A Comparative Study of Protein-Rich Extract Using Food Grade Extraction Procedure from Marine Algae, *Ulva lactuca* (Chlorophyta): Screening Through a Two-Level Factorial Experimental Design Strategy

Muhammad Idham Shukor, Deny Susanti, Normawaty Mohammad Nor, Nurul Iman Aminudin, and Muhamad Taher

INTRODUCTION

Chlorophyta,
Ulva lactuca

Capacity to
adopt different
phenotypes
(Perez et al., 2016)

Abundance of
secondary
metabolites

(Tabarsa et al., 2012)

Development of
a new optimised
method

27th September 2022, Yogyakarta, Indonesia

Methods & Procedures

Species Identification

- Sampling
- Freeze dry
- Storage 4 °C

Experimental design

- Two-Level Factorial
- 3 central point

Protein extraction protocol

- Conventional (food grade)
- Sequential extraction
- Purification (dialysis)

Protein determination

- Digestion
- Distillation
- Titration

Design of Experiment

Std	Run	Factor 1	Factor 2	Factor 3	Response
		A: Solute:solvent ratio (%)	B: Time (h)	C: Temperature (°C)	Protein Concentration (%)
1	7	1	1	30	0.808
2	4	1	1	30	0.401
3	12	1	1	30	0.048
4	13	10	1	30	15.085
5	19	10	1	30	15.263
6	15	10	1	30	15.815
7	23	1	3	30	0.041
8	24	1	3	30	1.832
9	27	1	3	30	1.585
10	22	10	3	30	14.448
11	11	10	3	30	14.940
12	3	10	3	30	15.947
13	6	1	1	70	0.602
14	18	1	1	70	0.411
15	20	1	1	70	0.0470
16	21	10	1	70	11.333
17	1	10	1	70	15.284
18	2	10	1	70	14.738

*Number of run continued

Solute-to-Solvent Ratio

1:100 mL – 10:100 mL

(Yaich et al., 2011; Holdt et al., 2011)

Extraction Time

1 h – 3 h

(Yaich et al., 2015; Wells et al., 2017)

Extraction Temperature

30 °C – 70 °C

(Polikovsky et al., 2016; Van der wal et al., 2013)

Results

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	58.36	7	8.34	54.42	< 0.0001	significant
A-Solute: solvent ratio	57.97	1	57.97	378.34	< 0.0001	
B-Time	0.1383	1	0.1383	0.9026	0.3547	
C-Temperature	0.1106	1	0.1106	0.7219	0.4067	
AB	0.1198	1	0.1198	0.7820	0.3882	
AC	0.0075	1	0.0075	0.0488	0.8277	
BC	0.0003	1	0.0003	0.0022	0.9634	
ABC	0.0174	1	0.0174	0.1138	0.7398	
Curvature	2.67	1	2.67	17.40	0.0006	
Pure Error	2.76	18	0.1532			
Cor Total	63.79	26				

Protein Range

0.047 % - 16.145 % (w/w)

Normal Plot of Residual

Design-Expert® Software

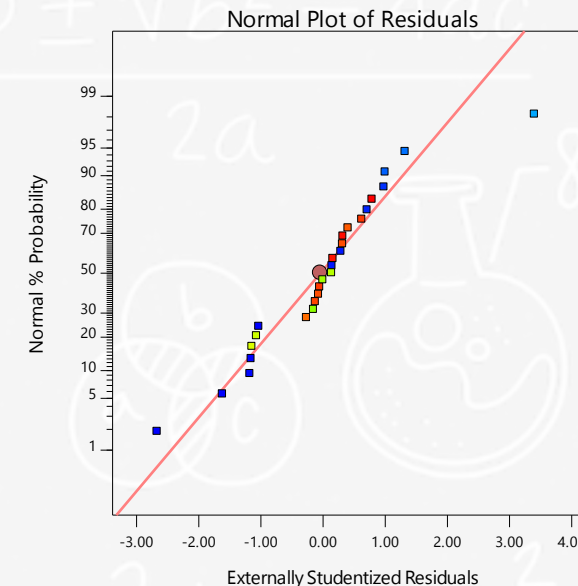**Sqrt(Protein Concentration (%))**
(adjusted for curvature)

Color points by value of
Sqrt(Protein Concentration (%)):
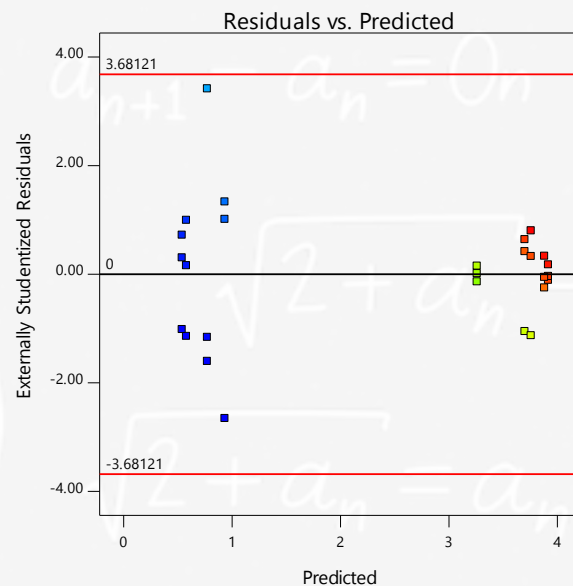
0.202 ▆ 4.018

Design-Expert® Software

Sqrt(Protein Concentration (%))
(adjusted for curvature)

Color points by value of
Sqrt(Protein Concentration (%)):

0.202 ▆ 4.018

One Factor Analysis

Design-Expert® Software
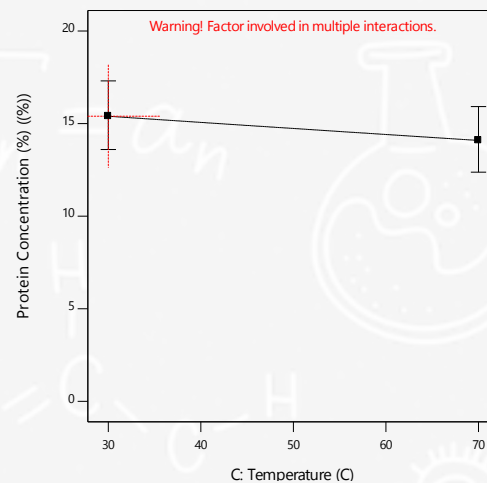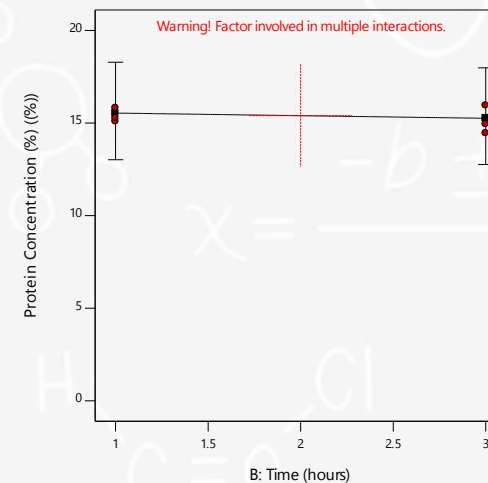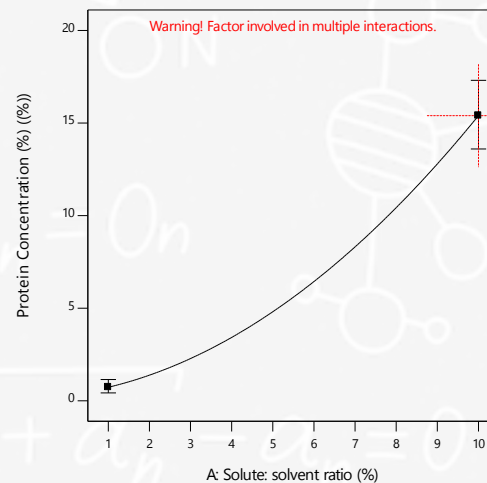Factor Coding: Actual
Original Scale

Protein Concentration (%) ((%))
(adjusted for curvature)

Actual Factors
A: Solute: solvent ratio = 10
B: Time = 2
C: Temperature = 30

Analysis of Optimum Region

Design-Expert® Software
Factor Coding: Actual
Original Scale

Protein Concentration (%) ((%))
(adjusted for curvature)
● Design points above predicted value
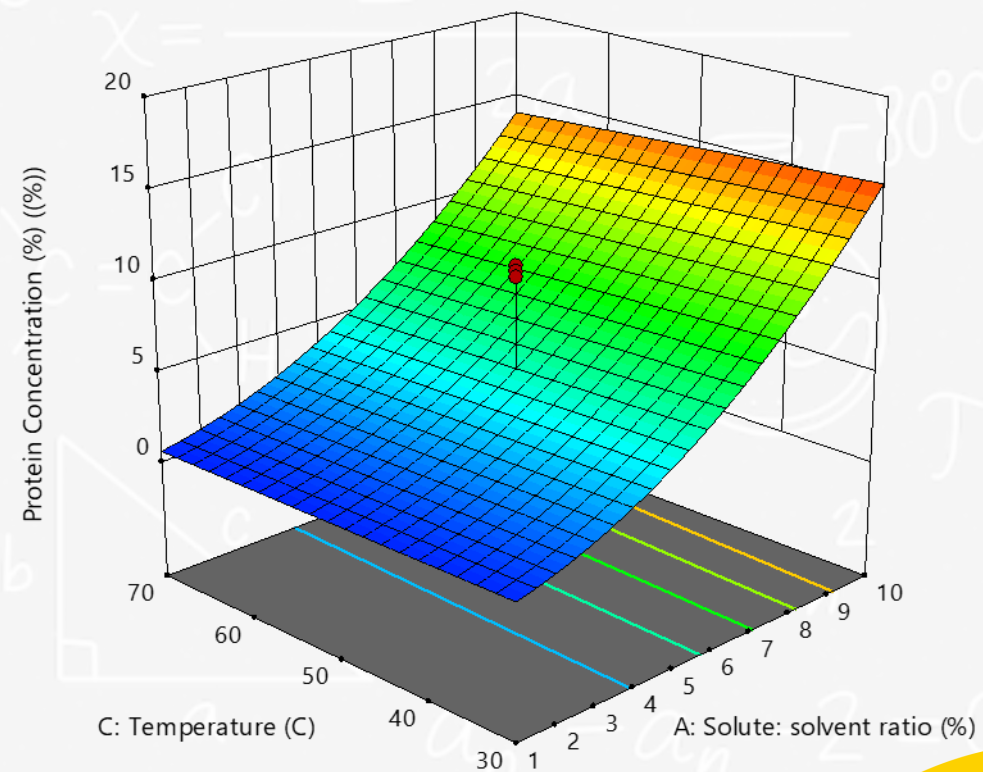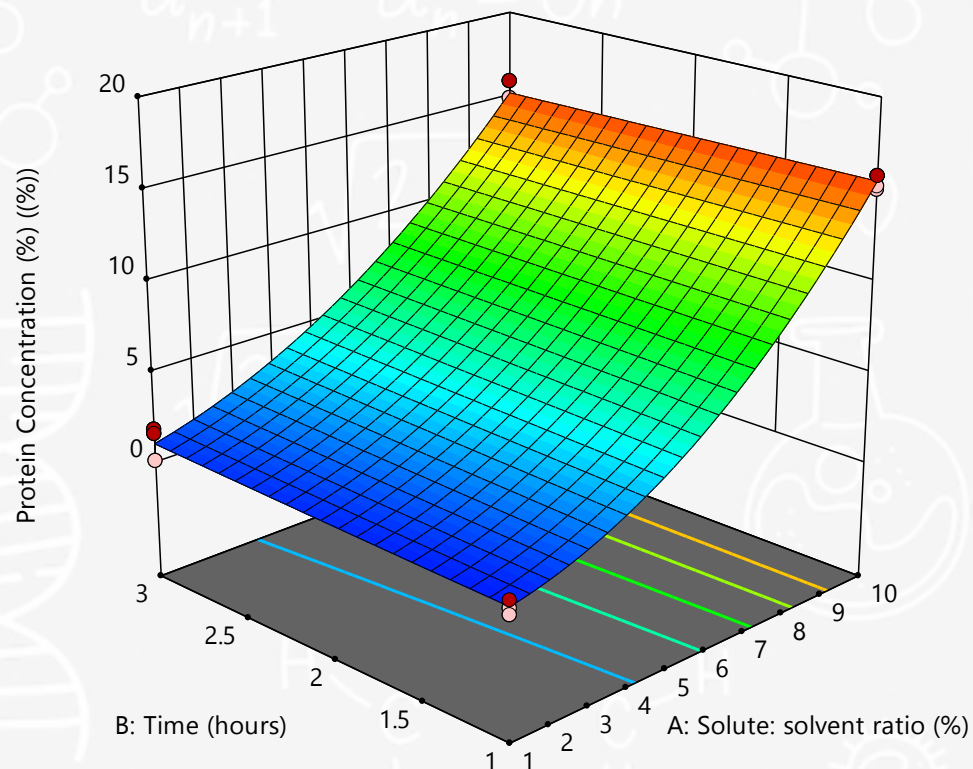○ Design points below predicted value

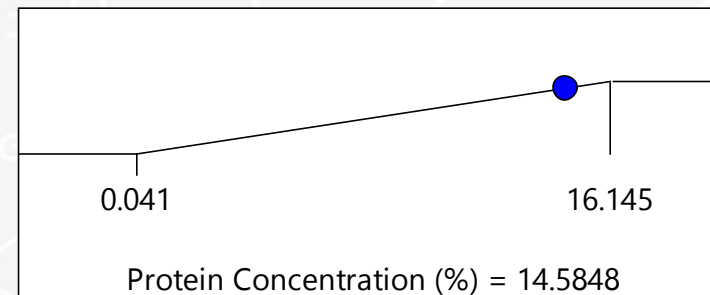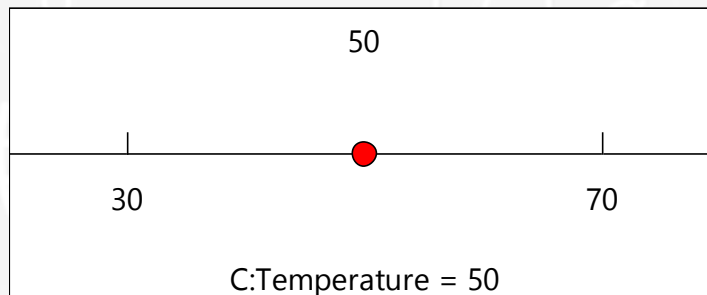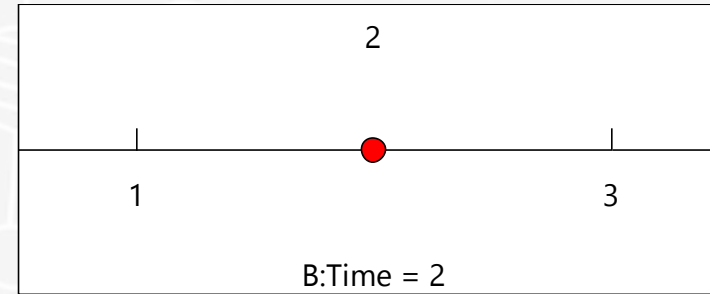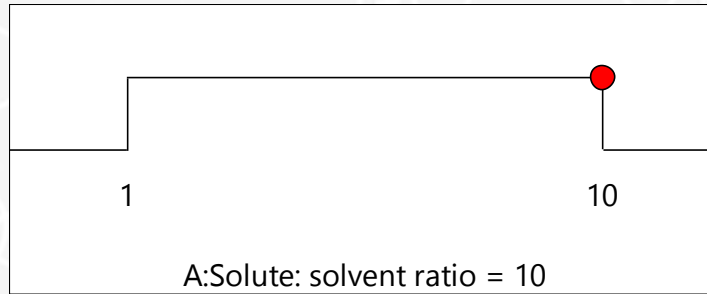0.041 ▮▮▮ 16.145

X1 = A: Solute: solvent ratio
X2 = B: Time

Actual Factor
C: Temperature = 30

Optimisation Ramp

A:Solute: solvent ratio = 10

B:Time = 2

C:Temperature = 50

Protein Concentration (%) = 14.5848

Desirability = 0.948

Conclusion

- Solute-to-solvent ratio had unnoticeable effects on the recovery yields ($p > 0.05$).

- The optimum yields at extraction time of 3 hours, temperature of 70 °C, solute-to-solvent ratio of 10:100 g/mL through Two-Level Factorial design experimental trials.

Acknowledgement

chemistry.uii.ac.id/ic3pe

27th September 2022, Yogyakarta, Indonesia

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