Recovery of phenolic compounds from edible algae using high hydrostatic pressure: an optimization approach

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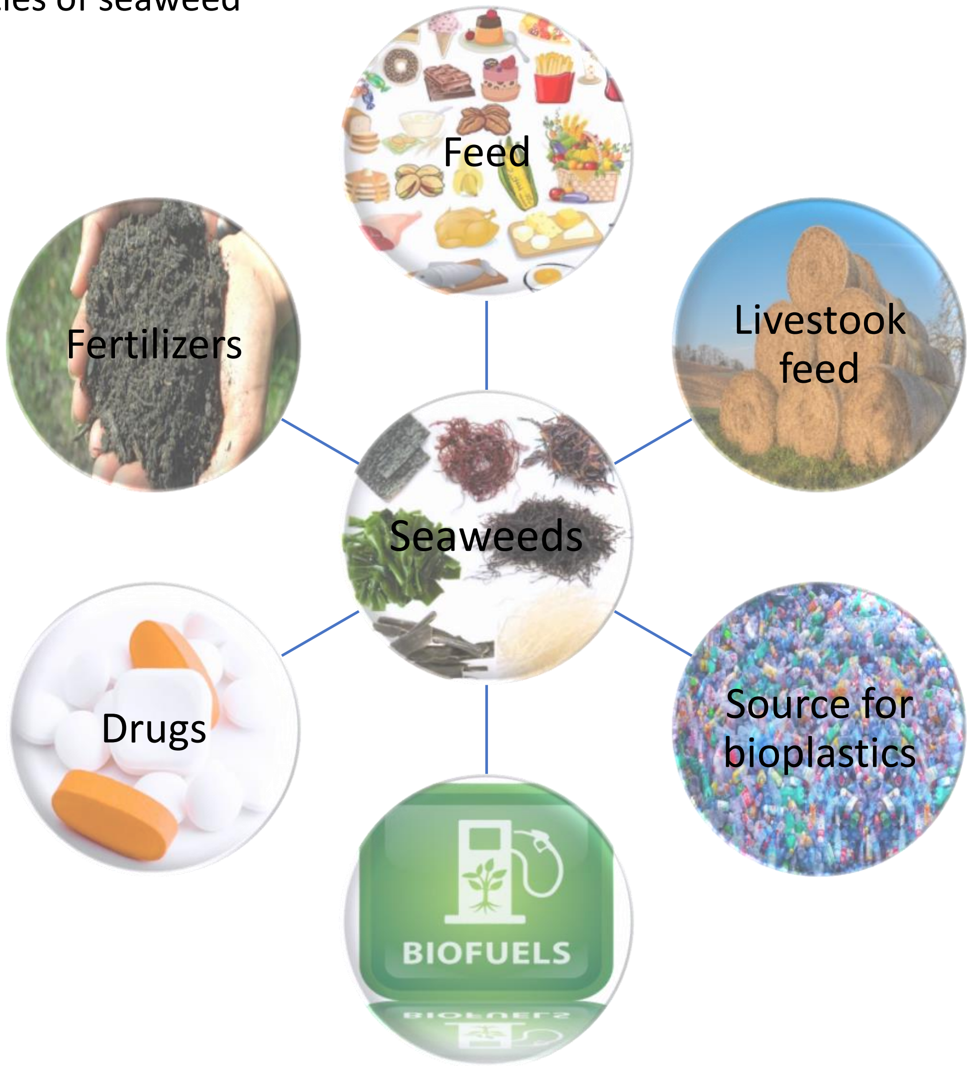
Introduction – Seaweed industry

Posibilities of seaweed



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Valorisation of seaweeds: obtaining extracts with pharmaceutical, cosmetic or food interest.

Introduction – Seaweed industry

Local common name	Scientific name	Uses
Kombu	Laminaria spp.	broths, snacks, with legumes
Kombu real	Saccharina latissima	with vegetables, cereals, fish ... or toast
Espagueti de mar	Himanthalia elongata	with vegetables, cereals, fish ... or fried
Wakame	Undaria pinnatifida	raw or boiled
Nori	Porphyra spp.	stews, vegetables, rice or sautéed
Dulse	Palmaria palmata	rehydrated
Alga percebe	Codium spp.	salads, sauces, dressings, creams, ceviche, sushi, croquettes, side dishes and rice dishes
Lechuga de mar	Ulva spp.	preserves

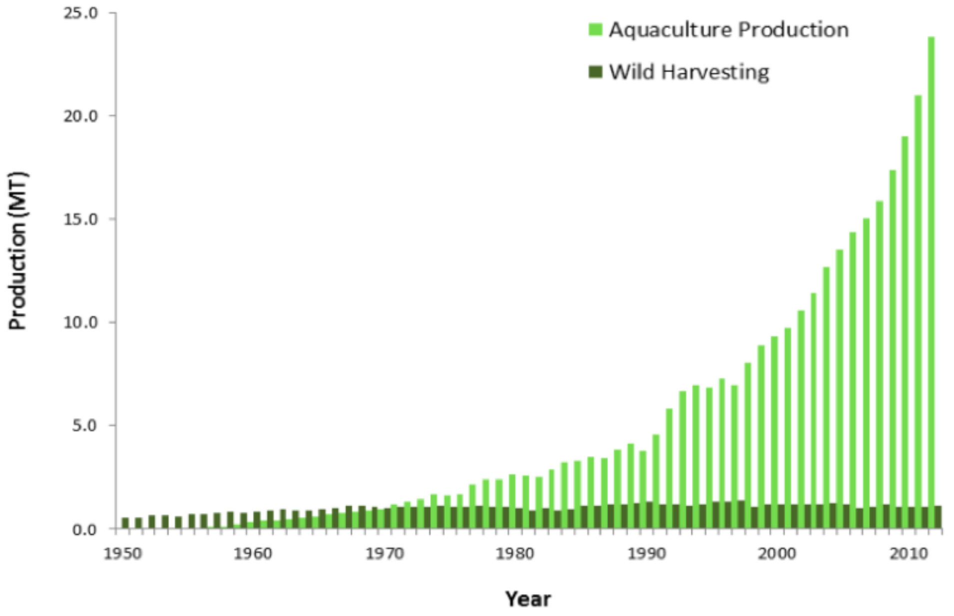

***Figure* 1. Global seaweed aquaculture production (1950-2014). FAO (2015)**

Aim

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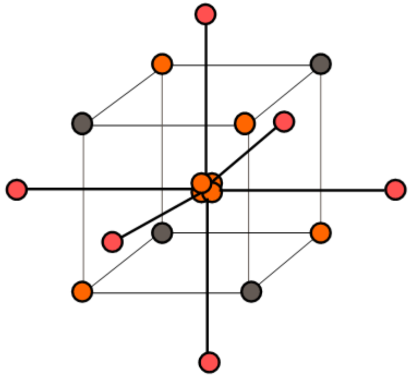
Project background

Algae used in this study:

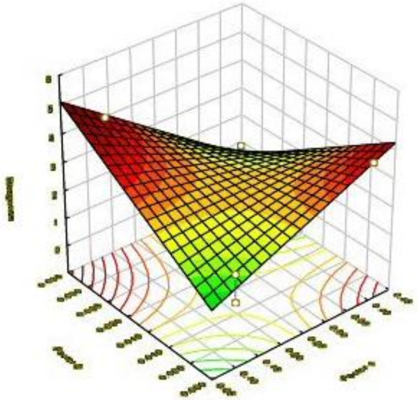
Laminaria spp.
Saccharina latissima
Himanthalia elongata
Undaria pinnatifida
Porphyra spp.
Palmaria palmata
Codium spp.
Ulva spp.



RSM design

Parameters studied:

- Concentration ethanol: 0 – 100 % aqueous-ethanol, v/v (solid - liquid ratio 30 g/L).
- Time: 10 – 110 min.
- Pressure: 100 – 600 mPa.



RSM results

Response surface methodology using a five-level central composite design combining the independent variables of processing time, pressure and solvent.

Phenolic and dry weight results

Aim - methods

Different extraction techniques

High hydrostatic pressures (HHP)

Microwave assisted extraction (MAE)

Extraction by supercritical fluids (SP)

Extraction with electric pulses (EP)

Ultrasound-assisted extraction (UAE)

Enzyme-assisted extraction (EAE)

Maceration (MA)

Non conventional techniques

Conventional techniques

Material and methods - HHP

Sample

Extracts lyophilisation

Powdered sample

HP extraction

model S-FL-085-09-W (Stansted Fluid Power Ltd., Harlow, UK)

High pressure extraction (HHP)

Centrifugation

Filtration

Chemical characterization

Material and methods - quantification

Extracts

HPLC-UV system

Mobile phase:
Formic acid (0,1 %)

Stationary phase:
Inertsil 100A ODS-3 reversed-phase column (5 µm, 4.6 × 150 mm) thermostatted at 35 °C

Folin-Ciocalteau technique

Same results with both techniques

Identification and quantification

Material and methods - RSM

Extraction Variables (independent):

X_1: Extraction time - t (min)
X_2: Pressure - P (mPa)
X_3: Solvent - % (Water-Ethanol)

Response variables (dependent):

Y_1: % of extraction yield
Y_2: mg of galic acid per g of dw seaweed

Response Surface Methodology (RSM)

$$Y = \underbrace{b_0}_{\text{Intercept}} + \underbrace{\sum_{i=1}^{n} b_i X_i}_{\text{Linear effect}} + \underbrace{\sum_{\substack{i=1 \\ j>i}}^{n-1} \sum_{j=2}^{n} b_{ij} X_i X_j}_{\text{Interactive effect}} + \underbrace{\sum_{i=1}^{n} b_{ii} X_i^2}_{\text{Quadratic effect}}$$

The optimization process here described can be solved with 31 experimental points plus some preliminary trials to centre the ranges of the variables involved.

The mathematical solutions produced allow to control the complete extraction process and can be used by the industry to select the conditions that makes the process more profitable.

Chemical study of 3 variables may involve MORE THAN 200 possible experimental combinations for each extraction technique

Results and discussion - RSM

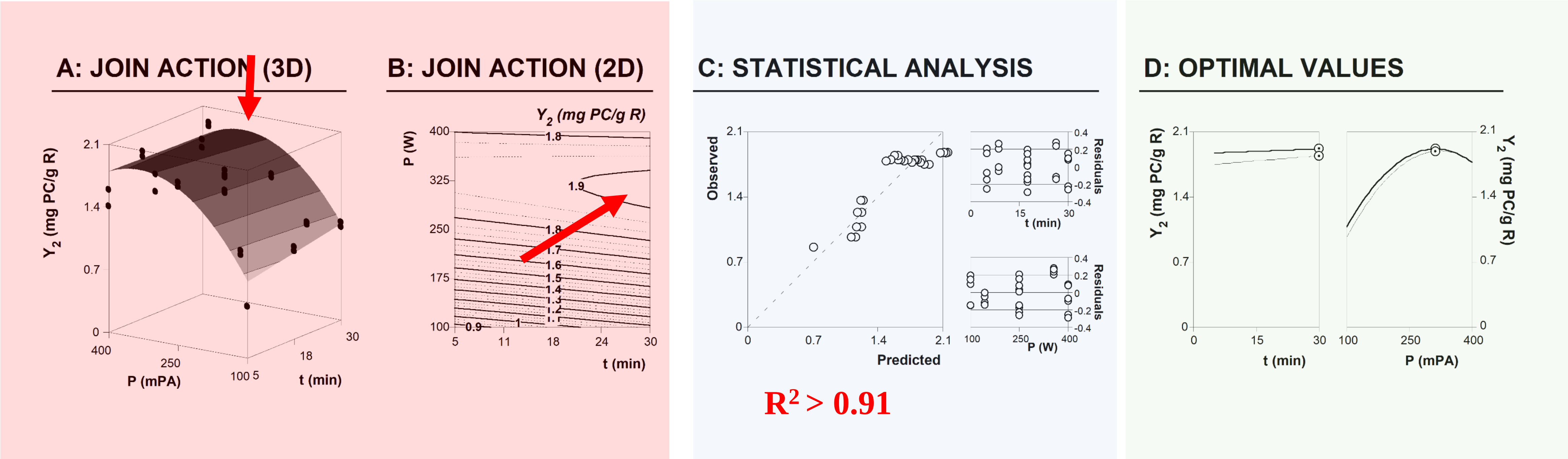
Table 1: Experimental RSM results of the CCCD for the optimization of the three main variables involved (X_1, X_2, and X_3) in the HPE for the two response value formats assessed (Y_1, and Y_2). Three replicates were performed for each condition for each technique. Samples used were Kombu (**KR**, *Laminaria spp.*), Kombu real (**KR**, *Saccharina latissimi*), Espagueti de mar (**EM**, *Himanthalia elongate*), Wakame (**W**, *Undaria pinnatifida*), Nori (**N**, *Porphyra spp.*), Dulse (**D**, Palmaria palmate), Alga percebe (**AP**, Codium spp.) and Lechuga de mar (**LM**, Ulva spp.).

VARIABLE CODED VALUES			EXTRACTED RESIDUES RESPONSES								TOTAL PHENOLIC CONTENT RESPONSES							
			K	*KR*	*EM*	*W*	*N*	*D*	*AP*	*LM*	*K*	*KR*	*EM*	*W*	*N*	*D*	*AP*	*LM*
X_1	X_2	X_3	%	%	%	%	%	%	%	%	mg PC/g	mg PC/g	mg PC/g	mg PC/g	mg PC/g	mg PC/g	mg PC/g	mg PC/g
-1	-1	-1	23.5	32.8	35.8	27.5	53.7	46.5	43.3	38.7	78.3	109.4	119.4	91.7	178.9	155.0	144.4	128.9
-1	-1	1	27.3	34.3	18.7	18.5	32.7	21.5	14.5	29.5	48.9	114.4	62.2	61.7	108.9	71.7	48.3	98.3
-1	1	-1	10.0	40.3	32.3	28.0	51.0	48.0	43.0	37.5	91.1	134.4	107.8	93.3	170.0	160.0	143.3	125.0
-1	1	1	14.5	26.3	15.2	18.7	22.2	20.2	20.7	27.7	48.3	87.8	50.6	62.2	73.9	67.2	68.9	92.2
1	-1	-1	30.0	43.3	36.0	27.5	51.8	47.3	45.0	41.3	100.0	144.4	120.0	91.7	172.8	157.8	150.0	137.8
1	-1	1	14.7	35.8	15.2	20.0	15.2	13.2	22.3	29.2	48.9	119.4	50.6	66.7	50.6	43.9	74.4	97.2
1	1	-1	27.3	39.2	33.3	27.8	52.0	47.2	40.3	37.3	91.1	130.6	111.1	92.8	173.3	157.2	134.4	124.4
1	1	1	10.0	29.5	15.0	16.2	29.7	23.8	25.5	30.5	33.3	98.3	50.0	53.9	98.9	79.4	85.0	101.7
-1.68	0	0	17.0	36.7	25.8	24.5	46.8	40.3	33.8	33.7	56.7	122.2	86.1	81.7	156.1	134.4	112.8	112.2
1.68	0	0	18.2	34.5	27.2	24.8	47.7	38.5	38.7	35.3	60.6	115.0	90.6	82.8	158.9	128.3	128.9	117.8
0	-1.68	0	18.3	34.5	25.7	23.7	50.7	42.7	32.2	36.0	61.1	115.0	85.6	78.9	168.9	142.2	107.2	120.0
0	1.68	0	0.0	26.7	22.2	0.0	0.0	38.8	31.2	31.0	0.0	88.9	73.9	0.0	0.0	129.4	103.9	103.3
0	0	-1.68	24.7	40.3	43.2	25.0	49.7	53.3	54.7	42.3	82.2	134.4	143.9	83.3	165.6	177.8	182.2	141.1
0	0	1.68	1.3	10.2	2.8	6.7	4.5	5.5	4.2	7.2	4.4	33.9	9.4	22.2	15.0	18.3	13.9	23.9
-1.68	-1.68	-1.68	29.3	40.0	36.5	23.2	48.0	40.8	51.0	33.7	97.8	133.3	121.7	77.2	160.0	136.1	170.0	112.2
-1.68	-1.68	1.68	1.7	6.3	2.5	3.2	3.5	5.0	2.0	5.7	5.6	21.1	8.3	10.6	11.7	16.7	6.7	18.9
-1.68	1.68	-1.68	27.3	41.3	45.7	17.0	50.2	56.3	48.7	38.7	91.1	137.8	152.2	56.7	167.2	187.8	162.2	128.9
-1.68	1.68	1.68	0.3	10.2	3.3	6.7	5.0	4.8	2.2	5.5	1.1	33.9	11.1	22.2	16.7	16.1	7.2	18.3
1.68	-1.68	-1.68	35.7	37.2	45.8	27.0	54.2	29.7	54.2	40.7	118.9	123.9	152.8	90.0	180.6	98.9	180.6	135.6
1.68	-1.68	1.68	0.2	5.8	1.7	4.2	4.5	2.0	1.5	5.5	0.6	19.4	5.6	13.9	51.1	6.7	5.0	18.3
1.68	1.68	-1.68	28.7	34.5	42.5	29.8	30.8	53.5	45.2	35.8	95.6	115.0	141.7	99.4	102.8	178.3	150.6	119.4
1.68	1.68	1.68	0.5	9.5	4.2	6.5	3.8	1.8	1.7	5.7	1.7	31.7	13.9	21.7	12.8	6.1	5.6	18.9
0	0	0	18.8	37.3	28.7	27.0	49.2	43.0	35.8	37.5	62.8	124.4	95.6	90.0	163.9	143.3	119.4	125.0
0	0	0	19.3	39.2	29.2	26.0	52.2	41.2	38.0	38.0	64.4	130.6	97.2	86.7	173.9	137.2	126.7	126.7
0	0	0	21.0	36.8	27.0	25.2	51.5	35.8	31.7	35.2	70.0	122.8	90.0	83.9	171.7	119.4	105.6	117.2
0	0	0	20.0	38.7	27.7	23.2	52.7	42.5	35.3	36.3	66.7	128.9	92.2	77.2	175.6	141.7	117.8	121.1
0	0	0	17.8	34.3	20.2	22.3	50.0	0.0	30.3	42.0	59.4	114.4	67.2	74.4	166.7	0.0	101.1	140.0
0	0	0	19.3	33.8	0.0	24.7	43.0	42.7	35.0	38.8	64.4	112.8	0.0	82.2	143.3	142.2	116.7	129.4

Results and discussion - RSM

Y_2: Phenolic Compounds Content

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{\substack{i=1 \\ j>i}}^{n-1} \sum_{j=2}^{n} b_{ij} X_i X_j + \sum_{i=1}^{n} b_{ii} X_i^2$$



Graphical results in terms of response value format *Y2* (mg PC/g R). Theoretical 3D response surface predicted with the second order polynomial equation and statistical description of the model

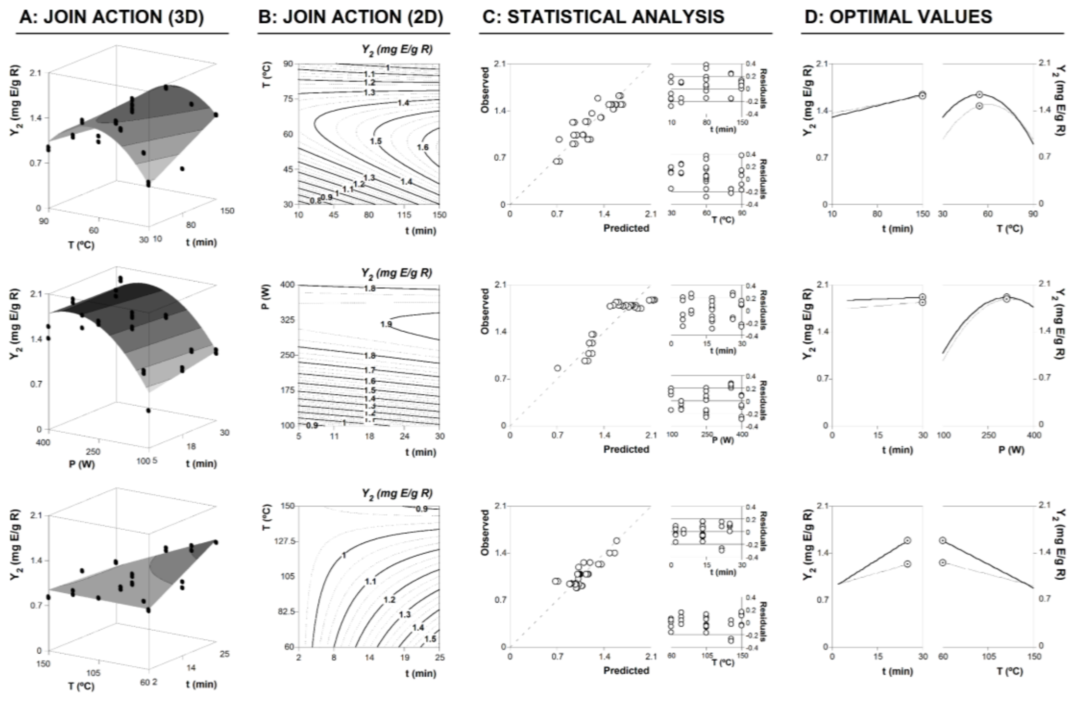
Kombu real

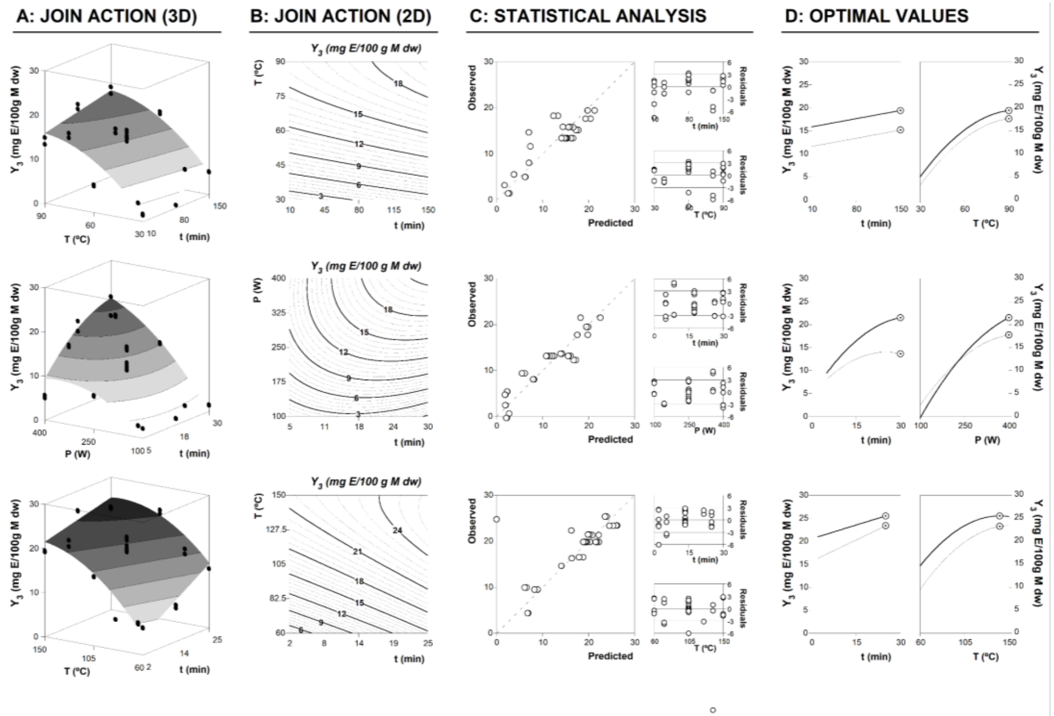
(*Saccharina latissima*)

Results and discussion - RSM

Y_2: Phenolic Compounds Content

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{\substack{i=1 \\ j>i}}^{n-1} \sum_{j=2}^{n} b_{ij} X_i X_j + \sum_{i=1}^{n} b_{ii} X_i^2$$

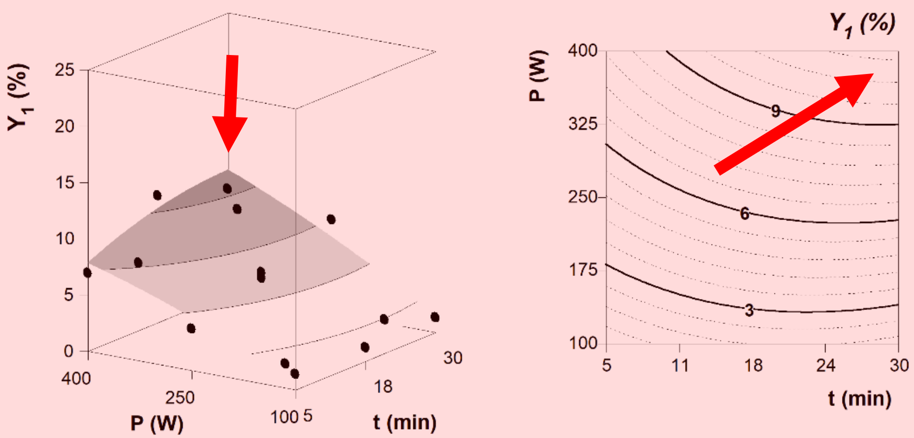


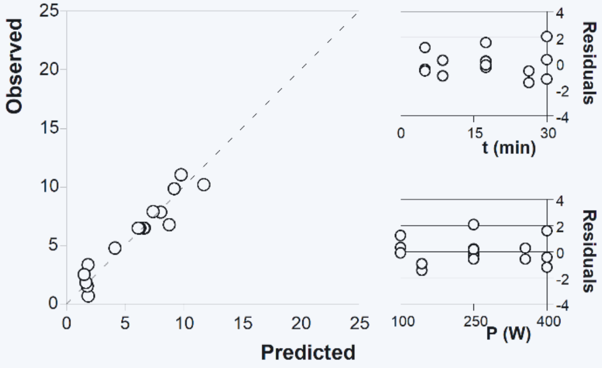


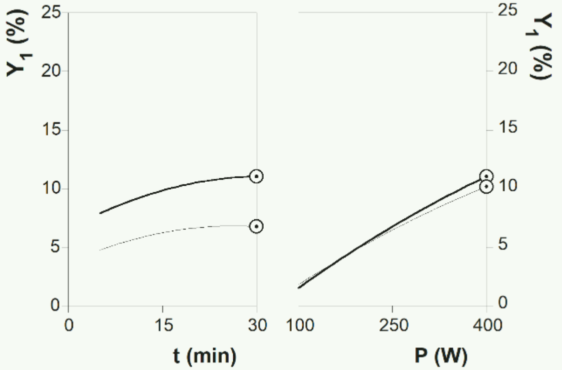
Results and discussion - RSM

Y_1: Extraction yield (%)

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{\substack{i=1 \\ j>i}}^{n-1} \sum_{j=2}^{n} b_{ij} X_i X_j + \sum_{i=1}^{n} b_{ii} X_i^2$$







$R^2 > 0.94$

Graphical results in terms of response value format *Y2* (%). Theoretical 3D response surface predicted with the second order polynomial equation and statistical description of the model

Kombu real

(*Saccharina latissima*)

Results and discussion - RSM

Table 3. Variable (X_1, X_2, and X_3) conditions in natural values that lead to optimal response values for RSM using a CCCD for HPE for the two individual response value formats (Y_1 and Y_2) and for the global optimal conditions. Samples used were Kombu (**K**, *Laminaria spp.*), Kombu real (**KR**, *Saccharina latissimi*), Espagueti de mar (**EM**, *Himanthalia elongate*), Wakame (**W**, *Undaria pinnatifida*), Nori (**N**, *Porphyra spp.*), Dulse (**D**, Palmaria palmate), Alga percebe (**AP**, Codium spp.) and Lechuga de mar (**LM**, Ulva spp.).

CRITERIA		OPTIMAL VARIABLE CONDITIONS			OPTIMUM RESPONSE	
		X_1: *t* (min)	X_2: *P* (mP)	X_3: *S* (%)		
K	Y_1	61	360	90	11.43±2.82	%
	Y_2	74	321	74	58.58±2.27	mg PC/g S dw
KR	Y_1	31	388	52	22.03±2.51	%
	Y_2	94	383	85	78.70±1.56	mg PC/g S dw
EM	Y_1	94	383	99	9.86±2.60	%
	Y_2	81	279	53	67.74±1.27	mg PC/g S dw
W	Y_1	98	111	69	20.17±2.96	%
	Y_2	36	232	36	54.27±2.08	mg PC/g S dw
N	Y_1	33	272	20	15.55±1.50	%
	Y_2	106	304	31	82.89±2.94	mg PC/g S dw
D	Y_1	40	463	99	8.84±1.35	%
	Y_2	100	274	100	82.27±1.99	mg PC/g S dw
AP	Y_1	47	148	27	18.25±1.53	%
	Y_2	30	175	89	62.18±1.27	mg PC/g S dw
LM	Y_1	41	370	50	21.18±1.00	%
	Y_2	91	398	90	11.95±2.73	mg PC/g S dw

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Thank you for your attention

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