Bioresource utilisation by sustainable technologies in new value-added biorefinery concepts — two case studies from food and forest industry

Anna Ekman a,1, Monica Campos b, Sofia Lindahl b,2, Michelle Co c,3, Pål Börjesson a, Eva Nordberg Karlsson d, Charlotta Turner b,*

a Lund University, Department of Technology and Society, Environmental and Energy Systems Studies, P.O. Box 118, SE-221 00 Lund, Sweden
b Lund University, Department of Chemistry, Centre for Analysis and Synthesis, P.O. Box 124, SE-221 00 Lund, Sweden
c Uppsala University, Department of Physical and Analytical Chemistry, P.O. Box 599, SE-751 24 Uppsala, Sweden
d Lund University, Department of Chemistry, Biotechnology, P.O. Box 124, SE-22100 Lund, Sweden

* Corresponding author. Tel.: +46 46 222 8125; fax: +46 46 222 8209.
E-mail addresses: Charlotta.Turner@chem.lu.se, charlotta.turner@me.com (C. Turner).
1 Current address: SIK, P.O. Box 5401, SE-402 29 Gothenburg, Sweden.
2 Current address: University of Oslo, Department of Chemistry, P.O. Box 1033, Blindern, NO-0315 Oslo, Norway.
3 Current address: Medical Products Agency, P.O. Box 26, SE-751 03 Uppsala, Sweden.

1. Introduction

Concern about climate change and scarcity of resources has brought environmental and sustainability issues into focus both on the political agenda and in the consciousness of the general public. One of the most important questions is the dependence of our economic output on fossil oil. This fossil feedstock is not only used for energy generation and transport, but also for the production of the major part of all materials and chemicals that we now rely on. One option to provide a more sustainable base for the economy would be the transition to a bio-economy in which the importance of biotechnology and biomass-based production to generate economic output is significantly greater than today (OECD, 2009). To make this transition not only possible but also sustainable, new technologies and efficient utilisation of biomass resources with good environmental performance in biorefineries will be necessary (Kamm and Kamm, 2007; Cherubini, 2010; de Jong et al., 2012). A biorefinery has parallels to a petroleum refinery in the sense that several products such as chemicals, biofuels and bio-based energy carriers are produced from one single raw material and that the production steps are integrated. In recent years much political focus has been on the development of biofuels, but also the market for bio-based chemicals is projected to increase significantly (Dornburg et al., 2008). To optimally utilise biomass resources, biorefineries that first extract valuable compounds using sustainable technologies and thereafter convert the biomass into other bio-based chemicals, biofuels and energy carriers should be implemented (Ragauskas et al., 2006; Clark et al., 2006). Technologies of importance will be those based on white biotechnology and on the principles of green chemistry (Hatti-Kaul et al., 2007).
Cultivating crops, for example with the purpose of production of biofuels gives a considerable contribution to environmental impact (Börjesson and Tufvesson, 2011). Utilising biomass residues as feedstock in biorefineries, is a promising option to further increase the sustainability of biorefineries, because this feedstock normally does not cause increased land-use competition with food production and potential, negative indirect land-use changes (see e.g. Searchinger et al., 2008; Berndes et al., 2011).

From a Swedish perspective, the utilisation of residues as raw material could increase profits both in the agriculture and in the forest industry. These are important industrial branches, which for several years have experienced decreasing real prices of their products in combination with increased prices of raw materials (Statistics Sweden, 2010a, 2010b). This has increased their motivation to find new value-added processes and products, especially targeting their low value by-products and waste streams, to increase their product portfolio. In addition to being inspired by university research, also political incentives influence the industry and the innovations realised (Fischer et al., 2003).

In this article an inventory of the key wastes and by-products from agriculture, food and forest industry sectors, feasible to use as input materials in biorefinery concepts in Sweden, are presented. Today, these raw materials are mainly used in the production of heat and power, animal feed or biogas by anaerobic digestion. However, it is known that these materials may also contain high-value compounds such as antioxidants, pigments and other molecules of interest. Antioxidants are compounds that prevent oxidation, for example, oxidative damage in biological systems caused by free radicals, and oxidation processes occurring in food, cosmetic and bulk chemical products (Halliwell, 1990). Some of the antioxidants are also valuable pigments that could pertinently replace chemically synthesized ones.

Two of the residues identified, onion waste from the food industry and birch bark from the forest industry, have been more thoroughly investigated with the aim to increase their current value as energy feedstock or animal feed to an additional value as feedstock for both energy and chemical compounds. In the first case, glycosylated antioxidants have been extracted from onion waste using pressurised hot water as a solvent. After the extraction, the quercetin glucosides are hydrolysed to the most active antioxidant form, quercetin, using thermostable β-glucosidase in a hot water process. Quercetin is known as a powerful antioxidant, for instance, in vitro studies have shown that quercetin may have a positive effect against cancer (Murakami et al., 2008), cardiovascular- (Cook and Samman, 1996) and neurodegenerative diseases (Ono et al., 2006).

In the second case, a mixture of antioxidants and betulin, a triterpenoid compound, have been extracted from birch bark using liquid carbon dioxide mixed with ethanol. Betulin has anti-inflammatory as well as anti-bacterial properties (Zuco et al., 2002; Yogeeswari and Sriram, 2005; Paduch et al., 2007), which is already recognised and betulin is used as additive in many cosmetic products. Furthermore, both in vitro and in vivo studies have proved that betulinic acid is a potent agent against a certain line of cancer and HIV (Pujjokia et al., 1994; Pisha et al., 1995; Cichewicz and Kouzi, 2004).

After processing, the remaining waste can be utilised for energy purposes as today, and in some cases be recycled as biofertilisers for cultivated land. Thus, the suggested extraction processes should be seen as complementary technologies, which could be integrated in the existing industry infrastructure.

2. Design of studied biorefinery concepts

This work has a trans-disciplinary approach and the methods applied cover analytical chemistry, biotechnology and environmental systems studies. The biorefinery concept according to this paper means value addition to by-products by technologies based on extraction and enzymatic conversion with environmentally compatible solvents and processes. An overview of the biorefinery concepts considered is shown in Fig. 1a and b.

This paper is divided into three following sections. Section 3 describes the work to find suitable raw materials that can be used as feedstock in future biorefinery applications. To enhance the value of the identified waste and by-products, novel technologies have been developed and existing technologies have been adapted to be more suitable for these specific types of materials. These technologies and how they have been applied to the two selected raw materials, onion waste and birch bark, are described in Section 4. Sustainability is one of the main drivers for the development of

---

**Fig. 1.** a. Schematic flowchart of an agricultural biorefinery concept using yellow onion as raw material. Products are shown in grey. Black dashed boxes show methodologies, and the value added processing step (including its raw material and product is boxed (grey dashed line). Waste residues after the extraction/hydrolysis step can be utilized in energy or fuel production, here exemplified by anaerobic digestion (dotted box). b. Schematic flowchart of a forest biorefinery concept using birch as raw material. Products are shown in grey. Black dashed boxes indicate methodologies. The value added processing step (including its raw material and product is boxed (grey dashed line). Waste residues after the extraction/hydrolysis step can be utilized in energy or fuel production, here exemplified by incineration (dotted box) currently in use for energy production. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
biorefineries, and sustainability comprises environmental, economic and social concerns. Section 5 presents a life cycle assessment (LCA) of the two novel technologies with focus on primary energy use and greenhouse gas (GHG) performance. The paper ends with the discussion and conclusions including some societal impacts from the proposed biorefinery systems.

3. Survey of potential wastes and by-products in Sweden

A screening survey has been conducted to find wastes and by-products from the food/agricultural and forest industries in Sweden, feasible as raw materials for biorefineries. The information obtained from the survey served as a basis for the selection of feedstock materials for the case studies included here. The availability of marketable, high-value compounds and sufficient volumes of residues were considered important parameters for their further development into biorefineries.

3.1. Data collection methods

In the literature, detailed statistics of residues from the fruit and vegetable industry is very scarce, in spite of the fact that an increasing number of researchers have discovered that extra value can be extracted from such waste, both energy and chemicals, making it an interesting resource (Laufenberg et al., 2003). However, some attention has been paid to the situation. For instance, all EU member states are required to provide statistics concerning the generation of industrial waste (SMED, 2006; Mahro and Timm, 2007; ARCADIS, 2009). The lack of statistics of vegetable industry waste might be due to various circumstances, e.g. the lack of terminology that makes it difficult to categorize different types of wastes (Mahro and Timm, 2007). Sometimes it is unclear which products can be seen as pure waste and which as by-products with a certain value. Because food waste does not cause any severe environmental threat, the handling and documentation of waste from the food industry has so far not received much attention (ARCADIS, 2009).

To obtain a better estimate, interviews were undertaken at Swedish companies processing fruit and vegetables. The survey was conducted in 2007 and companies were identified using the company register from Statistics Sweden (SCB). The companies were contacted by phone and answers were collected by phone or e-mail. The total amounts were calculated on the basis of the replies received. To assess the amounts of by-products from the forest industry, no interviews were necessary since the Swedish Forest Agency and the Swedish Forest Industries Organisation annually provide detailed statistics (Swedish Forest Industries Federation, 2009; Swedish Forest Agency, 2010; Swedish Energy Agency, 2011). These official statistics and published reports contained sufficient information and were used in the screening for potential raw materials from the forest industry.

3.2. Food industry and agriculture

From the company register 54 companies in relevant sectors and having a sufficient number of employees were asked to participate in the survey. Among these, the answer-frequency was approximately 44%. There is a high level of variation in the answers obtained from the interviews and therefore the estimates of volumes of residues are shown in relatively wide intervals (Table 1). However, the results served the purpose of identifying resources from which significant waste volumes are produced. From a quantitative perspective, the most abundant resources originated from cereal and potato production (Table 1). Potato waste is a material on which there is relatively limited information concerning potential extractables, with the exception of glycoalkaloids that are potential drug molecules often quite toxic to organisms (Friedman, 1997). Identification of other compounds would be valuable for further investigations. Cereal waste, on the other hand, has been more intensely investigated, and for this resource it is already known that, for instance, tocopherols (vitamin E, antioxidant) and plant sterols (beneficial for cardiovascular health) can be extracted (Ryan et al., 2007). Relatively large amounts of waste also originate from vegetable and fruit production (e.g. onions, oranges and apples) and in these cases polyphenolic compounds are interesting targets for extraction (Table 1). Numerous variants of polyphenolic compounds are present in various materials, and are frequently glycosylated on different positions of the backbone of the molecular structure. Quercetin and its glycosylated forms (Fig. 2a) are polyphenolic antioxidants identified as potentially extractable compounds from onion by-products (Table 1). Interest in this compound is based on health-promoting effects, supported by in vitro studies (Murakami et al., 2008; Cook and Samman, 1996; Ono et al., 2006). As our survey showed that onion by-products are available in Sweden in relatively large amounts, value addition by adding quercetin (a well defined molecular product) was set as an example for concept analysis of which a more detailed description is given in the following sections.

The amount of quercetin species varies in the yellow onion depending on sort and storage. In addition, the choice of extraction method will affect the yield of the product, but an estimate based on averaged data (0.2 g/kg (USDA, 2007)) predicts that this resource alone is sufficient for a yearly production of more than half a metric ton quercetin/year.

Today, the use of onion waste is in principle limited to compost, animal feed, or raw material in biogas-production (currently 50–75% of the onion waste (Ekman and Börjesson, 2007)). Our goal is to add value by implementing a sustainable method to first obtain the quercetin and then facilitate further use of the remaining waste for biogas production by anaerobic digestion. To achieve this, care has to be taken when selecting solvents and catalysts in this added step preceding the anaerobic digestion.

3.3. Forest industry

Sweden is the world’s second largest producer of processed forest products such as paper, pulp and sawn timber. The forest industry generates several by-products, which are used for a number of purposes such as the production of chipboard and plywood, chemicals or as a source of bioenergy (Swedish Forest Industries Federation, 2009). A large amount of by-products from the forest industry is today also used in energy generation, and bioenergy already plays an important role in the Swedish energy system due to a combination of the vast biomass resources and active governance to increase the share of renewable energy, for example, a carbon dioxide tax on fossil fuels. A large amount of forestry residues is used for internal energy generation in the forest industry. Even if it is desirable to use renewable energy from these sources, extraction of valuables in a step preceding energy production is interesting to increase the utilisation of the resource. The total use of logging residues, such as tops and branches for energy purposes is 7–8 TWh, but there is a potential to increase this without ecological damage (Jacobsson, 2005). Bark is another significant waste resource (Table 2), frequently separated into hardwood and softwood fractions. In this work, the chosen example concerns bark from birch. The amount of birch bark produced through decortication from forestry is estimated to be 1–2 Mm3/year (see Table 2). Data in the literature as well as our own results show that betulin (Fig. 2b), making up between less than 1% and 30% of the birch bark dry matter content, can be extracted before
Table 1
Survey of total amounts of by-products from the food/agricultural industry in Sweden combined with extractable, potentially high-value compounds found in the by-products as identified in the literature. The amounts of extractables are given as estimates and vary with the season and species, i.e. mg/g means 1–999 mg/g.

<table>
<thead>
<tr>
<th>Waste resource</th>
<th>Amount (tonnes/year)</th>
<th>Current use</th>
<th>Potential extractable compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Straw (wheat)</td>
<td>260,000–1,200,000(a)</td>
<td>Animal farming/energy production(a)</td>
<td>Wax products(b) (N.A.)</td>
</tr>
<tr>
<td>Cereal husks and other waste (mills)(b)</td>
<td>57,000(b)</td>
<td>Energy(b)</td>
<td>Policosanol (mg/kg)(b)</td>
</tr>
<tr>
<td>Potato juice (starch prod.)(c)</td>
<td>100,000–200,000(c)</td>
<td>Fertilizer</td>
<td>Tocopherols and plant sterols (mg/kg d.w.)(c)</td>
</tr>
<tr>
<td>Potato pulp (starch prod.)(c)</td>
<td>20,000–40,000(c)</td>
<td>Animal feed/Fiber production(d)</td>
<td>Proteins, amino acids, glycoalkaloids (mg/kg d.w.)</td>
</tr>
<tr>
<td>Potato waste (food production)(e)</td>
<td>40,000–50,000(e)</td>
<td>Biogas/Incineration(e)</td>
<td>Chlorogenic acid, glycoalkaloids, Phenolic acids (mg/100 g f.w.)(e)</td>
</tr>
<tr>
<td>Selected fruit and vegetable wastes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrot</td>
<td>5000–10,000(m)</td>
<td>Biogas/Animal feed</td>
<td>(\alpha)- and (\beta)-carotenes (mg/100 g f.w.)(m)</td>
</tr>
<tr>
<td>Onion</td>
<td>1000–5,000(m)</td>
<td>Animal feed/Biogas</td>
<td>Quercetin derivatives (mg/100 g f.w.)(m)</td>
</tr>
<tr>
<td>Orange (juice production)</td>
<td>5000–10,000(m)</td>
<td>Animal feed/Biogas</td>
<td>Naringenin derivatives (mg/100 g f.w.)(m)</td>
</tr>
<tr>
<td>Apple pomace/skin</td>
<td>500–1,500(m)</td>
<td>Animal feed/Soil improver</td>
<td>Hesperitin derivatives (mg/kg d.w.)(m)</td>
</tr>
<tr>
<td>Lettuce</td>
<td>1000–5000(m)</td>
<td>Compost</td>
<td>Vitamin C (mg/100 g f.w.)(m)</td>
</tr>
<tr>
<td>Brewery waste</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Draft/Brewers’ spent grain</td>
<td>60,000</td>
<td>Animal feed</td>
<td>Quercetin derivatives, cyanidin, ((\cdot\cdot\cdot\cdot))-epicatechin, ((\cdot\cdot\cdot\cdot))-catechin, phloridzin, chlorogenic acid (mg/100 g f.w.)(m)</td>
</tr>
<tr>
<td>Yeast</td>
<td>10,000</td>
<td>Animal feed</td>
<td>Quercetin derivatives, caffeyl derivatives (mg/100 g f.w.)(m)</td>
</tr>
</tbody>
</table>

\(d.w.\) = dry weight, \(f.w.\) = fresh weight, N.A. = not available.

\(a\) Börjesson (2007).
\(b\) Desvarte et al. (2006).
\(c\) Irmak et al. (2006).
\(d\) Dunford et al. (2010).
\(e\) Avfall Sverige (2008).
\(f\) Bryngelsson et al. (2002).
\(g\) Ryan et al. (2007).
\(h\) Normen et al. (2002).
\(i\) Ekman and Börjesson (2011).
\(j\) Bartova and Barta (2009).
\(k\) Meyer et al. (2009).
\(l\) Byg et al. (2012).
\(m\) Ekman and Börjesson (2007).
\(n\) Schieber et al. (2001a).
\(o\) Dao and Friedman (1994).
\(p\) Friedman and Dao (1992).
\(q\) Rodriguez De Sotillo et al. (1994).
\(r\) Alasalvar et al. (2001).
\(s\) Mustafa et al. (2012).
\(t\) Arscott and Tanumihardjo (2010).
\(u\) Slimestad et al. (2007).
\(v\) USDA (2007).
\(w\) Khan et al. (2010).
\(x\) Sawalha et al. (2009).
\(y\) Bermejo et al. (2011).
\(z\) Lu and Foo (1997).
\(aa\) Schieber et al. (2001b).
\(ab\) Crozier et al. (1997).
\(ac\) Ribas-Agusti et al. (2011).
\(ad\) Llorach et al. (2008).
\(ae\) Romani et al. (2002).
\(af\) Mussatto et al. (2006).
\(ag\) Tanguler and Erten (2008).

Fig. 2. (a) Chemical structure of quercetin (R, R’ = H), quercetin-3,4’-diglucoside (R’R = glucose), quercetin-4’-glucoside (R = H, R’ = glucose) and quercetin-3-glucoside (R = glucose, R’ = H). (b) Chemical structure of betulin.
4. Process development of biorefinery concepts

The aim of this process development is to not only choose processes that can add value to existing raw materials, but also to demonstrate methodologies with better environmental performance than conventional ones, which is in accordance with the principles for green chemistry (Anastas and Eghbali, 2010). Food and forest industries are the main producers of plant-based by-products and wastes in Sweden, as well as around the world. Birch bark and onion waste well represent feedstocks from these two sectors, and the former is a large-volume feedstock while the latter is of significantly smaller volume. Furthermore, both these by-products contain high-value compounds. The two case studies thus represent different aspects in terms of both industry sector and volume, which is of interest in a broad trans-disciplinary study like this.

The technologies comprised extraction (to obtain a betulin/antioxidant mixture from birch bark) or extraction combined with hydrolysis (to obtain quercetin from onion waste). The methodologies in the respective process were selected to take into account the use of more environmentally adapted solvents and techniques (both cases), as well as compatibility between the extraction and hydrolysis steps (the onion waste process). Biocatalysis was according to green chemistry principles chosen for the hydrolysis step. As the choices of commercial enzymes operating at different conditions are still limited, in house production and development of a selected enzyme was chosen (Fig. 3). Hence, in this case, pressurised hot water was selected as solvent and a thermostable enzyme as a catalyst for the extraction and conversion of quercetin from onion waste. In the other case, pressurised liquid carbon dioxide mixed with ethanol was chosen as a solvent for the extraction of betulin and antioxidants from birch bark.

4.1. Extraction and enzymatic hydrolysis targeting quercetin in onion waste

The conventional method used for extraction of quercetin from yellow onion is solid–liquid extraction with aqueous methanol.
should at least withstand temperatures of 85 °C. A response surface plot of PHWE yields of waste after hydrolysis will also not hinder further reprocessing raw material. Water is a cheap and readily available solvent that is non-flammable and non-toxic in contrast to the conventional extraction solvent methanol. Presence of enzyme in the waste after hydrolysis will also not hinder further refining of remaining raw material. A combination of the extraction and hydrolysis steps (Fig. 1a), promotes savings in material as well as in energy, but requires the use of compatible process conditions. In combination with PHWE an enzyme active and stable at high temperature is needed. A response surface plot of PHWE yields of quercetin-forms from yellow onion waste showed that the enzyme should at least withstand temperatures of 85 °C (Turner et al., 2006). Added advantages with high temperature processing also integrates better enzyme penetration and cell-wall degradation of the raw materials (Turner et al., 2007). Opposed to acid hydrolysis, enzymatic hydrolysis is selective, and thus the chosen enzyme needs to be specific for the glycosylation pattern on the target compound. Quercetin from onion waste is glucosylated and a glucosidase (EC 3.2.1.21) is suitable to obtain the aglycone form. Thermostable glucosidases, such as (TnBgl1A and TnBgl3B) from the hyperthermophilic bacterium Thermotoga neapolitana (growth optimum above 80 °C) meet both the temperature and specificity criteria, but were not commercially available. To obtain these enzymes, their genes were cloned and the enzymes produced in house (Turner et al., 2006).

4.1.1. In house production and development of glucosidase for improved hydrolysis

Numerous enzymes are available for researchers with their own cloning and production facilities, but the number of commercially available enzymes is significantly lower. In addition, as natural selection is not driven to select industrially relevant reactions, development in vitro is often required to improve the hydrolysis yield for desired processes (Fig. 3a). In this case, glycosylation at the 3-position in quercetin (Fig. 2a) is more difficult to hydrolyse enzymatically (Berrin et al., 2003; Turner et al., 2006). To improve hydrolysis at this position the gene encoding the β-glucosidase TnBgl1A was developed by mutagenesis (Lindahl et al., 2010; Khan et al., 2011), resulting in a variant, mutated at two positions, Fig. 3b, with improved the catalytic efficiency (Lindahl et al., 2010).

Lab-scale (0.5–3 L) cultivations in Escherichia coli (E. coli), for production of both wild-type and developed TnBgl1A resulted in approximately 40–50% target protein of the total protein in the cell, of which approximately 10% of the produced enzyme was in an active, soluble form (data not shown). Purification was accomplished by a two-step protocol, including heat treatment followed by immobilised, metal ion affinity chromatography (IMAC) utilizing a C-terminal His-tag. Heat treatment is a quick purification step (which selectively denatures heat-labile host cell proteins) resulting in approximately 70% purity that in many cases is sufficient for a commercial product. After the chromatographic step the purity of the biocatalyst exceeded 90%.

4.1.2. Pressurised hot water extraction and hydrolysis

The pure enzyme (>90%) was used in a biocatalytic hydrolysis step, which initially was separate from the extraction to allow the analysis of each step. Separation of the 2 steps allowed higher extraction temperature (120 °C, 50 bar) and shorter extraction time. The stability of the enzyme limited the temperature in this step to maximum 95 °C (Lindahl et al., 2010), but at this temperature, only limited incubation times were possible, as alpha-helical parts were reversibly affected from 90 °C and at 95 °C the half-life was 13 min. The yield of the combined extraction of 3 x 5 min and 10 min enzymatic hydrolysis at 90 °C was 13 mg quercetin/g onion dry weight (or 1.9 mg/g onion), using the wild type enzyme and an enzyme/onion dry weight ratio (g/g) of approximately 1/900 (calculated from data in Turner et al., 2006) but did not completely convert quercetin-3-glucoside to the aglycone. The yield was, however, similar or slightly higher than that obtained using the conventional, more laborious methanol/acid hydrolysis method (Turner et al., 2006). Using the mutant enzyme (Lindahl et al., 2010), complete conversion of quercetin-3-glucoside to the aglycone quercetin was obtained using a temperature of 95 °C for 5 min, but with a 2.5 times higher enzyme load.

To put this into perspective, the volume of onion waste is 1000–5000 tons fresh weight annually, corresponding to around 150–740 tons dry weight (Table 1). Dry weight percentage was obtained using a conversion factor of 6.75 (Turner et al., 2006). The quercetin-glucoside content is in the range of 100–1650 kg. Enzymatic conversion to quercetin aglycone, with the non-optimized enzyme load used in the analytical experiments (Turner et al., 2006; Lindahl et al., 2010) would lead to the use of 0.55 kg enzyme/kg quercetin aglycone and require roughly 55–900 kg enzyme per annum. A web-survey shows that the price of quercetin can be estimated to be in the range 10–150 $/kg (see alibaba.com, search-term quercetin). The high enzyme/product ratio necessitates limiting the cost of the enzyme to about 0.5–30 $/kg enzyme, which would cover 10% of the estimated price of the product. These estimates are, however, calculated directly from the analytical scale data, without attempting to optimize the enzyme load.

The current E. coli production profile, would in fed-batch mode of operation (assuming a final dry cell weight (dcw) of 50 g/L and recombinant protein of 3 g/L (calculating 10% of total protein as target protein, and a total protein corresponding to 0.5 x dcw) require a yearly cultivation volume in the range 20–300 m³ (to be compared with sizes of industrial scale fermentors, 1–50 m³). No up-scaling/optimization of either enzyme load or enzyme production (or production-system) has, however, yet been made, and improvements in these areas are achievable.

4.2. Liquid CO2/ethanol extraction of betulin and antioxidants from birch bark

Extraction of betulin from birch bark has conventionally been performed using an organic solvent e.g. dichloromethane, chloroform, acetone or methanol (Zhao et al., 2007). Betulin also has a relatively high solubility in ethanol, which increases with temperature (Zhao et al., 2007; Co et al., 2009). Birch bark also contains a variety of antioxidants, which are extractable by either water or ethanol at elevated temperature (Co et al., 2009). Hence, ethanol is a solvent that makes the extraction of both betulin and antioxidants possible. Ethanol is considered to be an environmentally sustainable solvent (Capello et al., 2007). However, the use of large amounts of ethanol in an extraction process requires an energy-intensive down-stream evaporation step.

One way to minimize the amount of ethanol used in the process is to instead use pressurised (i.e. liquid) CO2 containing 5–20 weight% ethanol. It is well known that liquid and supercritical
carbon dioxide are powerful solvents, enabling efficient extraction of lipids, oils, plant sterols and small organic compounds (Herrero et al., 2006; Pereira and Meireles, 2010). Since CO₂ is a gas at ambient temperature and atmospheric pressure, separation of products from the solvent is easy, and recycling of CO₂ a simple step of condensation and re-pressurisation. The use of supercritical carbon dioxide as solvent has been explored, as well as supercritical CO₂ with ethanol added as a co-solvent (Felfoldi-Gava et al., 2009).

Betulin was successfully extracted by both methods, the extraction yield being higher when using ethanol as a co-solvent.

In a previous study, Co et al. (2009) achieved complete extraction (ca 26 weight% of the bark, with bark being the outermost thin layer) of betulin in a small-scale analytical process that was significantly faster (minutes rather than hours) compared to conventional solid-liquid extraction. Pressurised hot ethanol was used as solvent and faster (minutes rather than hours) compared to conventional solid-liquid extraction. Pressurised hot ethanol was used as solvent and yielded 120°C in the extraction yield when using ethanol as a co-solvent.

Betulin was successfully extracted by both methods, the extraction yield being higher when using ethanol as a co-solvent.

For the design of a biorefinery, liquid CO₂ (50 bar, 16°C) with 20 weight% ethanol was used as extraction solvent, in a pilot-scale process. 200 kg of birch bark was processed, taken from a Swedish forest industry, Skutskär Stora Enso. Bark in this case contained not only the outermost betulin-rich layer but also inner more soft brown-colored layers. The birch bark extract produced was then investigated with regard to antioxidant composition, betulin content and the effect of birch-bark extracts as repellents against fallow deer (Alm Bergvall et al., 2013). Results obtained showed that 1 kg of birch bark extract (dry weight), derived from 56 kg of birch bark (dry weight corresponds to 120 kg of wet bark), contains about 150 g (dry weight) of betulin. It is, however, not known whether betulin alone is responsible for the repellent effect on fallow deer or whether other constituents, or a combination of betulin and other constituents in birch bark extract have this effect. Thus, both betulin and the whole birch bark extract are used in the calculation; roughly 1 kg of birch bark extract (dry weight) can be used to protect roughly 2500 pine trees from browsing of fallow deer, based on certain assumptions (Alm Bergvall et al., 2013). For the development of a final product, these loads must be further studied and optimised.

A conventional method, immersing dried birch bark in ethanol for several days, was used as comparison. Results obtained with the two methods are very similar in terms of output (Alm Bergvall et al., 2013); since in the proposed new method, liquid CO₂ acts mainly as a “carrier” for ethanol, not significantly changing its chemical properties as a solvent. However, the liquid CO₂ enables a significantly faster extraction, due to the higher diffusivity of the solvent (McHugh and Krukonis, 1986).

The extraction yield of betulin from the pilot scale run, was significantly lower than in the laboratory scale trials. This is mainly due to how the bark was collected as was discussed above, but also due to the equipment used. Hence further development on a large-scale and evaluation of the methods is necessary to improve the efficiency of the process. The process was, however, already at this stage sufficiently good to produce a product against browsing deer (Alm Bergvall et al., 2013).

5. Environmental evaluation

5.1. Life cycle assessment (LCA)

LCA is a comprehensive tool to calculate the environmental impact of a product or process, and the methodology is described in the standards ISO 14040-14044 (ISO, 2006).

In this study, LCA is used to compare the environmental impact of biorefinery systems that are either based on the extraction methods developed here or conventional extraction processes. The environmental impact categories included are global warming potential (GWP) and the demand for primary energy. The LCAs in this paper are gate-to-gate studies and so the environmental impact related to the production of the raw material, onion waste or birch bark, as well as the final use of products, are not included in the study. The input data used for the assessments is shown in Table 3.

5.2. Quercetin from onion waste

Two different onion waste-based biorefinery concepts were studied and the processes are shown in Fig. 4. The first one, named developed method, utilises PHWE followed by enzymatic hydrolysis of quercetin glucosides as described by Lindahl et al. (2010) for the extraction of quercetin. After the extraction, the remaining onion waste enters an anaerobic digestion unit in which methane and bio-fertilizer are produced. By the second method, called conventional method, quercetin is extracted by leaching with aqueous methanol and the hydrochloric acid catalysed hydrolysis of quercetin glucosides occur simultaneously. The onion and methanol mixture is then incinerated with heat recovery. Ash is, however, not recovered from incineration and used as fertilizer. The functional unit of the analysis is one tonne of onion waste. The input and output flows from the two biorefinery concepts are shown in Table 4. Data for extraction of quercetin is adapted from Lindahl et al. (2010) and for biogas production from Berglund and Börjesson (2006). Data for production of enzyme are taken from Nielsen et al. (2007), data for methanol from Fernlund (1996) and data for hydrochloric acid from Bousted (2005). Input data are shown in Table 3. Natural gas is used for the generation of heat and power (Ekman and Börjesson, 2011). The extraction process is not

Table 3

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Methanol</th>
<th>HCl</th>
<th>Ethanol</th>
<th>CO₂</th>
<th>Heat</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Energy Demand (MJ/kg or MJ/kg)</td>
<td>110</td>
<td>56</td>
<td>27</td>
<td>48</td>
<td>11.3</td>
<td>1.17*</td>
</tr>
<tr>
<td>GWP (kg CO₂-eq/kg or kg CO₂-eq/kg)</td>
<td>9</td>
<td>2.6</td>
<td>1.6</td>
<td>3.2</td>
<td>1.8</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.128</td>
</tr>
</tbody>
</table>

* Including a conversion efficiency of 90%.
yet tried in a scale larger than lab-scale and the LCA data is thus derived from a theoretical scale-up of this process as described in Lindahl et al. (2010).

From Table 4 and Fig. 5a and b it can be seen that the developed method requires significantly less energy and material input than the conventional method. For the new method, process energy contributes the most to environmental impact. In cases when enzyme cannot be reused or if a higher enzyme load per amount of product is needed, this would also give an increase both in emissions and energy demand. However, the emissions from the biorefinery concept based on conventional methods are significantly larger, mainly due to the consumption of fossil methanol. Even if the recovery of methanol is improved, thereby reducing the total emissions, this step still contributes significantly. Changing fossil methanol to renewable would improve the environmental performance but the total emissions would still be greater than for the developed method. The energy recovered from the concept based on the conventional method is mainly derived from methanol and thus not renewable. Upgraded biogas, which is the product from the biorefinery based on the developed method, has its origin in the onion waste and is thus renewable. This biogas can be used as a vehicle fuel or as feedstock in the chemical industry and is thus a more valuable product than heat.

Table 4
Summary of input and output flows from the biorefinery system of onion waste.

<table>
<thead>
<tr>
<th></th>
<th>Input</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developed method</td>
<td>Onion residues 1 tonne</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water 1.2 m³</td>
<td>1.25 GJ</td>
</tr>
<tr>
<td></td>
<td>Enzyme 0.3 kg/kg quercetin</td>
<td>1.3 kg</td>
</tr>
<tr>
<td></td>
<td>(reuse 20 times gives 0.02 kg/tonne onion)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heat 1.15 GJ</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Power 0.18 GJ</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biogas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₄</td>
<td>1.9 kg</td>
<td></td>
</tr>
<tr>
<td>Conventional method</td>
<td>Methanol 5.3 tonnes (6% process loss gives 0.32 tonnes/tonne onion)</td>
<td>1.3 kg</td>
</tr>
<tr>
<td></td>
<td>HCl 1.6 tonnes (6% process loss gives 0.1 tonnes/tonne onion)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heat 8.4 GJ</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat</td>
<td></td>
<td>6.34 GJ</td>
</tr>
</tbody>
</table>

Fig. 4. Description of the systems studied, the newly developed and conventional methods for production of quercetin extract from onion waste.

Fig. 5. a. Primary energy consumption in the biorefinery concepts for onion waste. The contribution from enzyme is minor and therefore not visible. b. GWP for the biorefinery concepts of onion waste. Expansion of system is not included. The contribution from enzyme is minor and therefore not visible.

5.3. Birch bark extract

The studied biorefinery systems based on birch bark are shown in Fig. 6. The conventional method for production of birch bark extract was leaching with ethanol and, by the new, developed method, extraction with liquid CO₂ and ethanol as a co-solvent. The FL is 1 tonne of birch bark with a moisture content of 40%. The bark contains the outer, white layer and the darker, inner layer as well as 13% of wood residue. The products produced in the biorefinery systems regardless of extraction method are birch bark extract (3% of the dry matter content in the bark) and the birch bark and wood residues that will be used for energy generation, in this case heat.

Before extraction, the birch bark was dried to about 15% moisture content, a process step that is equal for both methods. In the system named developed method, the dry bark is first soaked in ethanol and then extracted with liquid CO₂. The residence time in the extractor is approximately 2–3 h depending on the equipment. After extraction CO₂ is removed by distillation and the ethanol that is left in the extract is evaporated so that a solid birch bark extract is left. The extractor is washed with additional ethanol to remove the remaining extract.

In the system named conventional method, the dried bark is immersed in ethanol in an extraction vessel at room temperature where it remains for approximately three days. Ethanol and CO₂ are recycled and reused in both biorefinery systems, with an assumed loss of 6% for both ethanol and CO₂ in each system. For both the new and the conventional method, ethanol and CO₂ are produced from natural gas as described above. Input and output data is shown in Table 5. The process data are derived from pilot-scale trials in which 200 kg of birch bark was treated with the conventional and developed method respectively (SiOx Machines AB, Sollentuna,
The results from the LCA are shown in Fig. 7 a and b. The contribution when dried bark is used as energy carrier is excluded since this is equal for both concepts. As is seen in the figures, the developed method has a somewhat better environmental performance than the conventional method. The net primary energy consumption is approximately 6.5 GJ/tonne birch bark for the developed method and 11 GJ/tonne birch bark for the conventional method and the contribution to GWP is approximately 500 and 590 kg CO$_2$ equivalents/tonne birch bark respectively. The lower energy consumption for the developed method is due mainly to the smaller amount of ethanol that is consumed and the lower energy requirements for the evaporation of this ethanol. The amount of ethanol and energy consumed, as well as the origin of the ethanol and energy, are seen to be the most important parameters affecting environmental performance. If bio-based ethanol were used in the extraction the absolute contributions to GWP would decrease significantly, especially for the conventional method. If bio-based CO$_2$ from, for example upgrading of biogas, were used to replace fossil-based CO$_2$, the GWP for the new method would decrease further. If the birch bark after extraction is used to replace fossil-based energy for heat generation, the additional environmental gains from the system will increase.

### 6. Discussion on economic aspects

Biorefineries and an increased supply of bio-based products have the potential to contribute to economic growth, and increase our possibility to make choices concerning our consumption (Lise et al., 2008). Despite this, there are few examples when biorefineries have been implemented in reality and more studies of how this gap between research and commercialisation can be overcome will be needed. However, some factors have been identified as important, for example today’s low profit margins, the need for long-term stable political decisions and availability of sustainable raw materials at a reasonable cost (Ekman, 2012). The two case studies included here, quercetin from onion waste and betulin from birch bark, illustrate the complexity regarding the opportunities and challenges in the commercialisation of biorefineries.

The main competitors for onion waste are the biogas industry and producers of animal feed. The production of biogas is a relatively new industry in which plants are still facing low profit margins. To date, just a few plants show positive economy of scale and using onion waste as animal feed is normally financially better for farmers (Fagerström, 2011). One strategy to improve the profitability in the biogas industry and extract more value from the raw material (onion waste) would be to enter new markets by product innovation. Extraction of high-value compounds such as quercetin can be considered a product innovation strategy that, if successful, would induce economic growth both for the biogas industry and/or the food industries in which the raw material is produced.

There are not solely benefits associated with the extraction of quercetin but also several risks concerning an investment on the
scale necessary. One risk is the security of raw material supply. The actual amounts available are difficult to predict (see Table 1) and there is competition for the onion waste that influences its price. However, increased profits from the high-value products might increase the biogas industries’ willingness to pay for the material. Another risk is the production risk that is always present in the implementation of novel technologies from the experimental up to the commercial level. This risk can be mitigated by the establishment of a broader knowledge base through greater collaboration between industry and universities (Wu et al., 2010). Such collaboration could also result in the discovery of novel applications for quercetin, which would establish a new market and increase the demand for this product.

Birch bark is produced and used by the forest industry, mainly for generating process heat (Table 2). As long as the extraction of birch bark stays within the forest industry, this implies that the competition for the raw material is within the same industry and not between different actors as in the case of onion waste. This promotes an oligopolistic situation in the supply of betulin, which impairs market competitiveness. However, this could also imply cost efficiency in production, promoting new investments and more R&D. The amounts of birch bark available are significantly higher than the amounts of onion waste and this makes raw material supply a less severe risk than for the case of quercetin extraction. The opportunity cost for using birch bark in new applications will be correlated with the loss of an internal energy supply and the price of alternative fuels for energy generation. The risk associated with the investment in this case is due to the overall risks when a new technology is adopted and will be related to the costs for the scale-up of the process.

The extraction of betulin from birch bark can be seen both as a process innovation and a product innovation depending on the final target market for the product. If the market for betulin is, for instance, the cosmetics industry that is characterised by a strong international competition and entrance barriers and in which betulin is already an established component, process innovation is a strategy. Due to the significant amount of birch bark in Sweden, the betulin can be produced in major quantities at lower costs than competitors, thus facilitating the entrance into the market. Product innovation could be the case if the betulin or birch-bark extract can be used in novel applications such as a repellent for browsing animals where today’s alternatives are not sufficiently efficient (Torkelstam, 2007).

Other factors that affect the implementation of biorefineries are policy and legislation. For example, in Sweden, there are policy incentives for the development of cleaner technologies encouraging the development of “greener industries” and better utilisation of waste (Hammar and Löfgren, 2010). There are also policy incentives promoting biofuels, but there has been a shift in the attitudes of policy makers the last years and this has postponed investments. However, the use of by-products and wastes for biofuel production are still promoted by, for example, the EU (European Commission, 2009). For the biorefineries as described in this paper, biofuel policies may be of less importance since the main products are not in the first place intended for energy purposes even though biofuels such as biogas are important co-products. The extracted compounds can either be sold in pure form but they can also be used for further development of existing products from food and forest industries. Implementing a biorefinery concept of this type could also be a way of dealing with the disposal of waste that is otherwise a cost for companies.

Findings show that there is a positive interaction between university research, policy makers and private industry commercialization of innovations in chemistry and biotechnology (Malo, 2009). A firm may adopt university research in an innovative process depending on the size of the firm, the industrial environment and its own research and development intensity (Laursen and Salter, 2004). In recent years, university researchers have presented several examples of technologies that can be utilized by companies in biorefinery concepts. Even though the research performed at universities is often of a general character (concerning technological as well as economic aspects), evaluation by LCA has proven that this type of research can result in process development leading to reductions in resource requirements (and thus environmental benefits). The final stages in the R&D activities that directly relates to specific products, and their commercialisation, are however commonly performed by the companies themselves. Thus, university researchers can suggest technologies or processes that, for example, have potential to be used in a biorefinery and make this knowledge available. But, the companies must do the final step in finding a proper application and developing a marketable product (Henrik Ralvert, Fotovit AB, personal communication 2013-01-11). Thus, an increased co-operation between industries and university research is crucial in the development of successful biorefinery concepts from an economical and long-term sustainable point-of-view.

7. Discussion and conclusions

The establishment of innovative biorefinery concepts, producing high-value bio-products, is a necessary component in the transition to a bio-based economy in which biomass resources are utilised in the most efficient way. This will give both environmental and socio-economic benefits in the long term. However, deeper analyses are necessary to find biomass feedstock and applications with the lowest opportunity costs and best environmental performance. This paper presents a first attempt at such a broad analysis.

Residues from food and forest industries are considered promising feedstock for biorefinery concepts from a cost perspective, but also from an environmental sustainability perspective as they do not lead to increased land use competition and thereby potentially negative, indirect land-use changes. There is a significant biomass potential in the form of residues in the Swedish agriculture, food and forest industry sectors suitable for the extraction, including upgrading by biocatalytic processes, of various high-value chemical compounds, such as antioxidants, pigments, steroids, etc. Apart from using biomass feedstocks, which fulfils various sustainability criteria, the extraction methods and production processes need to be efficient and environmentally adapted to secure the life cycle performance of the final product.

Onion waste from the food industry and birch bark from the forest industry are two examples of biomass feedstock that could be used in future biorefinery concepts. Today, onion waste is used as animal feed and for biogas production and birch bark for heat and power production. However, current energy generation from onion waste and birch bark could be developed and complemented with an extraction application, to produce additional, high-value chemical compounds. Such innovative and new extraction processes are currently under development in university research project. From onion waste, the high-value compound quercetin can be extracted using pressurised hot water extraction coupled with enzymatic hydrolysis. This extraction method proved to have a better environmental performance than conventional methods since the use of fossil-based organic solvents and hydrochloric acid is avoided. Extraction of betulin from birch bark can also be achieved by various methods. From an environmental perspective, extraction by liquid CO₂ containing some ethanol proved to be better than conventional ethanol leaching.

Extraction of betulin from birch bark provides various possibilities for innovation in the forest industry that could have
significant socio-economic benefits. This is also the case for the extraction of quercetin from onion waste in the food industry. However, several different risks exist for the economic operators in the realisation of the innovations from a laboratory scale up to an industrial process and commercial scale. Examples of risks include the stability of the biomass supply, the accessibility and performance of the scaled-up process technology, the final markets for the end products, etc. The technological risk could be reduced by, for example, an increased co-operation between the industry and universities in applied research projects. A way to reduce the economic risk for the industries developing new biofuel concepts is by introducing long-term policy incentives promoting green chemicals and products. One example is investment subsidies for new and promising extraction and process technologies which are not fully commercial today. Such policy tools may become cost efficient in the longer term from an environmental and socio-economic perspective and crucial in the development of a bio-based economy.

Acknowledgements

We wish to acknowledge the financial support from the Swedish Research Council Formas for funding the SuReTech research collaboration (229-2009-1527). One of the authors (C.T.) also wishes to acknowledge the funding support received from the Swedish Research Council VR (2010-5439, 2010–333), Oscar & Lili Lamm’s Foundation and the Swedish Foundation for International Cooperation in Research and Higher Education (STINT, VR2009-7015). The authors also acknowledge the support from the FP7 framework program AMVLOMICS.

References


Alasalvar, C., Grigor, J.M., Zhang, D.L., Quantick, P.C., Shahidi, F., 2001. Comparison of the stability of the biomass supply, the accessibility and perfor-


Bartova, V., Bartu, J., 2009. Chemical composition and nutritional value of protein concentrates isolated from potato (Solanum tuberosum L.) fruit juice by precip-


Chen, T., Deng, C., Liu, R., 2010. Effect of selective condensation on the character-

Clark, J.H., Budarin, V., Deswarte, F.E.I., Hardy, J.J.E., Rose, P.M., 2006. The fractionation of valu-

Dao, L., Friedman, M., 1994. Chlorophyll, chlorogenic acid, glycoalkaloid, and pro-

Dias, A.C.P., Fernandes, P., Cabral, J.M.S., Pinheiro, H.M., 2002. Isolation of a biode-


Feldt, K., Hedin, E., 2004. Betulinic acid as anti-HIV principles from Syzigium-clavi-

Feldolf-Gava, A., Simandi, B., Plander, S., Szarka, S., Szoke, E., Kery, A., 2009. Betu-


Groskopf, L., Halkier, B.A., Pogson, S.B., 1999. Chemical composition of the needle-

Håkkinen, J.K., Hatakka, J., Jakkula, S., 1997. Chemical composition and nutritional value of protein concentrates isolated from potato (Solanum tuberosum L.) fruit juice by precip-

Høest, P., Juge, N., 2003. Substrate (aglycone) speci-


Juge, N., 2003. Substrate (aglycone) speci-

Kamal-Eldin, A., 1999. Effects of commercial pro-

Khosrowshahi, S., 2002. A comparative analysis of the stability of the biomass supply, the accessibility and perfor-


