# EFFECTS OF CONNECTIVE TISSUE PROTEINS ON CHEMICAL COMPOSITION AND EMULSION STABILITY OF LYONER TYPE SAUSAGE

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## Background

Processed meat products are important sources of proteins in human nutrition. Such composite meat products are often prepared from cuts high in connective tissue. The levels and type of specific no muscle animal protein ingredients used to formulate such products, however, vary greatly, resulting in wide variations in their protein quality and nutritive value. Therefore, an accurate assessment of the amounts of these proteins is essential for consumer information, regulatory purposes and international trade (ZARKADAS et al., 1993). It has generally been recognized that inexpensive cuts of meat and meat by-products high in connective tissue have relatively poor biological value. Chemical determination of collagen in composite meats can be employed to provide a rapid estimation of protein quality (ZARKADAS et al., 1993; EL, 1995). Collagen content was highly negatively correlated to essential amino acid content and rat PER with correlation coefficients of -0.99 and -0.98, respectively (LEE et al., 1978). Collagen is an incomplete protein. The value of collagen as human food is limited because of its low amino acid balance, being low in methionine and devoid of tryptophan (RAO & HENRICKSON, 1983). Regulations restricting collagenous connective tissue amounts in meat products have already been in force for some years in some countries. Some of them limit the connective tissue protein fraction of total (crude) protein content, while others restrict the proportion of connective tissue protein of total (crude) meat protein content; limits depend on the particular type of product. The determination of the connective tissue nitrogen content is based on chemical analysis of 4-hydroxyproline, the characteristic amino acid contained in the most important connective tissue proteins, collagen and elastin. Sausage manufactures presently use meat containing large quantities of connective tissue (collagen) to reduce processing costs. However, functional properties such as thermal shrinkage and gelatinization make the collagen additive levels in a food critical. The sausage products regulations allow the addition of up to 10% of skin, rind, tendon, and offal such as brain, liver, heart, stomach, tongue, kidney, bone marrow in sausages as hot dogs; therefore, it cannot be added to frankfurters and wieners (BRASIL, 2000). Collagen content ranges from 90% in tendon, to about 2% in most lean meat and to less than 1% in liver (BAILEY & LIGHT, 1989).

## Objectives

Determine the effect of levels 5, 10 and 15% commercial beef (epimysium/perimysium) and pork (rind) connective tissues replacement on chemical composition, pH, collagenous connective tissue protein and emulsion stability of Lyoner type sausages.

## Methods

**Lyoner sausage manufacture:** fresh beef connective tissue (BCT) containing both epimysium and perymisium was removed from commercial bovine hind muscles from a packing plant that utilized Skyner machine 7600 (Towsend). The BCT was packaged in vacuum plastic bags, frozen at –18°C and grinded (8mm plate) in a frozen state. The commercial pork rind was cooked in water (CPR) and chopped in cutter. The formulation of the control Lyoner type sausage (8Kg each batch) consisted of 41.1% beef, 12.5% mechanically deboned poultry meat, 19.1% pork backfat, 18.4% crushed ice, 4% textured soy protein concentrate (Proteimax TR-120), 1.5% salt, 0.015% sodium nitrite, 0.3% sodium tripolyphosphate, 0.05% sodium erythorbate, 1% spices and 2% manioc starch. The seven formulations containing 0% (control); 5%; 10% and 15% BCT or CPR were adjusted to 20% fat in the final product, altering beef, ice and backfat. CPR or BCT were added with lean beef at the beginning of the cutter's process. The products were prepared in the Meat Technology Centre of the Institute of Food Technology according to industrial standards. The batter was stuffed into 60 mm casings (CaseTech - K plus) and thermally processed to 72°C internal temperature. **Physico-chemical analysis**: moisture, protein (Kjeldahl method, factor 6.25), fat (diethyl ether extractable) and ash contents were determined in duplicate following the INSTITUTO ADOLFO LUTZ (1985) procedures. Duplicate pH readings of raw batter, cooked Lyoner and sterilized products were taken with a spear-tip electrode attached to HANNA HI9321 microprocessor pH-meter. Hydroxyproline analysis was carried out according to the method described by AOAC (1995). Hydroxyproline is quantitatively determined as measure of colagenous material. Collagenous connective tissue contains 12,5% hydroxyproline when nitrogen-to-protein factor of 6.25 is used. **Emulsion stability:** reported as percent fat and gelatin released from sterilized cans at 121°C (~150g cylindrical cans, nominal process value $F_o = 6.41$). **Statistical analysis**: all data underwent analysis of variance (ANOVA) and Tukey's Test to determine differences ($p<0.05$) between pairs of means, using GraphPad InStat tm, Copyright 1990-1993, V2.01.

## Results and Discussion

The pork rind absorbed 7.1% water in cooking process and resulted in a final chemical composition of 52.7% moisture, 22.0% protein, 24.9% fat, 0.2% ash, 2.37% 4-hydroxyproline, 18.9% collagenous connective tissue (CCT) and 85.9% collagenous connective tissue per total (crude) protein (CCT/P). Beef connective tissue after grind resulted in a comminuted product with 67.5% moisture, 19.1% protein, 12.2% fat, 0.7% ash, 0.79% 4-hydroxyproline, 6.3% CCT and 33.0% CCT/P. The final raw batter comminuting temperatures (cutter) ranged from 11.7 to 13.4°C. Chemical and collagenous composition, pH value and emulsion stability of Lyoner type sausage manufactured with 5, 10 and 15% beef connective tissue (BCT) or cooked pork rind (CPR) are presented in Table 1. For BCT treatments, it was observed a significant ($p<0.05$) increase on protein levels, ranging from 12.54 to 13.81%; no treatment differences were noted for moisture, fat and ash. Moisture levels varied from 59.65 to 58.34% for CPR treatments. As the quantities of added CPR increased, moisture content decreased and fat and protein contents increased ($p<0.05$). Fat and protein levels ranged from 20.26 to 21.67% and 12.54 to 14.56% respectively. Because of the higher protein rate of collagen (22.0%) than that of muscle (19.0%), the addition of collagen tends to increase the apparent lean meat (total protein) content of meat products. The mean collagenous connective tissue (CCT) values calculated from the amounts of 4-hydryproline found for BCT and CPR treatments, ranged from 1.68 to 2.40% and 1.68 to 4.10% of the total composite, respectively. The effect of added connective tissues was most significant for CPR replacements; all treatments were significantly different from each other. This reflected on collagenous connective tissue per total protein (CCT/P) content that ranged from 13.40 to 28.16%. The sausage added with BCT levels showed an increase on CCT/P, ranging from 13.40 to 17.65%. As shown in Figures 1 and 2, as the amount of bovine or pork connective tissue in the Lyoner increased, there was a significant linear increase ($R2 = 0.97$ and 0.99, BCT and CPR respectively) in the amount of collagenous connective tissue determined and a linear increase in collagenous connective tissue per total (crude) protein ($R2=0.8342$ and 0.9998, BCT and CPR). The raw batter, Lyoner

and conserve pH values showed a slight but significant increase with increasing BCT (6.01 to 6.10) or CPR (6.00 to 6.19) levels (Table 1). The small pH differences although statistically significant may not be of much relevance. All treatments added with connective tissue resulted in an significant increase on fat and gelatine release ($p<0.05$), the range was 0.51 to 2.83% and 0.51 to 2.10% for BCT and CPR treatments respectively, indicating a decrease on emulsion stability with increasing connective tissue levels, affecting functional properties, which are attributed to the lean meat proteins in a fine emulsion sausage like Lyoner.

## Conclusions

Lyoner type sausages replaced with beef connective tissue (epimysium and perimysium) and cooked pork rind increased total protein, collagenous connective tissue, pH and fat/gelatin release, affecting emulsion functionality. The pH difference from control (without connective tissue added) was too slight to be of much practical importance. The results were more pronounced for cooked pork rind replacements compared to beef connective tissue for all parameters evaluated except for emulsion stability that was slightly inferior. Since there is a straight correlation between connective tissue added and collagenous connective tissue content, new regulations on the composition of emulsion type meat products should establish analytical collagenous connective tissue protein values, and collagenous connective tissue protein fraction of the total protein content, by hydroxyproline determination.

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**Table 1**. Chemical and physical parameters means for Lyoner type sausage containing connective tissue proteins

| Parameters | Addition of connective tissue proteins | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Beef connective tissue (BCT)* | | | | Cooked pork rind (CPR) | | | |
| | Control | 5% | 10% | 15% | Control | 5% | 10% | 15% |
| **Chemical composition**[1] | | | | | | | | |
| Moisture (%) | 59.65$^{a}$ (0.13) | 58.70$^{a}$ (0.13) | 59.28$^{a}$ (0.01) | 59.23$^{a}$ (0.47) | 59.65$^{a}$ (0.13) | 58.87$^{a,b}$ (0.30) | 58.60$^{b}$ (0.23) | 58.34$^{b}$ (0.06) |
| Fat (%) | 21.26$^{a}$ (0.06) | 21.51$^{a}$ (0.43) | 20.14$^{a}$ (0.36) | 20.12$^{a}$ (0.39) | 21.26$^{b}$ (0.06) | 20.26$^{c}$ (0.03) | 21.26$^{b}$ (0.13) | 21.67$^{a}$ (0.07) |
| Total protein (%) | 12.54$^{b}$ (0.18) | 13.81$^{a}$ (0.20) | 13.78$^{a}$ (0.18) | 13.60$^{a}$ (0.05) | 12.54$^{b}$ (0.18) | 14.45$^{a}$ (0.61) | 14.07$^{a,b}$ (0.01) | 14.56$^{a}$ (0.42) |
| Ash (%) | 3.37$^{a}$ (0.07) | 3.35$^{a}$ (0.00) | 3.28$^{a}$ (0.05) | 3.30$^{a}$ (0.04) | 3.37$^{a}$ (0.07) | 3.42$^{a}$ (0.05) | 3.37$^{a}$ (0.00) | 3.30$^{a}$ (0.07) |
| Collagenous composition[1] | | | | | | | | |
| 4-Hydroxyproline (%) | 0.210$^{b}$ (0.013) | 0.226$^{a,b}$ (0.019) | 0.261$^{a,b}$ (0.009) | 0.300$^{a}$ (0.035) | 0.210$^{d}$ (0.013) | 0.332$^{c}$ (0.009) | 0.412$^{b}$ (0.015) | 0.512$^{a}$ (0.032) |
| Collagenous connective tissue (%) | 1.68$^{b}$ (0.10) | 1.80$^{a,b}$ (0.15) | 2.09$^{a,b}$ (0.07) | 2.40$^{a}$ (0.28) | 1.68$^{d}$ (0.10) | 2.66$^{c}$ (0.07) | 3.30$^{b}$ (0.12) | 4.10$^{a}$ (0.25) |
| Collagenous connective tissue per total protein (%) | 13.40 | 13.06 | 15.18 | 17.65 | 13.40 | 18.41 | 23.45 | 28.16 |
| pH value | | | | | | | | |
| Raw batter[1] | 6.05$^{b}$ (0.01) | 6.05$^{b}$ (0.01) | 6.06$^{a,b}$ (0.01) | 6.08$^{a}$ (0.01) | 6.05$^{c}$ (0.01) | 6.05$^{c}$ (0.01) | 6.10$^{b}$ (0.01) | 6.18$^{a}$ (0.02) |
| Pasteurized (Lyoner)[2] | 6.10$^{a}$ (0.01) | 6.10$^{a}$ (0.01) | 6.09$^{a}$ (0.01) | 6.10$^{a}$ (0.00) | 6.10$^{c}$ (0.01) | 6.11$^{c}$ (0.01) | 6.16$^{b}$ (0.01) | 6.19$^{a}$ (0.01) |
| Sterilized (Conserve)[2] | 6.01$^{c}$ (0.01) | 6.02$^{c}$ (0.01) | 6.04$^{b}$ (0.00) | 6.06$^{a}$ (0.00) | 6.00$^{d}$ (0.01) | 6.05$^{c}$ (0.01) | 6.09$^{b}$ (0.01) | 6.12$^{a}$ (0.01) |
| **Emulsion stability**[3] | | | | | | | | |
| Fat and gelatin release (%) | 0.51$^{d}$ (0.12) | 1.25$^{c}$ (0.39) | 1.81$^{b}$ (0.44) | 2.83$^{a}$ (0.26) | 0.51$^{d}$ (0.12) | 1.03$^{c}$ (0.19) | 1.57$^{b}$ (0.26) | 2.10$^{a}$ (0.62) |

$^{a, b, c}$Mean values in a row within treatment followed by different letters are significantly different ($p<0.05$) from each other
* Epimysium / perimysium fraction ( ) Standard deviation Number of replicates: [1]N=2; [2]N=4; [3]N=8

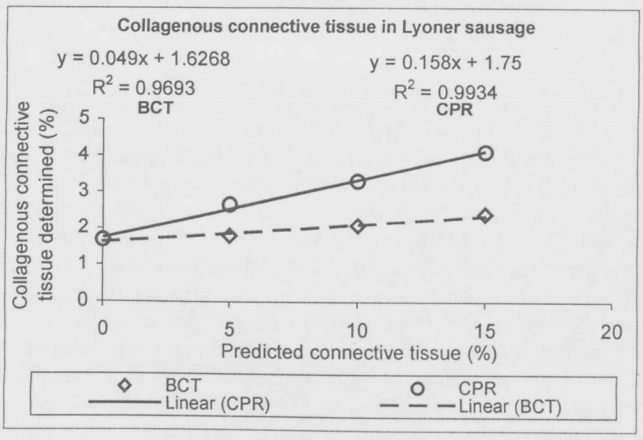


**Figure 1.** Relationship between predicted connective tissue and collagenous connective tissue determined in Lyoner type sausage containing amounts of bovine connective tissue (BCT) and cooked pork rind (CPR)

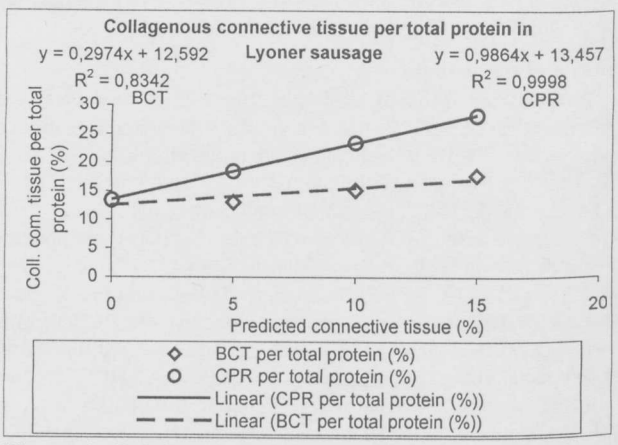


**Figure 2.** Relationship between predicted connective tissue added and collag. connective tissue per crude protein determined in Lyoner type sausage containing amounts of bovine connective tissue (BCT) and cooked pork rind (CPR)