RESPONSE SURFACE MODELING AND ANALYSIS OF ELASTIC MODULUS AND WATER SOLUBILITY OF THE COLLAGEN FILM

Xiaolong Wen[1], Aimin Jiang1*, Hui Yang[1], Lanfang Wu[1], Ming Tsao Chen[2] and H.W. Ockerman[3]

[1] College of Food Science, South China Agricultural University, Guangzhou 510642, PR China

[2] Department of Animal Science, National Chung-Hsing University, Taichung, Taiwan

[3] Department of Animal Science, The Ohio State University, Columbus, Ohio, USA

Abstract – In this study, on the basis of the single factor experiment, the Plackett-Burman design and response surface method were used to optimize the process parameters of the collagen film. The results showed that, the optimum technological conditions were 3 mL/100g 0.1 mol/L hydrochloric acid, 2% glycerol, 0.25% CMC-Na, 90% slurry, 4min soaking time in 0.25% glutaraldehyde solution, 40°C drying temperature, 20 h drying time. Under the optimal conditions, the elastic modulus (ES) and water solubility (WS) were 3710 g/mm 2 9.1%, respectively.

Key Words – pigskin, collagen film, elastic modulus, water solubility.

I. INTRODUCTION

Pigskin is a renewable nature source, it contains abundant collagen which is a biodegradable protein and has been used to prepare environmental friendly packaging materials such as sausage casing and films [1]. Several publications reported that the process parameters, such as the ratio of collagen to fat, plasticizer, conditions of alkali treatment and so on, will affect on the characteristics of collagen membrane [2-5]. In this study, on the basis of these reports, selected some important influencing factors and used the Plackett-Burman design and response surface method to optimizer the technological conditions for making edible film.

II. MATERIALS AND METHODS

Collagen was processed from pigskin which was purchased from the local market by the basic formulations which was reported elsewhere, to obtain a concentrated aqueous collagen slurry after the milling process described by Morgan *et al.* [6] and Deiber *et al.* [7] and the procedure has been made some modifications.

The process of the slurry for making collagen film according to the methods described by Shank [8], Cao *et al*. [9], with a slight modification. The water solubility WS of the collage film was measured according to a modified method describe by Ghasemlou *et al.* [10]. The textural properties of collagen film was assayed according to the methods described by Chen and Wu [11], and made some modifications, elastic modulus of the films was measured on a texture analyzer (TA.XT Plus, SMS UK). Statistical analysis: Design expert 6.0 statistical software package (Stat-EaseInc.,USA) was used to analyze the experimental data.

III. RESULTS AND DISCUSSION

On the basis of single-factor experiment, N =12's Plackett-Burman experimental design was employed to study the influence of process variables [0.1mol/L hydrochloric acid (factor A), glycerol (B), slurry (D), soaking time in 0.25% glutaraldehyde solution (E), CMC-Na (G), drying time (H), drying temperature (J)], The independent process variables were selected based on the results of preliminary single-factor experiments and it was coded at two levels (−1 and +1), the response value was the ES of the collagen film.

The design-expert 6.0 software was used to analyze the main effect factors, the results were shown in Table.1 (the ES as the response value, the p-value of the model was 0.0143, significantly, so the model can be used). From the Table.1, the order of significance of the factors were: glycerol, hydrochloric acid, CMC-Na, drying time, drying temperature, the slurry, soaking time in 0.25%

glutaraldehyde solution, so glycerol, hydrochloric acid and CMC-Na were used to a response surface method.

Table.1 The analysis results of the Plackett-Burman experiment.

Factor	Level		P-value	Significance
	-1	1		
A	2ml/100g	5ml/100g	0.0127	2
B	0	3.5%	0.003	1
D	70%	90%	0.0552	5
E	2min	5min	0.8528	7
G	0.5%	1%	0.041	3
H	20h	28h	0.0437	4
J	40℃	50℃	0.247	6

According to the experiment factors and their levels, used the Design-Expert software design for 17 experiments. 0.1mol/L hydrochloric acid (X1), usage of Glycerol (X2), usage of CMC-Na (X3) as independent variables, the slurry was 90%, drying time was 20 h, drying temperature was 40°C, soaking time in 0.25% glutaraldehyde solution was 4 min.

The final empirical models obtained in terms of coded factors were given below:

$$ES=3586.20-51.00X_1-42.50X_2+1151.25X_3-13.97X_1^2+69.52X_2^2-1133.97X_3^2+146.25X_1X_2-288.75X_1X_3-159.75X_2X_3$$

$$WS=16.82-0.74X_1+0.72X_2+6.07X_3+0.63X_1^2-0.22X_2^2-1.12X_3^2-0.43X_1X_2-0.018X_1X_3+0.79X_2X_3$$

Design-Expert software was used for the analysis of variance, verified the accuracy of this regression model and the significance of each parameters, ES (a) and WS (b) of the results were shown in Table 2. The results of the analysis of variance showed that their regression models were statistically significant ($P<0.05$), lack of fit value of the models was not significant ($P>0.05$), the values of the model correlation coefficient R^2 were 91.9, 92.62% respectively, the two models had a high imitation degree and correlation, well reflected the experiment values, so the developed quadratic models were significant to predict ES and WS of the films. In the model of the ES, the P-value of CMC-Na was less than 0.01, indicated that the usage of CMC-Na significantly influenced the value of the ES, followed by hydrochloric acid, glycerol had no significant effect. In the model of the WS, the P-value of CMC-Na was less than 0.0001, indicated that the usage of CMC-Na significantly influenced the value of the WS, hydrochloric acid and glycerol had no significant effect. From the contour plots, the interaction of hydrochloric acid, glycerol and CMC-Na were significant. As can be seen from the Figure 1-a, with the increase of CMC-Na, the ES exhibited a increased trend. Added the usage of CMC-Na, and contributed to the film was high strength and flexibility, but if the usage was too high, resulted in poor brittleness, due to the textural properties of the film decline, and the WS increase. With the increase of glycerol, the ES had no changes from the figure.

In Figure 1-b, with the increase of hydrochloric acid, the ES was on a declining trend, the usage of hydrochloric acid was associated with the protein solubility, molecular conformation and the intermolecular interactions, but if added too much will play an inhibiting role. However, the usage of glycerol had little effect on the ES of the film. From the Figure 1-c, with the increase of the usage of CMC-Na, the ES of the film showed an increasing tendency, increased the usage of hydrochloric acid, the ES of the film slowly decreased.

From the contour plots, the interaction of hydrochloric acid, glycerol and CMC-Na were significant. As can be seen from the Figure 1-d, with the increase of glycerol, the WS of the film showed an increasing trend, which there was a relationship with the water retention of glycerol. Increased the usage of hydrochloric acid, the WS of the film had a decreasing trend, the appropriate usage of hydrochloric acid will contribute to the molecular interaction, and reduce the WS. In Figure 1-e, with the increase of CMC-Na, the WS was on an obvious increasing trend, the suitable usage of CMC-Na will help to form a high strength and flexibility film, but if the usage was too much, will increase the WS, due to poor brittleness. With the increase of hydrochloric acid, the WS had slowly decrease. In Figure 1-f, added the usage of CMC-Na, the WS of the film had an obvious increase, but with the increase of glycerol, the WS of the film had a slowly increased.

To solve the optimal solution of the quadric polynomial regression model, the factor levels for the best ES and WS were 0.1mol/L hydrochloric acid: 2.08 mL/100g, glycerol: 2%, CMC-Na:

0.25%; and 0.1 mol/L, hydrochloric acid: 3.93 mL/100g, glycerol: 2%, CMC-Na: 0.25%, respectively. In order to verify the accuracy of the

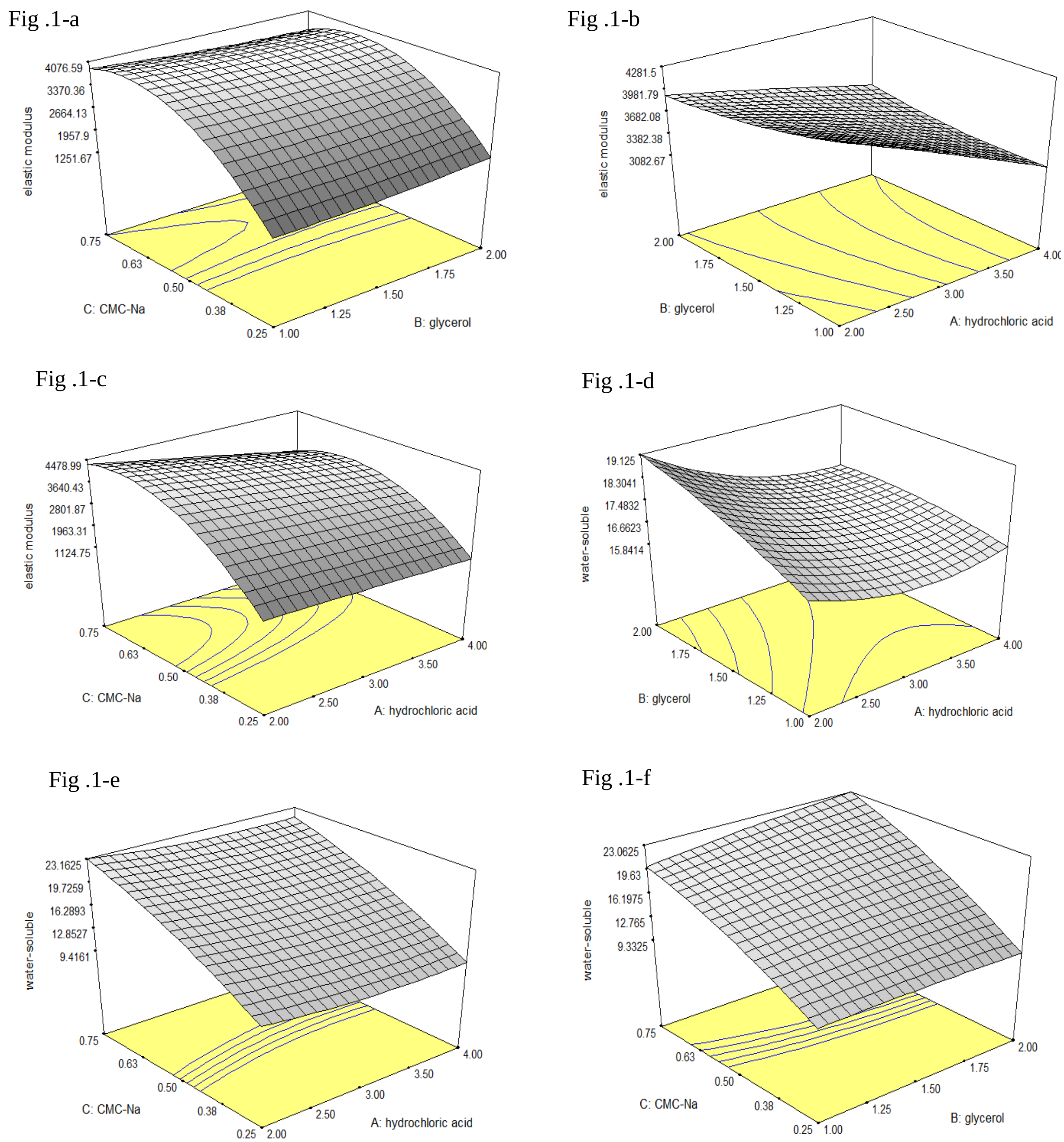

Fig.1 Experimental factors interaction and the contour plot of the ES and WS

model predicated values, according to the above technical parameters to make the films, compared with the films which prepared in the other conditions, the texture properties of the films were reasonable, the transparency and flexibility were better, and the actual measured values of the ES and WS were 3790 g/mm2 and 8.81%, had a smaller deviation with their respective predicted values.

In order to verify the accuracy of each equation, combined with the two indicators of the WS and ES, in the case of the other factors level were certain, the usage of hydrochloric acid was 3 ml/100 g, glycerol was 2%, CMC-Na was 0.25%, the WS of the film was 9.1%, the ES was 3710 g/mm2, and had a smaller deviation with their respective predicted values. Finally, the optimum technological conditions were determined as follow:

Table 2. Analysis of variance of the experimental results of the BBD

	Sum of squares		degree		Mean square		F-value		P-value> F	
	a	b	a	b	a	b	a	b	a	b
model	1.820E+007	313.78	9	9	2.023E+006	34.86	8.83	9.76	0.0045	0.0033
X_1	1.627E+006	4.44	1	1	1.627E+006	4.44	7.10	1.24	0.0322	0.3017
X_2	14450.00	4.15	1	1	14450.00	4.15	0.063	1.16	0.8089	0.317
X_3	1.060E+007	295	1	1	1.060E+007	295	46.27	82.6	0.0003	<0.0001
X_1^2	822.32	1.66	1	1	822.32	1.66	3.588E-003	0.47	0.9539	0.5168
X_2^2	20352.53	0.21	1	1	20352.53	0.21	0.089	0.058	0.7743	0.817
X_3^2	5.414E+006	5.29	1	1	5.414E+006	5.29	23.63	1.48	0.0018	0.2629
$X_1 \times X_2$	85556.25	0.75	1	1	85556.25	0.75	0.37	0.21	0.5605	0.661
$X_1 \times X_3$	3.335E+005	1.225E-003	1	1	3.335E+005	1.225E-003	1.46	3.430E-004	0.2669	0.9857
$X_2 \times X_3$	1.021E+005	2.51	1	1	1.021E+005	2.51	0.45	0.7	0.5259	0.4294
Lack of fit	3.321E+005	7.52	3	3	1.107E+005	2.51	0.35	0.57	0.7937	0.6620

a=modulus of elasticity, b=WS; the P value was significant level between each factors in the model. ($P < 0.05$) shown that the model or all factors were significantly correlated, $P < 0.01$ shown that they had highly significant correlation.

0.1 mol/L hydrochloric acid: 3 mL/100g, glycerol: 2%, CMC-Na: 0.25%, slurry: 90%, soaking time in 0.25% glutaraldehyde solution: 4 min, drying temperature: 40°C, drying time: 20 h.

IV. CONCLUSION

The results indicated that 0.1 mol/L hydrochloric acid: 3 mL/100g, glycerol: 2%, CMC-Na: 0.25%, slurry: 90%, soaking time in 0.25% glutaraldehyde solution: 4 min, drying temperature: 40°C, drying time: 20 h, the ES and WS of the film were very suitable. We will focus on effect of the alkali treatment on the properties of the collagen film in the future.

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