Production and characterization of protein-rich extracts from the red macroalga *Gelidium corneum* and its industrial agar extraction residues

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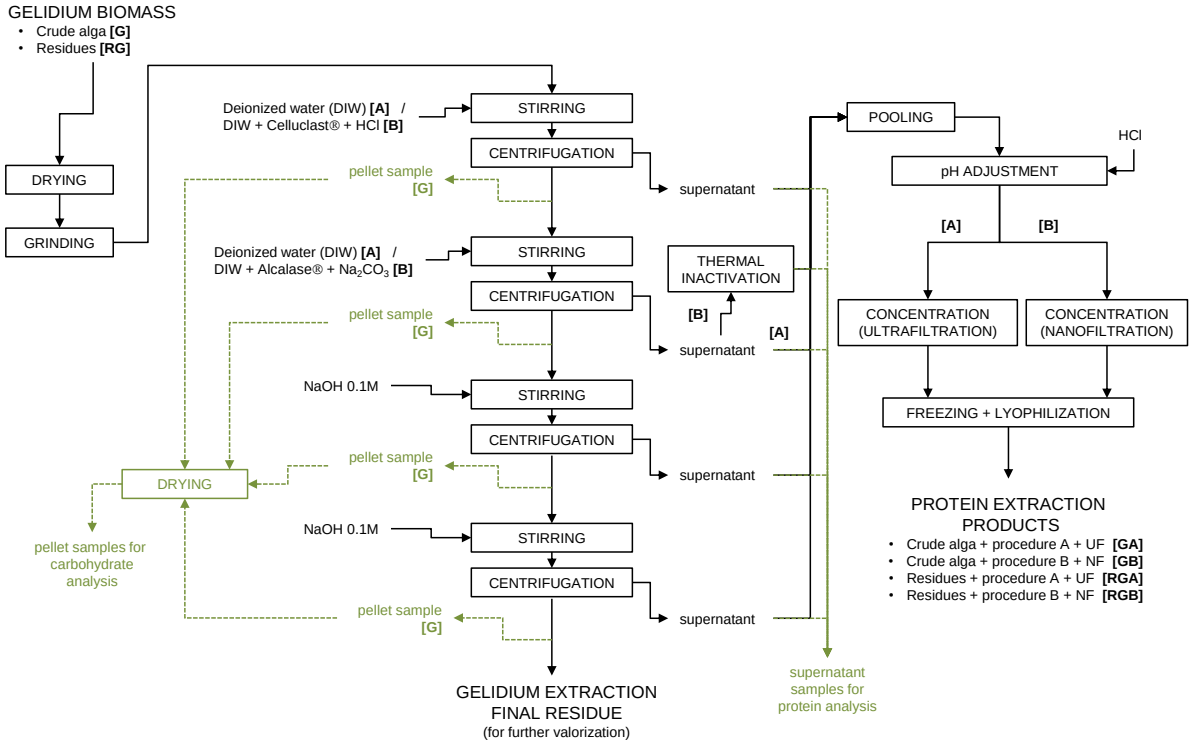

Fig. S1 Scheme of the extraction protocols applied to the *Gelidium corneum* biomasses.

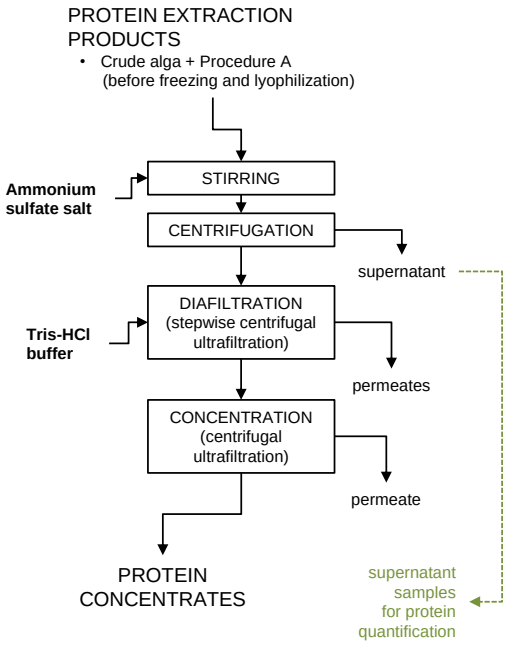

Fig. S2 Scheme of the ammonium sulfate protein precipitation protocols applied to the *Gelidium corneum* protein extraction products.

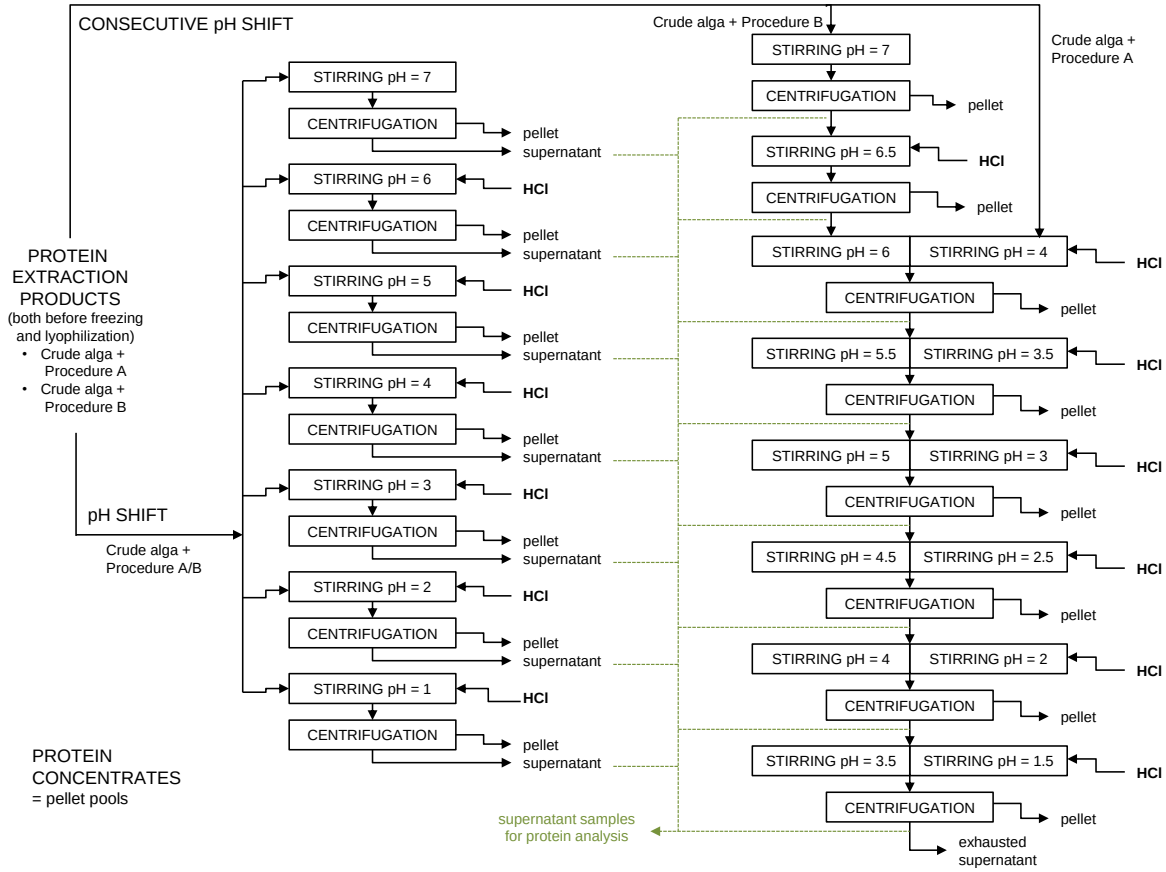

Fig. S3 Scheme of the pH-shift protein precipitation protocols applied to the *Gelidium corneum* protein extraction products.

TableS1 Composition of simulated digestion fluids used in the *in vitro* digestion protocol. The volumes (mL) and mass (mg) are calculated for a volume of 100 mL for each simulated digestion fluid.

	Oral phase	**Gastric phase**	**Intestinal phase**	
Inorganic components	**Saliva**	**Gastric juice**	**Duodenal juice**	**Bile**
	Volume (mL)	Volume (mL)	Volume (mL)	Volume (mL)
KCl (89.6 g L^{-1}) (Merck, 99.5%)	2.0	1.8	1.2	0.8
KSCN (20.0 g L^{-1}) (Sigma, P2713)	2.0	-	-	-
NaH_2PO_4 (88.8 g L^{-1}) (Merck, 99.5%)	2.0	0.6	-	-
Na_2SO_4 (57.0 g L^{-1}) (Merck, 90.0%)	2.0	-	-	-
NaCl (175.3 g L^{-1}) (Merck, 99.5%)	0.4	3.1	8.0	6.0
$NaHCO_3$ (84.7 g L^{-1}) (Merck, 99.5%)	4.0	-	1.33[a]	13.6
$CaCl_2.2H_2O$ (22.2 g L^{-1}) (Sigma, C3881)	-	3.6	0.144[a]	0.080[a]
NH_4Cl (30.6 g L^{-1}) (Riedel-de Haen, 99.5%)	-	2.0	-	-
KH_2PO_4 (8.0 g L^{-1}) (Merck, 99.5%)	-	-	2.0	-
$MgCl_2$ (5.0 g L^{-1}) (Riedel-de Haen, 99.5%)	-	-	2.0	-
Organic components	Volume (mL)	Volume (mL)	Volume (mL)	Volume (mL)
Urea (25 g L^{-1}) (Sigma, U5128)	1.6	0.7	0.8	2.0
Glucuronic acid (2 g L^{-1}) (Sigma, G5269)	-	2.0	-	-
Glucose (65 g L^{-1}) (Sigma, G5400)	-	2.0	-	-
Glucoseamine hydrochloride (33 g L^{-1}) (Sigma, G4875)	-	2.0	-	-
Bioactive components	Mass (mg)	Mass (mg)	Mass (mg)	Mass (mg)
α-amilase (Sigma, 86250)	90	-	-	-
Uric acid (Sigma, U2625)	3	-	-	-
Mucin (Sigma, M2378)	5	-	-	-
BSA (Sigma, A7906)	-	200	200	360
Pepsin (Sigma, P7125)	-	500	-	-
Pancreatin (Sigma, P8096)	-	600	1800	-
Lipase (Sigma, L3126)	-	-	300	-
Trypsin (Sigma, T6567)	-	-	3.2	-
α-Chymotrypsin (Sigma, C4129)	-	-	34.8	-
Bile (Sigma, B8631)	-	-	-	6000
Ultrapure water (Milli-Q)	Complete 100 mL volumetric flask			

[a] Volume added to each *in vitro* digestion reaction (mixture of simulated digestion fluid and food) - as precipitation may occur.

Table S2 Amino acid (AA) content profiles of protein-based ingredients, potentially for aquaculture diets. Indicated values should be read as average ± standard deviation (*Avr.*±*SD*; n=2).

Protein ingredient		Essential amino acids (EAA) g AA/100 g protein								Non-Essential amino acids (NEAA) g AA/100 g protein									Sums g AA/100 g protein
		His	Ile	Leu	Lys	Met	Phe	Thr	Val	Ala	Arg	Asn +Asp	Cys	Gln +Glu	Gly	Pro	Ser	Tyr	
Protein-based ingredients produced and/or analysed in the present study																			
G	*Avr.*	1.48	3.00	5.16	5.16	1.16	4.34	3.73	3.76	4.78	3.88	10.03	0.32	10.07	4.80	4.20	4.28	3.41	ΣAA = 73.6 ± 1.5
	SD	0.04	0.02	0.02	0.14	0.04	0.18	0.05	0.06	0.04	0.11	0.22	0.02	0.00	0.20	0.06	0.05	0.28	ΣEAA = 27.8 ± 0.5
GA	*Avr.*	0.80	2.11	4.29	2.53	1.25	2.88	2.82	2.96	4.59	2.78	7.24	<LOQ	8.69	3.87	2.77	4.30	2.61	ΣAA = 56.5 ± 1.5
	SD	0.05	0.06	0.11	0.03	0.04	0.18	0.06	0.08	0.09	0.11	0.01	<LOQ	0.09	0.27	0.11	0.05	0.17	ΣEAA = 19.6 ± 0.6
GB	*Avr.*	0.67	2.39	3.73	2.36	1.28	2.53	2.95	3.16	3.76	2.42	6.67	0.16	9.84	4.17	3.61	2.99	2.77	ΣAA = 55.5 ± 4.3
	SD	0.08	0.18	0.06	0.34	0.10	0.20	0.07	0.11	0.12	0.30	2.12	0.01	0.03	0.14	0.16	0.04	0.29	ΣEAA = 19.1 ± 1.2
RG	*Avr.*	1.67	3.72	6.71	5.29	1.50	5.88	3.82	4.59	5.52	4.70	10.81	0.07	11.06	6.16	6.05	4.57	4.14	ΣAA = 86.3 ± 2.2
	SD	0.06	0.20	0.14	0.20	0.03	0.06	0.08	0.20	0.31	0.09	0.12	0.00	0.20	0.14	0.05	0.19	0.17	ΣEAA = 33.2 ± 1.0
Protein-based ingredients characterized by Li and Wu (2020)																			
SPC	*Avr.*	3.08	5.59	8.58	7.47	1.74	5.99	4.85	5.66	5.10	8.59	13.64	2.02	22.65	5.32	5.16	7.16	4.25	ΣAA = 116.8 ± 0.7
	SD	0.03	0.04	0.03	0.05	0.05	0.09	0.03	0.04	0.04	0.00	0.02	0.02	0.11	0.04	0.05	0.04	0.02	ΣEAA = 43.0 ± 0.4
SBM	*Avr.*	2.48	4.38	7.45	6.07	1.30	4.76	3.85	4.49	4.26	6.91	11.27	1.51	17.25	4.96	5.27	4.63	3.62	ΣAA = 94.4 ± 0.9
	SD	0.06	0.06	0.09	0.06	0.01	0.06	0.02	0.10	0.05	0.04	0.08	0.01	0.12	0.05	0.05	0.05	0.02	ΣEAA = 31.9 ± 0.2
SM	*Avr.*	1.88	5.94	9.19	6.07	3.15	5.64	6.00	6.34	8.50	7.53	11.09	1.28	16.07	6.09	4.54	6.32	5.51	ΣAA = 111.1 ± 0.7
	SD	0.02	0.03	0.04	0.05	0.03	0.03	0.02	0.04	0.05	0.04	0.05	0.01	0.10	0.05	0.04	0.04	0.03	ΣEAA = 44.2 ± 0.3
FM	*Avr.*	2.17	3.82	7.08	7.02	3.13	3.64	3.96	4.75	5.81	5.88	8.96	1.08	14.30	6.83	5.97	4.04	3.03	ΣAA = 91.5 ± 0.7
	SD	0.03	0.02	0.03	0.04	0.06	0.03	0.04	0.04	0.04	0.03	0.05	0.01	0.06	0.05	0.06	0.03	0.04	ΣEAA = 35.6 ± 0.3

<LOQ – below limit of quantification

G–*Gelidium corneum* (crude alga); GA–water extract from *G. corneum*; GB–enzymatic extract from *G. corneum*; RG–industrial residues of *G. corneum* ; SPC–soy protein concentrate; SBM–soybean meal; SM–*Spirulina* meal; FM–fish meal (US menhaden).

Bibliography: Li P and Wu G (2020) Composition of amino acids and related nitrogenous nutrients in feedstuffs for animal diets. Amino Acids 52:523–542. https://doi.org/10.1007/s00726-020-02833-4