Electronic Supplementary Information

Uncovering the potential of aqueous solutions of Deep Eutectic Solvents on the extraction and purification of Collagen type I from Baltic Codfish (*Gadus morhua*)

Meena Bisht[a*], Margarida Martins[a], Ana C. R. V. Dias[b], Sónia P. M. Ventura[a*], João A. P. Coutinho[a]

[a]CICECO – Aveiro Institute of Materials, Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal.

[b]CESAM – Centre for Environmental and Marine Studies, Department of Environment and Planning, University of Aveiro, 3810-193 Aveiro, Portugal

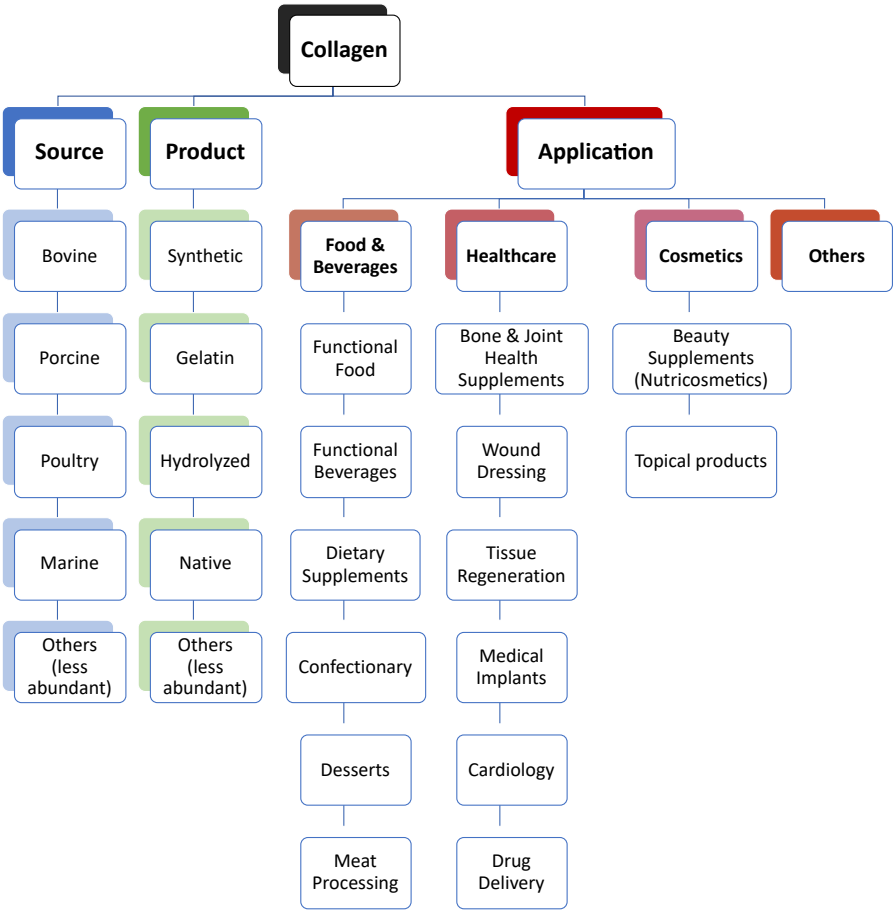

Fig. S1 Brief summary of sources, products and applications of collagen.

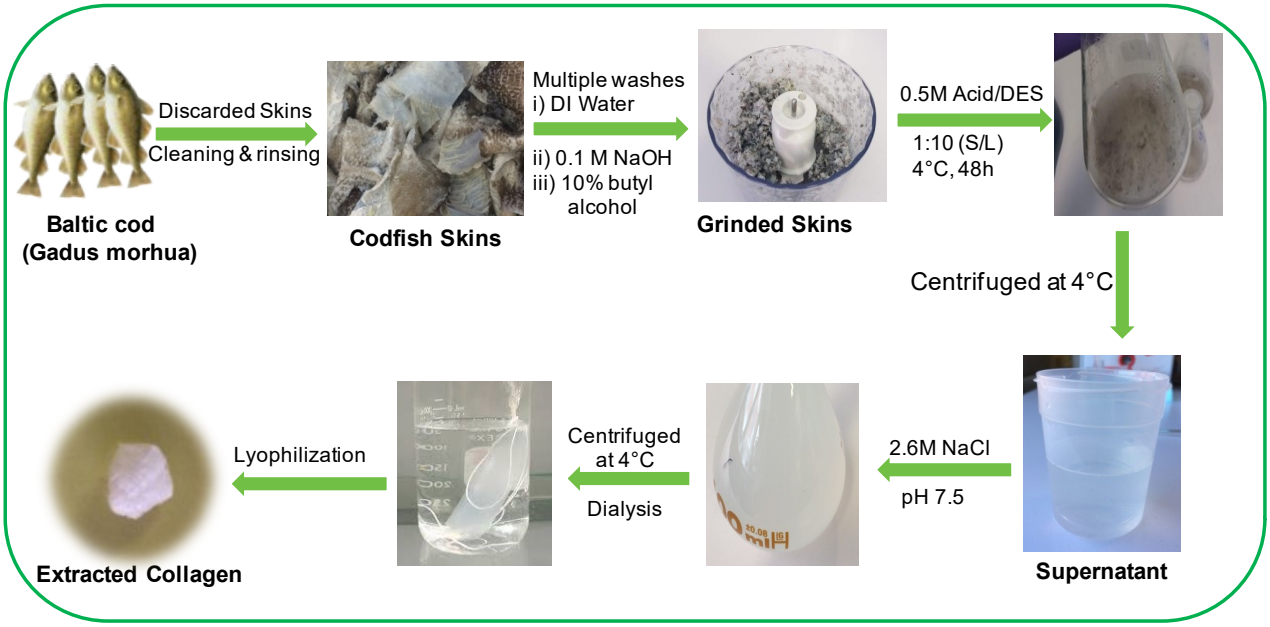

Fig. S2. Photographs showing the extraction of collagen type I from codfish skins using the conventional method with an aqueous solution of acetic acid.

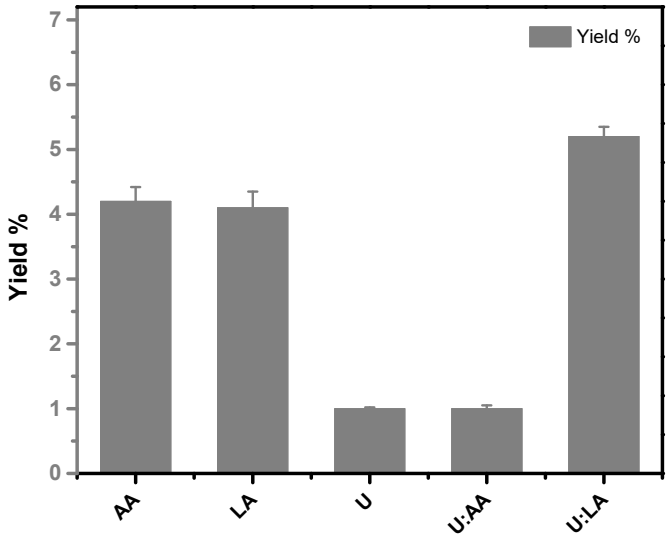

Fig. S3. Yield of extraction of collagen at 4 °C obtained by applying aqueous solutions of AA, LA, U and their corresponding eutectic mixtures at 0.5 M.

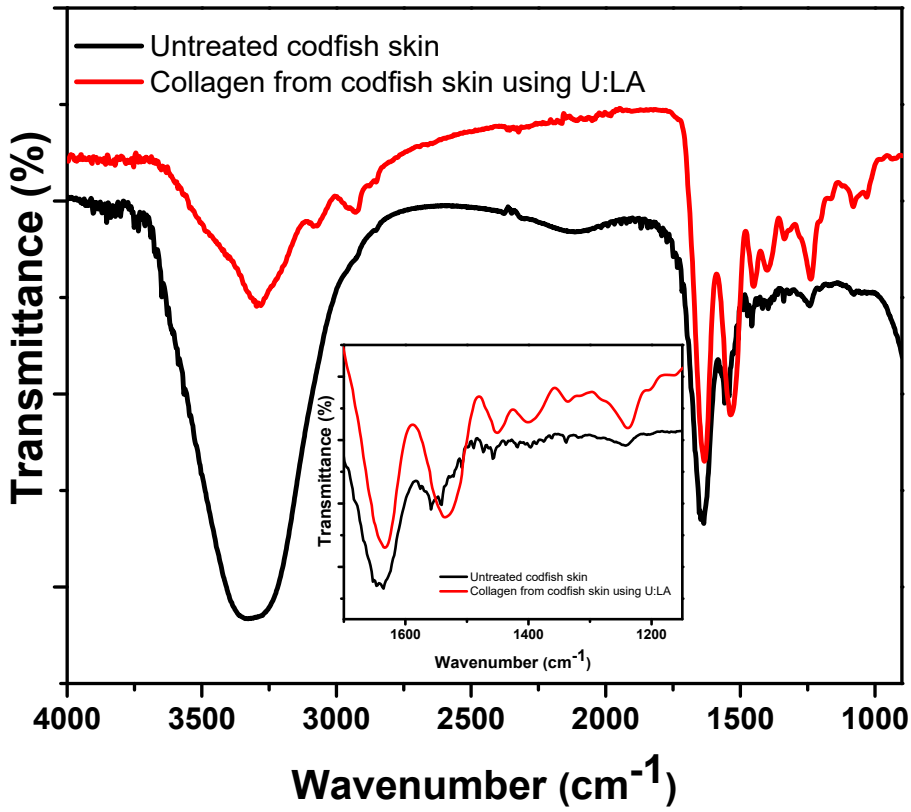

Fig. S4: FTIR spectra of untreated cod fish (raw cod fish skin) and collagen type I extracted from codfish skin using an aqueous solution of U:LA (1:2)

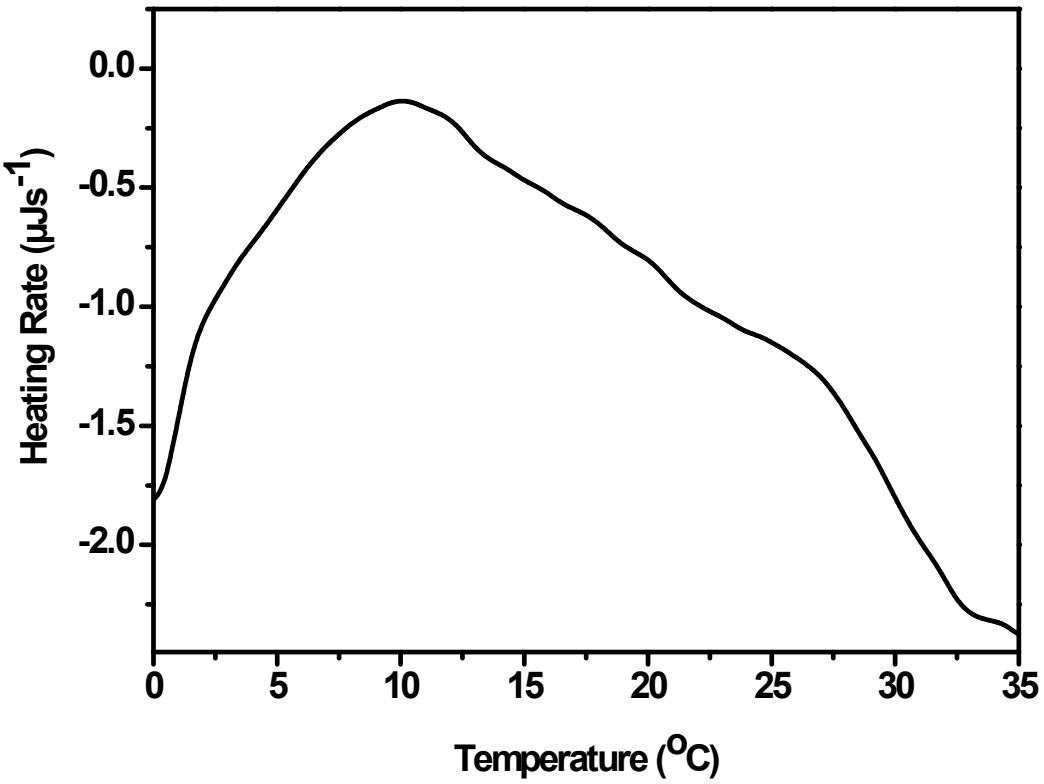

Fig. S5: DSC thermograms of collagen type I extracted from codfish skin using an aqueous solution of U:LA (1:2).

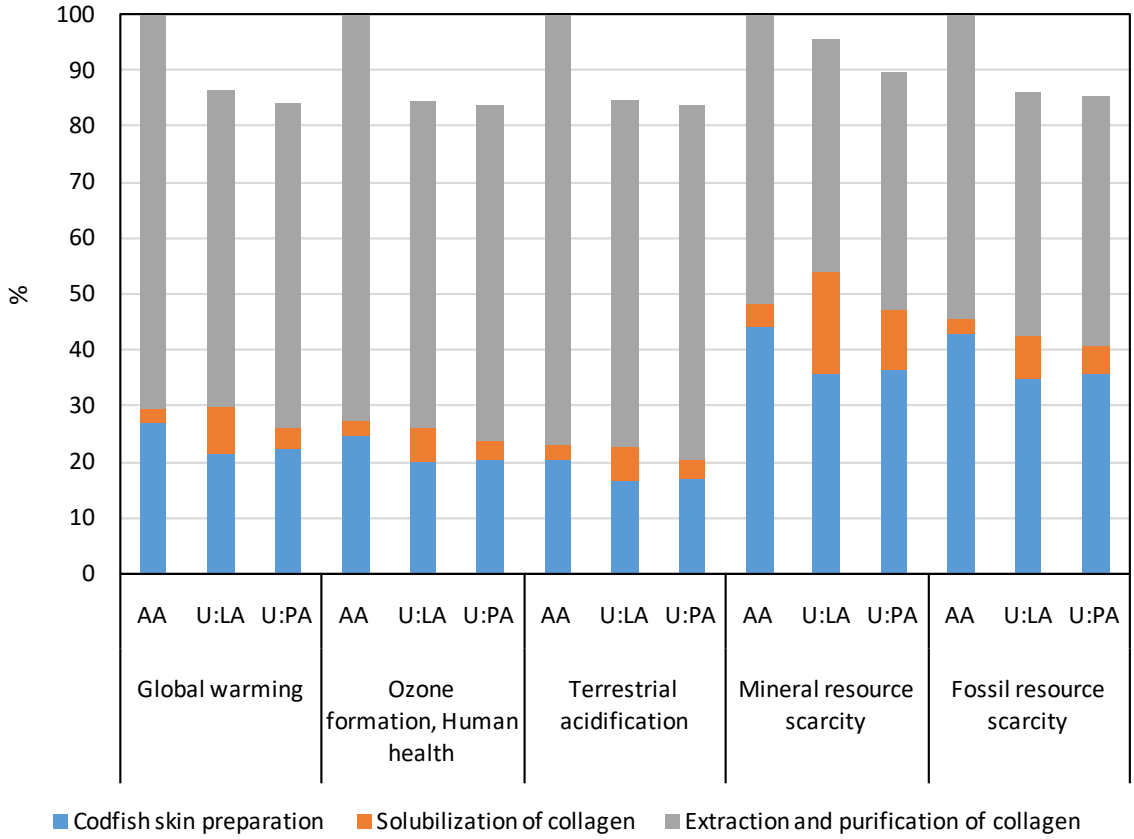

Fig. S6 LCA results: relative contribution of each step of the process to the total impacts for obtaining 1 g of collagen in the three systems evaluated with LCA: with AA (convencional solvent), U:LA (1:2) and U:PA (1:2). 100% was assigned to the system with the largest result in each impact category.

Table S1. Supplier, purity and water content (wt%) of the starting materials used in the preparation of acidic DES.

Compound	Supplier	Purity[a] (%)	Water Content (wt%)
Betaine	Acros Organics	98	1.3 ± 0.1
Urea	Panreac	> 99	0.0030 ± 0.0001
Cholinium chloride	Sigma	> 98	2.1 ± 0.4
Formic acid	Panreac	98	1.12 ± 0.08
DL-Lactic acid	Riedel de Haën	88-92	14.60 ± 0.05
Acetic acid	Fisher	99	0.10 ± 0.02
Propionic acid	Merck	> 99	0.09 ± 0.01

[a]as reported by the supplier.

Table S2. Inputs of chemicals, water and electricity for obtaining 1 g of collagen in the three systems evaluated with LCA: with AA, U:LA (1:2) and U:PA (1:2).

	Acetic acid	U:LA	U:PA
Codfish skin preparation			
Sodium hydroxide (g)	8.33	6.73	6.89
Butyl alcohol solution (L)	1.83	1.48	1.52
Water (mL)	8.33	6.73	6.89
Electricity (W.h)	0.85	0.69	0.70
Solubilization of collagen			
Acetic acid (g)	7.14	-	-
Urea (g)	-	5.77	5.91
Lactic acid (g)	-	17.31	-
Propanoic acid (g)	-	-	14.57
Water (mL)	230.95	169.23	176.38
Electricity (W.h)	67.05	54.15	55.43
Extaction and purification of collagen			
Sodium chloride (g)	36.19	29.23	29.92
Water (mL)	21.43	17.31	17.72
Electricity (W.h)	2503.97	2022.44	2070.21

Table S3. pH of aqueous solutions of acetic acid and DES at 0.5 M.

Solvents	pH
Acetic acid	2.564
CC:U (1:2)	7.760
CC:FA (1:2)	1.521
CC:AA (1:2)	2.012
CC:PA (1:2)	2.054
CC:LA (1:2)	1.471
Bet:FA (1:2)	2.604
Bet:AA (1:2)	3.109
Bet:PA (1:2)	3.242
Bet:LA (1:2)	2.224
U:FA (1:4)	1.640
U:AA (1:5)	2.244
U:PA (1:2)	2.480
U:LA (1:2)	2.114

Table S4. The absorption ratio between the 1240 and 1450 cm^{-1} bands.

Collagen sample	A1240	A1450	Ratio
Commercial collagen	0.355258	0.347228	1.023128
AA	0.252945	0.248312	1.018657
CC:PA (1:2)	0.159459	0.159787	0.997949
CC:LA (1:2)	0.149954	0.162049	0.925362
Bet:AA (1:2)	0.217435	0.235039	0.9251
Bet:PA (1:2)	0.252868	0.251088	1.007089
U:PA (1:2)	0.142611	0.149527	0.953747
U:LA (1:2)	0.125417	0.12389	1.01232

Table S5. LCA results: total impacts for obtaining 1 g of collagen in the three systems evaluated with LCA: with acetic acid, U:LA (1:2) and U:PA (1:2).

Impact category	Acetic acid	U:LA	U:PA
Global warming (kg CO_2 eq)	1.18	1.03	1.00
Ozone formation, human health (g NO_x eq)	2.53	2.15	2.13
Terrestrial acidification (g SO_2 eq)	4.65	3.94	3.90
Mineral resource scarcity (g Cu eq)	0.111	0.107	0.100
Fossil resource scarcity (kg oil eq)	0.490	0.423	0.420