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# Applications of various natural pigments to a plant-based meat analog

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ABSTRACT

To imitate the color of meat after cooking, natural pigments (red beet, monascus red, oleoresin paprika, sorghum, and cacao) were applied to a plant-based meat analog patty (PBMA). The target color ranges (external- and internal-cooked) for PBMA were set separately by evaluating the color values (L*, a*, and b*) of a Hanwoo (Korean native cattle) beef rib patty (HR). As a result of adding different levels of each pigment, the PBMA with cacao (1.0 mg/g) and sorghum (3.0–4.0 mg/g) pigments matched the external- and internal-cooked target ranges, respectively; however, adding a single pigment to PBMA insufficiently mimicked the external- and internal-cooked target ranges. To overcome the above limitations, red beet and cacao pigments were selected, and the color values of the PBMA with these pigments were optimized using response surface methodology. We obtained the optimized mixing ratio (0.4–1.5 mg/g of red beet and 1.1–1.3 mg/g of cacao pigments) and the sensory evaluation showed the color of PBMA with optimum pigments was most similar to HR, increasing appearance, overall acceptability, and purchase intention. These findings suggested that the cooked meat color can be mimicked by applying natural pigments to PBMA.

## 1. Introduction

Meat analogs, also known as meat alternatives, meat substitutes, imitation meat, and fake meat, are emerging food products (Sun, Ge, He, Gan, & Fang, 2021). Consumer demand and the global market for meat analogs have grown dramatically over the years (Godfray et al., 2018; Tuso, Stoll, & Li, 2015). Accordingly, its market size is expected to reach 3.5 billion dollars by 2026 from 1.6 billion dollars in 2019 (Boukid, 2021). However, this trend of replacing animal meat with meat analogs poses environmental, animal welfare, and public health concerns (Kumar et al., 2017). With the growth of the global population, the expected increase in meat consumption may lead to environmental and animal issues and the depletion of natural resources (Steinfeld & Gerber, 2010). Although animal meat has been a crucial protein source for humans throughout history, the excessive consumption of red meat poses health problems, including cardiovascular disease and cancer (Kaluza, Wolk, & Larsson, 2012; Mota, Boué, Guillou, Pierre, & Membré, 2019). For these reasons, research on meat analogs is being widely conducted in academia and industry.

Plant-based meat analogs are a type of meat analog made of plant proteins extracted from soybean, wheat, and other legumes (Bonny, Gardner, Pethick, & Hocquette, 2015). Due to the difference between animal and plant protein, previous research on plant-based meat analogs have been conducted to mimic the appearance, nutritional, physicochemical, rheological, and sensory properties of conventional meat (Kumar, 2016; Kyriakopoulou, Keppler, & van der Goot, 2021). Color, in particular, is one of the critical factors to determine product acceptance by consumers, thereby directly affecting consumer purchase decisions (Resurreccion, 2004). Myoglobin, a globin protein consisting of a heme group, is the main color pigment of animal meat, resulting in fresh meat's light, bright-red color (Suman & Joseph, 2013). During cooking, myoglobin is denatured by heat, resulting in a dull-brown color (Mancini & Hunt, 2005). Unlike animal meat, the color of plant-based meat analogs is yellow due to the absence of myoglobin in textured vegetable protein. In fact, the yellow color of plant-based meat analog makes it difficult to be perceived as real meat and to provide a meat-like sensory experience to consumers (He, Evans, Liu, & Shao, 2020). Therefore, color is an important hurdle to overcome for plant-based meat analogs.

Recently, there have been a few attempts to mimic the color of meat using natural colorants and leghemoglobin. For instance, beet juice extract was used in the "Beyond Meat™", tomato paste was used in the "MorningStar Farms™", and lycopene and carrot juice extract was used

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in the "Tofurky™". Moreover, soy leghemoglobin was utilized in the "Impossible Food™", conferring meat-like color due to its chemical and structural similarity to myoglobin (Bohrer, 2019). A few studies evaluated the color by applying one or two natural pigments to meat analogs; however, research on the color of meat analogs is still lacking in academia (Bakhsh et al., 2022; Wen, Kim, & Park, 2022). To the best of our knowledge, no studies have evaluated the cooked color of plant-based meat analog with various natural pigments and optimized color combinations to mimic real meat color. In this study, natural pigments (red beet, monascus, oleoresin paprika, sorghum, and cacao) were applied to a meat analog in different concentrations to evaluate whether they could mimic the color of cooked beef (Hanwoo, Korean native cattle). Furthermore, the mixing ratio of the pigments was optimized by use of the response surface methodology (RSM).

## 2. Materials and methods

### 2.1. Materials

Fresh Hanwoo (Korean native cattle) beef ribs were purchased from a local market. Non-colored plant-based meat analog batter was obtained from Nongshim Taekyung Co. Ltd. (Seoul, Korea). Its main ingredients included textured vegetable protein (soy protein), palm oil, canola oil, methylcellulose, apple extract, and salt. Commercial natural pigments from red beet, monascus red, sorghum, cacao (Jeys FI Co. Ltd., Seongnam, Korea), and oleoresin paprika (Esfood Co. Ltd., Pocheon, Korea) were purchased.

### 2.2. Sample preparation

#### 2.2.1. Preparation of meat and meat analog patties

Hanwoo beef ribs were minced using an electric meat grinder (DFG 450, Daehan Food Machine Co. Ltd., Siheung, Korea). For the Hanwoo beef rib patty (HR), 80 g of minced meat was shaped to a diameter of 9 cm and a thickness of 1.3 cm. After thawing the frozen plant-based meat analog batter, 20 g of each batter with different levels (0.0, 1.0, 2.0, 3.0, and 4.0 mg/g) of five natural pigments (red beet, monascus red, oleoresin paprika, sorghum, and cacao), respectively, was mixed by hand for 5 min. The plant-based meat analog patty (PBMA) was shaped to a diameter of 3.5 cm and to a thickness of 1.3 cm. Three patties of each batch were prepared, and all experiments were conducted in triplicate.

#### 2.2.2. Cooking

The HR and PBMA were cooked individually on a pan at 170–200 °C and were turned every 1 min until the internal temperature reached 75 °C. The internal temperature was measured by inserting a probe thermometer horizontally into the geometric center of the patty. After reaching the desired temperature, the patties were removed from the pan, immediately blotted with paper towels, and allowed to cool at room temperature for 20 min before measuring their color property.

### 2.3. Color

The color of cooked HR and PBMA were measured for L* (lightness), a* (redness), and b* (yellowness) values using a Minolta Colorimeter (Model CR-400, Minolta Co., Ltd., Osaka, Japan). The colorimeter was calibrated against a standard white plate (L* = 96.94, a* = 0.07, and b* = 2.33). Color determinations were randomly carried out at different locations of each external-cooked (grilled surface after cooking) and internal-cooked (cut in half parallel to the grilled surface after cooking) patties, and all measurements were conducted in triplicate. The appearances of cooked HR and PBMA were photographed using a digital camera (Olympus C-7070, Olympus Optical Co. Ltd., Tokyo, Japan). The photos showed the effects of cooking and pigment application on external- and internal-cooked patties.

### 2.4. Experimental design

The effect of red beet and cacao pigments on the color values (L*, a*, and b*) of PBMA was investigated using RSM with a central composite design. Ten experimental runs were performed with two replications on the central point and simple runs for the other points of the central composite design. Triplicate experiments were carried out for all determinations. Five levels of red beet (0.0, 1.0, 2.0, 3.0, and 4.0 mg/g) and cacao (0.0, 1.0, 2.0, 3.0, and 4.0 mg/g) pigments were used as two independent variables. The response variables were color values of external- and internal-cooked PBMA. The prediction of the response variable was given by a second-order polynomial model, with the equation as follows:

$$Y = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i} \sum_{j=i+1} \beta_{ij} X_i X_j$$

where Y is the response variables; $\beta_0$, $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ are the coefficients of the intercept, linear, quadratic, and interaction, respectively; and $X_{i\text{-}j}$ are independent variables. The fitness of the models was determined by analysis of variance (ANOVA), coefficient of determination ($R^2$), and lack of fit using the SAS software (version 9.1, SAS Institute Inc., Cary, NC, USA). The final interpretation of the models was plotted with SigmaPlot software (SigmaPlot 10.0, SPSS Inc., Chicago, IL, USA).

### 2.5. Sensory evaluation

The sensory analysis was conducted to compare the external- and internal-cooked HR with PBMA. The patties were cooked as described above and were served randomly to each panelist on a white polystyrene plate coded with 3 digits to minimize bias. Twenty-five untrained panelists (male and female) aged between 20 and 30 years old were evaluated for the sensory evaluation of external- and internal-cooked patties using a 9-point hedonic scale (1 = brown color or extremely dislike, 9 = red color or extremely like). The panelists were asked to evaluate the color, appearance, overall acceptability, and purchase intention of coded samples.

### 2.6. Statistical analysis

All data were analyzed using SPSS statistics (SPSS Inc., USA) except for RSM. Significant differences were analyzed using one-way ANOVA and Duncan's multiple range test with a significance level of 5%.

## 3. Results and discussion

### 3.1. Color of cooked PBMA and HR

The color values (L*, a*, and b*) of PBMA and HR are shown in Table 1. Comparing cooked HR and PBMA, the L* and b* values of PBMA were significantly higher than those of HR ($p < 0.05$). The a* value of external-cooked PBMA was significantly higher than that of the external-cooked HR; however, the a* value of the internal-cooked HR showed the reverse results ($p < 0.05$). Underlying the differences in color between PBMA and HR are their different components. The yellow-based color of PBMA could be attributed to the original color of protein isolate ingredients (Kyriakopoulou, Dekkers, & van der Goot, 2019), and the brown color of cooked HR was attributed to myoglobin. Accordingly, myoglobin, an oxygen and iron binding protein in fresh raw meat, exists in various redox states, such as deoxymyoglobin, oxymyoglobin, and metmyoglobin (Mancini & Hunt, 2005). When the meat is cooked, the globin is denatured and exposes the heme group, inducing oxidation. The denatured metmyoglobin promotes the formation of brown ferrihemochrome, resulting in the brown color of cooked meat (King & Whyte, 2006; Suman & Joseph, 2013). Thus, we confirmed that PBMA requires color adjustments to match the cooked color of HR as

**Table 1**
Color values (L*, a*, and b*) of cooked PBMA with different concentrations (%, w/w) of natural pigment, HR, and target color range.

| Treatment (mg/g) | | External-cooked | | | Internal-cooked | | |
|---|---|---|---|---|---|---|---|
| | | L* | a* | b* | L* | a* | b* |
| PBMA (Control) | 0 | 42.3 ± 0.4$^{b}$ | 14.2 ± 0.7$^{k}$ | 28.4 ± 0.1$^{c}$ | 65.0 ± 0.2$^{a}$ | 3.8 ± 0.2$^{m}$ | 24.2 ± 0.6$^{d}$ |
| Red beet | 1 | 47.6 ± 0.8$^{a}$ | 20.5 ± 0.5$^{i}$ | 25.9 ± 1.1$^{d}$ | 58.4 ± 0.4$^{b}$ | 16.7 ± 0.1$^{g}$ | 16.9 ± 0.3$^{h}$ |
| | 2 | 37.5 ± 0.3$^{d}$ | 21.9 ± 0.2$^{h}$ | 23.7 ± 0.2$^{e}$ | 53.8 ± 0.3$^{d}$ | 22.9 ± 0.5$^{d}$ | 12.7 ± 0.2$^{jkl}$ |
| | 3 | 36.6 ± 0.7$^{de}$ | 26.3 ± 0.9$^{c}$ | 20.4 ± 0.0$^{fg}$ | 52.6 ± 0.3$^{d}$ | 26.3 ± 0.6$^{c}$ | 12.5 ± 0.5$^{kl}$ |
| | 4 | 35.6 ± 0.2$^{e}$ | 28.6 ± 0.8$^{a}$ | 20.1 ± 0.3$^{fg}$ | 51.0 ± 0.2$^{e}$ | 27.9 ± 0.8$^{ab}$ | 12.6 ± 0.5$^{jkl}$ |
| Monascus red | 1 | 42.9 ± 0.5$^{b}$ | 23.9 ± 0.5$^{f}$ | 26.8 ± 0.2$^{d}$ | 56.5 ± 1.4$^{c}$ | 19.1 ± 1.6$^{f}$ | 22.5 ± 0.1$^{e}$ |
| | 2 | 36.3 ± 0.7$^{e}$ | 26.2 ± 0.3$^{cd}$ | 21.1 ± 0.1$^{f}$ | 49.9 ± 0.6$^{ef}$ | 26.8 ± 0.5$^{bc}$ | 21.5 ± 1.0$^{e}$ |
| | 3 | 35.9 ± 1.0$^{e}$ | 27.1 ± 0.6$^{b}$ | 21.1 ± 0.3$^{f}$ | 49.7 ± 0.9$^{ef}$ | 26.6 ± 1.1$^{c}$ | 21.3 ± 0.9$^{e}$ |
| | 4 | 33.8 ± 0.3$^{f}$ | 28.5 ± 0.4$^{a}$ | 18.3 ± 0.4$^{h}$ | 47.4 ± 0.2$^{g}$ | 28.4 ± 0.6$^{a}$ | 19.8 ± 0.1$^{f}$ |
| Oleoresin paprika | 1 | 42.5 ± 1.1$^{b}$ | 20.6 ± 0.6$^{i}$ | 32.7 ± 0.7$^{b}$ | 58.0 ± 0.3$^{b}$ | 17.0 ± 0.4$^{g}$ | 44.5 ± 1.8$^{c}$ |
| | 2 | 42.2 ± 0.6$^{bc}$ | 22.8 ± 0.4$^{g}$ | 33.2 ± 0.5$^{b}$ | 55.9 ± 1.1$^{c}$ | 18.9 ± 1.6$^{f}$ | 48.7 ± 1.9$^{b}$ |
| | 3 | 41.1 ± 0.6$^{c}$ | 25.1 ± 0.3$^{e}$ | 36.5 ± 1.1$^{a}$ | 55.7 ± 1.3$^{c}$ | 21.5 ± 0.9$^{e}$ | 51.1 ± 0.8$^{a}$ |
| | 4 | 41.9 ± 0.8$^{bc}$ | 25.4 ± 0.2$^{de}$ | 36.4 ± 1.3$^{a}$ | 56.3 ± 0.2$^{c}$ | 21.3 ± 1.1$^{e}$ | 49.4 ± 1.6$^{b}$ |
| Sorghum | 1 | 35.9 ± 0.5$^{e}$ | 15.4 ± 0.4$^{j}$ | 19.5 ± 1.5$^{g}$ | 52.4 ± 0.3$^{d}$ | 10.0 ± 0.2$^{j}$ | 18.7 ± 0.2$^{fg}$ |
| | 2 | 32.0 ± 1.5$^{g}$ | 15.5 ± 0.4$^{j}$ | 16.1 ± 0.8$^{i}$ | 48.6 ± 1.1$^{fg}$ | 11.2 ± 0.6$^{ij}$ | 18.1 ± 0.2$^{gh}$ |
| | 3 | 29.1 ± 0.3$^{hi}$ | 12.9 ± 0.2$^{l}$ | 11.2 ± 0.3$^{k}$ | 41.2 ± 1.5$^{i}$ | 12.6 ± 0.6$^{h}$ | 13.7 ± 0.4$^{ijk}$ |
| | 4 | 25.8 ± 0.4$^{j}$ | 10.8 ± 0.3$^{m}$ | 8.5 ± 0.2$^{l}$ | 37.0 ± 0.2$^{k}$ | 12.1 ± 0.4$^{hi}$ | 12.6 ± 0.9$^{jkl}$ |
| Cacao | 1 | 29.5 ± 0.8$^{h}$ | 8.7 ± 0.1$^{o}$ | 11.6 ± 0.2$^{k}$ | 45.9 ± 0.9$^{h}$ | 6.2 ± 0.2$^{k}$ | 14.5 ± 0.7$^{i}$ |
| | 2 | 24.6 ± 0.5$^{k}$ | 5.3 ± 0.5$^{p}$ | 5.6 ± 0.3$^{m}$ | 45.6 ± 0.4$^{h}$ | 5.3 ± 0.3$^{kl}$ | 14.2 ± 0.6$^{ij}$ |
| | 3 | 22.9 ± 0.5$^{l}$ | 3.6 ± 0.2$^{q}$ | 3.5 ± 0.5$^{n}$ | 39.1 ± 1.4$^{j}$ | 5.3 ± 0.2$^{kl}$ | 11.6 ± 1.2$^{lm}$ |
| | 4 | 23.0 ± 0.2$^{l}$ | 3.9 ± 0.0$^{q}$ | 3.4 ± 0.2$^{n}$ | 35.7 ± 1.5$^{k}$ | 4.8 ± 0.4$^{lm}$ | 10.7 ± 0.4$^{m}$ |
| HR | – | 28.1 ± 0.6$^{i}$ | 9.8 ± 0.4$^{n}$ | 12.8 ± 0.2$^{j}$ | 42.0 ± 0.3$^{i}$ | 11.0 ± 0.4$^{ij}$ | 13.2 ± 0.4$^{ijkl}$ |
| Target color range | | 24–32 | 8–11 | 11–15 | 36–48 | 9–13 | 11–15 |

L*, lightness; a*, redness; b*, yellowness; PBMA, plant-based meat analog patty; HR, Hanwoo (Korean native cattle) beef rib patty; Control, PBMA without pigment. Data are expressed as means ± SD (n = 3). $^{a\text{-}q}$ indicates significant differences at $p < 0.05$ by Duncan's multiple test.

follows: the L* and b* values of cooked PBMA needed to decrease, while the a* values needed to increase (internal-cooked) or decrease (external-cooked) accordingly. We set the target color range of PBMA based on the color values of cooked HR (Table 1). The range was set between 85% and 115% of the average color values (L*, a*, and b*). Several researchers have confirmed the beef color which is similar with the target color range (Ball et al., 2021; Yoo et al., 2020).

### 3.2. Effect of various pigments on the color of cooked PBMA

The color of cooked PBMA when exposed to the various pigments is shown in Table 1 and Fig. 1. For a better visualization, a colorimetric plane of cooked PBMA is provided in Fig. S1 to readily identify whether the target range is met. As the concentration of the pigments increased, L* values of external- and internal-cooked PBMA for all pigments (except for oleoresin paprika) significantly decreased ($p < 0.05$). The lowest values were found at 3.0 and 4.0 mg/g of cacao pigments (external-cooked), and 4.0 mg/g of sorghum and cacao pigments (internal-cooked). As the concentration of the pigments increased, the a* values of external- and internal-cooked PBMA with red beet, monascus red, and oleosin paprika pigments significantly increased, and the highest value was found at 4.0 mg/g of red beet and monascus red pigments ($p < 0.05$). As the concentration of the pigments increased, the b* values of external-cooked PBMA for all pigments (except for oleoresin paprika) significantly decreased ($p < 0.05$); however, the b* values for internal-cooked PBMA did not show a significant trend. Interestingly, PBMA with 1.0 mg/g of cacao and 3.0–4.0 mg/g of sorghum pigments matched with the target color range of external- and internal-cooked HR, respectively.

Accordingly, the difference in the color of PBMA with different pigments was due to specific constituents in each pigment and their changes with heating. For example, red beet pigment contains betacyanin such as betanin and isobetanin, representing pink to red color (Azeredo, 2009). It is known that betanin, a principal component of red beet, is hydrolyzed to betalamic acid (bright-yellow) and cyclodopa 5-O-glucoside (colorless) during heating, thereby decreasing a* value and increasing b* value (Chandran, Nisha, Singhal, & Pandit, 2014; Stintzing & Carle, 2004). In addition, monascus red pigment contains rubropuctamine and monascorubramine, expressing red color, and oleoresin paprika includes carotenoids such as capsanthin and capsorubin, representing orange to red color. These pigments were generally used for varied food products such as duck meat, tofu, and non-meat-based sausages due to their heat-stability (Agboyibor, Kong, Chen, Zhang, & Niu, 2018; Akramzadeh et al., 2018; del Rocío Gómez-García & Ochoa-Alejo, 2013). Regarding the brown pigments, the dark red-brown color of sorghum pigment is attributed to apigeninidin and luteolinidin, and the dark brown color of cacao pigment is owing to the conversion of procyanidin, a main polyphenol in cacao (Afoakwa, Paterson, Fowler, & Ryan, 2008; Dykes & Rooney, 2006). Several studies reported that sorghum and cacao pigments, due to its own color, had a significant effect on the cooked color of meat products such as beef and pork sausages, indicating that these pigments can provide meat analogs with desirable cooked-colors of meat (Choi, Kim, Choi, & Han, 2019; Xiong et al., 2022). Taken together, the addition of 1.0 mg/g of cacao and 3.0–4.0 mg/g of sorghum pigments could mimic the color of external- and internal-cooked beef, respectively. However, our observations revealed that the addition of a single pigments could not satisfy the external- and internal-cooked target color range simultaneously. Thus, a combination of two different pigments was attempted to overcome the limitation of the above approach. Among the pigments screened above, red beet and cacao pigments, which were expected to be acceptable for mixing (as shown in Fig. S1), were selected for RSM.

### 3.3. Model fitting and analysis

The experimental design and responses are shown in Table 2. The results of regression analysis and ANOVA, used for evaluating the statistical significance of the terms, are presented in Table 3. The regression models for color values (L*, a*, and b*) of external- and internal-cooked PBMA were significant ($p < 0.05$). The determination coefficient ($R^2$) ranged between 0.9029 and 0.9772 for all models, which indicates a high correlation between independent variables and responses (Little & Hills, 1978, pp. 63–76; Mendenhall, Beaver, & Beaver, 1975, p. 273). In addition, the lack of fit for all models was not significant ($p > 0.05$),

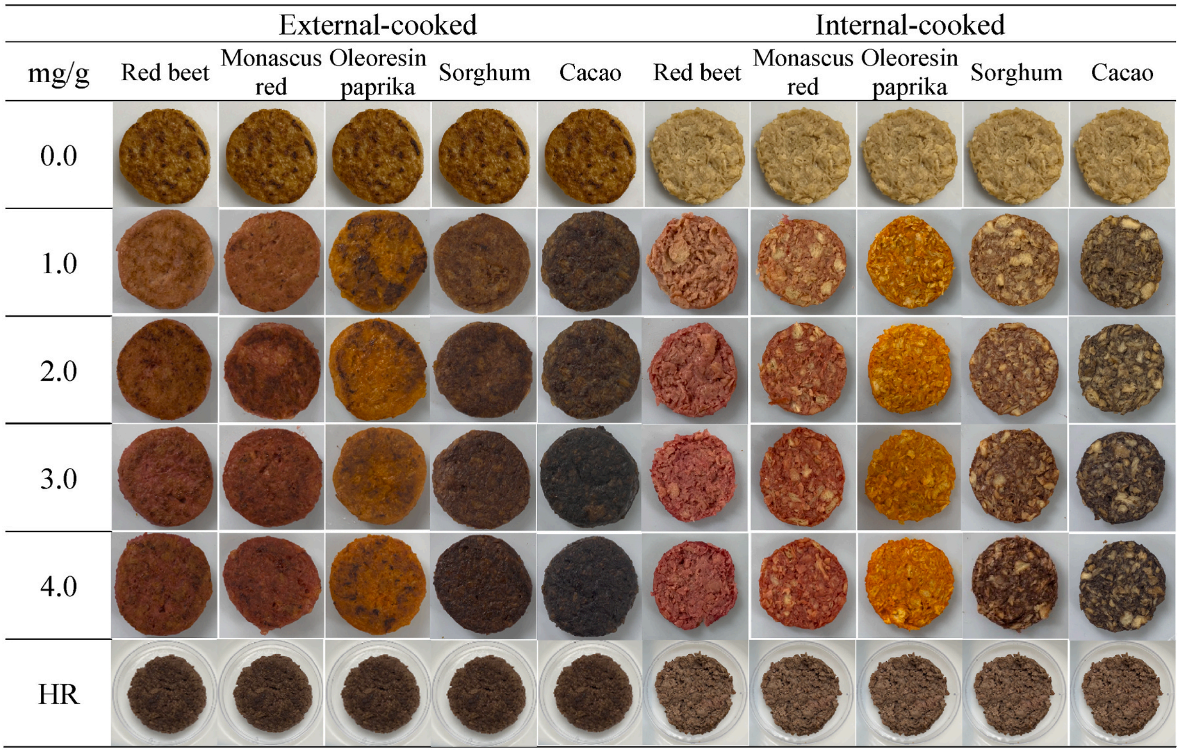


**Fig. 1.** Appearances of external- and internal-cooked PBMA with different concentrations of various pigments. L*, lightness; a*, redness; b*, yellowness; PBMA, plant-based meat analog patty.

**Table 2**
Experimental design and responses for color values (L*, a*, and b*) of PBMA with different concentrations of red beet and cacao.

| Sample no. | Variables | | Responses | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | External-cooked | | | Internal-cooked | | |
| | $X_1$ | $X_2$ | L* | a* | b* | L* | a* | b* |
| 1 | 1 | 1 | 24.67 ± 0.30 | 4.95 ± 0.40 | 4.52 ± 0.36 | 31.84 ± 0.37 | 10.40 ± 0.25 | 8.32 ± 0.64 |
| 2 | 1 | −1 | 31.84 ± 0.37 | 10.40 ± 0.25 | 8.32 ± 0.64 | 39.54 ± 0.56 | 15.22 ± 0.52 | 10.50 ± 0.33 |
| 3 | −1 | 1 | 23.65 ± 0.81 | 3.77 ± 0.14 | 3.80 ± 0.16 | 34.52 ± 0.76 | 7.51 ± 0.37 | 10.06 ± 0.48 |
| 4 | −1 | −1 | 32.79 ± 1.66 | 11.38 ± 0.37 | 12.01 ± 0.95 | 42.08 ± 0.17 | 10.24 ± 0.10 | 11.73 ± 0.14 |
| 5 | 0 | 0 | 26.47 ± 1.09 | 8.17 ± 0.85 | 6.23 ± 0.92 | 38.27 ± 1.02 | 11.04 ± 0.35 | 9.52 ± 0.23 |
| 6 | 0 | 0 | 25.88 ± 0.01 | 7.71 ± 0.55 | 6.53 ± 0.40 | 38.87 ± 1.93 | 11.89 ± 0.84 | 10.31 ± 1.43 |
| 7 | 2 | 0 | 26.11 ± 0.15 | 9.69 ± 0.99 | 6.48 ± 0.10 | 34.66 ± 1.70 | 16.07 ± 0.38 | 8.32 ± 0.08 |
| 8 | −2 | 0 | 37.49 ± 0.30 | 21.90 ± 0.15 | 23.69 ± 0.23 | 53.76 ± 0.28 | 22.89 ± 0.46 | 12.72 ± 0.16 |
| 9 | 0 | 2 | 23.01 ± 0.47 | 6.69 ± 0.91 | 5.52 ± 0.84 | 36.22 ± 0.44 | 11.64 ± 0.77 | 8.93 ± 0.60 |
| 10 | 0 | −2 | 24.61 ± 0.55 | 5.33 ± 0.55 | 5.59 ± 0.27 | 45.59 ± 0.38 | 5.33 ± 0.34 | 14.16 ± 0.55 |

$X_1$, Concentration of red beet (%); $X_2$, Concentration of cacao (%); L*, lightness; a*, redness; b*, yellowness; PBMA, plant-based analog patty.

**Table 3**
Variance analysis of regression model.

| | External-cooked | | | Internal-cooked | | |
|---|---|---|---|---|---|---|
| | L* | a* | b* | L* | a* | b* |
| Intercept | 38.4383* | 21.7850* | 26.9267* | 59.3083* | 14.5267* | 15.9867* |
| $X_1$ | 4.4226 | −4.1988 | −22.9845 | −47.6131 | 37.1833 | −23.8262 |
| $X_2$ | −84.7607* | −115.7155* | −147.9845* | −115.6464* | −77.9667* | −16.9929 |
| $X_1X_2$ | −29.2857 | 2.0804 | −0.1429 | 64.3661 | −5.6875 | 35.4196 |
| $X_1X_1$ | 49.2500 | 54.0000 | 110.2500 | −3.5000 | −52.2500 | −12.7500 |
| $X_2X_2$ | 92.9643* | 171.7054* | 214.1071* | 185.9911 | 158.4375* | 25.0446 |
| $R^2$ | 0.9772 | 0.9535 | 0.9612 | 0.9029 | 0.9441 | 0.9417 |
| *P* value | | | | | | |
| Regression | 0.0022 | 0.0090 | 0.0063 | 0.0373 | 0.0129 | 0.0140 |
| Lack of fit | 0.2453 | 0.1239 | 0.0777 | 0.0905 | 0.2189 | 0.4989 |

$X_1$, concentration of red beet (%); $X_2$, concentration of cacao (%); L*, lightness; a*, redness; b*, yellowness; RC, Regression coefficients; * Significant at $p < 0.05$.

indicating that the models were sufficiently accurate (Varnalis, Brennan, MacDougall, & Gilmour, 2004). These results demonstrated that the models showed good fits ($R^2 > 0.91$, *p*-value of lack of fit >0.05) with the experimental data.

### 3.4. Effects of variables on the color values of cooked (external and internal) PBMA

The color of external-cooked PBMA varied from 23.01 to 37.49 for L* values, 3.77 to 21.90 for a* values, and 3.80 to 23.69 for b* values. The cacao pigment had significant negative linear and positive quadratic effects for all color values ($p < 0.05$). The color of internal-cooked PBMA varied from 31.84 to 45.59 for L* values, 5.33 to 22.89 for a* values, and 8.32 to 14.16 for b* values. The cacao pigment had a significantly negative linear effect on the L* values and negative linear and positive quadratic effects on the a* values ($p < 0.05$), while having a negligible effect on b* values. The red beet pigment had no significant effects on all color values of cooked PBMA, with only cacao pigments having significant effects on cooked PBMA. Fig. 2 shows the response surface plots for the effects of red beet and cacao pigments on cooked PBMA color values. The addition of red beet pigment did not alter the color values of cooked PBMA, except for the a* values of internal-cooked PBMA. The a* values of internal-cooked PBMA decreased due to the red beet pigment; however, it was not statistically significant. All color values of cooked PBMA markedly decreased at low concentrations of cacao pigment but did not change at higher concentrations.

### 3.5. Predicted optimization and verification

To optimize the mixing ratio of the pigments for PBMA to imitate the color of HR, the plots of the responses (L*, a*, and b*) as functions of red beet and cacao concentration were superimposed (Fig. 3a). As a result, the region of external- and internal-cooked target color ranges partially overlapped. Hence, the optimized mixing ratio of the pigments was set at 0.4–1.5 mg/g of red beet and 1.1–1.3 mg/g of cacao pigments as the range of the independent variables corresponding to the part where the ranges of external- and internal-cooked color values overlapped. Verification experiments were thereafter carried out to evaluate the validity of the predicted RSM models. The conditions of the verification experiment were selected as 0.9 mg/g of red beets and 1.2 mg/g of cacao pigment, which are the central points within the optimal range of cooked PBMA. As can be seen in Table 4, the results obtained from the verification experiments were found to be close (less than a 5% difference) to the predicted values, and the appearances of the cooked PBMA also validated these results (Fig. 3b,c). The validated models successfully predicted the color values of PBMA by applying various combinations of red beet and cacao pigments. These observations indicate that the combined application of red beet and cacao pigments successfully achieved external- and internal-cooked target color range.

### 3.6. Sensory evaluation

The sensory evaluation results are shown in Table 5. There were no significant differences in the colors of external- and internal-cooked PBMA with optimum pigments compared to those of HR, indicating that the mixed pigments for PBMA successfully mimicked the HR color. In addition, PBMA with sorghum (3.0 mg/g) obtained higher scores than the HR, and PBMA with cacao (1.0 mg/g) obtained lower scores ($p < 0.05$). The consumers perceived that the PBMA with sorghum (3.0 mg/g) was redder and PBMA with cacao (1.0 mg/g) was more brownish than HR. Moreover, external- and internal-cooked PBMA with optimum pigments obtained similar scores to HR. It may be due to the similarity of the color between them. In particular, the appearance and purchase intention of the internal-cooked PBMA with optimum pigments was not significantly different from that of HR ($p > 0.05$). Taken together, these results confirmed that PBMA with the optimum pigments could be perceived most similarly to HR by consumers.

## 4. Conclusion

In this study, we evaluated the color values of PBMA with various natural pigments to mimic the color of HR. As a single pigment, 1.0 mg/g of cacao and 3.0–4.0 mg/g of sorghum matched the target range of external- and internal-cooked color, respectively. The optimum ratio of pigment mixture to match both external- and internal-cooked target color ranges was found to be 0.4–1.5 mg/g of red beet and 1.1–1.3 mg/g of cacao pigments, which was also confirmed by the results of sensory evaluation. This is the first report comparing the color values of PBMA with single or multiple pigments to those of real beef and can be used as fundamental information in the meat analog industry for imitating the cooked color of animal-based meat.

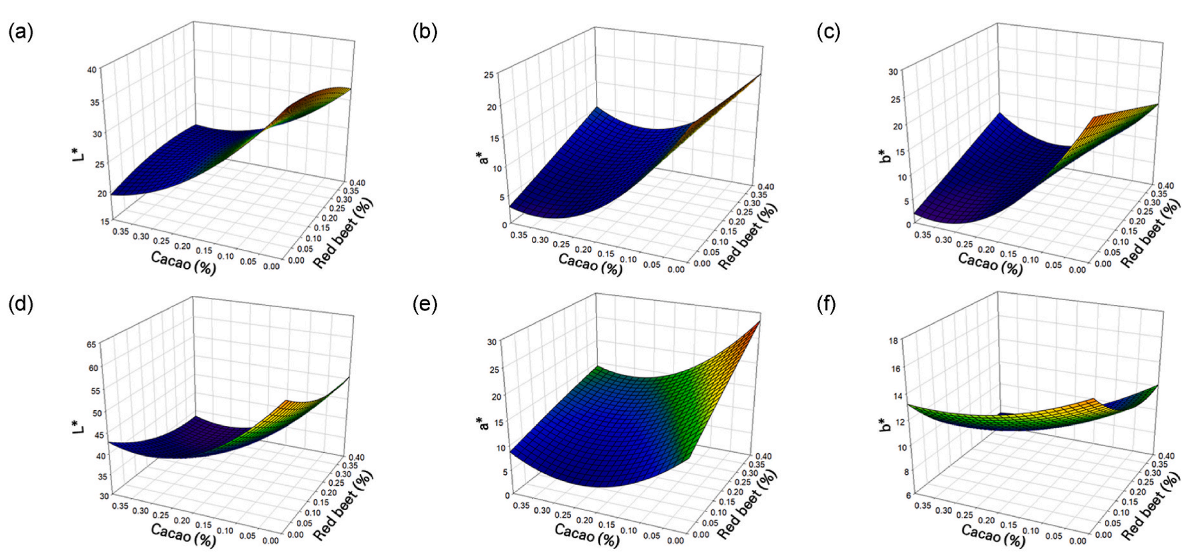


**Fig. 2.** Response surface plots for the effects of red beet and cacao on color values (L*, a*, and b*) of external-cooked (a–c), and internal-cooked (d–f) PBMA. L*, lightness; a*, redness; b*, yellowness; PBMA, plant-based meat analog patty. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

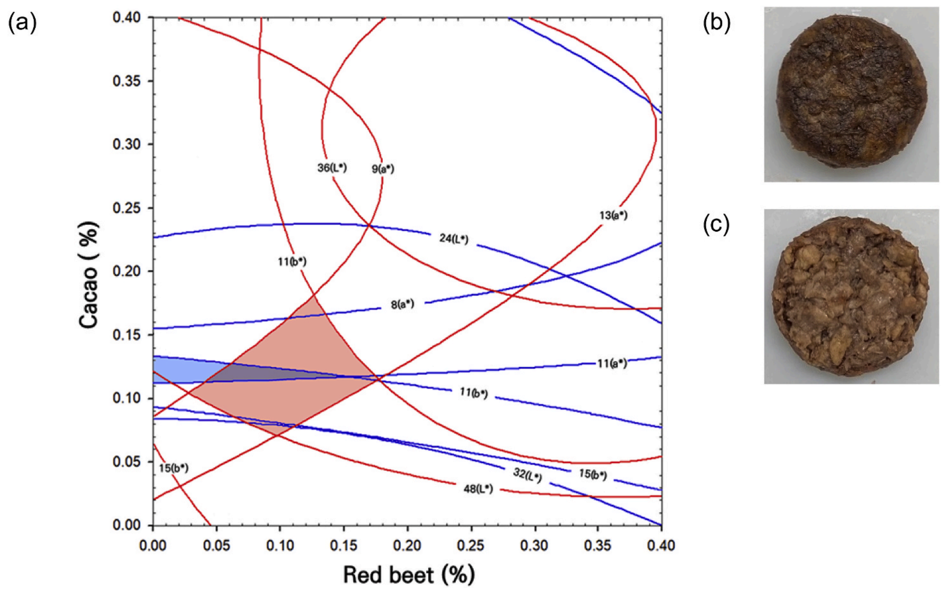


**Fig. 3.** Optimum region (a) for PBMA corresponds to the external-cooked (blue-shaded area), and internal-cooked (red-shaded area) target color range. The appearances of PBMA corresponding to the external-cooked (b) and internal-cooked (c) optimum region. PBMA, plant-based meat analog patty. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**Table 4**
The comparison of predicted and actual values.

| | Predicted value | | | Actual value | | |
|---|---|---|---|---|---|---|
| | L* | a* | b* | L* | a* | b* |
| External-cooked | 29.50 | 10.59 | 11.37 | 30.12 ± 0.86 | 10.23 ± 0.20 | 11.50 ± 0.71 |
| Internal-cooked | 44.31 | 10.19 | 12.31 | 44.67 ± 1.44 | 10.69 ± 0.47 | 12.65 ± 0.85 |

L*, lightness; a*, redness; b*, yellowness.

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## CRediT authorship contribution statement

**Kum Kang Ryu:** Investigation, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft. **Yu Kyeong Kang:** Investigation, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft. **Eun Woo Jeong:** Conceptualization, Investigation, Writing – review & editing. **Youjin Baek:** Conceptualization, Investigation, Writing – review & editing. **Kwang Yeon Lee:** Conceptualization, Investigation, Writing – review & editing. **Hyeon Gyu Lee:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

## Declaration of competing interest

The authors declare no conflict of interest.

## Data availability

Data will be made available on request.

**Table 5**
Sensory evaluation scores of HR and cooked PBMA with different pigments by untrained panelists (n = 25).

| Sensory attribute | | HR | Sorghum | Cacao | Optimum pigments (Red beet + Cacao) |
|---|---|---|---|---|---|
| External-cooked | Color | 2.60 ± 1.61$^{b}$ | 7.28 ± 1.28$^{a}$ | 1.64 ± 0.76$^{c}$ | 3.12 ± 1.76$^{b}$ |
| | Appearance | 7.36 ± 1.70$^{a}$ | 4.60 ± 1.71$^{c}$ | 6.24 ± 1.92$^{b}$ | 6.00 ± 1.87$^{b}$ |
| | Overall acceptability | 7.40 ± 1.32$^{a}$ | 4.56 ± 1.87$^{c}$ | 6.48 ± 1.53$^{ab}$ | 5.84 ± 1.82$^{b}$ |
| | Purchase intention | 7.32 ± 1.49$^{a}$ | 4.24 ± 1.67$^{c}$ | 6.24 ± 1.83$^{b}$ | 5.76 ± 1.76$^{b}$ |
| Internal-cooked | Color | 4.84 ± 1.70$^{b}$ | 6.60 ± 1.29$^{a}$ | 1.64 ± 0.99$^{c}$ | 4.80 ± 1.55$^{b}$ |
| | Appearance | 6.60 ± 1.50$^{a}$ | 4.48 ± 1.42$^{b}$ | 4.16 ± 2.06$^{b}$ | 5.76 ± 1.79$^{a}$ |
| | Overall acceptability | 6.60 ± 1.44$^{a}$ | 4.20 ± 1.47$^{c}$ | 4.24 ± 1.92$^{c}$ | 5.64 ± 1.91$^{b}$ |
| | Purchase intention | 6.40 ± 1.58$^{a}$ | 3.96 ± 1.62$^{b}$ | 4.24 ± 2.03$^{b}$ | 5.80 ± 2.02$^{a}$ |

Sensory evaluations were carried out using a 9-point hedonic scale (1 = brown color or extremely dislike, 9 = red color or extremely like). PBMA, plant-based meat analog patty; HR, Hanwoo (Korean native cattle) beef rib patty; Data are expressed as means ± SD (n = 25). $^{a-d}$ indicates significant differences within a row at $p < 0.05$ by Duncan's multiple test.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.114431.

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