Operators must demonstrate that they and their staff are technically competent to manage the site and have adequate financial provisions to cover the maintenance and aftercare of the landfill site. By 16 July 2002, all operators should have submitted to the competent authority site-conditioning plans setting out that they meet the requirements of this Directive.

The introduction of this Directive has a profound effect on the food industry. Since food waste is biodegradable, it will be diverted from landfills. Alternative routes must thus be sought. This Directive will therefore push the technology forward towards finding alternative uses for waste food, especially as the Directive specifically encourages the recovery and re-use of waste.

The Directive is implemented in England and Wales by the Landfill (England and Wales) Regulations 2002. The Regulations apply to all landfill sites that have been accepting waste since or after 16 July 2001. Existing landfills must demonstrate to the Environment Agency that they can comply with the Directive if they wish to operate beyond July 2002.

3.3. Regulation 1774/2002 on health rules concerning animal by-products not intended for human consumption:

The Regulation aims at allowing the use of raw material from animal processing in the animal food chain under food safety specifications, It also seeks to promote the management and valorisation of animal-by-products when possible. Animal by-products are defined in article 2 as "entire bodies or any part of an animal carcass, or any material of animal origin, not intended for human consumption". The Regulation obliges to collect, transport, store, handle, process, dispose of, place on the market, export, carry in transit and use animal by-products and products derived from them.

Animal by-products are divided into three categories:

- "Category 1 material - very high risk: this includes all body parts, including hides and skins, of animals suspected to be infected by TSE[6] as well as specified risk material, products derived from contaminated animals and catering waste from means of transport operating internationally. This material must be disposed of by incineration directly, be processed and incinerated, then landfilled according to existing regulations
- "Category 2 materials - high risk: this includes digestive tract content and manure, animal material from slaughterhouses wastewater treatment and contaminated products of animal origin. This material must be disposed of by incineration directly, processed and further i ncinerated or, for rendered fats and proteins further processed into fat derivatives for use as fertilisers or soil improvers, transformed to biogas or compost or landfilled according to existing regulations.
- "Category 3 material - low risk: this includes parts of slaughtered animals not intended for human consumption; rejected for human consumption but not affected by diseases; hides and skins; hooves and horns; pig bristles and feathers; non-ruminants' blood; animal by-products from products intended for human consumption; fish intended for fishmeal production; fish by-products from fish processing; and catering waste. This material can by directly disposed of by incineration, processed and further incinerated, processed in a processing plant, transformed in a technical plant, used as raw material in a pet-food plant, transformed in a biogas or composting plant or ensiled (for material of fish origin).

Further measures are provided in the Regulation for implementing reliable traceability and identification marking of specific materials. It also makes clear that intra-species recycling is illegal.

The Regulation will seriously affect the UK food industry after 31 December 2005, as landfilling of former foodstuff and catering waste will then become illegal. The Regulation has already been implemented in other EU countries. Former foodstuffs include waste generated from the production of ready-to-eat food, including scraps

[6] Transmissible spongiform encephalopathy

and dustbins from production lines. Raw meat and raw fish are already banned from landfills. Furthermore, after 31 October 2004, used cooking oils from catering establishments will no longer be permitted to be incorporated into animal feed, unless these have Hazard Analysis Critical Control Point (HACCP) procedures in place.

Many areas of the Regulation are still ambiguous and have not been clarified by the European Commission with respect to this Regulation. There is confusion for example on whether several products such as milk and milk based products and biscuits, bakery waste, pasta and chocolate should be classified in category 3.

Alternative routes to animal feed must be found for the disposal of animal waste, thus reducing the market potential of animal waste. Alternative disposal options include the production of biogas, biofuel, compost or technical products.

The Animal By-Products Regulations implementing the EU Regulation came into force in England and Wales in 2003.

3.4 Council Directive 2000/76/EC on the incineration of waste:

The aim of the Directive is to prevent or to limit as far as practicable the negative effects on the environment, in particular pollution by emissions into air, soil, surface water and groundwater, and the resulting risks to human health from the incineration and co-incineration of waste. A plant is only considered to be an incineration/ co-incineration plant if it burns waste as defined in the WFD. Co-incineration includes plants where waste is used as a fuel or where energy generation/production is the main purpose, such as power plants.

Stringent operational conditions, technical requirements and emission limit values are set out in the Directive for plants incinerating or co-incinerating waste within the EU. Considerable reductions must be achieved for acid gases such as nitrogen oxides, sulphur dioxide and hydrogen chloride as well as for heavy metals, persistent organic pollutants and certain carcinogens. The Directive also targets the incineration of non-hazardous waste.

The regulations apply immediately to all new incinerators and will apply to all existing installations from 28 December 2005. The Directive excludes plants burning animal carcasses and in many circumstances, vegetable waste.

The Waste Incineration Regulations came into force on 28 December 2002 in England. These set minimum technical requirements for some 2,600 incinerators and co-incinerators. Implementation will mainly be carried out under the IPPC regime.

3.5. Future Directive on Biowaste:

"Biodegradable waste" (or biowaste) is defined in article 2(m) of the Landfill Directive as "waste capable of undergoing anaerobic or aerobic decomposition, such as food and garden waste, and paper and cardboard". Traditionally this biowaste has been landfilled, incinerated or land spread. The decomposition of biowaste in landfills produces landfill gas and leachate; both have a high pollution potential.

The biological treatment of biodegradable waste is currently not covered by EU rules. This, according to the Commission, has resulted in the production and marketing of low quality compost from unsorted waste and "eco-dumping" (when low-quality compost is shipped to regions without proper treatment and application standards for biowaste).[7] The lack of well-defined drivers to support biological treatment of biological wastes is preventing the development of an economically sound recycling industry. However, methods such as mechanical-biological treatment of mixed or residual waste could play an important role for treating biowaste.

An EU initiative is thus required to improve the present situation for biowaste management and help meeting the targets of the Landfill Directive. As general principles, an improved management of biowaste in the EC should encourage, in this order:

a) the prevention and reduction of biowaste production

b) the reuse of biowaste

c) the recycling of separated biowaste into the original material

d) the composting or anaerobic digestion of separately collected biowaste not recycled into the original material

e) the mechanical/biological treatment of biowaste

f) the use of biowaste as a source of generating energy

The European Commission has committed itself to prepare a Directive on biowaste by the end of 2004 with the aim of establishing rules on safe use, recovery, recycling and disposal of this waste and of controlling potential contamination. The Commission has published a draft discussion document on biowaste in December 2003 setting out possible elements of this future Directive. General principles for biowaste collection, composting, anaerobic digestion, mechanical/biological treatment and use on land are described in this paper. The document supports recycling (especially to agricultural land) and waste minimisation and suggests that there will be a requirement for the separate collection of biodegradable waste. In Annex I of this document, food biowastes suitable for biological treatment are listed according to the 6-digit code of the European Waste Catalogue[8]. It is anticipated that standards for treated biowastes will be set up. The final adoption of the Biowaste Directive is expected in 2006.

4. Other Relevant Community Waste Legislation:

[7] Environment Directorate Draft Discussion for the ad hoc meeting on biowastes and sludges, 15-16 January 2004, Brussels

[8] Established by Commission Decision 2000/532/EC. the European Waste Catologue is available from www.europa.eu.int/eur-lex/en/lif/en_register_15103030.html

Although the Directives and Regulation described above will have the greatest effect on the food industry, there are numerous other pieces of EU waste legislation impacting the sector. These legal documents can be divided into several categories.

Firstly, there are various Directives dealing with the definition and classification of waste. These include the Waste Framework Directive (Council Directive 75/442/EEC) and Hazardous Waste Directive (Council Directive 91/689/EC).

Secondly, the Regulation on waste statistics (No. 2150/2002) considers how to quantify waste. This Regulation aims to establish a framework for the production of Community statistics on the generation, recovery and disposal of wastes.

Thirdly, many Directives focus on food waste management and disposal. Waste prevention (within the IPPC regime), landfilling and incineration legislation has already been described. Council Regulation No 2150/2002 on the supervision and shipments of waste within, into and out of the Community encompasses waste shipments.

The Directive on wastewater treatment (91/271/EC) and the Directive on the disposal of waste oils as amended (75/439/EEC) deal with wastewater treatment.

The Commission has also issued many Decisions pursuant to the entry into force of major Community Directives. For example, Council Decision 2003/33/EC defines the criteria for wastes for the different landfill categories as defined in the Landfill Directive. Decision 2001/25/EC follows the Animal By-Products Regulations and prohibits the use of certain animal by-products in animal feed.

Appendix 1 provides further information on current EU legislation as related to:

- "Legislation on solid & liquid wastes
- "Legislation for valorised products from food by-products
- "Special legislation in particular agro-food sectors

A table summarising the relevant legislation which has been adopted at **National** level for these Directives, set out in a table relating to the categories above can be found in Appendix 4 of the AWARENET Handbook.

5. Definition of "Waste":

Under the first issue of the WFD, 75/442/EEC, waste was defined as **"any substance or object which the holder disposes or is required to dispose of pursuant of the provisions of national law in force"** (article 1). Crucial to this definition of waste is the meaning of "disposal". This term was specified as "any act leading to the collection, sorting, transport and treatment of waste, its storage and tipping above or under ground as well as any transformation operations necessary for its reuse, recovery or recycling". Thus recyclable materials are classified as waste and subject

to the requirements of this Directive.

The WFD definition of waste was considered to be vague and ambiguous, leading to heterogeneous waste management policies throughout the European Community. The definition of waste was therefore refined in Directive 91/156/EEC amending Directive 75/442/EEC on waste. "Waste" is now defined as **"any substance or object (…) which the holder discards or intends or is required to discard." However, there are still problems arising from the lack of definition for "discard"**. Article 8(2)(iv) of the 6th Environmental Action Programme thus calls for a "clarification of the distinction between waste and non-waste". However, the Commission recognises that due to the subjective nature of waste, any definition would contain at least some degree of ambiguity. [9]

Furthermore, the definitions of recovery and disposal operations described in the WFD annexes are extremely general in nature, again leaving room for interpretation. These definitions play a key role in determining the set of procedures that must apply in waste treatment operations. As defined in the amended WFD, waste recovery includes operations that do not endanger human health and avoid harmful processes to the environment, such as oil re-refining or reuse, fuel or energy generation, spreading on land, composting or use of waste options within the food sector. Furthermore, many treatment operations, especially recycling operations are not covered by harmonised environmental requirements across the Community. Indeed, there is no generally applicable definition of recycling. [10] The lack of reliable definition for waste treatment may prevent Member States from developing and enforcing sound environmental waste treatment standards, leading waste to flow to countries with lower environmental standards. The implementation of IPPC across Member States will play a role in harmonising these operations through the application of Best Available Techniques, although the extent of the environmental performance of these activities will to an extent depend on the way BAT is implemented at a local level.

Neither is the term "secondary raw material" defined at European level, making it more difficult to distinguish when a product becomes waste, and when this waste becomes a secondary product. A substance should become waste the moment it leaves the place of production to be disposed of or to be put through one of the operations listed in Annex IIB of the WFD. Waste can then become a product if is recycled in accordance with the requirements for secondary raw materials (article 11 of the WFD) and if the recovery operation meets the criteria for public health and safety and for the protection of the environment. Problems have however arisen from the criteria used by various Member States to recognise non-waste. The Waste Management Policy Group of the organisation for Economic Co-operation and Development (OECD) has published a guidance document to indicate whether or not material can be regarded as waste. One criteria for determining when waste becomes a secondary product is "when recovery, or another comparable, process eliminates or sufficiently diminishes the threat posed to the environment by the original material (waste) and yields a material of sufficient beneficial use".

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[9] Communication from the Commission (2003) 301 Final, *Towards a thematic strategy for the prevention and recycling of waste*
[10] Communication from the Commission (2003) 301 Final, *Towards a thematic strategy for the prevention and recycling of waste*

Several European Court of Justice rulings have attempted to clarify when waste should no longer be treated as "waste" but as a "secondary product". Although these cases are not related to the food industry *per se*, they provide good examples that can be applied across all industry sectors.

Figure 1 on next page illustrates the relationship between waste and product according to EU legislation.

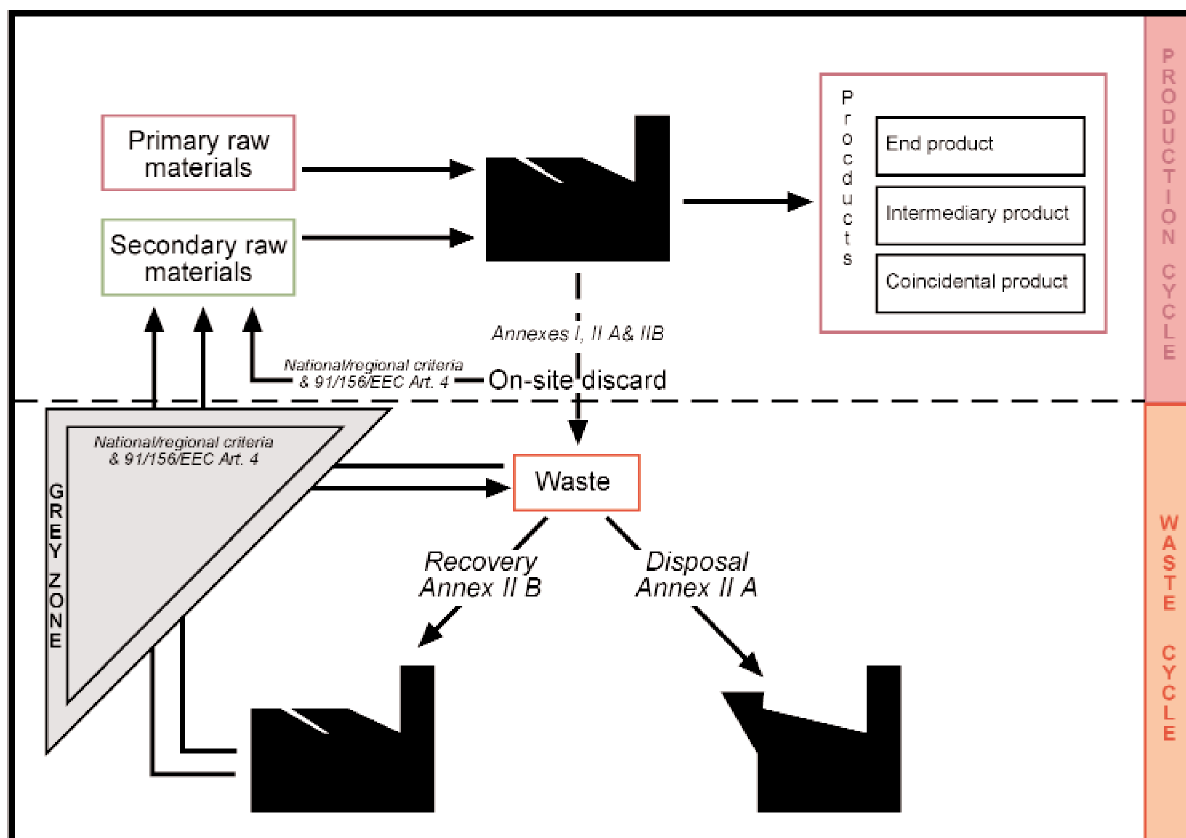
APPENDIX 1

Legislation on solid & liquid wastes

- "Council Directive 76/464/EEC on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community
- "Council Directive 80/68/EEC on measures and restrictions for the protection of groundwater against pollution caused by certain dangerous substances
- "Waste Framework Directive 91/156/EEC
- "Council Directive 91/271/EEC concerning urban waste-water treatment
- "Council Directive 91/689/EEC on hazardous waste
- "Council Regulation 259/93 on the supervision and control of shipments of waste
- "Council Directive 94/67/EEC on the incineration of hazardous waste
- "Commission Decision 96/350/EC for the adaptation of Annex IIA and IIB of Directive 75/442 on waste
- "Council Directive 1999/31/EC on the landfill of waste
- "Commission Decision 2000/532/EC establishing a list of wastes
- "Water Framework Directive 2000/60/EC
- "Council Directive 2000/76/EC on the incineration of waste
- "Regulation 2150/2002/EC on waste statistics
  Council Decision 2003/33/EC on criteria for acceptance of waste at landfills

Legislation relating to valorised products from food wastes

- "Regulation 1774/2002/EC on health rules concerning animal by-products not intended for human consumption
- "Directive 2003/30/EC on the promotion of the use of biofuels or other renewable fuels for transport
- "Council Directive 79/373/EEC on the marketing of compound foodstuffs
- "Council Directive 90/667/EEC on veterinary rules for the disposal and processing of animal waste
- "Council Directive 95/53/EC concerning the organisation of official inspections in the field of animal nutrition
- "Council Decision 1999/534/EC on measures on animal waste protecting against TSE
- "Council Decision 2000/766/EC on protection measures regarding TSE and feeding of *animal protein*



- "Decision 2001/25/EC prohibiting the use of certain animal by-products in animal feed
- "Regulation 999/2001/EC for prevention, control and eradication of TSE
- "Regulation 811/2003/EC on intra-species recycling ban for fish and burning and burial of animal by-products
- "Regulation 809/2003/EC on processing standards for category 3 material and manure used in composting plants
- "Regulation 810/2003/EC on processing standards for category 3 material and manure used in biogas plants

Special legislation in specific agro-food sectors

- "Council Directive 90/496 on Nutrition Labelling for Foodstuffs
- "Council Regulation 91/493 Laying Down Health Conditions for Production and Marketing of Council Directive 93/43 EEC on the Hygiene of Foodstuffs
- "Council Directive 94/435/EC on Sweeteners for Use in Foodstuffs
- "Council Directive 94/435/EC on Colours for Use in Foodstuffs
- "Council Directive 95/2/EC on Food Additives Other Than Colours and Sweeteners
- "Council Regulation 2200/96 on the Common Organisation of the Market in Fruit and Vegetables
- "Council Regulation 258/97/EEC covering Novel Foods and Novel Food Ingredients
- "Council Regulation 1493/99 on the Common Organisation of the Market in Wine

- "Commission Decision 1999/724 on Specific Health Conditions for Gelatine Intended for Human Consumption
- "Council Regulation 104/2000 on the Common Organisation of the Markets in Fishery and Aquaculture Products
- "Commission regulation (EC) No 1623/2000 of 25 July 2000 laying down detailed rules for implementing Regulation (EC) No 1493/1999 on the common organisation of the market in wine with regard to market
- "Council Directive 2000/13/EC on the Approximations of the Laws in Member States on labelling, presentation and advertising of foodstuffs
- "Commission Directive 2001/15 on Substances that May Be Added in Foods for Nutritional Purposes
- "Commission Decision 2001/471 on Regular Checks on Health Conditions for Production and Marketing of Fresh Meat and Fresh Poultry Meat
- "Council Regulation 178/2002 laying down General Principles and Requirements of Food Law
- "Commission Decision 2002/150 Authorising the Marketing of Coagulated Potato Proteins and Hydrolysates as Novel Food Ingredients

Consumer Issues and Sustainability

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Abstract

Various questions need to be investigated when investigating potential commercialisation of sustainable produced consumer products, including the following.

- How does the public conceptualise sustainability? Does this differ from expert views regarding sustainable practices?
- How does this relate to consumption of food / specific products?
- Are there individual and cross-cultural differences in the conceptualisation of, and attitudes towards sustainability?
- What are consumer beliefs regarding sustainable and unsustainable products and product features?
- How do core values influence attitude and opinion? (Is there a conflict? Are there regional and cultural differences?)

In particular, due consideration of these issues should be made when considering recycling food waste into new food products. Account should be taken of consumer acceptance of technological innovation applied during the production process, consumer perceptions of the motives of producers, and perceptions of risk. In particular, previous events such as the BSE crisis may negatively predispose consumers towards recycling food waste within the food chain. It is concluded that effective commercialisation strategies will focus on regaining consumer confidence in food production, as well as effectively communicating and involving consumers in the broader debate about sustainable food production, and how this might be achieved.

Introduction

In considering the issue of food waste recycling as an integral part of the food chain, some key questions regarding consumer attitudes to resultant novel products need to be asked. These include, for example, questions regarding our understanding of how the public conceptualises sustainability, and whether this differs from expert views regarding sustainable food production practices. It is also important to understand how these beliefs and attitudes relate to the consumption of specific foods and food products. Individual and cross-cultural differences in the conceptualisation of, and attitudes towards, sustainable production processes must also be assessed. Research should identify what are the salient consumer beliefs regarding sustainable and, conversely, unsustainable products and product features, and the way in which people's values influence attitudes and opinions. At the present time, however, there has been little systematic empirical investigation of these various issues and

questions. However, it may be possible to make some predictions of possible barriers and success factors regarding product commercialisation by examination of the existing relevant literature.

Consumer perceptions of risk

Consumer risk perceptions differs from those held by other stakeholders involved in food production and risk analysis. It has been well established that people's risk perceptions determine how they react to different hazards. Some factors (for example, whether a hazard is voluntary in terms of exposure or technological in origin) predict people's responses *across* different hazard domains (i.e. The extent to which risk is perceived to be involuntary increases the threat perception for all kinds of hazards). Other factors are domain specific (for example, people may have concerns about the potential for negative effects on animal welfare in the case of Bovine Spongiform Encephalopathy (BSE), which will not apply to other types of potential hazard such as food irradiation).

Psychological factors are important in influencing people's responses to a particular hazard. The technical risk estimates traditionally provided by experts have little influence on people's behaviours and responses. In comparison, people's risk perceptions are a far more influential determinant of their responses to different risks. For example, a risk that people perceive to be involuntary in terms of their personal exposure is more threatening than one that they choose to take, even if the probability of harm is the same, or possibly even less. For similar reasons, naturally occurring risks are less threatening than hazards that are technological in origin. People fear potentially catastrophic hazards more than those that affect a similar number of people, but at different times and places. Natural risks (for example, being struck by lightening) are perceived as less frightening than other equivalent risks which are technological in origin (Katsuya 2001; Slovic, 1993). Other concerns are very specific to particular hazard domains, and this is very much the case in relation to food (for example, see Miles and Frewer, 2001).

Public risk perceptions have been shown to be particularly important determinants of public responses to activities in the agri-food area. These include food safety (Fife-Schaw and Rowe, 2000; Verbeke, 2001), the biosciences, (Frewer *et al*, 1997), and the possible unintended negative environmental and health impacts of technology (Levidow and Marris 2001). All of these examples reflect the observation that public risk perception has been driven by the failure to provide information relevant to the actual concerns of consumers, but which instead focused on the technical risk estimates derived from expert knowledge.

From public misunderstanding of science to consumer engagement

Risk communication activities in the 1970s focused on changing public views on technology risk, particularly in the area of acceptance of emerging technologies. Hilgartner (1990) has described the process of attempting to align public views with

the risk analysis community as the "deficit model". Expert and elite organisations and institutions assumed that the publics are in some way *deficient* in their understanding of risk. If the lay public could understand science and its applications, technology, then concerns about the way associated risks were assessed and managed would disappear. For this reason, the goal of risk communication was to "rectify the knowledge gap" between the originators of scientific information and those receiving the information. Despite the best efforts of the popularisers of science and technology, the lay public remained deeply sceptical of the motives of scientists, regulators and industrialists (Bauer 1995).

As this approach did not appear to work in terms of developing technology acceptance, the next factor that was considered was that of public trust in regulatory institutions and industry. It was assumed by the policy community and industry that the acceptance of emerging technologies and other hazards was contingent on public trust in institutions with responsibility for regulating the associated risks (Siegrist 1999).

One approach to developing trust focused on greater public inclusion in the process of policy development. When people feel a lack of control over their exposure to potential hazards, risks are perceived as higher, so in cases where there is a lack of control, trust in risk assessment and risk management is likely to be a particularly important determinant of public confidence in food safety. A case in point is that of genetically modified foods, where consumer concern did not focus primarily on risk *per se*, but rather on the lack of personal control on the part of the consumer over consumption (Miles and Frewer, 2001).

It was reasoned by the policy community that more extensive public consultation and participation in risk management and other science and technology issues would restore public confidence in institutions with responsibility for public and consumer protection (see, for example, Renn, Webler and Wiederman, 1995; Rowe, Marsh and Frewer, 2004). Indeed, this appeared to reflect institutional recognition that consumers' attitudes towards different hazards are not only dependent on an analytical assessment of risk and benefit. Other factors, such as *ethical* and *moral* considerations, were recognised as potentially influential in establishing the acceptability or otherwise of a particular hazard or societal approval of the measures put into place to contain specific risks. Social inclusion is important if consumers are to build trust in both risk analysis and technology development and commercialisation, although consumer opinions should be seen to influence outcomes and technological developments otherwise the effect may be trust destroying rather than the converse.

The impact of food scares

Once consequence of various food "scares, for example, the GM controversies in the late 1990s, has resulted in increased consumer distrust of sustainability claims supporting "risky" technology push. The question that should be asked here is under what circumstances people will accept potentially controversial technologies applied

to production if there is a benefit to sustainability, and whether improved sustainability in itself represents a substantial enough benefit to offset consumer negativity towards GM production processes.

A further problem for those interested in improving sustainability through recycling waste products was the BSE scare, where consumer concern was underpinned by recycling of animal waste through the human food chain in the form of animal feed. As a consequence there may be consumer concerns about the use of reprocessed waste in novel food production.

It should also be remembered that food choice is as much a cultural, social and emotional process as it is a rational choice, and the purchase of products perceived as recycled waste may be problematic in terms of perceived quality reduction. Consumers also demand the enforcement of effective traceability systems and, as a consequence, are likely to demand the introduction of utilitarian labelling strategies focusing on both sustainability and food production. Consumer perceptions that food waste recycling is occurring in a non-transparent manner may compromise acceptability of the resultant products, particularly in the food area. Potentially negative emotional responses regarding the consumption of food waste may also be problematic.

The question that must be asked relates to understanding under what circumstances people will accept potentially controversial technologies applied to production if there is a benefit to sustainability, and whether improved sustainability in itself represents a substantial enough benefit to offset consumer negativity towards GM production processes. Against this, there is some evidence that genetic modification of food is more acceptable, at least for some consumers, if sustainability is perceived to improve as a consequence, and the resultant products are labelled as such (Miles and Frewer 2001).

I will now consider the example of the BSE scare in particular, as it has had a potential impact on consumer acceptance of other attempts to recycle food waste. Miles and Frewer (2001) have noted that public risk perceptions associated with the BSE crisis were driven by the failure of government and the industry to provide information relevant to the actual concerns of consumers. BSE related risk communication was based on technical risk assessments, ignoring key issues of concern to the public. These included worry about animal welfare, and effective communication regarding risk uncertainty. The latter was particularly salient given that consumers perceived uncertainty regarding the risks of BSE was being hidden by the authorities prior to 1996 in order to protect their own interests, and those of industry. Other consumer concerns focused on the use of technology in food production per se (for example, technology applied to animal husbandry), and the potential for unintended effects associated with the application of these processes to occur.

Frewer and Salter (2002) observe that, as a consequence of the BSE scare, the decline in the public's trust in science has passed a "threshold point" where the

legitimacy of scientific judgement is questioned. The rise of "consumer citizens" (who express informed choice *via* purchase behaviours), combined with the diminished role of the "expert" as a consequence of wide availability of specialist information, means that simply explaining that a product is safe and sustainable will not result in successful commercialisation. In addition, research must be conducted to prevent product developers misunderstanding consumer preferences regarding novel product development.

Best practice in developing sustainable production

It is important that industry develops a "code of conduct" regarding socio-economic impact of sustainable production processes. Such a code may not just serve humanitarian, ethical, environmental and other "non-competitive" goals, but *also* economic purposes. Quality and environmental policy are, for example, integrated parts of the ISO 14000 certification. Codes of conduct are likely to be particularly relevant when producers face a lack of trust from its stakeholders, when laws and regulations of the government are not specific regarding the issue at hand, and when people from different cultures meet and interact. In the absence of an international government or shared norms and values, codes of conduct may offer a solution that is clear to all actors involved in the issue at hand.

Ingelbleek (2003) has noted that such codes are of particular interest as part of strategies where firms adopt codes of conduct on food safety and/or sustainability. If customers percieve these attributes not as beneficial to themselves specifically, but as beneficial to society more generally, they may not be willing to pay for the development and implementation of the strategy as a consequence of increased prices in the retail sector. Thus codes of conduct regarding sustainable production may reflect *societal* value rather than *customer* value, but should not be associated with increased prices relative to similar products not produced in a sustainable way.

Conclusions

The development of effective commercialisation strategies regarding novel, sustainably produced food products must focus on regaining consumer confidence in food production and food technology, as well as effectively communicating and involving consumers in the broader debate sustainable food production, and how this might be achieved. The emphasis should be on understanding what *consumers* understand by sustainability, and how this might be introduced into product design. Adopting such a strategy may overcome consumer negativity linked to the application of food technologies to food production, which may be problematic in the context of food waste recycling, particularly if the resulting novel products are destined for human or animal consumption.

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Food surplus; reduction, recovery and recycling

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Introduction

Modern food production methods appear efficient. The reality is that the combination of large scale manufacturing and rigid supply chains create significant quantities of waste. This waste has a huge retail value, estimated to be between £8 and £16B a year and disposing of this material is becoming increasingly costly.

By considering this material as surplus food for human consumption which can be re-used within the production unit or recycled into meals there is the potential to reduce production costs and the environmental impact of this material as well as contribute to improvements in the health and welfare of the most vulnerable in society.

Waste from the food chain

The food sector accounts for over a third of the waste produced in the UK, a total of 17M Tonnes. Approx. 15% arises from food manufacturing and a further 21% from distribution, retailing and consumption (Fig 1). If one assumes that the biodegradable element of this waste stream has at least some potential for recovery and consumption by humans or animals then a report by C-Tech suggests this is around 1.9-3.8MT. The retail value of this surplus is approx. £7.6 and £16B assuming an average value of £4000 per tonne.

Data from the same report suggest that over 2/3 of this biodegradable portion of food surplus is already re-used or recycled (Fig 2). It is not clear what uses this includes but it is doubtful whether much of this recycling involves recovery for human consumption.

Source: "United Kingdom Food and Drink Processing Mass Balance" C-Tech Innovation Limited 2004, Biffaward Mass Balance Programme on Sustainable Resource Use

Source: "United Kingdom Food and Drink Processing Mass Balance" C-Tech Innovation Limited 2004, Biffaward Mass Balance Programme on Sustainable

Resource Use

Even with the comprehensive survey conducted by C-Tech it is difficult to be definitive about the actual % of food which is wasted through the food chain. Data from the USA suggests that around 20-27% of food is wasted and, in the USA, this is sufficient to feed 49M people every year. In the UK, FareShare estimates that:

- 4 million people in the UK cannot afford a healthy diet
- 1 in 7 people over 65 are at serious risk of malnourishment
- 3 out of 5 people living on the streets or in basic, insecure accommodation have no daily intake of fruit and vegetables.

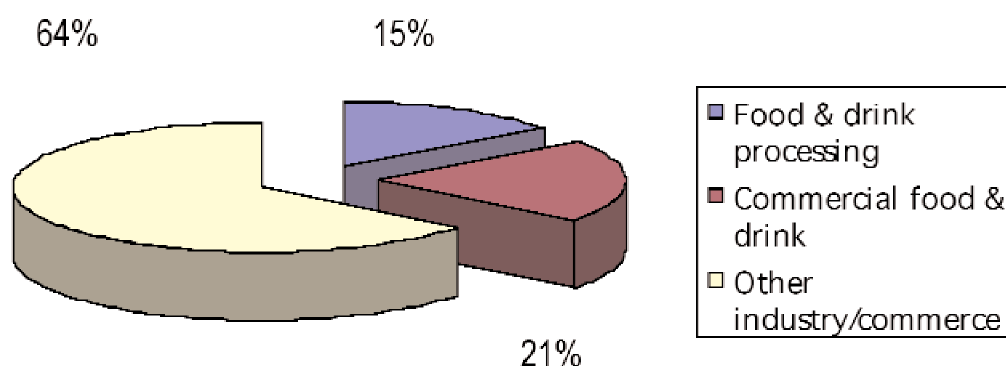

Figure 1. Waste from the food chain

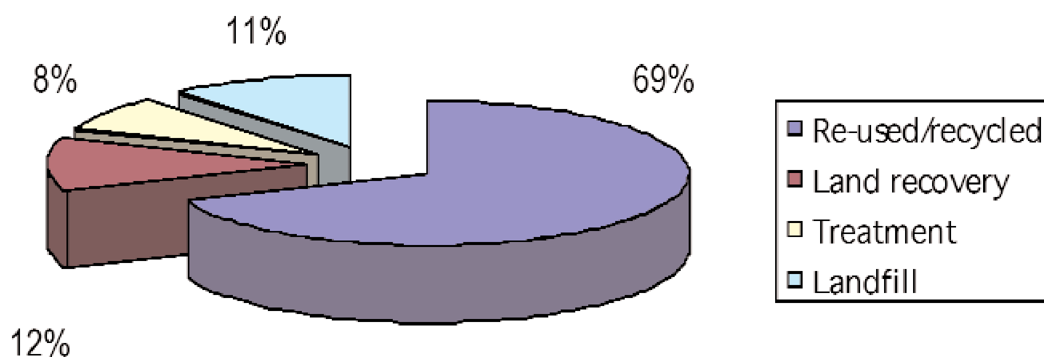

Figure 2. Disposal routes for biodegradable waste

Drivers

Apart from Corporate Social Responsibility what will drive companies to reduce this surplus and recover more of it for consumption? Cost is a major factor. Landfill costs in the US are now around £135 per tonne for this material and have already reached £85 a tonne in Ireland. In the UK, rendering costs are £65-80 a tonne and even landfill is already costing £35 a tonne.

More revealing is the retail value which is lost when surplus food has to be disposed of. For example, tinned tomatoes have a retail value of c. £1000 a tonne, breakfast cereals and marmalade are worth £4000 a tonne; values which may encourage a more pro-active approach to reducing this surplus.

Legislation is another driver. The Government is committed to reducing the volume of biodegradable waste which goes to landfill by 60% by 2016. In addition, waste minimisation is a key element of the Integrated Pollution Prevention and Control (IPPC) regulations currently impacting the food industry. Waste audits are required by 2005 or one year after an IPPC permit is first issued.

The Best Available Technology (BAT) requirements set out under IPPC promote a very clear hierarchy of recovery, re-use and reworking before disposal is considered though it is unfortunate that products and material which are surplus to requirements are considered to be ‘waste’.

Possible solutions; Second Harvest

In the USA, Second Harvest and several other charities collect and distribute surplus food at all stages in the food chain. Second Harvest alone distributes 500,000 tonnes a year some of which is turned into meals by volunteers and the homeless working in kitchens. Many major companies donate including Kellogs, Nestle, Unilever, McCain and Coca-Cola.

Partnering with Second Harvest provides liability protection, free pick up throughout the US, savings on inventory and disposal as well as product tracking. The issue of liability is, of course, a key one and the Bill Emerson Food Donation Act (1996) helped by:

setting a floor of gross negligence or intentional misconduct

protecting the donor company from liability when food is donated.

FareShare

The aim of FareShare in becoming involved with the issue of surplus food is:

The relief of poverty and the preservation and promotion of good nutrition and good health among people who are suffering from social, economic or emotional distress; in particular by:

- minimising food going to waste
- creating opportunities for people to improve their health and well-being by working in partnership with local charities to provide access to quality food and other services

- providing people with the chance to volunteer and contribute to their local communities as well as gain new skills.

The organisation is now distributing 1,500 tonnes of quality food from over 100 companies involved in 8 schemes across the UK. Over 1.5 million meals have been delivered and the health and well being of over 9,500 homeless and vulnerable people have been improved. FareShare are currently conducting a survey of companies involved in the food chain to establish the quantity of surplus food available and the barriers to recovery for human consumption.

A food recovery hierarchy

The cheapest and simplest solution to surplus food is not to produce the surplus in the first place (Table 1). Initial results of the survey by FareShare, albeit from a limited number of responses at the time of the conference, suggest that insufficient shelf life and the challenge of matching supply to demand are major reasons for surplus.

If surplus cannot be eliminated or reduced significantly then the next priority is to reduce and re-use material within the production unit, partly through more efficient transformations, better matching of supply to demand and then re-use. Separation of surplus for human consumption then enables this food to be recycled outside the production unit as either edible finished goods or edible raw materials which can be processed into meals.

If this proves difficult then animal feed is always an option though even this route is becoming increasingly proscribed by regulations. Beneficial treatment of surplus through composting or anaerobic digestion at least produces some benefits in terms of soil conditioning and fertilisation with landfill or destruction being the last resort.

Table 1; the food recovery hierarchy

Eliminate surplus

Reduce surplus and re-use within processing unit

Recover and recycle surplus outside processing unit
(edible finished goods and raw materials)

People first, animals second

Beneficial treatment

Disposal/destruction

A charter for food recovery

The potential to reduce costs and environmental impact as well as benefiting society is huge and there are some relatively simple steps which could be taken to encourage recovery and consumption of surplus food (Table 2). Technology has a

role to play though improvements in management practice are likely to have a far greater impact. Where technology can add most value is well up the process chain rather than at end of pipe.

Consideration of the recovery and re-use of surplus during the design of manufacturing processes and lines would help as would more efficient transformation of raw materials. The social sciences and software sector also have a role to play in enabling more accurate forecasting of demand.

Cleaning is a critical activity in any food manufacturing environment but can this be carried out with less food being wasted? Spills and overfills are another source of wasted material, some of which are due to operator error but improvements to vessel design and sensor technology could also contribute to fewer drops of raw materials and product. Liability is likely to be an issue for businesses and a Food Donation Act, similar to the Bill Emerson Act in the USA may accelerate food donation.

Table 2; A charter for the recovery of surplus food

Retailers and process industry	**Environment agency**
Edible product & ingredients will be recovered for human consumption	Change vocabulary & advice: 'surplus' not waste Promote food recovery hierarchy
Government	**Technology**
Food donation bill to restrict liability?	Design in surplus recovery More efficient transformations Less wasteful cleaning Sensors to reduce spills Better forecasting of demand

CONCLUSIONS

1. Modern food production and distribution is an inherently wasteful activity with around 25% of the material introduced to the food chain wasted.
2. Increasing costs, realisation of the lost retail value and the availability of reliable and cost effective recovery channels such as FareShare should encourage retailers and manufacturers to reduce the volume of material which is wasted.
3. A commitment to recover surplus food for human consumption either through re-use within the production unit or recovery and external recycling will have the most impact.
4. Government and its agencies have a role to play in changing attitudes by limiting use of the word waste, promoting the food recovery charter and hierarchy and considering introduction of a bill to limit the liability of donors.

5. Focusing on technological innovation within the production and distribution process is likely to be more beneficial than concentration on end of pipe solutions.

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High Value Products

Agri-Food Residues as a source of Phytochemicals

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Introduction

Epidemiological studies have related the dietary consumption of horticultural products, mainly fruits and vegetables, with a decrease in the incidence of cancer and cardiovascular disease mortality (Rimm *et al.*, 1996; Doll, 1990). This has been associated to the content of phytochemicals as one of the key factors. The main bioactive phytochemicals in fruit and vegetables include polyphenols, terpenoids, glucosinolates and other sulphur-containing compounds. Clinical studies support the role of the plant food phytochemicals as health-promoting food constituents (Kris-Etherton *et al*., 2002; Scalbert *et al*., 2002). The role of antioxidant phytochemicals in the prevention of these diseases has been mainly attributed to the prevention of LDL oxidation (Scalbert *et al*., 2002; Rankin *et al*., 1993) through a scavenging activity against peroxyl and hydroxyl radicals (Rankin *et al*., 1993).

These compounds are also responsible for different quality characteristics, including colour, flavour and aroma, of fruits and vegetables and related food products (wines, juices, etc.) (Tomás-Barberán and Espín, 2001). These are secondary metabolites that have an ecological role in plant tissues as insect feeding deterrents, UV-screens, and animal attraction to ensure pollination and seed dispersal among other functions (Harborne, 1982). By these reasons the phytochemicals are preferentially biosynthesised in the external plant tissues. These external parts are the main waste material during handling and processing of fruits and vegetables and constitute a good source for extraction of phytochemicals.

The main sources for waste production from the fruit and vegetable commercialisation and industrial transformation originate in the packing houses, were external leaves and low quality products are discarded, in the fresh-cut industries, where peels and external tissues are removed, and in the extraction industries, where the press-cake residues (pomaces) constitute an important residue.

Examples of the actual use of wastes from fruit processing industries to produce extracts that are available in the marked include orange, grape, apple and olive residues. The residues from orange juice extraction industries (orange albedo and flavedo and fruit segments) have already been exploited for many years for the

extraction of flavanones (hesperidin and related compounds) and pectin. Grape wastes from the wine-making industries (grape pomace and seeds) are also used industrially for the extraction of anthocyanin pigments, procyanidins and polyphenol extracts (Gabrielska *et al*., 1997; Lu and Foo, 1999). From the olive-oil extraction industries the residues can also be used for extraction of hydroxytyrosol (Visioli *et al*., 1999), the main phenolic antioxidant in olive oil and an efficient process for this purpose has been patented by researchers in the Instituto de la Grasa (CSIC) (Seville). From the cider industries, the apple pomaces are already used for extraction of pectins although phytochemical extracts have not yet been implemented. Recently research has been developed to use the tomato-juice production residues for the extraction of lycopene, one bioactive terpenoid pigment from tomato, and this has been studied by different groups using specific extraction techniques. The European Project MAXFUN is studying the possible use of the press-cake residues from the berry juice production (bilberry, black currant and grapes) for the extraction of phytochemicals.

The exploitation of residues from vegetable commercialisation and processing for extraction of phytochemicals is actually less developed.

The packing houses dealing with vegetables produce large amounts of wastes and residues (leaves, stems, etc.). Sometimes these by-products could reach 50% of the harvested material as in lettuce and cauliflower. These residues are very perishable products which management is not always easy and are responsible for environmental management problems in the industries. Minimizing their environmental impact has been the subject of an increasing concern in the past recent years.

In general, by-products from handling and commercialization of vegetables have been traditionally used as animal feedstuff (Martínez Teruel *et al*., 1998; Íñiguez *et al*., 2001), for fiber production (Femenia *et al*., 1981; Marconi *et al*., 2000) and fuel production (Hang, 1987). An interesting approach to give an added value to these materials is their use as sources of phytochemicals and natural antioxidant compounds, mainly phenolic compounds which in some cases have comparable activity to that of synthetic antioxidants (Azizah *et al*., 1999; Lu and Foo, 2000). A number of by-products have been previously studied as potential sources of antioxidants (onion, carrot, potato peel). However, as far as we know, the use of vegetables byproducts such as artichoke, cauliflower or lettuce as a possible source of antioxidant phenolics is scarcely reported.

Natural antioxidants are in great demand nowadays due to both consumers preference and health concerns associated with the use of synthetic antioxidants such as BHT and BHA (Oyeneho and Hettiarachchy, 1993; Azizah *et al*., 1999).

The changes of the modern lifestyle of life have produced an increase of consumption of the "ready to eat" foods (canned, refrigerated, etc) that generally contain small amounts of health-promoting compounds. Functional foods try to

contribute to a proper dietary habit by providing foodstuffs with ‘added-value’: adding new ingredients that increase their health-promoting properties by increasing bioavailability of active compounds, etc. (Roberfroid, 2000). In this context, addition of phenolic- enriched extracts derived from by-products could be a feasible strategy to develop functional foods and at the same time would contribute to valorize of these by-products.

In a recent project we have aimed to the identification of vegetable residues for the preparation of phytochemical extracts and the application of these extracts to the functionalisation of juices.

Results

Technological processes for phytochemicals extraction from residues.

The extraction processes used for the preparation of these phytochemical extracts have to meet some requirements. Firstly, it is preferred the use fresh raw materials for extraction, as a drying process, although would allow storage, would make unacceptable the increase in production costs. It is also necessary to use food compatible solvents as is the case of water, ethanol or mixtures of them. Thermal treatments are generally necessary to inactivate enzymes that can degrade the phytochemicals during the extraction process (Tomás-Barberán and Espín, 2001).

The extracts obtained need to be concentrated and spray drying or freeze drying are feasible technologies that could be applied depending on the price of the obtained extract in the market. In some cases a extract purification through non-ionic polymeric resins (of the type Amberlite XAD) can be used to concentrate the phytochemicals before drying (Tomás-Barberán *et al*., 1992). In these cases, the water extract (or a water solution of the ethanol-water extract) is filtered through the resin column and the phytochemicals are retained in the stationary phase. Then these compounds are eluted with ethanol, and this extract concentrated. The extracts obtained are generally prepared in dried form although liquid extracts is another possibility. These extracts can be used for the preparation of pills (dried extracts) or to prepare functional juices (Larrosa *et al*.; 2002) or other new foods such as soups, sauces, margarines, etc. to which liquid or dried phytochemical extracts can be added.

Vegetable wastes and residues as a source of phytochemicals.

The phytochemical composition of vegetables such as artichoke, cauliflower, lettuce, etc., as well as the effect of processing and storage on this composition (Gil-Izquierdo *et al*., 2001, Dupont *et al*., 2000) have been widely studied. These works have been focused mainly to improve the quality of the commercial products, however, scarce information is available regarding their corresponding by-products.

Artichoke by-products

The industries produce two types of artichoke by-products: vegetable stuff composed by outer bracts and stems which could be thermally treated (produced by the canning industries) or not (produced by fresh–handling industries) as well as the water used in the blanching process. These by-products have been studied concerning their application for animal feedstuff (Martínez Teruel *et al*., 1998) and fiber production (Goñi and Saura-Calixto, 1988; Femenia *et al*., 1998.).

The artichoke by-products are a very good source of antioxidant polyphenols with caffeic acid derivatives as main phenolic compounds (Llorach *et al*., 2002). The antioxidant activity has been proved with different assays showing special capacity to prevent the peroxidation of linoleic acid (Llorach *et al*., 2002).

Cauliflower by-products

Cauliflower by-products (*Brassica oleracea* L. var. botrytis) mainly consist of leaves and, in less amount, stems. Regarding the edible portion of the cauliflower, this is rather poor in phytochemicals and only small amounts of some hydroxycinnamic acid derivatives such as caffeic, sinapic, and ferulic acids were identified and quantified.

The HPLC analysis of cauliflower by-product extracts revealed the presence of both flavonoids and hydroxycinnamic acids (caffeic acid and sinapic acid). Different combinations of flavonols such as kaempferol and quercetin with sinapic acid and glucose have been identified being the main compounds kaempferol-3-O-sophoroside-7-O-glucoside and its sinapoyl derivative (kaempferol-3-O-(sinapoylsophoroside)-7-O-glucoside). Moreover, some flavonoids with an unusual high grade of glycosylation (5 sugars moieties) have been isolated and tentatively identified for the first time (Llorach *et al*., 2003a). To our knowledge, the characterization of flavonoids with more than four sugars has not been previously reported.

The cauliflower by-products showed a relevant antioxidant capacity, estimated from their ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II). In this way, 16 g (d.w.) of cauliflower by-products can provide the same antioxidant capacity than one cup of tea or one glass of red wine (Llorach *et al*., 2003b).

Lettuce by-products

By-products from lettuce (*Lactuca sativa* L.) varieties (Romaine, Iceberg and Baby) and one of chicory (*Cichorium endivia* L.) variety "escarole" have recently been used to evaluate their polyphenolic content as well as their antioxidant capacity showing interesting results (Llorach *et al*., 2004, in press). The phytochemical profile of lettuce by-products is composed by hydroxycinnamic acids (both caffeoylquinic and caffeoyltartaric acid derivatives) and flavonoids (both flavones and flavonols). The main hydroxycinnamic acid derivative identified was dicaffeoyltartaric acid (chicoric acid) followed by chlorogenic acid (5-O-caffeoylquinic acid). In addition different

isomers of isochlorogenic acid (3,5-O-dicaffeoylquinic acid) were identified. The flavone Luteolin-7-O-glucuronide was identified, and regarding the quercetin derivatives quercetin-3-O-glucoside, quercetin 3-O-glucuronide and quercetin 3-O-(6-O-malonyl)-glucoside have been identified. Regarding chicory byproducts the HPLC analyses of raw extracts showed a kaempferol 3-O-glucoside as the main flavonol and this compound has already been reported in chicory.

Lettuce by-products have shown a interesting antioxidant capacity both free radical scavenging activity and capacity to reduce Fe(III) to Fe(II) (Llorach *et al.*, 2004, in press).

Other byproducts have been considered a good source of phenolics compounds (Bonilla *et al.*, 1999; Lu y Foo, 1997). The overall values of artichoke by-products (18 g/kg dry weight) and cauliflower by-products (17 g/kg d.w.) were quite larger than that reported from grape marc (1 g/kg dw) (Bonilla *et al.*, 1999) and 2-fold higher than the apple pomace (7,24 g/kg dw) (Lu y Foo, 1997). Concerning the lettuce by-products the medium value (8 g/kg dw) was also higher than grape marc and similar to those reported from apple pomace.

The results obtained indicate that artichoke, cauliflower and lettuce by-products are an interesting and cheap source of antioxidant phenolics, especially when considering the huge amount of byproducts that are produced by both the fresh and fresh-cut industries.

Needs of research

The use of these extracts, however, presents some concerns. The first one is the market. Before producing these phytochemical extracts from agrifood residues it is essential to evaluate the potential market and price for these products. Another topic of concern is safety. It is essential to make sure that the pesticides and other agrochemicals are not concentrated in the extracts in the same way the phytochemicals are. It is therefore essential a routine analysis of pesticides in all these products. It is also important to establish the risk/benefit balance of using these phytochemical extracts for health-related purposes.

In addition it is necessary to control the content of the bioactive phytochemicals in the extracts by appropriate analytical methods. It is not unusual to find in the market pills, extracts and other preparations based on specific bioactive compounds in which the bioactive phytochemicals are only present as traces. This is the case of many grape extract preparations that claim a significant content of resveratrol, when the real content is very small or is even undetectable. It is necessary to give figures of the content of the main bioactive components.

The biological activity of these phytochemical extracts needs to be demonstrated by *in vivo* studies and clinical assays, as the bioavailability of many phytochemicals is rather small and in many cases the natural compounds are transformed into other

metabolites by the gut microflora, and these metabolites, but not the original phytochemicals, are then absorbed and circulate in plasma to reach the target tissues where the biological action takes place.

Conclusions

The wastes and residues of the fruit and vegetable industries, constitute an interesting source of phytochemicals that can be readily extracted by simple methods and can then be used for the preparation of different products (foods, pills or ingredients). It is however essential to guarantee the product safety (pesticides and risk assessment of increasing phytochemical concentration). The preparation of these extracts is technologically feasible and they can be obtained at relatively low cost. The biological activity of these extracts needs further research. The main objective of this field of research would be the recuperation of the health-promoting metabolites and quality-related compounds from horticultural products that are currently lost during handling and processing.

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Fish Waste and Functional Foods

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Abstract

Scientific research constantly provides new insights in the interaction between genetic predisposition, specific health risks and nutritional needs, and the functioning of separate nutrients. The role of food as an agent for improving health has been proposed as a new class of food- functional foods

Every year 30 million tons of such waste is dumped around the world, and Norway alone has been "wasting" 150,000 tons a year. Fish waste may be sources for of proteins of high biological value, unsaturated essential fatty acids, vitamins and antioxidants, minerals or trace metals and physiological beneficial amino acids and peptides.

Scientific data shows that the consumption of fish or fish oil containing omega-3 polyunsaturated fatty acids (PUFAs) reduces the risk of coronary heart disease, decreases mild hypertension, prevents certain cardiac arrhythmia, and sudden death, lowers the incidence of diabetes, and appears to alleviate symptoms of rheumatoid arthritis. It appears that omega-3 PUFAs play a vital role in the development and function of the nervous system (brain), photoreception (vision), and the reproductive system.

Additional components in seafood may be of importance for development of life style diseases. Potent peptides with high anti hypertensive activities and petides, which may modulate neuropeptide levels, have been isolated from fish waste. Protease inhibitors of the serpin family, or serine protease inhibitors, are a family of glycoproteins that include members involved in the control of blood coagulation, fibrinolysis, complement activation and inflammation processes, are also found. Calcium and vitamin D are other candidates. Antioxidants (tocopherols, ubiquinone, selenium, taurine, fish protein) have attracted special attention due to their possible prevention of low-density lipoprotein (LDL) oxidation.

Introduction

Scientific and technological developments in the field of food have marked a shift in the way people deal with food and health. Scientific research constantly provides new insights in the interaction between genetic predisposition, specific health risks and nutritional needs, and the functioning of separate nutrients. There is a growing awareness that the dietary source and form of food may affect the overall health of the consumer. With the help of these insights and the advancing (bio) technological possibilities in this field, it is possible to develop new biological active enriched foods. The role of food as an agent for improving health has initiated the development of new classes of food- functional foods. The term indicates a food that contains some health promoting components. Various so-called functional foods have appeared on the market. General nutritional advice such as 'eat a varied diet and not too much' or the 'five food groups', in the past is nowadays increasingly defined in terms of required intake of nutrients, whether or not geared to different target groups.

Volumes and Value of Fish Wastes

Fish wastes have a huge unexploited potential for value adding. Every year 18 – 30 million tons of waste is dumped around the world. The goal is to increase the use in foods, functional foods and biochemical products for human consumption. By-products from Norwegian fisheries, included fish farming, consist of viscera (liver, roe, stomachs, etc.), heads, backbones, cuts and rejected fish from processing (http://www.rubin.no/eng/). The by-products are generated when the fish is gutted, headed and further processed - either on-board in fishing vessels or in processing plants on shore. The Norwegian fisheries produce more than 550,000 tons of by-products annually, which is more than 20 % of all the fish caught and farmed in Norway. Today most of the by-products are used as raw materials for feed production; such as fishmeal, silage and feed for fur animals. About 150.000 tons are still dumped into the sea (Figure 2). The total value adding represents 1,25 billion NOK (2001). If we succeed to utilise more of the by-products as food for humans and as ingredients in foodstuff, health foods, pharmacy, cosmetics etc., the value adding may increase by 4-5 fold. Less than 10% of volume represents 50% of the added value (Figure1).

The current annual world production of aquaculture products is approximately 34 MMT (FAO, 1998). Total annual worldwide aquaculture landings are increasing rapidly from a total annual catch of 13.4 MMT in 1987 to 34.1 MMT in 1996 (FAO). However, as the aquaculture industry continues to grow, so do the problems associated with aquaculture waste.

Functional Ingredients from Fish Waste

A relationship between fish consumption and reduced mortality due to cardiovascular diseases was shown from the early 80's (Kromhaut *et al*., 1985, Marckmann and Gronbaek, 1999; Menotti *et al*., 1999; Mori *et al*., 1999). Curiously, in most of the references the positive effect has been attributed to the intake of marine fatty acids

alone, although lean and fatty fish, in most studies, gave the same protective effects.

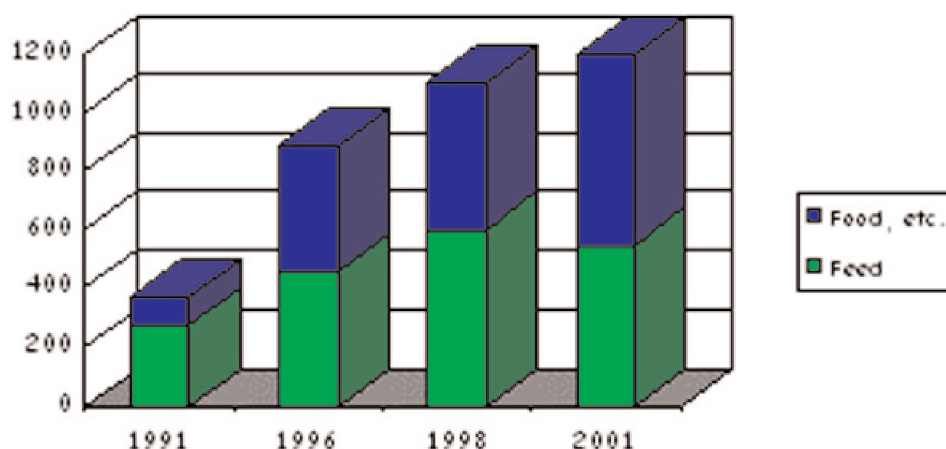

Figure 1. Value adding of marineby-products (1000 tons/tear)

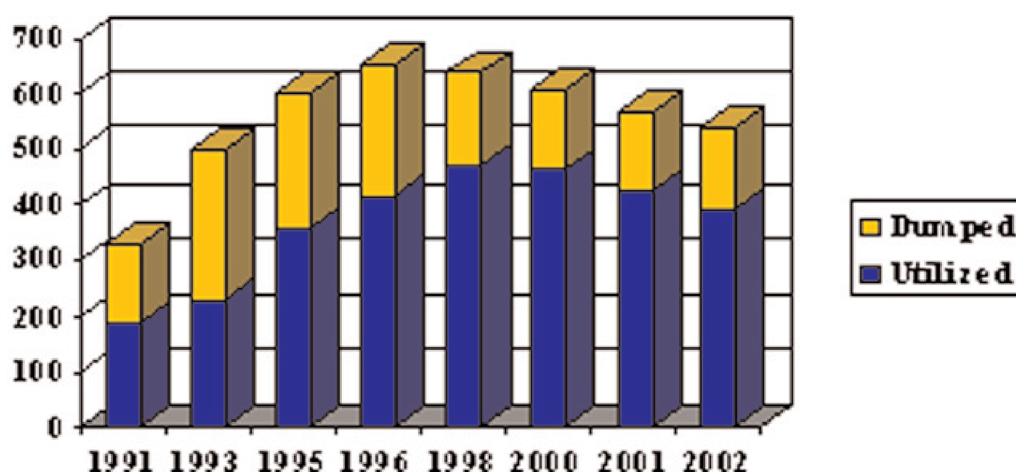

Figure 2. Value adding of marineby-products (1000 tons/tear)

Since late 70's it has been established that Greenland Eskimos living on their traditional marine diet, had a lower incidence of coronary heart disease (CHD) than when living in Denmark on a western diet (Dyerberg *et al*., 1978). All aspects of the Eskimo diet and their possible assosiation to development of CHD are still not fully explored. The Eskimos consume the bulk of their food raw or dried, seldom boiled or exposed to excessive heat (Berezovikova *et al*., 2001). Their traditional food habits includes extensive consumption of visceral organs, in this context regarded as waste (Elvevoll, 1988). The main task of modern processes is to make edible and stable products. Refining procedures for the removal of molecules that causes off- flavours or -taste to improve sensory attributes or safety of marine oils may destroy potent antioxidants or remove other components with potential beneficial effects. Modern meal preparing techniques may also lower the content of biologically active components. Losses of of low molecular weight compounds like taurine due to

preparing techniques are well known. Preliminary results from our lab indicates up to 50% leakage when preparing traditional (Norwegian) fish products (Dragnes and Elvevoll, 2003). Results from (Østerud *et al*., 1995, Brox *et al*., 2001, Ramirez-Tortosa *et al*., 1999) indicates that there are protective substances whose effect disappears when the products is subjected to rough processing conditions such as cooking or refining.

Of special interest when focusing on by- products; Eating practices that manages to maintain traditional food habits with extensive consumption of visceral organs may be beneficial for preventing atherosclerosis and hypertension (Nobmann *et al*., 1992). Visceral organs (liver, heart, kidney, gonads) are known as rich sources biological active molecules (Pedersen *et al*., 1999).

Beneficial Components

Seafood and fish waste may be sources for of proteins of high biological value, unsaturated essential fatty acids, vitamins and antioxidants, minerals or trace metals and physiological beneficial amino acids and peptides.

Scientific data shows that the consumption of fish or fish oil containing omega-3 PUFAs reduces the risk of coronary heart disease, decreases mild hypertension, prevents certain cardiac arrhythmia, and sudden death, lowers the incidence of diabetes, and appears to alleviate symptoms of rheumatoid arthritis. It appears that omega-3 PUFAs play a vital role in the development and function of the nervous system (brain), photoreception (vision), and the reproductive system (Simopoulos, 1999, Kirpal 2003). Additional components in seafood may be of importance for development of life style diseases like coronary heart diseases (CHD). Potent peptides with high anti hypertensive activities (ACE inhibitors) and petides which may modulate central neuropeptide levels have been isolated from hydrolysates from fish meat (Yoshikawa *et al*., 2000, Sorensen *et al*., 2004). Protease inhibitors of the serpin family, or serine protease inhibitors, are a family of glycoproteins that include members involved in the control of blood coagulation, fibrinolysis, complement activation and inflammation processes, are also found in seafood (Huang *et al*., 1995, Cao *et al*., 2001). Calcium, selenium, vitamin D, taurine and ubiquinone are other candidates from seafood for protection against CHD (Savige, 2001). Marine low molecular weight components antioxidants (tocopherols, CoQ10, selenium, taurine) have attracted special attention due to their possible prevention of low-density lipoprotein (LDL) oxidation (Kondo *et al*., 2000). Many proteins also exhibit antioxidative activity, fish proteins have also been shown to inhibit LDL oxidation in rat models.

Beneficial Components in Marine Oils Lost Due to Processing?

Fish oils are extracted from whole fish, fish liver (mainly cod liver) or by-products from the fisheries industry (mainly salmon). The traditional extraction technique involves heating or steam stripping of the raw material in order to release the lipids. Marine oils

are highly unsaturated and the application of high temperatures during extraction may cause undesired effects like; initiation of oxidation reactions, destruction of antioxidants and extraction of molecules that causes taste and smell in the oil fraction. It is inevitable that during heat extraction of the oil detectable changes occur in the different lipid component, as compared with their "virgin" state in the cells. A mechanical procedure applicable at lower temperatures, to avoid some of these undesired effects of temperature, has been developed (NIFA,1999).

Marine oils for human consumption are normally subject to an additional traditional oil refining process. The main objectives of this process are to remove pesticides and to make an edible and stable product. To achieve a stable, sensory acceptable and safe product the removal of a number of components (e.g. free fatty acids, phospholipids, pigments, sterols, transformation products, metals and possible toxic agents) are normally necessary. The conventional classical (caustic) refining operation consists of four main steps; degumming, deacidification (caustic neutralisation), bleaching or decolourization and deodorization. In addition optional steps like pre- cleaning (filter, sedimentation) or mixing of different batches, vinterization (dewaxing) and post –cleaning or polishing filtration may be applied. During some of the refining steps a number of chemical reactions (hydrolysis, autoxidation, isomerization, conjugation, polymerization, pyrolysis, dehydration) is likely to take place depending on the processing conditions. The process is designed to remove such products as well. Application and number of steps are also influenced by qualities of the oil e.g. unsaturation, accompanying substances, amount and nature of impurities, the past history – oxidative and hydrolytic damage suffered previously. Preferences are mainly based on quality criteria applied to oils, economic and environmental benefits in order to achieve a shortcut in the process sequence and less material loss.

In conclusion, removal of molecules to improve sensory attributes or safety of the marine oil may destroy potent antioxidants and may as well remove components with potential beneficial effects. There is an urgent need for development of new refining techniques for marine oils.

Marine Hydrolyses - Redused Cardiovascular Risk?

In this section, amino acids (taurine) and petides (ACE -inhibitors) are used as examples of possible additionally beneficial components from seafood and hence components in possible ingredients in functional foods. The components serve as examples when reviewing the literature. The products (functional ingredients, dietary supplements), due to refining costs, most probably, need to be relatively crude preparations (hydrolysates or free amino acids).

Differences in muscle osmolality, e.g. between marine and non-marine animals, are mainly due to nitrogenous solutes such as certain amino acids, among these, taurine (Abe, 2000). Seafood contains high levels of taurine (Laidlaw *et al*., 1990). The consumption of seafood is shown to give a rise in concentration serum taurine (Uhe *et al*., 1992, Stegink *et al*., 1970, Kim *et al*., 2003) and urinary excretion of taurine is

known as a marker for seafood consumption (Biosca *et al*., 1990).
The suggestion of a possible association between fish intake and reduced cardio vascular risk, through the beneficial effects of taurine in addition to and n-3 fatty acids has been put forward in (Mizushima *et al*., 1997, Yamori *et al*., 1994). In humans, taurine is regarded to be a conditionally essential amino acid as its physiological concentration can be partly regulated endogenously. The amino acid, taurine is known to have several positive effects on the cardiovascular system and a broad review is presented by Niittynen *et al*. (1999) Firstly, taurine has an antioxidant activity. This may reduce the production of proinflammatory products. Secondly, taurine has been shown to lower blood pressure in borderline hypertensive patients. It has also been reported that taurine can improve cardiac performance, reduce blood cholesterol values and suppress platelet aggregation.

The ability to form taurine is species dependent, and cats are unable to synthesise taurine. For this reason, cats have been used in animal studies to study availability of taurine due to processing. Frozen-preserved commercial diets have been shown to maintain plasma taurine concentration, whereas the heat-processed diet did not (Kim *et al*., 1996). It is reported that Cod protein subjected to technological processing showed lower digestibility, assimilate-ability and growth yield as compared with raw protein. A significant correlation was found between the results of the biological assessment of the nutritional value of processed protein and content of taurine in the liver and urine of rats, on the other hand (Lipka *et al*.,1993).

Although commonly used as a dietary supplement in the Far East, the potential advantages of dietary taurine consumption/supplementation have not been recognised in the Western World (Stapleton *et al*., 1998)

It has been documented that peptides from the digests of fish muscle possesses potent inhibitory activity against angiotensin I-converting enzyme (ACE) (Galardy *et al*., 1984, Kohama *et al*., 1996, Matsufuji *et al*., 1994, Yohshikawa *et al*., 2000, Sorensen *et al*., 2004). They possesses potent antihypertensive activities. For assessment of relative antihypertensive activities two peptides form fish to that of captopril (a common drug), they were orally administered to rats. When compared on molar basis accounted for 66% and 91% relative to that of captopril. It is of interest to note that of these peptides exert remarkably higher antihypertensive activities *in vivo* despite weaker *in vitro* ACE-inhibitory effects, which was ascertained by using captopril as the reference drug. Such peptides may be regarded as healthy components (through endogenous metabolism) of fish muscles and may be produced as ingredients or diet supplements.

Conclusion

1. Fish wastes - a huge unexploited potential (volume) for value adding.
2. Fish wastes - extended use as food, ingredients in functional food, in diet supplements should be exploited.

3. Fish wastes - The products, due to refining costs, need to be relatively crude preparations.
4. Fish wastes - A wide range of components in addition to unsaturated fatty acids should be studied and developed as products.
5. Fish wastes - There is a need for development of new refining techniques due to losses of availability trough traditional preparing and processing.

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Enzymatic Liberation of Functional Compounds from Vegatable Peel Matrix

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Introduction

Vegetables have long been associated with a healthy diet. Progress in research is now identifying compounds with potential health benefits (Puupponen-Pimiä *et al.*, 2003). Dietary fibre, vitamins, minerals, ω-3-fatty acids, and several sulphur compounds, present for example in garlic and related *Allium* plants, already are traditional ingredients with positive nutritional properties. Many vegetables are also good source of oligosaccharides, the best known example being inulin and fructooligosaccharides present at high concentrations in chicory roots and Jerusalem arti-choke parsley and leek, garlic, and onion. Prebiotic oligosaccharides can modulate colon microflora in a positive way (Gibson and Roberfroid, 1995). Plants also synthesize secondary metabolites which have only recently been recognized for their potential effects in protecting against various diseases. Ubiquituous plant sterols and phenols, such as flavonoids and phytoestrogens, as well as glucosinolates and their breakdown products specific for cruciferous vegetables are examples of plant secondary metabolites.

Especially phenolic compounds have attracted much interest recently, because they have a variety of beneficial biological properties. They are potent antioxidants, and exhibit various other physiological activities including anti-inflammatory, antimicrobial, antiallergic, anticarcinogenic and antihypertensive activities. High flavonoid consumption is associated with reduced risk of chronic diseases like cardiovascular diseases (Middleton and Kandaswami, 1994; Hertog *et al.*, 1995). There is now increasing interest in the extent to which such natural antioxidants in the diet can protect the body against the ravages of free radicals. Plant phenols can strongly prevent oxidation of low density lipoproteins *in vitro*, and they have either alone or in combination with e.g. dietary fibre a variety of other biological effects in numerous mammalian cell systems, as well *in vivo*.

In plants many of the phenolics act as protective compounds against plant pathogens, and they are often concentrated in the peels and skins. Industrial peeling of vegetables results in significant amounts of process waste. These by-products may, however, have functional value due to their high content of phenolic compound

(Nuutila *et al*., 2003) and other bioactive compounds. In addition, vegetable peels are a potential source of dietary fibres. Potato peels contain approximately 50% total dietary fibre on a dry weight basis (MacDougall and Selvendran, 2001).

In this work the effect of tailored mixtures of hydrolytic cell-wall degrading enzymes were used to treat vegetable peels in order to enhance the availability of phenolics and vitamins. In addition, the impact of the treatments on the content of insoluble and soluble dietary fibre were determined.

Materials and Methods

Vegetable peels

Potatoes (*Solanum tuberosum* cv Asterix), carrots (*Daucus carota* L. cv Fontana), Swedish turnips (*Brassica napus* L. cv Globus) and onions (*Allium cepa* L. cv Sturon) of the 2002 harvest were obtained in October from the commercial peelers in Finland. Potatoes, carrots and Swedish turnips were brushed in tap water, whereafter the peels were hand peeled by using a normal household peeling knife. Outer pigmented, papery peels of onions were dissected with a knife.

Enzyme preparations

Two different enzyme cocktails were prepared using commercial multienzyme preparations. Econase CE containing several cellulases was purchased from AB Enzymes Finland. Pectinex Ultra containing several pectin degrading activities was purchased from Novozymes, Denmark. Novozym 188 being a β-glucosidase enriched preparation was purchased from Novozymes. Lipase from Candida sp (Candida B1) was purchased from Biocatalysts. Protease (Neutrase) was purchased from Novozymes.

Cellulase activity was measured as overall cellulolytic activity (FPU) using filter paper as substrate (Ghose, 1987). Endopolygalacturonase activity was measured as described previously (Bailey and Pessa, 1990). β-glucosidase activity was measured with *p*-nitrophenyl-β-glucopyranolsyl as substrate Bailey and Linko, 1990). Protease activity was assayed with casein as substrate (Matsubara *et al*., 1958). The lipase activity was assayed by the olive oil emulsion method (Mustranta *et al*., 1993). Endoglucanase, mannanase, xylanase and β-glucanase activities of the enzyme preparations were analyzed using hydroxyethylcellulose (Ghose, 1987), locust bean gum (Ståhlbrand *et al*., 1993) and birchwood glucuronoxylan (Bailey *et al*., 1992) and β-glucan (Zurbriggen *et al*., 1990) as substrate, respectively. Pectin methylesterase was assayed with titration method (Suutarinen *et al*., 2000). Enzyme activity profiles of the enzyme preparations were determined (Table 1). The enzymes were blended as described in Table 2 and the overall activities in the cocktails were calculated.

Enzymatic treatments

Milled fresh peels were treated with the two enzyme cocktails. 100 g fresh peels were first homogenized with a blender bar (Bamix mono type M 133, 2-speed; ESGE AG, Mettlen, Switzerland) in a beaker for 30 s. Enzymes were suspended in 150 ml of water and the pH was checked (optimum pH 5-6). Peels were incubated with the enzyme cocktails at 40 ^oC for 20 h. The reference treatments were carried out correspondingly without the addition of the enzymes. After the treatments the reaction mixtures were freeze-dried (Christ Epsilon 2-25 DS, Martin Christ Gefrientrocknungsanlagen GmbH, Osterode am Hartz, Germany) for 39 hours and milled with Fritsch mill (Type 14.702, Fritsch GmbH, Idar-Oberstein, Germany) with a 0.5 mm sieve for the analysis of carotenoids, dietary fiber, total phenolics and antioxidativity. C-vitamin analysis was carried out from the fresh undried reaction mixtures. Papery onion peels were only milled before analysis.

Analyses

Vitamin C was analyzed from undried homogenized peels as dehydroascorbic acid according to Speek *et al*. (1984). HPLC analysis was carried out using Waters 6000 A solvent delivery system equipped with a Waters 712 autosampler and Waters 474 fluorescence detector (Waters Corporation, Milford, MA, USA). The system was controlled and data was treated using a Millennium 2.15 Workstation. Lutein,α- and β-carotene of freeze-dried samples were determined by HPLC from saponified sample (Konings and Roomans, 1997) Total phenolics was determined by modified Fohlin Ciocalteau method (Singleton and Rossi, 1965). Antioxidativity was analysed by spectrofotometric DPPH radical scavenger method (Malterud *et al*., 1996; Goupy *et al*., 1999).

Moisture contents were determined by the Karl Fischer method using Mettler Toledo DL18 Titrator (Mettler-Toledo GmbH, Greifensee, Switzerland), (Anonymous 2002). The protein content was determined by the Kjeldahl nitrogen method using copper sulphate as catalyst in the sulphuric acid digestion (420 ^oC) and an automated distillation/titration system (Tecator Auto 1030, Tecator AB, Höganäs, Sweden). A factor of 6.25 was used to convert nitrogen into protein. Fat was determined by gravimetry using an acid hydrolysis method according to the AOAC method 922.06 (2000). The ash content was determined by incineration in a programmable muffle oven from room temperature to 550 ^oC (4 h at peak temperature). All the results of above methods are mean values from two replicate determinations and they were calculated on a dry-weight basis. Carbohydrate content (%) was calculated by sub-stracting the sum of moisture, protein, fat and ash from 100%.

Dietary fibres were determined according to Asp *et al*. (1983). In the procedure, a peel sample of 0.3 g to 0.6 g was gelatinised by boiling for 15 min. with a heat-stable α-amylase and subsequently incubated with pepsin at acid pH for 1 hour and with pancreatin at neutral pH for another 1 hour. Insoluble dietary fibre was filtered off onto a crucible containing filter aid. Soluble fibre was precipitated from the aqueous filtrate with 4 volumes of ethanol and filtered. The two types of fibres were dried and weighed. After corrective analyses for undigested protein and for ash the contents for

dietary fibres were calculated.

Results

Characterization of the nutritional quality of the peels

The contents for major nutrients and ash in the untreated peels are presented in Table 3. Protein and fat contents in the peels were higher than those reported in edible parts except for protein in onion (Rastas *et al*., 1989). Carbohydrate contents in the peels were found to be close to those in the edible parts except for onion. As expected the untreated peels had high contents (22 - 72 %) of total dietary fibre (Table 3). Peels from Swedish turnip and onion contained more total fibre than peels from the two other vegetables (Table 3). In comparison to untreated peels, water treatment increased the contents of all fibres except for soluble fibre in carrot and Swedish turnip peels apparently due to the action of endogenic enzymes (Table 3, Fig. 1).

Peels of Swedish turnip had the highest vitamin C content (45 mg/100 g) of the studied peels whereas the vitamin A and carotene content was the highest in the carrots as could be expected (Table 3). Onion peels contained significant amounts of phenolics, i.e. 21 mg/g followed by potato and carrot peels (about 7 mg/g) and peels of Swedish turnip (below 2 mg/g) (Table 3). The tuber of coloured potato cultivars is reported to contain relatively high percentage of phenolic acids (79.1%) and a relatively low level of flavonoids, excluding anthocyanins (8.6%) (Lewis *et al*. (1998). Rodriguez-de-Sotillo *et al*. (1994) have studied the phenolics of potato peel wastes. Total concentration was found to be 48 mg/100g and four phenolic acids, chlorogenic acid, gallic acid, protocatechuic acid, and caffeic acid, were characterized as major components. Quercetin and its glycosides have been reported to be the major flavonoid in onion peels (Nuutila *et al*., 2003).

Effect of enzyme pre-treatment on the content of dietary fibre

The peels were treated with two different enzymes cocktails (Table 2). The mixture A contained extensive amounts of cellulose, xylan, mannan, glucan and pectin degrading hydrolases but was devoid of lipase and protease activities. β -glucosidase activity was also relatively low. The enzyme cocktail B was adjusted to the same FPU level but it contained 5-fold more polygalacturonase as compared to cocktail A. Furthermore it contained high levels of β-glucosidase, esterase and protease activities. After the treatment the treated peels with the treatment liquor were freeze-dried for subsequent analyses.

The effect of the enzyme treatments on the amount of dietary fibres was first determined. In the enzyme treatment A, with lower amounts of β-glucosidase, esterase and protease present, contents of insoluble fibre remained practically the same (potato and Swedish turnip peels) or slightly increased (carrot and onion peels) when compared with reference water treatment. The content of soluble fibre remained on the same level in Swedish turnip peels and increased in the other three peels (Table 5). Enzyme treatment B drastically decreased the amount of both insoluble and soluble fibres in potato and carrot peels. The observed decrease is expected to be

due to efficient hydrolysis of cell-wall polysaccharides and proteins to low DP products as a high contents of e.g. β-glucosidase and protease were present in the enzyme cocktail (Table 2). The prebiotic activity of these ow DP compounds remains to be studied.

Effect of enzyme treatment on vitamins and phenolics

The impact of enzyme treatment A on vitamin and carotenoid content was also analyzed. In all cases the vitamin C content was significantly decreased during the water treatment, i.e. treatment in similar conditions as the enzyme treatments but without any added enzymes (Tables 3-4). The decrease is expected to be caused by oxidative degradation of vitamin C at 40ºC. Possibly also endogenic enzymes may have affected the vitamin C content negatively. With the added enzyme cocktails no positive nor negative effect in the vitamin C content was observed as compared to the water treatment. Lutein content was also found to be decreased during the water treatment at 40ºC (Tables 3-4). A increase in the lutein content was found in the enzymatically treated potato, Swedish turnip and onion peels indicating that partial degradation of the peel matrix enhances the availability of lutein. When carrot peels were treated with enzyme cocktail a significant increase in the α-carotene, β-carotene and vitamin A concentration was observed (Table 4).

The amount of extractable phenolics in all peels except potato peels were increased as a result of the enzyme treatments. In the case of onion peels the increase was most pronounced, i.e. from about 24 mg/g to about 110 mg/g with both enzyme cocktails (Table 6). Significant increase was also observed with carrot peels, i.e. the phenolics were increased from 3 mg/g to 15-17 mg/g (Table 5). The increased availability of phenolics was also visualized in increased DPHH antioxidativity in the enzyme treated peels. (Table 5). In contrast to the other vegetable peels treated, the treatment of potato peels did not enhance the amount of detectable phenolics. Apparently the location of the phenolics is different in the potato peels as compared to the other peels studied. Thus, by partial hydrolysis and opening-up of the cell-wall matrix the extractability and availability of phenolics can be increased.

Conclusions

Cell-wall degrading enzymes are potential tools to liberate phenolics and other bioactive components from the cell-wall matrix in different plant products. The enzyme treatment was found to increase the available carotenoids in carrot peels. The amount of total phenolics in enzyme treated onion pigmented papery peels increased clearly (about fivefold) and in some extent in potato and carrot peels. This increase was also visualized in increased antioxidativity of the treated peels. The enzymatic treatment can easily be combined to other minimal processing technologies in order to upgrade current process by-products to functional food products.

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Table 1. Enzyme activity profiles of the enzyme preparations used in the enzyme cocktails for treatment of vegetable peels.

Enzyme	CEa FPU/ml	EGb Nkat/ml	XYLc nkat/ml	MANd nkat/ml	PGe nkat/ml	β-GLf nkat/ml	β-GLUg nkat/ml	PMEh nkat/ml	LIPi nkat/g	PROTj nkat/ml
Econase CE	77	16600	49000	2400	3200	106210	520	0	0	0
Pectinex Ultra SP-L	2	1837	2000	9000	334000	28670	43	1080	0	15
Novozym 188	0	290	3842	1880	11116	1819	5299	0	0	16
Candida lipase B1	0	0	0	0	200	0	2	0	1100000	0
Neutrase	0	38	52	0	178	3840	0	0	0	3850

a CE: overall cellulolytic activity, b EG: endoglucanase, c XYL: xylanase, d MAN:mannanase, e PG: endopolygalacturonase, f β-GL:β-glucanase, g β-GLU:β-glucosidase,h PME:pectinmethylesterase, i LIP:lipase, j PROT:protease.

Table 2. Calculated enzyme activities in the enzyme cocktails used for treatment of vegetable peels

Enzyme	CEa	EGb	XYLc	MANd	PGe	β-GLf	β-GLUg	PMEh	LIPi	PROTj
Cocktail	FPU/ml	nkat/ml	nkat/ml	nkat/ml	nkat/ml	nkat/ml	nkat/ml	nkat/ml	nkat/mg	nkat/ml
A	20	4320	12740	630	1030	27620	135	1	0	0
B	20	4430	14190	1340	5220	28310	2125	1	200	16

a CE: overall cellulolytic activity, b EG: endoglucanase, c XYL: xylanase, d MAN:mannanase, e PG: endopolygalacturonase, f b-GL:β-glucanase, g β-GLU:β-glucosidase,h PME:pectinmethylesterase, i LIP:lipase, j PROT:protease.

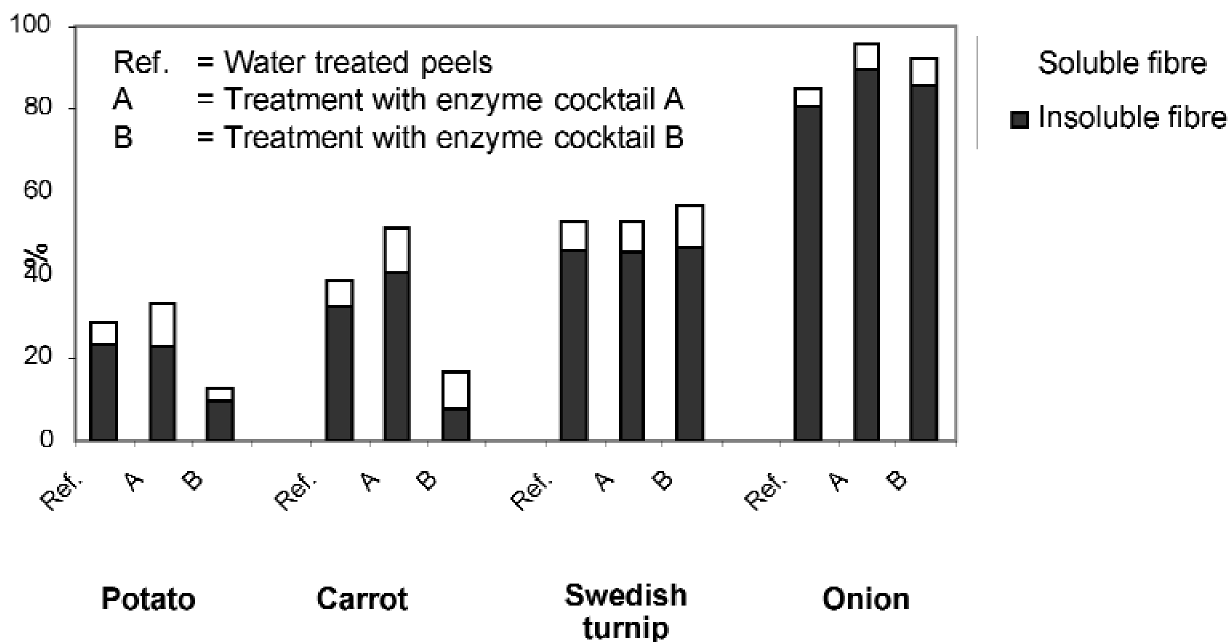

Figure 1. Dietary fibre content in the treated peels. REF= water treated peels, A= treatment with enzyme cocktail A, B= treatment with enzyme cocktail B.

Table 3. Analysis of the peel compositions. Analyses from freeze-dried samples with the exception of vitamin C measurement, which was carried out from fresh samples

Analyzed parameter	Potato	Carrot	Swedish turnip	Onion
Vitamin C (mg/100 g)	8.7	3.2	45	<0.5
Lutein (_g/100 g)	21	184	34	10
β-carotene (_g/100 g)	0	13439	0	0
β-carotene (_g/100 g)	0	21622	2013	0
Vitamin A RE/100 g	1.7	4740	339	0.8
Phenolics (mg/g)	6.8	7.3	1.6	21.4
Protein (% of d.w.)	11.2	7.3	16.0	2.0
Fat (% of d.w.)	2.2	7.1	4.2	3.4
Ash (% of d.w.)	6.6	13.7	13.0	6.1
Carbohydrates (% of d.w.)	80.0	71.9	66.8	88.5
Dietary fibres insoluble (% of d.w.)	19.7	22.9	32.0	70.3
Dietary fibres soluble (% of d.w.)	2.3	7.9	12.9	1.7
Total dietary fibres (% of d.w.)	22.0	30.8	44.9	72.0

d.w. means dry weight.

Table 4. Effect of enzyme cocktail A on vitamin C and carotenoid contents of freeze-dried reference and enzyme treated peels.

Analysis	Treatment	Potato	Carrot	Swedish turnip	Onion [b]
Vitamin C (mg/100 g) [a]	Water treatment [b]	0.6	<0.5	2.0	<0.5
	Enzyme treatment A[c]	<0.5	<0.5	3.8	<0.5
Lutein (_g/100 g)	Water treatment	5	42	1	2
	Enzyme treatment A	5	73	5	24
β-carotene (_g/100 g)	Water treatment	0	22945	71	0
	Enzyme treatment A	0	37664	226	0
β-carotene (_g/100 g)	Water treatment	0	38720	84	0
	Enzyme treatment A	0	67049	264	0
Vitamin A RE/100 g	Water treatment	0.4	8374	20	0.2
	Enzyme treatment A	0.5	14328	64	2.0

[a] Vitamin C was analysed from fresh peels; b onion peels were fresh (brown) papery

Table 5. Total phenolics and antioxidativity of freeze-dried peels.

Analysis	Treatment	Potato	Carrot	Swedish turnip	Onion [a]
Total phenolics (mg/g)	Water treatment	4.0	2.7	2.3	24.5
	Enzyme treatment A	4.9	14.9	4.7	107.0
	Enzyme treatment B	5.7	16.7	5.7	110.7
Antioxidativity (mg/mg DPHH)	Water treatment	23.5	112.4	401.6	5.7
	Enzyme treatment A	37.1	8.1	74.1	2.7
	Enzyme treatment B	36.6	19.5	85.1	3.3

a onion peels were fresh (brown papery).

Phyto Products Obtained by Supercritical CO_2 Extraction, an Environmentally Accepted Technology

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Introduction

Our developed World has a strong demand for creation healthy and less polluted environment using clean technologies and producing solvent residue free, nutritional foods. These food products must possess with natural colour, taste and self-live extensive properties as well as must contain biological active, health preventive compounds (e.g. antioxidants, vitamins).

Supercritical fluid extraction is one of the desirable technologies, which uses carbon dioxide for extraction of essential oils, fatty oils, pigments, and natural waxes from natural sources, mainly from herbs, spices and medicinal plants. According to the physico-chemical properties of supercritical CO_2, the extraction is carried out at moderate temperature (mainly between 31 - 60°C), therefore thermo-labile compounds can be obtained without any decomposition. The extract is absolutely solvent residual free as the CO_2 is in gaseous state at room temperature. Numerous, mainly apolar compounds can be extracted and/or fractionated as the solvating power of supercritical CO_2 changes within wide ranges with changes of the pressure and temperature during extraction. The extraction is carried out at relatively high pressure (between 74-500 bar) therefore the investment cost of such a plant is higher than that of the conventional units, although the process is easily controlled with low operating costs (McHugh and Krukonis, 1994; Mukhopadhyay, 2000; Pellerin, 1991).

Antioxidant, antimicrobial properties, pigment and volatile oil compositions of the extracts obtained from marjoram (*Origanum majorana* L.), thyme (*Thymus vulgaris* L.), caraway (*Carum carvi* L.), marigold (*Calendula officinalis* L.), feverfew (*Tanacetum parthenium* L.) and yarrow (*Achillea millefolium* L.) and industrial waste, tomato pomace were investigated. The effects of extraction parameters (temperature and pressure) on extraction yields, on carotenoid and tocopherol compositions were examined to optimise the technology for obtaining market demanded products. The antifungal and antibacterial properties of essential oils, traditional solvent- and

supercritical extracts were tested on food borne microbes. The antioxidant properties of marjoram supercritical and ethanolic extracts were compared and the ursolic acid compositions of the extracts were measured. The results support the notion that extracts obtained by supercritical CO_2 extraction may have roles as preservatives, natural antioxidants, colorants and flavour components in food and cosmetic systems.

Materials and methods

The raw materials used for the experiments were obtained from distributors or local merchants. The CO_2 used was 99.5 % (w/w) pure. Reagent-grade solvents were used for conventional Soxhlet extractions. Analytical grade reagents were used for chemical analysis.

Supercritical fluid extraction was carried out in a high-pressure apparatus equipped with a 5 L volume extractor vessel. The effects of process parameters (temperature and pressure) on the extraction yields, and on the yields of certain biological active compounds were revealed. Standard methods described in the Hungarian Pharmacopoea Ed. VII. were applied for the determination of the essential oil (by hydrodistillation), oleoresin (by Soxhlet extraction), and the moisture content of the samples. Soxhlet extraction was also carried out in a pilot plant apparatus (5 L volume) using 96% ethanol as a solvent.

The characteristic particle size and the distributions of the raw materials were investigated by Rosin-Rammler-Sperling-Bennet (RRSB) model.

The volatile oil compositions in essential oil and solvent extracts were quantified by GC and GC-MS methods. The fatty acid compositions were measured by HPLC. For determination of natural pigments like chlorophylls, carotenoids and the antioxidant compounds (triterpenoids, diterpenes and tocopherols) from marjoram and tomato pomace HPLC methods were used.

The antioxidant properties of marjoram herbs and extracts were revealed by using *i n vitro* screens in apolar and polar systems. In polar system the hydrogen-donating abilities of the samples were obtained in the presence of 1,1-diphenyl-2-picrilhydrazyl (DPPH) free radical and in the presence of instable $^{\bullet}OH$ radical in $H_2O_2/^{\bullet}OH$–luminol systems. The antioxidant capacities of ethanolic and supercritical extracts were investigated in apolar system by Rancimat method.

The antimicrobial activities of essential oils solvent extracts were revealed against food-poisoning fungi and bacterial strains. Developed agar-diffusion and dilution methods were used for determination of antimicrobial properties of the highly viscous supercritical extracts.

Results and discussion

Two different originated **marjoram** (*Origanum majorana* L.) herbs (Hungarian and Egyptian) were compared. Small amounts of essential oils and higher amounts of solvent extracts were obtained with n-hexane and ethanol, respectively. Pilot plant extractions were carried out with 96% ethanol and supercritical CO_2 solvents in 5 L extractors. Due to the less solvent power of $scCO_2$ smaller amounts of dark green, highly viscous extracts were obtained at 450 bar pressure and 50°C temperature than those extracted with ethanol. The effects of SFE parameters (temperature and pressure) on the yield of Hungarian marjoram were revealed. Applying a 32 factorial design and evaluating the experimental results the linear and quadric terms of pressure and the relation between temperature and pressure terms were highly significant on 95% significance level. The highest yield (3.8 g/ 100 g d.m.) was obtained at 400 bar and 60°C. Fractionated separation was carried out at 450 bar and 50°C extractor parameters with two separators in series. In the first separator higher molecule weighted compounds, pigments, di-, tri-, tetraterpenes, fatty acids and oils were collected, while the volatile compounds and linear alkanes, waxes were separated into the second separator. The essential oil compositions of Hungarian and Egyptian samples showed similarities. The main compounds were terpinen-4-ol and γ-terpinene, while in comparison of minor compounds slight differences were revealed. The extracts obtained by SFE and traditional solvent extraction contained the characteristic scented *cis*-sabinene-hydrate in small amounts, while terpinolene and palmitic-acid was found in relatively high amounts. Pigments as chlorophyll-a and –b and their degraded compounds pheophytin-a, and –b, while among the carotenoids lutein and β-carotene were quantified in the marjoram extracts. The extracts contained lower amounts of chlorophylls, while relatively high amounts of pheophytins due to the degradation of fresh material. Carotenoids were revealed at similar amounts in the extracts of apolar and polar solvents. Among the examined process parameters, the effect of pressure was highly significant on the yields of carotenoids and green pigments. The antioxidant properties of marjoram herb and extracts were revealed in *in vitro* tests in apolar and polar systems. In comparison of two herbs, the Egyptian herb had better properties and to compare the methods the ethanolic extracts possessed better antioxidant activities, which were comparable to synthetic antioxidant (butylated hydroxytoluene). Diterpene carnosol and triterpenoid ursolic acid as well-known antioxidant compounds were identified in the herbs and the extracts. The ethanolic extracts contained ursolic acid in higher amounts, while the carnosol was presented in higher amounts in apolar extracts. The antimicrobial properties of marjoram ethanolic and SFE extracts were mapped against food-poisoning fungi and bacteria strains. The SFE extracts showed strong inhibitory activities (minimal inhibition concentrations, MICs were 0.4-0.5 g extract/ 100 ml medium) against *Trichoderma viride, Aspergillus niger*, and *Penicillium cyclopium*. The effects on bacterial growth were revealed in the presences of marjoram extracts in different concentrations against *Escherichia coli*, *Pseudomonas fluorescence* and *Bacillus cereus*. The SFE extracts showed stronger activities than the ethanolic extracts, applying it in 0.4% concentration the inhibition were higher than 85%.

From **thyme** (*Thymus vulgaris* L.) herb relatively high amount of essential oil was obtained by hydrodistillation, however by traditional solvent extractions using ethanol almost ten times higher amount of extract was obtained than with *n*-hexane. Strongly scented, brownish-green waxy extract was produced by SFE at 400 bar and 60°C with higher yield than that of with *n*-hexane extraction. For comparison of the effects of process parameters, a 3^2 factorial design on the experimental results was applied, where the linear and quadric terms of pressure, the linear term of temperature and the relation between temperature and pressure terms were found to be significant on 95% significance level. According to the bigger characteristic particle size of thyme two times higher amount of $scCO_2$ solvent needed for overall extraction than that of marjoram SFE. The microbial properties of thyme essential oil, ethanolic and SFE extracts were compared. In the tests against *T. viride, A. niger*, and *P. cyclopium* the essential oil showed the strongest inhibition activity, in 0.025 g essential/100 ml medium total inhibitions were observed. Total inhibitions were measured against the three test fungi in the presences of 0.04% SFE and 1% ethanolic extracts. In the antibacterial tests against *E. coli, P. fluorescence* and *B. cereus* the minimal inhibitory concentration (MIC) were revealed. The MIC of essential oil was 0.1% against the three bacteria, while the MIC of SFE extract was 0.1% except against *P. fluorescence*, where 0.2% was needed for total inhibition. Only 12-40% inhibition activities were observed in the presence of 0.4% ethanolic extracts.

From the industrial waste, **tomato pomace** four samples were examined in the points of the effect of storage, the effects the applied solvents, the effects of process parameters on yields and the qualities of the products were widely studied. The extraction yields were obtained between wide ranges, however smaller amounts of extracts were measured with apolar solvents from better quality raw material, meanwhile with ethanol high amounts of highly viscous dark-red colored products were extracted. Among the examined process parameters, only the temperature had significant effect on the yield on 95% significance level. The highest amount of extract was obtained at 460 bar and 80°C without the degradation of the biological active compounds. The extracts were rich in carotenoids, the main compound was the health-preventive, highly valued lycopene. In the deep-frozen stored sample, the amount of carotenoids was ten times higher than that of in the air-dried and stored sample. The lycopene was found in the highest amounts in the carotenoid fraction of the apolar extracts (*n*-hexane and $scCO_2$). The yield of lycopene was increased with increasing the temperature and pressure of SFE. The extract obtained at 460 bar and 80°C contained the lycopene in the highest percentage (90%) among all the extracts obtained with different solvents. The amounts of tocopherols in the extracts were almost similar, relatively high amounts were recovered in the extracts obtained with apolar solvents. The highest tocopherol content was achieved by SFE at 300 bar and 80°C. The effects of linear terms of pressure and temperature were significant on the yield of tocopherols, however the effect of pressure was negative, therefore lower pressure increased the amount of tocopherols in the extracts. The types of tocopherols were found with big differences. The deep-frozen stored samples contained more α- and β-tocopherols than dried stored samples, the deep-frozen storage prevented better those compounds. It can be concluded that from an

industrial waste, tomato pomace high valued product can be obtained by SFE at higher pressure and temperature, which contains high amount of lycopene and tocopherols.

The antimicrobial properties of several other medicinal plants were also summarized. These plants were: caraway (*Carum carvi* L.), marigold (*Calendula officinalis* L.), feverfew (*Tanacetum parthenium* L.) and yarrow (*Achillea millefolium* L.). These herbs and extracts did not inhibited strongly the test microbes, however different inhibitions were revealed. Among the supercritical CO_2 extracts of the four medicinal plants different antifungal activities were observed. Against *Trichoderma viride*, *Aspergillus niger*, and *Penicillium cyclopium* feverfew extracts (MICs were > 1%) showed stronger inhibitions than the other extracts. Caraway extracts showed no inhibitions in the applied concentrations (1-5 g/100 ml medium). In the antibacterial tests the extracts showed significant strong inhibitions, especially in the higher applied concentration (0.4 g extract/100 ml medium) in the presences of food borne bacteria strains (*E. coli*, *P. fluorescence* and *B. cereus*). Surprisingly, caraway showed the strongest antibacterial activity among the four herbs (in 0.4% concentration, the inhibitions were > 97.5%). Also strong inhibitions were observed in the presences of the other three, marigold, yarrow and feverfew SFE extracts. In the applied 0.4% concentrations, higher than 73.4% inhibitions were obtained.

Conclusions

On the basic of the results above it can be concluded that the products obtained by $scCO_2$ extraction possess high biological values; therefore the usage is reasonable for well-defined purposes (food-, cosmetic or pharmaceutical industries). Natural oils, waxes, colours, aromas and flavours can be extracted and due to their antimicrobial and antioxidant properties longer shelf-live can be achieved.

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Nile Perch Skin and Bone Gelatin Extraction and Physico-Chemical Characterisation

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Abstract
Type A gelatins were extracted from skins and bones of young and adult Nile perch and analysed to determine their functional and chemical properties. Nile perch bone gelatin exhibited high ash content, suggesting a need for de-ionisation. The gelatin from both young and adult fish skins exhibited gel strength greater than 220 g. This was significantly higher than the gel strength for the bone gelatins (179 g and 134 g, respectively for young and adult fish). Gelatin from adult Nile perch skins also exhibited higher viscosity than bone and the young fish skin gelatins. Bone and skin gelatins had approximately the same amino acid composition, with a total imino acid content of about 21.5%. SDS PAGE revealed that skin gelatins had a higher content of polypeptides with molecular weight greater than β compared to bone gelatins. The differences in functional properties between the skin and bone gelatins appeared to be related to differences in molecular weight distribution of the gelatins.

Key Words: Nile perch, fish gelatin, bone gelatin, gel strength, imino acids, molecular weight distribution

1. Introduction

Nile perch (*Lates niloticus*) is a warm water fish species. It is the dominant fish species found in Lake Victoria, with annual catch estimated at 400,000 tonnes per year. It is estimated that 50% of the fish remains as waste during filleting (Shahidi, 1994) and 30% of the waste is in the form of bones and skins (Gòmez-Guillèn, Turnay, Fernández-Diaz, Ulmo, Lizarbe & Montero, 2002). Skins and bones are potential sources of gelatin and collagen. Recent outbreaks of Bovine spongiform encephalopathy (BSE) and increase in demand for kosher and halal foods have created a demand for fish gelatin for food applications.

The objective of this study was to extract gelatin from Nile perch bones and skins and to determine it's physico-chemical properties.

2. Materials and methods

2.1 **Raw materials**

Fish skins and skeletons were obtained from Nge-ge Fish Limited, Kampala, Uganda. The very small skins (skin thickness < 0.4 mm) and bones (skeleton length < 40 cm) from young fish and the very large skins (skin thickness > 1.5 mm) and bones (skeleton length > 95 cm) from adult fish were selected and used for the study. Moisture, lipid, ash and protein were determined by AOAC (1995) methods 950.46, 960.39, 900.2A and 928.08, respectively. The skins and skeletons for gelatin manufacture were frozen immediately upon delivery at the laboratory and thawed just before the gelatin extraction process.

2.2 **Preparation of gelatins**

Gelatins were prepared from skins and bones of Nile perch by the acid type (type A) pre-treatment followed by extraction at 50°C as described by Muyonga (2003).

2.3 **Analysis of gelatins**

The moisture, ash and fat content of the extracted gelatins were determined by the BSI 757 methods (BSI, 1975). Protein content was determined by Kjeldahl method (AOAC, 1995). The Bloom gel strength, viscosity and melting point were determined by the British Standard 757: 1975 methods (BSI, 1975). Corrected gel strength (assuming 87.5% protein) was calculated from the equation;

$$\text{Corrected Bloom} = \text{Bloom}_{measured} \times (87.5/(100 - \text{Moisture\%} - \text{Ash\%}))^2$$

Amino acid analysis was conducted using the Pico.Tag method (Bidlingmeyer, Cohen & Tarvin, 1984). Molecular weight distribution was determined using SDS-PAGE using the discontinous Tris-HCl/glycine buffer system (Laemmli, 1970), with 7.5% resolving gel and 4% stacking gel. The gels were scanned using a GS-300 transmittance/reflectance scanning densitometer (Hoefer Scientific Instruments, San Francisco, CA, USA).

2.4 **Statistical analysis**

Data for the different parameters were compared using analysis of variance (ANOVA) and means were separated using LSD. Except where stated otherwise, data from triplicate experiments were used.

3. Results and discussions

3.1 **Proximate composition of raw materials**

The protein content of the fish skins was found to be in the range 20 - 22% and that for bones was approximately 13% (Table 1). The protein content of the collagenous material represents the maximum possible yield of gelatin expected from them. This was higher for skins than for bones, but did not significantly vary with age for either skins or bones. The bones also generally contained higher ash and lower moisture than the skins. Skins from adult fish were found to contain more lipid than skins from

young fish skin, probably because the fish accumulate subcutaneous fat as they age. Ash content was also considerably higher for adult than for young fish skins, probably because of increased calcification of scales with age.

Table 1: Proximate composition of Nile perch skins and bones obtained from young and adult fish

	Skin		**Bone**	
	Young	**Adult**	**Young**	**Adult**
Moisture (%)	72.7 (1.3) a	68.4 (0.57) a	36.8 (2.6) b	36.3 (1.6) b
Protein (%)	20.3 (2.0) a	21.6 (1.3) a	13.2 (1.2) b	13.1 (1.3) b
Lipid (%)	4.96 (0.67) b	6.8 (0.3) a	7.1 (1.3) a	7. 8 (1.3) a
Ash (%)	3.7 (0.5) b	6.0 (0.2) b	38.4 (1. 8) a	39.1 (2.6) a

- Values in brackets are standard deviations of triplicate samples
- Values in the same row followed by same letter are not significantly different at α = 0.05

3.2 **Proximate composition of gelatins**

Table 2: Proximate composition of gelatins derived from skins and bones of young and adult Nile perch

	Skins		**Bones**	
	Young	**Adult**	**Young**	**Adult**
Protein (%)	88.8 (3.1) a	88.0 (4.7) a	83.3 (3.9) ab	78.4 (2.5) b
Moisture (%)	10.4 (0.9) a	10.5 (0.6) a	10.8 (0.4) a	10.3 (1.1) a
Ash (%)	1.7 (0.4) c	1.4 (0.4) c	8.4 (0.4) b	11.2 (0.5) a
Lipid (%)	0.0 (0.0) a	0.1 (0.0) a	0.2 (0.1) a	0.0 (0.0) a

- Values in brackets are standard deviations of triplicate samples
- Values in the same row followed by same letter are not significantly different at α= 0.05

The proximate composition of gelatin was found to vary with the type of tissue used as raw material but was unaffected by age of the fish (Table 2). Generally, the gelatin fat content was very low (< 0.5%). This showed that the pre-treatments used had eliminated fat as desired. The skin gelatins were low in ash (<2%). The bone gelatins, however, had much higher ash content (most in the range 3 - 10%), indicating that the leaching process was inadequate. Manufacture of fish bone gelatin may therefore, require an ion exchange step to remove the salts or improvement of the leaching process, for example, by application of a counter-current process.

3.3 **Gelatin functional properties**

Nile perch skin gelatins exhibited higher gel strength (222 and 229 g, respectively for

young and adult fish) than corresponding bone gelatins (179 and 134 g, respectively for young and adult fish) (Table 3). Adult fish skin gelatin also exhibited higher viscosity (42.3 mSt) than bone gelatins (28.2 and 30.0 mSt, respectively for young and adult fish). There was no significant difference ($p > 0.05$), however, between the viscosity of young Nile perch skin and the Nile perch bone gelatins. The melting temperature was found to be similar for adult fish skin and the bone gelatins but lower for young fish skin gelatin.

Table 3: Functional properties of gelatin extracted from different raw materials at 50°C

	Nile Perch Gelatins				Other Gelatins	
	Skins		Bones		Bovine	Commercial
					bone	Fish
	Young	Adult	Young	Adult		
Corrected Bloom (g)	222 (5) a	229 (10) a	179 (6) b	134 (12) c	221 a	216 a
Viscosity (mSt)	21.6 (2.2) b	42.3 (2.1) a	28.2 (2.8) b	30.0 (2.9) b	46 a	40 a
Melting Temperature (°C)	21.4 (0.3) c	26.3 (1.2) b	26.5 (0.7) b	25.5 (1.3) a	31.6 a	26.3 b

• Values in brackets represent standard deviation for triplicate samples
• Values in the same row followed by the same letter are not significantly different at $\alpha = 0.05$

3.4 **Molecular weight distribution**

SDS PAGE showed that the molecular weight distribution of Nile perch gelatins varied with the collagenous tissue used as raw material. Nile perch skin gelatins were generally found to contain higher proportions of the > β fraction than the Nile perch bone gelatins (Table 4). This is suggestive of higher incidence and/or stability of cross-links in the bone than in the skin collagen, resulting in more cleavage of peptide bonds during the manufacture of bone gelatins. It seems the thermal stable cross-links are more resistant to cleavage than the collagen peptide bonds.

Table 4: Molecular weight distribution of gelatins obtained from different raw materials at varying extraction temperatures

Sample source (extraction temperature)	**Proportion (%) of different fractions**			
	> β	**β - α**	**α**	**<α**
Young Nile perch skin	17.8 (2.9) b	25.9 (2.9) b	7.9 (1.0) c	48.4 (3.1) a
Adult Nile perch skin	19.7 (3.9) b	25.4 (3.1) b	22.3 (2.8) b	32.6 (4.1) b
Young Nile perch bone	3.3 (0.7) c	10.0 (1.4) c	28.4 (3.3) a	58.3 (6.7) a
Adult Nile perch bone)	5.0 (1.0) c	37.5 (2.9) a	30.0 (4.1) a	27.5 (4.1) b
Bovine bone	28.8 (3.8) a	27.1 (3.1) b	29.7 (3.6) a	14.4 (1.9) c
Commercial fish skin	18.4 (2.0) b	29.5 (3.6) b	27.3 (3.1) a	24.0 (2.7) b

• Values in brackets represent standard deviation for duplicate samples
• Values in the same column followed by the same letter are not significantly different at $\alpha = 0.05$

The functional properties of the gelatins studied were correlated to the proportion of the different molecular weight fractions. The lower content of high molecular weight fractions (> β) for bone gelatins was associated with lower viscosity and melting temperature exhibited by these gelatins in comparison to the adult fish skin gelatins.

The proportion of low molecular weight (< α) fraction (peptides) was higher for young than for the adult fish skin gelatin. This may be responsible for the lower viscosity and melting temperature for the young fish skin gelatin. According to Tavernier (1989), high incidence of low molecular weight peptides is associated with low viscosity, melting point, setting point and high setting time. In this study, viscosity and Bloom had a high positive correlation ($r^2 > 0.7$) to the > β fraction, while the α fraction was highly positively correlated ($r^2 > 0.7$) to melting point. Viscosity was also highly negatively correlated ($r^2 = -0.79$) to < α fraction.

Despite the difference in the level of peptides, the gel strength was similar for gelatins from young and adult fish skins. Earlier studies (Koepff, 1984; Graesser, 1985) also showed no simple correlation between gelatin gel strength and molecular weight distribution for high gel strength gelatins.

The melting point and viscosity of young Nile perch skin gelatin may partly be due to the very wide molecular weight distribution exhibited by this gelatin. According to Yau, Kirkland and Bly (1979), wide molecular weight distribution negatively affects some functional properties of macromolecules.

The bovine bone and commercial fish skin gelatins were found to exhibit functional properties quite similar to those of adult Nile perch skin gelatin, except for melting temperature, which was higher for bovine bone gelatin. The difference between the properties of fish gelatin and bovine bone gelatin may partly be attributed to differences in molecular weight distribution. The bovine bone gelatin and the commercial fish skin gelatin were found to be lower in peptides smaller than α-chain (Table 4) and had densitograms with fewer distinct fragments. Such densitograms are characteristic of alkali-processed gelatins (Koepff, 1984). The bovine bone gelatin also had a much higher content of γ components.

3.7. **Amino acid composition**

The amino acid composition of Nile perch gelatins was found to be similar for all the Nile perch gelatins (Table 5).

Table 5: Amino acid composition of gelatin from skins and bones of young and adult Nile perch

	Amino Acids g/100g Protein			
	Young fish skin gelatin	Adult fish skin gelatin	Young fish bone gelatin	Adult fish bone gelatin
Asp	5.26 (0.23)	5.29 (0.02)	4.67 (0.08)	5.17 (0.22)
Glu	9.41 (0.00)	9.41 (0.06)	9.41 (0.01)	9.42 (0.07)
Ser	3.00 (0.01)	3.08 (0.01)	3.02 (0.02)	3.13 (0.02)
Gly	23.65 (0.01)	23.76 (0.04)	23.51 (0.15)	23.55 (0.15)
His	1.02 (0.01)	1.01 (0.01)	1.04 (0.03)	1.04 (0.04)
Arg	8.14 (0.04)	8.31 (0.03)	7.94 (0.10)	8.17 (0.07)
Thr	2.71 (0.04)	2.80 (0.01)	2.81 (0.04)	2.86 (0.03)
Ala	10.53 (0.10)	10.56 (0.02)	10.46 (0.03)	10.32 (0.15)
Pro	12.47 (0.15)	12.81 (0.10)	12.27 (0.03)	12.00 (0.26)
Hyp	9.08 (0.02)	8.82 (0.04)	9.52 (0.02)	9.76 (0.05)
Tyr	0.55 (0.01)	0.55 (0.02)	0.60 (0.01)	0.62 (0.01)
Val	2.08 (0.02)	2.02 (0.01)	2.12 (0.01)	2.05 (0.02)
Met	1.74 (0.02)	1.32 (0.01)	1.75 (0.02)	1.45 (0.04)
Ile	0.98 (0.01)	0.95 (0.02)	1.11 (0.03)	1.00 (0.02)
Leu	2.28 (0.02)	2.21 (0.02)	2.40 (0.03)	2.30 (0.05)
Phe	2.09 (0.01)	2.09 (0.02)	2.24 (0.07)	2.15 (0.07)
Lys	3.60 (0.06)	3.56 (0.03)	3.43 (0.07)	3.58 (0.12)
Hyl	1.42 (0.04)	1.45 (0.10)	1.72 (0.01)	1.42 (0.11)

- Values in brackets represent standard deviation for triplicate samples
- Values for the different gelatins were not significantly different at $\alpha = 0.05$

The amino acid composition of Nile perch gelatins was found to differ from those reported for other species. Imino acid content of Nile perch gelatins (~ 21.5%) was higher than ~ 17% reported for cod gelatin (Grossman & Bergman, 1992; Gudmunsson & Hafsteinsson, 1997) but lower than ~ 25% (Grossman & Bergman, 1992) reported for tilapia and ~ 30% for mammalian (Poppe, 1992) gelatins.

4. Conclusions

1. Nile perch gelatins exhibit functional properties, which are more similar to mammalian gelatins than cold-water fish skin gelatins.

2. The superior functional properties of Nile perch gelatin compared to cold-water fish gelatins may be explained by their higher content of imino acids.

3. There is potential for exploitation of Nile perch processing waste for gelatin extraction. The potential is higher for Nile perch skins than bones because Nile perch skins give higher gelatin yield and the skin gelatin exhibits better functional properties than Nile perch bone gelatin.

5. Acknowledgements

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Characterization and Quantification of Polyphenolics from Grape Pomace by HPLC-DAD-MS/MS.

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Introduction

Grapes and products obtained therefrom, such as wine, grape juice, jams and raisins constitute an economically important factor. As can be seen from the annual world production of 61 Mio tons in 2002, grapes are the world´s largest fruit crop apart from oranges, with Italy, France, Spain and the US being among the most important producers. About 80% is used in wine making. Since approximately 20% of the weight of grapes processed remains as pomace, annually some 10 Mio tons of by-products result from wineries. Grape pomace composition and water contents may considerably vary, depending on grape variety and technology of vinification.

Since grape pomace is poorly digested as a feed, winery by-products have long been used as soil conditioner or for obtaining fertilizers. On the other hand, grape pomace represents a rich source of various high-value products such as ethanol, organic acids, grape seed oil, hydrocolloids and dietary fiber (Hang, 1988).

Furthermore, grape pomace is characterized by high phenolic contents because of poor extraction yields during wine making. Anthocyanins, catechins, flavonol glycosides, phenolic acids and alcohols and stilbenes are the principal phenolic constituents of grape pomace (Schieber *et al*., 2001). Anthocyanins from winery by-products are usually extracted with sulfite-containing water or acidified alcohols and used as natural food colorants (E 163).

Beginning with the ´French Paradox´ observations numerous studies have been initiated dealing with the antioxidative and health-promoting effects of plant secondary metabolites in grapes and wine, revealing the inhibition of human low-density lipoprotein oxidation by grape and wine phenolics (Teissedre *et al*., 1996). As a consequence, grape pomace is considered a valuable source of phenolic compounds which could be recovered as functional food ingredients. Despite detailed studies on the phenolic profile of grape pomace (Lu & Foo, 1999), its quantitative composition was mostly expressed as total phenolic contents and correlated with the

antioxidant activity of grape pomace extracts (Meyer *et al*., 1998). When individual substances were quantified, only few data concerning individual compounds or substance groups were reported.

A systematic comparison of the phenolic contents of the pomace derived from different grape varieties has not yet been presented. Therefore, the main objective of the present study was to determine the amounts of individual phenolic compounds in the skins and seeds of 14 different press residues originating from wine making. Such studies are of particular importance since polyphenols have been shown to differ considerably in their bioavailability and to exert differring biological activities *in vivo*. Thus, these data may contribute to facilitate the selection of suitable plant materials for the extraction of phytochemicals.

Materials and methods

Materials

Grape pomace samples were obtained from a local winery (´Felsengartenkellerei Besigheim e.G.´, Germany) and from the Institute for Special Crop Cultivation and Crop Physiology, Hohenheim University. Nine different samples from red wine production (vintages 2001 and 2002) were used for polyphenol analyses (cultivars ´Cabernet Mitos´, ´Lemberger´, ´Spätburgunder´, ´Trollinger´ (high-temperature short-time treatment of the red grape mash) and ´Schwarzriesling´ (rosé wine production)). Additionally, four pomace samples from white wine production (cultivars ´Kerner´, ´Müller-Thurgau´, ´Weisser Riesling´ and ´Merzling´; vintages 2001 and 2002) were also included in this study.

Methods

Sample preparation

Frozen grape pomace samples were manually separated into skins and seeds, lyophilized, and finely ground with a ball mill. After flushing with nitrogen in order to prevent oxidation during extraction, aliquots of the pulverized skins and seeds were extracted with methanol / 0.1% HCl (v/v) under stirring. The extracts were centrifuged, and the material was re-extracted with the same solvent. The combined supernatants were evaporated to dryness, and the residue was dissolved in acidified water (pH 3.0). Anthocyanins were analyzed by direct injection of the solutions. Non-anthocyanin phenolics of red grape skins were extracted four times with ethyl acetate before fractionation via solid phase extraction (SPE). The combined extracts were evaporated to dryness, dissolved in water and applied to the SPE cartridges. Seed and white grape skin extracts were directly used for solid phase extraction. Phenolic acids were subsequently eluted with deionized water and 0.01% HCl (Fraction I), anthoxanthines and stilbenes were eluted with ethyl acetate (Fraction II). The eluates were concentrated *in vacuo*, and the residues obtained were dissolved in suitable amounts of solvents, membrane-filtered and used for LC analyses (Oszmianski *et al*., 1988).

HPLC-DAD-MS/MS analysis

Polyphenol analyses were carried out on an Agilent HPLC series 1100. The separation was performed using a Phenomenex Aqua C18 column (250 ¥ 4.6 mm i.d.; 5 µm particle size), with a C18 ODS guard column (4.0 ¥ 3.0 mm i.d.). Peak assignment was performed by comparison of the retention times and UV/Vis spectra with those of standard compounds and by the molecular and pseudomolecular ions and their characteristic fragments in the MS^2 experiments obtained by the same HPLC system which was coupled on-line to a Bruker model 3000+ ion trap mass spectrometer fitted with an ESI source. Individual compounds were quantified using a calibration curve of the corresponding standard compounds. When reference compounds were not available, the calibration of structurally related substances was used including a molecular weight correction factor.

Results

Anthocyanins

Baseline separation of thirteen anthocyanins was achieved within 33 minutes, five of which were identified as the 3-*O*-monoglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, based on their UV-Vis and mass spectra, and by comparison with reference compounds. Additionally, eight further compounds were detected all of which represented acylated anthocyanins. Four of them were identified as 3-*O*-acetylglucosides of delphinidin, petunidin, peonidin, and malvidin, whereas four peaks were assigned to the 3-*O*-*p*-coumaroylglucosides of cyanidin, petunidin, peonidin, and malvidin, based on their molecular ions and on the corresponding anthocyanidin fragments produced in the MS^2 experiment. In contrast to the complex anthocyanin pattern of ´Cabernet Mitos´ peel extracts, the skins of all other cultivars were devoid of the 3-*O*-acetylglucosides of delphinidin and petunidin and the 3-*O*-coumaroylglucosides of cyanidin and petunidin. Moreover, the peels of ´Spätburgunder´ and ´Schwarzriesling´ were characterized by the complete absence of acylated anthocyanins (Kammerer *et al*., 2004).

Malvidin 3-*O*-glucoside was the predominant compound, mostly followed by peonidin 3-*O*-glucoside. Most strikingly, large amounts of anthocyanins were detected in the pomace samples of the cultivar ´Cabernet Mitos´. However, marked differences were observed between the anthocyanin contents of ´Cabernet´ skins from different vintages, where only 38 % of the total amounts of 2002 were found in the skins of 2001. Similar results were observed for ´Spätburgunder´ and ´Trollinger´, whereas the pomace of the poorly colored ´Lemberger´ grapes from the vintages 2001 and 2002 showed nearly the same total anthocyanin contents. Pigment contents ranged from 2.7 (´Trollinger´ 2001) to 131.9 g/kg DM (´Cabernet Mitos´ 2002).

Climatic and microclimatic factors must be made responsible for the great variability of pigment concentrations within the same cultivar. The data clearly demonstrate that thorough assessment of winery by-products is a prerequisite for a profitable

extraction of food colorants and bioactive compounds. Pomace of ´Cabernet Mitos´ proved to be a promising source of anthocyanins since the pigment concentration is much higher than reported for other grape varieties.

Phenolic acids

Among the hydroxybenzoates, gallic, protocatechuic, *p*-hydroxybenzoic, and syringic acids were identified by their UV spectra and by comparison of their retention times with reference compounds. Their pseudomolecular ions and the fragments released after collision-induced dissociation in the MS^2 experiment confirmed peak assignment. The hydroxycinnamates caffeic, *p*-coumaric, ferulic, and sinapic acids were identified accordingly. Additionally, caftaric, coutaric and fertaric acids were identified by their pseudomolecular ions at m/z 311, 295 and 325, respectively. CID in the MS^2 experiment revealed a loss of 132 Da, corresponding to a tartaric acid moiety and releasing caffeic, *p*-coumaric and ferulic acids, respectively. In most pomace samples trace amounts of 5-hydroxymethylfurfural were detected, probably resulting from the high-temperature short-time treatment of the red grape mash, which is commonly applied for an accelerated extraction during wine making.

Compared to the anthocyanins, the phenolic acids were present in considerably lower amounts, with caftaric acid being the predominant compound in all samples. Great variabilities in the phenolic acid contents of both different cultivars and samples of different vintages were observed. However, differences between vintage 2001 and 2002 were not consistent since only in some cases the contents were higher in 2002. It is assumed that different ripening stages of the grapes processed rather than climatic conditions are responsible for varying phenolic acid contents. However, differences were not as pronounced as described for the anthocyanins. Compared to peels of red grape varieties, phenolic acid contents of white grapes were generally lower. Their total contents ranged from 60.5 (´Schwarzriesling´ 2002) to 973.5 mg/kg DM (´Lemberger´ 2002) for red grape peels and from 104.7 (´Weisser Riesling´ 2001) to 227.0 mg/kg DM (´Merzling´ 2002) for white grape peels.

Most strikingly, levels were generally higher in the seeds compared to the skins for all white grape cultivars, while this was only the case with ´Spätburgunder´ (2002) and ´Schwarzriesling´ pomace. Furthermore, the phenolic profile of the skins and seeds differed significantly. While the red grape cultivars ´Cabernet Mitos´, ´Lemberger´ and ´Trollinger´ exhibited comparatively high proportions of the hydroxycinnamates caftaric, coutaric and fertaric acids in the seeds which probably result from adhering residual skin and pulp, all other seed extracts showed a predominance of gallic and protocatechuic acids.

Anthoxanthines and stilbenes

The flavan-3-ols catechin, epicatechin and the dimeric procyanidins B1 and B2 as well as epicatechingallate were detected in the skins and seeds. Among the flavonols quercetin (Q), Q 3-*O*-galactoside, Q 3-*O*-glucoside, Q 3-*O*-rhamnoside, kaempferol (K), K 3-*O*-glucoside, and isorhamnetin 3-*O*-glucoside were identified and quantified. Additionally, Q 3-*O*-glucuronide was tentatively identified based on its UV spectrum

and its mass spectral behavior, revealing a loss of 176 Da in the MS2 experiment, corresponding to a cleavage into the quercetin aglycone and a hexuronide moiety. Furthermore, two stilbenes, *trans*-resveratrol and its glucoside *trans*-polydatin, were identified by comparison with commercially available reference compounds.

Compared to the anthocyanins, anthoxanthines and stilbenes were minor compounds, with the exception of the flavan-3-ols in the seeds which ranged up to several grams per kg DM. Resveratrol and polydatin contents in the skins of all pomace samples ranged from 11.1 to 123.0 mg/kg DM for resveratrol and from 5.1 to 148.0 mg/kg DM for polydatin, showing only minor vintage-related differences with the exception of the ´Cabernet Mitos´ and the ´Spätburgunder´ pomaces. However, large variabilities were observed between the cultivars. Since stilbenes act as phytoalexins, their biosynthesis has been shown to be induced by abiotic stress such as ultraviolet light, and fungal infection, e.g. by Botrytis cinerea. Thus, different microclima and phytosanitary conditions of the grapes may account for the varying contents of stilbenes.

The total amounts of all identified anthoxanthines and stilbenes ranged from 297.3 (´Cabernet Mitos´, 2002) to 1857.8 mg/kg DM (´Lemberger´, 2002) for red grape peels and from 1560.2 (´Kerner´) to 6571.2 mg/kg DM (´Merzling´) for the white cultivars. White grape skins had generally higher yields than red ones, which again may be attributed to both cultivar-related variabilities and differences in red and white wine making techniques.

The seeds generally exhibited higher polyphenol contents than the skins. The phenolic profile was dominated by flavanols, whereas the flavonols and stilbenes were detected in minor amounts and must be attributed to mash constituents adhering to the seeds. Their contents ranged from 2.28 (´Trollinger´, 2002) to 18.76 g/kg DM (´Spätburgunder´, 2002). Differences between red and white grape seeds were not significant. This may be due to the fact that phenolic compounds are only poorly extracted from the grape seeds during vinification.

Conclusions

1. In accordance with previous reports, the results presented in this study demonstrate that grape pomaces have generally very high polyphenolic contents, making their utilization worthwhile and thus supporting sustainable agricultural production.

2. The data reveal great differences in both the anthocyanin, phenolic acid, anthoxanthine and stilbene contents of the press residues, primarily depending on cultivar and vintage.

3. This investigation underlines the necessity of a polyphenol screening since differences between the polyphenol subclasses were not uniform. Further studies need to be conducted taking into consideration the effects of different vinification techniques on the phenolic contents of the pomaces.

Acknowledgements

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Recent Developments of Marine Processing Waste Up-grading: Production of Hydrolysates with Biological Properties

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Abstract
In recent years, a large number of biologically active peptides has been isolated from bacterial, fungal, plant and animal sources or generated from proteins by enzymatic hydrolysis. The preparation of hydrolysates from fishery wastes and by-products (e.g. heads, frames, viscera) through enzymatic processes also shows to generate biologically active factors such as peptides inhibiting the angiotensin I-converting enzyme, thus exhibiting an antihypertensive effect or hormonal-regulating peptides such as (i) the small gastrointestinal peptides like gastrin and cholecystokinin (CCKs), and (ii) calcitonin and calcitonin gene related peptide (CGRP). *In vitro* and *in vivo* immunostimulatory activities are also detected in Shrimp hydrolysates. In addition to the biological activities described above, the presence of antioxidant compounds in marine by-products hydrolysates is reported.

All these by-products of fish and shellfish wastes are now considered in the context of the production of functionnal molecules exhibiting nutrition and health properties.

Key words: Marine by-products, hydrolysates, nutraceutics, biological activities

Introduction

The disposal or utilization of seafood processing wastes is a critical issue for the seafood industry. As an example, shrimp discard represents more than 30 million kg of waste material per year only for Norway. The remains are either discarded at sea or processed to animal feed although this material has a nutritional value almost as good as whole fish (Synowiecki & Al Khateeb, 2000; Ibrahim *et al*., 1994) and has an obvious potential as food ingredient (Imm & Lee, 1999; Shahidi & Synowiecki, 1991). In particular, these wastes constitute an important source of biologically active

molecules possessing peculiar properties and practical application promises in various areas among them nutraceutical field presents a rising interest. The preparation, through enzymatic processes, of hydrolysates of fishery wastes and co- products have been thoroughly studied during the two past decades. Published work deals mainly with enzyme technology (Guerard *et al*., 2001, Benjakul & Morissey, 1997; Martin & Porter, 1995 , Quaglia & Orban, 1987) or with the nutritional properties of the hydrolysates (Liaset & Espe, 2000 , Diniz & Martin, 1998 ; Shahidi *et al*., 1995).

A review by Kristinsson & Rasco (2000) pointed out the development of these enzyme technologies for producing new feeding stuffs, similar to that already obtained by enzymatic hydrolysis of milk or plant proteins and improving the functional properties of hydrolysates. Similarly, processing by-products from shellfish are made up primarily of protein residues from body sections such as heads and carapace, as well as minerals and chitin which constitute the exoskeleton of crab, shrimp and lobster.

A series of data concerning the identification of hydrolysates of useful (nutrition, growth, health, etc) biological activities have geen recently produced (Dufosse *et al*., 1997 ; Fouchereau-Peron *et al*., 1999, Ravallec-Plé *et al*., 2000, Bordenave *et al*, 2002; Gildberg & Stenberg, 2001). Several papers have also been recently published about the link between nutrition and free radicals. It seems that the alimentary absorption of anti-oxydants lower the appearance of some illnesses (Eastwood, 2001). On the other hand, small acid peptides from cod muscle and cod stomach hydrolysates stimulates superoxide anion production (" oxidative burst") in head kidney leukocytes from Atlantic salmon (Salmo salar) (Boegwald *et al*., 1996). Such stimulation has been obtained both after intraperitoneal injection *in vivo*, and after inoculating cell cultures *in vitro*.

All these observations clearly reveal the possibility of both economical and environmentally compatible improvements in health and nutrition fields in relation with the use of fish and shellfish by-products.

Results

Conditions of Hydrolysis and Characterisation of Hydrolysates.

A preliminary study on the catalytic efficiency of 19 industrial proteases was carried out on a standard substrate of tuna stomach proteins. As regards to the production of significant amounts of peptides in the desired range (3000 to 500 Da) and to final DH values, free amino-acid released, hydrolysate bitterness, the preparations of Alcalase® 2,4 L, Corolase PP, gave very interesting results.

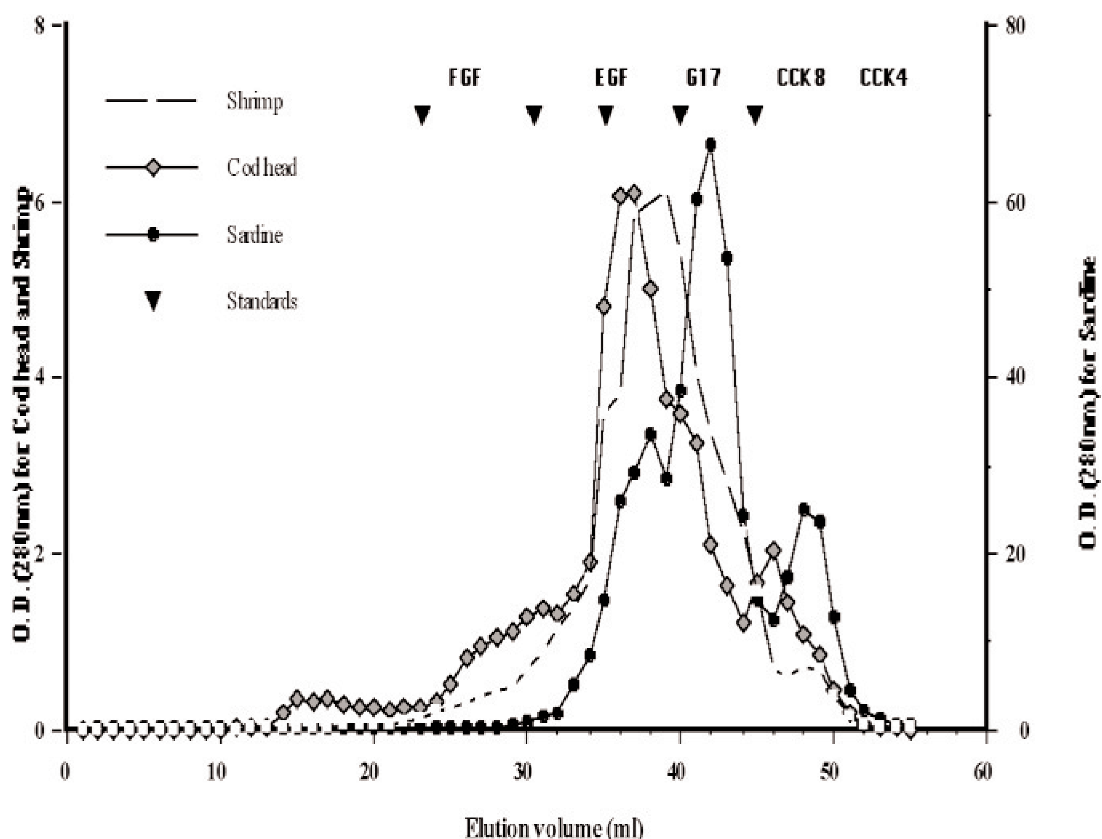

Figure 1. Gel filtration on Sephadex G-50 of shrimp waste, cod head and sardine hydrolysates fractions (Alcalase® 2,4 L). Markers: FGF (13.6 Kd),EGF (6 Kd), gastrin17 (2 Kd), CCK8 (1 Kd), CCK4 (0.6 Kd).

Viscera fraction, sardine wastes, hake wastes, shrimp wastes and tuna stomach were prepared using mainly Alcalase® 2,4 L and other enzyme preparations. Before being purified each extract was submitted to a preliminary purification by gel filtration. A convenient and rapid analytical tool for the identification, standardisation and comparison of the different hydrolysates produced was developed. The size exclusion chromatography (SEC) was applied in FPLC mode using a SUPERDEX PEPTIDE HR 10/30 column (Pharmacia).

Identification of Biological Activities in Hydrolysate Products.

Immunostimulating activities

Immunostimulatory effects of protein hydrolysates from shrimp waste and various cod by-products were investigated on the immune response of Atlantic Salmon, *Salmo salar* L.. Hydrolysates were tested for their ability to enhance the capacity of Atlantic salmon macrophages to produce O^-_2 (NBT-test) and to induce cytokine-like activity in leukocytes. The cytokine-assays used have been the measurement of the production of macrophage activating factor (MAF).

A shrimp waste hydrolysate (SW 98) primed the Atlantic salmon macrophages for increased respiratory burst activity. The effects were similar to, and in some cases

better than LPS from *A. salmonicida*. Similar results were obtained with extracellular superoxide anion production measured by the ferricytochrome c – method, and the two methods gave corresponding results. The variation of immuno stimulatory effects among different shrimp waste hydrolysates produced by the same procedure, indicates that variations in the raw materials may play an essential role.

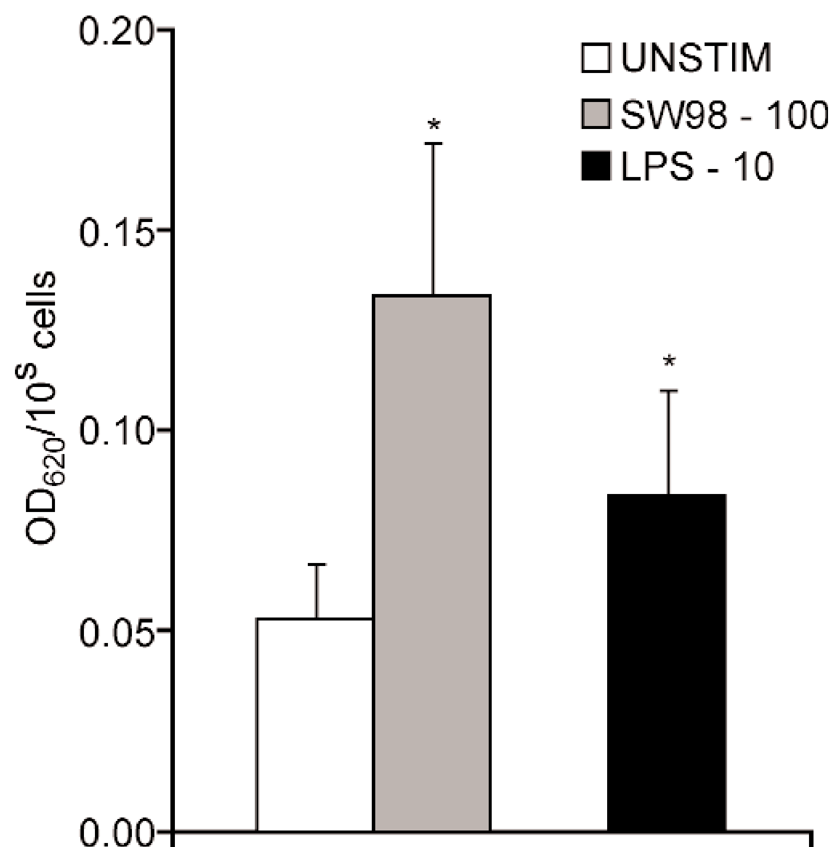

Figure 2. *In vitro* stimulation of immune response in Atlantic salmon macrophages by a shrimp waste hydrolysate as compared to stimulation by bacterial lipopolysacharides (LPS). Shrimp hydrolysate (100 mg/ml) provided a higher superoxide anion production by the macrophages than the LPS (10 mg/ml).

Inhibition of angiotensin converting enzyme (ACE)

The investigation on the angiotensin-converting enzyme (ACE) inhibitors was undertaken on whole fish hydrolysates and hydrolysate fractions. Hydrolysates comprised very complex mixture and were essentially composed of peptides. Shrimp hydrolysate fraction gave weak results in the ACE test whereas cod head and sardine hydrolysate fractions gave inhibition indexes reaching 90%. The most potent fish hydrolysate fractions were selected and fractionated in order to identify the active biomolecules.

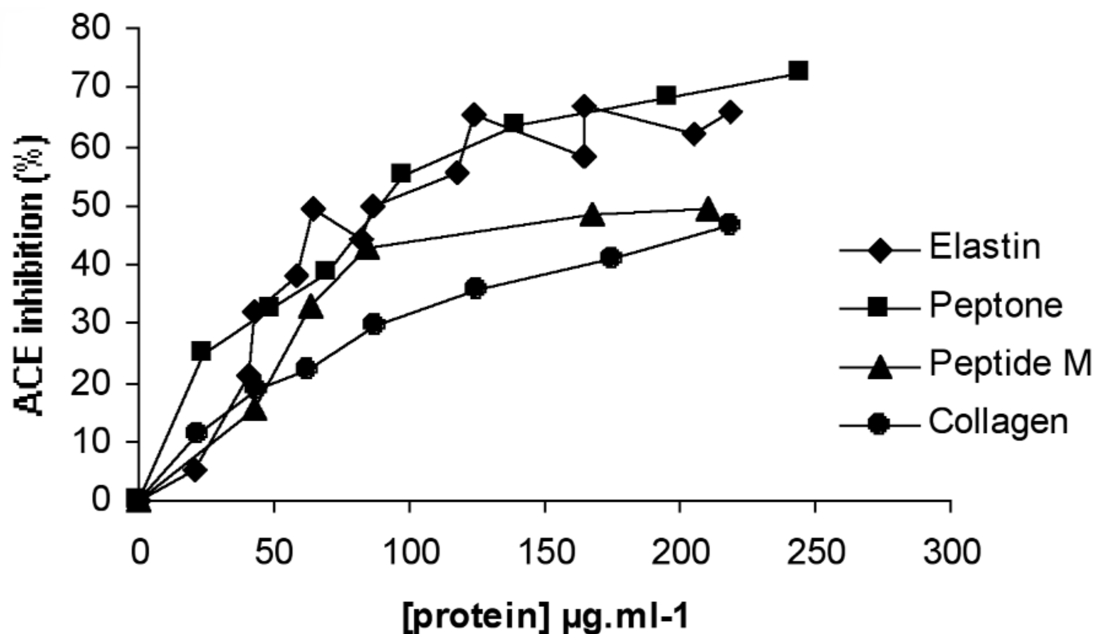

Figure 3. Graded concentrations of hydrolysates(on a total protein basis) comprised between 20 and 250 µg/ml were tested on the ACE activity. Results expressed in % ACE inhibition.

Calcitonin and calcitonin gene related peptides (CGRP) peptides

The identification of calcitonin and CGRP in hydrolysate fractions was carried out using radioimmunoassays and radioreceptor assays. No molecules biologically related to calcitonine were present in the hydrolysates. However, the cod and sardine viscera autolysates, sardine hydrolysates and the hake and tuna hydrolysates do contain peptidic fragments immunologically and biologically related to CGRP.

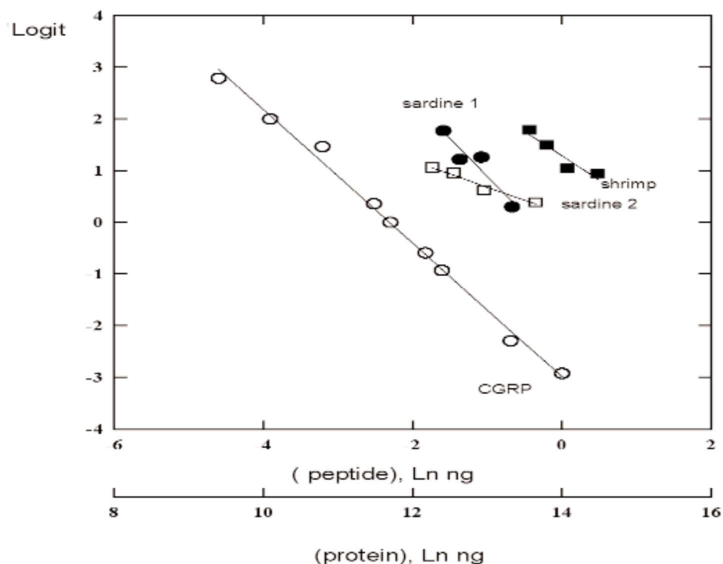

Figure 4. Effect of increasing concentrations of sardine and shrimp hydrolysates (alcalase treated) on the binding of 125I human CGRP to its antibody (anti human CGRP). Sardine hydrolysates were prepared using either 1% trypsin (batch 1) or alcalase (batch 2). Each hydrolysate (sardine 1, 2 and shrimp) contained CGRP immunorelated molecules with concentrations of about 50, 150 and 17 pg/mg of proteins, respectively.

The molecules purified from a sardine hydrolysate had an apparent molecular weight of about 6000 Da, interacted in the CGRP radioreceptor assay and inhibited the CGRP-stimulated adenylate cyclase activity.

Antioxidant and antiradical activities

The antioxidant & antiradical activities of the various hydrolysates were compared to to positive controls such as BHA and ascorbic acid. The most interesting results were obtained using the β-carotene linoleate model (Figure 5).

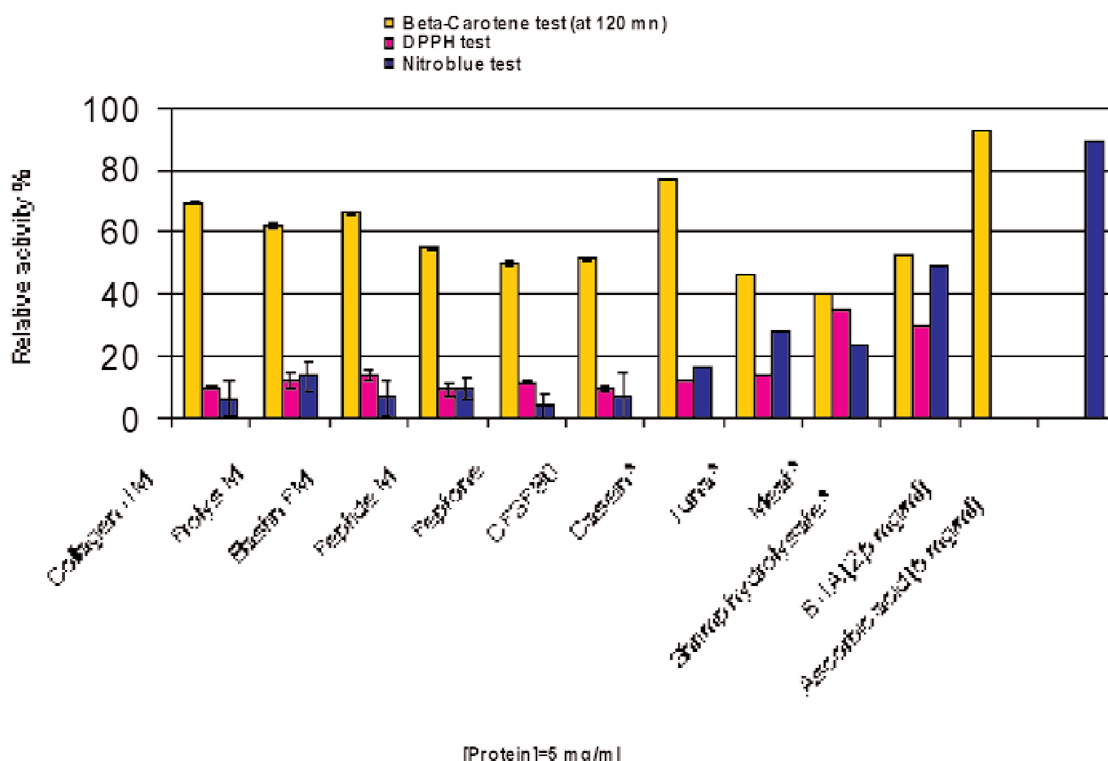

Figure 5. *In vitro* antioxidant properties of fish by-product hydrolysates evaluated according to their effect to prevent β-carotene bleaching after 120 min. incubation and to scavenge the free radical DPPH• (DPPH Test) and the superoxide anion (Nitro Blue Test). (Protein concentration = 5 mg/mL)

Conclusion

The preparation of hydrolysates from fishery wastes and by-products (e.g. heads, frames, viscera) through enzymatic processes using Alcalase 2,4 L and other proteases, have showed to contain biologically active factors such as *in vitro* and *in vivo* immuno stimulatory effects . Several other classes of molecules have been generated like peptides with opioid-like activity or anxiolytic properties, or peptides inhibiting the angiotensin I-converting enzyme, thus exhibiting an antihypertensive effect. The identification of hormonal-regulating peptides such as calcitonin and calcitonin gene related peptide (CGRP) in hydrolysate fractions was carried out by radioimmunoassays and radioreceptors and gave positive results. The presence of antioxidant compounds in marine by-products hydrolysates is also reported..

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Enhancement of the Radical Scavenging Activity of Tuna Waste Hydrolysate

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Introduction

The development of some non-enzymatic browning reactions, such as Maillard reaction (MR), has been associated to the formation of compounds with strong radical scavenging activity.

The MR is a complex reaction, since it is influenced by many factors such as temperature, pH, time, water activity, type and concentration of buffer, reactant source and sugar involved (Lingnert, 1990; Ames, 1990; Wijeckreme *et al*., 1997). Changing any of these factors will alter reaction rate, reaction pathways and reaction end-products.

Browning development in MR are generally used as an indicator of the reaction rate and MRPs formation, recently, there has been an attempt to correlate the antiradical activity of MRPs with the browning (Hayasa *et al*, 1989; Brands *et al*, 2000; Morales and Jimenez-Pérez, 2001).

During food processing and cooking at high temperatures the MR can produce mutagenic, DNA-damaging, and cytotoxic substances. This certainly concerns the so-called advanced Maillard products. The cytotoxic effet of MRP has been studied in different model systems and is associated with the use of high temperatures (Jing and Kitts, 2002).

The analysis of heating conditions at moderate temperatures is needed in order to maximise the antiradical activity without the production of the cytotoxic effect related to MRPs. The present investigation was undertaken in order to study the effect of sugar type and concentration on the increase of antiradical activity of MRPs obtained from a tuna waste hydrolysate.

Materials and methods

Materials.

A tuna hydrolysate was prepared using Alcalase 2,4, All reagents were of analytical grade and were purchased from Sigma/Aldrich (St Louis. Mo. USA).

Preparation of MRPs from sugar-protein hydrolysate system.

A mixture of tuna hydrolysate at 5 mg/mL of protein (according to Lowry assay) and ribose or glucose was prepared at 55°C, 65 hrs, pH 7, 0.05 M phosphate buffer.

Antiradical and Antioxidant activity.
The antiradical activity of MRPs was evaluated according to the procedure reported by Morales and Jimenez-Perez 2001. An aliquot of sample (200 µl) was added to 1 ml of a daily-prepared solution of 1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH) at a 74 mg/L concentration in ethanol. Mixture was shaken vigorously for 1 hr at 25°C. The sample was centrifuged at 10 000 x g for 5 min, then absorption of the supernatant was measured at 520 nm. Several experiments were performed in a preliminary step in order to determine the kinetics of DPPHo radical scavenging activity of samples. Then, the time required to reach the steady state was established. The DPPHo concentration in the reaction medium was calculated from the calibration curve, determined by linear regression : $[DPPH]_t = 0.0241 (A_{520\ nm}) + 0.022$ (r^2=0.9995). The Antiradical Activity (AA) of sample was expressed as percentage disappearance of DPPHo, $AA(\%)=(100-([DPPH]_t/[DPPH]_{H2O})*100)$ where $[DPPH]_{H2O}$ is the concentration of DPPH in the presence of water instead of hydrolysate.

Molecular weight distributions of MRP.
The HPLC equipment consisted of a Waters system with an integrated photodiode array detector model 996 and a fluorescence detector model 474. The HPLC was driven with the millenium32 program (Waters). Molecular weight of the reaction products of tuna hydrolysate and glucose or ribose was measured by size exclusion chromatography (SUPERDEX peptide HR 10/30, Pharmacia).

Cytotoxic effect
Cytotoxic effect of RMP was assessed using determination of cell viability. African green monkey (*Cercopithecus aethiops*) kidney cells (Vero cell line n°ATCC CCL-81) and Swiss albino mouse (*Mus musculus*) fibroblast cells (3T3-Swiss albino cell line n°ATCC CCL-92) were respectively grown in RPMI 1640 and in DMEM (BioWest, Nuaille France) containing 100 UI/ml Pénicilline, 100 ug/ml Streptomycine and 2 mM L-Glutamine (BioWest, Nuaille France), supplemented with 10 % fetal calf serum (FCS). Cells were grown at 37°C under 5% CO_2 and routinely passed before they reached confluence every 3 days.
Determination of 50% cytotoxic concentration (CC_{50}): Cytotoxicity of RMP was assessed using determination of cell viability performed by neutral red dye method as previously described by McLaren *et al*. and Langlois *et al*. . Briefly, 50 µL of media containing neutral red dye (0.02%) was added by well. After 4 hours incubation at 37 °C, the media was removed and the cells washed fourth times. Lysis buffer (50µL 1% acetate 50 % ethanol per well) before reading DO absorbance at 540 nm.

Results and discussion

Glucose and ribose were selected as sugar reactants due to differences in the reaction rates. The relationship between radical scavenging activity, browning intensity and sugar concentrations is shown in Figure 1.
The antiradical activity of the MRPs was 11 fold higher with ribose than when using glucose and a plateau was reached at a 40 mg/mL sugar concentration. Thus ribose was used for the further experiments. The high ribose reactivity could be related to

the acyclic form of the ribose thus making this sugar more reactive than the glucose. The rate of the reaction depends on the rate at which the sugar ring opens to the reducible, open-chained form (Davies and Labuza, 1997) Thus pentose sugars react more rapidly than hexose sugars.

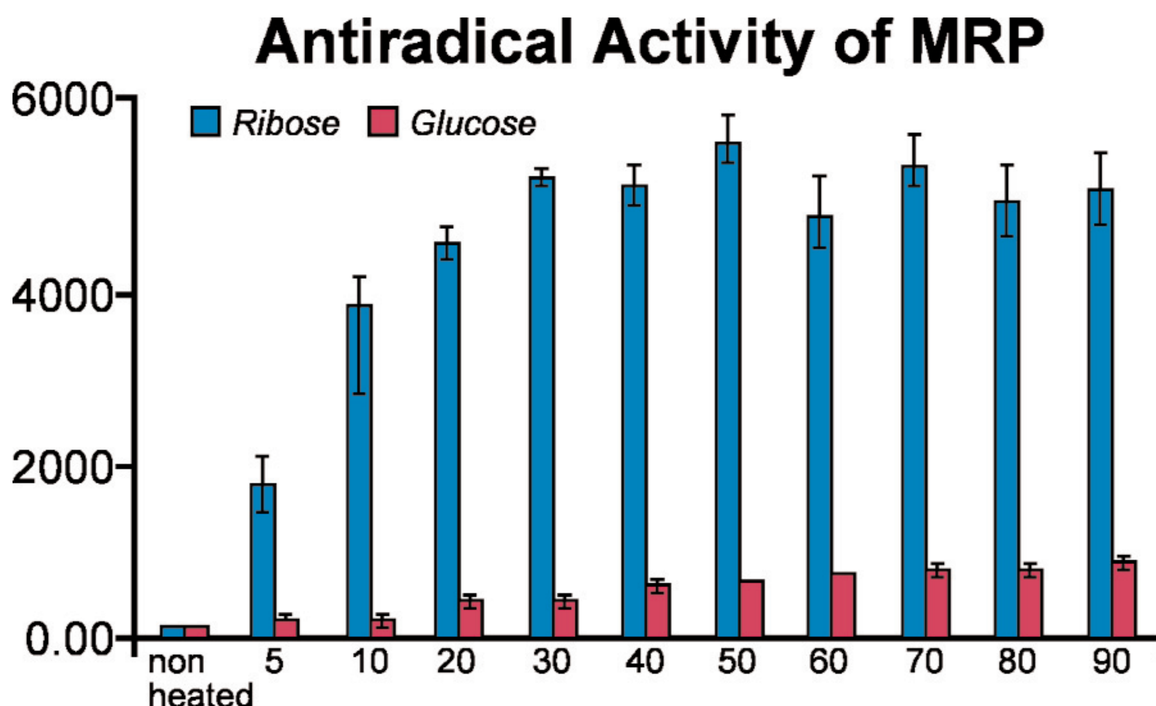
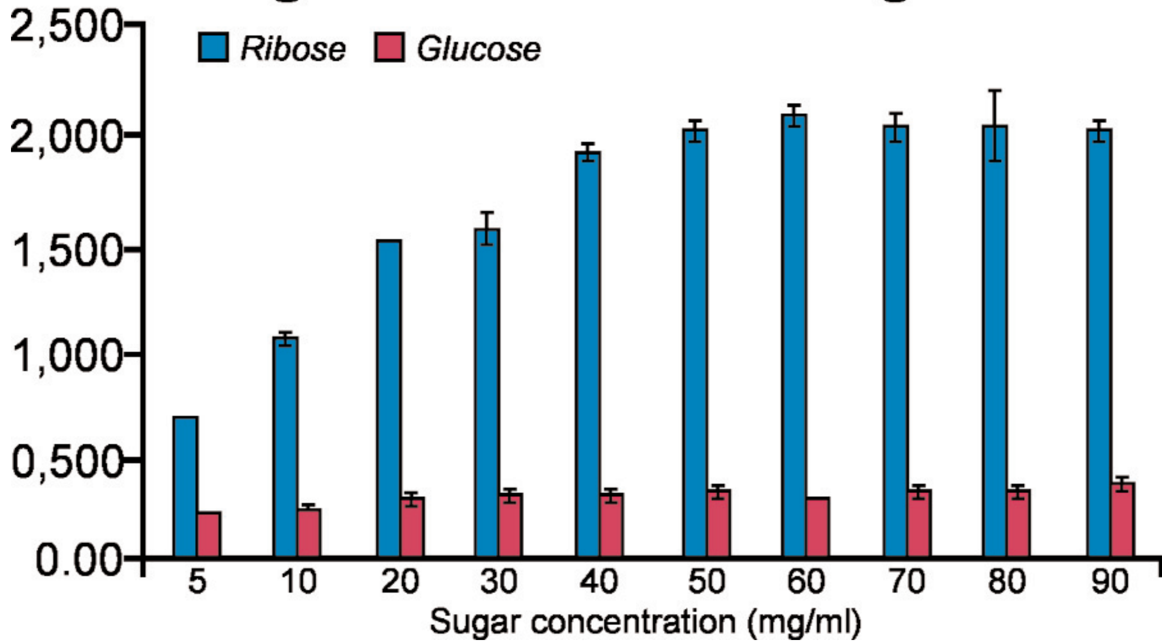


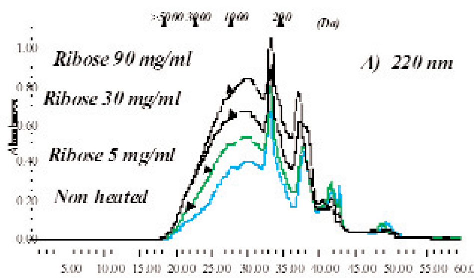
Figure 1. Effect of sugar concentration on radical scavenging activity. Heating conditions : 55°C, 17 hrs, pH 7, 0.05 M phosphate buffer.

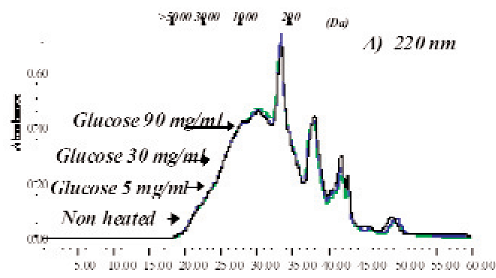


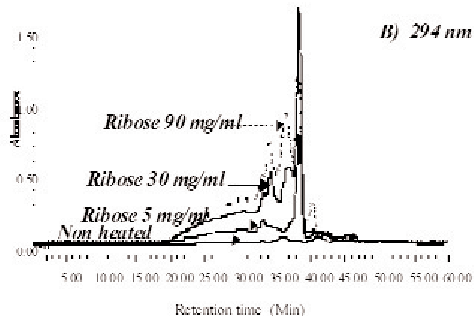
The progress of the browning was followed by monitoring absorbance at 420 nm (Figure 2). The brown colour development is largely due to the formation of chromophores, which have been widely studied in different model systems (Monti *et al*., 2001). The development of MRP colour was maximum at a 50 mg/mL ribose concentration. A correlation between antiradical activity and browning for both Ribose- and Glucose -hydrolysate was observed (r^2=0.86 and 0.92, respectively).

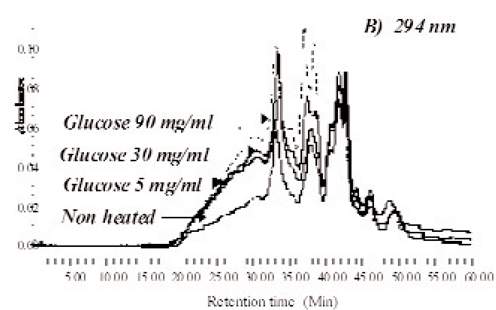
SEC-FPLC . MRP from ribose

SEC-FPLC . MRP from glucose









The MRPs were analysed using size exclusion chromatography (Figure 3). The study of chromatographic profiles obtained before and after the Maillard reaction of tuna hydrolysate and ribose found major changes in absorbency at 220 and 294 nm indicating major molecular rearrangements.

The chromatographic profiles obtained after the Maillard reaction of tuna hydrolysate and glucose showed minor molecular rearrangements.

Study of MRP potential cytotoxicity.

Table 1. Cytotoxic evaluation (Determination of 50% cytotoxic concentration (CC50)):

	3T3 Fibroblats	**Vero cells**
[MRP]	CC50 > 1g/L	CC50 > 0.7 g/L

The results of the cytotoxic evaluation of PRM are reported in Table 1. Cells were incubated with various concentrations of PRM (0-1000 µg/mL, 4 wells per concentration). Correlation curve was obtained between pinocytosis of neutral red (assessed by DO absorbance at 540 nm) and Neperian logarithm of PRM concentrations. The CC_{50} of the tested molecules was defined as the concentration that reduced the absorbance to 50% of that of controls. Results showed that PRM do not exhibit cytotoxic effect at concentration up to 500 mg/mL whatever is the cell type.

Conclusion

Radical scavenging activity of MRPs obtained from Ribose-Tuna Hydrolysate can be dramatically increased, at moderate temperatures without generating cytotoxic effect. These new developments may represent a viable alternative to current fish waste upgrading and may have promising applications in various areas, including the nutraceuticals and cosmetics fields.

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Recovery, Characterisation and Application of a Functional Food Ingredient Containing Carotenes and Oligogalacturonic Acids from Carrot Pomace

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Introduction

Carrot juices and derived products such as vitamin supplemented drinks represent an emerging market of growing economic importance. Due to the low yields associated with carrot juice production, up to 40 % of the raw material remains as pomace which is currently disposed as feed or fertiliser. Despite considerable improvements in processing techniques including the use of technical enzymes and decanter technology, substantial amounts of nutritionally valuable compounds such as carotenes and dietary fiber are retained in the pomace (Stoll *et al*., 2001; Chau *et al*., 2004). Therefore, the objective of the present study was to develop a process for the complete utilisation of carrot pomace on pilot-plant scale and to assess its application in a functional drink based on cloudy apple juice.

Materials and methods

Materials

Carrot pomace and enzyme preparations

Wet carrot pomace (18.5 % dry matter, cultivar ´Karotan´) was obtained from a juice processor (Gemüsesaft GmbH, Neuenstadt am Kocher, Germany) and stored in vacuum-sealed bags at -20 °C until use. The enzyme preparations Pectinex Ultra SP-L, Ultrazym AFP-L and Cellubrix L were donated by Novo Nordisk Ferment (Dittingen, Switzerland). Cytolase CL and Rohapect MA Plus were kindly provided by DSM Food Specialities (Lille Cedex, France) and AB Enzymes (Darmstadt, Germany), respectively. Citrus pectin type AU 202 was obtained from Herbstreith & Fox, Neuenbürg, Germany.

Methods

Enzymatic hydrolysis of carrot pomace

Carrot pomace was suspended in water at a ratio of 1:6 and comminuted in a colloid mill. Enzymatic hydrolysis was carried out using cellulolytic and pectinolytic enzyme

preparations under varying conditions of incubation (dosage, pH, temperature). The hydrolysate was passed through a finisher, homogenised and finally concentrated. The scale-up was performed in a 10 L pilot plant scale reaction vessel at 50 °C and pH 4.0 using each 750 ppm of Cytolase CL and Pectinex Ultra SP-L. The viscosity was recorded with a rotary viscosimeter before enzyme addition and at 30 min intervals during 3 hours of hydrolysis (Stoll *et al*., 2003b).

Characterisation of carotenes and oligogalacturonic acids
Sample preparation and HPLC analysis of carotenes were performed according to a modified procedure described previously (Marx *et al*., 2000). Oligo-galacturonic acids were characterised and quantified by on-line LC-ESI-MS (Stoll *et al*., 2003a).

Application of hydrolysed carrot pomace as a functional food ingredient
Cloudy apple juice and pomace hydrolysate were blended on pilot-plant scale (45 kg) to yield a 50 % fruit content and 12 mg/L total carotene. After deaeration in a colloid mill *in vacuo* for 10 min, the product was homogenised (180 bar) and deaerated again. Citrus pectin was added at concentrations of 0.5 and 1.0 g/kg. After addition of sucrose, ascorbic acid and citric acid, the blend was adjusted to the final concentration with water, pasteurised (90 °C, 60 s), filled into glass bottles, sealed under superheated steam, and finally water-cooled at room temperature. Stability of carotenes was evaluated after 20 and 24 weeks of storage under moderate and intense illumination at 23 °C and at room temperature, respectively (Stoll *et al*., 2003c). Turbidity and cloud stability were determined according to Reiter *et al*. (2003).

Results

Process for the recovery of a carotene-rich hydrolysate
The optimisation of enzymatic hydrolysis of carrot pomace was a major aspect of the present study. The progress of the reaction was monitored by viscosity measurements. A combination of pectinolytic and cellulolytic enzymes (ratio 1:1, 50 °C, pH 4.0) proved to be most efficient for complete cell wall degradation within 90 min. During scale-up to pilot-plant scale using these conditions the time required for hydrolysis was even reduced to 60 min. The finishing step after hydrolysis was established to remove coarse non-digested fibers. Sieves with mesh sizes of 0.5 and 0.8 mm were used yielding 13 and 10 % sieve residue, respectively. The use of 0.5 mm mesh resulted in a favourable particle size distribution with an increased number of particles <400 µm, while particles between 400 and 600 µm were depleted.

Microbial infestation by lactic acid bacteria as a part of the autochthonous flora of carrots was monitored by the determination of D- and L-lactic acid. After 3 hours of hydrolysis the L-lactic acid content did not exceed 6.7 mg/L, and D-lactic acid was completely absent.

The most valuable constituents of the hydrolysate are a- and b-carotene. Significant degradation of carotenes was not observed during hydrolysis, thus confirming the stability of carotene in its natural matrix (Marx *et al*., 2003). Losses of approximately

15 % after concentration resulted from residual carotenes on the surface of the pilot-plant equipment and are expected to be minimised by continuous industrial operation of the evaporator (Stoll *et al*., 2003b).

Application of hydrolysed carrot pomace as a functional food ingredient

Since β-carotene intake from fruits and vegetables is considered beneficial, the most obvious application for the carrot pomace hydrolysate is its utilisation as a functional food ingredient. Commercial products such as vitaminised drinks predominantly contain synthetic β-carotene formulations for provitamin A supplementation, which has been associated with adverse effects at high dosages. Furthermore, crystalline β-carotene formulations are usually based on gelatin to enhance water solubility, whereas for plant foodstuffs pectin is a more adequate hydrocolloid.

In the present study neither degradation of carotenes nor formation of *cis-isomers* was observed when applied as a functional food ingredient in cloudy apple juice. Even after six months of intense illumination total carotene contents remained unchanged. These findings support the protective role of the natural plant matrix and confirm that the physical state of carotenes seems to be the most important factor in carotene stability. As recently demonstrated, model preparations containing crystalline b-carotene showed pronounced stability during heating, whereas β-carotene dissolved in toluene resulted in isomerisation (Marx *et al*., 2003). Compared to vitamin-supplemented drinks composed of carrot juice as a natural source of β-carotene, drinks containing synthetic β-carotene formulations suffered from higher isomerisation rates which have been associated with nutritional consequences such as reduced provitamin A activity (Marx *et al*., 2000). For the consumer the problem arises that an exact calculation of the provitamin A intake cannot be realised. Whereas in beverages based on synthetic β-carotene overages need to be added to counteract losses and guarantee the labelled content during the specified shelf life, adjustment of the β-carotene content by application of the hydrolysed carrot pomace appears to be advantageous.

Apart from colour, cloud stability of functional drinks represents an important quality criterion which primarily determinates customers´ decision to buy such products. In the present study cloudy apple juice was chosen for the preparation of a functional drink because of its high cloud stability (Dietrich *et al*., 1996). A cloud-stabilising potential of pectin on pulp-containing fruit beverages was attributed among others to the increase in serum viscosity (Mensah-Wilson *et al*., 2000). Therefore, in preliminary laboratory scale experiments, citrus pectin was added in concentrations from 1 to 10 g/kg in 1 g/kg increments to evaluate cloud stability as well as mouthfeel. Pectin addition neither affected particle size distribution nor mean volume diameter D[4,3]. Based on these findings beverages containing 0, 0.5 and 1 g/kg pectin were prepared on pilot-plant scale.

After blending cloudy apple juice with the carrot pomace hydrolysate, the turbidity of the beverage prepared without pectin considerably increased from 630 nephelometric turbidimeter units (NTU) to 2560 NTU. The turbidity after centrifugation

remained unchanged for both beverages, resulting in lower relative turbidities. When adding 0.5 and 1 g/kg pectin, the turbidities of both beverage and serum slightly increased with a resulting increment of relative turbidity from 9.5 to 12 and 15.5 %, respectively. Hence the observed increase in turbidity upon addition of pectin might be attributed to the higher serum viscosity. Visual inspection of the sera revealed that fine cloud particles were still suspended. Surprisingly, despite the enzymatic degradation of the carrot pomace tissue and thus of the stabilising pectic substances, a satisfactory cloud stability of the hydrolysate in the beverages could be achieved (Stoll *et al*., 2003b).

Determination of oligogalacturonic acids

Degradation products of cell wall polysaccharides of higher plants, especially oligogalacturonic acids (OGAs) released from the homogalacturonan backbone of pectins, have been shown to affect a number of biological activities in plants such as induction of phytoalexins and regulation of growth and development (Ridley *et al*., 2001). Apart from this hormone-like function and the bifidogenic effect of pectic oligosaccharides, OGAs have recently attracted interest since they have been demonstrated to inhibit the adherence of bacteria to epithelial cells, the initial and crucial step of an infection, and might therefore be used as therapeutic agents (Guggenbichler *et al*., 1997). Furthermore, this adherence was also substantially reduced by carrot soup prepared according to Moro, which was introduced in 1908 in the therapy of diarrheal disease. However, the reports on the exact structure of the bioactive principles are conflicting. Whereas OGAs with a degree of polymerisation (dp) of 2-7 have been held responsible for the antiadhesive effect, more recent investigations have pointed out that the effect primarily depends on the presence of a terminal unsaturated uronic acid but not on the dp or the extent of esterification of the fragments.

Therefore, the carrot pomace hydrolysate was investigated for the presence of OGAs as a second putative functional food ingredient. OGAs were determined by LC-MS according to a method previously established (Stoll *et al*., 2002). Under the conditions initially applied, mainly monogalacturonic acid (1125 mg/L) was detected, whereas digalacturonic (75 mg/L) and trigalacturonic acids (54 mg/L) were present in lower amounts. After modification of enzymatic hydrolysis using a combination of Cytolase CL and Rohapect MA Plus, the resulting product contained extremely high amounts of dp 2 (684 mg/L) and dp 3 (1346 mg/L), while monogalacturonic acid (620 mg/L) was depleted (Stoll *et al*., 2003a).

Conclusions

1. A new process for the recovery of a carotene-rich hydrolysate from carrot pomace using established processing operations was developed, allowing the exploitation of this by-product on a large scale.

2. Outstanding stability of carotenes and satisfactory cloud stability of a model beverage based on cloudy apple juice indicate that hydrolysates from carrot

pomace represent a suitable natural alternative to synthetic β-carotene formulations for the supplementation of cloudy functional drinks. In contrast to a previous report by Henn and Kunz (1996), sensory quality is not adversely affected. Extended applications as a colouring food, e.g. in dried products, appear to be promising.

3. Due to the presence of oligogalacturonic acids, the hydrolysate may also serve as a source of antimicrobially active compounds which may find application as therapeutic agents.

Acknowledgements

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Fractionation and Partial Characterisation of Antioxidant Substances in Shrimp Waste Hydrolysate Modified by Ribose

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Introduction

Although the production of chitin and chitosan has been commercialised for decades, the proteic part of shellfish residues could be recovered and upgraded as a rich source of amino acids, peptides, proteins and other useful chemicals. The recovery of valuable fractions from shrimp processing discards and their enzymatic hydrolysis have already provided bioactive molecules such as immunostimulant compounds (Gildberg and Stenberg, 2001).

Since research regarding the antiradical activity of Maillard reaction products (MRP) has been performed mostly with sugar-amino acid models,little has been known about sugar-protein hydrolysate systems (Lingert *et al* 1981, Chuyen *et al*, 1998, Chevalier *et al*, 2001;Guerard and Sumaya-Martinez, 2003).

At an early stage of the Maillard reaction, the free amino groups react with carbonyl groups of sugar to form a reversible Schiff base, which rearranges to stable, covalently bonded Amadori products. In the beginning of the reaction, the formation of characteristic brightly colored pigments was reported in model systems with single amino acids and sugars (Gomyo *et al*. 1989). However, in the early stage of MR the radical scavenging activity was derived from the uncoloured reaction products, which are smaller than the brightly coloured pigments (Murakami *et al*, 2002). At intermediate stages highly-UV-absorbing and colourless compounds are continually formed. In the advanced phase of the reaction, Amadori products undergo further transformation to fluorescent, coloured substances and cross-linked polymers (Hodge, 1953; Ames, 1990). Formation of melanoidins and heterocycle compounds in the advanced stage of the Maillard reaction could explain the ability of glycated hydrolysate to react with radical compounds (Friedman, 1996).

The analysis of systems of sugar –amino acids by reversed-phase HPLC with diode array detection was used to develop chromatographic maps (Monti *et al*. 1999).

During food processing and cooking at high temperatures the MR can produce mutagenic, DNA-damaging, and cytotoxic substances. This certainly concerns the so-called advanced Maillard products. The cytotoxic effect of MRP has been studied in different model systems and is associated with the use of high temperatures (Jing and Kitts, 2002). Moreover, the use of moderates temperatures is needed in order to develop the antiradical activity without the production of the cytotoxic effect related to MRPs.

Materials and methods

Materials.

A shrimp waste hydrolysate was prepared using Alcalase 2,4, All reagents were of analytical grade and were purchased from Sigma/Aldrich (St Louis. Mo. USA).

Preparation of MRPs from sugar-protein hydrolysate system.

A mixture of shrimp waste hydrolysate at 5 mg/mL of protein (according to Lowry assay) and ribose was prepared at 55°C, 17 hrs, pH 6.5, 0.5 M phosphate buffer.

Antiradical and Antioxidant activity.

The antiradical activity of MRPs was evaluated according to the procedure reported by Morales and Jimenez-Perez (2001) and Vivas *et al*. (1997). The antioxidant activity was evaluated according to Marco G.(1968).

Molecular weight distributions of MRP.

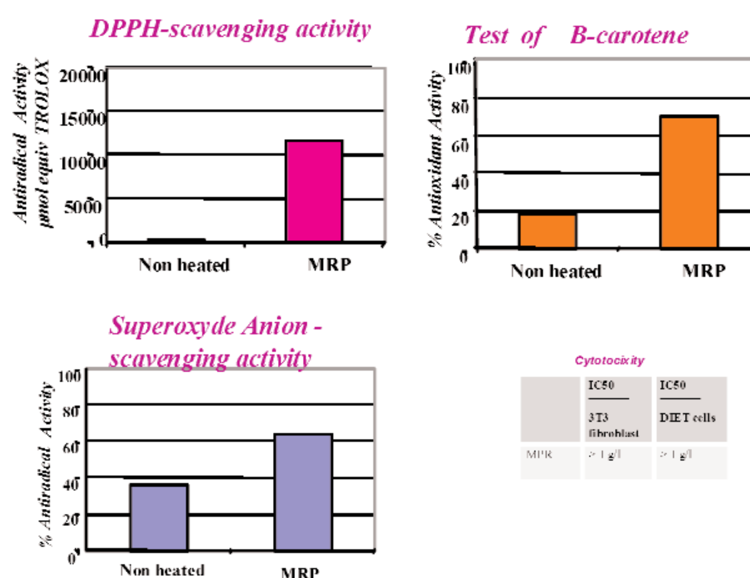
The HPLC equipment consisted of a Waters system with an integrated photodiode array detector model 996 and a fluorescence detector model 474. The HPLC was driven with the millenium32 program (Waters). Fractionation scheme in SEC-FPLC (SUPERDEX 75, Pharmacia) was developed in order to identify the fractions showing the antiradical activity. The different fractions were collected every 2 minutes, The active fractions were characterised by RP-HPLC (AQUASIL C18).

Cytotoxic assays.

Cytotoxic of RMP was assessed using determination of cell viability performed by neutral red dye method as previously described by Langlois *et al*.(1986).

Results and discussion

The Maillard reaction between shrimp waste hydrolysate and ribose produced a high increase in antioxidant/antiradical efficiency. The DPPH-scavenging activity of MRP was 25 fold higher than the unheated sample, the protection against the bleaching of β-carotene was triplicate and the inhibition of superoxide anion was twice as high in the presence of MRPs. The MRP do not exhibit cytotoxic effects when added to the culture medium at concentrations up to 1 g/L (Figure 1).



	IC50 3T3 fibroblast	IC50 DIET cells
MPR	> 1 g/l	> 1 g/l

Figure 1. Antioxidant/Antiradical activity and cytotoxic effect of MRPs

Fractionation scheme in SEC-FPLC (SUPERDEX peptide HR 10/30, Pharmacia), was developed in order to identify the fractions of MRP showing the antiradical activity. The different fractions were collected every 2 minutes and the antiradical activity (test DPPH) was examined (Figure 2). From the collection of MRPs using SEC-FPLC two major fractions were involved in the enhancement of the antiradical activity (test DPPH): Fraction I (> 1 000 Da) and Fraction II (< 1 000 Da), the antiradical activity of these fractions were 54 and 46 %, respectively, of the total activity.

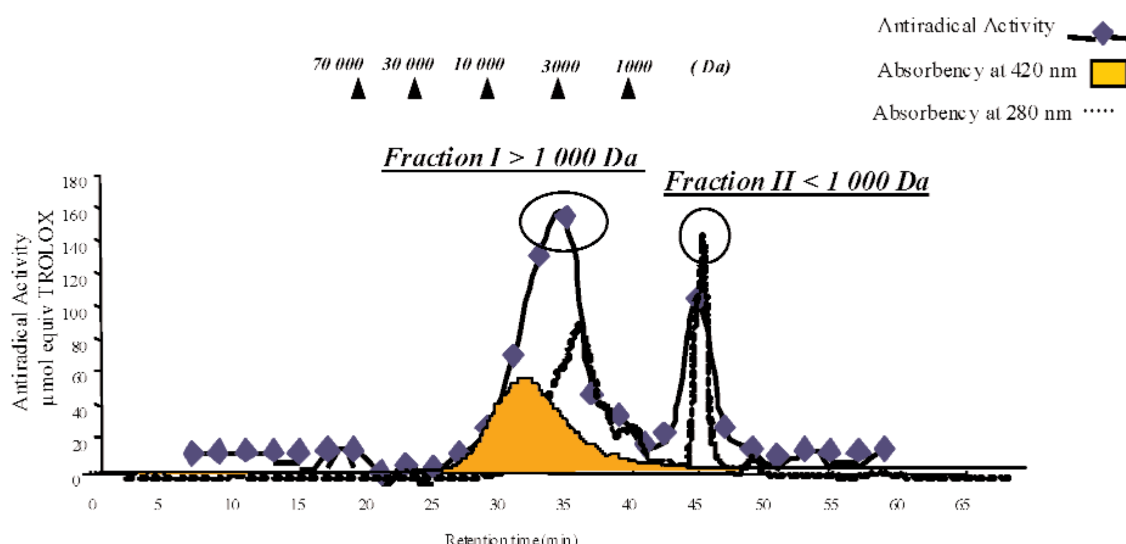


Figure 2. Elution of Rib-hydrolysate MRPs in the Superdex HR 75 (fractionation range 70-3kDa)

The elution of fractions in peak I by reversed phase HPLC (figure 3) gave a complex chromatogram with unretained material (retention time < 5 min) and convex broad bands (eluting between 20 and 30 min). Diode-array spectra indicated that unretained material absorbed in the visible region. The broad bands were designated as brown melanoidins (Bayle *et al*, 1996) and were due to high molecular weight material being spread out on the column by the gradient. The different shapes of the broad bands suggest different interactions between the material responsible for them and the HPLC stationary phase, which could be due to substances varying in structure and/or shape (Monti *et al*, 1998).

The elution of antioxidant peak II by reversed phase HPLC gave a simple chromatogram and one great resolved peak was observed (Figure 4). The HPLC retention time and diode –array spectrum from this peak were identical to 4-hydroxy-5-methyl-3(2)-furanone(HMFone). This compound is formed via 2,3 enolisation of the Amadori rearrangement product when the starting sugar is a pentose. The antioxidative activity of HMFone was reported in the glutathione –ribose model system at pH 7 and refluxed for 3 hours. Eiserich *et al* (1992) reported that the five-membered hetero-cyclic compounds such as furanones, oxazoles and thiazoles have a remarkable antioxidant activity through radical scavenging.

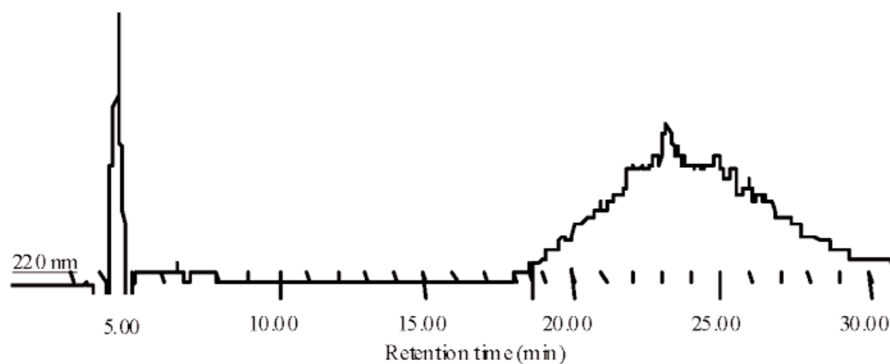


Figure 3. Elution of fraction I in RP-HPLC

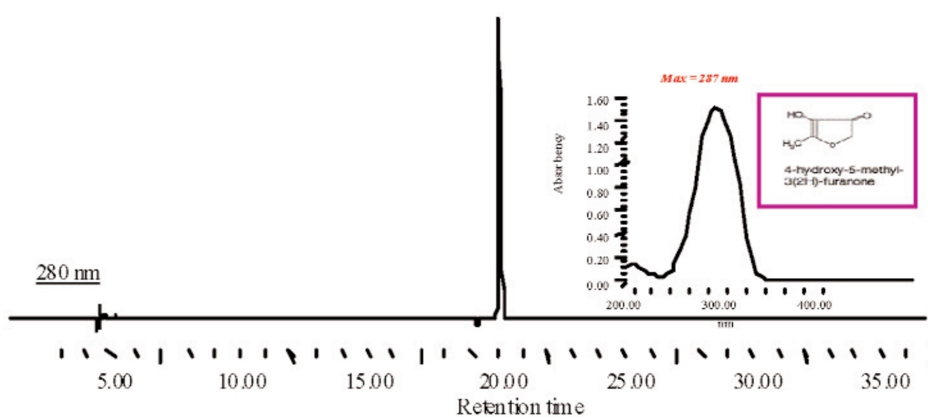


Figure 4. Elution of fraction II in RP-HPLC

Conclusion

The enhancement of antioxidant/antiradical activity of shrimp waste hydrolysate modified by ribose could be related to the presence of aromatic molecules (< 1000 Da) and brown pigments.

These antioxidant/antiradical fractions may represent a viable alternative to current shrimp waste valorisation without generating cytotoxic effects.

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Asparagus By-Product as Source of Functional Compounds

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Introduction

Asparagus is a plant food highly appreciated by consumer due its organoleptic, nutritional and functional properties, and it is one of the 30 most consumed vegetables in the world. It has traditionally been cultivated in Spain, and our country is currently the main producer within the European Union.
Asparagus is one of the nutritionally well-balanced vegetables in existence. Its wealth of nutrients, fiber, and very low sodium and calorie contents make asparagus a nutritionally wise choice fir today´s health-conscious consumer, and it is also an excellent source of vitamins A and C.(Ulrich *et al*, 2001).
Previously to the commercialisation of both fresh and processed asparagus, the spears are cut to a determined length, and sometimes they are also peeled. The waste generated during these steps of manipulation and processing of the samples can be until 60% of the stems. Thousands of tons of high quality asparagus parts are usually rejected as trims during canning and fresh packing operations. The large amount of byproducts from the canning process is partly attributed to the industry´s requirement that asparagus spears be trimmed to fit certain can sizes and not necessarily that the trims are low quality. It means that in the case of asparagus, the byproduct is likely the product, and it should not be considered just as waste.
Until now, this by-product has been used as animal food; however, the compositions of the soluble fraction and the cell wall evidence that asparagus by-product represents an important source of functional compounds. It has been reported that asparagus contains flavonoids (mainly rutin) and other phenolic compounds that possess strong antioxidant properties (Makris and Rossiter, 2001). Vinson *et al* (1998) analysed 23 vegetables commonly consumed and found that asparagus is the first in terms of total quality and quantity of antioxidants. These results are in consonance to those from Pellegrini *et al* (2003) who reported that asparagus and spinach are the vegetables with the highest antioxidant capacity. It is known that the antioxidant content of fruit and vegetables may contribute to the protection they offer from disease. In addition to these phytochemicals, asparagus byproducts represent a good source of compounds, such as hydroxycinnamic acids derivatives, which may be used because of their favourable technological or nutritional properties. The aim of the present work is the utilization of enzymatic and thermal techniques to obtain those functional compounds

Materials and methods

Materials

Plant material

By-product generated during the processing of white asparagus was used for the present work. This consists on the peels of the spears and the final portions of the stems that are discarded before asparagus canning. Waste volume mainly depends on the extent of lignification or fibrousness of the samples, and in this case represented about 50% of asparagus spears.

Chemicals

Ferulic acid, p-coumaric acid and rutin were purchased from Sigma-Aldrich. Driselase was purchased from Megazyme. XAD-column was from Pharmacia.
All chemicals and solvents used were HPLC-grade and purchased from Merck.

Methods

Alcoholic extraction: samples were extracted with 80% ethanol, and both alcohol soluble fraction (ASF) and residue (AIR) were investigated for their sugar and phenolic compositions.

Enzymatic treatment: AIR was incubated with Driselase (1g enzyme/10g AIR), for 48 h at room temperature. Soluble fraction was subjected to adsorption chromatography.

Thermal treatments: three thermal experiments were carried out on AIR. Experimental conditions were as follow:
1. Cooking, for 1h in a beaker of boiling water
2. Hydrothermal treatment of the sample, using saturated steam, at 100ºC, for 5 min
3. Hydrothermal treatment of the sample, using saturated steam at 210ºC, for 10min

Adsorption chromatography: selected fractions were applied to a column of XAD and eluted with water, methanol/water (50%) and methanol.

Analysis of phenolics by HPLC and Diode Array Detection (HPLC-DAD)

Phenolic compounds were detected and quantified by HPLC, as described by Waldron *et al* (1996) with some modification. The separation of phenolics was made by using a SYNERGI 4μHYDRO- RP80A reverse phase column (25 cm x 4.6 mm i.d., 4 μ m; Phenomenex, Macclesfield, Cheshire, UK) with gradient elution employing progressively increasing methanol-acetonitrile levels in 2 mL/L acetic acid.
The gradient profile for the separation of both soluble and wall-bound phenolics was formed using solvent A (10% (v/v) aqueous acetonitrile plus 2 mL/L acetic acid) and solvent B (40% methanol, 40% acetonitrile, 20% water, plus 2 mL/L acetic acid) in the following program: initially A 90%, B 10%; linear gradient over 17 min to A 57.5%, B 42.5%; held isocratically at A 57.5%, B 42.5% for further 6 min; linear gradient over 17 min to B 100%; linear gradient over 5 min to A 90%, B 10%; held isocratically at A 90%, B 10% for further 5 min. The flow rate was maintained at 1 ml/min.

Phenolics were detected using a Hewlett-Packard Series 1100 liquid chromatograph system equipped with an ultraviolet-visible detector and a Rheodyne injection valve (20µl-loop). Quantitation was by integration of peak-areas at 280 nm, with reference to calibrations made using known amounts of pure compounds.

Results

Alcoholic extraction

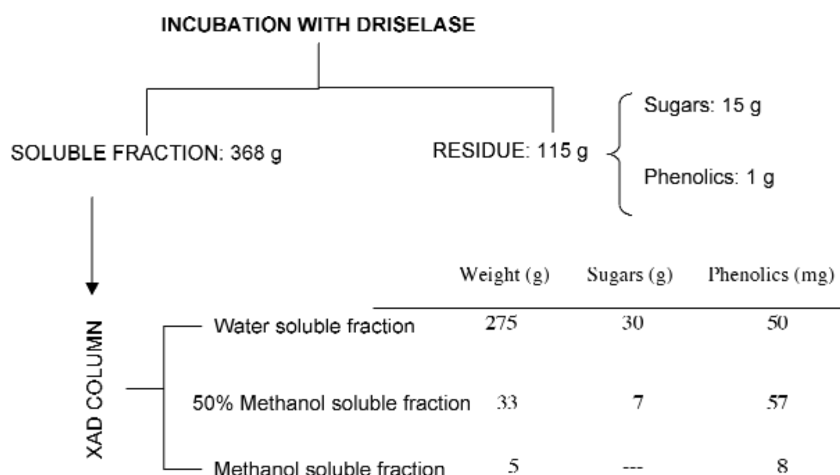
It has previously been reported the biological activity of methanolic extracts from asparagus (Nawfor *et al*, 2003), therefore these extracts could be a source of bioactive compounds. This study has showed that ethanolic extracts contain the same components that those released by methanol.

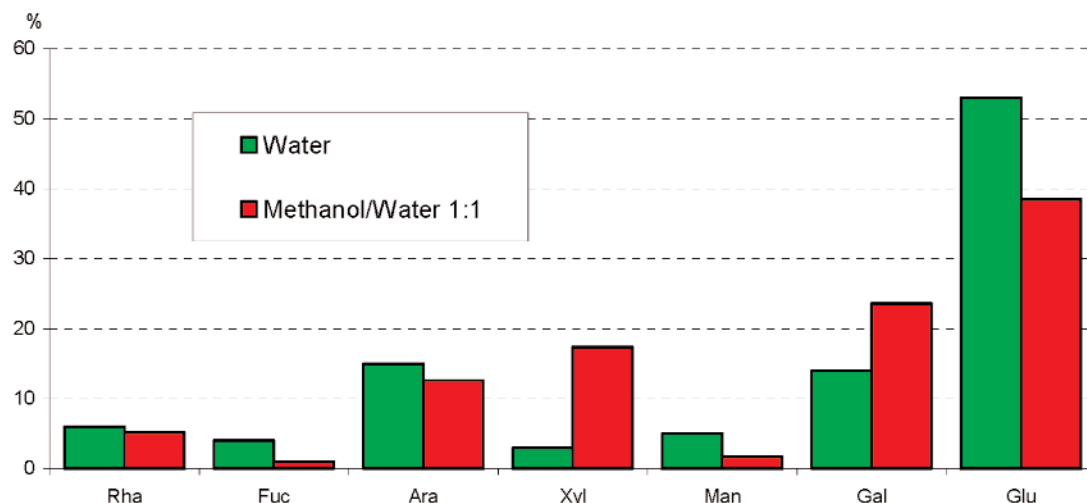
1 kg of dried asparagus byproduct was treated with 80% EtOH, and the suspension was filtered through glass filter for obtaining a soluble fraction (ASF) and a residue (AIR). After freeze-drying both fractions the yields were 225g and 750 g respectively. The ASF contained significant amounts of sugars and phenolics, mainly ferulic and coumaric glycosides. Adsorption chromatography was very efficient separating both components, as it can be observed in the table below. Phenolics were concentrated in the fraction eluted with methanol, while the highest sugar content was found in the water fraction.

XAD column fractions	Weight (g)	Sugars (g)	Phenolics (mg)
Water soluble fraction	152	45	----
50% Methanol soluble fraction	48	6	----
Methanol soluble fraction	13	2	450

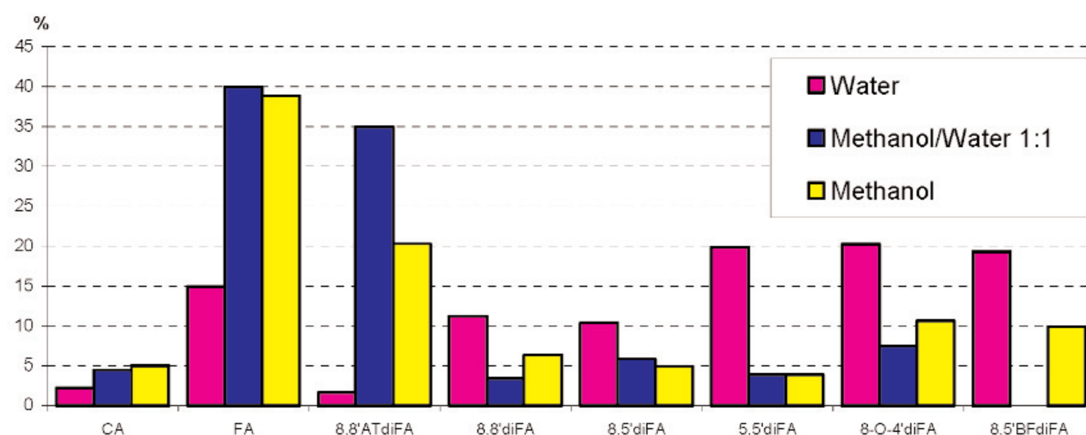
Enzymatic treatment

The residue obtained after treatment with 80% EtOH (AIR(was incubated with driselase in order to extracting remained sugars and phenolic compounds. Treatment with Driselase released around 50% AIR. After chromatography on XAD, it can be observed that main fraction was water soluble fraction, which contained mainly sugars. Phenolic contains were little in every fraction; however those represented higher percentages in the methanolic fractions than in the water one. It is remarkable the low content of sugars Analysis of sugar and phenolic profiles of these subfractions revealed that the first comes from degradation of arabinoxylans and glucans (see figure below).





Regarding the phenolics, their detailed study shown that ferulic derivatives were concentrated in water soluble fraction, and mainly consisted on ferulic acid and 8,8' ATdiFA, while other dehydrodimers were mainly located in the water soluble fraction. Data are summarized in next figure.



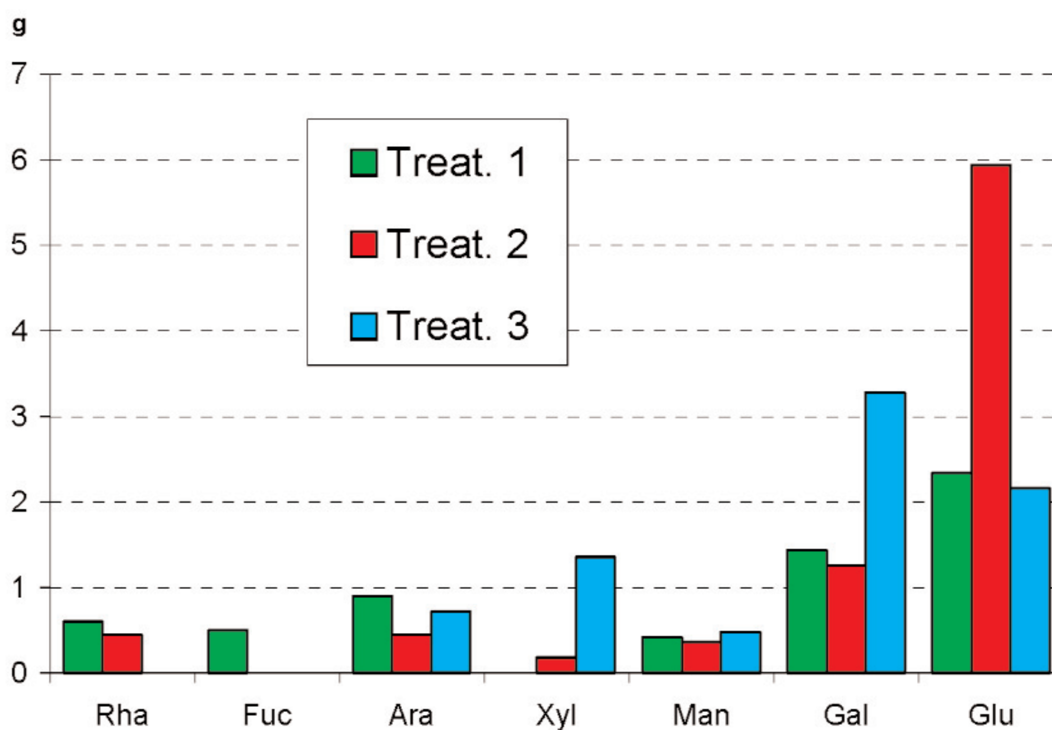
Thermal treatment

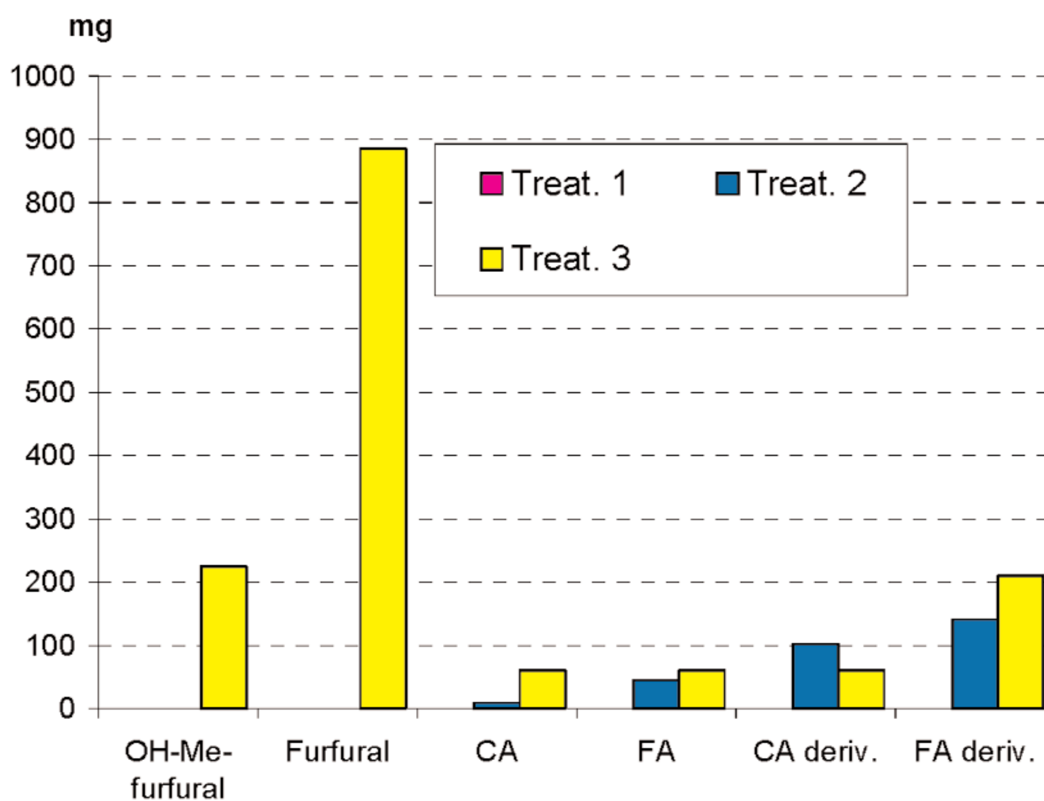
AIR was also subjected to different thermal treatments, at elevated temperatures and pressures. The results obtained from the three experiences described in materials and methods are shown in next table

	Weight (g)	Sugars (g)	Phenols (mg)
Treat.1	195	9	---
Treat.2	156	8	300
Treat.3	150	6	1500*

* 1080 mg corresponded to furfural

Detailed sugar and phenolic composition of the fractions obtained after thermal treatments are shown in the following figures:





Main sugars released by thermal treatments were Xylose, Galactose and Glucose. As it can be observed in the figure above, solubilization of xylose and galactose increased with the severity of thermal treatments, while highest amount of glucose was found in treatment 2.

Phenolics also increased with the severity of thermal conditions, obtaining different ferulic derivatives. In parallel, it was observed a decrease in the contents of insoluble phenolic compounds (data no shown). It is remarkable that the increase of phenolics released from treatment 3 was mostly attributed to the formation of furfural derivatives, while the amounts hidroxycinnamic acid derivatives coming from treatments 2 and 3 were similar.

Conclusions

Asparagus byproducts can be used as source of different functional compounds. The detection of significant amounts of hidroxycinnamic acids previously identified and quantified in asparagus product (Rodriguez-Arcos *et al*, 2002) evidence that the waste generated by processing this vegetable still contain bioactive compounds worth of being isolated. For this purpose is necessary to find a simple and unexpensive system that is attractive for the food-industry. The results of the present work indicate that both enzymatic and thermal treatments maybe be adequate for releasing and isolating ferulic and p-coumaric acid derivatives of application as additives and/or functional ingredients. Further investigations are being carried out in relation to the characterization of the phenolic compounds released from each of the treatments described in this paper.

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Bulk Products

Plant residues

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Introduction

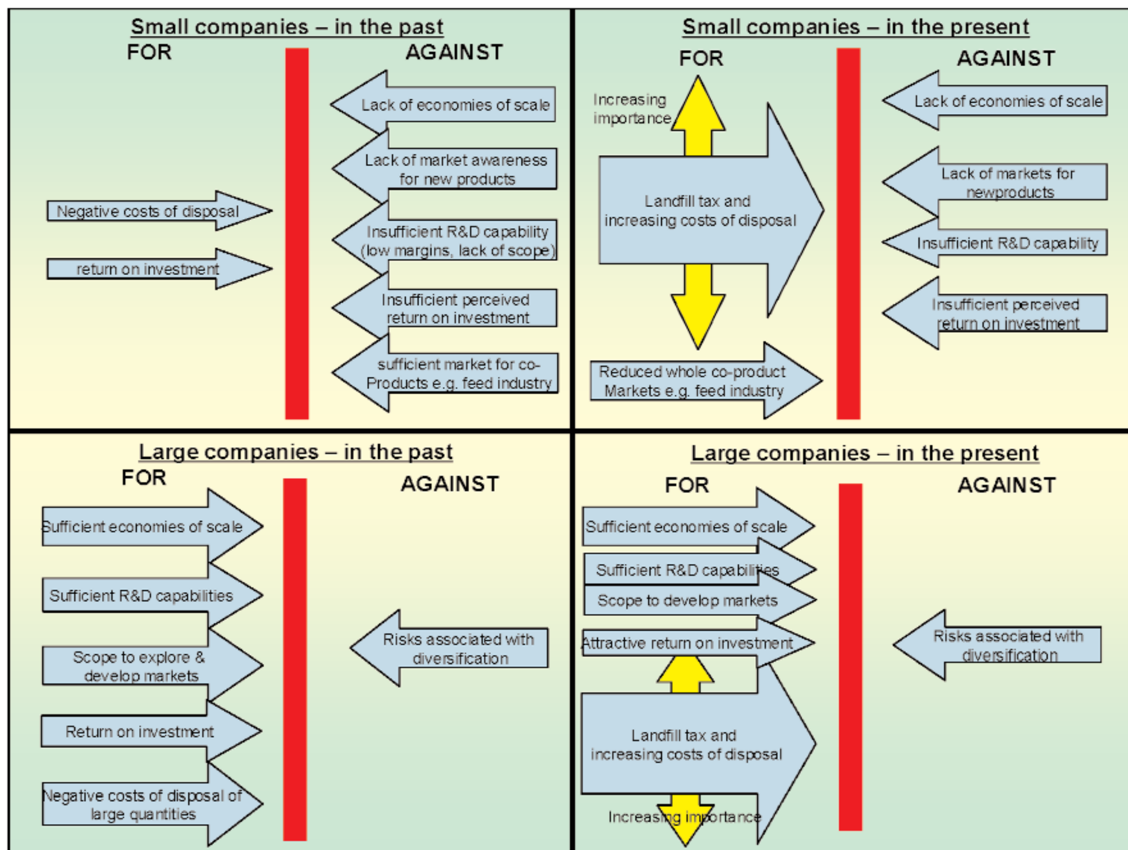
Fruit and vegetable processing has increased considerably during the last 25 years. This has reflected the increase in demand for pre-processed and packaged food, particularly ready meals. During the period that many modern processes were developed and implemented, disposal of waste was not the major issue it is today. Competitive advantage was often achieved by exploiting the benefits of economies of scale, and strategies consequently involved the centralization of processing activities. This resulted in localized production of large tonnages of waste co-products. These were often disposed of relatively cheaply by landfill, land-spreading, or selling as animal feed or for its production. However, subsequent to the Kyoto agreement, the issue of waste in our modern society has become more prominent since it contributes to many of the problems of global environmental sustainability. Vast quantities of food processing co-product wastes are produced throughout the EC. For example over 5 million tonnes of sugar beet pulp, 3.5 million tonnes in brewers grain and nearly half a million tonnes of onion peeling waste are produced annually. Further details may be found in the Awarenet Handbook (Awarenet, 2004). For most fruits and vegetables one can estimate the likely waste as approximately 30% or more of processed material. In some processes it may be up to 75%.

Drivers for change

It is clear that the drivers for, and hurdles against reducing food-processing waste are many and varied and have changed over time. The nature and extent of their influence on different firms will differ, and depend on the size of the organization and the precise nature of the waste streams. A simplified and subjectively evaluated view of the relative drivers for and against up-grading co-products is shown in the force diagram in Fig. 1. This is not a complete picture, but depicts the main issues, the evolution of which has resulted in increased pressures on industry to find new ways of reducing their waste streams. Those organizations in which the drivers for co-product exploitation have been previously out-weighed by drivers which discouraged it are now under the greatest pressure. This is due to recent EC and

national legislation concerning landfill (Sanders and Crosby, 2004). In particular, small and medium-sized organizations with little scope for R&D and new product development are hardest hit.

Figure 1. Force diagram of drivers for and against co-product exploitation, past and present.



Technical difficulties

Plant-based wastes from fruit and vegetable processing activities are often rich in potentially food-grade and non-food-grade components. Scientists and food technologists continue to expound the potential economic virtues of exploiting these "high value" components in order to find alternative uses for the co-products. However, there are several key areas of technical and scientific difficulty which serve to attenuate the economic rationale for up-grading co-products.

Microbiological instability

Many co-products have a high water content, particularly those derived from trimmings of fruits and vegetables. Some cereal waste co-products may also be quite moist, for example brewers' grain. These residues provide a high quality breeding ground for fungi and bacteria and will rapidly lose any potential as a source of food-grade materials as they start to undergo biodegradation. Such is the case when waste co-products are left for more than a few hours at ambient temperature. Hence there is an immediate requirement for stabilization using, for example, processes such as drying, refrigeration or freezing. This issue will not be considered significantly in

this paper but forms an important part of current research activities (see consideration of REPRO below).

Co-product heterogeneity

The key issue of concern to be considered here relates to the high degree of heterogeneity in food processing co-products. Interestingly, it is this very heterogeneity which often underlies the quality characteristics of the food products made by processes from which the co-products result (Waldron *et al*., 2003; Waldron, 2004). The undefined mixtures of components within the waste streams and their lack of uniformity prevent processors from readily identifying approaches to exploit these waste streams in a sustainable manner. The following section describes and discusses the different types of heterogeneity found in co-products derived from fruits, vegetables and cereals.

Heterogeneity of Fruit, Vegetable and Cereal Co-Products

Biological description of plant materials

The co-products which derive from plant tissues will have originated from intact plant materials and organs which entered the processing plant. These will include, for example, leaves, stems, roots, tubers, grains. These intact organs comprise a range of structures at different length scales (Fig.2). The plant organs occupy the largest length scale. Proceeding down the hierarchy, the organs comprise functionally distinct tissues such as those used for storage, transport and support. The tissues consist of cells, the characteristics of which will reflect their role in the tissue. For example, cells in storage tissues may be isodiametric, thin-walled and full of storage material such as starch and protein.

In contrast, cells involved in stem support may be elongated and lignified. Finally, the cells are surrounded by plant cell walls which provide the key strength and support for the plant organs. Hence, plant organs are composed of a range of structural entities at different length scales, ranging from the cm scale at the top, to the micron scale at the base. However, heterogeneity does not end here. The cell wall itself comprises a number of molecular components which are highly complex. The general structure of the wall is shown in Fig.3. It consists of a framework of cellulose microfibrils which are made predominantly of glucose chains, many of which are closely associated in regions of crystallinity.

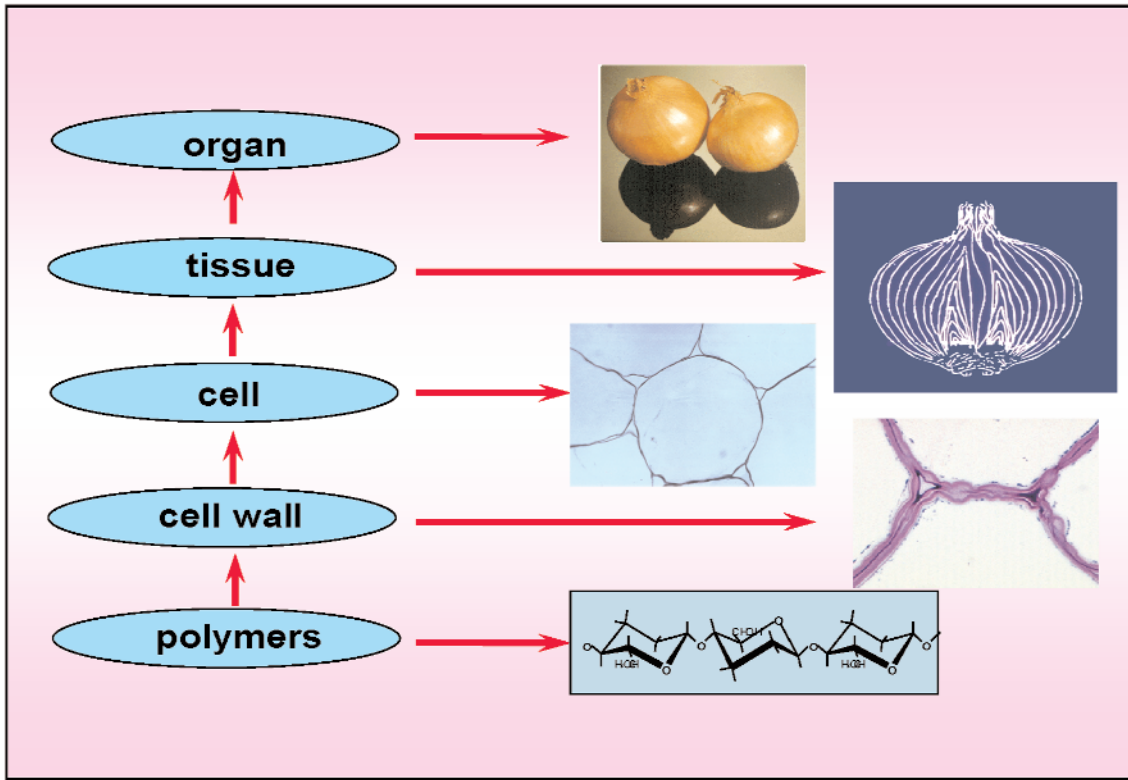


Figure 2. Hierarchy of structures in plant tissues

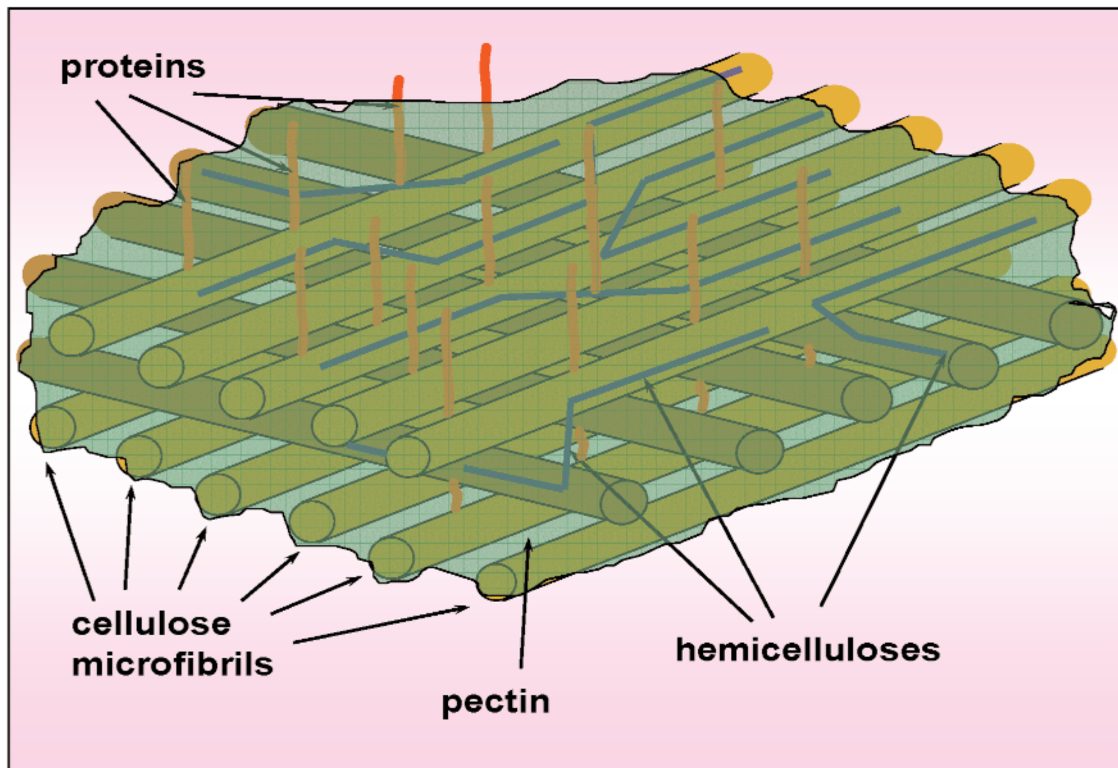


Figure 3. Generalised molecular structure of the plant cell wall.

Attached to these are a number of other complex molecules, including polysaccharides (the pectins and hemicelluloses), proteins and phenolics. The polysaccharides are generally heterogeneous in their structure, and the ratios of the different components tend to differ between different tissues and between different organs. They also differ between different plant types. Fig.4 shows the relative ratios of cell-wall components in walls from different plant tissues. In fruits and vegetables, the cell walls contain considerable quantities of pectic polysaccharides. In contrast, the cell walls of cereal endosperm walls, contain little pectin but a predominance of hemicellulosic polymers. These differences reflect the different functionalities of the tissues in the plant. The nature of the complexity at the different length scales will also change during plant growth and development, post-harvest treatments, and, of course, during the processing itself which can range from simple cutting through to thermal treatments and juice extraction. The residual co-products will be produced at different stages of the process line, and this will also lead to further complexity.

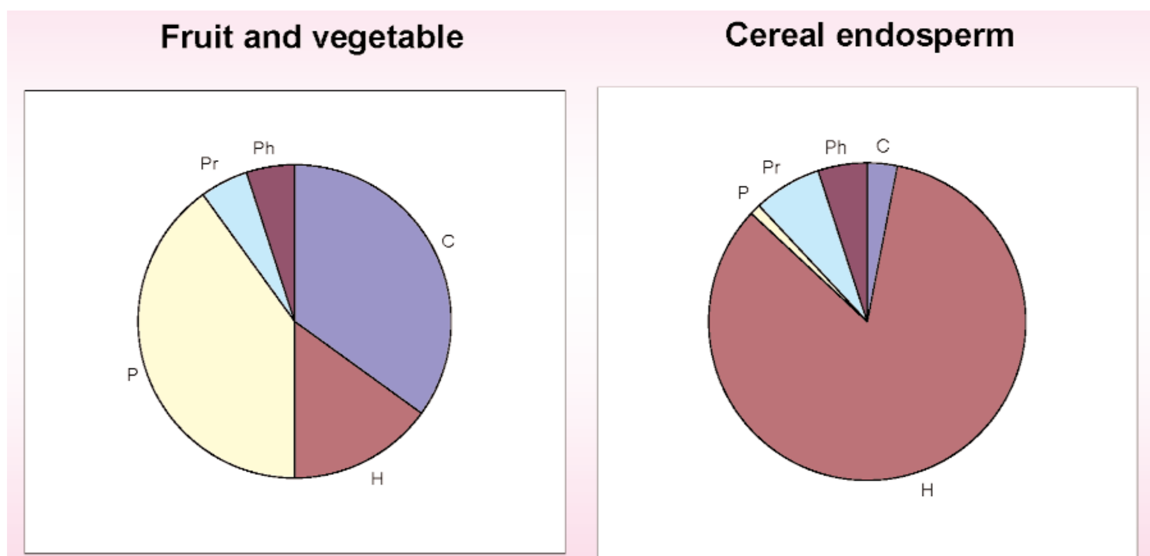


Figure 4. Relative quantities of cell wall components in different tissues

The complexity of the wall polymers is heightened further as a result of the changes they undergo during their lifetime. Whilst cellulose is known to be synthesized in the cell wall, the other components are synthesized within the cell and transported to the wall. There, they are further modified by enzyme activity. This series of events is summarized in Fig.5.

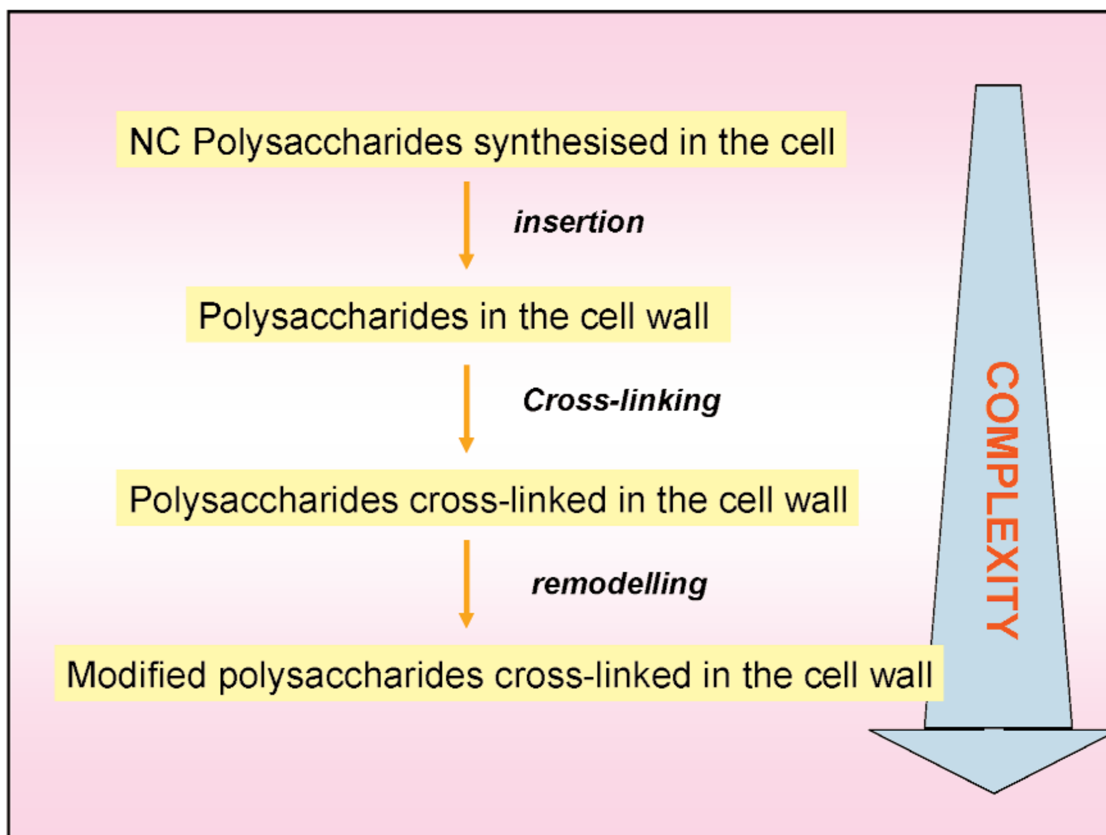
Figure 5. Biosynthesis and modification of cell-wall non-cellulosic (NC) polysaccharides

Potential routes for exploitation

Total residue use

How can these complex co-products be utilized? Historically, it has been found that the easiest routes involve exploitation of the whole residues. The animal feed industry provides an important example of such an approach, and numerous

co-products have provided animal nutrition for decades (Table1.; Crawshaw, 2001; Crawshaw, 2004). Unfortunately the apparent decline in animal husbandry and the



associated reduction in the use of animal feeds is one reason for the increasing problem of excess co-products. The advantage of such an approach is that as long as the co-products are used before they become microbiologically compromised or stabilized e.g. by dehydration, relatively little treatment is necessary. Of course, under certain circumstances some microbial activity may be beneficial in stabilizing the co-products and enhancing nutritional characteristics, for example lactate fermentation which forms the basis of silage production.

Table 1.
Plant-based co-products exploited in the manufacture of animal feed (Crawshaw, 2001)

- Apple pomace
- Bread waste
- Brewers Grain
- Citrus and tropical fruit residues
- Maize
- Potato waste – peel etc.
- Sugarbeet pulp and molasses
- Wheat fractions

Fractionation of residues

A more recent approach to exploiting co-products has involved the use of processing technologies to fractionate potentially high value components from residues, thereby turning what was effectively a waste stream into a product stream.

Extraction of intracellular contents

Most plant tissues contain potentially extractable components, such as residual starch and protein, and phytochemicals. The latter may often provide sources of additives, flavours, and food colourants; (Tomas-Barberan, 2004). This area of exploitation has been underpinned by the increasing evidence of health-promoting benefits of a range of phytochemicals and has led to an increasing number of companies trading in these components. There is no doubt that many fruit and vegetable co-products are rich in these biochemicals. However, their recovery may require significant purification or enrichment procedures which will add to the cost.

Exploitation of the residual cell wall material

Cell wall material provides a range of functional components. They can provide texturising agents either as intact structures, or via extracted rheologically-active hydrocolloids. For example, pectins have long been extracted from plant cell walls. Indeed, the pectin industry has relied on exploiting vast tonnages of residues left after the extraction of juice, particularly from citrus fruits and apples (Stevens, 1995). However, such polymers are generally low-value commodities, and their production may not yield a particularly high return unless novel functionalities can be provided. Cell-wall-derived components can also have nutritional and physiological benefits. Oligosaccharides derived from wall hydrolysis may be potential sources of probiotics. In addition, dietary cell-wall polymers have been associated with immunological functionality (Mueller and Anderer, 1990, Waldron and Selvendran, 1992). However, the nutritional benefit which is most associated with dietary cell walls concerns their role as sources of dietary fibre.

Dietary Fibre

An important area in which many co-products have been successfully exploited concerns the production of dietary fibre supplements and ingredients. The longstanding historical interest in this topic has developed from the observation that consumption of diets low in fibre is related to a large number of gastrointestinal and metabolic diseases in the developed world. Dietary fibre (DF) was defined originally as "that portion of food which is derived from the cellular walls of plant which is digested very poorly by human beings" (Trowell, 1972) i.e. the key structural components of plant-based foods. In the light of subsequent research the definition has been refined to include resistant starch, certain oligosaccarides, and there have even been suggestions that all material which is undigested should be included.

One of the main reasons that the DF ingredient industry has grown is the range of physiological functions that are attributed to DF (Table 2). Although there has been much research devoted to developing methods to quantify DF (total, soluble and insoluble), the values measured (e.g. non-starch polysaccharides) give little indication of

the functionalities other than a broad indication of potentially fermentable carbohydrate. Not all DF sources have all of the above physiological characteristics, and the lack of a clear relationship between quantification and functional properties prevents proper targeting of product development. This is in no small way due to the paucity of knowledge of DF structure-function relationships. There is a now a very great opportunity to exploit modern methods of analysis and characterisation of complex food systems, to evaluate the functionality of DF types in dietary studies. Reliable measures of DF functionality will provide the potential to exploit modern processing methods which can chemically and physically alter carbohydrate-based food materials, and add substantial value to residues and co-products which can provide a reliable source of functional DF. Such approaches require considerable multidisciplinary research activities which have been developed in a number of European centers of excellence over the last 10 years, often underpinned by integrative European Commission research projects.

Table 2

Key physiological functions and beneficial properties associated with some sources of dietary fibre

Possible function	Proposed mechanism(s)
Reducing the risk of atherosclerotic cardiovascular disease	• Lowering of blood cholesterol:
Reducing the risk of Cancer	• DF can act as a substrate for colonic fermentation resulting in the formation of benefical short-chain fatty acids which may help protect against colorectal cancer.
Controlling diabetes: type 2	• Soluble and insoluble DF can help to regulate blook glucose by reducing rate of post-prandial glucose absorption.
Reducing obesity	• fibre increases satiety perhaps by acting as a bulking agent and presenting a significant water-holding capacity (WHC).
Reduces constipation	• fermentable fibre produces increased faecal bacterial biomass • non-fermentable material can increase faecal WHC.

Tools and approaches to extract and modify cell-wall residues

There are numerous ways to modify cell-wall material, and these can be divided into three main categories (Table 3):
Table 3. Tools and approaches to extract and modify cell-wall residues

Tools	Examples
Chemical	Acid, alkali, salt
Physical	Thermal, milling, extrusion
Biochemical	Enzymes – in mixtures, individually, or precise synergy

Chemical and physical extraction processes have been in use for decades. Indeed, the use of hot acid for extracting pectin from citrus pressings and apple pomace was developed over a century ago. There is a large volume of literature on the use of such approaches in industry (Stevens, 1995), and most if not all have been developed empirically with much fine tuning. They generally lack precision, and the extracted products are often commodities. As a result, producing more of these from waste co-products is not particularly attractive. Part of the problem lies in the fact that the extracted polymers are quite heterogeneous and are difficult to tailor using chemical and physical procedures. However, the advent of biochemical approaches to modifying cell-wall polymers using cell-wall degrading enzymes may provide new opportunities. Cell-wall degrading enzymes have been used commercially for about 30 years. Most of the commercial preparations are fungal enzyme extracts which have been developed to augment industrial extraction processes which require general polysaccharide depolymerisation. These include the enhancement of citrus juice extraction, degradation of pectins to enhance juice concentration, and dissolution of flocculating polymers which can block filtration systems. Recent advances in molecular biology, particularly recombinant technology, are facilitating the economic production of industrial quantities of pure and specific enzymes. This provides the potential to develop a truly nanotechnological approach to deconstructing cell walls with some precision, and then tailoring the polymers to provide a range of functionalities. Fig.6 illustrates the way in which a limited number of enzymes can be used to modify complex polymers such as cross-linked arabinoxylans.

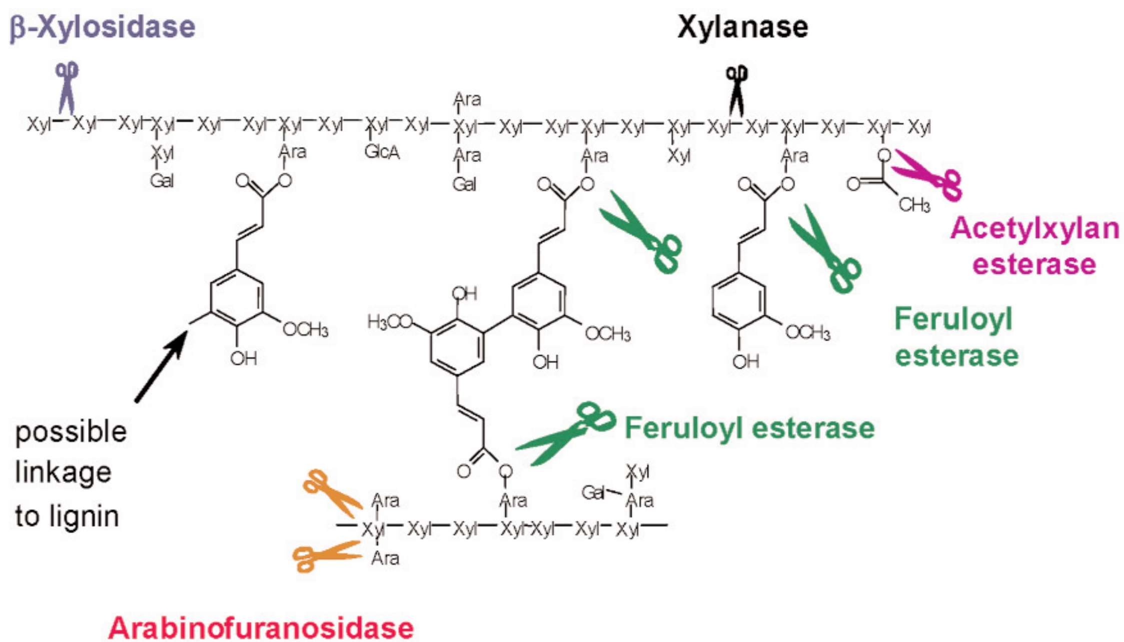


Figure 6. Use of enzymes to precisely modify polysaccharides

Although still in its infancy, the potential to use such methods to modify and functionally enhance residue-derived components is becoming a realistic possibility due to the co-evolution of several other research sectors. In particular, the improvements in chemical characterization using methods such as NMR, mass spectrometry in conjunction with high performance liquid chromatography, are helping in the elucidation of polysaccharide structure, and this has been augmented by novel methods of polymer visualization such as atomic force microscopy. These methods, in conjunction with the greater precision for evaluating physiological impact provide the basis for identifying structure-function relationships at different length scales. For example, the anti-tumor function of some _-glucans and certain pectic polysaccharides (Mueller and Anderer, 1990; Waldron and Selvendran, 1992).

Limiting criteria – barriers preventing waste upgrade

The overriding barrier to waste upgrade is economic. Organanisations producing co-products will, in order to be competitive, take the most economically viable route to dealing with these residues. The viability of a company will depend on a combination of factors including likely future earnings and cash-flow considerations. No organization will adopt a strategy which fails to provide acceptable future earnings over an economically appropriate time scale.

Risk associated with diversification strategies

In spite of the highly positive message from the areas of research discussed above, and the success stories described elsewhere in this proceedings, there are several key reasons why it is often very difficult to develop approaches to exploit wastes co-products:

The first, and simplest set of barriers can be considered in the light of the business management matrix developed by Igor Ansoff (1957): the Ansoff Matrix (Fig.7). This tool seems to be used far more in the US than in Europe. The matrix describes four different strategies for growth which relate to a firm's current and potential markets and products. These are:

- ***Market penetration:*** here, the firm increases its market share in its current market segments. This is a preferred approach since it exploits existing resources and capabilities and does more of what it is experienced at. This is particularly favorable in rapidly growing markets where new entrants are less likely to be a significant threat.

- ***Market Development:*** here the firm attempts to sell its existing products in new markets. This is considerably more difficult than market penetration because of the lack of experience in the new market segments. However, it benefits from the firm's previous experience in producing the products.

- ***Product development:*** this requires the firm to embark on developing new products for its current customers and markets. This is somewhat more risky than the previous strategy since it requires the firm to invest in R&D and often new plant and equipment. However, it benefits from the firm's previous in-depth knowledge of its markets which other competitors may not have, allowing it to target its product development with some precision.

- ***Diversification:*** this requires the firm to instigate both both market and product development. Here, it has to evaluate a new market sector(s) and then develop and produce new product(s) to satisfy that market. It has relatively low support from previous firm activities (depending on how new the products and markets are), and is therefore the most **difficult and risky** of the strategies.

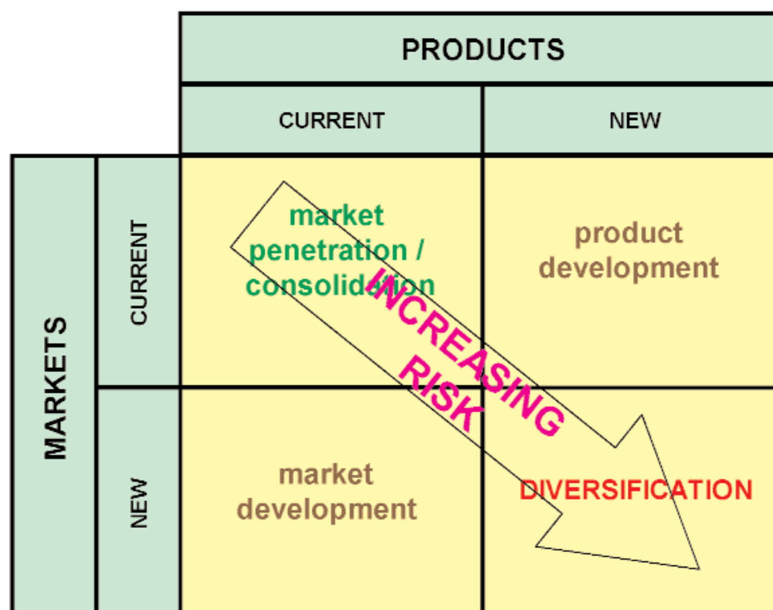


Figure 7. Ansoff Matrix (after Ansoff, 1957)

Upgrading waste materials is likely to require development of ***new*** products, usually for ***new*** markets and is one of the key reasons why ***waste is still a problem***. It also explains why most food processing organizations wish to retain a disposal system in which a third party takes their waste away from them.

Difficulties of exploiting new research and development

If co-product producers could use their co-products to economically create more of their main-stream products, *they would have already done so*. If there were simple transformations which could be used to enable co-products to be up-graded to economically-viable / profitable products, *it would have already happened*. New approaches will require the targeted use of research and development with a focus on whole-co-product utilization. It is therefore imperative that links in the **Knowledge Exploitation Chain** (Fig.8.) are pragmatically joined up. Unfortunately, there are some inherent barriers to effective exploitation of R&D:

(i) The food processing industry serves mature commodity markets which are generally associated with high turnover and low margins. This provides relatively little scope for firms to invest in R&D, and small processors often rely on technologies developed decades ago.

(ii) The timescale of R&D, particularly the "research" component is measured in years. If one considers basic research of the sort performed in universities and national research institutes, the time taken for research to be translated into new processes may be in excess of 5 years (more often, it is 10 or more). However, the economic vision of most private-sector firms and their owners / shareholders is relatively short, in the order of 12-15 months. Only the very largest of companies and multinational corporations are likely to be in a position to look far enough ahead to consider R&D timescale to be congruent with their own strategy. As a result, the prospect of investing in R&D is often poor.

(iii) The actors at different positions in the knowledge exploitation chain often have very different drivers. For example, firms at the right-hand end of the chain which might be in a position to exploit R&D and produce new processes will be influenced by "return on investment" criteria. They are not going to invest in the development of new products if they can not foresee a profitable return on their investment in R&D and plant in a reasonable (i.e. short) time. In contrast, at the left-hand end of the chain, where universities and institutes provide new knowledge which might provide the basis for new processes, they are often dominated by the evaluation of their research activities – usually in relation to high quality publications in research journals. In the UK, such evaluation falls under the auspices of the Research Assessment Exercise. They are unlikely to be driven by the requirements of actors further down the chain (Waldron, 2004). These different forms of evaluation contribute to widening the gaps within the knowledge exploitation chain and reduce the potential for successful R&D utilisation.

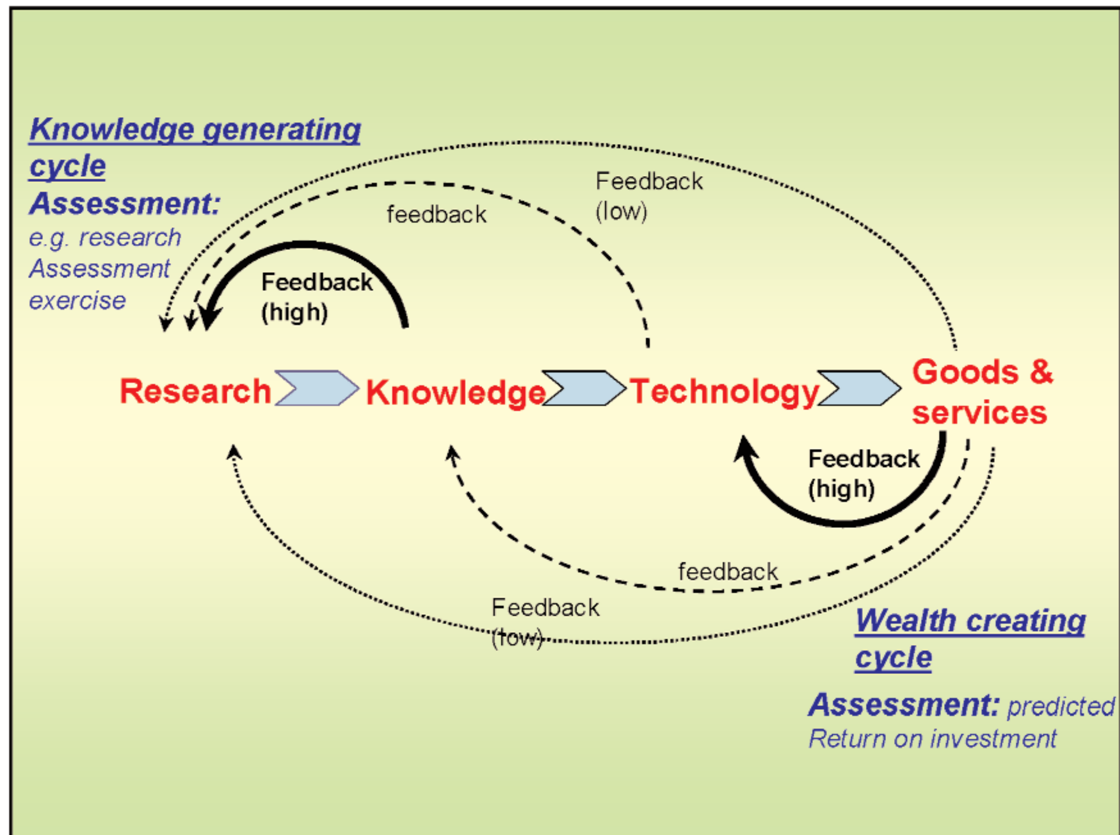


Figure 8. Knowledge Exploitation Chain

The way forward

Whether it is the food processors themselves or third parties which provide new processes to exploit co-products, it is clear that reaching a solution to waste will require the production of *new products* which have sufficient value in the global market which can *offset the current and future cost* of waste disposal. The approach(es) will have to consider the following options:

1) Stabilisation: In most cases, waste co-products require stabilization against microbiological degradation and loss of potential value.

2) Economies of scale: Processors need to produce sufficient quantities such that economies of scale provide a reasonable basis for exploitation. In this respect, agreements between companies which produce similar waste streams could be extremely beneficial.

3) Link up the knowledge exploitation chain: Effective exploitation and upgrading of co-products requires a high level of focused knowledge exploitation. Organisations seeking to upgrade waste co-products will need to integrate the knowledge-creation chain so as to focus research and development at all levels on aspects relevant to likely routes to economic exploitation. This will require closer interactions between

industries and public research organizations.
The benefits of integrating the chain are numerous:

- R&D activities towards the left-hand end of the chain will gain useful guidance as to where research is desperately needed. Due to the current focus on publication metrics for evaluating "basic research output" (Waldron, 1994) the exploitation issues are often poorly evaluated. An increased emphasis on solving problems of sustainability may help to create a research culture which takes a more problem-solving attitude.
- The huge base of knowledge which already exists will be more effectively exploited

4) Extract maximum return through whole-waste utilization: valorization of waste co-products will require exploitation of as many components that can be extracted across all levels of value, from potential high-value nutriceuticals through to relatively low-value compost. Research at all levels will help to increase the value of all outputs thereby increasing the exploitation of the co-product

These four approaches form the basis of an EC-STREP entitled REPRO, which is currently undergoing negotiations and which should start towards the end of 2004.

Acknowledgements

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Cheese Whey Utilisation

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Introduction

Milk is the only food designed for mammals by nature through evolution. Mammals have adapted to consume all other foods. Milk provides nutrition in the form of energy from the carbohydrate present in the form of lactose, nitrogen from the protein content and a rich source of calcium to build bones to name but a few. Milk also provides other important benefits. For example, there are many biologically activities associated with certain components in milk. Almost without exception, these biologically active components are exclusively to be found in the whey or serum fraction of milk. With this in mind, it is perhaps surprising that for many years, whey produced as a by product of cheese production was considered a waste material and was either dumped, sprayed on fields as fertiliser or at best, dried as cheese whey powder destined for the animal feed applications. Increasingly, over the last few decades, dairy companies have applied different technologies to process cheese whey resulting in its separation into its principle components, comprising fractions enriched in proteins, lactose and minerals. These technologies have been generally based around crystallisation, membrane and chromatographic processes. In the last decade, cheese whey processors have somewhat been a victim of their own success. The increasing volumes of edible lactose and whey protein concentrates flooding into the market have resulted in a downward trend in relative prices. Converting these semi-commodity products into added value products is the challenge facing the industry presently. This article will give a brief overview of traditional methods for valorisation of whey and then turn towards opportunities for real value creation that are embedded in the nutritional, functional (meaning structure-building) and physiological properties that are unique to whey components.

Whey production and processing

Whey is mainly generated during cheese production. This stream not surprisingly is referred to a cheese whey or sweet whey. The other source of whey is acid or casein whey produced during the industrial production of casein and caseinates from skimmed milk. There are some small but significant differences between these two sources but they will not be addressed in this article.

Cheese production results in the production of large quantities of cheese whey. Approximately 10 litres of milk are required to produce one kilogram of cheese. The relative distribution of the different milk components between a typical cheese (e.g. Gouda) and gouda whey can be seen in Table 1. As can be seen whey contains a significant part of milk solids including 20% of the total protein, >95% of the lactose and 50% of the calcium present in the original milk.

The total world production of liquid cheese whey is in the region of 145 million tons (Affertsholt and Nielsen, 2003). Of this 85 million tons can be considered to be industrially utilised and processed into higher added value products (Figure 1). The remaining 60 million tons or so is used directly in liquid animal feeds, sprayed on fields as fertiliser or just dumped. Looking in more detail at the industrially processed fraction, about 50 million tons is processed either into edible lactose or dried directly as cheese whey powder, approximately 30 million tons is processed into protein enriched concentrates, referred to as whey protein concentrates for products containing up to 80% protein and whey protein isolates for products containing > 90% protein. A further 6 million tons are processed into so-called demineralised blends. Care has to be taken when considering these figures (de Wit, 1998). They are based on liquid whey ' equivalents' which comprises about 6% dry solids. Therefore in Figure 1, in simple terms this means that the 30 million tons of WPC/WPI equates to about 3 million tons of dry whey solids. Looking at the global distribution of whey utilisation, it is perhaps surprising to learn of the approximately 60% of whey that is industrially utilised, this is built up from 80-90% utilisation in the US and Australasia and only 60% utilisation in Europe. Industrial processing of whey originating from the rest of the world is still at comparatively low levels. These figures are all the more surprising when the degree of valorisation is considered. In this area Europe may be considered in the vanguard with comparatively sophisticated approaches to extraction of the value-added components from cheese whey as will be described later in this article.

As mentioned above the categorisation of cheese whey as a waste product until recently is surprising given the interesting components that are to be found in it. The protein fraction of whey contains widely studied proteins such as beta-lactoglobulin, which is implicated in vitamin A transport, alpha-lactalbumin, involved in lactose synthesis, serum albumin, involved in fatty acid transportation, several molecules involved in passive immunity including immunoglobulins, lactoperoxidase and lactoferrin (Horton, 1995). The latter has also been implicated in a range of other functions including iron transportation, antioxidant and antiviral activities. The carbohydrate content of whey is almost exclusively comprised of lactose, a disaccharide of glucose and galactose.

In the broadest terms there ore two routes widely used to process cheese whey and extract added value from this raw material; an approach based on ultrafiltration or one based on demineralisation. The main steps in the ultrafiltration process and an indication of which components can be recovered at different stages is presented in Figure 2.

Ultrafiltration has gathered increasing popularity in recent years, to such an extent that the many of the major products recovered have become semi-commoditised. A costly chromatographic step is generally required to extract the bioactive components from the stream. This is of course optional and most whey processing plants do not implement such a process. This step can be positioned at the beginning or the end of the process. However, given that the value of the target components (e.g. lactoferrin, lactoperoxidase and immunoglobulins) is intimately linked to their biological activity, it is often wise to position their removal as far up stream as is possible. This ensures that they receive the lowest heat load possible, thereby retaining their native structure and preserving as much biological activity as is feasible. In such cases, it is critical that the chromatographic step does not result in pollution of the whey stream in any manner, for example through addition of salt or solvent since it is critical that feed stream can be further processed down stream. This largely limits the chromatographic techniques to ion-exchange-based technologies. Ultrafiltration systems are now essentially available off the shelf for this type of application. This technology is membrane based requiring the whey stream is passed under pressure over a membrane, which is permeable to low molecular weight components collectively referred to as the permeate but impermeable to high molecular weight components such as intact proteins (retentate). Typical components found in the permeate include lactose, soluble salts, peptones (peptide fragments of proteins)). The extent of the removal of the permeable components from the whey is dependent upon the scale of the UF system but is always limited if additional water is not added to further 'flush' the low molecular weight components from the proteins. This latter diafiltration step can boost the protein levels from 30-35% to any selected level up to 80%. In practice, there are specific standards of whey protein contents recognised and utilised in the food industry. These ingredients are referred to as whey protein concentrates (WPC) and they will be discussed later in this article. If higher levels of protein are required alternative membrane or chromatographic processes are required and can result in products comprised of 90% protein. These latter are classified as whey protein isolates (WPI) rather than WPCs.

The permeate from UF can be concentrated and dried as is or lactose and calcium can be crystallised from the concentrate. Nevertheless in terms of waste streams, the permeate poses the biggest problem. Removal of lactose results in delactosed permeate. This rest stream is difficult to dry due to its high mineral content. Often it is disposed of as liquid feed or combined with other carriers and dried.

The alternative process of de-mineralisation can address this issue to a certain extent. The process layout is similar in many respects to the UF process with an optional chromatography step early in the process but then followed by concentration and demineralisation. The latter process can be executed using various technologies but the most widely accepted in electo-membrane based and results in a de-mineralised, de-lactosed protein fraction and a waste stream of salt. The difference between this approach and UF is that essentially no lactose ends up in the waste stream. This means that there can be easier disposal to waste in many circumstances.

Applications of whey ingredients

Whey-derived ingredients can be found in many food and pharmaceutical applications (de Wit, 2001). An overview of these can be seen in Table 2. Permeate and WPCs are used in bakery products. WPCs are frequently used in processed meat, fish and poultry products as water binders and gelling agents. WPC35 is frequently used as a skimmed milk powder replacer in a wide range of dairy products, especially yoghurt and ice cream. Clinical and infant formulae are higher added value applications for whey proteins and lactose and hydrolysed whey proteins are used in production of hypoallergenic infant formula for babies allergic to cows milk. A significant amount of product development time is being spent on development of dietary supplements and function foods where whey proteins and hydrolysates thereof provide a rich source of bioactive ingredients. In addition, whey components have been shown to act as prebiotics for growth of probiotics and healthy intestinal flora. Finally, the application of lactose as a key ingredient in the pharmaceutical industry as a filler binder for tablets or a carrier for delivery of drugs via dry powder inhalation is frequently overlooked.

Valorisation of lactose from cheese whey

The relative added value of processing and refinement of lactose is presented in Figure 3. At the base of the pyramid is permeate. The value of permeate is related to the energy content in the form of lactose. Recovery of edible lactose via crystallisation from permeate provides a significant step up in value of about 3-5 times. Further value is added by upgrading from edible grade lactose to pharmaceutical grade for application as a pharmaceutical excipient in tablet and capsule manufacture. This step has more relation to meeting clinical GMP quality standards than changes in production processes per se. The cGMP standard is increasing required by the pharmaceutical industry from the excipient supplier as a license to do business.

There are different processes used in the pharmaceutical industry in the production of pharmaceutical tablets. The traditional process involves wet granulation, where the tablet ingredients are weighed and mixed in a blender, small quantities of water are added to moisten and agglomerate the mix. The mix is wet screened to remove large particles, dried and dry screened. After mixing in of lubricant to facilitate ejection of the tablet from the press, the mix can be compressed into tablets using machines with very high throughput. The lactose used in wet granulation is generally alpha lactose monohydrate, with its typical tomahawk-shaped crystals which are milled and/or sieved to provide reasonably homogeneous, improved flowability lactose preparations.

The alternative process is termed direct compression (DC). Here, the agglomeration process is undertaken by the lactose manufacturer. This means that the pharmaceutical company only dry blends the various ingredients before feeding to the tabletting presses. Here the key is the production of compressible lactose powders

that can comprise different crystal forms. Spray drying, roller drying or agglomeration and drying can be used to produce lactose for direct compression.

The highest value added pharmaceutical lactose preparations are those for application in dry powder inhalation (DPI). These generally comprise subfractions of preparations that are tailored to optimise delivery of drug from the dry powder inhaler device. DPI is a fast growing market (>10% CAGR) currently focused mainly in the chronic obstructive pulmonary disease sector, which includes diseases such as asthma. This means of drug deliver has several advantages over aerosol-based devices, so called metered dose inhalers (MDIs)). Whilst the latter have largely been reformulated away from ozone damaging CFCs towards hydrofluorocarbon alternatives, there are still issues relating to poor patient compliance (cold jet of expanding aerosol propellant stimulates the user to breathe out rather than in when it hits the back of the throat). Also, DPIs are more applicable to a wide range of drug classes. Proteins such as insulin and monoclonal antibodies may in future be delivered in this manner. Here inhalation obviates the requirement to inject protein-based drugs, which would be digested if taken orally.

The functioning of the lactose carrier in a dry powder inhaler is rather complex and fulfils several roles. Firstly, the lactose carrier is needed to ensure that the dose of active pharmaceutical ingredient (often in the microgram or milligram level) is achieved during packaging of the dosage in the capsule or blister pack at the pharmaceutical factory. Secondly, a weak adhesive mixture of the carrier and drug should be present to facilitate transport of the drug into the device when the device is primed for use. Finally, the adhesive mixture should be sufficiently weak to allow release of the active from the carrier into the airway to allow efficient delivery of active to the target area deep in the lung. A wide range of physical and chemical factors need to be addressed during the tailoring of a lactose carrier to a specific device:drug combination.

Applications of milk calcium

Calcium enrichment is a growth market, particularly in the Far East where natural sources of dietary calcium are less available. In Western countries, the market drive comes from the increasing occurrence of osteoporosis (brittle bone) disease. Many products can now be found on the market that are enriched in calcium in the form of carbonate, lactate, phosphate and citrate salts. Choice of calcium source is influenced by bioavailability, sensoric properties, such as taste and mouthfeel) calcium content and natural image. Milk calcium (calcium phosphate) can score on several of these criteria and can be crystallized or precipitated from cheese whey. Many animal and human studies have shown the good bioavailability of milk calcium (Bonjour, 1997). In addition, milk calcium delivers calcium and phosphate in proportion to that found in bone.

Applications of whey proteins

A value pyramid for the valorization of protein-containing ingredients from cheese whey is shown in Figure 4. The lowest value dried whey product is cheese whey powder (Affertsholt and Nielsen, 2003). Here the value is based on the protein and lactose content of the powder. The value of the cheese whey product can be increased approximately 10-fold by production of whey protein concentrate containing 30-35% protein (WPC35) using ultrafiltration. The value can be increased approximately a further 10 times by diafiltration to create a high end WPC80 or by raising the protein content still further to approximately 90% by a WPI process. The compositions of standard WPCs is shown in Table 3 and is compared to that of skimmed milk powder. The similarity between WPC35 and skimmed milk powder is obvious and is the basis of the easy exchangeability of these ingredients. The only compositional difference lies in the nitrogen levels present as real proteins as opposed to non-protein nitrogen (peptones and urea). However, the major part of the protein present in SMP is casein, a polymorphic protein with rather unique properties. In contrast, whey proteins are globular and are rather susceptible to exchange of pairings of sulphydryl groups in di-sulphide bonds which can lead to gelation of the proteins. This can influence the levels of exchange of these components that can be achieved in certain products otherwise detrimental changes in properties can be experienced. An example of this behaviour is seen in ice cream. It is standard practice to exchange up to 50% of the milk solids non fat (MSNF) in typical ice cream recipes with whey. However, exceeding this exchange level without undertaking other adjustments to the recipe can result in ice cream with a cold watery mouthfeel. This is now thought to relate to the incomplete displacement of the milk protein from the emulsion droplet surface by emulsifier during the ripening phase of the ice cream process. It is likely that the stronger surface films generated by whey proteins crosslinked by intermolecular disulphide bonds are involved in this effect and are certainly more difficult to displace by emulsifier that casein films formed in the SMP containing recipes. High protein content WPCs such as WPC80 account for a comparatively small part of the WPC market and usually are utilised in applications requiring additional functionality such as gelling.

Further value can be realised from the whey if key biologically active ingredients such as lactoferrin, lactperoxidase or immunoglobulins are extracted from the stream. These ingredients can command high prices in the market but are only present at the ppm level. As a result 99.9% of the solids of whey remain as by-product of their extraction. This means that separation processes for these components are only viable by producers that have large volumes of whey available for processing. Nevertheless, there are an increasing number of manufacturers supplying lactoferrin to the market and one can anticipate a time when availability will outstrip demand resulting in commoditisation of these ingredients. Here lies the largest threat to this business. This issue will be addressed in the following section.

Lactoferrin has been implicated in a wide range of functions and activities including iron transport and absorption, antimicrobial activity, immune modulation and

anti-oxidant activity. The antioxidant activity of lactoferrin has been demonstrated recently in a study of shelf life stability of spray-dried emulsions. The latter contain unsaturated fatty acids which are vulnerable to oxidation. This causes the development of an un-wanted rancid taste and a negative nutritional impact. Commercial vitamin mixtures mainly consisting of Vitamin E and A are well known anti-oxidants used to decrease the oxidation rate. These vitamins however can also act as a pro-oxidant if added in too large quantities. Lactoferrin exhibits anti-oxidant activity via its iron binding capability.

The role of iron in oxidation of food products is explained in Fenton chemistry, which describes the relation between trace amounts of iron and peroxide and oxidation. The so-called Haber-Weiss reaction is cited as the oxidative mechanism in which iron acts as a catalyst, resulting in the formation of an extremely reactive hydroxyl radical. The amount of iron to initiate a significant acceleration of the oxidation can be as low as 0,1 ppm. These amounts are found in commercial oils and can easily be reached in total nutritional formulae containing unsaturated fatty acids by other components (emulsifiers, proteins, etc). The addition of Lactoferrin to bind the iron can prevent the acceleration of the oxidation that this iron may catalyse.

This was investigated in a dietary fat powder containing 55% soya oil. The product has a linoleic acid content of 29%, resulting in a powder that is extremely susceptible to oxidation. Other ingredients present in the spray-dried fat concentrate are milk protein, malto dextrin and an emulsifier. To decrease the oxidation rate, a commercial antioxidant mixture comprising 150 ppm Ascorbylpalmitate and 30 ppm dl-α-Tocopherol (vitamin E) was used. In our test this anti-oxidant mixture was replaced with a cost equivalent dose of 100 ppm Lactoferrin. No other changes were made to either the process or recipe.

Oxidation of the fat was determined by the increase in the peroxide value. The threshold for noticing the rancid taste is in general 40 to 70 meq oxygen/kg fat. The resulting increase in peroxide value is shown in Figure 5 for a product without anti-oxidant, one with the commercial anti-oxidant and one with only lactoferrin.

As can be seen in Figure 5, both the commercial anti-oxidant mixture and Lactoferrin are effective in preventing generation of the rancid off-taste and decreasing oxidation rate. After 1 year the PO values of the Lactoferrin containing product were 28 and for the commercial anti-oxidant containing product 32 meq oxygen/kg fat. This demonstrates that lactoferrin is at least as effective at preventing rancid off-taste and decreasing oxidation rate as the commercial anti-oxidant mixture at a dosage that is comparable cost wise.

Prevention of commoditization of specialty ingredients

Lactoferrin is finding increased use in many applications. In the Far East many companies have developed infant formula products containing levels of lactoferrin equivalent to that found in mother’s milk. In addition, products such a yoghurts and

dietary supplements can be found that claim to boost the immune system. However, even a specialty ingredient such a lactoferrin can become semi-commoditized as levels of supply increase. This is increasingly a problem in the development of ingredients which provide a health benefit. Expensive clinical studies must be executed to support a specific application which needs to include a health claim. Companies need to be able to achieve a return on such research investments just as pharmaceutical companies. This can be achieved in much the same way as the latter by patenting technologies that allow differentiation of the specific product (e.g. lactoferrin) produced by one company compared to that produced by others. This can be illustrated with a recent case involving lactoferrin.

Food poisoning is a major problem with some 76 million cases reported in the US in 1999 (Mead *et al*., 1999). In total 320,000 resulted in hospitalisation and there were 5,200 that proved fatal for the victims. Not surprising, consumers are very concerned about food safety and are actively seeking safer products such as salmonella free chicken etc. It has now been shown that lactoferrin can be prepared in a so-called activated Lactoferrin form by combination with a number of other ingredients which comprise a patented formulation (Naidu *et al*., 2003). Tests have shown that this formulation not only results in inhibition of microbial growth, that has been well documented in the past, but also acts to detach bacteria from surfaces, such as meat tissue and blocks the exposed meat surface thus preventing re-attachment of fresh bacterial organisms. The effectiveness of activated lactoferrin in detachment of bacteria from the surface of meat has been demonstrated in a pilot meat processing system. In this pilot, beef steaks were subjected to normal treatments utilised in US slaughter houses, including water, steam and lactic acid washes. Scanning electron micrographs have shown distinct differences between the surface of a steak treated in the normal manner compared with steak exposed to exactly the same process but with the addition of a spray treatment with activated lactoferrin. The latter shows a ‘clean’ surface where the muscle filaments are clearly visible on the exposed meat surface. In contrast, in the absence of the additional activated lactoferrin spray step, there is a considerable amount of debris left on the meat surface. This FDA approved treatment is now in use in meat processing facilities in the US.

Conclusions

The valorisation of cheese whey is comparatively well developed. Protein concentrates and milled and sieved lactose products are now semi-commodity products. New technologies such as chromatography make it economically feasible to extract still more valuable components present at sub parts per million concentrations, whilst for example preserving the biological activity of the desired component. Nevertheless, the extraction of these ingredients and the proof of principle required to attract the high value in the market means that the companies working on them must protect their developments through for example patents to allow sufficient exploitation to allow the inventors to recoup their development costs.

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Table 1 The composition of milk, a typical cheese and cheese whey.

Component/ltr	Milkper litre	Gouda Cheese per 100g	Whey per 0.9 litre
Fat (g)	37	29	8
Protein (g)	34	26	8
Carbohydrate (g)	46	2	44
Ash (g)	7	2	5
of which Ca (g)	1.3	0.7	0.6
Water (g)	876	41	835
	1000	**100**	**900**

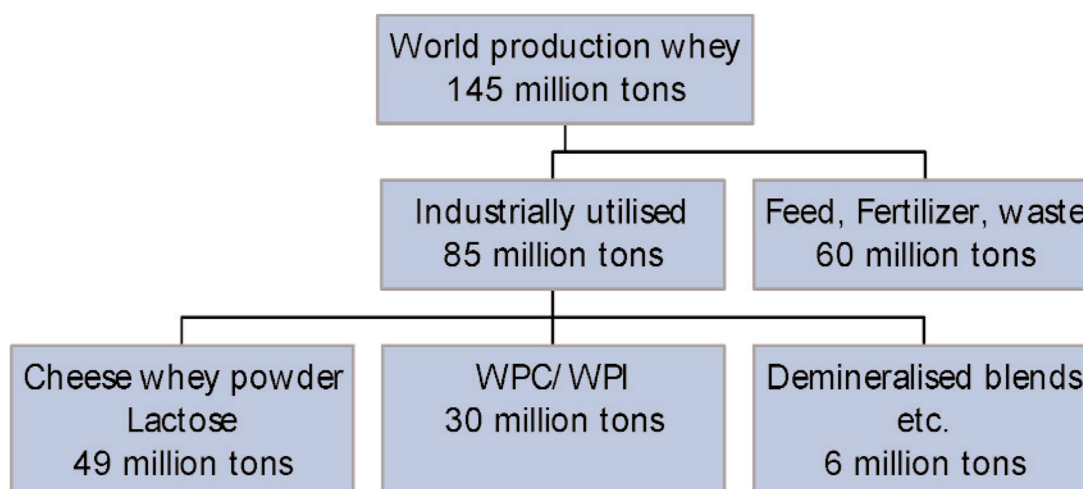
Table 2 Overview of applications of ingredients derived from cheese whey.

Baked products Bread Biscuits Cakes	**Dietetic Foods** Elderly Foods Clinical Foods Slimming Foods
Confectionary Chocolates Candies Aerated candies	**Pharmaceuticals** Wet granulation Direct compression Inhalers
Meat/Fish Products Hams Surimi Comminuted	**Nutraceuticals** Bioactive proteins Bioactive peptides Prebiotics
Infant Formula Pre-term Term Follow-on	**Dairy Products** Yoghurt Ice cream Drinks

Table 3The composition of skimmed milk powder and various whey protein concentrates (WPC)

Component (%)	WPC80	WPC60	WPC35	SMP
Total Protein	80	60	35	37
True Protein	76	57	30	35
NPN	4	3	5	2
Lactose	5	26	50	50
Minerals (Ash)	3	4	8	8
Fat	7	5	2	1
Moisture	5	5	5	4

Figure 1. The global utilization of cheese whey.



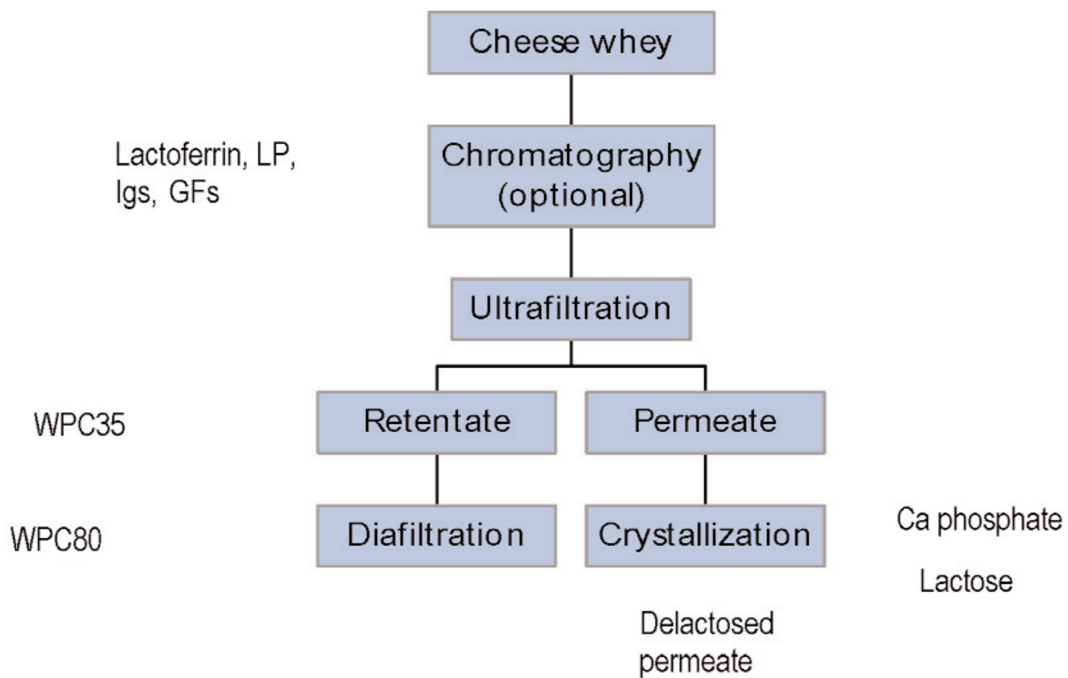


Figure 2. Schematic representation of processing of cheese whey using an ultrafiltration process.

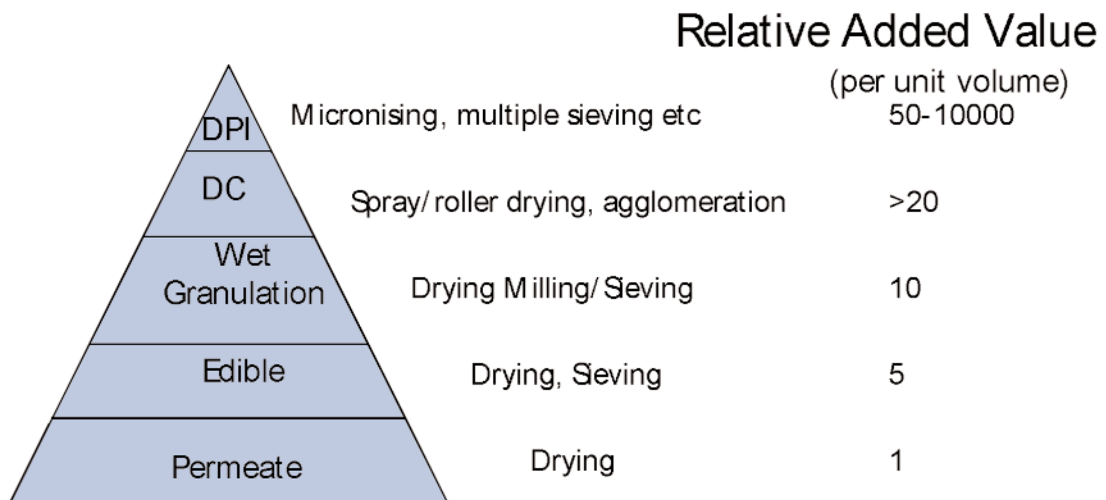


Figure 3. The relative added value pyramid for lactose ingredients derived from cheese whey.

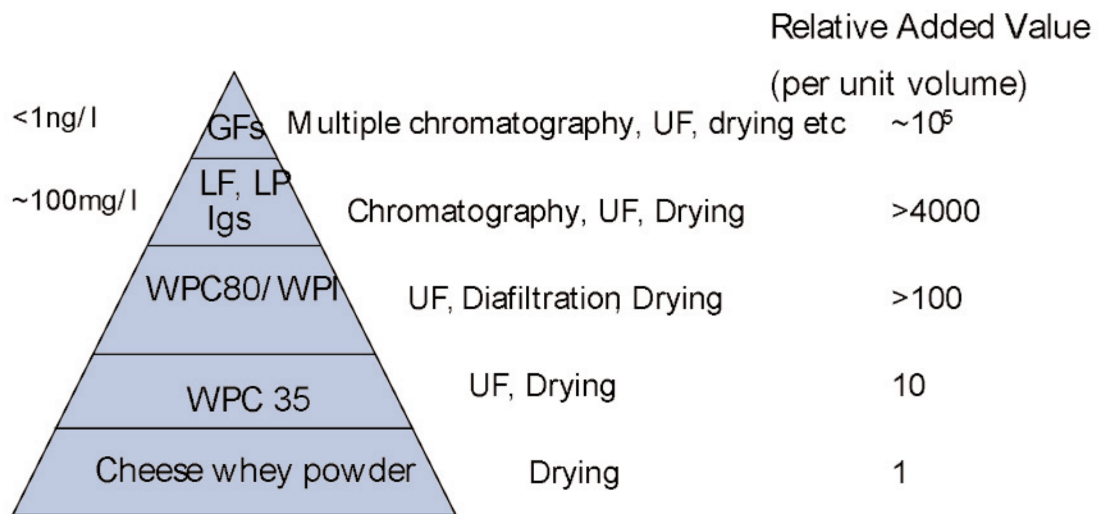


Figure 4. The relative added value pyramid for protein ingredients derived from cheese whey.

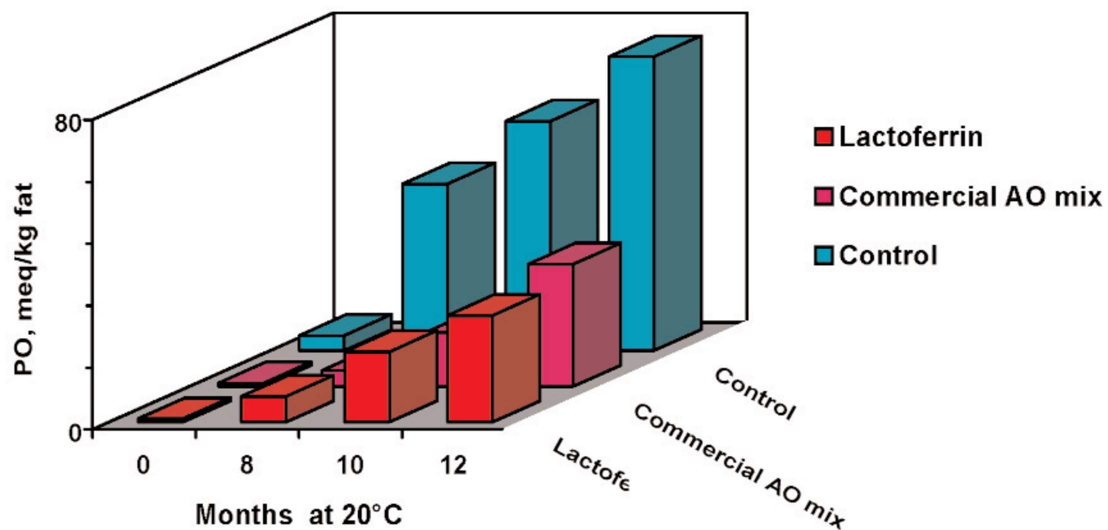


Figure 5. The results of a shelflife test measuring the rate of oxidation of a dietary fat powder in the absence of antioxidant, in the presence of a vitamin-based commercial antioxidant mixture (AO mix) and in the presence of 100ppm lactoferrin.

Recovery of pectin and polyphenolics from apple pomace and mango peels

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Introduction

Fruit juices and derived products such as nectars and drinks have experienced growing popularity within the last years. While grapes and apples are the most important fruits in the temperate zone, oranges, pineapples, bananas, watermelons, and mangos are the predominant fruits of tropical and subtropical areas. Per capita comsumption of juices is highest in Germany, amounting to more than 40 L in 2002. Fruits from moderate climates are usually characterised by a large edible portion and only small amounts of waste material such as peels and seeds, whereas considerably higher ratios of by-products arise from tropical and subtropical fruit processing. Disposal of waste poses a growing problem since the plant material is usually prone to microbial spoilage which limits further exploitation. Since on the other hand costs of drying, storage and shipment are economically limiting factors, efficient and inexpensive utilisation is becoming increasingly important. Apart from merely being a disposal problem, by-products of plant food processing have attracted intense interest since they have been shown to be a rich source of valuable compounds, in particular dietary fiber and organic micronutrients such as polyphenolics and carotenoids (Larrauri, 1999; Moure *et al*., 2001; Schieber *et al*., 2001b; Shrikhande, 2000). We here report the results of our studies on the recovery and characterisation of pectin and phenolic compounds from apple pomace and mango peels.

Materials and methods

Materials

Sources of apple pomace and mango peels

Acidic apple pomace extracts (pH 2.8; 3.1 °Bx) were provided by Herbstreith & Fox KG, Pektinfabrik Neuenbürg, Germany. The extracts were immediately frozen at -20 °C until use. Peruvian and Brazilian mango fruits (Mangifera indica L. cv. ´Tommy Atkins´) were obtained from the local market.

Methods

Recovery and characterisation of polyphenolics and pectin from apple pomace extracts

The apple pomace extract was applied to a column filled with an adsorber resin Amberlite XAD 16HP (Rohm & Haas, Frankfurt a.M., Germany) which retained the polyphenolics, while pectin and concomitant hydrophilic constituents (sugars, organic acids, minerals etc.) passed the resin. Phenolic compounds were recovered by elution with methanol. The organic solvent was evaporated in vacuo, and residues of water were removed by lyophilisation. Pectin was precipitated by addition of ethanol, separated by centrifugation, and freeze-dried (Schieber *et al*., 2003b). Colour parameters (L*a*b*) of pomace extracts were recorded before and after adsorptive removal of phenolic compounds using a Lambda 20 photometer equipped with an integration sphere RSA-PE-20 and WinCol software (Perkin Elmer, Dreieich, Germany). Polyphenolics were characterised by LC-DAD-MS according to a previously published method (Schieber *et al*., 2001a).

Extraction and characterisation of polyphenolics from mango peels

Mango peels were removed from the flesh with a stainless steel knife, immediately lyophilised, and finely ground. Phenolic compounds were extracted with aqueous acetone (80 %, v/v). Hydrolysable tannins and benzophenone derivatives were characterised in the crude extracts, while for the identification of flavonol and xanthone glycosides further purification by solid phase extraction with polyamide CC6 was required. The separation of polyphenolics was carried out using a 4 µm C18 Hydro-Synergi column (150 x 3.0 mm i.d.) from Phenomenex (Torrance, CA, USA). The mobile phase consisted of 2 % acetic acid in water (eluent A) and of 0.5 % acetic acid in water and acetonitrile (50:50, v/v; eluent B). Negative ion mass spectra were recorded in the range m/z 50-1000 for flavonol and xanthone glycosides and m/z 50-2000 for hydrolysable tannins and benzophenone derivatives, respectively (Schieber *et al*., 2003a; Berardini *et al*., 2004).

Extraction and characterisation of pectin from mango peels

Peels from 14 mango cultivars (´Tommy Atkins´, ´Kent´ [Brazil]; ´Ngowe´ [Kenia]; ´R2E2´ [Australia]; ´José´ [Réunion]; ´Minimango´ [Columbia]; ´Haden´, ´Heidi´ [Peru]; ´Manila´, ´Kaew´, ´Mon Duen Gao´, ´Maha Chanock´, ´Nam Dokmai´, ´Chock Anan´ [Thailand]) were screened for their pectin contents and quality. For this purpose, amounts of 10 g of ground peels were extracted with 25 % sulfuric acid at 90 °C for 2.5 hours. After centrifugation, the solubilised pectin was precipitated by addition of ethanol, separated by filtration, and finally lyophilised. Galacturonic acid contents and degree of esterification were determined titrimetrically.

Results

Colour characteristics of apple pomace extracts

In comparison with citrus pectins, apple pectins are characterised by superior gelling properties. However, the slightly brown hue of apple pectins caused by enzymatic browning may lead to limitations with respect to their use in very light-coloured products. From Table 1 it becomes evident that adsorptive removal of phenolic compounds was accompanied by a considerable increase in the lightness (L*) of apple pomace extracts, as exemplified for three samples. Since gelling properties were not adversely affected, extended fields of application may be developed for these refined apple pectins.

Table 1. L*a*b* values of pomace extracts before (-) and after (+) adsorptive removal of phenolic compounds

Sample	Adsorption	L*	a*	b*
1	_	61.4	5.7	27.9
	+	65.0	2.7	16.0
2	_	55.5	6.6	30.4
	+	65.7	2.6	16.9
3	_	69.6	5.6	32.0
	+	79.8	1.7	14.6

Composition of the phenolic fraction of the lyophilisate

Quantification of polyphenolics obtained after desorption from the resin and lyophilisation revealed that phloridzin, chlorogenic acid and quercetin 3-galactoside were the predominant compounds. Epicatechin, procyanidin B2 and phloretin xyloglucoside were also present in appreciable amounts, whereas other phenolics were minor constituents. The presence of the aglycones quercetin and phloretin results from the acidic conditions necessary for pectin extraction. In total, approximately 12 % of the lyophilisate could be assigned to polyphenolics (Schieber *et al*., 2003b). It is particularly noteworthy that the genuine profile of phenolic compounds usually found in apples is not significantly changed by the process. Since industrial scale-up has already been initiated, polyphenolics can be recovered in large amounts and may be used as natural antioxidants. Very recent investigations have also shown that apple polyphenol extracts used in Japan as a food additive and nutritional supplement can be considered safe (Shoji *et al*., 2004).

Pectin from mango peels

Due to the increasing tendency to maximise apple juice yields by the use of pectinolytic and cellulolytic enzymes, apple pomace resulting from mash liquefaction cannot be exploited for the recovery of pectin because the polysaccharides are partly depolymerised. On a long-term basis, enzymatic treatment might even lead to scarcity of apple pomace as a raw material. Therefore, alternative sources of pectins are urgently needed. Since mango peels are available in large amounts and have been demonstrated to contain high-quality pectin (Sudhakar and Maini, 2000) as well as dietary fiber (Larrauri *et al*., 1996), they are promising sources of valuable compounds and have been included in our studies on the recovery of functional food ingredients. The results of a cultivar screening of mango peel pectin are shown in

Table 2.

Table 2. Contents and quality characteristics of pectins extracted from 14 mango cultivars

Cultivar	Pectin (% dm)	Galacturonic acid (% dm)	Degree of esterification (%)
Tommy Atkins	19.2	86.0	65.6
Manila	19.8	75.8	57.6
Ngowe	21.2	87.2	62.9
R2E2	16.7	85.5	63.3
Kent	15.8	82.1	57.7
José	17.6	77.0	57.6
Minimango	16.3	86.6	56.3
Haden	17.6	86.4	60.7
Heidi	13.8	83.8	59.5
Kaew	16.3	80.2	57.2
Mon Duen Gao	12.7	85.5	57.8
Maha Chanock	12.9	82.0	62.8
Nam Dokmai	12.2	72.7	60.7
Chok Anan	12.6	83.2	60.9

Pectin contents ranged from 12.2 % (´Nam Dokmai´) to 21.2 % (´Ngowe´). Remarkably, in four of the six Thai mango cultivars relatively low pectin contents were determined. Pectins of all cultivars investigated showed very high galacturonic acid contents, ranging from 72.7 % to 87.2 %, and a high degree of esterification (>50 %). These findings demonstrate that mango peels are an interesting alternative to apple pomace and citrus peels for the recovery of pectin.

Phenolic compounds from mango peels

The separation of polyphenolics from mango peel extracts is shown in Figure 1. Among the large number of compounds detected, 29 were characterised by LC-MS. Based on multistage fragmentation experiments, compounds 1, 2, 6 and 9 were tentatively identified as maclurin derivatives and compound 5 as iriflophenone di-*O*-galloylglucoside (Berardini *et al*., 2004). Maclurins are considered key intermediates in the biosynthesis of xanthones, e.g. mangiferin (compound 3). Apart from mangiferin, hydrolysable tannins and benzophenone derivatives, several quercetin glycosides were also found, which is in agreement with previously published studies (Schieber *et al*., 2003a). For complete peak assignment, c.f. caption of Figure 1.

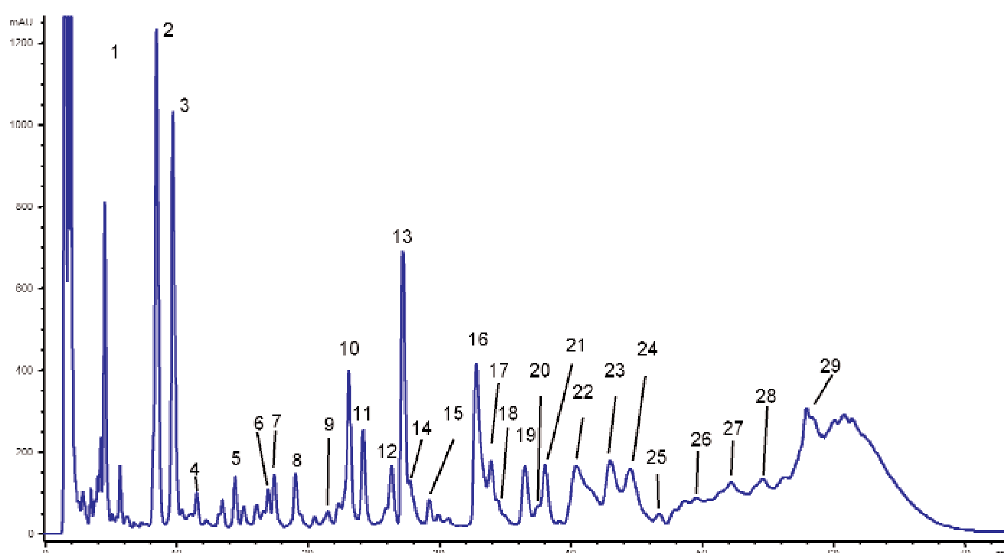

Figure 1. Separation of phenolic compounds from mango peels by HPLC (280 nm): (1) maclurin mono-*O*-galloyl-glucoside, (2) maclurin di-*O*-galloyl-glucoside, (3) mangiferin, (4,7,8) tetra-*O*-galloyl-glucose, (5) iriflophenone di-*O*-galloyl-glucoside, (6,9) maclurin tri-*O*-galloyl-glucoside, (10-12,14-15) quercetin glycosides, (13) penta-*O*-galloyl-glucose, (16-21) hexa-*O*-galloyl-glucose, (22-25) hepta-*O*-galloyl-glucose, (26-28) octa-*O*-galloyl-glucose, (29) nona-*O*-galloyl-glucose.

Conclusions

1. The new process for the combined recovery of pectin and polyphenolics from apple pomace can easily be integrated into the industrial pectin production, allowing the isolation of large amounts of polyphenolics which may be used as natural antioxidants and functional food ingredients.

2. Mango peels are a promising source of high-quality pectin and represent an interesting alternative to apple pomace and citrus peels. For exploitation on an industrial scale, immediate drying of peels is a prerequisite to avoid microbial spoilage and depolymerisation of pectin.

3. The presence of a large number of polyphenolics including hydrolysable tannins, benzophenone derivatives, and flavonol glycosides in mango peels has been demonstrated for the first time. Studies on their antioxidant and antimicrobial activities are currently under way.

Acknowledgements

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A systematic micro-dissection of brewers' spent grain

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Introduction

Context

Two major low-value co-products of the food processing industry are the bran of wheat (WB) after milling, and brewers' spent grain (BG) from barley, the main residue from brewing. Currently these are generally used for the production of low-value composts, livestock feed, or are disposed of in landfill as waste. Cost-effective deconstruction of these co-products into their polymeric, oligomeric and individual components, through mechanical and/or (bio)chemical means, combined with a reduction in biomass, could provide valuable streams for exploitation in a number of different applications.

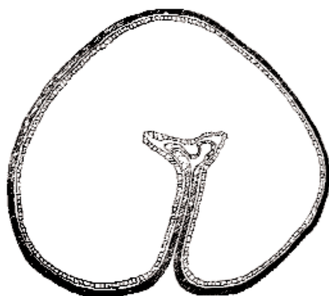
As part of a strategy to develop such an approach, it is necessary to understand the make-up of such bulk residues in relation to their biological, post-harvest and processing history. A detailed microscopic and chemical analysis of WB and BG revealed a wealth of structural and molecular components. A comparison between the two co-products highlighted not only the additional outer layers of the bran in BG, but also that its compositional complexity is more extensive than the wheat bran layers. In this poster, microscopy of BG is thus presented in greater detail. The presence of a number of potentially-useful components such as feruloylated arabinoxylan and protein was confirmed by microscopy and chemical analysis.

Comparative anatomy of wheat and barley grains

The difference in anatomy between wheat and barlely grains is illustrated in figures 1-4.

Figure 1. Unprocessed wheat (left) and barley (right) grains.

Figure 2. Longitudinal sections cut along the crease of each grain: wheat (left) and barley (right). The embryo (lower left) is distinct from the endosperm. When barley is malted, the embryo germinates and produces a shoot and roots. These shrivel as the malt is dried and are then partially or wholly abraded before sewing.



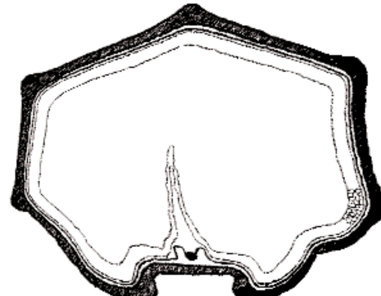
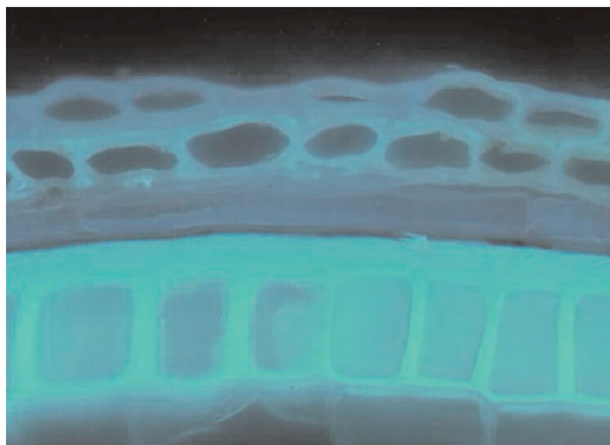
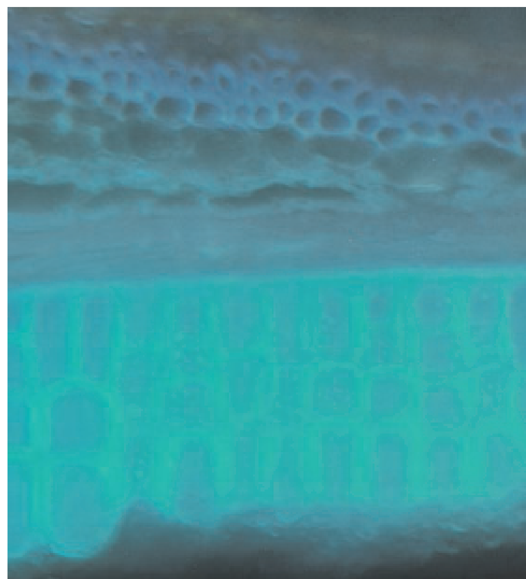


Figure 3. Diagram of transverse sections of wheat (left) and barley (right) (adapted from: Winton and Winton (1932)). The embryos are not shown but if sectioned would be seen at the top, between the outer layers and aleurone. In both cases, the endosperm forms the honeycomb-like bulk of the grain. Surrounding this in wheat are the aleurone (single layer of cells) and outer bran (dark layer), respectively. In barley, the aleurone forms a thicker triple layer of cells, surrounded by the outer bran and then the flowering glumes (dark layer).



Wheat



Barley

Figure 4. UV autofluorescence of transverse sections of bran layers. The lignified layers (particularly the barley glumes) appear blue and the aleurone is green. Fluorescence is caused by the phenolic acids (fig. 7 detail) linking cell-wall polymers.

Materials and methods

Production of wheat bran and brewers' spent grain

These were obtained from industrial producers (see Acknowledgements). WB was supplied dry whilst BG was supplied as frozen wet material. Figure 5 shows a schematic flow diagram of the processes applied to wheat and barley in the production of flour and beer, respectively. This shows how WB and BG are obtained as by-products. Although BG undergoes more extensive processing than WB, it contains a greater variety of the original components of the grain.

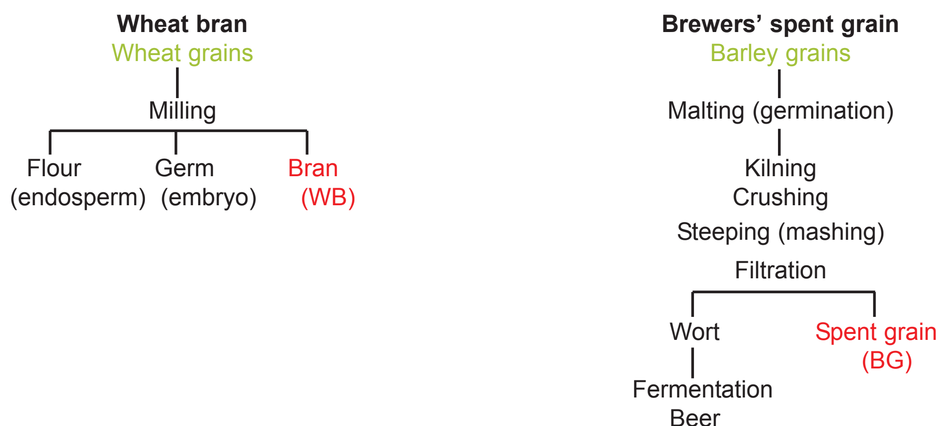


Figure 5. Schematic flow diagram of the processes applied to wheat and barley in the production of flour and beer, respectively.

Wheat bran **Brewers' spent grain**

Figure 6. Wheat bran and brewers' spent grain (dried).

Chemical analysis

Sugars were analysed as alditol acetates by GC and uronic acids by colorimetry; phenolic acids were analysed by HPLC; lignin was analysed by klasen method and other components by colorimetry or kjeldahl methods (Brillouet and Mercier (1981); DuPont and Selvendran (1987); Faulds *et al*. (2004); Valverde (1994)).

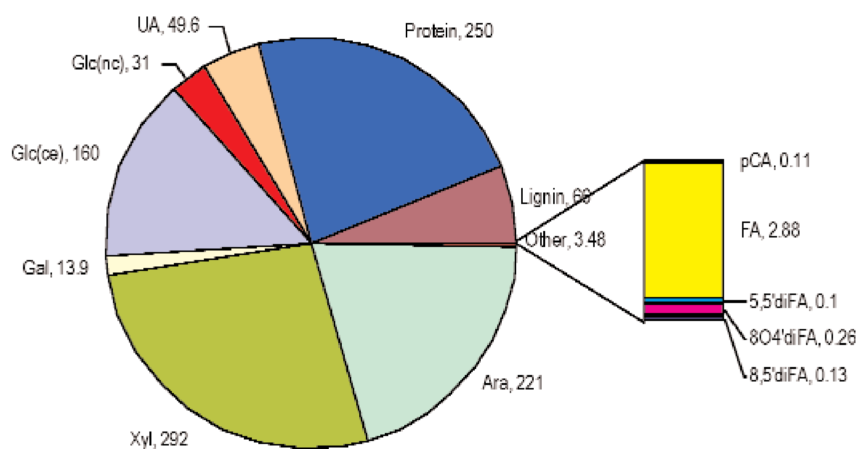
MIcroscopy

Light microscopy was performed using an Olympus BX60 microscope and images produced with AuQuis software. Fragments of BG were suspended in 5% ammonia solution for UV autofluorescence imaging.

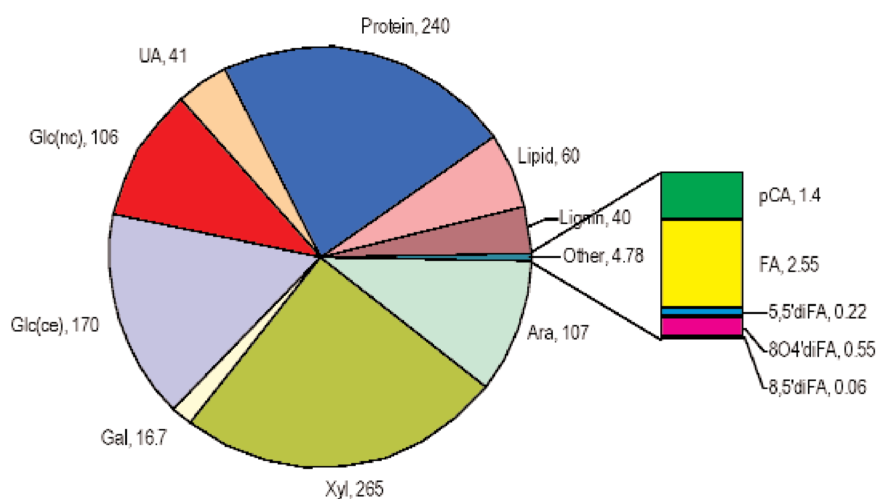
Results

Chemical analysis

Figure 7 shows a summary of detailed chemical analysis of alcohol-insoluble residue of WB and of BG (Brillouet and Mercier (1981); DuPont and Selvendran (1987); Faulds *et al*. (2004); Valverde (1994)). Amounts are expressed as µg per mg. The detail shows phenolic acid composition. Ara: arabinose; Xyl: xylose; Gal: galactose; Glc(ce): glucose (cellulose); Glc(nc): glucose (non-cellulose); UA: uronic acids; pCA: *p*-coumaric acid; FA: ferulic acid; 5,5'diFA: 5,5'-diferulic acid; 8*O*4'diFA: 8-*O*-4'-diferulic acid; 8,5'diFA: 8,5'-diferulic acid. Data on lipid content of WB is not available.



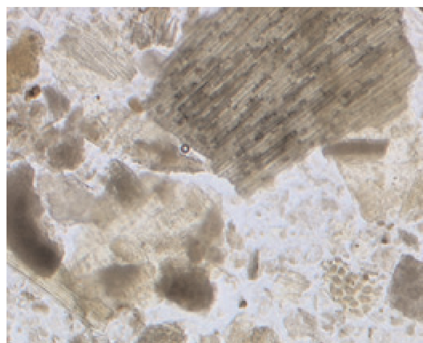
Wheat Bran



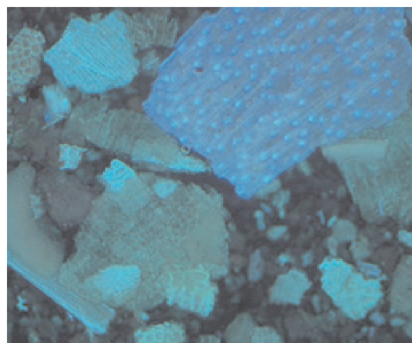
Brewers' Spent Grain

Figure 7. Chemical analysis of alcohol-insoluble residue of WB and of BG.

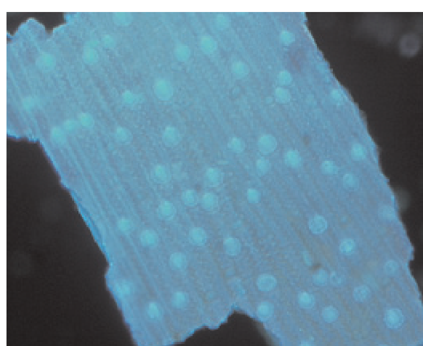
Microscopy



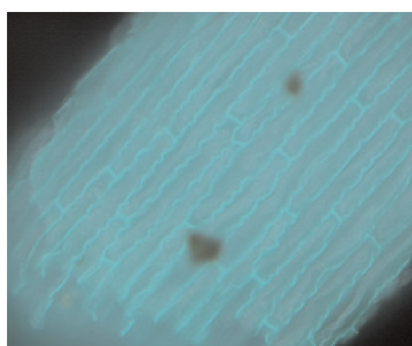
Bright field



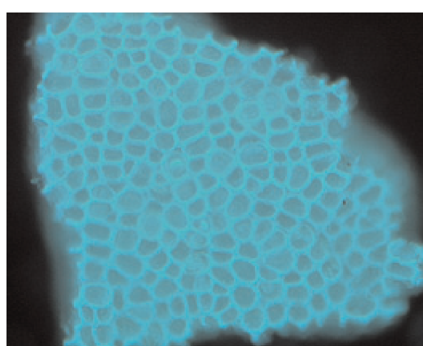
UV light



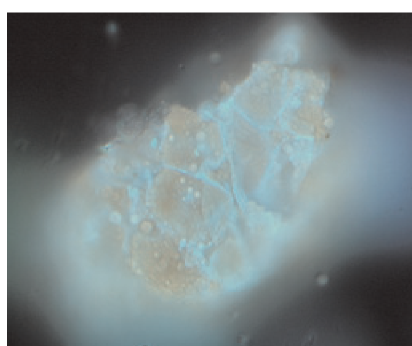
Flowering glume



Bran layer



Aleurone



Embryo

Figure 8. Under UV light, fragments of the different parts of the bran can be recognised by their distinctive autofluorescence. Material derived from the lignified glumes appears blue, whilst that originating from the aleurone is green. Lipid shows as orange-brown, particularly in fragments of embryo.

Acknowledgements

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Isolation and Characterisation of the Cell-Wall Materials of Korean Ginseng (*Panax ginseng* C.A.Meyer)

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Introduction

Korean ginseng (*Panax ginseng* C.A. Meyer) received a great deal of attention from the world as a oriental herbal medicine and health food (1). Many scientists have investigated and analyzed ginseng vigorously and have found new physiological or pharmacological effects of ginseng. But many people with herbal medicine still believe that ginseng is not doing its job as a single component but ginseng is doing rather its job as a complexes with other compounds (2).
The presense of phenolic compounds, associates with pectin, hemicellulose and cellulosecan contribute to changes in texture and biofuctional properties.
The purpose of this study was to isolate and characterise the polymers including phenolic acids from cell wall materials (CWM) of Korean ginseng (*Panax ginseng* C.A.Meyer), medicinal herb, with chemical methods. The amounts of uronic aicd , total sugars, and non-cellulosic neutral sugars of CWM from different parts of Korean ginseng were measured.

Materials and methods

Materials

Roots of *Panax ginseng* C.A. Meyer, which were harvested at Keumsan in Korea, were purchased commercially from Korea. They were grown for 5 years to maturity. Ginseng was washed, obtained only main root part, and immediately frozen in liquid nitrogen and stored –40 °C prior to chemical analysis.

Methods

Preparation of CWM

Samples(100g) were individually blended in 1.5% sodium lauryl sulfate (SDS) containing 5 mM $Na_2S_2O_5$ with an Ystral homogenizer (Ystral GmbH, Dottingen, Germany) for 5 min. A few drops of octanol were added to reduce foaming. The homogenate was filtered through a 100 μm nylon mesh (John Stannier and Co.,

Manchester, U.K.), and the residue was ball milled (Pascall, 0.5 L pot) at 0 °C in 0.5% SDS containing 3 mM $Na_2S_2O_5$ for 2 h at 60 rpm to remove the bulk of the remaining cell contents. After the homogenate had been filtered through 70 μm nylon mesh, the residue was suspended in cold water containing 3 mM $Na_2S_2O_5$ homogenized for 5 min, and refiltered. The procedure was repeated three times until the cell-wall residue was free of intracellular contents as assessed by light microscopy after staining in iodine/potassium iodide. The CWM was stored as a frozen suspension at –20 °C.

Sugar analysis

Neutral sugars were reduced with $NaBH_4$ and acetylated according to the method of Blakeney *et al*. (3) using 2-deoxyglucose (Sigma) as an internal standard. Alditol acetates were quantified by gas chromatography as described in Parr *et al*. (4). Uronic acids were determined colorimetrically according to a modification of the method of Blumenkrantz and Asboe Hansen (5).

Sequential extraction of CWM

Ginseng CWM was sequentially extracted as described by Waldron *et al*. (6).
To investigate this effect on cell wall chemistry of ginseng, CWM were prepared and were extracted sequentially with water, imidazole, CDTA(-1, -2), Na_2CO_3(-1, -2) , KOH(0.5, 1 and 4 M) to leave a residue.

Phenolic acid analysis

Cell wall phenolic acids(monomers and dimers) were identified and quantified by reverse phase HPLC with diode array detection (HPLC-DAD) as described by Waldron *et al*. (7).

Results

Sugar composition of CWM

Polymers extracted from CWM of main and fine root of ginseng had same values of uronic acid(UA) : neutral sugars(NS) ratio. The contents of total sugars in main root and fine root of ginseng were 1001.3, and 842.2 μg/mg CWM (Table 1). Arabinose and galactose were the main noncellulosic neutral sugars.

Table 1. Sugar composition of cell wall materials(CWM) from ginseng

Fraction	Yield (% CWM)	Cell wall sugars(μg /mg)								Total Sugar[a]	Ratio[b] UA:NS
		Rha	Fuc	Ara	Xyl	Man	Gal	Glu	UA		
Ginseng (main root)	4.6	10.9	3.0	100.3	14.6	13.4	89.9	273.9	495.3	1001.3	3
Ginseng (fine root)	5.8	9.4	2.8	81.2	19.6	13.1	67.6	259.2	389.2	842.1	3

[a] Values are expressed as mg of anhydrosugar/mg dry polymers.

[b] UA : NS, uronic acid : neutral sugar(arabinose + galactose).

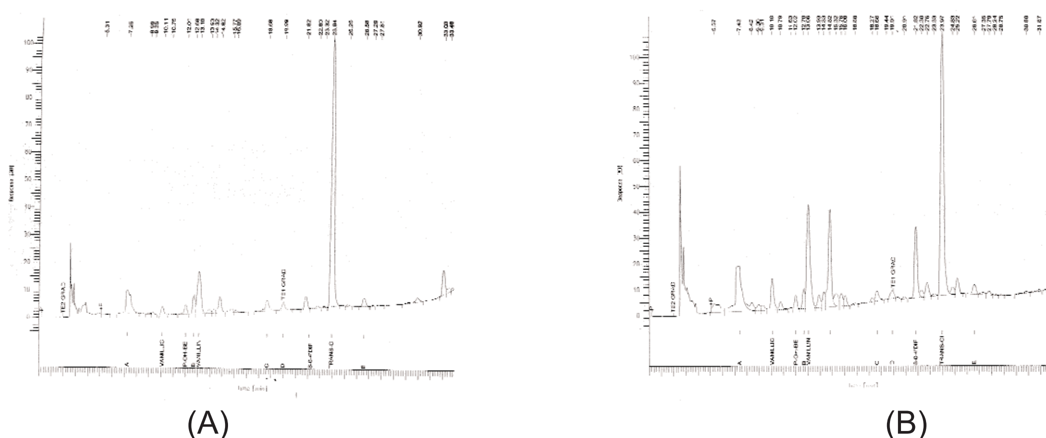
Phenolic composition of CWM

The chemical structure of five phenolic compounds analyzed from Korean ginseng CWM were identified to be vanillic acid, p-hydroxybenzaldehyde, vanillin, ferulic acid, and 8-O-4'diferulic acid by HPLC spectral data and LC-Mass results (Table 2, Fig. 1).

Table 2. Phenolic composition of AIR and alcohol extract from different parts of ginseng[a]

	Ginseng (main root)		Ginseng (fine root)	
	AIR	Alcohol Extract	AIR	Alcohol Extract
A	18.1	24.4	44.1	12.2
Vanillic acid	7.7	-	29.4	-
p-OH-benzaldehyde	2.9	6.6	3.4	4.5
B	11.4	19.9	10.8	27.2
Vanillin	26.2	53.5	53.6	44.7
Ferulic acid	10.8	-	58.5	-
C	8.7	37.1	8.4	34.6
D	4.9	23.5	7.7	12.2
8-O-4' diFA	15.2	-	109.8	-
E	6.8	-	7.4	-
F	6.8	96.0	-	69.0
Total phenolics	112.5	261.0	333.1	204.4

[a] Two replicates, mg /g of wall carbohydrate



(A) (B)

Figure. 1. HPLC profile of phenolics of main root CWM(A) and fine root CWM from ginseng

Sugar composition of fractions of CWM

The composition of major sugars in water and imidazole were galactose, arabinose, and in 0.5 M KOH, 1 M KOH, and 4 M KOH were glucose, arabinose, and galactose, respectively(Table 3).

Table 3. Sugar composition of fractions of cell wall materials(CWM) from ginseng obtained by sequential extraction with aqueous solvents

Fraction	Yield (% CWM)	Cell wall sugars(μg/mg)								Total Sugar[a]	Ratio[b] UA:NS
		Rha	Fuc	Ara	Xyl	Man	Gal	Glu	UA		
Water	0.5	3.6	5.0	113.5	1.8	12.8	129.8	18.9	148.2	433.6	1
Imidazole	1.3	7.6	2.6	117.4	3.0	42.9	169.6	54.0	577.7	674.8	2
CDTA-1	21.8	1.9	2.5	16.0	1.4	1.3	13.3	2.9	430.0	969.1	15
CDTA-2	12.0	3.4	3.6	7.2	1.5	1.4	5.9	2.2	82.3	107.5	6
Na2CO3-1	10.8	4.4	4.1	42.1	1.5	1.0	30.0	2.4	415.5	501.0	6
Na2CO3-2	3.3	9.4	2.5	96.1	2.7	1.5	77.9	3.2	533.3	726.6	3
0.5M KOH	1.6	8.5	6.0	115.0	50.2	4.8	97.1	177.2	271.9	730.7	1
1 M KOH	1.4	8.6	5.7	108.8	80.0	19.9	102.0	142.8	245.9	713.7	1
4 M KOH	4.5	3.0	10.1	76.3	112.9	47.7	107.8	209.8	140.3	707.9	1
Neutral Supt.	3.6	18.1	8.1	214.1	1.6	1.3	190.1	8.8	455.6	897.7	1
Final Resid	39.2	8.1	4.8	120.5	5.1	16.3	114.7	415.3	177.7	862.5	1

[a] Values are expressed as mg of anhydrosugar/mg dry polymers.

[b] UA : NS, uronic acid : neutral sugar(arabinose + galactose).

Phenolic composition of fraction of alcohol insoluble residue (AIR)

The wall-bound phenolics were released from ginseng AIR by sequential extraction with various solvents. 5 Kinds of major phenolic compounds and 5 kinds of unknown peaks were analyzed in sequential extracts of ginseng AIR (Table 4).

Table 4. Phenolic composition of fractions of AIR from ginseng obtained by sequential extraction with aqueous solvents[a]

	A	Vanillic acid	P-OH-benzal dehyde	B	Vanillin n	Ferulic acid	C	D	8-O-4' di FA	F
Water	155.4	31.3	57.1	27.8	19.8	91.8	24.5	33.0	24.2	79.0
Imidazole	80.4	11.7	50.7	12.7	14.0	29.2	15.1	0	17.7	99.1
CDTA-1	298.5	7.2	61.5	13.8	26.5	3.2	14.5	0	0	140.0
CDTA-2	735.5	11.7	33.7	12.8	15.7	1.5	8.4	0	0	84.7
Na_2CO_3-1	343.5	7.9	63.1	14.0	34.3	7.0	5.5	0	0	159.5
Na_2CO_3-2	72.5	7.3	51.3	9.5	11.7	22.0	21.0	0	0	79.5
0.5M KOH	31.3	0	64.2	0	5.0	135.0	16.5	0	0	49.3
1 M KOH	13.8	8.0	58.6	12.0	5.9	19.7	103.9	0	2.5	48.5
4 M KOH	61.5	6.3	113.9	38.2	32.6	12.9	10.8	0	5.0	163.0
Neutral Supt.	27.7	3.3	2.4	59.2	13.5	28.0	29.6	0	0	148.1
Final Resid	40.8	17.2	62.4	19.2	43.0	3.5	20.5	0	0	280.2

[a] Two replicates

Conclusions

1. Among phenolics, p-hydroxybenzaldehyde, vanillin, 8-O-4' diferulic acid were the first compounds identified from Panax ginseng C. A. Meyer. The content of 8-O-4' diferulic acid in fine root of ginseng was 109.8 μg/g CWM.

2. The composition of major sugars in water and imidazole were galactose, arabinose, and in 0.5 M KOH, 1 M KOH, and 4 M KOH were glucose, arabinose, and galactose, respectively.

3. High amounts of phenolics including unknown peak A were observed in high pectic polysaccaride fractions of CDTA-1, CDTA-2, and Na_2CO_3-1. The contents of ferulic acid(monomer) and 8-O-4'diferulic acid (dimer) were only concentrated in 1.0 M KOH and 4 M KOH soluble fractions.

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Low Molecular Weight Carbohydrates in Vegetable By-Products

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Introduction

By-products of plant food processing represent a major disposal problem for the industry concerned. The amount of vegetable residues generated after harvesting the edible portion of most crops can account for a large proportion depending on the plant (Goñi & Saura-Calixto, 1988; Femenia *et al*., 1998). Traditionally, agro-industrial waste has been used as a feed or as a fertilizer.
However, vegetable by-products are an important resource as a raw material for potential use in food additives or dietary supplements and as a source of extractable polysaccharides for industrial explotation. A major component of plant by-products is dietary fibre (Larrauri *et al*., 1996a; Larrauri *et al*., 1997a,b; Femenia *et al*., 1998; Larrauri, 1999). Dietary fibre with associated polyphenols has also been obtained from plant wastes (Larrauri *et al*.,1996b; Larrauri *et al*., 1997c,d; Saura-Calixto & Jiménez-Escrig, 2001). Others are minor bioactive components such as: carotenoids, phytoestrogens, etc (Llorach *et al*., 2002). Vegetable residues as a source of natural antioxidants (Moure *et al*., 2001) and functional compounds (Schieber *et al*., 2001), have recently been reviewed. Besides preparation of value-added products, the utilization of residues represents a good solution to avoid environmental pollution.
Yet, another group of interesting compounds that could be obtained from plant wastes are low molecular weight carbohydrates (LMWC; mono-, di- and oligosaccharides). Nevertheless, there are not many reports in the literature dealing with the use of vegetable residues as a raw material for the preparation of LMWC. Indigestible carbohydrates, such as non-digestible oligosaccharides, are currently considered prebiotics because of they reach the colon undigested, where they are fermented mainly by bifidobacteria and lactic acid bacteria, thus producing a health possitive effect (Rupérez, 1998a; Jan Van Loo *et al*., 1999; Roberfroid & Slavin, 2000; Rupérez & Bravo, 2001). The aim of this work was the extraction of LMWC from several vegetable residues from the food industry and their characterization by high performance liquid chromatography (HPLC), as a previous step to their potential utilization as prebiotic ingredients.

Materials and methods

Materials

Raw material

Vegetable by-products: 1) Stems of broccoli (*Brassica oleracea* var. italica), 2) leaves of cauliflower (*Brassica oleracea* var. botrytis), 3) floret stems of cauliflower, and 4) outer leaves of lettuce (*Lactuca sativa*) were directly obtained from a food industry. Freeze-dried samples were milled to a particle size of less than 1.0 mm before analysis.

Methods

All extractions and determinations were performed at least in triplicate and they are reported on a dry matter basis as mean values ± standard deviation.

Moisture content

Residual moisture content was determined by drying to constant weight at 105 ºC in an oven.

Extraction of low-molecular-weight carbohydrates from vegetable by-products

Low molecular weight carbohydrates (LMWC) from vegetable samples (500 mg) were extracted with ethanol (85%, v/v; 50 mL) in screw capped tubes, at 50ºC in a water bath with constant shaking for 1 h. After cooling to room temperature, samples were centrifuged (2,500 x g; 15 min). Aliquots (10 mL) of supernatants containing LMWC were evaporated to dryness in a R-114 Büchi vacuum rotatory evaporator with a B-480 Büchi water bath and temperature not exceeding 50ºC. The sample extracts were redissolved in Milli-Q water (1.5 mL) and filtered through 0.22 mm filters for aqueous solutions, just before HPLC analyses (Rupérez & Toledano, 2003).

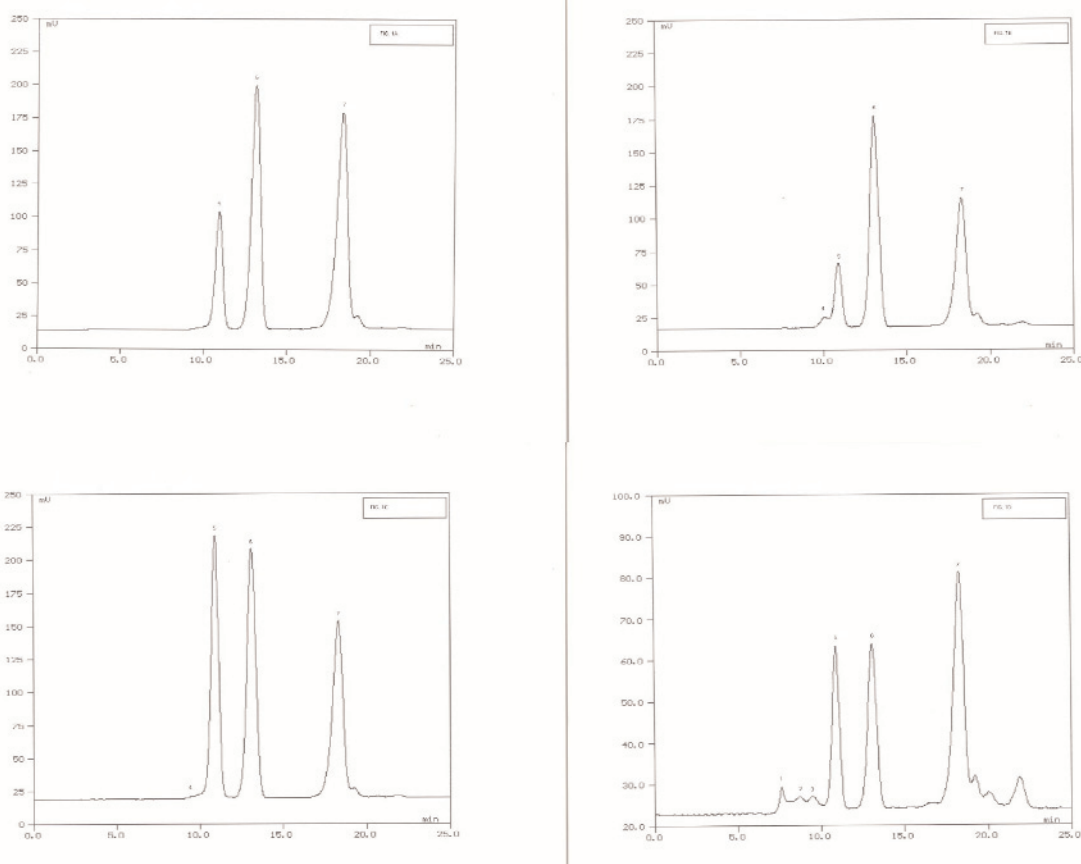
Low-molecular-weight carbohydrates determination by HPLC

LMWC (50 mL) were analysed by HPLC in an aqueous sample extract (100 mg/1.5 mL), on a Bio-Rad Aminex HPX-87P column (300 x 7.8 mm) with two Bio-Rad micro-guard cartridges (30 x 4.6 mm). The column was eluted isocratically with Milli-Q-filtered (0.45 mm) and degassed water at 85ºC, with a flow rate of 0.6 mL/min (Rupérez & Toledano, 2003). LMWC were identified and quantified by comparison with known standards (inulin, stachyose, raffinose, glucose, fructose from Sigma; sucrose from Merck). The following HPLC instruments were used: Kontron autosampler 360, Kontron ternary pump system 325, Waters differential refractometer R-401, Jones chromatography thermostatic oven, Kontron data system 450-MT2 and Hewlett-Packard deskjet 600 printer.

Results

Plant food residues have mainly been used as a source of dietary fibre and natural antioxidants, but vegetable by-products could also be utilized as a raw material for soluble sugar, polyol and oligosaccharide extraction. Celery by-products have recently been proposed as a rich source of mannitol (Rupérez & Toledano, 2003).

Residues from the food industry were extracted with hot ethanol to solubilize mono- di- and oligosaccharides. HPLC chromatograms of extracts from broccoli (A), cauliflower leaf (B), cauliflower floret stem (C) and lettuce residues (D) are shown in Fig. 1 A, B, C and D (1= inulin; 2= oligofructose; 3= stachyose; 4= raffinose; 5= sucrose; 6= glucose; 7= fructose).



Low-molecular-weight carbohydrates in vegetable extracts are shown in Table 1. Total sugar content ranged from 5.3% (lettuce) to approx. 20% dry weight in floret stems of cauliflower and broccoli leaves. Main soluble sugars in the residues were fructose (2.5-8.6%), glucose (1.5-7.9%) and sucrose (1.2-6.3%).

Table 1 HPLC determination of low-molecular-weight carbohydrates in vegetable by-products (% dry weight)

LMWC[1]	Sample			
	Broccoli	Cauliflower		Lettuce
		leaves	stems	
Inulin	0	0	0	0.13±0.05
Oligofructose	0	0	0	trace
Stachyose	0	0	0	trace
Raffinose	0	0.26±0.02	0.11±0.04	0
Sucrose	3.18±0.21	1.74±0.13	6.34±0.58	1.19±0.11
Glucose	7.91±0.57	6.86±0.61	7.18±0.56	1.48±0.11
Fructose	8.61±0.47	5.17±0.45	6.24±0.61	2.46±0.23
Total sugar	19.70±1.24	14.03±1.20	19.86±1.76	5.26±0.46

[1]LMWC = low-molecular-weight carbohydrates
Values are mean values ± standard deviation; a trace is less than 0.1% d.w.

Leaves and floret stems of cauliflower also contained a small amount of raffinose (0.1-0.3%) and waste of lettuce showed a trace of inulin, oligofructose and stachyose (Fig. 1 A,B,C,D). Food industry improvements in the processing of vegetables, such as the utilization of water steam instead of blanching to inactivate browning enzymes before deep-freezing, would probably increase the recovery of LMWC and other water soluble components from vegetable residues.

Conclusions

1. Vegetable by-products are proposed as an alternative natural source of soluble sugars and oligosaccharides. Oligosaccharides from vegetable wastes could be used as potential prebiotics in functional foods.

2. The remaining alcohol-insoluble residues from LMWC extraction of vegetable wastes could be further used for the preparation of dietary fibre.

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IN VIVO ASSAY OF OKARA (a waste in the process of making tofu)

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Introduction

The consumption of soy foods is increasing due to the benefits reported in nutrition and health. In the preparation of soymilk and tofu, okara is discarded as a by-product. In this process, soybean is soaked in water overnight, then blended and drained. The white soybean drink obtained is called "soymilk" and the insoluble residue is the okara. Okara is white and tastes similar to almond. The composition of the okara obtained depends on the soybean used and the method of tofu preparation (Japanese, Chinese style, etc) but, on a dry weight basis, it is on average composed of carbohydrate (50%), protein (30%), fat (10%) and ash (5%) (Ma *et al*., 1997). Due to the high amount of fibre, okara could be used as a supplement in human diets, especially in Western countries, which are lacking of the necessary fibre in their diet. Moreover, protein from okara could be a good source of low cost vegetable protein with a high nutritive value (Wang and Cavins, 1989). For the sake of heat treatment during soymilk and tofu preparation, okara led to denaturation of protein primary structure, thus improving its digestibility (Kamata *et al*., 1979; Ma *et al*., 1997). This research was conducted to investigate the possible *in vivo* health benefits of okara as a prebiotic.

Materials and methods

Raw material

Okara, a waste in the process of tofu preparation, was obtained from a local food industry. Okara was freeze-dried and milled to a particle size of less than 1mm before analysis.

Analytical methods
All determinations were performed at least in triplicate and they are reported on a dry matter basis as mean values ± standard deviation.
Moisture content of okara was determined by weight-loss after oven-drying to constant weight at 105 ºC (AOAC, 1995a).
Protein. Total nitrogen in freeze-dried okara was determined by the micro-Kjeldahl method (AOAC, 1995b) and protein was calculated as nitrogen x 5.71.
Fat content was obtained after extraction with petroleum ether by Soxhlet method (James, 1995).
Dietary fibre was analyzed by AOAC enzymatic-gravimetric method, fractionating into insoluble and soluble residues (AOAC, 1995c; AOAC, 1995d).
Ash content was measured as the residue obtained after incinerating at 550ºC for 3h (AOAC, 1995e).

Animals and diet
A total of twenty, 12 week-old, Wistar Hannover female rats were used in the experiment, divided into two groups of 10: the first one (control), was fed with a standard food and the second one, with a mixture of the standard food plus 10% of freeze-dried okara. The body weight of the rats and faeces were recorded weekly. The animals were fed for 4 weeks and at the end they were slaughtered under anaesthesia (diethyl ether), the blood was collected and the whole organs (spleen, kidneys, heart and liver) were rapidly removed. Faeces and organs were weighed and the somatic index (SI, organs/body weight) calculated.

Sampling and processing of caecal digesta
The caecum of rats was also weighed and its content kept for the control of pH and analysis by gas-liquid chromatography of the volatile organic acids (Tortuero *et al.*, 1997) released during the colonic fermentation of okara fibre. In order to determine the wet:dry ratio, another portion of the caecal content was either freeze-dried or dried in an oven at 105ºC overnight.

Results

Table 1 displays the composition of okara. It is quite remarkable the high amount of the indigestible fraction (73%), mainly composed of dietary fibre and protein. Fat content was 20%.

Table 1 Composition of okara (g/100g dry weight)

Okara	Indigestible fraction	Resistant Protein	Ashes in residue	Dietary fibre
IF	68.91±1.34	19.54±1.56	0.87±0.05	48.50±1.34
SF	3.80±0.07	2.25±0.35	1.00±0.03	0.55±0.01

Dietary fibre by AOAC method; IF=Insoluble fraction; SF=Soluble fraction
Table 2 shows the data from *in vivo* colonic fermentation of okara. The pH of the okara group increased and total SCFA content was higher compared to control. An increase of acetate was also observed in the okara diet, which is beneficial on health. No signifficant differences were observed in propionate or butyrate contents between both groups.

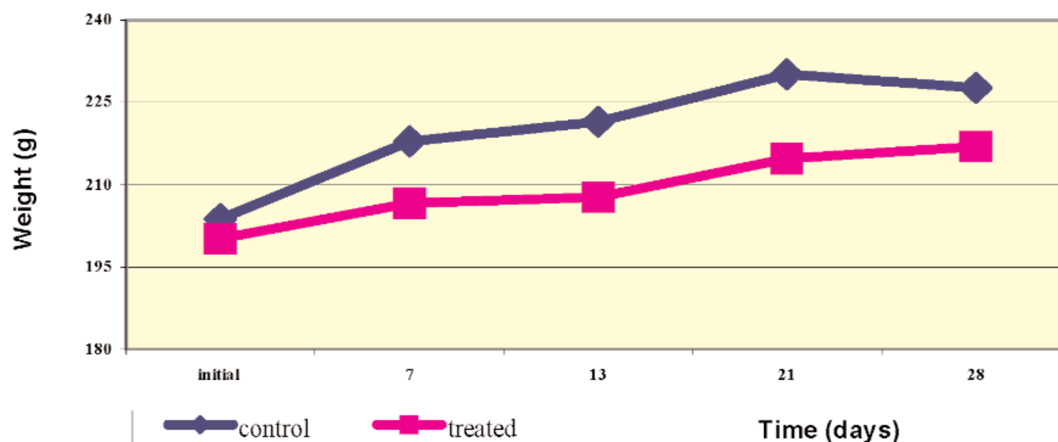
Table 2 Value of pH and short chain fatty acids (SCFA) of caecal contents in rats fed 10% okara for four weeks

	Control	Okara diet
pH	6.28 ± 0.28	6.04 ± 0.15
Total SCFA (mmol/g)	631.7 ± 97.1	823.3 ± 148.2
Molar proportion (%)		
Acetate	48.03 ± 0.33	50.04 ± 0.41
Propionate	24.08 ± 0.65	22.98 ± 0.86
Butyrate	23.94 ± 0.65	24.18 ± 0.81

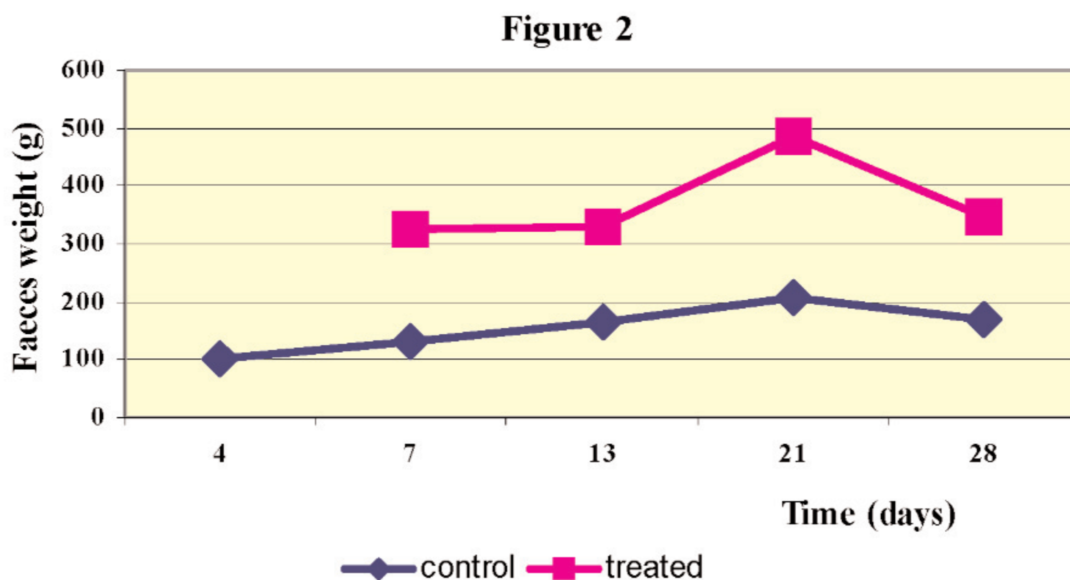
Values are means ± sd, n =10; traces (less than 2%) of iso-butyrate, valerate and iso-valerate were detected in control and treated groups

Significant differences were found in some of the parameters in the rats supplemented with fibre when compared to the control group. As regards Fig. 1, the body weight in the rats fed with okara was lower than in the rats fed with the standard

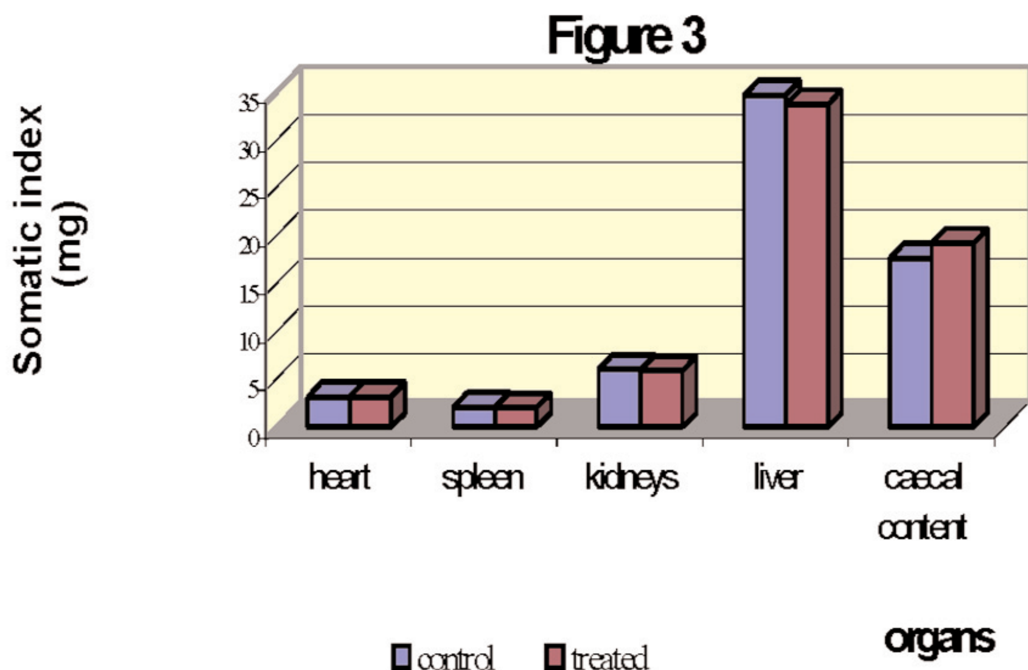
Figure 1



diet.
Okara fibre increases faecal weight (Fig. 2), due to the increase of moisture and intestinal peristalsis.



No significant differences were found in the somatic index of the organs, but a slight increase was observed in the caecal content of okara when compared to control group (Fig. 3).



Conclusions

1. Okara is an interesting source of dietary fibre and protein, therefore, it should not be regarded as a waste product but it might be used in human feeding.

2. The use of okara could contribute in the sustainable development, by using this waste.

Acknowledgements

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Whole-waste Utilisation and Future Concepts

Biological Hydrogen Production from Agro-Food By-Products

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Introduction

It is now generally recognized that for prevention of detrimental climate changes the emission of greenhouse gases needs to be reduced. The largest share in this respect comes from the CO_2 emission, which results from the use of fossil fuels for the production of electricity or other combustible fuels. To tackle the problem of CO_2 emission, a significant part of the worldwide energy R&D is aimed at employing a non-carbonaceous energy carrier, namely hydrogen. When hydrogen is used to provide energy, the concurrent oxidation product is water, which does not contribute to the greenhouse effect. Moreover, when hydrogen is used to power fuel cells, the high energy conversion efficiency of these fuel cells signifies important primary fuel savings thus promoting further reduction of CO_2 emission. However, whenever fossil fuels are the source for hydrogen production, the result will remain net CO_2 emission which will need to be discarded, e.g. by capture and sequestration in aquifers or the deep sub-soil. Moreover, utilization of fossil fuels is associated with exhaustion of reserves and depedence on supplies. These are the main reasons to investigate new approaches for the sustainable production of hydrogen where net CO_2 production is avoided and renewable resources are employed.
New approaches for the sustainable production of hydrogen should therefore be CO_2 neutral technologies. Here, two different primary resources can be distinguished: (i) water which is hydrolyzed to hydrogen and oxygen using renewable electricity (derived from wind, hydropower, or solar, nuclear, geothermal and photo-biochemical sources) and (ii) biomass which is oxidized to hydrogen and CO_2 by either thermochemical or biological conversions. All these approaches have a value of their own and since the expected demand for hydrogen will be enormous, they all will find their proper niche.

In this paper we will discuss biological hydrogen production in general and present data obtained using potato steam peels, an agro-food by-product stream, for hydrogen production. Similar as to most other initiatives, this approach is in the R&D phase. Important distinct advantages of this technology are the production of pure hydrogen and applicability on a small scale, which is of prime importance when using biomass. The envisaged bioprocess described below is aimed at converting locally produced biomass to hydrogen which can be used to supply the transport sector or the micro combined-heat-and-power devices which are in development for local application.

Biological hydrogen production

Microbial hydrogen production is a regular phenomenon found in two different groups of microorganisms. On the one hand, there are bacteria and (micro)-algae, which grow photo-autotrophically, i.e. using light as energy source and fixing CO_2 as carbon source. Through the utilization of light as primary energy source, water is split in hydrogen and oxygen by the enzyme hydrogenase. Until now, hydrogen production by autotrophic microorganisms has suffered from low yields with a light to hydrogen energy conversion efficiency of < 1.5% on a 400-950 nm solar spectrum basis (Akkerman *et al*., 2003).

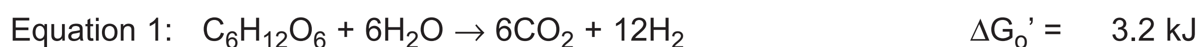
On the other hand, there are microorganisms, bacteria, which produce hydrogen during anaerobic, heterotrophic fermentation, i.e. utilization of organic compounds as energy and carbon source (Nandi and Sengupta, 1998). Reduction equivalents, generated during the oxidation of the organic compounds, are discarded by a.o. the reduction of protons employing the hydrogenase enzyme.

A distinct group of bacteria is able to grow photo-heterotrophically. These bacteria employ light as primary energy source and organic compounds as carbon source. In these bacteria, the nitrogenase enzyme, generally aimed at the fixation of nitrogen, catalyzes the production of hydrogen under anaerobic conditions, in the simultaneous absence of nitrogen and high concentrations of ammonium (ammonium renders the nitrogenase enzyme inactive).

The focus of this paper is on biological hydrogen production from biomass, by combining a heterotrophic and photo-heterotrophic fermentation step.

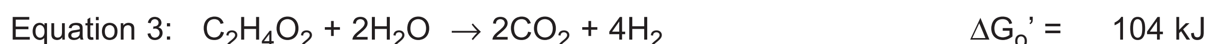
The maximum theoretical energy conversion efficiency achievable when glucose is converted to 12 moles of hydrogen is 99%. However, equation 1 shows that during this anaerobic oxidation of glucose, no energy is liberated for microbial growth. Therefore, biological hydrogen production will only occur when this reaction is shifted to the right, e.g. by immediate hydrogen removal and/or capture at extremely high temperatures. Alternatively, when glucose is first partially oxidized, e.g. according to equation 2, the liberated energy is more than sufficient to provide metabolic energy. In this case, 4 moles of hydrogen instead of 12 per mole of glucose are produced assuming no growth conditions. This efficiency is only achieved when glucose is oxidized to hydrogen, acetic acid and CO_2 (Van Niel *et al*., 2002). Many bacterial species show metabolic routes which enable alternative ways to dissipate electrons, e.g. by the reduction of pyruvate to lactate or alanine or acetyl-CoA to ethanol. In this way, the yield of hydrogen decreases. In a current review (de Vrije and Claassen, 2003) it was shown that thermophilic bacteria, growing at 70-80°C, have

superior performance with respect to yield of hydrogen production as compared to species growing at 35°C.
After the oxidation of glucose to hydrogen and acetate, a considerable amount of energy is still left in the by-product. From equation 3 it is obvious that further oxidation of acetic acid to hydrogen and CO_2 is unfavorable in terms of thermodynamics and, moreover, no energy is produced for bacterial growth. However, as during photo-heterotrophic fermentation light is used as energy source, the complete conversion of acetic acid to hydrogen and CO_2 can be established. Thus, the combination of a heterotrophic fermentation with a subsequent photo-heterotrophic fermentation (Fig. 1) will enable the complete conversion of glucose to 12 moles of hydrogen. Since the maximum conversion efficiency (99%) has to be corrected for energy and protons utilized for biomass production, the realistic overall energy conversion efficiency of glucose to hydrogen becomes approximately 69%.



Equation 1: $C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2$ $\Delta G_o' = 3.2$ kJ

Equation 2: $C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2C_2H_4O_2$ $\Delta G_o' = -206$ kJ



Equation 3: $C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 4H_2$ $\Delta G_o' = 104$ kJ

Feedstock for biohydrogen production

The novel approach to produce hydrogen is based on the combination of a thermophilic, heterotrophic and a photo-heterotrophic fermentation as shown in Figure 1. In the first fermentation, the feedstock is converted to hydrogen with the simultaneous production of organic acids (mainly acetic acid). In the second fermentation, the end-products of the thermophilic fermentation, i.e. the organic acids, serve as feedstock for the photosynthetic bacteria which are known for their preference to convert organic acids to hydrogen in the presence of light by the action of the nitrogenase enzyme. During growth of thermophilic bacteria, hydrogen production is directly linked to central metabolic pathways (Fig. 2). This is not the case during photo-heterotrophic growth. Here, electrons are liberated from the organic carbon source (in this case the organic acids) when it is oxidized. They are brought to a higher energy state in the photo-system and are stored in reduced ferredoxin. A redox balancing system shuttles them towards the nitrogenase enzyme, when the redox state of the cell is too low. The enzyme performs the hydrogenation reaction optimally when no nitrogen and/or high concentrations of ammonium are present. Besides electrons, the enzyme also needs ATP and protons.

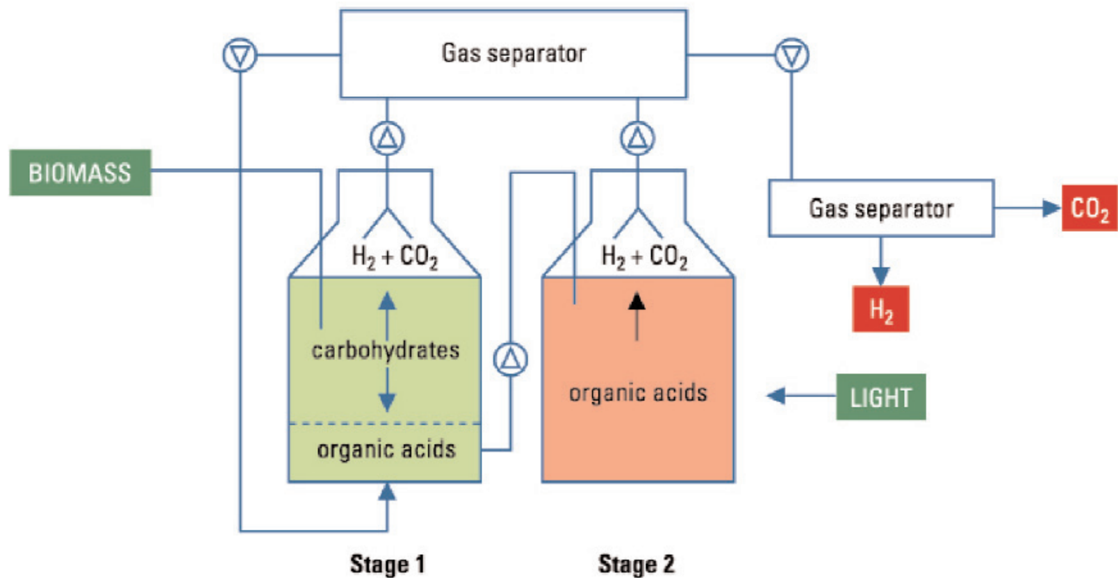


Figure 1. Outline of the bioprocess for production of hydrogen from biomass in a 2 stage fermentation. Stage 1 is for heterotrophic fermentation of carbohydrates to hydrogen, carbon dioxide and organic acids. In stage 2 the photo-heterotrophic fermentation of organic acids to hydrogen and carbon dioxide takes place.

The range of potential substrates which can be utilized by the broad range of hydrogen producing bacteria is extensive and open for exploration. From a thermodynamic point of view, the conversion of carbohydrates to hydrogen and organic acids is preferred because it yields the highest amount of hydrogen per mole of substrate in the central metabolic pathways as shown in Figure 2.
These carbohydrates can be monosaccharides but may also be polymers such as starch, cellulose or xylan. Besides carbohydrates also formate and peptides have, until now, been studied as substrates for heterotrophic hydrogen production (de Vrije and Claassen, 2003). Recently it has become clear that certain strains of extreme thermophilic bacteria can also oxidize amino acids to hydrogen. It is not clear whether specific amino acids, entering bacterial metabolism at the level of pyruvate are selected or whether this phenomenon is more general.

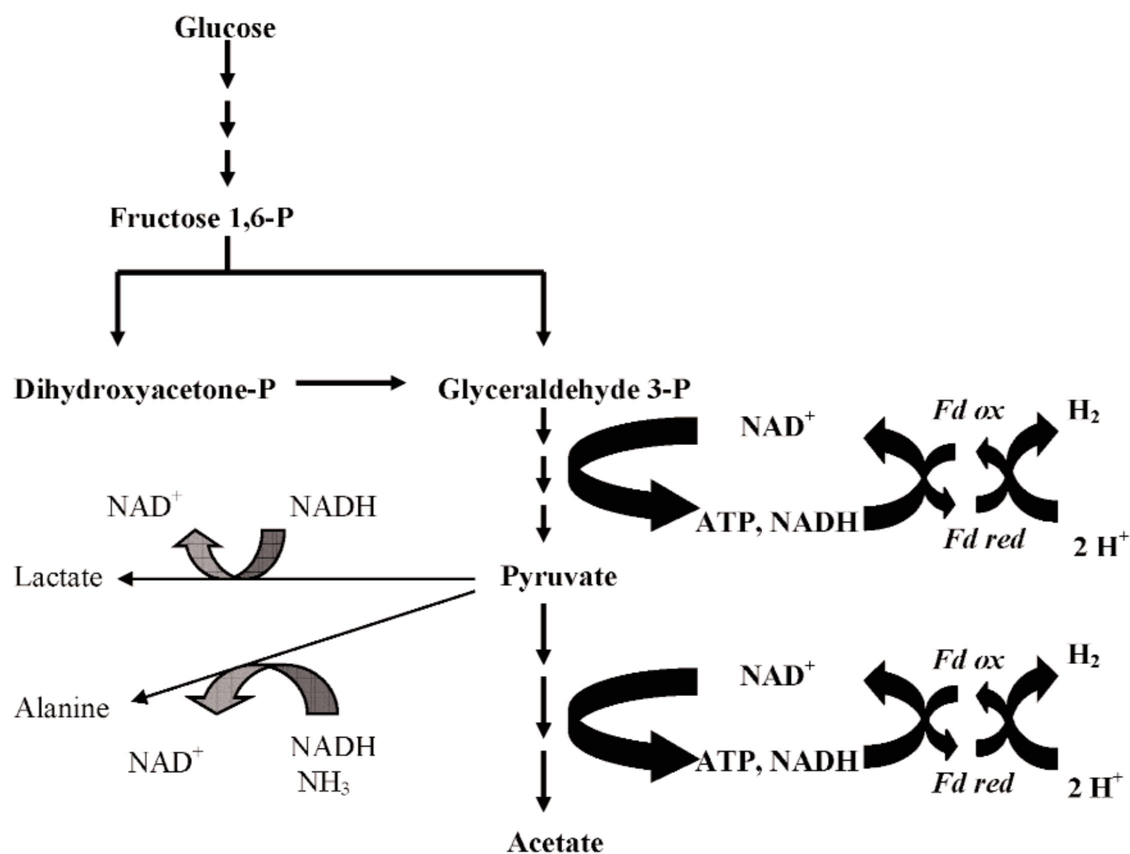


Figure 2. General metabolic pathway for hydrogen production from carbohydrates.

Other workers in the field have also recognized this great potential of hydrogen fermentation, i.e. the vast range of potential organic substrates. Noike and Mizuno (2000) and Yu *et al*. (2002) refer to several forms of organic waste streams, ranging from solid wastes like rice straw to waste water from a sugar factory and a rice winery, which have been successfully used for hydrogen production. Other organic feedstock ranges from domestic organic waste to more defined agro-industrial residues and finally to well defined products from energy crops. Several examples like sweet sorghum juice, hydrolysates of *Miscanthus*, paper sludge, and even domestic organic waste, have been tested with respect to applicability for biological hydrogen production (Claassen *et al*., 2002). This far, most experiments showed considerable hydrogen production, even with the limited number of thermophilic strains used. Lignocellulosic feedstock is generally regarded as a very promising feedstock because it is cheap and abundant. However, for obtaining high efficiencies, pretreatment for mobilization of fermentable sugars in hydrolysates is required. Presently, this is a costly procedure during which, as a result of the application of harsh conditions, potential inhibitory compounds are formed. Fermentation of hydrolysates obtained from *Miscanthus*, has yielded hydrogen, from glucose and xylose sugars simultaneously, but the overall efficiency needs to be increased (de Vrije *et al*., 2002).

Until now, in the experiments performed with the photo-heterotrophic fermentation using the purple non-sulfur bacterium *Rhodobacter capsulatus* NCIMB 11773, acetate was used as the model substrate. In principle, the microorganism can use a wide range of organic acids and alcohols for growth and hydrogen production (Hustede *et al*., 1993); (Gest *et al*., 1962); (Stevens *et al*., 1986). The fermentations gave substrate conversion efficiencies of maximally 50% and light energy to hydrogen energy conversion efficiencies of about 1.5%. These values will need further improvement for the process to be commercially feasible (80% and 7%, respectively, are required).
Currently, the main focus is on improving the light to hydrogen conversion efficiency. For this purpose, the flat panel photobioreactor design is used, as this configuration generally yields the highest photosynthetic efficiency in photofermentations (Richmond, 2000); (Janssen *et al*., 2003). Basic calculations have shown that at best, 10% of the (sun)light energy reaching the surface of a photobioreactor can be converted into hydrogen energy (Göbel, 1978); (Miyake, 1998).
The substrate to hydrogen conversion efficiency is investigated by using nitrogen limited cell cultures for minimizing growth and by investigating the carbon metabolism of the microorganism using *in vivo* ^{13}C-NMR techniques. The insight in the metabolism related to the formation and excretion of organic acids will be used to improve the acetate to hydrogen conversion ratio.

Potato steam peels

In this paper the utilization of potato steam peels for hydrogen production is presented. In the potato processing industry, a vast range of products, e.g. french fries or crisps, is produced and potato steam peels are a by-product, amounting to approximately 600 kton per year in the Netherlands. Presently, this by-product is sold to the fodder industry where it is, usually, combined with other wet organic by-products from the food industry to make a nutritious fodder. The utilization of potato steam peels for the production of hydrogen would provide an alternative market for this by-product. Furthermore, as potato steam peels are hydrolyzed prior to fermentation, a residual fraction is formed which is enriched in protein. This secondary by-product is expected to have improved properties in view of fodder production.
The composition of potato steam peels, mainly carbohydrates, protein and organic acids is extremely well suited for the two-stage bioprocess for hydrogen production. The carbohydrates are a prime substrate for the thermophilic bacteria as described above. The organic acids, already present in the initial substrate and additionally produced in the first fermentation, are the substrates of choice for the photo-heterotrophic fermentation.

Materials and methods

Pretreatment and enzymatic hydrolysis of the feedstock

Potato steam peels are obtained after treatment of potatoes for 10 – 20 sec at 200°C to remove the peel. This by-product, consisting of peels and purée, was milled and stored at -20°C prior to use. Enzymatic hydrolysis of potato steam peels was

performed using commercial α-amylase (BAN 480 L) and glucoamylase (AMG 300 L) enzyme preparations (Novozymes, Bagsvaerd, Denmark). Prior to hydrolysis the raw material was sterilized (121°C, 20 min). Potato steam peels (dry matter concentration of 12.6% w/v) were sequentially hydrolyzed by the two enzyme preparations at the following conditions: BAN, 1.8 μg enzyme/g dry matter, pH 6.0, temperature 70°C, incubation time 2 h; AMG, 2.9 μL enzyme/g dry matter, pH 4.3, temperature 60°C, incubation time 2 h. Hydrolysates were collected after removal of the remaining solids by centrifugation, adjusted to neutral pH and stored at -20°C. Hydrolysates were added to the bioreactor prior to the start of the cultivation.

Micro-organisms and medium composition

Heterotrophic fermentation

Caldicellulosiruptor saccharolyticus DSM 8903 was obtained from the Deutsche Sammlung von Mikroorganismen (DSM, Braunsweig Germany).
The medium used contained per Liter (unless stated otherwise): 10 g glucose, 0.75 g KH_2PO_4, 1.5 g K_2HPO_4, 0.75 g cysteine–HCl, 0.5 mg resazurine, DSM salts (0.9 g NH_4Cl, 0.9 g NaCl, 0.4 g $MgCl_2.6H_2O$, 2.5 mg $FeCl_3.6H_2O$), 1 g yeast extract and 1 mL trace element solution (SL-10). The trace element solution consisted of (per Liter): 10 mL 25% HCl, 1.5 g $FeCl_2.4H_2O$, 70 mg $ZnCl_2$, 100 mg $MnCl_2.4H_2O$, 6 mg H_3BO_3, 190 mg $CoCl_2.6H_2O$, 2 mg $CuCl_2.2H_2O$, 24 mg $NiCl_2.6H_2O$, 36 mg $Na_2MoO_4.2H_2O$. The medium was adjusted to pH 7.2, flushed with 100% nitrogen and autoclaved for 15 min at 120°C. Glucose and the trace element solution were autoclaved separately. Pre-cultures were cultivated in media with a glucose concentration of 4.0 g/L. The inoculum was 10% (^{v}/v) of culture in the exponential phase. For a 2 stage fermentation experiment, the cultivation on potato steam peels hydrolysate (approx. 10 g sugar/L) was done with the omission of NH_4Cl from the medium and a decrease of yeast extract to 0.5 g/L.
Batch cultures were grown in a jacketed 2 L bioreactor (Applikon, The Netherlands) at a working volume of 1 L. The fermentor was stirred with an agitation rate of 350 rpm and H_2 was removed by continuously sparging with N_2 (7 L/h). The pH was maintained at 7.0 with KOH. Cultivation temperature for *C. saccharolyticus* was 70°C.

Photo-heterotrophic fermentation

Rhodobacter capsulatus NCIMB 11773 was obtained from the National Collection of Industrial and Marine Bacteria (NCIMB, Aberdeen, UK). Pre-cultures were grown on a modified ASY medium (Miyake *et al*., 1984), called 'SYA' medium (Succinate Yeast extract Acetate). Instead of 1.32 g/L $(NH4)_2SO_4$, 4.4 g/L butyrate, 0.1% $NaHCO_3$ and 1 mg/L of yeast extract, 2.95 g/L succinate, 0.5 g/L yeast extract and 0.6 g/L acetate were added. Pre-cultures were flushed with nitrogen. Serum flasks were filled with 250 mL of SYA medium and flushed with nitrogen to obtain anaerobic conditions.
The medium applied in the reactor consisted for two thirds of effluent from the heterotrophic thermophilic fermentation and for one third of synthetic medium to prevent nutrient limitation. The final fermentation medium contained per L: 2.89 g acetate, 1.26 g lactate and 1.94 g glucose, 0.29 g KH_2PO_4, 0.24 g K_2HPO_4, 0.66 g $MgSO_4.7H_2O$, 33 mg $CaCl_2.2H_2O$, 6.7 mg $Na_2EDTA.2H_2O$, 3.9 mg $FeSO_4.7H_2O$,

0.93 mg H_3BO_4, 0.92 mg $MnSO_4.H_2O$, 0.25 mg $Na_2MoO_4.2H_2O$, 0.08 mg $ZnSO_4.7H_2O$, 0.013 mg $Cu(NO_3)2.3H_2O$ and 0.5 g yeast extract. The pH was set at 6.8 with KOH or HCl, and the medium was subsequently autoclaved at 121°C. Macronutrients and ASY components were autoclaved separately to prevent precipitation. The vitamin solution was filter sterilized.
The inoculum for the photo-heterotrophic fermentation was from a flat panel photobioreactor and added to an initial concentration of 0.65 g dry weight/L (OD_{660} = 1.95). The fermentation was performed in a 1.5 L stirred vessel fermentor (Applicon, The Netherlands) with an internal diameter of 12.5 cm. The liquid volume was 1.4 L and the stirring rate was 300 rpm. The pH was maintained at 7.0 with 1 M HCl. The temperature was maintained at 31°C using a water bath connected to a heat exchanger placed inside the bioreactor. After inoculation the reactor was flushed with argon.
The reactor was illuminated from two opposite sides with tungsten-halogen lamps (Philips Halotone, 300 W, R7S). Between the lamps and the reactor two 3-cm water filters were placed to remove far-red thermal radiation.

Analytical methods

Optical density measurement

Optical density of cultures of *C. saccharolyticus* was measured using a Pharmacia spectrophotometer and a wavelength of 580 nm. The optical density of bacterial suspensions of *Rhodobacter capsulatus* was determined at a wavelength of 660 nm using a 20 Genesys spectrophotometer (Spectronic Instruments, USA).

Bacterial protein content and elemental composition

The protein content of the thermophilic bacterial biomass (which is approx. 50% of the biomass dry weight) was determined according to Microbiuret (Goa, 1953). The elemental composition of *C. saccharolyticus* was assumed to be $CH_{1.8}O_{0.5}N_{0.2}$.

Dry weight determination

Part of the suspension of the photosynthetic bacteria was centrifuged for 10 min. at 10000 RCF (4°C) and thoroughly washed with de-mineralized water. The final pellet was dried to constant weight at 103 – 105°C. Finally, all organic matter was burned at 550°C overnight to obtain the ash content of the sample.

H_2 and CO_2 analysis

H_2 and CO_2 production in both bioreactors were monitored at regular intervals on a CP 4900 and a CP 9000 gas chromatograph (GC) equipped with a thermal conductivity detector (Varian, The Netherlands).
Hydrogen production in the heterotrophic fermentation was measured using a MolSieve $MSA^{HI}BF$ module, the temperature of the injector and column were 60 and 80°C, respectively. CO_2 production in the heterotrophic fermentation was monitored using a Pora Plot Q, PPQHIBF module. The temperature of the injector and column were both 80°C. N_2 was used as the carrier gas.
Hydrogen production in the photo-heterotrophic fermentation was measured using a Molecular Sieve 13X, 60/80 mesh (1.8 m x $^1/_4$" x 4 mm) packed column at 100°C with argon as carrier gas at a flow rate of 20 mL/min.

Glucose and reducing sugar determination
Glucose and maltose were determined by HPLC using a Waters Shodex ionpak KC811 column and detected by differential refractometry. The mobile phase was 3 mM H_2SO_4 at a flow of 1 mL/min. The column temperature was kept at 80°C. Starch and glucose were determined enzymatically (R-Biopharm, Darmstadt, Germany) and by a modified Trinder method (Sigma, the Netherlands), respectively. Reducing sugars were determined using the DNS method (Bernfield, 1955).
Organic acid analysis
Organic acid concentrations were determined by HPLC (similar to the sugar determination described above), or, alternatively, using a GC method. A glass packed column (10% Fluorad 431 on Supelco-port 100-120 Mesh) was used at 130°C together with a flame ionization detector at 280°C. Nitrogen, saturated with formic acid, was used as a carrier gas at a flow rate of 40 mL/min. If concentrations higher than 3 g/L (= 50 mM) were expected, samples were diluted with GA medium without acetate. Diluted samples were acidified to pH 2 with formic acid (3% v/v) at a 1:1 ratio and centrifuged for 5 minutes at 10000 rpm to remove the bacteria. Finally, the supernatant was injected in the GC column.
Protein content and ash determination of potato steam peels
The protein content of potato steam peels was calculated from the determined total nitrogen content (Kjeldahl method) using a conversion factor of 6.25. Ash was determined after combustion of the material at 525 °C for 4 h. Dry matter content was determined after drying at 105 °C for 24 h.

Results and discussion

Hydrogen production from potato steam peels hydrolysate

Potato steam peels
The physical composition of potato steam peels at the gate of the potato processing industry ranges from 12 to 18% dry weight, making this by-product stream a thick slurry. The chemical composition of potato steam peels is shown in Table 1. The determined components amount to 87% of the total dry matter. The remaining 13% dry matter consists of lipids and cell walls, or more generally, cell debris. The main component in the organic acid fraction, > 65% of the total organic acids, is lactic acid. Besides lactate, acetate and succinate are commonly observed.

Table 1. Composition of potato steam peels expressed as percentage of dry matter.

Component	Quantity, %
Starch	50.7
Glucose	1.2
Protein	13.3
Organic acids	13.6
Ash	8.2
cell debris	Not determined

Hydrolysis of potato steam peels

Even though starch is easily consumed for growth and hydrogen production by thermophilic bacteria, the rheological properties are disadvantageous for efficient process technology (van Groenestijn *et al*., 2002). Thus, potato steam peels were hydrolyzed using 2 different enzyme preparations. After hydrolysis with α-amylase, starch was solubilized to mainly oligosaccharides (hydrolysate 1) and after additional hydrolysis with glucoamylase, glucose was the main end-product (hydrolysate 2, Table 2). The hydrolysis efficiency of the starch was always high, amounting to more than 95%. Both hydrolysates were tested with respect to fermentability in cultures with *Caldicellulosiruptor saccharolyticus* and allowed the same initial yield in hydrogen and acetate production. This is an important finding in view of future process technology, because the omission of the second hydrolysis step using glucoamylase will reduce the number of process units. Furthermore, in the chain of events from potato steam peels production up to the thermophilic fermentation, the temperature remains above 70 ºC. The potential application of thermostable α-amylase preparations for the hydrolysis of the potato steam peels will significantly contribute to increased process efficiency.

Nitrogen analysis of the solid and liquid fraction after hydrolysis showed that roughly 80% of the initial protein was retained in the solid fraction. This means that in this fraction, the protein concentration had increased to over 30% of the dry matter. The increased ratio in protein over carbohydrates makes this by-product a promising feedstock for fodder production as the nutritious value is increased as compared to potato steam peels.

Table 2. Composition of hydrolysates made from potato steam peels using α-amylase (hydrolysate 1) and glucoamylase (hydrolysate 2) enzyme preparations.

Hydrolysate	**Component**	**Concentration** (mM)
Hydrolysate 1	Oligosaccharides	102.0
	Maltose	15.4
	Glucose	16.0
Hydrolysate 2	Maltose	4.4
	Glucose	376.0

Table 3. Production of hydrogen and organic acids by *Caldicellulosiruptor saccharolyticus* during growth on glucose or hydrolysate of potato steam peels. Glc: Glucose, Ac: acetic acid.

Medium composition /Carbon source	Production, mM			Molar ratio, Glc : H_2 : Ac	Maximum H_2 productivity, mmol/L.h
	hydrogen	acetate	lactate		
Complete /glucose	130	74	4	1 : 2.5 : 1.4	10.7
Complete /hydrolysate	218	89	0	1 : 4.2 : 1.7	11.7
KP_i+Cys /hydrolysate	103	45	12	1 : 4.0 : 1.7	2.2

Fermentation of potato steam peels hydrolysate

First, the total production of hydrogen and acetate in cultures of *C. saccharolyticus* growing on glucose was assessed. This was then compared to production in cultures growing on an equivalent amount of sugars in a potato steam peel hydrolysate. This hydrolysate was produced using α-amylase and

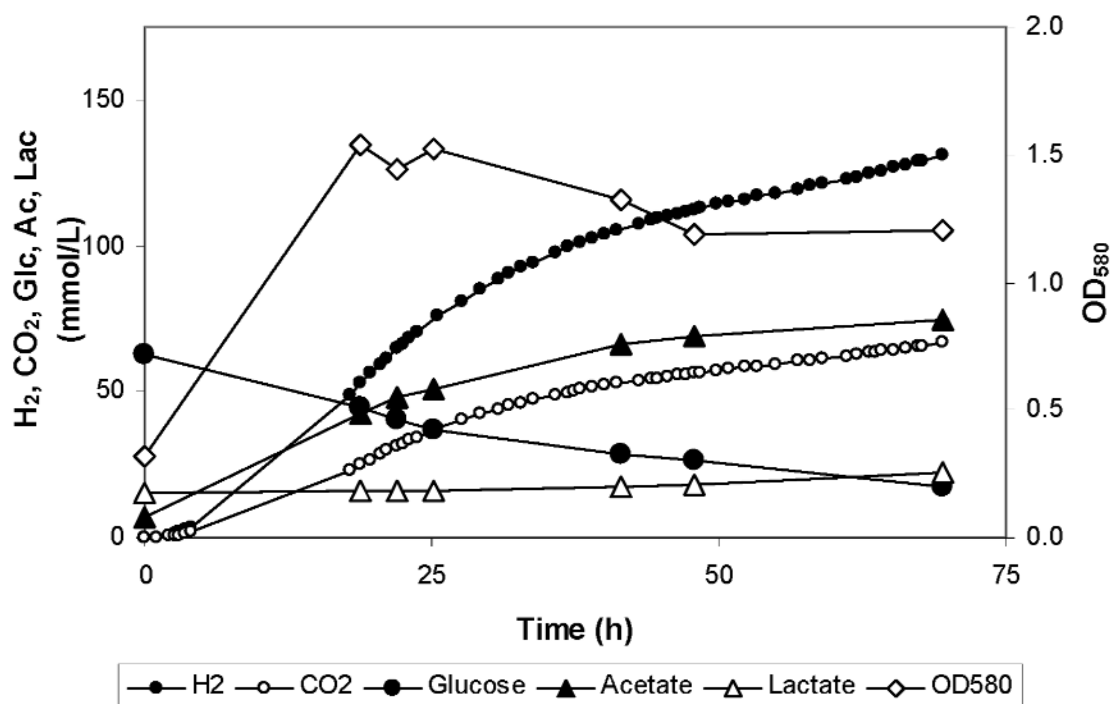


Figure 3. Growth and hydrogen production by *Caldicellulosiruptor saccharolyticus* during fermentation of hydrolysate of potato steam peels without NH_4^+.

glucoamylase (hydrolysate 2, Table 2). The duration of the fermentation of cultures growing on complete medium was circa 24 h after which the maximum optical density was reached. When the medium was simplified to reduce costs, by omission of yeast extract, NH_4^+ and Mg^{2+} salts, NaCl, $FeCl_3$ and trace elements, the fermentation proceeded at a much slower rate. During growth on hydrolysate the total production of hydrogen was higher than estimated from the consumed glucose (Table 3). This finding suggests that other components, e.g. residual oligosaccharides or proteins, in hydrolysate of potato steam peels are also metabolized to hydrogen contributing to a high hydrogen production, seemingly exceeding the maximal theoretical production of 4 mol H_2/mol glucose.

In Figure 3 the fermentation of potato steam peels hydrolysate by *C. saccharolyticus* is shown as a function of time. The hydrolysate was made using α-amylase and glucoamylase and in the medium yeast extract concentration was lowered to 0.5 g/L and NH_4^+ was omitted in view of the consecutive photo-heterotrophic fermentation. Initially the hydrogen production rate increased rapidly to > 4 mmol H_2/L.h, but decreased after 18 h of fermentation. Not all glucose was consumed, possible due to an as yet undefined limitation or to exhaustion of nitrogen as a result of the omission of NH_4^+. The culture was continuously sparged with nitrogen to remove hydrogen as soon as it was formed. This resulted in a hydrogen concentration of 1.5 % (^{v}/v) in the off-gas of the thermophilic reactor.

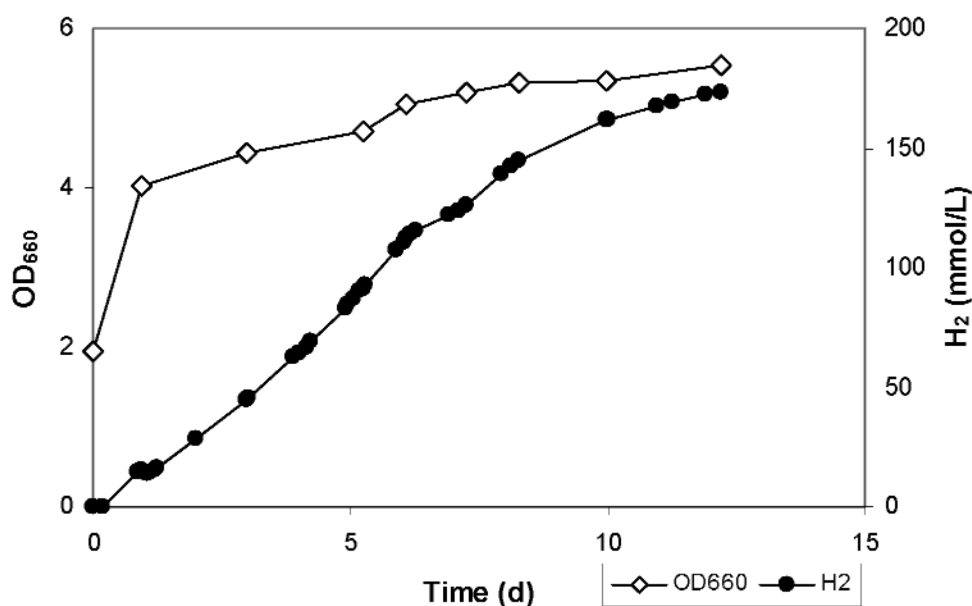


Figure 4. Growth and hydrogen production by *Rhodobacter capsulatus* during growth on the effluent of the thermophilic fermentation described in Figure 3.

The effluent from the thermophilic reactor was centrifuged, autoclaved, diluted and filtered (0.2 mm) before it was used as a substrate for the photo-heterotrophic fermentation with *Rhodobacter capsulatus*. The fermentation was done under a light intensity of 166.6 W m^{-2}. The fermentation liquid contained acetate (48.2 mM), lactate (14.0 mM) and glucose (10.8 mM). In Figure 4 the fermentation of organic acids by *Rhodobacter capsulatus* is shown as a function of time. During the first day of the fermentation the biomass density doubled. Apparently the effluent still contained sufficient dissolved nitrogen to allow photo-heterotrophic growth. Hydrogen production was not hampered by the presence of nitrogen-containing compounds and was 1.5 mmol H_2/L.h at its highest with a final concentration of >85% (^{v}/v) hydrogen in the off-gas. At the end of the first day a small feed of NH_4^+ was supplied to the bacteria at a feed rate of 0.052 mmol/h. Since the hydrogen production rate decreased, the feed rate was decreased to 0.015 mmol/h and the hydrogen production rate was restored. The addition of ammonia therefore needs to be studied further.
Because of the reactor configuration, the light regime could not be optimized and this fermentation suffered from light limitation. Therefore, the focus here is on the conversion of acetate to biomass and hydrogen. The most interesting aspect of this fermentation is the fact that hydrogen production occurred at a low specific growth rate. The highest H_2 production was observed on day 5: 1.5 mmol/L.h, which corresponds to an acetate consumption of 0.38 mmol/L.h. This implies that 13% of the acetate consumed is directed to biomass growth and 87% to hydrogen production.
The total hydrogen yield achieved in the thermophilic fermentation and the subsequent photofermentation showed an overall efficiency of 47%. Since the maximum achievable yield is estimated at 69%, based on 80% efficiency of the thermophilic fermentation (where one third of the hydrogen is produced) and 80% efficiency of the photo-heterotrophic fermentation, this 2 stage bioprocess seems very promising.

Conceptual design of a biohydrogen production plant

To investigate the technical and economical feasibility of biological hydrogen production from potato steam peels a conceptual design was made for an industrial production plant. In this production plant, the aim was to convert 6400 ton potato steam peels (dry weight) per year, being circa one-tenth of the annual production in the Netherlands, to hydrogen which meets the specifications for fuel cell application. The unit-operations in this conceptual design covered the hydrolysis, thermophilic and photo-heterotrophic fermentation, gasstripping, physical absorption and heat pumps. A simplified flow sheet is given in Figure 5.
Per hour 800 kg (dry weight) potato steam peels are introduced in the bioprocess, of which circa 400 kg/h is hydrolyzed to glucose. Protein-rich solids (containing most of the N and S) are separated from the hydrolysate and the glucose-rich liquid fraction is introduced in the first packed-bed reactor (van Groenestijn *et al.*, 2002) with thermophilic bacteria (volume 450 m^3). The gas mixture produced in this reactor is compressed and cooled to recover water and heat, which are both returned to the reactor. The final H_2/ CO_2/ H_2O gas mixture with circa 50% (^{v}/v) hydrogen is introduced in a PEM fuel cell.

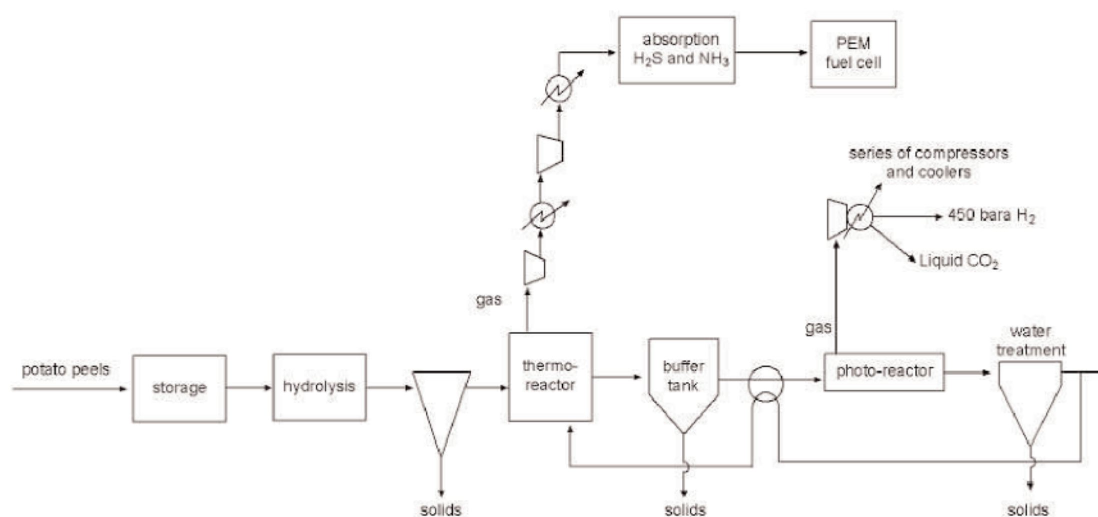


Figure 5. Simplified flow sheet of an industrial plant for the biological production of hydrogen from potato steam peels.

The effluent, containing acetate and some bacteria, is transported to a buffer tank. After removal of solids (bacteria), the clarified supernatant is transported to the second, tubular photo-bioreactor with an area of circa 10 ha, where photo-heterotrophic bacteria convert all organic acids to hydrogen and CO_2.

The gas produced in the photo-bioreactor contains more than 85% H_2 (^{v}/v), because CO_2 is largely absorbed in the water phase and released again in the thermo-reactor. This gas is compressed and cooled to H_2 at 450 bara, which can be used as transportation fuel, and to liquid CO_2, which can be used for sequestration.

The used water is pumped to a storage unit in which biological treatment takes place and all solids are removed.

Most process water with bicarbonate and cations for buffering, is returned to the thermo-reactor. In the bioprocess allowance for storage and buffer tanks is made because of the discontinuous character of the photo-heterotrophic fermentation (dependent on light). Also, a heat exchanger is included to transfer heat from the water effluent of the thermophilic fermentation to the water effluent of the photo-heterotrophic fermentation (35 °C).

The plant produces 57 kg H_2/h, of which 17 kg is produced in the first reactor by thermophilic bacteria using the carbohydrates in the feedstock. In the second reactor, the photo-heterotrophic bacteria convert the produced acetate as well as the organic acids initially present in the feedstock, to another 40 kg H_2/h. As a by-product from the hydrolysis and also from the fermentations, > 500 kg/h protein-rich solids are produced, which can be used as cattle feed.

In the conceptual design, liquid absorption for removal of H_2S and NH_3, if required, has been considered. Besides, heat is exchanged between demand and cooling, as much as possible e.g. by using the heat from the fuel cell to supply heat to the feed of the thermoreactor. Only 86 kW of external energy is required for pumps and

compressors (excluding the final compression to 450 bara), which is low as compared to the 1900 kW comprised in the hydrogen gas produced.

Table 4: Techno-economic evaluation of a conceptual design for biological hydrogen production from potato steam peels.

costs	**€/kg H_2**
Depreciation, maintenance, insurances and overhead	2.01
Personnel	0.17
Potato steam peels	0.68
Enzymes for hydrolysis	0.00
Caustic	0.11
H_2S absorbens	0.05
Electricity	0.13
Total cost	***3.10***

Cost analyses, which include capital, insurance, overhead, personnel, maintenance, raw material and electricity costs are shown in Table 4.
In this conceptual design, one ton of potato steam peels costs €50/ ton dry weight. The investments of the plant amount more than 6 million Euro. A preliminary estimate of the final production costs for hydrogen from potato steam peels amount to €3.10/kg H_2 which is equivalent to €22/GJ H_2.

Conclusions
Amongst the new initiatives to produce sustainable hydrogen, biological hydrogen production offers an elegant opportunity to convert wet biomass to pure hydrogen in small scale installations. The conceptual design of a bioprocess for biohydrogen production shows great promise and identifies potential improvements of the various process steps to achieve all desired conversion efficiencies and bioreactor volumetric capacities. The most important challenges are the increase in productivity by more efficient bioconversion and the decrease of investment cost by improved cost-effective bioreactor design.

Acknowledgements
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The Recycling of Food Wastes Within Aquatic Food Production Systems: Aquaculture - The Aquatic Blue Revolution Phenomenon

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Introduction

There is no doubt that the third millennium will herald marked changes in our global food production systems. The over-riding reason for these changes is the stark realization (after years of environmental abuse) that our planet has it's limits, both in terms of it's available natural resources (i.e., land, water, energy, nutrients, natural biota), and through its ability and capacity to harness and recycle these resources and sustain life as we currently know it. All too often our planet has been viewed as a limitless resource for the sole exploitation and enjoyment of mankind, rather than as a fragile living ecosystem of inter-dependent plants and animals. Through the activities of our modern societies (and the development of our towns, cities, agriculture and industries), we are now negatively impacting all things, from the air that we breath, the water that we drink, the food that we eat, the land that we live on, to the very weather of our planet and the well-being of all living things.

The upshot of the above is that in future all our food production systems, will have to become increasingly more environmentally and ecologically *responsible* if they are to be truly *sustainable* in the long run and be socially accepted as an economically viable means of producing food for an ever hungry population. With world population expected to reach 8 billion by 2030, pressure on the environment will continue to mount. The challenge of the coming years is to produce enough food to meet the needs of an additional 2 billion people while preserving and enhancing the natural resource base upon which the well-being of present and future generations depends.

The present paper is a follow-up to a presentation I gave in London over 25 years ago at the International Symposium on Effluent Treatment in the Biological Industries entitled *Nutritional evaluation of animal and food processing wastes* (Tacon, 1979). In particular, the present paper reviews the current and potential use of the major food waste streams by the aquaculture sector and discusses the potential future role which the sector could play in recycling available agricultural and fishery waste streams attempts into much needed high quality nutritionally-sound farmed aquatic food produce.

Aquaculture production

Definition
According to the Food and Agriculture Organization of the United Nations (FAO, 2004a), aquaculture is the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators etc. farming also implies individual or corporate ownership in the stock being cultivated. For statistical purposes, aquatic organisms which are harvested by an individual or corporate body which has owned them throughout their rearing period contribute to aquaculture while aquatic organisms which are exploitable by the public as a common property resource, with or without appropriate licenses, are the harvest of fisheries.

A long history
Aquaculture is not new, with the first known monograph *Treatise on Fish Culture* written by Fan Li in 473 BC. However, with the exception of China and the Asian and Pacific region, where fish culture has traditionally played an important role as a provider of much needed food fish, it was not until the twentieth century that aquaculture emerged on to the global arena as a major food-production sector and economic activity capable of rivaling the terrestrial animal-livestock farming sector (Tacon, 1003).

The fastest growing food sector
Total reported global aquaculture production reached a new high of 51.4 million metric tons (mmt) in 2002 (up by 6.1% from the previous year) or over half that of total global capture fisheries landings (94.5 mmt; FAO, 2004a). Valued at US $ 60 thousand million and based on the production of 250 different plant and animal species in over 180 countries, aquaculture has been the fastest growing segment of global food production for over three decades (Tacon, 2004a). Global aquaculture production has been growing at an average compound rate of 8.7% per year since 1970, compared with 2.9% per for total terrestrial meat production, and 1.2% for total capture fisheries landings (Figure 1).

Major species groups
Over half of total global aquaculture production in 2002 was in the form of finfish (25.73 mmt, valued at $ 31.95 thousand million), followed by molluscs (11.78 mmt, $ 10.51 thousand million), aquatic plants (11.59 mmt, $ 6.19 thousand million), crustaceans (2.13 mmt, $ 10.84 thousand million), amphibians and reptiles (123,473 mt, $ 469 million), and miscellaneous aquatic invertebrate animals (31,430 mt, $ 26 million; Figure 2 & 3).

Production by economic country grouping
Approximately 91.7% and 8.3% of total aquaculture production was produced within developing countries (47.12 mmt) and developed countries (4.26 mmt) in 2002, with total production valued at US $ 49.3 billion and 10.6 billion, respectively (FAO, 2004a). Moreover, over the last decade aquaculture production within developing countries has been growing over 7-fold faster than within developed countries; the latter almost totally due to the rapid reported development and growth of the aquaculture sector in China (Figure 4).

Although aquaculture production within developed countries represented only 8.3% of total aquaculture production by weight in 2002, they represented 17.7% of total production by value; the bulk of finfish production within these countries currently focused on the culture of higher value (in marketing terms) carnivorous finfish species (73.5% of the top twenty total cultivated finfish and crustacean species being carnivorous fish species; Figure 5). By contrast, finfish production within developing countries is still largely concentrated upon the production of lower value (in marketing terms) omnivorous/herbivorous and filter feeding fish for domestic consumption (Figure 6).

Production by geographic region and country
By region over 91.2% of total aquaculture production was produced within the Asian region (46.86 mmt, Figure 7) in 2002, followed by Europe (2.05 mmt or 4.0%), Latin America and the Caribbean (1.19 mmt or 2.3%), North America (0.67 mmt or 1.3%), Africa (0.46 mmt or 0.9%) and Oceania (0.15 mmt or 0.3%), as follows:

Asian region: 16.7-fold increase in production from 2.8 mmt in 1970 to 46.86 mmt in 2002, with the sector growing at a rate of 9.2%/year since 1970. China is by far the largest aquaculture producer in Asia and the world, producing 71.2% of total global aquaculture production in 2002 (Figure 11), with eight of the top ten aquaculture producing countries being located within the Asian region in 2002 (Table 1). By value aquaculture production within the region has increased over 5.2-fold from $ 9.4 billion in 1984 to $ 49.2 billion in 2002 (or 82.0% total global aquaculture production by value).

European region: 4.1-fold increase in production from 0.50 mmt in 1970 to 2.04 mmt in 2002, with the sector growing at a rate of 4.5%/year since 1970. Norway is currently the leading aquaculture producer in Europe at 553,933 mt or 27.1% of total European aquaculture production; Table 2). By value aquaculture production within the region has increased over 3.1-fold from $ 1.42 billion in 1984 to $ 4.49 billion in 2002 (or 7.5% total global aquaculture production by value).

Latin American and Caribbean region: 978-fold increase in production from 1,221 mt in 1970 to 1,194,506 mt in 2002, with the sector growing at a rate of 24.0% since 1970. Chile is by far the largest aquaculture producer in the region at 617,303 mt or 51.7% of production (Table 3). By value, aquaculture production within the region has increased over 9.8-fold from $ 337 million in 1984 to $ 3.32 billion in 2002 (or 6.5% total global aquaculture production by value).

North American region (USA and Canada): 3.9-fold increase from 172,272 mt in 1970 to 669,682 mt in 2002, with the sector growing at a rate of 4.3%/year since 1970; Table 4). By value, aquaculture production within the region has increased over 2-fold from $ 498 million in 1984 to $ 1.72 billion in 2002 (or 1.8% total global aquaculture production by value).

African region: 44.7-fold increase from 10,271 mt in 1970 to 459,897 mt in 2002, with the sector growing at a rate of 12.6%/year since 1970. Egypt is by far the largest aquaculture producer in the region at 376,296 mt or 81.8% of production (Table 5). By value, aquaculture production within the region has increased over 29-fold from $ 29 million in 1984 to $ 843 million in 2002 (or 1.4% total global aquaculture production by value).

Oceania: 18.4-fold increase from 8,421 mt in 1970 to 154,885 mt in 2002, with the sector growing at a rate of 9.5%/year since 1970. New Zealand is currently the largest producer by weight in the region at 86,583 mt (55.9% total production), although Australia is the largest producer by value at $ 256 million (Table 6). The total value of aquaculture production within the region has increased over 9-fold from $ 32 million in 1984 to $ 389 million in 2002 (or 0.6% total global aquaculture production by value; FAO, 2004a).

Farming systems and feeding methods

In general terms, the farming systems currently employed by finfish and crustacean farmers can be broadly divided into either extensive, semi-intensive or intensive farming systems. Feeding methods used by farmers within these farming systems can be broadly categorized as follows:

No feeding or nutrient application - typical of traditional extensive farming systems where finfish and/or crustacean growth and production is totally dependent upon the consumption of natural food organisms present within the culture environment (may include phytoplankton, zooplankton, benthic animals and plants, bacteria etc);

Fertilization or fertilizer nutrient application – typical of extensive and/or semi-intensive farming systems where chemical fertilizers and/or organic manures are applied to the culture environment to augment the endogenous (*in situ*) production of natural food organisms for the cultured finfish and/or crustacean species;

Fertilizer and/or supplementary feed nutrient application - typical of semi-intensive farming systems where finfish and/or crustacean growth is dependent upon the co-feeding of endogenously produced natural food organisms (the production of which is enhanced through the application of fertilizers) and/or external supply of supplementary feed inputs (the latter ranging from single unprocessed agricultural feed items, processed farm-made feed mashes, to industrially compounded commercial aquafeeds); and

Complete feed nutrient application - typical of intensive farming systems where finfish

and/or crustacean growth is totally dependent upon the external provision of a nutritionally complete diet for the entire culture period; the latter usually supplied in the form of a formulated commercial aquafeed or to a lesser extent, in the form of a farm-made aquafeed or fresh food item such as low-cost fish or seafood products.

The choice of the feeding method employed by individual farmers is largely dependent upon the intended farming system, fish/shrimp species grown, and fish/shrimp stocking density employed (and consequent natural availability per stocked animal), the resources available to the farmer in terms of inputs and financial, and the market value of the cultured species. Thus feeding methods typically may range from low cost extensive/semi-intensive fertilization/supplementary feeding methods (the latter usually employing locally available feed resources in the form of farm-made aquafeeds) in the case of small-scale farming operations, to the use of intensive fertilization/feeding methods (the latter usually in the form of industrially compounded aquafeeds) in the case of large-scale commercial farming operations.

Intensively fed species and aquafeed production by species

It has been estimated about 21.0 mmt or 40.9% of global aquaculture production in 2002 is currently dependent upon the supply and use of industrially compounded aquafeeds, including all non-filter feeding cyprinids, tilapia, miscellaneous freshwater fish species, diadromous fish species, marine fish species, and farmed crustacean species.

Major species groups currently dependent upon the use of compound aquafeeds in 2002, included:

- **Non-filter feeding carp species** (i.e. grass carp, common carp, crucian carp, white amur bream etc.), with an estimated 40% of total farmed carp production in 2002 currently using industrially compounded aquafeeds. Assuming a modest economic species Feed Conversion Ratio (FCR) of 2.0 for feeding carp (i.e. 2 units dry feed farm input to 1 unit wet fish farm output) and a total carp species (non-filter feeding species) production of 9.853 mmt in 2002 it is estimated that the total production of carp feeds was 8.276 mmt or 46.8% of total estimated global aquafeed production (Figure 8).

Major estimated carp feed markets (values given in mt)*

	2000	2001	2002	2003
China:	4,500,000	5,000,000	5,500,000	6,000,000
Indonesia:	200,000	250,000	275,000	300,000
Others:	300,000	350,000	400,000	500,000
Total*:	**5,000,000**	**5,600,000**	**6,175,000**	**6,800,000**

* Total/others: value could be considerably higher as Indian major carps in for example India and Bangladesh are still largely fed on farm-made aquafeed mashes

- **Salmonid species** (i.e. Atlantic salmon, rainbow trout, coho salmon etc), with an estimated 100% of total farmed salmonid production in 2002 currently using industrially compounded aquafeeds. Assuming an average economic species FCR of 1.3 for salmonids and a total salmonids production of 1.784 mmt in 2002 it is estimated that the total production of salmonid feeds was 2.319 mmt or 13.0% of total estimated global aquafeed production (Figure 8).

Major estimated salmonid feed markets (values given in mt)*

	2000	2001	2002	2003
Norway:	635,000	645,000	660,000	700,000
Chile:	480,000	655,000	625,000	675,000
UK:	182,000	197,000	208,000	220,000
Canada:	123,000	152,000	178,000	190,000
Faeroe Islands:	42,000	67,000	66,000	70,000
France:	57,000	64,000	63,000	65,000
USA:	64,000	60,000	50,000	60,000
Others1:	450,000	468,000	430,000	450,000
Total:	**2,033,000**	**2,308,000**	**2,280,000**	**2,430,000**

Others1: Value calculated by multiplying total salmonid production within remaining countries with a mean economic FCR of 1.3

- **Marine shrimp species** (i.e. Giant tiger prawn, fleshy prawn, whiteleg shrimp, banana prawn etc), with an estimated 85% of total farmed shrimp production in 2002 currently using industrially compounded aquafeeds. Assuming an average economic species FCR of 1.9 for marine shrimp and a total shrimp production of 1.292 mmt in 2002 it is estimated that the total production of shrimp feeds was 2.086 mmt or 11.7% of total estimated global aquafeed production (Figure 8).

Major estimated shrimp feed markets (values given in mt)

	2000	2001	2002	2003
China:	276,000	350,000	500,000	700,000
Thailand:	486,000	545,000	525,000	532,000
Indonesia:	152,000	200,000	250,000	300,000
Viet Nam:	65,000	80,000	150,000	220,000
India:	100,000	90,000	115,000	140,000
Brazil:	25,000	48,000	80,000	145,000
Ecuador:	105,000	98,000	110,000	100,000
Bangladesh:	75,000	60,000	80,000	90,000
Mexico:	55,500	70,000	80,000	90,000
Philippines:	30,000	50,000	60,000	70,000
Taiwan1:	50,000	55,000	57,000	60,000
Malaysia:	25,000	30,000	40,000	50,000
Honduras:	27,000	20,000	25,000	30,000
Colombia:	7,600	20,000	25,000	30,000
Venezuela:	15,000	20,000	25,000	30,000
Others:	50,000	75,000	100,000	150,000
Total:	**1,544,100**	**1,811,000**	**2,222,000**	**2,737,000**

Taiwan1:A large proportion of aquafeed production in Taiwan is exported to neighboring countries, including mainland China

- **Marine finfish species** (i.e. all unidentified reported marine fish species from China, Japanese amberjack, gilthead seabream, silver seabream, European seabss, Bastard halibut, croakers, groupers etc.), with an estimated 65% of total farmed marine finfish production in 2002 currently using industrially compounded aquafeeds. Assuming a modest economic species FCR of 2.0 for marine finfish and a total marine finfish production of 1.201 mmt in 2002 it is estimated that the total production of marine finfish feeds was 1.561 mmt or 8.7% of total estimated global aquafeed production (Figure 8). However, this figure may be lower since a large proportion of Chinese marine finfish production is still largely fed on low value trash fish and precise data feed production in China is difficult to come by.

Major estimated marine finfish feed markets (values given in mt)*

	2000	2001	2002	2003
China[1]:Still largely fed on trash fish ...little data				
Japan:	320,000	330,000	340,000	350,000
Greece:	135,000	135,000	125,000	130,000
Taiwan[2]:	70,000	80,000	90,000	100,000
Korea Rep:	45,000	60,000	75,000	100,000
Turkey:	66,000	57,000	52,000	55,000
Spain:	34,000	40,000	47,000	53,000
Others:[3]	66,000	75,000	75,000	80,000
Total:	**736,000**	**777,000**	**804,000**	**868,000**

[1]China: Although China is the worlds largest producer of farmed marine finfish (ca. 520,000 mt in 2003), the bulk of production is still largely fed on trash fish – however, the pelleted feed industry is starting to grow to meet domestic demands – potentially over 1 mmt in China alone

[2]Taiwan:A large proportion of aquafeed production in Taiwan is exported to neighboring countries, including mainland China

[3]Others: Value calculated by multiplying total marine finfish production within remaining countries with a mean economic FCR of 2.0 – with the assumption that only 50% of production is currently using pelleted aquafeeds

- **Tilapia** (i.e. Nile tilapia, unidentified reported tilapia species etc.), with an estimated 45% of total farmed tilapia production in 2002 currently using industrially compounded aquafeeds. Assuming a modest economic species FCR of 2.0 for tilapia and a total tilapia production of 1.50 mmt in 2002 it is estimated that the total production of tilapia feeds was 1.35 mmt or 7.6% of total estimated global aquafeed production (Figure 8);

Major estimated tilapia feed markets (values given in mt)*

	2000	2001	2002	2003
China:	566,000	604,000	636,000	650,000
Philippines:	139,000	160,000	184,000	206,000
Indonesia:	127,000	157,000	165,000	173,000
Thailand:	124,000	148,000	151,000	154,000
Taiwan:	33,000	55,000	57,000	58,000
Brazil:	32,000	36,000	42,000	48,000
Others[1]:	140,000	145,000	165,000	170,000
Total:	**1,161,000**	**1,305,000**	**1,400,000**	**1,459,000**

Others[1]: Value calculated by multiplying total tilapia production within remaining countries with a mean economic FCR of 2.0 – with the assumption that only 50% of production is currently using pelleted aquafeeds

- **Catfish** (i.e. Channel catfish, Torpedo shaped catfish, catfish hybrids etc), with an estimated 87% of total farmed catfish production in 2002 currently using industrially compounded aquafeeds. Assuming an average economic species FCR of 1.6 for catfish and a total catfish production of 0.515 mmt in 2002 it is estimated that the total production of catfish feeds was 0.717 mmt or 4.0% of total estimated global aquafeed production (Figure 8);

Major estimated catfish feed markets (values given in mt)*

	2000	2001	2002	2003
USA:	405,000	410,000	430,000	440,000
Thailand:	150,000	150,000	160,000	175,000
Indonesia:	30,000	50,000	75,000	100,000
Others[1]:	30,000	35,000	70,000	100,000
Total:	**615,000**	**645,000**	**735,000**	**815,000**

Others[1]: Value calculated by multiplying total catfish production within remaining countries with a mean economic FCR of 2.0 – with the assumption that only 50% of production is currently using pelleted aquafeeds

- **Freshwater crustacean species** (i.e. Chinese river crab, giant river prawn, red swamp crawfish etc.), with an estimated 43% of total farmed freshwater crustacean production in 2002 currently using industrially compounded aquafeeds. Assuming an modest economic species FCR of 2.4 for these crustaceans and a total species group production of 0.592 mmt in 2002 it is estimated that the total production of freshwater crustacean feeds was 0.611 mmt or 3.4% of total estimated global aquafeed production (Figure 8);

<u>Major estimated freshwater prawn feed markets</u> (values given in mt)*

	2000	2001	2002	2003
China:	100,000	130,000	115,000	125,000
Thailand:	10,000	15,000	30,000	50,000
India	20,000	30,000	40,000	50,000
Others[1]:	20,000	20,000	20,000	25,000
Total:	**150,000**	**195,000**	**205,000**	**250,000**

Others[1]: Value calculated by multiplying total freshwater prawn production within remaining countries with a mean economic FCR of 2.0 – with the assumption that only 50% of production is currently using pelleted aquafeeds

- **Milkfish** with an estimated 45% of total farmed milkfish production in 2002 currently using industrially compounded aquafeeds. Assuming a modest economic species FCR of 2.0 for milkfish and a total milkfish production of 0.528 mmt in 2002 it is estimated that the total production of milkfish feeds was 0.475 mmt or 2.7% of total estimated global aquafeed production (Figure 8);

<u>Major estimated milkfish feed markets</u> (values given in mt)*

	2000	2001	2002	2003
Philippines:	170,000	180,000	190,00	200,000
Indonesia:	175,000	170,000	180,000	185,000
Taiwan[1]	150,000	160,000	170,000	182,000
Total:	**495,000**	**510,000**	**540,000**	**567,000**

Taiwan[1]: A proportion is exported to neighboring countries

- **Eel** (i.e. Japanese eel, European eel etc.), with an estimated 82% of total farmed eel production in 2002 currently using industrially compounded aquafeeds. Assuming a modest economic species FCR of 2.0 for eel and a total eel production of 0.232 mmt in 2002 it is estimated that the total production of eel feeds was 0.380 mmt or 2.1% of total estimated global aquafeed production (Figure 8);

Major estimated milkfish feed markets (values given in mt)*

	2000	2001	2002	2003
China:	260,000	250,000	260,000	255,000
Taiwan:	60,000	60,000	70,000	70,000
Japan:	25,000	25,000	25,000	25,000
Others1:	20,000	20,000	10,000	15,000
Total:	**365,00**	**355,000**	**365,000**	**365,000**

Others[1]: Value calculated by multiplying total eel production within remaining countries with a mean economic FCR of 2.0 – with the assumption that only 50% of production is currently using pelleted aquafeeds

Global aquafeed production by country

In global terms, the estimated total production of compound aquafeeds in 2002 was approximately 17.77 mmt (Figure 8). Preliminary estimates for 2003 have put global aquafeed production at 18.36 mmt or about 3% of total compound animal feed production of 612 mmt (Gill, 2004a; Figure 9). China is currently the world's largest aquafeed producer, with total production of industrially compounded aquafeeds reported as 7.98 mmt in 2003 (Zhang Jian, American Soybean Association, Bejing, China – personal communication, May 28, 2004). The top compound aquafeed producers in 2003, together with their estimated total compound aquafeed production in 2003, are listed in Table 7. These countries currently produce over three quarters of total estimated global aquafeed production in 2003. However, it must be stated that the majority of the figures are still largely estimates and not official statistical information.

Table 7. Top estimated aquafeed manufactures by country in 2003

China*:	7.98 mmt
Indonesia:	1.033 mmt
Thailand:	0.800 mmt
Norway:	0.700 mmt
Chile:	0.700 mmt
USA:	0.500 mmt
Philippines:	0.500 mmt
Japan*:	0.467 mmt
Taiwan*:	0.388 mmt
Brazil:	0.367 mmt
India:	0.335 mmt
Viet Nam:	0.300 mmt
Sub-total	**14.07 mmt**

*Official/published statistical information

Food processing wastes currently recycled as feed inputs

Aquaculture has been an important recycler of agricultural food wastes, particularly within China and the Asian region, for over two millennia. Particular emphasis within developing countries has been placed on the important nutritional role played by aquaculture as a much needed provider of high quality and affordable food products for human consumption and recycler of locally available agricultural waste products as feed inputs (Tacon, 2001).

Agricultural food processing wastes which have been successfully recycled and used within farm-made and industrially compounded aquafeeds have included (in order of availability and importance):

- Cereal/milling byproducts - rice bran, rice polishings, broken rice, wheat bran, wheat middlings, wheat mill run, composted rice hulls/straw (used mainly within industrially compounded aquafeeds);
- Plant oilseed byproducts – fat extracted and non-extracted oilseed meals (soya, rape, cotton, mustard, groundnut, coconut, palm kernel; used both within industrial and farm-made aquafeeds; New *et al*. 1995);
- Miscellaneous food crop wastes and byproducts – discarded/spoiled fruit, tubers and roots, kitchen scraps, green fodder and grass cuttings (used mainly within farm-made aquafeeds; New *et al*. 1995);
- Brewing/fermentation byproducts: brewers grains, distillers solubles, extracted yeast products;
- Animal/rendered byproducts: meat meal, meat & bone meal, feather meal, poultry byproduct meal, fresh blood and blood meal, organ meals (liver, lung, kidney, heart), rumen and rumen contents, fats and tallows;
- Fishing/fishery wastes and byproducts: rendered fish/crustacean meals produced from fish canneries (tuna fishmeal), shrimp processing plants (shrimp head meal, krill meal), feed-grade industrial fisheries (sand eel, menhaden), and stabilized (fermented/ensiled) fish and seafood processing waste (used mainly within farm-made aquafeeds for high value carnivorous finfish and crustacean species).

Concluding remarks

It is important to highlight here that the increased use of adequately processed terrestrial animal by-product meals within compound aquafeeds would be seen as a means of safely recycling animal by-products from terrestrial warm-blooded farm animals through a completely different animal food chain, namely through cold-blooded aquatic animals - farmed finfish and crustaceans. Moreover, the dietary replacement of fishmeal and other marine resources within aquafeeds would turn the commercial aquaculture sector (i.e. for high-value carnivorous finfish/crustacean species) into a net fish producing industry rather than a net fish consuming industry

as it is now (Tacon, 2004b), but more importantly aquaculture would be seen by the public as a means of utilizing and converting hitherto non-food grade products and potential environmental health hazards (i.e. terms of disposal through dumping or incineration) into high-quality, nutritious, and safe food-grade aquatic farmed animal products.

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Sustainable use of Food Processing Wastes: Livestock Feed or Bioenergy?

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Introduction

The food processing industry produces large quantities of waste products. In the Netherlands the agricultural waste-streams are far larger than the total industrial waste-streams (Elzenga *et al*., 1996). Presently these agricultural residues are in use as fodder for livestock. In the Netherlands about 70 % of the fodder fed to pigs and cattle originates from waste-streams generated by the food processing industry (LEI, 1996). Comparable values are found on a global scale (Fadel, 1999). This livestock is more or less upgrading a waste-stream from not suitable for human consumption into a highly valued food commodity (meat). This implies that wastes from food processing industries form the basis for the production of important proteins in the human diet. Therefore the agricultural residues cannot be considered as worthless waste-streams, but have an important function in the food production system (as a basis for meat).

In principle, the available waste streams can also be used for other non-food purposes for instance as feedstock for bioenergy production. The amount of energy that can be obtained from these streams is substantial. It is estimated that in the Netherlands potentially 190 PJ can be obtained from these rest-streams (Faaij *et al*., 1997), on a global scale values of over 12 EJ are mentioned (Hall *et al*., 1993).

However, the use of these waste streams as an energy source will affect the food system, since an important source for livestock feed disappears.

This implies that using residues as energy source will affect the food production system. In the case of livestock the major source for livestock feed disappears. Since meat is an important source of proteins in the human diet, this loss of proteins must be compensated for other wise the food security of the population is affected. The magnitude of these adaptations has an important impact on the overall performance of the system. When a large amount of energy can be gained from the residues and the loss in meat production can be compensated for easily, the use of residues for energy generation has a large potential. However, when the impact of the adaptations overrule the energy gain, using residues for energy generation is not a realistic option.

This paper focuses on the question: what are the consequences of using waste streams for energy generation instead of using them for livestock feed. It studies the adaptations required in the food system to compensate for the loss of residues. The

magnitude of the trade-offs to the food system is determined through applying systems analysis. The values calculated are used in a comparison to land use consequences of bio-energy from agricultural residues and bio-energy from dedicated energy crops.

Method

System description

The present food production system is presented in Figure 1. In the primary production food crops are grown. The harvest of the primary production is converted into food items in the food industry (wheat into bread and pasta, soybeans into soy oil, sugar beet into sugar. etc). These food items are consumed by the population. Since most crops cannot be consumed as a whole residues emerge in this production process. These residues occur in various parts of the production chain. In primary production itself where only a part of the crop is sold to the food industry (wheat is sold, straw remains). In the food industry again only a part of the harvested product is used (sugar is sold, the beet pulp remains). In the present situation the remains of the human food production process are used as a basis for livestock fodder. They are fed as unprocessed residue directly to livestock or are processed in the livestock feed industry into concentrates. Livestock production results in meat, milk and eggs that are important sources of proteins in the human diet. The present system shows no interference with the energy system. If biomass is used as an energy source, this biomass originates from other sources like forests,. energy crop plantations etc.

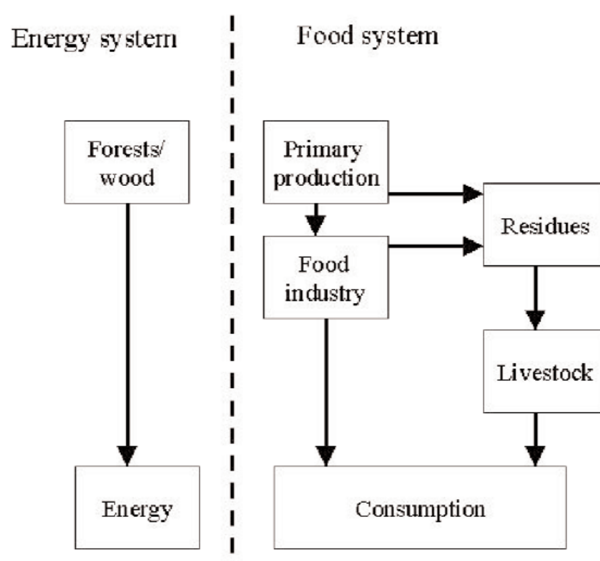

Figure 1. Schematical presentation of the present food-energy system (Energy crops system). Residues are used as livestock fodder and bio-energy is obtained from dedicated energy crops.

When the residues in the present food system are used for non-food purposes (energy) changes in the food system are likely to occur. In that case the main source for livestock feed disappears and since livestock fulfills an important function in the menu as protein source, replacement for these proteins has to be found. Here two options are analysed. First, a change of the population to vegetarian diets. A change to a vegetarian menu involves more than 'just leaving out the meat', in general the animal proteins in the menu are replaced with vegetable proteins from beans and pulses. This implies that extra protein crops have to be grown to fulfil the protein requirements of the population. Figure 2 shows this vegetarian system.

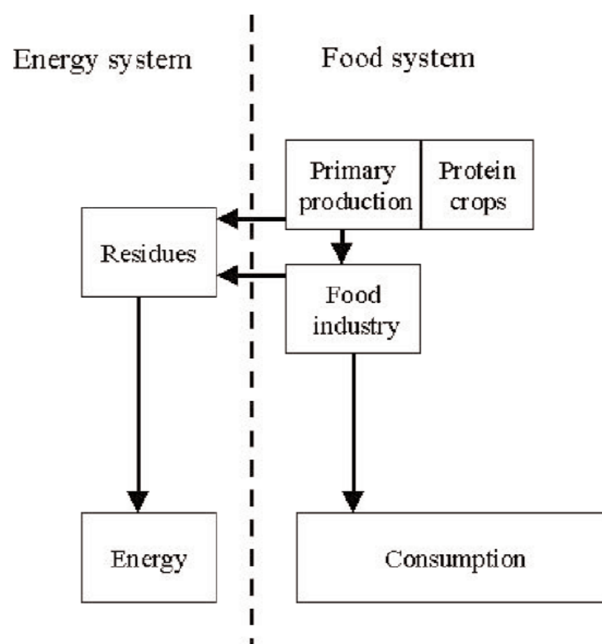

Figure 2. Schematical presentation of the Vegetarian System. Residues are used for energy generation and protein crops fulfil the protein requirements of the population.

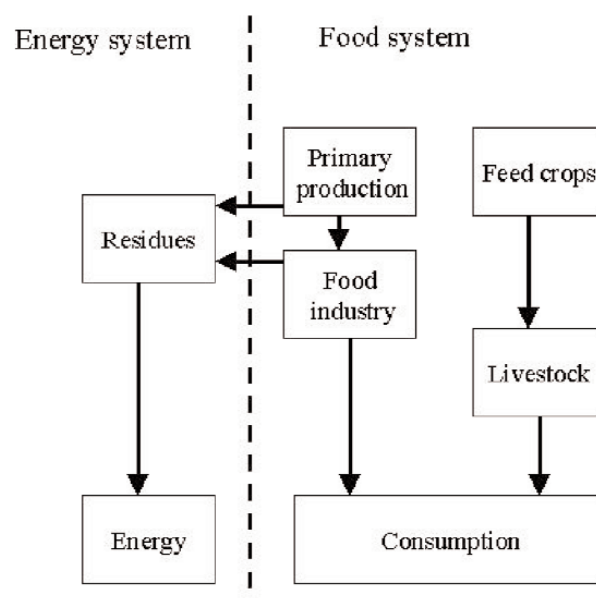

Figure 3. Schematical presentation of the Fodder Crop System. Residues are used for energy generation. Wheat is grown as a livestock fodder crop.

Another option is that special feed crops are grown to compensate for the loss of livestock fodder (Figure 3). By comparing the systems it becomes clear that the food system and the energy system are no longer independent. In the vegetarian system and in the livestock fodder system savings occur in the energy system (no forests/energy crops required for bioenergy), but compensation in the food system is needed to fulfill the food consumption requirements of the population. So the gain in the energy system (less energy plantations) involves a loss in the food system. Analysis of the magnitude of the gains and losses in the system is essential for evaluation of the sustainability of the options.

Quantifying the gains and losses in three systems

A wide variety of agricultural residues exists used for very different purposes like for livestock fodder, paper, soil improvement, etc. Within the context of this paper it is not possible to determine the trade-offs to all these systems. To obtain an impression of the magnitude of the trade-off's of using agricultural residues for energy generation, the effects on the food system are calculated for the largest waste-streams. The largest waste streams in the Netherlands concern oilseed cakes (from vegetable oil production) and molasses from sugar production, 34% and 25 % of the total respectively (Meeusen-van Onna *et al*, 1998). These residues are presently in use as feedstock for livestock fodder. When these residues are used for energy production, trade-offs in the meat-production system can be expected, since a source for livestock feed disappears. The trade-off's of using oil-seed cakes and molasses for energy generation therefore involve the growing of extra protein crops or the production of extra fodder crops. These trade-offs to the food system are quantified for the case that agricultural residues are used as pig fodder in a Dutch pig husbandry system (which is an intensive livestock production system). The answers to the following questions provide parts of the solution:

1. What is the magnitude of available residues in kg/person.
2. How much meat can be produced on basis of these residues
3. How much beans/pulses compensate for the animal protein in the menu
4. How much wheat compensates for the residues as livestock feed
5. How much energy can be gained from the residues considered
6. How much wood from biomass plantations compensates for energy in residues.
7. Finally how much acreage is needed for producing the beans, the wheat and the wood in the various systems.

1 Magnitude of the available residues

The available residues are calculated on basis of the present consumption per person. In the Netherlands 40 kg of sugar is consumed per person per year and 30

kg of vegetable oil (Catsberg & Kempen van Dommelen, 1997). Sugar is obtained from sugarbeet and vegetable oil mainly from soybean (LEI, 2000). Presently 1 Mg of sugarbeet produces 140 kg of sugar, 58 kg of dried pulp and 40 kg of molasses (Maassen & van Swaaij, 1999). Thus the consumption of 40 kg sugar results in: 17 kg of molasses and 11 kg of dried pulp (or 68 kg pressed pulp). The oil content of soybean is 20%, the consumption of 30 kg oil therefore generates a waste-stream of 120 kg of soybean cake.

2 How much meat can be produced on these residues

These residues can be used as fodder for pigs. In agricultural practice fodder quality is expressed in so called EVP ("Energy Value Pigs", Ew: Energie waarde varkens (in Dutch). For all waste-streams used as fodder this value is available in literature (CVB, 2001). Next to this the amount of fodder (expressed in EVPs) needed to raise a pig is known (CVB, 2001; Elferink, 2000). One pig 'yields' about 89 kg of pork (= meat sold to consumers). When losses in the production process are included on average about 4 EVP is needed for each kg of pork (Elferink, 2000). Table 1 shows the quality of the waste-streams considered as fodder for pigs and the total amount of fodder (expressed in EVP) that can be produced from the agricultural residues that come together with the sugar and vegetable oil consumption. The production of 30 kg vegetable oil and 40 kg sugar results in: 17 kg dried pulp (or 68 kg pressed pulp), 11 kg molasses and 120 kg soybean cake with a total 'fodder value' of 132 EVP, which is enough to produce 33 kg pork.

3 Beans as compensation for loss of proteins

For the Vegetarian System, the nutrients in the meat have to be replaced by vegetable alternatives to make the systems comparable. In here is focussed on the proteins. The protein content of pork is 22% (Voedingscentrum, 1997), 1 kg of meat therefore includes 0.22 kg of proteins. These proteins can be provided by beans and pulses, the protein content of these crops is about 20% (Voedingscentrum, 1997). So 1.1 kg of beans is equivalent to 1 kg of pork (with respect to its protein content). This implies that 36 kg of beans have to be produced to compensate for the loss of animal proteins in the diet.

4 Wheat as compensation for loss of fodder

For the Fodder Crops System fodder crops worth 132 EVP must be grown. The most frequent used fodder crop is wheat, the fodder quality of wheat is 1.1 EVP/kg wheat (CVB, 2001), so 120 kg wheat must be grown to make the systems comparable.

5 The value of the residues as an energy source

The agricultural residues can also be used for energy generation. Meeusen-van Onna *et al*, (1998) gives an overview of the heating values of the agricultural residues available in the Netherlands. These heating values are used as an indication of the

suitability of the residue as energy source. In practice this heating energy must be converted into an energy carrier, in this conversion process energy is lost. In this paper this loss is ignored and energy potentials are solely determined on heating values of the residues. This implies that energy gained is overestimated.

Table 1 also shows the quality of the residues as energy source and the total potential energy that can be gained from these residues is 2.2 GJ per person.

6 Wood as energy source

In the Energy Crops System energy is obtained from short rotation forestry systems (willow). The heating value of willow wood is 18 MJ/kg (Hall *et al*, 1993). To make systems comparable 2.2 GJ of biomass energy have to be produced, this would imply the production of 121 kg of wood.

7 Acreage required in the production systems

With the data calculated above the streams in the three systems can be quantified. However, since streams have different units, the systems can not be compared. (There is no scale to compare 120 kg wheat with 121 kg wood.) Therefore all inputs are recalculated to acreage (m2) required to grow the trade-offs. This is done by using present yield data: 7.0 Mg/ha for wheat (fodder crop, LEI, 2000), 3.0 Mg/ha for beans (replacement of the animal proteins, LEI 2000), and 15 Mg/ha for energy crops. The value for energy crops is an estimate. Since these crops are not yet grown on a large scale no statistical data on yields are available. Estimates in literature on yields of these crops show an enormous variation from 2 Mg/ha to over 60 Mg/ha (Berndes *et al*., 2003, Schelhaas & Nabuurs, 2001). Values between 10 and 20 Mg/ton/ha are frequently mentioned for biomass plantations in the temperate climate regions (Nonhebel, 2002: Hoogwijk, 2003).

Table 1 The amount of residues that result from the production of 30 kg of vegetable oil and 40 kg sugar. The quality of these residues as fodder and as energy source and the total amount of livestock fodder and energy that can be obtained from these residues.

Residue	**Quantity(kg)**	**Quality as fodder (EVP/kg)**	**Amount of Fodder (EVP)**	**Quality as energy source (MJ/kg)**	**Amount of energy (MJ)**
Dried beet pulp	17	1.0	17	-	
Pressed pulp	68	-		2	136
Molasses	11	0.7	7.7	12	132
Soybean-cake	120	0.9	108	16	1920
Total on waste-streams			**132**		**2188**

Results

Comparison of the systems

Table 2 shows the acreage required for the production of proteins and energy in the three systems. No acreage is attributed to the agricultural residues since it is assumed that they are ‘unwanted’ by-products of the sugar and vegetable oil consumption. This implies that in the Vegetarian System and the Fodder Crops System no land is attributed to energy and that in the Energy Crop System no land is attributed to the meat production. The production of 36 kg beans in the Vegetarian System requires 120 m2. In the Energy Crops System 80 m2 is needed for the production of 2.2 GJ energy (121 kg wood), and the production of 120 kg wheat in the Fodder Crops System requires 170 m2.

Table 2 Comparison of the acreage required for producing proteins {33 kg pork (on reststreams or 120 kg wheat as fodder) or 36 kg beans} and 2.2 GJ energy (on reststreams or 121 kg wood) in the three different food-energy production systems.

Energy crops		**Vegetarian**		**Fodder crops**	
	m^2		m^2		m^2
33 kg pork	0	36 kg beans	120	120 kg wheat	170
121 kg wood	80	2.2 GJ energy	0	2.2 GJ energy	0
Total	**80**	**Total**	**120**	**Total**	**170**

The large differences that occur between the systems are striking. The Energy Crops System and the Fodder Crops System produce the same commodities (energy and pork) but the Fodder Crops System requires nearly 100 m2 more to do so. The Vegetarian system also requires a larger acreage than the Energy Crops System (120 m2).

Discussion

The values used in the calculations above represent the typical situation in the Dutch food system anno 2000. Food systems are different all over the world and are determined by food consumption patterns and efficiencies in agriculture. In other countries food consumption patterns differ from the system studied here Gerbens-Leenes & Nonhebel, 2002) and these other consumption patterns will generate another residues, both in quantity as in quality. Further feed conversion factor (EVP/kg pork) used in here is typical for the Dutch pig husbandry system; this is an intensive high input production system. It can be expected that this value is higher in other systems (more fodder is required per kg meat). Finally the yield potentials of short rotation forestry are still a point of debate, estimates show a very large variation, leading to large uncertainties in acreage required for the production.

Therefore the acreage calculated here should not be interpreted at face value but is a tool for evaluating systems efficiencies and a method for determining the trade-off's between the energy system and the food system. It should be realised that the use of reststreams as livestock fodder in common practice in food systems all over the globe (Fadel, 1999).
It is striking that the Energy Crops System requires the smallest acreage to produce proteins and energy. It needs half the acreage of the Fodder Crops System, but also far less square metres than the Vegetarian System.
It is stressed that all data involve the consumption per person in The Netherlands. The differences between 80 m^2 and 170 m^2 seem small but on a national level it implies nearly 144.000 ha. The same accounts for the meat consumption 33 kg of meat per person is 530.000 Mg of meat on the national level. The 2.2 GJ of energy per person implies 35 PJ nationally.

Above is calculated that about 33 kg of meat per person per year can be produced from the waste-streams related to the consumption of oil and sugar. This would mean a daily consumption of nearly 90 grams. The present consumption (in The Netherlands) is in the order of 120 g/person/day. (The National Food Centre advises the consumption of 100 g meat/person/day (Voedingscentrum, 1997)). This implies that the residues from sugar and vegetable oil industry play a major role in the meat production and consumption. The use of these residues for other purposes (energy generation) will have large implications for the food system.
The use of the waste-streams as energy source resulted in 2.2 GJ per person per year, since total energy use per person in The Netherlands is in order of 200 GJ (ECN, 2003), the contribution of the waste-stream use to the total energy use is limited (1%). This implies that to obtain 1% of the energy required in the society nearly the 80% of the protein production is affected. The trade-off's to the food system are enormous and should be taken into account in further studies to agricultural residues as energy sources.
These trade-offs are caused by the fact that the nutritional value of meat in the diet is high. Refraining from meat consumption is only possible when compensation for the loss of proteins is found. The yields of beans and pulses crops are rather low: in the order of 3 Mg/ha, while the yields of energy crops are estimated on 15 Mg/ha (5 times as high). This difference in expected yield is the main cause for the difference in land use calculated.
Based on the analysis above it can be concluded that biomass for energy generation should be obtained from dedicated energy crops. Use of agricultural residues that can also be used for livestock fodder show too large trade-offs to the food system.

Analysis of other types of agricultural residues

The decision to focus on largest waste-streams has an effect on the results of this analysis. The waste-streams studied here do have a relative high fodder quality. To obtain an impression on validity of this analysis for waste-streams with lower fodder qualities an extra analysis is done. An important waste-stream mentioned in the

energy discussion is straw from grains (wheat, rice etc.). Large amounts are available (every kg grain generates 1 kg of straw). Straw is hardly used as a livestock fodder, in the agricultural tables mentioned earlier (CVB, 2001), straw is not even listed. In CVB (1986) and Duynie (2003) values of 0.4 EVP/kg are mentioned. Heating value of straw is 18 MJ/kg (Meeusen van Onna *et al*., 1998).

These data are used to determine the trade-offs to the food system when straw is used as energy source. The same method as mentioned earlier is conducted. 0.4 EVP/kg straw implies that 0.1 kg pork can be produced from 1 kg of straw, which is equivalent to 0.11 kg beans in the vegetarian system and that 0.36 kg wheat is needed for 0.4 EVP fodder in the fodder crops system. Since heating value of wood is the same as the heating value of straw 1 kg of straw, replaces 1 kg of wood in the energy crops system.

Table 3 shows the results for straw in comparison with molasses and soybean cake analysed earlier.

The table should be read as follows: when 1 kg molasses is used for energy generation 0.66 kg of wood is saved in the energy system. However, in the food system 0.19 kg of beans is required to compensate for the loss of proteins in a vegetarian diet or 0.64 kg of wheat to compensate for the loss of livestock fodder. When soybean cake is used for energy generation it replaces 0.88 kg wood in the energy system, but the trade-offs in the food system imply 0.24 kg beans or 0.81 kg wheat.

Table 3 The consequences of using various residues as energy source (explanation of this table in text).

	Savings in energy system	**Trade-off's in food system**	
Residue	Wood (kg)	Beans (kg)	Wheat (kg)
molasses	0.66	0.19	0.64
soybean-cake	0.88	0.24	0.81
straw	1.00	0.11	0.36

Figure 4 shows the same results but expressed in m^2 required for the production. When molasses are used for energy generation, this saves 0.4 m^2 of short rotation forestry in the energy system, but it requires 0.9 m^2 wheat for fodder or 0.6 m^2 of beans. This implies that using molasses, as source for energy generation is not sensible since the losses in the food system overrule the gains in the energy system. The same accounts for the soybean cake. Using soybean cake for energy generation saves 0.6 m^2 short rotation forestry, but requires 0.8 m^2 beans for human consumption or 1.2 m^2 wheat as fodder (a net loss of 0.2-0.6 m^2). Both in the Vegetarian System as in the Fodder Crops System net losses are calculated. However, a different picture is obtained for straw, using straw for energy purposes

saves 1 m^2 of wood, and losses 0.5 m^2 of wheat or 0.4 m2 of beans. This results in net gain in the combined energy/food system. The use of straw as energy source seems to have to lowest environmental impacts. However, as mentioned before straw is hardly used as fodder (due to its low fodder qualities). In the context of this paper, it is only analysed on the trade-off's as livestock fodder. In agricultural practice, straw serves as floor covering in stables and for soil improvements. For a proper analyses of the trade-off's of using straw as energy source the consequences of the need for other types of floor coverings and soil improvements should be considered. These analyses are beyond the scope of this paper.

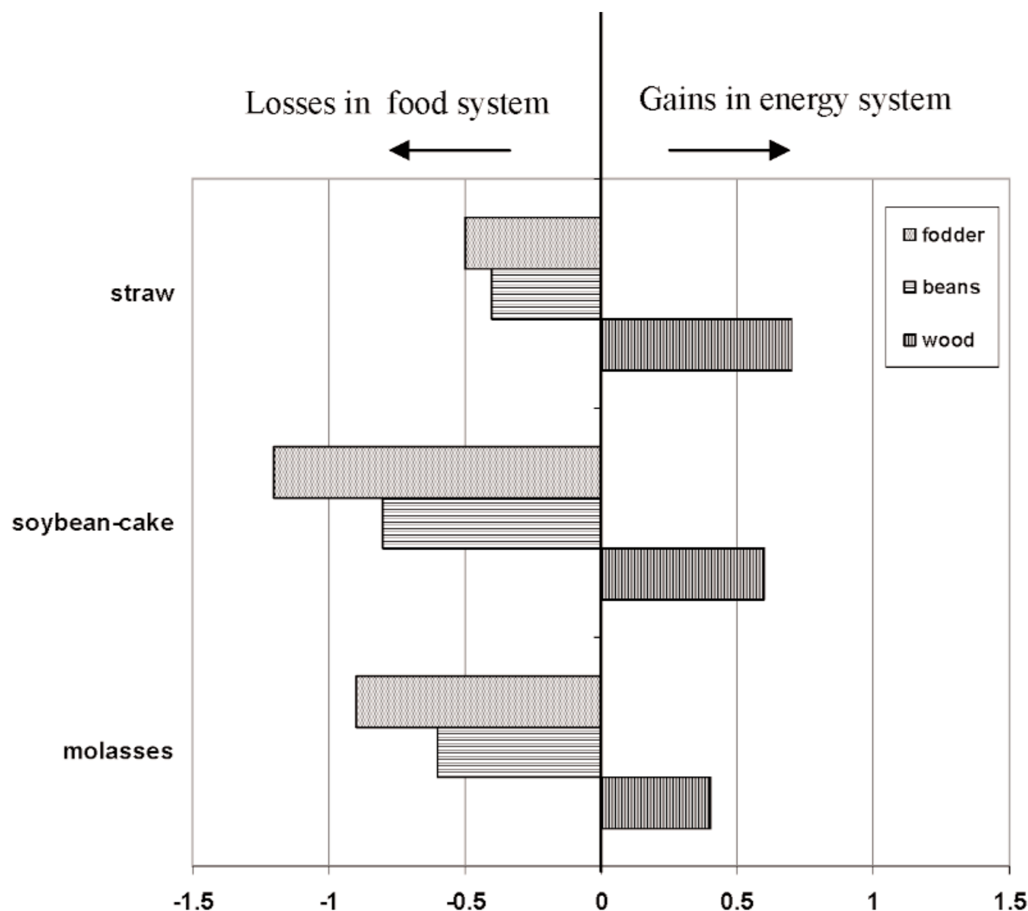

Figure 4 . The gains and losses (expressed in m2 needed for cultivation of the trade-off's) in the energy/food system when different types of agricultural residues are used.

Conclusion

The analysis above allows some general conclusions on use of agricultural residues for energy generation. When residues have a value as livestock fodder, use of these residues as energy source results tremendous trade-offs to the food system. In general the trade-offs in the food system overrule the gains in the energy system.

These trade-offs are due to the fact that loss of livestock fodder need to be compensated for to maintain a healthy diet for the population. Fodder crops or protein crops in combination with a change to a vegetarian lifestyle can fulfil this loss. Only the use of residues with very low fodder qualities results in a net gain in the total energy-food system. This implies that great care should be taken in using agricultural residues as an energy source and that producing bio-energy in biomass plantations seems preferable.

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Whole Utilization of Olive Oil Industry By-Product

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Introduction

The use of a modern two-phase processing technique in production of olive oil generates a new by-product that is a combination of liquid and solid waste, called "alperujo". In Spain, large volumes of waste, approximately 3,5-6 million tons/year, are generated (1). An integrated approach of this waste as fertilizer, or animal feed, or through recovery of residual oil and/or extraction of high added value products will contribute to diminish the environmental impact and will provide a way to make profitable the wastes from the olive mill plant.
The aim of the present study is browsing the viability of the transformation and integral recovery of alperujo by applying a simple steam explosion treatment. This system will allow obtaining high-added value compounds, such as hydroxityrosol; producing bioethanol and, finally, recovering the residual oil.

Materials and methods

Materials

Samples

Samples of "Alperujo", a very wet solid waste from two-phase decanters were supplied by the oil extraction factory "Oleícola El Tejar" (Córdoba, Spain). Alperujo was sampled at three different dates in the olive oil production season. The first sample was taken at the beginning of the olive oil production season; the second sample at the halfway mark of the season and the third sample at the end of the season.
These three waste samples were almost black in colour and had a smooth dough-like consistency. They were partially de-stoned, partially de-oiled (after secondary centrifugal processing to obtain the residual olive oil) and with a high content of water (71.5, 68.1 and 70.7% water, respectively).

Steam Treatment
The hydrothermal experiments were carried out in a flash hydrolysis laboratory pilot unit designed in the Instituto de la Grasa (Seville, Spain) (Figure 1). The reactor with 2 L of capacity (maximum operating pressure of 42 Kg/cm^2) was equipped with a quick-opening ball valve and an electronic device programmed for accurate control of steam time and temperature.

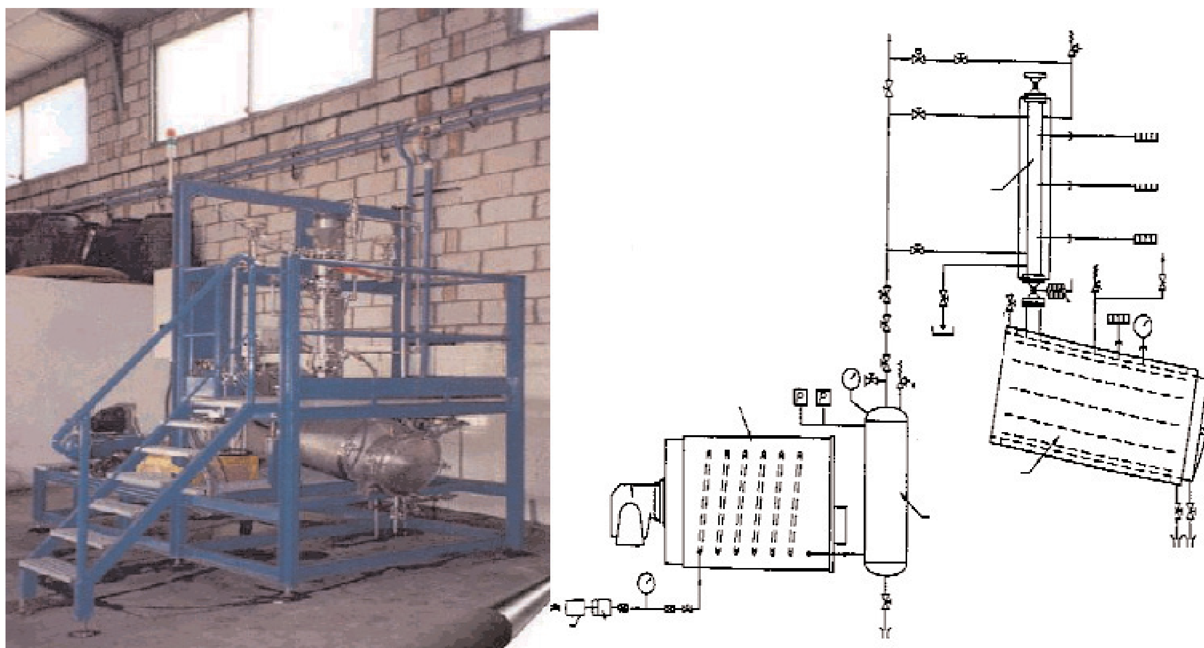

Figure 1. Flash hydrolysis laboratory pilot unit designed in the Instituto de la Grasa

Methods

The moist samples (250 g) were treated with saturated steam. The different experimental conditions used were in a range of temperatures of 160-240ºC, 5-10 min. We have also carried out different studies with and without catalysers, such as sulphuric and phosphoric acid.

Results

When a lignocellulosic material is treated with water or steam to temperatures in a range of 160-240ºC, an autohydrolysis process occurs (*2, 3*). Depending on the conditions used, there is a depolymerization of polysaccharides (mainly of hemicelluloses) and a breaking of the lignin-carbohydrate bonds, resulting in the solubilization of lignin fragments of low molecular weight. As a consequence of such treatment, the solid olive by-product was partially solubilised. This process made possible the releasing of several compounds that were toughly linked to alperujo matrix, and that could be worthy to be recovered. Some of these are fermentable sugars in form of monosaccharides and phenolic antioxidants, such as hydroxytyrosol, which are also abundant in olive fruits. The yield of hydoxytyrosol

increased with the severity of the treatment as well as by the addition of acid catalyser. It is remarkable that the for certain samples the treatments in presence of acid were much more effective, even in mild conditions of temperature and pressure. It can be concluded that the vapour conditions required for obtaining compounds of interest from the effluents generated in the olive oil industries are not too severe. So, the treatment of alperujo at 200ºC, for 5 min, adding phosphoric acid as catalyser, which is less corrosive, oxidant and expensive than the sulphuric acid, allow to get about 1-1.2g hydroxytyrosol / 100g of dry sample. In addition, many other substances such as sugars, mannitol, etc can be recovered in equivalent amounts.

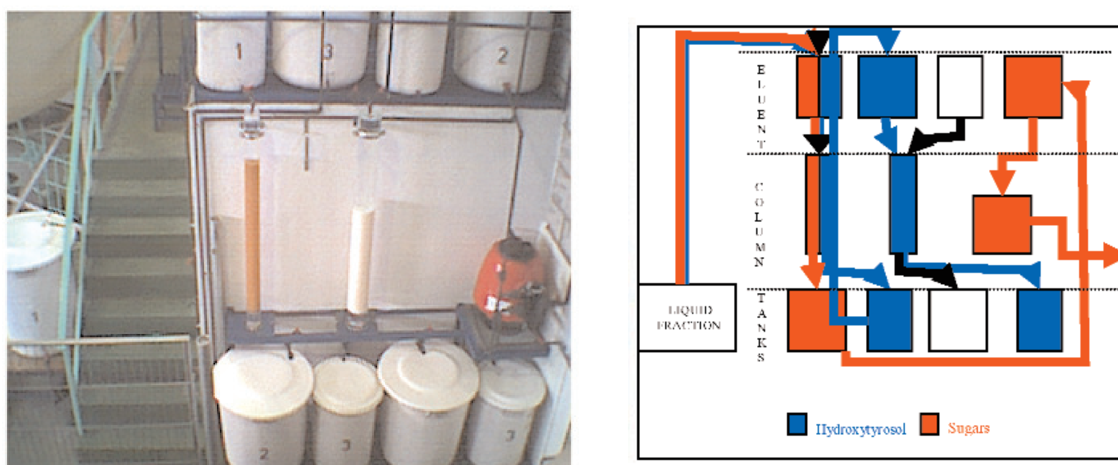

Figure 2: Scheme of pilot plant for the purification of hydroxytyrosol and detoxification of sucre liquors

The soluble fraction obtained by steam explosion of the simple, is submitted to purification by adsorption chromatography. For this purpose, it has been designed and constructed a pilot plant that basically consists on two columns filled with different adsorption resins (Figure 2). This purification process is protected under patent (4), and it is currently in exploitation. The first step if purification allows getting hydoxytyrosol with about 60-80% purity, while after the elution from the second column, the hydroxytyrosol reaches to 99.5% purity. The remained liquor, which is very rich in sugars, can be submitted to a process of detoxification in order to get the sugars ready for posterior fermentation, after eliminating or decreasing the concentration of inhibitory compounds.

During this process, a considerable reduction of the solid fraction also happened. In this solid is concentrated practically the total amount of the residual olive oil, whose concentration reaches about 15-20% of the fraction. Cellulose content also remains nearly to the initial values of the treated sample, which means about 30% of the solid fraction. These data would make feasible the extraction of the residual oil with organic solvents from the residue above described at affordable prices for the refined industries. Due its natural origin and no alteration during the process reported in this work, this refined olive oil could be use for human consumption. On the other hand, the hydrolysis of cellulose and other accompanying sugars represents a good source of compounds such as bio-ethanol, being a good alternative to other systems that use more complex samples and expensive pre-treatments systems.

Conclusions

1. We have developed a process that allows an integral recovery of solid waste from two-phase olive oil or "alperujo". This consists on a hydrothermal treatment where an autohydrolysis process occurs and the solid olive by-product was partially solubilised. Due this method makes easier the solid-liquid separation, the integral recovery includes both fractions.
2. The solid fraction is considerable reduced after the treatment and several components such oil, cellulose and proteins are concentrated. These can be used in human (refined oil) and animal (cellulosic residue) food and, finally, as substrate for composting.
3. From the water-soluble fraction besides recovering an important part of the antioxidant hydroxytyrosol, imbues olive oil stability, as well as being beneficial to health, with biological and antimicrobial properties, it will allow the recovery of mannitol and non-digestible oligosaccharides that can be used as promoters of the growing of colon bifidobacterium. Also this liquid fraction is rich in sugars, including monosaccharides (glucose) that are fermentable to bioethanol.
4. The system of purification of hydroxytyrosol, which is under patent, allows its production to industrial scale, with high degree of purity (over 99.6%) and low economic costs.

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Suggestions For Making Beet Sugar Industry More Eco-Compatible

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Introduction

In recent decades, a considerable research effort has been spent on the reduction of environmental impact of sugar production from sugar beet. The main directions of research are following:

- Identification of critical points of the process causing environmental problems;
- Utilisation of by-products or waste materials as secondary raw materials;
- Application of alternative, environment-friendly process technologies;
- Optimisation of energy and water use.

The block diagram in Fig. 1 illustrates the steps of the traditional sugar production process and the use of lime in the steps known as calco-carbonic purification stage. Limestone is obtained from mines thus creating ecological problems and spoiling the landscape. Carbonation sludge generated in sugar factories may be difficult to dispose of even if it is on a considerable scale utilized as soil improver in agriculture or as raw material in the cement production.

By-products as secondary raw materials

Utilization of beet pulp for paper production

The world output of paper has increased in the last 50 years about sixfold, reaching 300 millions tonnes per year. The main constituent of paper is cellulose; for the production of 1 kilogram of cellulose, it is necessary to process 7 kg of good-quality wood . Owing to a relatively low content of cellulose and lignin, and the content of pectins, beet pulp cannot be used as a direct substitute for wood. It can however be employed, after physical treatment, as so-called "organic filler" [1,2].
Sugar factories can produce ‘dried pulp’ (90% dry substance) obtained by mechanical pressing an subsequent high- or low-temperature drying, or ‘overpressed pulp’ having a dry matter content just above 25 %. The physical treatment of the pulp before its utilization in the slurry for the production of paper is obviously different in the two cases: the ‘dry pulp’ must be "dry" ground and/or micronized whilst the ‘overpressed pulp’ must be mixed with cellulose to form slurry which is then "wet"

ground. In both cases it is possible to make paper from slurry in which up to 15% is pulp replacing cellulose without appreciable modification of the physico-mechanical characteristics (Burst index, Elmendorf tear index, Breaking length, etc.) of the final product. The paper is not as white as that obtained from pure cellulose but is good enough for printing, photocopies, etc.

Utilization of carbonation sludge for the production of paper

One of the main components of paper, in addition to cellulose, is calcium carbonate. Its content, depending upon the different uses of paper, can can be as high as 20%. Owing to the massive use of lime, a paper mill considerably contributes to the environmental degradation. As a partial solution of this problem and the disposal of carbonation sludge from sugar production, the sludge can replace fresh lime for paper production. In order to satisfy quality requirements for a component of the paper slurry, the carbonation sludge must be dried and micronized before utilization. This idea has been tested in a laboratory and later, thanks to funding from the EC LIFE Project (contract No. 95/IT/A13/IT/393/VEN), also on an idustrial scale. Paper has been produced by completely substituting the calcium carbonate with carbonation sludge. The quality of the product was sufficient for printing and packaging applications [3,4].

Simultaneous utilization of beet pulp and carbonation sludge in paper production

Using both beet pulp and carbonation sludge, good-quality paper was obtained from a mix of materials containing up to 33% by-products coming from sugar factories [3]. It may be noted that a sugar factory processing 10,000 t of beet/day uses every year about 400 t of paper for packaging and 5 t of paper for printing and writing. If every sugar factory used only paper produced as described above using beet pulp and carbonation sludge, it would effectively recycle a considerable part of its by-products.

New environment-friendly process technologies

Lime-free sugar manufacturing process

Problems resulting from lime use in sugar production can be definitively solved by eliminating the traditional calco-carbonic juice purification. Its role in the production of commercial white sugar is to overcome the difficulties of processing raw juice which is rich in organic and inorganic substances (some of them thermolabile), has an acidic pH and is intensely turbid and coloured. The traditional purification process eliminates a part of non-sugar compounds (in particular thermolabile ones), makes the solution alkaline so decreasing the risk of sucrose inversion and yields juices that are limpid and low in colour.

Different process options have been studied with the aim of eliminating the calco-carbonic juice purification (in part, thanks to the funding obtained from EC INCO-COPERNICUS Project SUCLEAN, contract No. ERBIC15-CT96-0734). All the options described below have been experimentally tested both in the laboratory and in pilot plants but they still need industrial validation.

Direct crystallization of raw juice

Raw juice as such, without any treatment, can be concentrated at low temperature and crystallized by cooling in two or more steps to obtain raw sugar, which can subsequently be refined according to the criteria of traditional refining. Low purity of molasses can be attained owing to the presence of certain compounds decreasing the solubility of sucrose [5,6]. (When applying the calco-carbonic purification, these compounds are eliminated.) The total yield of crystallized sugar is comparable with that characteristic of the traditional process.

Crystallization of the micro-filtered raw juice

Commercial white sugar can be directly obtained from raw juice providing the juice is micro-filtered, treated by ion exchange to remove magnesium, concentrated at low temperature and crystallized by cooling in multiple steps. As shown in the diagram in Fig. 2, sugar obtained from the first step is commercial white sugar, while sugar obtained from the remaining steps must be recycled.

Microfiltration can be carried out using organic or ceramic membranes. The elimination of magnesium originating from beet is necessary to avoid its co-crystallization with sucrose. The total yield of crystallized sugar is comparable with the yield of the process employing calco-carbonic purification [7,8].

Chromatographic separation

In this process option, raw juice after micro-filtration and softening can be chromatographically separated in a SMB (Simulated Moving Bed) plant. The sugar-rich fraction can subsequently be concentrated at low temperature and crystallized by cooling in several steps. Commercial white sugar is obtained from the first two steps and sugar to be recycled from the following steps, before obtaining the final molasses [9].

Raw-juice crystallization combined with chromatographic separation

Raw juice after micro-filtration, magnesium elimination and concentration at low temperature, can be crystallized by cooling to obtain commercial white sugar. The mother liquor can then be separated by chromatography, decolourised by ion exchange and crystallized in five steps. Commercial white sugar is obtained from the two initial steps and sugar to be recycled - from the remaining three steps [10].

Optimisation of energy and water use

Energy

In general, energy use in a process plant can be studied using Pinch Analysis [11]. The established pinch-based approach cannot be directly applied to sugar plants for which so-called Extended Pinch Analysis has been developed [12]. By applying the extended approach to retrofit situations in traditional sugar factories, energy savings of up to 29% have been achieved [13].

Extended Pinch Analysis has been applied to the conceptual design of a small (1000 t beet per day) hypothetical sugar factory employing crystallization of micro-filtered raw juice. For such a factory, one can choose between various

power-plant technologies (steam turbine, gas turbine and piston engine) and various options of the juice-evaporation station (3 to 6 stages possibly combined with vapour recompression). If vapour is not recompressed, then the energy consumption is comparable to that of conventional sugar manufacturing, that is, about 200 kWh per 1 t beet. By applying a gas-turbine based power plant and five-stage evaporation station with vapour recompression, the energy consumption can be reduced by about 30% [14].

Water

A beet sugar factory is normally equipped with a water and wastewater system that can be decomposed into three subsystems:

- Main water and wastewater circuit in which hydraulic transport and washing of beet take place,
- Water circuit in the power plant and sugar manufacturing plant,
- Barometric circuit in which surplus vapours leaving the sugar manufacturing plant are condensed and waste heat is dissipated to the environment.

Soil present in beet supplied to the factory is separated during hydraulic transport and washing, and stays in water. The soil-water mixture is discharged from the main circuit. As it is also necessary to discharge a wastewater stream, the combined water loss must be compensated for by continuous supply of water liberated from beet (from the barometric circuit) and fresh water (from an outside source). The consumption of fresh water can be reduced by regenerating wastewater and recycling it to the main circuit.

Water consumption in conventional sugar factories is widely differentiated. Modern wastewater treatment technologies make it possible to use water very efficiently and even eliminate the intake of fresh water. However, in older factories the consumption of 25-45 kg water per 100 kg beet is still considered as normal.

To rationalise water use in a process plant, one can apply computer-aided methodologies among which the Water Pinch approach is perhaps most widely known [15]. Examples of application of Water Pinch to water management in the sugar industry indicate that limited modifications of the structure of water circuits accompanied by rearrangement of water flows may in some cases bring about considerable water savings [16].

The Water Pinch approach has been applied in a study of water use in a hypothetical sugar factory employing cooling crystallization of micro-filtered raw juice [17]. It was found that the novel process facilitates very low water consumption. In principle, no fresh water is needed during normal operation of the factory. Limited supply of water from an outside source may be required during production start-up or periods of hot weather when the return temperature of cooling water exceeds 20 °C.

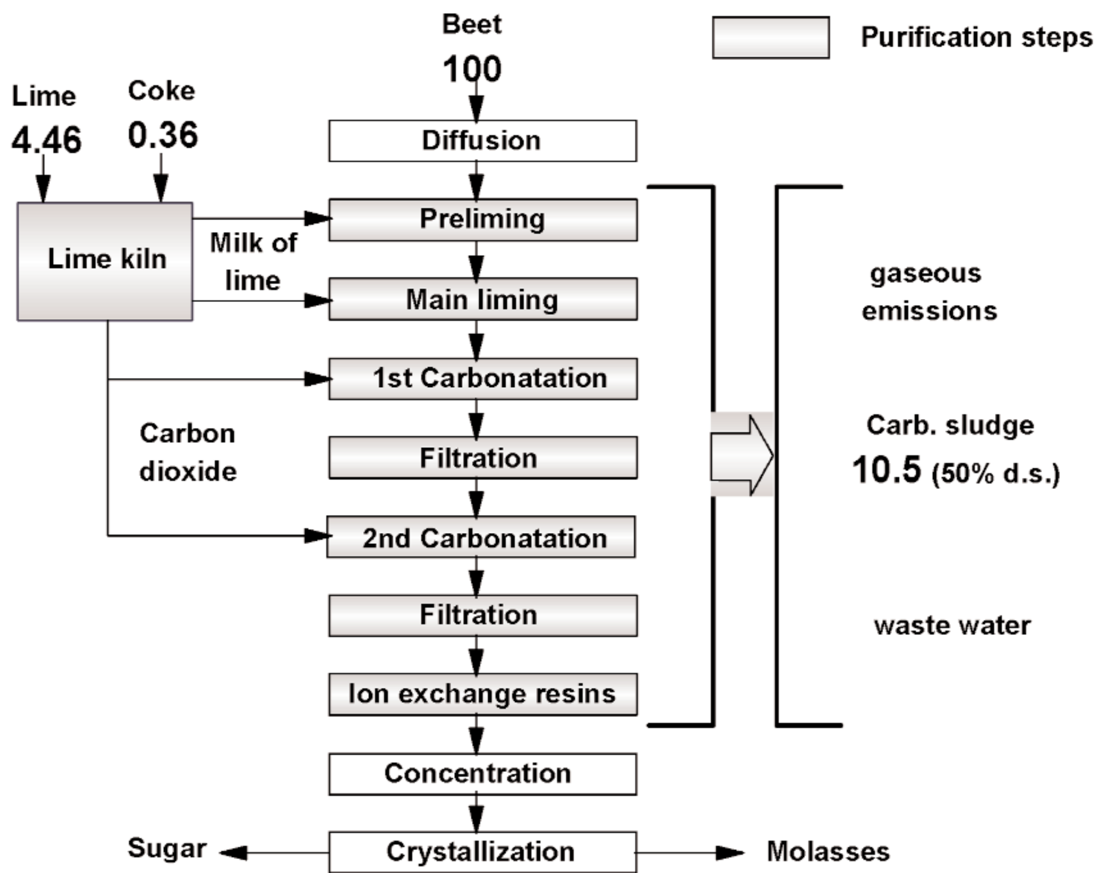

Fig. 1. Simplified flow diagram of a beet sugar factory

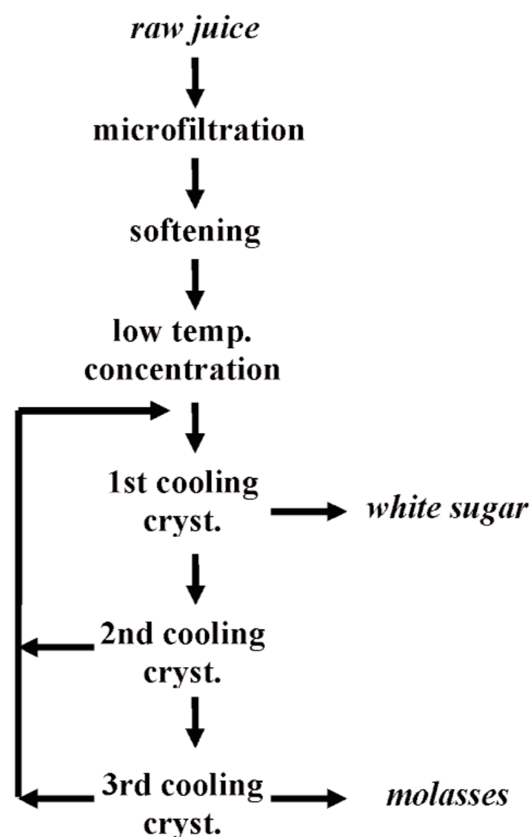

Fig. 2. Crystallization of micro-filtered raw juice.

Conclusions

The traditional beet sugar manufacturing technology has a considerable detrimental impact on the environment. This problem can only be partly solved through rational use of the by-products and improved use of the traditional technology. A major reduction of the environmental impact and an optimal solution of the problem can only be achieved by exploiting innovative technologies similar to those analysed in the present contribution.

Concerning the problems of energy and water saving, an approach based on "Water Pinch" and "Pinch Analysis" can be applied both to the traditional technology and the new ones. This makes it possible to optimise fuel and water consumption in any sugar factory.

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Future Concepts: Integration in Processing

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Introduction

One of the problems the food industry is facing is that, next to the production of food products, by-products and waste are produced. The current strategies are to minimize waste production in the first place, and furthermore to make use of by-products to turn them into valuable products (not necessarily food). The present paper addresses the question whether or not integration will help to solve these problems. Integration means that problems and their possible solutions are tackled from more than one viewpoint. In terms of science it means a multi- and perhaps interdisciplinary approach. The present author foresees integration at three levels when it comes to waste minimization and waste utilization. These are:

- integration of natural and social sciences to address societal issues related to industrial production of food
- integration over the food chain, especially needed because of the ongoing globalization
- integration in product- and process engineering

Integration of natural and social sciences

It should be realized that technology is a way to address societal questions by a science-based technical solution. If these societal issues are only considered from a technological point of view, one may arrive at an answer that is not really suited to the problem. In other words, it is very important to find not only the right answer but first and foremost to find the right question. The world is full of examples of beautiful technical answers to non-relevant questions. As a result, these answers will not be picked up by society. It is for this reason that societal questions should be studied in co-operation with social scientists. Incidentally, it is also of importance that societal questions are not only studied by social scientists. It is the very integration of the two that is important. The societal issue at stake is the industrial production of food. Developments over the past 100 years or so have led to the situation that most of the

food in the Western world is produced at large scale in factories. There is much debate among consumers concerning this food production. The problem of waste is certainly one of the issues here. When addressed properly, questions can be posed such as: do we want to maintain our current way of food production, or: are we prepared to give in a bit on food safety so that we do not have to throw away foods that have passed the best-before-date but are still perfectly all right? Especially a dialogue with consumers is very important here to see whether or not a technological solution is also desirable from a social point of view. Also, the question is whether or not a technical solution is economically feasible. One example where this integration is currently carried out is the so-called PROFETAS project (www.profetas.nl). PROFETAS is an acronym for PRotein Foods, Environment Technology And Society. The research question is whether or not the production of novel protein foods based on plant proteins is more sustainable than the production of meat products. To address this question, the project is divided in 15 subprojects. Basically, the questions are:

- Is the production of NPF's socially desirable? Are consumers willing to reduce meat consumption, and under what conditions? Is environmental sustainability a reason for consumers to change their attitude? Is it possible for farmers to change from cattle and livestock to vegetable protein sources? Does the production of NPF's indeed lead to a reduction of the environmental burden?
- Is the production of NPF's technically possible? Is it possible to produce attractive alternatives for meat products? This point is considered essential: if consumers do not want to accept NPF's because these products are not appealing the project is bound to fail
- Is the production of NPF's economically feasible? Is it possible to produce NPF's for a price that is competitive to meat products? Do world market prices allow all this?

The researchers active in PROFETAS are food technologists, nutritionists, economists, ecologists, consumer scientists, plant breeders, and agronomists. They are trying to work together. The lessons learned so far are that communication is essential but not at all straightforward, that there must be real commitment of everyone involved, and that to the point questions are posed. Integration requires experienced researchers; they must have expertise in research. One cannot ask PhD students to do this by themselves. It is still possible and necessary to do monodisciplinary research, but the point is that this research should fit to a higher goal. This way of integrating sciences really requires a paradigm shift.

Integration over the food chain

It is important to realize that the production of foods does not only take place in the food industry. The food industry is part of the food chain (Figure 1). It starts already at the stage of primary production, even including breeding. It is here that the quality of a product starts to take shape. This also applies to the production of by-products and waste. Waste production may already start here due to harvest losses, damage to the product, and the occurrence of pests and diseases. Raw materials are biological

materials, and usually (but not always) quality becomes less as the product moves along the supply chain. During transport to the food industry further losses may occur, for instance if the temperature is not strictly controlled, of if the produce is stored in too high a humidity. Then when the raw materials are processed, losses may occur and it becomes important to distinguish between unavoidable, wanted losses and avoidable unwanted losses. Avoidable losses could be called real waste (Somsen, 2004). Unavoidable losses are, for instance, potato peelings, cheese whey, egg shells. Avoidable losses occur for instance when the potato peelings are too thick or when too much casein enters the whey due to sub optimal rennet action. Waste also applies, to some extent at least, to inefficient energy and water use.

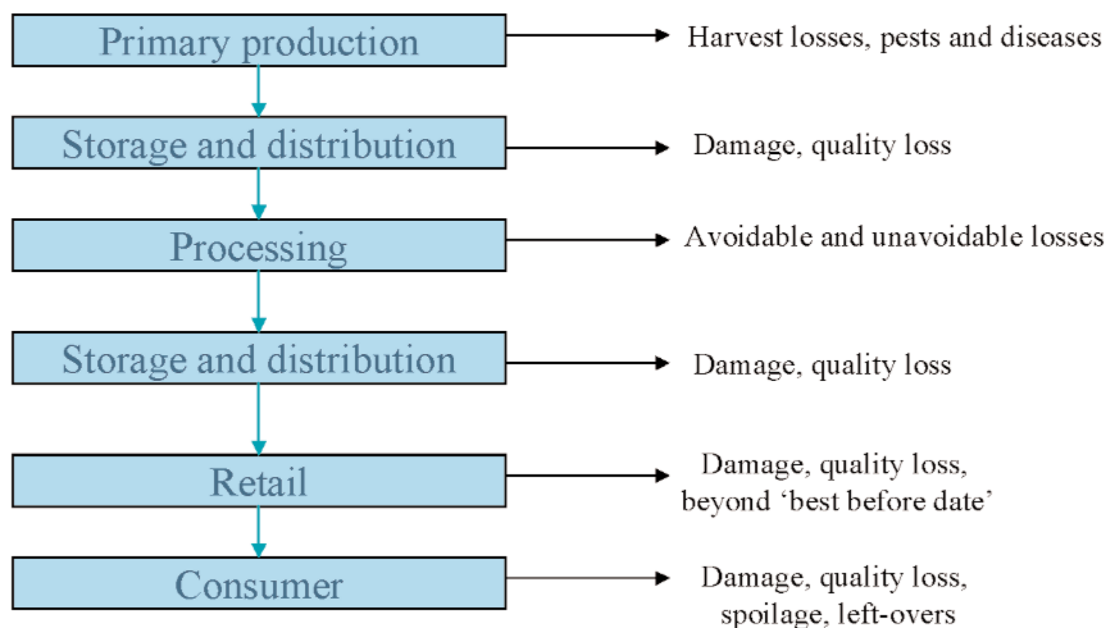

Figure 1. A schematic picture of the food supply chain.

Integration over the food chain becomes even more important due to globalisation. Foods, and raw materials for foods, come from all over the world, and storage and distribution conditions are essential to keep quality optimal. If not, unwanted losses will occur. For instance, if fresh produce is transported under controlled atmosphere, and this is not controlled at the optimal level, spoilage may easily occur and the produce may become unfit for consumption.

Integration in processing

Traditionally, processing methods in the food industry have not been selected to minimize waste production. The food industry in its infancy was mainly a scaling up of kitchen processes, and certainly not designed to reduce waste. This is not to say that the food industry is old-fashioned and not interested in waste reduction, but just to make clear that existing processes may need some rethinking from the point of view of waste reduction. This may include rethinking of the existing technology and a

paradigm shift in thinking of management. If waste reduction is to be taken seriously, managers have to see waste reduction as a normal way of improving quality of production, not as a burden. On the other hand, it should also be acknowledged that foods are complex materials and that some of the losses are indeed unavoidable. Food companies have as ultimate goal to manufacture products that satisfy the consumer. The question that needs to be addressed is whether production can be carried out with a minimum of waste production, while retaining the desired product quality. The two strategies to reach this goal are first to minimize waste production and second to turn unavoidable losses into valuable by-products. An appealing example of such an approach can be found in the dairy industry. On the one hand cheese production is optimised to obtain the maximum attainable yield from cheese milk. On the other hand, the unavoidable by-product of cheese, whey, can be completely utilised. Whey proteins are nowadays highly valued proteins, lactose is extensively used in the pharmaceutical industry, and even the salts can be used in the food industry. It is interesting to note that one also attempts to innovate by incorporating whey proteins into cheese, e.g. by ultrafiltration (Walstra *et al*, 1999). This is less easy than it seems. It leads to different product characteristics, and it is also hindered by legal regulations (cheese may only be called cheese if produced in the traditional way using rennet).

How to reach integration in processing? The factors involved are:

- raw materials properties
- types of technologies used
- end product specifications

The first thing to do is to critically evaluate the existing production process. Figure 2 gives a very schematic picture of what needs to be identified.

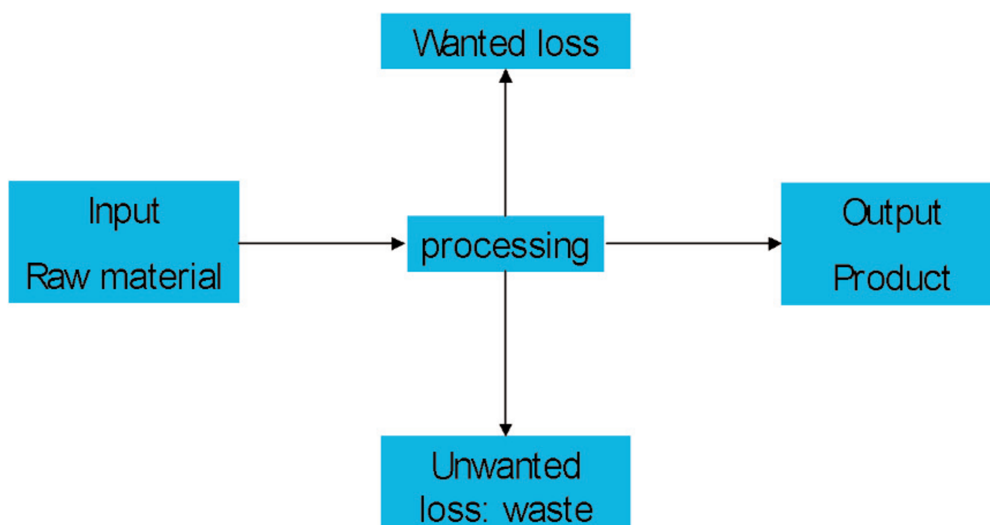

Figure 2. Schematic picture of waste and losses in the food industry (after Solmsen, 2004).

After the production process has been analysed in this way, the next phase is to consider whether the unwanted losses (which can be properly called waste) can be reduced. For instance, peeling of potatoes is a necessary step in production of French fries, and peelings are thus a wanted loss, but if they are too thick this leads to unwanted losses, and in fact to an inefficient process. Instead of optimising an existing process, it is perhaps also feasible to come to a better product and process design. Reduced waste production can also be seen as a quality attribute of a product. Furthermore, use of appropriate packaging technologies can reduce losses due to spoilage. Packaging is quite important in this respect to reduce waste of foods, but there is some ambiguity here: the packaging materials themselves lead to waste. It is quite a challenge to find an optimum balance. The search for biodegradable packaging materials is certainly worthwhile from this perspective.

A recently developed tool to reduce waste production is called Production Yield Analysis (PYA) (Somsen, 2004). It uses the so-called Yield Index:

$$\text{Yield Index} = \frac{\text{Production Yield}}{\text{Maximal Production Yield}}$$

The actual production yield for a process can be calculated from mass balances: how much material enters a process, how much comes out as product and how much as losses. This is a straightforward procedure. The maximal production yield is more difficult to calculate. It requires theoretical insight into product and process design. The use of models to calculate the maximal production yield is indispensable. The added advantage of doing this is that one obtains better insight in the applied processes. Figure 3 gives an example of what has been achieved in a potato processing company by applying the concept of yield index.

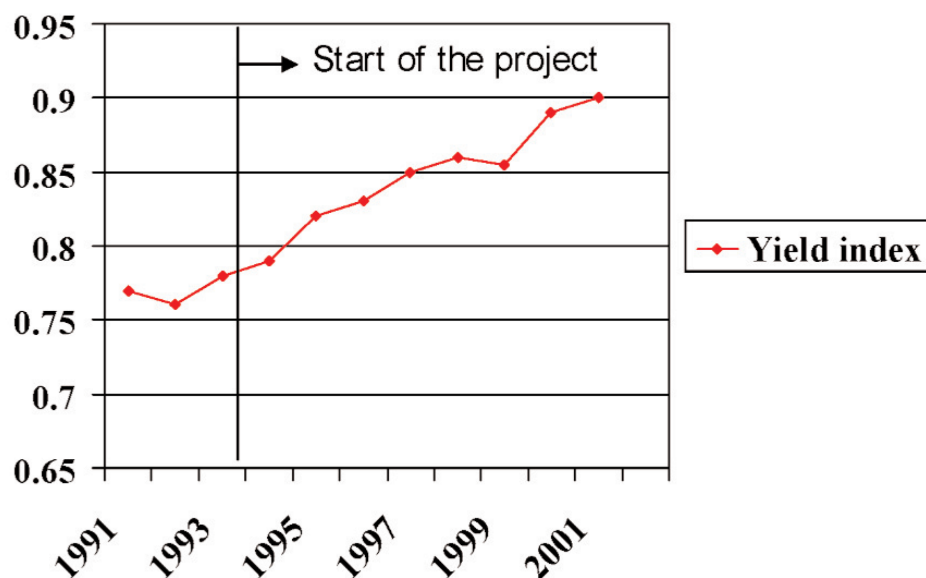

Figure 3. Example of the use of the Yield Index in a potato processing industry (Somsen, 2004).

Alternatively, if one is prepared to rethink product and process design, one may want to use engineering tools such as reverse engineering and TRIZZ. These techniques aim at achieving quality through engineering rather than taking quality as a result of engineering. Furthermore, there are nowadays relatively new technologies available such as hurdle technology, affinity chromatography, membrane technology, several non-thermal technologies, use of enzymes, and in the future nanotechnology (van Boekel, 1998). Considering the possibilities of such technologies may lead to new product and process design in which waste reduction is incorporated.

As for the reduction of energy use, an interesting tool is available, called exergy analysis. It is based on thermodyamic considerations. Energy can in principle not be destroyed according to the first law of thermodynamics. However, the quality of the energy, namely its potential to do work, can be lost. An exergy analysis of a production process provides insight into which part of the process perhaps destroys exergy unnecessarily, and then appropriate measures can be taken. Some examples of such an analysis can be found for sugar production (Tekin & Bayramoglu, 1998), and drying processes (Midilli & Kucuk, 2003, Syahrul *et al*, 2002).

Conclusions

Possible results of an integrated view on production processes and waste production are that a better understanding of product properties and processes is obtained, that product and process optimisation can been achieved, resulting in economical benefit, and last but not least that waste reduction has been accomplished. However, there are some associated problems to be overcome:

- a paradigm shift is needed in integrating social and natural sciences so as to address the right societal questions
- integration over the food chain requires trust and transparency in the chain; it must be clear who is taking responsibilities, and who is taking (and sharing!) the possible profit
- integration in processing requires involvement and commitment of management, and advanced knowledge of product and process engineering.

However, if these difficulties are overcome, this will definitely help to avoid end-of-pipe solutions, and will lead to new and innovative solutions.

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Workshop and Project Reports

AWARENET: Agro-Food Wastes Minimisation and Reduction Network

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Introduction

Despite of the economic importance of the food sector, as shown in Table 1, during the last EU Framework Programmes no global European vision addressing the environmental problems arising from agro-food wastes has been approached. Thus AWARENET is the first Thematic Network aiming at the Prevention, Minimisation and Reduction of Wastes from the European Agro-food Industry. Funded by the GROWTH Programme of the European Commission with a global budget of 1,47 M _ for a three-and-a-half-year duration (2001-2004), it focuses on 5 main agro-food sectors: meat, fish, dairy, wine and vegetables processing. Overall 34 members from 15 different European countries (Figure 1), including newly associated states, have contributed to the network results, gathered in the *Handbook for the prevention and minimisation of waste and valorisation of by-products in European agro-food industries.*

AWARENET is co-ordinated by the Spanish Technology Transfer Centre GAIKER and counts with the participation of agro-food wastes producers (manufacturing industries), waste valorisation industries, equipment manufacturers, consultancies and RORs and Universities. This wide background ensures a critical mass forum for information/expertise exchange and technology transfer.

The innovation of AWARENET is integrating the vision of the agro-food industrial waste problem from three different and critical points of view: regulatory issues, technology and market, with the final objective to propose a global R&D European strategy for agro-food industrial wastes.

Table 1. Food production values in Europe (Source: Eurostat, 1999 data)

NACE-Codes (3 digits)		ALL COUNTRIES - 1999		
Code	*Description*	*Total Quantity (kg)*	*Total Value (€)*	*Price (Euro/kg)*
15.10	Meat and meat products	44.230.121.954	86.579.097.344	1,96
15.20	Processed and preserved fish and fish products	4.907.718.264	12.493.550.809	2,55
15.30	Processed and preserved fruit and vegetables	27.182.883.657	25.386.041.634	0,93
15.40	Animal and vegetable oils and fats	24.428.897.857	13.330.657.166	0,55
15.50	Dairy products and ice cream	76.404.330.114	69.222.805.117	0,91
15.60	Grain mill products, starches and starch products	53.644.037.197	19.194.984.798	0,36
15.70	Prepared animal feeds	220.159.824.120	54.595.638.461	0,25
15.80	Other food products	57.926.390.901	95.502.680.600	1,65
15.90	Beverages	113.299.801.494	72.700.722.436	0,64

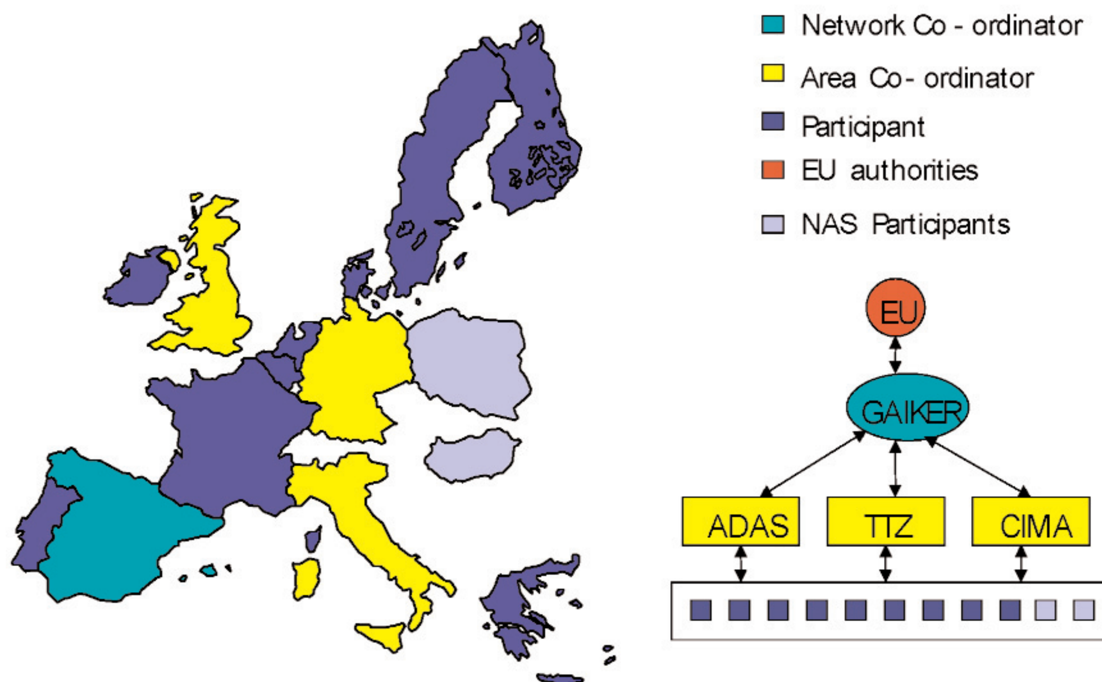

Figure 1. AWARENET consortium structure

Environmental constraints in the food processing industry

Food processing produces large amounts of waste, generally less hazardous than that from other industrial sectors like metallurgy, chemical industries, etc. and due to its natural origin mostly biodegradable. Furthermore, large amounts of effluent water, containing oxygen demanding substances, are also discarded. The non-hazardous content in agro-food wastes and wastewaters could be the reason for which this sector has not been subject to strict environmental regulations as other industrial sectors.

Implementation of environmental regulations on waste and wastewater management and good manufacturing practices have derived in the need for environmental management in food production processes as well. The most relevant key environmental issues in the food production sector are:

a) Raw material consumption
b) Water consumption
c) Effluent and solid waste treatment/discharge
d) Energy consumption
e) By-products

One key environmental problem is the high organic content of its effluents and residues, which implies a high treatment cost. In parallel, waste prevention, minimisation and valorisation are increasingly recognised as environmentally more desirable solutions for waste management. As these effluents and residues have a great number of organic compounds with a high nutritional value (proteins, oils, sugars, vitamins, colorants and antioxidants), their minimisation and valorisation has a double advantage: reduction of the polluting load of agro-food industries and contribution to the sustainable development of the agro-food sector through a rational use of the natural raw materials. It has also another important advantage: the cost saving, as the higher use of raw material in production processes implies a reduction in the amount of it needed, and a decrease in waste disposal taxes.

Materials and methods

Methods

To facilitate the co-ordination of such a wide working group four different inter-related working areas, with the corresponding area co-ordinator, were defined (Figure 2):

<u>Regulatory issues</u> (ADAS): gathering of current environmental situation of agro-food wastes (including the relative legislation in force), and realisation of an inventory of agro-food wastes in Europe (classification, amounts, etc).

<u>Technology</u> (TTZ): identification of critical points giving rise to wastes in the main production processes of the five target industrial sectors, practices used nowadays by

European SMEs for waste minimisation and upgrading, best available technologies and equipment currently available.

Market (CIMA): requirements and/or market opportunities for minimisation/ valorisation technologies and for existing or new products to be recovered from agro-food wastes.

European RTD strategy (GAIKER): conclusions of AWARENET activities for proposing a global European strategy for agro-food industrial wastes.

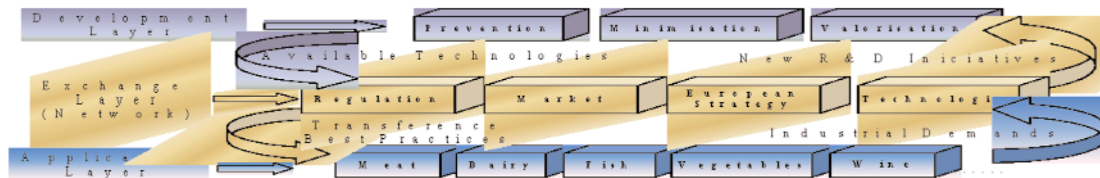

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Figure 2. Inter-relation of AWARENET working areas

Further to working areas distribution, members were arranged in discussion groups on different food processing subsectors considering their expertise. This favoured the outlining of a working matrix in which the inputs and tasks of each member were clearly stated and checked during the network life. In summary, the general approach of AWARENET network to deal with food waste and by-products issues has been the following:

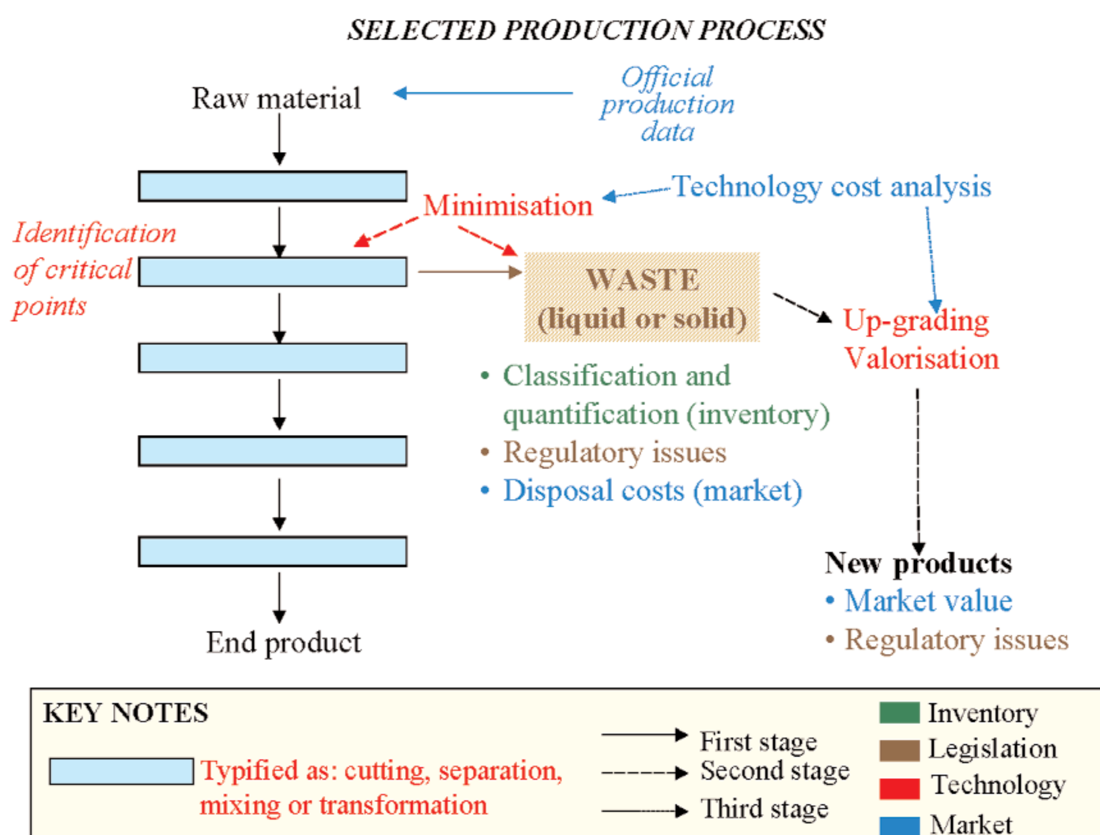

Figure 3. AWARENET working approach

Results

Regulatory issues

European Commission's legislation is a key driving force in the need for food waste management and valorisation. Thus, a thorough study on those regulations affecting the disposal, management, minimisation and valorisation of food solid and liquid wastes and by-products has been performed. Furthermore, implementation of these regulations in the European countries represented by AWARENET members has been accomplished. Apart from the Waste Framework Directive (91/176) and Urban Wastewater Treatment Directive (91/271) other Directives such as IPPC (96/61), landfilling (1999/31), promotion of biofuels (2003/30), animal waste (90/667) or Regulations like the one on animal by-products (1774/2002) are crucial for outlining future management of food waste and by-products issues. The restriction in landfilling organic wastes prompts the application and development of valorisation strategies.

Inventory of food wastes and by-products

A full accounting as "waste + by-products" has been realised by AWARENET members considering all those parts from the raw material not appearing as final product in the selected 19 production processes within the 5 main food sectors tackled by the network. These processes have been selected considering both

members expertise and the food subsector's significance in terms of economic revenues and food waste volumes and environmental needs. The waste and by-product percentage to be applied for each production process has been agreed by AWARENET members after process evaluation according to the figures in Table 2.

Consequently, a full matrix considering food processes and countries has been obtained from different data sources, mainly from Eurostat official data, and using a software developed on purpose within the network to facilitate data management. An overall figure of around 222 million tons/year of generated food waste and by-products has been obtained, which can serve to get an impression of the real significance of this issue. It is true that a great part of this volume goes to further valorisation at different value-added levels (spread on land, animal feed, composting, etc.), but still for the implementation of cost-effective or new production processes guiding updated figures are very helpful. Figure 4 offers an overview of the wastes and by-products generated by the different food processes evaluated and Figure 5 the estimated volumes of these substances in different European countries.

Table 2. % of food wastes and by-products in different processes

Production process	**% of wastes and by-products**
Fish canning	30-65
Fish filleting, curing, salting and smoking	50-75
Crustaceans processing	50-60
Molluscs processing	20-50
Beef slaughtering	40-52
Pig slaughtering	35
Poultry slaughtering	31-38
Milk, butter and cream production	Negligible
Yoghurt production	2-6
Fresh, soft and cooked cheese production	85-90
White wine production	20-30
Red wine production	20-30
Fruit and vegetables juice production	30-50
Fruit and vegetables processing and preservation	5-30
Vegetable oil production	40-70
Corn starch production	41-43
Potato starch production	80
Wheat starch production	50
Sugar production from sugar beet	86

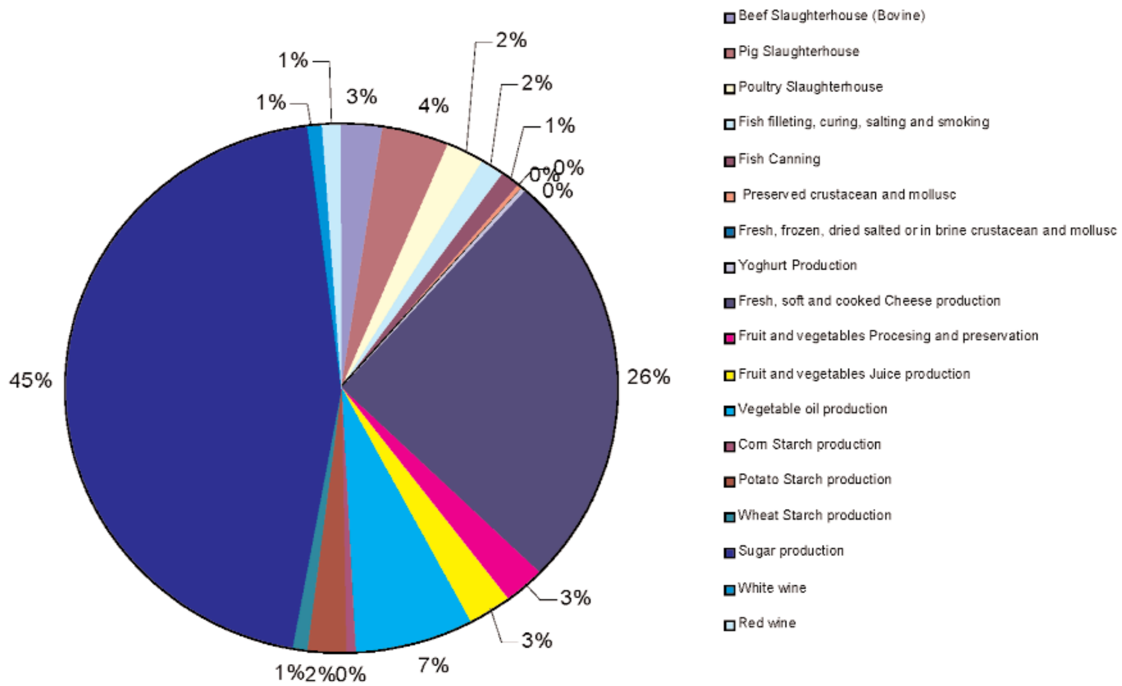

Figure 4. Contribution of different food production processes to food wastes and by-products in Europe

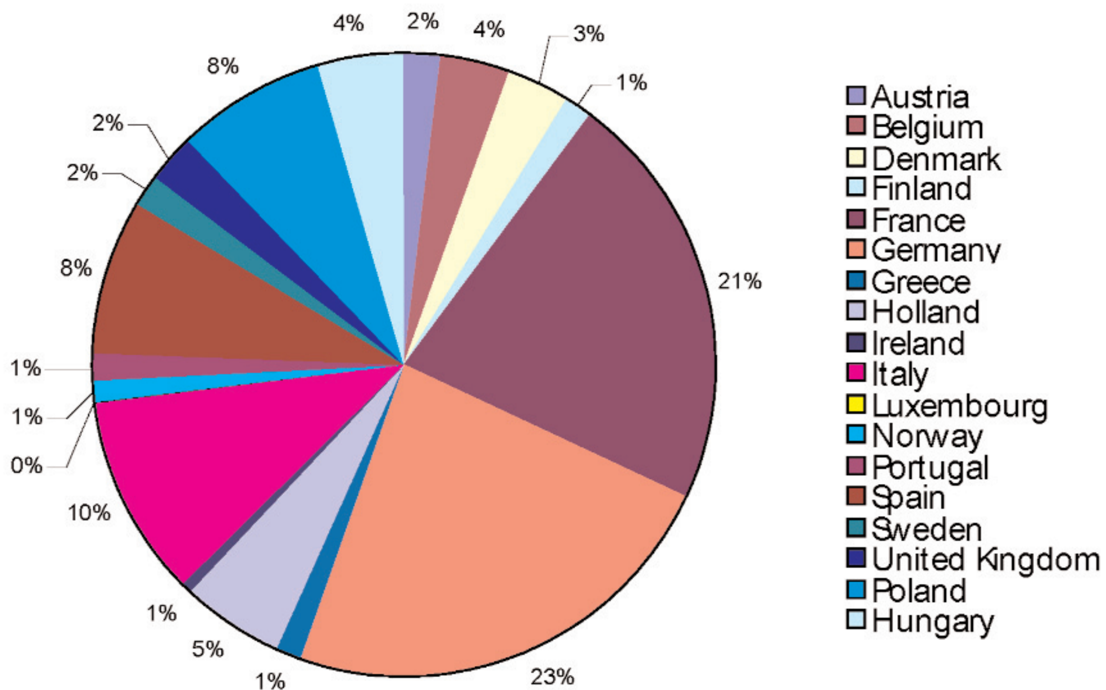

Figure 5. Food wastes and by-products distribution in Europe

Prevention, minimisation and valorisation technologies

The hierarchy of Pollution Prevention Technologies is normally as follows:

Source elimination or reduction	Zero cost or low costs
Reuse or recycling	Low costs
Waste treatment	Medium costs
Disposal	High cost

Consequently, in order to decrease the environmental impacts of food production processes and reduce the costs derived from the treatment and disposal of the wastes generated, actions should be focused on the less generation of wastes better than the treatment of them. Key actions address four main issues:

- Housekeeping: cleaning operations, maintenance of equipment, daily work practices
- Processes: optimisation
- Raw material: decreasing consumption or replacing some of them like hazardous cleaning agents, chemicals, and additives
- Technology: adopting new technologies or changing some equipment in order to reduce the generation of wastes and increase the efficiency of the process

Some of these actions imply no costs or low-costs and can decrease the environmental impact in a high extent such as good housekeeping. Others imply higher costs such as process optimisation and changes in equipment and technologies.

For this purpose, AWARENET technological issues have comprised the analysis of prevention and minimisation strategies in each of the 19 production processes. Main minimisation options include favouring the dry removal of solid substances and their ulterior valorisation, the recycling of process waters by the implementation of counter-current washing systems, the installation of water saving cleaning systems: water nozzling, triggered hoses, CIP systems… Besides, the recovery of valuable substances from liquid effluents (coagulation, membranes, ion exchange, fermentation, flotation, etc.) rather than their destruction in biological systems, giving rise to sludge, is favoured.

Furthermore, a thorough analysis of food waste valorisation technologies has been realised. A full range of technologies are described and classified under general types: mechanical separation, diffusional separation, chemical modification and biochemical modification. Besides, for each industrial sector and sub-sector evaluated within AWARENET individual valorisation casuistic has been analysed (Table 3).

Both for minimisation and valorisation technologies, analysis of IPPC-derived BAT concepts has been performed and the conclusions and suggestions to the IPTS working document are also included.
In fact, AWARENET has contributed to the BREF IPPC documents on *Slaughterhouses and animal by-products and Food, drink and milk processes.*

Market issues
Regarding market issues, all technology costs related to food waste and by-products management (avoidable costs in landfilling and treatment and valorisation costs for many options: composting, incineration, gasification, anaerobic digestion, biodiesel or bioethanol production…) have been evaluated. The hierarchy of added value of the different options is shown in Figure 6.

Hierarchy of added value:

- **Production of food products**
- **Chemical production**
- **Animal feed**
- **Fuel/energy recovery**
- **Composting or land application**
- **Sewer/landfill**

Higher value

Lower value

Negative value

Figure 6. Hierarchy of added value of food waste and by-products management options

Furthermore, a full list of products, some of them high-added value, deriving from food by-products with their guiding market values and applications is also presented in the handbook.

Table 3. Valorisation strategies in food processing

SECTOR	PROCESS	PRODUCTS from BY-PRODUCTS
Fish sector	Fish canning Fish filleting, curing, salting and smoking Crustaceans processing Molluscs processing	Fish meal and fish oil Polyunsaturated fatty acids Fish protein concentrate Fish protein hydrolysate Collagen Gelatine Direct animal feed Chitin/chitosan Calcium carbonate Foodstuffs
Meat sector	Beef slaughter Pork slaughter Poultry slaughter	Meat meal and fat Feather meal Protein hydrolysate Bone meal Blood plasma and blood cells Collagen Gelatine
Dairy sector	Milk, butter and cream production Yoghurt production Fresh, soft and cooked cheese production	Lactose Whey powder Whey protein Whey cheeses
Wine sector	White wine production Red wine production	Alcohol Natural pigments Tartaric acid Antioxidants Grape seed oil
Fruit and vegetables	Fruit and vegetable juice production Fruit and vegetables processing and preservation Vegetable oil production Corn starch production Potato starch production Wheat starch production Sugar beet production	Pectin Natural sweeteners Antioxidants Essential oils Proteins, acids, vitamin E , sterols Ethanol Fibres Lecithin Germ oil, germ meal Yeast Enzymes

Table 4. Commercial products from fish by-products market values

FISH SECTOR

Production Process	By-products	By-product components	Commercial products	Market Price (Euro/Kg or l)
Fish Canning & Fillleting	Head, Offal, Tail, Skin, Bone, Scrap		Raw	
			Fishmeal	0,5 - 1,3
			Fish silage	0,39
			Fish Protein	>1
			Fish Protein Hydrolysate	1-3,6
			Petfood	
			Fish Solubles	
			Food Ingredients	
			Glycerine, Soap	0,60
			Fish Glue	0,5-1
			Pearl Essence	
			Fish oil	0,2-0,67
			Fish oil raffinated	24
			Polyunsaturated fatty acids	
		Meat	Surimi	
		Skin and Bones	Collagen	15
			Gelatine	9
			Leather	13,35/skin
		Shark liver	Squalen	
		Shark cartilage	Chondroitin sulfate	
	Cooking and Wastewater			
			Flavour extract and powder	
			Fish proteins	0,33-110
			Fatty acids	7-19
	Shells		Construction material	0,015
Crustaceans	Shell and Viscera		Crustacean meal	0,435
	Shell		Chitin and Chitosan	15-100
			Astaxanthin	3000-12000

RTD needs

Last but not least, references to RTD needs linked to food wastes and by-products have also been evaluated. After a thorough analysis of EC funded projects in the last decade to check trends and deficiencies, the U.S.A. politics on food waste issues has been evaluated and compared to the sectorial RTD needs proposed by AWARENET members. General needs include:

- Reduce the water and/or water content of solid wastes
- Increase the shelf-life and value of solid wastes
- Pre-treament methods (sorting, sizing, etc)
- Convert the solid wastes into other by-products

Conclusions

In short, the Handbook resulting from AWARENET summarises the conclusions of the three-year progress of the network and aims to be useful for all those stakeholders, from food processors, recyclers, authorities, consumer groups, etc. close to food wastes and by-products management. The information aims to be extensive to those production processes not formerly covered by the network.

A copy of the handbook, as many other documents related to food wastes and by-products issues, is available at our Interest Group web site. Besides, AWARENET secretariat can give response to any issue regarding the network.

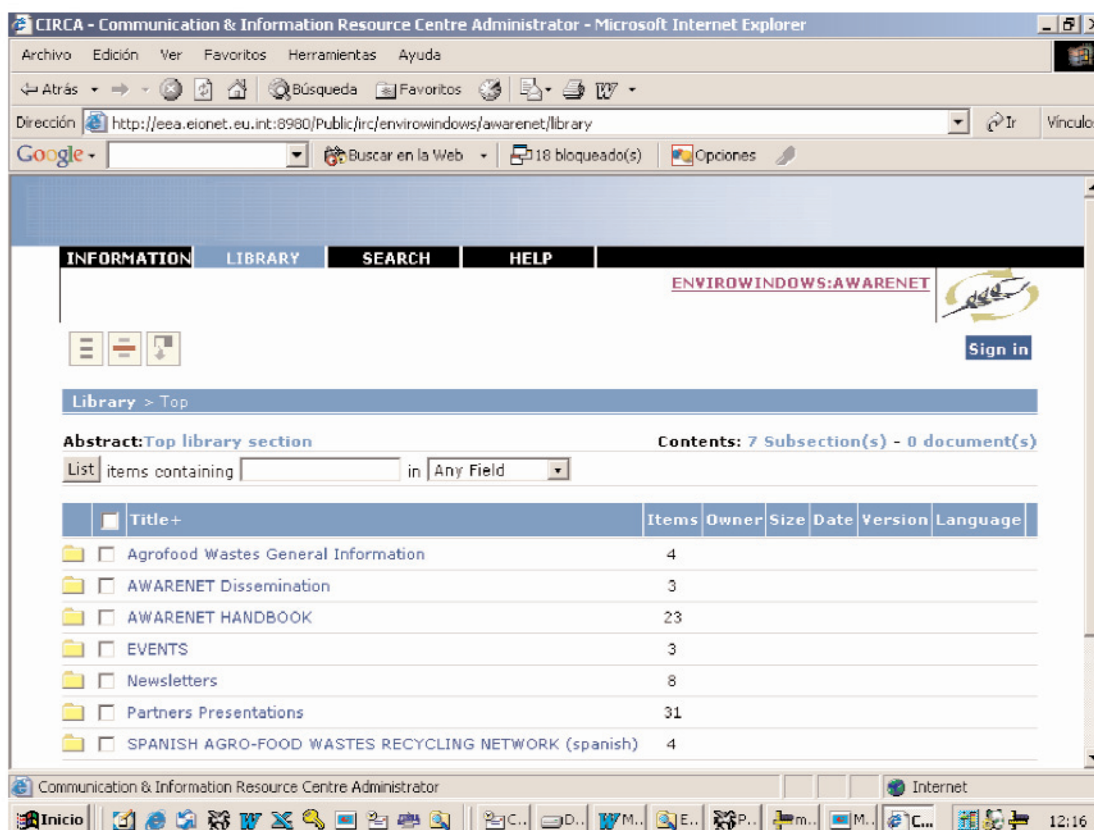

Figure 7. AWARENET Interest Group public access

Acknowledgements

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References

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Food Co-products as Feeds

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Introduction

The use of co-products from the food and drinks industries as animal feeds is a well-established practice. The oldest co-product feeds merchant in the UK, James & Son, has been marketing spent grains from London breweries since 1850, but the practice itself has a much longer history. Beer was first brewed some 5000 years ago in monastries and farms, and it is inconceivable that those early brewers did not make use of the extracted solids to feed their cattle. Such a tradition of use is a valuable feature in a world where many consumers appear to distrust the products of modern technology, especially in relation to the food chain. But tradition will not guarantee a future role unless feeding can continue to play a significant part in co-product valorization.

In the current situation, many food processors and manufacturers market a proportion of their production to the animal feed market. Distillers market both grains and the evaporated spent wash that remains after alcohol distillation. Fruit juice producers market their pulps and pomaces and sometimes remove other valuable fractions before they market, for instance, pectin-extracted pulp. Potato processors produce a wide variety of products, with every process likely to separate materials that fall either within or without the technical specification. Whilst the former group is marketed as human food, the off-spec material (often of similar nutritional value) is marketed as feed. The cereal fractionation industry deliberately separates its raw material into a number of specific fractions; viz: gluten, different grades of starch, and alcohol from the inseparable starch, with the remaining components recombined and marketed as a protein-rich feed. Arguably, the ultimate example is provided by the dairy industry that produces a huge range of products from milk, and whether they should be described as products or co-products is merely a matter of semantics – they are all intentional of value and in demand.

The Total Food conference highlighted a number of high value fractions that could be separated from co-products and provide an additional income stream. But these new processes may not utilize the full co-product supply nor obviate the use of the process residue as feed. Such novel processes may be regarded as merely an extension of the procedures currently in use. On the other hand, if the new process leads to contamination, physical destruction or an excessive increase in the moisture content of the residue, it may not be suitable for use as feed. In this case, the value of the new product would need to be substantially higher if the alternative process is to represent a commercial improvement on the current situation. An example was provided of a co-product that currently achieves a market price of £10 per tonne as a feed. If the novel product utilizes merely 3 per cent of the co-product, it would need to realise a price 33 times higher than the feed price and cover the disposal cost of the residue. That would imply a price of perhaps £375 per tonne, plus the novel processing cost, if the new product is to be competitive.

The Co-product Feeds Workshop considered it reasonable to suggest that any co-product development should attempt to retain the wholesome characteristics of the original raw material in the residue in order to ensure that, however different its composition, this material remains suitable for feeding. The Workshop was impressed by the potential of fermentation processes, such as bioethanol production, that not only yield an additional product but a yeast-enhanced co-product that has a higher commercial value in a world where protein feed may be in short supply.

The Workshop devoted some of its time to the discussion of fish and animal co-products, and to the impact of legislation. The avoidance of intra-species recycling was considered to be a sensible precaution that would help to prevent the recurrence of transmissible spongiform encephalopathies such as BSE. But it was noted that many fish are carnivorous and could make use of the co-products generated by the processing of other types of fish. The unfairness of the current ban on the use of fish co-product/fishmeal in ruminant diets, because the feed may have been 'contaminated' with meat products was identified , and hope was expressed that, following the development of a suitable diagnostic test, the ban would be lifted shortly. The waste involved in the removal of land animal co-products from the feed chain was also noted, with its implication that all of them represent an unacceptable health hazard. In the legislative area, the Workshop considered that of greatest concern was the confusion in official circles between products, by-products and wastes. This had been highlighted at the conference by the EU-sponsored Awarenet report that classified as waste a high proportion of materials that the food industry would regard as valuable fractions. Not only does such a classification threaten to burden the food industry with excessive bureaucracy, it may be self-fulfilling in that consumers may react negatively to the concept of animals fed on wastes and traditional feedingstuffs would then become wastes.

"Aid for Industry in Exploiting Research" – Workshop C

Williams, K. and Stockma, N.

Aims

- To understand the funding available for industrial R&D within the EU
- To identify opportunities for SME involvement
- To share best practice on engaging with SMEs

What Was Done?

- Presentation from Beta Technology detailing
  - Outline of Framework Programme 6
  - Funding themes
  - Budgets
  - Specific opportunities for SMEs
  - Opportunities for involvement of non-EU countries
  - What works?
  - Developments in FP7
- Round the table discussion detailing:
  - Delegates brought experiences from universities, research institutes, SMEs, member states and third countries
  - Knowledge shared on involving SMEs
  - Knowledge shared on industrial involvement in countries represented

Outputs

- Promoting successful engagement of SMEs
- EU Target for %involvement of SMEs in FP6 is 15%
- Current level of involvement is 8%

CRAFT

- 1 in 10 proposals funded
- Quality of proposals is poor
- Reality – 1 in 2 good proposals are being funded

SMEs

- "Rent an SME" is a waste of time
- SMEs must actively need the research output
- Need to identify "Elite SMEs"
- Interested, motivated & the technology champion
    - Innovative
    - Financially secure
- Existing relationships with SMEs advantageous

Increasing SME Involvement

- Reduced timescales to usable research outputs
- Shelter SME from bureaucratic burden
- Sector specific "IP for SMEs"

Suggestions & Solutions

- Create an EU wide network of elite SMEs
- Increasing SME involvement in Industry-Academia interactive programmes e.g. Food and Health Network
- Early access for SMEs to research proposals at the planning stage
- Early access for SMEs to research outputs