Response surface methodology for evaluation of blanching effects on antioxidant capacity and physical quality of ground pork product treated with mulberry leaves

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Abstract

Although hot water blanching has an important effect on inactivating microorganisms, the antioxidant capacity of foods might be lost by this process. The purpose of this work was to evaluate the predictive response surface models (RSM) and their mathematical equations for monitoring the quality of ground pork incorporated with mulberry leaves (12.5 g/kg) during blanching. The temperatures and times were modelled to create RSM mathematical equations based on the alteration of each parameter, including pH, texture characteristics, color values (L*, a* and b*), and antioxidant capacity. The results showed that pH values and hardness were affected by the blanching temperature, while the b* was affected by the blanching times. In contrast, the stringiness, and L* and a* were not altered by any blanching factors. Following the antioxidant activity, the percentage of DPPH radical-scavenging activity increased with an increase in all blanching factors. Considering the normal probability plots of each parameter along with the verification of the assumption of ANOVA, these results indicated that their predictive equations could be a prototype of RSM models for predicting the alterable physical quality and antioxidant capacity of ground pork incorporated with mulberry leaves during blanching.

Keywords: Response surface model (RSM), Blanching effect, Ground pork product treated with mulberry leaves

Introduction

Ground pork is a traditional raw meat material used in numerous food products such as meatballs, various types of sausages, and patties. Ground pork is more susceptible to microbial cross-contamination and oxidation due to the preparation by grinding with resultant alteration of the muscular lipid membrane that causes an adverse effect in organoleptic properties and induces the production of toxic compounds (Sojic et al., 2020; Villalobos-Delgado et al., 2017). The use of the synthetic antioxidants butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), is a popular method for decreasing the level of oxidation and improving the quality and shelf-life of ground pork. However, those chemical agents have been shown to have an adverse effect on the health of consumers (Biswas et al., 2012).

The implementation of natural preservatives for increasing antioxidant capacity by the use of various medicinal plants which have antimicrobial and antioxidant properties is a promising approach. *Morus alba* L. (mulberry) leaves are used in traditional medicine. *Morus alba* L. Leavf extracts possess the bioactive compounds gamma-aminobutyric acid, 1-deoxynojirimycin, flavonoids, alkaloids, essential oils, polysaccharides, vitamins, amino acids, trace elements, and chlorophyll (Cui et al., 2019; Cui et al., 2021). Zhang et al. (2016) revealed the potential effect of mulberry leaf extracts on the quality of raw ground beef during refrigerated storage. Abdeldaiem et al. (2017) reported that the methanolic extract of the mulberry plant could be used as a natural preservative to extend the shelf-life of minced beef for up to 14 days. Recently, Cui et al. (2021) suggested that ethanolic extract from *Morus alba L.* leaves could be used as a resource in chilled pork under retail preservation. However, the chemical method of solvent extraction might have complicated steps, high cost and toxic substances. Thus, mulberry leaves present an interesting option.

In meat processing, the conventional method of hot water blanching is used, which has the important effect of inactivating microorganisms and undesirable enzymes. Conversely, this process causes undesirable food product quality, especially a loss of nutrition and texture (Deag et al., 2019; Magra et al., 2006). Importantly, the conditions of time and temperature during blanching should be considered. Full factorial design and Response Surface Methodology (RSM) could be used to compare the effects of both temperature and time as this approach is an effective statistical technique for modelling multiple variables to create mathematical equations for predicting the quality of food products (Valinger et al., 2020).

Therefore, the purpose of this work was to investigate the blanching effects of temperature and time on the antioxidant activity and physicochemical properties of raw ground pork product treated with mulberry leaves using RSM, while the predictive modelling of each parameter could be a prototype for predicting the product quality of raw ground pork incorporated with mulberry leaves.

Methods and Materials

Preparation of raw ground pork incorporated with mulberry leaves

Fresh mulberry leaves were harvested from the agricultural farm of Rajamangala University of Technology Isan. The leaves were cut and cleaned thoroughly under running tap water. The pork was purchased from a Nakhon Ratchasima department store. Two kg of the pork meat was minced in a meat mincer and then mixed with sliced mulberry leaves (25 g), coriander root (15 g), pepper powder (15 g), seasoning sauce (15 g), soy sauce (25 g), seasoning powder (15 g), sugar (25 g), tapioca flour (25 g), and baking powder (15 g). The suitable level of mulberry leaves (12.5 g/kg) was selected from our preliminary study according to sensory evaluation (Data not shown). The mathematical models of blanching effects were carried out as in Table 1.

Table 1 The information of each independent factor and level used in the experiments

Independent factors (Unit)	Symbol of the variables	Levels of blanching temperature and time		
		-1	0	1
Temperature (°C)	X_1	70	80	90
Time (seconds)	X_2	40	50	60

The quality parameters, temperature and time, of the ground pork treated with mulberry leaves were modelled by RSM as shown in Equation (1). This was described by Montgomery (1997).

$$Y = beta_0 + beta_1 x_1 + beta_2 x_2 + beta_{11} x_1^2 + beta_{22} x_2^2 + beta_{12} x_1 x_2 + epsilon \quad (1)$$

The value of Y is the model response (pH, adhesiveness, stringiness, hardness, L*, a*, b*, and DPPH radical-scavenging activity), The values of X_1 and X_2 are independent variables. The variables $beta_{ij}$, $beta_{ii}$,

$beta_i$ and $beta_0$ represent the interaction, the quadratic effect, the linear effects as well as the regression coefficients for the intercept, respectively.

Measurement of pH, texture profiles and color

A digital pH meter (Fisher scientific model AB15) was used to measure the pH of the ground pork treated with mulberry leaves. Before analysis, the pH meter was calibrated by standard buffer solutions, such as pH 4 and pH 7. The texture profiles of all samples were measured by a texture analyzer (CT3 10K, Brookfield, USA). To analyze the color of the ground pork treated with mulberry leaves, each sample was measured using a Chroma meter CR-410 (Konica Minolta, Japan). The color parameters, L* (brightness), a* (redness / greenness), and b* (yellowness / blueness), were recorded.

Analysis of DPPH radical-scavenging activity (RSA)

In brief, the 2.5 kg of ground pork treated with mulberry leaves was homogenized with 22.5 mL of 0.85 % (w/v) NaCl for 1 min at a speed of 10000 rpm and the solution was filtered and used for analyzing antioxidant activity. The percentage of RSA was analyzed by measuring the direct DPPH-scavenging activity in the samples (Krüzselyi et al., 2020). DPPH (0.1 mM) was prepared in 80 % (v/v) ethanol. A sample solution (1 mL) was incubated with 3 mL of DPPH, while a control tube of DPPH was used to determine the maximum absorbance (3 mL DPPH + 1 mL ethanol). All mixtures were incubated at room temperature in the dark for 30 min. The absorbance of DPPH was measured at 515 nm. The percentage of RSA of each sample was calculated via Equation (2):

$$\% RSA = \left(\frac{A_{DPPH} - A_{sample}}{A_{DPPH}}\right) \times 100 \tag{2}$$

Statistical analysis and predictive model validations

A 3^2 full factorial design with two replicates was performed to predict the alterations in the various quality parameters of ground pork treated with mulberry leaves. Analysis of variance (ANOVA) of each parameter was used to estimate the significance ($P<0.05$) of the main effects, its interactions, regression coefficients and determination coefficients (R^2 values). Design-Expert® software (version 7.0) was performed to analyze the three-dimensional plots of RSM. The data of normal probability plots of residuals were considered for all physicochemical parameters of ground pork treated with mulberry leaves.

Results

The regression coefficients of temperature and time indicated that the significant parameters of ground pork treated with mulberry leaves were changed during blanching ($p<0.05$). Figure 1 shows that the RSM pH value significantly fitted the quadratic model ($p<0.05$) as expressed by Equation 3. At the same time, Figure 2A indicates the RSM hardness value significantly fitted the linear model ($P<0.05$) as expressed by Equation 4, while the RSM quadratic model of stringiness is shown in Figure 2B as calculated by Equation 5. The response surface diagram illustrating the blanching effects of temperature and time on the color of the ground pork treated with mulberry leaves, including L* (A), a* (B), and b* (C) is shown in Figure 3 ($p<0.05$), which are displayed in Equations 6, 7 and 8, respectively. In the case of antioxidant activity, the percentage of RSA significantly fitted the quadratic model ($p<0.05$) as presented by Equation 9, while its RSM model is shown in

Figure 4. As shown by the normal probability plots of the residuals for all parameters, shown in Figure 5, which are used to assess the RSM model validity, the points are narrowly scattered around a straight line.

$$pH = 3.31339+(0.11576\times X_1)-(0.054917\times X_2)+(5.87500\times 10^{-5}\times X_1\times X_2)-(7.1333\times 10^{-4}\times (X_1)^2)+(4.76667\times 10^{-4}\times (X_2)^2) \quad (3)$$

$$\text{Hardness (g)} = (-22.52778)+(0.62500\times X_1)+(0.075000\times X_2) \quad (4)$$

$$\text{Stringiness length (mm)} = 469.73333-(11.54612\times X_1)+(0.43467\times X_2)+(7.58750\times 10^{-3}\times X_1\times X_2)+(0.069125\times (X_1)^2)-(9.250\times 10^{-3}\times (X_2)^2) \quad (5)$$

$$L^* = 146.63944-(4.31054\times X_1)+(3.75233\times X_2)-(2.3125\times 10^{-3}\times X_1\times X_2)+(0.027733\times (X_1)^2-(0.034517\times (X_2)^2) \quad (6)$$

$$a^* = (-57.65778)+(1.37850\times X_1)+(0.42875\times X_2)-(2.40\times 10^{-3}\times X_1\times X_2)-(7.93333\times 10^{-3}\times (X_1)^2-(2.35833\times 10^{-3}\times (X_2)^2 \quad (7)$$

$$b^* = (-16.25778)+(0.60929\times X_1)-(0.31042\times X_2)-(4.71250\times 10^{-3}\times X_1\times X_2)-(2.33333\times 10^{-3}\times (X_1)^2+(7.24167\times 10^{-3}\times (X_2)^2 \quad (8)$$

$$\%RSA = (-1422.89778)+(27.99879\times X_1)+(11.82883\times X_2)-(0.058412\times X_1\times X_2)-(0.14893\times (X_1)^2-(0.067933\times (X_2)^2 \quad (9)$$

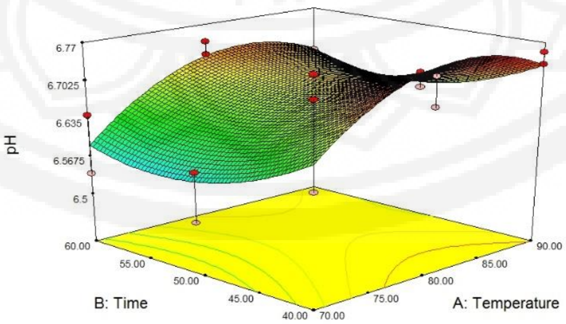

Figure 1 Response surface diagram illustrating the two blanching effects of temperature and time on pH values of the ground pork treated with mulberry leaves

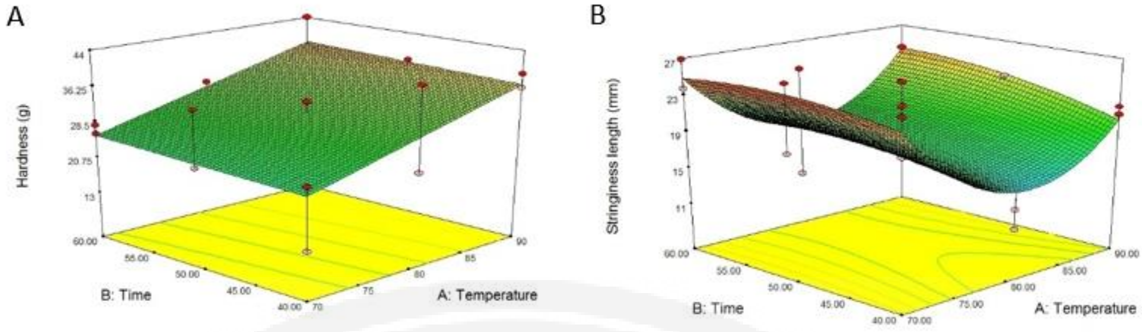

Figure 2 Response surface diagram illustrating the two blanching effects of temperature and time on hardness (A) and stringiness (B) of the ground pork treated with mulberry leaves

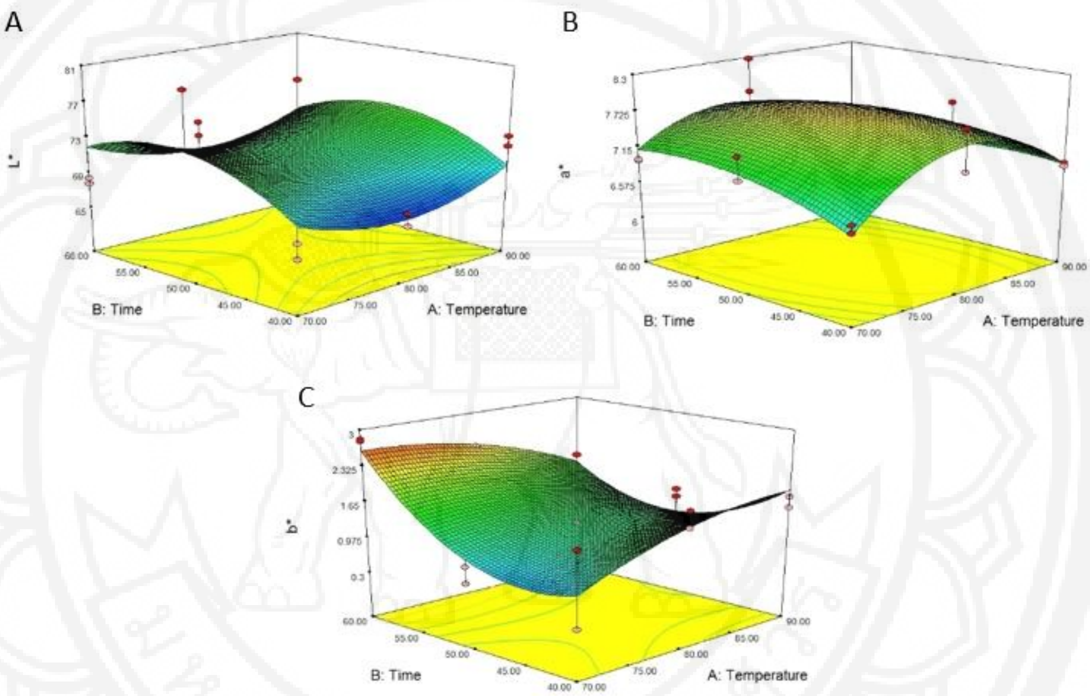

Figure 3 Response surface diagram illustrating the two blanching effects of temperature and time on color values, including L* (A), a* (B), and b* (C) of the ground pork treated with mulberry leaves

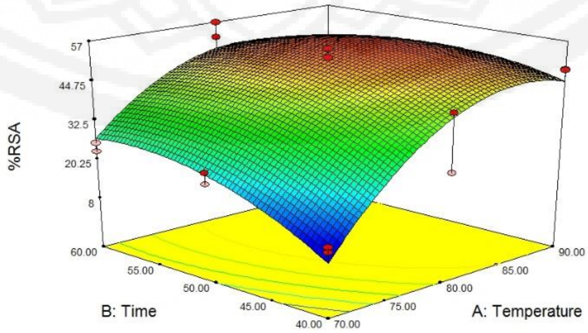

Figure 4 Response surface diagram illustrating the two blanching effects of temperature and time on the antioxidant activity of the ground pork treated with mulberry leaves

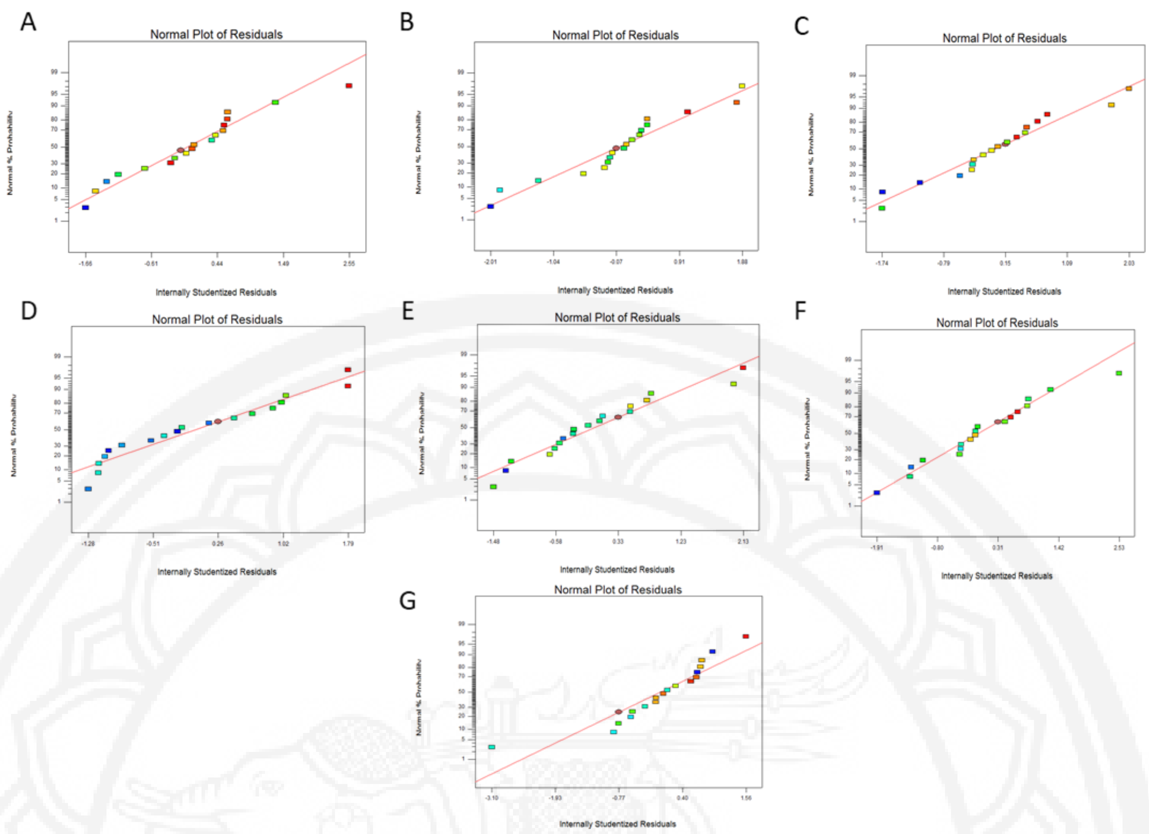

Figure 5 Normal probability plots of the residuals for the pH values (A), hardness (B), stringiness (C), L* (D), a* (E), b* (F), and %RSA (G) of the ground pork treated with mulberry leaves

Discussion and Conclusion

Based on ANOVA (data not shown), the pH value increased with an increase in the blanching temperature (Figure 1). Figure 2A indicates that the temperature increased while hardness increased. In contrast, the stringiness was not affected by time and temperature (Figure 2B). The color characteristics of all treatments were not affected by temperature (Figure 3), whereas the b* was affected by blanching times. This can be interpreted that heat treatment could have induced an alteration of the three-dimensional structure of meat protein and changed the color and texture properties. Past studies of cooked meat products indicated that heat-affected changes in myofibrillar protein, intramuscular connective tissue, muscle cytoskeleton, and protein oxidative damages were responsible for textural characteristics. As temperature increases, the protein structure starts to unfold. When the secondary and tertiary structures are lost, the unfolded protein can aggregate, and disulfide bonds scramble (Di et al., 2014; Murphy & Marks, 2000; Yu et al., 2017). In addition, Yu et al. (2017) also found that the color values of meat products can be changed by temperature and time levels. The interaction of the globin chain with the heme group affected its denaturation temperature along with oxymyoglobin and metmyoglobin. The myoglobin denaturation is responsible for a color change after heat processing. From the results of antioxidant activity, the percentage of RSA increased with an increase in all blanching factors. The thermal processing caused a significant loss of antioxidant activity owing to antioxidants leaching into the cooking water. Nevertheless, some literature reviews have revealed that thermal conditions do not decrease the overall antioxidant properties of food products (Al-Juhaimi et al., 2018; Nayak et al., 2015). To obtain this validity, the normal probability plots of each parameter were verified with the assumption of ANOVA values (Bolek & Ozdemir, 2017; Gasaluck & Mahidsanan, 2018; Yiga et al., 2021). Those predictive equations could be used as valid RSM models for monitoring the physical quality and antioxidant activity of ground pork products treated with mulberry leaves during blanching.

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