Food Science of Animal Products

Research Article

Color stability of dried pork meat slices containing bamboo leaf extract and *L*-cysteine under drying temperature variation

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Received: March 13, 2023; Revised: March 31, 2023; Accepted: April 9, 2023

Abstract: In this research, the color stability of dried pork meat slices (DPMS) containing 0.2% (*m*/*m*) bamboo leaf extract (BLE) and 0.5% (*m*/*m*) *L*-cysteine (Cys) was evaluated in the scope of air-drying temperature variation 55–75 °C. The results showed that the combined addition of BLE and Cys could reduce the range values of each color parameter of DPMS in the scopes (± 5 and ± 10 °C) of drying temperature variation 55–75 °C, such as the range value of lightness (L^*) from 2.81 to 1.10, redness (a^*) from 2.45 to 1.62, and yellowness (b^*) from 5.80 to 1.11 in the scope (65 ± 5) °C, especially the total color difference (ΔE) of less than 2 in 60–70 °C was below the limits discernible to the human eye. The two combined additives could significantly reduce the loss of monascus red pigment, thiobarbituric acid reactive substances (TBARs), carbonyl contents and the loss of sulfhydryl group in DPMS system during drying process ($P < 0.05$), inhibited the production of Maillard coloring substances ($P < 0.05$), and stabilized the relative content of myoglobin (deoxymyoglobin (DeoMb), oxymyoglobin (OMb) and methemoglobin (MetMb)) ($P < 0.05$). These results indicated that the combination of BLE and Cys could effectively improve the color stability of DPMS in the scope of drying temperature 60–70 °C, which displayed a great potential to enhance the color stability of the Chinese traditional meat products in large-scale production.

Keywords: dried minced pork slices; color stability; drying temperature variation; bamboo leaf extract; *L*-cysteine

Citation: J. Chen, Q. Luo, Y. Zhou, et al., Color stability of dried pork meat slices containing bamboo leaf extract and *L*-cysteine under drying temperature variation, Food Sci. Anim. Prod. 1 (2023) 9240009. https://doi.org/10.26599/FSAP.2023.9240009.

1 Introduction

Dried pork meat slices (DPMS, also known as Zhuroupu) is a traditional food with a long history in China, and its production process is complex, including slicing, seasoning, salting, sieving, drying and roasting [1]. Heat treatments are known to increase the oxidization from oxi- and myoglobin to metmyoglobin resulting in decreased redness (a^*) values, and the variation of air-drying temperature (50–70 °C) has an influence on the color of meat products, for example, total color change (ΔE) is higher for samples dried at 50 and 70 °C than for samples dried at 60 °C [2]. In fact, the air temperature of different locations in the blast drying room is uneven and fluctuant in large-scale production process, resulting in the unstable product quality such as color and texture. With the development of food e-commerce, the same quality grade of DPMS may come from different manufacturers, which results in non-uniform color of the whole quality because of the different drying temperature. Color not only plays a vital role in commercial acceptance of meat products [3], but also is the primary quality of DPMS. It is valuable to improve the color stability of DPMS from the perspective of its production process.

Bamboo is widely distributed throughout China. Its leaf is also used to improve the flavor and color of food [4]. According to the National Health Commission of China (GB 2760—2014), the bamboo leaf extract (BLE) is acceptable for addition to meat products as an excellent antioxidant. The combined addition of BLE and tea polyphenol can significantly reduce lipid oxidation and biogenic amine accumulation in sausages during storage [5], and the addition of BLE to clams can also inhibit lipid oxidation during frying and improve the color of these products [6]. The components of BLE such as flavonoids, phenolic acids and phenols, can inhibit the chain reaction of lipid autooxidation, chelate transient metal ions, remove cyanide and prevent the synthesis of nitrosamines in meat products [4]. It is worth exploring whether BLE is beneficial to improve the color stability of DPMS.

L-Cysteine (Cys) is widely used to improve color, flavor and texture in meat products [7], and has the potential to stabilize color by binding to hemoglobin or myoglobin sixth-position ligands to form complexes [3]. Cys can also improve the color of meat products via the combination with other antioxidants. For example, the combined addition of Cys and $NaNO_2$ can effectively increase the a^* value and nitroso pigment content in cured sausages [7]. Cys can bind with the polyphenols and thus synergistically inhibit the oxidation of myoglobin [8]. It can be inferred that the combination Cys with BLE (polyphenols) may stabilize the color of DPMS via reducing the myoglobin changes. However, to our knowledge, no literature is available regarding the effect of the combination on the DPMS color stability under the variation of air-drying temperature.

This work focused on the color stability of DPMS containing BLE and Cys under the variation of air-drying temperature, and further explored the mechanism of color stabilization in order to provide an approach to increasing the uniformity of DPMS color during process.

2 Materials and methods

2.1 Materials and chemicals

Chilled fresh pork hindquarters and pork back fat were supplied by Carrefour Group supermarkets (Hefei, China), The approximate composition of the hindquarters was consistent as follows: moisture (73.69 ± 0.22) %, crude protein (21.44 ± 0.02) %, crude fat (2.47 ± 0.06) % and ash (2.13 ± 0.04) % (n = 3), which was measured according to the method of the Chinese National Standards GB 5009.3—2016, GB 5009.5—2016, GB 5009.6—2016 and GB 5009.4—2016, respectively.

BLE (the content of bamboo leaf flavonoid is 10.2%) was purchased from Ruidi Pharmaceutical Co., Ltd. (Xi'an, China) and stored at 4 °C until use. Monascus red pigment (MRP), Cys (98.6%) and composite phosphate (99%) of food-grade were purchased from Zhejiang Yinuo Biotechnology Co., Ltd. (Hangzhou, China). Fish sauce (98.6%, food-grade) was purchased from Rixin Aquatic Products Co., Ltd. (Rongcheng, China). Glycerine (99.7%, food-grade) was purchased from Xinai Technology Co., Ltd. (Lianyungang, China). Sucrose, monosodium glutamate, pepper and egg were purchased from Carrefour Group supermarket (Hefei, China). MRP standard (98.0%) purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All other chemicals and reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China).

2.2 Preparation of DPMS

DPMS was prepared according to the method of Chen et al. [1] with a small modification. After the pork hindquarter was removed of the visible fat and connective tissue, a portion of the pork was cut into slices (length 6 cm, width 3 cm, and thickness 2 mm), the other was mixed with the back fat in the mass ratio of 3:1 (m/m) and ground into meat paste through a meat grinder (Guangzhou, China). Based on the appropriate addition level of *L*-Cys in minced meat products [7], the limited use of bamboo leaf antioxidants in meat products in GB 2760—2014, and the method of Zhang et al. [9], the formula of DPMS production was set as below: 200 g of the pork slices, 50 g of the meat paste, 0.5 g of BLE (0.2% of total pork meat weight), 1.25 g of Cys (0.5% of total pork meat weight), 55 g of sucrose, 12.5 g of fish sauce, 2.75 g of monosodium glutamate, 0.5 g of pepper, 0.375 g of complex phosphate, 0.05 g of MRP, 37.5 g of whole egg liquid and 2.5 g of glycerin. The samples with BLE and Cys were called the Q groups, while the control samples without BLE and Cys were referred to the CK groups. Basing on the generally air-drying temperature 55–75 °C in DPMS production factory, five air-drying temperature were tested respectively: 55 (CK_1, Q_1), 60 (CK_2, Q_2), 65 (CK_3, Q_3), 70 (CK_4, Q_4) and 75 °C (CK_5, Q_5). The dried slices were roasted at 230 °C for 3 min (flipping every 30 s), cooled at room temperature, sealed in a packaging bag, and then stored at 4 °C for testing within 2 days.

2.3 Stability of surface color and Δ*E* measurement

According to the method of Nkhata [10], the surface color of DPMS was determined via a ZE-7700 model chromometer (Nippon Denshoku, Japan). The reflection mode, D65 light source, 10° standard observer with a 10 mm diameter measurement area and the high-speed silicon photocell as the light receiver were used. The stability of surface color was expressed as the range of the mean value of each color parameter (lightness (L^*), redness (a^*) or yellowness (b^*)) in the air-drying temperature scopes of 55–65 ((60 ± 5) °C), 60–70 ((65 ± 5) °C), 65–75 ((70 ± 5) °C) and 55–75 °C ((65 ± 10) °C). The CK-$R_{60\pm5}$, CK-$R_{65\pm5}$, CK-$R_{70\pm5}$, CK-$R_{65\pm10}$, Q-$R_{60\pm5}$, Q-$R_{65\pm5}$, Q-$R_{70\pm5}$ and Q-$R_{65\pm10}$ represent the range of color parameter in the corresponding drying temperature scopes, respectively. ΔE was calculated using the mean of the corresponding color parameters between the given air-drying temperatures via an equation $\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ [10].

2.4 Detection of MRP retention rate

The content of MRP in DPMS was measured according to the method of the Chinese National Standards GB 5009.150—2016, a C_{18} column was used with an internal diameter of 4.6 mm and a particle size of 5 μm. MRP retention rate was calculated as follow:

$$\text{MRP retention rate}\ (\%) = \frac{S_1}{S_0} \times 100$$

Where S_0 indicates the content of MRP in raw material of DPMS (g/kg), and S_1 indicates the content of MRP in DPMS after air-drying and roasting (g/kg).

2.5 Detection of browning degree and fluorescence intensities of Maillard reaction

According to the method described by Geng et al. [11], the centrifuged supernatant was filtered through a 0.22 μm diameter microporous membrane to eliminate the effect of fat on the experiment. The filtrate was collected and was diluted 10-fold with ultrapure water, using for determining the browning degree and fluorescence intensity of the Maillard reaction. The browning degree was measured with a spectrometer (3J1-0015 UV-Vis, UH5300, Hitachi, Japan) through absorbance at 420 nm ($A_{420\ nm}$). Fluorescence intensities was determined by a fluorescence spectrophotometer (Hitachi, F-4500, Kyoto, Japan) at an excitation wavelength of 350 nm and an emission wavelength of 420 nm.

2.6 Detection of myoglobin forms

The myoglobin forms were detected according to Dai et al. [12]. In brief, 5 g of chopped DPMS was homogenized with 25 mL 0.04 mol/L phosphate buffer (pH 6.8) in a 50 mL centrifuge tube by a Fluko homogenizer (Shanghai, China) at 10 000 × g. After standing at 4 °C for 1 h, the tube was centrifuged for 25 min at 10 000 × g and 4 °C. The supernatant was filtered through 0.45 μm microporous filter. UV absorbance of the resulting filtrate was measured by microplate reader (BioTek, Vermont, USA) at 525, 545, 565 and 572 nm, respectively. The contents of oxymyoglobin (OMb), deoxymyoglobin (DeoMb), and metmyoglobin (MetMb) were calculated according to the following formulas, respectively.

$$\text{DeoMb content}\ (\%) = (0.369 \times R_1 + 1.140 \times R_2 - 0.941 \times R_3 + 0.015) \times 100$$

$$\text{OMb content}\ (\%) = (0.882 \times R_1 - 1.267 \times R_2 + 0.809 \times R_3 - 0.361) \times 100$$

$$\text{MetMb content}\ (\%) = (-2.541 \times R_1 + 0.777 \times R_2 + 0.800 \times R_3 + 1.098) \times 100$$

Where $R_1 = A_{572\ nm}/A_{525\ nm}$, $R_2 = A_{565\ nm}/A_{525\ nm}$, and $R_3 = A_{545\ nm}/A_{525\ nm}$, respectively.

2.7 Detection of protein oxidation in DPMS

The protein oxidation of DPMS was reflected by the carbonyls and sulfhydryl (–SH) contents of protein. The carbonyls content was determined according to the method of Rodriguez-Carpena et al. [13] and the sulfhydryl content was determined according to the method of Cheng et al. [14].

2.8 Detection of lipid oxidation in DPMS

According to the method of Rodriguez-Carpena et al. [13], a standard curve was prepared using 1,1,3,3-tetraethoxypropane as a standard for malondialdehyde (MDA) and the values of thiobarbituric acid reactive substances (TBARs) were expressed as milligrams of MDA per kg of DPMS sample.

2.9 Statistical analysis

Analyses of all samples were performed independently, and the data were presented as mean ± standard deviation (SD). Statistical analyses were performed using SPSS 22.0 analysis (IBM Inc., Armonk, NY, USA) and principal component analysis (PCA) was conducted by SIMCA 14.0 (Umetrics, Sweden). Data chart was made with OriginPro 2019 software (OriginLab Inc., Northampton, USA). Significant difference was assessed by use of Duncan's multiple range post hoc test. $P < 0.05$ indicates statistical significance.

3 Results and discussion

3.1 Stability of surface color and Δ*E*

As shown in Table 1, the L^* and b^* values of DPMS above 70 °C air-drying temperature (CK_4 and CK_5 groups) increased significantly ($P < 0.05$) compared with CK_1 group (55 °C), while the a^* value decreased with the increase of air-drying temperature ($P < 0.05$). The Δ*E* values of DPMS in the scopes of air-drying temperature variation ± 5 °C (55–65, 60–70, 65–75 °C) increased with the increase of air-drying temperature. The combined addition of BLE and Cys (Q groups) could improve a^* values ($P < 0.05$) of DPMS under 60–70 °C air-drying temperatures (Q_2, Q_3 and Q_4 groups), and could decrease the ranges (range values of CK-R and Q-R groups, as well as Δ*E* values between given air-drying temperatures) of L^*, a^*, b^* and Δ*E* values on the whole. In particular, as a reflection on the total color difference of a substance [9], Q-$\Delta E_{65\pm5}$ value of 1.93 within the scope from 60 to 70 °C was below the limits visible to the human eye in a large extent [12]. These results implied that the variation of air-drying temperature could result in the instability of DPMS color, and the combined addition of BLE and Cys could enhance the stability of DPMS color.

3.2 MRP retention rate

As a natural food colorant, MRP is used worldwide, especially in Asia [15–16]. As depicted in Table 2, MRP retention rates in DPMS without BLE and Cys (CK_1–CK_5 groups) were decreased successively with the increase of air-drying temperature ($P < 0.05$), while the retention rates of corresponding air-drying temperature could be significantly increased ($P < 0.05$) via the combined addition of BLE and Cys (Q_1–Q_5 groups). Meanwhile, the range values of the rate in Q_1–Q_5 groups were lower than that in CK_1–CK_5 groups under the corresponding air-drying temperature scope. These results indicated that high temperature could break the color stability of MRP and make red (a^* value) fade, and the combined BLE and Cys could compensate the heat-induced loss of MRP, thus improving the color stability of the DPMS.

A number of reactive hydroxyl radicals and superoxide anions are produced in food processing at high temperature [5,17], and these active components can bind to the two ends of the conjugated double bond, resulting in the transformation of chromophore structure and the discoloration of food [18]. In this study, high temperature of air-drying and subsequent roasting during DPMS processing could promote the formation of active components such as reactive hydroxyl radicals and superoxide anions, which could act on the conjugated double bond of MRP and induced the transformation of chromophore structure and the discoloration of DPMS.

Table 1 Color stability of DMPS with BLE and Cys under air-drying temperature variation (55–75 °C).

Group	L^*	a^*	b^*	Δ*E*
CK_1 (55 °C)	39.06 ± 0.86^{de}	19.91 ± 0.47^{ab}	18.14 ± 0.85^{e}	
CK_2 (60 °C)	39.01 ± 2.05^{de}	18.28 ± 0.73^{c}	19.31 ± 0.26^{d}	CK-$\Delta E_{60\pm5}$ = 3.41
CK_3 (65 °C)	37.76 ± 0.58^{e}	18.90 ± 0.18^{c}	21.13 ± 0.52^{c}	CK-$\Delta E_{65\pm5}$ = 6.70
CK_4 (70 °C)	41.82 ± 0.70^{b}	16.45 ± 1.28^{d}	25.11 ± 0.88^{a}	CK-$\Delta E_{70\pm5}$ = 7.55
CK_5 (75 °C)	43.28 ± 0.06^{a}	16.27 ± 0.67^{d}	25.56 ± 0.22^{a}	CK-$\Delta E_{65\pm10}$ = 9.28
Q_1 (55 °C)	39.90 ± 0.76^{cd}	20.83 ± 0.51^{a}	20.85 ± 0.40^{c}	
Q_2 (60 °C)	38.95 ± 0.33^{de}	20.05 ± 0.26^{ab}	21.66 ± 0.41^{c}	Q-$\Delta E_{60\pm5}$ = 2.99
Q_3 (65 °C)	37.85 ± 0.30^{e}	19.81 ± 0.67^{b}	22.77 ± 0.13^{b}	Q-$\Delta E_{65\pm5}$ = 1.93
Q_4 (70 °C)	38.56 ± 0.37^{de}	18.43 ± 0.18^{c}	22.64 ± 0.93^{b}	Q-$\Delta E_{70\pm5}$ = 4.25
Q_5 (75 °C)	40.92 ± 1.66^{bc}	16.88 ± 1.08^{d}	22.99 ± 1.37^{b}	Q-$\Delta E_{65\pm10}$ = 4.61
CK-$R_{60\pm5}$	1.30	1.63	2.99	
CK-$R_{65\pm5}$	2.81	2.45	5.80	
CK-$R_{70\pm5}$	5.52	2.63	4.43	
CK-$R_{65\pm10}$	5.52	3.64	7.42	
Q-$R_{60\pm5}$	2.05	1.02	1.92	
Q-$R_{65\pm5}$	1.10	1.62	1.11	
Q-$R_{70\pm5}$	3.07	2.93	0.35	
Q-$R_{65\pm10}$	3.07	3.95	2.14	

Note: Different letters a–e in the same column indicate significant difference ($n = 6$, $P < 0.05$). The $\Delta E_{60\pm5}$, $\Delta E_{65\pm5}$, $\Delta E_{70\pm5}$ and $\Delta E_{65\pm10}$ represent the calculated values using the mean of the corresponding color parameters between 55 and 65 °C, 60 and 70 °C, 65 and 75 °C (± 5 °C) as well as 55 and 75 °C (± 10 °C), respectively.

Table 2 MRP retention rate in DMPS and its range under air-drying temperature variation (55–75 °C).

Group	MRP retention rate (%)	Retention rate range (%)
CK_1 (55 °C)	57.32 ± 0.45^{d}	
CK_2 (60 °C)	53.29 ± 0.54^{e}	CK-$RR_{60±5}$ = 7.19
CK_3 (65 °C)	50.13 ± 0.36^{f}	CK-$RR_{65±5}$ = 14.80
CK_4 (70 °C)	38.49 ± 0.21^{g}	CK-$RR_{70±5}$ = 14.22
CK_5 (75 °C)	35.91 ± 0.31^{h}	CK-$RR_{65±10}$ = 21.41
Q_1 (55 °C)	64.63 ± 0.91^{a}	
Q_2 (60 °C)	64.35 ± 0.38^{a}	Q-$RR_{60±5}$ = 3.21
Q_3 (65 °C)	61.42 ± 0.53^{b}	Q-$RR_{65±5}$ = 4.12
Q_4 (70 °C)	60.23 ± 0.92^{c}	Q-$RR_{70±5}$ = 1.55
Q_5 (75 °C)	59.87 ± 0.25^{c}	Q-$RR_{65±10}$ = 4.76

Note: Different letters a–h in the same column indicate significant difference (n = 6, P < 0.05). The CK-$RR_{60±5}$, CK-$RR_{65±5}$, CK-$RR_{70±5}$, CK-$RR_{65±10}$, Q-$RR_{60±5}$, Q-$RR_{65±5}$, Q-$RR_{70±5}$ and Q-$RR_{65±10}$ represent the ranges of MRP retention rates in corresponding drying temperature ranges 55–65 ((60 ± 5) °C), 60–70 ((65 ± 5) °C), 65–75 ((70 ± 5) °C) and 55–75 °C ((65 ± 10) °C), respectively.

The increased MRP retention rate in DPMS could be related to the phenolic substances contained in BLE [4] and active –SH groups of Cys [7]. Due to the strong free radical scavenging ability of phenolic substances in BLE [5,17] and –SH groups, a large number of active hydroxyl radicals and superoxide anions caused by high temperature could be removed in DPMS system, which could weaken the attack of these reactive radicals on conjugated double bonds of MRP and could stabilize the structure of it. In addition, BLE can also reduce the oxidation of unsaturated double bonds during roasting [19], thus stabilizing the structure of MRP. These results meant that MRP retention rate in Q groups could be higher and more stable than that in CK groups under the variation of air-drying temperature (Table 2). This was also consistent with the change trend of ΔE value, indicating that the combination of BLE and Cys could improve the color stability during DPMS processing through action on MRP.

3.3 Browning degree and fluorescence intensity of Maillard reaction

Maillard reaction is a common non-enzymatic browning reaction between reducing sugars and free amino acids, which can produce melanoid and is closely related to color and appearance of products [20,21]. The formation of melanoid is easily influenced by the temperature [22]. Browning intensities is an indicator of melanoid formation, and fluorescence intensities is corresponding with the precursor substance of it [23].

As shown in Figure 1, the browning degree and the fluorescence intensity of Maillard reactants in Q groups were all significantly decreased compared with corresponding CK groups at the same temperature (P < 0.05). This result could be correlative with the low pH and low moisture content caused by the combined addition of BLE and Cys (Table 3), because the decrease of pH in acidic condition can effectively suppress protein degradation in pork meat during air-drying and mitigate the Maillard browning reaction [11], and the reduction of water content decelerates the browning intensity of the Maillard reaction by inhibiting the production from 5-hydroxymethylfurfural (an intermediate in the Maillard reaction) [24]. In addition, Cys could inhibit the formation of brown pigments at the end of Maillard reaction ($A_{420\ nm}$) [25], and could decelerate color formation by converting Maillard reaction intermediates and preventing the formation of certain intermediates [26,27]. To sum up, the compound of BLE and Cys could reduce the effect of Maillard reaction on the color stability of DPMS under air-drying temperature variation.

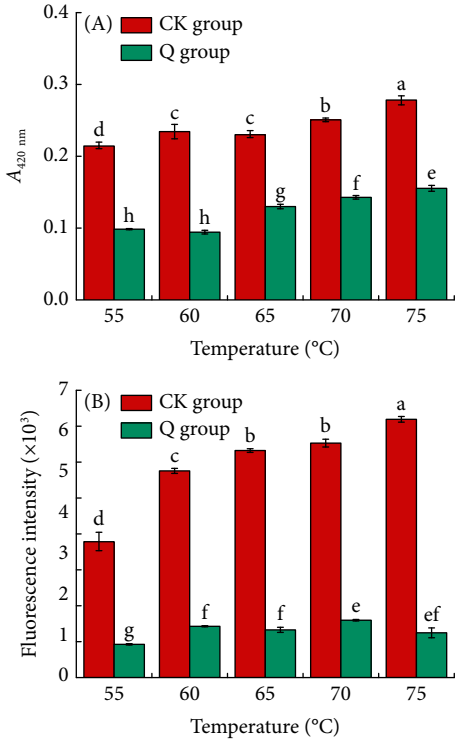

Figure 1 Browning intensity (A) and fluorescence intensity (B) of Maillard reaction in DMPS with BLE and Cys under air-drying temperature variations (55–75 °C). CK and Q indicate DMPS without and with the combined addition of 0.2% BLE and 0.5% Cys, respectively. Different letters a–h above the bars indicate significant difference (n = 6, P < 0.05).

Table 3 Moisture content and pH of DMPS with BLE and Cys under air-drying temperature variations (55–75 °C).

Group	Moisture content (%)	pH
CK_1 (55 °C)	21.46 ± 0.15^{a}	6.82 ± 0.05^{a}
CK_2 (60 °C)	17.31 ± 0.47^{d}	6.74 ± 0.16^{a}
CK_3 (65 °C)	17.66 ± 0.19^{cd}	6.76 ± 0.27^{a}
CK_4 (70 °C)	16.41 ± 0.12^{e}	6.88 ± 0.21^{a}
CK_5 (75 °C)	11.35 ± 0.74^{g}	6.76 ± 0.08^{a}
Q_1 (55 °C)	18.97 ± 0.08^{b}	5.94 ± 0.12^{b}
Q_2 (60 °C)	18.61 ± 0.06^{b}	6.01 ± 0.44^{b}
Q_3 (65 °C)	18.05 ± 0.14^{c}	5.89 ± 0.18^{b}
Q_4 (70 °C)	15.45 ± 0.29^{f}	5.87 ± 0.19^{b}
Q_5 (75 °C)	12.45 ± 0.35^{g}	5.96 ± 0.35^{b}

Note: Different letters a–g in the same column indicate significant difference (n = 6, P < 0.05).

3.4 Myoglobin forms

Typically, OMb and DeoMb are not highly stable and easily oxidize into brownish MetMb, and the aforementioned changes in meat and meat products can lead to discoloration [28]. Moreover, the

most obvious change of instrumental color is a decrease in redness (a^* parameter) and an increase in yellowness (b^* parameter) [29]. The increase in environmental temperature can enhance the autoxidation of OMb and DeoMb, which converted Fe^{2+} to Fe^{3+} in myoglobin, leading to an increase of MetMb [30] and the change in the color of meat products [29].

As shown in Table 4, the contents of DeoMb in CK_3–CK_5 groups significantly decreased ($P < 0.05$) above 65 °C air-drying temperature and the contents of MetMb increased ($P < 0.05$), while the combined addition of BLE and Cys (Q groups) could not prevent the variation trend (the decrease of DeoMb and the increase of MetMb) with increasing temperature. However, three structural forms of myoglobin (DeoMb, OMb and MetMb) in Q groups changed insignificantly ($P > 0.05$) under the variation of air-drying temperature compared with group CK, indicating that the combined addition of BLE and Cys could offset the volatility in myoglobin morphology caused by temperature variation. On the one hand, Cys contains the active group –SH, which can theoretically bind to the sixth ligand of iron porphyrin to maintain the iron in the theoretically ferrous state in myoglobin [7]. On the other hand, flavonoids, phenolic acids and other substances in BLE can block the autooxidation of meat products in the air [4]. Both composite effects could improve the ability of myoglobin to cope with the variation of air-drying temperature, thus enhancing the stability of DPMS's color.

Table 4 Relative content of myoglobin in DMPS with BLE and Cys under air-drying temperature variations (55–75 °C).

Group	DeoMb content (%)	OMb content (%)	MetMb content (%)
CK_1 (55 °C)	45.13 ± 0.79^a	4.35 ± 0.24^a	34.28 ± 1.07^b
CK_2 (60 °C)	44.79 ± 0.62^a	4.52 ± 0.46^a	34.36 ± 0.98^b
CK_3 (65 °C)	43.37 ± 0.52^b	4.31 ± 0.47^a	36.47 ± 0.89^{ab}
CK_4 (70 °C)	43.37 ± 0.54^b	4.13 ± 0.34^a	36.84 ± 0.90^a
CK_5 (75 °C)	42.78 ± 0.54^b	4.02 ± 0.22^a	37.66 ± 0.39^a
Q_1 (55 °C)	43.37 ± 0.78^b	4.13 ± 0.34^a	36.94 ± 0.89^a
Q_2 (60 °C)	43.27 ± 0.31^b	4.15 ± 0.28^a	36.98 ± 0.78^a
Q_3 (65 °C)	43.60 ± 0.80^b	4.06 ± 0.45^a	36.33 ± 0.92^{ab}
Q_4 (70 °C)	43.24 ± 0.40^b	3.92 ± 0.35^a	37.43 ± 0.94^a
Q_5 (75 °C)	43.49 ± 0.69^b	4.27 ± 0.40^a	36.60 ± 1.20^{ab}

Note: Different letters a–b in the same column indicate significant difference ($n = 6$, $P < 0.05$).

3.5 Protein oxidation

Protein oxidation is associated with the breakdown of the heme molecule and the subsequent release of free (non-heme) iron from the porphyrin ring [31], and the cross-linked aggregation of proteins induced by oxidation could also influence the light reflection and meat color [32], which has a direct effect on muscle food color [29]. And free iron can change the MRP from bright red to brown [33]. The degree of protein oxidation is closely related to temperature [34]. Therefore, it is feasible to keep the color stability of DPMS by regulating the proteins oxidation under the variation of air-drying temperature.

As shown in Figure 2A, the carbonyl contents of the Q groups were significantly reduced ($P < 0.05$) compared to the CK groups under the corresponding air-drying temperature, indicating a strong inhibitory effect of compound BLE and Cys. The oxidation of proteins can be related to a variety of factors, such as transient metal ions, iron ions released by heating degradation of myoglobin and free radical chain reactions mediated by lipid oxidation, especially carbonylation [35]. Flavonoids, phenolic acids and other substances in BLE have anti-oxidative effect [4], and these substances can delay protein oxidation by affecting the above factors. About for Cys, apart from its own –SH had anti-oxidative properties, their Maillard reaction with glucose was also effective in scavenging lipophilic free radicals and had chelating ability [36].

Sulfhydryl content is also one of the indicators of expressed protein-oxidation, the more sulfhydryl groups are lost and the more serious protein oxidation is [37]. High temperature can cause the protein structure to unfold and the sulfhydryl groups to be exposed [38]. As shown in Figure 2B, sulfhydryl contents of CK groups were significantly lower ($P < 0.05$) than that of Q groups at the corresponding air-drying temperature. This result suggested that the combined addition of BLE and Cys (Q groups) could increase sulfhydryl contents and inhibit heat-induced sulfhydryl loss in DPMS system. This effect could be not only correlative with tightly binding of flavonoids in BLE to alcohol-soluble proteins through hydrogen and hydrophobic interactions, inhibiting protein unfolding and reducing sulfhydryl groups exposure [39], but also related to –SH of Cys and its anti-oxidative effect [36]. Thus, the combined addition could improve the color stability (Table 1) of DPMS via inhibiting protein oxidation and correspond with the reduction of MetMb content (Table 4).

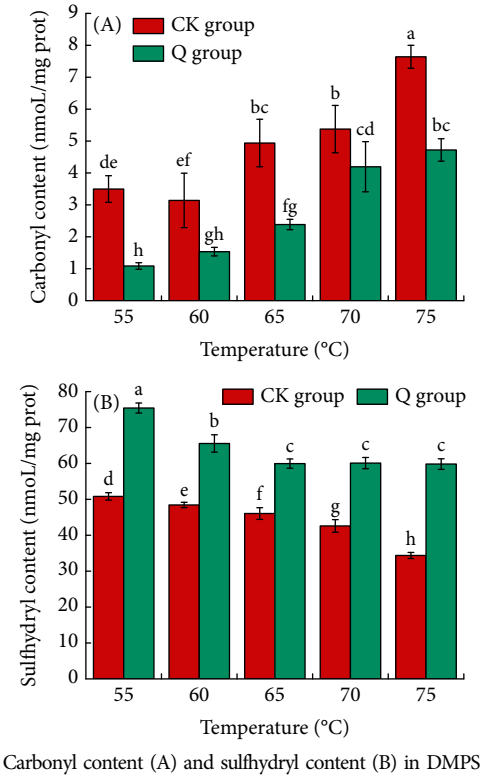

Figure 2 Carbonyl content (A) and sulfhydryl content (B) in DMPS with BLE and Cys under air-drying temperature variations (55–75 °C). CK and Q indicate DMPS without and with the combined addition of 0.2% BLE and 0.5% Cys, respectively. Different letters a–h above the bars indicate significant difference ($n = 6$, $P < 0.05$).

3.6 Lipid oxidation

TBARs reflects the level of lipid oxidation products [40] and it is

closely related to temperature [41]. As depicted in Figure 3, TBARs of DPMS in CK and Q groups indicated all the tendency to increase and then decrease with a rise of air-drying temperature within the scope of 55–75 °C, and a turning point of both groups all occurred at 70 °C. However, TBARs of Q-groups were lower than that of CK-groups ($P < 0.05$) in corresponding air-drying temperature except 55 °C, displaying the effective lipids anti-oxidation of BLE and Cys during DPMS processing.

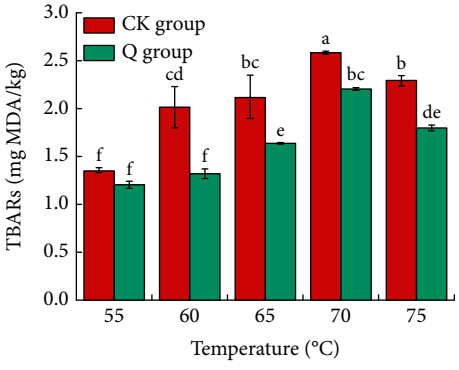

Figure 3 TBARs of myoglobin in DMPS with BLE and Cys under air-drying temperature variations (55–75 °C). CK and Q indicate DMPS without and with the combined addition of 0.2% BLE and 0.5% Cys, respectively. Different letters a–f above the bars indicate significant difference ($n = 6$, $P < 0.05$).

The current results demonstrated that lipid oxidation products were correlated to the color stability of meat products, and MDA could be the main contributor of redness changes in muscles [41]. A variety of plant extracts, such as tea polyphenols and bamboo leaves [5,42], had been shown to reduce lipid oxidation in meat products. Low TBARs values of Q-groups were correlative with anti-lipid oxidation effects of flavonoids, phenolic acids and other substances in BLE [4] and –SH groups of Cys [36], displaying the improvement of redness and color stability in DPMS. Moreover, pyrrole is the intermediate product formed by the reaction of lipid oxidation products with amino acid and protein residues, and is the precursor material of color, which has a yellow, red or brown appearance. Some pyrroles can also spontaneously polymerize to form dark brown melanoid, causing a decrease in a^* and an increase in b^* [43]. In this study, the production of pyrrole is reduced via decreasing the degree of lipid oxidation, thus improving the color stability during DPMS processing. The reduction of TBARs under high temperature (75 °C) could be attributed to the volatilizing of low-molecular-weight aldehydes [44]. In addition, the reduction in the degree of lipid oxidation can reduce the number of free radicals in the system and can interfere the oxidation of protein [45], and thus delaying the oxidation rate of proteins, especially insignificant increase ($P > 0.05$) of carbonyl contents and decrease of sulfhydryl contents in Q groups at 70–75 °C (Figure 2). These complex effects of reducing lipid oxidation could be conducive to the color stability of DPMS.

3.7 Multivariate data analysis

The effect of BLE and Cys on the color of DPMS under the variation of air-drying temperature could be explained by PCA. The first two principal components explained 83.6% of the total variation (principal components 1 and 2 explained 61.5% and 22.1%, respectively). As shown in Figure 4 and Figure 5, the spatial regions of all sample groups showed that the color of DPMS was unstable under the variation of air-drying temperature, while the sample points of Q groups were more clustered than that of CK groups, indicating high color stability of DPMS with BLE and Cys. Both exogenous additives have great influences not only on a^* by regulating myoglobin morphology, moisture content, MRP retention rate and sulfhydryl content, but also on b^* and L^* by regulating pH, Maillard reaction, carbonyl group and TBARs. MetMb content, carbonyl content, TBARs and moisture content were mainly responsible for the color stability of DPMS (variable importance for the projection (VIP) > 1).

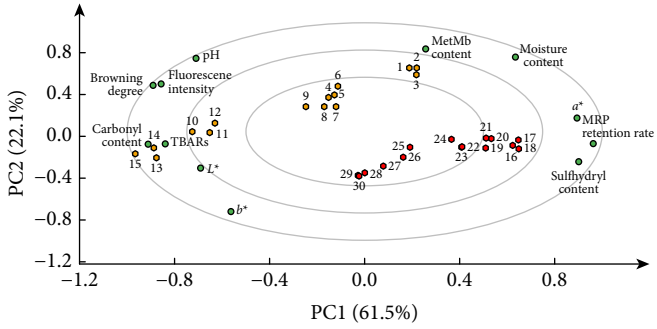

Figure 4 Score scatter plot of PCA in DMPS with BLE and Cys under air-drying temperature variations (55–75 °C). Multivariate data analysis (color, MRP retention rate, Maillard reaction and lipid oxidation, etc.) of DMPS with BLE and Cys under air-drying temperature variations. 1–3 and 16–18, DMPSs at 55 °C; 4–6 and 19–21, DMPSs at 60 °C; 7–9 and 23–25, DMPSs at 65 °C; 10–12 and 26–28, DMPSs at 70 °C; 13–15 and 28–30, DMPSs at 75 °C. Yellow is CK groups and red is Q groups.

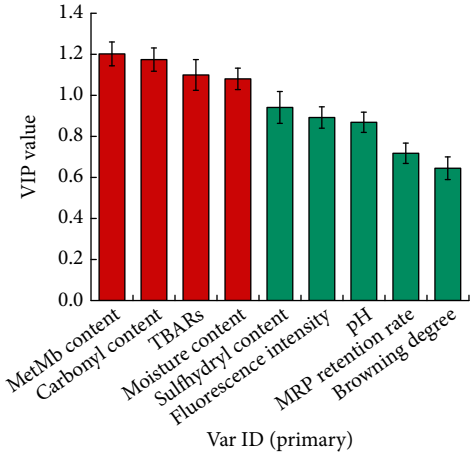

Figure 5 Variable importance for the projection (VIP) plot of MRP retention, Maillard reaction and lipid oxidation, etc. data (defined as X matrix) vs. color (L^*, a^* and b^* defined as Y matrix). The larger VIP values (> 1) were labeled in red, which indicate the important X variables for explaining Y variables.

Relationships between different parameters were determined by Pearson's correlation coefficient and summarized in Figure 6. The value of a^* was positively correlated with MRP retention rate ($r = 0.825$, $P < 0.01$), moisture content ($r = 0.628$, $P < 0.01$) and sulfhydryl content ($r = 0.705$, $P < 0.01$), and was negatively correlated with TBARs ($r = –0.835$, $P < 0.01$), pH ($r = –0.556$, $P < 0.01$), browning degree ($r = –0.712$, $P < 0.01$), fluorescence intensity ($r = –0.675$, $P < 0.01$) and carbonyl content ($r = –0.754$, $P < 0.01$). The value of b^* was positively correlated with carbonyl content ($r = 0.587$, $P < 0.01$) and TBARs ($r = 0.521$, $P < 0.01$), and was negatively correlated with MRP retention rate ($r = –0.508$, $P < 0.01$), MetMb content ($r = –0.661$, $P < 0.01$) and moisture content ($r = –0.769$, $P < 0.01$). The value of L^* was positively correlated with browning degree ($r = 0.489$, $P < 0.01$), fluorescence

intensity (r = 0.384, P < 0.05), carbonyl content (r = 0.619, P < 0.01) and TBARs (r = 0.408, P < 0.05), and was negatively correlated with MRP retention rate (r = –0.594, P < 0.01), sulfhydryl content (r = –0.467, P < 0.01) and moisture content (r = –0.741, P < 0.01). It is obvious that the color stability of DPMS mainly depended on the MRP retention rate (r = 0.825) and the degree of lipid oxidation (TBARs, r = –0.835) under the variation of air-drying temperature, although the instability of color during meat processing can be influenced by many factors such as the accumulation of MetMb [46], the fading of MRP and Maillard reaction [20,21].

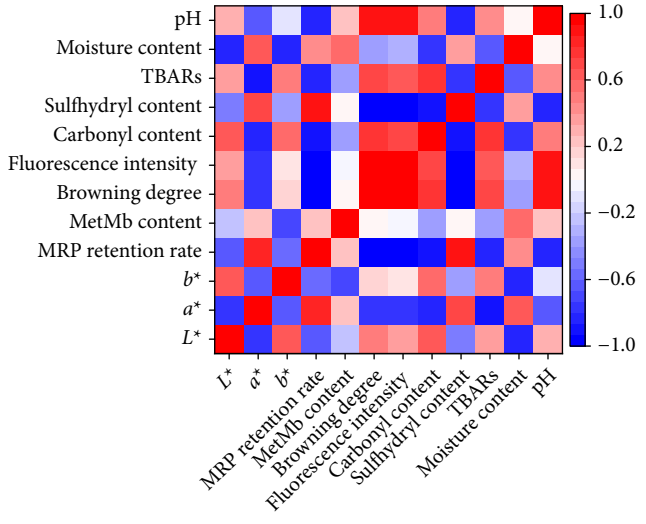

Figure 6 Pearson's correlation coefficients (r) between different parameters in DMPS with BLE and Cys under air-drying temperature variations (55–75 °C). The red color indicates that the two sets of data are positively correlated, while the blue color indicates that the two sets of data are negatively correlated. The darker the color, the greater the correlation coefficient.

The value of TBARs was correlated with retention rate of MRP (r = –0.778, P < 0.01), browning degree (r = 0.680, P < 0.01) and fluorescence intensity (r = 0.668, P < 0.01). Carbonyl content was correlated with MRP retention rate (r = –0.828, P < 0.01), browning (r = 0.780, P < 0.01) and fluorescence intensity (r = 0.690, P < 0.01). Sulfhydryl content was correlated with MRP retention rate (r = 0.884, P < 0.01), browning degree (r = –0.927, P < 0.01) and fluorescence intensity (r = –0.885, P < 0.01). These results implied that MRP retention rate was closely related to the protein/lipid oxidation and the degree of Maillard reaction, and thus influencing the color stability of DPMS during processing.

4 Conclusion

The combined addition of Cys and BLE could improve the color stability of DPMS in the scope of air-drying temperature variation 55–75 °C, especially ΔE in 60–70 °C (65 ± 5 °C) less than the limits visible to the human eye. The color stabilization effect was mainly attributed to the increased retention rate of MRP, the stabilized relative content of myoglobin (DeoMb, OMb and MetMb), the reduced production of Maillard coloring substances and the delayed oxidation of DPMS system, which induced by the two combined additives. These results could provide a feasible technical path to enhance the color stability of traditional DPMS under the variation of air-drying temperature, and improve the quality stability of DPMS in large-scale production and thus promote the development of the Chinese traditional meat products in the state of e-commerce.

Conflict of interest

The authors declare that there is no actual or potential conflict of interest.

Acknowledgements

This work was financially supported by the Major Science and Technology Project of Anhui Province (No. 202003a06020029). Thanks Miss Cui Cui for her experiment support.

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