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Author	Seonmin Lee[1], Kyung Jo[1], Seul-Ki-Chan Jeong[1], Yun-Sang Choi[2], and Samooel Jung[1]
Affiliation	[1]Division of Animal and Dairy Science, Chungnam National University, Daejeon 34134, Korea [2]Research Group of Food Processing, Korea Food Research Institute, Wanju 55365, Korea
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ORCID (All authors must have ORCID) https://orcid.org	Seonmin Lee (https://orcid.org/0000−0002−5713−1795) Kyung Jo (https://orcid.org/0000−0002−3006−5396) Seul-Ki-Chan Jeong (https://orcid.org/0000-0002-2163-8340) Yun-Sang Choi (https://orcid.org/0000−0001−8060−6237) Samooel Jung (https://orcid.org/0000−0002−8116−188X)
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CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Samooel Jung
Email address – this is where your proofs will be sent	samooel@cnu.ac.kr
Secondary Email address	
Postal address	34134
Cell phone number	+82-10-9380-1136
Office phone number	+82-42-821-5774
Fax number	+82-42-825-9754

Abstract

In this study, concentration levels of beet powder (BP) and caramel color (CC) were optimized to simulate beef color in meat analogs before and after cooking. The central composite design of response surface methodology (RSM) was used to set the levels of BP and CC, and the CIE *L**, *a**, and *b** values were selected as the responses for RSM. After optimization, myoglobin-free beef patties were prepared with three optimized levels of BP and CC. When raw, all the patties had the same color as natural beef; however, CIE *L**, *a**, and *b** values were statistically different from those of beef after cooking ($P<0.05$). Moreover, the use of BP and CC induced "browning" after the cooking process, with no excessive yellow color. Therefore, based on the overall desirability in the color optimization using RSM, the combination of BP (1.32%) and CC (1.08%) with the highest overall desirability can be used to simulate the color change of beef in meat analogs.

Keywords: meat analog, meat color, beet powder, caramel color

1. Introduction

The rapid increase in the world population and the limited production of food sources has led to the livestock industry becoming an unsustainable practice (Kumar et al., 2021). As more consumers become aware of environmental and animal welfare issues associated with the food they use, global demand for healthy alternative options has increased (Hwang et al., 2021). This trend has triggered changes in the food culture to develop sustainable meat analogs that mimic the sensorial and nutritional attributes of animal meats. The two major groups of meat analogs are cultured and plant-based. Cultured meats are generally described as “artificial,” “laboratory-grown,” or “synthetic” substitutes for meat, while plant-based analogs are composed of plant-origin proteins (Sun et al., 2021).

Among the various properties of meat and meat products, color is often regarded as the most important attribute for the consumers’ intent to purchase. Fresh meats are represented by diverse colors depending on the species, distribution of muscle fibers, and exposure to oxygen. Meat color mainly depends on the types and amount of a sarcoplasmic protein called myoglobin (King & Whyte, 2006). Upon exposure to high levels of oxygen, myoglobin converts into oxymyoglobin, which is bright cherry red in color. However, after slaughter, myoglobin exists as deoxymyoglobin exhibiting a purple-red color (Tomasevic et al., 2021). During the cooking process, the color of meat changes to brown due to the denaturation of proteins, primarily myoglobin (Sakai et al., 2022). It is desired that meat analog products undergo a similar color change before and after cooking. Cultured and plant-based meat analogs generally have a pale and beige or yellow color, respectively, due to the absence of myoglobin. Efforts have been made to mimic the red color of meat by the addition of natural pigments such as beet juice and soy leghemoglobin during the culturing of the tissues (Fraeye et al., 2020). However, it is still necessary to search for the ingredients and conditions that can simulate the thermally induced color changes of meat in its analogs are still required.

In a recent study, Sakai et al. (2022) showed that the use of beet pigments—often used to simulate red color in meat analogs—is associated with the appearance of a yellow-like brown color after thermal treatment when used in plant-based analogs. Herein, we simulated the color of raw and cooked beef patty samples by combining beet powder (BP) and caramel color (CC). We hypothesized that CC could partially simultaneously offset the high brightness in raw samples and the excessive yellowness in cooked samples that was caused by BP. We selected beef as reference material and utilized myoglobin-removed beef to produce patties incorporating BP and CC to investigate whether BP and CC can potentially replicate beef color even in the meat analogs based on animal tissues. This approach enabled us to assess the impact of BP and CC on the color characteristics of the patties in the absence of myoglobin. The novelty of this work is a multivariate statistical approach using response surface methodology (RSM) to optimize the levels of BP and CC to simulate the color of beef before and after cooking by considering the interactions between the two pigments.

2. Materials and methods

2.1. Sample preparation

Fresh beef (*semitendinosus*) and food grade BP and CC were purchased from a local market (Daejeon, Korea). Total of five carcasses (five batches) were obtained in five different days for the preparation of the source of beef with removed myoglobin (BRM). The excessive fats and connective tissues were removed, and the muscle was pulverized with a food mixer (C4 vv, Sirman SpA, Italy). To remove myoglobin (Lee et al., 2021), the ground beef was then mixed with an ice-cold buffer containing 0.1 M KCl, 2 mM $MgCl_2$, 1 mM ethylene glycol tetraacetic acid, and 10 mM potassium phosphate (pH 7.0) in a 1:3 (w/w) ratio. The mixture was homogenized at 13,000 rpm for 30 s and centrifuged at 5,000 × g for 10 min to separate the pellet. This procedure was repeated three times. Then, 0.1 M NaCl was added to the pellet in a ratio of 1:3 (w/w), and the homogenization and centrifugation were conducted three more times using the same conditions as described above. The pellet was washed with distilled water and used as BRM. The color attributes of beef before and after the removal of myoglobin are represented in Table 1.

2.2. Design of experiments

The central composite design of RSM was used to set the optimum levels and the interactions between the independent factors influencing responses. First, we designated two independent factors (BP and CC) to a response (CIE *L**, *a**, and *b** values before and after cooking). Then, a five-level two-factor central composite design was employed, including five replicates of the central points. The coded levels of the independent variables are represented in Supplementary Table 1.

2.3. Preparation of the beef patties

The BRM was used to produce beef patties with appropriate levels of BP and CC to replicate the color of beef (Supplementary Table 1). The BRM and the colors were thoroughly mixed using a food processor (FPM250, De'Longhi-Kenwood Appliances, Treviso, Italy) for 1 min. Then, the mixture was stuffed in a stainless can (5 cm in diameter) and vacuum-packed, followed by heating at 80℃ to reach the core temperature of 75℃. Then, the patty was cooled at 25℃, and the drip was removed to analyze the color. Five patties were prepared in independent days for each level to provide replicates for the color measurements and statistical analysis. Figures 1A and 1B show the appearance of the beef patties with five levels of BP and CC.

2.4. Color measurements

The color attributes (CIE L^*, a^*, and b^* values) were measured using a spectrophotometer (CM-5, Konica Minolta, Inc., Osaka, Japan) at two different parts of the cut inner surface (illumination area of 30 mm, illuminant D65, and standard observer of 10°). Blooming proceeded for 30 min before the color measurement. The average value of the two observation values was used as results for the analysis using Spectra Magic Software (SpectramagicTM NX, Konica Minolta).

2.5. Data analysis

Optimization of the concentration levels of the BP and CC was performed by RSM using Minitab 19 Statistical Software (Minitab Inc., State College, PA, USA). Polynomial regression models were used to predict the responses, as presented in Table 2. In the model, the regression coefficients of the intercept, linear, quadratic, the involved interaction, and the probability level of $P<0.05$ were employed using one-way analysis of variance (ANOVA) to statistically test their effects. The response surface and contour plots were obtained by the Minitab software.

The models were validated using the coefficient of determination (R^2), residual analysis, lack-of-fit test, and absolute average deviation (AAD). The first three analyses were conducted in Minitab software, and AAD was calculated using the following equation:

$$\mathrm{AAD} = \left\{ \left[\sum_{i=1}^{p} \left(|y_i \mathrm{exp} - y_i \mathrm{cal}| / y_i \mathrm{exp} \right) \right] / p \right\} \times 100$$

where p, $y_i$exp, and $y_i$cal indicate the experiment number, experimental response, and calculated response, respectively (Yolmeh & Jafari, 2017).

The levels (%) of beet powder and caramel color were optimized by the overall desirability features by setting nominal the best characteristics as the color of beef (L^*, a^*, and b^* value before and after cooking). T-tests were carried out to determine the significance of the differences between the color of beef and the optimized color of the patties.

3. Results and discussion

3.1. Color of beef before and after cooking

Table 3 shows the changes in the beef color before and after cooking based on the measured CIE *L**, *a**, and *b** values. Moreover, the appearance of the beef before and after cooking is represented in Figures 1C and 1D. After cooking, the *L** value increased from 34.00 (before cooking) to 45.05 ($P<0.05$) while the *a** value (21.76 before cooking, and 6.69 after cooking) and *b** value (18.33 before cooking, and 14.62 after cooking) decreased ($P<0.05$). These results indicate that the cooking process increased the lightness and decreased the redness and yellowness.

Myoglobin, a pigment responsible for the meat color, is an oxygen-binding protein (Tomasevic et al., 2021). When meat is exposed to oxygen after being cut, oxymyoglobin is formed under the presence of oxygen at normal atmospheric pressure, favoring the bright red color of fresh meat (King & Whyte, 2006). The surface of the inner cut of the beef patties was exposed to air for 30 min before the color measurement, resulting in the formation of oxymyoglobin as the predominant form of myoglobin in the analyzed parts of the raw beef. Conversely, the temperature of the beef patty's core reached 75°C during the cooking process. At this temperature, most oxy- and deoxymyoglobin contents denatured and transitioned into ferrohemochrome and then ferrihemochrome with a characteristic brown color (Suman & Joseph, 2013), resulting in a decrease in the redness. Notably, although we consider the thermally induced color changes as the main cause of "browning," the denaturation of myoglobin and other meat proteins can also form a gray color, which can increase the *L** value and decrease the *b** value. Although myoglobin accounts for only 0.5% of the total weight of red meat (King & Whyte, 2006), this protein pigment apparently changes meat color before and after cooking. The corresponding changes in the color should also be simulated in the meat analog.

3.2. Central composite design results and validation of the fitted model

Herein, the concentration levels of BP and CC were optimized using central composite design. We set the highest levels of BP and CC to 4.5% and 1.7%, respectively, based on our pilot study. Color measurements were carried out by setting a goal for the levels of BP and CC to reach the levels corresponding to raw and cooked natural beef. Table 2 shows the fitted polynomial equations for the responses (*L**, *a**, and *b** values before and after cooking). The fitted models were all quadratic by considering both the second-order and interaction equations of the dependent variables. All the models had *P* values less than 0.05, indicating that the models are significant. All terms in the models were also significant ($P<0.05$, data not shown).

In this study, R^2, *P* value of the lack-of-fit test, residual analysis, and AAD were used to validate the fitted models (Table 2). High R^2 and adjusted R^2 values were observed in all models, indicating the high accuracy of the applied models (Yomeh & Jafari, 2017). Moreover, the predicted R^2 values were 0.96, 0.92, and 0.93 for *L**, *a**, and *b** values, respectively, before cooking, and 0.98, 0.95, and 0.62 for *L**, *a**, and *b** values, respectively, after cooking. The difference between the predicted and adjusted R^2 was less than 0.2 in all cases, indicating the existence of a reasonable agreement. As Table 2 shows, all the *P* values in the lack-of-fit test were higher than 0.05, confirming that the polynomial equation can adequately describe the data.

In the residual analysis, normal probability plot and the residual errors versus run plot were obtained (Supplementary Figures 1 and 2) to confirm whether the residuals follow the normal distribution or not. In the normal probability plot, if the residual points follow a linear trend, the normal distribution of data is confirmed. Moreover, in the residual versus run plot, the residuals are arranged based on the order of the experimental runs; therefore, all data points should be randomly scattered to follow the normal distribution (Deepanraj et al., 2021). Herein, all the normal probability and residual versus run plots for the equations indicated that our data is normally distributed (Supplementary Figures 1 and 2).

Finally, we assessed AAD and statistical variability (Yomeh & Jafari, 2017). All the calculated AAD values were less than 11%, implying that the models had high accuracy as Zirebwa et al. (2014) reported. Therefore, according to the tests for model validation, the fitted models in Table 2 could be successfully employed for the optimization process.

Figures 2 and 3 show the response surface and contour plots for *L**, *a**, and *b** values before and after cooking. The response surface plots showed ridge surfaces. Observations showed that the level of BP had a direct correlation with *a** values before and after cooking (Figures 2B, 2E, 3B, and 3E) and *b** value after cooking (Figures 2F and 3F). CC had a direct relationship with *a** value before cooking (Figures 2B and 3B) and *b** values before and after cooking (Figures 2C, 2F, 3C, and 3F). The *L** value showed an inverse relationship with both the levels of BP and CC (Figures 2A, 2D, 3A, and 3D).

3.3. Optimization of the levels of BP and CC in beef patties

In RSM, desirability analysis was used to perform the multi-objective optimization considering a total of six responses: before and after cooking *L**, *a**, and *b** values. The independent variables BP and CC were employed to optimize the responses by setting the nominal best characteristics as the color of beef. We obtained three solutions with the highest overall desirability. The setting satisfying the highest desirability values close to 1 is considered to be the optimum condition. Table 4 shows three combinations of the BP and CC with the highest overall desirability. Although the overall desirability of 1 was set as the goal (Yolmeh & Jafari, 2017), the six responses that we optimized to simulate BP before and after cooking resulted in overall desirability values between 0.60 and 0.65 for the combined objectives.

Next, the validation of the optimum values was conducted to evaluate the performance properties of the input parameters. A set of experiments was conducted by setting the optimum levels of BP and CC using the same experimental steps to obtain the model, and the output color responses were measured (Table 3). Results showed that the AAD of the patties made by the optimized solutions was below 14%, compared to the predicted values (Supplementary Table 2), indicating that the fitted model successfully predicted the responses. There were no significant color differences between the patties made by the levels of three optimized solutions and real raw beef ($P>0.05$). This indicates that the optimized levels of BP and CC could well simulate the color of raw beef. However, after cooking, all the color parameters of the three patties had significant differences compared to real cooked beef ($P<0.05$) except for solution 3 where there was no significant difference between the L^* value of the patty and that of the real beef ($P>0.05$). This result implies that the combination of BP and CC could not accurately simulate the color of cooked beef. This might be related to the relatively low desirability (Table 4) for which we had to use six responses during the optimization. Nevertheless, the optimized levels of the three solutions showed similar trends in the L^* value (increasing) and the a^* value (decreasing) after cooking. However, the b^* value of the patties increased after cooking, while the b^* value of the real beef decreased after cooking. Moreover, as Figures 1C and 1D show, the patties that were made by the levels suggested by solutions 1, 2, and 3 exhibited a color appearance similar to real beef. The combination of BP and CC also successfully simulated the color change from red to brown upon cooking, judged by the reduction of the excessive yellow-like-brown color to some degree. A lower redness in the patties compared to beef was probably due to the color offset caused by the increase in the yellowness, simulating the thermally induced "browning" of beef. In Figure 1E, the appearance of oven-cooked patties is represented. Solution 3 had the most color similarity to beef, and its L^* value after cooking showed no significant difference from that of beef. Therefore, considering the results in both the instrumental color and the appearance of the patties, 1.30% of BP and 1.51% of CC can be the optimum condition to simulate beef color.

The use of BP or beet extract to induce the color change of meat analog is limited due to their excessive expression of yellowness. In Figure 1, the patties added with beet powder but no CC also exhibit a purple color before cooking and yellow- or pink-like brown after cooking in both before and after cooking. Hence, a combination of the beet powder and CC is used to overcome the limitation of beet pigments.

4. Conclusions

This study aimed at stimulating the thermally induced color changes that beef undergoes during cooking. Combinations of BP and CC were used to replicate the "browning" of beef. Five levels of BP and CC were obtained by the central composite design of RSM. The obtained models for the six responses (CIE L^*, a^*, and b^* values before and after cooking) had great accuracy in predicting the response when we monitored the coefficient of determination, P value in lack-of-fit test, residual analysis, and AAD. Using the desirability function, we obtained three solutions for optimized levels of BP and CC: 1.32% of BP and 1.08% of CC, 1.80% of BP and 1.01% CC, and 1.30% of BP and 1.51% of CC. The patties of which color was designed by combining the optimized levels of BP and CC were able to successfully simulate the color of raw beef. However, the cooked patties had different colors when compared with the cooked beef. Nevertheless, the combination of the BP and CC could well simulate the thermally induced increase in the L^* value and the decrease in the a^* value caused by cooking. Moreover, the appearances of the patties were also comparable with the beef before and after cooking. Therefore, the combination of 1.32% of BP and 1.08% CC, with the highest overall desirability in this study, can be used to simulate the changes in the beef color from red to brown during cooking.

Meat color depends on several factors, including the animal species, parts or cuts, distribution of the intermuscular fats, muscle fiber types, and quality characteristics of meat (pH and resulting water-holding capacity). Therefore, future studies are required to simulate the color of meat in the meat analogs depending on the target characteristics of the products.

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Table 1. The color attributes (CIE *L, *a**, and *b** values) of raw and cooked beef before and after removing myoglobin**

	Treatments	Before cooking	After cooking	SEM[1]
*L** value	(+) Myoglobin	34.00[B,b]	45.05[B,a]	1.127
	(–) Myoglobin	69.42[A,b]	71.81[A,a]	0.217
	SEM[1]	1.039	0.488	
*a** value	(+) Myoglobin	21.76[A,a]	6.69[A,b]	0.721
	(–) Myoglobin	2.89[B,a]	0.15[B,b]	0.079
	SEM[1]	0.705	0.170	
*b** value	(+) Myoglobin	18.33[a]	14.62[B,b]	0.900
	(–) Myoglobin	17.20[a]	16.70[A,b]	0.104
	SEM[1]	0.753	0.503	

(+) Myoglobin and (–) Myoglobin indicate the beef before and after removing myoglobin, respectively.

[1]Standard error of the least square mean

[A-B] Different uppercase letters indicate significant differences between means ($P < 0.05$).

[a-b] Different lowercase letters indicate significant differences between means ($P < 0.05$).

Table 2. The fitted polynomial equations for the response surfaces of the color (CIE *L, *a**, and *b** values) of the patties (before and after cooking) with the optimized composition of BP and CC**

Equation	R^2	Adjusted R^2	Lack-of-fit	AAD[1] (%)
Before cooking				
$L * \text{value} = 56.668 - 9.358X_1 - 16.622X_2 + 0.8057X_1^2 + 3.74X_2^2 + 1.347X_1X_2$	0.989	0.982	0.060	5.11
$a * \text{value} = 19.195 + 10.156X_1 - 13.368X_2 - 1.3606X_1^2 + 5.075X_2^2 - 1.579X_1X_2$	0.984	0.972	0.059	10.88
$b * \text{value} = 16.98 - 4.735X_1 + 10.513X_2 + 0.5755X_1^2 - 2.802X_2^2 - 1.07X_1X_2$	0.973	0.953	0.089	10.46
After cooking				
$L * \text{value} = 66.97 - 2.626X_1 - 16.98X_2 - 0.449X_1^2 + 1.827X_2^2 + 1.787X_1X_2$	0.992	0.986	0.767	2.43
$a * \text{value} = 4.841 + 3.087X_1 + 3.714X_2 + 0.0935X_1^2 - 0.092X_2^2 - 1.567X_1X_2$	0.990	0.983	0.645	10.47
$b * \text{value} = 18.00 + 1.487X_1 + 6.32X_2 - 0.151X_1^2 - 1.884X_2^2 - 0.638X_1X_2$	0.819	0.690	0.092	3.96

[1]absolute average deviation

Table 3. Color attributes (CIE *L, *a**, and *b** values) of beef *semitendinosus* and the patty (before and after cooking) that were made by the optimized composition according to the overall desirability**

	Treatments	Before cooking	After cooking	SEM[1]
*L** value	Beef	34.00^{b}	45.05^{B,a}	1.127
	Solution 1	36.46^{b}	48.97^{A,a}	0.409
	SEM[1]	1.039	0.598	
	Beef	34.00^{b}	45.05^{B,a}	1.127
	Solution 2	35.75^{b}	50.01^{A,a}	0.288
	SEM[1]	1.037	1.127	
	Beef	34.00^{b}	45.05^{a}	1.127
	Solution 3	33.72^{b}	46.04^{a}	0.309
	SEM[1]	1.038	0.536	
*a** value	Beef	21.76^{a}	6.69^{B,b}	0.721
	Solution 1	20.84^{a}	11.04^{A,b}	0.330
	SEM[1]	0.706	0.361	
	Beef	21.76^{a}	6.69^{B,b}	0.721
	Solution 2	23.68^{a}	11.38^{A,b}	0.350
	SEM[1]	0.705	0.381	
	Beef	21.76^{a}	6.69^{B,b}	0.721
	Solution 3	19.62^{a}	11.60^{A,b}	0.194
	SEM[1]	0.702	0.254	
*b** value	Beef	18.33^{a}	14.62^{B,b}	0.900
	Solution 1	19.39^{b}	25.18^{A,a}	0.440
	SEM[1]	0.758	0.654	
	Beef	18.33^{a}	14.62^{B,b}	0.900
	Solution 2	18.25^{b}	25.62^{A,a}	0.351
	SEM[1]	0.751	0.607	
	Beef	18.33^{a}	14.62^{B,b}	0.900
	Solution 3	20.51^{b}	26.64^{A,a}	0.357
	SEM[1]	0.751	0.611	

[1]Standard error of the least square mean

$^{A\text{-}B}$ Different uppercase letters indicate significant differences between means ($P < 0.05$).

$^{a\text{-}b}$ Different lowercase letters indicate significant differences between means ($P < 0.05$).

Table 4. Optimum levels of the variables to achieve the desired color of response variables (CIE *L, *a**, and *b** values of beef semitendinosus before and after cooking) with maximum overall desirability**

	BP (%)	CC (%)	Overall desirability
Solution 1	1.32	1.08	0.65
Solution 2	1.80	1.01	0.61
Solution 3	1.30	1.51	0.60

Figure Captions

Figure 1. Appearance of the patties before and after cooking.

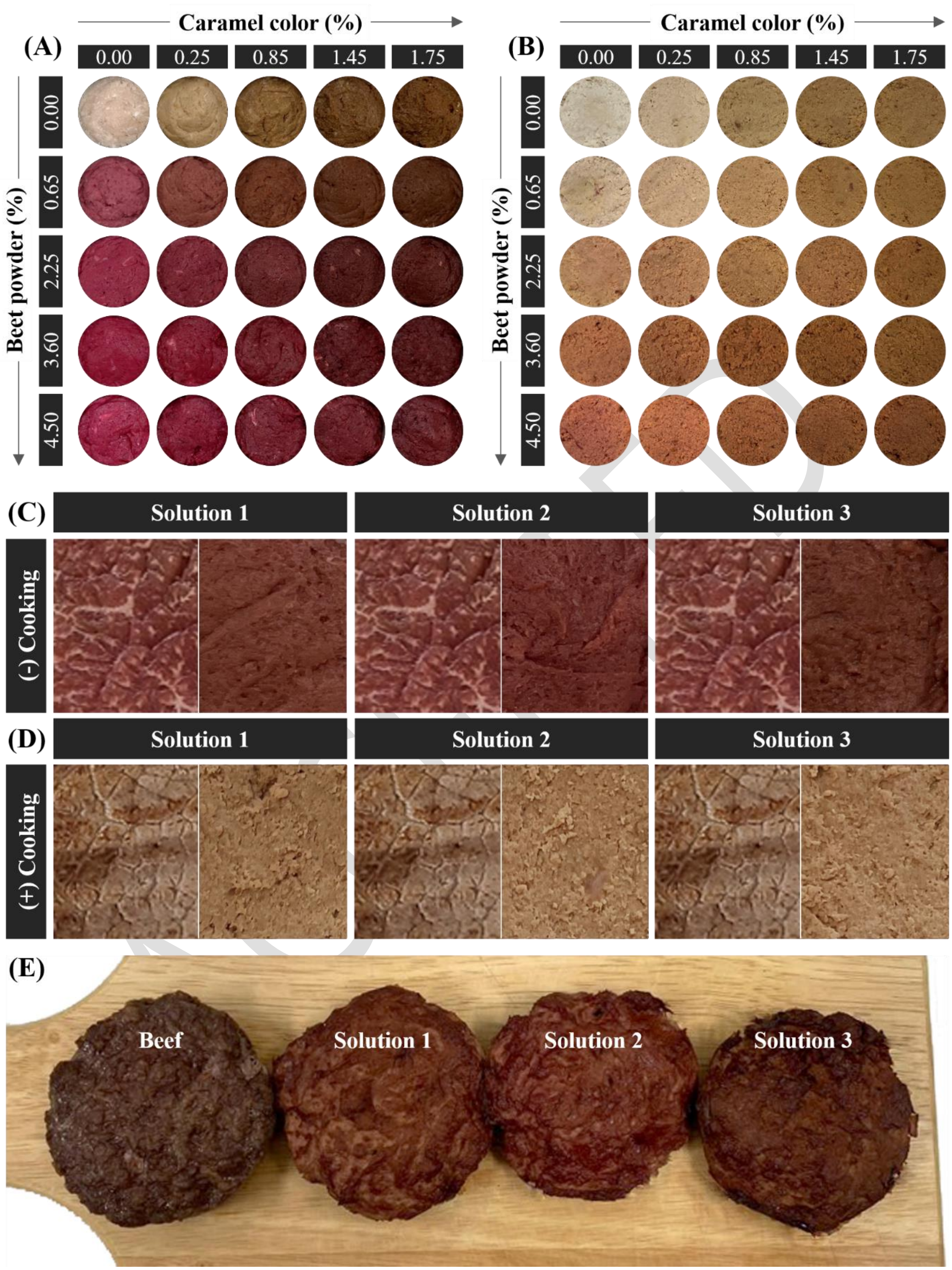
(A) and (B) represent the appearance of the patties before and after cooking, representatively; (C) and (D) represent the appearance of the patties that were made by the optimized composition according to the overall desirability before and after cooking, respectively; (E) shows the appearance of the oven-cooked beef patties that were made by the optimized composition.

Figure 2. Surface plots of the responses (CIE *L*, a, and *b** values of the patties) to the independent variables (levels of beet powder and caramel color).**

(A), (B), and (C) represent the surface plots of the responses as *L**, *a**, and *b** values of the patties before cooking, respectively; (D), (E), and (F) represent the surface plots of the responses as *L**, *a**, and *b** values of the patty after cooking, respectively.

Figure 3. Contour plots of the responses (CIE *L*, a, and *b** values of the patty) to the independent variables (levels of beet powder and caramel color).**

(A), (B), and (C) represent the contour plots of the responses as *L**, *a**, and *b** values of the patties before cooking, respectively; (D), (E), and (F) represent the surface plots of the responses as *L**, *a**, and *b** values of the patty after cooking, respectively.



(A)
Caramel color (%)
0.00
0.25
0.85
1.45
1.75
Beet powder (%)
0.00
0.65
2.25
3.60
4.50
(B)
Caramel color (%)
0.00
0.25
0.85
1.45
1.75
Beet powder (%)
0.00
0.65
2.25
3.60
4.50
(C)
Solution 1
Solution 2
Solution 3
(-) Cooking
(D)
Solution 1
Solution 2
Solution 3
(+) Cooking
(E)
Beef
Solution 1
Solution 2
Solution 3

Figure 1.

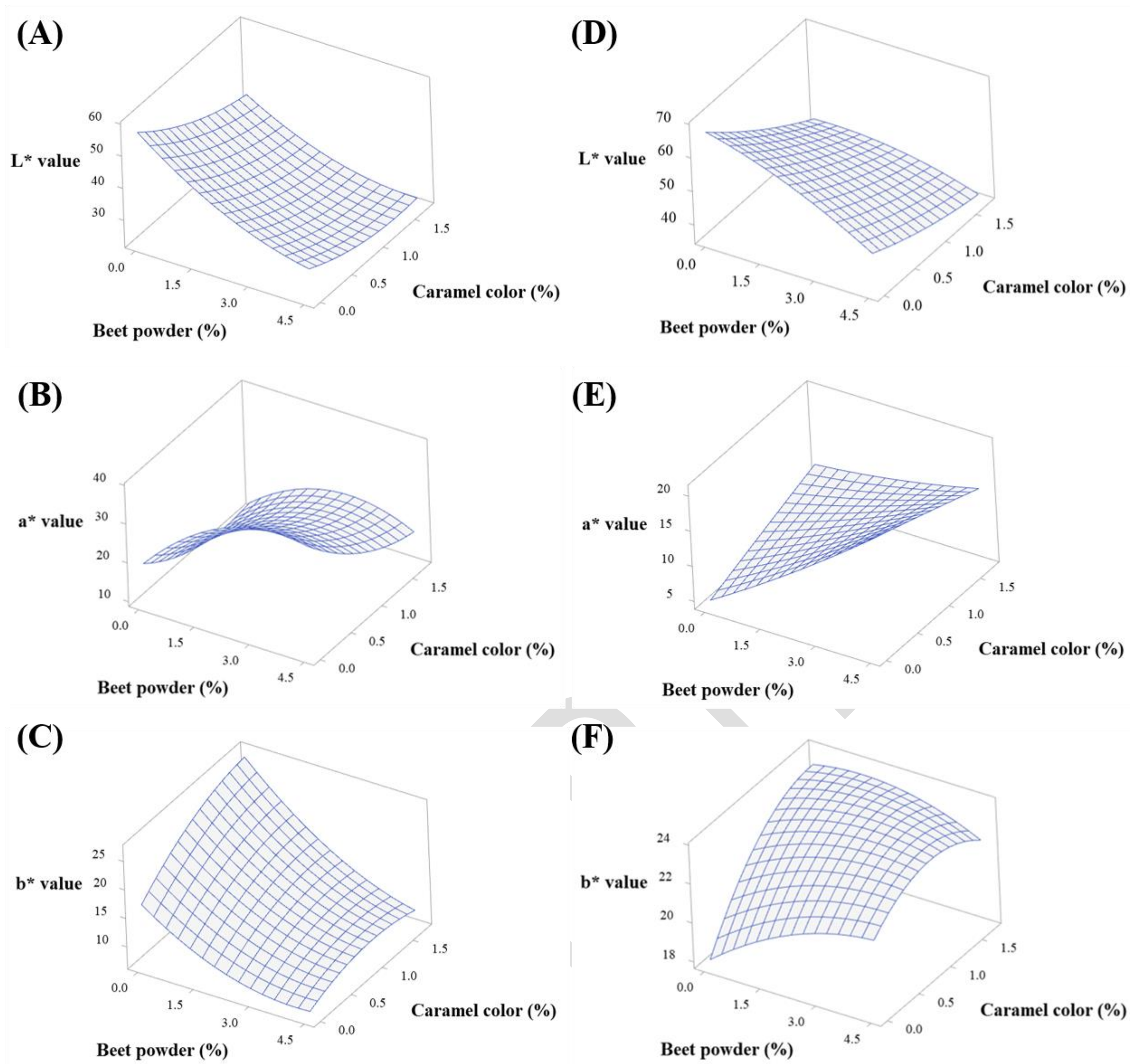

(A)
L* value
Beet powder (%)
Caramel color (%)
(B)
a* value
Beet powder (%)
Caramel color (%)
(C)
b* value
Beet powder (%)
Caramel color (%)
(D)
L* value
Beet powder (%)
Caramel color (%)
(E)
a* value
Beet powder (%)
Caramel color (%)
(F)
b* value
Beet powder (%)
Caramel color (%)

Figure 2.

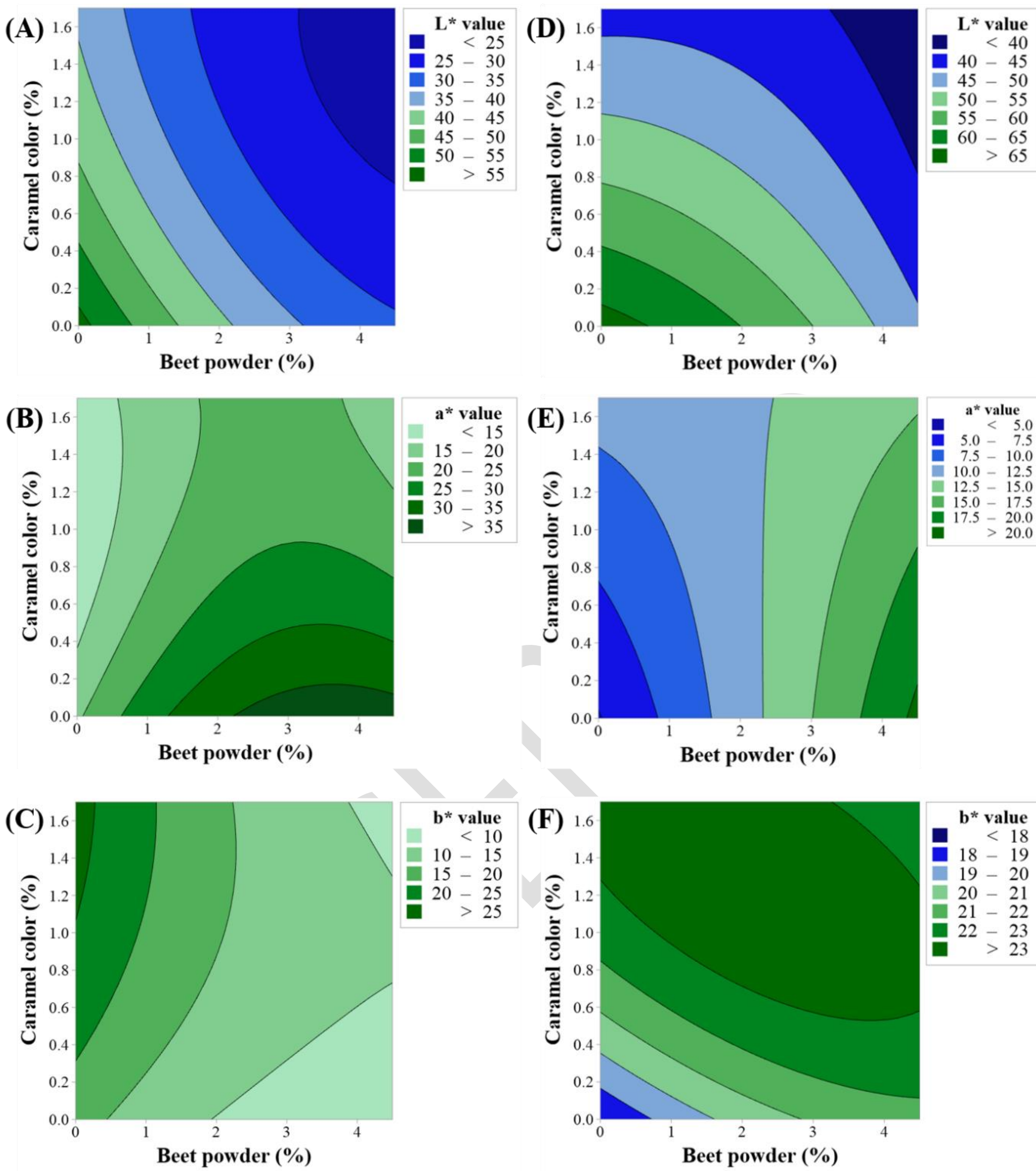

(A)
L* value
< 25
25 – 30
30 – 35
35 – 40
40 – 45
45 – 50
50 – 55
> 55
(B)
a* value
< 15
15 – 20
20 – 25
25 – 30
30 – 35
> 35
(C)
b* value
< 10
10 – 15
15 – 20
20 – 25
> 25
(D)
L* value
< 40
40 – 45
45 – 50
50 – 55
55 – 60
60 – 65
> 65
(E)
a* value
< 5.0
5.0 – 7.5
7.5 – 10.0
10.0 – 12.5
12.5 – 15.0
15.0 – 17.5
17.5 – 20.0
> 20.0
(F)
b* value
< 18
18 – 19
19 – 20
20 – 21
21 – 22
22 – 23
> 23
Caramel color (%)
Beet powder (%)

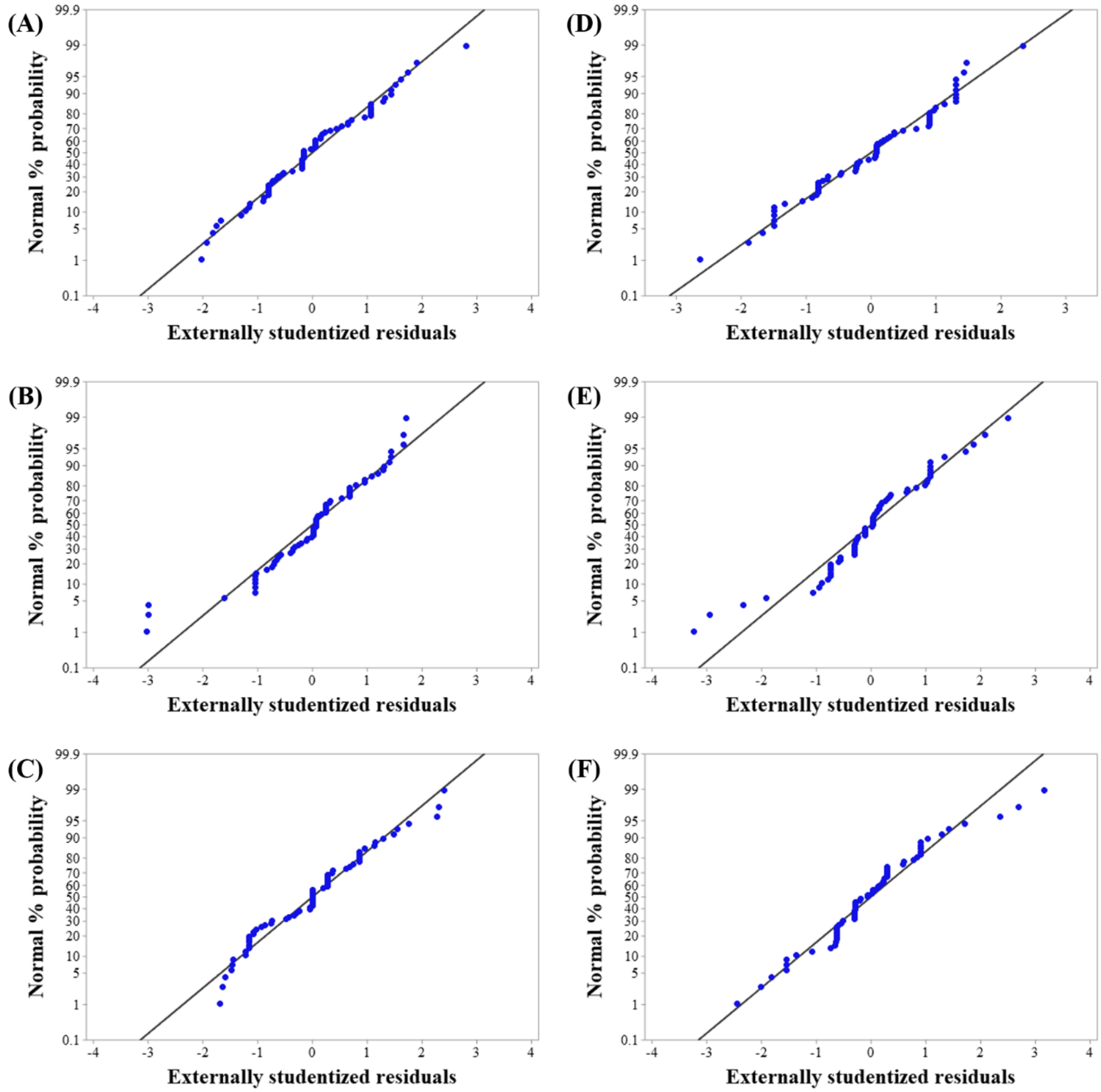
Figure 3.

Supplementary Table 1. The coded levels of independent variables (beet powder (BP) and caramel color (CC)) used for the central composite design

	Coded levels				
Independent variables	-1.414	-1	0	+1	+1.414
BP (wt%)	0.00	0.66	2.25	3.84	4.50
CC (wt%)	0.00	0.25	0.85	1.45	1.70

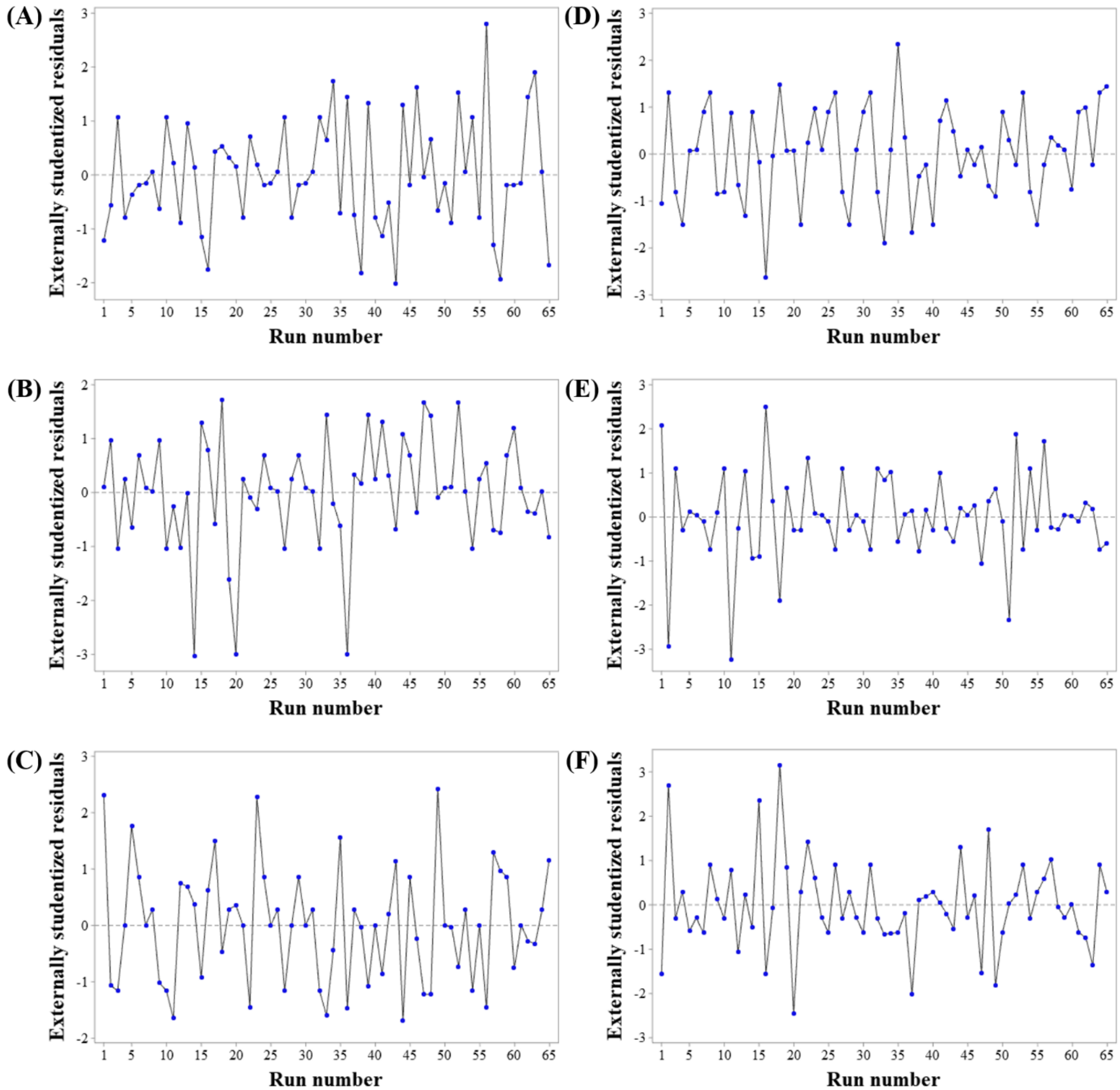
Supplementary Table 2. Average absolute deviation (%) of the optimized experimental responses to the predicted values

	Treatments	Before cooking	After cooking
*L** value	Solution 1	7.07	1.68
	Solution 2	12.03	2.58
	Solution 3	5.58	2.69
*a** value	Solution 1	7.07	7.11
	Solution 2	8.27	7.49
	Solution 3	6.74	4.74
*b** value	Solution 1	6.02	7.57
	Solution 2	13.10	9.10
	Solution 3	6.93	12.57



Supplementary Figure 1. Normal probability plots of the residuals.

(A), (B), and (C) represent the normal probability plots of the responses as L^*, a^*, and b^* values of the patty before cooking, respectively; (D), (E), and (F) represent the normal probability plots of the responses as L^*, a^*, and b^* values of the patty after cooking, respectively.



Supplementary Figure 2. Residual versus run plot of the residuals.
(A), (B), and (C) represent the residual versus run plots of the responses as *L**, *a**, and *b** values of the patty before cooking; (D), (E), and (F) represent the residual versus run plots of the responses as *L**, *a**, and *b** values of the patty after cooking, respectively.