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LCA FOR ENERGY SYSTEMS AND FOOD PRODUCTS

Life cycle assessment of culture media with alternative compositions for cultured meat production

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Abstract

Purpose Cultured meat is produced by cultivating animal cells in a bioreactor in a culture medium that provides nutrients and growth factors. Among other animal sera, fetal bovine serum (FBS) has traditionally been the most common used in the culture medium of mammalian cell cultures, i.e., 10% FBS medium that contains 10% FBS and 90% DMEM/F12 (v/v). As the aim of cultured meat is to replace livestock production, animal component-free culture media needs to be developed.

Methods We analyzed the environmental impact of replacing the 10% FBS culture medium with serum substitutes, i.e., growth factors, Essential 8™, protein hydrolysates from egg-white, eggshell membrane, poultry residues, pork plasma, and pea concentrate, and Tri-basal 2.0 + ITS medium that contains fibroblast growth factor (FGF-2), fetuin, bovine serum albumin (BSA), and insulin transferrin selenium (ITS). Life cycle assessment with a cradle-to-gate approach was used to quantify global warming potential, freshwater and marine eutrophication, terrestrial acidification, land use, water consumption, fossil resource scarcity, particulate matter formation, cumulative energy demand, and ozone formation of preparing 1-L culture medium. Sensitivity analysis was conducted to examine the impact changes under various production conditions including variations in the impact allocation strategy, production location, and energy sourcing.

Results and discussion The 2% FBS medium (2% FBS, 96% DMEM/F12, and 2% growth factors (v/v)) reduced all environmental impacts where marine eutrophication had the highest reduction (77%), while land use was the least affected with a reduction of 6%. The Tri-basal 2.0 + ITS and protein hydrolysates media reduced most of the analyzed environmental impacts. Protein hydrolysates from egg-white had the lowest environmental impacts reducing 81% global warming potential, 28% water consumption, 59% fossil scarcity, 87% eutrophying emissions, 91% terrestrial acidification, 82% particulate matter, and 70% ozone formation, compared to FBS-containing medium. Land use and energy demand were reduced the most by 17 and 37%, respectively, when the 10% FBS medium was replaced with the Tri-basal 2.0 + ITS medium.

Conclusions Changing the input of FBS in culture media from 10 to 2% (v/v) reduced all studied environmental impacts. Further reductions were achieved when FBS was totally replaced by basal media DMEM/F12, Essential 8™, protein hydrolysates, and recombinant growth factors. Land use was the least reduced, as it was driven by starch extraction to produce glucose for the DMEM/F12 basal medium. Culture medium with protein hydrolysates from egg-white achieved the highest impact reductions compared with the FBS-containing medium.

Keywords Serum-free · Protein hydrolysates · Cultured meat · Life cycle assessment · Environment · Cellular agriculture

1 Introduction

The environmental challenge of the current increase in food demand is pushing producers and policymakers to rethink the conventional agriculture production industry (Willett et al. 2019). Agriculture remains one of the main contributors to environmental burdens globally (Campbell et al. 2017), where livestock alone is responsible for approximately 20% of the global anthropogenic greenhouse gas (GHG) emissions (Xu et al. 2021). Cellular agriculture is introduced as an alternative path to mitigate the undesirable environmental consequences of livestock farming (Post et al. 2020; Moritz et al.

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2023). Previous research has demonstrated the advantages of cellular products, such as cultured meat, in overcoming some of the common burdens associated with livestock production, i.e., global warming, and land and water use (Tuomisto and Teixeira De Mattos 2011; Tuomisto et al. 2014, 2022; Mattick et al. 2015; Smetana et al. 2015; Sinke et al. 2023). The cultured meat technology refers to the biotechnological process of growing myocytes in a precise environment by obtaining parent cells from host livestock species and multiplying them in specific cultured media using controlled bioreactors (Zhang et al. 2020).

Standard culture medium formulations consist of components such as synthetic amino acids, inorganic salts, hydrolysis products, glucose, trace elements, and animal sera (Tuomisto et al. 2022). Fetal bovine serum (FBS) is currently the most common serum input to prepare culture media for cultured meat production (Caneparo et al. 2022). FBS has traditionally been used in mammalian cell cultures as a medium component, as it contains natural embryonic growth-promoting factors for enhancing cell growth and proliferation (Gstraunthaler et al. 2013; Sigma aldrich 2023). Current research has shown that FBS use in culture media can be eliminated (Subbiahanadar Chelladurai et al. 2021), while the use of macronutrients, like glucose, is essential, and their source is therefore of major interest. As the aim of cultured meat production is to replace livestock production, an animal-free cultured medium needs to be developed.

Extant studies indicate that culture media components may have a significant environmental impact in cultured meat production (Tuomisto and Teixeira De Mattos 2011; Mattick et al. 2015; Tuomisto et al. 2022; Sinke et al. 2023). This includes FBS sourcing from the animal production stream (van der Valk et al. 2010). Ethical considerations are associated with the slaughtering of pregnant cows and the poor well-being of fetal calves, where continuous suffering can be assumed during the FBS collection process (van der Valk et al. 2018; Kolkmann et al. 2022). To address these concerns together with the high costs of FBS, extant studies have developed and analyzed various FBS-free media that promote bovine cell growth and differentiation (Kolkmann et al. 2022; Messmer et al. 2022; Skrivergaard et al. 2023). Other serum-free media components, i.e., hydrolysates and recombinant growth factors, have provided promising results, as they have shown economic advantages and facilitated similar cell growth rates compared to FBS (Andreassen et al. 2020; Venkatesan et al. 2022; Skrivergaard et al. 2023).

The development of serum-free formulations for culture media is generally driven by the aim to reduce production costs (Fonoudi et al. 2020; O'Neill et al. 2021; Gomez Romero and Boyle 2023; Yamanaka et al. 2023) and to support cell proliferation and differentiation (Messmer et al. 2022; O'Neill et al. 2022; Stout et al. 2022; Nikkhah et al. 2023). Several studies on the life cycle assessment of cultured meat production included serum-free culture media, where results indicated lower environmental impacts of cultured meat compared to livestock meat, i.e., beef (Mattick et al. 2015; Sinke et al. 2023). However, only one study has assessed the environmental impacts of alternative culture media compositions compared to FBS-containing FBS media (Tuomisto et al. 2022). Tuomisto et al. (2022) demonstrated that replacing a culture medium containing FBS with serum-free Essential 8™ resulted in 28–44% lower environmental impacts. Essential 8™ is specially formulated for the growth and expansion of human pluripotent stem cells (Thermofisher 2023). Environmental assessment studies of switching to alternative culture media compositions and comparisons between various serum-free compositions are not yet widely available.

This work is driven by the objective to provide a comparative analysis of the environmental performance of a culture medium containing FBS and alternative FBS-free media containing serum substitutes, protein hydrolysates, and recombinant growth factors. We performed a life cycle assessment (LCA) including a sensitivity analysis to assess the environmental impacts of alternative culture media production.

2 Methods

2.1 Scope of the study

We conducted an attributional LCA of culture medium production. The study uses the cradle-to-gate approach. This includes upstream production processes until the product is ready for use. The functional unit is 1 L of culture medium. The SimaPro PhD 9.3.0.2 (Pré Sustainability, Amersfoort, the Netherlands) with ReCiPe 2016 Midpoint (H) (Huijbregts et al. 2017) and cumulative energy demand (CED) (Hischier et al. 2010) were used for the impact assessment. The following impact categories were evaluated based on their relevance to the studied product systems (Tuomisto et al. 2022), particularly the agricultural and chemical products: global warming potential, particulate matter formation, ozone formation, terrestrial acidification, freshwater eutrophication, marine eutrophication, land use, water consumption, and cumulative energy demand. In addition to the commonly assessed impact categories, the terrestrial ozone formation was included to indicate the level of nitrogen oxides (NO_x) emissions from livestock production to obtain FBS, crops production to obtain glucose, and the energy used to produce the ingredients of the basal medium DMEM/F12, protein hydrolysates, and growth factors.

2.2 System description

The standard culture medium used in this study (10% FBS medium) consists of 90% DMEM/F12 and 10% FBS. A 10%

FBS culture medium is commonly used in mammalian cell cultures for research purposes (van der Valk et al. 2018).

The DMEM/F12 basal medium includes glucose, synthetic amino acids, vitamins, inorganic salts, and other components (Tuomisto et al. 2022). The final dry mass of 1 L prepared solution is equivalent to 0.017 kg, in which glucose represents up to 26% (w/w). In this study, the glucose originated from starch derived from maize production. The inventory inputs for the preparation of the DMEM/F12 can be found in Supplementary Information, sheets 5–9.

FBS is a by-product of the beef production industry through fetal blood derivatives after the slaughter of pregnant cows (van der Valk et al. 2018). The blood obtained from the fetus is refrigerated to enhance blood clotting (Thermo fisher 2023a), with circa 200 km of cooled lorry transportation (Scherhaufer et al. 2020). The blood is centrifuged after blood cell coagulation for 30 min at 10,000 g to obtain 490 g of separated serum (49% of blood (w/w)) (Thermo fisher 2023b), with 2.6 kW power capacity (Thermo Scientific 2014). Finally, the serum is filtrated with nanofilters (pore size 0.1 µm) to sterilize liquids that include heat-labile components (Thermo fisher 2023b). The energy needed for the filtration was 0.54 kWh per m3 feed (Costa and de Pinho 2006). Serum and plasma have comparatively the same-sized fraction of fetal blood, where serum is the part of plasma that lacks coagulant factors (Mathew et al. 2022). FBS obtained from a fetus has a volume equivalent to 300 mL (Thermo fisher 2023a), with a density of 1.05 g/mL (Vitello et al. 2015). The annual beef production from a slaughterhouse corresponds to 15 2-year-old calves and three cows, where a calf weighs 647 kg and a cow weighs 665 kg (Van Paassen et al. 2019). In this study, 23% of the cows in a slaughterhouse were assumed to be pregnant (Nielsen et al. 2019). The quantity of FBS obtained from the slaughterhouse ($Serum_{Total}(kg)$) was calculated in Eq. (1):

$$Serum_{Total}(kg) = (d_{FBS} \times v_{FBS}) \times \%_P \times N_{Cow} \tag{1}$$

where d_{FBS} is the FBS density in kg/L, v_{FBS} is the volume of FBS solution obtained per one pregnant cow per liter, $\%_P$ is the percentage of cows that are pregnant, and N_C is the number of cows in the slaughterhouse. The quantity of FBS per kg of slaughtered cattle from the slaughterhouse ($FBS(kg)$) was calculated in Eq. (2):

$$FBS(kg) = \frac{Serum_{Total}(kg)}{\left(N_{Calf} \times W_{Calf}\right) + \left(N_{Cow} \times W_{Cow}\right)} \tag{2}$$

where N_{Calf} and N_{Cow} are the number of calves and cows in the slaughterhouse needed to produce beef, respectively; and W_{Calf} and W_{Cow} are the weights of a calf and a cow in the slaughterhouse, respectively. The inventory inputs for the production of FBS can be found in Supplementary Information, sheets 11 and 12. The inventory inputs for the preparation of the 10% FBS can be found in Supplementary Information, sheet 1.

2.3 Scenario development

All scenarios were experimentally conducted at the Nofima facility (Andreassen et al. 2020; Lundberg 2022), except for the Tri-basal 2.0 + ITS (Insulin-transferrin-selenium) solution, prepared separately at Aarhus University (Skrivergaard et al. 2023).

This study examined three paths of culture medium alternatives (Table 1):

(a) Reduced concentration of FBS: the FBS content was reduced from 10% in the baseline scenario down to 2% of culture medium volume (v/v), where the remaining 8% was replaced with the existing basal medium DMEM/F12 (6%) and serum substitute growth factors (2%). The relative cell proliferation was up to 100% of control cells after 48 h cultivation of bovine skeletal muscles when FBS volume was reduced to 2% and replaced with growth factors and the basal medium (Andreassen et al. 2020). Ultroser G was used as the serum substitute (Andreassen et al. 2020). Ultroser G is a semi-defined serum replacement designed to replace FBS (Sartorius 2024). It consists of lyophilized powder dissolved in ultra purified water (Sartorius 2021). Due to the lack of information on the specific ingredients of the lyophilized powder, growth factors that exist in the FBS were used in this study, i.e., fibroblast growth factor (FGF-2), insulin-like growth factor (IGF-1) and transforming growth factor (TGF). We considered the concentrations for the growth factors inside the FBS as a likely reference value to that in the serum substitute. FBS contained 111 ng IGF-1, 12.6 ng TGF-β, and 37.3 pg FGF-2 per ml FBS (Cytiva 2020). Ultroser G has a biological activity equivalent to 5 times that of FBS (Sartorius 2021). Hence, the concentrations of the growth factors in the serum substitute were considered 5 times the concentrations in FBS. In this work, growth factors were produced recombinantly in Escherichia coli *(E. coli)*. The production followed the protocol method described in Venkatesan et al. (2022), while the inventory input data were obtained from Trinidad et al. (2023) and altered to fit the scope of this study. The process begins with bacterial cell growth in a shaker flask at 37 °C with 1-L Luria–Bertani broth growth medium. The induction phase takes place with 0.6 mM IPTG at 17 °C, before cultures are centrifuged at 7000 g. The output cell biomass is suspended in a 1-L binding buffer for cell lysis. The binding buffer contains 100 mM HEPES, 500 mM NaCl, 5 mM imidazole, and 5% glycerol (v/v). Affinity

Table 1 Scenarios of the 10% FBS medium replacement. The glucose source to produce DMEM/F12 refers to maize starch in all scenarios. Percentages refer to % v/v. PH_ESM, culture medium with eggshell membrane hydrolysates; PH_EW, culture medium with egg-white hydrolysates; PH_Pea, culture medium with pea concentrate hydrolysates; PH_Pou, culture medium with poultry residue hydrolysates; PH_PP, culture medium with pork plasma hydrolysates

Scenario	1-L medium composition
Baseline	
10% FBS	90% DMEM/F12 + 10% FBS
Reduced FBS concentration	
2% FBS	96% DMEM/F12 + 2% serum substitute growth factors + 2% FBS
FBS replacement with protein hydrolysates (ph)	
PH_ESM	98% DMEM/F12 + 2% Essential 8™ + 550 mg eggshell membrane hydrolysates
PH_EW	98% DMEM/F12 + 2% Essential 8™ + 550 mg egg-whites hydrolysates
PH_Pea	98% DMEM/F12 + 2% Essential 8™ + 550 mg pea concentrates hydrolysates
PH_Pou	98% DMEM/F12 + 2% Essential 8™ + 550 mg poultry residue hydrolysates
PH_PP	98% DMEM/F12 + 2% Essential 8™ + 550 mg pork plasma hydrolysates
FBS replacement with Tri-basal 2.0 + ITS	
Tri-basal 2.0 + ITS	100% DMEM/F12 + 2 ng/mL FGF-2, 600 µg/mL Fetuin, 75 µg/mL BSA, 1 × ITS (10 µg/mL insulin, 5.5 µg/mL transferrin, 6.7 ng/mL selenium)

chromatography is used for protein purification. The obtained cell proteins are then washed with another 1-L buffer [100 mM HEPES, 500 mM NaCl, 30 mM imidazole, and 5% glycerol (v/v)], before the protein is eluted with 1-L elution buffer [100 mM HEPES, 500 mM NaCl, 250 mM imidazole, and 5% glycerol (v/v)]. The final product output referred to the purified growth factor with a mass ranging between 0.18 and 0.91 mg, depending on the yield of each growth factor. The inventory inputs for the production of all growth factors can be found in Supplementary Information, sheet 15–17. The ingredients of the serum substitute are described and found in Supplementary Information, sheet 10.

(b) FBS replacement with a combination of serum substitute and protein hydrolysates: the FBS-containing medium was replaced with Essential 8™ and protein hydrolysates produced from animal and plant by-products. The ingredients of Essential 8™ are described in Tuomisto et al. (2022). The inventory inputs for the preparation of Essential 8™ can be found in Supplementary Information, sheet 21. The scenarios included culture medium production with protein hydrolysates from poultry residues (PH_Pou), pork blood plasma (PH_PP), eggshell membrane (PH_ESM), egg-white (PH_EW), and pea concentrates (PH_Pea). The designed formulations of protein hydrolysate culture media had various cell growth relative to 100% growth of the control cells in bovine skeletal muscle cells after 48 h of incubation, where PH_PP had 59%, PH_ESM had 72%, PH_EW had 80%, PH_Pou had 50%, and PH_Pea had 70% of the control cells (Andreassen et al. 2020; Lundberg 2022).

The production processes of poultry residues and pea concentrates as raw materials for protein hydrolysates were obtained from LCI databases, i.e., Agri-footprint 5.0 (Van Paassen et al. 2019). The production process of egg-white and the associated amounts of energy and chemicals were obtained from Tsai et al. (2021). Eggshell residues were among the byproducts of the egg-white production process. The production process of the eggshell membrane followed the process developed by Utgård et al. (2016). The production process starts with the centrifugation of eggshell residues with energy equivalent to 28.8 MJ/m^3 (Deng et al. 2013), where the eggshell density is 2540 kg per m^3 (Owuamanam and Cree 2020). Eggshell residues are then separated in cyclone with an energy input of 98.6 kJ per kg of residues (Krokida et al. 2016), before they are sorted in a vibrating screening device with 0.5 kJ per kg of final eggshell membrane product (Deng et al. 2013), accounting for around 1.8% of the total eggs processed (w/w) (Mensah et al. 2021). The screening process results in the eggshell membrane product in addition to liquid egg-white wastes (Utgård et al. 2016). The production of pork plasma comes from blood collected from slaughterhouse for pig meat production (Van Paassen et al. 2019). The blood is transported into the processing site before it is centrifuged for 30 min at 10,000 g with 2.6 kW power capacity (Thermo Scientific 2014). The centrifugation is done to separate plasma that is then freeze-dried (Mok et al. 2021). The energy needed for the freeze-drying is 6.5 MJ per kg of removed water (Pardo and Leiva 2010). Removed water accounted for 82% and plasma powder accounted for 4% of total blood processed (w/w), respectively

(Andreassen et al. 2020). The inventory inputs for the production of raw materials used to produce protein hydrolysates can be found in Supplementary Information, sheet 14.

Protein hydrolysate production from all studied sources followed the same protocol applied at Nofima, Norway (Andreassen et al. 2020). Production begins with heating up a 4.5 L water bath up to 95 °C with energy input calculated as follows (Eq. 3) (Kobayashi et al. 2023):

$$E_t = C_p \times m \times \Delta T \tag{3}$$

where C_p is the specific heat capacity indicating the amount of energy needed to obtain a temperature change per unit mass of material, i.e., 4.186 J/g°C for water (Järviö et al. 2021a); ΔT is the temperature change needed to reach 95 °C, where the initial temperature was considered as 22 °C (Järviö et al. 2021a); and m is the mass of the solution to be heated. The solution containing the raw material undergoes enzymatic hydrolysis by adding up to 5 g enzymes per 500 g of added raw material, incubating at 50 °C while stirring (300 rpm) for 60 min, using an overhead stirrer with a power capacity of 0.08 kW (Radleys 2023). The process is followed by thermal inactivation by microwaving for 2 min using 2.9 kW power capacity. Finally, the product is centrifuged for 15 min at 4400 rpm using a centrifuge with 2.6 kW power capacity (Thermo Scientific 2014). The separated liquid goes through sterile filtration with energy input of 0.54 kWh per m^3 (Costa and de Pinho 2006). The final output is purified protein hydrolysate, in addition to waste streams including biowaste and wastewater (Andreassen et al. 2020). The inventory inputs for the preparation of culture media with protein hydrolysates can be found in Supplementary Information, sheet 13.

(c) Baseline medium replacement with Tri-basal 2.0 + ITS: The baseline culture medium was replaced with the Tri-basal 2.0 + ITS serum-free solution developed at Aarhus University, Denmark (Skrivergaard et al. 2023). The experimental results showed that the developed culture media had similar performance relative to serum media (i.e., 10% FBS) with regards to the proliferation of bovine and porcine satellite cells, in addition to C2C12 muscle cells, where around 1000 nuclei were counted after 2 days of incubation (Skrivergaard et al. 2023). The solution contained fetuin, bovine serum albumin (BSA), FGF-2 growth factor, and small fractions of ITS (Table 1). Fetuin production followed the common processes for recombinant protein production. Mammalian cells are obtained and fed with nutrients at 28 °C, followed by the fermentation stage supplied with glucose as the carbon source. After the growth of the mammalian cells, the solution is separated and purified via the ultrafiltration purification method. The processes and the input amounts of energy and chemicals were obtained from Järviö et al. (2021b). BSA preparation is conducted using the heat shock method (Raoufinia et al. 2016). The process begins by heating blood plasma to 60 °C, a temperature at which the albumin protein can resist while other pathogens are inactivated. The amount of energy needed (E_t) was calculated following Eq. (3), where C_p is 3.21×10^3 J/kg·K for bovine blood (Nahirnyak et al. 2006); ΔT is the temperature change needed to reach 60 °C, where initial temperature was considered as 22 °C (Järviö et al. 2021a). 0.04 M caprylic acid is added to stabilize in pH 5. Ultrafiltration is applied to purify the albumin concentrations leading up to 98% purity levels (Raoufinia et al. 2016).

The inventory inputs for producing the components of the Tri-basal 2.0 + ITS medium can be found in Supplementary Information, sheets 18–20.

2.4 Allocation

Economic allocation was preferred as the default allocation approach due to the different economic values of the main products and other by-products originating from the same processes. The inventory databases (i.e., ecoinvent and agri-footprint) provided the processes that use the economic allocation approach for the background data in addition to several raw materials, i.e., poultry residues and pea concentrates. The economic value of the FBS source (blood) averaged 0.4$ per kg of overall blood mass at the slaughterhouse gate (Le Féon et al. 2020). The economic value of beef and food-grade co-products was 13$ per kg of overall mass (Global product prices 2023). Considering the mass share of FBS to be 0.0035% based on Eq. (1), the allocation factor assigned to the blood produced at the slaughterhouse gate from beef production was thus equivalent to 0.00009%. For pork plasma production, the economic values of pork plasma powder and hemoglobin powder (a by-product of plasma powder production) on the market were 715$ per kg dry mass pork plasma powder (Kowalski et al. 2011; Pel-Freez 2023) and 180$ per kg dry mass hemoglobin powder (Laboratory sales 2023). Plasma powder had a 22% dry matter mass share; therefore, the allocation factor calculated for the pork plasma powder production was equivalent to 53%. Mass allocation was preferred for the egg processing site, as an estimate was not available for the economic values of eggshell residues.

2.5 Sensitivity analysis

A sensitivity analysis was carried out to examine the impact of inventory input variations on the environmental performance of culture medium production. In addition to the baseline scenario, the studied modifications on the inventory input were applied to the direct production processes of media replacements (i.e., protein hydrolysates and Tri-basal 2.0 + ITS).

The country grid mix was the default mean for direct electricity production. Selecting alternative energy sources used in the production processes was included in the aim of studying the potential environmental benefits or costs that these sources could bring to the system. In this study, we analyzed a complete switch into renewable energy sources (i.e., wind, solar, and hydro).

The production country can impact the environmental performance, which is partially driven by the electricity grid mixes and to what extent energy sources are relied upon during the production operations. To examine the sensitivity to various production locations, we analyzed Denmark and Norway in addition to Finland.

We applied mass allocation to assess the environmental changes associated with allocation parameter changes, this was needed considering the variation between economic values and dry matter mass values of the same variables (i.e., FBS, raw materials for protein hydrolysates, and Tri-basal 2.0 + ITS media). The changes in allocation covered the production of FBS and all raw materials in the FBS replacement scenarios.

2.6 Inventory data sources

Ecoinvent 3.8 (Ecoinvent 2021) and Agri-footprint (Van Paassen et al. 2019) were used to provide the LCI data. Agri-footprint provided the LCI data of the raw materials needed to produce lignocelluloses and protein hydrolysates. Literature and published protocols provided the sources of the activity data input (Table 2). Ecoinvent provided the LCI data for all the remaining variables. Global and European data were considered for the chemical production. European data were used for water sourcing, enzyme production, and waste treatment. Region-specific data were used for raw material, electricity, and energy production.

3 Results

3.1 Environmental impact of the baseline scenario—10% FBS

The results of the 10% FBS medium preparation indicated that the FBS—followed by amino acids and glucose—had the largest contribution to the environmental impacts (Fig. 1), i.e., global warming (83%), eutrophying emissions (88%), ozone formation (73%), fossil resource scarcity (61%), energy demand (49%), terrestrial acidification (91%), and particulate matter (83%). The contributions were driven by cattle production in the upstream of the FBS chain, which created more than 97% of the environmental impacts of FBS production. The use of water to obtain the 900 ml volume of DMEM/F12 basal medium was the largest contributor to the water consumption (47%). Glucose used for the basal medium had the largest contribution to land use, accounting up to 40%. This was driven by the land use requirements for maize starch production, where agricultural activities accounted for more than 95% of the total impact. Amino acids contributed similarly to the land use requirements, mainly due to the glucose inputs in amino acid production in the upstream production chain as inputs to the basal medium DMEM/F12.

3.2 Environmental impact of alternative culture medium scenarios

3.2.1 Culture medium with reduced FBS—2% FBS

The environmental impacts of the 2% FBS culture medium scenario showed higher contributions of glucose, amino acids, vitamins, and inorganic salts compared to the baseline scenario (10% FBS) (Fig. 2). This was due to 8% FBS being replaced with serum substitute growth factors and existing DMEM/F12 basal medium. However, FBS was still the largest contributor to global warming (48%), particulate matter (49%), ozone formation (34%), marine eutrophication (80%), and terrestrial acidification (68%). The serum substitute growth factor had the highest impact on freshwater eutrophication contributing around 54% compared to other culture medium ingredients. The eutrophying emissions to freshwater from the serum substitute growth factor were due to the use of distilled and ultra purified water while for the solubilizing the powder-like growth factors (Sartorius 2021).

3.2.2 Culture media with protein hydrolysates

Amino acid, glucose, and inorganic salt production were generally major contributors to most environmental impacts of the culture media with protein hydrolysates, whereas water consumption was mainly driven by the water used for the basal media (DMEM/F12) (Fig. 3). Energy consumption and raw material production had higher contribution to the environmental impacts of media with protein hydrolysates from eggshell membrane (PH_ESM), pea concentrates (PH_Pea), and poultry residues (PH_Pou), compared to culture media with protein hydrolysates from pork plasma (PH_PP) and egg-white (PH_EW). This reflects the lower protein hydrolysate yield of eggshell membrane (14.5%), poultry residues (19%), and pea concentrates (30%), compared to other raw materials. Glucose and amino acids had the largest land use impacts for all protein hydrolysate scenarios

Table 2 Life cycle inventory (LCI) data and sources for producing culture medium parameters, including baseline and alternative scenarios

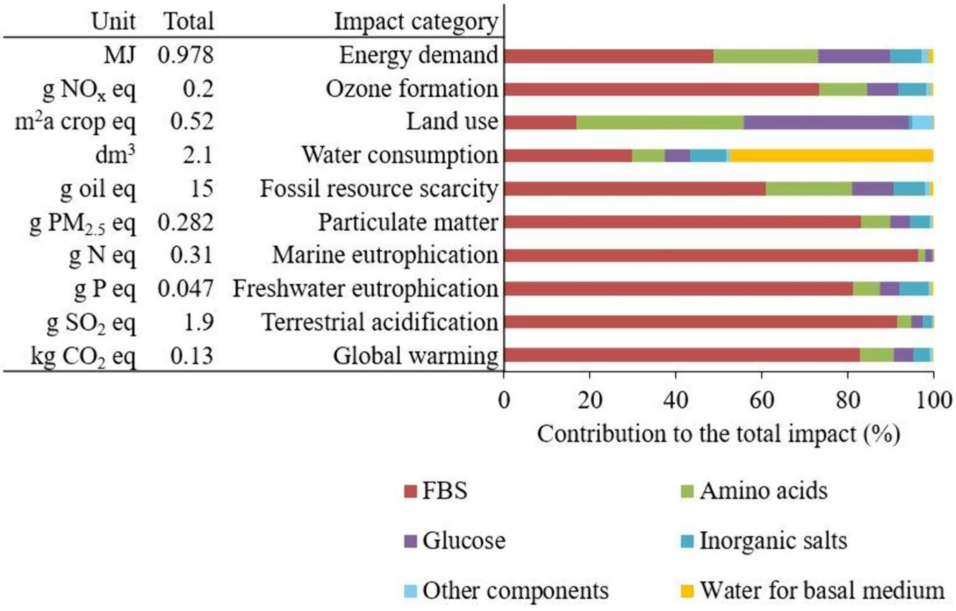
Inputs	Unit	Values	LCI data source
Production of 1 L DMEM/F12			
Amino acids	g	1.048	Tuomisto et al. (2022)
Glucose	g	4.5	Tuomisto et al. (2022)
Vitamins	mg	33.56	Tuomisto et al. (2022)
Inorganic salts	g	10.074	Tuomisto et al. (2022)
Other components	mg	58	Tuomisto et al. (2022)
Water	L	1	Tuomisto et al. (2022)
Production of 500 L FBS (1.05 kg/L)			
Fetal blood	kg	1000	Sharma and Sharma (2018), Mathew et al. (2022)
Electricity	MJ	10.512	Thermo Fisher (2023a)
Transportation	tkm	200	Scherhaufer et al. (2020)
Waste to treatment			
Biowaste	kg	140	Mathew et al. (2022)
Wastewater	L	370	Mathew et al. (2022)
Production of 1 ml serum substitute growth factors			
Ultrapure water	ml	1	Sartorius (2021)
IGF-1	ng	555	Cytiva (2020), Sartorius (2021), Trinidad et al. (2023)
FGF-2	pg	187	Cytiva (2020), Sartorius (2021), Trinidad et al. (2023)
TGF-β	ng	63	Cytiva (2020), Sartorius (2021), Trinidad et al. (2023)
Production of 1 L Essential 8™			
Amino acids	mg	30.8	Tuomisto et al. (2022)
Hydrolysis product	mg	64	Tuomisto et al. (2022)
Sodium borates	mg	543	Tuomisto et al. (2022)
DMEM/F12	L	1	Tuomisto et al. (2022)
Production of protein hydrolysates[a]			
Raw material			
Eggshell membrane	g	500	Mensah et al. (2021), Utgård et al. (2016), Andreassen et al. (2020)
Egg-white	g	500	Tsai et al. (2021), Andreassen et al. (2020)
Pea concentrates	g	500	Van Paassen et al. (2019), Lundberg (2022)
Poultry residues	g	500	Andreassen et al. (2020)
Pork plasma	g	500	Pardo and Leiva (2010), Mok et al. (2021), Andreassen et al. (2020)
Electricity	kJ	19,193.88	Andreassen et al. (2020)
Enzymes	g	5	Andreassen et al. (2020)
Water	kg	5.5	Andreassen et al. (2020)
Waste to treatment			
Biowaste[a]			Andreassen et al. (2020)
Wastewater[a]			Andreassen et al. (2020)
Municipal solid waste[a]			Andreassen et al. (2020)
Production of 1 L Tri-basal 2.0 + ITS			
DMEM/F12	L	1	Tuomisto et al. (2022)
FGF-2	µg	2	Venkatesan et al. (2022), Trinidad et al. (2023)
Fetuin	mg	600	Venkatesan et al. (2022), Järviö et al. (2021b), Harding (2008)
BSA	mg	75	Raoufinia et al. (2016), Harding (2008)
Insulin	mg	10	Tuomisto et al. (2022)
Transferrin	mg	5.5	Tuomisto et al. (2022)
Selenium			
Production of 1 kg glucose from maize starch			Wernet et al. (2016)
Electricity	kWh	0.416	
Enzymes	kg	0.00033	
Heat	MJ	1.15	

Table 2 (continued)

Inputs	Unit	Values	LCI data source
Maize starch	kg	0.9	
Steam	MJ	0.2	
Water	kg	0.3	
Emissions to air			
Nitrogen, atmospheric	kg	0.019	
Waste to treatment			
Wastewater	ml	2.7	

[a] Mass outputs of protein hydrolysate and related wastes depend on the raw material, choice of enzyme, and hydrolysis conditions used. Outputs for protein hydrolysates and wastes from each raw material can be found in the Supplementary Information, sheet 13

Fig. 1 Environmental impact results of 1 L 10% FBS medium preparation



(86–92% of total land used to produce culture with protein hydrolysates). This was mainly driven by the agricultural activities of starch cultivation for both inputs. Raw materials for culture media with protein hydrolysates from pea concentrates (PH_Pea) and poultry residues (PH_Pou) contributed the most to marine eutrophication with 66% and 34%, respectively. For protein hydrolysates from pea concentrates, pea cultivation was predominantly the driver of nitrogen emissions to marine ecosystems with more than 95% contribution, while chicken food production was the driver for marine eutrophication of protein hydrolysates from poultry residues (> 99%).

3.2.3 Tri-basal 2.0 + ITS culture medium

The environmental impacts of the Tri-basal 2.0 + ITS culture medium were driven by DMEM/F12 production (Fig. 4). Growth factors contributed most to marine eutrophication,

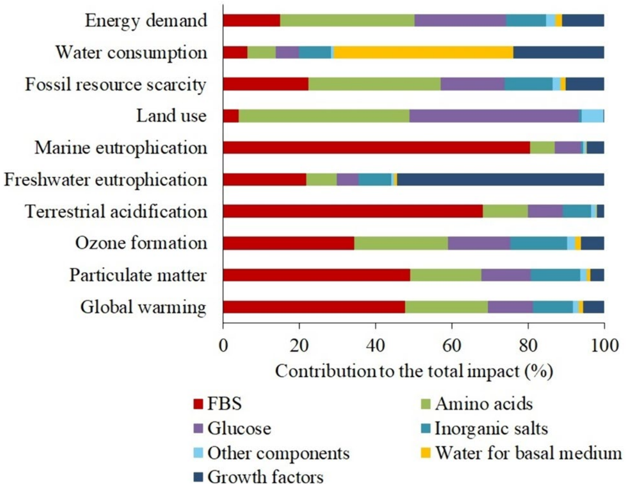


Fig. 2 Contribution of different components to the environmental impacts of 1-L culture medium consisting of 96% DMEM/F12, 2% growth factors, and 2% FBS (v/v)

reaching up to 29%. The environmental impacts of growth factors predominantly originated from recombinant fetuin production, with a more than 94% contribution. This was due to the mass differences of inputs to achieve the optimal efficiency for cultured cell proliferation, where recombinant fetuin constituted circa 86% of the mass among other growth factors. The glucose input as a carbon source was the major contributor to the environmental impacts of recombinant fetuin (26–88%).

3.3 Comparison of serum and serum-free culture media

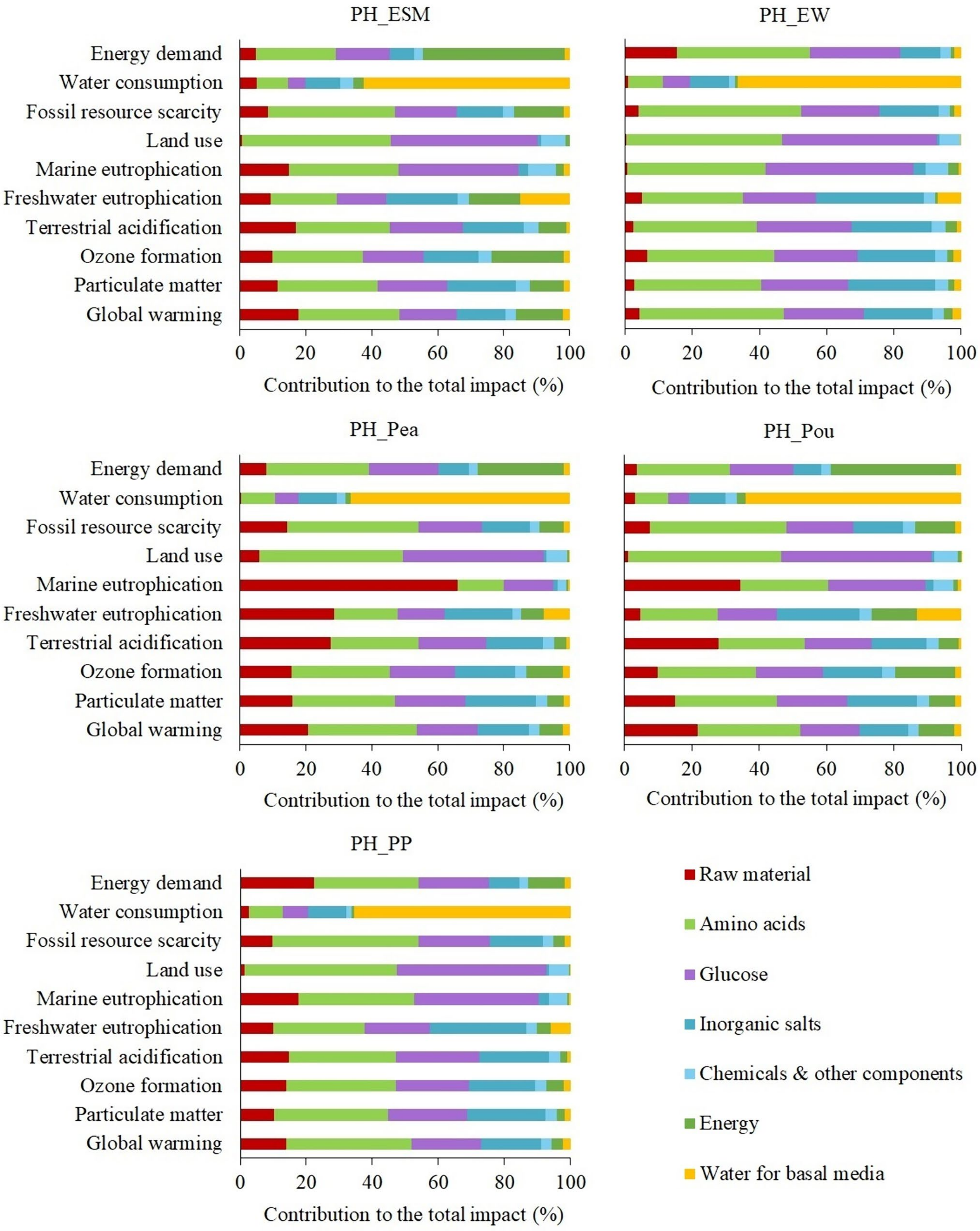
Replacing the baseline 10% FBS culture medium with serum-free alternatives reduced the environmental impacts in most categories (Fig. 5). Reducing FBS down to 2% (v/v) of the culture medium while adding 2% (v/v) recombinant growth factors decreased the overall environmental impacts, as FBS production was the main contributor to most impact categories. FBS reduction reduced marine eutrophication the most by 77%. The Tri-basal 2.0 + ITS culture medium reduced all impact categories. The components of the basal medium (amino acids, glucose, and inorganic salts) were the main contributors, while growth factors contributed, at most, 29% to the environmental implications of culture medium production. Among the protein hydrolysate culture media, PH_ESM reduced the impacts the least. The main factors were the required energy input for the raw material (i.e., eggshell membrane) and the lower protein hydrolysate concentration relative to the other studied raw materials. PH_ESM was the only protein hydrolysate culture medium scenario to exacerbate the cumulative energy demand compared to the baseline medium by less than 1%. However, most energy used for FBS production originated from fossil sources (95%), while 45% of the energy used for protein hydrolysate production from PH_ESM originated from nuclear, biomass, and other renewables. PH_EW significantly reduced most impact categories among the protein hydrolysate culture media, i.e., 81% of the global warming potential, 88% of terrestrial acidification and eutrophying emissions, 82% of particulate matter, 58% of fossil resource scarcity, 28% of water consumption, 16% of land use, 70% of ozone formation, and 21% of the energy demand. This was driven by the lower impact during raw material production and the hydrolysis processes compared to other alternative raw materials.

3.4 Sensitivity analysis

The culture media production for all scenarios was tested against several parameters. Culture media produced with protein hydrolysates were most affected by production location and energy sourcing. Other culture media compositions (i.e., 10% FBS, 2% FBS, and Tri-basal 2.0 + ITS) showed limited variations in their impact results (changes up to 5%). The results of the sensitivity analysis for all culture medium scenarios can be found in the Supplementary Information, sheet 24. The environmental impacts reported in previous sections showed the larger contribution of energy consumption on culture media with protein hydrolysates than with the other medium compositions. Additionally, the energy grid mix in each of the studied countries, i.e., Finland, Denmark, and Norway, drove the major variability associated with location changes of medium production. This led to the impact differences for culture media with protein hydrolysates that relied on energy consumption for protein hydrolysate production.

All culture media with protein hydrolysates had similar sensitivity results, except for PH_ESM, PH_EW, and PH_Pea, where additional energy was required for producing the raw materials. PH_ESM had the highest sensitivity overall among culture media produced with protein hydrolysates. The Finnish electricity sourcing relies on nuclear power which accounted more than 35% of the Finnish electricity mix, while hydro and wind power contributes to 18% and 16% to the country grid mix, respectively. Ten percent of the electricity generated originates from fossil-based sources, i.e., mainly coal (8.9%) (IEA 2022a). Switching to wind energy as the only source for electricity required for the culture medium with protein hydrolysates reduced all the environmental impacts, i.e., 11% of global warming, 25% of energy demand, 12% of fossil resource scarcity, and 20% of ozone formation. The use of hydropower to fuel the culture medium production with protein hydrolysates reduced most environmental impacts, except water consumption which increased by 66%. The increase in water consumption is driven by the evaporation of water from the surfaces of water reservoirs (Bello et al. 2018). The use of solar PV to generate electricity needed for the protein hydrolysates culture medium exacerbated some environmental impacts, i.e., freshwater eutrophication (9%), particulate matter (10%), global warming (0.9%), fossil resource scarcity (2%), and water consumption (4%). The increase in freshwater eutrophication when using solar PV energy was associated with phosphorus flows to the waterbodies following water requirements for solar panel production, installation, and maintenance. Maintenance is frequently required to rid the panel surfaces of accumulating dust (Ecoinvent 2021).

Location changes of the culture media production showed significant reductions in environmental impacts when production was switched to Norway and Denmark (Fig. 6). The change in the source of electricity generation was the main driver of the impact variations. Norwegian electricity consumption relies heavily on hydropower sources, reaching up to 88% of the Norwegian electricity grid mix, while 10% originates from wind energy sources. The Norwegian


PH_ESM
PH_EW
PH_Pea
PH_Pou
PH_PP
Energy demand
Water consumption
Fossil resource scarcity
Land use
Marine eutrophication
Freshwater eutrophication
Terrestrial acidification
Ozone formation
Particulate matter
Global warming
0
20
40
60
80
100
Contribution to the total impact (%)
🟥 Raw material
🟩 Amino acids
🟪 Glucose
🟦 Inorganic salts
🟦 Chemicals & other components
🟩 Energy
🟨 Water for basal media

◀**Fig. 3** Contribution of different components to the environmental impacts of 1-L culture medium consisting of 98% DMEM/F12, 2% Essential 8™ (v/v), and 500 mg protein hydrolysates. PH_ESM, culture medium with protein hydrolysates from eggshell membrane; PH_EW, culture medium with protein hydrolysates from egg-white; PH_Pea, culture medium with protein hydrolysates from pea concentrates; PH_Pou, culture medium with protein hydrolysates from poultry residues; PH_PP, culture medium with protein hydrolysates from pork plasma

production model achieved reductions in global warming (12%), fossil resource scarcity (12%), energy demand (26%), and ozone formation (20%), and freshwater eutrophication (15%) compared to the Finnish production model. The Norwegian production model exacerbated the water consumption by 58% compared to the Finnish model. The higher amounts of water used were driven by the reliance on hydropower sources to generate electricity in Norway (IEA 2022b). Danish electricity consumption mainly originates from renewables, i.e., wind (54%), biomass (18%), and solar (6%). Fossil fuels for electricity generation in Denmark are generally dominated by coal (13% of the total Danish electricity grid mix) (IEA 2022c). The culture medium production in Denmark reduced energy demand (8%) and water consumption (2%), while increasing the other studied impact categories, i.e., global warming (11%), ozone formation (9%), freshwater eutrophication (10%), and fossil resource scarcity (14%), compared to the Finnish production model. The exacerbation of freshwater eutrophication and fossil resource scarcity was driven by coal and natural gas use as part of the Danish grid mix (16% of Denmark electricity generation) (Fig. 6).

Changes to mass allocation increased the environmental impacts of the culture media enriched with FBS (Table 3). The increase in the environmental impact results was driven by changes in the allocation approach of the FBS as by product from beef production. The mass output of the FBS per 1 kg beef cattle from the slaughterhouse was equivalent to 0.017 g following Eq. 2. Hence, the mass allocation percentage for the FBS was 0.00358% (38 times the economic allocation percentage). This explains the higher mass allocation impact results from cattle production. Among the culture media containing protein hydrolysates, PH_PP and PH_Pou showed the highest impact increases. For pork plasma protein hydrolysates, the allocated percentage of impacts was 59% less when the production of plasma powder switched to the mass allocation approach. However, switching from economic allocation to mass allocation for raw materials, i.e., pig co-product feed grade, increased the allocated impacts by approximately 26-fold, leading to higher environmental impacts for culture medium preparation with pork plasma protein hydrolysates. The decrease in the environmental impacts of PH_Pea culture medium was driven by lower mass allocation impacts from pea farming compared to pea concentrate production. Tri-basal 2.0 + ITS medium was the least affected by allocation changes. Changes in allocation strategies were limited to BSA production that had limited mass contribution to the culture medium (less than 1%). Less impacts were allocated to BSA produced from blood plasma compared to the economic allocation scenario, where all the economic value was assigned to BSA and the remaining blood was considered waste.

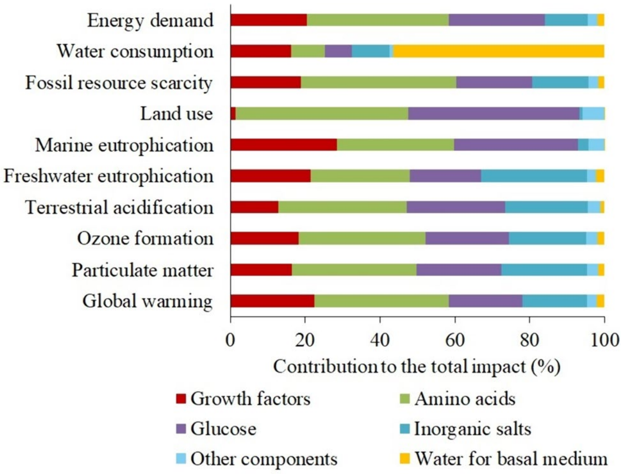


Fig. 4 Contribution of different components to the environmental impacts of 1 L Tri-basal 2.0 + ITS culture medium

4 Discussion

Despite the efforts to lessen the dependency on agriculture production, our results demonstrated the significance of the agricultural ingredient/by-product streams to the environmental impacts of protein hydrolysates, especially those produced from eggshell membranes and poultry residues. The egg production stream was the highest contributor to most environmental impacts of protein hydrolysates from eggshell membrane, contributing around 55% global warming, 50% particulate matter, 64% terrestrial acidification, 65% marine eutrophication, and 36% water consumption. For protein hydrolysates from poultry residues, the raw material (poultry residues) derived from chicken meat production was the highest contributor of global warming (64%), particulate matter (63%), marine eutrophication (85%), terrestrial acidification (80%), and water consumption (32%).

The electricity mix associated with the production location impacted the environmental performance of the culture media with protein hydrolysates. This was majorly driven by variations in the electricity grid mixes for powering the hydrolysis processes, where production in Denmark had the highest impact for most impact categories due to the higher consumption of fossil fuel-produced energy compared to Norway and

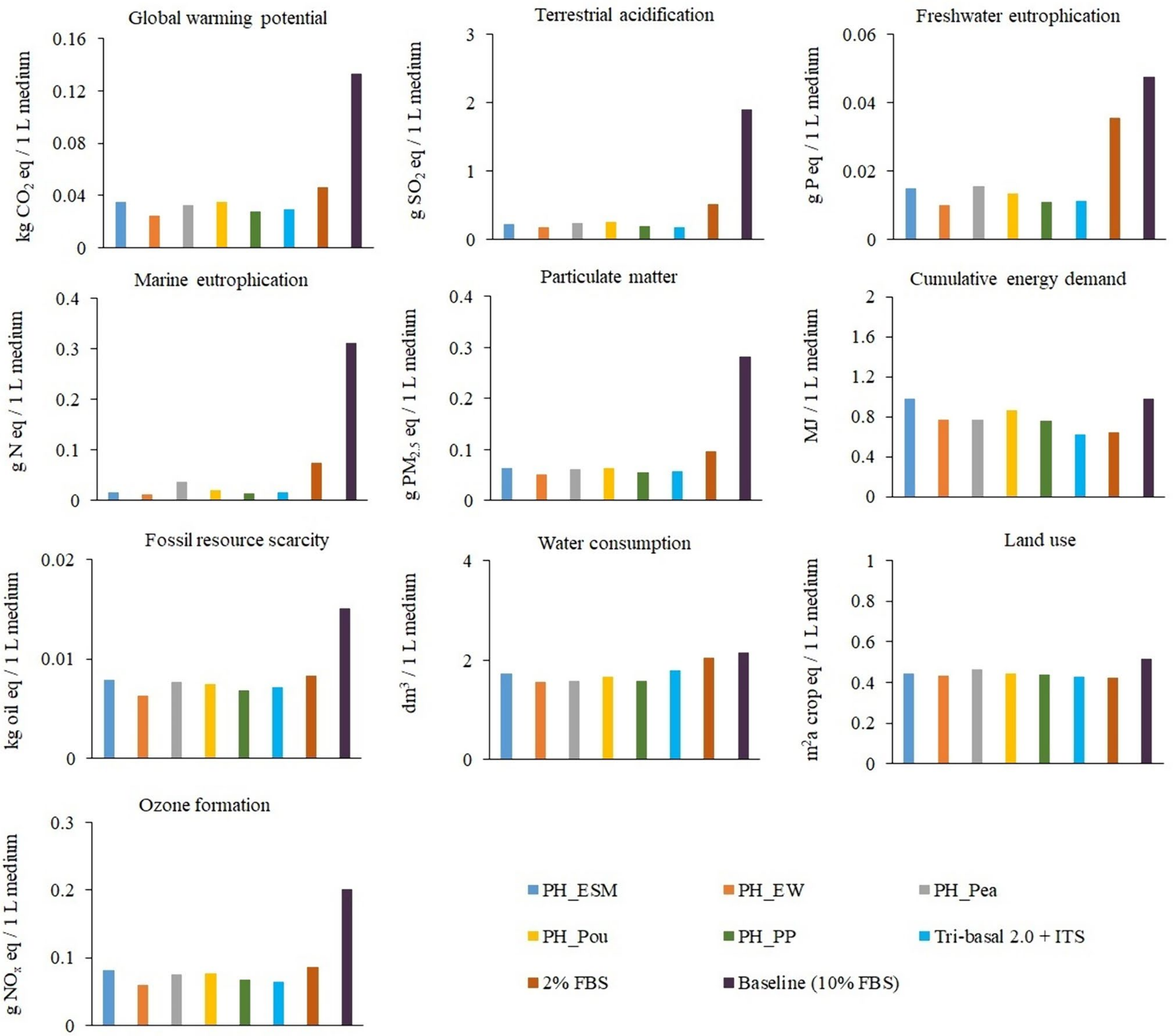


Fig. 5 Environmental impacts of baseline and alternative culture medium scenarios. Baseline medium refers to 10% FBS medium. PH_ESM, culture medium with protein hydrolysates from eggshell membrane; PH_EW, culture medium with protein hydrolysates from egg-white; PH_Pea, culture medium with protein hydrolysates from pea concentrates; PH_Pou, culture medium with protein hydrolysates from poultry residues; PH_PP, culture medium with protein hydrolysates from pork plasma

Finland. Despite the changes in the environmental impact results after the sensitivity analysis, the baseline scenario (serum medium, 10% FBS) still had the highest marks in all categories. The same conclusions apply to alternative energy sources used for protein hydrolysate production: the FBS culture medium still had the highest impacts independent of the changes in production conditions of serum-free media.

Our findings demonstrated the environmental advantage of egg-white over other FBS-free culture media with protein hydrolysates from egg-white had the highest impact reduction of most impact categories compared to the FBS-containing medium (10% FBS). The impact of culture media compositions on cultured meat development and cell growth is essential for determining the feasibility of the analysis. In addition, the skeletal bovine muscle cell measurements in the media with protein hydrolysates at the Nofima facilities showed that hydrolysates from egg-white sources had the highest cell growth (80%) relative to the control cells (Andreassen et al. 2020). The high cell growth associated with the use of egg-white hydrolysis (80%) relative to the control cells and the environmental advantages makes egg-whites a favorable raw material for the preparation of serum-free culture medium with protein hydrolysates. Egg-whites are food-grade by-products that could be used as promoting agents for the growth of bovine skeletal cells (Andreassen et al. 2020). However, egg-whites are

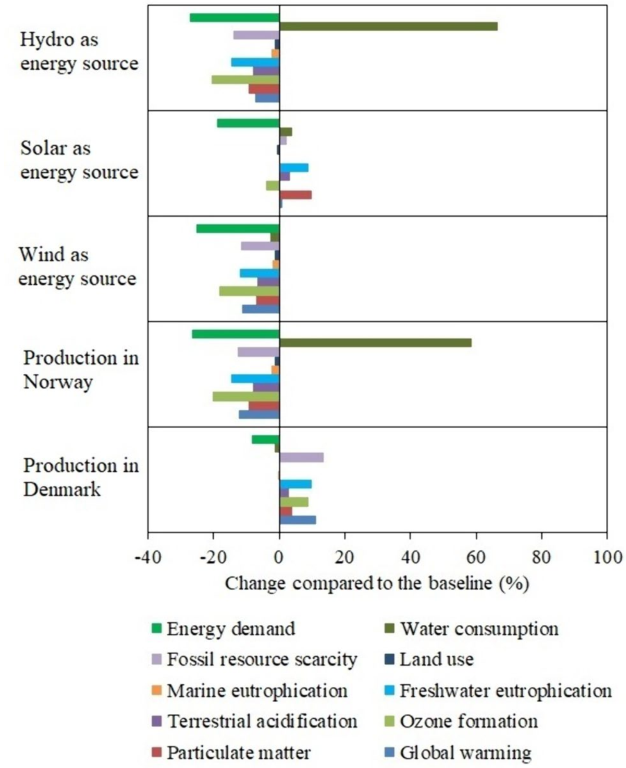


Fig. 6 Changes of environmental impacts associated with changes in production location and energy sources for the PH_ESM (culture medium with protein hydrolysates from eggshell membrane) scenario. Baseline scenario referred to PH_ESM production in Finland powered by the Finnish electricity mix

still livestock products even with less mass contribution to the culture medium compared to FBS-containing medium. Hence, protein hydrolysates from egg-whites does not help in getting a culture medium that is completely independent from animal-based ingredients.

The study successfully compared the environmental impacts of different culture medium compositions used for cultured meat production. However, it is worth mentioning that sources of some ingredients, i.e., amino acids, are not very recent. In this study, the source of the inventory for producing synthetic amino acids used in the basal medium (DMEM/F12) corresponds to Marinussen and Kool (2010), as this is still the only study that presents the detailed information on the ingredients of synthetic amino acid production publicly. In addition, even though the serum substitute that replaced FBS, i.e., Ultroser G, is known to be rich in growth factors, the content of the different growth factors and their concentration levels is not available to the public. Hence, this study considered the serum substitute to contain the same growth factors found in FBS, i.e., FGF-2, IGF-1, TGF-β with their respective concentrations. The environmental impact of the reduced serum scenario (2% FBS) is subject to change given the potential variations between the actual composition of Ultroser G serum substitute and that of the serum substitute considered in this study.

5 Conclusion

Cultured meat production is driven by the need to overcome certain ethical and environmental consequences of livestock farming. Culture media—a vital ingredient for the growth and proliferation of cells to produce cultured meat—is still reliant on livestock derivatives (i.e., FBS), raising the question of whether the cultured meat sector is contributing to its assigned objective. Extant studies show promising results for cell growth and proliferation using FBS-free media compositions compared to FBS-containing media.

Table 3 Percentage (%) changes of impacts after switching from economic allocation to the mass allocation approach for components of culture medium scenarios. The baseline medium refers to 10% FBS medium. PH_ESM, culture medium with protein hydrolysates from eggshell membrane; PH_EW, culture medium with protein hydrolysates from egg-white; PH_Pea, culture medium with protein hydrolysates from pea concentrates; PH_Pou, culture medium with protein hydrolysates from poultry residues; PH_PP, culture medium with protein hydrolysates from pork plasma

	Baseline	2% FBS	PH_ESM	PH_EW	PH_Pea	PH_Pou	PH_PP	Tri-basal 2.0 + ITS
Global warming	2763	1591	3	0.6	−11	34	63	−0.11
Particulate matter	3026	1784	2	0.3	−8	20	45	−0.11
Ozone formation	2345	1101	0.95	0.23	−8	14	23	−0.14
Terrestrial acidification	3463	2579	3	0.65	−14	36	96	−0.09
Freshwater eutrophication	2470	662	0.54	0.14	−15	8	9	−0.09
Marine eutrophication	2694	3087	5	1	−34	51	157	−0.59
Land use	632	153	0.16	0.03	−3	2	3	−0.01
Fossil resource scarcity	1363	499	0.63	0.14	−8	10	15	−0.06
Water consumption	1221	297	0.33	0.06	−0.18	4	6	−0.93
Energy demand	956	269	2	2	−3.5	4	−2	−0.18

In addition to culture medium with reduced FBS from 10 to 2% (v/v), several FBS-culture media was assessed, i.e., culture media with protein hydrolysates from several raw materials (egg-white, eggshell membrane, poultry residues, pork plasma, and pea concentrates), and the Tri-basal 2.0 + ITS that consisted of fetuin, growth factors, bovine serum albumin, and transferrin-insulin-selenium. The results of this paper showed that FBS-free culture media had environmental advantages over the FSB medium. Among the several FBS-free culture media that we analyzed, the culture medium with protein hydrolysates from egg-whites achieved the highest reduction for most impact categories compared to the FBS-containing medium (10% FBS).

In line with the general optimistic views on the benefits of FBS replacements for cultured meat production, our paper highlights the advantages of FBS-free media in reducing environmental burdens associated with the FBS production, by relying on alternative supplements, i.e., recombinant growth factors and protein hydrolysates.

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Author contributions Mohammad El Wali: methodology, software, validation, data curation, writing—original draft, visualization. Heini Karinen: conceptualization, methodology, software, validation, formal analysis, data curation, writing—review and editing. Sissel Beate Rønning: conceptualization, resources, writing—review and editing. Stig Skrivergaard: resources, writing—review and editing. Teodora Dorca-Preda: resources, writing—review and editing. Martin Krøyer Rasmussen: conceptualization, resources, writing—review and editing. Jette Feveile Young: conceptualization, resources, writing—review and editing. Margrethe Therkildsen: conceptualization, resources, writing—review and editing. Lisbeth Mogensen: conceptualization, resources, writing—review and editing. Toni Ryynänen: writing—review and editing, funding acquisition, project administration. Hanna L. Tuomisto: conceptualization, methodology, resources, writing—review and editing, supervision, project administration.

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Data 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.

Declarations

Competing interests The authors declare no competing interests.

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