Supplementary information

Comparison of carbon footprint and water scarcity footprint of milk protein produced by cellular agriculture and the dairy industry

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DATA 1 Additional process description details for mass and energy balance calculations

In **microfiltration**, the 0.2 µm pore size was chosen to ensure 100% fungus biomass rejection (sterile filtration). The recovery of the permeate was assumed to be 97.5%, and the target protein was assumed to be dissolved in solution, passing through the membrane. The estimation of electricity demand for microfiltration was 2 kWh/m^3 permeate (Hermann and Patel 2007).

The aim of **combined ultra- and diafiltration** was to concentrate the solution and remove residual impurities. A polyethersulfone (PES) membrane with a molecular weight cut-off (MWCO) of 10 kDa was chosen for both ultrafiltration and diafiltration. In mass balance calculations, 100% retention of proteins through the membrane and permeability of 85% for both water and impurities were assumed for the membrane performance. The operation mode for diafiltration was a continuous cross-current operation. The diavolume (the volume of diafiltration buffer introduced into the unit operation compared to the retentate volume) of 5 m^3 buffer solution/m^3 retentate was estimated to decrease the concentration of the impurities derived from bioreactor cultivation to a suitable level (EMD_Millipore 2013). The removal efficiency of impurities was roughly estimated by using the equation presented in (Millipore 2003). The estimation of electricity demand for ultra- and diafiltration was 19.7 kWh/m^3 (Cheryan 1998).

Protein was dried using a **spray drier** to a dry matter content of 95%. The estimate for heat demand was 4.9 GJ/tonne of evaporated water, and the estimate for electricity consumption was based on a heat to electricity consumption ratio of 26.9 (Baker and McKenzie 2005).

The performance of an industrial scale **chromatographic separation** of beta-lactoglobulin based on a simulated moving bed (SMB) unit system was estimated based on the available published data. No experimental data was available. In the chromatographic separation unit, the aim was to increase the concentration of the target protein to 95% (mass basis). The resin and chemicals required for the chromatographic separation depend on the proteins' characteristics. The target protein beta-lactoglobulin has a molecular mass of approximately 18 kg/mol, and the pH at its isoelectric point is pH 5.2 (Andersson and Mattiasson 2006). The contaminating proteins in the solution are not defined in the model. As the pH of the target protein's isoelectric point is below 7, anion exchange media is typically used in chromatographic separation. The buffer solution and other chemicals have been chosen based on (Ng and Snyder 2013), who reported chromatographic separation of beta-lactoglobulin from whey. Chemicals for chromatographic separation included a 0.02 mol/l sodium phosphate buffer solution (at pH 6.4) for equilibration and washing, a buffer solution including a varying concentration of sodium chloride for the elution of proteins and regeneration, and sodium hydroxide for final regeneration. The consumption of chemicals depends on the proteins being separated, the resin media utilized in the chromatograph, and the separation technology chosen. The chosen separation technology—simulated moving bed (SMB) technology—decreases the consumption of chemicals and buffer solution compared to conventional industrial-scale batch chromatographic separation (Andersson and Mattiasson 2006).

In this study, the consumption of chemicals per target protein and the volume of process water per target protein (kg chemical/target protein, dm^3 process water/kg target protein, Table 1) were estimated based on the data from Andersson and Mattiasson (2006), who reported the operation and performance parameters for separating lactoperoxidase and lactoferrin from whey protein concentrate by SMB and compared the results for conventional batch chromatographic separation. Although the separated protein is not the same, the data was used for two reasons. First, the mass balances over the SMB are coarse estimates because the side proteins were not known, and detailed data was not available from either SMB or batch chromatographic separations. Secondly, the chemicals and buffer solution utilized were the same in Andersson and Mattiasson (2006) and Ng and Snyder (2013) for separation for beta-lactoglobulin. The recovery of target protein was assumed to be 98%, based on Andersson and Mattiasson (2006). The estimation of electricity demand for the SMB system (0.05 kWh/m^3) was based on assumption of 1 bar pressure drop in a column, which according to Thang et al (2005) is a typical approximation for industrial chromatograph columns. Recycling of the chemicals was not considered in this study. However, at scale production, this practice would reduce waste streams and improve the environmental performance of DSP.

Table 1 Chemical and process water consumption in the SMB system

Chemical/process water	unit	Amount	Reference
Sodium hydroxide, NaOH (dry)	kg/kg target protein	0.3	Fonterra estimate
Sodium chloride, NaCl (dry)	kg/kg target protein	2.4	Andersson and Matiasson (2006)
Sodium phosphate, Na_2HPO_4 (dry)	kg/kg target protein	0.05	Andersson and Matiasson (2006)
Trisodium phosphate, NaH_2PO_4 (dry)	kg/kg target protein	0.24	Andersson and Matiasson (2006)
Process water	dm^3/kg target protein	145	Andersson and Matiasson (2006)

A diafiltration unit was required after the SMB unit to decrease the concentration of chemicals remaining in the product solution after the chromatographic separation. A similar diafiltration unit was considered as before the SMB: the same membrane was assumed, and the same performance parameters were utilized in the calculation, except for the diavolume. The diavolume of 7 m^3 buffer solution/m^3 retentate was estimated to decrease the concentration of the salt impurities derived from the SMB to a suitable level (EMD_Millipore 2013).

References (DATA 1)

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Data 2 Additional information about the datasets and factors used in the calculations

The datasets used in the calculations are divided into 9 life cycle steps in the reporting. These are carbon source, nitrogen source, other materials, process water, electricity, thermal energy, transportation, waste treatment, and avoided feed production. The ecoinvent database 3.7.1 (with the cut-off system model) is used as the source for the LCI datasets. The types of AWaRe factors used in each reporting category are shown in Table 2, while the datasets used in each reporting category and different locations are shown in Table 2. Finally, the collected AWaRe factors to represent an average value for the Queensland coastline in Australia, Victoria coastline/Melbourne area, and Alabama and Louisiana in United States (US) are shown in Table 4–Table 7 and Fig. 1-Fig. 4, respectively. It should be noted that the geographical areas of AWaRe factors are not equal between different FIDs, and the deviations may be large within the total area considered.

Table 2 Types of AWaRe factors used in each reporting life cycle step

Carbon source	Sugar production location, agri
Nitrogen source	For corn steep solids: sugar production location agri, for ammonia, plant location non-agri
Other materials	Plant location, non-agri (100km transport distance assumed)
Process water	Plant location, non-agri
Electricity	Plant location, non-agri
Thermal energy	Plant location, non-agri
Transportation	Same as the material transported
Waste treatment	Plant location, non-agri
Avoided feed production	Same as carbon source location, agri

Table 3 Datasets used, their use in different locations in the study, and the type of AWaRe factors used for each dataset

Variable	**Result category**	**Ecoinvent dataset name (3.7.1 cut-off)**	**Location in the study**	**AWaRe factor type**
Glucose	Carbon source	market for glucose, Global (GLO)	All	agri
Sucrose	Carbon source	market for sugar, from sugar beet, GLO	Germany	agri
		market for sugar, from sugarcane, GLO	Australia, US, New Zealand (NZ)	agri
Corn steep solids	Nitrogen source	ethanol production from maize, Rest of World (RoW)	All	agri
Ammonia	Nitrogen source	market for ammonia, anhydrous, liquid, Europe (referred as RER, which ecoinvent uses as the abbreviation for Europe)	Germany	non-agri
		market for ammonia, anhydrous, liquid, RoW	US, Australia, NZ	non-agri
Sulfur dioxide, SO_2	Other materials	market for sulfur dioxide, liquid, RER	Germany	non-agri
		market for sulfur dioxide, liquid, RoW	US, Australia, NZ	non-agri
Anti-foam agent	Other materials	market for chemicals, inorganic	All	non-agri
Potassium phosphate	Other materials	market for sodium phosphate, RoW	All	non-agri

Ammonium sulfate	Other materials	market for ammonium sulfate, RoW	US, Australia, NZ	non-agri
		market for ammonium sulfate, RER	Germany	non-agri
Magnesium sulfate	Other materials	market for magnesium sulfate, GLO	US, Australia, NZ	non-agri
		magnesium sulfate production, RER	Germany	non-agri
Calcium chloride	Other materials	market for calcium chloride, RoW	US, Australia, NZ	non-agri
		market for calcium chloride, RER	Germany	non-agri
Sodium hydroxide, NaOH	Other materials	chlor-alkali electrolysis, membrane cell, RER	Germany	non-agri
		chlor-alkali electrolysis, membrane cell, RoW	US, Australia, NZ	non-agri
Sodium chloride, NaCl	Other materials	market for sodium chloride, powder, GLO	All	non-agri
Sodium phosphate, Na_2HPO_4	Other materials	market for sodium phosphate, RER	Germany	non-agri
		market for sodium phosphate, RoW	US, Australia, NZ	non-agri
Trisodium phosphate, Na_3PO_4	Other materials	market for trisodium phosphate, GLO	All	non-agri
Water	Process water	market for tap water, Europe without Switzerland	Germany	non-agri
		market for tap water, RoW	US, Australia, NZ	non-agri
Electricity	Electricity	market for electricity, high voltage, DE	Germany	non-agri
		market for electricity, high voltage, AU	Australia	non-agri
		Market for electricity, high voltage, NZ	NZ	non-agri
		market for electricity, high voltage, US-SERC	US	non-agri
Steam	Thermal energy	market for heat, from steam, in chemical industry, RER	Germany	non-agri
		market for heat, from steam, in chemical industry, RoW	US, Australia, NZ	non-agri
		market for heat, district or industrial, natural gas	All	non-agri
Transport by truck/lorry	Transportation	market for transport, freight, lorry >32 metric ton, EURO6, RER	All	non-agri
Transport by train	Transportation	market for transport, freight train, Europe without Switzerland	All	non-agri
Transport by ship	Transportation	market for transport, freight, sea, container ship, GLO	All	non-agri
Biomass side stream, replacing animal feed	Avoided feed production	market for protein feed, 100% crude, GLO	All	agri
Wastewater treatment	Waste treatment	treatment of wastewater, average, capacity 1E9l/year, Europe without Switzerland	Germany	non-agri
Wastewater treatment	Waste treatment	treatment of wastewater, average, capacity 1E9l/year, RoW	US, Australia, NZ	non-agri
Biomass waste stream to composting	Waste treatment	treatment of biowaste, industrial composting, RoW	All	non-agri

Table 4 AWaRe factors - Queensland coastline average, collected from the Google Earth layer Aware v1.2 (January 2022)

FID	**Agri**	**Non-agri**
9944	1.3	0.9
9967	5.4	3.6
9902	4.7	3.7
9988	0.3	0.4
9989	10.8	10.3
10007	0.6	0.6
10039	3.5	2.3
10023	4.6	3.6
10069	18.6	14.2
10051	3.2	3.3
10052	8.7	6.4
10070	3	3.2
10086	41.8	18.8
10112	7.8	7.1
10130	39.6	18.3
10150	3.5	3.4
10208	15.9	14.3
10172	13.1	11.1
10173		4.1
10193	2.6	2.1
10174		12
10223	4.8	4.3
10236	2.3	2.1
10254	18.3	15.8
10272	2.3	2
10255		9.4
10273		2.3
10288		5.9
10301	4.6	4.5
10314	4	3.3
10322	6.2	4.9
10333	1.7	1.3
10343	1.3	1
10353	1.8	1.3
10365	1.3	1.1
10375	2.2	1.6
Queensland Coastline average	*7.7*	*5.7*

Table 5 AWaRe factors **-** Victoria coastline / Melbourne area average, collected from the Google Earth layer Aware v1.2 (January 2022)

FID	**Agri**	**Non-agri**
10615	4.4	2.8
10639	6.3	7.2
10638	2	2
10640	2.7	2.1
10616	1.2	0.9
10659	2.9	2
10657	2.9	2.5
10658	3.2	2.5
10637	2.8	2.1
10660	75.4	73.8
10661	3.5	3.2
10662	3	2.2
10641	1.5	1.2
10663	11.9	8.4
10617	1.8	1.2
Victoria coastline/Melbourne area average	*8.4*	*7.6*

Table 6 AWaRe factors **-** Alabama average, collected from the Google Earth layer Aware v1.2 (January 2022)

FID	Agri	Non-agri
6902	0.8	0.7
6930	0.7	0.6
6933	0.7	0.7
Alabama average	*0.7*	*0.7*

Table 7 AWaRe factors **-** Louisiana average, collected from the Google Earth layer Aware v1.2 (January 2022)

FID	Agri	Non-agri
7006	0.2	0.2
6964	0.8	0.7
6965	0.7	0.7
7002	1.1	0.9
6967	1.3	1.1
6966	1.3	0.9
6924	0.6	0.8
Louisiana average	*0.9*	*0.8*

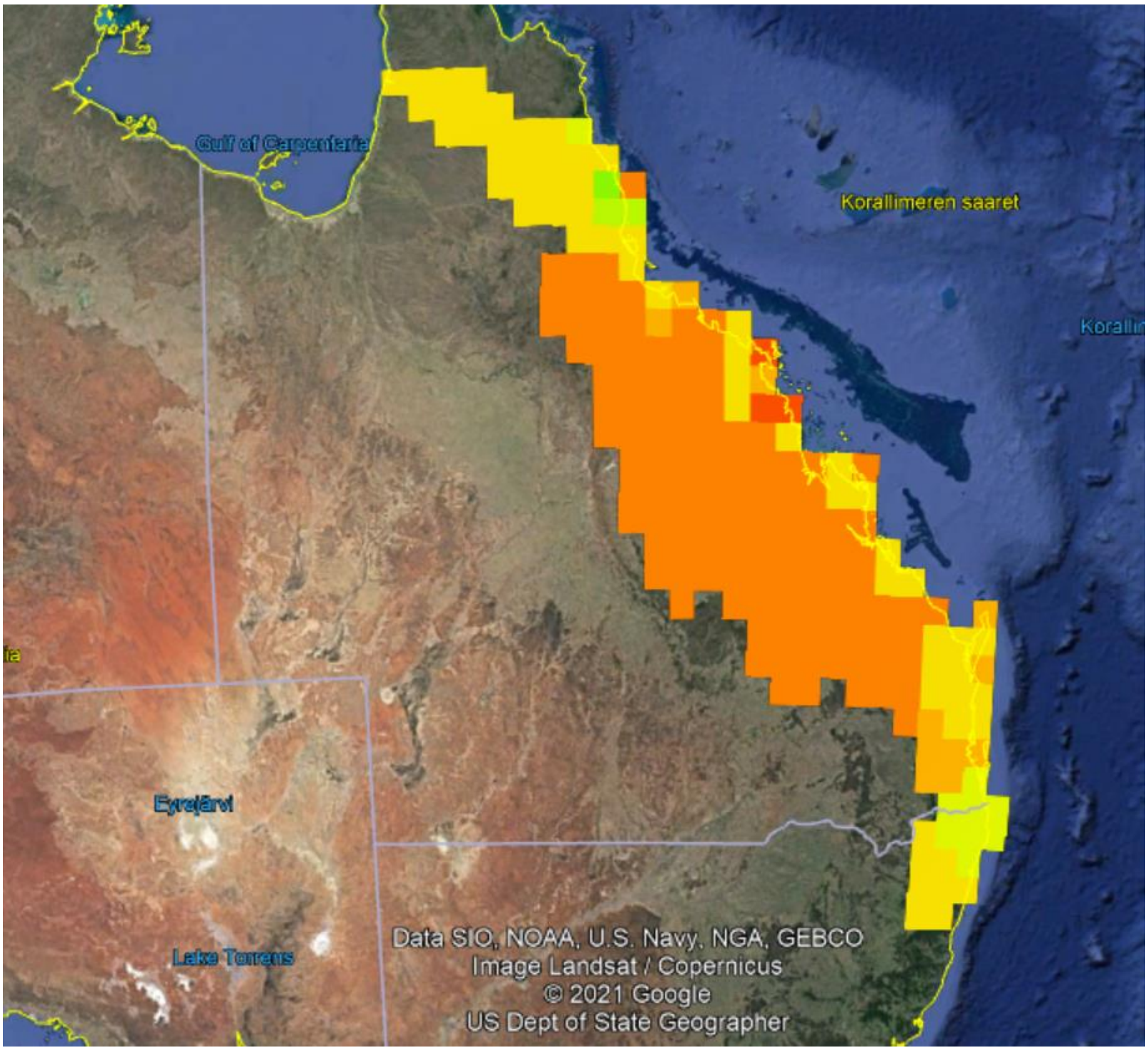

Fig. 1 Image of the Queensland coastline area that is considered in the AWaRe factors

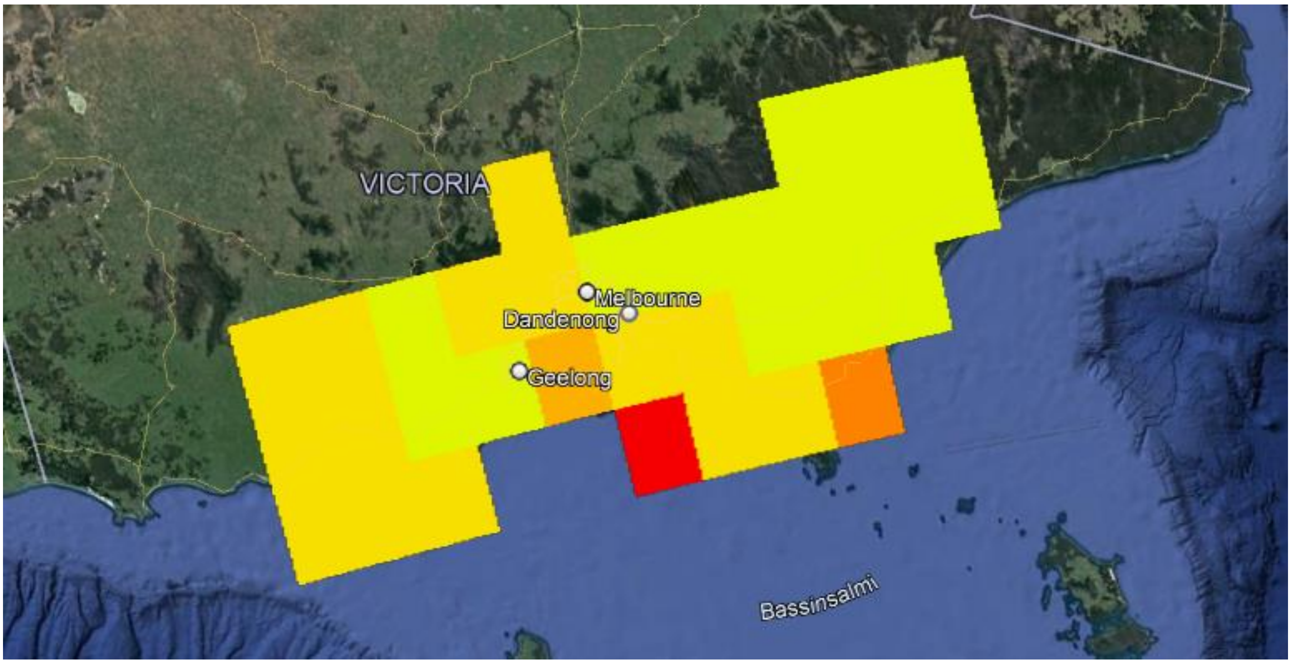

Fig. 2 Image of the Victoria coastline / Melbourne area that is considered in the AWaRe factors

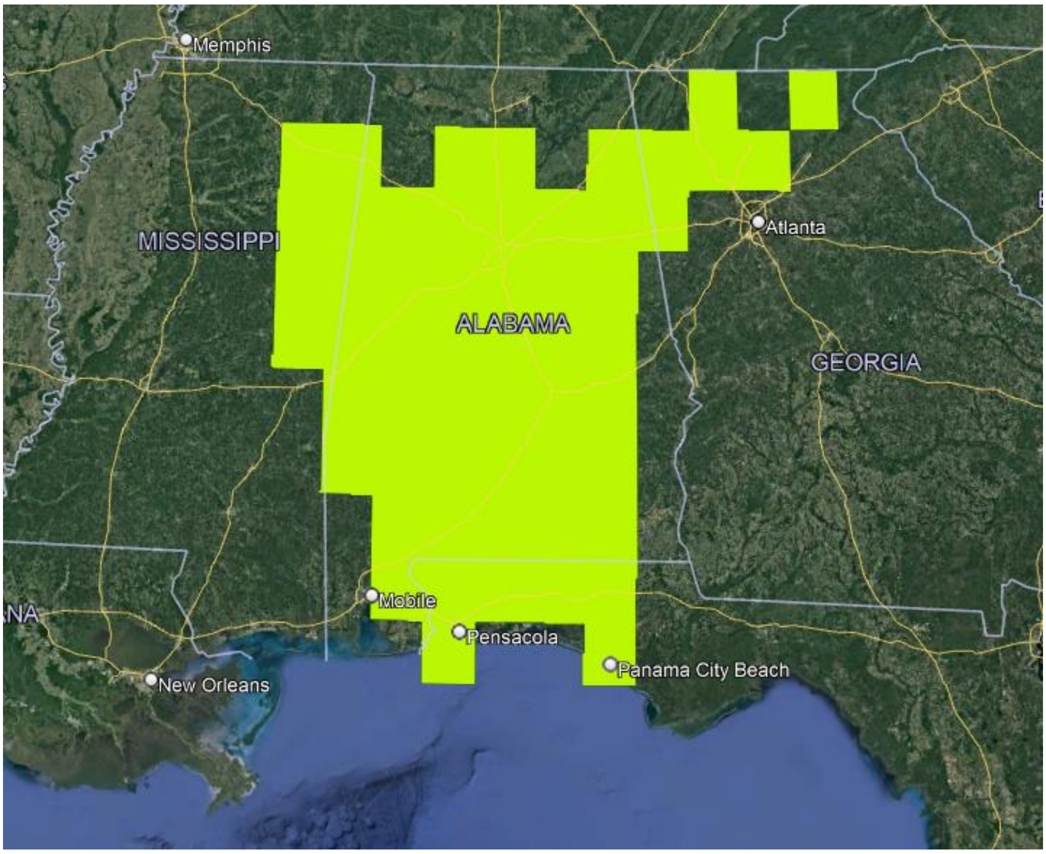

Fig. 3 Image of the Alabama area that is considered in the AWaRe factors

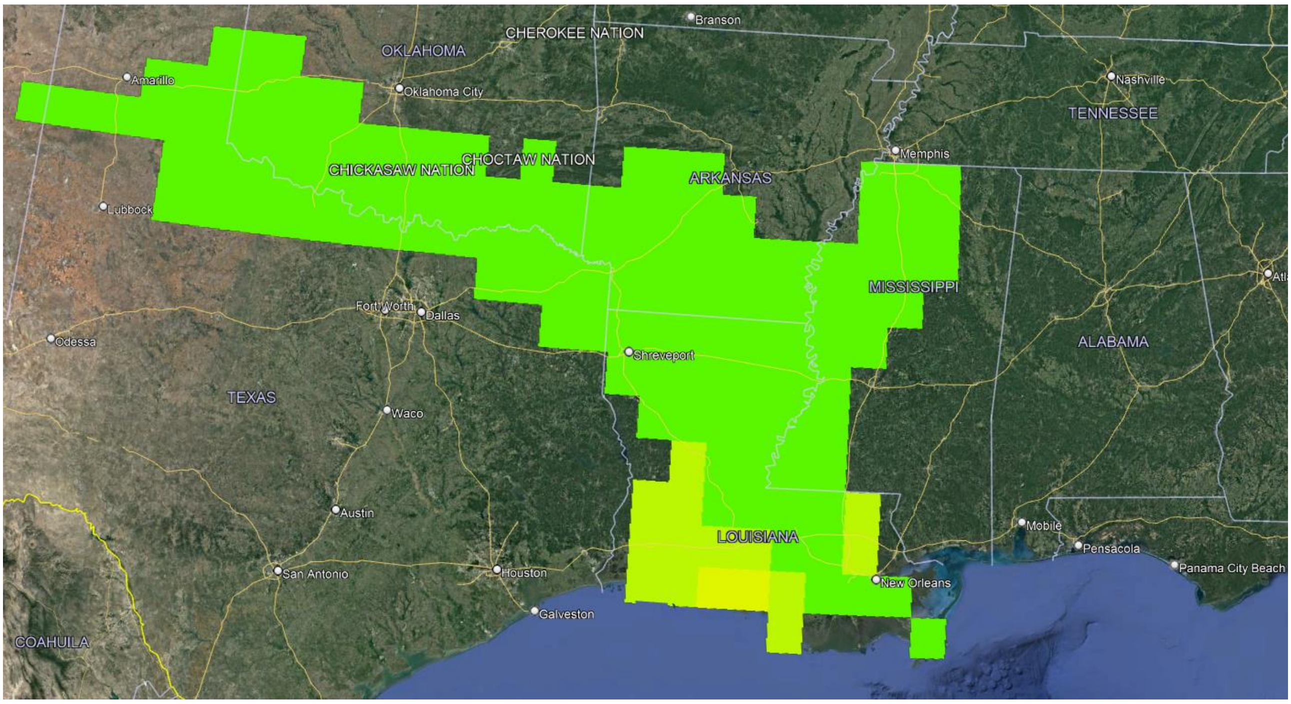

Fig. 4 Image of the Louisiana area that is considered in the AWaRe factors

Data 3 Carbon footprint and water scarcity footprint results

The results of carbon footprint and water scarcity footprint presented as numbers in Table 8 and Table 9, respectively.

Table 8 Carbon footprint results as tonne CO_2e per tonne of protein

	NEW ZEALAND				GERMANY				US ALABAMA				AUSTRALIA VICTORIA			
Scenario number	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Carbon source	5.35	3.65	5.35	5.76	5.35	2.13	5.35	5.76	5.35	3.65	5.35	5.76	5.35	3.65	5.35	5.76
Nitrogen source	0.92	0.92	0.92	0.99	0.76	0.76	0.76	0.82	0.92	0.92	0.92	0.99	0.92	0.92	0.92	0.99
Other materials	0.18	0.18	0.18	1.71	0.16	0.16	0.16	1.55	0.18	0.18	0.18	1.71	0.18	0.18	0.18	1.71
Process water	0.03	0.03	0.03	0.19	0.01	0.01	0.01	0.06	0.03	0.03	0.03	0.19	0.03	0.03	0.03	0.19
Electricity	0.88	0.88	0.87	0.98	4.86	4.86	4.82	5.42	4.87	4.87	4.83	5.43	8.09	8.09	8.02	9.03
Thermal energy	0.54	0.54	0.47	0.53	0.54	0.54	0.47	0.53	0.54	0.54	0.47	0.53	0.54	0.54	0.47	0.53
Transportation	0.18	0.17	0.18	0.22	0.04	0.04	0.04	0.07	0.04	0.21	0.04	0.07	0.15	0.15	0.15	0.19
Waste treatment	0.02	0.02	0.08	0.1	0.01	0.01	0.08	0.09	0.02	0.02	0.08	0.1	0.02	0.02	0.08	0.1
Avoided feed production	-0.86	-0.86	0	-0.92	-0.86	-0.86	0	-0.92	-0.86	-0.86	0	-0.92	-0.86	-0.86	0	-0.92
Net impact, t CO_2e/t protein	*7.2*	*5.5*	*8.1*	*9.6*	*10.9*	*7.7*	*11.7*	*13.4*	*11.1*	*9.6*	*11.9*	*13.9*	*14.4*	*12.7*	*15.2*	*17.6*

Table 9 Water scarcity results as m3 world eq. per ton of protein

	NEW ZEALAND				GERMANY				US ALABAMA				AUSTRALIA VICTORIA			
Scenario number	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Carbon source	926	5033	926	997	213	311	213	229	88	558	88	95	926	5033	926	997
Nitrogen source	27	27	27	30	18	18	18	19	11	11	11	12	122	122	122	132
Other materials	11	11	11	67	7	7	7	46	4	4	4	27	49	49	49	304
Process water	53	53	53	313	35	35	35	205	21	21	21	124	240	240	240	1409
Electricity	4	4	4	4	34	34	34	38	15	15	15	17	123	123	121	137
Thermal energy	1	1	1	1	1	1	1	1	0	0	0	0	6	6	5	5
Transportation	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
Waste treatment	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-1
Avoided feed production	-542	-542	0	-583	-125	-125	0	-134	-51	-51	0	-55	-542	-542	0	-583
Net impact, m3 world eq./t protein	*481*	*4587*	*1022*	*829*	*183*	*281*	*307*	*404*	*88*	*558*	*140*	*219*	*924*	*5031*	*1464*	*2401*