Supplementary Materials for

A sustainable waste-to-protein system to maximise waste resource utilisation for developing food- and feed-grade protein solutions

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Abbreviations

Abbreviation	Definition
ACNF	Advisory committee on novel foods
ANVISA	Brazilian Health Regulatory Agency
CF	Conversion efficiency
CFR	Code of federal regulations
CO_2	Carbon dioxide
DM	Dry mass
DW	Dry weight
EFSA	European food safety authority
DMEU	European Union Dry mass
FCR	Food conversion ratio
FDA	Food and drug administration
FRESH	Future ready food safety hub
FSA	Food standards agency
FSANZ	Food standards Australia and New Zealand
FSSAI	Food safety and standards authority India
FW	Fresh weight
GRAS	Generally recognized as safe
GWP100	Global warming potential, 100 years
iTOL	Interactive tree of life
LC	Lignocellulosic content
LCA	Life cycle assessment
MSWST	Municipal Solid Waste Supplementary Table
NCBI	National Center for Biotechnology Information
NPV	Net profit value
OFAS	Office of food additive safety
OFMSW	Organic Fraction of Municipal Solid Waste
PC	Protein content
PCD	Protein content dry weight
PDCAAS	Protein digestibility corrected amino acid score
R&D	Research and development
RPR	Residue to product ratio
SCoPAFF	Standing committee on plants, animals, food and feed
SFA	Singapore food agency
SI	Supplementary information
ST	Supplementary Table
TEA	Techno-economic analysis
U.S.C.	United States code
US	United states
USD	United States Dollar

Supplementary Information 1

Protein potential of the organic fraction of municipal solid waste (OFMSW). Corresponding database Supplementary Table ST1.

SI-1.1 Detailed Municipal Solid Waste (MSW) by country

213 countries were clustered into 11 regions: Africa, Caribbean, Central & West Asia, East Asia, Europe, Latin America, North America, Pacific, South Asia, South East Asia. Region, country, MSW collection rate (%), year of record, population, MSW generation (kg/year and kg/capita/day) were derived from online databases and papers and are detailed in Supplementary Table ST1.1 [1-4]. OFMSW (kg/capita/day) was derived from online databases (kg OFMSW/kg MSW) [1,4]. OFMSW chemical components were estimated including lipid, carbohydrate, and protein content (g/kg OFMSW), where the average chemical composition were derived from previous published studies for summer and winter [5]. Average annual lipid, carbohydrate and protein content (g/capita/day) were estimated from the mean average of the summer and winter lipid, carbohydrate and protein contents (g/capita/day). Corresponding database Supplementary Table ST1.1

SI-1.2 Average MSW by country

The average, standard deviation, maximum and minimum values for MSW generation (kg/capita/day), OFMSW generation (kg/capita/day), and lipid, carbohydrate and protein content (g/capita/day) were estimated from data collected for each country [1-5]. Corresponding database Supplementary Table ST1.2.

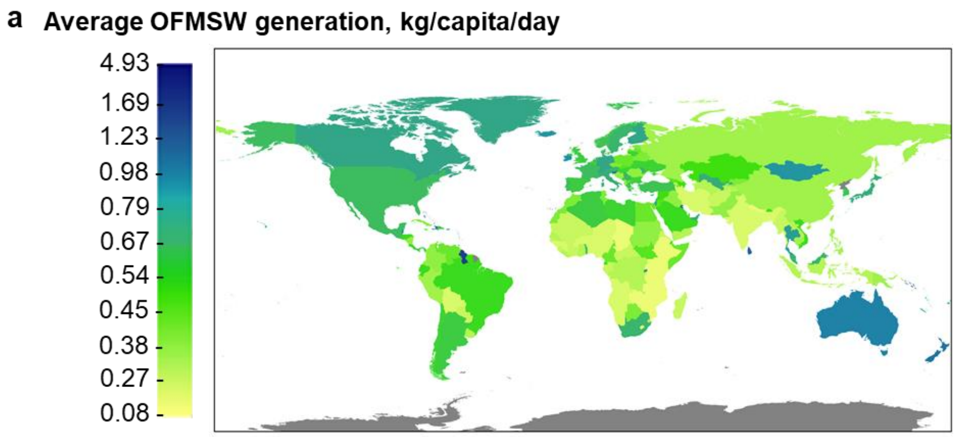
SI-1.3 Average MSW by region

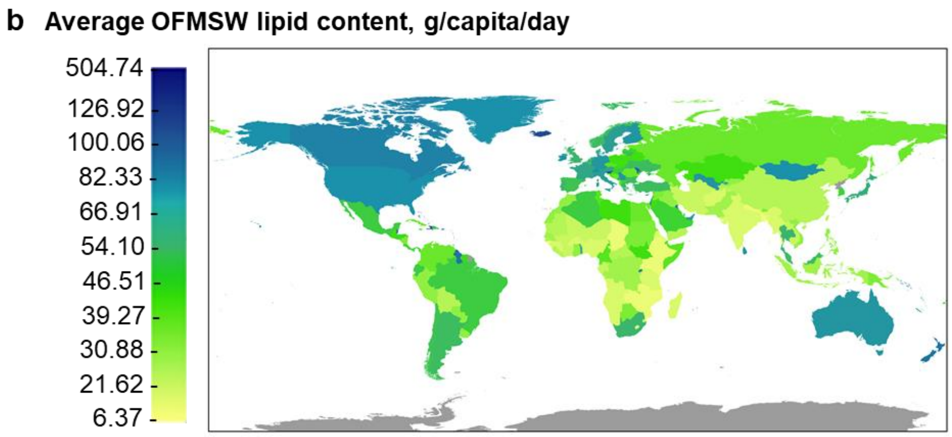
213 countries were clustered into 11 regions. Region, number of countries included in region, 2016 population, average MSW generation (g/capita/day), average OFMSW generation (g/capita/day), and average summer, winter and annual lipid, carbohydrate and protein content

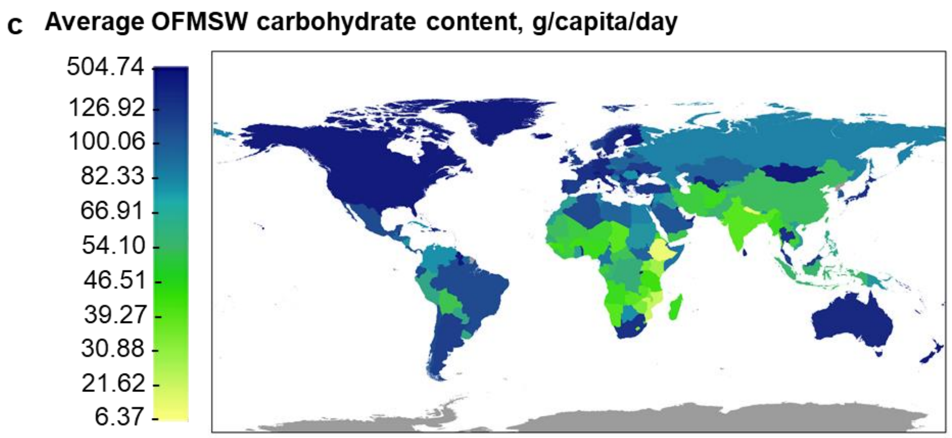
were derived from data collected in ST-1.1 [1-5]. The standard deviation is also presented for each average estimation. Corresponding database Supplementary Table ST1.3.

SI-1.4 OFMSW composition

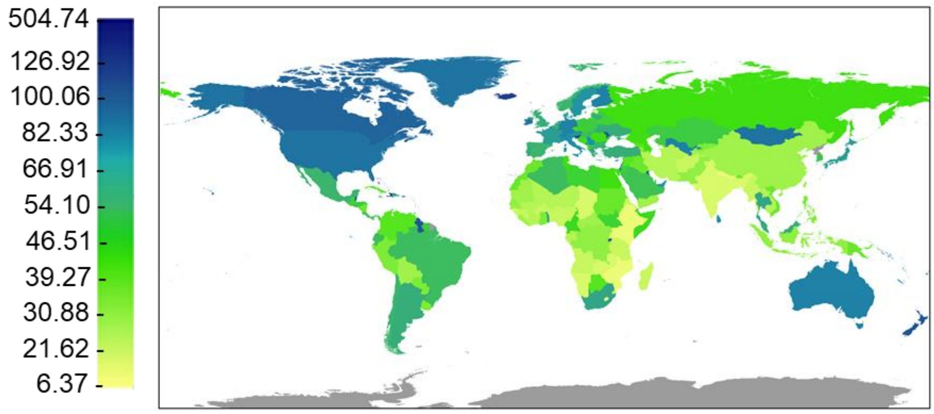
Regional OFMSW conversion factors (g OFMSW/g MSW) were derived from Kaza et al., (2018). Summer and winter lipid, carbohydrate, and protein contents (g/kg OFMSW) were derived from Esteves and Devlin (2010). Corresponding database Supplementary Table ST1.4







d Average OFMSW protein content, g/capita/day



Supplementary Information Figure 1 | Average Organic Fraction Municipal Solid Waste (OFMSW) generation (kg/capita/day) and OFMSW macronutrient composition (g/capita/day) was calculated for each country using data from literature [1-4]. **a** OFMSW generation was plotted according to a colour gradient scale ranging from low (minimum 0.08 kg/capita/day) to high (maximum 2.56 kg/capita/day). **b** OFMSW lipid content was plotted according to a colour gradient scale ranging from low (minimum 6.37 g/capita/day) to high (maximum 218.87 g/capita/day). **c** OFMSW carbohydrate content was plotted according to a colour gradient scale ranging from low (minimum 14.69 g/capita/day) to high (maximum 504.76 g/capita/day). **d** OFMSW protein content was plotted according to a colour gradient scale ranging from low (minimum 7.16 g/capita/day) to high (maximum 246.00 g/capita/day).

Supplementary Information 2

SI-2 Biochemical analysis of agricultural lignocellulosic residues

Crop products were classified into 11 product categories: brewing, cereal grains, fiber crops, fruits & berries, oil crops, pulses, roots & tubers, seeds & nuts, sugar crops, tobacco, vegetables based on biochemical analysis grouping and product type [6,7]. Annual yields (megatonnes/year) for each crop were analysed by country. Agricultural residue yields (megatonnes/year) were estimated based on the residue to product ratio ($RPR_{r,c}$) [6] and crop production $Yield_{c,j}$ (Eq.(S1)). Average cellulose, hemi-cellulose and lignin contents (% dry weight) of agricultural residues were collected from Phyllis database [7] to derive lignocellulosic resource potential for each region $Lignocellulose_j$ (Eq.(S1)).

$$Lignocellulose_j = \sum_{x,r} \alpha_{x,r}\, RPR_{r,c} Yield_{c,j} \quad \textbf{(S1)}$$

Where $RPR_{r,c}$ denotes the ratio of residue r to crop c,. $Lignocellulose_j$ is the lignocellulosic resource potential for region j, measured in megatonnes/year. $\alpha_{x,r}$represents the biochemical content (% dry weight) of lignocellulosic components x (lignin, hemicelluloses or cellulose) of residue r. Corresponding database Supplementary Table ST2.

Supplementary Information 3

SI-3 Microbial Protein

Reported microbial protein kingdom, genus and species, alternative names, national centre for biotechnology information (NCBI) number, reported protein production (% dry mass), trophic mechanism, and reported substrate were collected from literature. Reported substrates were catagorised into 7 classes: food-grade carbon source, food industry solid waste, food industry wastewater, lignocellulosic resource, petrochemical wastewater, waste gas CO_2, and waste gas methane.

A Newick tree was constructed from taxonomic classifications of species according to NCBI taxonomy database [8] and was uploaded to the interactive tree of life (iTOL) programme [9]. Average protein contents and substrate category were from values compiled from previous studies. Where a range of protein production values was obtained for a microbial species, average protein contents were calculated [10-51]. Corresponding database Supplementary Table ST3.

Supplementary Information 4

SI-4.1 Amino acid detailed

Amino acid content is presented for different waste-to-protein sources and benchmark comparison protein sources. Waste-to-protein sources include 7 orders of feed-grade insect: *Diptera* (true flies), *Hemiptera* (true bugs), *Lepidoptera* (butterflies and moths), *Blattodea* (cockroaches, termite), *Coleoptera* (beetles), *Hymenoptera* (sawflies, wasps, bees, ants), and *Orthoptera* (locusts, crickets and grasshoppers). *Hermetia illucens* and *Tenebrio molitor* were selected as subcategories of *Diptera* and *Coleoptera*, respectively, due to their extensive recent literature. Waste-to-protein sources also include 5 genera of feed-grade mycoprotein: *Pleurotus albidus*, *Spirulina sp.*, *Auricularia fucosuccinea*, *Agaricus blazei* and *Fusarium* sp.

Bench mark comparison proteins included 4 feed- and food-grade plant-based proteins (*Glycine max, Cannabis sativa, Pisum sativa,* and *Oryza sativa*), feed- and food-grade *Gallus gallus domesticus,* food-grade egg (https://fdc.nal.usda.gov), food-grade mycoprotein (*Fusarium venenatum*) and the recommended 70kg adult daily intake.

Feed-grade protein sources are highlighted in blue, and food-grade protein sources are highlighted in yellow. Food-certified protein sources are indicated with an asterisk '*'.

Protein source, substrate, crude protein content (g/kg DM) and essential, conditionally essential, non-essential amino acid content for 18 amino acids, excluding aspartate and glutamate (g/kg protein) and protein digestibility-corrected amino acid score (PDCAAS, %) were collected from literature [52-116]. Corresponding database Supplementary Table ST4.1.

SI-4b.2 Amino acid average

Average amino acid composition (g/kg protein) for 18 essential, conditionally essential, non-essential amino acids (excluding aspartate and glutamate) and protein digestibility corrected amino acid score (PDCAAS) were calculated using data from ST-4.1 for each protein source. Standard deviations are also presented for each protein source, calculated using data from ST4.1. Corresponding database Supplementary Table ST4.2.

Supplementary Information 5

Protein recovery potential of a waste-to-protein system. Corresponding database Supplementary Table ST5.

SI-5.1 OFMSW-to-insect

The global potential of feed-grade OFMSW waste input (megatonnes/year) was estimated based on Eq.(S2). Outputs (megatonnes/year) were determined by waste-to-protein conversion efficiency for three different species of insect (*Hermetia illucens, Archeta domesticus,* and *Tenebrio molitor*), Eq.(S5). Conversion efficiency (g protein/g input) was based on feed conversion ratio (g insect biomass/g OFMSW) and protein contents of insect outputs (g protein/g insect biomass) reported from literature [117].Corresponding database Supplementary Table ST5.1.

SI-5.2 Lignocellulosic-to-microbial protein

The global potential of food-grade lignocellulosic waste (megatonnes/year) was estimated based on Eq.(S3). Output protein (megatonnes/year) was estimated based average cellulose content (g cellulose/g lignocellulosic content), sugar extraction efficiency (g glucose/g cellulose) and microbial protein content (g protein/g microbial biomass) for three different microbial protein species (*Fusarium venenatum, Candida utilis,* and *Kluvymyces marxianus*) for glucose only and glucose and xylose, Eq.(S6) [118,119].

Estimates for lignocellulosic waste glucose only, and glucose and xylose were based on sugar extraction coefficients derived from previous published research where glucose was extracted from rice straw using food-grade ionic liquid [Ch][HSO4] in combination with food-grade Celluclast [118]. We assumed the same residues and same efficiency as rice straw glucose in our estimation. We assumed the same sugar extraction coefficient of xylose as lignocellulosic glucose i.e. 0.424 (g xylose/g hemicellulose). Conversion efficiency for lignocellulose-derived *F.venenatum* was based from previously published research [118]. Corresponding database Supplementary Table ST5.2.

SI-5.3 Food industry-to-biophysicochemical treatment

The global potential input of food industry examples (brewery and fishing) were estimated using Eq.(S4), (megatonnes/year).

Protein outputs (megatonnes/year) were estimated for three different biophysiochemical treatments (2% alcalase enzyme, hydrothermal treatment and sequential alkaline and dilute acid treatment). Conversion efficiencies obtained from literature were applied to estimate the protein contents of food industry waste (Eq.(S7)) [120-122]. Corresponding database Supplementary Table ST5.3.

SI-5.4 Input waste streams

Regional waste stream inputs were collected for OFMSW (megatonnes/year) [1]. Regional residue lignocellulosic content (megatonnes/year), lignocellulosic content, and holocellulosic content were derived from literature [6,7]. Global food industry waste (megatonnes/year, 2018) and protein content (g protein/g waste input) for fishing and brewery were based on previously published literature [120-122]. Corresponding database Supplementary Table ST5.4.

$$In^{OFMSW} = \sum_j OFMSW_j \quad \textbf{(S2)}$$

Where the variable In^{OFMSW} denotes the total global OFMSW potential which is determined by the (megatonnes/year) regional OFMSW $OFMSW_j$ (megatonnes/year) (SI-1) [1,5].The set j represents the different regions, defined as: Africa, Caribbean, Central and West Asia, East Asia, Europe, Latin America, North America, Pacific, South Asia, and South East Asia.

$$In^{Ligno} = \sum_j Lignocellulose_j \quad \textbf{(S3)}$$

Where the variable In^{Ligno} denotes the global potential of lignocellulosic agriculture residues (megatonnes/year) which is dependent on the regional agricultural residue $Lignocellulose_j$ (megatonnes/year) (SI-2) [6,123]. The set *j* represents the 11 different regions: Africa, Caribbean, Central and West Asia, East Asia, Europe, Latin America, North America, Pacific, South Asia, and South East Asia.

$$In^{FD} = \sum_{j,FD} W_{j,FD} \quad \textbf{(S4)}$$

Where the variable In^{FD} denotes the total input from global food and drink industry waste which is determined by the regional sector-specific waste $W_{j,FD}$ (megatonnes/year) ; set FD and j stand for specific food and drink sector and region, respectively; in Figure 5, FD includes fishing and aquaculture industry [124] and brewery industry [125].

$$Output_s = In^{OFMSW} (FCR_{s,} \times PC_s) \quad \textbf{(S5)}$$

The variable $Output_s$ represents the food-grade or feed-grade protein output of each insect species s (megatonnes/year) by converting OFMSW; it is determined by the global OFMSW resource availability (In^{OFMSW}), feed conversion ratio $FCR_{s,}$ (g insect outputs/kg substrate) and protein content (PC_s) for given species s (g protein/g biomass) In Figure 5, the set s refers to BSFL (*Hermetia illucens*), cricket *(Acheta domesticus)* or mealworm (*Tenebrio molitor*) [117].

$$Output_M = In^{Ligno} (Sugar\ extraction \times LC_M \times PC_M) \quad \textbf{(S6)}$$

The variable $Output_M$ represents the protein output (megatonnes/year) by converting lignocellulosic agriculture residues using different microbial species M ; $Sugar\ extraction$ represents the conversion coefficient for sugar extraction from lignocellulosic resources [118], LC_M represents coefficient to convert lignocellulosic sugar to microbial biomass (g biomass/kg substrate) and PC_M denotes protein content (g protein/g biomass) for given microbial species M. In Figure 5, M refers to *Fusarium venenatum*, *Candida utilis*,and *Kluvymyces marxianus* [118,119].

$$Output_{BC} = \sum_{j,FD} W_{j,FD} \times PC_{FD} \times CF_{BC,FD} \quad \textbf{(S7)}$$

The variable $Output_{BC}$ denotes the protein output (megatonnes/year) by converting food and drink industry waste using biophysiochemical technologies, which is determined by the regional waste availability $W_{j,FD}$, protein content of regional waste (PC_{FD}) and technology conversion efficiency ($CF_{BC,FD}$). $CF_{BC,FD}$ is a technology dependent conversion efficiency, which is derived from previous published research [120,121]; set BC refers to specific biophysicochemical technology including 2% alcalase enzyme treatment, hydrothermal pre-treatment, alkaline and dilute acid treatment to derive feed-grade protein from food-industrial waste streams.

Supplementary information 6

Growth cycle information from literature is presented for waste-to-protein holometabolous and hemimetabolous insect species, and *Gallus domesticus* (broiler chicken) and *Bos taurus* as a bench mark comparison. Corresponding database Supplementary Table ST6.

SI-6.1 Holometabolous species growth cycles

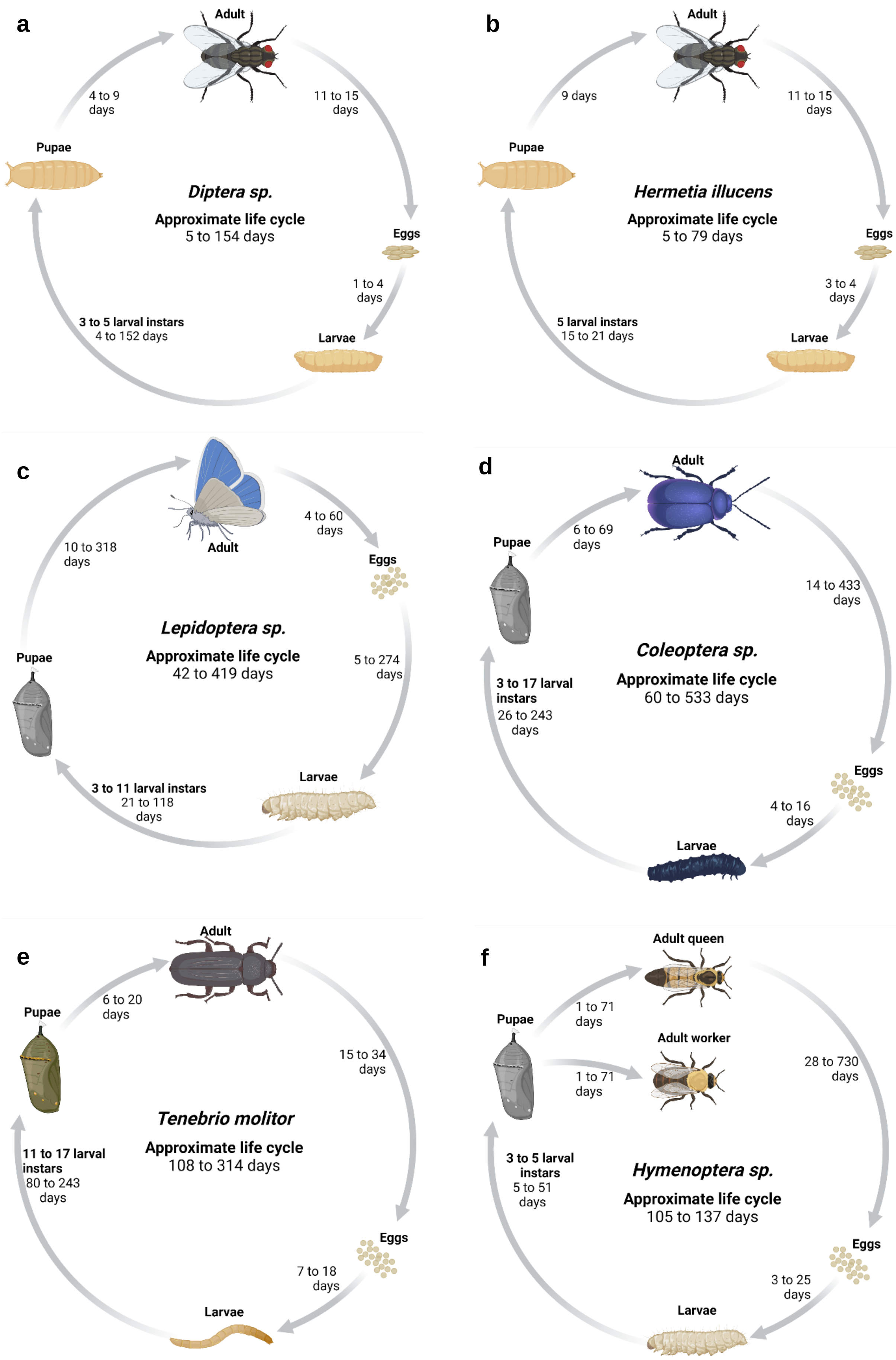
Species are sorted by order including: *Diptera* (true flies), *Lepidoptera* (butterflies and moths), *Coleoptera* (beetles) and *Hymenoptera* (sawflies, wasps, bees, ants). Number of larval instars, duration of egg incubation, larval, pupae and adult stages and total life span (days) are collected from literature. Data collected for species within an order were used to estimate a range for each order [126-157]. Corresponding database Supplementary Table ST6.1.

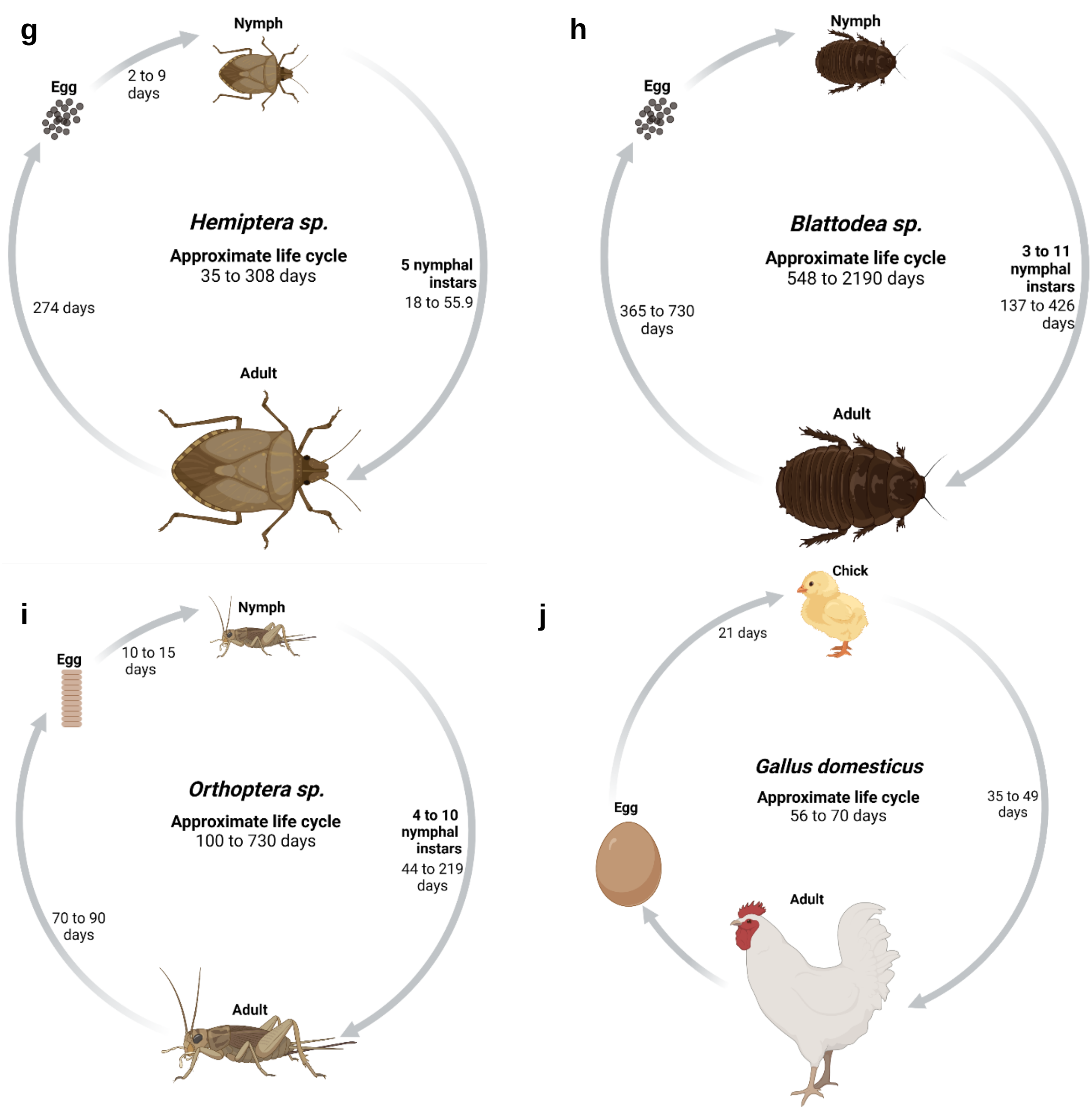
SI-6.2 Hemimetabolous species growth cycles

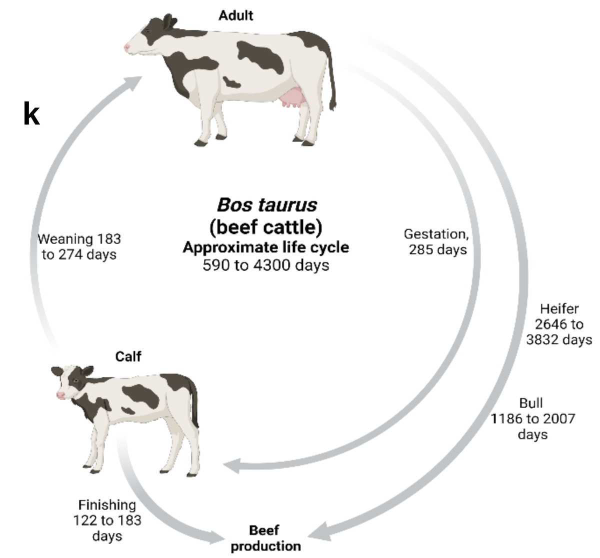
Species are sorted by order including: *Hemiptera* (true bugs), *Blattodea* (cockroaches, termite) and *Orthoptera* (locusts, crickets, grasshoppers). Number of larval instars, duration of egg incubation, nymphal, and adult stages and total life span (days) are collected from literature. Data collected for species within an order was used to estimate a range for each order [158-178]. Corresponding database Supplementary Table ST6.2.

SI-6.3 Animal-based protein growth cycles

Life span (days) collected from literature are provided for *Gallus domesticus*, and *Bos taurus* (beef cattle) as a bench mark for comparison with waste-to-protein insect species [179-184]. Corresponding database Supplementary Table ST6.3.


a
Adult
11 to 15 days
Eggs
1 to 4 days
Larvae
3 to 5 larval instars
4 to 152 days
Pupae
4 to 9 days
Diptera sp.
Approximate life cycle
5 to 154 days
b
Adult
11 to 15 days
Eggs
3 to 4 days
Larvae
5 larval instars
15 to 21 days
Pupae
9 days
Hermetia illucens
Approximate life cycle
5 to 79 days
c
Adult
4 to 60 days
Eggs
5 to 274 days
Larvae
3 to 11 larval instars
21 to 118 days
Pupae
10 to 318 days
Lepidoptera sp.
Approximate life cycle
42 to 419 days
d
Adult
14 to 433 days
Eggs
4 to 16 days
Larvae
3 to 17 larval instars
26 to 243 days
Pupae
6 to 69 days
Coleoptera sp.
Approximate life cycle
60 to 533 days
e
Adult
15 to 34 days
Eggs
7 to 18 days
Larvae
11 to 17 larval instars
80 to 243 days
Pupae
6 to 20 days
Tenebrio molitor
Approximate life cycle
108 to 314 days
f
Adult queen
Adult worker
28 to 730 days
Eggs
3 to 25 days
Larvae
3 to 5 larval instars
5 to 51 days
Pupae
1 to 71 days
1 to 71 days
Hymenoptera sp.
Approximate life cycle
105 to 137 days





Supplementary Information Figure 6 | Growth cycles of 9 proposed waste-to-protein insect orders including holometabolous **a** *Diptera* (including **b** *Hermetia illucens*), **c** *Lepidoptera*, **d** *Coleoptera* (including **e** *Tenebio molitor*) and **f** *Hymenoptera* and hemimetabolous **g** *Hemiptera*, **h** *Blattodea* and **i** *Orthoptera*. **j** *Gallus gallus domesticus* (broiler chicken) and **k** *Bos taurus* (beef cattle) are included as benchmark comparisons of animal-based protein sources. Detailed data can be found in Supplementary Information 6 [126-184]. Created with BioRender.com.

SI-6.4 Insect protein organisations

Insect protein organisations and businesses are listed in a database including: region and country of origin, insect species sold, feed- or food-grade, technology readiness level (TRL). TRL is catagorised as 1 to 3, 4 to 6 or 7 to 9, where 1 to 3 indicate research and development stage, 4 to 6 indicates pilot scale and 7 to 9 indicates commercial status. Notes are also included to indicate if organisations are non-governmental organisation (NGO) or utilising waste-to-protein. Corresponding database Supplementary Table ST6.4.

Supplementary Information 7

SI-7 Novel food and feed safety regulation

Supplementary Information Table 7.1 | Comparison of novel food and feed regulation for 9 different countries and regions including: the European union (EU), Australia, New Zealand, Canada, China, United States (US), India, Brazil, Singapore.

	EU	Australia/New Zealand	Canada	China
Novel food definition	Any food that was not used for human consumption to a significant degree within the Union before 15 May 1997[185]	Any non-traditional food that requires an assessment of the public health and safety[186]	A substance, including a microorganism that does not yet have a history of safe use as a food; A food that has been manufactured, prepared, preserved, or packaged by a process that has not been previously used for that food, and causes the food to undergo a major change; a major change; a food that is derived from a plant, animal or microorganism that has been genetically modified[187]	Food that has not been consumed traditionally in China, including: Animals, plants, or microorganisms; Substances derived from animals, plants, or microorganisms; Food substances which structure has been altered; Other newly developed food materials, such materials resulting from high-tech production methods (traditional consumption refers to known production and consumption of food material in the last 30 years and mentioned in the Pharmacopoeia of the People's Republic of China)[188]
History of Human Consumption Timeframe	Before 15 May 1997 within the EU; at least 25 years in a third country[185]	2-3 generations; 10-20 years in AU/NZ (guideline)[186]	“a number of generations”; evidence from other countries allowed[187]	In the last 30 years in China[188]
Legislation	Regulation (EU) 2015/2283[185]	Food Standard 1.5.1.[186]	Food and Drug Regulations (B.28.002)[187]	Food Safety Laws (2015); Administrative Measures for Safety Review of New Food Materials (2013)[188]
Government Organisation for Pre-Dossier Submission Consultancy	Unknown	Advisory Committee on Novel Foods (ACNF)[189]	Unknown	Unknown
Recipient Authority for Dossier Submission	European Commission (Member States informed)[190]	Food Standards Australia New Zealand (FSANZ)[189]	Health Canada’s Food Directorate[191]	Hygiene Supervision Center of The Health Administration Under the State Council[188]
Official Guidance Document Available?	Yes[190]	Yes[189]	Yes[191]	Yes[188]
Authority Responsible for Risk Assessment	European Food Safety Authority (EFSA), open to public comments[190]	Food Standards Australia New Zealand (FSANZ)[189]	Health Canada’s Food Directorate[191]	The Health Administration Under the State Council (Expert Assessment Committee on Novel Foods), open to public comments[188]
Authority Responsible for Final Decision-Making	European Commission, upon favourable vote from Member State representatives of the Standing Committee on Plants, Animals, Food and Feed (SCoPAFF)[190]	Food Standards Australia New Zealand (FSANZ) Request for review can be given by Australia and New Zealand Ministerial Forum on Food Regulation as well as The New Zealand Government[189]	Food Rulings Committee[191]	The Health Administration Under the State Council[188]
Estimated Time from Application Submission to Final Decision	7-24 months (within last two years)[192]	6-18 months[189]	410 days, 90% of the time (Performance Standard)[193]	2-3 years[194]

	US	India	Brazil	Singapore
Novel food definition	N/A	Food that: May not have a history of consumption by humans, or may not have a history of consumption in the region/ country of interest; or may not have any history of consumption of any ingredient used in it or the source from which it is derived; or a food or ingredient that is obtained by using new technology and/or innovative engineering process. This procedure may change the size, composition, or structure of the food or its ingredients – which may in turn change its nutritional value, metabolism, properties/ behavior or level of undesirable substances.[195]	Foods with no history of use in the country; foods containing novel ingredients with exceptions; foods containing substances already consumed that may be added or used at levels much higher than those currently observed in the foods that constitute part of a regular diet; and food offered in the form of capsules, pills, tablets and the like[196]	Foods and food ingredients that do not have a history of safe use, where safe use is defined as consumption as an ongoing part of the diet by a significant human population (e.g., the population of a country), for a period of at least 20 years and without reported adverse human health effects.[197]
History of Human Consumption Timeframe	Experience based on common use in food before 1958 for GRAS determination[198]	More than 15 years in India or more than 30 years globally[199]	Unknown	At least 20 years[197]
Legislation	Food additives: 21 U.S.C §342[200] GRAS: 21 CFR §170.30(b)[198]; 21 CFR §170.30(c)[201]; 21 CFR §170.30(f)[202];	Food Safety and Standards (Approval of Non-Specified Food and Food Ingredients) Regulations, 2017.[203]	Resolution 16/1999 and Resolution 17/1999[196]	Singapore Food Agency Act (2019); Sale of Food Act (1973)[197]
Government Organisation for Pre-Dossier Submission Consultancy	FDA’s Office of Food Additive Safety (OFAS)[204]	Unknown	Unknown	Future Ready Food Safety Hub (FRESH) FSA via monthly Novel Food Virtual Clinics to engage companies at early stages of R&D[197]
Recipient Authority for Dossier Submission	FDA (for food additive petition)[204] Self-determined (for GRAS notification)[205]	Food Safety and Standards Authority of India (FSSAI)[199]	Brazilian Health Regulatory Agency (ANVISA)[196]	Singapore Food Agency (SFA)[197]
Official Guidance Document Available?	Yes[204,205]	Yes[199]	Unknown	Yes[197]
Authority Responsible for Risk Assessment	FDA (for food additive petition)[204] GRAS panel consisting of experts to review publicly available scientific evidence[205]	Food Safety and Standards Authority of India (FSSAI)[199]	The Brazilian Health Regulatory Agency (ANVISA)[196]	Singapore Food Agency (SFA)[197]
Authority Responsible for Final Decision-Making	FDA (for food additive petition; voluntary GRAS notification can be made)[204,205]	Food Safety and Standards Authority of India (FSSAI)[199]	The Brazilian Health Regulatory Agency (ANVISA)[196]	Singapore Food Agency (SFA)[197]
Estimated Time from Application Submission to Acceptance	Typically, FDA responds to GRAS notification within 180 days; Average of 24 months for food additive petition[192]	Unknown	Unknown	9-12 months[197]

Supplementary Information 8

SI-8.1 Life cycle assessment (LCA) and techno-economic analyses (TEA)

Based on comprehensive review, data on life cycle assessment (LCA) and techno-economic analyses (TEA) have been collected for different waste-to-protein technologies and benchmark protein sources. Detailed data are presented in Supplementary Table ST-8.

Waste-to-protein covered in Supplementary Table ST-8 include 4 feed-grade insects (*Tenebrio molitor*, *Musca domestica*, *Hermetia illucens*, and *Protaetia brevitarsis seulensis)*, 7 feed-grade microbial protein sources (Hydrogen-oxidising bacteria sp., Methane-oxidising bacteria sp., *Tetraselmis suecica*, *Tisochrysis lutea*, *Arthrospira platensis*, *Chlorella sp.*, *Ascochloris sp.)* and 4 food-grade microbial protein technologies (*Cupriavidus necator* [206], *Spirulina platensis* [207], *Fusarium venenatum A3/5* from lignocellulosic resource and hydrogen-oxidising bacteria sp. Solein® from Solar Foods). It is worth noting that the food-grade microbial proteins listed above are still at the research and development stages.

Bench mark protein sources in Supplementary Table ST-8 cover commercialised or reported insect proteins and microbial proteins cultivated with non-waste substrates. These include 1 feed-grade insect (*Hermetia illucens*), 2 feed-grade microbial proteins (FeedKind® from Calysta, and *Chlorella vulgaris*), and 5 food-grade insects (*Tenebrio molitor, Hermetia illucens, Apis mellifera, Gryllus bimaculatus,* and *Acheta domesticus*). Additionally, traditional plant- and animal-sourced proteins have been also taken into account, involving soybean meal and fish meal as feed-grade proteins, cultured meat, food-certified Quorn™ Mycoprotein, and 10 food-grade plant-based proteins (soybean, tofu, bean, pea, nut, groundnut, other pulses, maize, rice, wheat), as well as 9 animal-based food proteins (chicken, egg, milk, cheese, beef, lamb, pork, fish, crustacean).

Supplementary Table ST-8 presents data collected for protein contents on a dry weight (%DW) or fresh weight (%FW) basis, oven-dried weight on a %FW basis, LCA system boundary, quantitative LCA and TEA results. 9 life cycle impact categories have been considered i.e. acidification, freshwater eutrophication, marine eutrophication, global warming potential (GWP100), ozone depletion, fossil resource depletion, photochemical oxidant formation, agricultural land occupation, and water use/depletion. To facilitate comparisons, LCA data have been compiled and recalculated on the basis of per kg of protein [206-236]. In economic analyses, capital cost, operational cost, total production cost, minimum selling price, and market price have been considered and compared based on per kg of protein [234,237-249]. Minimum selling price is defined as selling price of the protein product for which the net present value (NPV) is zero, which has been used to assess the economic viability of the protein technologies [234]. The total production cost ($E_{KPI=cost,s}$) is derived from Eq.(S8).

$$E_{KPI=cost,s} = CAPEX_s + OPEX_s \tag{S8}$$

Where the set s represents the protein species; the variable $E_{KPI=cost,s}$ denotes the total production costs of a given protein species s (USD/unit product), which is determined by of the capital cost, $CAPEX_s$ (USD/unit product) and operational cost, $OPEX_s$ (USD/unit product).

The LCA and TEA comparisons between different protein sources have been based on the equivalent units per kg protein, where the nutritional value (amino acid compositions) of different proteins were not considered. Thus, to facilitate comparison, LCA and TEA results collected from literatures were recalculated following the Eq.(S9).

$$E^*_{KPI,s} = \frac{E_{KPI,s}}{PC_s/DW_s} \tag{S9}$$

Where the variable $E^*_{KPI,s}$ denotes the comparable LCA or TEA results, based on per kg of protein for given protein species s, expressed as the key performance indicator KPI. The set

KPI contains 9 LCA and 3 TEA elements, including acidification, freshwater eutrophication, marine eutrophication, GWP100/global warming, ozone depletion, fossil resource depletion, photochemical oxidant formation, agricultural land occupation, water use/depletion, total production cost, minimum selling price, and market price. $E_{KPI,S}$ is the LCA or TEA data based on fresh weight. PC_s is defined as the protein contents of fresh weight for a given protein species s. DW_s stands for the oven-dried weight in % of fresh weight. The PC_s, DW_s, and other key assumptions are summarised in the Supplementary Information Table SI-T-8.1

Supplementary Information Table 8.1| Summary of protein content (PC_s, % fresh weight; PCD_s, % dry weight), oven-dried weight (DW_s, % fresh weight), and key assumptions.

	Protein source	PC_s (% fresh weight) / PCD_s (% dry weight) [a]	DW_s [b] (% fresh weight)	Data source and other key assumptions
Waste-to-protein	**Insect protein**			
	Tenebrio molitor	PC_s: 18.84%	37.16%	[221,232]
	Musca domestica	PCD_s: 47.90%.	NA	Substrate: mixture of poultry manure and house waste [236]
	Musca domestica	PCD_s: 63.65%.	NA	Substrate: pig manure, chicken manure, or mixture of sheep waste and fresh ruminant blood [221,223,224,246]
	Hermetia illucens (dried, defatted meal)	PCD_s: 100%	NA	Substrate: food wastes The protein content of dried, defatted meal is assumed to be 100%. Because this fresh meal mainly consists of water, fat, and protein. [228]
	Hermetia illucens (protein concentrate)	PC_s: 56.3%	NA	Substrate: by-products of food industry [230]
	Hermetia illucens (fresh insect puree)	PC_s: 17%	NA	Substrate: by-products of food industry [230]
	Hermetia illucens	PC_s: 48%	NA	Substrate: food wastes [225]
	Hermetia illucens (prepupae)	PC_s: 43.9%	NA	[245]
	Hermetia illucens	PC_s: 65%	NA	Substrate: agricultural by-products from starch manufacture and food by-product [235]
	Hermetia illucens	PC_s: 45.88%	NA	Substrate: chicken manure, brewery grains, potato peel, or expired food [213,221,223,246]
	Hermetia illucens	PCD_s: 52.80%	NA	Substrate: hen diet [208]
	Hermetia illucens	PCD_s: 53.40%	NA	Substrate: maize distillers [208]
	Hermetia illucens	PCD_s: 51.20%	NA	Substrate: okara [208]
	Hermetia illucens	PCD_s: 54.10%	NA	Substrate: brewery grains [208]
	Microbial protein			

	Hydrogen-oxidising bacteria sp.	PC_s: 65%	NA	The protein content of this hydrogen-based microbial protein ranges from 50-80%. Therefore, the mid-value (65%) is used as the protein content in fresh weight of this microbial protein. [242]
	Methane-oxidising bacteria sp.	PC_s: 20%	NA	[215]
	Arthrospira platensis	PC_s: 52.8%	96%	[229]
	Chlorella sp.			
	Ascochloris sp. ADW00	PC_s: 52.25%	95%	[229,244]
	Fusarium venenatum A3/5	PC_s: 12.59%	NA	[234]
Bench mark comparison	**Insect protein**			
	Tenebrio molitor	PC_s: 13.5%	NA	[227,228]
	Apis mellifera	PC_s: 10%	NA	[226]
	Microbial protein			
	Chlorella vulgaris	PC_s: 52.8%	96%	[229]
	Fusarium venenatum A3/5 (Quorn™ Mycoprotein)	PC_s: 11.7% PCD_s: 44%	25%	[234] [221]
	Plant-based protein			
	Glycine max (soybean meal)	PC_s: 45.55%	92.20%	[245,246]
	Glycine max (soybean)	PC_s: 36.49%	91.46%	[221]
	Phaseolus vulgaris (common bean)	PC_s: 23.58%	88.25%	[221]
	Zea mays (maize)	PC_s: 3.24%	24.00%	[221]
	Oryza sativa (rice)	PC_s: 6.75%	87.40%	[221]
	Triticum aestivum (wheat)	PC_s: 12.15%	89.06%	[221]

	Animal-based protein			
	Fish meal	PC_s: 70%	NA	[235]
	Fish meal	PC_s: 39.71%	93.00%	[245,246]
	Gallus domesticus (chicken)	PC_s: 17.45%	34.02%	[221]
	Egg protein concentrate	PC_s: 68%	85%	[229]
	Egg	PC_s: 12.56%	23.85%	[221]
	Milk	PC_s: 3.15%	11.87%	[221]
	Bos taurus (beef)	PC_s: 18.89%	36.65%	[221]
	Sus scrofa domesticus (pork)	PC_s: 16.31%	40.03%	[221]
	Oreochromis spp. (tilapia)	PC_s: 20.08%	21.92%	[221]
	Katsuwonus pelamis (skipjack tuna)	PC_s: 22.00%	29.42%	[221]

Note:
a. PC_s denotes the protein contents of fresh weight (% fresh weight) for a given protein species s; PCD_s represents the protein content of dry weight (% dry weight) for a given protein species s.
b. DW_s stands for the oven-dried weight (% fresh weight) for a given protein species s.
NA: data not available

SI-8.2 LCA and TEA of waste-to-protein

Base on the comprehensive literature review and analyses presented in Supplementary Table ST-8, we have compared the environmental profiles and economic viability between different protein species based on per kg of protein. Regardless of protein grade (feed- or food-grade) and their nutritional values (amino acid compositions), we have drawn the following conclusions.

LCA comparisons of waste-to-protein technologies and benchmark protein sources suggested that -

1. The environmental impacts of different insect proteins derived from wastes vary. Among 4 insect proteins produced via ‘waste-to-protein’ pathways in Supplementary Table ST-8, *Hermetia illucens* has attracted increasing research attention and represent the most environmentally sustainable option across most of the impact categories (GWP100: -1.40E+01 – 2.42E+01 kg CO_2 eq. per kg protein; Agricultural land occupation: -3.67E+01 – 1.78E+01 m^2a per kg protein; Water use/depletion: -7.2E-02 – 2.39E+00 m^3 per kg protein). In contrast, *Musca domestic* demonstrated higher environmental burdens compared with other insects, especially in energy profile (1.10E+00 – 1.13E+03 MJ per kg protein), agricultural land utilisation (4.71E-02 – 8.90E+01 m^2a per kg protein) and water use (5.14E-02 – 2.19E+03 m^3 per kg protein) categories.
2. Insect proteins produced from wastes demonstrated competitive environmental footprints in acidification, eutrophication, land use, and water use, compared with traditional plant-sourced proteins. For instance, the environmental scores of waste derived *Tenebrio molitor* (Freshwater eutrophication: 2.30E-02 - 2.74E-02 kg P eq. per

kg protein; GWP100: 5.25E+00 – 5.77E+00 kg CO_2 eq. per kg protein; Agricultural land occupation: 6.35E+00 – 8.49E+00 m^2a per kg protein) is close to these of soybean (Freshwater eutrophication: 1.60E-02 kg P eq. per kg protein; GWP100: 8.90E-01 – 3.74E+01 kg CO_2 eq. per kg protein; Agricultural land occupation: 5.24E+00 – 1.19E+01 m^2a per kg protein); while *Hermetia illucens* exhibits a better environmental performance than soybean in these categories. However, it should be noted that the energy consumption of waste-derived *Hermetia illucens* (mostly ranging from 7.19E+00 to 1.50E+02 MJ per kg protein) is slightly higher than traditional plant-based proteins on market (ranging from 5.33E+00 to 1.56E+01 MJ per kg protein), but lower than traditional animal-sourced proteins (ranging from 3.53E+01 to 2.99E+02 MJ per kg protein).

3. The sustainability of different microbial proteins also varies. Solein® (hydrogen-oxidising bacteria sp.) from Solar Foods outperformed other microbial protein species in most environmental impact categories (GWP100: 3.91E-03 - 4.21E-02 kg CO_2 eq. per kg protein; Agricultural land occupation: 5.22E-05 – 1.27E-03 m^2a per kg protein; Water use/depletion: 2.34E-05 – 1.71E-04 m^3 per kg protein). Furthermore, Solein® from Solar Foods is generally recognised as food-grade [250], although more work should be undertaken to confirm its food safety produced via ‘waste-to-protein’ pathways. Additionally, microbial proteins produced via electricity from grid showed higher GWP100 burdens, ranging from 1.29E+01 to 4.64E+02 kg CO_2 eq. per kg protein, in comparison with that utilising renewable energy (solar, wind), ranging from 3.91E-03 to 4.26E+00 kg CO_2 eq. per kg protein, indicating that the environmental burdens derived from fossil fuel consumption for energy input cannot be neglected.
4. The environmental credits derived from carbon capture and utilisation e.g. waste gas CO_2 as substrate for microbial proteins cultivation can benefit the sustainability of

protein sources. Based on the LCA profile for *Tetraselmis suecica* and *Tisochrysis lutea* [218], the assumed 'zero-burden' substrate - flue gas (a recycled waste-product obtained from the burning of used vegetable oils) demonstrates superior environmental performance (GWP100: 3.84E+01 - 4.84E+01 kg CO_2 eq. per kg protein; Fossil resources depletion: 3.65E+02 – 5.94E+02 MJ per kg protein; Water use/depletion: 1.31E+01 – 2.09E+01 m^3 per kg protein) to pure CO_2 from cylinder (GWP100: 5.96E+01 – 6.61E+01 kg CO_2 eq. per kg protein; Fossil resources depletion: 5.96E+02 – 8.15E+02 MJ per kg protein; Water use/depletion: 1.65E+01 – 2.42E+01 m^3 per kg protein). This result suggests the significant environmental advantages of 'waste-to-protein' technologies. However, the previous research followed an economic allocation approach to partition the environmental impacts between co-products which led to 'zero-burden' flue gas but underestimate the potential environmental benefits of waste-to-protein. If following a carbon counting approach to track the carbon captured, utilised and sequestered in microbial fermentation, a negative environmental 'credit' could be allocated to microbial protein, which would significantly enhance the environmental sustainability profiles.

5. Microbial proteins derived from wastes represent environmentally superior systems to plant- and animal-sourced proteins across almost all impact categories, except for the fossil resources depletion/energy use. The energy use for microbial proteins ranges from 2.11E+01 to 6.32E+03 MJ per kg protein, which is higher than both traditional plant-based protein (ranging from 5.33E+00 to 1.56E+01 MJ per kg protein) and animal protein (ranging from 3.53E+01 to 2.99E+02 MJ per kg protein). Quorn™ mycoprotein derived from *Fusarium venenatum A3/5* is a commercially produced food-grade microbial protein; *Fusarium venenatum A3/5* cultivated through fermentation of lignocellulosic sugar sources was reported to deliver sustainable footprint [234] including

impacts on GWP100 (2.37E+01 kg CO_2 eq. per kg protein), acidification (1.65E-01 kg SO_2 eq. per kg protein), freshwater eutrophication (1.30E-02 kg P eq. per kg protein), agricultural land occupation (4.39E+00 m^2a per kg protein) and water use/depletion (2.23E+00 m^3 per kg protein). This microbial protein has similar environmental impacts of organic broiler in GWP100 (2.66E+01 kg CO_2 eq. per kg protein) and freshwater eutrophication (1.16E-02 kg P eq. per kg protein), but much lower scores in other categories, indicating its high potential as a protein alternative.

Techno-economic analyses results indicated that -

1. Insect proteins produced from waste demonstrate great competitiveness from the economic perspective. For example, the market price of *Hermetia illucens* (1.94-2.41 USD per kg protein) is cheaper than that of rice (6.02 USD per kg protein) and is close to soybean and wheat (1.33 and 2.27 USD per kg protein, respectively). It is obvious that this insect market price range is lower than that of animal-based proteins (15.4-76.3 USD per kg protein). Nevertheless, it should be noted that the food safety of waste derived insect protein is still under certification. Therefore, the final market price of commercialised waste derived insect protein might increase to some extent, due to the requirement for additional processes to ensure the food safety.
2. The price of different microbial proteins varies significantly. According to Supplementary Table ST-8, it can be difficult for microbial proteins to compete with both plant and animal-sourced proteins due to a relatively high selling price. The feed-grade hydrogen-based microbial protein in García ‘s work [242] (5.69-25 USD per kg protein) has shown to be less economically beneficial than soybean meal (0.754-1.98 USD per kg protein) and fishmeal (3.02-4.01 USD per kg protein). Food-grade Quorn™ mycoprotein product (*Fusarium venenatum A3/5*) derived from lignocellulosic sugar

sources [234] is predicted with a minimum selling price of 173.02 USD per kg protein, which is twice the market price of beef (76.3 USD per kg protein) and six times more than chicken (27.7 USD per kg protein).

The following research gaps have merged from the literature review on LCA and TEA studies of 'waste-to-protein' systems -

1. Further research efforts could be devoted on holistic yet robust analyses of environmental profiles of novel protein sources, in particular on insect and microbial proteins, which represent a clear knowledge gap. Most of the LCA studies published thus far focused on global warming (GWP100), arable land use, and water use impact categories; whereas less research attention has been given to other important impact categories - including fossil resources depletion, acidification, eutrophication, ozone depletion, and photochemical oxidant formation. Furthermore, previous LCA research lacks explicit interpretation of sensitivity and uncertainty in LCA findings. An interesting research direction is to further explore the LCA data quality based on statistical methods to enable robust evidences for decision-making and comparative assertions on novel protein technologies.
2. Limited publicly available TEA studies hinder the understanding of the scalability and viability of waste-to-protein technologies. Computational experiments based on process design and simulation would save empirical efforts at lab or pilot scales and guide research and development to focus on performance-limiting steps. Thus, waste-to-protein process simulation and optimisation represent another research frontier to accelerate novel protein technology scaling-up.

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