GRAQ GRUPO DE REAÇÃO E ANÁLISES QUÍMICAS

LAQV requimte LABORATÓRIO ASSOCIADO PARA A QUÍMICA VERDE

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C. Neves[1,2], Tiago Vale[1], Stephanie Morais[1], C. Soares[1]*, C. Grosso[1], V.F. Domingues[1], M. Vieira[2,3], C. Delerue-Matos[1]

*cds@isep.ipp.pt

1 REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida 431, 4249-015, Porto, Portugal
2 Ciências Químicas e das Biomoléculas/CISA, Escola Superior de Saúde—Instituto Politécnico do Porto, Rua Doutor António Bernardino de Almeida 400, 4200-072 Porto, Portugal
3 TBIO, Center for Translational Health and Medical Biotechnology, R. Dr. António Bernardino de Almeida 400, 4200-072 Porto

Exploring the Bioactive Potential of *Gracilaria gracilis*: An Extraction Optimization Study Using Response Surface Methodology

Introduction

Algae, essential for ecosystem health, span microalgae and macroalgae (seaweeds). Their composition varies with type, season, and location, but seaweeds offer nutrition, including fiber, minerals, omega-3s, and proteins [1]. Algae also combat stress with antioxidants and are rich in diverse bioactive compounds like iodine [2]. Now, antioxidants play a vital role in food preservation by combating oxidative damage caused by free radicals [3]. Extracting these compounds is intricate, depending on factors like solvent and temperature [4].

The main objective of this study is to determine the optimal extraction conditions for *Gracilaria gracilis,* aiming to maximize the extraction efficiency of antioxidants, potentially providing a natural and sustainable alternative to synthetic antioxidants commonly used in the food industry.

Figure 1: *Gracilaria gracilis*, algae under study

In this study, *G. gracilis* was selected due to its potential antioxidant properties.

Materials and Methods

Seaweed preparation

The algae from the brand Alga+ was rehydrated, dehydrated, and ground to obtain powdered samples.

Solid-Liquid Extraction

The solid-liquid extraction method was employed using deionized water as the solvent. Factors such as temperature, algal mass to solvent ratio, and extraction time were carefully controlled during extraction.

Experimental Design

The Box-Behnken and Central Composite designs were used as statistical approaches to optimize the extraction conditions for maximum antioxidant yield. Experimental runs were conducted based on the design matrix, and the resulting data were analyzed using Design-Expert 11.0.0 software.

Total Phenolic Compounds

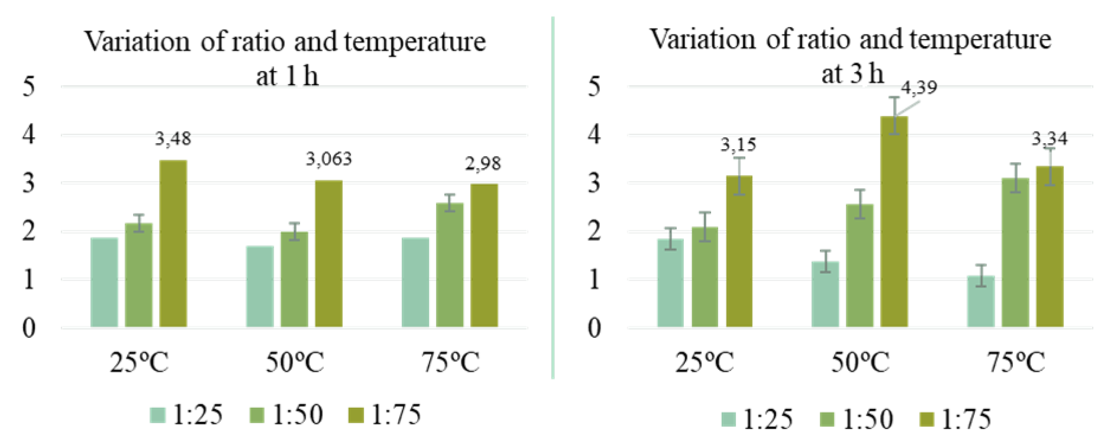
The total phenolic compounds (TPC) in the algae extracts were determined using the Folin-Ciocalteu colorimetric method. Results are presented in mg GAE/ g dw.

Results

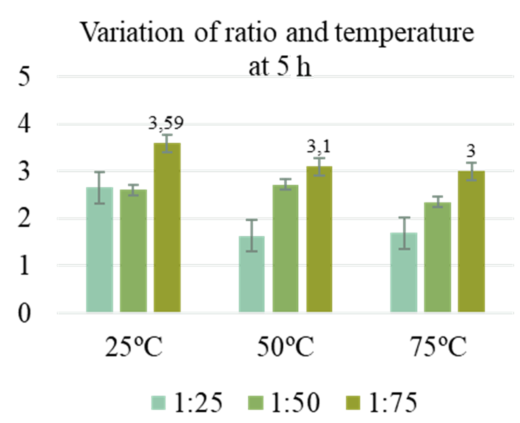
The algae were subjected to extractions considering the following factors:

Table 1: Extraction conditions and according to minimum and maximum values.

Factor	Name	Minimum	Maximum
A	Temperature	25	75
B	Ratio	25	75
C	Time	1	5



Graphs 1 and 2: Variation of ratio (biomass:solvent) and temperature at 1 and 3 hours for *G. gracilis*.



Graph 3: Variation of ratio (biomass:solvent) and temperature at 5 hours for *G.gracilis*.

Table 2: ANOVA for Reduced Quadractic model using the BBD for *G. gracilis*.

Source	Sum of Squares	df	Mean Square	F-value	*p*-value	
Model	5.45	4	1.36	43.57	< 0.0001	significant
A	0.0216	1	0.0216	0.6918	0.4218	
B	5.14	1	5.14	164.25	< 0.0001	
AB	0.2233	1	0.2233	7.14	0.0203	
B²	0.0687	1	0.0687	2.20	0.1640	
Residual	0.3752	12	0.0313			
Lack of Fit	0.3002	8	0.0375	2.00	0.2625	not significant
Pure Error	0.0750	4	0.0187			
Cor Total	5.82	16				

Table 3: ANOVA for Reduced Cubic model for the CCD for *G. gracilis*

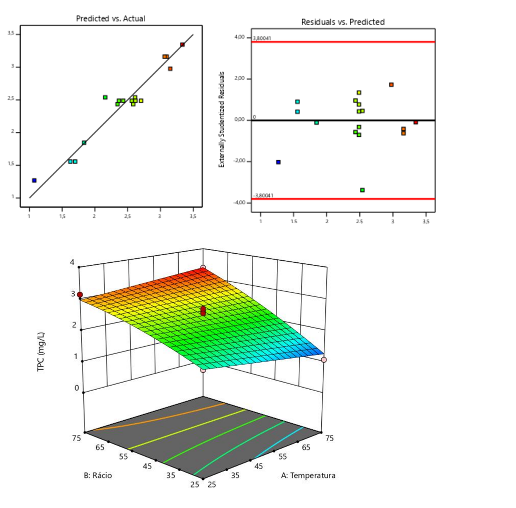
Source	Sum of Squares	df	Mean Square	F-value	*p*-value	
Model	8.73	7	1.25	17.14	< 0.0001	significant
A	0.5080	1	0.5080	6.98	0.0215	
B	4.52	1	4.52	62.11	< 0.0001	
AB	0.0017	1	0.0017	0.0227	0.8827	
A²	0.0204	1	0.0204	0.2796	0.6066	
B²	0.1342	1	0.1342	1.84	0.1996	
A²B	1.26	1	1.26	17.35	0.0013	
AB²	0.9287	1	0.9287	12.76	0.0038	
Residual	0.8734	12	0.0728			
Lack of Fit	0.7576	7	0.1082	4.67	0.0544	not significant
Pure Error	0.1158	5	0.0232			
Cor Total	9.61	19				

Table 4: Fit Statistics of ANOVA for Reduced Quadractic model in BBD for *G. gracilis*

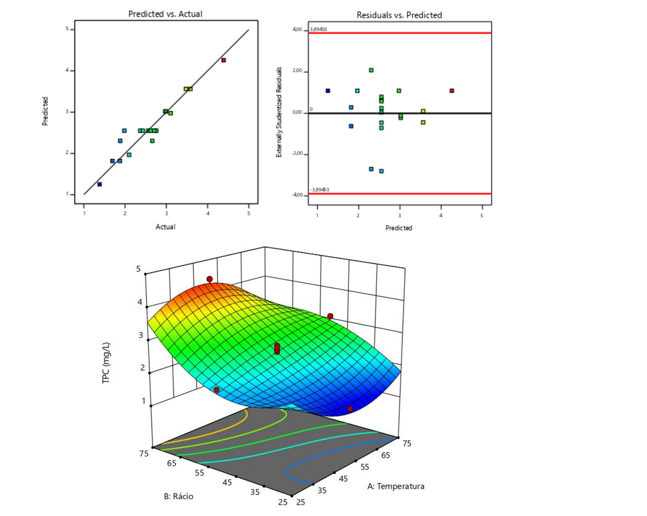
Std. Dev.	0.1768		R²	0.9356
Mean	2.43		Adjusted R²	0.9141
C.V. %	7.29		Predicted R²	0.8326
			Adeq Precision	21.6373

Table 5: Fit Statistics of ANOVA for Reduced Cubic models in CCD for *G. gracilis*

Std. Dev.	0.2698		R²	0.9091
Mean	2.61		Adjusted R²	0.8560
C.V. %	10.32		Predicted R²	0.6269
			Adeq Precision	17.6231



Graphs 4 and 5: Diagnostic graphs for Predicted vs Actual and Predicted vs Residual and model graph for the factor AB (temperature × ratio) of ANOVA for Reduced Quadractic model for BBD for *G. gracilis*



Graphs 6 and 7: Diagnostic graphs for Predicted vs Actual and Predicted vs Residual and model graph for the factor AB (temperature × ratio) of ANOVA for Reduced Cubic model for CCD for *G. gracilis*

Table 6: Constrains of the optimal conditions for *G. gracilis*

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:Temperature	is in range	25	75	1	1	3
B:Ratio	is in range	25	75	1	1	3
C:Time	is in range	1	5	1	1	3
TPC	maximize	1.383	4.39	1	1	3

Table 7 and 8: Selected optimal conditions by ANOVA for Reduced Cubic (BBD) and ANOVA for Reduced Quadractic (CCD) models, respectively, for *G. gracilis*

BDD model

Number	Temperature	Ratio	Time	TPC	Desirability
1	74.993	74.684	*3.507*	3.335	1.000

CCD model

Number	Temperature	Ratio	Time	TPC	Desirability
4	46.474	75.000	*1.120*	4.278	0.963

Conclusions

- In conclusion, this study optimized extraction conditions to enhance antioxidant yield from *G. gracilis* algae. Results highlighted species-specific responsiveness to parameters, emphasizing tailored approaches. Notably, lower temperatures favored extraction efficiency, with peak TPC yield (4.39 mg GAE/g dw) at 50ºC, 1:75 ratio, and 3-hour duration, aligning with prior cold-water extraction success.
- TPC analysis across conditions provided insights into temperature, ratio, and time effects, aiding standardized protocols for food applications. However, variable outcomes due to solvent, method, and focus require context via a comparative literature review.
- Future research will explore alternative extraction methods like MAE, PLE, and UAE, refining the process. Similar approaches will optimize extraction for diverse seaweed species.
- In summary, this study advances algae antioxidant extraction by refining conditions, contributing to sustainable, innovative bioactive integration in the food sector.

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PROJETO Nº C644915664-00000026

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