The 5th International Symposium on Processing of Food, Vegetables and Fruits 2023

Optimizing pressurized hot water technology to assist the recovery of residual crude agar from the algae industry waste stream with tailorable physicochemical properties and gel texture

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June 2023

Background and problematics

- Brief summary of macroalgae classification and agar production process
- Outline of the notion of food industry waste stream management and valorization
- A short overview of pressurized hot water technology (subcritical water)
- The optimization method and results from Box-Behnken design and response surface methodology (RSM)

Classification of macroalgae (Seaweeds)

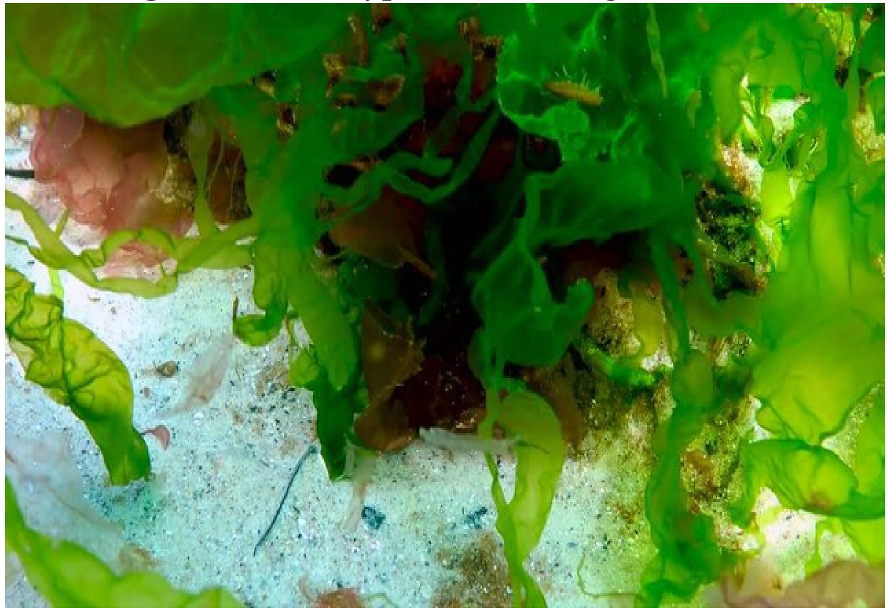
Algae: diverse group of photosynthetic and aquatic plant-like organisms that range from unicellular to large multicellular forms.

Macroalgae: Multicellular marine algae

Fig. 1: Different ypes of macroalgae or seaweed

Rhodophycae
(Red algae)
4000-6000 species
Pigment: Phycobiliprotein
Hydrocolloids: Agar / Carraghenan

Phaeophycae
(Brown algae)
≈ 1500 species
Pigment: fucoxanthin
Hydrocollid: Alginate



Chlorophycae
(Green algae)
≈ 1100 species
Pigment: Chlorophyll b

Agar

- Vegetable gelatin,
- Thermoreversible marine hydrocolloid.
- 40 – 45% of the dry red algae weigth
- 94% gross fiber

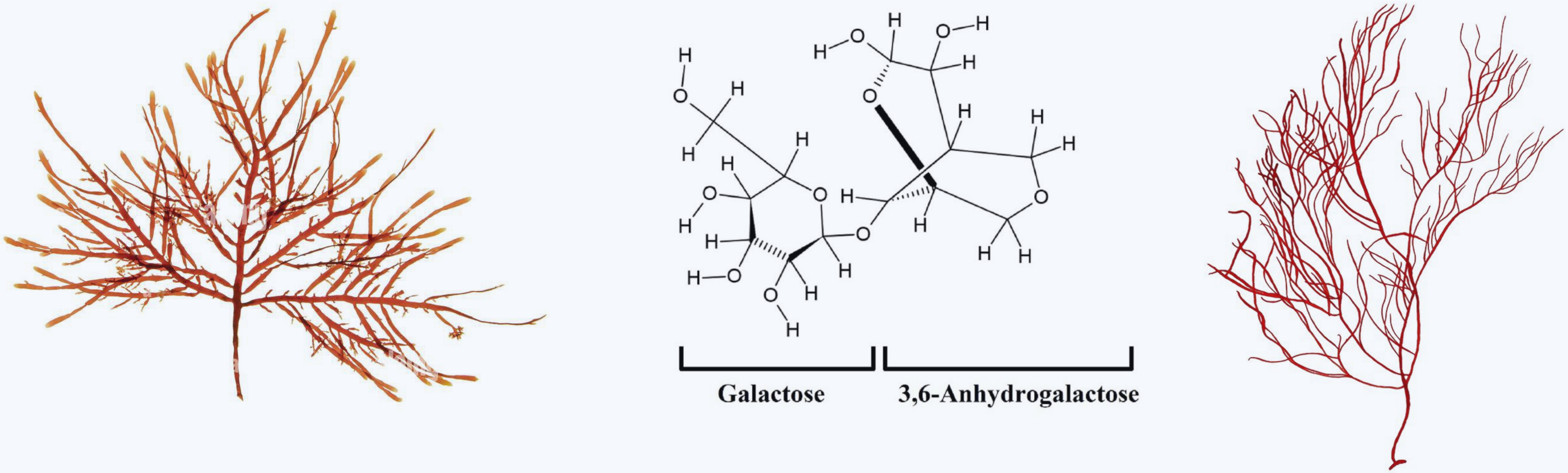
Agarose

- (1-4)-linked 3,6-anhydro-α-L-galactopyrannose (1–3)-linked-β-D-galactose unit.
- Coil structure at 85 ◦C, which pseudoequilibrates upon cooling
- Generates a 3D double helix structure or gel capable of immobilizing water molecules

Agaropectin

- Same backbone
- Heterogeneous anionic groups: sulfate, pyruvate, glucuronate.

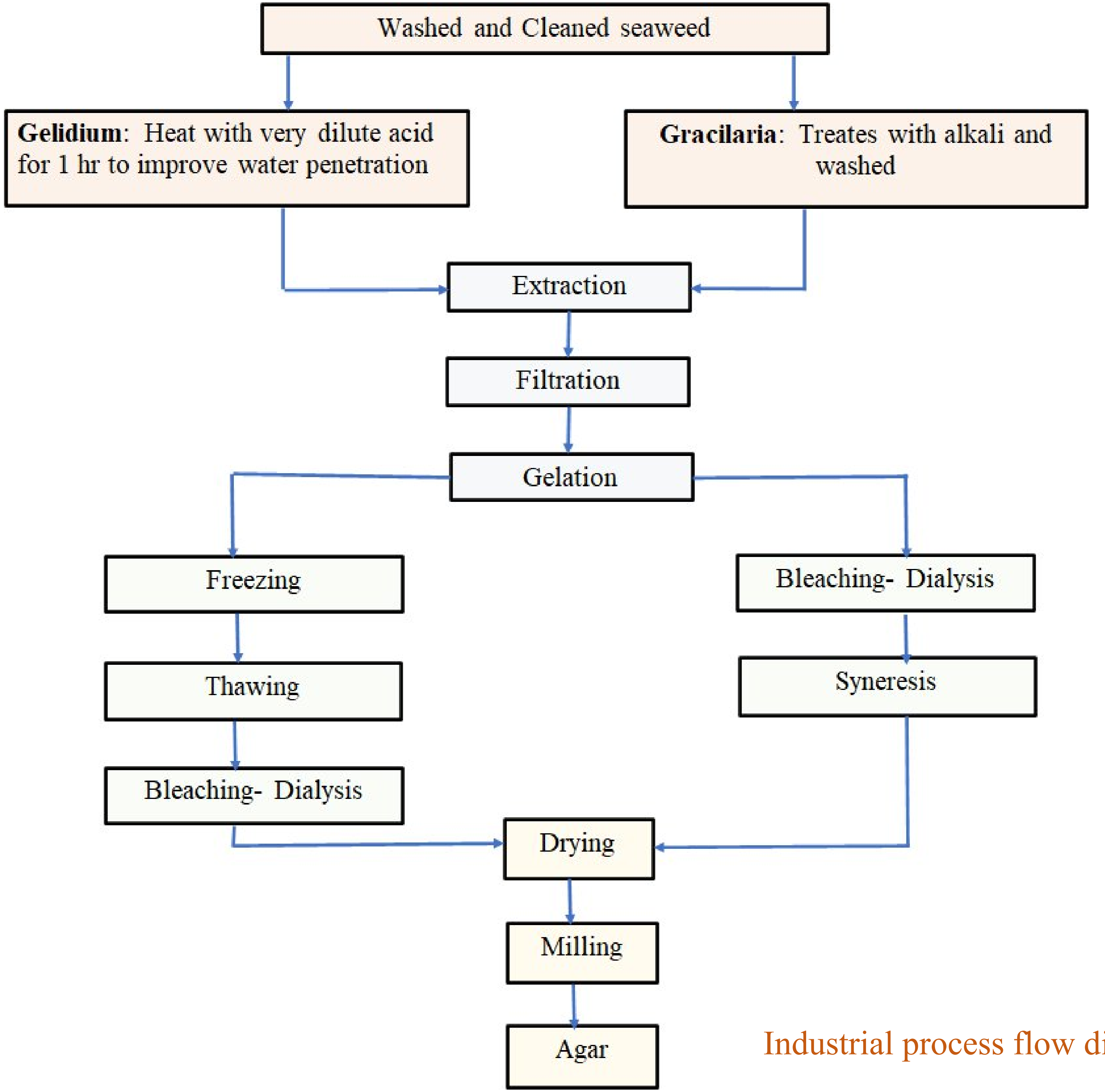
Fig. 2: Main seaweed source of agar



Gelidium spp.

Molecular structure of agarose backbone: D-Galactose and 3,6-anhydrogalactose

Gracilaria spp.

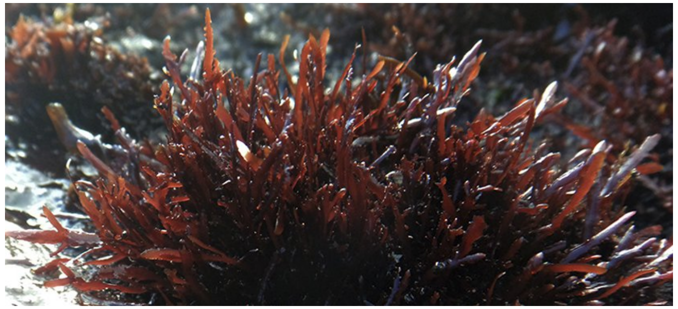


McHugh, D. J. (2003). A guide to the seaweed industry. *FAO fisheries technical paper*, *441*, 105.

Industrial process flow diagram for extracting agar from red seaweed

Case study: Exploitation of the Food Industry Waste Stream via Sustainable Biorefining

ALGWAS-BIOR Project | University of Burgos (ubu.es)



Case study: Red algae industry (*Gelidium sesquipedale*)

Hydrocolloid-centric industrial extraction (Hispanagar)

Commercial food grade agar 40 – 45%

Post-extraction algae waste stream 60 – 55%.
2000 to 2400 kg/day of valuable extracted waste stream.

- Phenolic compounds
- Antioxidant
- Polysaccharides
- Protein
- Dietary fibers
- Vital minerals
- Vitamins

Management strategy of the generated industrial waste stream

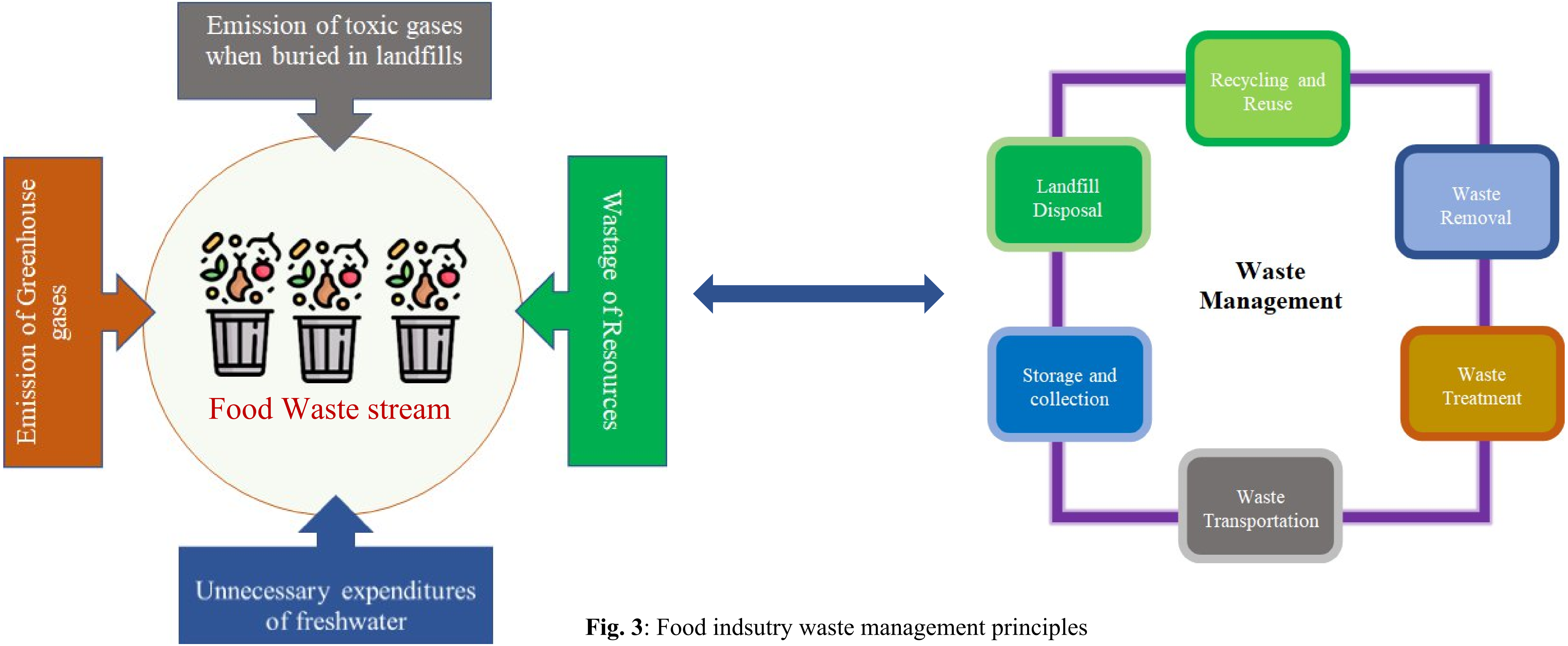


Fig. 3: Food indsutry waste management principles

- Emerging green technologies could play a substantial role in the green recovery of a range of bioproducts from the Agri-Food waste stream through the concept of biorefining.

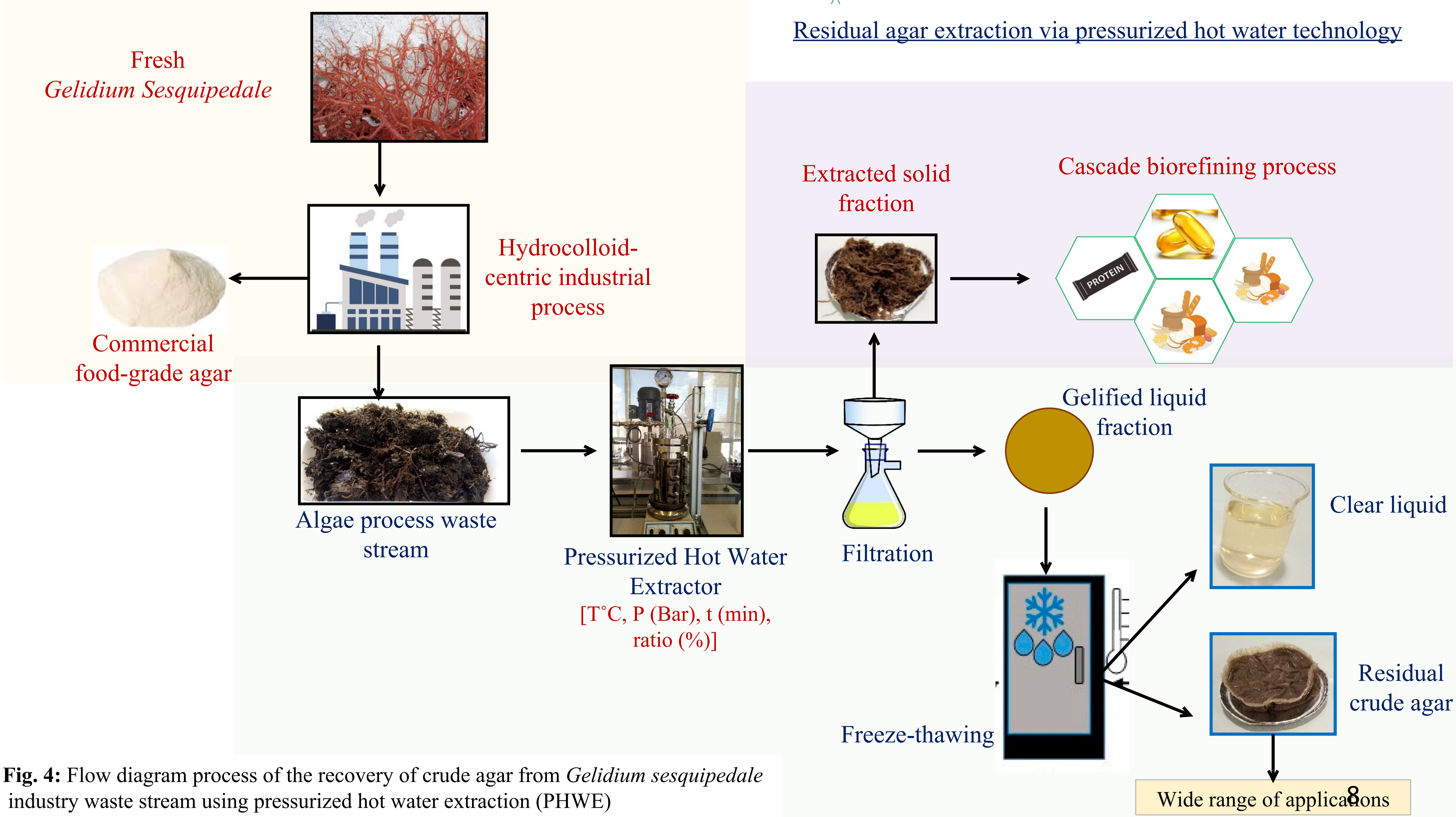


Fig. 4: Flow diagram process of the recovery of crude agar from *Gelidium sesquipedale* industry waste stream using pressurized hot water extraction (PHWE)

Use of pressurized hot water (subcritical water) technology

What is subcritical water? Water is heated at temperature above its boiling point (100°C) but below its critical point (374°C) under high pressure to keep the water in its condensed phase.

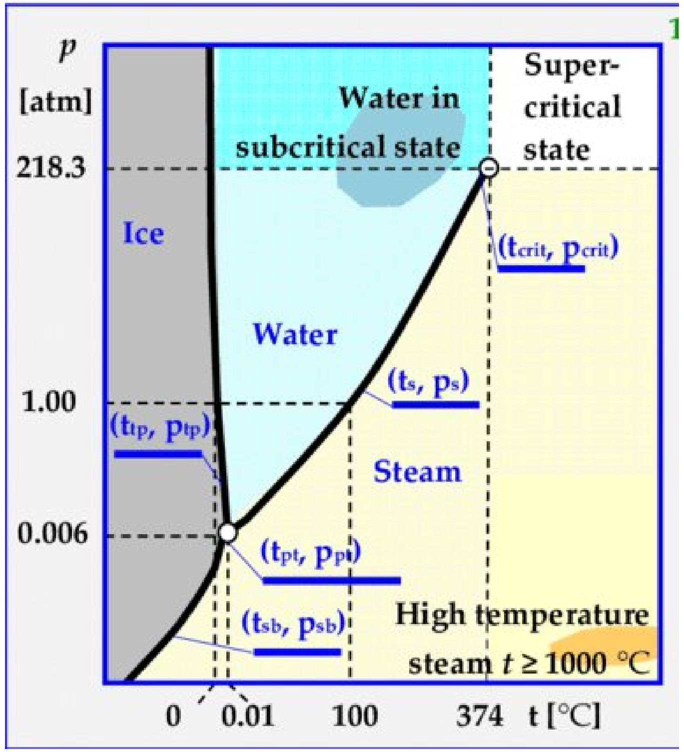


Fig. 5: Water phase diagram and its properties in normal, high temperature under pressure

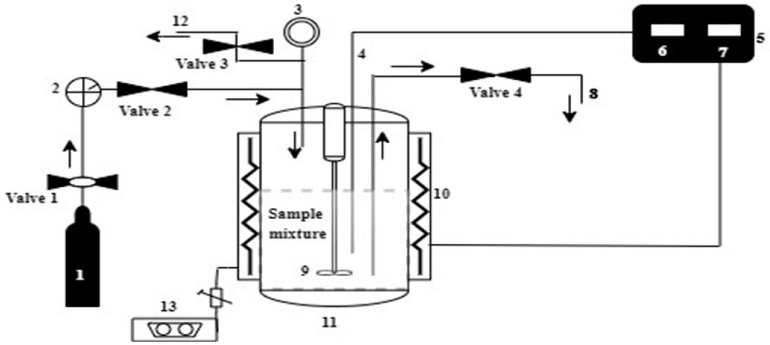


Fig- 6: Discontinuous subcritical water extractor, capacity 500 mL.

Severity factor

$$\log(R_0) = log\left\{t \times e^{\left[\frac{(T-100)}{14.75}\right]}\right\}$$

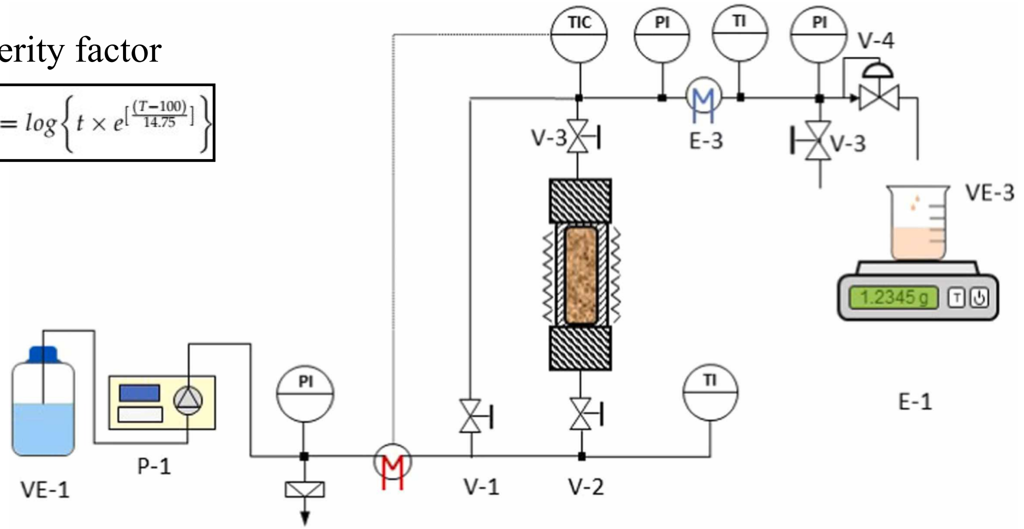
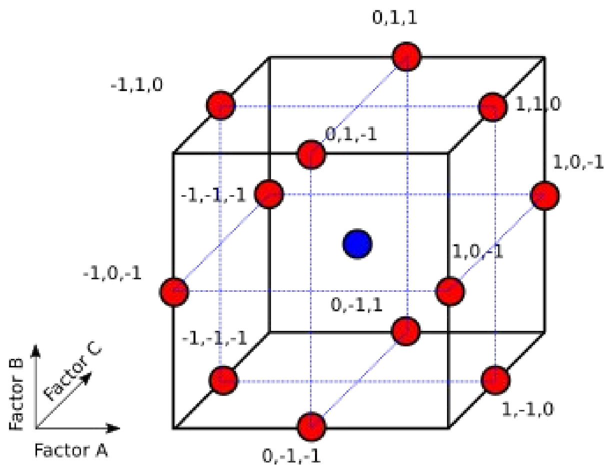


Fig. 7: Laboratory scale semicontinuous subcritical fluid extractor available in the laboratories of the BIOIND research group. Maximum specifications: p = 50 Mpa and T = 300 C. A liquid pump allows use of co-solvent. Several extracors with different capacities (50-100 mL).

Process optimization: Box-Behnken design

- Array used to construct a response surface (RSM).
- For each factor, 3 levels are required.
- The levels of the factors are at the midpoints of the edges (Red dots) and in the center point (Blue dot).



$$y = f(x_1, x_2,\ x_3, \ldots\ldots\ldots, x_k) \quad \textbf{1)}$$

Where y is the specific experimental response and xi the experimental factors.

- Economical detection of main effects.
- Rotatable
- No corner points of hypercube
- Three level multifactor experiments
- Avoid extreme combined factor, preventing potential loss of data.

❑ 2nd order polynomial model

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} x_i x_j + \varepsilon \quad \textbf{2)}$$

Where, x_1, x_2, x_3,……..x_k are the input factor that influence the response y, β_0, β_i, β_{ii}, β_{ij}, (i = 1, 2, 3,………k; and j= 1, 2, 3, ………k) are the unknown parameters and ϵ being the randon error. The β coefficients, which should be determined in the second-order model, are acquired by the least square method.

Table 1: Experimental factors and associated levels

Factors	Symbols	Levels −1	Levels 0	Levels +1
Reaction temperature (°C)	**A**	80	105	130
Reactor pressure (bar)	**B**	1	35.5	70
Extraction time (min)	**C**	40	97.5	150
Solid content (%)	**D**	3	6.5	10

	R^2	P-Value
Yield (%)	94.71	0.0008
Gelling temperature	91.1	0.0001
Meling temperature	88.76	0.0000
Gel strength (g/cm^2)	84.27	0.0001
3.6-Anhydrogalactose (%)	67.77	0.0094
Sulfate content (%)	79.8	0.0005

Table 2: Experimental patterns of the BBD reporting the different factors and responses

	Factor A	*Factor B*	*Factor C*	*Factor D*	*Response 1*	*Response 2*	*Response 3*	*Response 4*	*Response 5*	*Response 6*
Run	Temperature (°C)	Pressure (bar)	Time (min)	Solid Content (% w:v)	Yield (%)	Gel Strength (g/cm^2)	Melting Temp. (°C)	Gelling Temp. (°C)	3,6-ANG (%)	Sulfate content (%)
1	105	37.5	45	3	14.9	25	84.2	35	21.1	2.3
2	80	70	97.5	6.5	14.1	198	83.5	31	34.8	2.8
3	105	35.5	45	10	12.4	286	84.5	35	22.7	4
4	105	1	97.5	10	11.8	235	84.5	35.1	27.4	4.3
5	130	35.5	97.5	3	16.7	30	75	30	22	2.7
6	105[a]	35.5[a]	97.5[a]	6.5[a]	13.3[a]	216.1[a]	80.0[a]	34.0[a]	26.3[a]	4.7[a]
7	105	70	97.5	3	15.1	202	81.5	32.3	36.6	1.7
8	130	35.5	97.5	10	12.5	12.7	73	28.5	37	4.8
9	105	70	97.5	10	10.7	174.1	82.9	33.5	37.1	4
10	80	35.5	97.5	10	11	320.2	83	34	22.5	3.6
11	130	70	97.5	6.5	12	8	68	29.2	32.6	7
12	130	1	97.5	6.5	14.9	50.8	73.6	29.8	25.3	8.4
13	105	70	45	6.5	13.9	112	84.6	34.7	27.5	2.7
14	130	35.5	45	6.5	13.8	189	77.1	33	32.2	10
15	105	1	45	6.5	13.7	229	85.5	34	22.4	4.2
16	105	1	150	6.5	13.3	289.3	83.7	35	29.4	4.9
17	80	1	97.5	6.5	11.1	277.7	82.5	35	21.3	3.5
18	105[a]	35.5[a]	97.5[a]	6.5[a]	13.1[a]	218.0[a]	83.6[a]	34.0[a]	25.6[a]	4.8[a]
19	105	35.5	150	3	16.1	210	81.5	33.5	29.9	2.8
20	80	35.5	97.5	3	14.2	226.2	80.6	32.5	19.2	1.5
21	105	1	97.5	3	14.1	307.3	82	33.5	25.6	2.6
22	130[b]	37.5[b]	150[b]	6.5[b]	12.6	–	–	–	39.8	6.0
23	105	70	150	6.5	11.5	28.7	69	28.7	38.6	3.9
24	80	37.5	45	6.5	12.8	35	86	35	21.2	3.2
25	105	35.5	150	10	10	29.4	73.9	29.4	25.3	6
26	105[a]	35.5[a]	97.5[a]	6.5[a]	13.1[a]	34.0[a]	82.0[a]	34.0[a]	25.8[a]	4.7[a]
27	80	35.5	150	6.5	12.5	34	85	34	24.8	3.2

Center points

a) Represent the factors and responses obtained at the center points in the Box-Behnken design and allowed experimental replications.
b) Responses 2, 3, and 4 were not obtained due to the non-gelling property of the recover solid extracts at high A factor value (temperature = 130 °C) and high C factor value (extraction time = 150 min) (the pressure was set to 37.5 bar and the solid concentration to 6.5 wt%).

Results: physicochemical properties

- ➢ 930, 872 and 770 cm^{-1} characteristic of 3,6-AGN

- ➢ Reduction of both melting and gelling temperatures with severity factor.
- ➢ Positive relationship between the strength of the crude agar gel and its melting and gelling temperatures

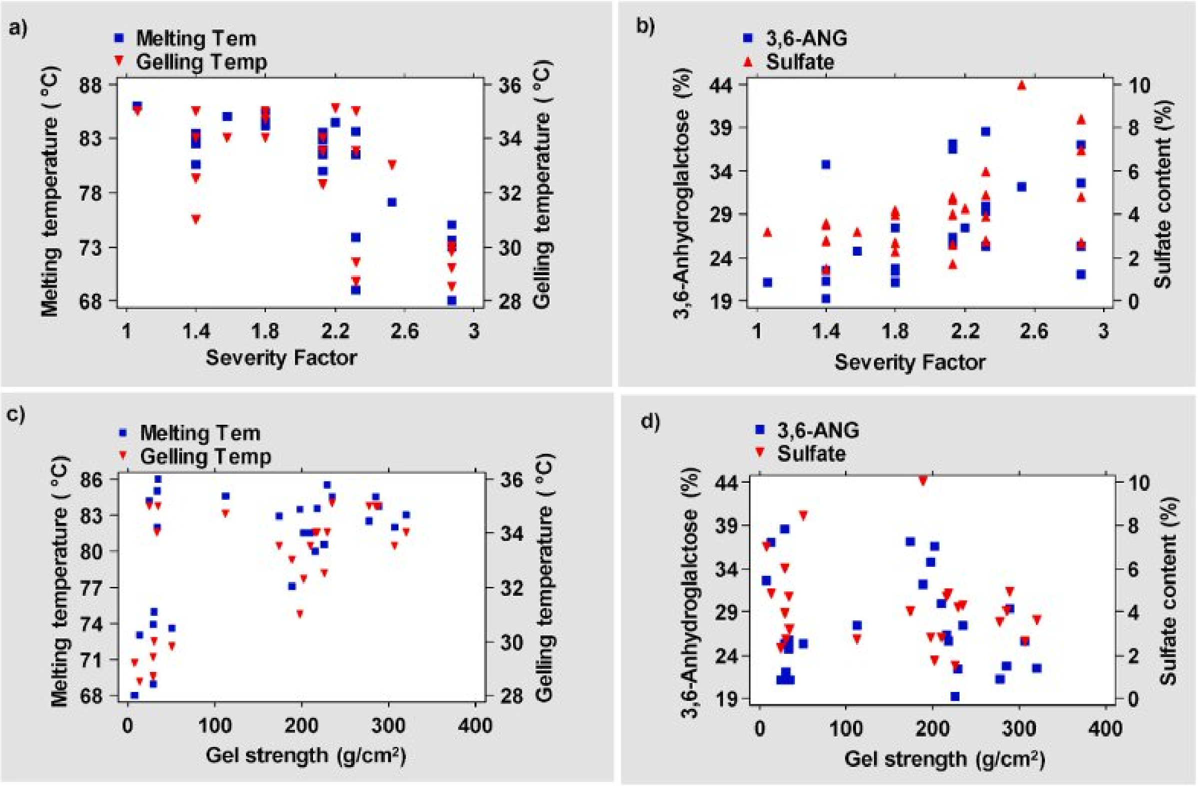


Fig. 8: Linear correlation between the severity factor of the PHWE process, and different physicochlemical properties of the recovered measured from gels containing 1.5% (w: v) agar.

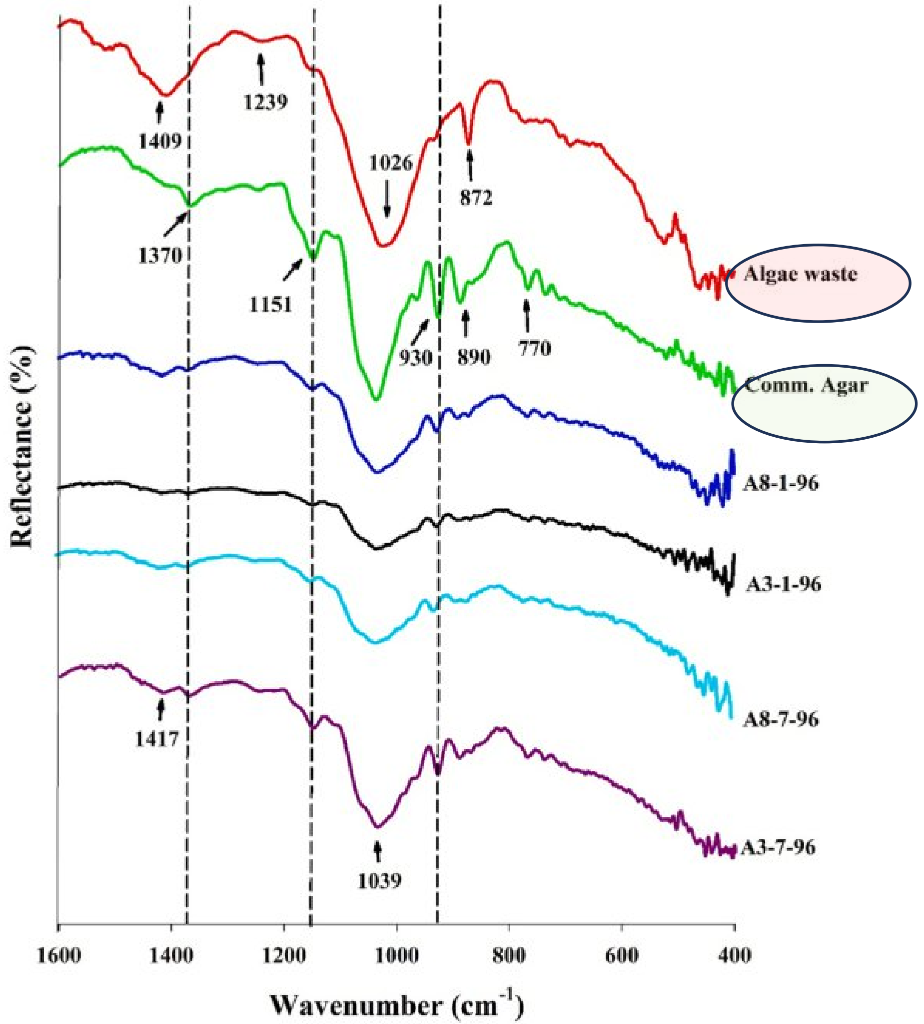


Fig. 9: FT-IR spectra showing the bands below 1600 cm^{-1} of the algae waste stream, commercial agar and crude agar recovered via PHWE.

Results: physicochemical properties

- The higher the solid concentration ➡ lower the yield.
- Highest yield ➡ at temperatures above 110 ◦C and extended extraction time (150 min), low algae concentration of 3% (w: v).

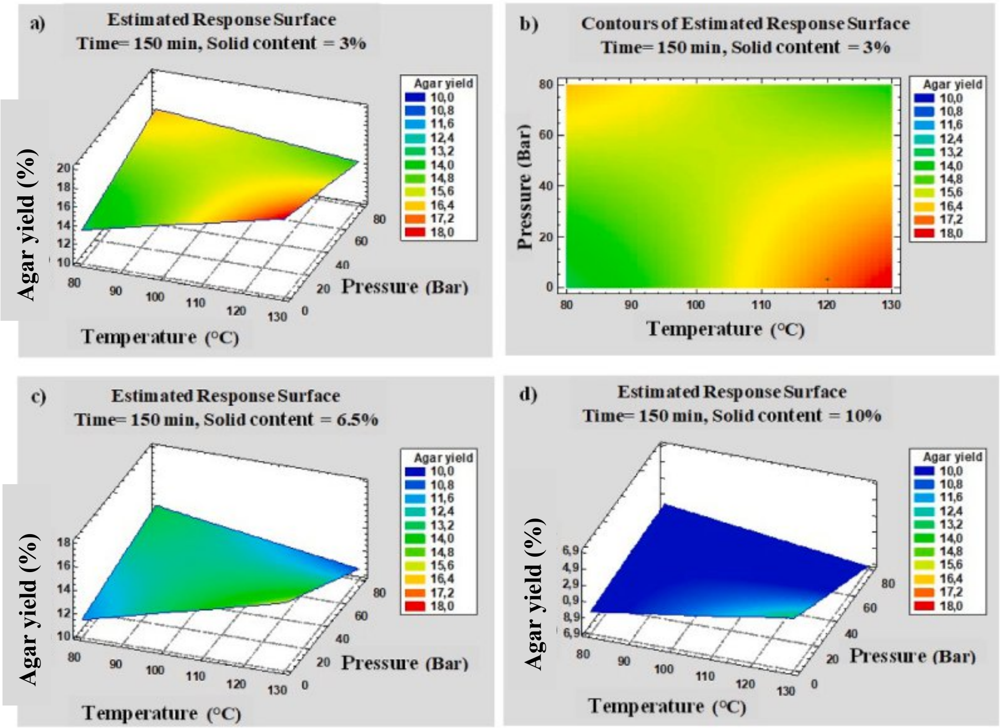


Fig. 10: 3D-response Surface (a, b, c) and contour plots (b) showing the variation of the agar yield in function of the extraction parameters.

- High value of 41.016% of 3,6-anhydrogalactose.
- Optimal operating conditions: Temperature of 130˚C, Pressure ≈ 70 bar, and reaction time of 149.84 min, and a solid concentration of 7.65% (w: v).

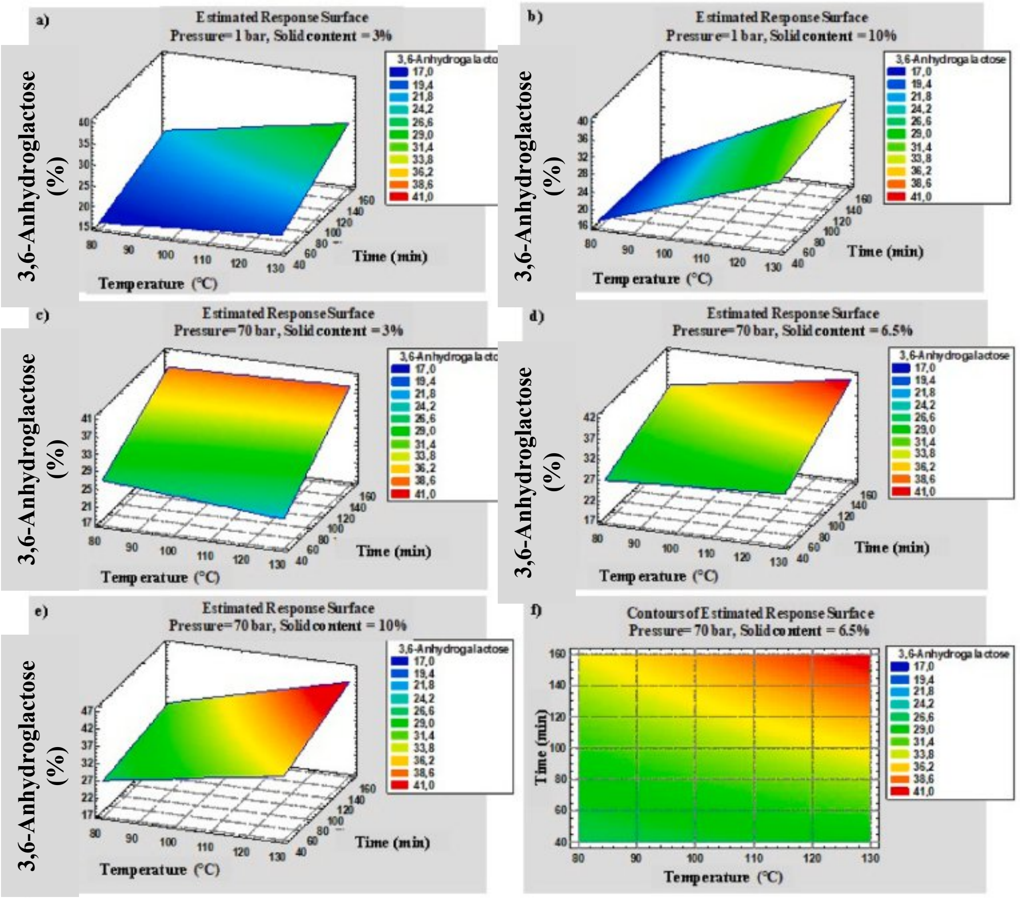


Fig. 11: 3D-response surface and contour plots showing the variation of the 3,6-anhydrogalactose content in function of the PHWE parameters.

Results: physicochemical properties

The 2nd order polynomial regression of the fitted quadratic model for each response was given in the following equations:

$\textit{Agar yield} = 4.42075 + 0.0859443 \times A + 0.203961 \times B + 0.0244244 \times C + 0.0928512 \times D - 0.00171014 \times AB - 0.00434783 \times BD - 0.00489796 \times CD$	$\textit{Gel strength} = -514.48 + 11.6774 \times A - 1.1715 \times B + 3.35271 \times C + 50.6173 \times D - 0.0740056 \times A^2 - 0.484354 \times CD$
$\textit{Gelling Temperature} = 11.1814 + 0.513465 \times A + 0.0628019 \times B + 0.00608082 \times C - 0.0309524 \times D - 0.00280504 \times A^2 - 0.000966184 \times BC$	$\textit{Melting temperature} = 38.6693 + 1.15411 \times A - 0.0302068 \times B - 0.0452656 \times C - 0.0714286 \times D - 0.0065023 \times A^2$
$3,6-\textit{Anhydrogalactose} = -1.66648 + 0.150333 \times A + 0.134783 \times B + 0.0646032 \times C + 0.419048 \times D$	$\textit{Sulfate content} = -9.47575 + 0.0703333 \times A - 0.0140097 \times B + 0.000634921 \times C + 1.98333 \times D - 0.128571 \times D^2$

Optimizimg the texture profile of the PHWE recovered crude agar gel

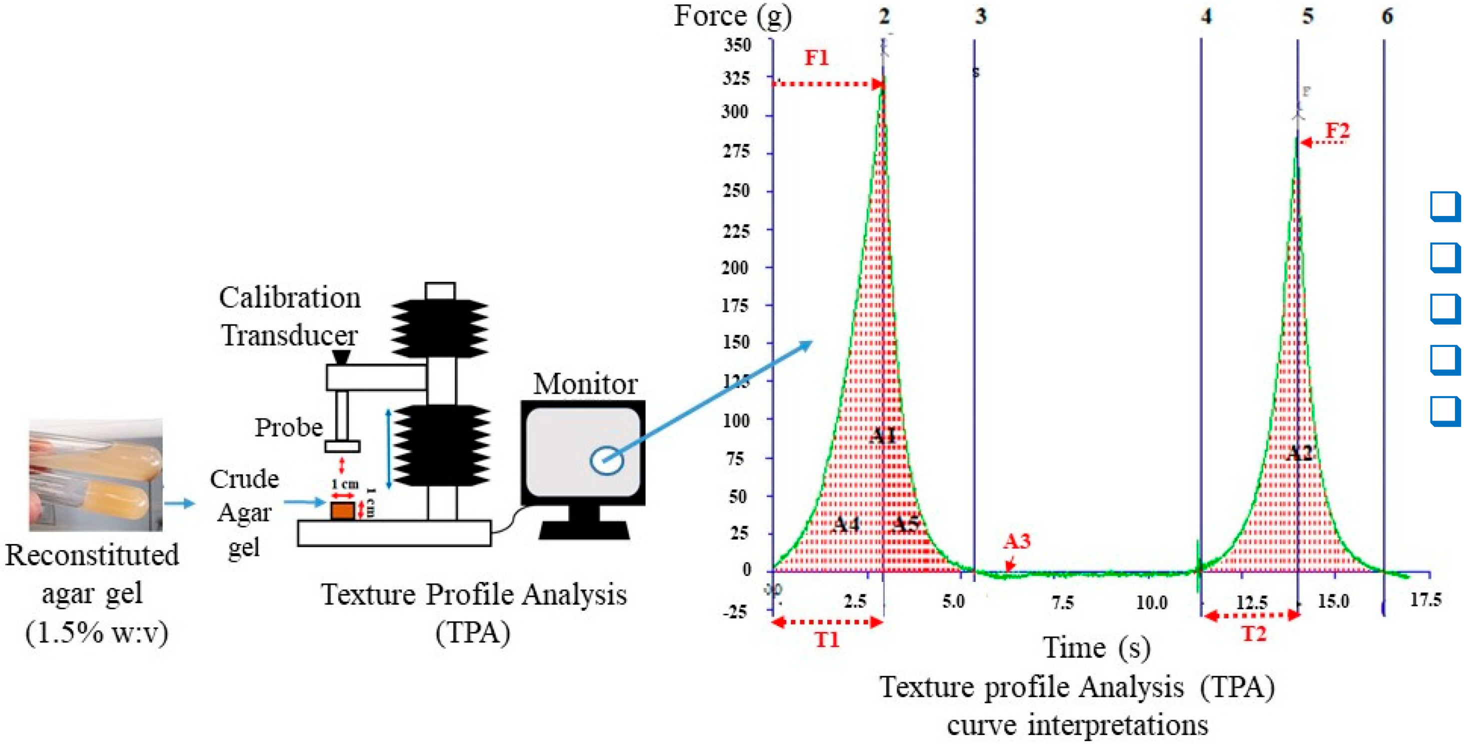


Fig. 12: Diagram of the texture profile analysis set-up, and related double compression curves used to determine the texture parameters of the PHWE recovered residual agar gel from the waste stream.

- Hardness (g)
- Springiness
- Cohesiveness
- Adhesiveness (g.s-1)
- Gumminess (g)

- A two factor interaction (2FI) model has been developed based on the following simplified equation:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i x_j,$$

where *Y* is the predicted response surface function, *β*0 is the model constant, *βi* is the slope or linear effect of the input factor *xi*, *βii* is the quadratic effect of input factor *xi*, and *βij* is the linear by a linear interaction effect between the input factor *xi* and factor *xj*

Table 3: The experimental pattern of the Box-Behnken design reporting the different process factors and response values

	Factor A	Factor B	Factor C	Factor D		Response 1	Response 2	Response 3	Response 4	Response 5	
Run	Temperature (°C)	Pressure (bar)	Time (min)	Solid Content (w:v%)	Severity Factor	Yield (%)	Hardness (g)	Adhesiveness (g.s^{-1})	Cohesiveness	Springiness (mm)	Gumminess (g)
1	105 (0)	35.5 (0)	45 (−1)	3 (−1)	1.80 [c]	14.9	460.2	−8.55	0.62	0.944	284.65
2	80 (−1)	70 (1)	97.5 (0)	6.5 (0)	1.40 [c]	14.1	427.01	−9.83	0.47	0.93	232.58
3	105 (0)	35.5 (0)	45 (−1)	10 (1)	1.80 [c]	12.4	447.76	−11.5	0.7	0.938	300.87
4	105 (0)	1 (−1)	97.5 (0)	10 (1)	2.13 [c]	11.8	380.58	−15.64	0.59	0.944	289.89
5	130 (1) [b]	35.5 (0) [b]	97.5 (0) [b]	3 (−1) [b]	2.87 [c]	16.7	–	–	–	–	–
6	105 (0) [a]	35.5 (0) [a]	97.5 (0) [a]	6.5 (0) [a]	2.13 [c]	13.3	370.60	−12.88	0.59	0.928	231.27
7	105 (0)	70 (1)	97.5 (0)	3 (−1)	2.13 [c]	15.1	391.32	−7.89	0.62	0.933	253.21
8	130 (1) [b]	35.5 (0) [b]	97.5 (0) [b]	10 (1) [b]	2.87 [c]	12.5	–	–	–	–	–
9	105 (0)	70 (1)	97.5 (0)	10 (1)	2.13 [c]	10.7	461.80	−18.68	0.52	0.869	240.61
10	80 (−1)	35.5 (0)	97.5 (0)	10 (1)	1.40 [c]	11.0	601.45	−12.18	0.66	0.945	394.76
11	130 (1) [b]	70 (1) [b]	97.5 (0) [b]	6.5 (0) [b]	2.87 [c]	12.0	–	–	–	–	–
12	130 (1) [b]	1 (−1) [b]	97.5 (0) [b]	6.5 (0) [b]	2.87 [c]	14.9	–	–	–	–	–
13	105 (0)	70 (1)	45 (−1)	6.5 (0)	1.80 [c]	13.9	465.38	−8.09	0.67	0.937	292.96
14	130 (1) [b]	35.5 (0) [b]	45 (−1) [b]	6.5 (0) [b]	2.54 [c]	13.8	–	–	–	–	–
15	105 (0)	1 (−1)	45 (−1)	6.5 (0)	1.80 [c]	13.7	345.79	−11.72	0.58	0.941	243.89
16	105 (0)	1 (−1)	150 (1)	6.5 (0)	2.32 [c]	13.3	375.02	−11.32	0.75	0.942	238.38
17	80 (−1)	1 (−1)	97.5 (0)	6.5 (0)	1.40 [c]	11.1	503.23	−9.6	0.67	0.939	238.33
18	105 (0) [a]	35.5 (0) [a]	97.5 (0) [a]	6.5 (0) [a]	2.13 [c]	13.1	400.36	−12.9	0.60	0.925	229.20
19	105 (0)	35.5 (0)	150 (1)	3 (−1)	2.32 [c]	16.1	357.7	−9.2	0.74	0.942	223.80
20	80 (−1)	35.5 (0)	97.5 (0)	3 (−1)	1.40 [c]	14.2	394.74	−6.2	0.57	0.899	193.95
21	105 (0)	1 (−1)	97.5 (0)	3 (−1)	2.14 [c]	14.1	461.32	−8.33	0.69	0.942	280.59
22	130 (1) [b]	35.5 (0) [b]	150 (1) [b]	6.5 (0) [b]	3.06 [c]	12.6	–	–	–	–	–
23	105 (0)	70 (1)	150 (1)	6.5 (0)	2.32 [c]	11.5	225.30	−18.96	0.51	0.889	146.54
24	80 (−1)	37.5 (0)	45 (−1)	6.5 (0)	1.06 [c]	12.8	374.60	−9.52	0.55	0.867	240.21
25	105 (0)	35.5 (0)	150 (1)	10 (1)	2.32 [c]	10.0	232.12	−18.86	0.52	0.898	149.21
26	105 (0) [a]	35.5 (0) [a]	97.5 (0) [a]	6.5 (0) [a]	2.14 [c]	13.1	400.11	−12.78	0.60	0.930	229.02
27	80 (−1)	35.5 (0)	150 (1)	6.5 (0)	1.59 [c]	12.5	493.19	−15.6	0.68	0.930	309.95

Unsupported conditions   Center points

Results: texture profile

- High severity factor (SF) ➡ lower hardness values
- Weak positive relationship between the SF and gel springiness
- Moderate negative impact of the SF on adhesiveness.

Table : Coded regression equations based on the polynomial two-factor interaction model of the different textural responses of the pressurized hot water extraction recovered agar gel from the algae industry waste stream.

$$\textbf{Gel hardness} = 385.024 - 69.846A - 9.513B - 66.12375C - 18.535D - 125.41275AC - 154.39AD - 67.3275BC$$

$$\textbf{Gel cohesiveness} = 0.62466667 + 0.02466667A - 0.03625B - 0.00625C - 0.0425D + 0.06375AB - 0.07125AC - 0.0875AD - 0.0825BC - 0.075CD$$

$$\textbf{Gel gumminess} = 242.608667 - 25.613A - 12.518B - 45.555C - 7.70875D - 80.2AC - 108.11375AD - 36.2275BC$$

$$\textbf{Gel adhesiveness} = -12.248 - 1.93966667A - 0.648B - 2.456C - 3.669D - 2.8175BC - 1.6775CD$$

$$\textbf{Gel springiness} = 0.9278 + 0.00946667A - 0.015B - 0.011125C - 0.014D - 0.042625AC - 0.037AD - 0.0165BD$$

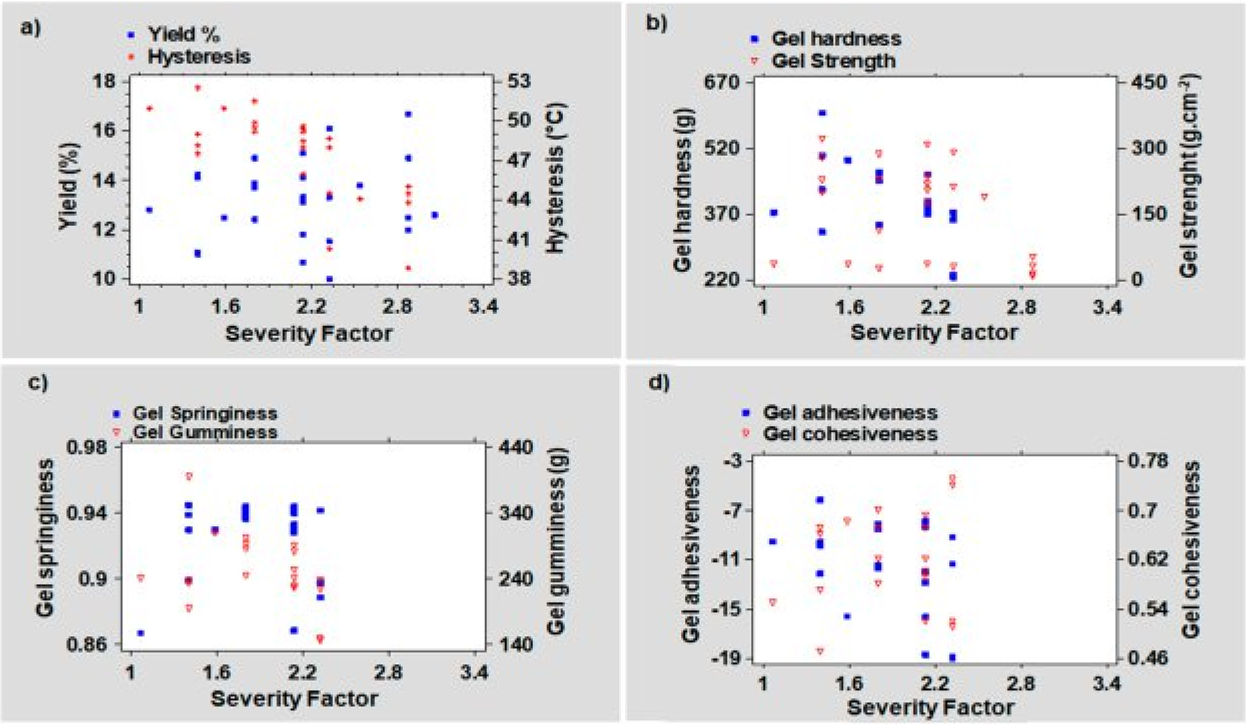


Fig. 13: Correlations between the severity factor of the pressurized hot water extraction treatment and (**a**) the agar yield (%) and hysteresis (°C), (**b**) the agar gel hardness (g) and gel strength (g.cm-2), (**c**) the agar gel hardness (g) and gel strength (g.cm-2), and (**d**) the gel adhesiveness and cohesiveness

Results: texture profile

- Elevated temperatures and low algae-to-water ratios were required to recover harder agar gels.
- Increasing the pressure to 70 bar with a short operating time resulted in a visible expansion of the favorable experimental region

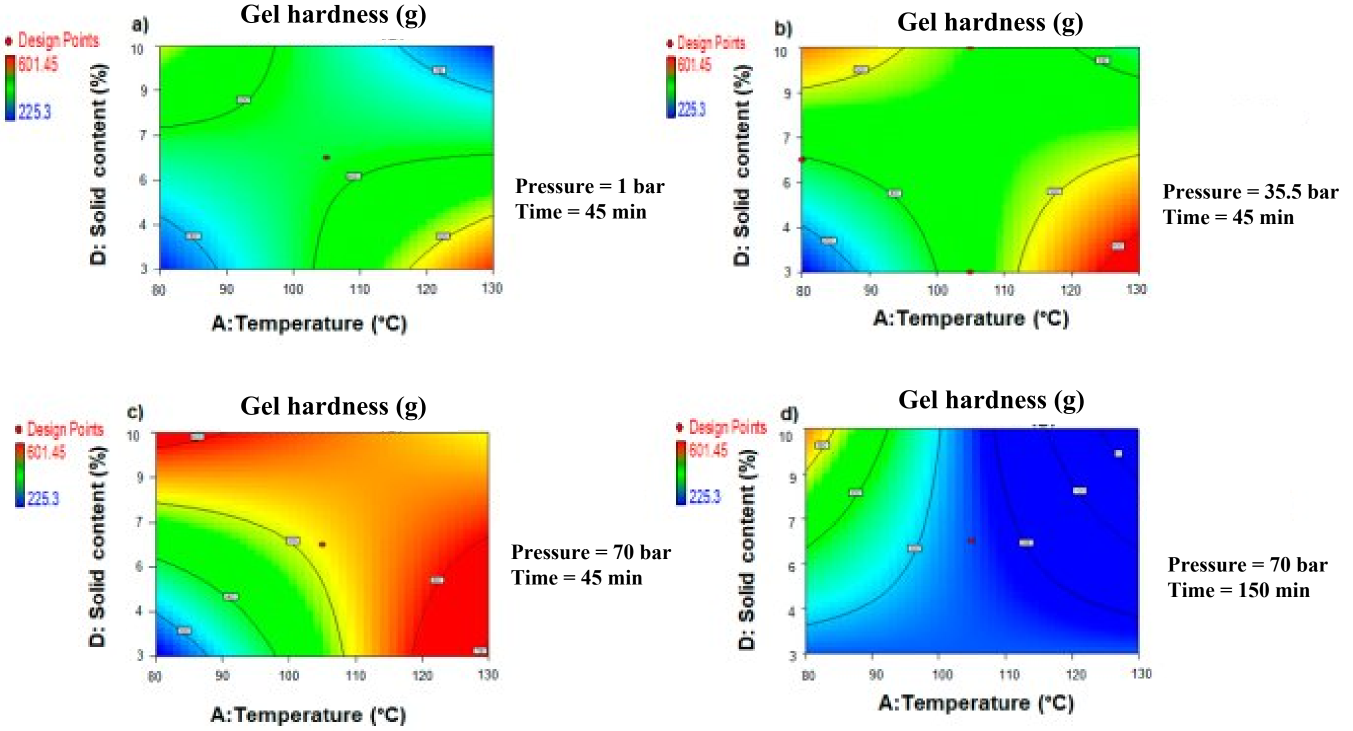


Fig. 14: Response surface contour plots showing the combined influences of the pressurized hot water extraction (PHWE) temperature (A) and solid content (D) on the hardness of the recovered agar gel at fixed pressure (B) and the operating time (D). (**a**) B = 1 bar and C = 45 min. (**b**) B = 35.5 bar and C = 45 min. (**c**) B = 70 bar and C = 45 min. (**d**) B = 70 bar and C = 45 min.

- High cohesive gel required elevated operating temperatures associated with short recovery times, depending on the pressure.
- High temperatures > 120 ◦C combined with an algae concentration < 8% (*w:v*), subsequent increase in pressure to 35.5 bar seemed to improve the gel homogeneity.

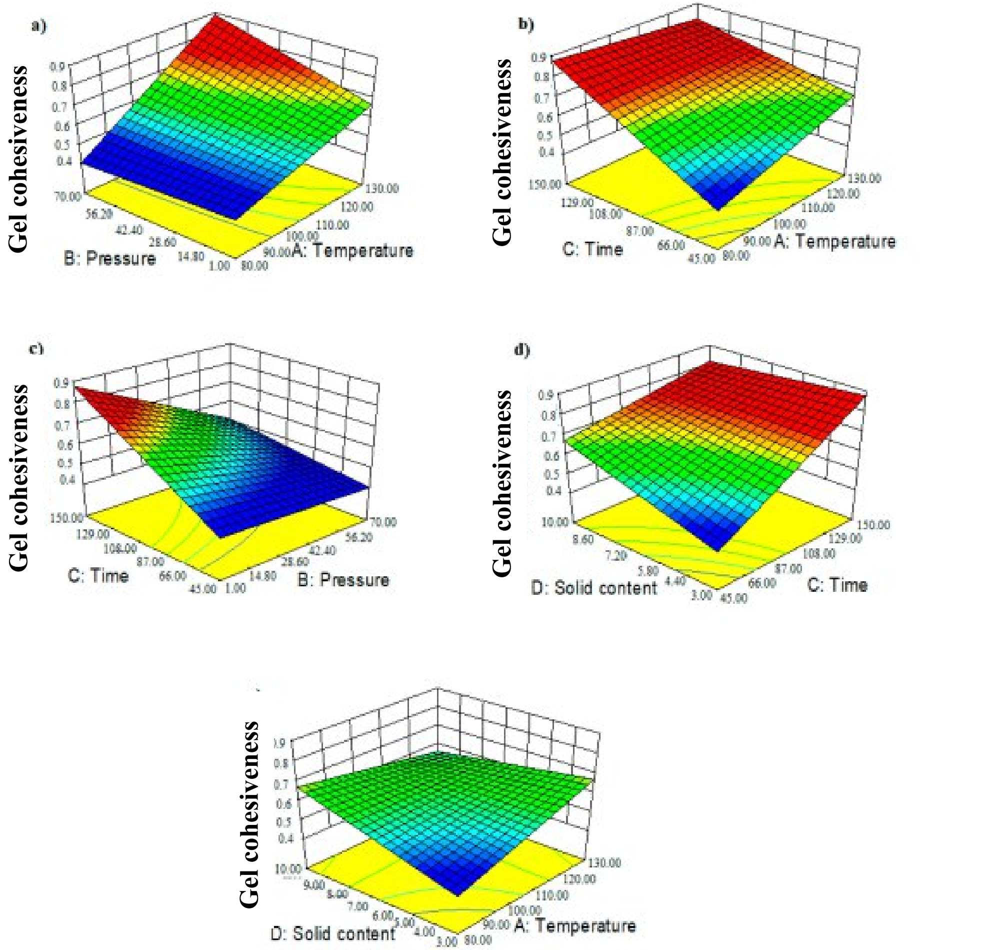


Fig. 15: Three-dimensional (3D) response surface contour plots showing the combined effects of the (**a**) the pressurized hot water extraction (PHWE) temperature and pressure, (**b**) the recovery time and temperature, (**c**) the recovery time and pressure, (**d**) the solid content and recovery time and (**e**) the solid content and temperature on the recovered agar gel cohesiveness.

Results: texture profile

- Increasing the temperature resulted in high level of gumminess when the algae-to-water ratios < 3% *w:v*.
- Gumminess was enhanced when high temperatures ≈ 130 ◦C combined with reaction times < 100 min.

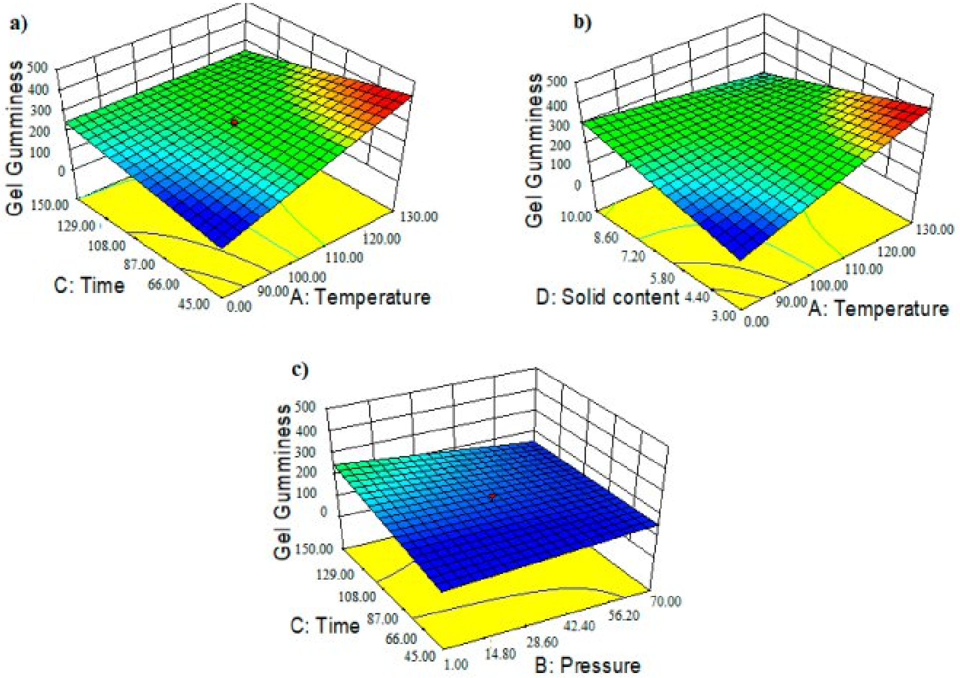


Fig. 16: Three-dimensional (3D) response surface contour plots showing the combined effects of (**a**) the operating time and temperature of the pressurized hot water extraction (PHWE), (**b**) the solid content and temperature and (**c**) time and pressure on the recovered agar gel.

- Higher elastic gels at temperatures > 100 ◦C, regardless of time and pressure.
- At pressure = 70 bar, raising ratio to 10% (*w:v*) decreased the springiness.
- Temperatures < 85 ◦C combined with time > 130 min = only conditions for acceptable elasticity.

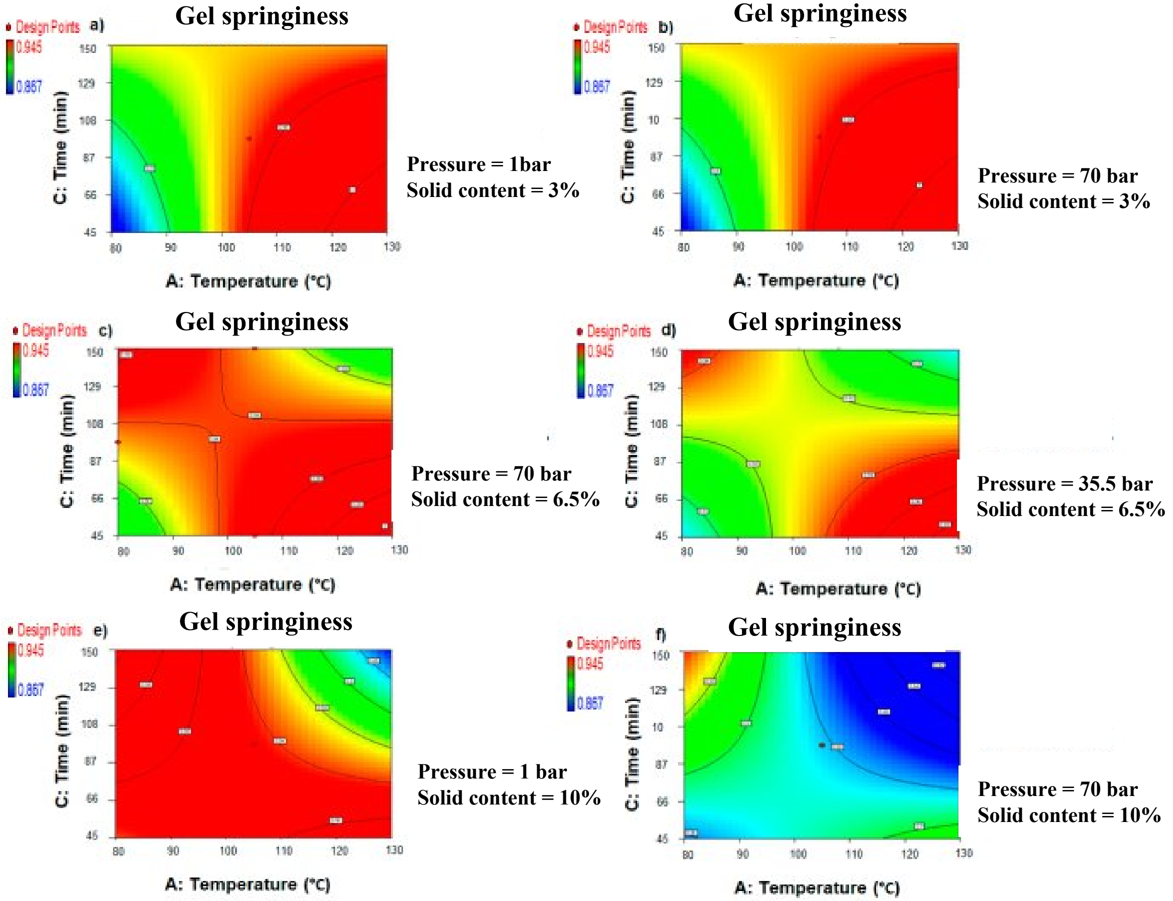


Fig. 17: Response surface contour plots showing the combined influences of the pressurized hot water extraction (PHWE) temperature (A) and operating time (C), on the springiness of the recovered agar gels at fixed pressure (B) and solid content (D). (**a**) B = 1bar and D = 3% (*w:v*). (**b**) B = 70 bar and D = 3% (*w:v*), (**c**) B = 1 bar and D = 6.5% (*w:v*). (**d**) B = 35.5 bar and D = 6.5% (*w:v*), (**e**) B = 1 bar and D = 10% (*w:v*) and (**f**) B = 70 bar and D = 10% (*w:v*).

Results: texture profile

- ❖ The partial desirability function (*di*) for each response which runs from **0**, the least desirable response, to **1**, the optimal response.

- ❖ The global multiresponse desirability (*D*), which is defined as the average weighted geometric of *n* individual desirability functions, was then obtained from the following equation.

$$D = \left[\prod_{i=1}^{n} d_i^{\,Pi}\right]^{\frac{1}{n}}$$

where *Pi* is the weight of the response, normalized so that $\sum_i^n Pi = 1$.

- ➢ The proficiency of each response by comparing their proximity to 1.
- ➢ Therefore, the combined multiresponse desirability of 0.786 was found to be attractive

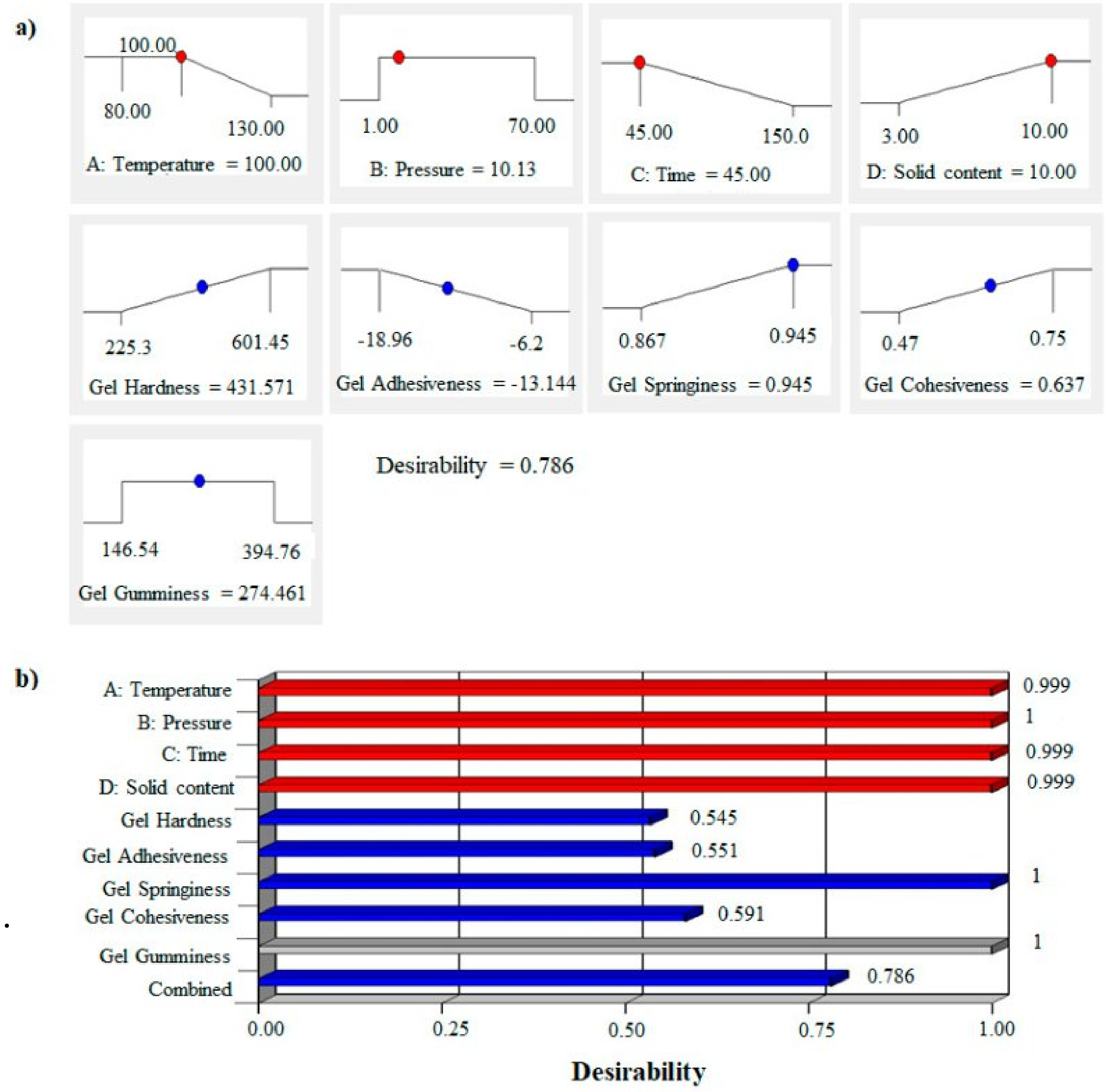


Fig. 18: (**a**) Ramp function graph of pressurized hot water extraction process parameters targeting a minimization of the temperature and recovery time while maximizing the algae-to-water percent ratio. (**b**) Bar graph of desirability for combined optimization of the PHWE process.

Conclusion:

- Coupling a Box-Behnken design with a response surface methodology was a suitable statistical approach for optimizing the pressurized hot water extraction (PHWE) technology.

- In the framework of circular economy and industrial symbiosis the selectivity of PHWE is an advantageous strategy for adding-value to the agro-industry waste stream.

- The adjustability of the physicochemical properties and texture profiles of the recovered agar will expand the spectrum of applications.

- Using PHWE at mild conditions of temperature was the first step of a subsequent integral cascade biorefining of the discarded food industry waste stream.

Thank you for your attention!

This work was supported by the European Commission, Horizon 2020 program through the Marie-Curie Individual Fellowship (H2020-MSCA-IF-2019), with regards to the ALGWAS-BIOR project (Grant agreementnumber 898804).

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