Formulation of chicken sausages with broiler blood proteins and dye

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Abstract Dye and proteins recovered from broiler blood were used in the formulation of chicken sausages in substitution of soy proteins and synthetic dyes. Three formulations of chicken sausages were prepared: standard, liquid plasma, and freeze-dried plasma. The application of protein and blood dye in the formulation of sausages and sensory analysis followed the Brazilian legislation. Physicochemical, microbiological and sensory attributes of sausages were evaluated during 60. All the formulations met the physicochemical and microbiological requirements. Considering the sensory analysis, sausages prepared using freeze-dried plasma presented significant difference from the standard sausage. There was no significant difference in the acceptance between the standard and liquid plasma sausages. Therefore, the use of such byproducts can add value to meat products without any adverse effect consumers acceptability.

Keywords Broilers · Protein · Dye · Sausage · Physicochemical · Microbiological

Introduction

Blood of animals intended for human consumption are byproducts obtained in large volumes and often considered as a waste. However, the blood has a high potential for reutilization in the food industry, minimizing environmental risks or reducing its management costs. Protein and dye obtained from the blood of some animals are used in many countries for incorporation into food as a source of low-cost protein for human nutrition. Economic and ecologic advantages are the incentives for the use of blood and its derivatives as food ingredients (Hurtado et al. 2011; Roselino et al. 2017).

The use of blood proteins in the food industry is a promising alternative to valorize this byproduct.

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Hygienically collected blood is a cheap raw material that can be used as a food ingredient without being allergenic. Moreover, considering that blood is naturally found in meat, the introduction of blood derivatives in meat products would not be considered as an addition of an external ingredient, especially when it comes from the same animal species (e.g., porcine blood for pork meat products). According to Hurtado et al. (2011), plasma can be seen as an economical, healthy and ecological source of functional proteins without having allergenic substances, which allows improving texture and sensorial characteristics and, at the same time, allows increasing nutritional value of products. For example, the consumption of “Morcilla de Burgos” is quite popular in North of Spain, although the ingredients used in the formulation of these blood sausages differ according to the region (Santos et al. 2003).

The demands for high-quality food products are always increasing (Silva et al. 2018). The substitution of caloric substances, fats and synthetic dyes by the natural ones is at the forefront of investigation in food-related areas (Aslinash et al. 2018; Zabot et al. 2016). In meat processing, the main advantages of substitution of synthetic dyes and proteins are the increasing of the nutritive value of products (reduction of harmful synthetic dyes), the reduction of costs, and the reduction of the “off-flavor” caused by soy protein. However, some drawbacks for the expansion of this substitution are related to the characteristics of broiler byproducts (and other byproducts in meat processing industries), which can change depending on the slaughterhouses and on the processing technologies, thus influencing the physicochemical and microbiological characteristics of final products (Mir et al. 2017; Kopec et al. 2013; Hurtado et al. 2012; Youssef and Barbut 2011; Mandal et al. 2001). New technologies are being studied to ensure the quality and acceptance of the final products (Salehi and Kashaninejad 2017). For example, freeze-dried foods are products with high added-value because they maintain most of their original nutrients, with the advantage of being dehydrated materials.

Based on this brief context, the aim of this study was to recover proteins and dye from broilers blood and to apply them in sausage formulations as a replacer of soy protein and synthetic dyes. Liquid plasma and freeze-dried plasma have been used in the formulations. In this short communication, the responses evaluated were the content of lipids, protein, ash, moisture, and microbiological and sensory analyses.

Materials and methods

Blood sampling and anticoagulant addition

Blood broilers were collected from animals approved by the Brazilian Federal Inspection in an industrial processing chicken meat plant located in Erechim-Brazil. Blood samples were transported to the laboratory in refrigerated boxes 24 h after extraction to avoid variability due to storage time. The anticoagulant was mixed with the broiler blood immediately after its collection in the meat plant (Table 1). After its addition, none sign of coagulation was seen neither during storage nor during centrifugal fractionation. All the procedures for obtaining the sausages were carried out in the Meat Pilot Plant of the Regional Integrated University (URI-Erechim, Brazil).

Table 1 Preparation of anticoagulant and formulations of standard sausage, liquid plasma sausage, and freeze-dried plasma sausages

Ingredient		Proportion (g/kg broiler blood)		
Broiler blood		1000		
Salt of cure		3.2		
Fine salt		30		
Sodium tripolyphosphate		5		
Antioxidant		3.2		
Formulation (kg)	Standard sausage	Liquid plasma sausage	Freeze-dried plasma sausage	
Mechanically separated meat	1000	800	1000	
Water	68.5	0	68.5	
Salt (sodiumchloride)	31.9	31.9	31.9	
Sugar	4.5	4.5	4.5	
Cassava starch	24.3	24.3	24.3	
Texturedsoyprotein	30.4	30.4	30.4	
Isolatedsoyprotein	31	0	0	
Condiments	12	12	12	
Tripolyphosphate	4.2	4.2	4.2	
Antioxidant	5.7	5.7	5.7	
Salt of cure	4.2	4.2	4.2	
Syntheticdyes	1.5	0	0	
Blooddye	0	1.5	1.5	
Liquid plasma	0	300	0	
Freeze-dried plasma	0	0	31	

Blood fractionation and freeze-drying

For blood fractionation, the centrifugation was performed at 6500 rpm and 5 °C for 10 min (Thermo Scientific, SL 40/40R, USA). The fractions of plasma and hemoglobin were obtained, being approximately 90 wt% and 10 wt%, respectively. Liquid plasma consisted of samples of fractionated blood plasma in a liquid state.

For obtaining freeze-dried plasma, part of the fractionated liquid plasma was submitted to freezing at − 80 °C in an ultra-freezer (Sanyo, MDF-U53VC, Brazil) during 12 h in petri dishes. Thereafter, the samples were placed inside the drying chamber of the freeze-dryer (Edwards, E-C, China) in operation for a total period of 36 h.

Formulation of sausages

The formulation of sausages followed the Technical Regulation of Identity and Quality of Sausages, according to the Ministry of Agriculture, Livestock and Food Supply from Brazil (MAPA 2000). Three chicken sausage formulations were prepared, according to quantities presented in Table 1. The first one was called standard, which was similar to that used industrially (with soy proteins and synthetic dyes). The second one was called the liquid plasma, which had a partial substitution of mechanically separated meat by liquid plasma (without adding isolated soy protein and water). The third one was called freeze-dried plasma, which had a substitution of isolated soy protein and synthetic dyes by freeze-dried plasma.

For preparing the sausages, the raw material and mechanically separated meat were ground (disc 8 mm) in a grinder (Visa, Brusque, Brazil). The ingredients were added to the cutter (Frigomaq, Chapecó, Brazil) with ice water and homogenized until the temperature reached 5 °C. Afterward, textured soy protein was added and homogenized again for 2 min. The product was embedded in cellulose casings, tied and subjected to the cooking process. Cooking in hot water tank (Frigomaq, Chapecó, SC, Brazil) was carried out in the following steps. Firstly, the product was placed in a tank with water at 55 °C for 20 min. Thereafter, water was warmed to 75 °C and the time was monitored until the internal side of the sausage reached 72 °C. After the cooking process, the product was cooled in ice water to facilitate removal of the casing. The product was packed in a plastic bag and stored under refrigeration (4 ± 1 °C). All the industrial ingredients used for preparing the chicken sausages were kindly provided by an industrial processing chicken meat plant located in Erechim-Brazil.

Microbiological and physicochemical analyses

Analysis of thermotolerant coliforms, such as *Staphylococcus aureus* and *Salmonella* in the sausages were carried out between the 0 and 60th days. The chicken sausages were also characterized in terms of lipids (Soxhlet method), protein (Kjeldahl method), fixed mineral residue (ash, method of incineration in a muffle furnace at 550 °C) and moisture contents (with sand, indirect gravimetric method at 105 °C). All the analyses were performed in triplicate and were performed in accordance with the methods of the AOAC (1990) and IAL (2005).

Sensory analysis

Sensory evaluation was performed to verify the acceptability of formulated sausages and to compare with the standard one (with soy protein addition). Thirty-three untrained tasters gently analyzed the chicken sausage samples. The preference ordering test was performed in individual cabins with controlled temperature and luminosity where each taster received a cube of each sausage sample (1 cm thick slices) in containers properly coded randomly. The results were submitted to the Friedman test (p value < 0.05).

Results and discussion

Chicken sausages: microbiological and physicochemical analyses

Table 2 presents the results of the microbiological analysis for three sausage formulations. All the samples of chicken sausage did not present contamination by thermotolerant coliforms, *Staphylococcus aureus*, and *Salmonella* for the 60 day of analysis, according to the Brazilian legislation standards (ANVISA 2001).

Silva et al. (2013) and Silva et al. (2014) reported that microbial counts of smoked goat blood sausage were lower than the limits recommended by the Brazilian legislation. It characterized the sausage as a suitable product for human consumption. The high microbiological quality could be attributed to the use of high-quality raw materials and the Good Manufacturing Practices employed during all processing stages.

The results of the physicochemical analysis are presented in Fig. 1. According to MAPA (2000), all the three samples of chicken sausage are in accordance with the standards recommended in the Brazilian legislation standards for sausages. In mass basis, the lipids, protein, and ash ranged from 20.8 to 23.6%, from 12.1 to 12.5% and from 2.8 to 3.4%, respectively.

Table 2 Microbiological analyses for the three formulations of sausages (standard sausage, liquid plasma sausage, and freeze-dried plasma sausage)

	Brazilian Legislation ^a	Standard sausage		Liquid plasma sausage		Freeze-dried plasma sausage	
		0 day	60 days	0 day	60 days	0 day	60 days
Thermotolerant coliforms	1.0×10^3 MPN/g	< 3.0 MPN/g	< 3.0 MPN/g	< 3.0 MPN/g	< 3.0 MPN/g	< 3.0 MPN/g	< 3.0 MPN/g
<i>Staphylococcus aureus</i>	3.0×10^3 CFU/g	2.5×10^2 CFU/g	5.0×10^1 CFU/g	2.2×10^3 CFU/g	Absence	Absence	3.2×10^2 CFU/g
<i>Salmonella</i>	Absence in 25 g	Absence	Absence	Absence	Absence	Absence	Absence
Friedman test (<i>p</i> value < 0.05)		42		24		14	

MPN most probable number, CFU colony forming units

^aANVISA (2001)

Fig. 1 Physicochemical analyses for the formulations of sausages (standard sausage, liquid plasma sausage, and freeze-dried plasma sausage)

Fontes et al. (2015) formulated mortadellas by replacing different levels (0, 5, 10, 15 and 20 wt%) of meat by carbon monoxide-treated porcine blood. Results for moisture (68.9 wt%), ash (2.9 wt%) and protein (16.1 wt%) were similar if compared with the formulation using liquid plasma sausage. Likewise, in the production of “Morcilla de Burgos”, Santos et al. (2003) analyzed the physicochemical and sensory properties of this traditional Spanish product. Values for moisture (62.2 wt%), ash (4.3 wt%), protein (13.1 wt%) and lipids (28.7 wt%) corroborate the findings obtained in this work.

Nowak and Von Mueffling (2009) developed a treatment for combining porcine blood corpuscle concentrate (BCC) and porcine collagenous connective tissue (rind) to prepare salami-type sausage. In such case, two formulations of sausages were microbiologically stable and had positive sensory, chemical, and physical properties. The sausages formulated with blood byproducts meet all standards for being commercialized worldwide.

Sensory analysis of sausages

According to the Friedman test, the standard, liquid plasma and freeze-dried plasma sausages presented sum of totals of 42, 24 and 14, respectively. Taking into account the sensory analysis, differences (*p* value < 0.05) between the samples of sausages were found: the freeze-dried plasma sausage differed from the other two sausages. The critical difference between the ordering totals in relation to 5% is 22. Therefore, all the samples that differ from each other by a value higher than 22 are significantly different. Therefore, based on this referenced value, the standard and liquid plasma sausages produced in this work did not differ statistically.

The acceptance is not a rule for all products that contain byproducts from slaughterhouses. For example, in frankfurters, Hurtado et al. (2012) reported that the addition of

porcine plasma to sausages significantly modified their flavor. Although these modifications were rated as slightly worse than controls, the overall acceptance was scored as 6.3 from 10 as a maximum scale.

Overall, most of the studies that deal with subjects similar to that one presented in this work indicate several feasibilities of using blood byproducts in meat formulations. In such trend, Nowak et al. (2007) reported that it is feasible to use blood cell concentrates (BCC) in a meat product both from the microbiological and the sensory (color) point of view. Approximately 10 wt% to 12 wt% of a standardized BCC can be used in blood sausages. These authors (Nowak et al. 2007) concluded that s-BCC is a useful ingredient for meat products. This inference is also reported by some other articles, especially for the formulation of different products, as mortadella, liver pate (Hamzeh et al. 2016) and beef sausages (Martinez and Machado 2016).

Conclusion

Formulations of chicken sausages with broiler blood as a replacer of soy protein and synthetic dyes met the Brazilian legislation standards. The microbiological analysis revealed that the standard, liquid plasma and freeze-dried plasma sausages presented values of colony forming units of *Staphylococcus aureus* and most probable number of thermotolerant coliforms lower than the maximum limiting values established by the Brazilian legislation. Furthermore, the absence of *Salmonella* was reached in all samples. In the sensory evaluation, none difference was observed between two samples: standard sausage and liquid plasma sausage. Therefore, it is feasible to produce a chicken sausage with the addition of liquid plasma and hemoglobin as a byproduct from the meat industry (replacement of soy protein and synthetic dyes) without influencing the quality of the product.

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