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Optimization of Calcium Extraction from *Stichopus horrens* using Sulphuric Acid

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Introduction

- Alternate source for medicinal ingredients.
- Studies show that sea cucumber (*Stichopus horrens*) have many benefits:
 - Good dietary delicacy (Gagaran and Cheema, 2017).
 - Used to treat osteoarthritis (Chen, 2003).
 - Antifungal properties (Van Den Hoek and Bayoumi, 2017).
- Have remarkable healing properties (Subramaniam et al., 2013).
- Improve amount of calcium yield by testing and altering variables of extraction.



Figure 1: *Stichopus horrens*.

Materials and Methods

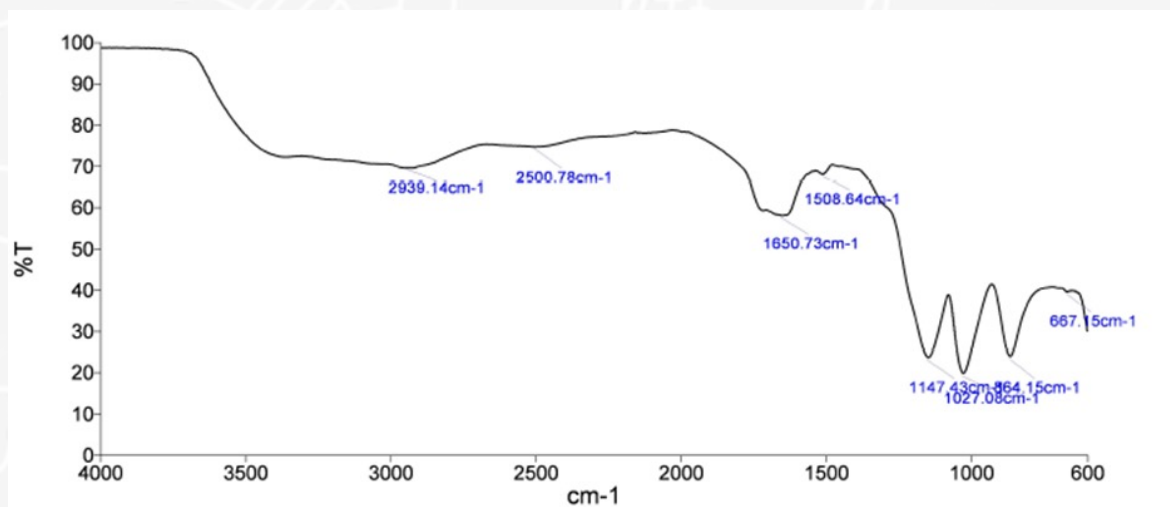
- Sample Preparation .
 - Purchased from Pulau Pangkor.
 - Innards was discarded and stored in -40 °C refrigerator.
 - Sample dried using oven for 5 days at 50 °C
- Sample extraction
 - Ground dried samples mixed with sulphuric acid
 - Follow variables set from Two-Level Factorial Design
- FT-IR analysis
- AAS analysis
- Statistical analysis



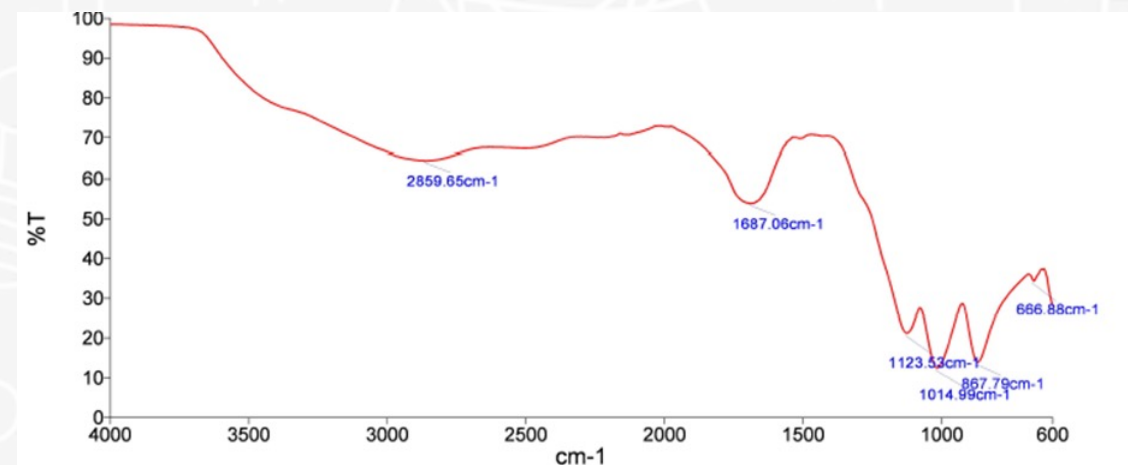
Figure 2: Calcium sulphate extract.

Results and Discussions

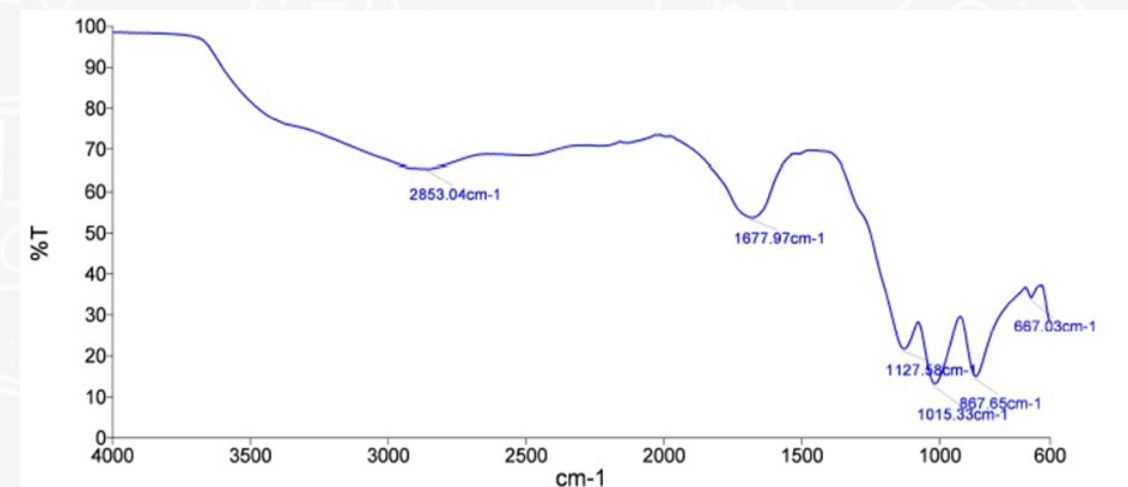
- FT-IR analysis for Calcium Sulphate
 - Strong absorption band around 1128 cm⁻¹ spectrum indicates the presence of calcium sulphate (Al Dabas et al., 2014).



(a)



(b)



(c)

Figure 3: FT-IR reading for sulphate compounds. (a) Sample that was runs with minimum variables. (b) with maximum variables. (c) with center point.

Result and discussion

- Screening using Two-Level Factorial design

TABLE 1. Simplified experimental design with results of each run using Two-level factorial design.

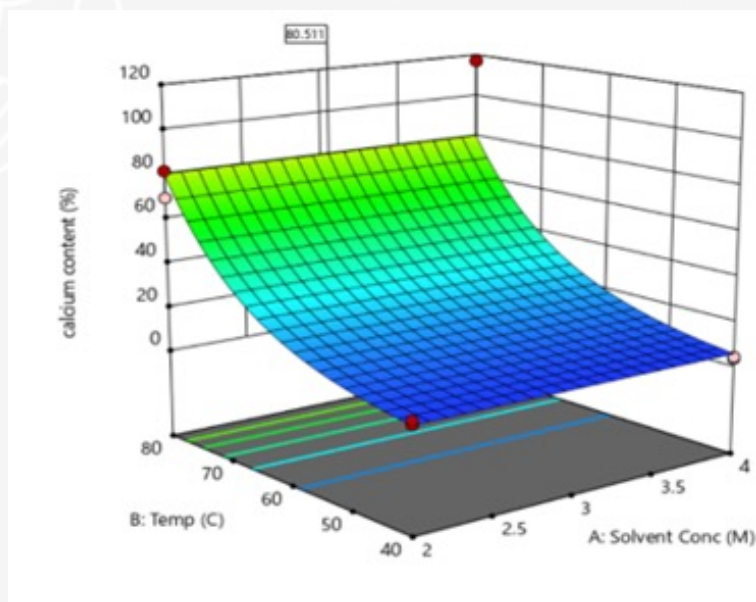
Run	Solvent concentration (M)	Temperature (C)	Duration (min)	Calcium content (mg/100 mg)
1	4	80	140	117.08
2	4	40	80	5.4
3	3	60	110	24.29
4	4	40	140	3.55
5	3	60	110	25.29
6	2	40	80	5.74
7	3	60	110	20.07
8	2	80	80	49.87
9	2	80	140	69.63
10	2	80	80	62.09
11	2	40	140	7.64
12	2	40	140	5.97
13	4	80	140	66.25
14	4	80	80	44.69
15	2	40	80	2.77
16	4	80	80	36.05
17	4	40	80	2.27
18	4	40	140	4.12
19	3	60	110	16.69
20	2	80	140	81.72

TABLE 2. ANOVA results for Two-level factorial design

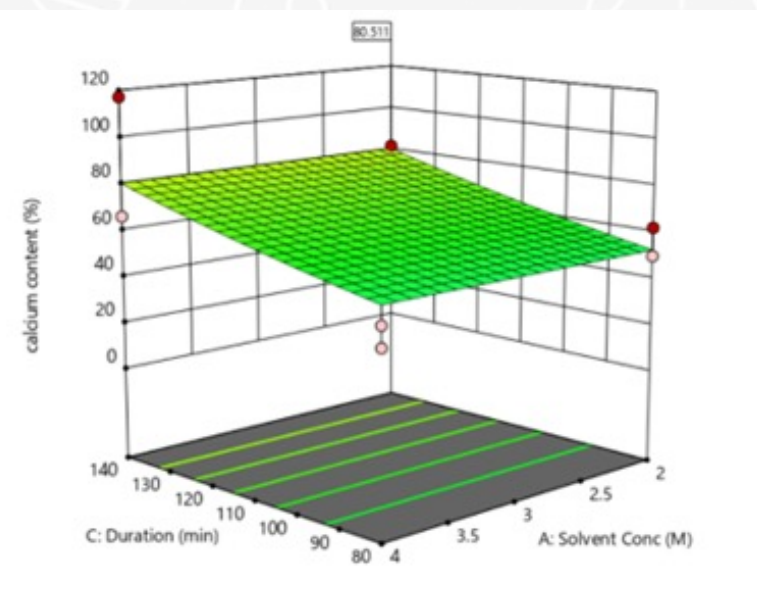
Source	Sum of Square	df	Mean Square	F-value	p-value	
Model	28.95	2	14.47	150.23	<0.0001	Significant
B-Temperature	28.22	1	28.22	292.91	<0.0001	
C-Duration	0.7270	1	0.7270	7.55	0.0143	Not significant
Curvature	0.2140	1	0.2140	2.22	0.1556	
Residual	1.54	16	0.0964			
Lack of Fit	0.5274	5	0.1055	1.14	0.3940	
Pure Error	1.01	11	0.0922			
Cor Total	30.71	19				

Result and discussion

- Effects of factors on 3D model



(a)



(b)

Figure 4: 3D Surface plots for factors interaction. (a) Temperature vs solvent concentration (b) Duration vs solvent concentration.

Result and discussion

- Determination of Optimum Condition

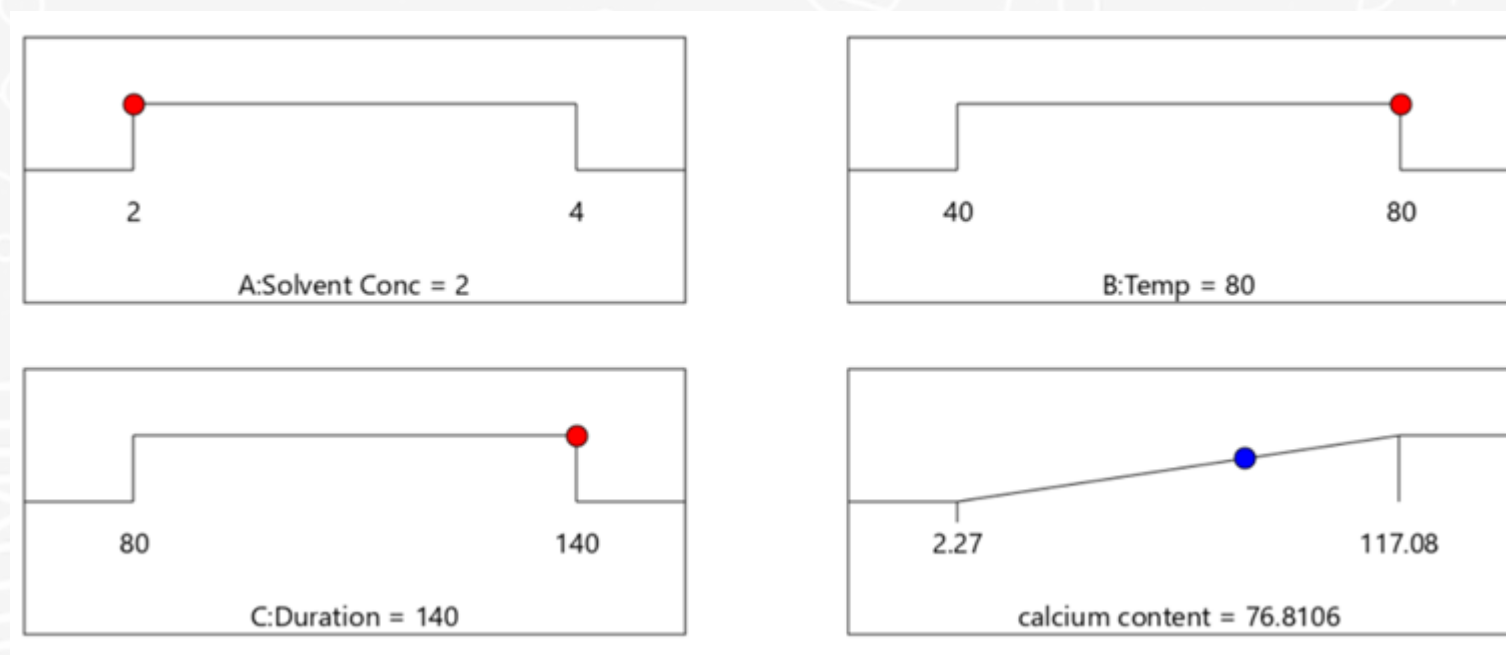


Figure 5: Desirability ramp for optimization of numerical values.

Conclusion

- Calcium yield heavily influence by temperature and duration.
- Sulphuric acid concentration does not affect the calcium yield.
- Two-level factorial design can efficiently determines the most optimal conditions for extraction.
- Extraction for calcium using sulphuric acid and waterbath shaker is an effective method.

Acknowledgements

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References

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