Effects of temperature and NaCl percentage on lipid oxidation in pork muscle and exploration of the controlling method using response surface methodology (RSM)

Jianhao Zhang [1], Guofeng Jin [1], Lichao He [1]

[1] College of Food Science and Technology, Nanjing Agricultural University, Nanjing, China

Abstract—**Main and interactive effects of temperature (15, 20, 25, 30 or 35 ℃) and NaCl (1%, 3% or 5%) on lipid oxidation in pork belly muscle were investigated through single-factor and response surface experiments, respectively, by measuring POV and TBARS of muscles, and the regulation method of primary lipid oxidation was also optimized. The results indicated that, within the range of temperature lower than 40 ℃, and NaCl added level lower than 5.0% (w/w), both temperature and NaCl showed prooxidant effects (p<0.05). The activation energy (E_a, 92.35 kJ/mol) for POV was higher than that (65.66 kJ/mol) for TBARS. Adding NaCl (< 5%, w/w) could decrease both the E_a, and when NaCl was added at levels of 3.1% and 3.4%, respectively, the corresponding E_a for primary and secondary lipid oxidation were the lowest. Temperature and NaCl had significant (p<0.001) interactive effect on primary lipid oxidation rate. Elevating temperature could significantly (p<0.05) and linearly (y=-0.067x+5.113) decrease the threshold value of NaCl concentration influencing lipid oxidation in pork belly muscles. When temperature was 35 ℃ and NaCl level was 2.77%, the primary oxidation rate was the fastest. These results suggested that raising temperature and decreasing salt content could accelerate primary lipid oxidation rate in belly muscle, which will make the change inflexions of POV and TBARS advanced, and thus provide more times for decreasing oxidation indices and improving flavour quality of products by secondary or more further oxidation.**

Keywords—**Belly muscle; Temperature; NaCl; POV and TBARS; Controlling; Response surface methodology.**

I. INTRODUCTION

Lipid oxidation is an important degradative change in muscle foods during processing and storing. In many cases, lipid oxidation is considered a major cause of quality deterioration in stored foods[1,2]. However, on the other hand, it is generally accepted that lipid oxidation is also essential for the development of aroma of many dry-cured meat products, such as dry-cured hams and dry-cured sausages etc[3]. Although the significance of lipid oxidation to aroma formation of some famous traditional meat products has been recognized unanimously, the studies devoted to this area are still limited compared to those devoted to antioxidation of meat and meat products.

The oxidative changes of muscle foods is usually affected by both processing conditions and food additives. The effect of temperature and NaCl on lipid oxidation have been widely in various foods . However, for the temperature effect on lipid oxidation, most of the reported studies were on the vegetable and soybean oils and few were focused on muscle lipids[4,5,6]. NaCl has been proposed to have a dual effect on lipid oxidation in meat, which mainly determined by its concentration in meat. Rhee[7] have reported that NaCl activated lipid oxidation at concentrations lower than 2%, but above which the lipid oxidation in minced pork muscle was inhibited. However, whether this conclusion is universally applicable for all of the pork products regardless of the differences in other treatment conditions, such as temperature, have not been elucidated.

It has been found that temperature and NaCl exist interaction for many biochemical reaction in meat. Lytras, *et al*[8] have found that the ionic strength in meat could influence the temperature at which denaturation of myoglobin occurs. But, up to now, information on the interaction between temperature and NaCl for lipid oxidation is very limited.

Therefore, the aim of this study was to evaluate the effects of temperature, NaCl as well as their interaction on the lipid oxidation in pork belly muscle, and based on these results, to further explore an empirical controlling method of lipid oxidation using response surface methodology (RSM).

II. MATERIALS AND METHODS

2.1. Experiment 1 (effect of temperature on lipid oxidation)

Six kilogram minced muscles were randomly divided into four equal portions. Each portion was further divided into 15 equal samples. A total of 60

samples, each sample weighing 100 g, were obtained. Then each portion was randomly assigned to one of the following treatments: oxidized for 5 days at 15, 20, 25 or 30 ℃, respectively. For all the five treatments, relative humidity was uniformly set at 75%. Each treatment was done in triplicate. During the 5 days oxidation period, three replicate muscle samples were randomly taken every day for POV and TBARS determination.

2.2. Experiment 2 (effect of NaCl on lipid oxidation kinetics)

A total amount of 13.5 kg fresh minced belly muscles were divided into three equal portions, and then NaCl was added to the muscles of each portion to achieve final concentrations of 1.0%, 3.0% and 5.0%, respectively. The muscles of each portion were mixed together for 2 min and divided into 45 samples, each weighing 100 g, thereafter all these samples were equally divided into three subgroups (15 samples per subgroup) and each one was assinged to one of the following three different treatments: oxidized at 17 ℃, 27 ℃ or 37 ℃, respectively, for 5 days, the relative humidity was set as 75%. During the 5 days oxidation period, three samples were randomly taken from each subgroup every day for POV and TBARS determination.

2.3. Response surface experiment

The response surface experiment was designed based on central composite design (CCD) procedure, and Experimental range and levels of the two independent variables in terms of the actual and coded values are given in Table 1.

Table 1 Experimental range levels of two independent variables in terms of actual values

Variables	Coded variable levels				
	-1.414	-1	0	1	+1.414
Temperature (℃)	10.86	15	25	35	39.14
NaCl content (%)	0.17	1	3	5	5.83

III. RESULTS AND DISCUSSION

3.1. Effect of temperature on lipid oxidation rate

Fig. 1 shows the changes in peroxide values (PV) during five days oxidation period of different groups of belly muscles that incubated at different temperatures. As can be seen that the typical bell-shaped curves of PV changes were observed for all the treatments. The main difference among all the treatments was that the time when PV reached the peak in belly muscles decreased significantly with the temperatures increasing (p<0.05).

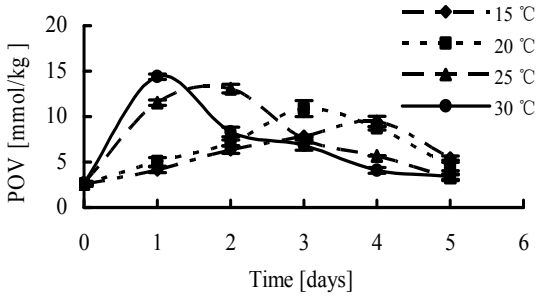

Fig. 1 Changes of peroxide value in different belly muscles oxidized at different temperatures

To better understand the effect of temperature on lipid primary oxidation rate in belly muscle, the temperature dependence of the reaction rate was analyzed by the Arrhenius equation, and the Arrhenius plot was given in Fig. 2. From the Arrhenius plot, the activation energy (E_a) for primary oxidation of lipid in belly muscle was determined as 92.35 kJ/mol, which was within the range of the E_a values (24-240 kJ/mol) that previously reported for lipid oxidation of edible oils[9, 10]. In addition, the well fitting degree of the Arrhenius equation (Fig. 2) also indicated that the magnitude of the primary oxidation of lipid in pork muscle was closely related with temperature (R^2=0.98, p<0.05).

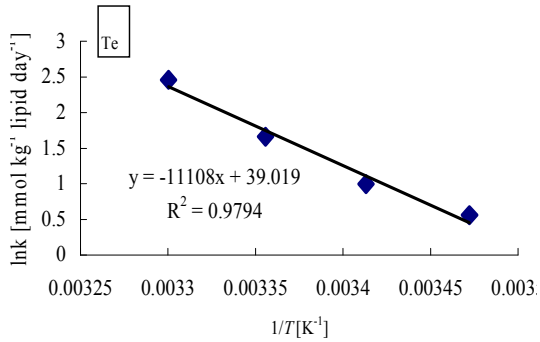

Fig. 2 Arrhenius plots for the peroxide values of lipid in pork belly muscle

The effect of temperature on TBARS changes in belly muscles is shown in Fig. 3. For the 15 ℃ and 20

℃ treatments, the TBARS increased sustainably over the whole period of time studied (p<0.05). However, for 25 ℃ and 30 ℃ treatments, TBARS increased significantly (p<0.05) up to the peaks on day 4 and day 3, respectively, and thereafter decreased sharply with time. This indicated that increasing temperature could also significantly (p<0.05) promote the secondary oxidation of lipid in belly muscle but not result in high TBARS level in the final products, in spite of the well known prooxidant effect of temperature.

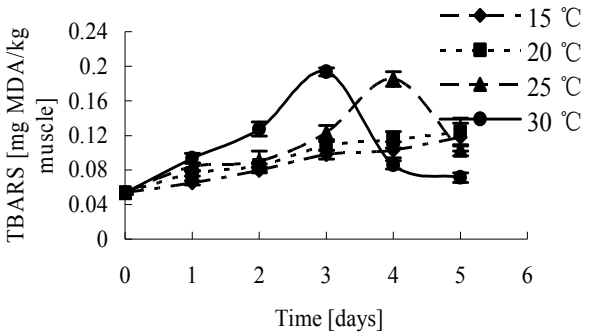

Fig. 3 Changes of TBARS in different belly muscles oxidized at different temperatures

The E_a for TBARS formation in the belly muscle was determined as 65.66 kJ/mol through the Arrhenius plot (Fig. 4). This value was lower than that (92.35 kJ/mol) for hydroperoxides formation, suggesting that increasing temperature had more significant promoting effect on the decomposition of hydroperoxides than on their formation.

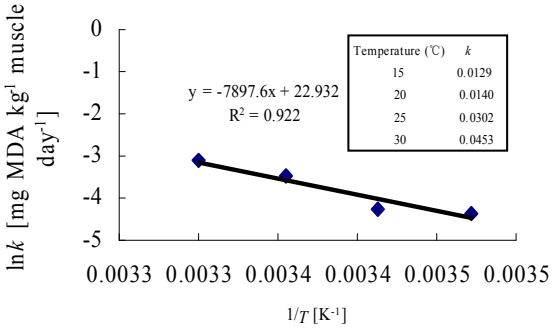

Fig. 4 Arrhenius plots for the TBARS values of lipid in pork belly muscle

3.2. Effect of NaCl on lipid oxidation kinetics

Fig. 5 shows the E_a for lipid primary oxidation in the belly muscles treated with different levels (1.0%, 3.0% and 5.0%) of NaCl. From this figure (Fig. 5), it can be seen that adding NaCl (< 5.0%) to the belly muscles could significantly decrease the E_a for lipid primary oxidation (p<0.05). When NaCl added level was lower than 3.0%, the E_a for lipid primary oxidation decreased significantly with the increase of NaCl added level (p<0.05). However, when the level of NaCl was above 3.0%, then the E_a for lipid primary oxidation began increased with NaCl added level increasing. This result indicated that when the NaCl added level was 3.0% or close to 3.0%, the corresponding E_a for lipid primary oxidation could be minimal. The regression analysis results of E_a value versus NaCl added level also revealed that when the corresponding E_a for lipid primary oxidation was minimal (53.179 kJ/mol), the corresponding NaCl added level was 3.1%.

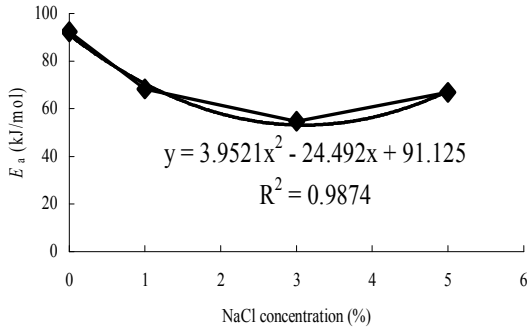

Fig. 5 Changes in E_a for POV with NaCl content changes in belly muscles

Similarly to the primary oxidation, the E_a for lipid secondary oxidation also decreased initially with NaCl added level increasing from 0.0% to 3.0%, and increased thereafter with NaCl added level increasing (Fig. 6). But when NaCl added was 5.0%, the E_a (56.01 kJ/mol) for lipid secondary oxidation was still lower than that (65.66 kJ/mol) of the control which was not added NaCl. The regression analysis results of E_a value versus NaCl added level revealed that when the E_a for secondary lipid oxidation was minimal (53.179 kJ/mol), the corresponding NaCl added level was 3.4%.

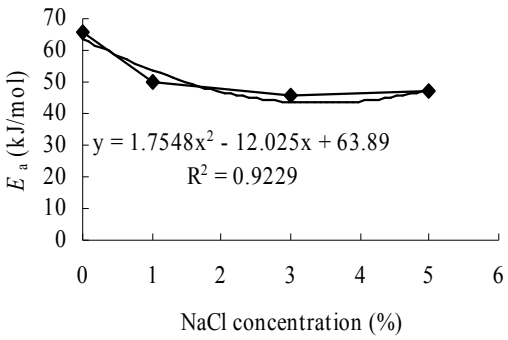

Fig. 6 Changes in E_a for TBARS with NaCl content changes in belly muscles

3.3. Response surface experiment results

Results from the response surface experiment are summarized in Table 2. And the quadratic polynomial regression equation in terms of actual factors is listed as follow:

$$Y=4.52-0.10X_1-0.44X_2+5.75E-003X_1X_2+ 3.18E-004X_1^2+0.04X_2^2 \quad \text{(Eq. 1)}$$

where Y is the T_{max} (the time when peroxide value reached the peak); X_1, X_2 are the actual values of temperature and NaCl concentration, respectively.

3.3.1. Interaction of temperature and NaCl

Fig. 7 shows the interaction between temperature and NaCl on primary lipid oxidation rate (T_{max}).

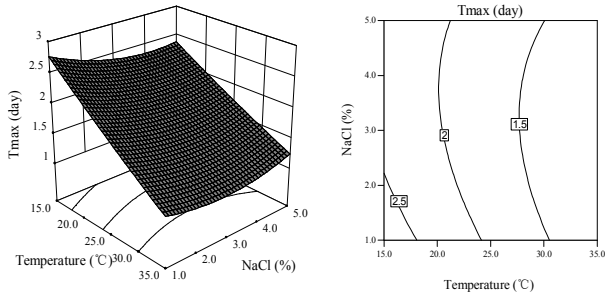

Fig. 7 Response surface and contour plot of the interactive effect of temperature and NaCl concentration on the T_{max}

It can be seen that, when added NaCl into the meat samples, the lipid oxidation rate still increased with the increase of temperature. But the increase rate was gradually decreased with NaCl content increasing. For the effect of NaCl on lipid oxidation, the threshold value of NaCl concentration affecting lipid oxidation also decreased with temperature increasing. Compared to the lipid oxidation rate decrease with NaCl content increasing, the decrease of NaCl threshold value with temperature increasing was more rapid. This may indicate that, when temperature and NaCl were interacted on each other in pork belly muscle, the NaCl effect on lipid oxidation was more affected by temperature than was the temperature effect on lipid oxidation affected by NaCl content.

3.3.2. Controlling method optimization

From the regression equation Eq. 1, the optimal conditions for the Tmax were obtained as follows: temperature 35 ℃, NaCl concentration 2.77%. Under the optimum conditions, the minimal Tmax was estimated to be 1.03 days.

IV. CONCLUSIONS

In conclusion, the results of this study show that lipid oxidation rate in pork belly muscle increased significantly with NaCl content increasing within the studied range. However, to what extent NaCl functioned as prooxidant of lipid was significantly affected by the variations in temperature. With temperature increasing, the threshold value of NaCl affecting lipid oxidation in pork belly muscle gradually decreased. Therefore, as the optimized result from RSM experiment suggested, high temperature (35 ℃) and medium level of NaCl content (2.77%) could effectively accelerate lipid primary oxidation (minimal T_{max}=1.03 days), which will consequently endow the subsequent oxidation steps with enough time. Under this condition, more lipid oxidation-derived flavour compounds will generate and the TBARS will also be decreased. This is very favorable for dry-cured meat products processing. And through this, the processing times of dry-cured meat products are hopeful to be shortened on the basis of ensuring the flavour quality and safety of final products.

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Table 2 Central composite design arrangement and experimantal response

Run	Temperature (℃)	NaCl content (%)	Peroxide value(mmol/kg)[b]				Equation $Y=ax^2+bx+c$	T_{max} ($-b/2a$) (day)
			1st day	2nd day	3rd day	4th day		
1	25.00	3.00	9.93±0.19	13.33±0.24	7.22±0.05	5.84±0.12	$y=-1.20x^2+4.14x+7.70$ $R^2=0.69$	1.73
2	25.00	3.00	10.05±0.11	12.76±0.37	7.15±0.22	5.86±0.03	$y=-1.00x^2+3.18x+8.50$ $R^2=0.72$	1.59
3	10.86	3.00	2.08±0.47	3.87±0.14	4.60±0.14	3.09±0.31	$y=-0.83x^2+4.52x-1.67$ $R^2=0.98$	2.72
4	25.00	0.17	3.42±0.19	7.70±0.06	6.47±0.13	5.70±0.12	$y=-1.26x^2+5.51x+1.53$ $R^2=0.99$	2.19
5	15.00	5.00	7.02±0.03	8.38±0.24	10.39±0.69	5.12±0.24	$y=-1.66x^2+7.92x+0.36$ $R^2=0.79$	2.39
6	15.00	1.00	3.22±0.07	3.96±0.36	6.21±0.26	3.77±0.31	$y=-0.80x^2+4.37x-0.66$ $R^2=0.63$	2.73
7	25.00	3.00	9.86±0.30	13.03±0.49	7.02±0.16	5.86±0.12	$y=-1.08x^2+3.61x+8.03$ $R^2=0.68$	1.67
8	35.00	5.00	2.81±0.02	2.96±0.07	2.45±0.03	2.25±0.04	$y=-0.09x^2+0.22x+2.73$ $R^2=0.85$	1.22
9	35.00	1.00	1.89±0.19	2.04±0.11	1.60±0.08	1.54±0.25	$y=-0.05x^2+0.11x+1.88$ $R^2=0.72$	1.10
10	25.00	3.00	9.80±0.30	12.95±0.61	7.10±0.17	5.89±0.08	$y=-1.09x^2+3.69x+7.88$ $R^2=0.69$	1.69
11	25.00	5.83	9.27±0.46	13.14±0.13	7.51±0.52	4.52±0.22	$y=-1.72x^2+6.59x+5.01$ $R^2=0.81$	1.92
12	25.00	3.00	9.86±0.30	13.25±0.29	7.24±0.09	5.69±0.27	$y=-1.24x^2+4.32x+7.47$ $R^2=0.71$	1.74
13	39.14	3.00	2.40±0.10	2.71±0.29	1.61±0.01	1.44±0.19	$y=-0.12x^2+0.20x+2.44$ $R^2=0.76$	0.83