

Accepted Manuscript

**Title: Cochineal Carmine Adsorbed on Layered Zinc Hydroxide Salt:  
Responsible for the Reddish-Pink Color of Cooked Hams Without  
Adding Curing Salts**



**Authors:** C. Canan, A. C. T. Cursino, G. C. Ongaratto, D L. Kalschne, L. H. Silva, F. R. Cardoso

Manuscript number: **PCCC-2401-1277**

To appear in: Progress in Color, Colorants and Coatings

Received: 16 January 2024

Final Revised: 6 May 2024

Accepted: 11 May 2024

Please cite this article as:

C. Canan, A. C. T. Cursino, G. C. Ongaratto, D L. Kalschne, L. H. Silva, F. R. Cardoso, Cochineal Carmine Adsorbed on Layered Zinc Hydroxide Salt: Responsible for the Reddish-Pink Color of Cooked Hams Without Adding Curing Salts, Prog. Color, Colorants, Coat., 17 (2024) XX-XXX.

This is a PDF file of the unedited manuscript that has been accepted for publication. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form

**Cochineal Carmine Adsorbed on Layered Zinc Hydroxide Salt: Responsible for the  
Reddish-Pink Color of Cooked Hams Without Adding Curing Salts**

C. Canan<sup>\*1</sup>, A. C. T. Cursino<sup>2</sup>, G. C. Ongaratto<sup>1</sup>, D L. Kalschne<sup>1</sup>, L. H. Silva<sup>3</sup>, F. R.  
Cardoso<sup>1</sup>

<sup>1</sup> Departamento de Alimentos, Programa de Pós-graduação em Tecnologia de Alimentos,  
Universidade Tecnológica Federal do Paraná, P.O. Box: 85884-000, Medianeira, Brasil.

<sup>2</sup> Departamento de Química, Universidade Tecnológica Federal do Paraná, P.O. Box:  
85884-000, Medianeira, Brasil.

<sup>3</sup> Departamento de Física, Universidade Tecnológica Federal do Paraná, P.O. Box:  
85884-000, Medianeira, Brasil.

**Abstract**

Color is one of the main attributes used to select or reject meat products, with the characteristic reddish-pink color of cured meats being developed by adding nitrate salts, which are rejected by many consumers due to their harmful effects on health. This study aimed to apply a hybrid dye (ZHN-carmine) synthesized from layered zinc hydroxide salt (ZHN) to produce a reddish-pink color in sliced cooked ham and to evaluate color stability during storage. The ham samples were prepared with cochineal carmine and hybrid dye (ZHN-carmine), sliced, vacuum-packed, and non-vacuum-packed, and exposed to white fluorescent light (1100 LUX) ( $5 \pm 1$  °C). The instrumental color measurement was performed at 0, 1, 4, 6, 8, 11, 13, and 15 day intervals. Distinction between the vacuum-packed ham samples and the non-vacuum-packed ham samples was possible based on a\* value (red color intensity), which showed the importance of oxygen

removal for red color stability. The reddish-pink color was more intense in the ham added with ZNH-carmine, and no color reduction was observed over the days, irrespective of it being vacuum-packed or not. Cochineal carmine adsorbed onto layered zinc hydroxide salt may be a potential replacement for curing salts regarding color formation in vacuum-packed or non-vacuum-packed cooked ham.

**Keywords:** zinc hydroxy nitrate; natural dyes; stability; meat products; nitrite/nitrate.

## Introduction

Pork production worldwide is estimated at 114.2 million tons for 2024, 3% more than the previous year [1]. The per capita consumption in Brazil was 13.42 kg, ranking third among the world's largest consumers [2]. Eighty-nine percent of pork is consumed as processed products such as cooked ham, fresh sausages, and bologna [3].

Food selection and approval by consumers usually start with appearance, and color is the most important sensory attribute [4, 5]. Consumers are generally also attracted by safety, nutritious, and healthier foods [6]. For cured meat, red color development and fixing, and to ensure *Clostridium botulinum* safety, nitrates and nitrites stand out [5]. Despite their multifunctionality, the use of curing salts is highly argued due to their toxic effects on human health, which lead to carcinogenic substances formation [7]. Their replacement is consequently a challenge for the food industry. Thus, adding an extra additive is believed to be needed to maintain the color stability and microbiological safety of cooked meat products without curing salts. Research to produce stable natural dyes is essential for developing safe additives that provide a natural color compatible

with traditional cured and cooked meat products found commercially [4]. Carminic acid is the most widely used natural dye in meat products, along with curing salts to obtain a reddish-pink color, well-accepted in several countries and by international legislation [7]. Some studies are concerned about carmine dye toxicity, mainly due to its use in meat products and by environmental dissemination in the composition of effluents [8]. However, carmine dye acceptable daily intake (ADI) is 5 mg per kg body weight per day, which is a low dosage [9]. For example, 60 kg people had to eat more than 3.5 kg of meat products per day containing a percentage of 0.0006 % of pure carmine to reach the ADI, which is impractical. Moreover, the carmine used by the slaughterhouse industry is generally diluted at 3% solution, implying an even greater food consumption to reach the ADI. In this context, associating carmine, traditionally used in meat products, with a safe ADI plus the possibility of removing nitrite - an additive with proven toxic effects, represents a safe purpose.

However, carmine has been reported to be a sensitive dye as it undergoes photodegradation when exposed to light [9]. Several technologies aim to achieve resistant and chemically stable dyes. In this sense, non-toxic hybrid dye techniques [10], nanocarriers [11], enzymatic techniques [12], and encapsulation [13] have been highlighted.

To increase cochineal carmine dye stability applied on meat products to counter interferences caused by processing and storage, Ongaratto et al. [14] developed a hybrid dye from cochineal carmine that showed greater stability due to the dye's adsorption on lamellar zinc hydroxide salt. These compounds are built by stacking units known as lamellae, linked by interlamellar ions' electrostatic interactions [15]. Lamellar compounds

increase stability against photodegradation [16], an essential characteristic of natural dyes, combined with low toxicity levels, low production costs, and ease of obtaining and handling [17,18]. The use of lamellar compounds has been studied in pharmaceutical, cosmetic, and nutraceutical fields [19]. They have been used in vitamins acting as multifunctional food supplements [20] and on the chemical and biological protection of food-grade nisin [21], proving the safety of such a technique that may be extended to the food sector.

Some studies have been found in the literature that evaluated the replacement of curing salts in meat products by sorbate and benzoate [22], phytic acid [23], celery, sodium lactate [24], Japanese radish [25], besides the use of celery associated with high hydrostatic pressure [26] or the association of vegetable juice powder (celery powder vegetable; juice PWD NAT, Chr. Hansen Inc.) and *Staphylococcus carnosus* starter culture [27], and plasma-treated winter mushroom powder [28]. Jo et al. [28] present in their review other plant sources of nitrates and nitrites, such as beet, lettuce, Swiss chard, fermented spinach, and parsley extract, with pathogen-inhibiting preservatives function. The use of cochineal carmine as a dye in meat products was evaluated by Ongaratto et al. [14] and Ruiz-Capillas et al. [24]. Therefore, studies assisting meat product processing companies with new, safe, and viable additives to replace curing salts are crucial.

In this study, inorganic matrices such as layered zinc hydroxide salt adsorbed with natural cochineal carmine dye (ZHN-carmine) were obtained and applied in a nitrite-free ham production. Color stability was evaluated during storage of vacuum-packed and non-vacuum-packed hams, and the color was compared to that of ham produced with traditional cochineal carmine dye.

## 2. Experimental

### 2.1. Hybrid dye (ZHN-carmin)

Hybrid dye was obtained by an ion exchange reaction between zinc hydroxy nitrate (ZHN) and cochineal carmine dye, as described by Ongaratto et al. [14]. The synthesis for ZHN preparation was performed using a 100 mmol L<sup>-1</sup> zinc nitrate aqueous solution (Dinâmica, Indaiatuba, São Paulo, Brazil) added with 1 mol L<sup>-1</sup> sodium hydroxide solution (Dinâmica, Indaiatuba, São Paulo, Brazil) until pH 6.8 was reached. The solution remained under agitation (Magnetic shaker, PC 410D, Corning, USA) for 24 h. Afterward, the solid was separated by centrifugation using a relative centrifugal force (RFC) of 3354 g for 5 min (Cientec, CT 5000R, Belo Horizonte, Minas Gerais, Brazil) at 25 °C. The obtained solid (ZHN) was oven-dried, macerated, and stored in a hermetically closed container at room temperature (25 ± 1 °C) for ion exchange with cochineal carmine dye.

For ion exchange, an aqueous solution with 4 mmol cochineal carmine (Carmine WS 52 %, Globenatural, Chorrillos, Peru), pH corrected to 7.0 with 2 mol L<sup>-1</sup> hydrochloric acid (Dinâmica, Indaiatuba, São Paulo, Brazil) was added to 2.4 mmol ZHN. The dispersion remained under magnetic stirring (Corning, 6796-420D, New York, USA) at 70 °C for 7 days. After that, the solid was separated by centrifugation at 3354 g for 5 min at 25 °C and washed with distilled water until obtaining a light-pink residual water. The solid was dried in a desiccator, macerated, and stored at room temperature. The obtained hybrid dye was named ZHN-carmin.

## 2.2. Hybrid dye applied in ham

Cochineal carmine and hybrid dye (ZHN-carmine) were used separately on cooked hams to compare their behavior in terms of color intensity and stability during storage. The hams were produced following the Brazilian Identity and Quality Standards [29] and the Brazilian legislation on permitted food additives for meat products [30]. The base formulation was composed of: pork leg (76.90 %), ice (19.20 %), sodium tripolyphosphate (Ibrac<sup>®</sup>) (0.50 %), salt (Diana<sup>®</sup>) (1.85 %), condiment (Conditec<sup>®</sup>) (0.58%), sodium erythorbate (Ibrac<sup>®</sup>) (0.17 %), carrageenan (Ibrac<sup>®</sup>) (0.50 %), isolated soy protein (Proteíco<sup>®</sup>) (0.29%). Ham identified as formulation 1 was added of cochineal carmine (Globenatural<sup>®</sup>) (0.0006 %), and ham identified as formulation 2 was added of hybrid dye (ZHN-carmine) (0.0033 %). The cochineal carmine percentage was based on values practiced by slaughterhouses. The total hybrid dye (ZHN-carmine) added mass was higher when considering the total cochineal carmine added mass. However, the amount of cochineal carmine was similar in both formulations, and the percentage difference was due intercalation compound.

For ham preparation, chilled boneless pork legs ( $7 \pm 1$  °C) purchased from a local market were trimmed, i.e., subcutaneous and intermuscular fat and connective tissue removed. Both brines were prepared by adding sodium tripolyphosphate to water, followed by its complete dissolution (Mechanical stirrer, 713 D, Fisatom, Brazil). Then, the other additives and ingredients were added, including cochineal carmine dyes or hybrid dyes (ZHN-carmine). Before being added to the brine, the hybrid dye (ZHN-carmine) added to formulation 2 was submitted to ultrasonic treatment (Sonics Vibra Cell, VC-505, Newtown, USA) at 37 kHz frequency and 100% amplitude for 4 min at 25

°C, to disaggregate and disperse better the adsorbed dye [14, 31]. Such a procedure was not needed for cochineal carmine added in formulation 1 due to its high solubility in water. Ice was added to the brine to keep the temperature at  $3 \pm 1$  °C during preparation. For the brine to be absorbed into the meat and its weight to increase by 20 %, formulations were vacuum-tumbled separately (Dorit, VV-T-10, Switzerland) for 30 min at 4 °C, with a 0.6 - 0.7 bar pressure and 20 rpm. After curing under refrigeration at  $5 \pm 1$  °C for 24 hours, the meat pieces were again vacuum-tumbled for 30 min. They were packed in polyethene bags, molded in stainless molds, and placed in a steam oven (Unimatic 1000, Eller, Italy) for 1 h at 60 °C, 1 h at 65 °C, 1 h at 70 °C and finally, maintaining oven temperature at 80 °C. The hams were considered cooked when they reached 69 °C internal temperature (geometric center of each ham, corresponding to its thickest part). Cooking time was approximately 4 h. After that, still in the cooking oven, they were subjected to thermal shock with running water for 30 min and then stored under refrigeration after reaching  $5 \pm 1$  °C. They were sliced and packed in low-density transparent polyethylene packaging, with and without vacuum (Selovac, 200B, São Paulo, Brazil), kept under refrigeration at  $5 \pm 1$  °C and exposed to a white fluorescent light source at a 40 cm distance, 1100 ( $\pm 50$ ) LUX for 15 days. The lux condition was chosen based on the study by Marchesi et al. For lux measurements, a digital lux meter (LD-400, Instrutherm, São Paulo, Brazil) was used on the shelves displaying ham for sale in local supermarkets.

### **2.3. Ham color evaluation**

Ham produced with cochineal carmine (1) or hybrid dye (ZHN-carmine) (2),



sliced, vacuum-packed (a) or non-vacuum-packed (b), and exposed to UV radiation were evaluated using instrumental color measurements at nine distinct points (delimited into portions of thin dough) using the CIELAB system with a colorimeter (CR 400, Konica Minolta, Osaka, Japan), calibrated with a D65 standard illuminant and a 10° angle. L\* (luminosity; 100 = white, 0 = black), a\* (redness; +, red; -, green), and b\* (yellow; +, yellow; -, blue) values were obtained. Analyses were performed on days 0, 1, 4, 6, 8, 11, 13, and 15. The total color difference ( $\Delta E$ ) [Equation (1)] was estimated at 0 and 15 days.

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Where  $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$  are the derivatives of the corresponding parameters, respectively.

#### 2.4. Statistical analysis

The instrumental color measurement results were expressed as mean  $\pm$  standard deviation of the mean (SDM), followed by the analysis of variance (one-way, ANOVA) and Tukey's test ( $p < 0.05$ ) and principal component analysis (PCA) using the Statistica 7.0 software (Statsoft, Tulsa, USA).

### 3. Results and Discussion

The hybrid dye (ZHN-carmin) with 44 % stoichiometric yield was characterized by X-ray diffraction (XRD), Fourier-Transform Infrared Spectroscopy (FTIR),

Thermogravimetric (TGA), and differential scanning calorimetry (DSC) as previously detailed by Ongaratto et al. [14].

### 3.1. Ham color evaluation

The appearance images of ham samples added with cochineal carmine (1) and hybrid dye (ZHN-carmine) (2) and of vacuum-packed (a) and non-vacuum-packed (b) hams at 0 and 15 days of storage are shown in Figure 1.

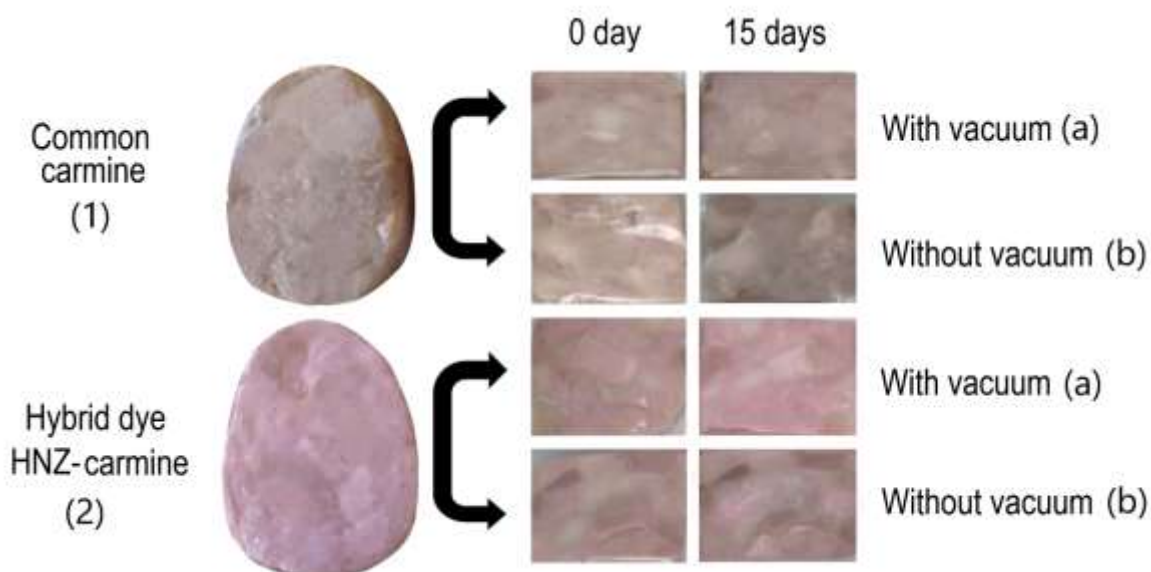


Figure 1. Ham samples added with cochineal carmine (1) and hybrid dye (ZHN-carmine) (2) and of vacuum-packed (a) and non-vacuum-packed (b) hams at 0 and 15 days of storage.

$L^*$ ,  $a^*$ , and  $b^*$  color parameters results of ham added with cochineal carmine (1) and ham added with hybrid dye (ZHN-carmine) (2) vacuum-packed (a) and non-vacuum-packed (b) exposed to UV radiation under refrigeration temperature are shown in Table 1.

**Table 1.** Colorimetric analysis results for different ham formulations.

Parameters	Formulation	Storage period (days)							
		0	1	4	6	8	11	13	15
L*	1(a)	65.20±0.89a AB	63.53±1.60a A	63.30±1.42aB	63.97±0.94aA	62.93±2.28aA	63.23±2.32aB	62.98±1.77aB	63.41±1.76aB
	1(b)	64.08±2.11a B	64.87±2.27aA	65.32±2.38aAB	63.99±2.29aA	65.10±2.22aA	64.51±1.78aAB	64.35±1.46aAB	64.15±1.47aAB
	2(a)	64.44±1.15a B	63.21±1.56aA	64.66±1.33aAB	63.36±1.35aA	64.52±1.82aA	63.63±1.36aB	63.62±1.47aB	64.10±1.19aAB
	2(b)	66.67±0.95a A	65.55±2.46aA	66.29±2.38aA	65.45±2.51aA	65.34±1.36aA	66.58±2.11aA	66.07±1.42aA	66.21±2.33aA
a*	1(a)	4.42±0.20cB	3.16±0.16dB	3.50±0.25dC	3.71±0.27dC	5.17±0.29bC	6.24±0.43aC	6.30±0.37aC	6.56±0.40aC
	1(b)	4.80±0.30aB	2.88±0.27bB	2.40±0.23cD	2.56±0.22bC	2.75±0.17bC	2.71±0.28bcD	2.84±0.13bD	2.52±0.18bC
	2(a)	7.84±0.43cA	7.65±0.35cA	8.30±0.54cA	9.78±0.75bA	10.30±0.72abA	10.74±0.49aA	10.87±0.45aA	10.98±0.49aA
	2(b)	7.79±0.37aA	7.40±0.52aB	7.32±0.37abB	7.03±0.38bB	7.27±0.41aB	7.41±0.58abB	7.54±0.36aB	7.32±0.46aB
b*	1(a)	9.85±0.52aA	9.60±0.44aAB	10.17±0.43aA	8.20±0.67bAB	8.12±0.39bB	7.90±0.46bAB	7.17±0.45bB	7.77±0.34bAB
	1(b)	9.39±0.68abAB	9.86±0.73aA	9.95±0.61aA	8.77±0.47bC	9.47±0.29aB	8.52±0.63bcA	8.52±0.58bC	7.66±0.67cAB
	2(a)	8.71±0.51aB	8.62±0.48aC	9.10±0.45aB	7.57±0.15bB	7.47±0.23bC	6.86±0.60bC	7.42±0.22bB	7.16±0.49bB
	2(b)	8.72±0.64abB	9.04±0.63aBC	8.89±0.28aB	8.51±0.35aC	7.91±0.47cBC	7.54±0.58dBC	7.90±0.44cD	7.97±0.59bcdA

1(a) Vacuum-packed cochineal carmine ham; 1(b) Non-vacuum packed ham with cochineal carmine; 2(a) Vacuum-packed hybrid dye (ZNH-carmine) ham; 2(b) Non-vacuum-packed Hybrid dye ham (ZNH-carmine). Means ± Standard Deviations (n = 9) followed by uppercase superscripts differed in columns, and lowercase superscripts differed in rows ( $p \leq 0.05$ ) by Tukey's Test.

The two formulations of hams added with cochineal carmine (1a and 1b) and ZNH-carmine hybrid dye (2a and 2b) exposed to UV radiation showed changes in color parameters during storage due to the addition of different coloring agents (Table 1). Similarly, alterations were observed with the same dyes used in mortadella studied by

Ongaratto et al. [14], who observed a more intense color with lower  $L^*$  values and higher  $a^*$  values for mortadella added with ZHN-carmine hybrid dye, an ideal alternative to obtain the stable reddish-pink color characteristic of cooked meat products. On the other hand,  $L^*$  values showed no specific trend. Although they were not significantly different from each other, Figure 2 showed higher  $L^*$  parameter values for sample 2(b) and lower values for sample 1(a), which is close to sample 2(a).

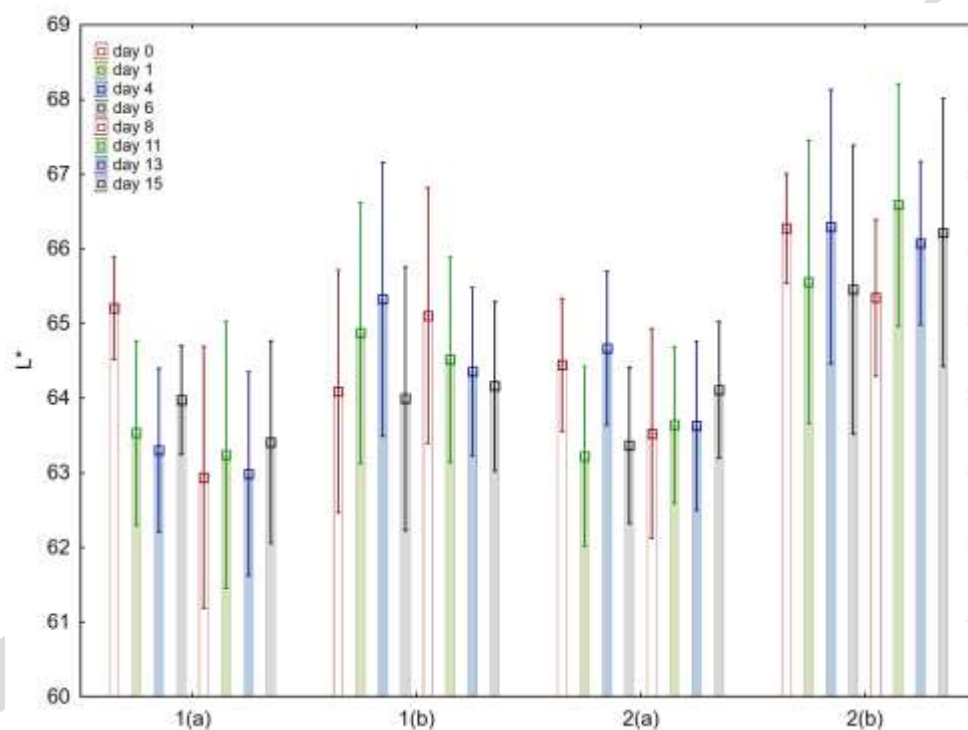


Figure 2. Changes to lightness ( $L^*$ ) color parameters of ham added with cochineal carmine (1) and with hybrid dye (ZHN-carmine) (2). Measurements were taken at 0, 1, 2, 4, 6, 8, 11, 13, and 15 days. 1(a) Vacuum-packed cochineal carmine ham; 1(b) Non-vacuum packed ham with cochineal carmine; 2(a) Vacuum-packed hybrid dye (ZNH-carmine) ham; 2(b) Non-vacuum-packed Hybrid dye ham (ZNH-carmine).

Thus, it may be inferred that sample 2(b) was lighter than the other samples, and

samples 1(a) and 2(a) were darker in color than sample 1(b). These results were similar to the color analysis from [3] in fresh sausage. The authors evaluated the sausage not added with dye, the one added with carmine, and the other two formulations with different microencapsulated jabuticaba extract concentrations and obtained virtually stable  $L^*$  values during 15 days of storage at  $1 \pm 1$  °C. Similarly, Salueña et al. [33] reported that  $L^*$  value did not change significantly during the meat's oxygenation and oxidation, and the meat color may be represented over a plane of constant  $L^*$  changing mainly  $a^*$  values. For  $a^*$  values, it was noted that the formulation (2a and 2b) added with hybrid dye (ZNH-carmine) had a more intense red color, evidenced by the highest positive  $a^*$  value (Figure 3), which reached 69% higher at day 0 when compared to formulation (1a and 1b) using cochineal carmine, resulting in a significant difference ( $p > 0.05$ ) between hams (1a and 1b) and (2a and 2b).

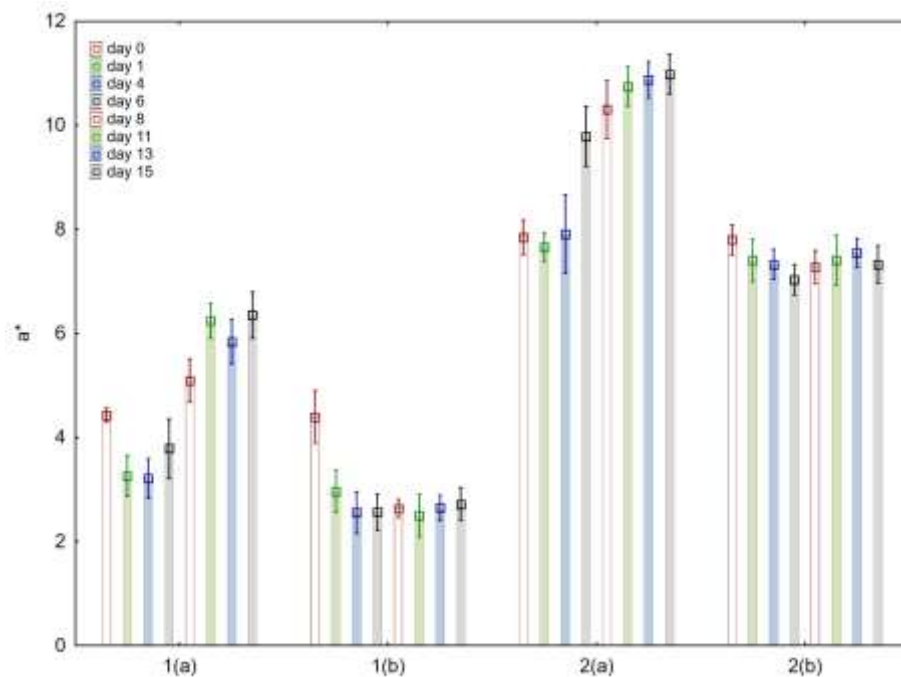


Figure 3. Changes in lightness ( $a^*$ ) color parameters of hams added with cochineal carmine (1) and with hybrid dye (ZHN-carminine) (2). Measurements were taken at 0, 1, 2, 4, 6, 8, 11, 13, and 15 days. 1(a) Vacuum-packed cochineal carmine ham; 1(b) Non-vacuum packed ham with cochineal carmine; 2(a) Vacuum-packed hybrid dye (ZNH-carminine) ham; 2(b) Non-vacuum-packed Hybrid dye ham (ZNH-carminine).

Vacuum-packed ham 1(a) had a 48.4% increase for  $a^*$  value during the 15 day-storage period, whereas non-vacuum-packed ham 1(b) had a 52.5 % reduction ( $p > 0.05$ ), changing the ham's reddish-pink color. Baldin et al. [3] reported that samples of fresh sausage added with carmine and stored under refrigeration lost their characteristic red color, obtaining 13.4  $a^*$  values on the first day of storage and 8.0 after 15 days, reaching a 40% red color reduction. Furthermore, Boyles and Sobeck [34], in their study on red organic food dyes photostability, observed a carminic acid degradation by loss of absorbance, showing a faster initial red color loss in one day when compared to the other dyes studied (Red 40 and betanin). According to the cited authors, the decrease in red

color stability may have been impacted by the oxygen environment, corroborating with the result found in this study for ham 1b. On the other hand, the hams formulated with the hybrid dye (2a and 2b vacuum-packed and non-vacuum-packed, respectively) remained stable for  $a^*$  values, with no red color loss during storage, even when exposed to UV radiation. Furthermore, ham 2a had a 40 %  $a^*$  value increase. In contrast, ham 2b only maintained the initial color stable without any significant difference ( $p > 0.05$ ) between 0 and 15 days. Such  $a^*$  parameter behavior highlights the hybrid dye (ZHN-carmine)'s high red color stability compared to the cochineal carmine added in the formulation (1).

Overall,  $b^*$  values had less significant variations than the other color parameters between samples and storage times (Figure 4), corroborating with the results from Salueña et al. [33], who showed greater stability for  $b^*$  value in meat than  $a^*$  values.

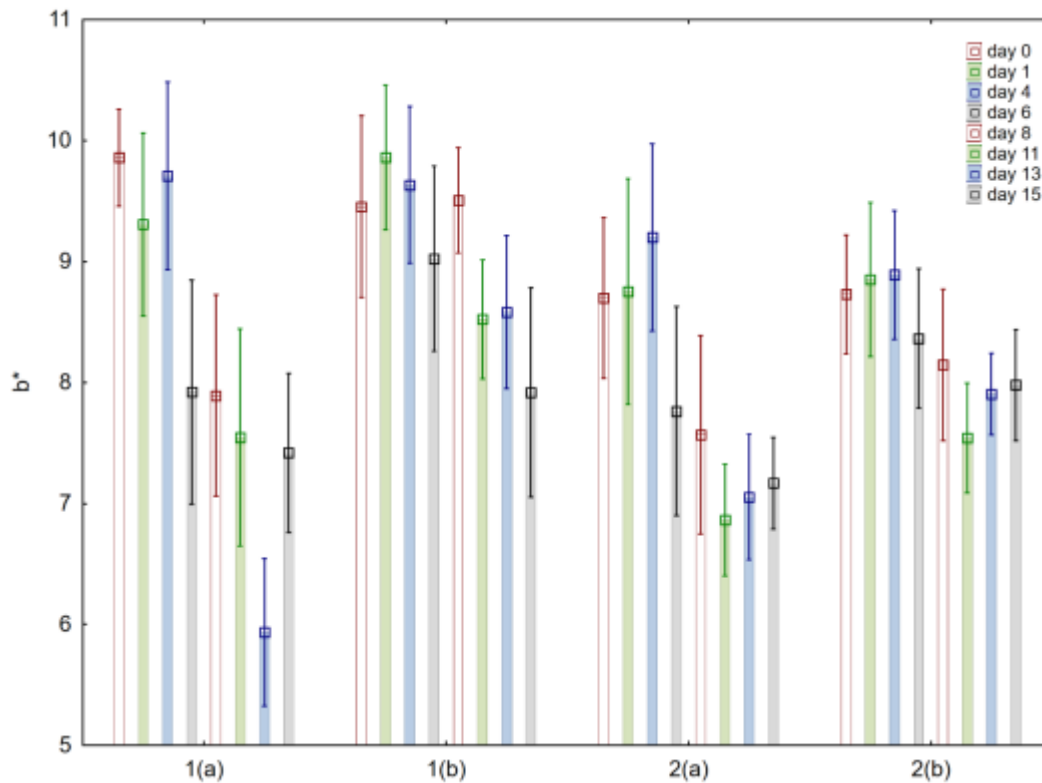


Figure 4. Changes in lightness ( $b^*$ ) color parameters of ham added with cochineal carmine (1) and with hybrid dye (ZHN-carminine) (2). Measurements were taken at 0, 1, 2, 4, 6, 8, 11, 13, and 15 days. 1(a) Vacuum-packed cochineal carmine ham; 1(b) Non-vacuum packed ham with cochineal carmine; 2(a) Vacuum-packed hybrid dye (ZNH-carminine) ham; 2(b) Non-vacuum-packed Hybrid dye ham (ZNH-carminine).

On day 0 and on the evaluated times up to 15 days,  $b^*$  values were positive and usually higher for samples (1), indicating a greater predominance of yellow color in samples added with cochineal carmine. For the storage period, all samples had the stability maintained at the  $b^*$  value until day 4, after which a slight decrease (less than 20 %) was observed for all samples. The results for the ham formulated with hybrid dye (ZNH-carminine) (2) were similar to the color analysis ones from Delgado-Pando et al. (2019) who, when studying ham with reduced salt content, however, using sodium nitrite,



obtained L\* values between 65.13 and 68.43, a\* values between 8.55 and 9.71 and b\* values between 7.79 and 9.44, highlighting the hybrid dye (ZNH-carmine) importance in providing the reddish-pink color given by the curing salts. The total color change ( $\Delta E$ ) over the entire storage period (0 to 15 days) was also determined to evaluate the color difference between samples better.  $\Delta E$  values were 3.54, 2.86, 3.53, and 1.00 for samples 1(a), 1(b), 2(a), and 2(b), respectively. It was verified that the smallest color variation relative to the studied product during its storage time occurred for sample 2(b), which was prepared with a non-vacuum-packed hybrid dye (ZHN-carmine). Conversely, sample 2(a) showed a greater color variation, intensifying the red coloration and highlighting the importance of hybrid dye for the stability of cooked meat products' characteristic color. On the other hand, the variations for samples 1(a) and 2(a) were very close, and both had the greatest color variation between 0 and 15 days. The average variation was due to sample 1(b). Thus, it may be verified that the ham formulations added with cochineal carmine (1a and 1b) obtained the greatest color variations compared to those added with hybrid dye, where the oxygen instability of carmine dye was noted since sample 1(b) lost its characteristic pink color. However, sample 1(a) showed greater dye stability as it was vacuum-packed. It was also found that the vacuum-packed ham samples had a similar color variation. When PCA was evaluated, a two-dimensional consensual solution justified 77.03 % of the variance (Figure 5).

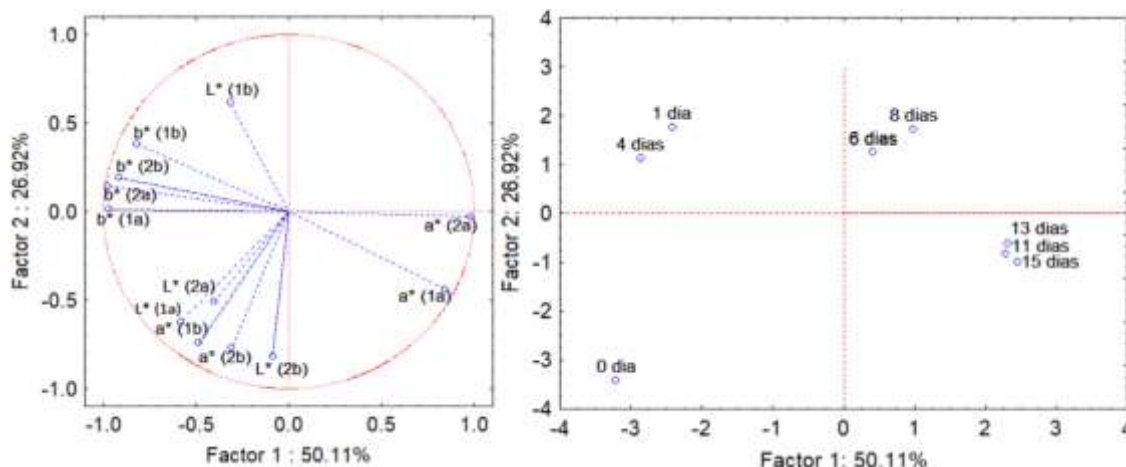


Figure 5. Ham samples' Factor 1 versus Factor 2 (A) loadings and (B) scores plots. 1(a) Vacuum-packed cochineal carmine ham; 1(b) Non-vacuum packed ham with cochineal carmine; 2(a) Vacuum-packed hybrid dye (ZNH-carmine) ham; 2(b) Non-vacuum-packed Hybrid dye ham (ZNH-carmine).

Factor 1 accounted for 50.11 % of the variance, discriminating vacuum-packed ham samples (right) from non-vacuum-packed ham samples (left) based on a\* value (red color intensity). It demonstrated the importance of oxygen removal by referencing the stability of red color in the ham, which was partially lost in the presence of oxygen. Factor 2, with 26.92% of the variance, distinguished ham sample 1b (positioned at the top) from the other samples (1a, 2a, and 2b; placed at the bottom) based on the L\* value. Such behavior of the sample containing cochineal carmine showed the need for oxygen removal to prevent dye oxidation. A lesser need for oxygen removal found between the ham and the packaging was noted when using the hybrid dye (ZNH-carmine). This is highly beneficial for the product's appearance and consumer acceptance, considering that ham pieces are often sliced and portioned by supermarket staff members, who pack the product in plastic or styrofoam trays, covering them with cling film, thus remaining in an

oxygen-rich atmosphere. For storage periods score plots evaluated by factor 1, a segmentation of the samples was observed on day 0 compared to a period longer than 11 days, mainly associated with the  $a^*$  value increase for vacuum-packed samples and a slight  $b^*$  value decrease for all samples. For factor 2, a segmentation of the samples at day 0 with the samples ranging between 1 and 8 days was observed. It appears to be linked to a slight decrease in  $L^*$  value from day 1 until day 8. From day 11 onwards, it returned to values closer to those observed on day 0.

#### 4. Conclusion

The hybrid ZNH-carmin dye obtained by cochineal carmine adsorption into the layered zinc hydroxide salt may be considered an efficient, viable, and safe replacement for the dyes commonly used in cooked hams, considering that the reddish-pink color was more intense in the sample added with ZNH-carmin. No color reduction was observed over the days, irrespective of it being vacuum-packed or not. Meanwhile, the non-vacuum-packed ham added with cochineal carmine significantly decreased the characteristic red color.

#### 5. References

1. USDA. Livestock and poultry: world markets and trade. United States Department of Agriculture and Foreign Agricultural Service. 2024. p. 1–16. Available from: [https://apps.fas.usda.gov/psdonline/circulars/livestock\\_poultry.pdf](https://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf)
2. OECD-FAO AO. Meat consumption. OECD. 2023. Available from: <https://data.oecd.org/agoutput/meat-consumption.htm>

3. Baldin JC, Michelin EC, Polizer YJ, Rodrigues I, de Godoy SHS, Fregonesi RP, et al. Microencapsulated jabuticaba (*Myrciaria cauliflora*) extract added to fresh sausage as natural dye with antioxidant and antimicrobial activity. *Meat Sci.* 2016; 118: 15-21. <https://doi.org/10.1016/j.meatsci.2016.03.016>
4. Bohrer BM. An investigation of the formulation and nutritional composition of modern meat analogue products. *Food Sci Hum Wellness.* 2019; 8(4): 320-329. <https://doi.org/10.1016/j.fshw.2019.11.006>
5. Dey S, Nagababu BH. Applications of food color and bio-preservatives in the food and its effect on the human health. *Food Chem Adv.* 2022; 1: 100019. <https://doi.org/10.1016/j.focha.2022.100019>
6. Martins N, Roriz CL, Morales P, Barros L, Ferreira ICFR. Food colorants: Challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. *Trends Food Sci Technol.* 2016; 52: 1-15. <https://doi.org/10.1016/j.tifs.2016.03.009>
7. Pöhl H. Applications of Different Curing Approaches and Natural Colorants in Meat Products. In: *Handbook on Natural Pigments in Food and Beverages: Industrial Applications for Improving Food Color.* 1st ed. Woodhead Publishing Series in Food Science, Technology and Nutrition; 2016. p. 209-225.
8. Iammarino M, Mentana A, Centonze D, Palermo C, Mangiacotti M, Chiaravalle AE. Dye use in fresh meat preparations and meat products: a survey by a validated method based on HPLC-UV-diode array detection as a contribution to risk assessment. *Int J Food Sci Technol.* 2020; 55(3): 1126-1135. <https://doi.org/10.1111/ijfs.14275>

9. Müller-Maatsch J, Gras C. The “Carmine Problem” and Potential Alternatives. In: Carl R, Schweiggert RM, editors. Handbook on Natural Pigments in Food and Beverages: Industrial Applications for Improving Food Color. Elsevier; 2016. p. 385-428.
10. Lima E, Bosch P, Loera S, Ibarra IA, Laguna H, Lara V. Non-toxic hybrid pigments: Sequestering betanidin chromophores on inorganic matrices. *Appl Clay Sci.* 2009; 42(3-4): 478-482. <https://doi.org/10.1016/j.clay.2008.06.005>
11. Zhao X, Zhang X, Tie S, Hou S, Wang H, Song Y, et al. Facile synthesis of nanocarriers from chitosan and pectin with improved stability and biocompatibility for anthocyanins delivery: An in vitro and in vivo study. *Food Hydrocoll.* 2020; 109: 106114. <https://doi.org/10.1016/j.foodhyd.2020.106114>
12. Liu J, Zhuang Y, Hu Y, Xue S, Li H, Chen L, et al. Improving the color stability and antioxidation activity of blueberry anthocyanins by enzymatic acylation with p-coumaric acid and caffeic acid. *Lwt.* 2020; 130: 109673. <https://doi.org/10.1016/j.lwt.2020.109673>
13. Antigo JLD, Stafussa AP, Bergamasco R de C, Madrona GS. Chia seed mucilage as a potential encapsulating agent of a natural food dye. *J Food Eng.* 2020; 285: 110101. <https://doi.org/10.1016/j.jfoodeng.2020.110101>
14. Ongaratto GC, Oro G, Kalschne DL, Trindade Cursino AC, Canan C. Cochineal carmine adsorbed on layered zinc hydroxide salt applied on mortadella to improve color stability. *Curr Res Food Sci.* 2021; 4: 758-64. <https://doi.org/10.1016/j.crfs.2021.10.006>
15. Marangoni R, Ramos LP, Wypych F. New multifunctional materials obtained by

- the intercalation of anionic dyes into layered zinc hydroxide nitrate followed by dispersion into poly(vinyl alcohol) (PVA). *J Colloid Interface Sci.* 2009; 330(2): 303-309. <https://doi.org/10.1016/j.jcis.2008.10.081>
16. Zimmermann A, Jaeger S, Zawadzki SF, Wypych F. Synthetic zinc layered hydroxide salts intercalated with anionic azo dyes as fillers into high-density polyethylene composites: First insights. *J Polym Res.* 2013; 20(224): 1-11. <https://doi.org/10.1007/s10965-013-0224-3>
17. Forano C. Environmental Remediation Involving Layered Double Hydroxides. In: Wypych F, Satyanarayana KG, editors. *Clay Surfaces - Fundamentals and Applications.* 2004. p. 425-58.
18. Fernandes FM, Baradari H, Sanchez C. Integrative strategies to hybrid lamellar compounds: An integration challenge. *Appl Clay Sci.* 2014; 100: 2-21. <https://doi.org/10.1016/j.clay.2014.05.013>
19. Hwang SH, Han YS, Choy JH. Intercalation of functional organic molecules with pharmaceutical, cosmeceutical and nutraceutical functions into layered double hydroxides and zinc basic salts. *Bull Korean Chem Soc.* 2001; 22(9): 1019-22. <https://doi.org/10.5012/bkcs.2001.22.9.1019>
20. Cursino ACT, Zanotelli NC, Giona RM, de Oliveira Basso RL, Mezalira DZ. Multifunctional food supplements based on layered zinc hydroxide salts intercalated with vitamin anions and adsorbed with vanillin. *J Food Sci Technol.* 2021; 58: 3963-3971. <https://doi.org/10.1007/s13197-020-04859-8>
21. Velázquez-Carriles CA, Carbajal-Arizaga GG, Silva-Jara JM, Reyes-Becerril MC, Aguilar-Uscanga BR, Macías-Rodríguez ME. Chemical and biological protection

- of food grade nisin through their partial intercalation in laminar hydroxide salts. *J Food Sci Technol*. 2020; 57: 3252-3258. <https://doi.org/10.1007/s13197-020-04356-y>
22. Alnoman M, Udombijitkul P, Paredes-Sabja D, Sarker MR. The inhibitory effects of sorbate and benzoate against *Clostridium perfringens* type A isolates. *Food Microbiol* . 2015; 48: 89-98. <http://dx.doi.org/10.1016/j.fm.2014.12.007>
23. Bloot APM, Kalschne DL, Nogues DRN, Amaral JS, Flores ELM, Colla E, et al. Phytic Acid against *Clostridium perfringens* Type A: A Food Matrix Study. *Foods*. 2022; 11(3): 406. <https://doi.org/10.3390/foods11030406>
24. Ruiz-Capillas C, Herrero AM, Tahmouzi S, Razavi SH, Triki M, Rodríguez-Salas L, et al. Properties of reformulated hot dog sausage without added nitrites during chilled storage. *Food Sci Technol Int*. 2016; 22(1): 21-30. <https://doi.org/10.1177/1082013214562919>
25. Guimarães AS, Guimarães JS, Rodrigues LM, Fontes PR, Ramos A de LS, Ramos EM. Assessment of Japanese radish derivatives as nitrite substitute on the physicochemical properties, sensorial profile, and consumer acceptability of restructured cooked hams. *Meat Sci*. 2022; 192: 108897. <https://doi.org/10.1016/j.meatsci.2022.108897>
26. Myers K, Cannon J, Montoya D, Dickson J, Lonergan S, Sebranek J. Effects of high hydrostatic pressure and varying concentrations of sodium nitrite from traditional and vegetable-based sources on the growth of *Listeria monocytogenes* on ready-to-eat (RTE) sliced ham. *Meat Sci*. 2013; 94(1): 69-76. <https://doi.org/10.1016/j.meatsci.2012.12.019>

27. Sindelar JJ, Cordray JC, Olson DG, Sebranek JG, Love JA. Investigating quality attributes and consumer acceptance of uncured, no-nitrate/nitrite-added commercial hams, bacons, and frankfurters. *J Food Sci.* 2007; 72(8): S551-9. <https://doi.org/10.1111/j.1750-3841.2007.00486.x>
28. Jo K, Lee S, Jo C, Jeon HJ, Choe JH, Choi YS, et al. Utility of winter mushroom treated by atmospheric non-thermal plasma as an alternative for synthetic nitrite and phosphate in ground ham. *Meat Sci.* 2020; 166: 108151. <https://doi.org/10.1016/j.meatsci.2020.108151>
29. Brazil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa Nº 20, de 31 de julho de 2000. Regulamento técnico de identidade e qualidade de presunto cozido; 2000 p. 1-22. Available from: <https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=03/08/2000&jornal=1&pagina=55&totalArquivos=88>
30. Brazil. Agência Nacional de Vigilância Sanitária. Resolução da Diretoria Colegiada-RDC nº 272 Brazil: Estabelece os aditivos alimentares autorizados para uso em carnes e produtos cárneos; 2019 p. 194. Available from: <https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=18/03/2019&jornal=515&pagina=194>
31. Silva MLN, Marangoni R, Da Silva AH, Wypych F, Schreiner WH. Poly(vinyl alcohol) composites containing layered hydroxide salts, intercalated with anionic azo dyes (tropaeolin 0 and tropaeolin 0). *Polimeros.* 2013; 23(2): 248-56. <https://dx.doi.org/10.4322/S0104-14282013005000026>
32. Marchesi CM, Cichoski AJ, Zanoelo EF, Dariva C. Influência das condições de



armazenamento sobre os pigmentos cárneos e a cor do salame italiano fatiado.

Ciência e Tecnol Aliment. 2006; 26(3): 697-704.

[https://www.scielo.br/j/cta/a/wnD9ScNb3FVpd8VqSBZwm6j/?format=pdf&lang=](https://www.scielo.br/j/cta/a/wnD9ScNb3FVpd8VqSBZwm6j/?format=pdf&lang=pt)

[pt](https://www.scielo.br/j/cta/a/wnD9ScNb3FVpd8VqSBZwm6j/?format=pdf&lang=pt)

33. Salueña BH, Gamasa CS, Rubial JMD, Odriozola CA. CIELAB color paths during meat shelf life. Meat Sci. 2019; 157: 107889. <https://doi.org/10.1016/j.meatsci.2019.107889>
34. Boyles C, Sobeck SJS. Photostability of organic red food dyes. Food Chem. 2020; 315: 126249. <https://doi.org/10.1016/j.foodchem.2020.126249>
35. Delgado-Pando G, Allen P, Kerry JP, O'Sullivan MG, Hamill RM. Optimising the acceptability of reduced-salt ham with flavourings using a mixture design. Meat Sci. 2019; 156: 1-10. <https://doi.org/10.1016/j.meatsci.2019.05.010>