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Alkali Pre-Treatment Optimization of Sardine Scales

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ABSTRACT: Response surface methodology (RSM) was adopted for pre-treating optimization. Concentration of NaOH (X_1) and treatment time (X_2) were chosen for independent variables. Dependent variables were protein content (Y_1) and hydroxyproline content (Y_2). Optimal conditions were X_1=0,5% and X_2=4h , and predicted values of multiple response optimal conditions were Y_1=0,32 mg/l and Y_2=2,67mg/l. That result was in agreement with the predicted value, which indicates that the model used was adequate for pre-treatment.

KEYWORDS: Sardine scales, Alkali pre-treatment, Optimization, Surface response methodology.

I. INTRODUCTION

The collagen from fish scales can be used in food applications to replace mammalian collagen which is not only at risk of contamination with bovine spongiform encephalopathy (BSE), but also got some resistance from koshers and halal.
Fish scales are dermally derived, specifically in the mesoderm, and biocomposites of highly ordered type I collagen fibers and hydroxyapatite [1] which are stiff and not easy to swell like the bone [2]. Utilization of fish scales for collagen or gelatin extraction has been reported of carp, black drum, sheepshead seabream, red sea bream, Japanese sea bass, red sea bream, red tilapia and sardine [3-10]. Therefore, this extraction needed to be applied in alkali pre-treatment before. In fish scales gelatin or collagen production, this step is continued to swelling step using low concentration of alkali solution [1, 12].
The response surface methodology (RSM) is an important tool that allows following of the evolution and the optimization of processes from an appropriate experimental design of a limited number of experiments. In our study the application of response surface methodology was used to optimize the alkali pre-treatment condition of sardine scales. This alkali pre-treatment should be able to remove non-collagenous protein effectively, but must generate the lowest hydroxyproline loss.
Therefore, the aim of this work is to optimize the alkali pre-treatment of the sardine scales using response surface methodology [13]. RSM has effectiveness in the optimization and monitoring of food manufacturing processing. The basic principle of RSM is to determinate model equations that describe interrelations between the independent variables and the dependent variables [14].

II. MATERIALS AND METHODS

1. Raw material

Fish scales were mechanically separated from fresh sardine (*Sardina pilchardus*) and washed with chilled tap water (to remove the impurities adhering to the surface), then placed in polyethylene bags and stored at -25°C until analysis.

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2. Alkaline pre-treatment of sardine scales

The sardine scales were treated with 1 volume (w/v) of alkali solution (NaOH) at concentrations of 0.26%, 0.3%, 0,4% and 0,54% under stirring for different times (3.18 h, 4 h, 6 h, 8 h and 8.82 h) at 4°C. The solution was changed every 2 hours. After the alkali treatment, the scales were washed with distilled water at 4°C and filtered with two layers of cheesecloth. The filtrate was collected and ready for hydroxyproline content and protein content determination.

3. Protein content

The protein content in each treatment solutions was determined by the Bradford's method [15].

4. Hydroxyproline content

The hydroxyproline content was determined by the method of Bergman and Loxly (1963) [16].

5. Experimental design and statistical analysis

A central composite design of response surface methodology [13] with two variables was used to study the response pattern and to determine the optimum combination of the variables. The variables optimized were NaOH concentration (%, X_1) and treatment time (h, X_2), each at five coded levels -1.41,-1, 0, 1 and 1.41 as shown in Table 1. Protein content (mg/l, Y_1) and Hydroxyproline content (mg/l, Y_2) were dependent variables.

Central composite design (CCD) in the experimental design consisted of 2^2 factorial points, four axial points and three replicates of the central point (Table 2).

Response functions describing variations of dependent variables with two independent variables (Xi and Xj) can be written as follows:

$$Y= \beta_0+\beta_i \cdot X_i +\beta_j X_j +\beta_{ii} X^2_i +\beta_{jj} X^2_j +\beta_{ij} X_i X_j$$

Where Y is the dependent variable (protein content and hydroxyproline content), X_i and X_j are the input variables which affect the response, X_i^2 and X_j^2 are the square effects, X_iX_j is the interaction effect, β_0 is the offset term, β_i and β_j are the linear effects, β_{ii} and β_{jj} are the squared effects and β_{ij} is the interaction effect. Multiple responses optimization was calculated by desirability function of MINITAB 16 statistical software, in order to search the condition simultaneously satisfying two dependent variables (Y_1 and Y_2).

Table 1: Independent variables and their levels in the 2-factor, 5-level central composite design (CCD) for alkali pre-treatment of sardine scale.

Independent variables	Symbol	Range and levels				
		-1,414	-1	0	+1	+1,414
Concentration of NaOH (%)	X_1	0.26	0.3	0.4	0.5	0.54
Treatment time (h)	X_2	3.18	4	6	8	8.82

III. RESULTS AND DISCUSSION

1. Response surface plots and the effect of factors

Fig.1 shows the estimated response function and the effect of the independent variables (X_1; X_2) on the dependent variables (Y_1 and Y_2). Two independent variables of X_1 (concentration of NaOH) and X_2 (treatment time) are major factors for alkali pre-treatment from sardine scales.

Fig.1A depicts the effect of independent variables on Y_1 (protein content). At higher coded values of NaOH concentration and treatment time, protein content increased with an increase in NaOH concentration and treatment time, while at lower coded values of NaOH concentration and treatment time, protein content decreased with increase of NaOH concentration and treatment time. It could be concluded that at low or high levels of NaOH concentration and treatment time, recovery of non-collagenous proteins are favored.

Fig.1B shows that increase of NaOH concentration and treatment time during alkali pre-treatment leads to an increase of hydroxyproline content, although at a faster rate with treatment time than with NaOH concentration.

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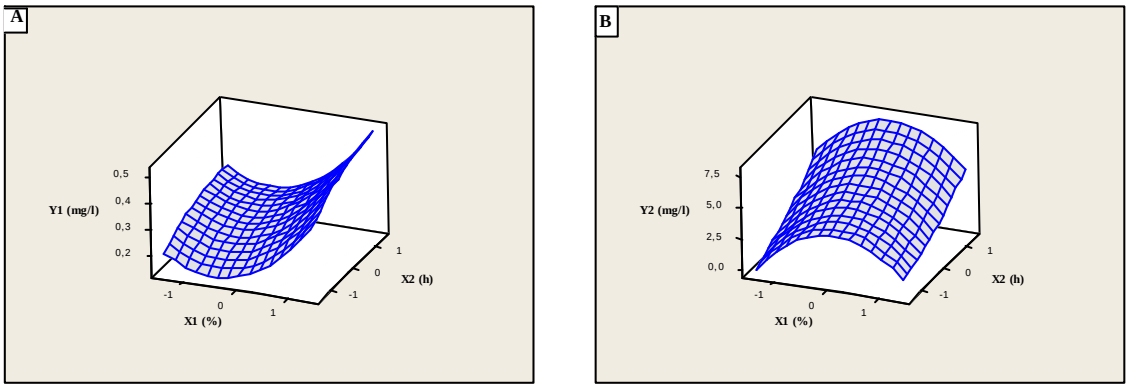

Fig 1: Response surfaces showing effect of NaOH concentration (%,X_1) and treatment time (h,X_2) on (A) protein content and (B) Hydroxyproline content.

2. Diagnostic checking of the fitted models

All 11 experimental runs were evaluated using the Response Surface Regression (RSREG) procedure and the results of dependent variables (Y) for each point are shown in Table 2.

Table 2 :The actual design of experiments and response of alkaline pre-treatment

Runs	Independent variables				Responses	
	X_1	X_2	[NaOH] (%)	Treatment time (h)	Y_1	Y_2
1	-1	-1	0.3	4	**0,20**	**2,03**
2	1	-1	0.5	8	**0,31**	**2,78**
3	-1	1	0.3	4	**0,27**	**4,74**
4	1	1	0.5	8	**0,40**	**5,13**
5	-1.414	0	0.26	3.18	**0,27**	**1,95**
6	+1.414	0	0.54	8.82	**0,50**	**2,43**
7	0	-1.414	0.4	3.18	**0,16**	**3,42**
8	0	+1.414	0.4	8.82	**0,27**	**8,20**
9	0	0	0.4	6	**0,24**	**5,15**
10	0	0	0.4	6	**0,25**	**5,06**
11	0	0	0.4	6	**0,25**	**5,14**

Y_1: protein content (mg/l), Y_2: Hydroxyproline content (mg/l), X_1: concentration of NaOH (%), X_2: treatment time (h).

By using t-statistic on the predicted model, the coefficients and P-values on all the variables of linear (X_1, X_2), quadratic(X_{12}, X_{22}) and interactions were calculated and shown in Table 3.

Table 3 : Estimated coefficients of the fitted quadratic polynomial equation for different response based on t-statistic.

Term	Y_1		Y_2	
	Coefficient	P-value	Coefficient	P-value
Constant	5.11667	0.000	0.24600	0.000
X_1	0.22735	0.125	0.07065	0.000
X_2	1.47749	0.000	0.03944	0.000

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$X_1.X_1$	-1.54583	0.000	0.06825	0.000
$X_2.X_2$	0.26417	0.132	-0.01675	0.009
$X_1.X_2$	-0.09000	0.628	0.00500	0.448

Not significant at P < 95%. All other coefficients were significant at P < 95%
Y_1 (protein content, mg/l), Y_2 (hydroxyproline content, mg/l), X_1 (concentration of NaOH, %), X_2 (treatment time, h)

All the interaction coefficients were not significant (> 0,05) in all models. On the other hand, all the quadratic coefficients except the X_2X_2 term of Y_2 were highly significant at p <0,05. The X_1 term of Y_2 was not significant in case of linear coefficients and the other linear coefficients were significant (Table 3).

The determination coefficients (R^2) for Y_1 and Y_2 were higher than 0.90, indicating that the regression model explained the reaction well. The fitted models are shown in table 4.

Table 4: Response surface model for alkali pre-treatment of sardine scales

responses	Quadratic polynomial model	R^2	P-value
Y_1	Y_1=0,24+0,07X_1+0,03X_2+0,06X_1X_1-0,01X_2X_2+0,005X_1X_2	0,9979	0,000
Y_2	Y_2=5,11+0,22X_1+1,47X1,45X_1X_1+0,26X_2X_2-0,09X_1X_2	0,9996	0,000

3. Analysis of variance

The analysis of variance (ANOVA) for the quadratic polynomial model was used to indicate the adequacy of the fitted model. Table 5 shows ANOVA for the models that explain the response of two dependent variables, Y_1 (protein content) and Y_2 (hydroxyproline content). Interaction terms for all the dependent variables (Y_1 and Y_2) were not significant (P=0,49 and P=0,27; respectively) at 95% probability level, whereas linear term (X_1;X_2), quadratic term (X_{11};X_{22}) and total regression model were highly significant (P< 0,05) at 95% probability level.

Table 5. Analysis of variance (ANOVA) for response of dependent variables (Y_1 andY_2)

Responses	Source	DF	SS	MS	F-value	P-value
Y_1	**Regression**	**5**	0.0870	0.0179	84.66	0.000
	Linear	**2**	0.0523	0.0261	127.41	0.000
	Square	**2**	0.0345	0.0172	83.99	0.000
	Interaction	**1**	0.0001	0.0001	0.49	0.517
	Residual Error	**5**	0.0010	0.0001	-	-
	Lack-of-Fit	**3**	0.0009	0.0001	9.61	0.096
	Pure Error	**2**	0.0001	0.0001	-	-
	Total	**10**	0.0880	-	-	-
Y_2	**Regression**	**5**	34.5982	6.9196	56.67	0.000
	Linear	**2**	17.8774	8.9387	73.21	0.000
	Square	**2**	16.6884	8.3442	68.34	0.000
	Interaction	**1**	0.0324	0.0324	0.27	0.628
	Residual Error	**5**	0.6105	0.1221	-	-

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Lack-of-Fit	**3**	0.6056	0.2019	82.96	0.012
Pure Error	**2**	0.0049	0.0024	-	-
Total	**10**	35.2087	-	-	-

FD: degree of freedom, SS: sum of Square, MS: Mean square, Y_1 (protein content), Y_2 (hydroxyproline content)

The check of model adequacy was performed by a normality test (Anderson-Darling normality test) for error terms using residuals of the dependent variables, Y_1 and Y_2 (Fig. 2).

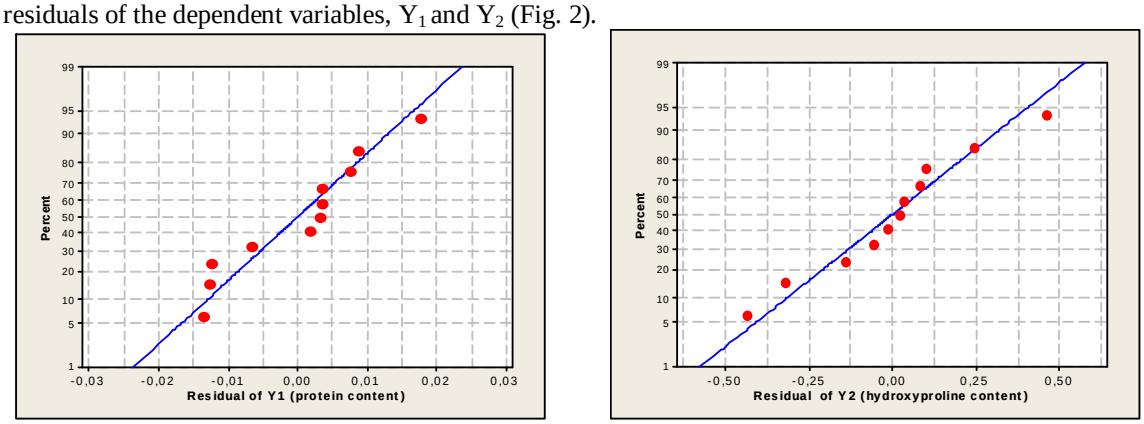

Fig 2: Normal probability plots for error terms using residuals of the dependent variables by Anderson-Darling Normality test

The error terms of two dependent variables had the normal distribution as the Anderson-Darling normality test. Therefore, response surface model represented as quadratic polynomial equation was statistically significant.

4. Optimization using desirability function approach

As it's shown in fig.3, the individual desirability values, the overall desirability D and predicted value are calculated by Minitab. The factors obtained at the maximum point of Y_1 and the minimum points of Y_2 are calculated as X_1=0,5% and X_2=4h which are known as estimated condition (Fig.3).

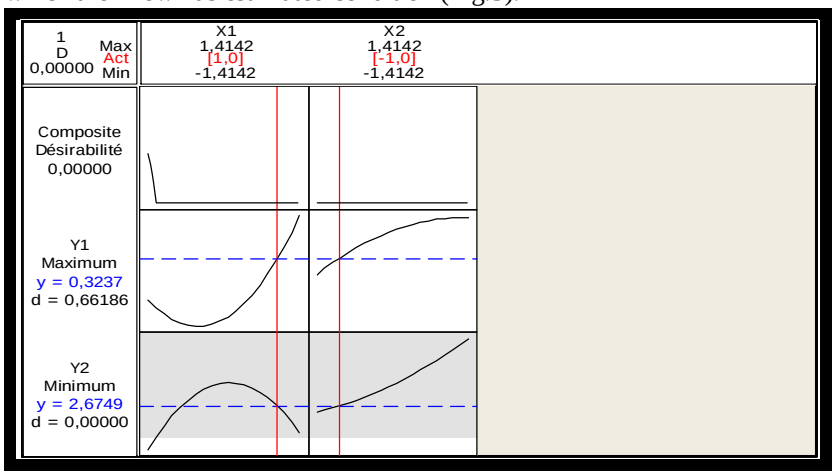

Fig 3: Optimization plot

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5. Verification of optimal parameters

Validation experiments are applied at the estimated condition. The results are in agreement with the predicted value, it's also confirmed that the model used in this experiment is appropriate (Table 6).

Table 6: Test results for verification of the results of alkali pre-treatment

Optimal Condition		Predicted values		Verification experiment	
X_1 (%)	X_2 (h)	Y_1 (%)	Y_2 (%)	Y_1 (%)	Y_2 (%)
0,5	4	0,32	2,67	**0,46±0,34**	**3,43±1,1**

IV. CONCLUSION

Response surface methodology has been realized for the determination of the optimal conditions for alkali pre-treatment. The resulting model led to the optimal conditions for removing non-collagenous proteins with minimum collagen loss.

The alkali treatment for sardine scales is significantly influenced by NaOH concentration and the treatment time. Linear and quadratic effects of these two variables affect protein content and hydroxyproline content in alkali solutions. This process could be considered as a sustainable alternative for the industry since it allowed decrease the cost of whole collagen extraction process.

REFERENCES

[1.] Gómez-Guillén, M.C., Giménez, B., López-Caballero, M.E., and Montero, M.P., "Functional and bioactive properties of collagen and gelatin from alternative sources: A review", Food Hydrocolloids, Vol.25, Issue 8., pp.1813-1827, 2011.

[2.] Zhang, F., Xu, S., and Wang, Z., "Pre-treatment optimization and properties of gelatin from freshwater fish scales", Food and Bioproducts Processing, Vol.89, Issue 3., pp.185-193, 2010.

[3.] Liu, D., Wei, G., Li, T., Hu, J., Lu, N., Regenstein, J.M., and Zhou, P., "Effects of alkaline pretreatment and acid extraction conditions on the acid-soluble collagen from grass carp (Ctenopharyngodon idella) skin", Food Chemistry, Vol.172, pp.836-843, 2015.

[4.] Liu, D., Zhou, P., Li, T., and Regenstein, J.M., "Comparison of acid-soluble collagens from the skins and scales of four carp species", Food Hydrocolloids, Vol. 41, pp.290-297, 2014.

[5.] Liu, D., Liang, L., Regentein, J.M., and Zhou, P., "Extraction and characterisation of pepsin-solubilised collagen from fins, scales, skins, bones and swim bladders of bighead carp (Hypophthalmichthys nobilis)", Food Chemistry, vol.133, Issue 4., pp.1441-1448, 2012.

[6.] Duan, R., Zhang, J., Du, X., Yao, X., and Konno, K., "Properties of collagen from skin, scale and bone of Carp (Cyprinus carpio)", Food Chemistry, Vol.112, Issue 3., pp.702-706, 2009.

[7.] Ogawa, M., Portier, R.J., Moody, M.W., Bell, J., Schexnayder, M.A., and Losso, J.N., "Biochemical properties of bone and scale collagens isolated from the subtropical fish black drum (Pogonia cromis) and sheepshead seabream (Archosargus probatocephalus)", Food Chemistry, Vol.88, Issue 4., pp.495-501, 2004.

[8.] Nagai, T., Izumi, M., and Ishii, M., "Fish scale collagen. Preparation and partial Characterization", International Journal of Food Science and Technology, Vol.39, Issue 3., pp.239-244, 2004.

[9.] Ikoma, T., Kobayashi, H., Tanaka, J., Walsh, D., and Mann, S., "Physical properties of type I collagen extracted from fish scales of Pagrus major and Oreochromis niloticas", International Journal of Biological Macromolecules, Vol.32, Issue 3-5., pp.199-204, 2003.

[10.] Nomura, Y., Sakia, H., Ishii, Y., and Shiraj, K., "Preparation and some properties of type I collagen from fish scales", Bioscience Biotechnology Biochemistry, Vol.60, Issue 12., pp.2092-2094, 1996.

[11.] Zhou, P., and Regenstein, J.M., "Effects of alkaline and acid pretreatments on alaska pollock skin gelatin extraction", Journal of Food Science, Vol.70, Issue 6., pp.C392-C396, 2005.

[12.] Wangtueai, S., and Noomhorm, A., "Processing optimization and characterization of gelatin from lizardfish (Saurida spp.) scales", Food Science and Technology, Vol.42, Issue 4., pp.825-834, 2009.

[13.] Box, G.E.P., and Wilson, K.B., "On the experimental attainment of optimum conditions", Journal of the Royal Statistical Society Series B", Vol.13, Issue 1., pp.1-45, 1951.

[14.] Edwards, I.M, and Jutan, A., "Optimization and control using response surface methods", Computer and Chemical Engineering, Vol.21, Issue 4.,pp. 441-453, 1997.

[15.] Bradford, M.M., "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding", Analytical Biochemistry, Vol.72, Issue 1-2., pp.248-254, 1976.

[16.] Bergman, I., and Loxley, R., "Two improved and simplified methods for the spectrophotometric determination of hydroxyproline", Analytical Chemistry, Vol.35, Issue 12., pp.1961-1965, 1963.