

Effect of storage time on the quality of chicken sausages produced with fat replacement by collagen gel extracted from chicken feet

Íris B. S. Araújo,^{*,1} Darlinne Amanda S. Lima,[†] Sérgio F. Pereira,[†] Rafaella P. Paseto,[†] and Marta S. Madruga[†]

**Department of Management and Agroindustrial Technology, Federal University of Paraíba, 58220-000 Bananeiras/PB, Brazil; and [†]Department of Food Engineering, Federal University of Paraíba, 58051-900 João Pessoa/PB, Brazil*

ABSTRACT The objective of this study was to evaluate the use of collagen gel extracted from chicken feet on chicken sausages during 42 d of refrigerated storage. Three chicken sausages were processed: standard (**SS**); replacing 50% fat with commercial collagen powder (**SC**); replacing 50% fat with chicken foot collagen gel (**SG**). Sausages were stored at 4°C and analyzed every 14 d, for proximate composition, fatty acid profile, thiobarbituric acid reactive substances (**TBARS**) number, antioxidant activity, electrophoresis, instrumental color, water

holding capacity (**WHC**), texture profile analysis, and quantitative descriptive analysis. Sausages **SC** and **SG** had similar behavior to the standard in the sensorial parameters of appearance and color over 28 d of refrigerated storage. **SG** had the highest **WHC** (81.05%), the lowest **TBARS** value (0.38 mg MDA/kg), and the highest antioxidant activity in addition to having the best atherogenicity and thrombogenicity index compared with **SC** treatment, making collagen gel viable to replace fat and control the effects of lipid oxidation.

Key words: cardiovascular diseases, Dyslipidemia, low-fat sausages, TBARS, lipid oxidation

2021 Poultry Science 100:1262–1272

<https://doi.org/10.1016/j.psj.2020.10.029>

INTRODUCTION

Brazil is the world's largest exporter of chicken meat. In 2018, the country remained ahead of the United States, which is the largest producer in the foreign market. In 2019, in the first 2 mo Brazil already had a 2% increase in sales, with China being the largest importer of chicken meat (ABPA, 2019; USDA, 2019). The main factors in the consumption of chicken meat are its nutritional quality due to the advantages such as low content of saturated fatty acids and low cost of production, which implies directly in the selling price (Khulal et al., 2016).

With the growing increase in chicken meat market, the volume of by-products also increased. Many of these by-products are underused, although they have high biological value. Among these by-products, chicken feet stand out for their high content of collagen, which can

be extracted and used by food industry, with application as moisture retainer, gelling agent, stabilizer, or thickener (Li et al., 2007; Moraes and Cunha, 2013; Araújo et al., 2019).

Depending on how it has been extracted or hydrolyzed collagen may also present bioactive properties such as chelating activity of minerals, antioxidants, antimicrobial, antifreeze, antihypertensive (Toldrá et al., 2012; Zhang et al., 2013). Once inserted into food, it can improve texture and, in accordance with the bioactive properties that the molecule may play, can maintain product stability during storage.

In meat products, collagen can be used to perform technological functions usually performed by the fat used in the formulations. Sausages, for example, are viewed by the population in nutritional terms as high-fat foods, and their high consumption may contribute to the incidence of cardiovascular disease. Sousa et al. (2017), by replacing Frankfurt sausage fat with hydrolyzed collagen at different levels, found the viability of up to 50% substitution without causing damage to sausage quality and improving aspects such as the water holding capacity (**WHC**) of sausages, which made them more succulent.

© 2020 Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received March 1, 2020.

Accepted October 27, 2020.

¹Corresponding author: iris@cchsa.ufpb.br

The use of collagen from chicken feet as a fat substitute, due to its bioactive and technological properties, can have advantages in the quality of meat products when compared with other fat substitutes. Araujo et al. (2018) extracted collagen from chicken feet under different conditions of time and enzyme concentration and found that the collagen presented in the best condition had good water holding and emulsifying capacity. Araujo et al. (2019) used collagen from chicken feet in sausages and when comparing with other sources of fat substitutes, sausages with chicken legs collagen had lower atherogenicity and thrombogenicity index, in addition to improving the emulsion stability and WHC. Kiliç and Özer (2017), replacing beef fat by interesterified palm kernel oil in quality of sausages found that during storage, the fat substitute could not contain the effects of oxidation, presenting high values of thiobarbituric acid reactive substances (TBARS) at 30 d of storage.

Brazilian law allows for a maximum fat content of 30% in sausages, which is used to improve sensory characteristics and reduce costs (Brazil, 2000). The World Health Organization (WHO, 2015) recommends fat intake for less than 10% of total daily energy. On the other hand, fat reduction measures in industrialized products must be adopted so that practical consumer products continue to be offered to consumers. The use of alternative sources such as collagen, in addition to increasing the protein content of food, can bring benefits during refrigerated storage, as they may perform antioxidant activity, in addition to other properties (Soladoye et al., 2015). Based on the perspective presented, the present work aimed to verify the effect of storage time for 42 d at 4°C on the quality of chicken sausages produced by replacing fat with collagen extracted from chicken legs.

MATERIALS AND METHODS

Collagen Extraction From Chicken Feet

Collagen gel was extracted in feet from broilers slaughtered between the ages of 42 and 47 d. They were purchased in the local market of João Pessoa/PB, Brazil. The collagen extraction was performed by acid and enzymatic hydrolysis process, with acetic acid and pepsin at 4°C and 12 h of hydrolysis time, in accordance with the methodology adapted from Shimokomaki et al. (1981) and Simões et al. (2014), which was detailed by Araujo et al. (2018).

The process was started by immersing the chicken feet in acetic acid solution 0.5 mol for 24 h in the ratio of 1:10 (w/v). The material was then homogenized in Ultra-Turrax (IKA, model T25) for 10 min at 5,000 × g. In hydrolysis, pepsin was used for 12 h at 4°C with continuous agitation. To stop the hydrolysis process, the pH was elevated to 7.5 and centrifuged for 30 min at 10,000 × g at 4°C. The precipitate was discarded and the supernatant was precipitated with NaCl to a concentration of 3 mol/L and centrifuged at 10,000 × g for

30 min at 4°C. The supernatant was discarded and the precipitate obtained was dialyzed with 0.5 mol/L acetic acid solution for 72 h with daily solution exchange. The collagen gel obtained was kept under freezing (−18°C) until its use in chicken sausages.

Sausage Processing

Three sausage formulations were processed in accordance with the inputs and quantities listed in Table 1, one of them being a standard formulation (SS), with 100% pork backfat; formulation with 50% pork backfat substitution for commercial hydrolyzed collagen powder of porcine origin (SC) (Germina, Parnamirim/RN, Brazil); and another with 50% fat reduction by collagen gel extracted from chicken feet (SG). The description of sausage processing is in the previous heading. A 50% fat replacement with collagen was used in this study because this was the best formulation obtained by Sousa et al. (2017) studying different levels of fat replacement with collagen powder. Chicken meat (equal parts of breast and drumstick) and backfat were thawed for 24 h at 7°C, weighed, comminuted, and emulsified in a cutter (CR-4 L; Skymsem, Brazil) together with the other ingredients for 5 min. Emulsions were encased into a cellulose casing with a 32-mm diameter (Viscofan, Spain) using a manual sausage stuffer, manually bundled and cooked in a water bath for 30 min, at an internal temperature of 72°C. After cooking, the casings were manually removed, and the sausages were cooled in cold water and vacuum-packed in high-density polyethylene bags.

Sausages were stored under refrigeration at 4 ± 1°C for a period of 42 d and analyzed at 14-day intervals for chemical parameters (chemical composition, collagen, fatty acid profile, TBARS number, electrophoretic profile and activity antioxidant by 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and ferric reducing antioxidant power [FRAP]), physical (instrumental color, WHC, and texture profile), and sensory (appearance, texture, aroma, and taste) parameters. The entire processing was replicated 3 times in corresponding independent production batches.

Table 1. Formulations of low-fat chicken sausages by collagen powder and collagen gel extracted from chicken feet.

Ingredients	Formulations (%) ¹		
	SS	SC	SG
Chicken meat	65	65	65
Pork backfat	15	7.5	7.5
Chicken feet collagen	-	7.5	-
Commercial collagen	-	-	7.5
Water (ice)	15	15	15
Additives (nitrite salt, phosphates, sodium erythorbate, glutamate monosodium, spices)	5	5	5

¹SS: standard sausage, SC: 50% fat replacement sausage with commercial powder collagen, SG: 50% Fat replacement sausage with chicken feet collagen gel.

Effects of the Use of Collagen as a Fat Substitute on the Sensory, Chemical, and Physical Characteristics of Sausages During Storage

Quantitative Descriptive Analysis (QDA). The Quantitative Descriptive Analysis (QDA) of low-fat sausages was performed in accordance with the methodology proposed by Meilgaard (1999). The test was conducted with prior approval by the Ethics and Research Committee with Human Beings (CAAE: 65402717.8.0000.5188), to meet the ethical and scientific requirements laid down in Resolution No. 466 of the National Health Council (Brazil, 2012). The test was conducted according Stone and Sidel (2004) with 11 trained panelists, and the final evaluation was performed on day 0, 14, and 28 of refrigerated storage (4°C), through 3 sessions per day, each treatment in 3 repetitions. All samples as they were were within the microbiological standards of Brazilian legislation (Brazil, 2001). The samples were served monadic, randomly, through the intensity of the sensory attributes measured in an unstructured 9 cm scale. The panelists were previously selected by interviews and screening tests of selection, such as identification of basic tastes, odor recognition and triangular tests made with emulsified chicken meat products. These panelists developed the terminology of the descriptors used to characterize the sausages sensorially. Test products were served as illustrative stimuli for the consensus language development. The next step consisted of training to skill with unstructured scales, the table of descriptors and the evaluation of sausages from this study. References were used for generating of the sensory terminologies: Pink color, Visual Succulence, Softness, Succulence, Chewiness, Cooked Chicken Aroma, Rancidity Aroma, and Cooked Chicken Flavor.

The sensory attributes were Pink color, Visual Succulence, Softness, Succulence, Chewiness, Cooked Chicken Aroma, Rancidity Aroma, and Cooked Chicken Flavor. No sensory analyzes were performed on day 42 to ensure the safety of the food to the panelists because despite being microbiologically analyzed, the sausages SC and SG showed superficial slime, probably due to the collagen that was being exuded in storage. The preparation of sausages was performed in accordance with the methodology proposed by Morin et al. (2002), in which cooked sausages were heated to 50°C and immediately served to panelists, coded with random 3-digit numbers. Crackers and water were served between the samples.

Chemical Analysis. Sausages were subjected to moisture, protein, and ash analyzes in accordance with the methodology proposed by AOAC (2007). Lipid content was determined according to Folch et al. (1957). Collagen content was also determined by hydroxyproline analysis (AOAC, 2007). The number of TBARS was determined in accordance with the method proposed by Rosmini et al. (1996). All analyzes were performed in triplicate.

From the lipid extracts obtained in the lipid analysis at all storage times, the fatty acids methylation was performed (Hartman and Lago, 1973). The identification and quantification of fatty acid esters was performed by injection in gas chromatograph (VARIAN 430-GC), in accordance with the methodology detailed by Araújo et al. (2019). The quantification of fatty acids was expressed as percentage of area for calculations of the sum of saturated fatty acids (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), the atherogenicity index (AI), which represents the stimulus potential for platelet aggregation (Tonial et al., 2011), calculated by the expression $AI = [C12: 0 + 4 (C14: 0) + C16: 0] / (\Sigma SFA + \Sigma PUFA)$ and the thrombogenicity index (TI) was also calculated in accordance with the expression $TI = (C14: 0 + C16: 0 + C18: 0) / [(0.5 \times \Sigma MUFA) + (0.5 \times PUFA \omega-6) + (3 \times PUFA \omega-3) + (PUFA \omega-3/PUFA \omega-6)]$. Injections for each sausage sample were performed in triplicate.

Antioxidant Activity. The capture effect of the DPPH radical on chicken sausages was analyzed in accordance with the adapted methodology of Chandini et al. (2008) and Chakka et al. (2015). The antioxidant activity by the FRAP method was determined in accordance with the methodology proposed by Benzie and Strain (1996) and Pulido et al. (2000). All antioxidant activity analyzes were performed in triplicate.

Electrophoresis. Chicken sausages were subjected to polyacrylamide gel electrophoresis (SDS-PAGE) in accordance with the methodology of Laemmli (1970), adapted by Daguer et al. (2010), with protein extraction with urea 6 mol/L. The running conditions were 12% separation gel, 4% sample application gel, 30 A and 125V current. The Full-Range Molecular Weight Marker (12 - 225 kDa, GE Healthcare Life Sciences) was used. Gels were stained in a solution containing 10% (v/v) acetic acid, 40% (v/v) methyl alcohol, and 1% Coomassie Brilliant Blue R-250.

Physical Parameters. The analysis of WHC was performed in accordance with the adapted methodology of Huff-Lonergan and Lonergan (2005). Sausage color parameters were determined using a Konica Minolta CR-400 colorimeter (Minolta Chromameter Co., Ltd., Osaka, Japan) operating on the CIELab system, operating with D65 illuminant and 10° observer. The parameters L^* , a^* , and b^* were measured on the inner surface of sliced sausages at 4 randomly selected locations for each treatment.

Sausages were subjected to texture profile analysis using a TA-XT2i Texturometer (Stable Micro Systems, Surrey, UK) coupled to a 6-mm cylindrical stainless steel (P/6) probe in accordance with the detailed methodology of Sousa et al. (2017), for the parameters of hardness, adhesiveness, resilience, cohesiveness, and gumminess.

Statistical Analysis

Data obtained were subjected to two-way analysis of variance ($P < 0.05$) to analyze treatment and time

effects (Steel et al., 1996) using the Assistat statistical software version 7.6 Beta (Silva, 2016). The averages obtained were compared by Tukey test. The averages of the analyzes were also submitted to the principal component analysis (PCA), performed through the MVSP statistical software (KCS, 2007).

RESULTS AND DISCUSSION

Effects of Storage Time on Sensory, Physicochemical Characteristics of Chicken Sausages Produced With Fat Replacement by Collagen Extracted From Chicken Feet

Quantitative Descriptive Analysis. The means and standard deviations of the sensory parameters analyzed

by the QDA are showed in Figure 1. Regarding appearance, in the pink color (Figure 1A), the SC sausage, with the addition of commercial collagen, had higher pink color scores on day 0 and 28 during storage. Differences in pink color can be attributed to the differences in colors and aspects of the fat and its substitutes used in the sausages. The standard SS sausage kept the pink color stable in the first 14 d, with little variation during storage. SG sausage with collagen gel decreased the pink color during storage, with a drop in the first 14 d, followed by stability until day 28. The downward trend observed for the pink color was also seen for the instrumental color parameter a^* , related to the red color intensity of the sausages, in SC and SG sausages in 14 d of storage, in accordance with Table 2.

In the sensory texture parameters of the sausages, it was verified that the SG treatments was softer (Figures 1B–1D). On day 28, it was observed that the

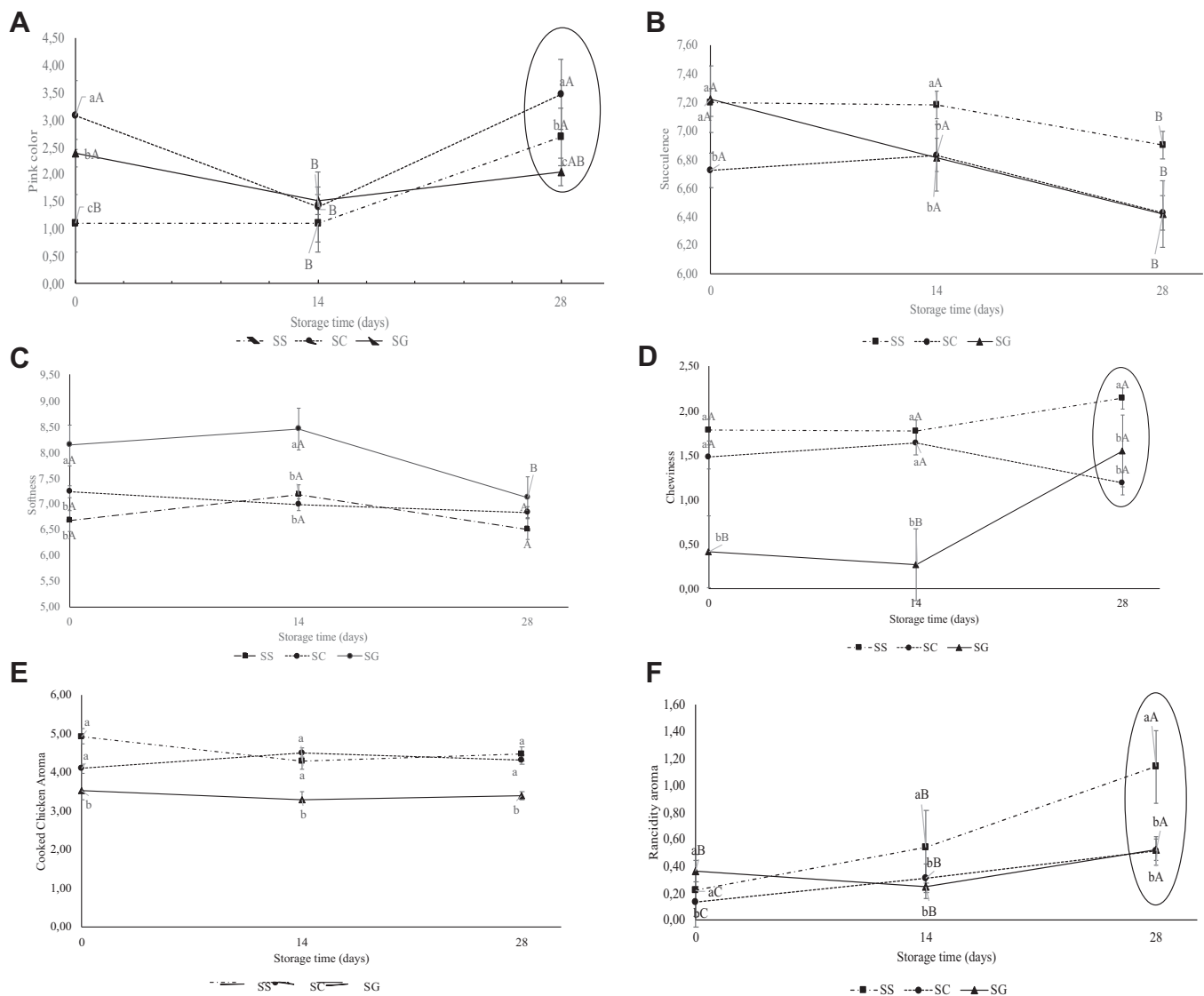


Figure 1. Sensory parameters from chicken sausages by quantitative descriptive analysis: Pink color (A), Succulence (B), Softness (C), Chewiness (D), Cooked chicken aroma (E), and Rancidity aroma. Different lowercase letters in the same storage time denote significant differences among sausages formulations ($P < 0,05$). Different capital letters in the same treatment show significant differences in the storage time. Abbreviations: SS, standard sausages; SC, sausages with hydrolyzed collagen; SG, sausages with collagen gel from chicken feet.

Table 2. Means and standard deviations of chemical, physical, and texture profile parameters of collagen-substituted chicken sausages in refrigerated storage.

Parameter	Treatment	Storage time (days)			
		0	14	28	42
Protein ¹	SS	59.41 ± 0.37 ^{b,B}	64.16 ± 0.32 ^{b,A}	61.94 ± 1.44 ^{b,A,B}	50.93 ± 1.38 ^{b,C}
	SC	70.56 ± 0.22 ^{a,B}	72.73 ± 0.41 ^{a,A,B}	74.22 ± 0.52 ^{a,A}	60.85 ± 0.65 ^{a,C}
	SG	70.60 ± 1.15 ^{a,A}	71.59 ± 0.32 ^{a,A}	73.07 ± 0.39 ^{a,A}	48.55 ± 0.53 ^{b,B}
Collagen ¹ (g/100g)	SS	2.39 ± 0.05 ^{b,C}	4.69 ± 0.03 ^{b,A}	3.66 ± 0.03 ^{c,B}	3.81 ± 0.08 ^{c,B}
	SC	9.85 ± 0.71 ^{a,B}	10.58 ± 0.01 ^{a,A}	10.08 ± 0.42 ^{a,A,B}	10.09 ± 0.09 ^{a,A,B}
	SG	2.96 ± 0.01 ^{b,C}	4.96 ± 0.17 ^{b,A}	4.20 ± 0.05 ^{b,B}	4.49 ± 0.03 ^{b,A,B}
L*	SS	74.82 ± 0.08 ^{b,B}	76.10 ± 0.21 ^{a,A}	73.71 ± 0.23 ^{b,C}	76.70 ± 0.43 ^{a,A}
	SC	74.25 ± 0.02 ^{c,A}	74.14 ± 0.24 ^{b,A}	73.11 ± 0.09 ^{c,B}	73.26 ± 0.18 ^{c,B}
	SG	76.45 ± 0.12 ^{a,A}	74.04 ± 0.17 ^{b,B}	76.28 ± 0.12 ^{a,A}	75.83 ± 0.35 ^{b,A}
a*	SS	5.72 ± 0.08 ^{c,B}	6.18 ± 0.08 ^{a,A}	4.85 ± 0.11 ^{b,C}	5.29 ± 0.11 ^{b,B}
	SC	6.75 ± 0.02 ^{a,A}	5.62 ± 0.22 ^{b,B}	5.35 ± 0.20 ^{a,B}	6.43 ± 0.07 ^{a,A}
	SG	6.10 ± 0.07 ^{b,A}	4.77 ± 0.19 ^{c,C}	5.20 ± 0.19 ^{a,B}	5.95 ± 0.41 ^{a,b,A}
b*	SS	22.71 ± 0.01 ^{b,B}	24.50 ± 0.17 ^{a,A}	24.29 ± 0.20 ^{b,A}	22.86 ± 0.34 ^{b,B}
	SC	23.68 ± 0.15 ^{a,B}	24.37 ± 0.14 ^{a,B}	25.43 ± 0.09 ^{a,A}	25.58 ± 0.35 ^{a,A}
	SG	23.55 ± 0.20 ^{a,B}	23.05 ± 0.81 ^{b,B}	25.31 ± 0.56 ^{a,A}	22.41 ± 0.62 ^{b,B}
WHC	SS	75.99 ± 0.73 ^{a,b,A}	74.79 ± 1.10 ^{b,C}	77.81 ± 0.58 ^{a,B}	79.56 ± 0.95 ^{b,A}
	SC	75.19 ± 1.73 ^{b,B}	75.95 ± 1.09 ^{a,B}	76.46 ± 1.17 ^{a,B}	79.24 ± 1.21 ^{b,A}
	SG	76.82 ± 0.25 ^{a,B}	76.49 ± 1.14 ^{a,B}	74.78 ± 1.06 ^{b,C}	81.05 ± 0.68 ^{a,A}
Hardness (g)	SS	537.26 ± 29.58 ^{a,A}	521.81 ± 24.37 ^{a,A}	521.90 ± 7.08 ^{a,A}	330.92 ± 9.07 ^{b,B}
	SC	425.59 ± 15.11 ^{b,B}	488.06 ± 20.10 ^{a,A}	225.01 ± 1.04 ^{b,D}	365.65 ± 2.04 ^{a,C}
	SG	120.89 ± 23.45 ^{c,C}	192.30 ± 5.43 ^{b,B}	243.66 ± 10.17 ^{b,A}	130.72 ± 21.04 ^{c,C}
Adhesiveness	SS	-69.98 ± 7.30 ^{a,B}	-246.95 ± 12.65 ^{a,A}	-69.31 ± 2.44 ^{b,B}	-73.58 ± 2.92 ^{b,B}
	SC	-64.19 ± 0.89 ^{a,C}	-215.44 ± 0.79 ^{b,A}	-88.05 ± 1.89 ^{a,B}	-91.92 ± 4.44 ^{a,B}
	SG	-7.85 ± 2.84 ^{b,B}	-244.65 ± 8.03 ^{a,A}	-3.01 ± 1.78 ^{c,B}	-8.35 ± 0.83 ^{c,B}
Resilience	SS	0.7119 ± 0.01 ^{a,B}	0.2304 ± 0.01 ^{b,C}	0.6243 ± 0.03 ^{b,B}	0.8659 ± 0.14 ^{a,A}
	SC	0.6229 ± 0.03 ^{a,A}	0.2399 ± 0.02 ^{b,C}	0.4755 ± 0.02 ^{c,B}	0.6686 ± 0.01 ^{b,A}
	SG	0.6890 ± 0.01 ^{a,B}	0.7781 ± 0.01 ^{a,A,B}	0.8084 ± 0.06 ^{a,A}	0.6957 ± 0.01 ^{b,B}
Cohesiveness	SS	0.4379 ± 0.01 ^{a,B}	0.1522 ± 0.01 ^{b,C}	0.5369 ± 0.01 ^{a,A}	0.4447 ± 0.05 ^{a,B}
	SC	0.4028 ± 0.03 ^{a,B}	0.1650 ± 0.01 ^{b,D}	0.3194 ± 0.01 ^{c,C}	0.5083 ± 0.01 ^{a,A}
	SG	0.4388 ± 0.01 ^{a,A}	0.3099 ± 0.01 ^{a,B}	0.3875 ± 0.09 ^{b,A}	0.3705 ± 0.01 ^{b,A,B}
Gumminess	SS	226.00 ± 3.39 ^{a,A}	74.73 ± 3.45 ^{a,D}	121.87 ± 8.97 ^{a,C}	140.74 ± 9.35 ^{b,B}
	SC	123.20 ± 6.93 ^{b,B}	78.61 ± 1.94 ^{a,C}	76.83 ± 2.52 ^{b,C}	178.37 ± 2.58 ^{a,A}
	SG	40.37 ± 3.37 ^{c,B}	53.17 ± 9.92 ^{b,B}	133.88 ± 6.01 ^{a,A}	44.66 ± 6.35 ^{c,B}

abc,ABCD Different lower-case letters indicate significant statistical effect of rows and different uppercase letters indicate significant statistical effect on columns ($P < 0.05$).

Abbreviations: SS, standard sausage; SC, 50% fat replacement sausage with commercial powder collagen; SG, 50% fat replacement sausage with chicken leg collagen gel.

¹Calculated on a dry basis.

treatments did not differ from each other ($P < 0.05$). Regarding storage time, SS and SC remained constant at 28 d, whereas SG decreased its softness between day 14 and 28. Sausage succulence, related to water release during chewing, had the same behavior regarding storage time, remaining stable between 0 and 14 d and decreasing between 14 and 28 d of storage (Figure 1B). Morin et al. (2002), in a study with low-fat sausages using barley β -glucans as fat substitutes, in QDA was obtained that standard sausage juiciness values close to this research and observed a decrease of this value when the fat was replaced by carboxymethylcellulose because of loss of water retention. Probably collagen gel extracted from chicken has a greater ability to retain water than hydrolyzed collagen powder, in accordance with Table 2 for WHC of sausages. It is also a good fat substitute compared with commonly used carbohydrates.

No significant time effects were observed for the sausage chewiness parameter; however, when comparing the treatments, the SG sausage presented the lowest chewable values at all storage times (Figure 1D). The addition of collagen gel contradicts the effects observed by Seo et al. (2015), when evaluating the sensory texture of starch-added sausages with corn starch, chicken

breast, and surimi as fat substitutes during refrigerated storage.

Regarding the aroma of sausages, the parameter cooked chicken aroma had similar behavior, where the sausage SC obtained values similar to the SS standard (Figure 1E). SG treatment, with the addition of collagen gel, presented lower averages of cooked chicken aroma and flavor in all storage times. Probably the acetic acid used in the collagen gel extraction may have caused aroma and taste inhibition. This comment was made by 7 of the 11 panelists (63.6%). Another factor is that fat reduction decreases aroma and taste scores in meat products, due to their characteristic fatty acids, as verified by Venturini et al. (2011), by sensorial evaluation in cured chicken low-fat sausages. There were no significant variations in chicken flavor; however, the chicken flavor of sausages increased with storage time.

The rancidity aroma of chicken sausages was poorly perceived by panelists during the sessions, but it was observed to increase during refrigerated storage (Figure 1F). At all times analyzed, it appears the difference among SS and other treatments ($P < 0.05$), probably due to its higher fat content that SC and the SG. Between latter treatments, during storage (day 14 and

28) they did not differ. With the exception of day 0, low-fat sausages had a lower rancidity aroma than SS sausage and did not differ from each other ($P < 0.05$). The reduction in rancidity aroma may have been due to both the reduction in fat added to the formulation and a probable antioxidant effect of collagen. De Paula et al. (2017), when analyzing the effect of different packages on the cold storage of Brazilian Tuscan sausage, found a strong correlation of rancidity aroma with lipid and protein oxidation.

Chemical and Physical Analysis. Table 2 contains the averages for the chemical, physical, and instrumental texture profile parameters of the sausages during the 42 d of storage. The protein content on dry basis was lower for standard SS treatment at all storage times. This can be justified by the replacement of fat by protein sources in SC and SG. It was also observed that for the collagen content, the highest averages were obtained for the SC treatment at all storage times. The differences obtained probably resulted from the introduction of collagen, which is more concentrated in powder form, compared with gel extracted from chicken feet. Regarding storage time, it was found that both protein and collagen content increased between 0 and 14 d, followed by a steady fall until day 42. Protein decrease may be associated with possible oxidation, which according to Estévez et al. (2011) and Soladoye et al. (2015) can modify some amino acids such as methionine, cysteine, lysine, threonine, and arginine. Another factor that can justify this behavior is that part of the water-soluble proteins can be exuded by exudation during storage.

Regarding the color parameters, in the luminosity parameter, it was observed that there was oscillation between the samples and during the storage time. From day 14, there was an increase in L^* as a function of time for SG treatment and a decrease in SC, which may have probably happened due to the use of collagen in gel form (SG), with crystalline aspects in comparison with powdered collagen used in SC, due to possible differences in the form of extraction. Serdaroglu et al. (2016), when studying the effects of replacing fat with a gelatin emulsion in hamburgers, found that the gel-added samples showed higher luminosity, justifying that the presence of gelatin increases the L^* value due to the greater light reflection than the animal fat globules, which are bigger. The exception occurred on day 14, where coincidentally there was the sharpest decrease in fat in SS treatment.

The color parameters a^* and b^* , when comparing the effects between the treatments, had the same behavior, where the fat-reduced samples, in general, presented higher values when compared with the SS treatment, except for the day 14, which was similar. The SG sausage was inferior to the other treatments. Regarding time, it was observed that there was a decrease of a^* and increase of b^* , mainly between day 0 and 28. The decrease of a^* value may be associated with lipid oxidation, which implies metmyoglobin accumulation, as shown by observed by Sohaib et al. (2017) on chicken steaks

throughout storage. The values of a^* were close to those found by Jin et al. (2016), in emulsified chicken sausages, whereas b^* values were close to those obtained by Bolger et al. (2016), in vitamin E-enriched chicken sausages.

Regarding the WHC of sausages (Table 2), it was observed that among the treatments, those who had fat reduction (SC and SG) had WHC values similar or higher than the SS standard ($P < 0.05$). Serdaroglu et al. (2016) state that collagen gels have the ability to bind water molecules and retain fat in the matrix, which in practical terms may make the low-fat product closer to standard. With respect to time, it was verified in all treatments the increase of retention over 42 d. The values obtained in this study were higher than those found by Méndez-Zamora et al. (2015), in low-fat Frankfurt-type sausages with inulin and pectin addition, probably due to the properties of collagen gelling and binding to water and fat.

In the texture profile analysis, the SS and SC treatments presented higher hardness values compared with SG, in accordance with Table 2, and during the storage time only the collagen gel sausage increased the hardness value. It is emphasized that the behavior explained for softness in ADQ is opposite to the behavior obtained for instrumental hardness. Sousa et al. (2017), by introducing various levels of collagen powder for fat substitution in chicken sausages, found that sausages lost softness along with the addition of collagen powder in the formulation.

For the adhesiveness, it was observed that, except for day 14, SG presented smaller area compared with the other treatments, being the sausage less adhesive. Regarding storage time, an increase between 0 and 14 d was observed in all treatments, followed by a reduction up to 42 d of storage. Regarding resilience, variations were observed between samples, where the treatment SG presented higher averages up to the 28th day of storage, decreasing on day 42. The values of this study were close to those obtained by Prestes et al. (2015), in low-fat chicken mortadella with substitution of fat by starch.

In the cohesiveness parameter, it was observed that on day 0 there were no differences between treatments, but during storage the standard sausage showed oscillations, whereas the sausages SC and SG showed increasing effect on cohesiveness, which represents a positive effect, because probably collagen made more internal bonds in the sausages, giving greater stability. The values were similar to those found by Jin et al. (2016), in emulsified pork sausages, which ranged from 0.48 to 2.21%. Regarding the bud, an effect similar to hardness was observed (Table 3), which also increased with storage time. Gumminess is the energy of chewing and is increasingly needed as sausages get harder. The obtained bud values were close to those found by Serdaroglu et al. (2016), in a meat emulsion added with a gelled emulsion, with values between 3.01 and 13.82 N.

SDS-PAGE Electrophoresis. The electrophoretic profile of the sausages at all storage times is shown in

Table 3. Means of the sum and indexes of fatty acids obtained as a percentage of area for collagen gel-replaced chicken sausages during storage.

Parameters	Treatments	Storage time (days)			
		0	14	28	42
ΣSFA	SS	36.37 ± 0.90 ^{a,A}	37.50 ± 0.14 ^{a,A}	36.91 ± 0.75 ^{b,A}	30.82 ± 0.59 ^{b,B}
	SC	36.49 ± 0.58 ^{a,B}	30.95 ± 0.54 ^{b,C}	39.49 ± 0.94 ^{a,A}	40.19 ± 0.42 ^{a,A}
	SG	37.93 ± 0.20 ^{a,B}	32.37 ± 0.62 ^{b,B}	42.67 ± 0.22 ^{a,A}	40.30 ± 0.45 ^{a,A}
ΣMUFA	SS	37.82 ± 0.75 ^{a,B}	37.31 ± 0.13 ^{b,B}	37.86 ± 0.76 ^{a,B}	43.61 ± 0.84 ^{a,A}
	SC	38.14 ± 0.35 ^{a,B}	42.26 ± 0.70 ^{a,A}	37.95 ± 0.54 ^{a,B}	42.05 ± 0.81 ^{a,A}
	SG	38.24 ± 0.12 ^{a,B}	43.81 ± 0.57 ^{a,A}	34.19 ± 0.54 ^{b,B}	35.88 ± 0.56 ^{b,B}
ΣPUFA	SS	22.82 ± 0.30 ^{b,A}	24.73 ± 0.01 ^{a,A}	25.21 ± 0.52 ^{a,A}	25.76 ± 0.61 ^{a,A}
	SC	25.15 ± 0.30 ^{a,A}	25.17 ± 0.02 ^{a,A}	27.17 ± 0.48 ^{a,A}	19.97 ± 0.94 ^{b,B}
	SG	23.80 ± 0.32 ^{b,A}	23.82 ± 0.02 ^{b,A}	23.10 ± 0.77 ^{b,A}	23.78 ± 0.50 ^{a,A}
ΣMUFA/ΣSFA	SS	1.04 ± 0.05 ^{a,B}	1.02 ± 0.01 ^{b,B}	1.02 ± 0.01 ^{a,B}	1.41 ± 0.05 ^{a,A}
	SC	1.05 ± 0.02 ^{a,A}	1.34 ± 0.02 ^{a,A}	0.83 ± 0.02 ^{b,A}	1.05 ± 0.37 ^{b,A}
	SG	1.01 ± 0.01 ^{a,B}	1.24 ± 0.02 ^{a,A}	0.80 ± 0.01 ^{b,B}	0.89 ± 0.01 ^{b,B}
ΣPUFA/ΣSFA	SS	0.63 ± 0.02 ^{b,B}	0.68 ± 0.01 ^{a,B}	0.68 ± 0.01 ^{a,B}	0.84 ± 0.05 ^{a,A}
	SC	0.69 ± 0.02 ^{a,A}	0.65 ± 0.14 ^{a,A}	0.69 ± 0.06 ^{a,A}	0.48 ± 0.07 ^{b,A}
	SG	0.63 ± 0.02 ^{b,A}	0.25 ± 0.01 ^{b,B}	0.54 ± 0.02 ^{b,A}	0.50 ± 0.12 ^{b,A}
AI	SS	0.70 ± 0.02 ^{a,A}	0.52 ± 0.02 ^{b,B}	0.90 ± 0.04 ^{a,A}	0.49 ± 0.08 ^{b,B}
	SC	0.71 ± 0.02 ^{a,A}	0.37 ± 0.07 ^{c,B}	0.90 ± 0.18 ^{a,A}	0.79 ± 0.06 ^{a,A}
	SG	0.72 ± 0.01 ^{a,A}	0.66 ± 0.01 ^{a,A}	0.77 ± 0.02 ^{b,A}	0.59 ± 0.03 ^{b,B}
TI	SS	1.18 ± 0.05 ^{a,A}	1.17 ± 0.01 ^{a,A}	1.10 ± 0.09 ^{a,A}	0.68 ± 0.02 ^{b,B}
	SC	1.15 ± 0.03 ^{a,A}	0.77 ± 0.06 ^{b,B}	1.27 ± 0.51 ^{a,A}	1.30 ± 0.11 ^{a,A}
	SG	1.19 ± 0.03 ^{a,A}	1.14 ± 0.02 ^{a,A}	1.08 ± 0.05 ^{a,A}	0.68 ± 0.15 ^{b,B}

^{ab,AB}Different lowercase letters indicate significant statistical effect of rows and different uppercase letters indicate significant statistical effect on columns ($P < 0.05$).

Abbreviations: SS, standard sausage; SC, 50% fat replacement sausage with commercial powder collagen; SG, 50% fat replacement sausage with chicken leg collagen gel; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AI, atherogenicity index; TI, thrombogenicity index.

Figure 2. Regarding the effects of the storage of sausages, it was observed in all treatments, especially in SS and SC, a higher intensity of the lower molecular weight bands as the refrigeration time advance, which may be the effect of storage on protein fragmentation. In SG sausage this effect was observed, but with lighter bands. The last bands of the electrophoretic profile, smaller than the 31 kDa band, which become lighter on day 28, may correspond to the myosin chains, which according to [Feng et al. \(2016\)](#) can be reduced by becoming protein polymers during storage.

Effects of Storage Time on the Oxidation of Chicken Sausages Produced by Replacing Fat With Collagen Extracted From Chicken Feet

Fatty Acid Profile, Lipid Content, Number of TBARS, and Antioxidant Activity. The averages and deviations of the sum of fatty acid percentages are listed in [Table 3](#). It was observed in all treatments during storage the predominance of saturated and monounsaturated fatty acids, reaching values close to 70% of the total fatty

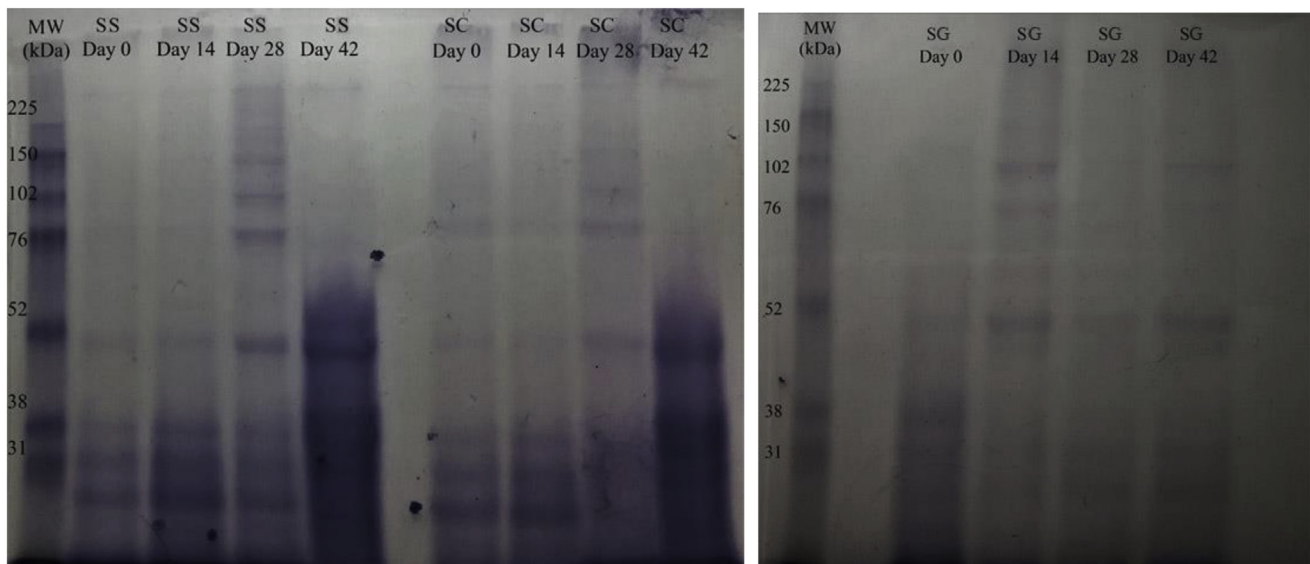


Figure 2. SDS-PAGE electrophoresis for low-fat chicken sausages with fat replacement by collagen in cold storage. Abbreviations: MW, molecular weight (kDa).

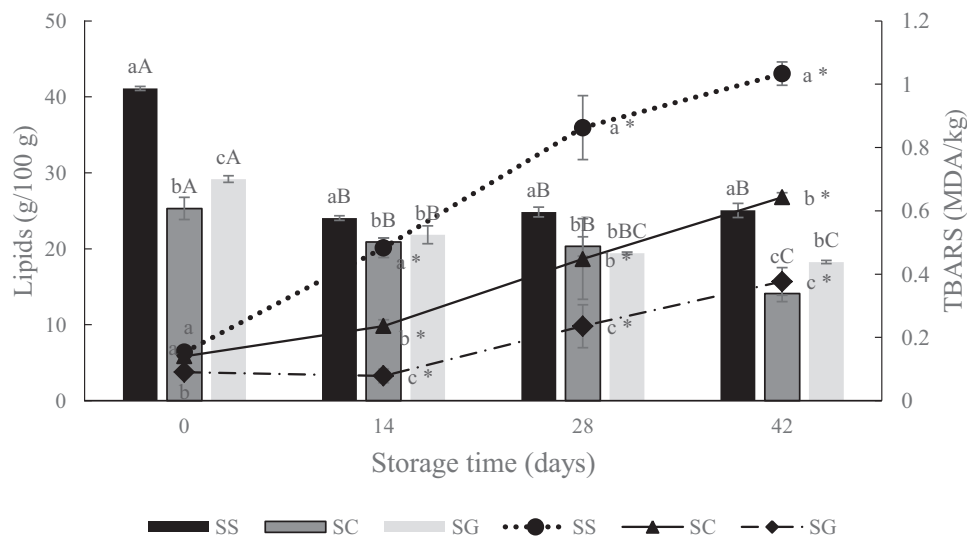


Figure 3. Graphical representation of the means obtained for lipid content and TBARS number of chicken sausages with fat replacement by collagen in refrigerated storage. Bars: means of lipid content; Rows: Averages the number of TBARS over storage. Different lowercase letters indicate differences between treatments, and uppercase letters indicate differences with respect to time ($P < 0.05$). * Significant differences over time for TBARS plots ($P < 0.05$). Abbreviations: SS: Standard Sausage, SC: 50% Fat replacement sausage with commercial powder collagen, SG: 50% Fat replacement sausage with chicken leg collagen gel; TBARS, thiobarbituric acid reactive substances.

acids, a common profile for chicken meat, as seen by [Yilmaz et al. \(2002\)](#), in low-fat chicken sausages. Regarding saturated fatty acids, it was found that between treatments on day 0 there was no statistical difference; however, during storage the percentage of SS treatment decreased compared to other treatments, with similar behavior for monounsaturated fatty acids between day 0 and 14. Regarding polyunsaturated fatty acids, it was observed that treatments SS and SG kept their percentages during storage, whereas SC sausage, with commercial collagen, decreased the sum between day 28 and 42. The values of Σ PUFA were close to those found by [Morais et al. \(2013\)](#) when replacing pork backfat by soy oil in sausages with alligator meat.

Regarding the Σ MUFA/ Σ SFA ratio, it was observed in relation to the storage time that SS treatment increased, SC treatment conserved, and SG decreased between day 14 and 42. Among treatments, SS presented higher values from day 28. The values obtained were close to those found by [Morais et al. \(2013\)](#) in alligator meat sausage with 50% reduction in fat, which obtained an average value of 1.33%. In relation to Σ PUFA/ Σ SFA, a similar behavior was verified between the treatments, with values obtained close to those found by [Berasategi et al. \(2011\)](#), in omega-3 enriched Bologna sausages, which found values between 0.49 and 0.84%.

It was observed that the AI of SS and SG sausages decreased during storage; however, the opposite behavior was obtained in the standard SS sausage. From day 28, SG sausage presented lower AI values, which may be a sign of lower risk of cardiovascular disease. The AI values obtained were slightly higher than those found by [Stajic et al. \(2011\)](#), which obtained values between 0.46 and 0.61 in Sremska sausages in 14 d of storage. Thrombogenicity index showed a similar reduction behavior over time, being the SS and SG treatments inferior to the SC treatment, with values close to

those found by [Berasategi et al. \(2011\)](#) in Bologna sausages.

Initially for lipids, it was verified in the bar graph of [Figure 2](#) that, proportionally to the substitution performed, the values obtained for the SC and SG treatments correspond to approximately half of the values obtained for the standard SS sausage. Regarding the storage effect, it was observed in all treatments that the lipid content was decreasing, especially between day 0 and 14, during which the standard sausage had the largest reduction, being approximately 41.6%. Probably, the reduction may be justified by a possible lipid oxidation, which is related to the increased rancidity aroma ([Figure 1F](#)) or by the fat quantification method chosen for this study. The values obtained were close to those obtained by [Seo et al. \(2015\)](#) in low-fat sausages for the control treatment (35.70% on a dry basis) and in the fat substitution treatment with 10% corn starch, 15% chicken breast, and 20% surimi.

Regarding the number of TBARS, the values obtained over the 42 d of refrigerated storage are distributed graphically in [Figure 3](#), plotted in line graphs. An inversely proportional relationship was observed between the lipid content and the number of TBARS during storage because while decreasing the lipid content, the malonaldehyde concentration of the sausages increased. As expected, at all times the SS treatment presented higher means of TBARS. The SG sample, with the addition of chicken foot collagen gel, presented lower averages than the other treatments, probably due to the fact that collagen gel has antioxidant potential. Regarding the sensory analyzed rancidity, although there were significant differences between treatments, the scores were low, indicating little perceived rancidity on the part of the panelists. This relationship is according with the conclusion reached by [Ockerman and Kesh \(1982\)](#), who reported that the flavor of fat oxidation,

Table 4. Means and standard deviations of DPPH radical capture activity and ferric reducing antioxidant power (FRAP) of collagen-substituted chicken sausages in refrigerated storage.

Parâmetros	Tratamentos	Storage time (days)			
		0	14	28	42
DPPH (% of inhibition)	SS	8.02 ± 0.52 ^{b,B}	7.81 ± 0.33 ^{b,B}	7.26 ± 0.39 ^{b,B}	16.47 ± 0.29 ^{a,A}
	SC	10.01 ± 0.43 ^{a,B}	8.65 ± 0.27 ^{b,C}	10.09 ± 0.91 ^{a,B}	17.94 ± 1.38 ^{a,A}
	SG	10.13 ± 0.64 ^{a,B}	11.06 ± 0.31 ^{a,B}	11.40 ± 0.07 ^{a,B}	16.11 ± 0.57 ^{a,A}
FRAP (mg Trolox/g)	SS	20.69 ± 0.01 ^{a,D}	29.39 ± 0.09 ^{a,C}	37.95 ± 0.31 ^{a,B}	43.17 ± 0.94 ^{a,A}
	SC	11.99 ± 0.01 ^{c,C}	19.06 ± 0.14 ^{b,B}	36.30 ± 0.05 ^{a,A}	33.25 ± 0.13 ^{b,A}
	SG	16.60 ± 0.40 ^{b,C}	14.98 ± 0.08 ^{c,C}	36.05 ± 0.16 ^{a,B}	41.85 ± 0.83 ^{a,A}

^{abc,ABC} Different lowercase letters indicate significant statistical effect of rows and different uppercase letters indicate significant statistical effect on columns ($P < 0.05$).

Abbreviations: SS, standard sausage; SC, 50% fat replacement sausage with commercial powder collagen; SG, 50% fat replacement sausage with chicken leg collagen gel.

resulting from the deterioration of fatty acids, would be perceived in sensory evaluation tests when reaching above 1.0 mg of malonaldehyde, kg of meat, which did not occur until 28 d of storage. Seo et al. (2015), when replacing pork backfat in chicken sausages by cornstarch obtained TBARS values between 0.46 and 1.59 mg MDA/kg sample, higher than this study.

Lower TBARS values may be related to a possible collagen antioxidant activity used in chicken sausages. The averages and deviations obtained for the antioxidant activity related to the capture of the DPPH radical and the iron reducing power (FRAP), expressed as Trolox equivalent, are listed in Table 4. Regarding the DPPH radical, it was observed that the treatments with the addition of collagen-replacing fat presented higher inhibition percentage, seen in all storage times.

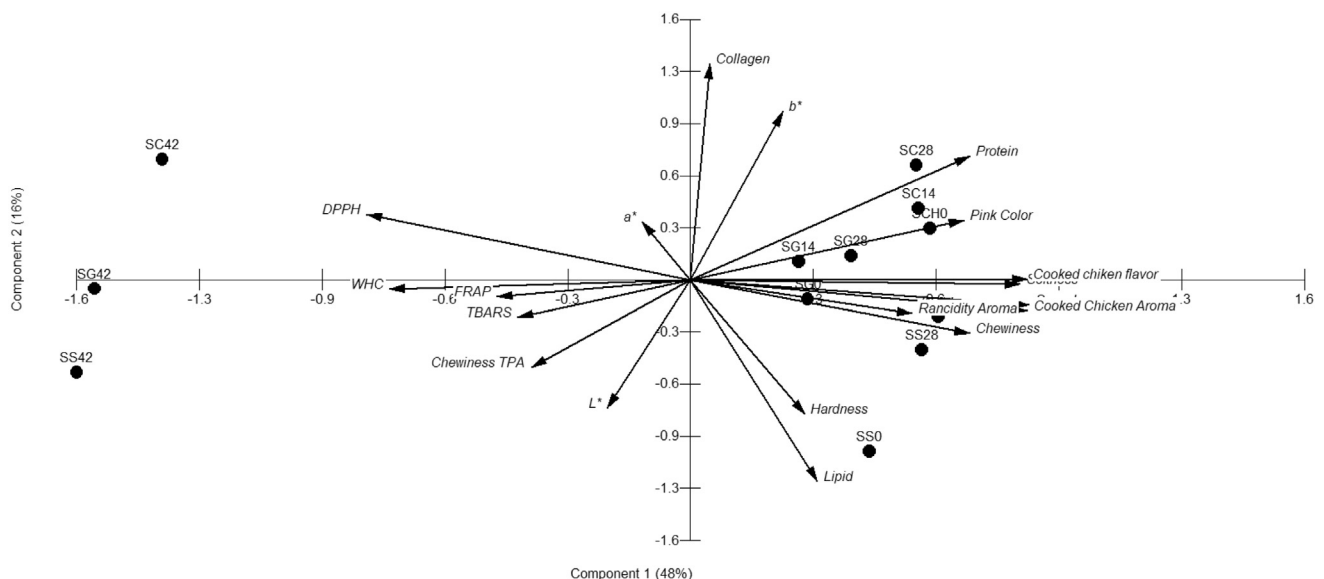
In storage time, it was observed that the treatments were stable up to 28 d of storage, with a significant increase between 28 and 42 d. Regarding the sausage reducing power tests (FRAP), it was observed that, unlike DPPH analyzes, the samples oscillated between times, with the SG sausage activity closer or similar to the SS standard. Mejri et al. (2017) obtained higher iron reduction activities in fermented sausages during

storage, using low molecular weight peptides, which verified that such activity was attributed to the 3 kDa peptide fractions during the refrigeration period.

Principal Component Analysis

In the PCA presented in Figure 4, it was observed that in relation to the sensory parameters, the collagen gel-added sausages occupied positions distributed in the first and fourth quadrants, close to the softness, water exudation (SG0 and SG14), and salty taste (SG28). Standard treatment at time 0 correlated with lipid content. The SS and SC sausages presented greater proximity and possible correlation with the chicken flavor and aroma parameters, which corroborates the behavior described in the QDA.

In the other attributes analyzed in the sausages (Figure 4), it was found that all treatments at times 0, 14, and 28 were arranged in the first and fourth quadrants, except for the SS treatment at time 28, which correlated most strongly with the parameter, chewability. The treatments at time 42 remained together in the second quadrant, being better correlated with the number of TBARS and the antioxidant activity

**Figure 4.** Principal component analysis for quality parameters of chicken sausages with fat replacement by collagen in refrigerated storage.

parameters (DPPH, FRAP). At this time, SG sausage correlated most strongly with WHC. Tomaschunas et al. (2013), when studying sensory changes in inulin and citrus fiber substitution Lyon liver sausages, verified by PCA that the samples with higher fat content correlated with the characteristic meat aroma and the juiciness of the sausages.

CONCLUSION

Regarding the storage of sausages, it was observed that up to 28 d the sausages maintained the hardness parameters, the protein content and few variations in the sensory texture parameters. Fat reduction by collagen had advantages in reducing the number of TBARS, being the collagen gel sample better in antioxidant activity and atherogenicity and thrombogenicity indices, when compared with the hydrolyzed collagen sausage. Refrigerated storage for 28 d was important to maintain a greater number of desirable quality parameters. Substitution of fat by collagen was a viable alternative to maintain the main quality characteristics combined with the offer of a healthier product. In addition, collagen gel represented a viable alternative for better use of waste from the refrigeration industry, maintaining sausage quality for 28 d at 4°C. For best results, storage studies with other lower levels of collagen gel replacement fat are recommended.

DISCLOSURES

There are no conflicts of interest between the authors of the article entitled "Effect of storage time on the quality of chicken sausages produced with fat replacement by collagen gel extracted from chicken feet" submitted and approved in this Journal.

REFERENCES

- ABPA. Associação Brasileira de Proteína Animal. 2019. Novas habilitações devem impulsionar vendas à China. Accessed Sept. 2019. <http://abpa-br.com.br/noticia/artigos/todas/novas-habilitacoes-devem-impulsionar-vendas-a-china-2909>.
- AOAC. 2007. Official Methods of Analysis of AOAC International. Association of Official Analysis Chemists International, Gaithersburg, Maryland. CD-ROM.
- Araújo, I. B. S., T. K. A. Bezerra, E. S. Nascimento, C. A. A. Gadelha, T. Santi-Gadelha, and M. S. Madruga. 2018. Optimal conditions for obtaining collagen from chicken feet and its characterization. *Food Sci. Technol.* 38:167–173.
- Araújo, I. B. S., D. A. S. Lima, S. F. Pereira, and M. S. Madruga. 2019. Quality of low-fat chicken sausages with added chicken feet collagen. *Poult. Sci.* 98:1064–1074.
- Benzie, I. F. F., and J. J. Strain. 1996. The Ferric Reducing Ability of Plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* 239:70–76.
- Berasategi, I., S. Legarra, M. G. I. Ciriano, S. Rehech, M. I. Calvo, R. Y. Caverro, I. Navarro-Blasco, D. Ansorena, and I. Astiasarán. 2011. "High in omega-3 fatty acids" bologna-type sausages stabilized with an aqueous-ethanol extract of *Melissa officinalis*. *Meat Sci.* 88:705–711.
- Bolger, Z., N. P. Brunton, J. G. Lyng, and J. F. Monahan. 2016. Quality attributes and retention of vitamin E in reduced salt chicken sausages fortified with vitamin E. *J. Food Sci. Technol.* 53:3948–3959.
- Brazil. 2001. Ministry of Health. National Health Surveillance Agency (ANVISA), Resolution RDC n° 12 of January 2. Technical Regulation on Microbiological Standards for Food. Official diary. Brasília DF. 2001. Accessed Apr. 2020. http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2001/anexos/anexos_res0012_02_01_2001.pdf.
- Brazil. 2012. Ministry of Health. National Research Ethics Commission. Health National Council. Official Gazette. Brasília, Federal District, 2012. Accessed Apr. 2020. https://bvsms.saude.gov.br/bvs/saudelegis/cns/2013/res0466_12_12_2012.html.
- Brazil. 2000. Ministry of Agriculture and Supply Secretary of Agricultural Defense. Normative Instruction SDA No. 4, of March 31, 2000. Technical Regulations on Identity and Quality of Mechanically Separated Meat (CMS), Mortadela, Sausage and Sausage. Accessed Nov. 2020. <https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=05/04/2000&jornal=1&pagina=54&totalArquivos=73>.
- Chakka, A. K., M. Elias, R. Jini, P. Z. Sakhare, and N. Bhaskar. 2015. In-vitro antioxidant and antibacterial properties of fermentatively and enzymatically prepared chicken liver protein hydrolysates. *J. Food Sci. Technol.* 52:8059–8067.
- Chandini, S., P. Ganesan, and N. Bhaskar. 2008. In vitro antioxidant activities of three selected brown seaweeds of India. *Food Chem.* 107:707–713.
- Daguer, H., M. P. Stephan, and L. S. Bersot. 2010. Perfil eletroforético de lombo suíno adicionado de proteínas não cárneas. *Ciência Rural* 40:434–440.
- De Paula, R., J. M. Soares, P. F. Da Silva, G. T. Backes, R. L. Cansian, H. Treichel, and E. Valduga. 2017. Assessment of different packaging structures in the stability of refrigerated fresh Brazilian Toscana sausage. *Int. Food Res. J.* 24:173–181.
- Estévez, M., S. Ventanas, and M. Heinonen. 2011. Formation of Strecker aldehydes between protein carbonyls— α -aminoacidic and γ -glutamic semialdehydes—and leucine and isoleucine. *Food Chem.* 128:1051–1057.
- Feng, X., C. Li, X. Jia, Y. Guo, N. Lei, R. M. Hackman, L. Chen, and G. Zhou. 2016. Influence of sodium nitrite on protein oxidation and nitrosation of sausages subjected to processing and storage. *Meat Sci.* 116:260–267.
- Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:497–509.
- Hartman, L., and R. C. A. Lago. 1973. Rapid preparation of fatty acids methyl esters. *Lab. Pract.* 22:475–476.
- Huff-Lonergan, E., and S. M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: the role of postmortem biochemical and structural changes. *Meat Sci.* 71:194–204.
- Jin, S. K., S. R. Ha, S. J. Hur, and J. S. Choi. 2016. Effect of the ratio of Raw material components on the Physico-chemical characteristics of emulsion-type pork sausages. *Asian-Australasian J. Anim. Sci.* 29:263–270.
- KCS. 2007. MVSP Plus: Manual User's. Kovach Computing Services, Wales, UK.
- Khulal, U., J. Zhao, W. Hu, and Q. Chen. 2016. Nondestructive quantifying total volatile basic nitrogen (TVB-N) content in chicken using hyperspectral imaging (HSI) technique combined with different data dimension reduction algorithms. *Food Chem* 197(Pt B):1191–1199.
- Kılıç, B., and C. Ö. Özer. 2017. Effects of replacement of beef fat with interesterified palm kernel oil on the quality characteristics of Turkish dry-fermented sausage. *Meat Sci.* 131:18–24.
- Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of the bacteriophage T4. *Nature* 227:680–685.
- Li, B., F. Chen, X. Wang, B. Ji, and Y. Wu. 2007. Isolation and identification of antioxidative peptides from porcine collagen hydrolysate by consecutive chromatography and electrospray ionization-mass spectrometry. *Food Chem.* 102:1135–1143.
- Meilgaard, M. C., B. T. Carr, and G. V. Civille. 1999. Sensory Evaluation Techniques. 3rd ed. CRC Press, New York, NY.
- Mejri, L., R. Vázquez-Villanueva, M. Hassouna, M. L. Marina, and M. C. García. 2017. Identification of peptides with antioxidant and antihypertensive capacities by RP-HPLC-Q-TOF-MS in dry

- fermented camel sausages inoculated with different starter cultures and ripening times. *Food Res. Int.* 100:708–716.
- Méndez-Zamora, G., J. A. García-Macías, E. Santellano-Estrada, A. Chávez-Martínez, L. A. Durán-Meléndez, R. Silva-Vázquez, and A. Quintero-Ramos. 2015. Fat reduction in the formulation of frankfurter sausages using inulin and pectin. *Food Sci. Technol.* 35:25–31.
- Moraes, M. C., and R. L. Cunha. 2013. Gelation property and water holding capacity of heat-treated collagen at different temperature and pH values. *Food Res. Int.* 50:213–223.
- Morais, C. S. N., N. N. Morais, J. Vicente-Neto, E. M. Ramos, J. Almeida, C. Roseiro, C. Santos, L. T. Gama, and M. C. Bressan. 2013. Mortadella sausage manufactured with Caiman yacare (*Caiman crocodilus yacare*) meat, pork backfat, and soybean oil. *Meat Sci.* 95:403–411.
- Morin, L. A., F. Temelli, and L. McMullen. 2002. Physical and sensory characteristics of reduced-fat Breakfast sausages formulated with barley b-Glucan. *J. Food Sci.* 67:2382–2391.
- Ockerman, H. W., and J. C. Kesh. 1982. Dried pork as influenced by nitrate, packaging method and storage. *J. Food Sci.* 47:1631–1634.
- Prestes, R. C., L. B. Silva, A. M. P. Torri, E. H. Kubota, C. S. Rosa, S. S. Roman, A. P. Kempka, and I. M. Demiate. 2015. Sensory and physicochemical evaluation of low-fat chicken mortadella with added native and modified starches. *J. Food Sci. Technol.* 52:4360–4368.
- Pulido, R., L. Bravo, and F. Saura-Calixto. 2000. Antioxidant activity of dietary as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food Chem.* 48:3396–3402.
- Rosmini, M. R., F. Perlo, J. A. Pérez-Alvarez, M. J. Pagán-Moreno, A. Gago-Gago, F. López-Santoveña, and V. Aranda-Catalá. 1996. TBA test by extractive method applied to paté. *Meat Sci.* 42:103–110.
- Seo, H. W., G. H. Kang, S. H. Cho, V. H. Ba, and P. N. Seong. 2015. Quality properties of sausages made with replacement of pork with corn starch, chicken breast and surimi during refrigerated storage. *Korean J. Food Sci. Anim. Resour.* 35:638–645.
- Serdaroğlu, M., B. Nacak, M. Karabyıkoğlu, and G. Keser. 2016. Effects of partial beef fat replacement with gelled emulsion on functional and quality properties of model system meat emulsions. *Korean J. Food Sci. Anim. Resour.* 36:744–751.
- Shimokomaki, M., V. C. Duance, and A. J. Bailey. 1981. Identification of two further collagenous fractions from cartilage. *Biosci. Reports* 1:561–570.
- Silva, F. A. S., and C. A. V. de Azevedo. 2016. The Assistat Software Version 7.7 and its use in the analysis of experimental data. *Afr. J. Agr. Res.* 11:3733–3740.
- Simões, G. S., E. T. F. Silveira, S. R. Oliveira, E. Poleze, J. R. D. Allison, E. I. Ida, and M. Shimokomaki. 2014. Optimum conditions for extracting collagen from the tunica albuginea of immunologically castrated pig testes and the functional properties of the isolated collagen. *Meat Sci.* 96:1460–1468.
- Sohaib, M., F. M. Anjum, M. S. Arshad, M. Imran, A. Imran, and S. Hussain. 2017. Oxidative stability and lipid oxidation flavoring volatiles in antioxidants treated chicken meat patties during storage. *Lipids Health Dis.* 16:17–27.
- Soladoye, O. P., M. L. Juárez, J. L. Aalhus, P. Shand, and M. Estevez. 2015. Protein oxidation in processed meat: Mechanisms and potential Implications on Human Health. *Compr. Rev. Food Sci. Food Saf.* 14:106–122.
- Soladoye, O. P., J. Saldo, L. Peiro, A. Rovira, and M. M. Mur. 2015. Antioxidant and Angiotensin 1 Converting enzyme inhibitory functions from chicken collagen hydrolysates. *J. Nutr. Food Sci.* 5:369.
- Sousa, S. C., S. P. Fragoso, C. R. A. Penna, N. M. O. Arcanjo, F. A. P. Silva, V. C. S. Ferreira, M. D. S. Barreto, and I. B. S. Araújo. 2017. Quality parameters of frankfurter-type sausages with partial replacement of fat by hydrolyzed collagen. *LWT - Food Sci. Technol.* 76:320–325.
- Stajic, S., M. Perunović, S. Šobajčić, and D. Vranić. 2011. Cholesterol content and atherogenicity of fermented sausages made of pork meat from various breeds. *Proc. Food Sci.* 1:568–575.
- Steel, R. G. D., J. H. Torrie, and D. Dickey. 1996. Principles and Procedures of Statistics. McGraw-Hill, New York.
- Stone, H., and J. L. Sidel. 2004. Sensory Evaluation Practices. 3rd ed. Elsevier Academic Press, San Diego, CA.
- Toldrá, F., M. C. Aristoy, L. Mora, and M. Reig. 2012. Innovations in value-addition of edible meat by-products. *Meat Sci.* 92:290–296.
- Tomaschunas, M., R. Zorb, J. Fischer, E. Kohn, J. Hinrichs, and M. Busch-Stockfisch. 2013. Changes in sensory properties and consumer acceptance of reduced fat pork Lyon-style and liver sausages containing inulin and citrus fiber as fat replacers. *Meat Sci.* 95:629–640.
- Tonial, I. B., C. E. C. Bravo, N. E. Souza, M. Matsushita, W. M. Furuya, and J. V. Visentainer. 2011. Qualidade nutricional dos lipídios de tilápias (*Oreochromis niloticus*) alimentadas com ração suplementada com óleo de soja. *Revista Alimentos e Nutrição.* 22:103–112.
- USDA. 2019. United States Department of Agriculture: Foreign Agricultural Service. <https://apps.fas.usda.gov/psdonline/app/index.html#/app/advQuery>.
- Venturini, A. C., A. D. Cavenaghi, C. J. C. Castillo, and E. M. Quiñones. 2011. Sensory and microbiological evaluation of uncured fresh chicken sausage with reduced fat content. *Food Sci. Technol.* 31:629–634.
- WHO. 2015. World Health Organization, OPAS estabelece critérios para definir níveis excessivos de açúcar, sal e gorduras em alimentos e bebidas processados. Accessed May 2020. http://www.paho.org/bireme/index.php?option=com_contentandview=articleandid=320:opas-estabelece-criterios-para-definir-niveis-excessivos-de-acucar-sal-e-gorduras-em-alimentos-e-bebidas-processadosandItemid=183.
- Yilmaz, I., O. Simsek, and M. Isikli. 2002. Fatty acid composition and quality characteristics of low-fat cooked sausages made with beef and chicken meat, tomato juice and sunflower oil. *Meat Sci.* 62:253–258.
- Zhang, F., L. Fang, C. Wang, L. Shi, T. Chang, T. Yang, H. Yang, and M. Cui. 2013. Effects of starches on the textural, rheological, and color properties of surimi-beef gels with microbial transglutaminase. *Meat Sci.* 93:533–537.