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Microwave-assisted extraction of pectin from unused pumpkin biomass

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INTRODUCTION

The research was focused on the seeded oil pumpkin (*Cucurbita pepo* L. var. *Styriaca*) biomass as an agricultural waste, a significant and not yet utilized source of pectin. After harvesting seeds, the residual biomass has a limited application and is usually left in the field. For the valorisation of the pumpkin biomass, the objectives of our previous studies were aimed to the overall chemical composition of the pumpkin biomass and of its different tissues (1). Several methods for isolation of polysaccharide have been reported (1,2).

The microwave-assisted extraction techniques have been employed as complementary methods to extract polysaccharides from vegetable sources. The effect of variables - (A) microwave heating time, (B) liquid/solid ratio, (C) extraction temperature - on the yield and quality of extracted pectin from the pumpkin biomass was investigated. The **response surface methodology** was used to optimize the effects of processing parameters.

MATERIALS AND METHODS

Raw materials:
Biomass represents the graded residue of Styrian oil pumkin fruit after separation of seeds (2.4 % Klason lignin, 3.5 % uronic acid, 7.9 % ash)(1).

Isolation

Pumpkin biomass
1. Swell 1h in water
2. Adjust pH to 2.5 with HCl
3. Microwave heating (Milestone StartSYNTH)
4. Filter

Filtrate
5. Adjust pH to 5.6 with KOH
6. Precipitate with ethanol
7. Filter

Wet pectin
8. Dialysis
9. Freeze-dried

Pumpkin pectin

Experimental data were analyzed using Design-Expert 8.0.7.1 statistical package including ANOVA to obtain the interaction between the process variables and the response. The three variable levels ***Box–Behnken response surface experimental design*** (BBD) was employed.

RESPONS SURFACE METHODOLOGY

Levels of extraction variables used in Box-Behnken design.

Variable		Levels		
Liquid/solid ratio	A	30	40	50
Extraction time (min)	B	2	6	10
Extraction temperature (C)	C	80	100	120

Sequential model fitting:

1. Response = *yield*

Source	Model Analysis Prob>F	Lack of fit Prob>F	R-square Press
Linear	< 0.0001	0.0714	5.37
Quadratic	0.1692	0.0627	11.72
Cubic	0.0627	aliased	aliased

2. Response = *molecular weight*

Source	Model Analysis Prob>F	Lack of fit Prob>F	R-square Press
Linear	0.0003	0.0580	$2.41 \cdot 10^{-4}$
Quadratic	0.1534	0.0583	$6.64 \cdot 10^{-4}$
Cubic	0.0583	aliased	aliased

EXPERIMENTAL DESIGN ANALYSIS

Analysis of variance for the pectin ***yield***

Source	F Value	P value Prob>F
Model	23.32	< 0.0001
A		< 0.0001
B		0.0141
C		0.0007

Model is significant and is suitable for use

In this case variables A, B, C are significant model terms.

Analysis of variance for the pectin ***molecular weight***

Source	F Value	P value Prob>F
Model	15.88	0.0003
A	34.86	< 0.0001
B	0.46	0.5096
C	12.33	0.0049

Model is significant and is suitable for use

In this case variables A and C are significant model terms. Variable B (extraction time) is not significant.

REFERENCES

1. Košťálová Z., Hromádková Z., Ebringerová A. Chemical Papers 63 (2009) 406–413.
2. Košťálová Z., Hromádková Z, Ebringerová A. Industrial Crops and Products 31 (2010) 370–377.
3. Yamaguchi, F. et al. Bioscience, Biotechnology, and Biochemistry 59 (1995) 2130-2131.

RESULTS

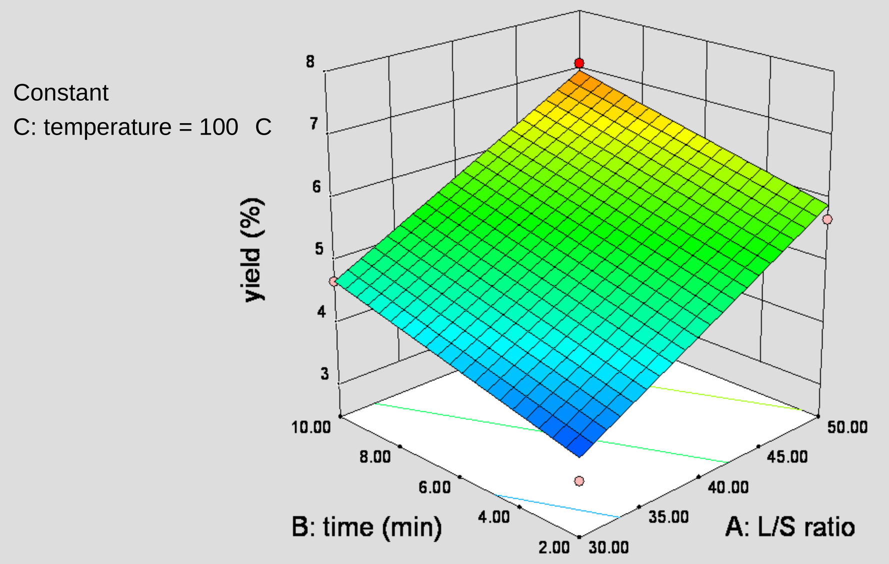
The equation obtained in terms of coded factors:

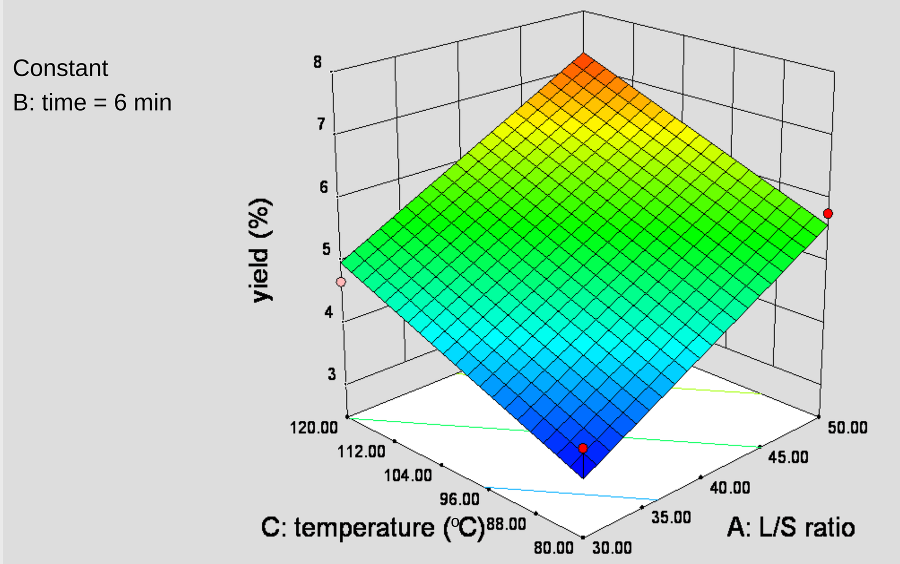
Yield = 5.32+1.14*A+0.52*B+0.84*C

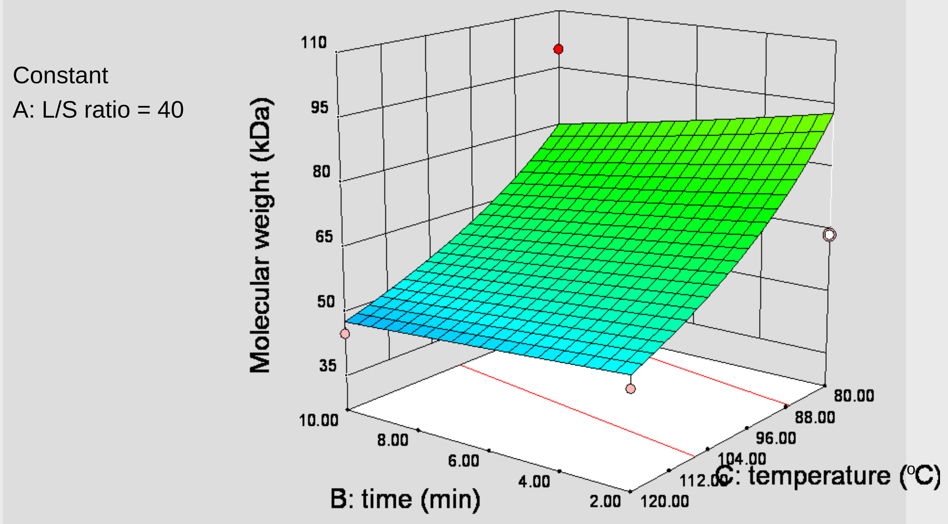
Yield (as % of pectin from air dried raw material)

Influence of variables on yield

Response surface plots representing the effect A) L/S ratio, B) time of sonication and C) temperature on extraction yield.







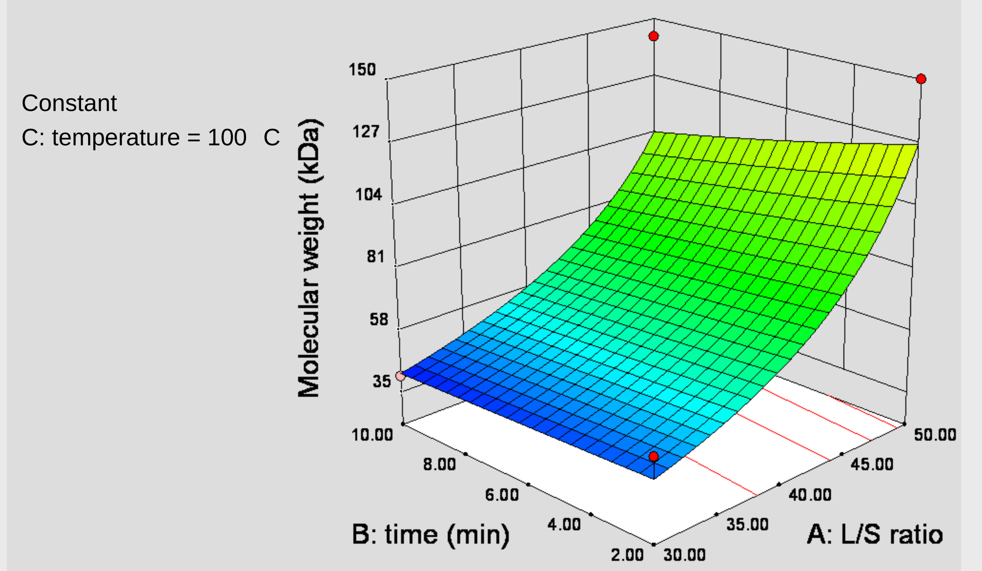
The equation obtained in terms of coded factors:

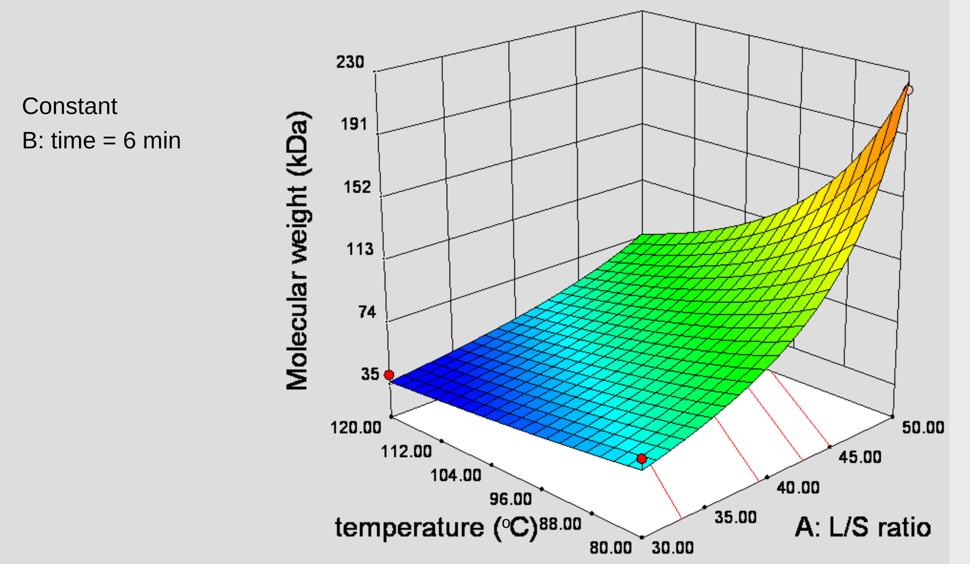
$M_w = 0.016 - 7.116 \cdot 10^{-3} \cdot A + 8.215 \cdot 10^{-4} \cdot B + 4.233 \cdot 10^{-3} \cdot C$

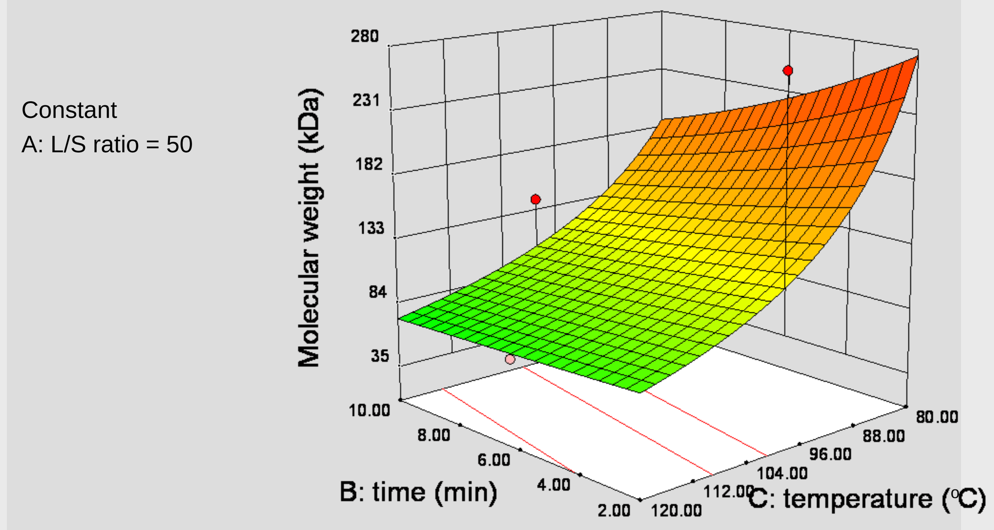
M_w (weight averaged molecular weight)

Influence of variables on molecular weight

Response surface plots representing the effect A) L/S ratio, B) time of sonication and C) temperature on molecular weight







* Molecular weight was determined by HPSEC chromatography

OPTIMALIZATION

Optimalization criteria:
- Yield → maximum (lower importance)
- Molecular weight → target to 100 kDa with limits 80 -120 kDa (higher importance)

The medium-molecular-weight pectin has still lower viscosity, higher solubility and retains biological activity (3).

Optimal conditions:
L/S ratio = 50, time= 10 min
temperature = 102.2 C

Predicted results:
Yield = 7 %, M_w= 100 kDa

CONCLUSION

- Probability value (P<0.0003) demonstrates a very high significance for both regression models.
- The coefficient of determination (R^2) for the yield model is 0.8641 and M_w model is 0.8125.
- Yield of polysaccharides was increased linearly with increasing all variables.
- Molecular weight of polysaccharides increase rapidly in L/S ratio = 50.
- The increasing temperature degraded the pectin matrix to small molecular weight.
- The time of sonication had only a very small effect to both responses.
- Optimal condition L/S ratio = 50, time= 10 min and temperature = 102.2 C was determined.
- Verification of optimal conditions is in progress.