Supplementary materials to

Ex-ante life cycle assessment of commercial-scale cultivated meat production in 2030

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Data availability statement

Most data generated or analysed during this study are included in this published article and its supplementary materials files. In some cases these are aggregated data, summarized as averages or scenarios, in order to account for variation and to ensure data confidentiality. In a few cases, confidential company data that could not be aggregated are used. In these cases it is mentioned in-text that confidential data are used and these are not included in the article or supplementary materials.

Contents

Appendix A - Overview of previous Life Cycle Assessments about cultivated meat

A few unique studies have been done to date to assess the (projected) environmental impacts of CM production:

- Tuomisto and Teixeira de Mattos (2011) assess a hypothetical large-scale production system that uses cyanobacteria hydrolysate as main input for culture media. Three different countries of production are considered.
- Tuomisto et al. (2014) assess a few hypothetical large-scale production systems with cyanobacteria, wheat and corn hydrolysate as main input for culture media. Worst and best case scenarios are considered.
- Mattick et al. (2015) assess a hypothetical mature production process with detailed modeling based on Chinese Hamster Ovary (CHO) cell cultures. Hydrolysate as well as defined basal media are included.
- Tuomisto et al. (2022) assess a bench-scale production process for CM in hollow fiber bioreactors using experimental data from mouse myoblast C2C12 cells. Various scenarios are included, among which for different cell metabolism, different media formulations and different energy sources.

Building on these unique studies, a couple of additional (scenario) analyses have been done (Smetana et al. 2015, 2018; Lynch and Pierrehumbert 2019). These have also been included as far as possible in this appendix.

The main study characteristics and results are summarized in Table A.1 and Table A.2 respectively.

Table A.1 - Overview of published environmental assessments on cultivated meat production known to date

Authors (year)	LCA type	System(s) under study	Functional unit(s)	Life cycle stages considered	Environmental indicators[a]	Main feedstock for culture media	Main energy sources	Data sources
Tuomisto and Teixeira de Mattos (2011)	Comparative ex-ante LCA of CM, beef, sheep, pork and poultry	Large-scale (30 x 1,000 L) CM production facilities in Thailand, Spain and California.	1,000 kg of cultured meat (30%dm, 19%protein)	Cyanobacteria cultivation, sterilization and muscle cell cultivation (excl. biomass transportation and bioreactor production, excl. Energy for heating/cooling)	Energy use, Climate change, Land use, Water use	Cyanobacteria hydrolysate	Grid electricity from Thailand, California (USA) and Europe. No heating considered as this is created by metabolic heat.	Secondary for cyanobacteria cultivation, materials for bioreactors, hypothetical (theoretical) for CM production processes. Secondary for beef, sheep, pork and poultry.
Tuomisto et al. (2014)	Comparative ex-ante LCA of CM, beef, sheep, pork and poultry	Large-scale (exact sizes not reported) CM production facility in Europe, cyanobacteria production in Spain	1,000 kg of cultured meat (30%dm, 19%protein)	Feedstock cultivation, sterilization and muscle cell cultivation (incl. biomass transportation and bioreactor production, incl. energy for cooling and heating)	Energy use, Climate change, Land use, Water use (indirect and blue water)	Cyanobacteria, wheat and corn hydrolysate	Average European electricity. Electrical heating.	Secondary for cyanobacteria, wheat and corn cultivation, hypothetical (theoretical) for CM production processes. Secondary for beef, sheep, pork and poultry.
Mattick et al. (2015)	Comparative ex-ante LCA of CM, beef, pork and poultry	Large-scale (6 x 15,000 L) CM production facility in California. Data on tissue cultivation derived from studies on Chinese Hamster Ovary (CHO) cell cultivation.	1 kg of CHO cell biomass (17%dm, 7%protein)	Facility operation, proliferation, differentiation and cleaning	CML 2001: Energy use, Climate change, Eutrophication potential. Ecological Footprint: Land use	Glucose, glutamine, soy hydrolysate and basal media	Electricity and heat from fossil mix consisting of mainly natural gas, followed by coal and steam.	Secondary for inputs and fundamental CHO cultivation process characteristics. Hypothetical (theoretical) for large-scale production. Secondary for beef, pork and poultry.
Smetana et al. (2015)	Basic cradle-to-plate assessment of various meat substitutes	-	1 kg of ready-to-eat product (26%protein)	Cyanobacteria cultivation, CM production, storage, transport, cooling, cooking	Energy use, Climate change, Land use	Cyanobacteria hydrolysate	Unspecified, assumed to be identical to Tuomisto and Teixeira de Mattos (2011)	Secondary data mostly based on Tuomisto and Teixeira de Mattos (2011). Further assumptions unclear. The values for Energy use in this assessment are much higher than in Tuomisto and Teixeira de Mattos (2011).

Authors (year)	LCA type	System(s) under study	Functional unit(s)	Life cycle stages considered	Environmental indicators[a]	Main feedstock for culture media	Main energy sources	Data sources
Smetana et al. (2018)	Sensitivity analysis based on existing LCA studies	Utilization of agri-food waste as input for CM production	1 kg of product	-	Energy use, Climate change, Land use, Water use	Bacteria fed on agri-food side and waste streams	Unspecified non-renewable energy.	Secondary data. CM production based on Tuomisto and Teixeira de Mattos (2011) and Mattick et al. (2015).
Lynch and Pierrehumbert (2019)	Sensitivity analysis based on existing LCA studies	Selection of different characterisation method (GWP1000y instead of GWP100y) in combination with different consumption pathways	-	See 'Data sources'	Climate change (Warming impact in ΔK)	See 'Data sources'	See 'Data sources'	Secondary data. CM production based on Tuomisto and Teixeira de Mattos (2011), Tuomisto et al. (2014) and Mattick et al. (2015).
Tuomisto et al. (2022)	Ex-ante LCA of CM	Bench-scale (largest size 21.3L) CM production in hollow fiber reactors with C2C12 cells, medium formulations DMEM/F12+FBS and Serum-free media. Different energy sources included (among which seawater cooling)	1 kg of CM (slurry, 30%dm, 20%protein)	Production of inputs (both medium and other), preparation of culture media, proliferation and differentiation of cells, separation and recycling of spent media, waste disposal	Cumulative Energy Demand (CED), Fossil resource scarcity, Freshwater eutrophication, Global warming, Land use, Ozone formation, Particulate matter, Terrestrial acidification, Water consumption	Single pharmaceutical-grade ingredients included in DMEM/F12 and Essential 8 media formulations, Fetal Bovine Serum (FBS)	Average UK electricity mix for the baseline scenario. Wind and solar energy for sensitivity analyses.	Primary, experimental data for a.o. medium consumption and cell biomass increase. Secondary data and calculations for a.o. energy consumption, CHO cell metabolism and medium ingredients production.
This study	Comparative attributional ex-ante LCA of CM, beef, pork and chicken	Commercial scale CM production in 2030. Facility producing 10 kton/year, working volume of biggest proliferation reactor 10,000L.	1 kg of meat cells (20%-30%dm, 18%-25%protein)	Agricultural feedstock production, medium ingredient and edible scaffold production, gate-to-gate processes including medium preparation, sterilization, seed train, proliferation, differentiation on edible scaffold, harvesting	All ReCiPe Midpoint (2016) impact categories, Cumulative Energy Demand (CED), Feed Conversion Ratio (FCR), greenhouse gas emission profile	Glucose, soy hydrolysate, amino acids from microbial and chemical production, microbial recombinant protein production	Electricity: Global average mix in 2030 and 100% renewable (solar/wind). Heat: natural gas-fired CHP and 100% renewable (geothermal).	Lab-scale primary data from CM producers, full-scale primary data from processes in comparable technologies and data from computational models, supplemented with data from literature. Important data have been cross-checked with experts.

[a]*See Table A.2 for the impact assessment (IA) methods used for characterisation*

Table A.2 - Overview of impact assessment (IA) results of the LCA studies on cultivated meat known to date (per kg edible product)

Publication	Scenario	Impact category *Unless stated otherwise, Energy use method is Cumulative Energy Demand (CED) v 1.11 (Frischknecht et al. 2007) and all other methods from ReCiPe 2016 (H) v1.1 (Huijbregts et al. 2016)*								
		Energy use (MJ)	**Climate change (kg CO_2-eq.)**	**Land use (m^2a)**	**Water use (m^3)**	**Particulate matter (kg $PM_{2.5}$-eq.)**	**Terrestrial acidification (kg SO_2-eq.)**	**Freshwater eutrophication (kg P-eq.)**	**Ozone formation (kg NOx-eq.)**	**Fossil resource scarcity (kg oil-eq.)**
Tuomisto and Teixeira de Mattos (2011)		*Primary Energy Use (method unclear)*	*GWP100y (IPCC 2006)*	*Land for feedstock cultivation (method unclear)*	*Freshwater use (blue + green) (Milà I Canals et al. 2009)*					
	Thailand	25.20	1.89	0.19	0.38					
	California	31.80	2.24	0.23	0.37					
	Spain	31.70	1.90	0.23	0.52					
Tuomisto et al. (2014)		*Primary Energy Use (calculated with energy conversion factors)*	*GWP100y (IPCC 2006)*	*Land for feedstock cultivation (method unclear)*	*Water scarcity (Pfister et al. 2009)*					
	Cyanobacteria-best case	38.70	2.27	0.46	0.52					
	Wheat-best case	35.50	3.27	2.60	0.33					
	Corn-best case	34.50	2.96	2.82	0.84					
	Cyanobacteria-worst case	60.90	3.38	0.46	0.52					
	Wheat-worst case	57.70	4.38	2.60	0.33					
	Corn-worst case	56.70	4.07	2.82	0.84					
Mattick et al. (2015)		*CED v1.? (Frischknecht et al. 2007)*	*CML 2001 (Guinée et al. 2002)*	*Ecological Footprint (Frischknecht et al. 2007)*			*CML 2001 (Guinée et al. 2002)*	*CML 2001 (Guinée et al. 2002) (g PO4-eq.)*		
	Baseline	106.00	7.50	5.50			0.07	7.9		
	Lower end (90% CI)	43.46	3.15	2.92				-39		
	Upper end (90% CI)	315.88	22.28	8.47				54		
Smetana et al. (2015)		*ReCiPe 2008 (Goedkoop et al. 2013)*	*ReCiPe 2008 (Goedkoop et al. 2013)*	*ReCiPe 2008 (Goedkoop et al. 2013)*						
	Low	290.70	23.90	0.39						
	High	373.00	24.64	0.77						
Tuomisto et al. (2022)	CMB	532.78	25.19	6.89	0.54	0.04	0.12	0.01	0.05	7.60
	CMB128	231.34	12.64	3.36	0.32	0.02	0.06	0.00	0.03	3.83
	CMC	94.09	4.88	1.84	0.12	0.01	0.03	0.00	0.01	1.33
This study	Baseline-conventional energy	277.55	14.34	2.41	0.07	0.01	0.03	0.001	0.02	4.23
	Baseline-renewable energy	163.83	2.82	2.48	0.09	0.01	0.02	0.001	0.01	0.76
	Low-conventional energy	187.88	9.60	2.25	0.06	0.01	0.02	0.001	0.02	2.90
	Low-renewable energy	116.48	2.21	2.29	0.07	0.01	0.01	0.001	0.01	0.61
	High-conventional energy	481.70	24.80	3.47	0.13	0.02	0.05	0.003	0.04	7.66
	High-renewable energy	287.81	5.00	3.59	0.15	0.01	0.03	0.003	0.02	1.53

Appendix B - Complete impact assessment results for CM and conventional meats

The impact assessment results for the CM baseline model in the three energy scenarios is provided in Table B.1. The comparison of ambitious benchmarks of both CM and conventional meats is provided in Table B.2. Both the full range of indicators from ReCiPe 2016 and Cumulative Energy Demand (CED) are included.

Table B.1 - Impact assessment results for the baseline scenario of CM

Scenario		**Baseline scenario (mid medium)**		
Impact category	Unit	Ambitious benchmark	Renewable Scope 1&2	Global average energy
Global warming	kg CO_2-eq.	2.82E+00	4.04E+00	1.43E+01
Stratospheric ozone depletion	kg CFC_{11}-eq.	5.64E-06	6.20E-06	1.10E-05
Ionizing radiation	kBq Co-60-eq.	1.69E-02	2.80E-02	1.23E-01
Ozone formation, Human health	kg NO_x-eq.	8.05E-03	9.68E-03	2.35E-02
Fine particulate matter formation	kg $PM_{2.5}$-eq.	6.29E-03	6.55E-03	8.77E-03
Ozone formation, Terrestrial ecosystems	kg NO_x-eq.	8.38E-03	1.00E-02	2.39E-02
Terrestrial acidification	kg SO_2-eq.	1.75E-02	1.86E-02	2.81E-02
Freshwater eutrophication	kg P-eq.	1.00E-03	1.05E-03	1.48E-03
Marine eutrophication	kg N-eq.	1.29E-03	1.29E-03	1.27E-03
Terrestrial ecotoxicity	kg 1,4-DCB-eq.	5.84E+01	5.40E+01	1.63E+01
Freshwater ecotoxicity	kg 1,4-DCB-eq.	6.60E-02	6.59E-02	6.51E-02
Marine ecotoxicity	kg 1,4-DCB-eq.	5.04E-02	4.86E-02	3.33E-02
Human carcinogenic toxicity	kg 1,4-DCB-eq.	1.27E-01	1.21E-01	7.20E-02
Human non-carcinogenic toxicity	kg 1,4-DCB-eq.	2.25E+00	2.26E+00	2.34E+00
Land use	m^2a crop-eq.	2.48E+00	2.47E+00	2.41E+00
Mineral resource scarcity	kg Cu-eq.	3.96E-02	3.74E-02	1.85E-02
Fossil resource scarcity	kg oil-eq.	7.62E-01	1.14E+00	4.32E+00
Water consumption	m^3	8.57E-02	8.42E-02	7.12E-02
Cumulative energy demand	MJ	1.64E+02	1.76E+02	2.78E+02

Table B.2 - Comparison of ambitious benchmarks for 2030 of CM and conventional meats

Indicator	Unit	CM	Chicken	Pork	Beef (dairy cattle)	Beef (beef cattle)
Global warming	kg CO_2-eq.	2.82E+00	2.74E+00	5.08E+00	8.85E+00	3.49E+01
Stratospheric ozone depletion	kg CFC_{11}-eq.	5.64E-06	2.08E-05	4.32E-05	8.84E-05	4.77E-04
Ionizing radiation	kBq CO-60-eq.	1.69E-02	1.02E-02	1.10E-02	7.21E-03	3.36E-02
Ozone formation, Human health	kg NO_x-eq.	8.05E-03	7.68E-03	8.44E-03	6.59E-03	2.81E-02
Fine particulate matter formation	kg $PM_{2.5}$-eq.	6.29E-03	7.91E-03	1.08E-02	2.17E-02	9.97E-02
Ozone formation, Terrestrial ecosystems	kg NO_x-eq.	8.38E-03	7.77E-03	8.53E-03	6.68E-03	2.84E-02
Terrestrial acidification	kg SO_2-eq.	1.75E-02	5.71E-02	7.98E-02	1.70E-01	7.84E-01
Freshwater eutrophication	kg P-eq.	1.00E-03	5.77E-04	7.32E-04	3.66E-04	1.74E-03
Marine eutrophication	kg N-eq.	1.29E-03	5.25E-03	1.03E-02	2.27E-02	1.44E-01
Terrestrial ecotoxicity	kg 1,4-DCB-eq.	5.84E+01	4.91E+00	6.84E+00	3.58E+00	1.28E+01
Freshwater ecotoxicity	kg 1,4-DCB-eq.	6.60E-02	2.82E-01	3.24E-01	1.29E-01	6.24E-01
Marine ecotoxicity	kg 1,4-DCB-eq.	5.04E-02	5.25E-02	6.17E-02	3.11E-02	1.32E-01
Human carcinogenic toxicity	kg 1,4-DCB-eq.	1.27E-01	2.90E-03	3.97E-03	3.78E-03	8.21E-03
Human non-carcinogenic toxicity	kg 1,4-DCB-eq.	2.25E+00	4.51E+00	1.04E+01	1.57E+01	6.55E+01
Land use	m^2a crop-eq.	2.48E+00	6.80E+00	7.52E+00	5.46E+00	2.43E+01
Mineral resource scarcity	kg Cu-eq.	3.96E-02	2.24E-03	2.52E-03	2.31E-03	1.03E-02
Fossil resource scarcity	kg oil-eq.	7.62E-01	3.61E-01	4.17E-01	4.14E-01	1.88E+00
Water consumption	m^3	8.57E-02	6.72E-02	4.58E-02	7.45E-02	2.53E-01
Cumulative Energy Demand (CED)	MJ	1.64E+02	2.29E+01	2.65E+01	2.55E+01	1.04E+02

Appendix C - Acknowledgement of organizations that contributed with data and/or cross checks of data ranges

In Table C.1, the organizations that agreed to be acknowledged are summed up. A few organizations did not want to be acknowledged or did not respond, for various reasons.

Table C.1 – Organizations that provided data and/or cross checks of data ranges

Company or institute	Expertise
A*star	Cultivated meat research institute (Avian)
Aleph Farms	Cultivated meat production (Bovine)
Avant Meats	Cultivated meat production (Fish)
Mosa Meat	Cultivated meat production (Bovine)
Shiok Meats	Cultivated meat production (Crustacean)
Wild Type	Cultivated meat production (Fish)
Akron Bioproducts	Recombinant proteins, scaffolds, cell banking systems
Black & Veatch	Consulting engineering and design-build services
Buhler	Extrusion and feed pre-mix
Cell-trainer Biotech	Consulting engineering
CE Delft	Sustainability research and consultancy firm
Evides	Water production and treatment
The Good Food Institute	NGO in the field of alternative protein research and innovation
Merck[a]	Cell culture media and other process related products (e.g. equipment and filters)
OSPIN	Bioreactors and tissue chambers for cell expansion and differentiation
Laurus Bio	Recombinant proteins
Warner Advisors	Consulting engineering

[a]*Merck KGaA, Darmstadt, Germany. https://www.emdgroup.com/en/research/innovation-center/innovation-fields/cultured-meat.html*

Appendix D - Data and data quality assessment

Main process design parameters values, sources and data quality

The main process design parameters are provided in Table D.1. The main sources for inputs into the LCA model are provided in Table D.2. The type and number of sources, data quality and whether an independent cross-check has been carried out is mentioned. The data quality assessment classification is as follows:

0 No data available at this moment

1 Primary data from representative process and scale

2 Primary data from representative process with extrapolation for scale

3 Primary data from similar process and scale

4 Secondary data from literature

5 Estimate or calculation based on expert judgment

Culture medium (high-level) composition and quantity is provided in Table 1 of the main report. Energy use is provided in Table D.3 and composition of the electricity mix for the global average energy scenario is provided in Table D.4. Estimated byproducts in wastewater are provided in Table D.5. Material use for equipment modeling are provided in Table D.6. Some quantitative data on model inputs is confidential and therefore not included, for example the estimated energy use for recombinant protein production.

Table D.1 - Main process design parameters, their sources and data quality

Main process parameters	Value	Source	Data quality	# of data sources used	Independent cross-check
Annual production of commercial facility in 2030	10 kton	CM and supply chain companies	5	12	No
Species and cell type	Various species, non-GMO cell lines	CM companies	1	7	Yes
Type of production	Semi-continuous production with 3 intermediate harvests	Literature (Specht 2020), confirmed by bioprocessing companies	4	1	Yes
Size of largest proliferation vessel (working volume)	10,000 L	CM and supply chain companies estimate (median)	5	7	Yes
Size and amount of bioreactors at facility (working volumes)	107 x 50 L Stirred-Tank Reactor (STR), 130 x 10,000 L STR; 430 x 2,000 L Perfusion Reactor (PR)	Calculated, based on production line presented in Specht (2020) and project-specific parameters	4/5	-	Yes

Main process parameters	Value	Source	Data quality	# of data sources used	Independent cross-check
Duration of production from inoculum to harvest	42 days (30 days for ~25 doublings + 2 days for additional harvests + 10 days of differentiation and maturation)	Specht (2020), cross-check by bioprocessing companies	4	1	Yes
Maximum cell density during proliferation	$50*10^6$ cells/ml	CM and supply chain companies (median)	2	7	Yes
Avg. cell volume	3,500 μm^3/cell	CM companies (median)	1	4	Yes
Doubling time	30 hours	Conservative round-up from Specht (2020): 28 days. Feasibility validated by companies.	4/2	1	Yes
Oxygen uptake rate (OUR)	4 pmol cell-1 day-1	Average based on literature (Wagner et al. 2011; Tuomisto and de Mattos 2011; Mattick et al. 2015; Super et al. 2016) and 1 CM company	4/2	5	Yes
Aeration rate	0.1 vvm	Conservative estimate based on numbers from (Tuomisto and de Mattos 2011; Mattick et al. 2015; Humbird 2021), rounded upwards to account for higher cell densities	4	3	No
Production of meat per production run	3,080 kg	Calculated, based on production line presented in Specht (2020) and project-specific parameters	5	-	No
Number of production runs needed for producing 10 kton	3,243	Calculated (annual production/production in one production run)	5	-	No
Density of meat	881 kg/m^3	Specht (2020)	4	N.a.	No

Table D.2 - Main sources for process inputs and data quality

Main model inputs and their production	Source	Data quality	# of data points used	Independent cross-check
Energy use for production (heating, cooling, mixing, aeration, pumping)	Calculations by bioprocess engineers with extrapolations by authors	5	1	Yes
Energy use for cleaning: Clean/Steam-In-Place (CIP/SIP)	Calculations by bioprocess engineers with extrapolations by authors	3	1	No
Energy use for HVAC[a]	Calculations by authors	4	1	No
Energy production	Ecoinvent LCA database, modelled after global stated policies scenario in World Energy Outlook 2030 (IEA 2019)	1	n.a.	n.a.
Purified water use for cleaning the bioreactors	Bioprocess engineering companies	3	2	Yes
Purified water production	Water companies	1	1	No
Culture medium composition and quantity	Companies and research organizations	2	10	Yes
Medium recycling rate	CM and supply chain companies	0 (no consensus)	6	Yes

Main model inputs and their production	Source	Data quality	# of data points used	Independent cross-check
Hydrolysate	Colantoni et al. (2017) NB: Soy direct land use change (dLUC) set to 0 (as is done for conventional products)	4	1	No
Amino acids	Data from Marinussen and Kool (2010), Mattick (2014), Mattick et al. (2015). Amino acids modelled: - L-Glutamine - L-Threonine - L-Lysine - D,L-Methionine	4	1	No
Recombinant proteins	Amino acid production data from Marinussen and Kool (2010), Mattick (2014), Mattick et al. (2015) used as the basis. Water and electricity use adapted for recombinant protein fermentation with data from two producing companies.	2	3	Yes
Other medium ingredients	Ecoinvent and Agri-footprint LCA databases	1	N.a.	N.a.
Transport of medium ingredients	Based on standard Ecoinvent values for global markets	1	N.a.	N.a.
Scaffold use	CM and supply chain companies	5	8 companies (total) of which 3 with future estimate of quantity of scaffold used	No
Scaffold production (hydrogel)	De Marco et al. (2017)	4	1	No
Bioreactor production	Tuomisto et al. (2014) and calculations by authors	4	2	Yes
Storage and mixing tanks use	Calculations by authors	5	1	No
Storage and mixing tanks production	Calculations by authors based on industry documentation	4	1	No
Filters for filtration use	Supplying companies	2	1	Yes
Filters for filtration production	Supplying companies	1	1	No

[a]*Heating, Ventilation and Air Conditioning*

Table D.3 - Energy demand of one year of operation of the facility producing 10 kton of CM (MWh)

Process stage	Heat		Electricity				Total	
	Initial heating	Maintaining temperature (heat exchanger)	Mixing (agitation)	Aeration	Pumping	Other	Heat	Electricity
Small-scale proliferation	436	8,780	230	249	12	0	436	9,270
Large-scale proliferation	8,729	175,599	4,602	4,970	230	0	8,729	185,401
Differentiation and maturation	5,893	14,302	3,113	3,923	272	0	5,893	21,610
Cleaning of reactors	6,223	0	0	0	7	0	6,223	7
Harvesting (centrifuge)	0	0	0	0	0	259	0	259
Filtration of medium	0	0	0	0	492	0	0	492
HVAC[a] of building	1,813	0	0	0	0	6,071	1,813	6,071
Cell banking	0	0	0	0	0	3	0	3
Total	**23,094**	**198,681**	**7,945**	**9,141**	**1,013**	**6,333**	**23,094**	**223,113**

[a]*Heating, Ventilation and Air Conditioning*

Table D.4 - Global average electricity mix for 2030 (IEA 2019)

Source	Share
Coal	29%
Gas	24%
Oil	3%
Nuclear	9%
Hydro	15%
Wind	9%
Solar	9%
Other renewable	3%

Table D.5 - Production of metabolic byproducts in wastewater, indication in ton/year for the whole facility (calculations based on Mattick et al. 2015)

Substance	Medium scenario		
	Low	Mid	High
Lactate	2,567	3,209	4,011
Alanine	0	1	9
Ammonia	7	16	37

Table D.6 - Equipment material demand (per 1 unit) (calculations based on Tuomisto and de Mattos 2011 and industry documentation)

Equipment type	Steel (kg)	Insulation (kg)	PVC (kg)	Electronics (kg)
Stirred-tank Reactor (STR) small	50.5	0.7	5.0	2.0
Stirred-tank Reactor (STR) large	1,688.7	7.6	5.0	2.0
Perfusion Reactor (PR)	532.5	3.3	5.0	2.0
Storage and mixing tanks for culture medium	3,964.6	0.0	5.0	2.0

Data collection procedure for culture medium usage

Data collection for the culture medium scenarios took place over the course of 2019 – 2022. There were two main rounds of data collection (the first in 2019 – 2020 and the second in 2022), which were followed by additional communication with individual parties for verification or better understanding of data.

First round of data collection

In the first round of data collection, the following specific questions were asked regarding production of meat from a specific amount of medium. In all cases we explicitly asked for answers regarding the current situation (in that case 2019), and the anticipated situation 10 years into the future.

- What is the estimated output of wet cellular mass relative to the volumetric capacity of your largest proliferation vessel (kg/L)?
    - What is the %dry mass in this output?
    - What total volume of medium per batch is used to create this mass?

- What culture medium do you expect will be used in the future/are you aiming for (e.g. basal medium, hydrolysate, yeast extract, premix based on a variety of feedstocks)?
    - What do you expect to be the quality grade requirements (pharma, food or feed grade)?
    - What do you expect to be the main feedstocks and production processes for medium production?
    - If you expect it to be a combination of ingredients from different sources, please list the percentages of these ingredients (e.g. xx% basal medium, xx% yeast extract and xx% food-grade single ingredients)
- Do you expect your feed conversion efficiency to go up with increase in production scale?
    - Have you been able to calculate a feed conversion ratio so far? If so, what is it?
    - If so, please specify how you calculate the feed conversion ratio (e.g. dry matter in:dry matter out or glutamine in:final product out).
    - Can you give an indication of a realistically achievable feed conversion efficiency (please refer to the source: literature, own lab tests, etc.)?
- Do you currently recycle part of your medium? If so, what component(s) are retained and at what efficiency? Please also list your expectations towards the future.
    - Please list any metabolites (e.g. lactate, ammonia) you are currently measuring during your bioprocess.
- Does your medium contain any growth factors or recombinant proteins (including insulin, transferrin, etc)? Please list on a level of aggregation that you feel comfortable with (total --> compound specific). In the end, it is important that we gain insight into the total quantity/volumes and costs per kg/ton final product. A higher level of detail means a more accurate analysis.
    - Are any of these produced in-house? Please list on a level of aggregation you are comfortable with.
    - If so, what are the cost savings per quantity of protein?
    - If you are able to say, are any growth factors used species-specific? If so, has there been any efficiency gain in a measured parameter (e.g. proliferation, differentiation, etc) using the species-specific protein? Please list any information here.

Most parties filled in most of the questions, to varying degrees of accuracy. In addition, a data sheet was included and (partially) filled in by multiple parties, which resulted in additional insights in media composition and additives.

The parties indicated they currently used DMEM/F12, in cases supplemented with various elements (recombinant proteins, amino acids, salts, growth factors). Based on interviews with (industry) experts, it is assumed that DMEM/F12 is not used in the future, but instead the medium components are delivered to the factory as a dry powdered mix (DPM). All parties indicated serum-free media would be the standard in the future and most parties indicated they were aiming to include hydrolysates.

In order to calculate the total amount of ingredients in the DPM, the primary data from companies was translated to its individual ingredients. Three medium scenarios were defined based on the range of data received (see CE Delft, 2021). The mid-medium scenario was compared to the scenarios presented in Specht (2020) and the amount of medium was found to be between the low- and average media use scenarios from Specht (2020).

The report was sent for checking to the parties involved before publishing, which did not result in any changes.

Second round of data collection

For the second round of data collection, the same parties from the first round were contacted to provide information for an update of the medium scenarios. Two reasons for this were that a few years had gone by (delivering additional insights for medium use, also at slightly larger scales than bench-top), and additional analysis showed that the low medium scenario from CE Delft (2021) resulted in cell mass with a relatively low dry matter (dm) content, which could not be generalized across different cell types and was too low to be comparable to conventional meat.

The approach for data collection was different in the second round. A table with three scenarios was presented to the parties (Table D.7). These scenarios were largely similar to those presented in CE Delft (2021), but the low medium scenario was adapted to match the ‘enhanced catabolism cell types’-medium scenario in Humbird (2021), corrected for a lower dm content (20% instead of 30%).

General and specific questions were asked to the parties. The specific questions relate to the data provided by individual parties and are therefore excluded here due to confidentiality.

General questions:

Our questions are regarding estimated culture medium usage for the year 2030. The table below shows the scenarios that we developed based on received data. Please note that the unit is in g/kg meat cells (excluding scaffold, so 100% meat cells).

- Regarding amino acids and sugars (note: these values are higher than used in the current report, because the current numbers could not be generalized across different cell types and therefore we used literature data to define the lower range):
    - Is the amount of amino acids what you would expect?
    - Is the amount of sugars what you would expect?
    - If for both, lower amounts than the 'low' scenario are possible, are you able you substantiate with data or calculations?
- Do you expect albumin will be used?
- Does anything seem off in the values/ranges from your perspective?

Table D.7 – Second round of data collection (Culture medium scenarios: total g of ingredients needed for producing one kg of cultivated meat cells, therefore excluding any scaffolding material)

Components	Low-medium scenario[a] (g)	Baseline scenario (g)	High-medium scenario (g)	Main ingredients
Amino acids (total), of which:	259	322	400	L-glutamine, L-Arginine hydrochloride
Amino acids from hydrolysate	*194*	*241*	*300*	
Amino acids from conventional production	*65*	*80*	*100*	
Sugars (total), of which:	240	310	400	Glucose, pyruvate
Sugars: Glucose	*239*	*308*	*396*	
Sugars: Pyruvate	*1*	*2*	*4*	
Recombinant proteins	1	7	50	Albumin (mainly), insulin, transferrin
Salts	40	80	160	Sodium chloride
Buffering agent	10	32	100	HEPES
Vitamins	0.2	2	20	i-Inositol, Choline chloride
Growth factors	<<1	<<1	<<1	
Water	7,500	12,649	40,000	Ultrapure water
Total (g)	**8,548**	**14,033**	**41,930**	
Total medium for 1 kg meat cells (L)	**9**	**15**	**43**	

Note: Approximate (rounded) values; values may not add up.

[a]Amino acids and sugars consumption based on data assumptions for enhanced catabolism cell types in Humbird (2021), 20%dm meat. Data received previously indicated much lower numbers for both sugars and amino acids, but these showed a discrepancy with current literature and could not be validated.

Some important conclusions that came from this second round of data collection were:

- Amino acids quantities were within range of expectations
- Sugars quantities were slightly below expectations, and were expected to be higher than amino acids quantities (even when accounting for the fact that when hydrolysates are used, higher quantities of amino acids will be needed than when only single amino acids are used)
- Some experimental data was provided to substantiate lower medium use than the low medium scenario, but this could not be generalized across cell types and it was uncertain whether this could also be achieved at larger scales than lab-scale
- Albumin was unlikely to be included in future medium formulations for most parties, and already excluded currently for some parties
- Concentrations of solids were too high, resulting in a medium too viscous for cell culture, and therefore additional water would be needed (although scenarios were reported that indicated certain fed-batch feeding strategies could reduce the amount of water in the medium needed down to around 20L)
- Total minimum amount of solids needed is 600 – 800 g/kg meat cells

The additional information was used to determine new low, mid and high medium scenarios (see main paper, Table 1).

A mass balance check was performed for the three medium scenarios and for the upper and lower bound of expected protein content in the final product, to check whether more carbon was supplied than produced and what the carbon consumption efficiency would be in the different scenarios (Table D.8). Average carbon contents of amino acids, glucose and protein of 41%, 40% and 51% respectively were used for the calculations. This verified that in theory, ample carbon is supplied to produce the required cell mass.

Table D.8 – Carbon consumption efficiency (C out in meat cells : C supplied in sugars and amino acids in the medium)

Protein in final product	Low medium	Mid medium	High medium
18% protein	46%	35%	27%
25% protein	64%	49%	37%

The amounts of ingredients needed for 1 kg of meat cells was also compared to Humbird (2021) and Tuomisto et al. (2022) (Table D.9). In general, the consumption of amino acids and glucose will be dependent on a cell's metabolism, which is known to vary between species and cell type (O'Neill et al. 2022). Despite this variability, both the amount of amino acids and of glucose in this study fall within the range of the compared studies, which model data from CHO and C2C12 cells, respectively. The amino acid consumption in the baseline (mid medium) scenario in this study and in the scenarios including hydrolysates in Humbird (2021) are slightly higher than in the optimized scenarios of Tuomisto et al. (2022). This could be explained by the fact that Tuomisto et al. (2022) use defined media made with single amino acids, and no hydrolysates (DMEM/F12 + FBS or Essential 8). In these defined media, the ratio of amino acids:glucose is almost 1:3. When hydrolysates are used as a (partial) source of amino acids, current evidence from both CM producers and literature shows that relatively more amino acids are needed, because the media composition is not defined and not optimal (Table D.9). Glucose consumption in the baseline (mid medium) scenario in this study falls between the optimized scenarios of Tuomisto et al. (2022) and the enhanced catabolism scenario in Humbird (2021) (which is a more probable cell type for commercial cell cultures than wild-type).

Table D.9 – Comparison of amino acids and glucose mass balance between this study and other, recent studies (data from other studies recalculated to g/kg CM using publicly available information)

Aspect	**This study**			**Tuomisto et al. (2022)**			**Humbird (2021) Scenarios for inclusion of hydrolysates**	
	Low medium	Mid Medium	High medium	CMB	CMB128	CMC	Wild-type catabolism	Enhanced catabolism
Amino acids (g/kg CM)	200	283	400	448	197	196	453	388
Sugars (g/kg CM)	320	400	500	1270	557	557	816	360
Dry matter content	20%-30%	20%-30%	20%-30%	30%	30%	30%	30%	30%
Protein content	18%-25%	18%-25%	18%-25%	20%	20%	20%	21%	21%

Data collection and assumptions for energy consumption

Data on energy consumption were retrieved from engineering consultants in the field working for CM companies to develop scaled-up facility designs. Energy consumption for this study relies on modeling of similar processes and scales in SuperPro designer software. See Figure 2 (main paper) for the production line characteristics. The primary assumption was that metabolic heat generated was similar to other cell/protein production processes. Further assumptions are that biomass production is proportional to oxygen uptake rate (OUR) and that the cells are the final product. System characteristics used for the model, such as cell densities, average OUR and aeration rate (vvm), are provided in Table D.1. Table D.10 provides the model energy

consumption results for one production line, including relevant assumptions and data modifications. Total energy consumption of the facility is a multiplication of the energy consumption of one production line times the amount of production lines at the facility (3,243, see Table D.1).

Table D.10 – Energy consumption estimates for one production line

Production stage	Bio-reactor	Total time in reactors	Culture medium consum-ption	Aspect	Quantity (kWh)	Modelling outcomes and assumptions
Small-scale proliferation	1 x 250 ml flask and 1 x 50 L STR (working volume)	10 days	382 L	Initial heating	135	Conservative assumption: 5% of large-scale proliferation energy use.
				Maintaining temperature	2,707	Idem
				Mixing	71	Idem
				Aeration	77	Idem
				Pumping	5	Idem
Large-scale proliferation	1 x 10,000 L STR (working volume)	12 days (10 + 1 + 1)	86,000 L	Initial heating	2,691	Heating culture medium from 10ºC to 37ºC
				Maintaining temperature	54,146	Average cooling load to bioreactor jacket to remove excess metabolic heat in order to maintain temperature at 37ºC: 180 kW continuously (0.6 Mbtu/hr). This is based on calculations for metabolic heat production of the cells at the stated cell densities and oxygen uptake rates (OUR). Original numbers provided were for cells with a volume of 5,000 um, for which peak heat rate was calculated to be 260 kW continuously (0.9 MBtu/hr). For cells with a volume of 3,500 um, this is estimated to be 70% of that (3,500/5,000), as metabolic heat production is linearly related to (molar) mass at constant OUR (Altwasser et al. 2017).
				Mixing	1,419	Motor of 5 kW operating continuously
				Aeration	1,532	Compressor motor of 5 kW operating continuously
				Pumping	100	Pump of 0.5kW operating continuously
Differentiation and maturation	4 x 2,500 L PR (working volume)	10 days each	22,000 L (4 * 5,500 L)	Initial heating	1,817	Heating culture medium from 10 ºC to 37 ºC
				Maintaining temperature	4,410	Average cooling load to bioreactor jacket to remove excess metabolic heat in order to maintain temperature at 37ºC: 18 kW continuously (60 kbtu/hr). Model assumes no growth during differentiation phases and therefore minimal metabolic heat production.
				Mixing	960	Motor of 4 kW operating continuously
				Aeration	1,210	Compressor motor of 5 kW operating continuously
				Pumping	84	Pump of 0.4kW operating continuously
Cleaning of reactors	All reactors above except flask	2 days	Water: 10,000 L	Initial heating	1,919	Heating CIP water from 10ºC to 50ºC. Assumes 0.5L cleaning water for 1L of working volume over 5 stages of cleaning – total of 10,000L cleaning water. Additional SIP step assumping 100 kg of steam per cubic meter of working volume for one hour and electric steam generation.
				Pumping	nominal	
Medium sterilization through filtration			108,382 L	Pumping	29	0.27 Wh per liter medium

In addition, HVAC power consumption was included. This is based on an average power consumption of 0.3 kW/m^2 facility size (number from primary data collection). 77% of this is electricity and 23% is heat/steam (Tschudi et al., 2001). Facility size is estimated at 3000 m^2, or 3 times the floorspace occupied by equipment.

Total annual energy consumption is reported in Table D.3.

Appendix E - Nutritional composition of CM and conventional meats

Average (macro)nutritional compositions are provided in Table E.1. For CM, a few parties were able to provide dry matter content, but information about macronutrients was not provided. Therefore deliberately broad ranges based on various conventional meat products are assumed and reported here in the row for CM.

Table E.1 - Average (macro)nutritional composition of the protein products under study

Product	Water	Protein	Carbohydrates	Other
	% of total weight			
CM	70-80	18-25	0	0-12
Beef	74	23	0	3
Pork	72	22	2	4
Chicken	74	23	0	3

Appendix F - Conventional meats ambitious benchmarks theoretical background

Ambitious benchmarks were created for conventional meats in order to lead to highly robust conclusions regarding sustainability claims of CM. A summary of the improvements modeled for conventional meats is provided in Table F.1. The improvements are discussed per topic below.

Food additives such as 3NOP have been developed that claim a reduction of methane emissions from enteric fermentation. Measured reductions vary widely and range from a negligible to over 90% (Klop 2016; Dijkstra et al. 2018; Honan et al. 2021; van Gastelen et al. 2022). In this study, a reduction of 15% was modeled, while adding 1.5 grams of enzymes per day to feed regimes. Sustainable energy was used for production of these enzymes.

Soy products contribute significantly to the carbon footprint and biodiversity impacts of pork and chicken (and to a lesser extent beef). This is for a large part due to land use change (LUC). Certification of LUC-free soy is available in the market, and while the efficiency of certifications vary, improvements in these schemes are made. Therefore it was assumed that LUC associated with soy will be zero in 2030 (both in m2a and in kg CO2), for both conventional and cultivated meats.

Ammonia (NH3) contributes substantially to multiple environmental indicators and this is most significantly so in cattle (beef and dairy) production. Ammonia emissions can be reduced through additional outdoor grazing. Outdoor grazing was increased in the models by adding 50% of the difference between the current Dutch average and the required amount for organic production, in hours of outdoor grazing per year. This results in 5.4% reduction in ammonia emissions (calculations based on Hoving et al. 2014).

Energy used for ambitious benchmarks of conventional meat production was assumed to be sustainable, both at the farm and at feed production plants.

These changes result in a carbon footprint that is 15%, 26% and 53% lower for the 2030 benchmark for beef, pork and chicken respectively.

Table F.1 - Summary of improvements modeled for the ambitious benchmarks of conventional meats

Product	Based on database and process	Adjusted for
Beef (beef cattle)	Agri-footprint: Beef meat, fresh, from beef cattle, at slaughterhouse, PEF compliant/IE Economic	– Methane emissions from enteric fermentation: -15%, additional input: enzymes. – Additional outdoor grazing resulting in ~5.4% lower NH_3 emissions. – Sustainable energy (electricity and heat) at farm and in feed compound production and soybean production.
Beef (dairy cattle)	Agri-footprint: Beef meat, fresh, from dairy cattle, at slaughterhouse, PEF compliant/NL Economic	– Methane emissions from enteric fermentation: -15%, additional input: enzymes. – Additional outdoor grazing resulting in ~5.4% lower NH_3 emissions. – Sustainable energy (electricity and heat) at farm and in feed compound production and soybean production.
Pork	Agri-footprint: Pig meat, fresh, at slaughterhouse/NL Economic	– No LUC or associated GHG emissions related to soy in feed. – Sustainable energy (electricity and heat) at farm and in feed compound production and soybean production.
Chicken	Agri-footprint: Chicken meat, fresh, at slaughterhouse/NL Economic	– No LUC or associated GHG emissions related to soy in feed. – Sustainable energy (electricity and heat) at farm and in feed compound production and soybean production.

Appendix G - Description of sensitivity analyses and sensitivity analyses results

A. Cell culture medium

See paragraph 2.3.3. in the main text.

Results are provided in Table G.1.

B. Cell density

Maximum cell density during proliferation stages is a parameter for which many companies are optimizing. This is highly dependent on the adopted product system and bioreactor types. In this study, we have adopted a median maximum cell density expected to be achieved by CM companies in commercial-scale production. In the stirred-tank reactor (STR) system that we model, it may be feasible to increase cell densities by a factor of 1.4 (to $7.1*10^7$ cells/ml, the higher density scenario), but not much more, according to experts in the field. At the baseline cell volumes, this density is close to the theoretical maximum cell volume fraction of 0.25 (Humbird 2021). It is plausible that certain large-scale production systems will not manage to operate at the cell densities that we model in the baseline scenario, and therefore model lower densities by a factor of 10 ($10*10^6$ cells/ml). In other reactor systems, cell densities up to 10^9 cells/ml are currently already feasible, albeit not yet at very large scales (Allan et al. 2019). However, limitations regarding metabolite formation and oxygen availability can be expected in those situations.

Higher cell densities would mean that more meat cells can be grown in a reactor of the same volume. It affects the energy demand for heating and cooling, as cultures with higher cell densities need less heating and more cooling, and cultures with lower cell densities need more heating and less cooling (per unit of volume). It is assumed that the total cooling load needed remains unchanged. Lower cell densities mean that more water has to be added to the growing medium to fill up the reactors, and more reactors are needed to produce the same amount of CM, with subsequently more energy and water used for cleaning. Changes in cell density during proliferation stages have no effect on assumed cell density during differentiation and maturation.

Results are provided in Table G.2.

C. Production run time

The production run time depends on the doubling time (for proliferation stages), on the desired maturity of cells in the final product (for differentiation and maturation), and the size of the largest proliferation vessel. Based on primary data we have decided to vary the total production run time by -25% and +25%. This does not necessarily cover all data received, but we feel that this does provide a solid basis for companies to interpret the results in comparison to their own specific process design.

For a shorter proliferation time, the cell doubling time is reduced from 30 to 22,5 days, and for a longer proliferation time increased from 30 to 37,5 days.
Shorter residence time in the reactors reduces overall energy demand, lowers medium demand during differentiation and maturation (we assume a linear relation), and results in a smaller number of reactors needed to produce the same amount of CM. Longer residence time affects these aspects reversely. We assume medium use in proliferation stages is not affected, as the same quantity of cellular biomass has to be produced, thus demanding the same amount of ingredients.

Results are provided in Table G.3.

D. Cell volume

Average cell volume differs per species type and cell type. For example, fat cells are much larger than muscle cells, and within the different types of muscle cells there is large variation. Also, small animals tend to have

smaller cells than large animals. As the companies involved in this study produce a range of species and cell types, we used an average cell volume for the baseline scenario and determined smaller cell volume (500 μm3) and larger cell volume (5,000 μm3) based on primary data and literature.

The effects of smaller and larger cell volume are similar to that of lower and higher cell densities, respectively, as total cell mass harvested from one unit of volume decreases accordingly.

Results are provided in Table G.4.

E. Amount of harvests from semi-continuous process (single batch up to 10 harvests)

In the semi-continuous production process modeled in the baseline scenario, three harvests are made from the largest proliferation vessel, in one production run (see paragraph 2.3.1). In this sensitivity analysis, the effect of having less or more harvests from the largest proliferation vessel during one production run is assessed: one (batch production), five or ten harvests. This influences the number of production runs needed and the time per production run. With an increasing number of harvests, the production runs last longer, but the number of production runs decreases such that the combined operating time of the bioreactors in the facility decreases. The number of bioreactors needed also decreases. Energy demand increases- or decreases accordingly too, mainly because stand-by cooling load, aeration, and agitation are continuous and therefore in part related to combined operating time. There is no effect on culture medium use, with the exception that for the single batch scenario more water is added to the reactor to ensure adequate working volume. With an increasing number of harvests, the risk of contamination and therefore spoiled batches increases, but this was out of scope in this study.

Results are provided in Table G.5.

F. Smart cooling

Cooling energy is expected to be a major driver for energy demand in CM production. In this study, it is the dominant driver for facility energy use. In the baseline scenario, an active cooling system (using a refrigeration cycle) is modeled. This is the most conservative approach. More passive cooling (for example using cooling water and an air fin cooler) is also an option for many locations. By optimizing the cooling system to geographical location and ambient temperatures, a smart combination of active and passive cooling can lead to a

reduced electricity demand for cooling. The compressor is by far the main driver of energy use in the cooling system and therefore implementing more passive cooling can drastically reduce the electricity demand. For this sensitivity analysis, the electricity demand for cooling energy is set at 50% of the baseline scenario, representative of a location where a refrigeration cycle is needed for half of the year at maximum.

Results are provided in Table G.6.

Table G.1 - Results for sensitivity analyses on production run time

Scenario		**A1: Shorter production run time (-25%: 32 days, three harvests)**			**A2: Longer production run time (+25%: 52 days, three harvests)**		
Impact category	**Unit**	**Ambitious benchmark**	**Renewable Scope 1&2**	**Global average energy**	**Ambitious benchmark**	**Renewable Scope 1&2**	**Global average energy**
Global warming	kg CO_2-eq.	2.63E+00	3.73E+00	1.36E+01	3.00E+00	4.34E+00	1.50E+01
Stratospheric ozone depletion	kg CFC_{11}-eq.	5.12E-06	5.63E-06	1.02E-05	6.15E-06	6.77E-06	1.17E-05
Ionizing radiation	kBq Co-60-eq.	1.57E-02	2.58E-02	1.17E-01	1.79E-02	3.01E-02	1.28E-01
Ozone formation, Human health	kg NO_x-eq.	7.49E-03	8.97E-03	2.23E-02	8.56E-03	1.04E-02	2.47E-02
Fine particulate matter formation	kg $PM_{2.5}$-eq.	5.88E-03	6.12E-03	8.24E-03	6.67E-03	6.96E-03	9.25E-03
Ozone formation, Terrestrial ecosystems	kg NO_x-eq.	7.80E-03	9.29E-03	2.26E-02	8.92E-03	1.07E-02	2.51E-02
Terrestrial acidification	kg SO_2-eq.	1.62E-02	1.73E-02	2.63E-02	1.87E-02	1.99E-02	2.97E-02
Freshwater eutrophication	kg P-eq.	9.14E-04	9.58E-04	1.36E-03	1.09E-03	1.15E-03	1.58E-03
Marine eutrophication	kg N-eq.	1.17E-03	1.16E-03	1.14E-03	1.41E-03	1.41E-03	1.39E-03
Terrestrial ecotoxicity	kg 1,4-DCB-eq.	5.53E+01	5.13E+01	1.51E+01	6.11E+01	5.63E+01	1.74E+01
Freshwater ecotoxicity	kg 1,4-DCB-eq.	5.95E-02	5.95E-02	5.87E-02	7.23E-02	7.22E-02	7.14E-02
Marine ecotoxicity	kg 1,4-DCB-eq.	4.73E-02	4.57E-02	3.09E-02	5.33E-02	5.14E-02	3.55E-02
Human carcinogenic toxicity	kg 1,4-DCB-eq.	1.20E-01	1.14E-01	6.74E-02	1.33E-01	1.27E-01	7.62E-02
Human non-carcinogenic toxicity	kg 1,4-DCB-eq.	2.14E+00	2.15E+00	2.22E+00	2.34E+00	2.35E+00	2.44E+00
Land use	m^2a crop-eq.	2.26E+00	2.25E+00	2.19E+00	2.69E+00	2.69E+00	2.62E+00
Mineral resource scarcity	kg Cu-eq.	3.74E-02	3.54E-02	1.73E-02	4.15E-02	3.91E-02	1.97E-02
Fossil resource scarcity	kg oil-eq.	7.09E-01	1.05E+00	4.09E+00	8.12E-01	1.23E+00	4.51E+00
Water consumption	m^3	7.99E-02	7.85E-02	6.61E-02	9.11E-02	8.95E-02	7.61E-02

Table G.2 - Results for sensitivity analyses on cell density

Scenario		B1: Higher cell density (x4: 7.1E7 cells/ml)			B2: Lower cell density (x10: 5E6 cells/ml)		
Impact category	**Unit**	**Ambitious benchmark**	**Renewable Scope 1&2**	**Global average energy**	**Ambitious benchmark**	**Renewable Scope 1&2**	**Global average energy**
Global warming	kg CO_2-eq.	2,78E+00	4,00E+00	1,40E+01	3,86E+00	5,26E+00	2,09E+01
Stratospheric ozone depletion	kg CFC_{11}-eq.	5,62E-06	6,18E-06	1,08E-05	6,59E-06	7,23E-06	1,44E-05
Ionizing radiation	kBq Co-60-eq.	1,66E-02	2,77E-02	1,21E-01	2,38E-02	3,65E-02	1,80E-01
Ozone formation, Human health	kg NO_x-eq.	7,93E-03	9,56E-03	2,31E-02	1,09E-02	1,28E-02	3,38E-02
Fine particulate matter formation	kg $PM_{2.5}$-eq.	6,19E-03	6,45E-03	8,61E-03	8,73E-03	9,03E-03	1,24E-02
Ozone formation, Terrestrial ecosystems	kg NO_x-eq.	8,26E-03	9,90E-03	2,34E-02	1,14E-02	1,32E-02	3,43E-02
Terrestrial acidification	kg SO_2-eq.	1,73E-02	1,84E-02	2,76E-02	2,31E-02	2,43E-02	3,86E-02
Freshwater eutrophication	kg P-eq.	9,90E-04	1,04E-03	1,45E-03	1,76E-03	1,81E-03	2,45E-03
Marine eutrophication	kg N-eq.	1,29E-03	1,28E-03	1,26E-03	1,52E-03	1,51E-03	1,48E-03
Terrestrial ecotoxicity	kg 1,4-DCB-eq.	5,72E+01	5,28E+01	1,61E+01	8,47E+01	7,97E+01	2,28E+01
Freshwater ecotoxicity	kg 1,4-DCB-eq.	6,59E-02	6,58E-02	6,50E-02	7,49E-02	7,48E-02	7,36E-02
Marine ecotoxicity	kg 1,4-DCB-eq.	4,95E-02	4,78E-02	3,28E-02	6,99E-02	6,79E-02	4,48E-02
Human carcinogenic toxicity	kg 1,4-DCB-eq.	1,23E-01	1,18E-01	7,00E-02	2,04E-01	1,97E-01	1,23E-01
Human non-carcinogenic toxicity	kg 1,4-DCB-eq.	2,20E+00	2,21E+00	2,29E+00	3,33E+00	3,34E+00	3,46E+00
Land use	m^2a crop-eq.	2,47E+00	2,46E+00	2,40E+00	2,93E+00	2,93E+00	2,83E+00
Mineral resource scarcity	kg Cu-eq.	3,87E-02	3,65E-02	1,81E-02	6,04E-02	5,79E-02	2,95E-02
Fossil resource scarcity	kg oil-eq.	7,52E-01	1,13E+00	4,22E+00	1,04E+00	1,47E+00	6,30E+00
Water consumption	m^3	8,45E-02	8,30E-02	7,03E-02	1,18E-01	1,17E-01	9,71E-02

Table G.3 - Results for sensitivity analyses on cell volume

Scenario		C1: Larger cell volume (5,000 µm3)			C2: Smaller cell volume (500 µm3)		
Impact category	**Unit**	**Ambitious benchmark**	**Renewable Scope 1&2**	**Global average energy**	**Ambitious benchmark**	**Renewable Scope 1&2**	**Global average energy**
Global warming	kg CO_2-eq.	2.78E+00	4.01E+00	1.41E+01	3.57E+00	4.96E+00	1.88E+01
Stratospheric ozone depletion	kg CFC_{11}-eq.	5.62E-06	6.19E-06	1.08E-05	6.45E-06	7.09E-06	1.35E-05
Ionizing radiation	kBq Co-60-eq.	1.66E-02	2.77E-02	1.21E-01	2.19E-02	3.45E-02	1.62E-01
Ozone formation, Human health	kg NOx-eq.	7.95E-03	9.58E-03	2.31E-02	1.01E-02	1.19E-02	3.05E-02
Fine particulate matter formation	kg PM2.5-eq.	6.20E-03	6.47E-03	8.63E-03	8.01E-03	8.31E-03	1.13E-02
Ozone formation, Terrestrial ecosystems	kg NOx-eq.	8.28E-03	9.91E-03	2.35E-02	1.05E-02	1.24E-02	3.10E-02
Terrestrial acidification	kg SO_2-eq.	1.73E-02	1.84E-02	2.77E-02	2.15E-02	2.28E-02	3.54E-02
Freshwater eutrophication	kg P-eq.	9.91E-04	1.04E-03	1.45E-03	1.63E-03	1.69E-03	2.25E-03
Marine eutrophication	kg N-eq.	1.29E-03	1.28E-03	1.26E-03	1.50E-03	1.49E-03	1.46E-03
Terrestrial ecotoxicity	kg 1,4-DCB-eq.	5.74E+01	5.30E+01	1.61E+01	7.62E+01	7.12E+01	2.10E+01
Freshwater ecotoxicity	kg 1,4-DCB-eq.	6.59E-02	6.58E-02	6.50E-02	7.42E-02	7.41E-02	7.31E-02
Marine ecotoxicity	kg 1,4-DCB-eq.	4.97E-02	4.79E-02	3.29E-02	6.39E-02	6.19E-02	4.17E-02
Human carcinogenic toxicity	kg 1,4-DCB-eq.	1.24E-01	1.18E-01	7.01E-02	1.79E-01	1.72E-01	1.07E-01
Human non-carcinogenic toxicity	kg 1,4-DCB-eq.	2.20E+00	2.21E+00	2.29E+00	2.97E+00	2.98E+00	3.09E+00
Land use	m^2a crop-eq.	2.47E+00	2.46E+00	2.40E+00	2.85E+00	2.85E+00	2.77E+00
Mineral resource scarcity	kg Cu-eq.	3.88E-02	3.66E-02	1.81E-02	5.37E-02	5.12E-02	2.61E-02
Fossil resource scarcity	kg oil-eq.	7.53E-01	1.13E+00	4.24E+00	9.62E-01	1.39E+00	5.68E+00
Water consumption	m^3	8.46E-02	8.31E-02	7.04E-02	1.10E-01	1.08E-01	9.08E-02

Table G.4 - Results for sensitivity analyses on culture medium use

Scenario		**D1: Low medium[a]**			**D2: High medium[b]**		
Impact category	**Unit**	**Ambitious benchmark**	**Renewable Scope 1&2**	**Global average energy**	**Ambitious benchmark**	**Renewable Scope 1&2**	**Global average energy**
Global warming	kg CO_2-eq.	2.33E+00	2.89E+00	1.29E+01	5.00E+00	1.38E+01	2.48E+01
Stratospheric ozone depletion	kg CFC_{11}-eq.	4.31E-06	4.56E-06	9.20E-06	7.93E-06	1.20E-05	1.70E-05
Ionizing radiation	kBq Co-60-eq.	1.38E-02	1.88E-02	1.12E-01	3.01E-02	1.12E-01	2.12E-01
Ozone formation, Human health	kg NO_x-eq.	6.59E-03	7.33E-03	2.09E-02	1.50E-02	2.69E-02	4.16E-02
Fine particulate matter formation	kg $PM_{2.5}$-eq.	5.28E-03	5.40E-03	7.56E-03	1.16E-02	1.35E-02	1.59E-02
Ozone formation, Terrestrial ecosystems	kg NO_x-eq.	6.83E-03	7.58E-03	2.11E-02	1.63E-02	2.82E-02	4.29E-02
Terrestrial acidification	kg SO_2-eq.	1.44E-02	1.49E-02	2.41E-02	2.99E-02	3.80E-02	4.79E-02
Freshwater eutrophication	kg P-eq.	5.89E-04	6.12E-04	1.03E-03	2.55E-03	2.91E-03	3.35E-03
Marine eutrophication	kg N-eq.	9.52E-04	9.51E-04	9.29E-04	1.83E-03	1.81E-03	1.78E-03
Terrestrial ecotoxicity	kg 1,4-DCB-eq.	5.22E+01	5.02E+01	1.32E+01	1.00E+02	6.76E+01	2.84E+01
Freshwater ecotoxicity	kg 1,4-DCB-eq.	4.76E-02	4.75E-02	4.68E-02	9.48E-02	9.41E-02	9.33E-02
Marine ecotoxicity	kg 1,4-DCB-eq.	4.32E-02	4.25E-02	2.71E-02	8.46E-02	7.11E-02	5.57E-02
Human carcinogenic toxicity	kg 1,4-DCB-eq.	1.13E-01	1.10E-01	6.20E-02	2.16E-01	1.74E-01	1.23E-01
Human non-carcinogenic toxicity	kg 1,4-DCB-eq.	2.00E+00	2.00E+00	2.08E+00	4.20E+00	4.27E+00	4.35E+00
Land use	m^2a crop-eq.	1.89E+00	1.88E+00	1.82E+00	3.59E+00	3.53E+00	3.47E+00
Mineral resource scarcity	kg Cu-eq.	3.51E-02	3.41E-02	1.56E-02	6.75E-02	5.12E-02	3.16E-02
Fossil resource scarcity	kg oil-eq.	6.12E-01	7.88E-01	3.85E+00	1.53E+00	4.22E+00	7.66E+00
Water consumption	m^3	6.99E-02	6.92E-02	5.64E-02	1.51E-01	1.40E-01	1.27E-01
Cumulative energy demand	MJ	1.42E+02	1.48E+02	2.48E+02	2.88E+02	3.76E+02	4.82E+02

[a]More efficient medium usage, removal of albumin, largely reduced HEPES use); [b]Less efficient medium usage, full use of albumin and HEPES

Table G.5 - Results for sensitivity analyses on amount of harvests

Scenario		E1: More harvests from proliferation vessel[a]			E2: Less harvests from proliferation vessel[b]			E3: More harvests from proliferation vessel[c]		
Impact category	**Unit**	**Ambitious benchmark**	**Renewable Scope 1&2**	**Global average energy**	**Ambitious benchmark**	**Renewable Scope 1&2**	**Global average energy**	**Ambitious benchmark**	**Renewable Scope 1&2**	**Global average energy**
Global warming	kg CO_2-eq.	2.54E+00	3.77E+00	1.22E+01	3.61E+00	4.83E+00	2.04E+01	2.30E+00	3.53E+00	1.04E+01
Stratospheric ozone depletion	kg CFC_{11}-eq.	5.52E-06	6.08E-06	9.97E-06	5.97E-06	6.54E-06	1.38E-05	5.42E-06	5.98E-06	9.12E-06
Ionizing radiation	kBq Co-60-eq.	1.52E-02	2.63E-02	1.04E-01	2.16E-02	3.28E-02	1.79E-01	1.38E-02	2.49E-02	8.73E-02
Ozone formation, Human health	kg NOx-eq.	7.28E-03	8.91E-03	2.02E-02	1.02E-02	1.19E-02	3.30E-02	6.62E-03	8.25E-03	1.74E-02
Fine particulate matter formation	kg PM2.5-eq.	5.62E-03	5.89E-03	7.70E-03	8.21E-03	8.47E-03	1.18E-02	5.05E-03	5.31E-03	6.77E-03
Ozone formation, Terrestrial ecosystems	kg NOx-eq.	7.58E-03	9.22E-03	2.06E-02	1.07E-02	1.23E-02	3.35E-02	6.89E-03	8.52E-03	1.77E-02
Terrestrial acidification	kg SO_2-eq.	1.61E-02	1.72E-02	2.49E-02	2.17E-02	2.28E-02	3.72E-02	1.48E-02	1.59E-02	2.21E-02
Freshwater eutrophication	kg P-eq.	9.67E-04	1.02E-03	1.36E-03	1.11E-03	1.16E-03	1.81E-03	9.35E-04	9.84E-04	1.26E-03
Marine eutrophication	kg N-eq.	1.28E-03	1.28E-03	1.26E-03	1.32E-03	1.32E-03	1.29E-03	1.27E-03	1.27E-03	1.25E-03
Terrestrial ecotoxicity	kg 1,4-DCB-eq.	5.01E+01	4.57E+01	1.50E+01	8.23E+01	7.79E+01	2.02E+01	4.28E+01	3.85E+01	1.38E+01
Freshwater ecotoxicity	kg 1,4-DCB-eq.	6.53E-02	6.53E-02	6.46E-02	6.77E-02	6.76E-02	6.64E-02	6.48E-02	6.47E-02	6.42E-02
Marine ecotoxicity	kg 1,4-DCB-eq.	4.45E-02	4.28E-02	3.04E-02	6.72E-02	6.54E-02	4.16E-02	3.95E-02	3.78E-02	2.79E-02
Human carcinogenic toxicity	kg 1,4-DCB-eq.	1.09E-01	1.03E-01	6.35E-02	1.77E-01	1.72E-01	9.65E-02	9.38E-02	8.81E-02	5.61E-02
Human non-carcinogenic toxicity	kg 1,4-DCB-eq.	1.89E+00	1.90E+00	1.97E+00	3.27E+00	3.28E+00	3.40E+00	1.58E+00	1.59E+00	1.65E+00
Land use	m^2a crop-eq.	2.39E+00	2.39E+00	2.34E+00	2.72E+00	2.71E+00	2.61E+00	2.32E+00	2.32E+00	2.28E+00
Mineral resource scarcity	kg Cu-eq.	3.42E-02	3.20E-02	1.66E-02	5.51E-02	5.29E-02	2.41E-02	2.95E-02	2.73E-02	1.49E-02
Fossil resource scarcity	kg oil-eq.	6.92E-01	1.07E+00	3.68E+00	9.62E-01	1.34E+00	6.14E+00	6.32E-01	1.01E+00	3.13E+00
Water consumption	m^3	7.80E-02	7.65E-02	6.59E-02	1.08E-01	1.06E-01	8.62E-02	7.14E-02	6.99E-02	6.14E-02

[a]5 harvests; [b]1 harvest - batch process; [c]10 harvest - going towards continuous process

Table G.6 - Results for sensitivity analyses on smart cooling

Scenario			**F1: Smart cooling[a]**	
Impact category	**Unit**	**Ambitious benchmark**	**Renewable Scope 1&2**	**Global average energy**
Global warming	kg CO_2-eq.	2.21E+00	3.44E+00	9.60E+00
Stratospheric ozone depletion	kg CFC_{11}-eq.	5.39E-06	5.95E-06	8.77E-06
Ionizing radiation	kBq Co-60-eq.	1.32E-02	2.44E-02	8.03E-02
Ozone formation, Human health	kg NO_x-eq.	6.35E-03	7.99E-03	1.62E-02
Fine particulate matter formation	kg $PM_{2.5}$-eq.	4.83E-03	5.09E-03	6.40E-03
Ozone formation, Terrestrial ecosystems	kg NO_x-eq.	6.61E-03	8.25E-03	1.65E-02
Terrestrial acidification	kg SO_2-eq.	1.43E-02	1.54E-02	2.10E-02
Freshwater eutrophication	kg P-eq.	9.47E-04	9.97E-04	1.24E-03
Marine eutrophication	kg N-eq.	1.27E-03	1.27E-03	1.25E-03
Terrestrial ecotoxicity	kg 1,4-DCB-eq.	3.99E+01	3.55E+01	1.35E+01
Freshwater ecotoxicity	kg 1,4-DCB-eq.	6.46E-02	6.45E-02	6.41E-02
Marine ecotoxicity	kg 1,4-DCB-eq.	3.74E-02	3.57E-02	2.69E-02
Human carcinogenic toxicity	kg 1,4-DCB-eq.	8.96E-02	8.39E-02	5.52E-02
Human non-carcinogenic toxicity	kg 1,4-DCB-eq.	1.46E+00	1.47E+00	1.52E+00
Land use	m^2a crop-eq.	2.29E+00	2.29E+00	2.25E+00
Mineral resource scarcity	kg Cu-eq.	2.79E-02	2.57E-02	1.47E-02
Fossil resource scarcity	kg oil-eq.	6.09E-01	9.89E-01	2.90E+00
Water consumption	m^3	6.89E-02	6.74E-02	5.99E-02
Cumulative energy demand	MJ	1.16E+02	1.28E+02	1.88E+02

[a]*Active + passive, 50% electricity reduction for cooling*

Appendix H – Ex-ante LCA additional literature review

Ex-ante LCA does not predict the future, but rather explores potential scenarios. Particularly useful are methodologies in LCA that use scenarios to assess future impacts associated with large-scale implementation of lab- or pilot-scale technologies (Cucurachi et al. 2018). The application of scenarios in ex-ante technology assessments allows for testing of policy interventions, investments, design changes and changes in socio-, techno- and economic systems. The results can also be used to estimate the validity of sustainability claims of future production and gain insights and understanding of specific design choices (van der Giesen et al. 2020).

Three topics in particular need extra attention when conducting ex-ante LCA (Cucurachi et al. 2018). The first is determining the functionality and the functional unit (FU). Novel technologies may aim to replace existing technologies based on a perceived main function, but rarely have an identical set of functionalities. The functional unit was explicitly defined broadly in this study, as many different high-protein products are compared to each other and the CM model is a general average of different products. Secondly, the lack of data that is representative of future (large-scale) production is inherent in ex-ante assessment since future systems do not exist yet and may influence the incumbent system. In this study, scenarios and conservative estimates for the baseline CM model, and ambitious benchmarks as well as global average footprints were shown for conventional meats, to treat some of this uncertainty. Thirdly, the lack of knowledge on the environmental impact of novel substances can cause skewed results, where the novel technology performs very well simply because characterization factors for its emissions are not available. As CM is in principle identical to conventional meats, and its production takes place in highly controlled environments, the skewing of results is assumed to be minimal.

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