

## Development of low fat chicken mortadella using collagen as a fat substitute

<sup>1</sup>\*Prestes, R. C., <sup>2</sup>Leite, C. V. G., <sup>1</sup>Kubota, E. H., <sup>1</sup>Rosa, C. S. and <sup>2</sup>Kempka, A. P.

<sup>1</sup>*Universidade Federal de Santa Maria, Department of Food Science and Technology, Avenida Roraima, 1000, 97105-900, Santa Maria RS, Brazil*

<sup>2</sup>*Universidade do Estado de Santa Catarina, Department of Food Engineering, BR 282 Km 573.7, s/n, 89870-000, Pinhalzinho SC, Brazil*

### **Article history**

*Received: 12 December 2013*

*Received in revised form:*

*10 February 2014*

*Accepted: 11 January 2014*

### **Keywords**

*Fat substitutes*

*Consumer product safety*

*Collagen*

*Functional food*

### **Abstract**

This study aimed to develop a formulation of mortadella with reduced fat (light) using different types of bovine collagen and fat substitutes. Natural collagen fiber and collagen fiber powder were tested following a 2<sup>2</sup> factorial design with a central point. A standard formulation without reduced fat and without collagen was also developed for comparison. The following tests were performed: physicochemical characterization (moisture, ash, protein, lipids and A<sub>w</sub>); cooling, freezing and reheating losses; water holding capacity; texture (shear and compressive strength); color coordinates (L\*, a\*, b\*); microbiological evaluation (coliforms at 45°C/g, coagulase positive *Staphylococcus aureus*/g; sulphite reducing clostridia to 46°C/g; and *Salmonella*/25 g) and sensory evaluation (multiple comparison test). Statistical analysis was performed using analysis of variance (ANOVA), Tukey's test and response surface test. There was no significant difference ( $p > 0.05$ ), except for ash and compressive strength for the tested samples. The other evaluated parameters (L\*, a\*, b\*, pH, freezing and reheating losses, ability to retain water, moisture, protein, lipids and shear force) showed significant differences ( $p < 0.05$ ). The best results were obtained for formulation F2 (2.0% collagen fiber powder). In the sensory evaluation, the products were considered equal to the standard regarding texture ( $p > 0.05$ ), which indicates that the use of collagen is a promising development for this type of product.

© All Rights Reserved

### **Introduction**

Consumers who are more concerned about health issues are seeking to reduce their fat intake by consuming foods that are either fat-free or with low levels of fat. However, the reduction of fat in meat products presents a number of difficulties such as poorer appearance, flavor, texture of the final product, and also lower product acceptance by consumers. In the search for technological solutions to prevent these problems, manufacturers have introduced several modifications in formulations to mitigate the undesirable effects of fat reduction. Among these modifications are the use of carbohydrates, fiber, and non-meat protein of vegetable and animal origin, which also contribute to improving the texture of the product. One of these ingredients is collagen, which is widely available and which is used primarily for high water retention capacity (Sams, 2001).

Collagen fiber is obtained from native collagen that is extracted from the inner layers of bovine leather (Santana *et al.*, 2012). This fiber undergoes a chemical process (alkali treatment with calcium hydroxide) and subsequent degreasing and drying at low temperatures. Collagen fiber powder is obtained

by a process similar to that of collagen fiber, but it is subjected to high temperatures and subsequent grinding.

Due to its characteristics and properties such as low viscosity in aqueous solution, neutral odor, colorless, transparent, emulsifying and stabilizing properties, foaming and movies, solubility, dispersibility, wettability, compressibility, carrier substances and low allergenicity, collagen presents numerous industrial applications (Denis *et al.*, 2008; Karim & Bhat, 2008; Goméz-Guillén *et al.*, 2011).

This study aimed to develop a formulation of low fat (light) chicken mortadella using different types of bovine collagen and fat substitutes.

### **Materials and Method**

The experiments were performed in the laboratories of the State University of Santa Catarina (Pinhalzinho, SC, Brazil). The tested collagens were donated by Novaprom Food Ingredients (Lins, SP, Brazil) and were coded as 'fiber' and 'powder', corresponding respectively to Novapro® natural collagen fiber (particle size between 1.80 and 1.92 mm and 99.00% protein according to information

\*Corresponding author.

Email: [rosacrisprestes@hotmail.com](mailto:rosacrisprestes@hotmail.com)

Tel: +55 55 32208254

from the supplier) and Novapro® collagen fiber powder (particle size between 0.45 and 0.57 mm and 96.38% protein according to information from the supplier). For the development of the formulations a 2<sup>2</sup> factorial design with a center point was used and a standard formulation (S) was also developed (without the addition of collagen and without fat reduction), as can be seen in Table 1.

The mortadella was prepared in a cutter (Visa, Brusque, SC, Brazil) with a 3 kg capacity. The raw meat that was used was skinless and fat-free chicken thighs and drumsticks from frozen chickens that were donated by the Aurora Central Food Cooperative (São Miguel do Oeste, SC, Brazil). The base formulation (items that did not differ for all the formulations) consisted of the following ingredients and additives: skinless chicken drumsticks and thighs (48.27%); cassava starch (5.00%) (Fecularia São Miguel, São Miguel do Oeste, SC, Brazil); soy protein isolate (4.00%) (Solae, Esteio, RS, Brazil); salt (2.20%) (Diana, São Paulo, SP, Brazil); sodium erythorbate (0.10%) (Wenda, São Paulo, SP, Brazil); sodium nitrite (0.015%) (BASF, São Paulo, SP, Brazil); 3% cochineal carmine (0.01%) (CHR Hansen, São Paulo, SP, Brazil); sodium polyphosphate (0.50%) (Kerry, São Paulo, SP, Brazil); monosodium glutamate (0.10%) (Ajinomoto, São Paulo, SP, Brazil); dehydrated glucose (0.50%) (Corn Products, São Paulo, SP, Brazil); acidity regulator (0.50%) (Nutract, Chapecó, SC, Brazil); semi-refined kappa-carrageenan (0.50%) (FMC, São Paulo, SP, Brazil); and flavoring (0.30%) (Kraki, São Paulo, SP, Brazil). The other ingredients that differed in % according to the experimental design are shown in Table 1.

The pieces were embedded in artificial casings (five-layer bi-oriented nylon/poly with a thickness of 0.12 mm) and subjected to cooking in water to a temperature of 75°C internally, and then cooled in a bath with cold water and ice (4°C) until they reached 4°C. The mortadellas were subsequently stored under refrigeration (5°C) until analysis. The following determinations were performed: moisture, ash, protein, lipids and pH, as per the official method (AOAC, 1990; IAL, 2005). Freeze-thaw losses (FTL) were performed according to the methodology of Lee *et al.* (2002) with some modifications; the samples were cut into rectangles approximately 1 cm in height and were divided into four parts. The samples were then weighed and packed individually in plastic containers (polyethylene) and taken to be frozen at -18°C. After 24 hours of freezing, the pieces were thawed at room temperature (20°C) for 4 hours and then packed in filter paper, 12.5 cm in diameter. Then, the samples were pressed between two glass plates

Table 1. 2<sup>2</sup> factorial design with central point used for the development of formulations with reduced fat and added collagen (real and coded values)

Formulation/Treatment Ingredients (%) <sup>a</sup>	Chicken Fat	Water	Natural Collagen fiber (NCF)	Collagen fiber powder (CP) <sup>c</sup>
S <sup>b</sup>	26.0	10.0	-	-
F1	18.0	18.0	2.0 (+1)	0.0 (-1)
F2	18.0	18.0	0.0 (-1)	2.0 (+1)
F3	18.0	18.0	2.0 (+1)	2.0 (+1)
F4	18.0	18.0	0.0 (-1)	0.0 (-1)
CP <sup>c</sup>	18.0	18.0	.0 (0)	1.0 (0)

<sup>a</sup> Refers to the percentage of the ingredient added to the final product.

<sup>b</sup> Formulation coded as standard (S).

<sup>c</sup> Formulation that corresponded to central point (CP).

at 2000 g for 5 minutes. After pressing, they were removed from the filter paper and re-weighed and the percentage of water that was lost was determined by the difference in weight percentage.

Losses due to reheating (RL) were performed according to the methodology proposed by Hachmeister and Herald (1998) and the samples were cut into uniform sizes of 2.0 x 2.0 x 6.0 cm and weighed. They were then immersed in about 300 mL of boiling water in a 500 mL beaker, covered with watch glass, and kept for 6 minutes. Then they were drained on a paper towel and refrigerated (5°C) for 6 minutes. The percentage of loss due to reheating was given by the difference in weight percentage. To measure the shear force (SF) and compressive strength (CS) a methodology adapted from Desmond *et al.* (2000) was used, with a universal texturometer, (Kratos Model IKCL2-USB, Cotia, SP, Brazil), with a cell load of 5 kgf, maximum load on the Y axis of 3 kgf, maximum displacement on the X axis of 8.32 mm, speed of 10 mm·min<sup>-1</sup> and load break of 20.0% of the total load. The samples were cut into cylinders of about 1.0 cm height and diameter. The values of maximum peak compression were converted from kgf to N. The color coordinates of lightness (L\*) and chromaticity (a\* being the ratio of green to red, and b\* the blue to yellow index) were obtained using a Hunter Lab colorimeter (EZ 4500L Miniscan, Hunterlab, Reston, VA, USA) calibrated with white as standard (Y = 93, x = 0.3136 and y = 0.3321). Readings were carried out using slices of mortadella approximately 5 mm thick (5°C).

For the evaluation of syneresis, ten cubes (2.0 cm per side) were vacuum packed (Selovac, São Paulo, SP, Brazil) and stored under refrigeration (7°C) (Refrimate, Venâncio Aires, RS, Brazil). The packaging laminate consisted of a blend of polyethylene terephthalate (PET) and linear low-density polyethylene (LDPE) without a barrier and approximately 0.12 mm thick. Every two days the samples were left out of the refrigerator for two hours and only on the seventh day were they opened for evaluation of loss of liquid, i.e. in the period of 7

days they were removed from the refrigerator three times. After a seven-day period of repetition of the described procedures, the package was opened and the cubes were dried and weighed. The objective of this procedure was to simulate inappropriate refrigeration conditions. The syneresis percentage was calculated by weight difference.

The water holding capacity (WHC) was evaluated according to a methodology adapted from Ockerman & Organisciak (1978); the method consisted in taking samples of mortadella 3 cm in diameter and 2.5 cm in height and weighing them. The samples were compressed (50%) using a 3 kg plate for 15 minutes (a glass plate was used to protect the sample). This evaluation was conducted at a room temperature of 20°C and the product was at 5°C. The samples were then dried with a paper towel and weighed again. The percentage of water retained was determined by the difference in weight percentage.

Microbiological analysis was performed according to the standards required by the ANVISA (2001) (coliforms at 45°C/g, coagulase positive *Staphylococcus aureus*/g, sulphite reducing clostridia to 46°C/g and *Salmonella* sp/ 25g). For sensory evaluation, multiple comparison tests were used to assess whether there was a difference between the standard sample (S) and other tests (F1, F2, F3 and F4) in relation to texture. The principle of the test consisted in providing a slice of the standard sample, specified with the letter S, and other slices of coded samples, following the factorial design. The testers were asked to taste the samples and compare them with the standard across a scale (1 = best, 2 = equal, 3 = worse) and in a second stage the degree of difference between the standard sample and the coded samples was assessed using the following scale (0 = none, 1 = slight, 2 = average, 3 = very, 4 = extreme) (Dutcosky, 1996; IAL, 2005). Fifty-six untrained testers participated in the evaluation and the experimental procedures were duly approved by the State University of Santa Catarina.

Three repetitions were performed for each experiment and the analyses were carried out in triplicate, at least. The results were submitted to analysis of variance (ANOVA) and Tukey's test with a significance level of 95% ( $p < 0.05$ ), using Statistica® 8.0 (StatSoft Inc., Tulsa, OK, USA) software. The graphics and calculations of the effects were also obtained using the aforementioned computer program and Microsoft® Excel 2003 (Microsoft Brazil, São Paulo, SP, Brazil) software.

The quality parameters of greatest importance for industrial usage were analyzed. Only models that were considered to be adequate are presented in this

article ( $R^2 > 0.90$ , and in the ANOVA table the value of F calculated for the regression was greater than the tabulated values; the value of F calculated for the residue was lower).

## Results

Evaluating the results (Table 2), a significant difference ( $p < 0.05$ ) can be seen in the values for moisture, protein, lipids and pH. Apart from ash content, there was no significant difference ( $p > 0.05$ ) for the analyzed samples of mortadella. The results for moisture content ranged from 47.55% (S) to 55.39% (F2). Regarding the values found for protein (10.01 to 14.61%), the most noteworthy were for the formulations F3 (2.0 % NCF + 2.0 % CFP) and CP (1.0% NCF + 1.0% CFP), where the simultaneous use of both types of collagen was beneficial in providing an increased protein content in the mortadella, compared with the results obtained for the formulations S and F4 (without the addition of collagen).

There was no statistically significant difference ( $p > 0.05$ ) for the ash values, indicating that the addition of collagen in the form of natural fiber or fiber powder did not affect this parameter. The pH values were between 6.71 and 6.82. Pietrasik and Janz (2010) found pH values from 6.30 to 6.63 and Chin *et al.* (1999) found values from 6.37 to 6.5, in both cases for low fat mortadella. Table 3 shows that there was significant difference between samples ( $p < 0.05$ ) regarding the determination of water holding capacity (WHC), losses due to freeze-thaw (FTL), losses due to reheating (RL) and syneresis. In terms of WHC, the results ranged from 97.42% (CP) to 98.46% (F1). Formulations F1 and F3 showed higher WHC, and consequently greater resistance to the release of liquid and fat.

The results of the evaluations of losses, which consisted of submitting the mortadella to unfavorable conditions such as heating (in the case of using the mortadella for fresh consumption) and freeze-thaw conditions (in the case of a product for export), are shown in Table 3. Evaluating the percentages of freeze-thaw losses (FTL) and losses due to reheating (RL), it can be seen that the formulation which presented the lowest losses was F1, which only used collagen fiber powder. It was found that the losses were higher when both types of collagen were simultaneously used at maximum concentrations (F3), than when collagen fiber powder was used on its own (F1).

The model below (Equation 1) was used in the construction of response surface (allowing

Table 2. Results for levels of moisture, protein, lipids, ash and pH for the standard formulation (S) and formulations with reduced fat (F1, F2, F3, F4 and CP)

Formulation/Treatment <sup>a</sup>	Moisture (%)	Protein (%)	Lipids (%)	Ash (%)	pH
S (Standard)	47.55 <sup>c</sup> ± 1.12	10.01 <sup>c</sup> ± 0.26	36.00 <sup>c</sup> ± 0.17	3.94 <sup>c</sup> ± 0.03	6.71 <sup>c</sup> ± 0.026
F1 (0.0% NCF + 2.0% CFP)	55.39 <sup>bc</sup> ± 0.30	12.60 <sup>b</sup> ± 0.77	27.00 <sup>b</sup> ± 0.13	3.97 <sup>b</sup> ± 0.06	6.78 <sup>b</sup> ± 0.01
F2 (2.0% NCF + 0.0% CFP)	54.25 <sup>b</sup> ± 0.23	12.81 <sup>b</sup> ± 0.52	29.34 <sup>ab</sup> ± 0.47	4.09 <sup>b</sup> ± 0.01	6.82 <sup>b</sup> ± 0.04
F3 (2.0% NCF + 2.0% CFP)	54.94 <sup>bc</sup> ± 0.02	14.60 <sup>b</sup> ± 0.17	28.32 <sup>ab</sup> ± 0.33	4.34 <sup>b</sup> ± 0.39	6.81 <sup>b</sup> ± 0.03
F4 (0.0% NCF + 0.0% CFP)	55.45 <sup>bc</sup> ± 0.26	11.79 <sup>b</sup> ± 0.13	29.84 <sup>ab</sup> ± 1.08	4.06 <sup>b</sup> ± 0.02	6.73 <sup>b</sup> ± 0.03
CP (1.0% NCF + 1.0% CFP)	55.52 <sup>c</sup> ± 0.19	13.81 <sup>b</sup> ± 0.12	31.46 <sup>b</sup> ± 0.59	4.11 <sup>b</sup> ± 0.01	6.79 <sup>b</sup> ± 0.02

<sup>a</sup> Means ± standard deviation shown with different letters differ significantly vertically ( $p < 0.05$ ).

NCF and CFP correspond to natural collagen fiber and collagen fiber powder, respectively.

Table 3. Results for water holding capacity (WHC), freeze-thaw loss (FTL), reheating loss (RL) and syneresis pattern for the standard formulation (S) and for the reduced fat formulations (F1, F2, F3, F4 and CP)

Formulation/Treatment <sup>a</sup>	WHD (%)	FTL (%)	RL (%)	Syneresis (%)
S (Standard)	98.43 <sup>b</sup> ± 0.30	2.95 <sup>b</sup> ± 0.19	2.20 <sup>b</sup> ± 0.02	0.46 <sup>ab</sup> ± 0.07
F1 (0.0% NCF + 2.0% CFP)	98.46 <sup>b</sup> ± 0.04	1.93 <sup>b</sup> ± 0.02	1.41 <sup>b</sup> ± 0.16	0.95 <sup>bc</sup> ± 0.15
F2 (2.0% NCF + 0.0% CFP)	97.89 <sup>b</sup> ± 0.05	3.52 <sup>b</sup> ± 0.01	2.21 <sup>b</sup> ± 0.54	1.17 <sup>b</sup> ± 0.04
F3 (2.0% NCF + 2.0% CFP)	98.22 <sup>ab</sup> ± 0.03	2.66 <sup>b</sup> ± 0.19	3.32 <sup>b</sup> ± 0.05	0.32 <sup>b</sup> ± 0.05
F4 (0.0% NCF + 0.0% CFP)	97.47 <sup>b</sup> ± 0.25	2.66 <sup>b</sup> ± 0.04	1.24 <sup>b</sup> ± 0.09	0.98 <sup>b</sup> ± 0.09
CP (1.0% NCF + 1.0% CFP)	97.42 <sup>b</sup> ± 0.35	2.11 <sup>b</sup> ± 0.14	1.57 <sup>b</sup> ± 0.09	1.11 <sup>b</sup> ± 0.26

<sup>a</sup> Means ± standard deviation shown with different letters differ significantly vertically ( $p < 0.05$ ).

NCF and CFP correspond to natural collagen fiber and collagen fiber powder, respectively.

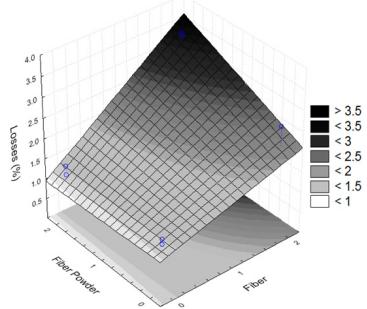


Figure 1. Response surface for the dependent variable, losses due to reheating (%) according to the independent variables of natural collagen fiber (Fiber) (%) and collagen fiber powder (Fiber Powder) (%)

the visualization of the behavior of % loss due to reheating (L) as a function of the significant variable (natural collagen fiber (F) and collagen fiber powder (P)).

$$L = 1.929 + 0.763F + 0.278P + 0.277FP \quad (1)$$

The model was predictive ( $R^2 = 0.914$ ) and it was used in the construction of response surface (Figure 1), allowing the visualization of the behavior of the reheating losses of the mortadella. In relation to syneresis, the results ranged from 0.32% (F3) to 1.17% (F2). Pietrasik and Janz (2010) found syneresis values of up to 3.65% for low fat mortadella.

Table 4 shows the results for the mortadella samples for the following parameters: compression strength (CS), shear force (SF) and color ( $L^*$ ,  $a^*$  and  $b^*$ ). There was significant difference ( $p < 0.05$ ) for all evaluations. For SF, the results ranged from 2.01 N (F1) to 2.90 N (F3), indicating a higher resistance to cutting due to the addition of a mixture of collagen fiber and collagen fiber powder. It was also observed that the addition of collagen resulted in lower values

Table 4. Results obtained for shear force (SF), compression strength (CS),  $a^*$  (green to red ratio),  $L^*$  (brightness) and  $b^*$  (blue to yellow index) for the standard formulation (S) and for the reduced fat formulations (F1, F2, F3, and F4 CP)

Formulation/Treatment <sup>a</sup>	SF (N)	CS (N)	$a^*$	$L^*$	$b^*$
S (Standard)	2.44 <sup>abc</sup> ± 0.23	16.71 <sup>b</sup> ± 1.49	9.28 <sup>b</sup> ± 0.08	65.18 <sup>b</sup> ± 0.30	12.23 <sup>b</sup> ± 0.12
F1 (0.0% NCF + 2.0% CFP)	2.01 <sup>b</sup> ± 0.15	13.30 <sup>b</sup> ± 0.92	9.69 <sup>b</sup> ± 0.67	65.96 <sup>b</sup> ± 0.23	12.68 <sup>b</sup> ± 0.10
F2 (2.0% NCF + 0.0% CFP)	2.27 <sup>ab</sup> ± 0.16	15.63 <sup>bc</sup> ± 0.47	9.70 <sup>b</sup> ± 0.07	63.88 <sup>b</sup> ± 0.48	12.29 <sup>b</sup> ± 0.07
F3 (2.0% NCF + 2.0% CFP)	2.90 <sup>a</sup> ± 0.41	15.32 <sup>b</sup> ± 0.14	9.49 <sup>b</sup> ± 0.11	65.54 <sup>bc</sup> ± 0.20	12.97 <sup>b</sup> ± 0.12
F4 (0.0% NCF + 0.0% CFP)	2.32 <sup>ab</sup> ± 0.23	17.32 <sup>b</sup> ± 0.12	8.92 <sup>b</sup> ± 0.06	69.27 <sup>d</sup> ± 0.62	12.03 <sup>b</sup> ± 0.04
CP (1.0% NCF + 1.0% CFP)	2.61 <sup>bc</sup> ± 0.29	16.00 <sup>b</sup> ± 0.35	9.80 <sup>b</sup> ± 0.02	66.02 <sup>c</sup> ± 0.27	12.01 <sup>b</sup> ± 0.06

<sup>a</sup> Means ± standard deviation shown with different letters differ significantly vertically ( $p < 0.05$ ).

NCF and CFP correspond to natural collagen fiber and collagen fiber powder, respectively.

Table 5. Results for multiple comparison test for developed formulations of chicken mortadella (F1, F2, F3, F4 and CP)

Formulation/Treatment <sup>a</sup>	Comparison <sup>b</sup>	Degree of Difference <sup>c</sup>
F1 (0.0% NCF + 2.0% CFP)	1.88 <sup>b</sup> ± 0.81	1.44 <sup>b</sup> ± 1.09
F2 (2.0% NCF + 0.0% CFP)	1.50 <sup>b</sup> ± 0.63	1.13 <sup>b</sup> ± 0.89
F3 (2.0% NCF + 2.0% CFP)	1.93 <sup>b</sup> ± 0.89	1.47 <sup>b</sup> ± 0.89
F4 (0.0% NCF + 0.0% CFP)	1.81 <sup>b</sup> ± 0.83	1.25 <sup>b</sup> ± 0.93
CP (1.0% NCF + 1.0% CFP)	1.69 <sup>b</sup> ± 0.79	1.63 <sup>b</sup> ± 1.20

<sup>a</sup> Means ± standard deviation shown with different letters differ significantly vertically ( $p < 0.05$ ).

<sup>b</sup> Evaluation criteria for comparison: 1 = best-1, 2 = equal, 3 = worse than the standard regarding texture.

<sup>c</sup> Criteria for degree of difference: 0 = no difference from standard, 1 = slight, 2 = average and 4 = very different from the standard in relation to texture.

NCF and CFP correspond to natural collagen fiber and collagen fiber powder, respectively.

of compression strength (F1, F2, F3, and CP).

The results for the color parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) can be seen in Table 4. It can be noted that the use of collagen at a higher level reduced the  $L^*$  values (comparing the F4 treatment without collagen with the F1, F2, F3, and CP treatments), and increased the  $a^*$  and  $b^*$  values. Chin *et al.* (1999) also found increased  $b^*$  values in mortadella with added mixtures of soy protein and konjac.

In the microbiological evaluation, all the samples were in accordance with the standards established by the relevant legislation (ANVISA, 2001), and in the sensory evaluation, the samples were considered equal to the standard ( $p < 0.05$ ). When the testers were asked about the degree of difference of the samples, the average obtained on the scale was 1.384, indicating little difference between the samples and the standard (Table 5).

## Discussion

Comparing the moisture results for formulation S (standard, without reducing fat) with the other samples it was found that the highest moisture values were found for the treatments with reduced fat, which coincided with the results of Figueiredo *et al.* (2002), who obtained higher moisture values in sausages prepared with fat substitutes (xanthan gum, and whey protein concentrate). Pietrasik and Janz (2010) found moisture contents of 61.40% to 71.60% in low fat bologna-type mortadella, and according to these authors, the moisture content is proportional to the water content that is added to these products,

which is greater, due to the removal of fat.

Regarding the values found for proteins, Olivo and Shimokomaki (2001) and Prestes *et al.* (2013) also reported an increase in the total protein content in meat products added bovine collagen. The S and F4 formulations did not meet the minimum protein level set for this type of product (MAPA, 2000). The values found for lipids indicated that only formulation F1 (2.0% CFP) could be classified as light because it showed a minimum reduction of 25% of energy or total fat compared to the standard set by legislation (ANVISA, 1998).

Some of the differences found for pH levels can be justified by the production system (handling, food, genetic, etc.) as mentioned by other authors (Novello *et al.*, 2008; Youssef and Barbut, 2011). In relation to losses and water retaining capacity, because the collagen fiber powder had a greater surface area, due to smaller particle size, there was a greater interaction in the product, which allowed greater water retention and improved synergy with the myofibrillar proteins and fat, which can also be demonstrated by the higher moisture levels, as previously mentioned (Table 1). Higher WHC values may also indicate a greater resistance of the product, due to a more cohesive protein matrix.

Lower losses due to freeze-thaw and reheating were found when using collagen fiber powder, which can be explained by its greater interaction with the ingredients and additives in the formulations, which allowed the formation of a more resistant and cohesive protein. Schilling *et al.* (2004) observed higher fluid losses than those found in this study; around 10.95% for hams produced with 3% native collagen and raw meat with PSE (pale, soft and exsudative) characteristics. According to these authors, the use of different levels of collagen should be explored in order to determine the optimum improvement of the quality of the product. According to Pietrasik *et al.* (2010), non-meat proteins exhibit similar behaviour to proteins present in meat, promoting water retention, higher binding, and occupying the interstitial spaces of the gel matrix. However, as mentioned by Sams (2001) and Pearson and Dutson (1997), when collagen is added at certain concentrations it can have a negative effect that causes shrinkage, especially when these products are subjected to high temperatures, due to a low stability in relation to heat (Karim and Bhat, 2008), which can interfere with the binding of the meat. Through the response surface it was possible to see that the lowest concentrations of collagen fiber associated with the highest concentrations of collagen fiber powder resulted in lower losses due to reheating, which represented a technological advantage for the

product.

It was observed that the lowest level of syneresis was found for preparation F3 (2.0% NCF + 2.0% CFP), indicating that synergy occurred, with the mixture of the collagens and the structure of the protein matrix, which showed a higher entrapment of water and fat. When the natural collagen fiber was used alone (F2) it produced the worst results. Added collagen, in certain concentrations, can have a negative effect, causing shrinkage, especially when these products are submitted to high temperatures (Karim and Bhat, 2008). This has been confirmed by Hernández-Briones *et al.* (2009), who investigated fish gelatine gels at high concentrations (5% to 10%). The water release caused by the protein-protein interaction is very strong. Yang *et al.* (2007) also observed this behavior in relation to chicken meat protein (myosin), where the gelation of the pure myosin was better than for the myosin-gelatin mixture. Damoradan *et al.* (2010) also confirm that there is a limit to the concentration of added collagen because there is a tendency for the formation of protein-protein bonds that are stronger than the interactions with the protein of the meat. These concentrations may also vary, especially if polysaccharides are added. Above this concentration limit, the gel is forced out of the structure.

The samples that showed lower WHC also had higher levels of syneresis (F2 and CP), indicating the lower resistance of the collagen fiber when used alone or in combination with collagen fiber powder. This result could also be explained by the greater tendency to shrinkage of natural collagen fiber compared to collagen fiber powder, and as a consequence the ease of loss of fluid from the structure when the product is subjected to unfavorable conditions. When the collagen fiber was added individually it resulted in a higher compressive strength and higher shear force in the samples due to their physical form and larger particle size (treatment F2 compared to F1). Figueiredo *et al.* (2002) also found that the use of a greater proportion of whey protein concentrate as a fat substitute in sausages resulted in higher compressive strength and greater hardness of the products. Furthermore, the addition of collagen in the formulation can interfere with the lightness of the product, since there is a decrease in the concentration of myoglobin, which results in an opaque product (Youssef and Barbut, 2011).

Lightness ( $L^*$ ) is related to the degree of clarity of color, indicating whether the colors are bright or dark. The light source is pointed at the sample and the response represents the proportion of reflected light. Higher  $a^*$  values indicate a redder color and lower values indicate a greener color; whereas,

higher  $b^*$  values indicate yellowness and lower  $b^*$  values indicate a bluer color (Ramos and Gomide, 2007). The a/b ratio is also useful to show the color changes from pink to a brown or brownish product. The interaction of myoglobin with collagen causes a dilution in myoglobin and consequent changes in the absorption, leaving it near to 400 nm. The reduction of  $L^*$  values and increased  $b^*$  values result in a more opaque product. Lower  $L^*$  values and higher  $b^*$  values can be explained by the reduction in the concentration of myoglobin in meat (Youssef and Barbut, 2011).

In the sensory evaluation, the samples were considered to be equal to the standard ( $p > 0.05$ ) in relation to texture, indicating that the addition of the tested collagens did not impair this important sensory parameter (Table 5). Olivo and Shimokomaki (2001), Prestes et al. (2012), Prestes et al. (2013) noted that the addition of bovine collagen did not result in changes in flavor or the characteristics expected in meat products. Figueiredo et al. (2002) reported a lower level of acceptance of the texture of sausages with fat substitutes when compared to the standard. These authors explained that the absence of fat impaired the softness that was expected for this type of product.

## Conclusion

It was concluded that by replacing fat with collagen it is possible to obtain a reduced fat (light) formulation of chicken mortadella. The formulation F1 (2.0% collagen fiber powder) reached the mandatory parameters required by legislation (minimum reduction of 25.0% in fat). In the sensory evaluation, the developed products were considered to be equal to the standard with respect to texture ( $p > 0.05$ ), which indicates that the use of collagen is a promising development for this type of product. The addition of collagen fiber powder showed a better performance than the natural collagen fiber in relation to the various evaluated technological parameters, mainly in relation to losses, which demonstrated the strong interaction between the collagen fiber powder in the structure of the product and the possibility of the application of this type of collagen in other types of functional meat products.

## References

- ANVISA. 1998. Agência Nacional de Vigilância Sanitária. Portaria no. 27 de 13 de janeiro de 1998. Aprova o Regulamento Técnico sobre a informação nutricional complementar. Diário Oficial da União de 16/01/1998, Brasília, p.68.
- ANVISA. 2001. Agência Nacional de Vigilância Sanitária. Resolução da Direção Colegiada no.12, de 02 de janeiro de 2001. Aprova o Regulamento Técnico sobre padrões microbiológicos para alimentos. Diário Oficial da União de 10/01/2001, Brasília, p.68.
- AOAC. 1990. Official Methods of Analysis of the Association of Analytical Chemists. 40<sup>th</sup> ed. Washington.
- Chin K.B., Keeton J.T., Longnecker M.T. and Lamkey, J.W. 1999. Utilization of soy protein isolate and konjac blends in a low fat bologna (model system). Meat Science 53 (1): 45-47.
- Damoradan S., Parkin K. and Fennema, O.R. 2010. Química de Alimentos de Fennema.p. 719-759. Porto Alegre: Artmed.
- Denis, A., Brambati, N., Dessaувages, B., Guedji, S., Ridoux, C., Meffre, N. and Autier, C. 2008. Molecular weight determination of hydrolyzed collagens. Food Hydrocolloids 22 (6): 989-994.
- Desmond E.M., Kenny T.A., Ward P. and Sun, D. 2000. Effect of rapid and conventional cooling methods on the quality of cooked ham joints. Meat Science 56 (3): 271-277.
- Dutcosky, S.D. 1996. Análise Sensorial de Alimentos. p.73-216. Curitiba: Champagnat.
- Figueiredo, V.O., Gaspar, A., Borges, S.V. and Modesta, R.C.D. 2002. Influência de substitutos de gordura animal sobre a qualidade da salsicha Tipo Viena. Journal Food Technology 5 (75): 11-17.
- Gómez-Guillén, M.C., Giménez, B., López-Caballero, M.E. and Montero, M.P. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. 2011. Food Hydrocolloids 25 (8): 1813-1827.
- Hachmeister, K.A. and Herald, T.J. 1998. Thermal and rheological properties and textural attributes of reduced-fat turkey batters. Poultry Science 77 (4): 632-638.
- Hernández-Briones, B., Velázquez, G., Vázquez, M. and Ramírez, J.A. 2009. Effects of adding fish gelatin on Alaska pollock surimi gels. Food Hydrocolloids 23 (8): 2446-2449.
- IAL. 2005. Instituto Adolfo Lutz. Agência Nacional de Vigilância Sanitária. Métodos Físico-Químicos para Análises de Alimentos. Brasília: Ministério da Saúde, Agência Nacional de Vigilância Sanitária.
- Karim, A.A. and Bhat, R. 2008. Gelatin alternatives for the food industry: recent developments, challenges and prospects. Food Science and Technology 19 (12): 644-656.
- Lee, M.H., Baek, M.H., Cha, D.S., Park, H.J. and Lim, S.T. 2002. Freeze-thaw stabilization of sweet potato starch gel by polysaccharide gums. Food Hydrocolloids 16 (4): 345-352.
- MAPA. 2000. Ministério da Agricultura Pecuária e Abastecimento. Regulamento Técnico de Identidade e Qualidade de Mortadela. Instrução Normativa no. 20, de 31 de julho de 2000. Diário Oficial da União de 03/08/2000, Brasília, 2000.
- Novello, D., Ost, P.R., Neumann, M. and Pellegrini, L.G. 2008. Avaliação bromatológica e perfil de ácidos

- graxos da carne de frango de corte alimentados com rações contendo farinha de carne e ossos. Ambiência 4 (3): 355-366.
- Ockerman, H.W. and Organisciak, C.S. 1978. Diffusion of curing brine in tumbled and non-tumbled porcine muscle tissue. Journal Food Protection 41:178–181.
- Olivo, R. and Shimokomaki, M. 2001. Carnes: No Caminho da Pesquisa. p.5-72. Cocal do Sul: Imprint.
- Pearson, A.M. and Dutson, T.R. 1997. Advances in meat research. p.244-255. Blackie Academic & Professional: London and New York.
- Pietrasik, J. and Janz, J.A.M. 2010. Utilization of pea flour, starch rich and fiber rich fractions in low fat bologna. Meat Science 43 (2): 601-608.
- Prestes, R.C., Carneiro, E,B,B. and Demiate, I.M. 2012. Hydrolyzed collagen, modified starch and guar gum addition in turkey ham. Ciência Rural 42 (7): 1307-1313.
- Prestes, R.C., Graboski, A., Roman, S.S., Kempka, A.P., Tonazzzo, G., Demiate, I.M. and Di Luccio, M.2013. Effects of the addition of collagen and degree of comminution in the quality of chicken ham. Journal Applied Poultry Research 22 (4): 885-903.
- Ramos, E.M. and Gomide, L.A.M. 2007. Avaliação da qualidade de carnes: fundamentos e metodologias. p.287-374. Viçosa: Ed. UFV.
- Santana, R.C., Sato, A.C.K. and Cunha, R.S. 2012. Emulsions stabilized by heat-treated collagen fibers. Food Hydrocolloids 26 (1): 73-81.
- Sams, A.R. 2001. Poultry Meat Processing. p.181-280. New York: CRC Press.
- Schilling, M.W., Marriott, N.G., Acton, J.C., Anderson-Cook, C., Alvarado, C.Z. and Wang, H. 2004. Utilization of response surface modeling to evaluate the effects of non-meat adjuncts and combinations of PSE and RFN pork on water holding capacity and cooked color in the production of boneless cured pork. Meat Science 66 (2): 371-381.
- Yang, Y.L., Zhou, G.H., Xu, X.L. and Wang, Y. 2007. Rheological properties of myosin-gelatin mixtures. Journal Food Science 72 (5): 270-275.
- Youssef, M.K. and Barbut, S. 2011. Effects of two types of soy protein isolates, native and preheated whey protein isolates on emulsified meat batters prepared at different protein levels. Meat Science 87 (1): 54-80.
- Zorba, O. and Kurt, S. 2006. Optimization of emulsion characteristics of beef, chicken and turkey meat mixtures in model system using mixture design. Meat Science 73 (4): 611-618.