

Lipid and protein oxidation of emulsified chicken patties prepared using abdominal fat and skin

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ABSTRACT Skin (SK) and abdominal fat (AF) are by-products that are abundantly produced in poultry slaughterhouses. Skin is used in meat products, especially in sausages, but its use is related to microbiological contamination and susceptibility to oxidation. The aim of the present study was to evaluate the effect of SK replacement with AF on the quality characteristics of emulsified patties stored under freezing conditions (-18°C) for 90 D. The AF showed higher lipid content than did the SK, but the SK had a higher predominance of polyunsaturated fatty acids. Regarding the partial chemical composition, the treatments showed differences

in moisture, lipids, and total collagen. The values for para-anisidine and carbonyl compounds at the end of 90 D of storage were not influenced by the incorporation of SK or AF. The judges also did not observe differences in the color and aroma of the emulsified products during the 90 D. Furthermore, volatile compounds considered oxidation markers were not detected at the end of the storage period. Given these results, emulsified patties made with SK or AF undergo, to a similar degree, low levels of lipid and protein oxidation when stored under freezing conditions (-18°C) for 90 D, which allows the use of some of these lipid sources in meat products.

Key words: by-product, meat emulsion, storage, volatile compounds

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INTRODUCTION

The poultry sector has gained prominence in recent years because of its growth, with a further estimated increase in production of 25% by the year 2025 (Barbut, 2015). Given the high yield, poultry farming has led to the generation of considerable quantities of by-products from slaughter. Approximately, 22–33% of poultry production consists of by-products that include viscera, head, blood, feet, feathers, and skin (SK) (Erge and Zorba, 2018).

Abdominal fat (AF) is one of the by-products of chicken slaughter and has been little used in the food industry. Abdominal fat represents 2.5% of the total weight of the slaughtered chicken and is usually used in energy generation as biofuel and in the production of soap or animal feed (Centenaro et al., 2008). However, chicken AF has polyunsaturated fatty acids (PUFA), such as oleic and linoleic fatty acids, making it a potential ingredient

in the preparation of meat products for improving nutritional value (Chiu and Gioielli, 2002).

Chicken SK, in turn, is considered a source rich in proteins and lipids, where approximately 20% of fatty acids are unsaturated (Dalziel et al., 2015). Research has confirmed the antioxidant bioactivity of chicken SK peptides obtained through enzymatic hydrolysis (Onuh et al., 2014), which also makes it an interesting ingredient for the development of meat products.

A patty is a meat product that is popularly known and consumed by almost all countries, mainly because of its practicality and convenience for the consumer. Normally, patties are not defined as emulsified products; however, the Technical Regulation of Identity and Quality of Hamburgers (Brasil, 2000) does not restrict its classification according to the degree of meat comminution used, which makes it possible to use processing techniques such as emulsion, for example.

Meat emulsion is a system composed of 2 or more immiscible phases, such as oil-water. It is a technique that can be applied to make the product as homogeneous as possible, in addition to adding value to the final product because it is considered a sophisticated technique (Jiang and Xiong, 2015).

Normative Instruction no. 20, from July 31, 2000 (Brasil, 2000), does not define the origin of fat that can be used in the preparation of patties; thus, the processing

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industries generally use the SK derived from the slaughter of poultry. However, the Ministry of Agriculture, Livestock, and Food Supply suggests the development of studies with replacement of chicken SK with AF, given the observed degree of microbiological contamination of SK and of oxidation processes because of the predominance of unsaturated fatty acids, which are higher in poultry than in other animal sources such as ox meat and mutton meat (Mottram et al., 2001).

Oxidative processes are responsible for the loss of quality in meats and meat products (Wang et al., 2018). Lipid oxidation occurs through free radical reactions that, in search of stability, attack the unsaturated bonds of fatty acids and can form different products that lead to changes in color, aroma, and texture of the food item (Falowo et al., 2014). Free radicals can also attack proteins, triggering oxidation with the formation of distinct products responsible for changing important parameters in meat quality, including changes in the nutritional value resulting from the loss of essential amino acids and decreased protein digestibility (Ferreira et al., 2018).

Given the above, the objective of this study was to evaluate the effect of the addition of SK and AF on the lipid and protein oxidation of emulsified patties stored under freezing conditions (-18°C) for 90 D.

MATERIALS AND METHODS

Experimental Design and Materials

The effect of fat type (SK or AF) on the chemical characteristics and oxidative stability of emulsified chicken patties was evaluated using a completely randomized design under a 2×4 factorial arrangement, analyzing the effects of the source of fat at 4 storage times (0, 30, 60, and 90 D).

Breasts, SK, and AF were obtained from commercial Cobb broilers, male and female, with an age at slaughter of between 42 and 48 D, in a slaughterhouse certified by the Federal Inspection Seal in the state of Paraíba, Brazil. The other ingredients used to prepare the emulsified products were purchased from the local market of the city of João Pessoa, state of Paraíba, Brazil.

The raw materials were collected from the commercial processing line of the slaughterhouses. Breasts were obtained after deboning and cutting, SK was obtained after removal from the carcass, and AF was collected from the abdominal cavity after the carcass cooling stage. After collection, the raw materials were stored at 4°C for 24 h and frozen at -18°C for 48 h until the emulsified products were prepared.

Preparation of Emulsified Chicken Patties

Two emulsified patty formulations were processed as described in Table 1. Initially, the chicken breasts were ground in an industrial grinder with a 6-mm disc (Model MC 160; Ibrasmak, São Paulo, Brazil). Subsequently, the other ingredients were added according to the

Table 1. Emulsified chicken patties formulations elaborated with chicken skin and abdominal fat.

Raw material (%)	Formulations	
	PSK	PAF
Chicken breast	70.0	70.0
Chicken skin	15.0	-
Chicken abdominal fat	-	15.0
Cold water	15.0	15.0
Ingredients (g/kg)		
Onion dried	40.0	40.0
Garlic dried	20.0	20.0
Sodium chloride	20.0	20.0
Cumin	10.0	10.0
Chili dried	1.00	1.00
Glutamate monosodium	1.00	1.00

Abbreviations: PAF, emulsified chicken patties with 15% abdominal fat; PSK, emulsified chicken patties with 15% skin.

formulations, and to obtain a homogeneous product, the mixture was emulsified in a cutter (Modelo 90,510; G. Paniz, Caxias do Sul, Brazil) for approximately 6 min, not allowing the meat mass to exceed 12°C . Next, the emulsified patties were molded, using approximately 100 g of meat, in a plastic mold (10 cm diameter) and placed in styrofoam trays covered with low-density polyethylene film. The products were frozen (-18°C) in a commercial freezer, and the analyses were performed at 30-day intervals totaling 90 D of storage.

Fatty Acids Profile of the Chicken SK, AF, and Emulsified Chicken Patties

The total lipids present in SK, AF, and emulsified chicken patties were extracted using the method proposed by Folch, et al. (1957). Fatty acid esters were obtained to determine the fatty acid profile according to the method described by Hartman and Lago (1973), using a 7-mL aliquot with a concentration of 0.5 g/mL fat. Identification and quantification of fatty acid esters was performed using a gas chromatograph (VARIAN 430—GC, Walnut Creek, CA) coupled with a flame ionization detector and fused silica capillary column (SPTM—2560; Supelco, Bellefonte, PA), $100\text{ m} \times 0.25\text{ mm}$, with a $0.20\text{-}\mu\text{m}$ film thickness. Helium was used as the carrier gas at a flow rate of 1 mL/min. The following conditions were used: injector temperature 240°C ; initial oven temperature 100°C , increasing at a rate of $2.5^{\circ}\text{C min}^{-1}$ until reaching 245°C , which was maintained for 30 min, with a total run time of 88 min; and detector temperature 250°C . The following were the flow rates for the auxiliary gases: helium 25 mL min^{-1} , hydrogen 30 mL min^{-1} , and synthetic air 300 mL min^{-1} . Aliquots of 1.0 μL of the esterified extract were injected in a split/splitless injector (split 1:100). The chromatograms were recorded using Galaxie Chromatography Data System software. To identify the fatty acids, the retention times of the methyl esters of the samples were compared with Supelco ME19-Kit (Fatty Acid Methyl Esters C6 - C24) standards. The results were expressed as percent area (%). The atherogenicity

Table 2. Characterization total lipids of the chicken skin and abdominal fat.

Parameter	SK	AF	P-value
Lipids ¹	39.42 ± 0.32	71.80 ± 0.50	<0.001
Fatty acids ²			
Saturated			
C12:0	-	0.02 ± 0.00	
C14:0	1.46 ± 0.03	1.06 ± 0.05	<0.001
C15:0	0.05 ± 0.01	0.06 ± 0.01	0.295
C16:0	19.66 ± 0.12	20.96 ± 0.18	<0.001
C17:0	0.19 ± 0.01	0.20 ± 0.03	0.323
C18:0	5.61 ± 0.04	5.88 ± 0.04	<0.001
C20:0	0.18 ± 0.01	0.17 ± 0.08	0.855
C22:0	0.21 ± 0.02	0.19 ± 0.00	0.082
C24:0	0.08 ± 0.02	0.03 ± 0.01	0.002
Σ SFA	27.43 ± 0.04	28.56 ± 0.15	<0.001
Monounsaturated			
C14:1n5	0.06 ± 0.01	0.09 ± 0.01	0.019
C15:1n5	0.01 ± 0.00	0.02 ± 0.01	0.057
C16:1n7	4.18 ± 0.07	4.47 ± 0.08	0.002
C17:1n7	0.10 ± 0.01	0.21 ± 0.02	<0.001
C18:1n9	34.29 ± 0.11	34.79 ± 0.17	0.003
C20:1n9	0.32 ± 0.02	0.29 ± 0.08	0.586
C22:1n9	0.20 ± 0.01	0.14 ± 0.01	<0.001
C24:1n9	0.07 ± 0.00	0.02 ± 0.01	<0.001
Σ MUFA	39.24 ± 0.08	40.03 ± 0.07	<0.001
Polyunsaturated			
C18:2n6c	30.11 ± 0.10	28.47 ± 0.09	<0.001
C18:3n6	2.66 ± 0.04	2.61 ± 0.07	0.308
C20:2	0.02 ± 0.00	0.02 ± 0.01	0.849
C20:3n6	0.02 ± 0.00	0.01 ± 0.00	0.205
C20:3n3	0.03 ± 0.00	0.01 ± 0.00	0.003
C20:4n6	0.37 ± 0.02	0.14 ± 0.01	<0.001
C20:5	-	0.01 ± 0.00	
C22:2	0.02 ± 0.01	0.02 ± 0.01	0.814
C22:6n3	0.12 ± 0.02	0.10 ± 0.02	0.135
Σ PUFA	33.34 ± 0.05	31.40 ± 0.13	<0.001
PUFA/SFA	1.22 ± 0.00	1.10 ± 0.01	<0.001
AI	0.32 ± 0.00	0.32 ± 0.00	0.817
TI	0.71 ± 0.00	0.76 ± 0.01	<0.001

P < 0.05 indicate that there is a significant difference between formulations.

Abbreviations: AF, abdominal fat; AI, atherogenicity index; MUFA, monounsaturated fatty acid; PAF, emulsified chicken patties with 15% abdominal fat; PSK, emulsified chicken patties with 15% skin; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SK, skin; TI, thrombogenicity index.

¹Data expressed as g/100 g sample.

²Data expressed as percentage of area (%).

index (AI) and thrombogenicity index (TI) were calculated as described by Ulbricht and Southgate (1991).

Determination of the Partial Chemical Composition

The moisture, ash, total protein, collagen, and pH were determined according to the methodologies of Association of Official Analytical Chemists (2000) described in items n° 950.46.41, 920.153, 928.08, 990.26, and 947.05, respectively. Total fat was quantified using the method proposed by Folch et al. (1957). The total collagen content was quantified by measuring the hydroxyproline concentration, according to Association of Official Analytical Chemists procedure 990.26 (2000). The hydroxyproline concentration was calculated from a standard curve of hydroxyproline with concentrations ranging from 0.6 to 3.6 µg/mL. Total collagen content was calculated by multiplying the

hydroxyproline level by the conversion factor of 8.0; the final result was expressed as grams of total collagen per 100 g of sample.

Evaluation of the Oxidative Stability of Emulsified Chicken Patties

Peroxide Value The peroxide value (PV) was determined according to the method described by Carvalho et al. (2002). A 15-mL aliquot of the lipid extract obtained by the method of Folch et al. (1957) was added to 22.5 mL of pure acetic acid and 0.75 mL of saturated KI solution. After leaving it to stand in the dark, 22.5 mL of distilled water and 3 mL of 1% starch solution were added to the initial mixture. The procedure ended with titration of the mixture with 0.01 N sodium thio-sulphate. The PV was expressed in mEqO₂/kg.

Para-anisidine Value The para-anisidine value (pAV) was determined according to the method adapted from IUPAC (1987). Approximately 0.5 g of fat present in the lipid fractions obtained by the method of Folch et al. (1957) was diluted in iso-octane, and the absorbance was measured at 350 nm (A₁). Then, a 1-mL aliquot of para-anisidine (2.5 mg/mL) was added to the mixture (sample + iso-octane), followed by homogenization and then allowed to react in the dark for 10 min. A second absorbance reading (A₂) was performed at 350 nm. The final result was calculated from the equation pAV = [(1.2*(A₂-A₁)/m)]*25, where "m" is the lipid mass.

Thiobarbituric Acid Reactive Substances The thiobarbituric acid reactive substances concentration was determined according to the method described by Rosmini et al. (1996) with modifications. The results were expressed as mg MDA/kg of sample and calculated using a standard curve of 1,1,3,3-tetramethoxypropane with concentrations ranging from 0.2 to 2.6 µg/mL.

Quantification of Total Carbonyl Compounds

Carbonyl compounds were quantified according to the modified methodology of Oliver et al. (1987). The amount of total carbonyls was expressed in nmoles of carbonyls per mg of protein from readings at 370 nm. Protein was quantified with the aid of a standard albumin curve (0.1 mg/mL to 1 mg/mL) from readings at 280 nm.

Analysis of Volatile Compounds Volatiles were extracted by solid-phase microextraction (SPME) with an SPME device (Supelco). The fiber used was 65-µm polydimethylsiloxane/divinylbenzene, activated according to the manufacturer's recommendations (250°C/30 min). Approximately 2 g of the sample of the cooked and ground emulsified patties was placed in a 20-mL hermetically sealed glass vial with a screw-cap containing a Teflon-lined septum. After reaching equilibrium (60°C/5 min), the fiber was exposed to the headspace for 60 min for extraction. After this period, the SPME device was moved from the sample vial and inserted directly into the injection port of the mass spectrometer (Agilent Technologies 5977B, Little Falls, DE) coupled to a 7890B gas chromatographer, responsible for separating and

identifying the volatiles collected by the SPME. The following conditions were used: initial oven temperature 40°C/2 min, increasing at 4°C min⁻¹ until 280°C, and maintained for 10 min, totaling 72 min of run time. The injector temperature was set at 250°C. Helium was used as the carrier gas at a flow rate of 1.0 mL/min in the 1:10 split injection system. The transfer line temperature was 170°C. The mass spectrometer was operated in the electron impact mode (70 eV), and the mass scanning range was from 35 to 350 AMU at a sampling rate of 3.33 scans/s. The compounds were identified by analyzing the fragmentation patterns in the mass spectra, which was confirmed by comparing their mass spectra with those in the database provided by the National Institute of Standards & Technology, USA, as well as their linear retention indices with those of known compounds. The results were expressed as percent area (%).

Sensory Analysis In the sensory analysis, the color and aroma parameters of the products were evaluated during the 90 D of storage by approximately 70 untrained judges who reported regularly consuming patties. A hedonic scale of 9 points was used, ranging from disliked it very much (1) to liked it very much (9). The emulsified chicken patties were cooked on a preheated (175°C) hotplate (CKSTGR 3007, Oster, China) for approximately 6 min, that is, 3 min on each side until reaching an internal temperature of 75°C. To evaluate color, cylindrical samples (2.5 cm in diameter and 1.0 cm in height) were obtained from each emulsified patty and served in 50-mL plastic cups. For aroma, approximately 5 g of the cooked and ground samples were placed in 50-mL Falcon tubes sealed and wrapped in aluminum foil according to the method described by Ferreira et al. (2016). The samples were coded with 3 random numbers.

Instrumental Color of Emulsified Chicken Patties

The instrumental color was determined by reading the parameters L* (lightness), a* (redness) and b* (yellowness) in 6 different portions of the emulsified product using a Minolta digital colorimeter (Model CR Miner, Mahwah/NJ). Before the readings, the instrument was calibrated. The total colorimetric difference (ΔE) was calculated using the equation $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. The hue angle was calculated using the equation $\text{Hue} = \arctan(b^*/a^*)$.

Texture Profile Analysis and Shear Force

The texture profile was determined using a TA-XT2i texturometer (Stable Micro Systems, Surrey, UK) equipped with a P/35 cylindrical probe. The samples were obtained using a cylindrical cutter (2.5 cm diameter \times 1.0 cm height). The evaluated parameters were hardness (N), gumminess (N), chewiness (N), elasticity (dimensionless), cohesiveness (dimensionless), and resilience (dimensionless), according to the methodology proposed by Bourne (2002). The following conditions were used: pretest speed 2.0 mm/s; test speed 2.0 mm/s; post-test speed 5.0 mm/s;

compression distance 4 mm; and trigger force: 5 g. For shear force (SF), the samples were cut in a rectangular shape (2.5 cm long \times 1.0 cm height \times 1.0 cm wide) and analyzed using a TA-XT2i texturometer (Stable Micro Systems) equipped with a Warner Bratzler blade operating at a speed of 10 mm/s, penetration depth of 20 mm and contact force of 5 g. The texture profile analysis (TPA) and SF data were analyzed using Texture Expert software for Windows 1.20 (Stable Micro Systems \ TE32 L \ \ version 6.1.4.0 England).

Statistical Analysis

Data were processed using IBM SPSS Statistics 2.3 software. For the SK and AF lipid profile and characterization of the emulsified patties, Student *t* test was used. The time effect was evaluated using 2-way ANOVA, with the lipid source and storage time being the variables established as fixed factors. Means were then compared by Tukey's test at the 5% significance level.

RESULTS AND DISCUSSION

Lipid Profile of the Chicken SK and AF

Abdominal fat had a lipid content approximately 45% higher than that of SK, as shown in Table 2. The fat concentration obtained for SK is consistent with the results reported by Feddern et al. (2010). No studies of AF lipid content were found; however, the observed results are

Table 3. Characterization (mean \pm standard deviation) of emulsified chicken patties elaborated with chicken skin and abdominal fat.

Parameter	Formulations		<i>P</i> -value
	PSK	PAF	
Physicochemical composition			
Moisture ¹	70.28 \pm 1.17	64.64 \pm 0.26	<0.001
Ash ¹	2.54 \pm 0.30	2.12 \pm 0.70	0.311
Protein ¹	16.01 \pm 0.26	15.68 \pm 0.49	0.282
Lipids ¹	5.76 \pm 0.38	11.26 \pm 0.51	<0.001
Collagen ¹	0.48 \pm 0.01	0.17 \pm 0.00	<0.001
pH	6.06 \pm 0.01	6.05 \pm 0.01	0.114
Instrumental color			
L*	59.39 \pm 0.17	60.42 \pm 0.42	0.001
A*	1.59 \pm 0.04	1.58 \pm 0.07	0.806
B*	19.58 \pm 0.35	19.80 \pm 0.20	0.224
Hue	85.35 \pm 0.01	85.43 \pm 0.07	0.329
ΔE	1.07 \pm 0.22		
Texture profile			
Hardness ²	32.16 \pm 5.41	18.86 \pm 4.39	<0.001
Springiness ³	0.88 \pm 0.03	0.85 \pm 0.03	0.202
Cohesiveness ³	0.81 \pm 0.01	0.81 \pm 0.04	0.609
Gumminess ²	25.99 \pm 4.64	11.95 \pm 1.32	<0.001
Chewiness ²	23.69 \pm 4.12	10.21 \pm 1.09	<0.001
Resilience ³	0.38 \pm 0.01	0.35 \pm 0.02	0.020

ΔE : total colorimetric difference.

P < 0.05 indicate that there is a significant difference between formulations.

Abbreviations: PAF, emulsified chicken patties with 15% abdominal fat; PSK, emulsified chicken patties with 15% skin.

¹Data expressed as g/100 g sample.

²Data expressed as Newton (N).

³Dimensionless parameters.

Table 4. Fatty acids profile and nutritional quality index of emulsified chicken patties elaborated with chicken skin and abdominal fat stored under freezing (-18°C) during 90 D.

Fatty acid ¹	Time (days)	Formulations				
		PSK	PAF	F	T	FxT
Saturated						
C14:0	0	6.02 ± 1.81 ^{a,A}	2.27 ± 0.26 ^{b,A}	0.002	0.001	0.023
	90	2.13 ± 1.01 ^{a,B}	1.38 ± 0.25 ^{a,B}			
C16:0	0	20.15 ± 1.82 ^{a,A}	20.69 ± 0.57 ^{a,A}	0.945	0.415	0.359
	90	20.21 ± 0.26 ^{a,A}	19.74 ± 0.22 ^{a,A}			
C18:0	0	5.45 ± 0.32 ^{a,B}	5.93 ± 0.20 ^{a,A}	0.093	0.013	0.012
	90	7.94 ± 1.47 ^{a,A}	5.92 ± 0.22 ^{a,A}			
C24:0	0	0.34 ± 0.08	-			
	90	-	-			
Σ SFA	0	31.96 ± 0.41 ^{a,A}	28.90 ± 1.00 ^{b,A}	<0.001	0.001	0.824
	90	30.28 ± 0.80 ^{a,B}	27.05 ± 0.60 ^{b,B}			
Monounsaturated						
C16:1n7	0	3.77 ± 0.24 ^{a,A}	3.14 ± 0.35 ^{b,A}	0.045	<0.001	0.051
	90	2.50 ± 0.15 ^{b,B}	2.49 ± 0.33 ^{b,B}			
C17:1n7c	0	-	-			
	90	-	0.11 ± 0.04			
C18:1n9c	0	35.12 ± 0.46 ^{a,A}	36.16 ± 1.22 ^{a,B}	0.007	0.001	0.209
	90	36.97 ± 0.84 ^{b,A}	39.41 ± 1.45 ^{a,A}			
C22:1n9	0	-	-	0.014	<0.001	0.014
	90	0.33 ± 0.15 ^a	0.10 ± 0.03 ^b			
Σ MUFA	0	38.89 ± 0.61 ^{a,B}	39.29 ± 1.56 ^{a,B}	0.040	0.008	0.129
	90	39.80 ± 0.68 ^{a,A}	42.10 ± 1.38 ^{a,A}			
Polyunsaturated						
C18:2n6c	0	25.71 ± 0.94 ^{b,B}	29.89 ± 2.12 ^{a,A}	0.012	0.035	0.029
	90	28.96 ± 1.40 ^{a,A}	29.33 ± 0.82 ^{a,A}			
C18:3n6	0	1.47 ± 0.39 ^{a,A}	1.74 ± 0.54 ^{a,A}	0.178	0.229	0.630
	90	0.96 ± 0.66 ^{a,A}	1.51 ± 0.57 ^{a,A}			
C20:2	0	0.54 ± 0.33	-			
	90	-	-			
C20:4n6c	0	0.61 ± 0.25 ^a	0.17 ± 0.13 ^b	0.015	<0.001	0.015
	90	-	-			
C22:2	0	0.26 ± 0.13	-			
	90	-	-			
C22:6n3	0	0.56 ± 0.15	-			
	90	-	-			
Σ PUFA	0	29.15 ± 0.90 ^{a,A}	31.80 ± 2.53 ^{a,A}	0.054	0.913	0.319
	90	29.92 ± 1.24 ^{a,A}	30.85 ± 0.87 ^{a,A}			
PUFA/SFA	0	0.91 ± 0.04 ^{b,A}	1.10 ± 0.13 ^{a,A}	0.001	0.185	0.628
	90	0.99 ± 0.07 ^{b,A}	1.14 ± 0.02 ^{a,A}			
AI	0	0.66 ± 0.08 ^{a,A}	0.42 ± 0.03 ^{b,A}	<0.001	<0.001	0.009
	90	0.41 ± 0.06 ^{a,B}	0.35 ± 0.02 ^{a,B}			
TI	0	0.90 ± 0.02 ^{a,A}	0.81 ± 0.04 ^{b,A}	<0.001	0.008	0.006
	90	0.87 ± 0.03 ^{a,A}	0.74 ± 0.02 ^{b,B}			

Means with different lowercase letters for the same line were significantly different between formulations ($P < 0.05$). Means with different upper case letters for the same column were significantly different between times ($P < 0.05$).

F: P -value for formulation; T: P -value for time; F x T: P -value for interaction between formulation and time.

Abbreviations: AI, atherogenicity index; MUFA, monounsaturated fatty acid; PAF, emulsified chicken patties with 15% abdominal fat; PSK, emulsified chicken patties with 15% skin; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TI, thrombogenicity index.

¹Data expressed as percentage (%).

expected because there is a greater accumulation of fat in subcutaneous tissues and around viscera, as is the case for AF (Fagundes et al., 2017).

The fatty acid profiles for SK and AF were similar (Table 2). Saturated fatty acids (SFA) were less abundant in both SK and AF, with lauric acid (C12:0) detected only in AF. Monounsaturated fatty acids (MUFA) were more abundant in the 2 by-products, especially oleic acid (C18:1). Polyunsaturated fatty acids, mainly linoleic acid (C18:2), were also abundant in SK and AF, with SK exhibiting a higher content (30.11%) than AF (28.47%).

The total unsaturated fatty acid content was above 65% for the 2 by-products, demonstrating agreement with the study by Feddern et al. (2010), which reported 69.6% unsaturated fatty acids in chicken SK. However, SK showed a higher PUFA content, which induces oxidation reactions and justifies SK substitution with AF in meat products.

Regarding nutritional quality indicators, there was no significant difference for AI (Table 2). However, AF presented a higher TI (0.76) than did SK (0.71). The results obtained for the AI and TI agree with what was observed by Feddern et al. (2010) in chicken SK. Furthermore, the

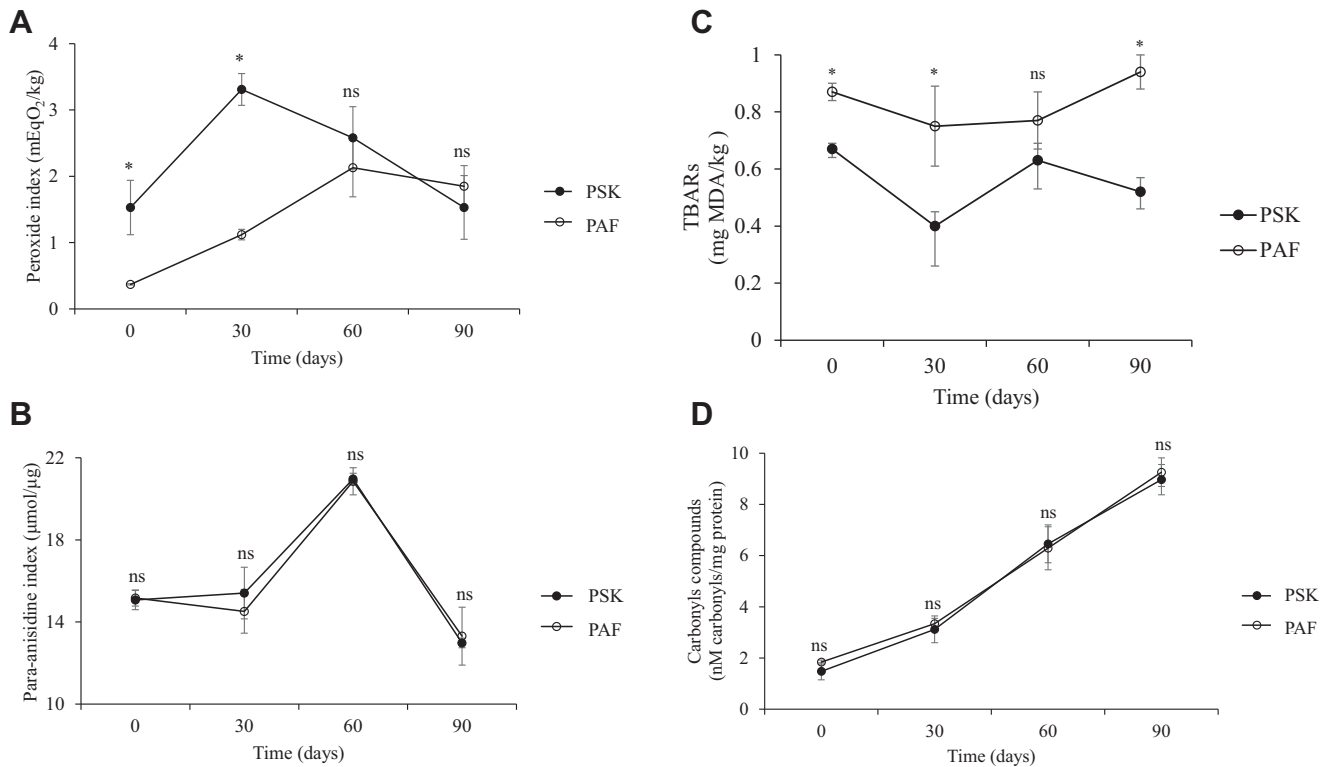


Figure 1. Evolution of (A) peroxide values, (B) TBAR, (C) pAV and (D) carbonyl compounds of emulsified chicken patties processed with chicken skin and abdominal fat and stored under freezing conditions (-18°C) for 90 D. Abbreviations: PSK, emulsified chicken patties with 15% skin; PAF, emulsified chicken patties with 15% abdominal fat; pAV, para-anisidine value; TABR, thiobarbituric acid reactive substances. * denotes a significant difference ($P < 0.05$) between formulations. ns denotes no significant difference ($P > 0.05$) between formulations.

AI and TI for the 2 treatments did not exceed the acceptable levels of 0.5 for the AI and 1.00 for the TI (Ulbricht and Southgate, 1991).

Characterization of Emulsified Chicken Patties

The physicochemical composition of the emulsified patties is provided in Table 3. There was no effect of the fat source used in the processing of emulsified products for the parameters protein, pH, a^* , b^* , and hue ($P > 0.05$). However, moisture content, lipid content, collagen, and L^* varied according to the treatment ($P < 0.05$).

The emulsified chicken patties with 15% SK (PSK) formulation had a higher moisture (70.28 g/100 g) and collagen (0.48 g/100 g) content than did the emulsified chicken patties with 15% AF (PAF) formulation because of the composition of SK, which contains more water and structural proteins. Regarding total fat, the PAF formulation was almost double that obtained for the PSK formulation. This result was expected given the values found for SK and AF. Regarding lightness (L^*), the PAF formulation was lighter when compared with the PSK formulation, which may be explained by the greater lightness observed in AF. Regarding color, the total colorimetric difference (ΔE) between formulations was 1.07 (Table 3), a value between 0.5-1.5, indicating that visually, and there was no difference in color between

the products according to the scale proposed by Cruz-Romero et al. (2007).

In the TPA of the emulsified patties (Table 3), the lipid source influenced the parameters hardness, gumminess, chewiness, and resilience ($P < 0.05$). For hardness, gumminess, and chewiness, the PSK formulation showed twice the values observed for the PAF formulation, indicating that the SK promoted a product that required greater force during chewing so that it disintegrates to the point of being swallowed. This characteristic was also observed by Fagundes et al. (2017) when evaluating the substitution of pork fat with a mixture of pork SK and canola oil in patties. The authors observed that the use of pig SK increased the aforementioned parameters and suggested that the results were because of the presence of collagen in SK, which is responsible for the formation of a resistant gel.

The fatty acid profile at the beginning and end of storage (times 0 and 90) are shown in Table 4. The fatty acids found in higher amounts were the same for SK and AF (oleic, linoleic, and palmitic acids), reflecting the behavior of the lipid source used for processing the emulsified product. During the 90 D of storage, the type of fat used did not influence the PUFA content, indicating that there were few oxidative reactions in the products, which are factors that contribute to the reduction in PUFA contents. Regarding SFAs and MUFAs in the samples, there was an effect of fat type and time. The SFA content decreased during storage,

Table 5. Volatile compounds (% area) detected in emulsified chicken patties elaborated with chicken skin and abdominal fat stored under freezing (-18°C) during 90 D.

Volatile compounds (% area)	Time (days)	Formulations	
		PSK	PAF
Acids¹			
Butyric acid	0	0.81	0.86
	90	-	-
Aldehydes¹			
3-methyl butanal	0	0.82	1.51
	90	-	-
Pentanal	0	-	1.03
	90	-	-
Hexanal	0	1.11	1.76
	90	-	-
Cuminal	0	9.73	10.57
	90	27.19	27.59
Total aldehydes	0	11.66	14.87
	90	27.19	27.59
Sulphur compounds¹			
Methyl-mercaptan	0	1.10	0.81
	90	-	-
2-propenethiol	0	6.37	10.35
	90	-	0.33
Propyl-mercaptan	0	4.46	4.42
	90	-	-
Allyl sulfide	0	0.96	0.72
	90	-	-
3,4-dimethyl-tiopheno	0	0.65	0.56
	90	-	-
Methyl-propenyl-disulfide	0	1.44	0.77
	90	-	-
Total sulphur compounds	0	14.99	17.62
	90	0.00	0.33
Terpenes¹			
α -thujene	0	2.38	2.16
	90	0.78	0.82
β -pinene	0	51.76	42.87
	90	18.88	19.75
β -myrcene	0	-	0.45
	90	0.74	0.70
3-carene	0	-	-
	90	1.88	2.01
o-cymene	0	3.45	3.28
	90	17.47	15.68
Limonene	0	0.66	1.61
	90	3.92	4.41
γ -terpinene	0	10.62	10.55
	90	26.57	24.53
α -terpinen-7-al	0	1.73	0.56
	90	0.90	1.17
γ -terpinen-7-al	0	0.90	4.45
	90	0.59	1.96
4-thujanol	0	1.03	0.72
	90	1.08	1.06
	90	-	-
Iso-cariofileno	0	-	-
	90	0.27	-
Total Terpenes	0	72.53	66.65
	90	72.81	72.08

Abbreviations: PSK, emulsified chicken patties with 15% skin; PAF, emulsified chicken patties with 15% abdominal fat.

¹Data expressed as percentage (%).

mainly because of the reduction in myristic acid, and the PSK formulation showed the highest proportion at 90 D (30.28%), contrary to what was observed for MUFAs, which were present in higher quantities in the PAF formulation (42.10%). These results can be explained by what Liu et al. (2018) observed in their experiments. The researchers evaluated the oxidative stability of saturated and unsaturated lard fractions and observed that

the unsaturated fraction exhibited greater lipid oxidation stability than did the saturated fraction.

Regarding the AI and TI (Table 4), the PSK formulation showed higher results for the 2 index, indicating that this formulation has higher levels of atherogenic and thrombogenic fatty acids (Ulbricht and Southgate, 1991). The decrease in the index over the storage period is because of reduced SFA content and increased MUFA content, as discussed above.

Oxidative Stability of Emulsified Chicken Patties

The results for lipid oxidation analyzed through the formation of peroxides and aldehydes are provided in Figure 1. For the PV (Figure 1A), the PSK formulation accumulated a greater amount of peroxides at 30 D, followed by a decrease until the end of storage, which probably occurred because of the degradation of hydroperoxides into secondary oxidation products (Guyon et al., 2016). For the PAF formulation, the greatest accumulation of hydroperoxides was observed after the 60th day of storage, remaining the same until day 90 ($P > 0.05$). This difference in behavior may be explained by the different fatty acid profiles of SK and AF, as SK had a higher PUFA content than did AF (Table 2), which may contribute to the early occurrence of lipid oxidation reactions. Notably, despite the aforementioned behavior, the amount of peroxides formed in the PSK and PAF formulations was statistically equal at the end of storage ($P > 0.05$) and did not reach the rancidity threshold of 10 mEqO₂/kg (Cagdas and Kumcuoglu, 2014).

Regarding the formation of the secondary aldehyde compounds quantified by pAV analysis, there was no difference between the formulations ($P > 0.05$) (Figure 1B). The highest aldehyde formation in the samples was observed after 60 D of storage, which agrees with the observation for hydroperoxide formation previously discussed, where this same period marked a decrease in the primary compound of lipid oxidation, indicating that these compounds were decomposed into secondary products such as aldehydes.

For the formation of malondialdehyde (Figure 1C), the use of SK or AF had an effect until the 60th day, when the formation in the formulations was statistically equal ($P > 0.05$), corroborating the results observed in the pAV analysis. The PAF formulation showed the highest MDA values throughout the entire storage period; however, the 2 treatments remained statistically equal from the beginning to the end of the storage period, demonstrating good oxidative stability of the processed product and indicating that the use of SK or AF does not induce high oxidative stress in the lipids present. A similar behavior was observed by De Carli et al. (2018) in chicken patties refrigerated for up to 24 wk. Furthermore, the quantification of MDA in emulsified patties did not reach the rancidity threshold of

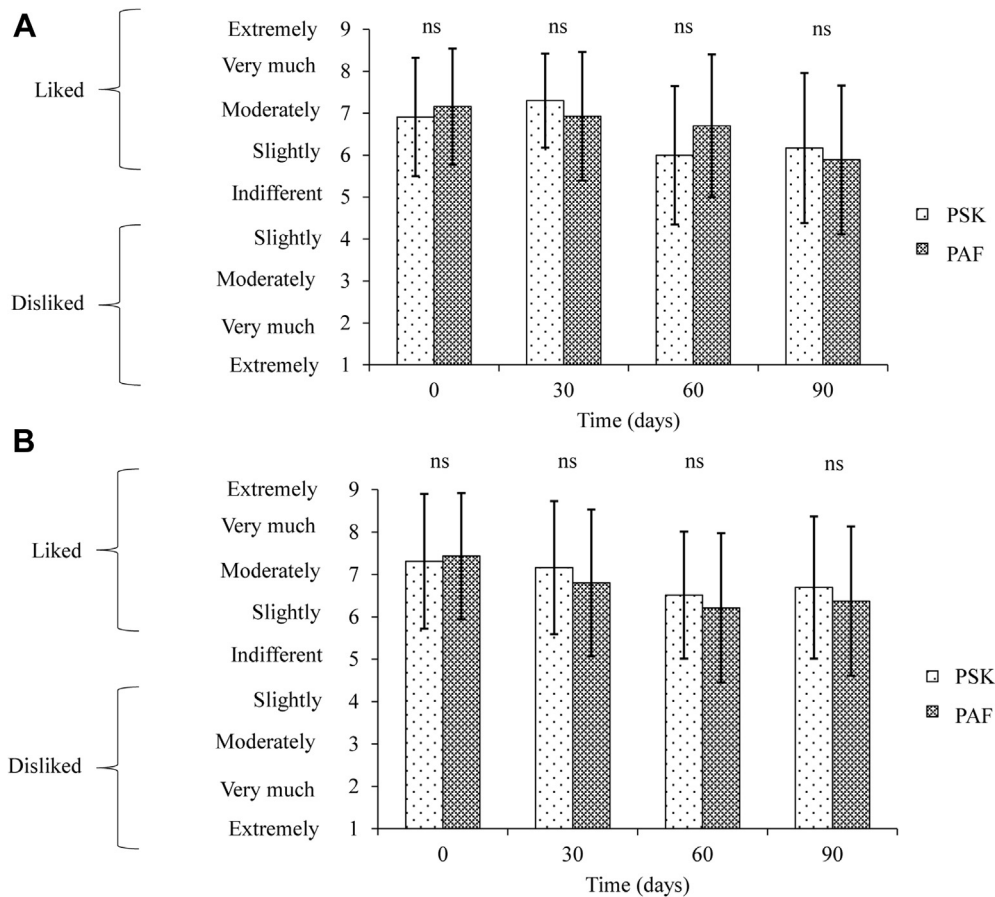


Figure 2. (A) Color and (B) flavor of emulsified chicken patties processed with chicken skin and abdominal fat and stored under freezing conditions (-18°C) for 90 D. Abbreviations: PSK, emulsified chicken patties with 15% skin; PAF, emulsified chicken patties with 15% abdominal fat. ns on top of bars denotes no significant difference ($P > 0.05$) between formulations and time of storage.

2 mg MDA/kg (Campo et al., 2006) in any period evaluated.

The results for protein oxidation can be observed in Figure 1D. There was a significant increase in total carbonyl compounds over the 90 D, with higher accumulation at the end of storage for both formulations. A similar behavior was observed by De Carli et al. (2018), who detected up to 5.59 nmol/mg of protein in chicken patties. Although a significant difference was observed during storage in the formation of carbonyl compounds, it was not possible to detect an effect of the use of SK or AF on protein oxidation ($P > 0.05$).

A total of 22 volatile compounds were identified in the emulsified chicken patties, which are presented in Table 5. Such compounds include 1 acid, 6 aldehydes, 6 sulfur compounds, and 9 terpenes. The only acid detected in both samples was butyric acid, which was only observed at the beginning of storage.

Aldehydes were detected in high amounts in the formulations. This group is seen as an indicator of lipid oxidation, especially pentanal and hexanal compounds (Sohaib et al., 2017), which were identified in the emulsified products in low quantities when compared with cuminaldehyde, which is characteristic of cumin (*Cuminum cyminum* L.). This result confirms the data previously discussed in regard to the low oxidation levels of the processed products.

Volatile sulfur compounds were found in significant quantities only at the beginning of storage for both formulations, which was expected because the volatile sulfur compounds have high volatility and are rapidly released from the product (Nam et al., 2007). This result is in line with what was observed by Sohaib et al. (2017) in chicken patties stored for 7 D under refrigeration.

Terpenes were the most predominant volatiles in the formulations. According to Yang et al. (2018), terpenes are responsible for the aroma of meat products, because they are present in large proportions in various spices, such as pepper, which has a high content of β -pinene and limonene, and cumin (*C. cyminum* L.), which has high terpinene content (Sowbhagya et al., 2011).

The perception of consumers regarding the color and aroma of emulsified products (Figure 2) during storage corroborates the results obtained in the oxidation analysis. In general, there was no significant difference between the 2 formulations for any of the evaluated times, which is related to low levels of lipid oxidation and a similar volatile profile among the samples. In general, the scores assigned by the judges varied between “liked it slightly” (6) and “liked it moderately” (7), where the lowest scores were given at the end of storage, characterizing the beginning of the deterioration of the aroma, which may result in the development of warmed over flavor (Bailey and Um, 1992). These results are consistent

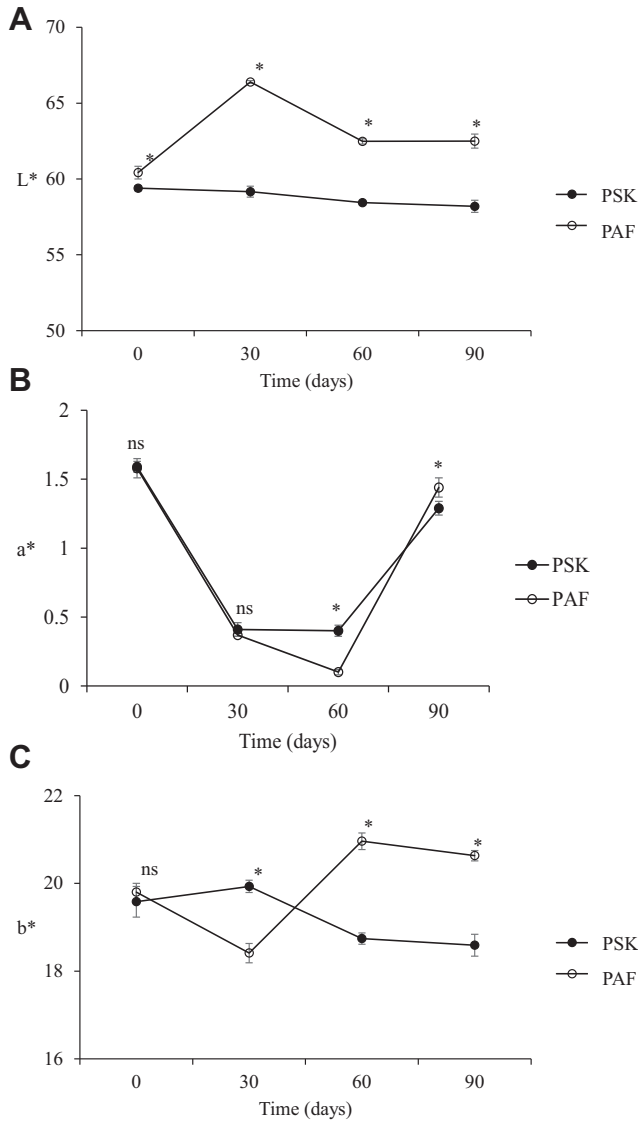


Figure 3. Evolution of (A) lightness, (B) redness and (C) yellowness in chicken patties processed with chicken skin and abdominal fat and stored under freezing conditions (-18°C) for 90 D. Abbreviations: PSK, emulsified chicken patties with 15% skin; PAF, emulsified chicken patties with 15% abdominal fat. * denotes a significant difference ($P < 0.05$) between formulations. ns denotes no significant difference ($P > 0.05$) between formulations.

with those observed by [Ferreira et al. \(2016\)](#) in chicken patties stored for 14 D under refrigeration.

Changes in Color, Hardness, and SF of Emulsified Chicken Patties During Storage

Figure 3 shows the color results (L^* , a^* , and b^*) of the emulsified chicken patties throughout storage. Regarding lightness (**Figure 3A**), the PAF formulation had a higher value, with a considerable increase at 30 D, followed by a decrease at the end of storage. For the PSK formulation, lightness showed a significant decrease between days 30 and 60, remaining constant until the end of storage, which may be related to oxidative processes.

The a^* values varied significantly between the formulations ($P < 0.05$) (**Figure 3B**). Both showed a statistically

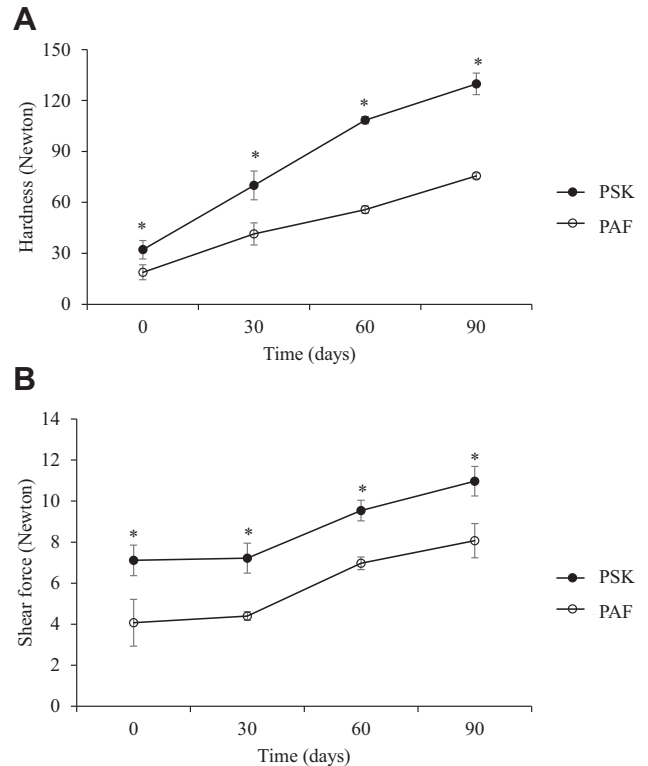


Figure 4. Evolution of hardness (A) and shear force (B) of emulsified chicken patties processed with chicken skin and abdominal fat and stored under freezing conditions (-18°C) for 90 D. Footnote: PSK, emulsified chicken patties with 15% skin; PAF, emulsified chicken patties with 15% abdominal fat. * denotes a significant difference ($P < 0.05$) between formulations. ns denotes no significant difference ($P > 0.05$) between formulations.

similar decrease until the 30th day of storage. Between days 30 and 60, the PSK formulation maintained constant values, while values for the PAF formulation continued decreasing until the 60th day. Then, the values for both formulations increased until the 90th day. Considering the beginning and end of storage, the greatest reduction was exhibited by the PSK formulation (18.87%) when compared with the PAF formulation (8.86%). In general, redness decreases during storage because of changes in the product caused by the activation of oxidative processes or changes in the pigments because of cold temperatures ([Ganhão et al., 2010](#); [Sohaib et al., 2017](#)).

For the b^* parameter, the formulations exhibited opposite trends (**Figure 3C**). After processing, while the values for the PSK formulation increased, those for the PAF formulation decreased, which may be because of the lipid source used because AF has a slightly yellowish color.

Regarding the SF and hardness (**Figure 4**), the formulations had similar results. For the 2 parameters, the values increased over the storage period, but the PSK formulation presented higher values at the end of storage. For this formulation, the SF and hardness showed increases of 35.19 and 75.21% throughout storage, respectively. The PAF formulation showed lower values for SF and hardness, which indicates that the SK used in the preparation of the emulsified patties promoted

greater hardness of the product, which required greater force to be cut. This result is related to the collagen content present in SK and is in line with what was observed by Sousa et al. (2017) in chicken sausages made with collagen powder as a fat substitute.

CONCLUSION

The use of SK or AF in the preparation of emulsified chicken patties had no great influences on the lipid and protein oxidation rate when the products were stored under freezing conditions (-18°C) for a period of 90 D, indicating that the use of these by-products in meat products is feasible as a result of their oxidative stability. However, further analysis such as free thiols and disulfide bonds are required to validate protein oxidation results. When considering the results obtained for the TPA and SF, the use of AF, which has greater tenderness and succulence and lower chewiness, is indicated. In addition, further studies assessing a combination of SK and AF are needed.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found <https://doi.org/10.1016/j.psj.2019.11.027>.

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