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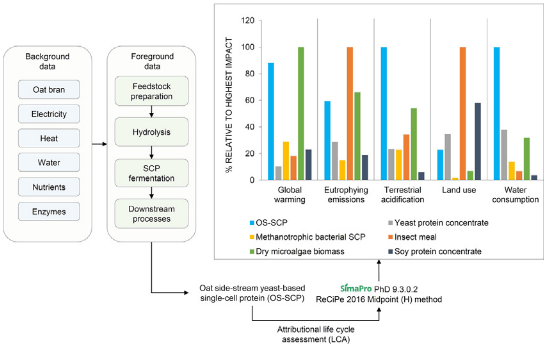
HIGHLIGHTS

- LCA of yeast-based SCP fish feed grown in oat side-streams in Finland was performed.
- Energy production had the highest contribution to the studied environmental impacts.
- Yeast-based SCP had lower land use but higher other impacts than soy protein.
- Bacterial SCP was only novel feed having all impacts lower than yeast-based SCP.

GRAPHICAL ABSTRACT



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ABSTRACT

Production of fish meal and plant-based feed proteins continues to increase to meet the growing demand for seafood, leading to impacts on marine and terrestrial ecosystems. Microbial proteins such as single-cell proteins (SCPs) have been introduced as feed alternatives since they can replace current fish feed ingredients, e.g., soybean, which are associated with negative environmental impacts. Microbial protein production also enables utilization of grain processing side-streams as feedstock sources. This study assesses the environmental impacts of yeast-based SCP using oat side-stream as feedstock (OS-SCP). Life-cycle assessment with a cradle-to-gate approach was used to quantify global warming, freshwater eutrophication, marine eutrophication, terrestrial acidification, land use, and water consumption of OS-SCP production in Finland. Dried and wet side-streams of oat were compared with each other to identify differences in energy consumption and transportation effects. Sensitivity analysis was performed to examine the difference in impacts at various locations and fermentation times. Benchmarking was used to evaluate the environmental impacts of OS-SCP and other feed products, including both conventional and novel protein products. Results highlight the importance of energy sources in quantifying the environmental performance of OS-SCP production. OS-SCP produced with dried side-streams resulted in higher global warming (16.3 %) and water consumption (7.5 %) than OS-SCP produced from wet side-streams, reflecting the energy and water requirements for the drying process. Compared with conventional products, such as soy protein concentrates, OS-SCP resulted in 61 % less land use, while exacerbating the

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Abbreviations: DMB, Dry microalgae biomass; FE, Freshwater eutrophication; GW, Global warming; IM, Insect meal; LCA, Life-cycle assessment; LCI, Life-cycle inventory; LU, Land use; ME, Marine eutrophication; OS-SCP, Oat side-stream yeast-based single-cell protein; SCP, Single-cell protein; SoyPC, Soy protein concentrate; TA, Terrestrial acidification; WC, Water consumption; YPC, Yeast protein concentrate.

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environmental impacts in all the other categories. OS-SCP had more impact on global warming (205–754 %), water consumption (166–1401 %), freshwater eutrophication (118–333 %), and terrestrial acidification (85–340 %) than other novel products, including yeast protein concentrate, methanotrophic bacterial SCP, and insect meal, while lowering global warming (11 %) and freshwater eutrophication (20 %) compared with dry microalgae biomass.

1. Introduction

The challenge of providing adequate nutrition for the growing and increasingly wealthier global population has been widely recognized. Many argue that reduction of meat consumption is unavoidable (Willett et al., 2019). Seafood provides beneficial nutrients, including high-quality protein, and its environmental impacts are often substantially lower than terrestrial animal-sourced foods (Poore and Nemecek, 2018). While the catch from wild fisheries has been stable, the aquaculture industry has been expanding and further growth is expected to meet future demand. Increased fish supply requires increased fish feed (Li et al., 2020). Fish feed is costly and is an environmental hot spot of aquaculture systems (Ghamkhar and Hicks, 2020; Jones et al., 2020). Fish feed can greatly exacerbate land use, global warming, and acidifying emissions contributing up to 72 % to these categories in the fish farming value chain, mainly due to the intensive use of crops-based and fish-based feed ingredients (Samuel-Fitwi et al., 2013). Therefore, reductions in cost and environmental impact of fish feed production are essential for sustainable fish-farming. Traditionally, fish meal was the main protein source for fish feed; however, plant-based protein is increasingly replacing it due to concerns regarding the production of fish meal. Because of the difference in the quality of protein, often only a certain amount of fish meal can be replaced by plant-based proteins (Cottrell et al., 2020). The provision of plant-based proteins, commonly produced from soy, wheat, corn, and rapeseed, involve issues related to land-use change (e.g., deforestation), water use, and competition with direct food consumption (Agboola et al., 2021).

Novel protein feed ingredients, such as microbial products, have been considered as alternative protein sources. Compared with plant-based proteins, the production of these alternative proteins tends to require less land and water and is more resilient to climate fluctuations (Øverland and Skrede, 2017). Single-cell proteins (SCPs) based on fungi (including yeast), microalgae, and bacteria have been extensively studied recently for their high protein content, high protein yield, and good protein quality (Jones et al., 2020). The general SCP production process includes media preparation, fermentation, and downstream stages, such as separation and drying. For sustainable production, preparation of the media can be key as it affects the overall production process design (Sharif et al., 2021), which consequently influences the environmental performance and cost of the final product (Nasseri et al., 2011). An advantage of SCPs is that a broad range of substrates, including agricultural and industrial side-streams, are suitable for their production (Anupama, 2000). Generation of food waste or side-streams is unavoidable in the processing of food. Use of waste material as substrate is particularly attractive as it valorizes waste streams and reduces waste treatment costs and associated environmental emissions (Bekatorou et al., 2006). The biorefining of food processing side-streams, such as sugarcane molasses (Yan et al., 2018), apple pomace (Gullón et al., 2008), grape pomace (Botella et al., 2007), banana peels and pulp (Naranjo et al., 2014), orange peel (Boukroufa et al., 2015), date waste (Hashempour-Baltork et al., 2020), and corncob (Samanta et al., 2015) for use as substrate has been explored. The application of staple food processing side-streams as feedstock has also been explored. This includes wheat bran (Yunus et al., 2015), rice bran (Pruksasri et al., 2019), rice straw (Upcraft et al., 2021), and potato starch processing waste (Liu et al., 2014). Side-streams from the processing of staple food, such as oat in Finland, can be reliable feedstock. Unlike wheat or corn, oat grows well in the north above a latitude of 60 degrees. However, cereal by-products or side-streams of cereal processing that include oats are not yet used efficiently in northern latitudes (Valoppi et al., 2021). The Finnish food industry used 143 million kilograms of oats in 2021 (Natural Resources Institute Finland, 2021). Finland is not self-sufficient in feed protein with around 87,000 tons being imported in 2021 (Trademap, 2022), and there is room for an increase in the utilization of oat side-streams for feed production.

The use of food waste and processing side-streams tends to reduce some environmental impacts, such as land and water use, compared with a conventional carbon sources. However, the biorefining of feedstock may be energy intensive and require enzymes, which could cause different types of environmental impacts. To avoid environmental burden shifting, the environmental impacts of SCP production with side-streams need to be assessed. Life-cycle assessment (LCA), which evaluates the environmental impacts of a product for its entire life cycle, is a useful tool for this purpose because of its ability to assess a broad range of environmental impacts. Previous LCA studies on SCPs are limited. Couture et al. (2019) applied LCA to compare yeast-based SCP, bacterial meal, and soy-based protein as a protein ingredient for fish feed, where wheat was considered as the substrate for SCP fermentation. Tallentire et al. (2018) also included SCP grown on wheat substrate as an alternative protein source for chicken feed in their LCA study. Spiller et al. (2020), Smetana et al. (2015), and Upcraft et al. (2021) conducted LCA of SCPs, in which potato processing wastewater, sugar beet molasses, and rice straw, respectively, were used as feedstock. To the authors' knowledge, no LCA study has investigated the environmental impacts of SCP production on oat processing side-streams.

The aim of this study was to perform an LCA of yeast-based SCP using an oat side-stream as feedstock (OS-SCP) to examine its environmental impacts. This paper provides insight into the major question: Is OS-SCP an environmentally viable alternative for the aquafeed value chain? This study strives to answer this question by conducting a hot-spot analysis, a scenario analysis, and a sensitivity analysis to assess the environmental gains and losses of OS-SCP production relative to other feed protein products and to determine the potential future improvements and the robustness of results. The results were compared with soy protein concentrate and other novel protein feed ingredients to examine the relative environmental performance of the OS-SCP product.

2. Materials and methods

2.1. Life-cycle assessment

2.1.1. Scope of the study

Attributional LCA of yeast-based OS-SCP production was conducted in this study. The LCA was based on experimental data obtained from Matis in Iceland and data from the literature and technical reports. The study uses system boundaries from cradle-to-gate, including the environmental impacts of the processes upstream of the production chain until the stage where the product is ready for use. The processes included are production of energy and other inputs, oat cultivation, generation of the oat side-stream, biomass refining, fermentation, and the downstream processes. Machinery cleaning and capital inputs including the facility and machinery manufacturing are outside the scope of study, therefore, their associated environmental impacts are not included.

A large-scale production system was assumed to enable fair comparison with other protein sources for fish feed available in the published literature. The functional unit used for impact evaluation was 1 kg of dried OS-SCP product. Impacts per kilogram of protein content were also calculated and used for comparison with other protein products. The SimaPro PhD

9.3.0.2 with ReCiPe 2016 Midpoint (H) method was used for the calculation. ReCiPe 2016 is one of the most recent impact assessment methods, providing the state-of-art means to address environmental concerns at the midpoint level (Huijbregts et al., 2017). The impact categories of global warming (GW), freshwater eutrophication (FE), marine eutrophication (ME), terrestrial acidification (TA), land use (LU), and water consumption

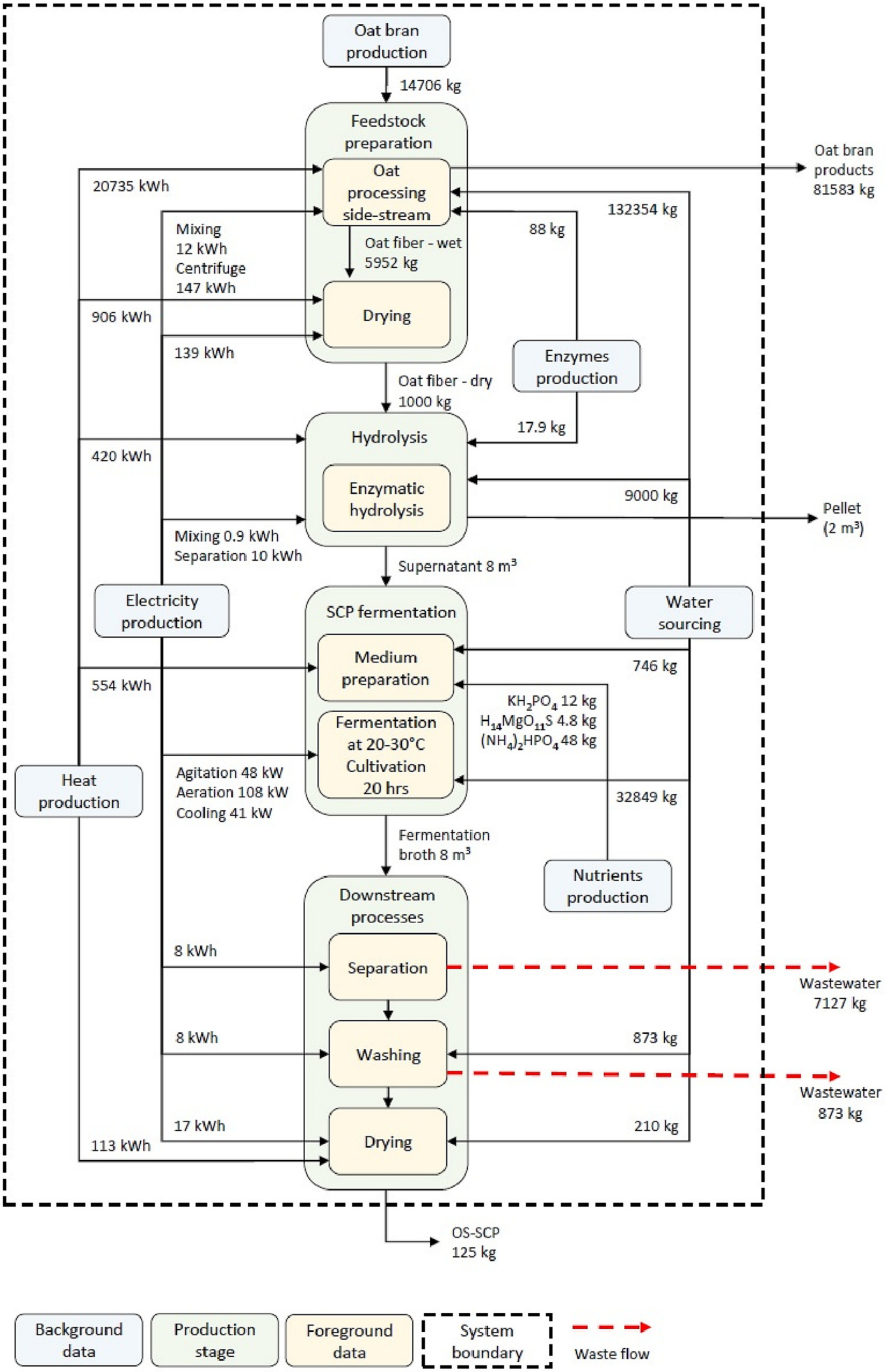

Fig. 1. Unit process diagram and system boundary of the OS-SCP production.

(WC) were evaluated by considering previous studies on similar products (Couture et al., 2019; Maiolo et al., 2020; Tallentire et al., 2018).

2.1.2. System description

An oat side-stream from oat bran processing for products such as oat protein concentrate and beta-glucan was investigated as a carbon source for yeast-based OS-SCP production (Fig. 1). The processing included heating and electricity for mixing and centrifugation. Heating energy was calculated using the mathematical formulation developed by Piccinno et al. (2016):

$$Q_{react} = \frac{Q_{heat} + Q_{loss}}{\eta_{heat}} \tag{1}$$

where Q_{react} is the heating energy required, Q_{heat} is the sum of energy for raising the temperature, Q_{loss} is the heat loss on the reactor surface, and η_{heat} is the efficiency of the heating device. Given that the reaction takes place in a solution, Q_{heat} was calculated as follows:

$$Q_{heat} = C_p \times m_{mix} \times (T_r - T_0) \tag{2}$$

where C_p is the specific heat capacity that indicates the amount of energy needed to obtain a temperature change per unit mass of material, m_{mix} is the mass of the reaction mixture, T_r is the starting temperature, and T_0 is the ambient temperature. To calculate the Q_{loss}, we referred to the same condition reported in Piccinno et al. (2016), that is, the reactor is insulated from the surrounding surface and the insulation is the only limiting factor. Hence, Q_{loss} was calculated as follows:

$$Q_{loss} = A \times \frac{k_a}{s} \times (T_r - T_{out}) \times t \tag{3}$$

where A is the surface area of the reactor, k_a is the thermal conductivity of the insulation material, s is the thickness of the insulation, T_r is the temperature inside the reactor, T_{out} is the temperature outside the reactor (Table 1), and t is the time of the reaction which is around 20 h. The electricity required for mixing was calculated following the work done by Piccinno et al. (2016):

$$E_{mix} = \frac{N_p \times \rho_{mix} \times N^3 \times d^5 \times t}{\eta_{stir}} \tag{4}$$

where E_{mix} is the required energy for mixing, N_p is the power number associated with the impeller and is constant throughout the mixing process, ρ_{mix} is the density of the reaction, N is the rotational speed of the agitator, d is the diameter of the impeller, t is the time of the reaction, and η_{stir} is the efficiency of the agitator (Table 1). The electricity needed for the centrifugation was calculated by referring to the electricity demand per unit of substance (1 kWh per 100 kg) reported in Fasaei et al. (2018). The outcome of the processing stage included wet oat fiber fractions that were then dried and taken for the hydrolysis stage.

Dried, fine-milled oat-based fiber fraction with a mass of 1000 kg was resuspended in water to a final concentration of 15 % (*w*/*v*) and pH was confirmed to be approximately 6. Deionized water was added to ensure a reaction mixture that is free from potentially possible contaminants. Three enzymes were added at a final concentration of 0.5 % of the substrate biomass (*w*/w), i.e. BAN® α-amylases (*endo*-); Amylase® α-amylases (*exo*-); and Ultraflo® Max β-glucanase, xylanase. All enzymes were purchased from Novozymes. The enzyme reaction was mixed by shaking with an electricity input following Eq. (4).

The reaction was then incubated in the incubate at 50 °C for 4 h. For this, heat was applied to raise the reaction to the required temperature. The heating energy required was calculated following Eq. (1). After incubation, solid and liquid fractions were separated by centrifugation at 800 *g* for 10 min with an electricity input equivalent to 10 kWh (Fasaei et al., 2018), resulting in approximately 70 % of the original water volume as saccharified liquid and approximately 30 % as spent fiber pellet. The liquid fraction contained approximately 3 % glucose, along with other small sugars and larger carbohydrates, and was used directly as culture medium for yeast-based OS-SCP, after sterilization by autoclaving. The spent fiber pellets contained 40 % protein and 36 % carbohydrate (of dry weight) compared with 32 % protein and 58 % carbohydrate in the original dried fiber fraction. The growth medium for submerged fermentation was prepared with the supernatant supplemented with monopotassium phosphate (1.5 kg/m^3), magnesium sulfate heptahydrate (0.6 kg/m^3), and diammonium phosphate (6 kg/m^3) (Liu, 2020). The medium was heated to be ready for the fermentation process. The amount of heat required for medium preparation was calculated according to the mathematical formulation reported by Järviö et al. (2021) as follows:

$$Q_{total} = Q_{heating} + Q_{vaporizing} \tag{5}$$

where Q_{total} is the total quantity of energy needed. $Q_{heating}$ is the quantity of energy for heating up water to 393.15 K (equivalent to 120 °C), and is calculated as follows:

$$Q_{heating} = m \times c_p \times dT \tag{6}$$

where m is the mass of the substance, c_p is the specific heat of the substance, and dT is the temperature rise of the substance. $Q_{vaporizing}$ is the quantity of energy for vaporizing the water at target water temperature 393.15 K, and is calculated as follows:

$$Q_{vapurizing} = dh_{vap} \times m \tag{7}$$

where dh_{vap} is the heat of vaporization (kJ/kg) (Table 1).

The fermentation process occurred in a 10 m^3 bioreactor tank at 20–30 °C with a cultivation time equals to 20 h. The medium went through

Table 1
Values of the parameters used for inventory data calculations.

Variable	Abbreviation	Value	Unit	Source
Specific heat capacity	C_p	4.2	$\frac{kJ}{kg*K}$	Järviö et al. (2021)
Reaction temperature	T_r	393.15	K	Piccinno et al. (2016)
Ambient temperature	T_0	298.15	K	Piccinno et al. (2016)
Surface area	A	27.381	m^2	Piccinno et al. (2016)
Thermal conductivity	k_a	0.042	$\frac{W}{m*K}$	Piccinno et al. (2016)
Thickness	s	0.075	m	Piccinno et al. (2016)
Temperature outside reactor	T_{out}	298.5	K	Piccinno et al. (2016)
Efficiency of heating	η_{heat}	79	%	Piccinno et al. (2016)
Power number of impeller	N_p	0.79	–	Piccinno et al. (2016)
Rotational speed of the agitator	N	0.658	$1/s$	Piccinno et al. (2016)
Diameter of impeller	d	0.803	m	Piccinno et al. (2016)
Efficiency of agitator	η_{stir}	90	%	Piccinno et al. (2016)
Heat of vaporization	dh_{vap}	2256.4	$\frac{kJ}{kg}$	Järviö et al. (2021)
Temperature of cooling water	$T_{cw,\ in\ rct}$	5	°C	Harding (2008)
Temperature of water out of reactor	$T_{cw,\ out\ rct}$	30	°C	Harding (2008)

agitation and aeration processes (Harding, 2008; Meyer et al., 2017). The medium was then cooled by adding cooling water. The calculation of the amount of cooling water required followed the mathematical formulation reported by Harding (2008), where the amount of water needed is dependent on the temperature of the cooling water in the reactor ($T_{cw,\ in\ rct}$), the temperature of cooling water out of the reactor ($T_{cw,\ out\ rct}$), the specific heat of water ($C_{p,\ w}$), and the energy to cool the reactor ($E_{rct,\ cw}$) (Table 1):

$$M_{cw,rct} = \frac{E_{rct,cw}}{C_{p,w}\left(T_{cw,out\ rct} - T_{cw,in\ rct}\right)} \tag{8}$$

The fermentation process resulted in a total of 8000 kg of broth including the biomass fractions. In the downstream processes, the obtained biomass was first separated from water through centrifugation (Fasaei et al., 2018). Another separation process took place following a washing session of the biomass cells while and demanded the same electricity input required for the centrifugation process. After that, biomass cells were dried under heat and electricity inputs (Santonja et al., 2020). A fluidized bed dryer was used for all downstream processes including centrifugation and drying. The final output referred to the produced batch of yeast-based OS-SCP equivalent to 125 kg of dry weight yeast-based OS-SCP.

The waste resulting from the processes and their management was not included into the system boundary of the production model. The inventory parameters that were taken from the life-cycle inventory databases and used for the calculations are provided in Supplementary Information, Section 1.

2.1.3. Allocation

The which was the main impact contributor, was used for all scenarios leading to different applications and economic values in the market, and therefore, the economic allocation was used. Allocation for the oat side-stream was estimated based on the literature (Heusala et al., 2020). Major products derived from oats processing include oat oil, oat starch, beta glucan, and oat proteins. The mixed fractions that result from the processing of oat products are not utilized. However, the economic allocation of these mixed fractions was calculated to be 0.4 %, based on the energy content (Heusala et al., 2020). The mass allocation was used for the allocation of the liquid (supernatant) and solid outputs of the enzymatic hydrolysis. The economic value of both solid and liquid fractions was unknown in the market, and thus, there was no concrete basis to assume any economic value for either output.

2.1.4. Scenarios

This work considered two production scenarios of the OS-SCP. Dry oat side-stream scenario refers to the baseline scenario – OS-SCP (dry) – where the oat side-stream was dried at the oat processing facility before being transported to where the enzymatic hydrolysis took place to reduce the transportation burden. Since drying processes are generally energy intensive, associated environmental impacts could be considerable. Hence, the impacts of another production scenario where the oat side-stream was transported in its wet form was also evaluated – OS-SCP (wet) (Table 2). The mass of the wet oat-side stream after the feedstock preparation stage was around 5882 kg. The changes in the production process for the OS-SCP (wet) scenario refers to the elimination of the drying process of the oat side-streams. By eliminating the drying process, the hydrolysis stage required around 54 % less water relative to the OS-SCP (dry) scenario to reach the required glucose concentration level. The inventory input of the OS-SCP (wet) is given in Supplementary Information, Section 1.

Table 2
Scenarios for the production of yeast-based OS-SCP.

Variables	Scenarios	
	OS-SCP (dry)	OS-SCP (wet)
Processing stage	Obtained oat side-stream is dried using electricity and heat input before being transported to the hydrolysis stage	Obtained oat side-stream is transported to the hydrolysis stage in its wet form without the drying process

2.2. Oat side-stream transportation

The allocated environmental impacts of oat side-stream generation are expected to be small due to its lower value relative to other products in the production system. The trade-off between the environmental burdens of the drying and transport of the side-stream was examined to determine the maximum transport distance without exceeding the impacts caused by the drying process for cases where the oat processing and the OS-SCP production facilities are at different sites. The impact of transportation was calculated based on the use of Euro 6 freight trucks. In the current situation, the oat processing site and the OS-SCP production site were assumed to be at the same location. A continuous increase in the distance between the two sites was assumed reaching up to 100 km to test the dynamics of the trade-off relationship between the impacts of dry-based and wet-based OS-SCPs.

2.3. Other types of feedstocks

First generation feedstock – wheat flour and corn flour – are also studied as alternative feedstock for a comparison with the oat side-stream. Differences in glucose yield from biorefining are considered based on data from the literature. The glucose yield of supernatant with wheat flour as feedstock was equivalent to 12 %, following the biorefining process at a temperature of 30 °C and pH 6 (Åkerberg et al., 1998). Supernatant from corn flour as feedstock had higher glucose yields equivalent to 41 % at a temperature of 35 °C and pH 4.5 (Zhang et al., 2002). However, OS-SCP yields are assumed not to be affected by the different feedstock. Achieving glucose concentrations of supernatants from alternative feedstocks similar to that from the oat side-streams required dilution with extra water prior to the fermentation process. The inventory input of the medium preparation using wheat and corn flour as feedstock is given in Supplementary Information, Section 3.

2.4. Sensitivity analysis

Sensitivity analysis was carried out to determine the sensitivities of assumptions used in this study (Table 3). Firstly, the economic allocation was chosen to estimate the impacts of the oat side-stream. Since the value of side-streams is generally much lower than that of the main products, economic allocation seems more practical than mass allocation. However, the price of the oat side-stream is uncertain, as the production is not currently on the market, and an arbitrary price range (±50 %) was considered in economic allocation to check the sensitivity.

It was assumed that the production of the OS-SCP occurred in Finland; however, it can be similarly produced in any oat-producing region. To realize the sensitivity of location, OS-SCP production in Canada (Quebec) and Australia was additionally studied in terms of their adequate production of oats and potential fish feed markets as well as the difference in their electricity mix. The analysis was based on an assumption that locations of production of the oat side-stream and OS-SCP are close to each other; and therefore, the burden of transportation between the facilities was not considered. The inventory input of OS-SCP production in Australia and Canada (Quebec) are given in Supplementary Information, Section 2.

Many factors could affect the optimal fermentation time. For the base case, 20 h was selected; however, it could be longer and that would increase the operational electricity consumption for the fermentation process. To determine the influence of fermentation time on the overall environmental performance, 30 and 40 h for the fermentation time were also examined.

2.5. Benchmarking

To evaluate the environmental impacts of the OS-SCP studied here, the LCA results were compared with those of other potential novel protein

Table 3
The sensitivity analysis scenarios (baseline and two alternatives) under the changes of (a) economic allocation, (b) production location, and (c) fermentation time.

Sensitivity analysis	Changed parameter	Baseline	Alternative 1	Alternative 2	Explanation
(a)	Economic allocation	0.4 %	0.2 %	0.6 %	Market value of the fractions from processed oat side-stream to be used for the OS-SCP production refer to the value calculated by Heusala et al. (2020) for the baseline scenario, while an arbitrary price change of −50 % and + 50 % was considered for alternative 1 and 2, respectively.
(b)	Production location	Finland	Canada (Quebec)	Australia	Location of OS-SCP production was considered to be in Finland for the baseline scenario. Two additional production locations were considered for alternative 1 (Quebec, Canada) and alternative 2 (Australia). These are oat production countries that differ in electricity mix patterns.
(c)	Cultivation time	20 h	30 h	40 h	Cultivation time for the fermentation was 20 h for the baseline scenario. The sensitivity of extra time probability was examined by adding 10 h to alternative 1, and 20 h to alternative 2.

sources for fish feed, yeast protein concentrate (YPC), methanotrophic bacterial SCP, insect meal (IM) from *Hermetia illucens* larvae, and dry microalgae biomass (DMB) from *Tetraselmis suecica*, as well as a conventional protein source, soy protein concentrate (SoyPC). *Hermetia illucens* has a rich nutritional profile with 55 % DM protein, 35 % DM fat, and a well-balanced essential amino acid profile (Bußler et al., 2016), making it a suitable protein source for fish diets with no negative impacts on the fish health and growth (Magalhães et al., 2017; Sogari et al., 2019). The inventory data for these alternatives were estimated based on previous studies (Maiolo et al., 2020; Tallentire et al., 2018) and the Agri-footprint database (Table 4). In the DMB production model, 80 % of the carbon dioxide that was fed to the microalgae as a carbon supply was not consumed but reportedly emitted to the atmosphere as CO_2 emissions. (Maiolo et al., 2020). However, since that the carbon dioxide originated from biogenic sources, the emissions were not accounted in the analysis. Like the OS-SCP production system, machinery cleaning and capital inputs including the facility and machinery manufacturing, are beyond the scope of this study, and therefore, their associated environmental impacts are not included. The LCA analysis of the alternative feed proteins followed the same method applied for the analysis of OS-SCP, i.e. is the ReCiPe 2016 Midpoint (H) using the SimaPro PhD 9.3.0.2. The geographical location of the production processes was set in Finland to maintain the consistency of the benchmarking analysis. The inventory parameters that were taken from the life-cycle inventory databases and used for the calculations are provided in Supplementary Information, Section 4.

2.6. Inventory data sources

Ecoinvent 3.8 (Ecoinvent, 2021), Agri-footprint 5.0 (Van Paassen et al., 2019), and Agribalyse 3 (Agribalyse, 2022) provided background data. Finland was assumed for the location of OS-SCP production. However, due to the limited availability of region-specific data in the databases, European average and other regions' data were used when regional data were unavailable. Finnish average electricity mix was assumed to be used based on Ecoinvent 3.8 data. European average data were used for heat, water and chemicals (Ecoinvent 3.8). Input of oat bran used the Finnish dataset (Agribalyse 3), whereas wheat flour and corn flour input used “the rest of the world” data (Ecoinvent 3.8.).

For the location sensitivity analysis, Australian and Canadian (Quebec) electricity mix were used for Australia and Canada-Quebec, respectively, based on the Ecoinvent 3.8 data. Input of heat and water for Canada-Quebec used the Canada-Quebec specific data based on the Ecoinvent 3.8 data, whereas in the case of Australia, the “rest of the world” data was used also through the Ecoinvent 3.8. Like the Finland production model, the input of oat bran used the French data for both locations (Agribalyse 3).

For the alternative protein feed products, the input of heat, electricity, chemicals, enzymes, and water data used the same geographical datasets used for the OS-SCP (Ecoinvent 3.8), as the production location is the same – Finland. For the Soy protein concentrate, data for the soybean meal was taken from the Netherlands dataset (Agri-foot print 5).

3. Results

3.1. Hot-spot analysis of OS-SCP production scenarios

The environmental results indicated higher impacts of the dried side-stream scenario than the wet side-stream scenario (Fig. 2). The dried side-stream scenario caused a 14 % higher impact than the wet side-stream scenario in terms of GW due to the energy required for the side-stream drying. Electricity used for the drying caused a slight increase in FE (4 %), and the heat and electricity usage for drying contributed to TA of the dried oat side-stream scenario (3 %). For WC, in addition to direct and indirect use of water in the drying process, the increased amount of water needed for biorefining of the dried oat side-stream, which was accounted for in the medium production stage, caused 7 % higher WC in the dried side-stream scenario than in the wet side-stream scenario. The negative impact in the downstream processes stage was due to the disposal of liquid after the OS-SCP biomass separation. Overall, the difference between the dry and wet side-stream scenarios was small (0.4–3 %) except for GW and WC.

The environmental impacts of the fermentation stage were the highest for GW, FE, and WC (Fig. 2). The electricity requirements for mixing, aeration, and cooling during fermentation were the dominant contributors to GW and FE at 68 % and 66 %, respectively. Water consumption for cooling during the fermentation process also greatly contributed (55 %) to WC. The impact of the medium production stage was the highest for ME (85 %), TA (60 %), and LU (57 %). The impacts of enzyme production were the main cause in this stage for most of the impact categories with contribution proportions of 77 % for FE, 98 % for ME, 88 % for LU, and 60 % for WC. The exceptions were GW and TA, where medium sterilization and diammonium phosphate production were the main contributors, at 44 % and 57 %, respectively. Significant contributions of the feedstock production stage were also observed for ME, TA, LU, and GW of the dried oat side-stream scenario. Oat cultivation contributed the most to TE (87 %), ME (94 %), and LU (87 %). Within feedstock production, the drying process of the wet oat side-stream contributed heavily to GW (74 %) and FE (63 %), while water use, enzyme production, and electricity use for oat side-stream processing and the drying process had a combined contribution of around 70 % to the WC impact category.

3.2. Environmental impacts of oat side-stream transportation

Fig. 3 shows the difference in environmental impacts between dried and wet oat side-stream transport from an oat processing facility to an OS-SCP production site depending on the transport distance. The y-axis is the environmental impact relative to the impact of the drying process. When 100 %, the transport impact of the wet oat side-stream is equivalent to the impact of the drying process in the dried oat side-stream scenario. The environmental benefit of eliminating the drying process is offset by the impact of transport at a transport distance of around 30, 40, and 50 km for FE, LU, and TA, respectively. The wet oat side-stream can be transported without exceeding the impacts of drying for a much longer distance in the case of ME, GW, and WC.

Table 4
The list of benchmark feed proteins, their abbreviations, and the corresponding inventory data sources and values.

Product	Abbreviation	Protein content	Inventory data sources	Inventory data	Unit	Amount
Soy protein concentrate	SoyPC	66 %	Agri-footprint 5, Blaufuss and Trushenski (2012)	Input		
				Soybean meal	kg	1000
				Ethanol	kg	9
				Electricity	MJ	684
				Heat	MJ	4581
				Water	dm^3	170
				Output		
				SoyPC (97.4 %)*	kg	634
				Soybean fines (1.8 %)*	kg	75
				Soybean molasses (0.8 %)*	kg	313
				wastewater treatment	kg	154
Methanotrophic bacterial single-cell protein	Methanotrophic bacterial SCP	72.9 %	Tallentire et al. (2018)	Input		
				Water	kg	8.51
				Flue gas	m^3	1700
				Oxygen	m^3	2015
				Ammonia	kg	138
				Phosphoric acid	kg	42
				Magnesium sulphate	kg	18
				Iron sulfate	kg	1
				Copper sulphate	kg	1
				Potassium nitrate	kg	4
				Electricity (processing)	kWh	1438
				Heat (drying)	MJ	26.76
				Output		
				Methanotrophic bacterial SCP	kg	1000
Yeast protein concentrate	YPC	67.6 %	Tallentire et al. (2018)	Input		
				Dry wheat grain	kg	3030
				Water	kg	10,000
				Sulfuric acid	kg	100
				Diammonium phosphate	kg	5.06
				Enzymes	kg	200
				Electricity (processing and dehydration)	MJ	1480
				Heat (processing and drying)	MJ	9722
				Output		
				YPC (6 %)*	kg	152
				Bioethanol (82 %)*	kg	1000
				DDGS (12 %)*	kg	988
				Waste		
				Water	kg	9942
Insect meal	IM	50 %	Maiolo et al. (2020)	Input		
				Rye middlings	kg	5787
				Wheat bran	kg	213
				Sodium chloride	kg	1.7
				Sodium hydrochloride	kg	1.7
				Water	m^3	6.3
				Energy (heating, ventilation, insect processing)	kWh	3366.7
				Output		
				IM	kg	1000
				Insect oil	kg	333.3
				Waste		
				Water	m^3	3
Dry microalgae biomass	DMB	40 %	Maiolo et al. (2020)	Input		
				Sodium nitrate	kg	427
				monosodium phosphate	kg	27.4
				Carbon dioxide, liquid	kg	9020
				Sodium hydrochloride	kg	25.1
				hydrochloric acid	kg	4.1
				Energy (cultivation, harvesting, and drying)	kWh	12,098
				Output		
				DMB	kg	1000
				Emissions in air		
				Carbon dioxide	kg	7220
				Water loss	m^3	4

* Economic allocation applied.

3.3. Impacts of feedstock types

The environmental impacts of SCP production with different types of feedstocks are shown in Fig. 4. The type of feedstock affects the impacts in the feedstock production stage. The impacts were substantially smaller for the oat side-streams in most cases, except for GW of the dried oat side-stream scenario due to the energy requirement for the drying process. The difference among the feedstock types is caused by the difference in crop cultivation practice, grain processing methods, and glucose yields. Due to its low economic value, the oat side-streams gained environmental advantages when the economic allocation was used, as in this study. The impacts of the medium production stage were also noticeably affected. The changes in the feedstock amounts heading to the hydrolysis stage led to changes in the amounts of enzymes required, as the enzyme requirements were calculated as percentage of feedstock substances (Pihlajaniemi et al., 2020). The difference in the values of enzyme input to the mixture,

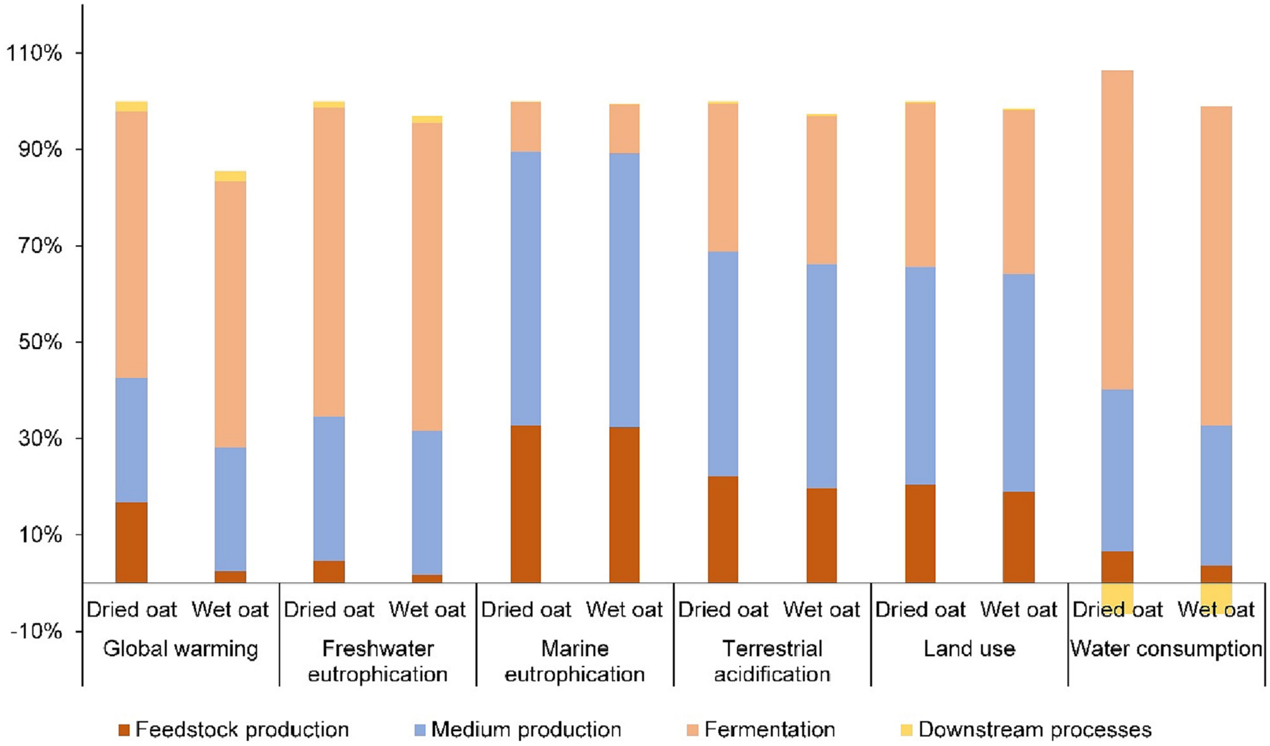

Fig. 2. Relative environmental impacts of OS-SCP production scenarios and contributions of each production stage.

which was proportional to the amount of feedstock, was the main cause of the difference. The relatively small difference in TA between the oat side-streams and other feedstock was because the same amount of diammonium phosphate, which was the main impact contributor, was used for all scenarios.

3.4. Sensitivity analysis

The economic allocation values of the oat side-stream only affected the impacts caused during the feedstock production stage (Fig. 5a). The categories for which energy consumption was the major contributor, such as GW

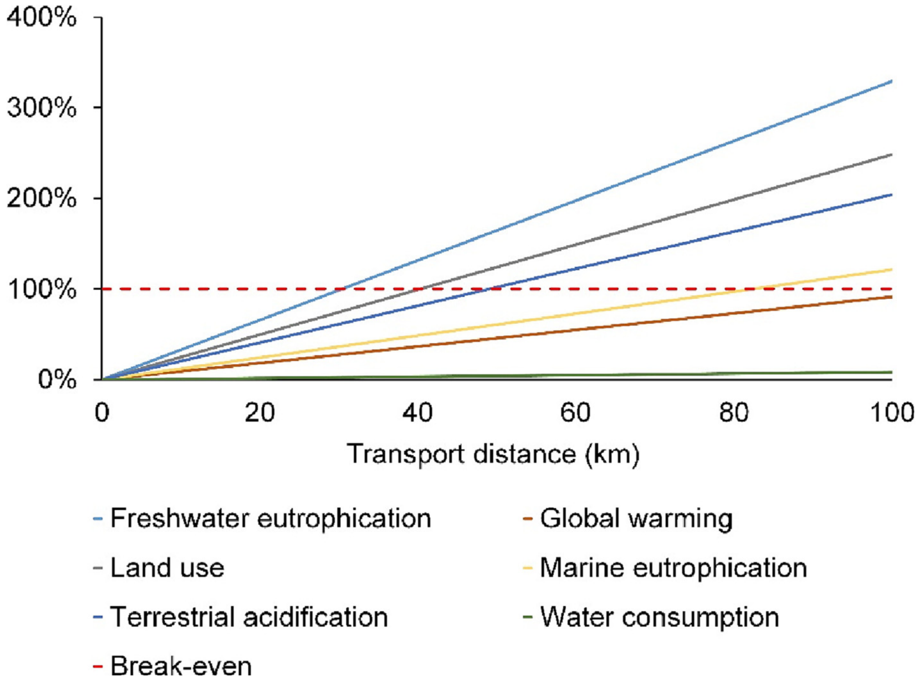

Fig. 3. Impacts of transport of the wet oat side-stream relative to those of the drying process for the dried oat side-stream.

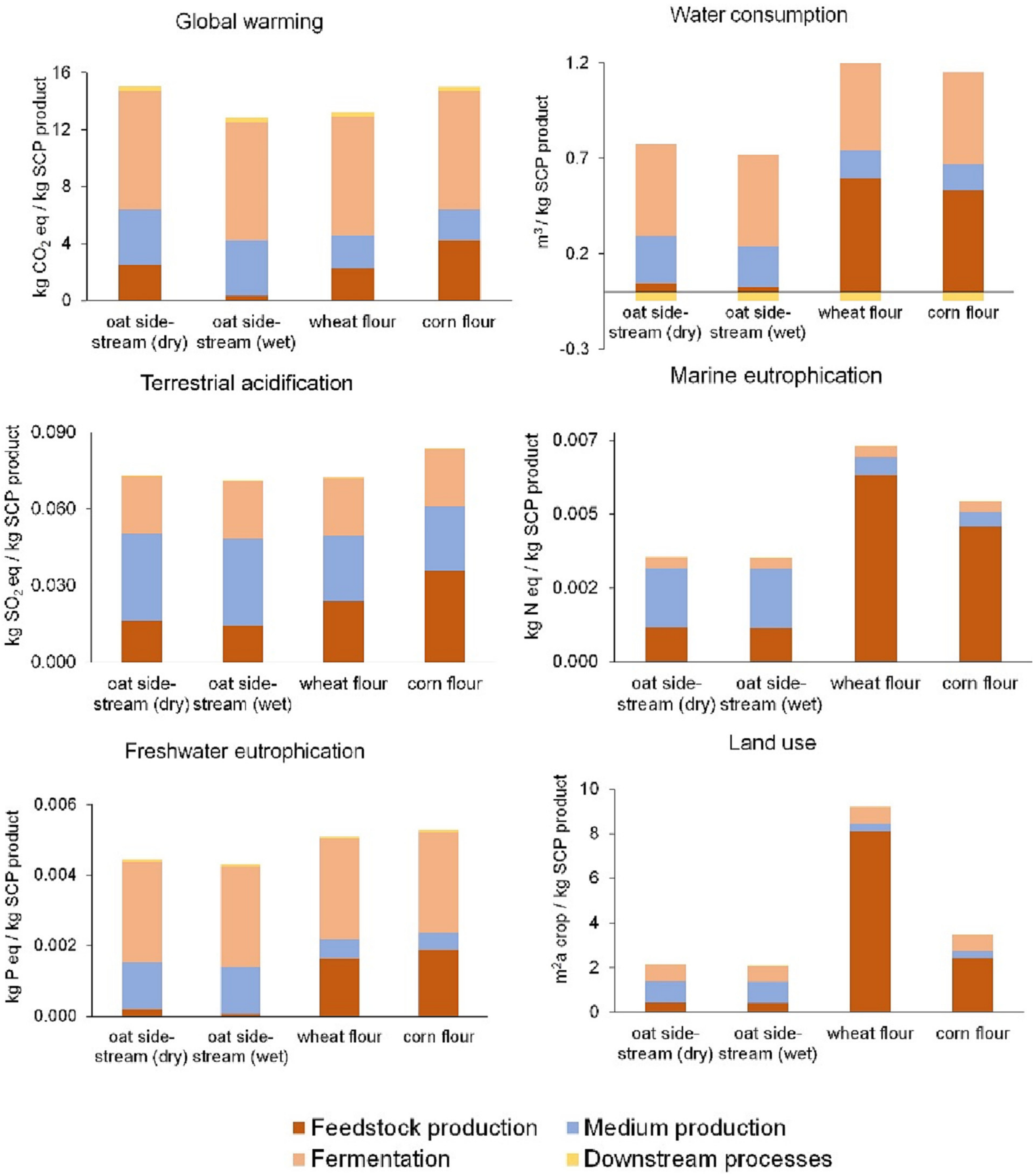

Fig. 4. Environmental impacts of single-cell protein production with different types of feedstocks.

and FE, were confirmed not to be sensitive to the allocation values. After the economic allocation was applied, ME was most affected by a 16 % increase of total impact given 50 % higher economic values, and a 16 % decrease given 50 % lower economic values. The variations of the ME levels were driven mainly by the feedstock preparation changes, with the contribution ranging between 17 % and 49 % with a − 50 % and + 50 % economic allocation scenarios respectively. The ME impact on the feedstock preparation stage was predominantly caused by the oat bran production, with a contribution of 97 %. The location of OS-SCP production showed a stronger influence (Fig. 5b). The most affected was the fermentation stage for all impact categories. This was due to the high electricity requirements, the impacts of which were related to the region's electricity mix. Since the proportion of electricity generated by fossil fuel is large in Australia compared with Finland and Canada, GW, FE, ME, and TA were highest for Australia, while LU was highest for Finland and WC for Canada. The sharp increase in the FE category for Australia was due primarily to the use of hard coal and lignite for electricity production; around 60 % of eutrophication originates from the treatment of spoil from hard coal mining, and the rest, 40 %, comes from spoil treatment from lignite mining. The impacts caused by oat cultivation also led to difference between the selected locations for ME and LU. Changing fermentation time affects the impacts during the fermentation stage, which is energy-intensive (Fig. 5c). The most affected impact categories were GW and FE, where the contributions of electricity consumption were the highest and all categories were noticeably affected.

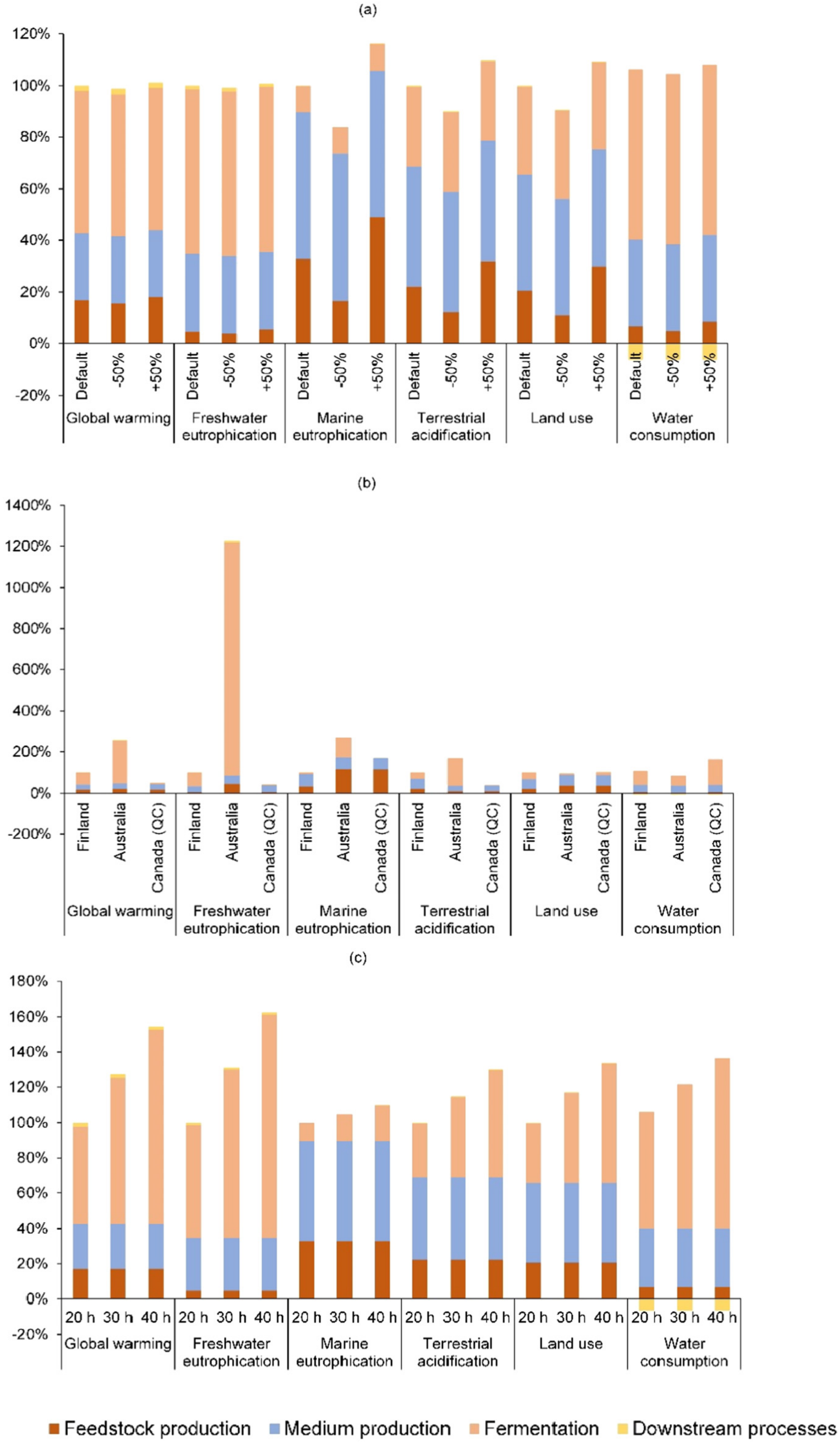

Fig. 5. Results of sensitivity analysis based on (a) economic allocation values, (b) location, and (c) fermentation time.
QC, Quebec.

3.5. Benchmarking

Fig. 6 shows the environmental impacts of different types of potential protein sources for fish feed per kilogram of protein content. The detailed analysis was done based on protein content for the benchmarking.

The environmental performance of the OS-SCP with the wet oat side-streams was better than that with the dried oat side-stream; however, the differences are relatively insignificant compared with other protein products. GW from other protein products was substantially lower except for DMB, that was 13 % higher than OS-SCP (dry) (Fig. 6a). The DMB also caused higher FE (by 24 %) than that of the OS-SCPs (Fig. 6b). The main contributor was the energy used to produce carbon dioxide for feed in the case of DMB, which accounted for 63 % of the total FE impact. For ME, IM had the highest impact due to the rye-based feed for insects that contributed up to 97 % of the total impact (Fig. 6c). The OS-SCPs caused higher FE than the rest while achieving lower impact than DMB (by around

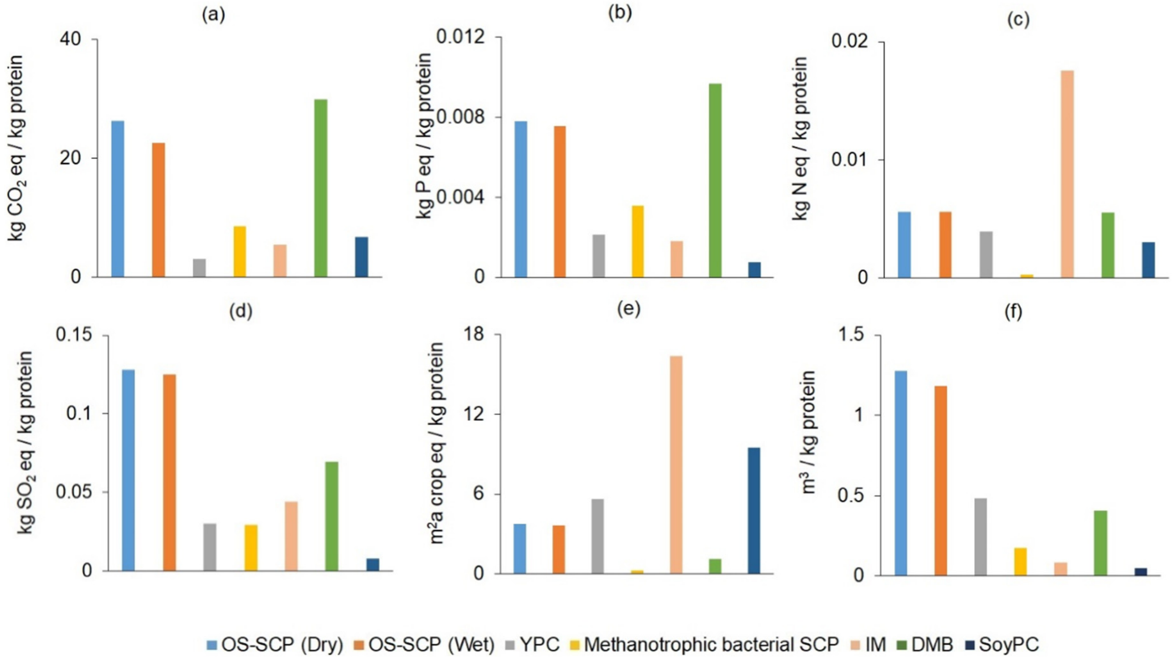

Fig. 6. Environmental impact results per kilogram protein content for (a) global warming, (b) freshwater eutrophication, (c) marine eutrophication, (d) terrestrial acidification, (e) land use, and (f) water consumption.
*(SCP – single cell protein, YPC – yeast protein concentrate, SoyPC – soy protein concentrate, IM – insect meal, DMB – dry microalgae biomass).

19 %) (Fig. 6d). The higher FE of the DMB was mainly caused by the feeding of carbon dioxide to the microalgae, which accounted for 59 % of the total impact outcome. TA and WC were highest for the OS-SCPs (Fig. 6d and f). The main contributor to TA was diammonium phosphate production (26 %), while cooling water and enzyme production were the largest contributors to WC (56 %). Marked differences among the alternative protein products were observed in LU (Fig. 6e). Those using agricultural products in the production process had a large impact on LU. The main contributors were the production of wheat, rye, and soybeans for YPC, IM, and SoyPC, respectively. Although the OS-SCPs also used agricultural products – oats – due to the allocation of the impacts the resulting LU impact was relatively small, with oat production contributed <2 % to the LU impact category for the OS-SCP production. It was, however, still much higher than that for methanotrophic bacterial SCP and DMB, where no direct input of grains and crops were required for the production of feedstock.

4. Discussion

4.1. Environmental sustainability of OS-SCP

The results showed some undesirable consequences of OS-SCP production for most of the impact categories, affecting the overall sustainability of the product. However, environmental advantages can be realized in lowering land use requirements compared with other novel and conventional feed proteins examined and with SCP products from other cereal feedstocks. The achieved reduction of land use offers new areas for natural vegetation that can act as carbon stocks and accelerate carbon sequestration rates (Cook-Patton et al., 2020). This, by extension, can reduce the global warming potential levels and offset part of the carbon equivalent emissions from the OS-SCP production processes. Also, saving land from crop production activities to produce fish feed can improve the biodiversity and natural ecosystem levels, as damages to the ecosystem often correlate with human activities and the spread of croplands (Tilman et al., 2017).

The cultivation of oats is driven by the production of mainstream products including oat oil, oat proteins, beta glucan, and oat starch (Heusala et al., 2020). If the impacts of oat cultivation are eliminated from the analysis, impact results can be reduced by up to 31 % for ME, 16 % for TA, and 18 % for LU. However, with regard to the comparison with other studied feed protein products, the overall conclusions do not change. The environmental impact of OS-SCP production neither exceeds nor goes below the impact category levels of all the other products except for YPC, where the results indicated lower ME levels than the YPC product.

4.2. Credibility and generalization of OS-SCP

Comparisons between different types of protein sources for fish feed are not straightforward. Here the impacts were analyzed based on the protein content of products. However, the protein quality and other nutrients in the products are different, likely affecting the growth of fish. The amount of alternative protein sources that can replace conventional fish feed ingredients depends on factors other than protein content. Cottrell et al. (2020) analyzed previous studies of various protein sources to replace fish meal in fish feed and showed that the plausibility of the replacement could vary depending on the source of protein and nutritional content. Multiple replacement challenges related to economic scale-up for microalgae-based, protein quality for yeast-based, and palatability issues for methanotrophic bacterial SCPs are still unresolved (Jones et al., 2020; Moran et al., 2018; Ritala et al., 2017). Besides the possibility of providing novel feed as supplements, the nutrition benefits that these feed can provide should also meet the common recommendations if they are served as complete diets, including protein (18–50 %), carbohydrates (15–20 %), lipids or fats (10–25 %), ash (<8.5 %), phosphorus (<1.5 %), and trace amounts of vitamins and minerals (Craig et al., 2017). The final output of OS-SCP had 57 % protein, making it a protein-rich product that can be used as a protein supplement in fish feed diets. There are other methods to produce alternative protein products and the ones studied here may not be the most environmentally sustainable. Some methods are independent of outdoor agriculture and rely on autotrophic bacteria in producing microbial proteins using carbon dioxide (Pikaar et al., 2018), or methane as a carbon source (Tallentire et al., 2018). These SCP products had lower impacts on most categories than the studied OS-SCP including global warming (52–67 %), freshwater eutrophication (50–54 %), and land use (67–92 %) (Järviö et al., 2021; Tallentire et al., 2018). The environmental advantages reflect the lower heat and electricity demands needed for OS-SCP production in drying side-streams and for medium preparation. Moreover, most novel feed – including OS-SCP – are not mature products and there is the potential to improve their environmental performance with technological advancements.

The production of OS-SCP relied on the input of natural gas for heating and nutrient production for the medium preparation, contributing to environmental impacts such as GW and TA. Unlike the production route of 1st generation SCP, the production of 2nd generation SCP can lessen the dependency on natural gas and synthetic chemicals via the integration of renewable energy systems, anaerobic digestion, recovery of nutrients, biogas cleaning, carbon capture technologies, and fermentation (Khoshnevisan et al., 2022). This production route goes in line with the circular economy objective of brining side-streams back into the economic system (Areniello et al., 2023).

In the study of YPC, methanotrophic bacterial SCP, and SoyPC for fish feed conducted by Couture et al. (2019), improved performance was found for YPC and methanotrophic bacterial SCP compared with SoyPC for the impact categories of climate change, acidification, freshwater and marine eutrophication, land occupation, water consumption, and primary production requirements. In contrast, the impact category with the highest impact for SoyPC among these three products was LU in this study. YPC was the highest for ME, TA, and WC, and methanotrophic bacterial SCP was the highest for GW and FE. These differences were likely due to different processes assumed to produce each protein product and/or the location of production. For example, YPC was produced in Norway and methanotrophic bacterial SCP and SoyPC were produced in the USA in Couture et al. (2019). In our study, all were assumed to be produced in Finland. The sensitivity analysis in this study showed that the environmental impacts can vary considerably depending on the production location. This was mainly driven by the electricity grid mix where countries with high fossil fuel consumption (i.e., Australia) had higher impacts than countries where renewable energy sources contributed to at least half of the electricity consumption. Results showed that >80 % of GW and FE in Australia originated from lignite and coal mining activities to produce fossil fuel. Sillman et al. (2020) demonstrated the significance of renewable energy sources in lowering overall environmental impacts of microbial proteins via the concept of Power-to-Food (PtF) in several countries (i.e., Finland, Cyprus, and Germany). Regional transitions to renewable sources for electricity production can help reduce the differences of environmental impacts observed in the sensitivity analysis for OS-SCP production. Maiolo et al. (2020) investigated multiple production scenarios for IM and methanotrophic bacterial SCP and showed marked differences in environmental impacts.

Since the use of an oat side-stream as substrate for SCP production is still in the early stage, there is high potential for system optimization and reduction of associated environmental impacts. As shown in the sensitivity analysis, the electricity sources strongly affect the environmental performance of OS-SCP production. The production location and energy sources need to be carefully selected. Russia, Canada, Poland, Finland, and Australia were among the top five oat producing countries worldwide in 2021, together accounting for 52 % of global production (FAOSTAT, 2022). Unlike Finland or Canada, where electricity is largely generated from renewable sources (50–62 %), electricity production in Russia, Australia and Poland is still heavily dependent on fossil fuels (64–83 %) (Ritchie and Roser, 2022). The local production of OS-SCP highlights these countries' high global warming potential and freshwater eutrophication levels, as numerically shown in the sensitivity analysis of this study. If electricity consumption can be reduced, especially during the fermentation stage, it would effectively lower the impacts. Hence, the optimal conditions for an OS-SCP production site correlates heavily with electricity consumption and sources.

Due to the unavailability of Australia-specific oat production processes in the Life Cycle Inventory (LCI) database used in this study, the "rest of the world" data were used; hence, these impacts may vary greatly when region-specific data are used.

4.3. Limitations of the study and future work

Since the OS-SCP production has not been established on an industrial scale, assessment for large-scale production was performed based on laboratory-scale data and literature data, assuming a linear relationship for material use, such as enzymes and nutrients, in the culture media. Literature data for a similar scale and theoretical calculations were used for other purposes, such as the calculations of biomass yield and consumption of electricity and water. Due to a lack of local-specific data, some of the data used in this study were from other regions with a similar condition, or an average of a greater region. While environmental assessment of the system with more precise data is recommended when such data become available for more accuracy, this study can be useful to determine the hot spots of the system in order to improve the environmental performance of the OS-SCP production.

The types and amount of enzyme used and medium compositions that affect the biomass quality and yield are other factors to be considered for environmental optimization. The yield could also depend on the system scale and the strains of yeast. Genetic modifications of yeast may also improve biomass yield, efficiency of the use of substrates, OS-SCP nutritional contents, and fermentation parameters such as duration. They could broaden the technological options for the downstream processes such as flocculation (Ravindra et al., 2009; Ritala et al., 2017). These genetic modifications also may contribute to reducing the impacts of the production system.

The duration of fermentation could influence the impacts of the OS-SCP due to the extended operational electricity usage as shown in the sensitivity analysis. A limitation of this study was that although the extension of fermentation time could increase the OS-SCP yield, which affects the environmental impacts per unit mass of OS-SCP, the yield difference was not taken into account. In fact, the laboratory-scale experiment at Matis showed a 25–50 % increase in yield by extending the fermentation time from 20 to 40 h. This was not considered in the sensitivity analysis as the yield used in the default setting of this study was an average value from several literature sources, which may already be close to the optimized yield. The total impact differences due to the different fermentation time could be smaller than the results shown in this study when a 25–50 % increase in yield is considered.

The oat side-stream as well as other side-streams from industrial processes are often discarded. Using these streams to generate valuable products eliminates the environmental emissions of disposal processes such as landfilling. In this study, the environmental benefits of eliminating waste were not quantified. Wastes can generally occur after the fermentation processes during the separation stage in the form of water flows. Those flows include nutrient fractions such as nitrogen and phosphorus that can reach up to 1 % of the final product (Järviö et al., 2021). These nutrients can serve as organic fertilizers if treated back to the economic system. When the associated impacts are eliminated, the environmental performance of the OS-SCP relative to alternative protein sources may improve depending on the future value of these effluents.

Plant-based alternatives to a protein source have long been applied in fish feed. While they reduce some environmental impacts compared with animal-based protein, impaired growth performance and fish health and increased feed and nutrient waste have been identified. The use of SCPs as an alternative protein source is relatively new and studies on their impacts on fish growth and feed waste are currently limited. Several fish growth studies with fish feed that includes yeast as one of the protein sources have been published (Betiku et al., 2018; Hauptman et al., 2014; Leeper et al., 2022; Vidakovic et al., 2016). However, since the experimental conditions in each study are different, e.g. fish feed recipes, strains of yeasts, types and life stages of fish, and proportions of replacements, straightforward conclusions cannot be drawn. The use of functional units that account for fish growth and quality is recommended for comparing environmental impacts among alternative protein sources when the data become available. SCPs produced by different strains of yeast may have different effects on fish growth. Ritala et al. (2017) concluded in their review paper that for salmon and shrimps, *Candida utilis* was a better SCP source than, for instance, *Saccharomyces cerevisiae*. Currently, the yeast strains examined for fish feed are limited. Further investigation of the differences in yeast strains may enhance the value of SCP in fish feed.

The scalability of the production system is important for the SCP to be marketed. The provision of an oat side-stream could affect the scalability. As the amount of side-stream depends on the demand for the main products, its availability cannot be easily controlled. As determined in this study, if long-distance transport of the side-stream is necessary, drying of the side-stream, which increases the environmental impact, would be required.

The analysis at Matis showed that biomass remaining after oat side-stream hydrolysis contained a high protein content (~40 %), which may be used as another protein source for fish feed. Since its suitability has not been studied thus far, it was not considered as a fish feed ingredient in this study. If it is included, the protein yield of the SCP production system with the oat side-stream would increase and reduce the environmental impact per protein content for the system. In addition, the economic value of these biomass fractions is vague. The economic allocation of the biomass solid fractions can bring environmental advantages to OS-SCP production if the economic value contribution is relatively higher than the mass contribution percentage (>30 %).

The environmental performance of the OS-SCP production system may be further improved by modifying the processes, e.g. recycling water, and through the application of simultaneous saccharification and fermentation (SSF). While SSF may simplify the system, special care is needed as the risk of contamination can increase.

The economic aspect of OS-SCP production is also important to be successful in the market. Since the cost of protein is an economic burden for fish feed (Jones et al., 2020), reduction of the cost by using the side-streams as substrate can be advantageous. Economic analysis needs to be performed to quantitatively evaluate the economic benefit of OS-SCP production with the side-stream.

5. Conclusion

As the demand for farmed fish is projected to increase, the demand for good quality fish feed will also increase. To meet future demand, production of diverse types of novel protein ingredients has been studied, since replacing feed ingredients that can be human food, such as SoyPC, may be preferable to tackle the current and future human food crisis. Many of these ingredients are still in the early stage of development and need further optimization and scale-up of the production systems. The yeast-based OS-SCP can be one such future protein source. However, further research is required on scaling up and to improve environmental performance. Despite the undesirable consequences on global warming, water consumption, acidification and eutrophication emissions compared with other fish feed products, the adoption of OS-SCP can deliver environmental benefits to the ecosystem and biodiversity via land use reduction. This can also offer opportunities for new carbon sink areas to offset the rise of global warming caused directly by OS-SCP production processes.

CRediT authorship contribution statement

Yumi Kobayashi: Conceptualization, Methodology, Software, Validation, Formal analysis, Data curation, Writing – original draft, Visualization. **Mohammad EL-Wali:** Writing – review & editing. **Hörður Guðmundsson:** Resources. **Elísabet Eik Guðmundsdóttir:** Resources, Writing – review & editing. **Ólafur H. Friðjónsson:** Conceptualization, Resources, Writing – review & editing. **Eva Nordberg Karlsson:** Conceptualization, Resources, Writing – review & editing. **Marja Roitto:** Conceptualization, Writing – review & editing, Project administration, Funding acquisition. **Hanna L. Tuomisto:** Conceptualization, Writing – review & editing, Supervision, Project administration.

Data and materials availability

The authors declare that all data supporting the findings of this study can be found in the article and/or its Supplementary Information file.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162318.

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