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Research Article Effect of Alkaline Treatment Conditions to the Rehydration of Bovine Omasum for Hotpot

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Abstract: Optimization of process condition of bovine omasum is a valuable attempt to guide the industrial production of maodu for hotpot. The quantitative effects of concentration of NaOH, treatment temperature and treatment time on the rehydration effect of bovine omasum for Hotpot using Box-Behnken design. The experimental data obtained were fitted to a second-order polynomial equation using multiple regression analysis and also analyzed by appropriate statistical methods. By solving the regression equation and also by analyzing the response surface contour plots, the optimal alkaline treatment conditions to the rehydration of bovine omasum were determined: a concentration of NaOH of 0.77 g/100 mL, a treatment temperature of 26.8° C and a treatment time of 33.2 min.

Keywords: Alkaline treatment, bovine omasum, box-behnken design, hotpot, rehydration

INTRODUCTION

Cattle are compound stomach ruminant which has four stomach rooms named rumen, reticulum, omasum and abomasums. Bovine tripe refers to the bovine ruminant stomach, particularly the rumen and the reticulum, which are the parts of the ruminant stomach most widely used in processed meat products (Zarkadas *et al.*, 1996; Conti-Silva *et al.*, 2011; Silva *et al.*, 2010; Vaz and Arêas, 2010; Anandh *et al.*, 2008; Florek *et al.*, 2012; Dubost *et al.*, 2013; Malvestiti *et al.*, 2007). However, the omasum is not used for human food at abroad, probably due to cultural reasons.

The renowned "numb and spicy hotpot" was originated in Chongqing, China. "maodu" is a popular dish in Chongqing hotpot, which has a characteristics of crisp and less slag. Bovine omasum was as the raw material for maodu production (Wu et al., 2012; Deng, 2009; Zhao, 2011; Zhou et al., 2012; Guo et al., 2013). Some methods such as alkaline treatment should be taken to tenderize its collagen and elastin fibres. It is necessary to take some measures for improving tenderness and cook yield of maodu. Alkaline Treatment are commonly used for this process. The processing technology was mainly based on working experience without detailed standards or guidance, which led to limitations in quality control and industry application of this traditional meat product. The low quality and the short shelf-life still limit its further growth in the Chinese market. Increasing the application of slaughter by-products has economic

importance in beef industry and research on processing technology of omasum is one of the major directions, but this field is lack of research both here and abroad.

Response Surface Methodology (RSM) is an affective statistical technique for optimizing complex processes. Box-Behnken Design (BBD), one of RSM, only have three levels and need fewer experiments. It's more efficient and easier to arrange and interpret experiments in comparison with others and widely used by many researches.

In this study, the main objective was to optimize the alkaline treatment conditions to the rehydration of bovine omasum for Chongqing Hotpot.

MATERIALS AND METHODS

Experimental materials and chemicals: Salted bovine omasum was a generous gift of Chongqing Liangran Food Processing Co. (Chongqing, China) (Fig. 1a). All chemicals used in this investigation were analytical grade and purchased from Beijing Chemicals Co. (Beijing, China).

Producing progress of maodu for Chongqing hotpot: Maodu for Chongqing hotpot is processed as follows: slaughter, cut the stomach, remove the residues, cut the other useless parts, clean, salt for long-term preservation, boil, rehydrate, cut the tripe into pieces, improves the water-holding capacity and refrigerate.

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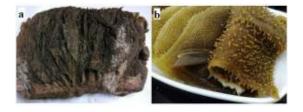


Fig. 1: (a) Appearance of salted bovine omasum, (b) maodu production

Table 1: Independent variable values of the process and their corresponding levels

	Symbol		Level		
Independent	Codified	Un-codified	-1	0	1
Concentration of NaOH (g/100 mL)	X ₁	x ₁	0.20	0.60	1.00
Temperature (°C)	X_2	X ₂	15	25	35
Time (min)	X3	X3	10	30	50

Table 2: Box-Behnken Design (BBD) 3 variables with the observed responses and predicted values for the weight gain rate of maodu

	Code	Coded variable levels ^a		Weight gain rate of maodu (%)		
Run	\mathbf{X}_1	X2	X3	Actual values	Predicted values	
1	-1	-1	0	48.78	42.45	
2	-1	0	-1	52.83	56.29	
3	-1	0	1	64.35	71.17	
4	-1	1	0	74.91	70.96	
5	0	-1	-1	40.67	43.54	
6	0	-1	1	63.88	63.39	
7	0	1	-1	71.25	71.74	
8	0	1	1	80.93	78.06	
9	1	-1	0	69.34	73.29	
10	1	0	-1	88.66	81.84	
11	1	0	1	96.61	93.15	
12	1	1	0	81.33	87.66	
13	0	0	0	106.62	105.89	
14	0	0	0	105.72	105.89	
15	0	0	0	105.32	105.89	
$X_1 =$	= (x ₁ - 0.6	$(0.4, X_2)$	$=(x_2 - 25)$	$/10, X_3 = (x_3 - 30)$	/20	

Alkaline treatment was used for rehydration of bovine omasum, followed by washed with water for some times until the pH value of 11.0-11.5 in 2 h. After that, the bovine omasum was plaed in a screen mesh to drain water. The weight gain rate of bovine omasum was calculated as follows:

Weight gain rate of bovine omasum (%) =
$$\frac{W_2 - W_1}{W_1} \times 100\%$$
 (1)

where,

- W_1 : The weight of bovine omasum before alkaline treatment, which was treated by boiling and draining water
- W_2 : Weight of bovine omasum after alkaline treatment, washing by water and draining water (g)

Experimental design: A three level, four variable Box-Behnken factorial Design (BBD) was applied to determine the best combination of extraction variables for the weight gain rate of bovine omasum. Three alkaline treatment variables considered for this research were X_1 (concentration of NaOH), X_2 (treatment temperature) and X_3 (treatment time) and the proper range of three variables were determined on the basis of single-factor experiment for the maodu production. The coded and uncoded (actual) levels of the independent variables are given in Table 1. Table 2 listed the whole design consisted of 15 experimental points, three replicates (treatment 13, 14 and 15, respectively) at the centre of the design were used to allow for estimation of a pure error sum of squares. The triplicates were performed at all design points in randomized order.

Experimental data were fitted to a quadratic polynomial model and regression coefficients obtained. The non-linear computer-generated quadratic model used in the response surface was as follows:

$$Y = \beta_0 + \sum_{i=0}^{3} \beta_i X_i + \sum_{i=0}^{3} \beta_{ii} X_i^2 + \sum_{i=0}^{3} \sum_{j=0}^{3} \beta_{ii} X_i X_j$$
(2)

where,

Y	: The measured response associated with
	each factor lever combination
$egin{array}{c} eta_0 \ eta_i \end{array}$: An intercept
β_i	: Regression coefficients computed from
	the observed experimental values of Y
X_i	: The coded levels of independent variables
$X_i X_j$ and X	$_{i}^{2}$: The interaction and quadratic terms,
-	respectively

Statistical analyses: Data were expressed as means Standard Errors (SE) of three replicated determinations. The responses obtained from each set of experimental design (Table 2) were subjected to multiple non-linear regressions using Design expert 8.0 software (Stat-Ease, Minneapolis, MN, USA). The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2 and the significances of the regression confident were checked by F-test and pvalue.

RESULTS AND DISCUSSION

Fitting the model: A regression analysis (Table 3) was carried out to fit mathematical models to the experimental data aiming at an optimal region for the responses studied. Predicted response *Y* for the weight gain rate of could be expressed by the following second-order polynomial equation in terms of coded values:

Table 3: Analysis of variance for extraction variables as linear, quadratic terms and interactions on response variables

qua	iuratic terms	and interac	tions on res	polise variat	103
Source	S.S.	df	M.S.	F value	Prob.>F
Model	5940.68	9	660.08	13.42	0.0053
Residual	245.96	5	49.19		
Lack of fit	245.07	3	81.69	184.26	0.0054
Pure error	0.89	2	0.44		
Cor total	6186.63	14			
		$R^2 =$	$R^2_{Adj} =$	CV =	
		0.9602	0.8887	9.14	
S.S.: Sum of	f square; M.S	S.: Mean squ	uare		

Table 4: Estimated regression model of relationship between response variables (weight gain rate of maodu) and independent

		2,3/			
Variables	S.S.	df	M.S.	F-value	p-value Prob.> F
X ₁	1129.79	1	1129.79	22.970	0.0049
X_2	919.13	1	919.13	18.680	0.0076
$\overline{X_3}$	342.70	1	342.70	6.970	0.0460
$X_1 X_1$	617.62	1	617.62	12.560	0.0165
$X_2 X_2$	2191.65	1	2191.65	44.550	0.0011
$X_3 X_3$	1110.29	1	1110.29	22.570	0.0051
$X_1 X_2$	49.98	1	49.98	1.020	0.3597
$X_1 X_3$	3.19	1	3.19	0.065	0.8092
$X_2 X_3$	45.77	1	45.77	0.930	0.3791
000	с M	C M			

S.S.: Sum of square; M.S.: Mean square

variables (X_1, X_2, X_3)

 $Y = 105.88667 + 11.88375X_1 + 10.71875X_2 +$ $6.54500X_3 - 3.53500X_1X_2 - 0.8925X_1X_3 -$ $3.38250X_2X_3 - 12.93333X_1^2 - 24.36333X_2^2 -$ $17.34083X_3^2$ (3)

where,

- Y : The weight gain rate of bovine omasum (%)
- X_1, X_2 and X_3 : The coded variables for concentration of NaOH, temperature and time

Table 3 listed the Analysis of Variance (ANOVA) for the fitted quadratic polynomial model of the weight

gain rate of maodu. F-test suggested that model had a very high model F-value (F = 13.42) and a very low p-value (p = 0.0053 < 0.01), indicating this model was highly significant.

The lack of fit measures the failure of the model to represent the data in the experimental domain at points which are not included in the regression. As showed in Table 3, F-value and p-value of the lack of fit were 184.26 and 0.0054, respectively, which implied it was highly significant relative to the pure error and indicated that the proposed second-order model is not the best fit for those responses but gives a tendency (Pérez *et al.*, 2013).

The significance of each coefficient was determined using p-value in Table 4. The p-value is used as a tool to check the significance of each coefficient and the interaction strength between each independent variable. The corresponding variables would be more significant at greater F-value and smaller p-value. The data in the Table 3 indicated that 4 independent variables $(X_1, X_2 \text{ and } X_3)$ and 3 quadratic terms $(X_1^2, X_2^2 \text{ and } X_3^2)$ significantly (p<0.05) affected the weight gain rate of bovine omasum.

Analysis of response surface: Three-dimensional response surface plots as presented in Fig. 1 to 3 are very useful to see interaction effects of the factors on the responses. These types of plots show effects of two factors on the response at a time. In all the presented figures, the other two factors were kept at level zero.

As expected, the weight gain rate of bovine omasum first increased and then decreased when x_1 changed in the range from 0.20 to 1.00 g/100 mL and the highest yield was obtained at a concentration of NaOH of 0.77 g/100 mL when x_2 was constant.

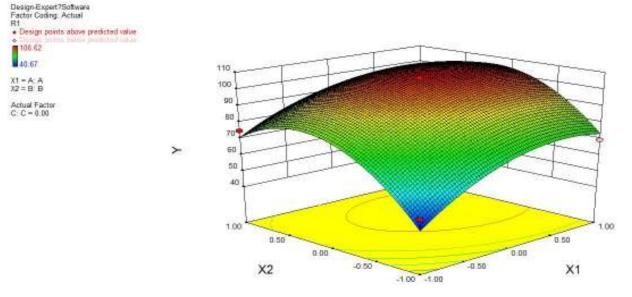
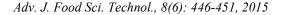


Fig. 2: Response surface showing the effect of concentration of NaOH (X₁) and treatment temperature (X₂) on weight gain rate of bovine omasum (Y)



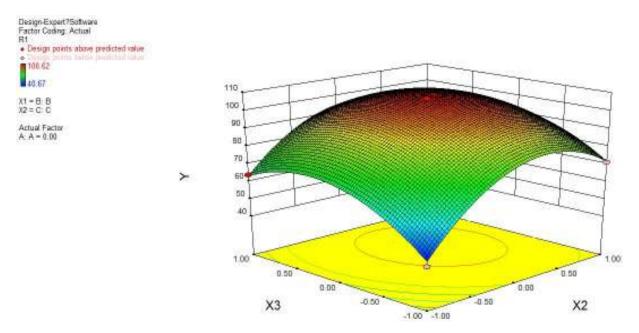


Fig. 3: Response surface showing the effect of concentration of NaOH (X1) and treatment time (X3) on weight gain rate of bovine omasum (Y)

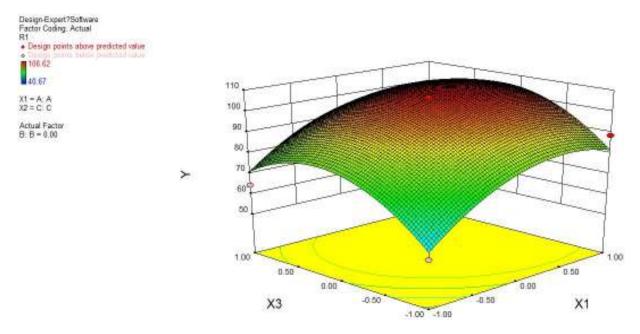


Fig. 4: Response surface showing the effect of treatment temperature (X₂) and treatment time (X₃) on weight gain rate of bovine omasum (Y)

The weight gain rate of bovine omasum also first increased and then decreased when x_2 was increased in the range from 15 to 35°C and the highest weight gain rate of bovine omasum was obtained at a treatment temperature of 26.8°C when x_1 was constant. x_1 exhibited a significant effect whereas x_2 showed a weaker effect on weight gain rate of bovine omasum and the interaction between x_1 and x_2 didn't impacted the weight gain rate of bovine omasum significantly (p>0.05) (Fig. 2). The weight gain rate of bovine

omasum first increased and then decreased when x_3 was increased from 10 to 50 min and the highest weight gain rate of bovine omasum was obtained at a treatment time of 33.2 min when x_1 was constant. x_1 exhibited a significant effect whereas x_3 showed a weaker effect on the weight gain rate of bovine omasum and the interaction between x_1 and x_3 did not impact the weight gain rate of bovine omasum significantly (p>0.05) (Fig. 3). Figure 4 show that X_2 exhibited a significant effect whereas X_3 showed a weaker effect on the weight gain rate of bovine omasum and the interaction between X_2 and X_3 did not impact the weight gain rate of bovine omasum significantly (p>0.05).

The experiment was carried out under the optimal (within the experimental conditions range): concentration of NaOH 0.77 g/100 mL, treatment temperature of 26.8°C and treatment time of 33.2 min. Under the optimal conditions, the model predicted a maximum response of 109.92 (%). To ensure the predicted result was not biased toward the practical value, experimental rechecking was performed using this deduced optimal condition. A mean value of 109.76±1.07 (%) (N = 3), obtained from real experiments, demonstrated the validation of the RSM model. The good correlation between these results confirmed that the response model was adequate for reflecting the expected optimization. The results of analysis indicated that the experimental values were in good agreement with the predicted ones and also suggested that the models of Eq. (3) are satisfactory and accurate.

Alkali solution treatment is the process of softening the dehydration materials in water, soaking them in alkali solution and removing alkali by water rinsing. It aims to restore dehydration materials to freshness. This treatment is more suitable for animal raw materials (bovine omasum, bovine tendon, dried scuttle-fish, dried squids, etc.) instead of water treatment (Fu *et al.*, 2007). There is a layer of liquid membrane on the surface of such animal materials and water molecule can hardly enter their inner side of the membrane. Alkali can lead to loss of membrane, which will promote the penetration of water and finally achieve rehydration.

After alkali solution treatment, the bovine omasum's volume was expanded and its moisture content increased (Fig. 1b). When the bovine omasum was immersed in 0.77 g/100 mL NaOH solution, its pH was above 12. But as a food, its pH should not exceed 10.5 according to the quality standards seted by chinease government departments, including NY5268-2004 (pollution-free food-maodu) and DBS50/001-2011 (Local food safety standard: bovine omasum for Chongqing hot pot). When we adjusted the pH to 10, the weight loss rate of bovine omasum reached 40% after placed for 12 h. So further investigation on improvement of the water-holding capacity of bovine omasum is warranted. The best combing ratio of mixed phosphates (including Sodium Tripolyphosphate, and Sodium Pvrophosphate Sodium Hexametaphosphate) and their concentration for the water-holding of bovine omasum will be investigated. In addition, the pH value of bovine omasum was alkaline, distinctive from other meat products, which had acidic pH value. Common preservatives for meat products such as nisin, potassium sorbate, sodium acetate and sodium dehydroacetate show good

anticorrosive activity under acidic condition. So, the anticorrosion problems of bovine omasum should be made a thorough study.

CONCLUSION

Alkaline treatment of bovine omasum is a key step for the production of maodu as a raw material for hotpot. The quantitative effects of concentration of NaOH, treatment temperature and treatment time on the rehydration effect of bovine omasum could satisfy the standardized production and further sales growth of maodu in Chinese hotpot industry. Also, increasing the application of slaughter by-products has economic importance in beef industry.

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