LCA Report

Life Cycle Assessment of animal-free whey protein production by fermentation

Version 1.0

For :
Bon Vivant

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December 2022

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Title	Life Cycle Assessment of animal-free whey protein production by fermentation
Ordered by	BON VIVANT
Citation	Hamelin L, Cellier C (2022). Life Cycle Assessment of animal-free whey protein production by fermentation. LCA Report Version 1.0.
Disclaimer	The information contained in this report is based upon sources and estimations judged reliable by the authors. Hamelin Lab Consulting and Processium decline any responsibility for the consequences of direct or indirect use of the information and results contained in this document. The study does not make any comparison between competing animal-free whey protein products. The study compares, however, two scenarios: one (i) where 2160 tonnes/year of animal-free whey protein are supplied through the specific fermentation process studied herein and one (ii) where this quantity is instead supplied by cow milk. These systems are made equivalent by considering that the other functions (carbohydrates, lipids, minerals) also supplied in the milk system have to be compensated in the animal-free system. The study presented in this report is in its first version and was made at the light of the requirements of the ISO 14040 and 14044 standards (2006) relating to LCA. It was intended to provide early science-based guidance for decision-making, but not to fully comply with the ISO standards, which, on the basis of the results of the present study, may be mandated in a next study. Among others, a critical review carried out by a panel of independent experts with the procedure described in clause 6 of ISO 14044 was not performed. Accordingly, the study cannot be used in comparative assertions intended to be disclosed to the public.

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List of abbreviations

Non-exhaustive list of acronyms and abbreviations frequently used through this report:

C, N, P, H	Carbon, nitrogen, phosphorus, hydrogen
CFC-11	Trichlorofluoromethane, an ozone depleting substance
CH4	Methane
CO2	Carbon dioxide
CTUe	Comparative Toxic Unit for ecosystems
CTUh	Comparative Toxic Unit for humans
DM	Dry matter
EF	Environmental Footprint Life Cycle Impact Assessment Method
eq.	equivalent
GHG	Greenhouse gas
GLO	Global market
ILCD	International Reference Life Cycle Data
IPCC	Intergovernmental Panel on Climate Change
ISO	International Standard Organization
LCA	Life Cycle Assessment
LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Assessment
LHV	Lower Heating Value
NMVOC	Non methane volatile organic compounds
Pt	Point; dimensionless index used as metric for the land use change impact
RER	Rest of Europe market
TEA	Techno-economic analysis
ww	Wet weight

Definitions

Whey	It consists of milk without most of the fat & caseins, and without some of the minerals (calcium, phosphate) that were included in caseins micelles. Whey dry mass is made of lactose and soluble proteins. If further processing is foreseen (e.g. to WPC or WPI), often, remaining minerals are removed from whey before drying.
Whey Protein Concentrates (WPC) and Whey Protein Isolate (WPI)	Whey protein concentrates (WPC) or Isolates (WPI) are obtained mainly by ultrafiltration of whey: carbohydrates, minerals and small organics are removed (50 to > 90% removal). Chromatography techniques may be used for highest quality (WPI).
Animal-free whey	Whey proteins that are identically produced by micro-organisms through precision fermentation.
Precision fermentation	Fermentation plus precision biology. A process allowing to program micro-organisms to produce almost any complex organic molecule.

1. Goal and Scope definition

1.1 Goal definition

1.1.1 Context of the study

BON VIVANT intends to develop and industrialize a fermentation-based process to produce whey proteins for food applications, without the use of animal production (e.g. dairy farming, sheep rearing, etc.). This will from here onwards be referred to as the BV process. The yeast strain that will be used in the precision fermentation process is under development, and partners are involved for the process scale-up from lab to pilot and then semi-industrial scale.

In order to supply evidence-based arguments on the environmental performance of its project, guide innovation and future investments, BON VIVANT wants a life cycle assessment to be performed on the consequences of implementing the BV process (versus not implementing it).

The study is thus carried out to have a first quantitative base of estimates of the environmental implications of introducing the BV process to the market. It can be seen as a first LCA screening to be further refined if the environmental performance of the BV market appears promising from this first analysis.

1.1.2 Objectives

This study aims to assess the environmental consequences of producing 2160 tonnes/year (fresh weight) of animal-free whey protein, obtained through the BV process.

This product has a variety of application potential, but the focus is here on substituting animal dairy protein.

At this stage, the purpose is not to study the consequences of introducing animal-free whey protein in a specific market segment, but to investigate the environmental impacts of its production. Therefore, the present study is cradle-to-gate, i.e. it examines all environmental impacts up to, and including, the production of the product.

As Life Cycle Assessment is a comparative approach, the animal-free whey protein product studied herein is compared against a comparator. The choice of the comparator is not obvious, without targeting a specific application. Hence, a broad comparator is needed.

Here, cow milk was selected as comparator. Cow milk is indeed a key raw feedstock used for several products of the dairy industry. The vision is to here to replace milk for these products.

More specifically, it is considered that if the animal-free whey protein product studied herein is not produced, an equivalent amount of whey protein (in terms of content only, not refined to whey protein concentrate nor whey protein isolate or hydrolysate) would be supplied from milk. This however means that the others products obtained from dairy milk (Figure 1) need to be supplied by other means, as further detailed in later sections.

This LCA study allows:

- Having the quantified, cradle-to-gate environmental impacts of the supply of animal-free whey protein
- Identifying the activities contributing the most to the different environmental impacts, and hence the options and levers for improvement
- Comparing the cradle-to-gate environmental impacts of the supply of animal-free whey protein and dairy milk protein to market

As this LCA is concerned with the consequences of investments to be made in the future, marginal data rather than average data are used. This means that an effort is made to capture the suppliers reacting to a demand change, rather than reflecting those already in place. To illustrate this, a fictive electricity mix consisting, at a given point of time, of 50% coal, 30% wind and 20% solar can be considered. Average data will consider 50% coal, 30% wind, and 20% solar for this mix. Marginal data will examine data series (either based on the past or a projection), and derive the rate of change over the time period considered, and consider the supplier mix based on its ability to react to demand (so only those whose production has/will increase over the period analysed are considered). By doing this, constrained suppliers (e.g. hydropower in many regions of the World where all the exploitable areas have been used) will not be part of the supplier mix. This also often happens with suppliers slow to react to a demand change in many regions of the world (e.g. delay for permit, etc.), e.g. nuclear power in the case of electricity. This is further detailed in [1] and in [2] for the specific case of electricity mixes.

1.1.3 Audience

The study is intended for internal use at BON VIVANT and bilateral use with a number of relevant stakeholders, including, but not limited to, investors, governmental decision makers /environmental agencies, and potential customers for the whey product.

1.1.4 Critical Review

This version of the study does not include a critical review by a panel of experts, in accordance with clause 6 of ISO 14044 (2006). This is intended for a later stage.

1.2 Purpose and general scope

1.2.1 System definition

The analysis considers two systems:

i. Animal-free whey: This system takes its point of departure in considering the implementation of a facility producing 2160 tonnes (ww) of animal-free whey protein per year, with the BV process. This quantity is based on a techno-economic analysis (TEA), as further detailed. This system is considered as the main focus of the present study, and is the one on which a greater effort for building life cycle inventories was made.

ii. Cow milk: This system considers that a demand of 2160 tonnes/year of whey protein is met by cow milk. This system is, in this study, considered as a comparator, that could ideally be, to the extent possible, replaced by the Animal-Free Whey system. Because of its comparator status, and because of the acknowledged quality of the Ecoinvent database where the milk inventory was withdrawn (section 1.2.5), a lower effort was made on gathering new life cycle inventories for this system.

Yet, these systems are not equivalent, as the Cow milk system also supplies lipids, carbohydrates, minerals and vitamins, as illustrated in Figure 1.

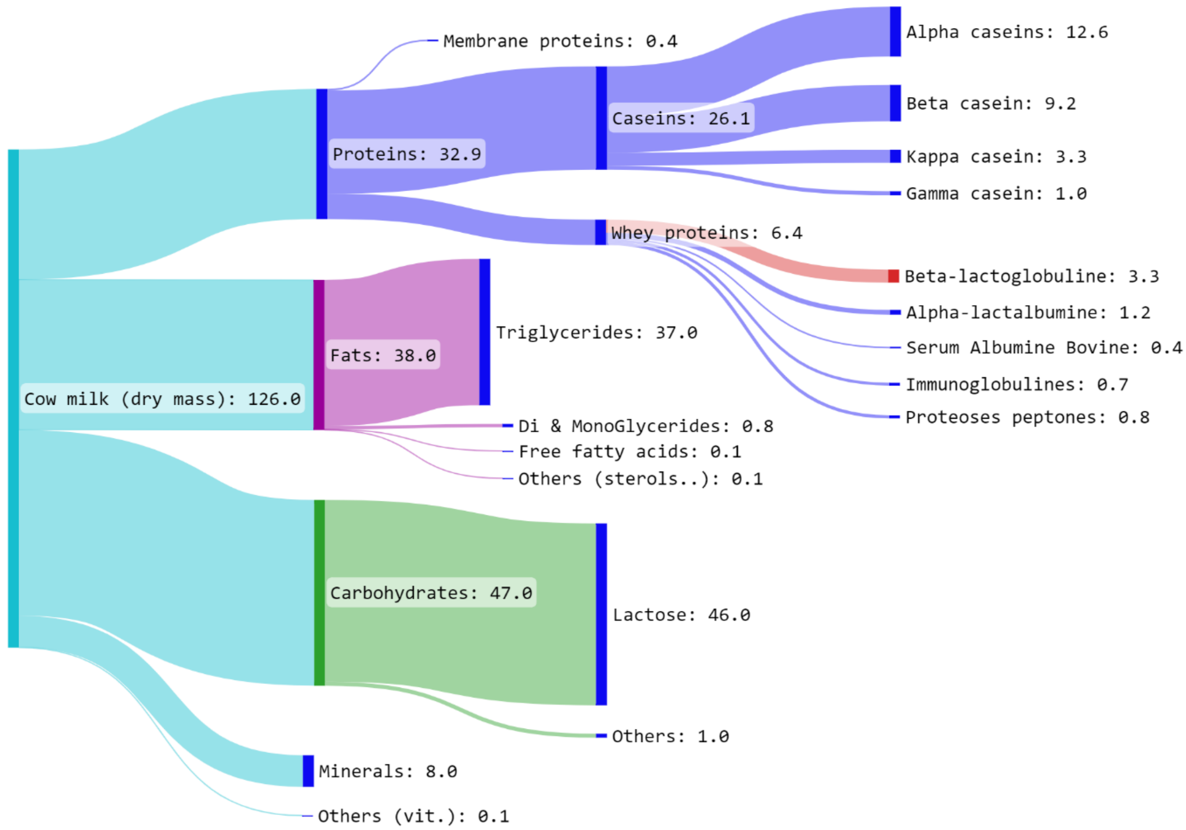

Figure 1. Average cow milk composition from the dairy industry, adapted from [3]. vit.: vitamins. Values in g per kg milk.

To ensure the same functionality, the Animal-free whey system must therefore supply an equivalent of the fats, carbohydrates, minerals and vitamins generated alongside the 2160 tonnes whey (ww) in the Cow milk system. These will here be referred to as “compensatory products”. On the basis of Figure 1, this is estimated to:

- 3086 tonnes carbohydrates/y (ww)
- 2495 tonnes lipids/y (ww)
- 525 tonnes minerals/y (ww)
- 7 tonnes vitamins/y (ww)

The suppliers considered for these compensatory products in the Animal-Free Whey system are as shown in Table 1. Maize and rapeseed oil are respectively considered as marginal suppliers for carbohydrates and lipids, on the basis of [4]. Monocalcium phosphate and vitamins for animal feed are selected as suppliers of minerals and vitamins, respectively, on the basis of the availability of life cycle inventories, as will be later described.

Table 1. Suppliers of compensatory products in the Animal-Free Whey System

Compensatory product	Amount needed in the Animal-free whey protein system (Tonnes ww / y)	Supplier of compensatory product	Details	Amount to supply from supplier (tonnes ww/y)
Carbohydrates	3086	Maize	Considering 0.57 kg carbohydrates per kg maize [4]	5437 t ww maize/y
Lipids (fats)	2495	Rapeseed oil	Considering 0.995 kg lipids per kg rapeseed oil [4]	2508 t ww rapeseed oil / y
Minerals	525	Monocalcium Phosphate	Considering a 1:1 substitution	525 t ww monocalcium phosphate / y
Vitamins	7	Vitamin mix for animal feed	Considering a 1:1 substitution	7 t vitamin mix for animal feed / Y

There is, of course, uncertainty related to the choices of suppliers for the compensatory products. The importance of this uncertainty can among others be seized through sensitivity analyses.

1.2.2 Multi-functionality

Besides the intended (or main) product or service, systems often supply (secondary) co-products as well. This is referred to as multi-functionality in ISO 14044 [5]. According to the standard, the technique consisting of partitioning the environmental impacts of a system between the main and secondary products (on the basis of physical, monetary, or other relationships that can be established between these), which is referred to as "allocation", should be avoided whenever possible.

Here, allocation is avoided through a technique called system expansion, consisting of including the functions and fate of all co-products within the system, that is, the market they are sold to, and the product or service they would replace as a consequence. This is further described in e.g. [1].

The co-products generated in the Animal-Free Whey system and their use is further described.

1.2.3 System boundaries

The study is a so-called "cradle-to-gate" LCA, i.e. it encompasses all activities up to the production of the whey protein (and exclude downstream activities related to the consumption of the whey protein). This allows, in a first stage, to focus on the field of action of Bon Vivant. The activities included in the two studied systems are illustrated in Figure 2.

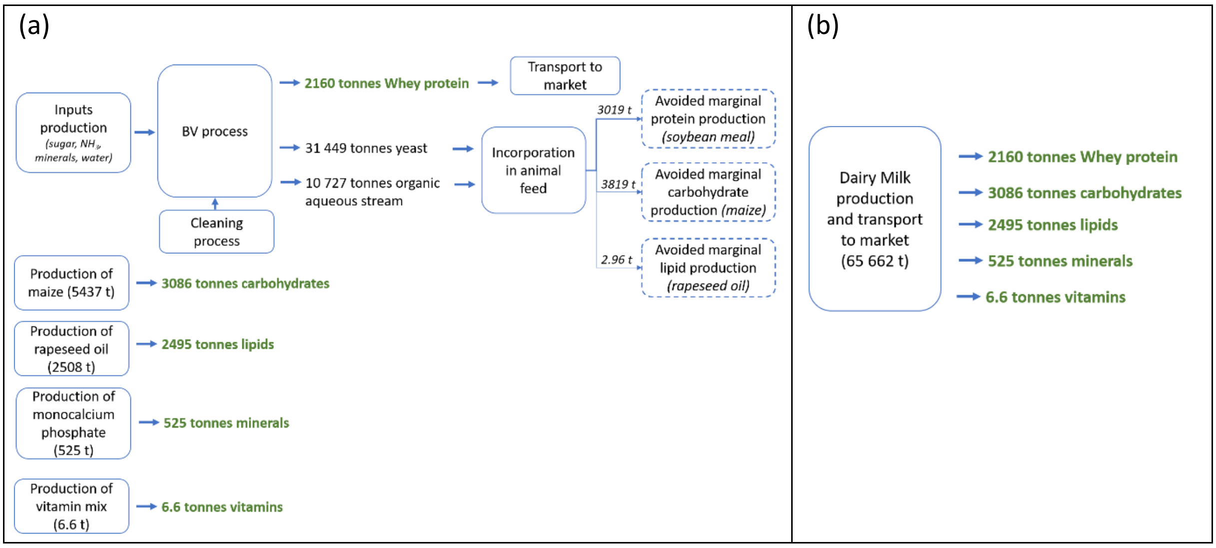

Figure 2. System boundary considered for (a) Animal-free Whey system and (b) the Cow milk system. Dotted lines indicate avoided processes, while full lines are induced process. In green: the services being supplied (these need to be equal in both systems). Flows are expressed on an annual basis, and for a wet mass, unless otherwise specified. This baseline case considers that co-products are used within animal feed. In a nutshell: system (a) supplies 2160 tonnes whey protein/y, so system (b) needs to supplies this from dairy milk. However, in system (b), this comes with a certain amount of carbohydrates, lipids, minerals, vitamins. Therefore, these need to be supplied from 'compensatory products' in (a). It should be noted that being each of the box (process), several sub-activities may be included, not represented herein to ensure tractability.

1.2.4 Functional unit, temporal and geographical scopes

The functional unit, i.e. the reference to which all the input and output data are mathematically related to, is defined as follows:

"Supplying an annual amount of whey protein corresponding to 2000 t (ww) of pure β-lactoglobuline"

All impact results will therefore be expressed upon that basis. Yet, as previously detailed, the comparison performed herein also involve the supply of additional services to ensure system equivalency.

The temporal scope considered herein for the Animal-Free Whey protein production is France, i.e. it is considered that the Animal-Free Whey protein production occurs in France. This defines among others the type of electricity and heat supply to consider, as well as eventual site-specific legislations and environmental conditions (e.g. in the case of local application to soils). Sensitivity analyses considering another temporal scope can be made to assess the importance of the location of the production site.

Yet, all the inputs demanded by the system do not necessarily stem from France; they are purchased on the local, European, or global market according to the product type, as will be detailed in the inventory section.

The temporal scope is medium- to long-term. The study considers a technology for which investments are to be made in the future, and that would be operating at least 30 years. The vision is that the data used herein should, to the extent possible, reflect this temporal scope (2023 – 2050).

1.2.5 Data and tools

The foreground life cycle inventory (LCI) data of this study, i.e. those that are the key object of this study and are made especially for its purpose, relate to the production of Animal-Free Whey protein through the BV process. These data, or more specifically the input and output needed to produce the functional unit, were obtained from a techno-economic study made prior to this LCA study, and are available as a Confidential Appendix.

The background LCI data, i.e. generic activity data such as the electricity mix, specific crop or fertilizer production, tap water production were withdrawn from the Ecoinvent v3.6 database [6,7], selecting the ‘consequential’ database. These dataset include land use changes (for crops; further discussion on this can be found in [4]).

The overall LCA analysis was facilitated with the SimaPro LCA software, v9.1.

1.2.6 Life Cycle Impact Assessment

Life Cycle Impact Assessment is the phase of the LCA where all substances flows from the inventory are translated into specific environmental impacts. The Life Cycle Impact Assessment (LCIA) method selected to do this is the Environmental Footprint (EF) method [8,9]. This method is one of the most updated methodologies available, and is recommended by the European Commission [10].

The EF method allows to calculate the environmental impacts for 16 different impact categories (Table 2). However, the maturity level is not the same for each indicator, as indicated in Table 2. Because of the uncertainties linked to the results obtained from toxicity and ecotoxicity indicators, the authors of these indicators as well as the United Nations Programme of the Environment (UNEP) indicate that result differences are to be considered as significant at logarithmic scale only (below that, differences between to system should not be interpreted as significant) [11,12].

Table 2. The 16 impact categories of the EF method, the metric used to express them, and their recommendation level. I: recommended and satisfactory (green); II. Recommended but in need of some improvement (yellow); III. Recommended, but to be applied with caution (white); III/interim. As III, but differences in results are to be considered as significant at logarithmic scale only (salmon).

Impact Category	Issue reflected	Indicator	Unit	Recom-mendation Level in [8]
Climate Change	Modification of climatic balances, and in particular the natural phenomenon of the greenhouse effect, due to the anthropogenic increase in certain gases in the atmosphere (the main ones are CO_2, CH_4, N_2O and fluorinated gases). Model based on the IPCC 2021 [13] 2021 (Assessment 6 Report) for the 8 key substances reported in IPCC 2021 Table 7.15, else based on IPCC 2013[14] (the LCA software was updated for these 8 substances only). Based on GWP_{100}, with consideration of feedback loops. Updated GWP_{100}, in kg CO_2 eq per kg substance: CO_2, fossil: 1 CH_4, fossil: 29.8 CH_4, non-fossil: 27.0 N_2O: 273 HFC-32, fossil: 771 HFC-134a: 1526 CFC-11: 6226 PFC-14: 7380 It should be noted that the EF method considers a GWP_{100} of zero for biogenic CO_2, as will be later detailed.	Radiative forcing as Global Warming Potential (GWP)	Kg CO_2 eq.	I
Ozone depletion	Phenomenon of destruction of the stratospheric ozone layer due in particular to CFC gases. This layer, by absorbing harmful ultraviolet rays, act as a protection for living organisms.	Ozone Depletion Potential	Kg CFC-11 eq.	I
Respiratory Inorganics (Particulate Matter)	Airborne fine particle pollution, which can have serious health consequences following their infiltration into the respiratory tract. In addition to particles from combustion, nitrogen oxides (NOx),	Human health effects associated with exposure to PM2.5	Disease Incidence	I

	sulphur and ammonia are also gases associated with the production of particles.			
Photochemical Ozone Formation (smog)	Ozone pollution, or “smog” generated by the emission of volatile organic compounds and NOx in the lower layers of the atmosphere. Ozone is a strong oxidant, it causes respiratory problems and limits plant growth.	Tropospheric ozone concentration increase	Kg NMVOC eq.	II
Ionising radiation	Quantification of the impacts of ionizing radiation on the population	Human exposure efficiency relative to U^{235}	kBq U^{235} eq	II
Acidification	Natural phenomenon which is amplified by the increase in atmospheric pollutants, including NH_3, NO_x and SO_2. This effect results in a decrease in absorption of mineral elements by the vegetation.	Accumulated exceedance	mol H+ eq	II
Eutrophication, terrestrial	Excessive nitrogen enrichment of a terrestrial environment following the deposition of nitrogenous compounds after their emission in the atmosphere.	Fraction of nutrients reaching freshwater end compartment	mol N eq	II
Eutrophication, freshwater	Excessive enrichment of an aquatic environment in phosphate nutrients. In the aquatic environment, this enrichment can cause a overabundant development of plant biomass whose subsequent decomposition consumes, in in part or in whole, the oxygen dissolved in the water and reduces the biodiversity of the aquatic environment.		Kg P eq	II
Eutrophication, marine	Excessive enrichment of an aquatic environment in nitrogenous nutrients.	Fraction of nutrients reaching marine end compartment	Kg N eq	II
Land Use	Indicator of soil quality based on the model LANCA V2.2. It aggregates 4 indicators: biotic production, erosion resistance, mechanical filtration, groundwater replenishment	Soil quality index (dimensionless)	Pt [a]	III
Water use	Model Relative Available Water Remaining (AWARE) which reflects the remaining available water after the demand for humans and aquatic ecosystems has been satisfied.	User deprivation potential (deprivation weighted water consumption)	m^3 depriv. water	III
Resource use, minerals and metals	Indicator for measuring the use of metallic and mineral resources, quantified in kg of antimony-equivalent (Sb-eq) per kg extraction.	Abiotic resource depletion (Abiotic depletion potential, ultimate reserves)	Kg Sb eq	III
Resource use, fossil	Indicator for measuring the use of energetic resources (fossil and nuclear)	Abiotic resource depletion, fossil fuels (Abiotic depletion potential, fossil, including uranium)	MJ	III
Human toxicity, cancer	Impact categories representing toxic effects on human beings, in terms of morbidity, of emissions of substances into the environment (USETox model).	Comparative Toxic Unit for humans (CTUh)	CTUh	III / interim
Human toxicity, non-cancer			CTUh	III / interim
Ecotoxicity, freshwater	Impact category representing toxic effects on freshwater ecosystems, in terms of fraction of potentially affected species, of emissions of substances into the environment	Comparative Toxic Unit for ecosystems (CTUe)	CTUe	III / interim

	(USETox model).			

[a] *dimensionless index in [kg biotic production / (m^2 land occupation and transformation*y)] [kg soil / (m^2*y)] [m^3 water/(m^2*y)] [m^3 groundwater/ (m^2*y)]*

1.2.7 Biogenic Carbon accounting

For the foreground inventories (here essentially the Animal-Free Whey protein production), two categories of carbon dioxide emissions to the atmosphere are distinguished: fossil emissions (i.e. those stemming from fossil hydrocarbons) and biogenic emissions (i.e. those stemming from biomass and its transformation). It is often considered that biogenic CO_2 emissions are part of a "short-cycle" where the exchanges between the different C pools from and to the atmosphere, taking place within a few months to a few decades, are seen as stable and to cancel one another (i.e. all the carbon dioxide bio-physically absorbed by plants from the atmosphere through photosynthesis is returned to the atmosphere as CO_2 when the plants are used). This is often referred to as "neutral" C (or the 0 / 0 method; [15]), and is the accounting method used in the EF method (i.e. a GWP_{100} of zero for biogenic CO_2), and thus herein. In contrast, fossil CO_2 emissions are associated to "long cycles", needing millions of years to reach an equilibrium.

There are various approaches to deal with biogenic carbon, the advantages and short-comings of each will not be discussed herein, but have been discussed in several publications (e.g. [15–17]).

1.2.8 Data Quality Requirements

This is not specified in this report, though efforts were made to consider data as reliable, complete, and representative of the time and geographical scopes considered as possible.

Different data quality requirements can be used, for instance those proposed by the pedigree matrix of [18] (widely used in LCA studies), as well as the ILCD (International Life Cycle Data system) entry level quality requirements for data compliance, described in [9].

1.2.9 Uncertainty Analysis

LCAs involve a high volume of data and estimation methods, all based upon a number of parameters that carry uncertainty. This uncertainty propagates through the different calculations that are made, to get a final impact result that is not a single value, but a value within an interval. As a result of this uncertainty, it may not be possible to conclude that e.g. A is better than B simply because it has a lower impact. Uncertainty analysis sheds light on the extent to which two results are really different, and the certainty of the conclusions that are made on the basis of the results.

1.2.10 Sensitivity Analysis

Sensitivity (i.e. how much the result is affected by a change in one or several parameters) can be performed either as perturbation analysis (changing values one-at-the-time by e.g. 10%; [19]), or scenario analysis (e.g. changing the type of electricity used). The latter is performed in this study.

2. Life Cycle Inventory

2.1 Electricity mix

The French Electricity mix of the Ecoinvent 3.6 consequential database was used, process "Electricity, medium voltage {FR}| market for | Conseq, U". The medium voltage electricity is here the high voltage electricity along with the transmission network and sulfur hexafluoride, a key compound in electric utilities. The electricity composition of the French (high voltage) mix is presented in Table 3. It should be noted that, since this is based upon consequential data, it is 1) based upon predictions (here from the European Commission, until 2050 [20]), and upon the rate of change from one year to the other. This is why Table 3 does not include nuclear; it does not mean nuclear is not part of the projection, just that the rate of change is below zero (so there is a decrease rather than an increase in nuclear). Here, this reflects the slow deployment of this technology (permit, etc.), and as a result, that it is not the supplier that will react to a demand change.

Table 3. Composition of the French electricity mix considered by the selected Ecoinvent process (high voltage) from the consequentiual database

Technology	% in the electricity mix
Geothermal	2%
Hydro, run-of-river	1%
Wind, on-shore	62%
Wind, off-shore	22%
Wood	13%

Other electricity mixes are illustrated in Table 4 as comparators. These are relevant to consider in the case another geographical scope (i.e. where the production of the animal-free whey protein occurs) is studied.

Table 4. Comparative electricity mixes from the Ecoinvent v3.6 consequential database, high voltage. Not used herein, shown for illustrative purposes only.

Technology	USA / Southeastern Electric Reliabilty Council (SERC)	Brazil	China / State Grid Corporation of China (SGCC)
Geothermal	4%	0%	0%
Hydro, reservoir	2%	61%	0%
Hydro, run-of-river	9%	0%	8%
Wind, on-shore	22%	28%	13%
Wind, off-shore	0%	0%	0%
Wood	0%	5%	8%
Natural Gas	29%	0%	12%
Nuclear	0%	6%	18%
Hard Coal	0%	0%	45%

2.1.1 Animal-free whey production process

The Life Cycle Inventory of the animal-free whey protein was, as earlier mentioned, based upon a techno-economic study made prior to this LCA study, and is presented in Table 5.

*Table 5. Life Cycle Inventory of the Animal-Free Whey protein production, at production site gate**

	Amount	Unit	***Comment and specification of the Ecoinvent process used***
Main product: animal-free whey protein			
kg product	**1.00**	kg	
Input - Material/fuels			
Sugar, from sugarbeet	6.49	kg	*Ecoinvent process : Sugar, from sugar beet {GLO}| market for | Conseq, U. Note: another sugar source can be subsequently explored.*
NH3 (anhydrous)	0.69	kg	*Ecoinvent process : Ammonia, liquid {RER}| market for | Conseq, U*
Minerals, potassium	0.12	kg	*Ecoinvent process: Potassium sulfate, as K2O {GLO}| market for | Conseq, U*
Minerals, phosphate	0.18	kg	*Ecoinvent process: Phosphate fertiliser, as P2O5 {GLO}| market for | Conseq, U. Sodium phosphate could have been used, but this is intended for the detergent industry*
Water	16	kg	*Ecoinvent process: Water, decarbonised, at user {GLO}| market for | Conseq, U*
Input - Energy			
Steam	0.008	GJ	*Ecoinvent process: Heat, from steam, in chemical industry {RER}| market for heat, from steam, in chemical industry | Conseq, U. Process in GJ. Steam approximated as heat (GJ) considering 3.2 GJ/t steam*
Electricity	0.021	Mwh	*Ecoinvent process: Electricity, medium voltage {FR}| market for | Conseq, U*
Output (other than product of interest)			
Yeast	14.56	kg	*Yeasts or host micro-organisms residues composed of proteins, carbohydrates, fibres and minerals*
Organic aqueous stream	4.97	kg	*Other media residues from the culture media, mostly composed of carbohydrates and other carbon sources*
Discharge to soil and water			
			Considered to be none; the process is considered tight to these losses.
Emissions to air			
Biogenic CO2	12.95	kg	*From the fermentation, estimated based on stoichiometry.*

*Acronyms for countries are provided according to ISO 3166-1 alpha-2 country codes. Group of countries (GLO, RER) are as provided in the table of acronyms

To meet the functional unit, an amount of 2160 tonnes (ww) is needed, and this will generate 31449 tonnes of yeast and 10727 tonnes (ww) of organic aqueous stream.

2.1.2 Compensatory ingredients

The amount of necessary compensatory ingredients is presented in Table 1. The specific Ecoinvent process considered for their life cycle inventory is presented in Table 6.

Table 6. Ecoinvent processes used to model the compensatory ingredients

Compensatory ingredient	Ecoinvent process considered, unless otherwise specified
Maize	Maize grain {GLO}| market for | Conseq, U
Rapeseed oil	Rape oil, crude {CH}| market for | Conseq, U
Monocalcium Phosphate	Monocalcium phosphate, animal feed, at retailer gate/FR U*
Vitamin mix for animal feed	Vitamin, animal feed, at retailer gate/FR U*

*Processes from Agribalyse 3.0.1, for the animal feed market. These were not available in the Ecoinvent database, and were judged, among all processes available, as the best proxies to represent the minerals and vitamins, respectively, to be used as compensatory products for the milk no longer supplied in the animal-free whey protein scenario.

2.1.3 Valorization of co-products from animal-free whey production

There are different valorization options for the generated co-products, for instance as ingredients for inclusion within compound animal feed, as organic fertilizer, or as energy (e.g. via anaerobic digestion). Yet, recently proposed hierarchisation of priorities for organic resources suggest to first use it for human (if possible) or animal alimentation, to the extent possible (Figure 3). For this reason, valorization within animal feed is here considered as the baseline.

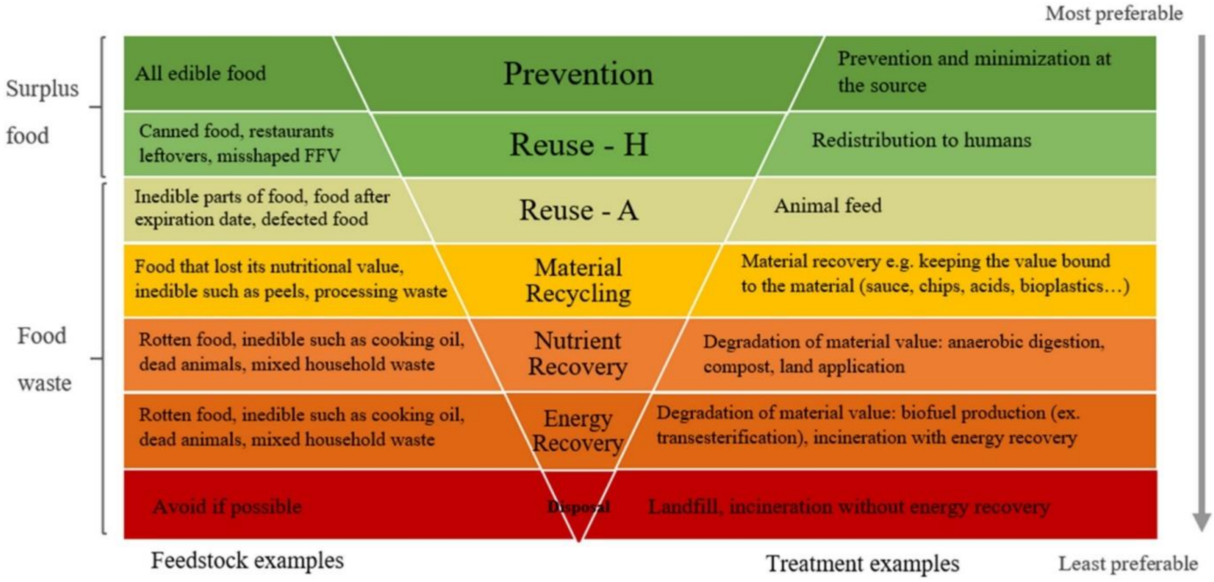

Figure 3. Proposed prioritization hierarchy for organic waste in [21], reproduced with permission of the authors. This hierarchy was also adopted by the European Joint Research Centre of the European Commission [22]

2.1.3.1 Valorisation of yeast and OAS as feed

The use of new ingredients such as the yeast and OAS produced herein within animal feed implies that conventional ingredients will not be produced. Avoided feed crops were estimated following the Scandinavian Feed Unit (SFU) proxy [23], following the methodology described in [4], which is an adaptation of the equations presented in [24]. This methodology considers the content in crude protein, lipid, ash, dry matter, as well as the digestibility of the ingredient's organic matter, and is applied generically independently of the specific animal species it is intended for.

Accordingly, the inclusion of yeast and OAS co-products in animal diets was assumed to displace a mix of three ingredients: (i) soybean meal, (ii) palm oil, (iii) maize. These ingredients are respectively the most competitive (i.e. marginal) source of (i) feed proteins, (ii) feed carbohydrates and (iii) feed lipids, as further detailed in [4].

The composition considered for the yeast and OAS is detailed in Table 7, while the composition of conventional ingredients is as in [4], and presented in Table 8.

Table 7. Biochemical composition of the yeast and OAS considered to calculate the amount of conventional feed ingredient avoided

Biochemical composition	Yeast	OAS	Comment
Water (% WW)	82%	90%	From TEA
Dry matter (% WW)	18%	10%	From TEA
Ashes (% DM)	0.30%	29%	From TEA
Cellulose (% DM)	58.90%*	0%	From TEA
Hemicellulose (% DM)			
Lignin (% DM)			
Protein (% DM)	38.9%	0%	From TEA
Lipids (% DM)	1.1%	0%	From TEA
Starch (% DM)			
Sugars (% DM)	0.3%	26%	From TEA
Other (% DM)	0.5%	45%	From TEA
Crude Fiber (%DM)	5.7%	0.5%	Proxy from data in [4]. For yeast: average of brewer yeast, corn spent grains, wheat spent grain. For OAS: average of wine sediment and apple pomace.
Digestibility	86%	43.3%	For yeast: taken from CVB Feed table (average from: 'wheat yeast concentrate', 'brewer yeast liquid-CP'). For OAS, same as for Crude Fiber, with data in [4]

*Polysaccharides fully attributed to cellulose. This does not affect the calculation of SFU, as neither cellulose, hemicellulose nor lignin intervene in the calculation.

Table 8. Composition of conventional ingredients used, retrieved from [4]

STREAM	Soybean meal	Maize	Palm oil
Water (% WW)	12%	14%	1%
Dry matter (% WW)	88%	86%	99.5%
Ashes (% DM)	7%	1%	0%
Cellulose (% DM)	8%	2%	0%
Hemicellulose (% DM)	5%	7%	0%
Lignin (% DM)	1%	0%	0%
Protein (% DM)	52%	8%	0%
Lipids (% DM)	2%	4%	100%
Starch (% DM)	6%	65%	0%
Sugars (% DM)	9%	1%	0%
Other (% DM)	11%	11%	0%
Crude Fiber (%DM)	6.8%	2.0%	0.0%
Digestibility	88%	92%	89%

On this basis, the displaced ingredients have been calculated, with the results shown in

Table 9.

*Table 9. Avoided conventional ingredients by introducing the yeast and OAS co-products in animal feed. In kg ww ingredient per kg ww co-product. Based on the values from above tables, and following the SFU methodology described in [4]**

	Avoided ingredients (kg ww / kg ww co-products)		
	Soybean meal	Maize	Palm oil
Yeast	0.096	0.117	0.000094
Organic aqueous stream	0	0.013	0

* The number of reported digits is not to be seen as an indication of precision

2.2 Cow milk production system

The life cycle inventory for cow milk was taken from the Ecoinvent 3.6 consequential database, being the most complete, transparent and used database for LCA worldwide [25,26]. The specific process selected, namely "Cow milk {GLO}| market for | Conseq, U", is detailed in Table 10. It includes the production of the milk itself and the transportation to market.

*Table 10. Details of the Ecoinvent process "Cow milk {GLO}| market for | Conseq, U" used as benchmark**

	Amount	Unit	Comment and specification of the Ecoinvent process used
Main product: Global cow milk			
kg product	**1.00**	kg	
Input - Material/fuels			
Cow milk {CA-QC}	0.00472	kg	*Ecoinvent process: Cow milk {CA-QC}| milk production, from cow | Conseq, U*
Cow milk {ROW}	0.99527	kg	*Cow milk {RoW}| milk production, from cow | Conseq, U*
Transport, train	0.1114	tkm	*Ecoinvent process: Transport, freight train {GLO}| market group for | Conseq, U*
Transport, light commercial vehicle	0.0295	tkm	*Ecoinvent process: Transport, freight, light commercial vehicle {GLO}| market group for transport, freight, light commercial vehicle | Conseq, U*
Transport, light commercial vehicle	0.4625	tkm	*Ecoinvent process: Transport, freight, lorry, unspecified {GLO}| market group for transport, freight, lorry, unspecified | Conseq, U*
Transport, sea	0.5644	tkm	*Ecoinvent process: Transport, freight, sea, transoceanic ship {GLO}| market for | Conseq, U*

*Acronyms for countries are provided according to ISO 3166-1 alpha-2 country codes. Group of countries (GLO, RER) are as provided in the table of acronyms. QC: Quebec.

As it can be seen from Table 10, the cow milk production is represented as being from "the rest of the world" from more than 99% (while less than 1% is represented by milk produced in Canada). This process (namely: *Cow milk {RoW}| milk production, from cow | Conseq, U)*, in turns, includes:

- The feed production (silage, grains, protein, energy concentrate and minerals)
- Wood chips used for bedding
- Housing operation (use of energy for lightning, slurry agitators, milking machines, etc., use of water for drinking and cleaning, use of cleaning chemicals, materials for the infrastructure)
- The avoided meat (resulting from calves produced alongside the milk)
- The avoided mineral fertilizers (resulting from the use of manure)
- On-farm transport (car)
- Housing emissions including enteric fermentation.
- Manure management emissions (44% managed as solid, 56% as liquid manure)

This process ends with the milk at farm gate, ready to be delivered.

3. Results and Interpretation

LCA results interpretation is the last of the four phases of an LCA, according to ISO 14044 [5]. It consists of (i) identifying the significant issues (on the basis of LCIA results and a contribution analysis), (ii) an evaluation step, and (iii) drawing conclusions, on the basis of (i) and (ii).

3.1 Life Cycle Impact Assessment Results

The characterized results are presented in

Table 11. Interim impacts (Table 2; human- and ecotoxicity impacts) are not presented, as the differences between the two systems were not in the logarithmic scale. For the animal-free whey protein scenario, the result is given as total = Direct + rest. In other words, the total is the emissions from the protein production alone (as presented in Table 5) (direct) + all other emissions (compensatory products, etc.) (rest).

Table 11. Life Cycle Impact Assessment Results: Cow milk vs animal-free whey protein to supply the functional unit. Results for the animal-free whey protein include a breakdown between direct emissions (i.e. those of producing the protein) and all other emissions (rest). Results for the animal-free whey protein excludes transport and cleaning. Human- and ecotoxicity impacts are not shown as the differences were not logarithmic, hence the differences cannot be considered relevant. All impact results expressed per functional unit.

		Benchmark (cow milk)	**Animal-free Whey Protein Scenario**			**Performance vs benchmark**
Impact category	**Unit**	**EcoInvent 3.6**	**Total**	**Direct***	**Rest****	
Climate change	kg CO2 eq	1.61E+08	**5.97E+06**	1.62E+07	-1.02E+07	-96%
Ozone depletion	kg CFC11 eq	5.16E+00	**3.01E+00**	2.18E+00	8.29E-01	-42%
Respiratory inorganics	disease inc.	8.10E+00	**3.67E+00**	2.29E+00	1.38E+00	-55%
Eutrophication freshwater	kg P eq	3.29E+04	**1.03E+04**	6.09E+03	4.22E+03	-69%
Eutrophication terrestrial	mol N eq	4.84E+06	**1.81E+06**	1.16E+06	6.51E+05	-62%
Eutrophication marine	kg N eq	8.05E+05	**1.43E+05**	3.02E+04	1.12E+05	-82%
Acidification terrestrial and freshwater	mol H+ eq	1.19E+06	**4.46E+05**	2.92E+05	1.53E+05	-63%
Photochemical ozone formation	kg NMVOC eq	2.65E+05	**5.48E+04**	4.96E+04	5.15E+03	-79%
Ionising radiation	kBq U-235 eq	2.02E+06	**2.53E+05**	1.28E+05	1.25E+05	-87%
Water scarcity	m3 depriv.	8.12E+07	**9.13E+05**	-3.07E+06	3.98E+06	-99%
Land use	Pt	1.81E+10	**1.38E+09**	4.46E+08	9.36E+08	-92%
Resource use. mineral and metals	kg Sb eq	1.83E+02	**1.30E+02**	4.61E+01	8.41E+01	-29%
Resource use. energy carriers	MJ	4.90E+08	**2.44E+08**	2.13E+08	3.17E+07	-50%

* Emissions from the animal-free whey protein production alone; ** All other emissions

For both systems (cow milk and animal-free whey protein), the net result is the sum of (positive) impacts and (negative) credits. In Table 12, this is detailed for the animal-free whey protein system, showing both the positive and negative impacts of the net score displayed in

Table 11.

Table 12 also details the breakdown of activities contributing to both the positive share (white) and negative share (salmon) of the analysed impacts.

Most of the time, the negative share is due to the activities avoided in the foreground system, here the soybean, maize, and oil no longer produced as the co-products (yeast and OAS) are incorporated in animal feed. In other words, negative score are explained by the three last columns of Table 12. This leads to think that the selected pathway for co-products management is really important, as it contributes with net negative impacts (benefits). However, only looking at percentages can be misleading. In fact, comparing the positive (not net) score of Table 12 with the net scores for the milk benchmark, it can be seen that the impact results are still lower for the animal-free whey protein (and it should be noted that the positive scores of Table 12 do include the 'positive' co-products processing impact).

However, there are four notable exceptions where negative scores are not associated with the ingredients avoided as co-products are used in animal feed. For instance, in climate change, rapeseed oil is negative. This reflects that the oil is produced alongside rapeseed meal, and this meal avoids an alternative on the market. According to the data used, it avoids both a marginal protein and carbohydrate source for animal feed. Here, the credit is mostly because of the avoided marginal protein. This is the so-called oil paradox and would be observed with any other oil, just with different magnitudes, and is further elaborated in e.g. [27].

For ionizing radiation, maize grain production is net negative. This is because of the heat needed to dry the maize (from the global market). A portion of this heat comes from the co-generation of heat and power. Therefore, an additional demand from such heat involves an additional amount of co-generated power, that does not need to be produced from the marginal power source. As this is the global market, a portion of the marginal power is from countries involving nuclear. With this avoided nuclear comes the avoided treatment of tailing from uranium mining (key substance Radon-222), hence the negative score on ionising radiation for maize. The last two exceptions appear for the impact water scarcity, where both the production of the animal-free whey protein and rapeseed oil are net negative. For the latter, it is as for climate change; producing rapeseed oil comes along with rapeseed meal, and this meal avoids an alternative on the market (marginal protein and carbohydrate). The avoided carbohydrate source for animal feed, in particular, involves crops that require irrigation. Avoiding the production of these carbohydrate crops involves avoiding this irrigation, hence the explanation on the negative score for water scarcity. For the animal-free whey protein production process, the reason is similar. Here, it is because of the sugar production, as, along with it, comes the production of sugar beet pulp, used in animal feed where it replaces marginal carbohydrates, and again the irrigation required by these crops.

Table 12. Breakdown for the animal-free protein production system (not process) alone. Detail, for each impact, of the positive and negative contributions to the net impact result, as well as a breakdown of the activities contributing to ***the positive impact portion (in white)*** *and those contributing to* ***the negative impact portion (in salmon).*** *The sum of both the white and salmon activities is 100%. Impact results are expressed per functional unit.*

		Impact result			Activities contribution to positive (white) and negative (salmon) portion of the net impact								
		Sum+	Sum-	**Total (net)**	Animal-free whey protein production	Maize grain production. global	Rape oil production. crude. {CH}	Monocalcium phosphate	Vitamin	Processing co-products in compound feed	Avoided soybean meal {GLO}	Avoided Maize grain {GLO}	Avoided Palm oil. refined {GLO}
Climate change	kg CO2 eq	2.18E+07	-1.58E+07	**5.97E+06**	74%	14%	24%	3%	0%	8%	62%	14%	0.1%
Ozone depletion	kg CFC11 eq	3.21E+00	-2.06E-01	**3.01E+00**	68%	5%	20%	3%	0%	4%	42%	57%	0.2%
Ionising radiation. HH	kBq U-235 eq	3.26E+05	-7.21E+04	**2.53E+05**	39%	48%	17%	23%	1%	13%	52%	7%	0.1%
Photochemical ozone formation. HH	kg NMVOC eq	7.94E+04	-2.46E+04	**5.48E+04**	63%	14%	4%	4%	0%	15%	69%	31%	0.1%
Respiratory inorganics	disease inc.	3.85E+00	-1.84E-01	**3.67E+00**	60%	7%	26%	2%	0%	5%	1%	99.6%	0.4%
Acidification terrestrial and freshwater	mol H+ eq	4.85E+05	-3.94E+04	**4.46E+05**	60%	8%	26%	2%	0%	3%	28%	72%	0.1%
Eutrophication freshwater	kg P eq	1.32E+04	-2.92E+03	**1.03E+04**	46%	21%	8%	6%	0%	19%	34%	66%	0.0%
Eutrophication marine	kg N eq	1.76E+05	-3.29E+04	**1.43E+05**	17%	11%	69%	1%	0%	2%	59%	41%	0.1%
Eutrophication terrestrial	mol N eq	1.95E+06	-1.37E+05	**1.81E+06**	60%	7%	30%	1%	0%	3%	27%	73%	0.1%
Land use	Pt	3.19E+09	-1.81E+09	**1.38E+09**	14%	19%	40%	1%	0%	26%	77%	23%	0.0%
Water scarcity	m3 depriv.	3.22E+07	-3.13E+07	**9.13E+05**	10%	89%	23%	9%	0%	2%	2%	65%	0.0%
Resource use. energy carriers	MJ	2.81E+08	-3.69E+07	**2.44E+08**	76%	9%	6%	4%	0%	6%	54%	46%	0.0%
Resource use. mineral and metals	kg Sb eq	1.42E+02	-1.22E+01	**1.30E+02**	32%	8%	24%	12%	0%	24%	37%	63%	0.0%

The results of Table 12 are further analysed in Table 13, Table 14, and Table 15, where a detailed contribution analysis is performed on the positive impacts. In accordance with [9], all activities contributing to at least 80% of the (positive) impact are further analysed, by uncovering which sub-activities contribute to the impact, and which substances.

For instance, for climate change, it can be seen in column B that 74% of the impact is due to the animal-free whey protein production, and 14% to maize grain (compensatory product). Then, column C shows what are the activities responsible for the impact of these two processes. For instance, the climate change impact of animal-free whey protein production is due to sugar production (54%), steam production (19%) and ammonia production (13%). The last column shows which substance is the key contributor of the impact of each activity identified in column C. For example, for sugar beet production, 75% of the climate impact is due to fossil CO_2 (itself mostly emitted during the production of the nitric acid used for producing the needed fertilizers).

It can be seen from Table 13, Table 14, and Table 15 that for each impact, the most important contributing processes to the impact is the production of animal-free whey protein. The only exception is for marine eutrophication, where rapeseed oil (compensating product) is the most important contributor.

3.1.1 Key levers of improvements

The reason why the production of animal-free whey protein is impactful is often due to the production of sugar (from sugar beet), except for freshwater eutrophication (there it is mostly due to electricity), ionising radiation (there it is mostly due to anhydrous ammonia production) and resource use, mineral and metals (where it is also mostly due to electricity). This involves that there might be a level of environmental improvement in exploring <u>different types of sugar sourcing</u>, and/or in <u>ensuring the implementation of specific agro-ecological practices</u> in the production of the sugar source, especially with regards to fertilization. Such practices could include the integration of the sugar production with the animal-whey protein production, where the co-products could instead be co-digested with e.g. animal manure and the nutrients recycled back to the sugar production, thereby reducing the amount of mineral fertilizers used. Another way to reduce the amount of fertilizers being used could be an association of the sugar beet with legumes in the cultivation system. Reducing the amount of mineral fertilizers being used would mitigate most impacts (climate change and all those linked to reducing N losses listed below; freshwater eutrophication could also be mitigated if the use of mineral fertilizers is also reduced). Emerging technologies such as electric tractors could also be an opportunity [28,29] to mitigate the ozone depletion impact, photochemical ozone formation (smog), the use of fossil resources and to a less important extent climate change. More importantly, applying mitigation measures to control the losses of nitrogen (in particular NH_3 and NOx) to air during fertilization could improve several impacts: respiratory inorganics, terrestrial- and marine eutrophication, acidification, and photochemical ozone formation. This topic has been widely studied for several decades, and a variety of solutions exists, some being summarized in e.g. [30,31] (association with biochar, acidification of the fertilizers, deep injection, type of fertilizer, nitrogen inhibitors, time of application, etc.).

Other impacts, such as those related to compensatory products (rapeseed, maize), or those related to electricity, heat and ammonia production, are more difficult to act on. It should be highlighted that even if the foreground system (as well as some background processes) involve renewable electricity, this is also not without impact (e.g. on freshwater eutrophication and mineral resource use for wind mills, marine eutrophication, photochemical ozone formation and land use for wood-based electricity).

Table 13. Contribution analysis for level I (recommended and satisfactory) and level II (recommended but in need of some improvements) impacts. Results in column A are expressed per functional unit. Column B presents the process explaining at least 80% of the impact in column A, and their contribution. The activities explaining at least 80% of the impact of processes in B are reported in column C, with their contribution. The last column presents the substance contributing the most to each of the activities identified in column C.

Impact	Total midpoint impact, per Functional Unit A		Contributing process (positive impact) (to explain 80% of the total midpoint impact in A) B		Activity-level contribution (to explain 80% of the contributing process in B; first level only) C		Major elementary flow explaining impact of activity in C	
	Value	Unit	%	process	%	activity	%	flow
Climate change	5,97E+06	kg CO2 eq	74%	Animal-free whey protein production	54%	Sugar, from sugar beet {GLO}	75%	CO2, fossil (due to **nitric acid production** behind potassium nitrate and urea ammonium nitrate fertilizers, and second to **diesel for harvesting**)
					19%	Heat, from steam, in chemical industry {RER}	94%	CO2, fossil (due to natural gas production, to get **high pressure gas**)
					13%	Ammonia, anhydrous	94%	CO2, fossil (losses during the **ammonia synthesis** process)
			14%	Maize grain	53%	Maize grain RoW	70%	CO2, fossil (major process: heat for **drying maize**)
					33%	Maize grain US	68%	CO2, fossil (major process: heat for **drying maize**)
Ozone depletion	3,01E+00	kg CFC-11 eq	68%	Animal-free whey protein production	51%	Sugar, from sugar beet {GLO}	54%	Methane, bromotrifluoro-, Halon 1301 (from **petroleum production** needed for diesel in tractors)
					31%	Ammonia, anhydrous	59%	Methane, bromotrifluoro-, Halon 1301 (from **petroleum production** needed for the heavy fuel oil used in the synthesis process)
			20%	Rapeseed oil	100%	Rape oil, crude {CH}| rape oil mill operation	43%	Methane, tetrachloro-, CFC-10 (for **trichloromethane production** needed for the acetamide-anillide-compound used as agrochemical input for rapeseed cultivation)
Respiratory inorganics (Particulate matter)	3,67E+00	disease incidence	63%	Animal-free whey protein production	76%	Sugar, from sugar beet {GLO}	89%	NH3 (from cultivation, mostly due to **fertilizers application**)
					9%	Ammonia, anhydrous	83%	Particulates, < 2.5 um (from **heavy fuel oil production**, which is used in the synthesis process)
			14%	Maize grain	46%	Maize grain RoW	58%	NH3 (from cultivation, due to **fertilizers application**)
					31%	Maize grain US	59%	NH3 (from cultivation, due to **fertilizers application**)
					18%	Maize grain AR	94%	NH3 (from cultivation, due to **fertilizers application**)
Eutrophication, freshwater	1,03E+04	kg P eq	46%	Animal-free whey protein production	36%	Electricity, medium voltage {FR}| market for	100%	Phosphate (**treatment of sulfidic tailing** from cooper used for wind mills)
					16%	Sugar, from sugar beet {GLO}	98%	Phosphate (**losses during cultivation**, associated to fertilizers application)
					15%	Heat, from steam, in chemical industry {RER}	100%	Phosphate (**treatment of spoil from lignite mining**, as lignite is used in the heat production mix)
					15%	Phosphate fertilizer, as P2O5	98%	Phosphate (losses during production of **phosphate fertilizer**)
			21%	Maize grain	59%	Maize grain RoW	81%	Phosphate (spoil from **lignite mining**, used to produced heat needed for maize drying)
					38%	Maize grain US	79%	Phosphate (spoil from **lignite mining**, used to produced heat needed for maize drying)
			19%	Processing co-products	95%	Electricity, medium voltage {FR}| market for	100%	Phosphate (**treatment of sulfidic tailing** from cooper used for wind mills)
Eutrophication, terrestrial	1,81E+06	mol N eq	60%	Animal-free whey protein production	92%	Sugar, from sugar beet {GLO}	92%	NH3 (from cultivation, mostly due to **fertilizers application**)
			30%	Rapeseed oil	100%	Rape oil, crude {CH}| rape oil mill operation	93%	NH3 (from cultivation, mostly due to **fertilizers application**)
Eutrophication, marine	1,43E+05	kg N eq	69%	Rapeseed oil	100%	Rape oil, crude {CH}| rape oil mill operation	94%	Nitrate (from cultivation, mostly due to **fertilizers application**)
			17%	Animal-free whey protein production	74%	Sugar, from sugar beet {GLO}	34%	Nox (from cultivation, mostly due to **fertilizers application**)
					12%	Electricity, medium voltage {FR}| market for	84%	Nox (from **wood-based electricity** portion)

Table 14. Contribution analysis for remaining level II (recommended but in need of some improvements) impacts. Results in column A are expressed per functional unit. Column B presents the process explaining at least 80% of the impact in column A, and their contribution. The activities explaining at least 80% of the impact of processes in B are reported in column C, with their contribution. The last column presents the substance contributing the most to each of the activities identified in column C.

Impact	Total midpoint impact, per Functional Unit A		Contributing process (positive impact) (to explain 80% of the total midpoint impact in A) B		Activity-level contribution (to explain 80% of the contributing process in B; first level only) C		Major elementary flow explaining impact of activity in C	
	Value	Unit	%	process	%	activity	%	flow
Acidification	4,46E+05	mol H+ eq	60%	Animal-free whey protein	85%	Sugar, from sugar beet {GLO}	90%	NH3 (from cultivation, mostly due to **fertilizers application**)
			26%	Rapeseed oil	100%	Rape oil, crude {CH}| rape oil mill operation	94%	NH3 (from cultivation, mostly due to **fertilizers application**)
Photochemical ozone formation, human health	5,48E+04	kg NMVOC eq	63%	Animal-free whey protein production	52%	Sugar, from sugar beet {GLO}	76%	NOx (mostly **from harvesting**, but also from fertilizers application)
					21%	Electricity, medium voltage {FR}| market for	75%	NOx (due to **wood-based electricity** portion)
					10%	Ammonia, anhydrous	67%	NOx (from **liquid ammonia synthesis** process)
			14%	Maize grain	47%	Maize grain RoW	78%	NOx (major process: heat needed for **drying of maize**)
					30%	Maize grain US	77%	NOx (major process: heat needed for **drying of maize**)
					8%	Transport, freight, sea, transoceanic ship {GLO}	86%	NOx (emitted as a result of **maritime fuel burning**)
			15%	Processing co-products	97%	Electricity, medium voltage {FR}| market for	75%	Nox (due to **wood-based electricity** portion)
Ionising radiation	2,53E+05	kBq U-235 eq	39%	Animal-free whey protein production	50%	Ammonia, anhydrous	92%	Carbon-14 (plasma torch **incineration of low-level radioactive waste** from petroleum production, for heavy fuel oil to produce ammonia)
					25%	Phosphate fertilizer, as P2O5	27%	Carbon-14 (**treatment of tailing from uranium mining**, due to nuclear electricity, for the portion of the phosphate fertilizer produced in 'the rest of the world')
					14%	Electricity, medium voltage {FR}| market for	73%	Carbon-14 (plasma torch **incineration of low-level radioactive waste** from light fuel oil production used in the construction of the transmission network)
			23%	Monocalcium phosphate	94%	Phosphoric acid, industrial grade, without water, in 85% solution state {GLO}	44%	Carbon-14 (plasma torch **incineration of low-level radioactive waste** from the nuclear electricity portion)
			17%	Rapeseed oil	100%	Rape oil, crude {CH}| rape oil mill operation	94%	Carbon-14 (plasma torch **incineration of low-level radioactive waste** from petroleum production, for diesel in tractors)
			13%	Processing co-products	92%	Electricity, medium voltage {FR}| market for	73%	Carbon-14 (plasma torch **incineration of low-level radioactive waste** from light fuel oil production used in the construction of the transmission network)

Table 15. Contribution analysis for level III (Recommended, but to be applied with caution) impacts. Results in column A are expressed per functional unit. Column B presents the process explaining at least 80% of the impact in column A, and their contribution. The activities explaining at least 80% of the impact of processes in B are reported in column C, with their contribution. The last column presents the substance contributing the most to each of the activities identified in column C.

Impact	Total midpoint impact, per Functional Unit A		Contributing process (positive impact) (to explain 80% of the total midpoint impact in A) B		Activity-level contribution (to explain 80% of the contributing process in B; first level only) C		Major elementary flow explaining impact of activity in C	
	Value	Unit	%	process	%	activity	%	flow
Water scarcity	9,13E+05	m3 world eq	89%	Maize grain	54%	Maize grain {RoW}| production	15%	Water, **turbine use**, unspecified natural origin, **CN**
					45%	Maize grain {US}| production	21%	Water, **well, US**
Land use	1,38E+09	Pt	40%	Rapeseed oil	100%	Rape oil, crude {CH}| rape oil mill operation	21%	Transformation, from annual crop to **rapeseed production in Europe** (/Switzerland)
			26%	Processing co-products	99%	Electricity, medium voltage {FR}| market for	95%	Occupation, forest, intensive (from wood chips production of **wood-based electricity**)
			19%	Maize grain	56%	Maize grain {RoW}| production	56%	Transformation, to annual crop (here **maize production**)
					38%	Maize grain {US}| production	56%	Transformation, to annual crop (here **maize production**)
Resource use, minerals and metals	1,30E+02	kg Sb eq	32%	Animal-free whey protein	65%	Electricity, medium voltage {FR}| market for	10%	From 'Copper, 0.99% in sulfide, Cu 0.36% and Mo 8.2E-3% in crude ore' used for **wind turbine production**
			24%	Rapeseed oil	99%	Rape oil, crude {CH}| rape oil mill operation	15%	Gold, Au 6.7E-4%, in ore (used to produced the **platinum needed as catalyst for nitric acid**)
			24%	Processing co-products	94%	Electricity, medium voltage {FR}| market for	10%	From 'Copper, 0.99% in sulfide, Cu 0.36% and Mo 8.2E-3% in crude ore' used for **wind turbine production**
Resource use, fossils	2,44E+08	MJ	76%	Animal-free whey protein production	47%	Sugar, from sugar beet {GLO}	67%	Gas, natural
					27%	Ammonia, anhydrous	56%	Gas, natural
					13%	Heat, from steam, in chemical industry {RER}	52%	Gas, natural
			9%	Maize grain	54%	Maize grain {RoW}| production	29%	Coal, hard
					32%	Maize grain {US}| production	33%	Oil, crude

3.1.2 Separate analysis of the animal-free whey protein production process (not system)

In Table 12, it can be observed that the animal-free whey protein production process represents more than 50% of the positive impacts for 7 (out of 13) of the impacts. In Table 16, the animal-free whey protein production process (not system) is thus analysed separately, in order to identify eventual levers to improve the environmental performance of this process.

As it can be seen, sugar production (here from the global sugar beet market) is dominant for 7 (out of 13) impacts, i.e. it contributes to ≥50% of the impact. This applies for climate change, ozone depletion, respiratory inorganics, eutrophication (terrestrial & marine), acidification and photochemical ozone depletion. Sugar production is also an important contributor (47%) for the impact Resource use, energy carriers.

This confirms the previous analysis, namely that the sugar sourcing represents an important lever for improving the environmental performance. However, it should be highlighted that even residual sources (e.g. sugar beet molasses) are not impact free, as they would have otherwise been used for something else (e.g. animal feed).

Anhydrous ammonia is important for ionizing radiation (50% contribution) and water scarcity (47% contribution). For the former, this is, as shown in Table 14, due to the portion of nuclear energy used to produce it (as it is sourced from the European market); the range of action for mitigation is here more limited. For the latter, it is explained by the use of water for the stream reforming process needed to produce liquid ammonia and water cooling for partial oxidation[1].

The use of heat (steam) does contribute to all impacts, but never represent more than 15%. This may therefore not be the first priority in terms of action to implement in order to reduce the environmental impacts of the animal-free whey protein production process.

The use of electricity is important for freshwater eutrophication (36%), land use (86%) and resource use (mineral & metals). For the former, this is due to the treatment of sulfidic tailing from copper used in wind mills; for the latter, it is explained by the wood-based electricity portion of the mix.

[1] The process considers that for 1 kg liquid ammonia stemming from the European market, 0.85kg stems from the steam reforming process and 0.15kg stems from partial oxidation

*Table 16. Results breakdown for animal-free whey protein production detailing the specific contribution of sugar production, ammonia and needed heat to the total impact *. Contributions greater than 50% in bold.*

Impact category	Unit	Sugar	Ammonia	Heat (steam)	Electricity	Rest
Climate change	kg CO2 eq	**54%**	19%	13%	10%	5%
Ozone depletion	kg CFC11 eq	**51%**	31%	9%	5%	3%
Ionising radiation, HH	kBq U-235 eq		**50%**	11%	14%	25%
Photochemical ozone formation, HH	kg NMVOC eq	**52%**	10%	8%	21%	8%
Respiratory inorganics	disease inc.	**76%**	9%	2%	8%	6%
Acidification terrestrial and freshwater	mol H+ eq	**85%**	4%	3%	4%	5%
Eutrophication freshwater	kg P eq	16%	8%	15%	36%	25%
Eutrophication marine	kg N eq	**74%**	5%	5%	12%	4%
Eutrophication terrestrial	mol N eq	**92%**	1%	1%	4%	1%
Land use	Pt		2%	5%	**86%**	6%
Water scarcity	m3 depriv.		47%	2%	7%	44%
Resource use, energy carriers	MJ	47%	27%	13%	6%	6%
Resource use, mineral and metals	kg Sb eq	4%	15%	0%	**65%**	16%

*Empty fields mean that the activity had a contribution with a different sign (e.g. negative) than the total net (e.g. positive) given in Table 11. Total may be different than 100% due to rounding.

3.2 Sensitivity analysis

3.2.1 Inclusion of transport, equipment and cleaning

Results in

Table 11 do not include transport to market, infrastructure, and cleaning for the animal-free whey protein, while it does for cow milk (section 2.2). An attempt was thus made to include these through a very rough conservative proxy. This proxy relies on the work of [32]. According to the values found in this study, ratios between ‘equipment’, ‘cleaning’, and ‘transport’ versus production of the milk were derived. These are shown in Table 17.

Table 17. Ratio production :cleaning/equipment/transport considered on the basis of [32], and share of the impact upon which these ratios should be applied for the animal-free protein production process.

Impact	**Unit**	**Ratio to production activity**			**Share related to site**
		Cleaning	**Equipment**	**Transport**	
Climate change	kg CO2 eq	0.51	0.01	0.05	22%
Ozone depletion	kg CFC11 eq	0.35	0.00	0.06	15%
Respiratory inorganics	disease inc.	0.86	0.08	0.22	48%
Eutrophication freshwater	kg P eq	26.64	0.10	0.22	29%
Eutrophication terrestrial	mol N eq	5.73	0.02	0.29	10%
Eutrophication marine	kg N eq	5.73	0.02	0.29	-126%
Acidification terrestrial and freshwater	mol H+ eq	0.89	0.03	0.20	84%
Photochemical ozone formation, HH	kg NMVOC eq	0.69	0.02	0.31	7%
Ionising radiation, HH	kBq U-235 eq	0.44	0.00	0.00	51%
Water scarcity	m3 depriv.	1.31	0.00	0.01	17%
Land use	Pt	0.84	0.03	0.09	6%
Resource use, mineral and metals	kg Sb eq	0.73	0.66	0.02	37%
Resource use, energy carriers	MJ	0.41	0.01	0.03	174%

The values of Table 17 were added to the direct animal-free whey protein results of Table 11, but only to the share related to the production site (i.e. excluding inputs as minerals, sugar, or ammonia imported from elsewhere[2]). This share is shown in the last column of Table 17. Finally, a conservative uncertainty margin of 25% was applied for all impacts[3]. Results are shown in Table 18.

[2] More precisely, the share is calculated as follows: impacts of water, heat and electricity out of the total net impact (for the animal-free whey protein production process).

[3] Example for Climate Change. The estimation of impacts when transport, equipment and cleaning are used is calculated as follows: (1.62E+07) x (1+(0.51+0.01+0.05) x22%) x (1+25%). Values stem from Table 11 and Table 17, on which the 25% uncertainty margin is applied.

Table 18. Life Cycle Impact Assessment results including a transport and cleaning proxy for animal-free whey protein

		Benchmark (cow milk)	Animal-free Whey Protein Scenario			Performance vs benchmark
Impact category	**Unit**	**EcoInvent 3.6**	**Total**	**Direct***	**Rest****	
Climate change	kg CO2 eq	1.61E+08	**1.26E+07**	2.28E+07	-1.02E+07	-92%
Ozone depletion	kg CFC11 eq	5.16E+00	**3.72E+00**	2.89E+00	8.29E-01	-28%
Respiratory inorganics	disease inc.	8.10E+00	**4.57E+00**	3.19E+00	1.38E+00	-44%
Eutrophication freshwater	kg P eq	3.29E+04	**1.16E+05**	1.12E+05	4.22E+03	252%
Eutrophication terrestrial	mol N eq	4.84E+06	**2.59E+06**	1.94E+06	6.51E+05	-46%
Eutrophication marine	kg N eq	8.05E+05	**1.88E+05**	7.57E+04	1.12E+05	-77%
Acidification terrestrial and freshwater	mol H+ eq	1.19E+06	**5.49E+05**	3.95E+05	1.53E+05	-54%
Photochemical ozone formation, HH	kg NMVOC eq	2.65E+05	**8.56E+04**	8.05E+04	5.15E+03	-68%
Ionising radiation, HH	kBq U-235 eq	2.02E+06	**3.20E+05**	1.95E+05	1.25E+05	-84%
Water scarcity	m3 depriv.	8.12E+07	**3.81E+06**	-1.72E+05	3.98E+06	-95%
Land use	Pt	1.81E+10	**2.43E+09**	1.49E+09	9.36E+08	-87%
Resource use, mineral and metals	kg Sb eq	1.83E+02	**1.94E+02**	1.10E+02	8.41E+01	6%
Resource use, energy carriers	MJ	4.90E+08	**3.21E+08**	2.89E+08	3.17E+07	-35%

* Emissions from the animal-free whey protein production alone; ** All other emissions

According to this conservative estimation, the inclusion of cleaning, equipment, transport and uncertainty still indicate overall benefits in producing animal-free whey protein in comparison to milk protein. This, however, is no longer true for eutrophication freshwater, and to some extent for resource use (mineral and metals). For the former, it reflects the important cleaning:production ratio used herein, on the basis of [32]. It is not clear, in [32], what this high terrestrial eutrophication is due to. One hypothesis is the protein residues flushed out in the rinsing phase, as well as the phosphorus in the detergents used for the cleaning. Here, it is important to highlight that the values from [32] were just used as a conservative proxy to judge whether the rigorous inclusion of transport, cleaning and equipment would change the conclusions. On the basis of this conservative sensitivity analysis, it appears it would not change the conclusions, but that it is worth investigating into more details.

3.2.2 Sucarcane instead of sugar beet

A second sensitivity analysis was performed considering the use of sugarcane (global market) instead of sugar beet (also global market) as used herein (Table 19). It shows little difference, indicating the need for a specific local agroecological solution, in order to improve the impact of the sugar sourcing. Sligthly less improvements are observed for 8 of the 13 impacts.

Table 19. Sensitivity analysis with sugarcane instead of sugar beet as sugar source

		Benchmark (cow milk)	Animal-free Whey Protein Scenario, baseline with sugar beet as sugar source		Animal-free Whey Protein Scenario, sugarcane as sugar source	
Impact category	**Unit**	**EcoInvent 3.6**	**Total**	**Performance vs benchmark**	**Total**	**Performance vs benchmark**
Climate change	kg CO2 eq	1.61E+08	**5.97E+06**	-96%	**1.08E+07**	-93%
Ozone depletion	kg CFC11 eq	5.16E+00	**3.01E+00**	-42%	**2.49E+00**	-52%
Respiratory inorganics	disease inc.	8.10E+00	**3.67E+00**	-55%	**5.21E+00**	-36%
Eutrophication freshwater	kg P eq	3.29E+04	**1.03E+04**	-69%	**5.88E+03**	-82%
Eutrophication terrestrial	mol N eq	4.84E+06	**1.81E+06**	-62%	**1.27E+06**	-74%
Eutrophication marine	kg N eq	8.05E+05	**1.43E+05**	-82%	**1.76E+05**	-78%
Acidification terrestrial and freshwater	mol H+ eq	1.19E+06	**4.46E+05**	-63%	**3.02E+05**	-75%
Photochemical ozone formation	kg NMVOC eq	2.65E+05	**5.48E+04**	-79%	**7.19E+04**	-73%
Ionising radiation	kBq U-235 eq	2.02E+06	**2.53E+05**	-87%	**7.41E+05**	-63%
Water scarcity	m3 depriv.	8.12E+07	**9.13E+05**	-99%	**5.72E+07**	-30%
Land use	Pt	1.81E+10	**1.38E+09**	-92%	**3.55E+09**	-80%
Resource use. mineral and metals	kg Sb eq	1.83E+02	**1.30E+02**	-29%	**1.70E+02**	-7%
Resource use. energy carriers	MJ	4.90E+08	**2.44E+08**	-50%	**1.60E+08**	-67%

3.3 Evaluation : Sensitivity, completeness, and consistency checks

According to ISO 14044 (2006), the evaluation step consists of three activities, namely sensitivity, completeness and consistency checks, and these need to be supplemented by uncertainty analysis, sensitivity analysis and data quality analysis for studies “intended to be used for a comparative assertion intended to be disclosed to the public”.

3.3.1 Sensitivity Check

The objective of the sensitivity check is to assess the reliability of the final results and conclusions by determining how they are affected by uncertainties in data, allocation methods, and LCIA-methods, among others (ISO 14044, 2006). Sensitivity is assessed on three levels: modelling approach, data quality, uncertainty in data and LCIA-methods.

This study handled multi-functionality by system expansion (section 1.2.2), therefore no allocation methods were used. Nevertheless, the consequential approach to modelling the life cycle inventory in this LCA is fundamental to the results obtained where animal-free whey protein to supply the services described in section 1.2.4 leads to an improvement for all impact categories, in comparison to the use of cow milk. Large part of the impacts’ reduction (negative scores) come from the management of co-products, here translating in the avoidance of ingredients for animal feed. This was implemented by means of the system expansion which in itself follows the consequential rationale, and the assumption of full elasticity of supply. This means that if the demand increases with one unit, the suppliers will react by increasing supply with one unit, and conversely when demand decreases. This is here seen as a reasonable assumption, in the perspective of attempting to anticipate the long-term consequences of changes (here in implementing animal-free whey protein versus not implementing it). To anticipate short-term consequences, price effects and rebound effect should be included, and that would require a more sophisticated modelling approach.

However, as earlier emphasized, these negative scores where not determining for animal-free whey protein to present lower impact than the cow milk counterpart.

The LCIA method used is Environmental Footprint (EF), which is an update of the ILCD method (itself a consensus of several LCA experts across Europe). The characterisation models used for several impacts builds upon similar theoretical fundaments as those used in other popular LCIA methods such as

ReCiPe, Impact2002+, CML. For climate change, it is important to highlight that all methods reply upon the IPCC characterization factors for GWP_{100} (here updated, to the extent possible, to reflect the latest characterization factors of the latest IPCC update, namely the one of Assessment Report 6). It is also important to highlight that the EF method is an update of the ILCD, itself elaborated on a consensus building on all LCIA methods existing at the time the exercise was made. Therefore, the choice of impact assessment method is not likely to affect the conclusions.

Results from the contribution analysis (Table 13, Table 14, Table 15) show that the results are more affected by some specific data. These are the data for which a small variation (+/- 10%) creates a noticeable change in the final impact results. This implies that particular efforts to gather robust values for these data are needed. A perturbation analysis to test which data are sensitive was not performed herein, but at the light of (Table 13, Table 14, Table 15), the potentially sensitive data could include the amount of heat for drying maize, the amount of phosphate emissions related to spoil from lignite mining (used for the heat to dry maize), the nitrogen emissions related to fertilizer application, the emission of Halon 1301 linked to petroleum production, the carbon-14 emission from incineration of low-level radioactive waste, the proportion of wood-based electricity, among-others.

3.3.2 Completeness check

The objective of a completeness check is to ensure that the information provided in the difference phases of the LCA are sufficient in order to interpret the results (ISO 14044 2006).

In general, the system boundaries and inventory data are described comprehensively in this report. The LCI covers relevant flows for animal-free whey production activities in the foreground system as well as the supply chains of these to the fullest possible, in the background system. The background database, the consequential Ecoinvent v3.6, does not include overhead and services. A database like Exiobase would be needed in the background to include such activities. Finally, although the animal-free whey protein production inventory covers most materials, energy and emissions simulated by the techno-economic analysis, no detailed inventory were made for transport to market, equipment and cleaning. The possible influence of these missing activities was accounted for in the sensitivity analysis.

The EF method covers 16 impacts, and all but 3 were calculated (i.e. those where logarithmic differences are necessary for concluding on an actual difference were excluded), which is considered to provide the most comprehensive picture possible.

3.3.3 Consistency check

The objective of the consistency check is to verify that assumptions, methods and data are consistent with the goal and scope. Especially the consistency regarding data quality along the product chain, regional/temporal differences, system boundaries and LCIA are important (ISO 14044).

Data was collected according to the goal and scope of the analysis. The input needed (and resulting output) for the production of the animal-free whey protein production were obtained from a state-of-the-art TEA. This involve a variety of hypothesis, but these are rather linked to the process than the geography where the process is implemented. Here, the key importance of the geography lies in the type of electricity being used for production, and water withdrawn. For the background, in general, Ecoinvent markets and processes representative for 'rest of the world' (global) were used as much as possible. Temporal consistency for the background data is less consistent (some data are projections, other are past statistics), but best available data has been used.

Regarding the system boundaries and modelling approach, the system boundary follows a consequential approach, while the background dataset used are so-called marginal data from the consequential Ecoinvent v3.6 database; accordingly, it is considered that there is consistency between the modelling approach used and the type of data considered.

3.4 Sensitivity Analysis Results

Sensitivity analysis were performed to (i) (roughly) add the impact of cleaning, equipment and transport (to animal-free whey protein production) and (ii) test the impact of a different sugar source. Both showed no differences in the conclusion for most impacts, except for freshwater eutrophication in the case of the former. This indicates the need for documenting more accurately the cleaning impact and eventual losses of phosphorus-containing residues/detergents.

A more extensive sensitivity analysis should be added for compliance with ISO 14040/44, if the study is "intended to be used for a comparative assertion intended to be disclosed to the public". This could include a more in-depth analysis of different sourcing for sugar, different production location (and thus electricity mixes), or different valorisation of the co-products, for example. This should also include perturbation analysis to identify sensitive data.

3.5 Uncertainty Analysis Results

This was not performed, but should be added for compliance with ISO 14040/44, if the study is "intended to be used for a comparative assertion intended to be disclosed to the public".

3.6 Evaluation of data quality

This was not performed, but should be added for compliance with ISO 14040/44, if the study is "intended to be used for a comparative assertion intended to be disclosed to the public".

3.7 Limitations

As earlier stated, the goal of this study was to provide a first assessment on the environmental consequences of implementing the animal-free whey protein production process studied herein. As such, this study embeds a few limitations. One is linked, as emphasized in the sensitivity analysis, with the inclusion of more accurate estimates on emissions and input from and to the environment in relation with the cleaning, transport and use of infrastructure involved in the animal-free whey production process. Moreover, another element that should be studied more in-depth is the land-use changes (and related impacts) related to the sugar source. Here, this was considered on the basis of the Ecoinvent data, but as shown in e.g. [4], Ecoinvent has some inconsistencies when it comes to and use, and differences between specific land use change inclusion methods and the Ecoinvent data can be significant.

4. Conclusion

This life cycle assessment was carried out to have <u>a first</u> quantitative base of estimates of the environmental implications of introducing the BV process to the market. This first assessment results showed that reductions, in comparison to supplying whey protein with cow milk, can be anticipated by implementing the BV process (in comparison to not implementing it and supplying whey protein with cow milk). These reductions range between 29% to 99%, depending on the impact. A wide range of impacts were quantified, including 13 impacts in total. Climate change and water use were the impacts where the largest reductions were observed. By including, through a conservative proxy (as no specific data were available), transport to market and cleaning for the BV process, these conclusions were maintained, except for freshwater eutrophication and resource use (metals and minerals), where an increase of the impact was observed. For the latter, the increase was rather negligible, while for the former, it indicates the possibility of phosphorus losses from residues and detergent during cleaning, and the importance to prevent these.

5. Appendixes

This report is accompanied by confidential analyses, including the results of the techno-economic study, the life cycle inventory and the results analysis of the life cycle impact assessment.

6. References

[1] Brandao M, Martin M, Cowie A, Hamelin L, Zamagni A. Consequential Life Cycle Assessment: What, How, and Why? 2017. https://doi.org/10.1016/B978-0-12-409548-9.10068-5.

[2] Vandepaer L, Treyer K, Mutel C, Bauer C, Amor B. The integration of long-term marginal electricity supply mixes in the ecoinvent consequential database version 3.4 and examination of modeling choices. Int J Life Cycle Assess 2019;24:1409–28. https://doi.org/10.1007/s11367-018-1571-4.

[3] Vuillemard J-C. Science et technologie du lait. 3e édition. Presses de l'Université Laval; 2018.

[4] Javourez U, Rosero Delgado EA, Hamelin L. Upgrading agrifood co-products via solid fermentation yields environmental benefits under specific conditions only. Nat Food 2022;3:911–20. https://doi.org/10.1038/s43016-022-00621-9.

[5] ISO. ISO 14044: Environmental management — Life cycle assessment — Requirements and guidelines. International Organization for Standardization, Geneva, Switzerland; 2006. https://doi.org/10.1136/bmj.332.7555.1418.

[6] Wernet G, Bauer C, Steubing B, Reinhard J, Moreno-Ruiz E, Weidema B. The ecoinvent database version 3 (part I): overview and methodology. The International Journal of Life Cycle Assessment 2016;21:1218–30. https://doi.org/10.1007/s11367-016-1087-8.

[7] Weidema BP, Bauer Ch, Hischier R, Mutel Ch, Nemecek T, Reinhard J, et al. The ecoinvent database: Overview and methodology, Data quality guideline for the ecoinvent database version 3 2013.

[8] Fazio S, Biganzioli F, De Laurentiis DL, Zampori L, Sala S, Diaconu E. Supporting information to the characterisation factors of recommended EF Life Cycle Impact Assessment methods, version 2, from ILCD to EF 3.0. Ispra: 2018.

[9] European Commission. PEFCR Guidance document, - Guidance for the development of Product Environmental Footprint Category Rules (PEFCRs), version 6.3 of December 2017. 2018.

[10] Recommendation on the use of Environmental Footprint methods n.d. https://environment.ec.europa.eu/publications/recommendation-use-environmental-footprint-methods_en (accessed November 18, 2022).

[11] Fantke P, Bijster M, Hauschild MZ, Huijbregts M, Jolliet O, Kounina A, et al. USEtox® 2.0 Documentation (Version 1.00) 2017. https://doi.org/10.11581/DTU:00000011.

[12] Global Guidance for Life Cycle Impact Assessment Indicators Volume 2 - Life Cycle Initiative 2019. https://www.lifecycleinitiative.org/training-resources/global-guidance-for-life-cycle-impact-assessment-indicators-volume-2/, https://www.lifecycleinitiative.org/training-resources/global-guidance-for-life-cycle-impact-assessment-indicators-volume-2/ (accessed November 18, 2022).

[13] Chen D, Rojas M, Samset BH, Cobb K, Diongue-Niang A, Edwards P, et al. Framing, Context, and Methods. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. in Press, IPCC; 2021.

[14] IPCC. Climate Change 2013. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press; 2013.

[15] Carbon footprint assessment of a wood multi-residential building considering biogenic carbon 2022. https://doi.org/10.21203/rs.3.rs-2273783/v1.

[16] De Rosa M, Pizzol M, Schmidt J. How methodological choices affect LCA climate impact results: the case of structural timber. Int J Life Cycle Assess 2018;23:147–58. https://doi.org/10.1007/s11367-017-1312-0.

[17] Haberl H, Sprinz D, Bonazountas M, Cocco P, Desaubies Y, Henze M, et al. Correcting a fundamental error in greenhouse gas accounting related to bioenergy. Energy Policy 2012;45:18–23. https://doi.org/10.1016/j.enpol.2012.02.051.

[18] Weidema BP, Wesnæs MS. Data quality management for life cycle inventories—an example of using data quality indicators. Journal of Cleaner Production 1996;4:167–74. https://doi.org/10.1016/S0959-6526(96)00043-1.

[19] Bisinella V, Conradsen K, Christensen TH, Astrup TF. A global approach for sparse representation of uncertainty in Life Cycle Assessments of waste management systems. Int J Life Cycle Assess 2016;21:378–94. https://doi.org/10.1007/s11367-015-1014-4.

[20] European Commission. EU Reference Scenario 2016. Energy, transport and GHG emissions trends to 2050. Brussels, Belgium: 2016.
[21] Teigiserova DA, Hamelin L, Thomsen M. Towards transparent valorization of food surplus, waste and loss: Clarifying definitions, food waste hierarchy, and role in the circular economy. Science of The Total Environment 2020;706:136033. https://doi.org/10.1016/j.scitotenv.2019.136033.
[22] Food waste measurement n.d. https://food.ec.europa.eu/safety/food-waste/eu-actions-against-food-waste/food-waste-measurement_en (accessed November 18, 2022).
[23] Møller J, Thøgersen R, Helleshøj ME, Riis Weisbjerg M, Søegaard K, Hvelplund T. Fodermiddeltabel - Sammensætning og foderværdi af fodermidler til kvæg - Forskning - Aarhus Universitet. Dansk; 2005.
[24] Tonini D, Hamelin L, Astrup TF. Environmental implications of the use of agro-industrial residues for biorefineries: application of a deterministic model for indirect land-use changes. GCB Bioenergy 2016;8:690–706. https://doi.org/10.1111/gcbb.12290.
[25] openLCA Nexus: The source for LCA data sets n.d. https://nexus.openlca.org/databases (accessed January 2, 2023).
[26] Pauer E, Wohner B, Tacker M. The Influence of Database Selection on Environmental Impact Results. Life Cycle Assessment of Packaging Using GaBi, Ecoinvent 3.6, and the Environmental Footprint Database. Sustainability 2020;12:9948. https://doi.org/10.3390/su12239948.
[27] Schmidt JH. Life cycle assessment of five vegetable oils. Journal of Cleaner Production 2015;87:130–8. https://doi.org/10.1016/j.jclepro.2014.10.011.
[28] Un-Noor F, Wu G, Perugu H, Collier S, Yoon S, Barth M, et al. Off-Road Construction and Agricultural Equipment Electrification: Review, Challenges, and Opportunities. Vehicles 2022;4:780–807. https://doi.org/10.3390/vehicles4030044.
[29] Beltrami D, Iora P, Tribioli L, Uberti S. Electrification of Compact Off-Highway Vehicles—Overview of the Current State of the Art and Trends. Energies 2021;14:5565. https://doi.org/10.3390/en14175565.
[30] Anas M, Liao F, Verma KK, Sarwar MA, Mahmood A, Chen Z-L, et al. Fate of nitrogen in agriculture and environment: agronomic, eco-physiological and molecular approaches to improve nitrogen use efficiency. Biological Research 2020;53:47. https://doi.org/10.1186/s40659-020-00312-4.
[31] Jones C. Management to Minimize Nitrogen Fertilizer Volatilization n.d.
[32] Gésan-Guiziou G, Sobańtka AP, Omont S, Froelich D, Rabiller-Baudry M, Thueux F, et al. Life Cycle Assessment of a milk protein fractionation process: Contribution of the production and the cleaning stages at unit process level. Separation and Purification Technology 2019;224:591–610. https://doi.org/10.1016/j.seppur.2019.05.008.