

Natural colorants improved the physicochemical and sensorial properties of frozen Brazilian sausage (*linguiça*) with reduced nitrite

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ABSTRACT: The use of natural colorants in meat products can keep its coloring attractive as well as meet the demand from consumers interested in products that are healthier. Nitrite is added as a preservative to these products and it is also a precursor of the generation of a specifically desired color in meat products. However, its use has been associated with the development of certain kinds of cancer. The objective of this study was to evaluate the effect of reduced levels of nitrite in Brazilian sausage (*linguiça*) with natural colorants on the physicochemical and sensorial properties following frozen storage. Nine treatments were tested: 0.015 % nitrite (C150), 0.0075 % nitrite (C75) and 0 % nitrite (C0), 0.0075 % nitrite and 1.5 % microencapsulated betalain (BME75), 0 % nitrite and 1.5 % microencapsulated betalain (BME0), 0.0075 % nitrite and 0.19 % commercial betalain (BCP75), 0 % nitrite and 0.19 % commercial betalain (BCP0), 0.0075 % nitrite and 0.02 % of cochineal carmine (CC75) and 0 % nitrite and 0.02 % of cochineal carmine (CC0). Oxidative stability, residual nitrite and instrumental color were the determinant factors. Sensory analysis was directed at color, texture, taste and global acceptance. Natural colorants did not have any antioxidant effect. Sausages prepared with betalain and 0.0075 % nitrite showed the highest a* values. The addition of colorants improved both color and overall acceptance. The addition of betalain and cochineal carmine with 0.0075 % nitrite proved to be effective in achieving high acceptability in Brazilian sausage (*linguiça*).

Keywords: betalain, sensory evaluation, cochineal carmine, lipid oxidation

Introduction

Sodium nitrite is a salt commonly used to cure meats in the production of ham, bacon and sausages. It acts by preserving meat products, inhibiting the germination of spores of *Clostridium botulinum* and resisting the growth of *Listeria monocytogenes*, among others (Feng et al., 2016). Nitrite also has antioxidant properties which influence the reduction in rancidity of the product (Sucu and Turp, 2018). Nitrite is known for its contribution to the development of the reddish-pink color and the flavor characteristics of cured meat products (Alahakoon et al., 2015; Bedale et al., 2016; Feng et al., 2016). However, nitrite is also connected to increased risk in certain types of cancer (Jin et al., 2012; Zhang et al., 2018). It is considered the most feared food additive and, is thus, avoided by consumers (Bedale et al., 2016). Research has been carried out in recent years to reduce nitrite in these products, and thereby decrease the potential risks to humans (Alahakoon et al., 2015; Alirezalu et al., 2018; Feng et al., 2016; Jin et al., 2012; Ruiz-Capillas et al., 2014).

On the other hand, meat color is the main factor in consumer's purchasing decisions because it is considered to be a good indicator of meat freshness and quality (Domingo et al., 2015). In this regard, food coloring is used to maintain an attractive red color in meat products; however, only natural colorants should be considered for such application, since 'natural' is associated with an

image of good health and quality (Martínez et al., 2006). The most commonly used natural colorant in meat products is cochineal carmine. It is soluble in water and its color depends on the pH. Moreover, it is stable when confronted by changes in temperature and light and is resistant to oxidation (Borges et al., 2012).

Betalains are plant pigments that belong to the *Caryophyllales* order (Strack et al., 2003). According to their chemical structure, betalains are divided into two groups: the red-violet betacyanins and the yellow betaxanthins. The root of red beets (*Beta vulgaris L. ssp. vulgaris*) is the main source of betanin (Nemzer et al., 2011), which contains about 300-600 mg betanin kg⁻¹ (Kanner et al., 2001) and high amounts of nitrate, containing more than 2,500 mg nitrate kg⁻¹ (Sucu and Turp, 2018).

The meat processing industry is searching for alternative technologies to increase the shelf-life of meat products by utilizing additives from natural sources (Lorenzo et al., 2018a, b, c). In view of this, the replacement of nitrite from artificial origin with natural compounds in meat products is a trend that has been on the increase over the last few years (Hwang et al., 2017; Jin et al., 2014; Sucu and Turp, 2018). The reduction in temperature of storage (frozen) or vacuum packaging are procedures that have been adopted to ensure microbiological safety and prolong the commercial life of low-nitrite content meat products (Lago et al., 2019).

The objective of this work was to assess the effect of the betalain and cochineal carmine, as natural colorants, on the physicochemical properties, lipid oxidation and sensorial acceptance of frozen Brazilian sausage (*linguiça*) with reduced nitrite.

Materials and Methods

Brazilian sausage (*linguiça*) production

Brazilian sausage (*linguiça*) is a raw meat product widely consumed in Brazil, usually sold to the consumer under frozen or fresh conditions; this product needs to be cooked before consumption. It is not an emulsified product such as frankfurter sausage or mortadella sausage. The raw materials, pork ham (19.13 % of protein and 5.33 % of fat) and pork fat trimmings (15.49 % of protein and 22.68 % of fat) were obtained from the slaughterhouse (Ipoã, São Paulo, Brazil – latitude 23°31'34" S; longitude 46°20'38" W; altitude 745 m) 48 h after slaughter, and are ground using a 10 mm diameter disc. All formulations had the following ingredients: pork ham (68 %), pig fat trimmings (15 %), sodium lactate (2.0 %), condiment for Brazilian sausage (0.5 %), sodium chloride (0.8 %), sodium hexametaphosphate (0.2 %), natural garlic (0.4 %), sodium erythorbate (0.4 %), white pepper (0.195 %) and ascorbic acid (0.02 %).

Microencapsulated betalain (BME) was produced by the Laboratory for Physical Measurement at IBILCE (Institute of Biosciences, Humanities and Exact Sciences) through the homogenization of beetroot with 70 % ethanol, which was then filtered and evaporated under vacuum to constant volume (15° Brix). This extract was homogenized in a solution of 35 % of total solids with enzymatically modified starch and maltodextrin DE 10 at a ratio of 80:20, respectively. Next, the finalized solution was atomized in a spray drier to obtain microcapsules in the final concentration of betalain (0.034 % w w⁻¹). The commercial betalain (BCP) used was B-35-WSP (Chr. Hansen's Laboratorium Denmark A/S., Horsholm, Denmark), where the concentration of betalain was 0.25 to 0.28 % (w w⁻¹). Another colorant used was cochineal carmine (CC), namely, CC-3000WS (Chr. Hansen's Laboratorium Denmark A/S., Horsholm, Denmark), in which the concentration of caminic acid was 2.8 to 3.2 % (w w⁻¹).

Nine treatments were processed and the difference between treatments was the amount of sodium nitrite, colorant and water added (Table 1). The ground meat and

other ingredients were blended for 10 min and this meat mixture was stuffed into natural pork casings 34 mm in diameter and tied into approximately 12 cm skins. The Brazilian sausages (*linguiça*) were individually vacuum packed in nylon-polyethylene bags and were frozen and stored at -18 ± 2 °C for up to 90 days for evaluation. For the analysis, the Brazilian sausages (*linguiça*) were thawed for 24 h at 4 ± 2 °C. All treatments were replicated independently three times. For each replicate, 30 sausages were produced per treatment. Samples at day 0 were taken for pH, chemical composition and sensorial analysis; whereas samples at 0, 30, 60 and 90 days of storage for TBARS, residual nitrite and color parameters.

Proximate composition

Moisture, ash and protein contents were determined following the official method of the Association of Official Analytical Chemistry (AOAC, 2007). Fat content was assessed using the method described by Bligh and Dyer (1959). The analyses were performed in triplicate for each treatment and results were expressed in percentages.

Oxidative stability (TBARS), residual nitrite and pH values

Lipid oxidation was determined according to the method previously described by Vyncke (1970) with modifications proposed by Zipser and Watts (1962). The betalain is a colorant soluble in water, so it can be present in filtered extracts before the addition of a thiobarbituric acid solution. Therefore, the filtrate obtained in the determination of oxidative stability was subject to two different conditions: 1) following the methodology mentioned, adding thiobarbituric acid solution in acetic acid (90 % v v⁻¹), and 2) with the addition of acetic acid only (90 % v v⁻¹) without the presence of thiobarbituric acid. Both were read at 532 nm, and the values of condition 2 were deducted from condition 1, in order to isolate the interference of betalain in the results. Residual nitrite analysis was performed using the diazotization reaction of nitrites with sulfanilic acid and coupling with alpha-naphthylamine hydrochloride in an acidic medium, forming alpha-naphthylamino-pazobenzene-β-sulfonic acid, which shows a pink coloration as described by the Association of Official Analytical Chemists (AOAC, 2000). The results were determined spectrophotometrically at 540 nm and by a standard curve at different concentrations of sodium nitrite. The assays were performed in C150, C75, BME75, BCP75 and CC75 treatments and the

Table 1 – Difference between treatments of frozen Brazilian sausages (*linguiça*) (%).

Treatment	C150	C75	C0	BME75	BME0	BCP75	BCP0	CC75	CC0
Water	12.47	12.4775	12.485	10.9775	10.985	12.2875	12.295	12.4575	12.465
NaNO ₂ ¹	0.015	0.0075	-	0.0075	-	0.0075	-	0.0075	-
CBM ²	-	-	-	1.5	1.5	-	-	-	-
CCB ³	-	-	-	-	-	0.19	0.19	-	-
CCL ⁴	-	-	-	-	-	-	-	0.02	0.02

¹Sodium nitrite; ²Microencapsulated betalain; ³Commercial betalain; ⁴Cochineal carmine.

pH was determined using a PG 1800 pH meter with a penetration probe and four readings were taken for each treatment. All the determinations were made in triplicate.

Color measurement

Color measurements were determined on the outer surface of the uncooked sausage (*linguiça*) using a ColorFlex45/0 colorimeter (Hunterlab, Reston, United States) and calibrated using the black glasses and white tiles provided. Samples were analyzed directly over the 0.75-inch aperture, with D65 illumination configurations, and a 10° viewing angle, using the Universal Software program version 4.10. The CIELab system of color specification was used and the parameters obtained were L* (lightness), a* (redness) and b* (yellowness). Ten determinations were made for each treatment, avoiding areas that had an excess of fat.

Sensory analysis

The effective acceptance test was carried out on a group of eighty consumers, who answered a questionnaire on characterisation of the sensory panel – 71.25 % said they liked Brazilian sausage (*linguiça*) a lot, even though 70.89 % consume it only once a week. A hedonic scale of nine points ranging from 1 – “disliked extremely” to 9 – “liked extremely” was used to evaluate the color, texture and flavor attributes as well as overall acceptance. The samples were cooked in an FELS3 electric oven until the geometric centre of the sausage reached 72 ± 2 °C, controlled by a digital thermocouple. After cooking, the samples were cut into 2 cm (height) cylinders and kept at 60 °C in a water bath at 90 °C until the analysis. They were delivered in a monadic manner (Meilgaard et al., 1999) and followed a complete block design. This study was approved by the Ethics Committee of the Institute of Biosciences and Exact Sciences, UNESP (n° 58983516.2.0000.5466).

Statistical analyses

Nine treatments (C150, C75, C0, BME75, BME0, BCP75, BCP0, CC75 and CC0) of Brazilian sausage (*linguiça*) were manufactured in triplicate (n = 3), and analyzed during the frozen storage period (at 0, 30, 60 and 90 days). For the statistical analysis of the results, analysis of variance (ANOVA) was carried out using the

General Linear Model (GLM). The time of storage and the treatment were considered to be constant effects while manufacture repetition was a random effect. For the sensory analysis, the treatment was considered to be the constant effect and consumers as the random effect. Statistical analyses were carried out using the statistical SPSS software package (IBM SPSS Statistics, version 23).

Results and Discussion

Proximate composition and pH

Nitrite significantly affected ($p < 0.05$) the moisture content of the Brazilian sausage (*linguiça*) from control samples and those manufactured with microencapsulated betalain (Table 2), whereas samples prepared with commercial betalain and cochineal carmine were not affected. Our findings are in agreement with those observed by Alahakoon et al. (2015) who found that the use of nitrite salts in meat reduces its moisture content and water activity. The amount of protein varied from 15.31 to 18.36 %, fat content between 5.35 and 8.97 % and the amount of ash ranged from 2.40 to 2.83 %. These variations are inherent in the raw meat used.

On the other hand, it was also found that the addition of natural colorants (betalains and cochineal carmine) and the use of nitrite (0 to 0.015 %) did not affect ($p > 0.05$) the pH values of Brazilian sausages (Table 2). Similar results were found by Sucu and Turp (2018) who found that the addition of beetroot powder as a nitrite alternative did not affect the pH values of Turkish fermented beef sausage. However, Choi et al. (2017) and Hwang et al. (2017) noticed that the inclusion of red beet showed a decrease in pH values of emulsion type sausages.

Oxidative stability

Nitrite shows antioxidant effects on frozen Brazilian sausages (*linguiça*) at 60 days, where C150 presents TBARS values lower than C0 and BCP75, and BME75 were lower than BME0 (Figure 1). At day zero, the results found for controls with nitrite (C150 and C75) were similar ($p < 0.05$) to the control which had no added nitrite (C0). At 30 and 90 days, no differences to TBARS values among treatments were found ($p > 0.05$) (Figure 1). The inclusion of beetroot powder could show an antioxidant activity due to betalain

Table 2 – Proximate analysis and pH of frozen Brazilian sausages (*linguiça*).

	Treatments									SEM	p-value
	C150	C75	C0	BME75	BME0	BCP75	BCP0	CC75	CC0		
Proximate analysis (%)											
Moisture	70.30 ^{bc}	70.38 ^{bc}	73.04 ^a	69.18 ^d	70.38 ^{bc}	69.61 ^{cd}	70.28 ^{bc}	71.17 ^b	71.14 ^b	0.237	0.000
Protein	16.23 ^{bcd}	16.76 ^{bc}	18.36 ^a	15.31 ^d	15.32 ^d	16.29 ^{bc}	16.89 ^b	15.82 ^{cd}	16.31 ^{bc}	0.217	0.000
Fat	8.97 ^a	7.16 ^b	5.35 ^c	7.31 ^{ab}	6.11 ^{bc}	7.37 ^{ab}	6.68 ^{bc}	6.91 ^{bc}	7.73 ^{ab}	0.245	0.001
Ash	2.40 ^b	2.69 ^{ab}	2.78 ^a	2.69 ^{ab}	2.69 ^{ab}	2.77 ^a	2.83 ^a	2.75 ^a	2.45 ^b	0.036	0.003
pH	5.89	5.93	5.71	5.91	5.85	5.82	5.77	5.87	5.78	0.018	0.067

C150 = 0.015 % nitrite, C75 = 0.0075 % nitrite, C0 = 0 % nitrite. BME75 = 1.5 % microencapsulated betalain and 0.0075 % nitrite, BME0 = 1.5 % microencapsulated betalain and 0 % nitrite, BCP75 = 0.19 % commercial betalain and 0.0075 % nitrite, BCP0 = 0.19 % commercial betalain and 0 % nitrite, CC75 = 0.02 % cochineal carmine and 0.0075 % nitrite, CC0 = 0.02 % cochineal carmine and 0 % nitrite. ^{a-d}Mean values in the same line not followed by a common letter differ significantly ($p < 0.05$). SEM = standard error of the mean.

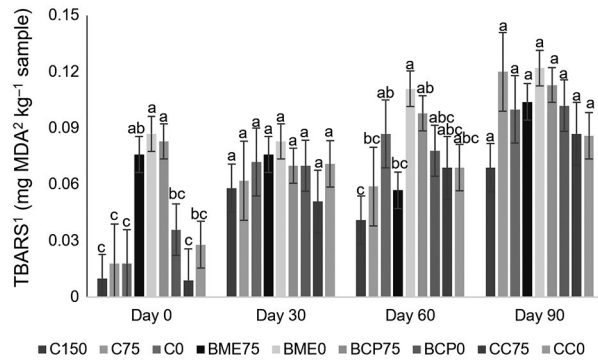


Figure 1 – TBARS values of Brazilian sausage (*linguiça*) during frozen storage. C150 = 0.015 % nitrite, C75 = 0.0075 % nitrite, C0 = 0 % nitrite, BME75 = 1.5 % microencapsulated betalain and 0.0075 % nitrite, BME0 = 1.5 % microencapsulated betalain and 0 % nitrite, BCP75 = 0.19 % commercial betalain and 0.0075 % nitrite, BCP0 = 0.19 % commercial betalain and 0 % nitrite, CC75 = 0.02 % cochineal carmine and 0.0075 % nitrite, CC0 = 0.02 % cochineal carmine and 0 % nitrite. ^{a-c}Mean values on the same day not followed by a common letter differ significantly ($p < 0.05$) Errors bars show the standard error. ¹TBARS = Thiobarbituric acid reactive substances. ²MDA = Malondialdehyde.

content, which presents antioxidant and radical-scavenging activities (Georgiev et al., 2010). The use of betalain and cochineal carmine did not have any antioxidant effect on Brazilian sausage (*linguiça*). According to Stagnari et al. (2014), 100 g of red beet contain between 62.04 and 118.92 mg of betalains.

Our results are in agreement with the data found by Sucu and Turp (2018) who did not observe any significant differences in TBARS values at the end of storage time in Turkish fermented beef sausage manufactured with sodium nitrite and three levels of beetroot powder. Moreover, according to O'Neill et al. (1998), the threshold for detecting rancid odor in pork is between 0.5 and 2.0 mg TBARS kg^{-1} of the sample. The TBARS results obtained in the present study did not exceed the limit compromising the sensory quality of the product in all the periods analysed.

Residual nitrite content

The residual nitrite values (Figure 2) after 0 and 30 days of storage were the highest in C150. A similar result was found by Jin et al. (2012) who used different concentrations of nitrite (100 and 150 mg kg^{-1}) and purple-fleshed sweet potato powder in cooked pork sausage. A drop in residual nitrite amount was found in all treatments during the storage time ($p < 0.05$).

The results of the present study showed that, on day 0, the samples presented a mean reduction of 68 % of the initial concentrations after production, ranging from 24.10 and 77.69 mg kg^{-1} nitrite (Figure 2). BME75 and the control (C150 and C75) had a reduction of approximately 50 % of the nitrite content compared to the initial amount

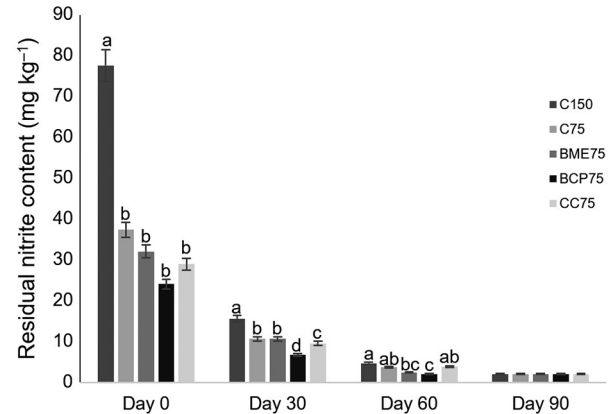


Figure 2 – Residual nitrite content of Brazilian sausages (*linguiça*) during frozen storage. C150 = 0.015 % nitrite, C75 = 0.0075 % nitrite, BME75 = 1.5 % microencapsulated betalain and 0.0075 % nitrite, BCP75 = 0.19 % commercial betalain and 0.0075 % nitrite, CC75 = 0.02 % cochineal carmine and 0.0075 % nitrite. ^{a-c}Mean values in the same day not followed by a common letter differ significantly ($p < 0.05$). Error bars show the standard error.

added. The reduction of nitrite values was lower than those reported by Xi et al. (2012) who observed that residual nitrite content was around 75 % of the initial levels after the production process.

After 30 days of storage, the amount of nitrite presented in the C150 had a reduction of 90 % of the initial concentration added at the formulation stage in the C75 and BME75 the reduction of nitrite was approximately 86 %, whereas in the BCP75 and CC75 the reduction was 91 and 87 %, respectively. After 60 days of storage, all treatments had values ≤ 5 % of the amount of nitrite added, ranging from 2.60 to 4.74 mg kg^{-1} nitrite for BCP75 and CC75, respectively. At the end of storage time, it was not possible to quantify the nitrite in the samples (below limit of detection). According to Sucu and Turp (2018), the residual nitrite amount differed according to the processing conditions, type of product, the presence of sodium ascorbate, and other factors (non-heme proteins, myoglobin and lipids).

Color measurement

The inclusion of betalain and cochineal carmine decreased lightness and yellowness and increased redness values (Table 3). There were no significant differences in luminosity ($p < 0.05$) compared to the control. Thus, the reduction of nitrite had no effect on this parameter. The control with nitrite at 0.0075 % (C75) and the control with no added nitrite (C0) presented the highest luminosity values during the storage time. This outcome agrees with the data found by Ruiz-Capillas et al. (2014) who noticed that the addition of nitrite showed a higher luminosity than the treatments with added celery as a natural source of nitrite and *urucum* and cochineal carmine as colorants in hot dog sausages. Moreover, Bloukas et al. (1999) when studying the effect of adding natural colorants and nitrite

Table 3 – Color parameters (L*, a* and b*) of frozen Brazilian sausages (*linguiça*).

	C150	C75	C0	BME75	BME0	BCP75	BCP0	CC75	CC0	SEM	p-value
L*											
0	53.58 ^{abAB}	54.41 ^{ba}	54.22 ^{ba}	50.39 ^{baB}	50.81 ^{baB}	51.94 ^{baB}	51.72 ^{baB}	53.19 ^{baB}	54.04 ^{ba}	0.289	0.001
30	58.20 ^{abAB}	58.60 ^{ba}	58.72 ^{ba}	54.53 ^{acD}	55.55 ^{abCD}	54.12 ^{abD}	53.14 ^{abD}	54.49 ^{abCD}	57.13 ^{abABC}	0.258	0.000
60	57.55 ^{abAB}	58.69 ^{ba}	57.52 ^{abAB}	54.54 ^{acD}	55.21 ^{abCD}	54.34 ^{abCD}	53.82 ^{abD}	56.76 ^{abABC}	58.10 ^{ba}	0.234	0.000
90	57.26 ^{abAB}	57.39 ^{abAB}	58.86 ^{ba}	54.44 ^{ab}	54.81 ^{ab}	55.32 ^{ab}	55.52 ^{ab}	56.86 ^{abAB}	56.48 ^{abAB}	0.263	0.000
SEM	0.372	0.372	0.360	0.461	0.466	0.352	0.429	0.401	0.346		
p-value	0.000	0.000	0.000	0.000	0.000	0.005	0.019	0.001	0.000		
a*											
0	10.11 ^{bEF}	10.07 ^{cEF}	9.28 ^{af}	13.93 ^{AB}	12.79 ^{abC}	14.80 ^{abA}	13.67 ^{abAB}	11.73 ^{bCD}	11.01 ^{aDE}	0.168	0.000
30	11.43 ^{ac}	11.27 ^{abC}	8.31 ^{abd}	14.60 ^{AB}	11.90 ^{abC}	15.61 ^{aA}	13.35 ^{ab}	13.99 ^{ab}	11.07 ^{ac}	0.168	0.000
60	10.64 ^{abE}	10.39 ^{bcE}	8.40 ^{abf}	14.02 ^{AB}	11.80 ^{bd}	15.05 ^{abA}	12.88 ^{abCD}	12.98 ^{abC}	10.01 ^{bE}	0.160	0.000
90	11.57 ^{abC}	11.46 ^{abC}	7.71 ^{bd}	13.89 ^A	11.38 ^{bc}	14.33 ^{ba}	12.31 ^{bb}	13.73 ^{aA}	10.79 ^{abC}	0.166	0.000
SEM	0.172	0.141	0.161	0.148	0.138	0.131	0.144	0.168	0.132		
p-value	0.005	0.001	0.010	0.437	0.004	0.004	0.007	0.000	0.019		
b*											
0	12.97 ^{aA}	12.88 ^A	12.83 ^{AB}	11.37 ^C	11.87 ^{ac}	10.17 ^{ad}	9.59 ^{abd}	11.96 ^{BC}	11.79 ^C	0.101	0.000
30	11.76 ^{baBC}	12.10 ^{AB}	12.19 ^A	10.76 ^{CD}	10.59 ^{bd}	9.09 ^{be}	9.43 ^{abE}	11.08 ^{BCD}	11.67 ^{ABC}	0.106	0.000
60	11.65 ^{baBC}	11.99 ^{AB}	12.22 ^A	11.05 ^{BC}	10.75 ^{bc}	9.60 ^{abd}	8.67 ^{bd}	11.60 ^{ABC}	11.68 ^{ABC}	0.111	0.000
90	11.83 ^{baB}	12.41 ^A	12.38 ^A	10.65 ^{CD}	10.12 ^{bd}	9.08 ^{be}	9.74 ^{abE}	11.21 ^{BC}	11.83 ^{AB}	0.113	0.000
SEM	0.122	0.127	0.119	0.132	0.124	0.112	0.120	0.132	0.101		
p-value	0.000	0.053	0.199	0.216	0.000	0.001	0.016	0.070	0.934		

C150 = 0.015 % nitrite, C75 = 0.0075 % nitrite, C0 = 0 % nitrite. BME75 = 1.5 % microencapsulated betalain and 0.0075 % nitrite, BME0 = 1.5 % microencapsulated betalain and 0 % nitrite, BCP75 = 0.19 % commercial betalain and 0.0075 % nitrite, BCP0 = 0.19 % commercial betalain and 0 % nitrite, CC75 = 0.02 % cochineal carmine and 0.0075 % nitrite, CC0 = 0.02 % cochineal carmine and 0 % nitrite. ^{A-F}Mean values in the same line not followed by a common letter differ significantly ($p < 0.05$). SEM = standard error of the mean. ^{a-c}Mean values in the same column not followed by a common letter differ significantly ($p < 0.05$).

to the color of frankfurter sausage, it was observed that the highest L* values were found in the control treatment, with the addition of nitrite only, whereas the samples with added carmine or betalain showed the lowest luminosity. The values of L* increased over time for all the treatments analyzed and the same trend was observed by Sucu and Turp (2018) in fermented sausages manufactured with the addition of beetroot powder in different concentrations (as a source of nitrate) and reduction of nitrite. Jin et al. (2014) also observed that L* values for the red beet-added group increased during storage time ($p < 0.05$); control treatments did not present any effect of storage on lightness ($p > 0.05$). On the contrary, Hwang et al. (2017) observed a decrease in L* of low-fat frankfurter sausages by the addition of red beet extract. In Brazilian sausages (*linguiça*) (at day 0), the control samples without the addition of colorants (C150, C75 and C0) presented similar redness values, possibly due to an incomplete curing reaction, with a* values ranging from 9.28 to 10.11. In this regard, Bloukas et al. (1999) noticed that, initially, nitrite is reduced to nitric oxide, and this process involves the oxidation of myoglobin to metmyoglobin, producing a brown color. Subsequently, the nitric oxide reacted with the myoglobin of the meat, forming nitric oxide myoglobin, a bright red compound which remains stable when exposed to heat.

After 30, 60 and 90 days of storage, C0 presented the lowest a* values ($p < 0.05$), corroborating the role of the nitrite in the final coloring of Brazilian sausage (*linguiça*) (Table 3). On the other hand, C150 and C75 were similar ($p > 0.05$), reporting that amounts below

0.015 % nitrite are sufficient to generate the desired color of cured products. The addition of the betalain colorant increased the redness ($p > 0.05$) in Brazilian sausage (*linguiça*) with nitrite at 0.0075 % producing the highest a* values in all periods analysed (BCP75). These results show that the addition of betalain colorant is effective in improving the color of Brazilian sausage and keeping it attractive for up to 90 days of frozen storage. Moreover, BME0, BCP0 and CC0 had higher or similar values than the C150 and C75 controls and higher than the C0 treatment (Table 3), showing that the use of the colorants assisted in the final color of the meat product, even with no added nitrite. Bloukas et al. (1999) found that carminic acid, betanin and paprika extract significantly increased the redness in Frankfurter sausage and their interaction with nitrite resulted in an increase in a* values. The authors also claimed that the interaction of nitrite with natural colorants positively affected the intensity of the red color of the sausage, resulting in a more acceptable product for consumers. Other authors (Jin et al., 2014; Sucu and Turp, 2018) have also noticed that a* values increased with an increased level of red beetroot added. There was a reduction in a* values over time in the C0, BME0, BCP75, BCP0 and CC0 treatments. The reduction found in the treatments after the addition of betalain may be due to the degradation of this pigment by light, oxygen, pH and temperature (Martínez et al., 2006). In addition, the encapsulation process of betalain did not help to improve its stability in Brazilian sausage (*linguiça*) due to the carriers' material used being soluble in water.

Thus, the microencapsulated betalain showed behavior similar to commercial betalain.

C0, C75 and C150 did not show any differences ($p > 0.05$) for b^* values during the storage time (Table 3). The lower b^* values ($p < 0.05$) for Brazilian sausages (*linguiça*) were found in BCP75 and BC0. This finding agrees with that noticed by Sucu and Turp (2018) who found that the inclusion of beetroot powder significantly decreased b^* values on day 0.

Sensory analysis

As regards color attributes (Table 4), Brazilian sausage (*linguiça*) manufactured with natural colorants and nitrite presented the highest scores (7.57, 7.26, 7.22 and 7.07 for BCP75, CC75, C150 and BME75, respectively). In contrast, the C0 control had the lowest score for color, with a mean between the terms "slightly dislike" and "moderately dislike" and was no different from the BME0, which had no addition of nitrite. This outcome is in agreement with the data noticed by Jin et al. (2014), who found higher color scores in emulsified sausages prepared with red beet addition and nitrite compared to control, whereas no significant differences were observed in the other treatments in terms of flavor, tenderness and juiciness. In addition, Sucu and Turp (2018) reported that the inclusion of beetroot powder significantly ($p < 0.05$) increased the color scores of the Turkish fermented beef sausages.

Texture attribute presented significant differences between treatments, presenting the lowest scores in Brazilian sausages (*linguiça*) prepared without sodium nitrite and natural colorants. The addition of natural colorants significantly increased ($p < 0.05$) the overall acceptance of the Brazilian sausage (*linguiça*) even with a 50 % reduction in nitrite, since the best scores were obtained for BCP75, CC75 and C150 treatments. However,

the treatments that had no added nitrite presented the lowest scores for the overall acceptance, even with the addition of betalain and cochineal carmine. This finding agrees with that found by Sucu and Turp (2018) who observed that the overall acceptance scores of sausages prepared with 0.24 % and 0.35 % of beetroot powder were higher than those of the control treatment. In addition, Choi et al. (2017) also found significant differences in the overall acceptability of meat emulsions prepared with 10 % fermented red beet compared to those of the control treatment containing nitrite.

The C0, without addition of colorant and nitrite, presented the lowest scores for all the attributes examined (Table 4) and was, therefore, less acceptable compared to the others. Ruiz-Capillas et al. (2014) reported a sensory evaluation of three treatments for hot-dog type sausages - with nitrite, with no added nitrite but with cochineal carmine and no added nitrite with annatto, and their results showed that the treatment with annatto was less acceptable than the other treatments since the color is the attribute that mostly contributed to overall acceptability.

Conclusion

The addition of betalain and cochineal carmine in frozen Brazilian sausage (*linguiça*) improved the color when the nitrite was reduced or not added. The physicochemical and sensorial properties (color, flavor and overall acceptance) of Brazilian sausage (*linguiça*) were compromised in the cases of no added nitrite and natural colorants. No decrease in the oxidative stability of frozen Brazilian sausage (*linguiça*) manufactured with reduced nitrite was observed. The betalain proved to be a natural alternative for reducing nitrite levels without compromising the acceptability of frozen Brazilian sausages (*linguiça*).

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Authors' Contributions

Conceptualization and data acquisition: Barretto, T.L.; Bis-Souza, C.V.; Barretto, A.C.S. **Data analysis and writing and editing:** Bellucci, E.R.B.; Barretto, T.L.; Lorenzo, J.M.; Bis-Souza, C.V.; Barba, F.J.; Barretto, A.C.S.

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Table 4 – Sensory acceptance of frozen Brazilian sausages (*linguiça*).

Treatments	Sensory attributes			
	Color	Texture	Flavor	Overall acceptance
C150	7.22 ^{ab}	7.61 ^a	7.60 ^{ab}	7.57 ^a
C75	6.80 ^b	7.06 ^{bcd}	7.24 ^{bc}	7.10 ^b
C0	3.96 ^d	6.56 ^d	6.85 ^c	6.04 ^d
BME75	7.07 ^{ab}	7.46 ^{ab}	7.64 ^{ab}	7.41 ^{ab}
BME0	4.61 ^{cd}	7.01 ^{bcd}	7.20 ^{bc}	6.54 ^c
BCP75	7.57 ^a	7.49 ^{ab}	7.74 ^a	7.65 ^a
BCP0	4.76 ^c	7.11 ^{abc}	7.25 ^{bc}	6.57 ^c
CC75	7.26 ^{ab}	7.57 ^a	7.74 ^a	7.55 ^a
CC0	5.05 ^c	6.91 ^{cd}	7.24 ^{bc}	6.49 ^c
SEM	0.077	0.048	0.045	0.046
p-value	0.000	0.000	0.000	0.000

C150 = 0.015 % nitrite, C75 = 0.0075 % nitrite, C0 = 0 % nitrite. BME75 = 1.5 % microencapsulated betalain and 0.0075 % nitrite, BME0 = 1.5 % microencapsulated betalain and 0 % nitrite, BCP75 = 0.19 % commercial betalain and 0.0075 % nitrite, BCP0 = 0.19 % commercial betalain and 0 % nitrite, CC75 = 0.02 % cochineal carmine and 0.0075 % nitrite, CC0 = 0.02 % cochineal carmine and 0 % nitrite. ^{a-d}Mean values in the same column not followed by a common letter differ significantly ($p < 0.05$). SEM = standard error of the mean.

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