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ENERGY DEMAND FOR PROTEIN PRODUCTION BY CELLULAR AGRICULTURE IN COMPARISON TO THE MILK CHAIN

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Tiivistelmä – Referat – Abstract

Cellular agriculture (CA) utilizes cells to produce alternatives for conventional animal or plant-based foods. Concepts for CA are available, but their feasibility for large scale production is not yet well documented. The aim of this work was to compare CA and conventional milk chain in terms of energy demand. Enzyme production was included to the analysis as a benchmark to precision fermentation. In the life cycle analysis (LCA), life cycle inventory was compiled from scientific literature for CA and enzyme production. Inventory data for milk powder production was taken from Valio Carbo Calculator. A functional unit of 1 kg protein was applied. Cumulative energy demand for each product was re-calculated applying a cradle-to-gate system boundary. Background data was compiled from Ecoinvent and Agri-footprint databases and analyzed with OpenLCA.

Milk protein in general has lower requirement in non-renewable and renewable CED than CA alternatives. Main contributors to CED in CA were electricity, thermal energy, carbon source and in some scenarios cleaning-in-place (CIP). CED values were 185 MJ/FU, 179 MJ/FU – 1007 MJ/FU and 410 MJ/FU – 166 385 MJ/FU for milk protein production, CA, and enzyme production, respectively. Moreover, enzyme production revealed that CED of CA has potential to have much higher values than reported on CA production. In the future, studies with continuous fermentation utilizing different by-products and including more impact factors should be pursued.

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life cycle assessment, cellular agriculture, milk chain, cumulative energy demand, protein

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Tiivistelmä – Referat – Abstract

Solumaataloudessa soluja käytetään ruoantuotantoon tavallisen maatalouden sijaan. Solumaatalouden konsepteja on kehitetty jo pitkään, mutta niiden soveltaminen teollisessa mittakaavassa on vielä puutteellista. Tämän työn tarkoituksena on verrata solumaatalouden ja maitoketjun energiantarvetta tuotettuun proteiiniin nähden käyttäen elinkaariarviota. Entsyymituotanto otettiin mukaan analyysiin vertailuarvoksi tarkkuusfermentaatiolle. Elinkaariarvion inventaarion etualantiedot kerättiin tieteellisistä julkaisuista solumaataloudelle ja entsyymituotannolle, mutta maitoketjun osalta käytettiin Valio Carbo Calculatorista saatua dataa. Taustatiedot kerättiin Ecoinvent- ja Agri-footprint tietokannoista. Toiminnallisena yksikkönä (FU) toimi 1 kg proteiinia. Vaikutusarviointina kumulatiivinen energiantarve (CED) laskettiin jokaiselle toiminnalliselle yksikölle. Analyysiin käytettiin OpenLCA-ohjelmistoa, ja se tehtiin kehdosta portille selvitysrajauksena (cardle to gate).

Maitoproteiinin CED oli lähes joka tapauksessa matalampi uusiutuvien ja uusiutumattomien energianlähteiden osalta verrattuna solumaatalouden ja entsyymituotannon tarpeisiin. Suurimmat vaikutukset CED:n kasvuun oli sähköllä, lämpöenergialla, hiilen lähteellä ja joissakin tapauksissa CIP-kiertopesulla. CED arvot olivat maidolle 185 MJ/FU, solumaataloudelle 179MJ/FU – 1007 MJ/FU ja entsyymeille 410 MJ/FU – 166 385 MJ/FU. Lisäksi entsyymituotannon korkean kumulatiivisen energiantarpeen perusteella voidaan päätellä, että solumaataloudessa on potentiaalia suurempiin energiantarpeisiin kuin mitä voidaan päätellä nykyisten tietojen pohjalta. Jatkotutkimuksia tarvitaan jatkuvan fermentaation, sivuvirtojen hyödyntämisen ja useampien vaikutusarviointien osalta.

Avainsanat – Nyckelord – Keywords

elinkaariarvio, solumaatalous, maitoketju, kumulatiivinen energiantarve, proteiini

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PREFACE

This Master’s thesis was carried out in 2022 as a collaboration with Future Sustainable Food Systems group from the Helsinki Institute of Sustainability Science HELSUS at the University of Helsinki and Valio Ltd. And this Master’s thesis will be a part of my Master studies at Dairy technology and sciences in the department of Food and Nutrition in the Faculty of Agriculture and Forestry at the University of Helsinki.

I feel gratitude for my excellent supervisors Dilek Ercili-Cura (Valio), Natasha Järviö (UH), and Hanna Tuomisto (UH) for always being available when asked and being flexible during this journey. I also want to thank project member Aleksi Astaptsev (Valio) for helping with the calculations and providing crucial data about the milk chain. I also wish to thank my steering group at the University of Helsinki and Valio: Niina Valkonen (Valio), Pekka Varmanen (UH), Juha Nousiainen (Valio), Saara Pöyri (Valio) and Riitta Partanen (Valio) for good discussions and comments during my thesis. I also want to thank Enni Kerola (Valio) for conversations during my thesis process.

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1 INTRODUCTION

Current state of food production

Food production causes extensive burden on the environment mainly via land use, biodiversity loss, climate change and water scarcity. In fact, it has been estimated that food production uses 50 % of habitable land and causes 26 % of human made greenhouse gasses (Poore and Nemecek, 2018; FAO, 2019). Agricultural practices have caused 80 % of global deforestation, from which forestry and grazing have been major drivers in decrease of total biomass on earth (Bar-On, Phillips and Milo, 2018; Ercili-Cura *et al.*, 2021). On contrary, biomass of humans and livestock has been steadily growing and has led to a point where animal-based food production uses 77 % of agricultural land while providing 37 % of current protein demand (Bar-On, Phillips and Milo, 2018; Ercili-Cura *et al.*, 2021). Moreover, land-use change together with emissions from livestock accounts for 15-20 % of human made greenhouse gasses (Poore and Nemecek, 2018; Xu *et al.*, 2021). Land use in food production remains as a major environmental burden, so a need for alternative routes for a more sustainable protein production is needed.

Cellular agriculture

Cellular agriculture (CA) is a field that includes the use of cells and host organisms that can be utilized to produce agricultural products that are traditionally made with conventional methods such as animal-based food production or plant-based crops (Ercili-Cura *et al.*, 2021). Cellular agriculture is based on microbes such as fungi (i.e., yeasts and filamentous fungi), bacteria, and algae (Ercili-Cura *et al.*, 2021). Their carbon source can be provided from crops, side-streams of agri-food industries or even from methane and carbon dioxide (Ritala *et al*. 2017; Gilpin et al., 2017; Voutilainen, Pihlajaniemi and Parviainen, 2021; Järviö *et al.*, 2021a). Cellular agriculture can be divided into biomass fermentation and precision fermentation. Biomass fermentation includes production of single-cell proteins and cell cultured meat. Precision fermentation consists of recombinant food proteins, ingredients, and enzymes (Good Food Institute, 2020; Rischer, Szilvay and Oksman-Caldentey, 2020).

Biomass fermentation has been used in feed and food production for decades. Yeasts including torula yeast (*Candida utilis)* were cultivated in Germany for human consumption during 1940s from wood industry side-streams (Harris, 1949). Microbial protein-based feed from methane was successfully produced with *Methylophilus methylotrophus* in 1970s by Imperial Chemical Industries (Senior, 1980). Most known mycoprotein is Quorn™ which is processed biomass of *Fusarium venenatum,* and it has been produced since 1985 for human consumption (Wiebe, 2004). In the last decade, hydrogen oxidizing bacteria (HOB) has been studied as a potential way to produce food for humans (Sillman *et al.*, 2020; Järviö *et al.*, 2021a). Also, agricultural land use in food production could be bypassed with alternative food production routes such as cellular agriculture relying on HOB utilizing hydrogen and carbon dioxide as main energy and carbon sources, respectively (Ercili-Cura *et al.*, 2021; Järviö *et al.*, 2021a; Sillman *et al.*, 2020).

Precision fermentation has been competitive way to produce commodities such as insulin and chymosin in pharma and enzyme industry in 1990s, followed by cosmetics like collagen and vitamins in early 2000s. If the trend continues, production of food products such as ingredients and proteins will become feasible within a decade (Tubb and Seba, 2021). Currently, precision fermentation can be used to produce food proteins such as ovalbumin or whey proteins that are comparable to animal-based proteins (Ito and Matsudomi, 2005; Järviö *et al.*, 2021b; Perfect Day, 2021). Moreover, enzymes such as chymosin produced with precision fermentation have been on the markets for over three decades. They are more studied, and the processes could have beneficial information for large-scale food precision fermentation processes. Therefore, enzyme production was chosen as a part of this thesis because precision fermentation used in CA for production of food proteins consists of similar production steps as in production of enzymes.

Environmental impacts

It seems likely, that CA will be a part of the food system in the future, but it will still require science-based evaluation especially in the terms of energy demand (Tubb and Seba, 2021; Ercili-Cura *et al.*, 2021). Also, recent studies have come to a careful conclusion that precision fermentation can be a feasible way to produce food for humans while producing less environmental impacts when compared to animal-based counterparts (Järviö *et al.*, 2021b; Perfect Day, 2021). How much energy is needed together with the availability of renewable energy sources can shape the role of CA in the future food production systems.

In current knowledge, most of energy is used in production of electricity and conventional carbon sources (Gilpin et al., 2017; Järviö et al., 2021b; Perfect Day, 2021). However, the number of studies from CA is very limited. More research and empirical data from energy usage especially on large scale production is needed to produce more accurate studies (Järviö *et al.*, 2021a). Furthermore, lack of markets for side-streams of CA, such as microbial biomass, forces the by-products to be processed in waste treatment. This increases environmental burdens of protein production, which could be avoided by better utilization of side-streams. There is not empirical data for this and thus, assessment regarding future scenarios can also be done *ex ante* (Cucurachi et al., 2019).

Dairy consumption is rising globally, and it is predicted that milk production will rise 1.7 % annually (OECD-FAO, 2019; FAO, 2019). In fact, 12 % of global protein demand comes from dairy (Smith *et al*., 2022). Milk powders are also widely used in food industry, which makes them a comparable standard with powdered proteins made with CA. The energy demand of milk powder production is highly dependable on feed type, thermal energy and managing system. Also, adoption of new technologies and use of specialized cattle breeds can affect to the energy usage (Finnegan *et al*., 2017; Wang et al., 2019; Berton et al., 2020).

Not many studies include energy demand but published studies that have provided foreground data can be used to recalculated energy demands of these processes.

Objective

Objective of this thesis is to compare energy demand of CA-based food production processes and conventional powdered milk protein production. Powdered milk or milk proteins are well established, widely used food ingredients. They can be directly replaced with CA-based counterparts e.g., milk proteins produced by precision fermentation. Cumulative energy demand (CED) as impact category was chosen because, although in general, CA doesn’t require high land-areas or as much water as livestock produced food, CA-based processes are generally perceived as energy intensive (Ercili-Cura *et al*., 2021; Järviö *et al*., 2021b; Perfect Day, 2021). At least one comparative LCA between CA and milk protein production has been made by Behm *et al*. (2022) where they compared carbon footprint and water scarcity footprint. Energy demand also reveals the feasibility of these processes in greater extent than for example usage of GWP as an impact factor. Goal of this thesis was to compile and normalize LCI from published reports for CA (and enzyme production as an industrial benchmark to CA) and provide a comparison between milk chain- and CA-based protein production in terms of energy consumption.

To increase the amount of data, studies about enzyme production with precision fermentation are also considered in this thesis. Milk production data is provided by Valio farms and Valio production facilities.

2 EXPERIMENTAL RESEARCH

2.1 Life cycle assessment

There are many myths and conceptions in food systems that can be tackled with well gathered, analyzed, and presented information. One way to do it is a life cycle assessment (LCA). It considers every input and output of each production step, starting from resource extraction to the end of the product's life cycle. It links these inputs and outputs to different environmental impact categories (Teixeira, 2015; Cucurachi et al., 2019). For instance, climate change can be quantified by adding up GHG emissions of all production steps and converting them to carbon dioxide equivalents using emission conversion factors describing the GWP of each gas for 100 years timeframe. LCA can additionally be used to quantify the total required energy demands of the system, expressed in MJ/FU product (Frischknecht *et al.*, 2007). LCA has been developed over four decades ago and it has become a leading way for organizations and companies to assess environmental impacts of their goods, processes, and services (Teixeira, 2015; Cucurachi *et al*., 2019). LCA was standardized in 2006 in ISO 14040 and ISO 14044. There are two main groups of LCA studies which are descriptive and comparative, where the first one is made to identify environmental impacts of the selected product system and second compares two different systems (Baldini, Gardoni and Guarino, 2017).

Life cycle assessment should begin by addressing a goal for the assessment and its scope. In this phase is decided what the assessment considers (i.e., is it a product process or service), and why the assessment is done. The purpose and plan of the study should be stated as clearly as possible (Cucurachi *et al*., 2019). Processes included in the analysis are usually expressed as cradle-to-grave, cradle-to-gate or gate-to-gate, as well as cradle-to-cradle in circular economy. These are part of the system boundary definition, which define what is assessed and what is omitted. Part of scope definition is deciding the functional unit (FU), which is the quantitative unit to which the environmental impacts are related (Cucurachi *et al*., 2019). FU can be, for example, 1kg of energy corrected milk (ECM) or 1kg powdered milk protein. The chosen FU should reflect the function of the product and fit with the goal and scope of the study. For example, a FU of 1 kg of ECM can be used when comparing the environmental impacts of milk while 1 kg of protein is more suitable when comparing different protein powders that contain different concentrations of protein content (Baldini, Gardoni and Guarino, 2017; Cucurachi *et al.*, 2019).

Life cycle inventory (LCI) is the second step of the LCA and usually follows when the scope of the study has been decided. In LCI, data necessary for the assessment is gathered, relationships are identified, and inputs and outputs are quantified (Cucurachi *et al*., 2019). LCI can be divided to foreground and background data. Background data is average data gathered from databases such as Ecoinvent, GaBi and food specific databases such as World Food LCA Databases. Foreground data, also known as primary data, is collected by the LCA practitioner and usually includes empirical data from research or companies etc., (Cucurachi *et al*., 2019). Choice of database can affect to the outcome of impact assessments. For example, Ecoinvent 3.6 often leads to larger environmental impacts than GaBi database, partly because of the larger number of background processes in the ecoinvent 3.6 (Pauer, Wohner and Tacker, 2020).

In life cycle impact assessment (LCIA) LCI data is aggregated, impact categories are assessed and LCIA methods fitting for the selected impact categories are used. Overall, there is a plethora of different impact categories, and they should be selected based on goal of the study. Impact categories can be presented as a single environmental indicator, i.e., midpoint result or aggregated to endpoint indicators (Huijbregts *et al.*, 2017). Generally, environmental flows based on LCI data are aggregated into different impact categories with software, such as SimaPro, GaBi or OpenLCA, but Microsoft Excel can also be used. Examples of LCIA methods are ReCiPe, CML, CED and IMPACT 2002+ (Poore and Nemecek 2018; EDA, 2020).

Interpretation is the last phase of LCA, and it includes the interpretation of inventory and impact results. Inventory interpretation includes the critical evaluation of the FU and what it is analyzing, an evaluation of systems boundaries in terms of what was left outside of the scope and what was included, and why this was done. How did the allocation affect the results and why certain allocations were used? What impact method or methods were used and how does that affect to the results? Which database was used, and are there known issues or limitations, like a lack of data for some geographical area? Lastly, the representativity of the background data is evaluated (Cucurachi *et al*., 2019).

2.1.1 Goal and scope

The goal of the life cycle assessment was to assess cumulative energy demand for cradle-to-gate protein production in selected CA processes and milk systems (Table 1). This comparative LCA for CA and milk products was applied using OpenLCA 1.11.0, and LCI data was taken from published articles for CA and enzyme calculations and from Valio carbo database for milk protein production calculations (Table 1). Background data was acquired from ecoinvent 3.7 and agri-footprint 5.0 databases. CED was calculated for all LCIs with Cumulative energy demand (CED) v.1.1 method by ecoinvent. FU is 1kg of protein. Protein is in dried form in all cases except mycoprotein (myco1, myco2G, myco2S) and cellulase (enzyme5). Protein production is assumed to take place in Finland. The study design described above is illustrated in Figure 1.

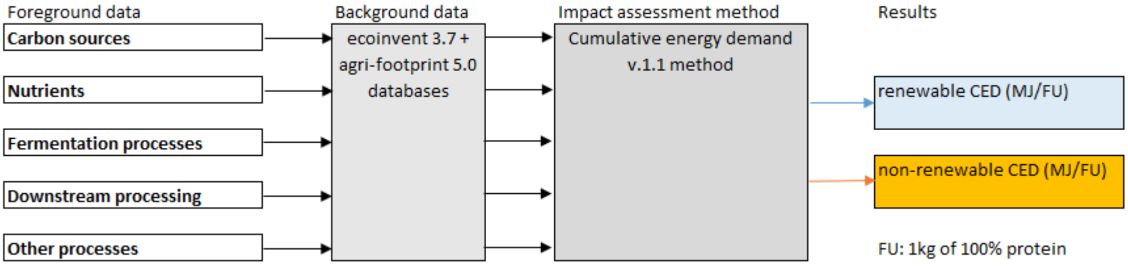

Figure 1. Study design. Some examples of foreground inputs are on the left side, which are then combined with background data from ecoinvent and agri-footprint databases. This information is then combined with CED v1.1 method to obtain renewable and non-renewable CEDs. Foreground inputs are different for all products, and they are collected to supplementary data (Tables S1-S4).

2.1.2 System descriptions and data collection

LCI data was taken from published reports during May in 2022 and processed for delivering thorough comparison of selected production systems for CED. Publications with LCI data for cellular agriculture (biomass and precision fermentation including enzyme production) were searched using Scopus, Web of Science, Google scholar and Connected papers. LCIs from collected papers were further processed with OpenLCA by using ecoinvent v3.7 and agri-footprint 5.0 databases. List of LCIs from CA and enzyme processes can be found at table S1 and table S2. LCI list for milk in in table S3 and auxiliary processes are listed in table S4. System descriptions of selected CA processes and milk chain are reported below together with system boundaries (Figure 4 and Figure 5). Abbreviations for CA, enzyme and milk protein production are opened in the Table 1.

Table 1. CA, milk chain and enzyme processes included in the LCA.

abbreviation	product	main carbon source	allocation	LCI collected from
myco1	mycoprotein	molasses	no allocation	Smetana *et al*., 2015
myco2G	mycoprotein	glucose	no allocation	Upcraft *et al*., 2021
myco2S	mycoprotein	straw	no allocation	Upcraft *et al*., 2021
hob1	hob biomass	CO_2	no allocation	Sillman *et al*., 2020
hob2	hob biomass	CO_2	no allocation	Järviö *et al*., 2021a
rova	recombinant ovalbumin	glucose	no allocation	Järviö *et al*., 2021b
rovabm*	recombinant ovalbumin	glucose	protein	Järviö *et al*., 2021b
enzyme1	β-galactosidase	lactose**	no allocation	Feijoo *et al*., 2017
enzyme2	xylanase	dried barley	no allocation	Cimenlik *et al*., 2021
enzyme3	peroxygenase	-	no allocation	Bello *et al*., 2021
enzyme4	hydroxymethylfurfural oxidase	-	no allocation	Bello *et al*., 2021
enzyme5	cellulase	glucose	no allocation	Gilpin *et al*., 2017
milk	milk protein	feed mix	mass	Valio Carbo, 2022

* Based on protein-based allocation between ovalbumin and microbial biomass.

**modelled using liquid whey from cheese production.

Biomass fermentation

F. venenatum is best known and most studied organism in mycoprotein production for human consumption (Whittaker *et al*., 2020). LCIs of mycoprotein production with *F. venenatum* is based on articles by Smetana *et al*. (2015) and Upcraft *et al*. (2021). These papers do not model the use of glucose syrup from starch, but use different carbon sources (e.g., molasses from sugar beet and glucose form rice straw) when compared to mycoprotein production by Quorn Foods (Wiebe, 2002; Finnigan *et al*., 2017; Whittaker *et al*., 2020). Additionally, amount of produced glucose from straw used in Upcraft *et al*., (2021) is also modelled as glucose from maize starch in this thesis (myco2G). Background information for glucose production from maize starch was acquired from ecoinvent v.3.71 database. Smetana *et al*. (2015) compiled LCI and system boundaries based on Raats (2007) and Finnigan *et al*. (2010). The latter is not currently available and Raats (2007) is a training thesis conducted by master student from University of Groningen, using environmental process analysis. In short, LCI from Smetana *et al*. (2015) is simplified when compared to Upcraft *et al*. (2021). However, it is still included because LCI for CA are very limited, and it is widely referred.

Mycoprotein manufacturing includes fermentation, heating, and separation. These are mentioned in Upcraft *et al*. (2021) but not in Smetana *et al*. (2015). However, there are some differences in background data because Upcraft *et al*. (2021) has lower electricity need of

25 kWh for production of 1 kg of protein in comparison to 59 kWh in Smetana *et al.* (2015). Wastewater treatment (WWT) is not considered in Smetana *et al.* (2015) but it is a part of Upcraft *et al.* (2021) LCI in feed production and downstream processing. Furthermore, Smetana *et al.*, (2015) is cradle-to-plate LCA, which includes ready-to-eat meal with 10 % protein. This has some increase in transportation and electricity inputs, which are not specified in the original article, so they cannot be excluded.

Glucose production from straw in Upcraft *et al.* (2021) was modelled with food-safe ionic liquid and cellulase hydrolyzation in myco2G. 0.038 kg enzyme per kilo of mycoprotein was needed. In ecoinvent's market for enzymes, 4.16 kg of potato starch is used to produce 1 kg of alpha-amylase, glucoamylase or cellulase in average with bacteria. LCI for ionic liquid is opened in table S1. Electricity demand for different processes is not separated in the original paper, so the same amount of electricity for straw and glucose-based processes is used, which will probably overshoot the need for electricity for myco2G. Straw treatment in myco2S was calculated based on LCI in Upcraft *et al.* (2021) by using ecoinvent database. In the database, market for straw was used where raw material is mainly comprised of rye straw side-stream from rye production instead of rice in Upcraft *et al.* (2021).

Other product evaluated within biomass fermentation is biomass of HOB, from which two sets of LCIs were used from Sillman *et al.* (2020) and Järviö *et al.* (2021a). Both LCAs include CO_2 capture and on-site electrolyzer, though Sillman *et al.* (2020) modelled the electrolyzer as in-situ within the bioreactor. Downstream processing is conducted as pasteurization followed by separation and drum drying in Järviö *et al.* (2021a), whereas Sillman *et al.* (2020) uses centrifugation followed by evaporation. Electricity consumption for the whole process is 30 kWh and 17 kWh, respectively. Also, both have HOB biomass as FU with protein contents of 65 % and 60 %. In this thesis, the LCIA is calculated for 1kg of 100 % protein.

Precision fermentation – food proteins produced by CA

Only one publication regarding precision fermentation that published its LCI was Järviö *et al.* (2021b). In this publication, recombinant ovalbumin (rova) production started with cultivation of genetically engineered *T. reesei* strain at 28 °C. Preculture of inoculant is moved to main fermenter for continuous protein production. Hydrolysed maize as a carbon source, nutrients, such as nitrogen and minerals were added to the cultivation media in preculture and during fermentation. For the whole system, five bioreactors (of sizes 0.06, 0.6, 9, 63 and 125 m3) were modelled for a production capacity of 100,000 kg/year. Approximately 50 CIP cleaning rounds were assumed for bioreactors. Fermentation was followed by filter press, where biomass solids were separated from proteins in liquid phase. Biomass had 58.3 % moisture content. Produced proteins where then ultrafiltrated where 35.6 kg of water was removed per 1 kg of protein. Retentate was heated and dried with spray drier to create a powdered protein product with 92 % protein content. Heat recovery during heating and drying steps was assumed. In this thesis, LCI was scaled for 100 % protein content instead of 92 %.

Two allocation scenarios rova and rovabm were modelled where all the environmental burden is put on ovalbumin protein or divided with biomass protein and ovalbumin protein in 33.8 % allocation factor for the biomass, respectively. Electricity consumption for rova and rovabm is 11 kWh and 6 kWh for 1 kg of ovalbumin, respectively.

Main production steps for downstream processes of CA products are shown in Figure 2.

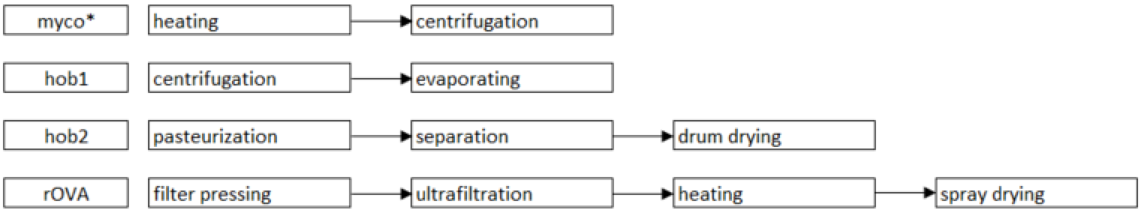

Figure 2. Downstreaming processes of CA products
rova and rovabm have the same downstream processing.
*myco includes myco1 and myco2. Myco1 did not have mentions about downstream processing, so same or very similar downstream processing than with myco2 is assumed. Myco2S and myco2G have same downstream processing including heated centrifugation of biomass and centrifugation of mycoprotein mass, which is based on Upcraft *et al*. (2021).

Precision fermentation – enzyme production

As industrial scale data for CA concepts is scarce, published reports on LCA of various enzymes were used. Five enzyme production processes with published LCI were chosen. Enzyme activity and mass is not comparable, because the mass of one active unit of enzyme can vary highly (Bello *et al*., 2021). Therefore, comparison of enzymes is also done in mass. Downstream processes between enzymes have large differences, which are collected to Figure 3.

β-galactosidase process was divided to three sections of storage area, fermentation section and downstream processing which are included in the LCI (Feijoo *et al*., 2017). Also, CIP with NaOH and H_2SO_4 as detergents was modelled as an ancillary process for fermentation and downstream processes. *Saccharomyces cerevisiae* as a host organism which expressed lacA gene from *Aspergillus niger* was used. Downstream processes included microfiltration, homogenizing, centrifugation, polishing filtration, ultrafiltration, ion exchange, diafiltration and freeze drying. The FU in LCI is 1kg of dried β-galactosidase. Reported electricity consumption is 260 kWh per kg of enzyme and based on water content of LCI the scale of bioreactor is around 50 m^2.

Cimenlik *et al*. (2021), published a pre-print from LCA where they are comparing solid state and suspended culture fermentations in xylanase production. LCI from suspended culture utilized in this thesis. Based on water content of LCI, the scale of bioreactor is around 50 m^2 and reported electricity consumption is 16345 kWh per kg of protein. However, this publication is not peer reviewed, which makes the data not as reliable.

Peroxygenase and hydroxymethylfurfural oxidase production are taken from Bello *et al*. (2021). They assessed an LCA based on laboratory-scale experiments which were extrapolated to 100 000 L. Peroxygenase was extrapolated from 6 L to 100.000 L and it used 5066 kWh/kg of enzyme and included centrifugation, vacuum pump, freezing or cooling, microfiltration, ultrafiltration, and chromatography. Hydroxymethylfurfural oxidase was extrapolated from 25 L to 100.000L and it used 3741 kWh/kg of enzyme, and it included microfiltration, freezing, microfiltration and ultrafiltration as downstream processing.

In Gilpin *et al*. (2017), *T. reesei* is used as a host organism to produce cellulase from maize glucose in 300.000 L batches. Produced cellulase is taken into cellulose hydrolysis at bioethanol production plant thus, no purification steps were considered. According to LCI, 6,3 kWh of electricity was needed to produce 1 kg of enzyme.

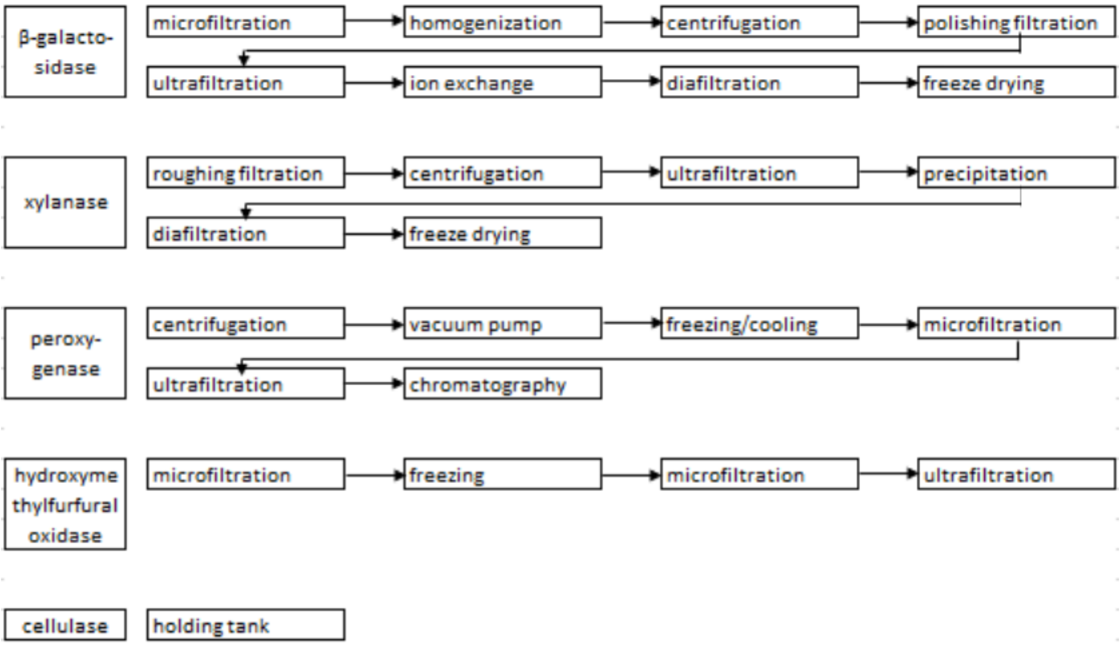

Figure 3. Enzyme production downstream processes. Enzymes from β-galactosidase to cellulase which represent the enzymes from enzyme 1 to enzyme 5, respectively.

System boundaries for CA and enzyme-based protein production are shown in Figure 4.

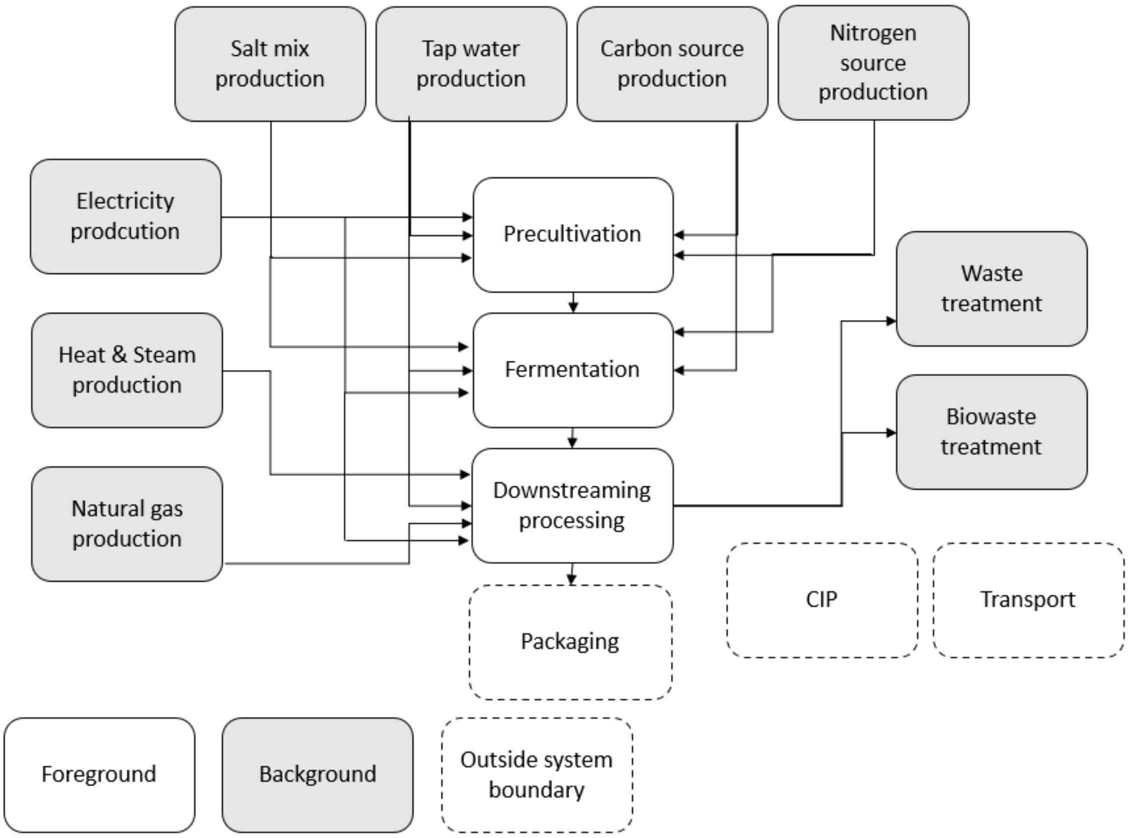

Figure 4. System boundaries of CA and enzyme production.

Milk chain and milk protein production

Milk is produced at Finnish Valio farms where information has been gathered from over 1300 farms and 1113 of these have provided sufficient data for LCA about CED to be made. Moreover, empirical data about energy usage from milk powder production is obtained from Valio Seinäjoki plant. This will give a good picture about Finnish milk production from cradle-to-factory gate. Milk production boundaries are shown in Figure 5.

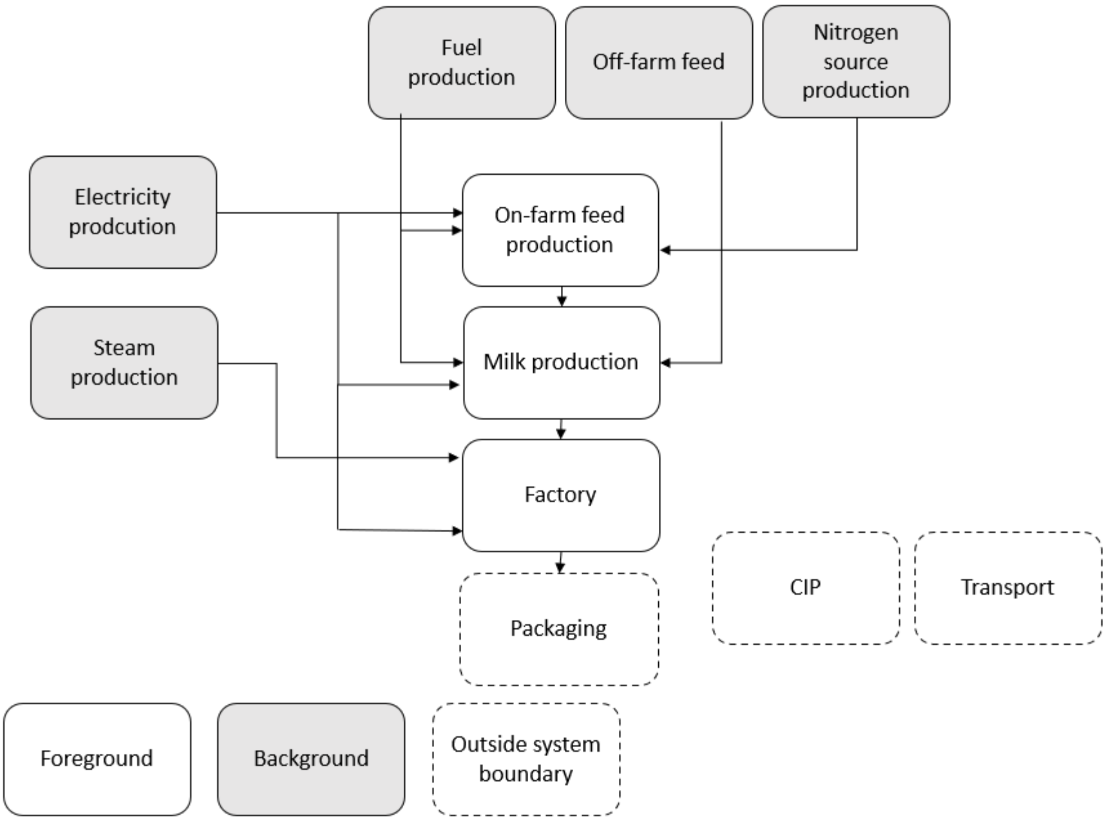

Figure 5. Milk chain boundaries.

Primary data is taken straight from 1113 Valio farms and from processing plant at Seinäjoki. Energy consumption primary data from farm includes fuel-based energy, electrical energy, and thermal energy usage per 1 kg of protein in raw milk. Allocation factors were provided prior to LCA calculations by Valio. For farms fuel energy, mass allocation for 75 % was first done between feed products based on feed usage, because 25 % of the feed is sold or stored and therefore, not used in feeding. Next, all energy sources were energy allocated for 99 % and 1 % to milk and meat, respectively. Finally, dry-mass allocation was used for dry matter in a way that 28 % of energy consumption was allocated for protein and rest for fat and lactose. In-farm produced feed and fertilizers are included in the primary data and energy usage of off-farm produced feed is calculated based on average values from feed production in Valio farms. CED for bought fertilizers is calculated based on ecoinvent fertilizers. Milk is produced at Finnish farms with an average size of 65 cows per farm.

Processing plant includes needed electricity and steam for powdered milk products (e.g., protein powders, protein and fat powders, milk powders and lactose free milk powders) at Seinäjoki factory in 2021. Mean protein content for all powdered products was 35 %, and it was used for calculating energy usage for 1 kg of powdered protein. Transportation of milk is omitted from primary data. On average raw milk is transported for 50-200 km in lorries that can contain over 40 000 liters of raw milk (Aleksi Astaptsev, Valio, Personal communication 17.6.2022). LCI for CED analysis was compiled from primary data taken from the farms and calculated using same methodology as with CA and enzyme LCIAs. LCI is collected on Table S3.

Table 2 includes carbon source, electricity, steam, and CIP data from all LCIs that have been used as foreground data.

Table 2. Summed values for carbon source (kg), electricity (kWh), steam (kg) and CIP (kg) for CA, enzyme production and milk protein production per 1kg of protein. Data taken from Tables S1-S4. CIP is not taken into account in the main CED comparison calculations.

	Carbon source	Carbon source (kg)	Electricity total (kWh)	Steam (kg)	CIP Base	kg	Acid	kg
myco1	molasses	30.00	59.22	-	-	-	-	-
myco2G	glucose	8.89	24.68	-	-	-	-	-
myco2S	straw	74.29	24.68	-	NaOH	0.16	H3PO4	0.39
hob1	CO2	2.93	17.08	-	-	-	-	-
hob2	CO2	2.85	29.28	11.66	NaOH	0.00	HNO3	0.00
Rova	glucose	2.54	10.54	-	NaOH	0.02	HNO3	0.01
Rovabm	glucose	1.55	6.42	-	NaOH	0.01	HNO3	0.01
enzyme1	lactose	88.90	260.45	489.00	NaOH	2941.10	H2SO4	3829.00
enzyme2	barley	72.40	16345.06	-	NaOH	4.34	H3PO4	7.25
enzyme3	-	-	5065.60	88.41	-	-	-	-
enzyme4	-	-	3741.15	210.39	-	-	-	-
enzyme5	glucose	4.70	6.30	-	-	-	-	-
milk	feed mix	13.93	5.46	7.47	-	-	-	-

Background data

For described systems, Ecoinvent was used for all LCI calculations except dried barley production (FI), crude maize germ oil (pressing) (NL), maize steepwater (NL), and liquid whey (NL) were produced in Finland (FI) or Netherlands (NL) and taken from agri-footprint 5.0

database. Finnish production of grass, barley grain, oat grain and rapeseed meal for milk protein production system are based on agri-footprint 5.0 and further adjusted by Natasha Järviö. Potassium phosphate production was not included in the databases, so it was calculated in the same way as Gilpin *et al*. (2017) did from phosphoric acid and potassium hydroxide and it is included in the Table S4. In every LCI, it was assumed that average Finnish energy grid from ecoinvent is used for medium voltage electricity production.

2.1.3 Analysis methods

OpenLCA 1.11.0 was used for calculating LCIA, sensitivity analysis and Monte Carlo (MC) analysis from LCI shown in Tables S1 - S4. Protein based allocation for rovabm and milk was used because protein production is the primary function of the evaluated products. Cumulative energy demand v.1.1 method by ecoinvent was used to analyse the cumulative energy demand (CED). CED takes in account all energy that is withdrawn from the nature and used along the life cycle (Frischknecht *et al*., 2007). It consists of direct, indirect, or grey energy where the latter includes construction and raw material manufacturing. For example, CED considers the losses of energy in transmission lines from the power plant to the raw material extraction and cooling of the product, all the way to the end usage and waste management, if cradle-to-grave life cycle assessment is prepared. CED consists of non-renewable and renewable resources. Non-renewables include fossil-based energy sources, nuclear energy, and primary forests. Renewables can be categorized as biomass, wind, solar, geothermal and water. Within these, biomass also includes carbon sources such as glucose or feed and the energy that is embedded in them. Comparing energy requirements with CA and livestock especially in biomass will give better understanding in the whole processes because in CA it is possible to bypass biomass as energy source whereas in livestock that is necessity. CED v.1.1 by ecoinvent is shown in Table 3.

Table 3. Cumulative energy demand (CED) impact assessment method and included energy types, subcategories, and energy sources. Table modified from Frischknecht *et al*. (2007).

type	subcategory	energy source included
non-renewable resources	fossil	hard coal, lignite, crude oil, natural gas, coal mining off-gas, peat
	nuclear	uranium
	primary forest	wood and biomass from primary forests
renewable resources	biomass	wood, food products, biomass from agriculture, e.g., straw
	wind	wind energy
	solar	solar energy (for heat and electricity)
	geothermal	geothermal energy (100-300m)
	water	run-of-river hydro power, reservoir hydro power

Sensitivity analyses were made by increasing the inputs for electricity, carbon source and CIP detergents. Based on Järviö *et al*. (2021b), 20 % increasement in input values was used. In other scenario electricity input in Finnish average electricity grid (FAEG) was modelled to contain only renewable energy sources, which are mainly hydro and wind power. Ratio between renewable electricity sources was kept the same and it was named as renewable electricity grid (REG).

MC uncertainty analysis was iterated for 100 times. Amount of data points in ecoinvent database is very hard to calculate or even unknown, and would change with each process, so reliable confidence intervals cannot be calculated. Therefore, in this thesis MC is mainly used for seeing the uncertainty ranges for each process. Moreover, calculating multiple scenarios with high number of iterations from background data that is not as accurate as empirical data sets from specific processes could be, leads to multiplying this inaccuracy which leads to misleading results (Heijungs, 2020). Also, calculating high number or iterations on a laptop is time-consuming without bringing much more insight to this kind of calculations. 100 iterations were also used by Järviö et al. (2021a).

For understanding the possible differences between analysed processes, the titers of fermentation broth were compared by looking the productivity values in the publications. The titers are calculated based on amount of produced protein after downstream processing divided by produced fermentation broth. Enzyme 2 was not included in the titer calculations because there was not enough data about the mass flows. 30 % of biomass loss is assumed with myco1 and myco2 products during heated centrifugation step (Upcraft *et al*., 2021). Myco2G and myco2S have the same productivity values so they are reported as myco2. Enzyme5 titer is taken from U.S Natural Renewable Energy Laboratory process (Humbird *et al.*, 2011), which is used to model the production system in Gilpin et al. (2017).

Ratio between fermentation and downstream processing is calculated only for hob2, because it was the only CA LCI together with hob1, that reported all the processes in such a detail that that they were possible to divide between fermentation and downstream processing. Hob2 was used because it is based on pilot scale while hob1 is more theoretical. Ratio between farm and factory-gates was also calculated for milk protein.

2.2 Results

Results for CA and milk chain produced protein in non-renewable CED and renewable CED are presented in Figure 6. Enzyme production in presented in Figure 7. Non-renewable CED per kg of protein was highest with mycoprotein (380 – 740 MJ/FU), followed by HOB (224 – 328 MJ/FU), recombinant ovalbumin (223 MJ/FU), milk (141 MJ/FU), and recombinant ovalbumin with allocation (133 MJ/FU). Renewable CED was highest for mycoproteins (227 – 267 MJ/FU), whereas ovalbumin (46 MJ/FU with allocation and 76 MJ/FU without allocation), HOB (40 – 62 MJ/FU) and milk (44 MJ/FU) were closer to each other. Highest variance in MC analysis was observed for mycoproteins in both CED results. In milk chain 68 % of non-renewable CED was used at farm and rest at factory gate. Renewable CED for farm and factory gate was 92% and 8%, respectively.

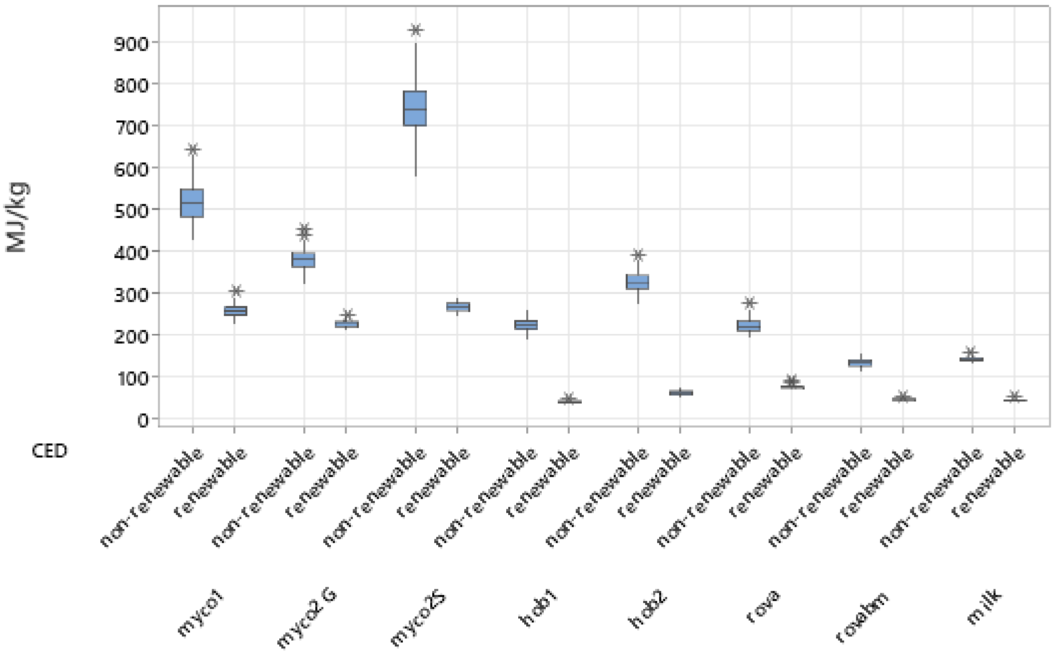

Figure 6. Non-renewable CED for CA and milk chain produced proteins iterated by 100 times in MC.

High differences between CED values from hundreds of MJ/FU to hundreds of thousands MJ/FU were observed between the LCA results of enzymes (Figure 7). Non-renewable CED varied from ~132 000 MJ/FU for enzyme2 (xylanase) to 30 000 – 40 000 MJ/FU for enzyme4 (hydroxymethylfurfural oxidase) and enzyme3 (peroxygenase), respectively. The lowest non-renewable CED was for enzyme1 (β-galactosidase) and enzyme 5 (cellulase) with ~5000 MJ/FU and ~300 MJ/FU, respectively. Renewable CED was the highest for enzyme2 (~34000 MJ/FU), followed by enzyme1, enzyme3, enzyme4 and enzyme5 from

~11000 to 109 MJ/FU. Enzyme2 had the highest variation in MC results followed by enzyme3 and enzyme4.

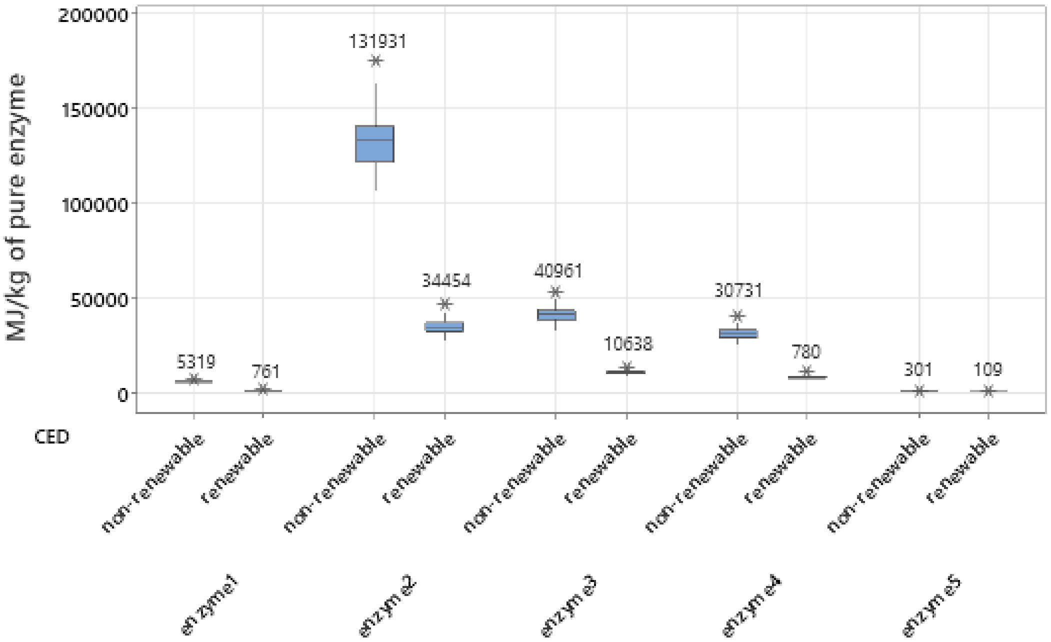

Figure 7. Enzyme production CED iterated by 100 times in MC. Mean MJ/kg values are written above the boxes for each process.

Difference between CEDs of proteins and enzymes produced by precision fermentation was observed. To explain this almost three orders of magnitude difference, the productivity values in publications were looked. Protein titers after downstream processing are shown in figure 8. Protein titres for enzyme1, enzyme3 and enzyme4 are ranging from 0.05 – 0.97 g/L, which makes them very low when compared to others. Enzyme5 has the highest titre of 40 g/L. Mycoproteins with continuous fermentation have protein titers from 2.00 to 2.58 g/L. HOB protein titres range from 15.00 to 26.32 and therefore, have highest variance. rova protein titer is 18.79 g/L.

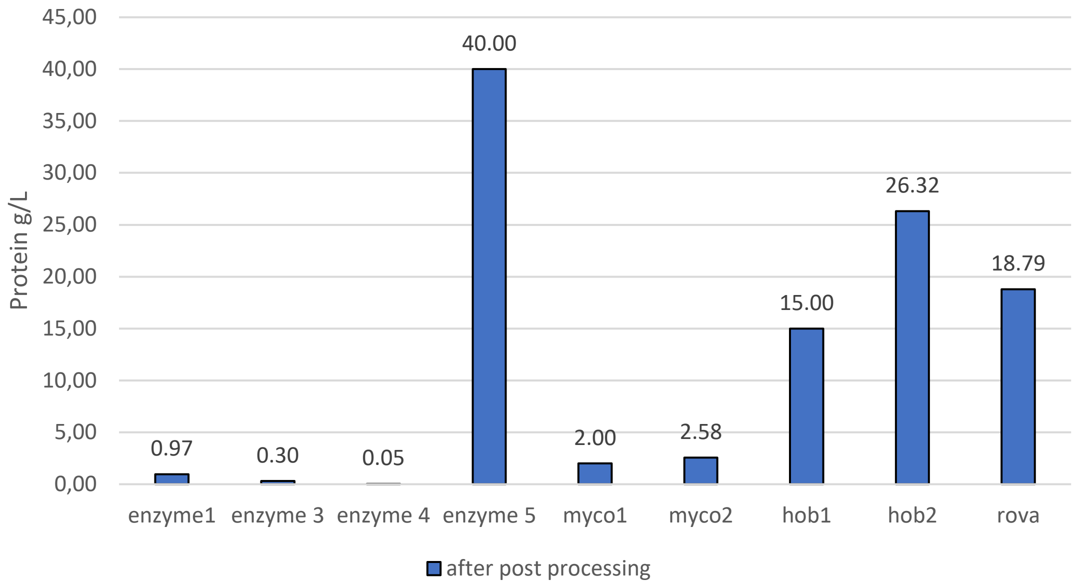

Figure 8. Protein titers (g/L) after downstream processing. Amount of protein per produced broth from fermenter is shown as g/L. Information about mass flows in enzyme2 production was not accessible. Enzyme5 titer is taken from U.S Natural Renewable Energy Laboratory process (Humbird *et al*., 2011).

Sensitivity analysis was done by changing the electricity source to renewables. The results are shown in Figure 9 for average non-renewable and renewable CED of mycoprotein (myco), hob (hob), rova, rovabm, milk, and enzymes. Non-renewable CED dropped and renewable CED was increased in all scenarios. However, total CED was also decreased in all cases, for example, total CED of enzymes was decreased by 57 % followed by hob, myco, rova and milk with 39 %, 28 %, 24 % and 19% decrease in total CED, respectively. In numbers, mycoprotein decreased from 797 MJ/FU to 576 MJ/FU, HOB decreased from 327 MJ/FU to 199 MJ/FU, rova decreased from 300MJ/FU to 228 MJ/FU, rovamb decreased from 180 MJ/FU to 138 MJ/FU, milk decreased from 185 MJ/FU to 150 MJ/FU and enzymes decreased from 65648 MJ/FU to 28193 MJ/FU.

Sensitivity analysis was also done for CA and enzyme processes with CIP included (Figure S1.). This revealed that the electricity is the major contributor to non-renewable CED in all cases except for enzyme1. In fact, increase of 20 % in electricity inputs caused over 19 % increase in non-renewable CED for enzyme2, enzyme3 and enzyme4. Enzyme5 was also increased for 13,3%. In CA the results were more subtle, but myco1 increased 17,6 % and hob1 and hob2 for 13,0 % and 14,1 %, respectively. Non-renewable CED increased only for a 10 % or less with myco2G, myco2S and rova. Renewable CED was increased for ~19-20 % with HOB scenarios and with enzyme2, enzyme3, and enzyme4. Carbon source did not have significant impact on non-renewable CED except for myco2G (8,3 %), but it increased renewable CED with enzyme5 for 17 % and both myco2 scenarios and rova for 13-15 %.

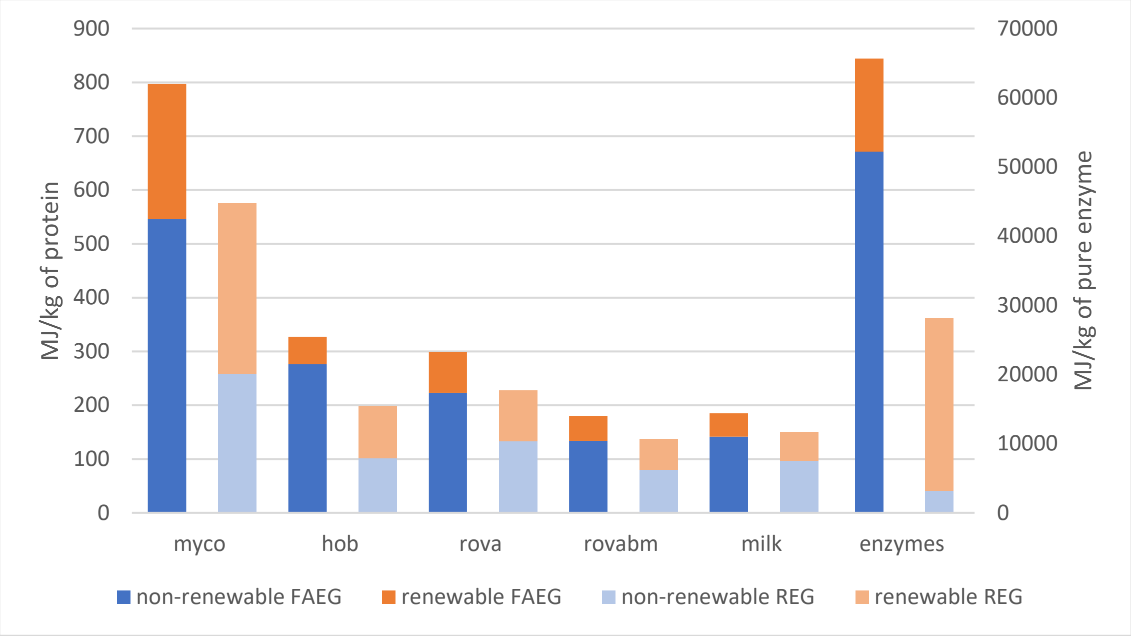

Figure 9. Total CED of all processes with average value for each product type using Finnish average electricity grid (FAEG) or Finnish only renewable electricity grid (REG). From left to right, myco is average from myco1 myco2G and myco2S. hob is average from hob1 and hob2. rova and rovabm are shown separately. Enzymes on the right side, are presented as an average from enzyme results, except for enzyme5, which was excluded because it did not include downstream processing like other scenarios.

Ratio between fermentation and downstream processing in CA and farm and factory (drying step) gates in milk production is shown in Figure 10. In HOB CA fermentation had 91 % of total CED leaving 9 % for downstream processing, which means 355 MJ/FU and 35 MJ/FU in total CED, respectively. From HOB downstream processing 98 % is from drying. In milk protein, 74 % of total CED came from farm gate and 26 % from drying step, which means 134 MJ/FU and 47 MJ/FU, respectively. Enzyme1 had same percentage between fermentation and downstream processing as milk with farm and factory. However, there were differences in percentages between enzyme1 and milk in renewable and non-renewable CED shown in Figure 6 and Figure 7.

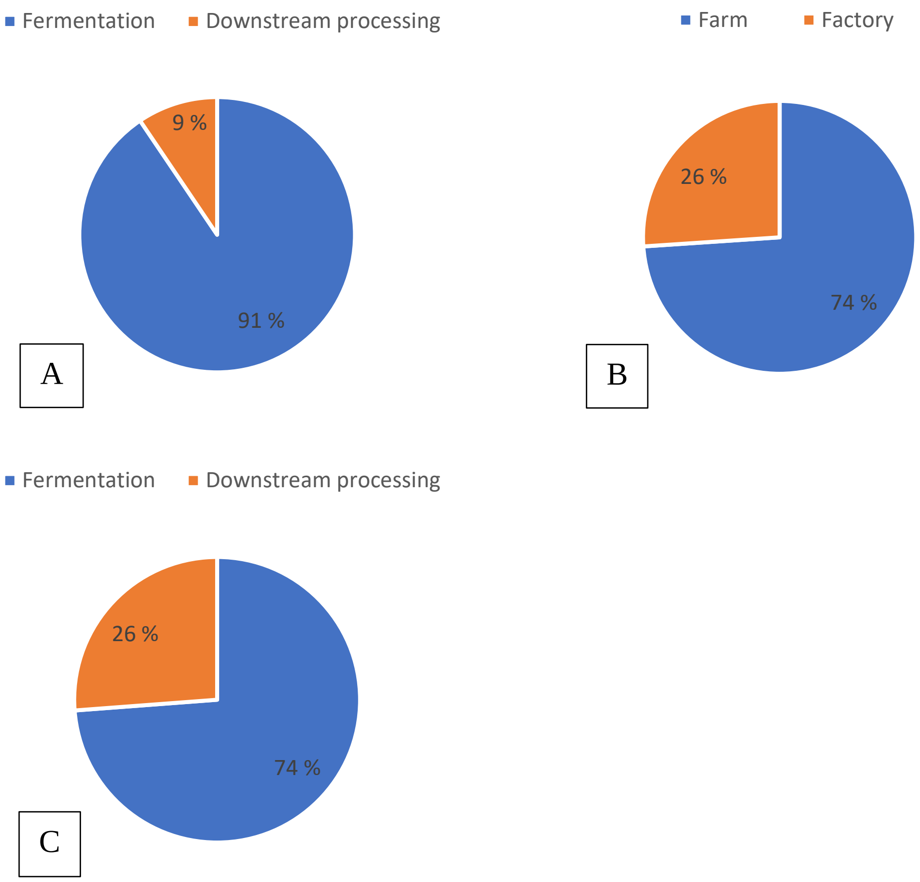

Figure 10. Total CED ratio between fermentation and downstream processing in HOB protein production (A), ratio between farm and factory in milk protein production (B) and ratio between fermentation and downstream processing in enzyme1 production (C). Pasteurization step is included in the fermentation step of HOB protein production.

2.3 Discussion

Based on the results, milk protein has generally lower requirement in non-renewable and renewable CED than CA (Figure 6, Figure 9). A compilation of average values from CA-based proteins, enzymes and powdered milk are shown in Figure 9, which also included sensitivity analysis for electricity inputs.

According to Figure 6, non-renewable CED of powdered milk protein is ~70 % less than average mycoprotein, approximately half of average HOB and ~35 % less than rova. Only when rova protein is allocated with protein from biomass (rovabm) milk protein has 5 % higher non-renewable CED than that of precision fermentation. Milk protein at farm gate has 95 MJ/FU of non-renewable CED. Non-renewable energy comes from fossil-based energy sources, nuclear energy, and primary forests.

Renewable energy includes wind energy, solar energy, geothermal energy, hydro power but also biomass, which includes wood, food products, and biomass from agriculture like straw. Therefore, carbon sources such as glucose, straw and whey will also contribute to renewable CED because there is embedded energy in the biomass that is stored during the photosynthesis (Frischknecht *et al.*, 2007). Ultimately, renewable CED is most usable when whole food production systems are studied. For example, biomass production with agriculture requires lot of land to transform sunlight to energy, but could the land be used more efficiently in terms of energy usage? Also, if fossil fuels are changed to renewable energy sources, heat-loss during energy transformation and transfer in whole process will be lower, which is also shown in Figure 9 as lower total CED between FAEG and REG.

The highest renewable CED was found for mycoprotein which has the highest inputs in electricity, but also the highest carbon feed inputs when compared to other CA products. Renewable CED for myco1 is most sensitive to changes in electricity, whereas renewable CED for myco2 is mostly affected by changes in amount of carbon sources (Figure S1). Moreover, HOB in average had 51 MJ/FU of renewable CED, which is close to powdered milk protein (44 MJ/FU) (Figure 9). However, CO_2 will contribute to renewable CED only through used electricity source, unlike other carbon sources like cow feed or straw from myco2, which explains that renewable CED of HOB is not as sensitive to increase of carbon input (Figure S1). Renewable CED of HOB can be explained by high amount of needed energy from Finnish electricity grid, which had a 47 % share of energy produced with renewables in 2017 (Statistics Finland 2018). However, wood use as a renewable energy source is calculated differently in CED v.1.1 than in Statistics Finland, where usage of primary forests is differentiated from the use of other wood sources and not seen as a renewable energy source (Frischknecht *et al.*, 2007). This will probably give lower share of renewable electricity source for average Finnish grid in ecoinvent, that what it is reported by Statistics Finland (Frischknecht *et al.*, 2007; Statistics Finland 2015). Renewable CED of powdered milk protein (44 MJ/FU) was lowest when compared to CA-based protein production (Figure 9). Milk protein at farm gate before processing had 40 MJ/FU of renewable CED caused mainly by production of cow feed and electricity and represented 92 % of total renewable CED for the milk protein production.

In short, primary energy demand (PED) used by Perfect Day (2021), in its LCA, is energy in its raw form before conversion to electricity or heating. Same way as CED, PED takes in to account all energy, direct and indirect, transformations and transportations of raw materials and products (Perfect Day, 2021). CED also contains calculations with similar idea of primary energy, but in this case CED is calculated based on CED v.1.1 method by ecoinvent and it is divided to renewable CED and non-renewable CED, whereas PED in Perfect Day (2021) contains only non-renewable primary energy. In LCA made by Perfect Day (2021), recombinant whey containing 90 % protein with *T. reesei* as a host organism has PED of 243 MJ/kg without allocations. Perfect day also used mass allocation scenario where primary energy demand allocated for whey protein was reduced to 56.3 MJ/kg, which is only 23 % of the no allocation scenario. If Perfect Day scenario with 90% protein content with and without allocation would be applied to the case of rova with Finnish localization, it would roughly mean 270 MJ/kg and 62MJ/kg in terms of total CED, respectively. This is very close to Perfect Day results of non-renewable PED.

When compared to CA or enzyme results in here, this value is exceptionally low. Moreover, the biomass of genetically modified *T.reesei* cannot be utilized at the moment at least in EU, which can make the valorization of this side-stream more difficult in near future.

Milk protein

Raw milk primary energy in Valio process was between 1,7 and 3,0 MJ/ECM (kg) (Aleksi Astaptsev, Valio, Personal communication 17.6.2022). Milk contained 2,8 % protein, which is lower when compared to global averages (3.2-3.3 %). This means that more milk must be produced when compared to global average per 1 kg of milk protein. Values acquired from Valio had allocation by dry mass which caused 28 % of CED burden to be allocated for dry protein. 78 % of dry weight consisted of lactose and fat. It is worth noting that in this allocation method, lactose, fat, and protein are valued only based on the mass and not for example on nutritional quality or value.

Based on literature, fat and protein corrected milk with biophysical allocation uses 81 to 152 MJ/kg of milk protein in CED (Berton *et al.*, 2020; Berton *et al.*, 2021). However, the energy demand can range between 76 MJ/kg and 262 MJ/kg for fat and protein corrected milk based on different LCA studies (Todde *et al.*, 2018a; Todde *et al.*, 2018b; Wang *et al.*, 2018; Wang *et al.*, 2019; Rotz *et al.*, 2020; Tricky and Kissinger, 2022). In addition to impact method, allocation method and functional unit, energy demand of milk production is mostly affected by feed type and managing system (Wang et al., 2019; Berton *et al.*, 2020). Moreover, pure protein content calculated from skimmed milk powder production in factory-to-factory gate uses around 68 to 77 MJ/Kg protein in total CED (Finnegan *et al.*, 2017; Yan and Holden, 2018). In this thesis, milk protein needed 134MJ/FU at farm gate and 47MJ/FU in factory-to-factory-gate, which makes 182 MJ/FU in total. This means that drying step CED is more efficient than reported on literature, and farm step CED is in the same range as similar biophysical allocation reported LCAs.

Milk production had multifunctional problems that were solved with allocations. First, an energy allocation between milk and meat was used, resulting in 99% of impacts for milk produced. Next was mass allocations of 28 % for the protein fraction of the raw milk as explained above. In downstream processing, used energy at Seinäjoki plant was mass allocated for powdered product protein content (35 %).

Milk protein production in comparison to CA

In milk protein production based on Figure 10, total CED used at farm and factory were 134 MJ/kg and 47MJ/kg, respectively. Hob2 had 355 MJ/FU at fermentation and 34 MJ/FU at drying step and enzyme1 had 4412 MJ/FU at fermentation and 1567 MJ/FU in downstream processing. In milk protein, LCI for drying milk powder is taken from Seinäjoki factory in a large-scale spray drying processing and powdered milks have 35 % of protein in average. HOB LCI is taken from pilot scale production using drum drying, and it is calculated based on 65 % protein content, which means that less biomass is needed to be dried to achieve 100 % dry protein. Pilot scale production is extrapolated to larger scale ex-ante and therefore, there will most probably be differences in large scale production or even different drying method. However, based on current calculations drying step in milk protein production requires only roughly 12 MJ/FU more energy which can be regarded as a small difference. This is interesting because it is widely acclaimed that spray drying is more energy intensive drying method than drum drying. When compared to hob scenarios, total CED of 264 MJ/FU is needed for hob1 and 390 MJ/FU for hob2. Milk protein production uses 67 % of hob1 total CED and 47 % of hob2 total CED and therefore, requires significantly less energy when modelled with these scenarios. Additionally, enzyme1 fermentation and downstream processing was compared with hob2 and milk, and the percentage of total CED used in the downstream processing was surprisingly 26 %, which is the same as is the CED of milk drying in milk protein production.

Comparing of biomass before drying to milk before drying can bring valuable information, because some CA products can be manufactured as a fresh products like milk alternatives. This is the case of mycoproteins, which use 777 MJ/FU for myco1 and 607 MJ/FU for myco2G. When straw is used as a carbon source as with myco2S, up to 1007 MJ/FU is needed which is more than 7-fold increase when compared to milk protein without drying taken into consideration. These values are surprisingly high when compared to hob2, which needed 355 MJ/FU before drying step.

Precision fermentation was also studied based on rova, which represents recombinant food proteins. Total CED of rova was 300 MJ/FU which is roughly 40 % more than with milk protein production.

In terms of calculations, rovabm was only one which had protein allocation between produced protein and excluded protein biomass. This resulted in 180 MJ/FU of total CED which is close to milk protein (182 MJ/FU). In the future if CA is more broadly used, there is high probability that the side-streams are more widely valorized. For example, it has been proposed that microbial biomass that is discarded in precision fermentation could be used as a feed and pet food or in novel materials such as composites, foams, and leather-like fabrics (Rischer, Szilvay and Oksman-Caldentey, 2020; Järviö *et al*., 2021b; Perfect Day 2021).

Electricity in CA and milk protein production

Total electricity requirements for the core processes in LCIs of CA and milk protein production has some differences (Table 4). Milk and rovamb has lowest electricity requirements of 5 kWh and 6 kWh respectively, followed by rova with 11 kWh, hob1 with 17 kWh and myco2 with 25 kWh. Hob2 and myco1 have 30 kWh and 59 kWh, respectively, which makes them most demanding in electricity requirements per kg of protein.

LCI of biomass fermentation processes is shown in Table S1. Based on LCIs, mycoprotein needs 25 – 59 kWh of electricity per 1 kg of protein. Electricity requirement including direct air capture of CO_2 (DAC) for hob1 and hob2 were 17 kWh and 30 kWh per kg of protein, respectively. When these are compared to Voutilainen (2021), where dry biomass produced with Pekilo, Torula and *F. venenatum* needs 1.1, 1.7 and 2.3 kWh/kg of 100 % protein, respectively, the difference is significant. However, Raats (2007) reported the energy use for producing 1kg of Quorn (100 % protein) to be 28.9 – 72.5 kWh where 37.8 kWh would be the energy usage for optimal production scenario. High electricity demand of HOB can be justified because it needs high amount of energy for on-site feed production. However, some of the high demand for electricity in mycoprotein could be explained by the fact that it is based on *F. venenatum* mycoprotein, which has been manufactured on large-scale since 1980s', whereas other CA scenarios are based on pilot scale production. LCI especially for myco2 is based on Upcraft *et al.*, 2021, which is based on Quorn production and is very precise on mass flows. Hob1 is based on literature where hob2 has more data gathered based on experiments and pilot scale production. Hob2 included transportation, CIP and WWT, which makes it more thorough in terms of input values, even though transportation and CIP were not included in this LCA. Hob1 and hob2 used 60 % and 65% protein content, respectively per kg of product, which will relatively increase CED for hob2 slightly more than with hob1 when compared to input values.

Table 4. Electricity inputs of processes from LCIs for 1kg of protein.

Product	Electricity total (kWh)
myco1	59
myco2G	25
myco2S	25
hob1	17
hob2	29
rova	11
rovabm	6
enzyme1	260
enzyme2	16345
enzyme3	5066
enzyme4	3741
enzyme5	6
milk	5

Ovalbumin production needs 7.0 – 10.5 kWh of electricity per kg of protein depending on allocation method (Table S1). According to a study by Voutilainen (2021), recombinant protein production needs 7.5 kWh/kg of protein, which is in the same range. According to LCA assigned by Perfect day, 1kg of dried whey product needs 13kWh of electricity, which is a bit higher, and instead of enzymatic hydrolysis of starch used in rova, it includes in-house starch hydrolysis to glucose at 120 C in pressurized and acidic environment (Perfect Day, 2021). This environment could also be used as a sterilization processing of the feed, which is always needed, but not always included in LCA analyses. Based on Perfect Day LCA, this process could be made without a high demand of electricity (Perfect Day, 2021).

Sensitivity analysis

Changing the electricity source from mixed electricity mix to renewable electricity mix lowers the total CED which is mainly caused by less heat loss during transformation and transportation of fossil-based energy sources to electricity. This can be seen in case of CA, milk, and enzymes in the figure 9. Changing only the electricity source also partly shows which different protein production methods are the most electricity dependent when compared to other energy sources such as steam or natural gas.

Non-renewable CED was decreased roughly 63 % in hob, 52 % in myco, 40 % in rova and 31 % in milk. Renewable CED was increased by 47 % in hob, 20 % in myco and 19 % in rova and 18 % in milk.

High decrease in non-renewable CED and high increase in renewable CED of hob is expected can be explained by higher share of electricity when compared to other CA scenarios. But non-renewable CED in HOB decreased even more in Järviö *et al*. (2021a), because they modelled the steam production to be done at the factory with electricity, so it was regarded as electricity input as well. In fact, 91% of total CED needed for hob-biomass production in Järviö *et al*. (2021a) was related to hydropower produced electricity.

In enzymes, non-renewable CED decreased from 52235 MJ/FU to 3203 MJ/FU, while renewable CED increased from 13413 MJ/FU to 24990 MJ/FU, which stands for 93 decrease and 46 % increase, respectively. All enzymes except enzyme1 were very sensitive to electricity changes (Figure S1), but when CIP was not considered with enzyme1, the largest inputs remained as electricity and whey protein. Enzyme3 and enzyme4 had only electricity and steam inputs in their LCI, which also explains the dramatic drop of 57 % in total CED of enzymes when changed from FAEG to REG (Figure 9).

Enzyme production

Enzymes were included in this thesis even though it is problematic to compared enzymes in mass because enzymes are manufactured based on their activity instead of amount of biomass. In the production, this can result in size differences of different protein molecules and slower metabolism routes such as folding and post-processing which are not optimal for mass production but instead more optimal for proteins with certain characteristics. Enzymes are also needed in low quantities so there hasn’t been as big demand for price reduction if compared to for example, food protein production. Moreover, purification through downstream processing can be needed especially on pharma scale, but in food and wood sector there can be some impurities and side-activities. All in all, some food and wood industry enzymes were picked for LCI of this thesis because enzymes have been made with precision fermentation in large scale for decades and therefore, they are included in the thesis.

Enzymes in the LCI such as, xylanases and β-glucanases are used in food industry of bakery and dairy, respectively (Feijoo *et al.,* 2017; Cimenlik *et al.,* 2021). Next, cellulases are mainly used in wood and paper industry, and lastly, enzymes such as peroxygenases and hydroxymethylfurfural oxidases are studied as more environmental and economical solutions for biotransformation of lignocelluosic biomass (Gilpin *et al.,* 2017; Bello *et al.,* 2021).

As scaled production of CA-based proteins has not been realized yet, an idea of using these large-scale enzyme production studies as a benchmark to industrial CA processes was applied. Moreover, it was found that not many public datasets are available for large scale enzyme production. For instance, Kim *et al*. (2009) integrated their LCI to GlaxoSmithKline in-house LCA database FLASK™. Some LCA studies like Nielsen *et al*. (2007) use confidential company information and Olofsson *et al*. (2017) acquired LCI data from Novozymes and kept it confidential. Therefore, these cannot be used to replicate the study, used for other studies nor compare the results as reliably as with the studies that have published LCIs. Moreover, according to Bello *et* al. (2021), there is no common framework and no accessible inventories available. Ultimately, it was found that no company has published foreground data about energy requirements of enzyme production.

Based on Figures 7 and 8, it could be deduced that when amount of titer, i.e., concentration of target protein in the fermentation broth, rises, value of CED decreases. Enzyme5 is originally based on NREL process, where reactor size is 300 m^3 and protein harvest titer is 40g/L (Humbird *et al*., 2011). According to studies, titers as high as 100 g/L are realistic for cellulolytic enzymes produced by *T. reesei* (Landowski et al., 2016).

This can give some explanation to low CED values when compared to other enzymes which have reactor sizes from 50 m^3 for enzyme1 and enzyme2 (based on water content in TableS2) to 100 m^3 in enzyme3 and enzyme4 (Bello *et al*., 2021). Process titers of enzymes 1, 3 and 4 are between 0.05 g/L and 0.97 g/L which are the lowest of all (Figure 8). This can be partly explained by low production yield, high amount of purification steps and difficulties in production of high number of bioactive molecules that are, recombinantly produced in production of enzyme1, 3 and 4, and thus foreign for the host organism, and therefore possibly toxic to it. This is not the case with enzyme5 which is naturally produced by *T. reesei* in conditions that are high in cellulose (Landowski et al., 2016). Batch size is also different between enzyme LCIs. Generally, higher batch-size is related to lower CED values. Enzyme2 has lowest batch size, followed by enzyme3 and enzyme4 and finally by enzyme5 (Feijoo *et al*., 2017; Cimenlik *et al*., 2021; Bello *et al*., 2021; Gilpin *et al*., 2017). However, this isn't the whole truth because enzyme1 with low batch size also has a low CED when compared to enzymes 2-4. CA production has much higher titers, when compared to enzyme1, enzyme3 and enzyme4. Batch-fermented hob1 and hob2 have 15 and 26 g/L, respectively. Also, rova has titer of 19 g/L. Mycoprotein has lowest titers ranging from 2 to 3 with myco1 and myco2, respectively. One reason is that mycoprotein process is continuous, so titers are naturally lower when compared to batch fermentation.

Non-renewable CED results of enzyme2, enzyme3 and enzyme4 are very sensitive to changes in electricity (Figure S1). With enzyme5, non-renewable CED was increased by ~13,5 % and ~5,5 % when electricity or carbon inputs were increased by 20 %, respectively. This means enzyme5 is most sensitive to differences to electricity input, but also carbon input will affect to non-renewable CED, but the effect is not as significant. Non-renewable CED is mostly affected because most of the electricity is produced with fossil fuels in average Finnish electricity grid.

One other major factor affecting the total CED input was the extent of downstream processing. Processes with the highest number of downstream processes in general required the highest non-renewable CED and when the number of processes decreased, the non-renewable CED decreased accordingly. Amount, of downstream processes (in brackets) is highest for enzyme1 (8), followed by enzyme2 (6), enzyme3 (6), enzyme4 (4) and enzyme5 (0). However, enzyme1 doesn't follow this rule and has less non-renewable CED than enzyme4. Processes are shown in Figure 3. Moreover, enzyme1 and enzyme2 use freeze drying, whereas enzyme3 and enzyme4 are processed to liquid form with chromatography and ultrafiltration as a last processing step, respectively (Bello *et al.*, 2021; Cimenlik *et al.*, 2021; Feijoo *et al.*, 2017). Enzyme5 is also in liquid form, and it is kept in a holding tank, until it is used for ethanol production (Gilpin *et al.*, 2017). Enzyme5 is not a representative example in this respect that it does not contain any steps for downstream processing. But when compared to CA processes like hob2 in Figure 10. over 91 % of total CED comes from fermentation, which seems to be the most energy demanding process in CA. Based on relatively low CED of enzyme1, the difference isn't as big, because downstream processes need more electricity than fermentation (Table S2).

Sensitivity analysis revealed that both non-renewable CED and renewable CED of enzyme1 production is highly sensitive to changes in CIP detergents (Figure S1). Detergents used for CIP in enzyme2 are just 0.148 % for base and 0.189 % for acid when they are compared to enzyme1 CIP detergents. Moreover, myco2S has 0.005 % and 0.010 % of base and acid when compared to enzyme1, respectively. Surprisingly, CIP detergents were not included in five LCIs without any explanation so they cannot be compared and thus CIP is not considered in this thesis, but only in this sensitivity analysis. CIP is essential for production of enzymes, CA, or milk proteins to avoid microbial contaminations, but also to enable extensive purification steps to obtain pure enzymes (Feijoo *et al.*, 2017). However, the amount of used detergents in enzyme1 production is very extensive. In fact, CIP protocol for enzyme1 uses 5870 kg of 50 % sodium hydroxide and 7658 kg of 50 % nitric acid per 1 kg of enzyme

(Table S2), but it is hard to justify these numbers when comparing to other publications with similar usage of purification steps. On the other hand, if enzyme1 is closer to the reality, there is a high change that energy demand will rise significantly.

The use of CED as an environmental impact category is limited in LCA studies regarding enzymes. A study by Nielsen *et al*. (2007) found that the primary energy use is between 20 – 130 MJ for kg of enzyme product including filling materials. Gilpin *et al*. (2017) reported similar finding with CED that was between 52 – 81 MJ/kg of enzyme which is directed from fermentation tanks to bioethanol processing without further downstream processes. Production of immobilized enzymes was reported to be between 117 – 207 MJ/kg of enzyme, but no percentage for pure enzyme content was given (Kim *et al*., 2009). Non-formulated enzyme production was studied by Agostinho *et al*. (2015), and in their assessment, non-renewable energy demand was 1664 MJ/kg of cellulase enzyme.

Downstream processes have variations, but to find out how energy demanding the fermenting phase is, electricity consumption of fermenters was compared. In Gilpin *et al*. (2017), CIP was not included and there was no post processing so electricity consumption of 6,3 kWh, can be chiefly addressed to fermentation processes. In other enzyme studies, fermenters, compressors, pumps, and agitation needed 121,6 kWh in Feijoo *et al*. (2017) and 18,8 kWh in Gonzalez-Garcia *et al*. (2018). Furthermore, significant amount of electricity from 15333 kWh in Cimenlik *et al*. (2021), to 3613 and 4924 kWh in Bello *et al*. (2021) is required for fermenters, which is many times higher than other processes combined. However, it should be noted that Cimenlik *et al.* (2021) is not yet published as peer-reviewed version which increases uncertainties. Not only these differences between electricity demand raise uncertainties related to inputs needed for enzyme production, but this finding underlines the need of empirical and specific data for large-scale processing. Also, when the differences are this high, there is a possibility that the meaning of 1 kg of enzyme in different studies included in Table 1 can change from pure enzyme to enzyme product with filler materials or impurities, even though they all can be understood as 1 kg of pure/powdered enzyme based on the original studies. Unfortunately, clear description of the final products was often missing or not clearly stated in articles. Based on patents by Novozymes A/S and Henkel AG & KGaA, enzyme granules contain at least 2 to 5 wt % and 0.075 to 3.5 wt % of enzyme, respectively (Henkel AG, 2013; Novozymes A/S, 2022). This would mean that in the studies where the FU is 1 kg of enzyme product, majority of the product (99.915 – 95 %) can be something else than enzyme, like formulation materials. Therefore, the impact of producing pure enzyme is reduced significantly. This is the case with formulated enzymes such as used in Nielsen *et al.* (2007) and Kim *et al*. (2009).

Enzymes in the LCI of this thesis are from Feijoo *et al*. (2017), who had 1 kg of β-Glucosidase as an output, Cimenlik *et al*. (2021) who reported 99+% purity for xylanase in their LCA, Bello *et al*. (2021) who used units, but had pure enzyme as an output of their LCI and, Gilpin *et al*. (2017) who used a term of 1 kg of cellulase enzyme. Therefore, all LCIs for the enzymes are probably very close of being 100 % enzyme, but definitions are not exact.

Furthermore, Gilpin *et al*. (2017) does not have formulation, but their reported CED is still as low as 52 – 81 MJ/kg of pure enzyme. In this thesis, electricity, and thermal inputs for enzyme5, which are based on Gilpin *et al*. (2017) are same as with other modelled enzymes, which increased non-renewable CED and renewable CED to 301 MJ/FU and 109 MJ/FU, respectively. Originally Gilpin *et al*. (2017) reported energy demand with one CED value, which is assumably summed up values of non-renewable and renewable CEDs, which leads to a loss of information (Frischknecht *et al.*, 2007). In the process modelled by Gilpin *et al.* (2017), heating and electricity was provided with organic waste feedstocks which provide no increase in CED because the energy in them is dealt with cut-off approach. This means the primary process where this organic waste was created has already used the embedded energy and therefore is not included to Gilpin *et al*., (2017) process (Frischknecht *et al*., 2007).

Additionally, there is market for enzymes in ecoinvent 3.7, which includes average inputs for production of alpha-amylase, glucoamylase and cellulase. It is not indicated if the enzyme is formulated or not, but it needs 4.16 kg of potato starch and 6.3 kWh of electricity per 1kg of enzyme. Surprisingly, this is same amount of electricity input as in Gilpin *et al*. (2017) even though they used different sources for the input data. When non-renewable and renewable CED is calculated for 1 kg of this average database enzyme, it results as 148 MJ/kg and 94 MJ/kg, respectively (data not shown). Comparing the results with previously published literature reveals much higher values in terms of CED for enzyme production in this thesis (Kim *et al*., 2009; Nielsen *et al.*, 2007). It may be sourced from the lack of proper definition of an enzyme product. However, this was not possible to be identified. This high CED values for enzyme production in the case of enzyme2, has not been published previously, which raises question about reliability of energy demand based on LCI of enzyme2 because it is not peer-reviewed. However, in the case of enzyme1 and enzyme5 the energy demand is lower, but still can be up to 5000 MJ/FU. This raises a question if everything taken into consideration when LCIs in precision fermentation are build? However, there are discrepancies to be clarified regarding data on the enzymes as well so a clear picture cannot be drawn based on it.

Energy sources

Energy can be used in different forms by utilizing electricity, fuels, steam etc. and previously mainly electricity inputs are considered. However, thermal energy like steam or heat generated by natural gas is calculated separately in LCIA with different downstream processes, that doesn't for example, include Finnish average energy mix for steam generation. These all are energy inputs with different ratio of conversion losses and background processes. For instance, steam as an energy input is only included in hob2 and milk protein production, which can result as a different amount of used energy when compared for example electricity, that is used in-house to produce steam. This can also affect to the ratio between renewable and non-renewable CED. These differences are shown in Table 2 and in supplementary tables S1-S4.

Around 47 % of Finnish energy mix was produced with renewables in 2017 from which around 34 % with wood derived sources. Therefore, biomass accounted for around 16 % of renewable energy sources in Finland. However, distinction between primary forests and other wood sources is not mentioned in Statistics Finland 2018. This needs to be stated, because forests having over 100 years old trees have been in 19 % decline since year 1996, mostly because of forestry, and the trend is continuing (Aalto *et al.*, 2023). This type of forest, that has been relatively undisturbed by human activity is regarded as primary forest, and thus non-renewable in the ecoinvent database (Frischknecht *et al.*, 2007). Unfortunately, data about the forest types and their usage is limited and moreover, more than half of forests in Finland are in private ownership (Aalto *et al.*, 2023). Therefore, conclusions about energy usage between non-renewable and renewable cannot be drawn, but it is worth noting that some percents of biomass energy that is regarded as renewable is in fact non-renewable.

Carbon and nitrogen sources

The production of carbon sources can have a tremendous impact on CED, especially if usage of carbon as a feed is high, like with mycoprotein. In this thesis, glucose as a carbon source was used in myco2G, whereas myco2S used glucose produced from straw and myco1 used sugar beet molasses. In these inventories, amount of glucose was 1,12 kg per 1 kg of mycoprotein in myco2G and amount of molasses was 3 kg per 1 kg of mycoprotein in myco1 (Table S1). Therefore, ratio between glucose and molasses when comparing these papers was 0,37. Whereas glucose is the most abundant monosaccharide, composition of sugar beet molasses is mainly sucrose (60 %), ash, organic nitrogen compounds (10 %) and nitrogen salts (2 %), which is one reason why its demand as a feed is so much higher than with solely easily metabolized glucose (Sjölin *et al.*, 2020).

One way to reduce energy requirements for glucose and nitrogen sources is to use side-streams from other industries. However, not all side-streams can be used as a carbon source and one important question is that which carbon sources are safe to be used in the fermentation of food or feed product (Upcraft *et al.*, 2021)? Gilpin *et al.* (2017) compared pretreated softwood, molasses from sugar cane, and starch-based glucose as a carbon source. They stoichiometrically balanced the carbon feed stocks and assumed that entire reactive carbon source is consumed during fermentation. However, it has been studied that carbon source affects the proliferation rates of microbes and quality of protein. Toxicology studies might also be needed when source of feed is changed, because different metabolic pathways can be used and toxins can result as a by-product (Whittaker *et al.*, 2020). Thus, it is not straightforward to replace one source with the other for empirical comparisons.

Similarly to Quorn production, nitrogen source is ammonium in myco2G and myco2S, but myco1 has nitrogen fertilizer. Moreover, molasses as well as straw include additional nitrogen. In general, nitrogen as feedstock has greater impact in terms of environmental impact factors but it does not represent high impact in CED.

Benefits and restrictions of methods

Monte Carlo runs can vary from 100 to 10 000 runs per LCA, and it will increase the precision of calculations. However, data used in LCA calculations can be based in small samples and too high number of runs (>10 000) will be done in expense of accuracy and thus, can lead to results that seems accurate but have ignored the inaccurate parameters or input data. Especially, when datasets have lots of assumptions and databases are used, there is a high risk of uncertainty and if MC iterations are run for thousands of times, falsely overly significant results will occur. Smaller number of runs can increase the precision but still leaves higher standard deviation which is beneficial in understanding the quality of original data (Heijungs, 2020). Therefore, 100 iterations were used in this thesis. This was done even though it has been argued by Heijungs (2020), that when the sample size is unknown, such as with most databases, MC shouldn't be used at all. MC is still a good tool to monitor uncertainty ranges for each process, while keeping the number of iterations relatively low.

Low number of iterations is also enough to find out the range where CED will most likely fall with these inputs (Heijungs, 2020).

Databases have inputs from multiple sources. For instance, Ecoinvent average Finnish electricity mix is valid for the year 2017 medium voltage shares for electricity technologies. Share of renewables was approximately 47 % for Finnish production in 2017, mostly from hydro, wind, and wood-based biomass, and with similar portfolio over 50 % in 2020 (Statistics Finland, 2018; 2021). Therefore, share of renewable energy is higher than reported here.

A short mentioning about other environmental impact factors

Using CED as an only environmental indicator should be avoided (Jungbluth *et al.*, 2007). Values may be calculated with different methodologies, but they will give a scale of cellular agriculture environmental impacts. Therefore, a table consisting environmental indicators from original papers are presented here (Table 5). Furthermore, in time it can also be that impact factors that are thought to be the most relevant regarded to food production are usually improved, such as GWP in milk chain (FAO, 2019). This can cause burden shifting between impact categories and, it has been studied for instance, by Sabia et al., (2020), that farms using high amount of feed concentrates produce less GHG emissions but have highest impact on total biodiversity loss. However, total biodiversity loss as an impact factor is rarely included in LCA studies (Baldini, Gardoni and Guarino, 2017).

Table 5. Environmental impacts of the studied proteins (as 1 kg of protein). Valio milk calculations are based and calculated from ECM (3.2 % protein) whereas Poore & Nemecek (2018) are based and calculated from FPCM (3.3 % protein).

Product	global warming potential ($kg\ CO_2$-eq kg^{-1})	land use (m^2a crop kg^{-1})	eutrophication ($kg\ PO_4^{3-}$-eq kg^{-1})	acidification ($kg\ SO_2$-eq kg^{-1})	water scarcity (m^3)
myco1	58.500	8.150	-	-	-
myco2	23.660	4.390	0.013	0.165	2.232
hob1*	1.000	0.060	<0.001	-	3.750
hob2	6.740	0.071	0.004	0.032	6.000
rova	10.403	4.903	0.004	0.037	11.491
raw milk**	33.125 (96.970)	(272.727)	(324.242)	(606.060)	(303.030)

*solar energy; **Valio Carbo (2022) values and Global mean values from Poore and Nemecek (2018) in brackets for raw milk, which was used to calculate values for protein. 31.25 * ECM and 30.30 * FPCM were used.

Sources: Myco1 (Based on Smetana et al., 2015), myco2 (Based on Upcraft et al., 2021), hob1 (Based on Sillman et al., 2020), hob2 (Based on Järviö et al., 2021a), rova (Based on Järviö et al., 2021b), raw milk (Poore & Nemecek, 2018; Valio Carbo, 2021)

3 CONCLUSIONS

According to presented data, protein production via conventional milk chain requires lower electricity input and cumulative energy demand per kg of protein compared to that of selected cellular agriculture-based protein production processes. The main factors that affect CED were identified as electricity composition, carbon source, and possibly CIP. CED from different sources of carbon can be affected by using side-streams from different industrial processes like straw or whey. Furthermore, downstream processes can also affect to the CED, but it seems that fermentation part, including carbon source, nitrogen source and electricity inputs to fermentation requires highest amount of CED. Milk chain had highest relative CED at farm gate mainly because of fuels and electricity used in feed production but also with farm operations. Over 90% of renewable energy was used at the farm gate, and only 32 % of non-renewable cumulative energy demand was generated during the processing steps.

Inclusion of enzymes showed the complexity of interpolations from lab to pilot to industrial scale operations. However, technology for CA-based food production is still at its infancy and it is possible that the energy demand will decrease as technology is further developed. Moreover, number of studies is limited, and more research is needed. Technology for enzyme production varies a lot and higher number of public studies would give much needed insight. Data between different LCA studies on enzyme production and especially between cellular agriculture and enzyme production has large variance. In short, drawing a simple conclusion is very laborious or even impossible. However, based on high CED of enzyme production it was revealed that CED of CA has a potential to have much higher values than reported on CA production, but on the other hand, CED of protein production with CA can be very close to milk protein production. Yield also varies substantially when different types of proteins with different downstream needs are produced. Much improvement can be made in optimizing and developing CA in food production. Moreover, when the scale of production increases, hot spots of energy usage can shift (Bello *et al*. 2021).

Even though energy demand would still seem to be higher than with conventional food production, using cellular agriculture to produce raw-materials for animal-based food production could lower especially the need for land use but also, the other environmental impacts as well. On the other hand, if the animal-based food production could be by-passed and energy could be used directly to produce for instance, recombinant naturally lactose free milk protein by CA, there wouldn’t be a need to use the energy, for example, to the conventional production of lactose. All in all, in the future, more studies with continuous fermentation utilizing different by-products and including more impact factors should be pursued.

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5 SUPPLEMENTARY

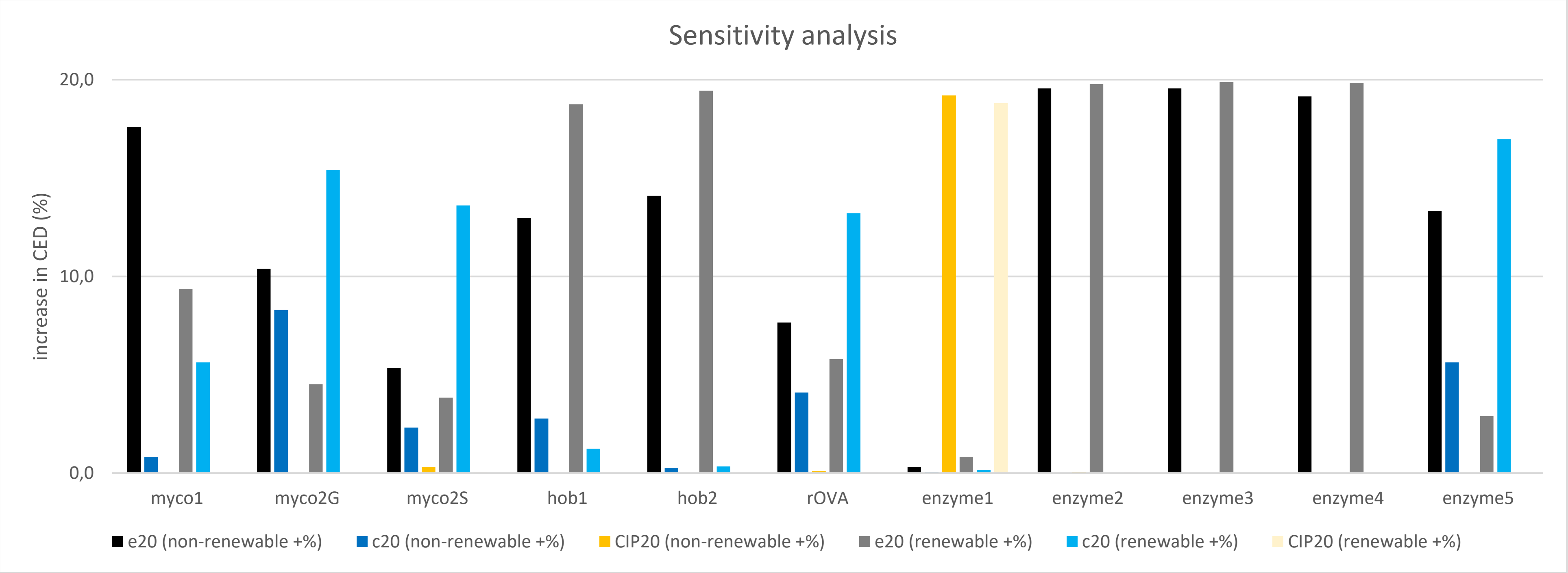

Figure S1. Sensitivity analysis of CA and enzyme production. Electricity inputs are increased by 20 % (e20) and carbon feed inputs such as glucose, barley or CO_2 are increased by 20 % (c20). Base and acid inputs of CIP are increased by 20 % in CIP20. CIP analysis is not done to myco1, myco2G, hob1, enzyme3, enzyme4 and enzyme5 because they were not included in the original LCI. Furthermore, CIP is out of boundaries in the final study, but it is included to this sensitivity analysis presented in supplementary data.

Table S1. LCI for CA products. CED is calculated for 1 kg of 100% protein product without CIP or transportation.

myco1 (Based on Smetana et al., 2015)

inputs

Flow	Amount	Unit	Provider
electricity, medium voltage	213.200	MJ	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
molasses, from sugar beet	30.000	kg	market for molasses, from sugar beet | molasses, from sugar beet | APOS, U - GLO
organic nitrogen fertiliser, as N	0.690	kg	market for organic nitrogen fertiliser, as N | organic nitrogen fertiliser, as N | APOS, U - GLO
tap water	400.000	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
transport, tractor and trailer, agricultural	2154.500	kg*km	market for transport, tractor and trailer, agricultural | APOS, U - CH
outputs			
mycoprotein	1.000	kg	

myco2G (Based on Upcraft et al., 2021)

inputs

Flow	Amount	Unit	Provider
ammonia, anhydrous, liquid	0.337	kg	market for ammonia, anhydrous, liquid | ammonia, anhydrous, liquid | APOS, U - RER
electricity, medium voltage	24.682	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
glucose	8.886	kg	market for glucose | glucose | APOS, U - GLO
phosphoric acid, industrial grade, without water, in 85% solution state	0.392	kg	market for phosphoric acid, industrial grade, without water, in 85% solution state | APOS, U - GLO
tap water	84.934	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
outputs			
mycoprotein	1.000	kg	

myco2S (Based on Upcraft et al., 2021)

inputs

Flow	Amount	Unit	Provider
[Ch][HSO4]	0.419	kg	[Ch][HSO4] - FI (Table S4)
ammonia, anhydrous, liquid	0.337	kg	market for ammonia, anhydrous, liquid | ammonia, anhydrous, liquid | APOS, U - RER
calcium chloride	0.005	kg	market for calcium chloride | calcium chloride | APOS, U - RoW
copper sulfate	2.9E-4	kg	market for copper sulfate | copper sulfate | APOS, U - GLO
electricity, medium voltage	24.682	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
enzymes	0.307	kg	market for enzymes | enzymes | APOS, U - GLO
lime, hydrated, packed	0.030	kg	market for lime, hydrated, packed | lime, hydrated, packed | APOS, U - RER
magnesium sulfate	0.032	kg	market for magnesium sulfate | magnesium sulfate | APOS, U - GLO
phosphoric acid, industrial grade, without water, in 85% solution state	0.461	kg	market for phosphoric acid, industrial grade, without water, in 85% solution state | APOS, U - GLO
potassium sulfate	0.079	kg	market for potassium sulfate | potassium sulfate | APOS, U - RoW
refrigerant R134a	0.273	kg	market for refrigerant R134a | refrigerant R134a | APOS, U - GLO
sodium hydroxide, without water, in 50% solution state	0.322	kg	market for sodium hydroxide, without water, in 50% solution state | APOS, U - GLO
straw	74.285	kg	market for straw | straw | APOS, U - RER
tap water	131.349	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	0.391	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
zinc monosulfate	0.002	kg	market for zinc monosulfate | zinc monosulfate | APOS, U - RoW
outputs			
mycoprotein	1.000	kg	

hob1 (Based on Sillman et al., 2020)

fermentation inputs

Flow	Amount	Unit	Provider
ammonia, anhydrous, liquid	0.160	kg	market for ammonia, anhydrous, liquid | ammonia, anhydrous, liquid | APOS, U - RER
CO2	1.760	kg	Direct air capture for hob1 (Table S4)
electricity, medium voltage	9.860	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
phosphoric acid, industrial grade, without water, in 85% solution state	0.370	kg	market for phosphoric acid, industrial grade, without water, in 85% solution state | APOS, U - GLO
sulfite	0.349	kg	market for sulfite | sulfite | APOS, U - RER
tap water	0.110	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	0.065	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
fermentation outputs			
Biomass slurry	1.000	kg	
downstream processing inputs			
Biomass slurry	1.667	kg	fermentation inputs
electricity, medium voltage	0.650	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
heat, district or industrial, natural gas	4.850	kWh	market for heat, district or industrial, natural gas | APOS, U - Europe without Switzerland
downstream processing outputs			
HOB Biomass	1.000	kg	

hob2 (Based on Järviö et al., 2021a)

inputs

Flow	Amount	Unit	Provider
ammonia, anhydrous, liquid	0.923	kg	market for ammonia, anhydrous, liquid | ammonia, anhydrous, liquid | APOS, U - RER
CO2	2.850	kg	Direct air capture for hob2 (Table S4)
electricity, medium voltage	0.128	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
electricity, medium voltage	28.874	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
electricity, medium voltage	0.080	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
electricity, medium voltage	0.194	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
nitric acid, without water, in 50% solution state	0.003	kg	market for nitric acid, without water, in 50% solution state | APOS, U - RER w/o RU
sodium hydroxide, without water, in 50% solution state	0.006	kg	market for sodium hydroxide, without water, in 50% solution state | APOS, U - GLO
steam, in chemical industry	7.984	kg	market for steam, in chemical industry | steam, in chemical industry | APOS, U - RER
steam, in chemical industry	3.676	kg	market for steam, in chemical industry | steam, in chemical industry | APOS, U - RER
tap water	1.171	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	36.551	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	0.009	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
transport, freight, aircraft, unspecified	0.107	t*km	market for transport, freight, aircraft, unspecified | APOS, U - GLO
transport, freight, lorry, unspecified	0.308	t*km	market for transport, freight, lorry, unspecified | APOS, U - RER
outputs			
HOB biomass	1.000	kg	

rova (Based on Järviö et al., 2021b)

inputs

Flow	Amount	Unit	Provider
ammonia, anhydrous, liquid	1.110	kg	market for ammonia, anhydrous, liquid | ammonia, anhydrous, liquid | APOS, U - RER
ammonium sulfate	0.511	kg	market for ammonium sulfate | ammonium sulfate | APOS, U - RER
calcium chloride	0.020	kg	market for calcium chloride | calcium chloride | APOS, U - RER
cobalt oxide	9.400E-5	kg	market for cobalt oxide | cobalt oxide | APOS, U - GLO
electricity, medium voltage	4.330	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
electricity, medium voltage	0.152	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
electricity, medium voltage	0.011	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
electricity, medium voltage	6.050	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
glucose	2.540	kg	market for glucose | glucose | APOS, U - GLO
heat, district or industrial, natural gas	0.620	kWh	market for heat, district or industrial, natural gas | APOS, U - Europe without Switzerland
iron sulfate	1.270E-4	kg	market for iron sulfate | iron sulfate | APOS, U - RER
magnesium sulfate	0.015	kg	market for magnesium sulfate | magnesium sulfate | APOS, U - GLO
manganese sulfate	4.070E-5	kg	market for manganese sulfate | manganese sulfate | APOS, U - GLO
nitric acid, without water, in 50% solution state	0.022	kg	market for nitric acid, without water, in 50% solution state | APOS, U - RER w/o RU
polydimethylsiloxane	0.044	kg	market for polydimethylsiloxane | polydimethylsiloxane | APOS, U - GLO
sodium hydroxide, without water, in 50% solution state	0.043	kg	market for sodium hydroxide, without water, in 50% solution state | APOS, U - GLO
sodium phosphate	0.380	kg	market for sodium phosphate | sodium phosphate | APOS, U - RER
sulfuric acid	1.950E-5	kg	market for sulfuric acid | sulfuric acid | APOS, U - RER
tap water	39.300	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	0.065	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	48.600	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
zinc oxide	1.620E-5	kg	market for zinc oxide | zinc oxide | APOS, U - GLO
outputs			
rova	1.000	kg	

rovabm (Based on Järviö et al., 2021b)

inputs

Flow	Amount	Unit	Provider
ammonia, anhydrous, liquid	0.734	kg	market for ammonia, anhydrous, liquid | ammonia, anhydrous, liquid | APOS, U - RER
ammonium sulfate	0.338	kg	market for ammonium sulfate | ammonium sulfate | APOS, U - RER
calcium chloride	0.013	kg	market for calcium chloride | calcium chloride | APOS, U - RER
cobalt oxide	6.2E-5	kg	market for cobalt oxide | cobalt oxide | APOS, U - GLO
electricity, medium voltage	2.866	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
electricity, medium voltage	0.101	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
electricity, medium voltage	0.007	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
electricity, medium voltage	4.005	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
glucose	1.681	kg	market for glucose | glucose | APOS, U - GLO
heat, district or industrial, natural gas	0.410	kWh	market for heat, district or industrial, natural gas | APOS, U - Europe without Switzerland
iron sulfate	8.4E-5	kg	market for iron sulfate | iron sulfate | APOS, U - RER
magnesium sulfate	0.010	kg	market for magnesium sulfate | magnesium sulfate | APOS, U - GLO
manganese sulfate	2.7E-5	kg	market for manganese sulfate | manganese sulfate | APOS, U - GLO
nitric acid, without water, in 50% solution state	0.014	kg	market for nitric acid, without water, in 50% solution state | APOS, U - RER w/o RU
polydimethylsiloxane	0.029	kg	market for polydimethylsiloxane | polydimethylsiloxane | APOS, U - GLO
sodium hydroxide, without water, in 50% solution state	0.029	kg	market for sodium hydroxide, without water, in 50% solution state | APOS, U - GLO
sodium phosphate	0.252	kg	market for sodium phosphate | sodium phosphate | APOS, U - RER
sulfuric acid	1.3E-5	kg	market for sulfuric acid | sulfuric acid | APOS, U - RER
tap water	26.017	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	0.043	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	32.173	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
zinc oxide	1.1E-5	kg	market for zinc oxide | zinc oxide | APOS, U - GLO
outputs			
rovabm	1.000	kg	

Table S2. LCI for enzyme products. 1778 kg of liquid whey contains approximately 88,900 kg of lactose. CED is calculated for 1 kg of 100% protein product without CIP or transportation

enzyme1 (Based on Feijoo et al., 2017)

fermentation inputs

Flow	Amount	Unit	Provider
ammonium sulfate	5.330	kg	market for ammonium sulfate | ammonium sulfate | APOS, U - RER
electricity, medium voltage	34.900	Wh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
electricity, medium voltage	121.600	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
Liquid whey (Gouda 48+), at processing/NL Economic	1778.000	kg	Cheese (Gouda 48+), at processing Economic - NL
magnesium sulfate	851.000	g	market for magnesium sulfate | magnesium sulfate | APOS, U - GLO
steam, in chemical industry	489.000	kg	market for steam, in chemical industry | steam, in chemical industry | APOS, U - RER
tap water	317.000	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	41769.000	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	1714.000	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
urea	5.330	kg	market for urea | urea | APOS, U - RER
downstream processing inputs			
electricity, medium voltage	138.850	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
sodium chloride, powder	16.041	kg	market for sodium chloride, powder | sodium chloride, powder | APOS, U - GLO
sodium hydroxide, without water, in 50% solution state	12.200	kg	market for sodium hydroxide, without water, in 50% solution state | APOS, U - GLO
sodium hydroxide, without water, in 50% solution state	5870.000	kg	market for sodium hydroxide, without water, in 50% solution state | APOS, U - GLO
sulfuric acid	3829.000	kg	market for sulfuric acid | sulfuric acid | APOS, U - RER
tap water	28.200	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	9525.000	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	1900.000	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	776.958	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
outputs			
B-galactosidase	1.000	kg	

enzyme2 (Based on Cimenlik et al., 2021)

inputs

Flow	Amount	Unit	Provider
ammonium sulfate	66.700	kg	market for ammonium sulfate | ammonium sulfate | APOS, U - RER
Barley grain, dried, at farm/FI Mass	72.400	kg	Barley grain, dried, at farm Mass - FI
calcium chloride	0.150	kg	market for calcium chloride | calcium chloride | APOS, U - RER
electricity, medium voltage	58842.200	MJ	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
glucose	0.910	kg	market for glucose | glucose | APOS, U - GLO
magnesium sulfate	2.200	kg	market for magnesium sulfate | magnesium sulfate | APOS, U - GLO
phosphoric acid, industrial grade, without water, in 85% solution state	8.528	kg	market for phosphoric acid, industrial grade, without water, in 85% solution state | APOS, U - GLO
potato starch	0.190	kg	market for potato starch | potato starch | APOS, U - GLO
sodium hydroxide, without water, in 50% solution state	1.440	kg	market for sodium hydroxide, without water, in 50% solution state | APOS, U - GLO
sodium hydroxide, without water, in 50% solution state	7.240	kg	market for sodium hydroxide, without water, in 50% solution state | APOS, U - GLO
water, deionised	5654.200	kg	market for water, deionised | water, deionised | APOS, U - Europe without Switzerland
outputs			
Xylanase	1.000	kg	

enzyme3 (Based on Bello et al., 2021)

inputs

Flow	Amount	Unit	Provider
electricity, medium voltage	153487.580	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
steam, in chemical industry	2678.900	kg	market for steam, in chemical industry | steam, in chemical industry | APOS, U - RER
tap water	2379484.800	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
outputs			
CglUPO	30.300	kg	

enzyme4 (Based on Bello et al., 2021)

inputs

Flow	Amount	Unit	Provider
electricity, medium voltage	17471.160	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
steam, in chemical industry	982.520	kg	market for steam, in chemical industry | steam, in chemical industry | APOS, U - RER
tap water	269069.640	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
outputs			
HMFO	4.670	kg	

enzyme5 (Based on Gilpin et al., 2017)

inputs

Flow	Amount	Unit	Provider
ammonia, anhydrous, liquid	0.189	kg	market for ammonia, anhydrous, liquid | ammonia, anhydrous, liquid | APOS, U - RER
ammonium sulfate	0.037	kg	market for ammonium sulfate | ammonium sulfate | APOS, U - RER
calcium chloride	0.011	kg	market for calcium chloride | calcium chloride | APOS, U - RER
cooling energy	59.800	MJ	market for cooling energy | cooling energy | APOS, U - GLO
Crude maize germ oil (pressing), at processing/NL Mass	0.026	kg	Crude maize germ oil (pressing), at processing Mass - NL
electricity, medium voltage	6.300	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
glucose	4.700	kg	market for glucose | glucose | APOS, U - GLO
heat, central or small-scale, biomethane	2.900	MJ	market for heat, central or small-scale, biomethane | APOS, U - Europe without Switzerland
magnesium sulfate	0.008	kg	market for magnesium sulfate | magnesium sulfate | APOS, U - GLO
Maize steepwater wet, at processing/NL Mass	0.269	kg	Maize steeped, at processing Mass - NL
potassium phosphate	0.053	kg	production of potassium phosphate
sulfur dioxide, liquid	0.028	kg	market for sulfur dioxide, liquid | sulfur dioxide, liquid | APOS, U - RER
tap water	19.000	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
outputs			
cellulase	1.000	kg	

Table S3. LCI for milk powder protein production (Based on Valio Carbo, 2022). CED is calculated for 1 kg of 100% protein product without CIP or transportation.

Farm inputs			
Flow	Amount	Unit	Provider
organic nitrogen fertilizer, as N	0.309	kg	market for organic nitrogen fertiliser, as N | organic nitrogen fertiliser, as N | APOS, U - GLO
diesel, burned in agricultural machinery	0.383	kWh	market for diesel, burned in agricultural machinery | diesel, burned in agricultural machinery | APOS, U - GLO
light fuel oil	0.531	kg	market for light fuel oil | light fuel oil | APOS, U - Europe without Switzerland
petrol, unleaded, burned in machinery	0.062	kWh	market for petrol, unleaded, burned in machinery | petrol, unleaded, burned in machinery | APOS, U - GLO
Grass*	8.165	kg	Grass, at dairy farm/FI Economic (of project Oatmilk)
Barley grain*	2.676	kg	Barley grain, at farm/FI Economic (of project Agri-footprint - economic allocation)
Oat grain*	1.070	kg	Oat grain, at farm/FI Economic (of project Agri-footprint - economic allocation)
Rapeseed meal (off-site)*	2.022	kg	Rapeseed meal, consumption mix, at feed compound plant/FI Economic (FULLY FINNISH) (of project Oatmilk)
electricity, medium voltage	4.144	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
Factory inputs			
electricity, medium voltage	1.313	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
steam, in chemical industry	5.554	kg	market for steam, in chemical industry | steam, in chemical industry | APOS, U - RER
outputs			
milk powder (100% protein)	1.000	kg	

*Grass, barley grain, oat grain and rapeseed meal are based on Finnish production from project Oatmilk and project Agri-footprint (Järviö, 2022).

Table S4. LCI for auxiliary processes.

[Ch][HSO4] - FI (Based on Upcraft et al., 2021)

inputs

Flow	Amount	Unit	Provider
electricity, medium voltage	4.340E-4	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
ethylene oxide	0.363	kg	market for ethylene oxide | ethylene oxide | APOS, U - RER
tap water	0.149	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	0.188	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
trimethylamine	0.487	kg	market for trimethylamine | trimethylamine | APOS, U - RoW
outputs			
[Ch][HSO4]	1.000	kg	

Direct air capture for hob1 (Based on Sillman et al., 2020)

inputs

Flow	Amount	Unit	Provider
chemical, organic	0.004	kg	market for chemical, organic | chemical, organic | APOS, U - GLO
electricity, medium voltage	0.710	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
heat, district or industrial, natural gas	3.780	kWh	market for heat, district or industrial, natural gas | APOS, U - Europe without Switzerland
outputs			
CO2	1.760	kg	

Direct air capture for hob2 (Based on Järviö et al., 2021a)

inputs

Flow	Amount	Unit	Provider
electricity, medium voltage	52.300	kWh	market for electricity, medium voltage | electricity, medium voltage | APOS, U - FI
outputs			
CO2	281.000	kg	

production of potassium phosphate (Based on Gilpin et al., 2017)

Flow	Amount	Unit	Provider
phosphoric acid, industrial grade, without water, in 85% solution state	0.659	kg	market for phosphoric acid, industrial grade, without water, in 85% solution state | APOS, U - GLO
potassium hydroxide	1.290	kg	market for potassium hydroxide | potassium hydroxide | APOS, U - GLO
tap water	0.099	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
tap water	0.000	kg	market for tap water | tap water | APOS, U - Europe without Switzerland
outputs			
potassium phosphate	1.000	kg	