Physicochemical and sensory characteristics of mortadella manufactured with *Caiman yacare* (*Caiman yacare*) meat and natural food colorants

Características físico-químicas e sensoriais da mortadela fabricada com carne de Caiman yacare (Caiman yacare) e corantes alimentares naturais

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ABSTRACT
This study evaluated the effects of the natural food colorants from annatto (*Bixa orellana*) (0.3%, 0.6%, and 0.9%) and cochineal carmine (*Dactylopius coccus*) (0.05%, 0.10% and 0.15%) on the physicochemical and sensory characteristics of mortadella made with *Caiman yacare* (*Caiman yacare*) meat. All mortadella formulations were prepared in accordance with Brazilian law. There were significant differences ($P < 0.05$) between treatments in pH, color parameters C* and h*, and texture profile. The scores determined in the sensory evaluation also showed differences in color, flavor, texture, and overall impression of mortadella formulations with different concentrations of both natural food colorants ($P < 0.05$). The mortadella containing 0.15% cochineal carmine received the highest scores for color (7.91), flavor (7.49), texture (7.43) and overall impression (7.58), thus presenting the best physicochemical and sensory characteristics among all formulations studied.

Keywords: emulsions, sustainability, meat processing.

RESUMO
Este estudo avaliou os efeitos dos corantes alimentares naturais de anato (Bixa orellana) (0,3%, 0,6% e 0,9%) e carmim cochonilha (Dactylopius coccus) (0,05%, 0,10% e 0,15%) sobre as características físico-químicas e sensoriais da mortadela feita com carne de Caiman yacare (Caiman yacare). Todas as formulações de mortadela foram preparadas de acordo com a legislação brasileira. Houve diferenças significativas ($P < 0,05$) entre os tratamentos de pH, parâmetros de cor C* e h* e perfil de textura. As pontuações determinadas na avaliação sensorial também mostraram diferenças na cor, sabor, textura e impressão geral das formulações de mortadela com diferentes concentrações de ambos os corantes alimentares naturais ($P < 0,05$). A mortadela contendo 0,15% de carmina de cochonilha recebeu os maiores escores de cor (7,91), sabor (7,49), textura (7,43) e impressão geral (7,58), apresentando as melhores características físico-químicas e sensoriais entre todas as formulações estudadas.

Palavras-chave: emulsões, sustentabilidade, processamento de carne.

1 INTRODUCTION

The consumption of protein from wild animals such as *Caiman yacare* meat (*Caiman yacare*) has become a trend in the national and international markets in recent years (Vicente-Neto et al., 2010; Morais et al., 2013).

Since the 1990s, the state of Mato Grosso has developed a productive chain for *Caiman yacare*, and it stands out in having the largest commercial farming of these species. It is also the largest producer of *Caiman yacare* meat, so it has a great impact on the local economy each year (Leitner & Toledo, 2010).

Alternatives to such meat in the diet of all consumers, regardless of social class, have been studied by several authors (Vieira, 2010; Paulino et al., 2011; Paulino, 2012;

Amorim et al., 2022) leading to the development of various products, including mortadella, by using retailing meat from commercial cuts (Morais et al., 2013).

Meat from *Cayman yacare* has become a product of great commercial value in the production chain because it has pleasant sensory attributes. Health benefits include low levels of inter- and intramuscular fat and saturated fatty acids, and the meat has high concentrations of polyunsaturated fatty acids (Romanelli, Caseri, & Lopes Filho, 2002; Vicente Neto et al., 2006; Leitner & Toledo, 2010; Vicente Neto et al., 2010; Morais et al., 2013).

Caiman yacare meat is a white meat with high luminosity values (L*> 54.0) and low a* values due to its low myoglobin content (Romanelli, 1995; Romanelli et al., 2002; Vicente Neto, 2005; Rodrigues et al., 2007; Faustman, Sun, Mancini, & Suman, 2010; Canto et al., 2012; Morais, 2013). Thus, to achieve the typical color of processed meat products such as mortadellas, which is known for its reddish color and typical seasonings (Conceição & Gonçalves, 2009; Yunes et al., 2013), food colorings must be added to provide a color that is attractive to the eye of the consumer.

Manufacturers of meat products prefer to use natural food colorings, especially annatto and cochineal carmine because they are stable to light, heat, and the presence of oxidizing agents. These colorings also work very well on high-protein foods and present low risk of toxicity (Spellmeier & Stülp, 2009; Volp, Renhe, & Steingueta, 2009; Zarringhalami, Sahari, & Hamidi-Esfehani, 2009).

Cochineal carmine (*Dactylopius coccus*) has carminic acid as an active ingredient and shows a wide range of tones, effectively replacing synthetic food colorants (Carvalho, Collins, & Carvalho, 2001; Oliveira, Pereira, Nagem, Pinto, & Santos, 2002; Spellmeier & Stülp, 2009; Volp et al., 2009; Aun et al., 2011). Annatto (*Bixa orellana*) provides a red-orange-yellow color and is obtained by the leaching of seeds with edible vegetable oils (bixin) or alkaline solutions (norbixin) (Zarringhalami et al., 2009).

The objective of this study was to evaluate the effects of the natural food colorants from annatto (*Bixa orellana*) and cochineal carmine (*Dactylopius coccus*) on the physicochemical and sensory characteristics of mortadellas made with *Caiman yacare* meat (*Caiman yacare*).

2 MATERIALS AND METHODS

2.1 RAW MATERIAL

For preparation of the emulsified product mortadella type, *Caiman yacare* meat

trimmings, pork fat, soybean oil, and water-soluble natural food colorants from cochineal carmine and annatto were used.

The pre-slaughter, slaughter, inspection and management of the animals from which the raw materials were obtained was performed in accordance with the current standards of animal welfare and health inspection in Brazil (Ministério da Agricultura, Pecuária e Abastecimento, 2008).

The *Caiman yacare* meat trimmings were purchased in a local market from a breeding facility located in the municipality of Caceres in Mato Grosso – Brazil, registered in the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA - MT) under number 1/51/92/0197-0. The animals were raised in an intensive system, as described by Vicente-Neto et al. (2010), and they were slaughtered in a commercial slaughterhouse (SIF 2452) when their live weight was between 5 and 7 kg. The *Caiman yacare* meat used had an average pH of 5.5-5.6 with an average composition of 76.10% moisture, 21.13% protein, 1.09% ash and 0.53% fat.

The trimmings were minced twice in a meat grinder - I PB 10 (Beccaro Ltda, Rio Claro, Brazil) with an 8 mm disc, and they were stored in vacuum-sealed packaging at -18 °C by 5 hours, until product manufacturing.

Pork fat used in the formulations came from the dorsal subcutaneous region of commercial pigs (Landrace x Duroc cross-breed) raised in an intensive system with a diet based on corn, soybean flour, vitamins and minerals. The pigs were slaughtered at 5 months of age in a commercial slaughterhouse (SIF 3551). The pork fat was minced in a meat grinder - I PB 10 (Beccaro Ltda, Rio Claro, Brazil) with an 5 mm disc and stored in vacuum-sealed packaging at -18 °C by 5 hours, until product manufacturing.

The soybean oil purchased in the local market was produced from soybean seeds (*Glycine max*) that were genetically modified with a gene from *Agrobacterium* sp. (Bunge, MT-RND) and processed in an industrial plant located in the southeastern region of the state of Mato Grosso. The soybean oil was stored in polyethylene terephthalate packages with a light barrier at room temperature and used 50 days after production.

The natural food colorants were cochineal carmine (*Dactylopius coccus*) water-soluble liquid (pH 10.50 - 12.00, carminic acid content 2.80 - 3.20 g/100 g), and annatto (*Bixa orellana*) water-soluble liquid (pH 12.00 - 13.70, minimum norbixin content of 0.90 g/100 g), at concentrations of up to 1%, as recommended by the manufacturer (Duas Rodas Industrial, Jaraguá do Sul, Brazil).

2.2 MANUFACTURING OF MORTADELLAS

The mortadellas were made at the Laboratory of Analysis of Meat and Derivatives in the Department of Food Science at the Federal University of Lavras - MG. The following formulation was used: 70% *Caiman yacare* meat, 22.5% pork fat, and 7.5% soybean oil for each treatment (proportion meat/lipid 70/30). In the control treatment, 0% natural food colorant was used (No natural food colorant). The treatments with addition of natural food colorants were designated as 0.05%, 0.10% and 0.15% for cochineal carmine natural food colorant; and with annatto natural food colorant were designated as 0.3%, 0.6% and 0.9%. The other ingredients used in the manufacture of mortadella were added as a proportion of the total the amount of meat-lipid mixture, in the same amount for all treatments: water/ice (20%), cassava starch (5%), textured soybean protein (4%), salt (1.4%), pigskin emulsion (1%), ready-mix for mortadellas (nitrite, ascorbic acid, polyphosphates and spices) (1%), and garlic powder (0.4%).

To prepare the pigskin emulsion was done which descripted by Morais et al., (2013). The pigskin emulsion was kept under refrigeration (5 °C) until the manufacturing of the mortadella.

The ingredients were added to the cutter (Filizola SA, São Paulo, Brazil) in the following order: a) meat and salts (salt and ready-mix for mortadellas), b) half of the water / ice, lipid fraction, and pigskin emulsion, c) the other half of the water / ice, lipid fraction, and pigskin emulsion, d) cassava starch, textured soy protein, and garlic powder, and e) natural food colorant. Mixing was carried out of each ingredient was added for 1 minute to obtain a homogeneous paste. The temperature of the batter was kept below 13 °C throughout the process.

The meat emulsion was manually embedded (Filling Machine Jamar EB-06, Sao Paulo, Brazil) into 45 mm cellulosic casings in 500 g portions. Then, the mortadellas were cooked in water at a cooking cycle of 55 °C/30 minutes, 65 °C/30 minutes, 75 °C/30 minutes, and 85 °C/30 minutes so that the center of each portion reached 73 °C. At the end of the cooking process, the mortadellas were cooled in water at 5 °C for 30 minutes. Then, the products were kept at 4 °C until the laboratory analyses were conducted. Each formulation was prepared in triplicate (3 batches of 1.5 kg, with a total of 4.5 kg per treatment, one unit of 0.5 kg of each bath was used in the analyses). The mortadellas were evaluated at 1 day after processing, with the exception of the sensory analyses, which were performed at 7 days after processing.

2.3 LABORATORY ANALYSES

The chemical composition, pH, index of lipid oxidation, color parameters, and texture profile were analyzed at the Department of Food Science, Federal University of Lavras. The sensory evaluation was performed in the Laboratory of Bromatology at the Federal Institute of Education, Science and Technology of Mato Grosso - IFMT *Campus* Cuiabá - Bela Vista.

2.3.1 Proximate composition

To determine the proximate composition, samples were homogenized in a multiprocessor until a homogeneous mass was obtained. Analysis of the moisture, fat, protein, and ash of the mortadellas was performed in triplicate using AOAC (1995) methods 950.46, 960.39, 928.08, and 920.153, respectively.

2.3.2 Determination of pH and index of lipid oxidation - TBARS

The pH values of mortadellas were measured by inserting a glass electrode coupled to a portable digital potentiometer HI 99163 Meat pH Meter (Hanna Instruments, Woonsocket, USA) was performed in triplicate according to the AOAC methodology (1995).

Lipid oxidation was determined in triplicate by the TBARS method - thiobarbituric acid reactive substances (Raharjo, Sofos, & Schmidt, 1992) - with minor modifications, as described by De Carli, Terra, Fries, Menezes, and Palezi (2013).

2.3.3 Color parameters

The color measurements of the mortadella samples were carried out with a Minolta colorimeter (CM-700d, D65, Konica Minolta Sensing Inc., Osaka, Japan) was performed in triplicate as described by AMSA (2012) using according to the CIE Lab color system. The saturation (C*) and hue angles (h*) were calculated from a* and b* values using the following formulas: $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$ and $h^* = \tan\text{-}1\ (b^*/a^*)$.

2.3.4 Texture Profile Analysis

Texture Profile Analysis (TPA) was performed on a TA.XT. Plus texture analyzer (Stable Micro Systems Inc., Surrey, England) as described by Mitchell et al. (2013), all parameters of TPA was performed in triplicate. The deformation curves as a function of time were used to calculate hardness, cohesiveness, fracturability, adhesiveness,

springiness and chewiness within the software package Texture Expert Exceed (Texture Analyser Stable Micro System Inc., Surrey, England).

2.3.5 Sensory evaluation

Prior to sensory analysis, the mortadellas made with *Caiman yacare* meat containing natural food colorants were subjected to microbiological analyses (Salmonella, fecal coliforms at 45 ºC, sulfite-reducing clostridia / *Clostridium perfringens*, and coagulase positive staphylococci) in the Laboratory for Analysis of Animal Products - LAPOA / MT, as required by Brazilian legislation (Agencia Nacional de Vigilância Sanitária, 2001) to ensure food security. All samples submitted to microbiological analyses were within the standards established by law and were suitable for consumption (Agencia Nacional de Vigilância Sanitária, 2001).

The sensory panel was composed of untrained judges, who were asked to evaluate the sensory attributes (color, flavor, texture, and overall impression) of the product by using a nine-point hedonic scale, where 1 = extremely dislike; 2 = very dislike; 3 = moderately dislike; 4 = slightly dislike; 5 = neither liked nor dislike; 6 = slightly like; 7 = moderately like; 8 = very like and 9 = extremely like.

The samples were cut (2 mm thickness), individually placed in white disposable plastic cups (50 ml) on a white tray coded with random three-digit numbers, and provided to each judge along with an evaluation card and a glass of water. The sensory evaluation was carried out in individual booths with white light, and the judges were instructed to cleanse their palates with water between samples.

To check how much the judges liked or disliked the samples were subjected to three (3) acceptance tests (sensory panel):

Sensory Panel 1 - Mortadella made with *Caiman yacare* meat with different concentrations of annatto natural food colorant, evaluated by a panel consisting of 103 (one hundred and three) untrained judges, both males and females, with an age ranging of 14 to 54 years old;

Sensory Panel 2 - Mortadella made with *Caiman yacare* meat with different concentrations of cochineal carmine natural food colorant evaluated by a panel consisting of 103 (one hundred and three) untrained judges, both males and females, with an age ranging of 14 to 54 years old.

Sensory Panel 3 - Based on the statistical analysis of sensory panels 1 and 2, the best treatment for each panel was selected and compared with mortadella formulation

without food colorant addition, with a panel consisting of 104 (one hundred and four) untrained judges, both males and females, with an age ranging of 14 to 55 years old.

2.3.6 Statistical analysis

The data from the proximate composition from 7 (seven) formulations (No natural food colorant: 0%; cochineal carmine natural food colorant: 0.05; 0.10; 0.15%; and annatto natural food colorant: 0.3; 0.6; 0.9% were analyzed by descriptive statistics using the statistical computer package SISVAR 4.0 (Ferreira, 2000). One mortadella unit individually of 0.5 kg obtained from each batch was considered an experimental unit for each treatment.

The results for pH, TBARS lipid oxidation, and CIELab objective color obtained from 3 (three) formulations prepared with different concentrations of cochineal carmine natural food colorant (0.05%, 0.10%, and 0.15%) were studied as a completely randomized design. One mortadella unit individually of 0.5 kg obtained from each batch was considered an experimental unit for each treatment. These variables were analyzed with the GLM procedure by using the statistical package SAS (2003) and the differences were determined by Tukey's test at 5% significance, using the following model: $Y = \mu + Ci + e$; where: μ = the overall mean, Ci = effect of the concentration of cochineal carmine natural food colorant (i = 1 to 3), and e = the experimental error.

Likewise, the results for pH, TBARS lipid oxidation, and CIELab objective color obtained from 3 (three) formulations prepared with different concentrations of annatto natural food colorant (0.3%, 0.6%, and 0.9%) were studied as a completely randomized design. One mortadella unit individually of 0.5 kg obtained from each batch was considered an experimental unit for each treatment. The data were analyzed with the GLM procedure by using the statistical package SAS (2003) and the differences were determined by Tukey's test at 5% significance, using the following model: $Y = \mu + Ai + e$; where: μ = the overall mean, Ai = effect of the concentration of annatto natural food colorant (i = 1 to 3), and e = the experimental error.

The data for pH, TBARS lipid oxidation, and CIELab objective color obtained from mortadella without (0%) natural food colorant, mortadella with concentration of 0.15% cochineal carmine natural food colorant and mortadella with concentration of 0.9% annatto natural food colorant were studied as a completely randomized design. One mortadella unit individually of 0.5 kg obtained from each batch was considered an experimental unit for each treatment. The data were analyzed with the GLM procedure

by using the statistical package SAS (2003) and the differences were determined by Tukey's test at 5% significance, using the following model: Y = μ + Ti + e; where: μ = the overall mean, Ti = effect of the addition or not of natural food colorant (i = 1 to 3), and e = the experimental error.

The data for Texture Profile Analysis (TPA) obtained from mortadellas without natural food colorant (0%), mortadella with different concentration of cochineal carmine natural food colorant (0.05%; 0.10% and 0.15%) and mortadella with different concentration of annatto natural food colorant (0.3%; 0.6% and 0.9%) were studied as a completely randomized design. One mortadella unit individually of 0.5 kg obtained from each batch was considered an experimental unit for each treatment. The data were analyzed with the GLM procedure by using the statistical package SAS (2003) and the differences were determined by Tukey's test at 5% significance, using the following model: Y = μ + Ti + e; where: μ = the overall mean, Ti = effect of the concentration of natural food colorant (i = 1 to 7), and e = the experimental error.

The results of the sensory panel 1 and 2 were subjected to the GLM procedure (SAS Institute, 2003) using the following model: Y = μ + Ci + Pj + e; where: μ = the overall mean, Ci = effect of the concentration of natural food colorant (i = 1 to 3), Pj = the effect of the taster (j = 1 to 103), and e = the experimental error. The means were compared using Tukey's test ($P < 0.05$).

The data of the sensory panel 3 were subjected to the GLM procedure (SAS Institute, 2003) using the following model: Y = μ + Ti + Pj + e; where: μ = the overall mean, Ti = effect of the addition or not of natural food colorant (i = 1 to 3), Pj = the effect of the taster (j = 1 to 104), and e = the experimental error. The means were compared using Tukey's test ($P < 0.05$).

3 RESULTS

3.1 PROXIMATE COMPOSITION

The average proximate composition of mortadellas made with *Caiman yacare* meat containing different concentrations of natural food colorants are in accordance with the legal standards (Table 1).

Table 1. Proximate composition of mortadella made with *Caiman yacare* meat with or without addition of the natural food colorants cochineal carmine (*Dactylopius coccus*) and annatto (*Bixa orellana*).

Variable	Mortadella		
	No Food Colorant	Carmine	Annatto
Moisture (%)	61.04±0.09	60.89±0.23	61.18±0.11
Crude Protein (%)	12.86±0.60	13.25±0.34	13.11±0.11
Ether extract (%)	16.66±0.07	15.58±3.62	14.87±0.82
Ash (%)	2.25±0.10	2.19±0.34	2.25±0.06
Carbohydrates (%)	7.19±0.66	8.09±3.32	8.59±1.01

Means followed by standard deviation.

3.2 DETERMINATION OF PH AND INDEX OF LIPID OXIDATION TBARS

There was a significant difference ($P < 0.05$) in the pH values of the mortadella formulations containing the natural food colorant carmine cochineal, and the higher pH value (6.19) was observed for the concentration of 0.15% of food colorant (Table 2).

Table 2. Mean pH values, C* (saturation index), h* (hue angle), and TBARS of mortadella formulation made with different concentrations of the natural food colorant cochineal carmine (*Dactylopius coccus*).

Variable	Cochineal carmine concentration (%)			SEM[1]	*P*-trat[2]
	0.05	**0.10**	**0.15**		
pH	5.98[c]	6.08[b]	6.19[a]	0.006	<0.000
C*	11.83[c]	16.19[b]	18.82[a]	0.041	<0.000
h*	36.19[a]	19.55[b]	10.74[c]	0.020	<0.000
TBARS (mg malonaldehyde /kg)	0.38[a]	0.41[a]	0.41[a]	0.027	0.692

[1]SEM –Standard error of the mean. [2]*P*-trat – Estimation of the treatment effect. Means followed by different letters in the same row differ according to Tukey's test (P<0.05).

Table 3. Mean pH values, C* (saturation index), h* (hue angle) and TBARS of mortadella formulations made with different concentrations of the natural food colorant annatto (*Bixa orellana*).

Variable	Annatto concentration (%)			SEM[1]	*P*-trat[2]
	0.3	**0.6**	**0.9**		
pH	6.18[a]	6.21[a]	6.23[a]	0.017	0.220
C*	15.72[c]	20.85[b]	26.15[a]	0.169	<0.000
h*	82.95[a]	81.67[b]	79.49[c]	0.240	<0.000
TBARS (mg malonaldehyde /kg)	0.49[a]	0.41[a]	0.41[a]	0.035	0.246

[1]SEM –Standard error of the mean. [2]*P*-trat – Estimation of the treatment effect. Means followed by different letters in the same row differ according to Tukey's test (P<0.05

There was a significant difference ($P < 0.05$) in pH values of both mortadella formulations without natural food colorant addition and with different natural food colorants, and higher pH values (6.23) were observed for the mortadellas containing annatto food colorant (Table 4).

Table 4. Mean pH values, C* (saturation index), h* (hue angle), and TBARS of mortadella formulations without natural food colorant addition; with 0.15% natural food colorant cochineal carmine (*Dactylopius coccus*); and with 0.9% natural food colorant annatto (*Bixa orellana*).

Variable	Natural Food Colorants			SEM[1]	*P*-trat[2]
	No Food Colorant	Carmine 0.15%	Annatto 0.9%		
pH	5.70[b]	6.19[a]	6.23[a]	0.014	<0.000
C*	12.72[c]	18.73[b]	26.15[a]	0.295	<0.000
h*	84.81[a]	10.74[c]	79.49[b]	0.167	<0.000
TBARS (mg malonaldehyde /kg)	0.40[a]	0.41[a]	0.41[a]	0.018	0.931

[1]SEM –Standard error of the mean. [2]*P*-trat – Estimation of the treatment effect. Means followed by different letters in the same row differ according to Tukey's test (P<0.05).

3.3 COLOR CIE-LAB – COLOR SATURATION (C*) AND HUE ANGLE (H*)

Significant differences ($P < 0.05$) were observed for both color saturation index (C *) and hue angle (h*) with the different concentrations of the same food colorant (Tables 2 and 3), and in formulations with or without addition of different natural food colorants (Table 4).

The food colorants addition affected the color coordinates a* and b* such that a higher concentration of both cochineal carmine and annatto food colorants resulted in higher C* values and lower h* values (Tables 2 and 3).

3.4 TEXTURE PROFILE ANALYSIS - TPA

Significant differences ($P < 0.05$) were observed for the parameters hardness, fracturability, adhesiveness and chewiness of mortadella formulation without natural food colorant addition and with different concentrations of the natural food colorants cochineal carmine and annatto (Table 5).

The mortadella formulation containing 0.9% annatto food colorant showed higher means for hardness (1.80 kg), fracturability (1.40 kg), and adhesiveness (0.34 kg / mm). In contrast, mortadellas without natural food colorant addition showed higher values for cohesiveness (2.56) and chewiness (23.21 kg / mm) than the other treatments (Table 5).

Table 5. Mean TPA values of mortadella formulations containing different concentrations of natural food colorants.

Variable	Natural Food Colorant								
	No Food Colorant	Carmine			Annatto			SEM[1]	*P*-trat[2]
		0.05%	0.10%	0.15%	0.3%	0.6%	0.9%		
Hardness (kg)	1.78^{a}	1.56^{a}	1.58^{a}	1.59^{a}	1.41^{ab}	1.05^{b}	1.80^{a}	0.000	0.108
Fracturability (kg)	1.12^{ab}	1.23^{a}	1.18^{ab}	1.23^{a}	1.11^{ab}	0.78^{b}	1.40^{a}	0.002	0.090
Cohesiveness	2.56^{a}	2.50^{a}	2.43^{a}	2.52^{a}	2.55^{a}	2.53^{a}	2.50^{a}	0.496	0.045
Adhesiveness (kg/mm)	0.026^{ab}	0.020^{b}	0.025^{ab}	0.022^{ab}	0.030^{ab}	0.020^{b}	0.034^{a}	0.019	0.003
Springiness (mm/g)	5.04^{a}	4.97^{a}	4.55^{a}	4.82^{a}	5.05^{a}	4.58^{a}	5.01^{a}	0.100	0.117
Chewiness (kg/mm)	23.21^{a}	19.30^{a}	17.42^{ab}	19.37^{a}	18.26^{ab}	12.09^{b}	22.23^{a}	0.000	1.398

[1]SEM –Standard error of the mean. [2]*P*-trat – Estimation of the treatment effect. Means followed by different letters in the same row differ according to Tukey's test (P<0.05).

3.5 SENSORY ANALYSIS

In the first sensory evaluation, the assessors identified differences ($P < 0.05$) in color, flavor, texture, and overall impression for the different natural food colorant concentrations (Table 6).

Table 6. Sensory scores of mortadella formulations containing different concentrations of the natural food colorant cochineal carmine (*Dactylopius coccus*).

Attributes[1]	**Carmine Concentration**			**SEM**[2]	***P*-trat**[3]
	0.05%	**0.10%**	**0.15%**		
Color	5.50[b]	6.97[a]	7.37[a]	0.149	<0.001
Flavor	6.67[b]	7.14[ab]	7.25[a]	0.145	<0.001
Texture	6.76[b]	7.40[a]	7.41[a]	0.116	<0.001
Overall Impression	6.42[b]	7.32[a]	7.16[a]	0.145	<0.001

[1] Scores from 1 (extremely dislike) to 9 (extremely liked). [2]SEM –Standard error of the mean. [3]*P*-trat – Estimation of the treatment effect. Means followed by different letters in the same row differ according to Tukey's test (P<0.05).

Significant differences ($P > 0.05$) were observed for color, texture, and overall impression of the mortadellas containing annatto natural food colorant (Table 7).

Table 7. Sensory scores of mortadella formulations containing different concentrations of annatto (*Bixa ollerana*) food colorant.

Attributes[1]	**Annatto Concentration**			**SEM**[2]	***P*-trat**[3]
	0.3%	**0.6%**	**0.9%**		
Color	4.23[b]	4.66[a]	4.72[a]	0.098	<0.001
Flavor	6.04[a]	6.32[a]	6.41[a]	0.135	<0.001
Texture	6.31[b]	6.63[a]	6.82[a]	0.094	<0.001
Overall Impression	5.69[b]	6.19[a]	6.23[a]	0.117	<0.001

[1]Scores from 1 (extremely dislike) to 9 (extremely liked). [2]SEM –Standard error of the mean. [3]*P*-trat – Estimation of the treatment effect. Means followed by different letters in the same row differ according to Tukey's test (P<0.05).

Significant differences ($P < 0.05$) were observed for the sensory attributes color, flavor, texture, and overall impression of mortadella formulations without natural food colorant addition; with addition of 0.9% annatto; and with addition of 0.15% cochineal carmine (Table 8).

Table 8. Sensory scores of mortadella formulations without natural food colorant addition; with addition of 0.15% cochineal carmine *(Dactylopius coccus*); and with addition of 0.9% annatto (*Bixa ollerana*).

Attributes[1]	Samples			SEM[2]	*P*-trat[3]
	Carmine 0.15%	No Food Colorant	Annatto 0.9%		
Color	7.91[a]	4.98[b]	5.49[b]	0.160	<0.001
Flavor	7.49[a]	7.20[b]	6.91[b]	0.130	<0.001
Texture	7.43[a]	7.04[b]	7.00[b]	0.116	<0.001
Overall Impression	7.58[a]	6.65[b]	6.88[b]	0.129	<0.001

[1]Scores from 1 (extremely dislike) to 9 (extremely liked). [2]SEM –Standard error of the mean. [3]*P*-trat – Estimation of the treatment effect. Means followed by different letters in the same row differ according to Tukey's test (P<0.05).

4 DISCUSSION

4.1 PROXIMATE COMPOSITION

According to the Technical Regulation of Identity and Quality of mortadellas (Ministério da Agricultura do Abastecimento e da Reforma Agrária, 2000), mortadella shall present a maximum of 65% moisture, 30% fat, and at least 12% protein. It was observed in our study that all formulations made with *Caiman yacare* meat (Table 1) were in accordance with the legal standards. The products had chemical compositions similar to those observed other studies conducted in Brazil using emulsified meat products from different animals (Mercadante, Capitani, Decker, & Castro, 2010; Trindade, Thomazine, Oliveira, Balieiro, & Favaro-Trindade, 2010; Guerra et al., 2011; Trindade et al., 2011; Morais et al., 2013).

4.2 PH

As shown in Tables 2 and 3, the pH of the mortadella samples containing cochineal carmine and annatto increased progressively with increasing natural food colorants concentration. Possibly, the higher pH values observed in the samples with natural food colorants are because both natural food colorants are solubilized in alkaline solutions (pH 10 to 13.70), which may have contributed to the increase in pH of the mortadella samples. This fact can be confirmed by checking the mean pH of the mortadellas made without natural food colorant addition (5.70), which is shown in Table 4.

Even with the addition of natural food colorants (pH > 10.0) that caused the pH elevation of mortadellas in the present study, it remained at approximately 6.0 (close to neutral), as established by Brazilian legislation (Ministério da Agricultura, Secretaria

Nacional de Defesa Agropecuária, 1981). Several authors reported similar results in marketed mortadellas (Conceição & Gonçalves, 2009), sausages and mortadellas with pork fat replaced by soybean oil (Choi et al., 2010; Trindade et al., 2010 Trindade et al., 2011; Morais et al., 2013), goat mortadella from discarded meat (Guerra et al., 2011), sausages with different natural pigments as substitutes for sodium erythorbate (Mercadante et al., 2010), and Frankfurt-type sausages with poultry meat and collagen fibers (Pereira et al., 2011).

4.3 LIPID OXIDATION (TBARS)

Brazilian legislation does not establish a maximum malonaldehyde content / kg of meat products, but unpleasant odor (rancidness) and the slime of deterioration are associated with malonaldehyde concentrations between 0.5 and 1.36 mg / kg. However, TBARS values between 0.6 and 2.0 mg malonaldehyde / kg indicate changes in lipid oxidation that are barely perceptible by sensory evaluation (Terra et al., 2006; Choi et al., 2010; Mercadante et al., 2010; Trindade et al., 2010; Morais et al., 2013). Values higher than 1.59 mg malonaldehyde / kg can be harmful to human health (Terra et al., 2006).

The malonaldehyde values found in this study (Table 2, 3 and 4) were below 0.5 mg / kg. Thus, they are considered too low to be perceived by sensory analysis and too low to indicate lipid oxidation (Terra et al., 2006; Choi et al., 2010; Mercadante et al., 2010; Trindade et al., 2010).

4.4 OBJECTIVE COLOR

The saturation index (C*) and hue angle (h*) are calculated from the color coordinates a* and b* and are used to determine the color saturation and intensity of red color. High C* values indicate greater intensity of red color in the sample, whereas high h* values indicate less red intensity (AMSA, 2012).

In the formulation with cochineal carmine (Table 2), the sample containing 0.15% natural food colorant showed the best C* (18.82) and h* (10.74) values when compared with concentrations of 0.05% (C*=11.83, h*=36.19) and 0.10% (C*=16.19, h*=19.55). This indicates that the concentration of 0.15% cochineal carmine conferred higher saturation and greater red intensity to the samples, characterizing the typical color of emulsified products.

Similar behavior was observed for mortadellas containing annatto food colorant (Table 3). The concentration of 0.9% annatto showed the best C* value (26.15) and h*

value (79.49) when compared to concentrations of 0.3% (C* =15.72, h*= 82.95) and 0.6% (C* =20.85, h*= 81.67).

4.5 TEXTURE PROFILE ANALYSIS

The higher values found for hardness, fracturability, and adhesiveness in the mortadella formulation with 0.9% annatto may have occurred due to the higher pH value (6.23) observed in all formulations. The high pH may have favored the gelation of collagen present in the meat trimmings segments of alligator emulsion and added collagen, collagen generally increases the hardness and juiciness of emulsified meats and meat products (Trindade et al., 2011; Prestes, 2013.).

The texture profile analysis of the current study showed that hardness, fracturability, cohesiveness and adhesiveness values were similar to those reported by Morais et al. (2013) in mortadellas made with caiman meat with replacement of 25% pork fat by soybean oil.

4.6 SENSORY ANALYSIS

The scores found for the color attribute (7.91) of mortadellas containing 0.15% cochineal carmine (Table 8) reflect a consumer preference for mortadella with light red color, tending toward rosy. A similar result was observed by Uyhara et al. (2008) for the sensory acceptance of sausages made with Nile tilapia with dyes.

The mortadella containing 0.15% cochineal carmine (Table 8) received higher scores for color (7.91), flavor (7.49), texture (7.43) and overall impression (7.58). The higher scores observed for all sensory attributes of mortadellas with 0.15% cochineal carmine natural food colorant (Table 8) reflect the preference of the consumers for mortadellas with a reddish color. This may be because consumers consider the red color to be the most attractive color for mortadellas, possibly because it is associated with fresh meat (Gomide et al., 2013).

5 CONCLUSION

The mortadella made with *Caiman yacare* meat and 0.15% of the natural food colorant cochineal carmine presented the best physicochemical and sensory characteristics.

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