SALT REDUCTION IN COOKED HAM: EFFECT OF TUMBLING AND SALT LEVEL ON PROTEIN EXTRACTION AND SALT DIFFUSION

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Abstract – The purpose of this study was to investigate the effect of two reduced NaCl contents (1% and 1.2%), combined with three tumbling types (T1, T2 and T3) on salt diffusion and protein extraction in cooked ham production. For each trial, 25 pork deboned thighs were processed. Before and after tumbling, *Semimembranosus* muscles and exudates were analyzed for salt and protein content. Moreover, composition and thermal behavior of myofibrillar protein by SDS-PAGE and DSC respectively were evaluated. No significant influence of salt level on salt diffusion and myofibrillar extraction during massage was observed. On the contrary, tumbling affects (P<0.05) salt-soluble protein extraction. The friction massage with high work-to-rest ratio was the most effective for the extraction of myofibrillar proteins and additionally it allowed non-denaturated actin and myosin extraction.

Key Words – DSC, Myofibrillar proteins, SDS-PAGE

I. INTRODUCTION

In meat industry salt is used as preserving agent, flavoring and it is also responsible for textural properties of processed meat. It activates proteins to increase hydration and water-binding capacity; moreover, it is responsible for protein extraction during tumbling, which leads to the formation of sticky exudates [1, 2]. However, due to the role of sodium in the development of hypertension in sodium-sensitive individuals, public health and regulatory authorities have recommended a reduced dietary intake of sodium chloride [3].

It is well known that myofibrillar proteins are mainly responsible for textural properties of processed meat products; in particular myosin and actin contribute to the development of gelation process during the cooking phase [2]. The production of low salt cooked ham creates the problem of a sufficient extraction of myofibrillar proteins, needed to obtain a compact texture. The aim of the present work is to investigate different tumbling in order to obtain a uniform salt diffusion and a good protein extraction in low salt cooked ham, reaching a salt reduction greater than 25% if compared to the average value (1.8%) of Italian cooked ham.

II. MATERIALS AND METHODS

Experimental design

A factorial experimental design (3x2) was adopted to study three different tumbling (T1, T2 and T3) combined with two salt levels (1% and 1.2%, expressed as g NaCl/100g cooked ham).

Tumbling T1 and tumbling T2 were performed with the same friction massage, modifying work-to-rest ratio (T1=7'/53', T2=10'/50'). This system is smooth and acts through friction between the thighs. Tumbling T3 provides an intense mechanical action, because the machine rotates in vertical direction and thighs are before lifted to the upper part of the machine and then they fall down. It provides a series of nine cycles (15'of work and 45' of rest) followed by a protracted rest (15 hours) in maceration phase. All tumbling were carried out for 48h, at 7°C, under vacuum. Control was as follows: tumbling T1 and 1.8% salt.

For each trial 25 heavy pig thighs were processed at SSICA pilot plant. The raw meat, taken at a local slaughterhouse, has been deboned before processing. A brine for high quality cooked hams was prepared with a standard formulation of ingredients and additives. It was adopted a processing as "open thighs", with a 25% brine injection (calculated on thigh weight). NaCl content in the brine was estimated to obtain 3 salt levels between 1.0% and 1.8% in the end product. Then, the processed thighs were cooked until 69°C (core temperature).

Analyses

For each trial, five thighs, chosen according to weight and pH representative of the batch, were analyzed.

A portion of *Semimembranosus* muscle (SM) was removed before and after brine injection. In addition, the extraction process was monitored during tumbling, making five withdrawals of exudates at different established times (0, 3, 21, 24, 48h). Each sample was analyzed for total protein using the AOAC procedure [4]. NaCl was determined as chloride ion by Volhard method [5]. Sodium content was examined through Cation Analysis Kit by Capillary Electrophoresis System P/ACE MDQ (Baeckman Coulter). Water Holding Capacity (WHC) was determined on raw material samples [6]. Sarcoplasmic and myofibrillar proteins were extracted from meat and exudate samples as described by Barbieri & Rivaldi [7]. Total and myofibrillar proteins were expressed as g/100g (meat or exudate). SDS-PAGE of myofibrillar protein extracts was performed according to the method of Laemmli [8] modified by Barbieri & Rivaldi [7]. Protein bands intensity was evaluated on the gels using the Quantity One software (Biorad). By means of Differential Scanner Calorimeter Pyris1-DSC (Perkin Elmer) enthalpy of denaturation (ΔH) and transition temperature of proteins were determined. Ten milligrams of meat or exudate were scanned from 20°C to 100°C at 10°C/min rate.
Data were evaluated through One-Way Variance analysis (ANOVA); averages were compared by Least Significant Difference (LSD) test ($P<0.05$), using SPSS for Windows v13.0 (SPSS Inc. Chicago, IL).

III. RESULTS AND DISCUSSION

Raw material selection
The thighs to be processed were selected in a narrow weight and pH range: the average weight was 8.95±0.51 kg and the average pH was 5.73± 0.08. The thighs were homogenous for WHC (0.420±0.02) and natural content of sodium (0.06±0.01 g/100g).

Salt diffusion during tumbling
Figure 1 shows the variation of salt content in exudates during tumbling; the displayed bars are the average of the two assessed reduced salt levels, at the same times, *vs* tumbling type.

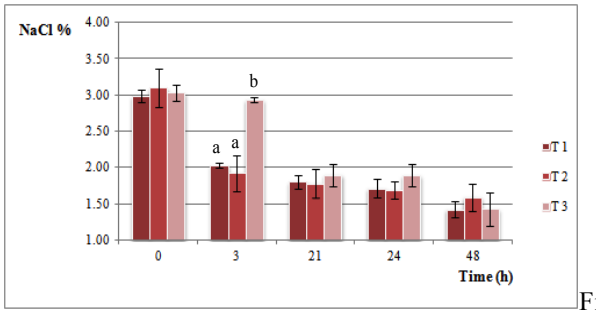

Figure 1. Effect of tumbling type on salt content in exudates.
Different letters on the bars at the same time indicate significantly different means.

At three hours from the beginning of the process, the type of tumbling affects salt absorption ($P<0.05$). Salt penetrated more quickly for T1 and T2, but at other tumbling times, until the end of the process, salt content of exudates was not different ($P\geq0.05$) for T1, T2 and T3.

Table 1. Effect of tumbling type on NaCl content in meat and exudates at the end of process. Comparison with one - way ANOVA.

Tumbling time = 48h					
NaCl (%)		T1	T2	T3	sign p
Meat	1.2	1.37±0.12*	1.67±0.05	1.59±0.14	n.s.
	1.0	1.25±0.05	1.45±0.10	1.23±0.06	n.s.
Exud.	1.2	1.45±0.02	1.75±0.01	1.63±0.03	n.s.
	1.0	1.45±0.02	1.43±0.01	1.22±0.07	n.s.

*mean±std. dev.

During tumbling, the muscular structure is loosened as a result of mechanical treatment, favoring the distribution and absorption of the brine. Therefore, the content of salt in meat and in exudates at the end of process (Table 1), showed that the reduced ionic strength of the brine was effective for salt diffusion. No differences ($P\geq0.05$) were found in meat and exudates, for the assayed salt levels, at the end of tumbling if T1, T2 and T3
are compared.

Total and myofibrillar protein extraction
Analyses of exudates give us information about the extraction of protein during tumbling. Figures 2a and 2b report the increasing trend of total (T.P.) and myofibrillar (M.P.) protein extraction in

exudates. The bars combine the average values of extraction for the two salt levels tested: averages at the same tumbling times were compared by ANOVA. The type of tumbling significantly affects protein extraction (P<0.05).

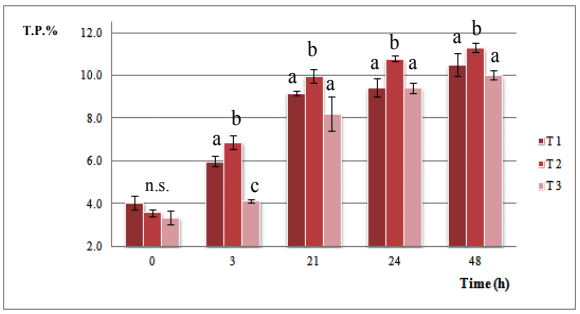

Figure 2a. Effect of tumbling on T.P. in exudates. Different letters on the bars at the same time indicate significantly different means.

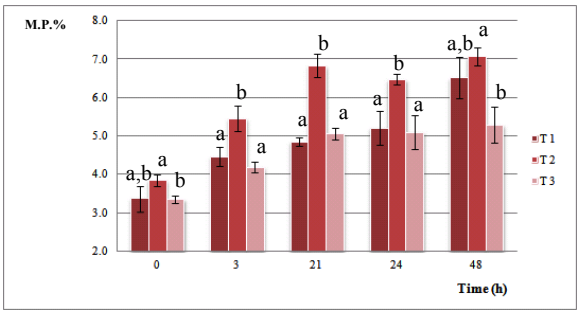

Figure 2b. Effect of tumbling on M.P. in exudates. Different letters on the bars at the same time indicate significantly different means.

Friction massage (T1 and T2) is more effective (P<0.05) than fall tumbling (T3) for T.P., while T2 is the most effective (P<0.05) for M.P. extraction. The friction massage positively affects (P<0.05) protein extraction with main reference to myofibrillar proteins; tumbling T3 was found as the least effective.

Table 2. Effect of salt level on total and myofibrillar protein extraction in exudates and meat at the end of tumbling T1.

		Salt			
		1.8%	1.2%	1.0%	sign p
Exud. 48h	T.P.	10.51±0.03*	10.88±0.61	10.35±0.56	n.s.
	M.P.	6.7±0.13	6.50±0.24	6.02±0.28	n.s.
Meat 48h	T.P.	16.02±1.18	16.42±1.13	16.86±1.69	n.s.
	M.P.	8.35±0.71	7.88±2.27	6.78±2.11	n.s.

*mean ±std.dev.

Table 2 points out that salt level doesn't significantly affect protein extraction. Even the most reduced salt level (1%) together with a 48h tumbling time allowed a good T.P. extraction, while the type of applied tumbling is a key factor to achieve M.P. extraction (Figure 2b).

SDS-PAGE analysis

SDS-PAGE of myofibrillar extracts of tumbling exudates and meat shows the change of protein composition during time (Figure 3).

Troponins T, I, C (38, 23, 20 kDa) and the three light chains of myosin (25,18,15 kDa) bands are detectable in the samples taken after 15' tumbling time. The presence of high molecular weight myofibrillar protein begins after at least 3 hours of tumbling. Myosin and actin are critical for protein gelation during cooking phase; this allows muscles to stick together, providing a compact product. Desmin is an interfilamental constituent of myofibril and represents an index of the damage of muscles tissue [7]. Tropomiosin is an actin-associated protein, which regulates the actin interaction with myosin, according to calcium concentration [9]. Electrophoretic profile of proteins showed that, during tumbling, myofibrillar proteins appear in exudates after 3h: we suppose that the ionic strength was sufficient even with the lower salt level. Figure 3 shows the increase in relative intensity of the same bands in exudates during tumbling, in particular myosin heavy chain (220 kDa), α-actinin (100 kDa), desmin (54 kDa), actin (45 kDa) and tropmiosin (35 kDa).

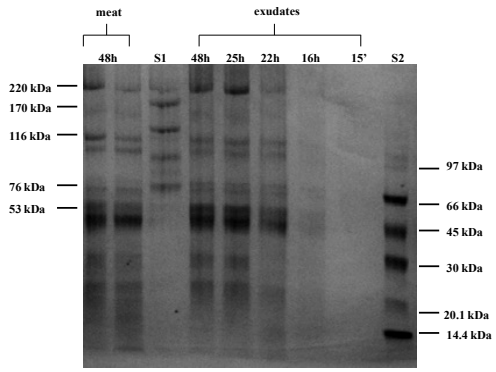

Figure 3. SDS-PAGE of myofibrillar extracts of meat (at the end of T2) and exudates (during T2) at 1 % salt. S1 and S2 are high and low weight molecular markers

respectively.

The relative intensity of these bands increases in exudate extracts and decreases in meat extracts after 48 hours of tumbling (data not shown).

DSC analysis

The changes in thermograms of meat and exudate samples (Figure 4) show the typical pathway of meat with three main transition regions due to the thermal effects associated with denaturation of myosin, sarcoplasmic proteins and actin (54°C, 68°C and 73°C) [10]. Enthalpy of denaturation of samples of meat before processing and exudates at the end of tumbling were calculated. The presence of non-denaturated myofibrillar proteins in exudate at the end of tumbling, in particular myosin and actin, is very important because they are essential for protein gelation during the cooking phase, to obtain a product with good sensory and rheological properties.

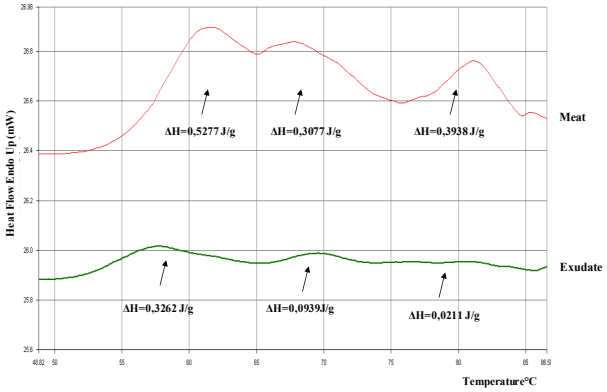

Figure 4. DSC profile of meat (before processing) and of an exudate at the end of tumbling T2 (salt 1 %).

IV. CONCLUSION

The study of the tumbling phase shows that an appropriate massage allows an effective protein extraction even with reduced ionic strength brine. Tumbling prolonged up to 48 hours ensures homogeneous distribution of salt in thighs. There are no significant effects due to the level of added salt, while myofibrillar protein extraction is affected by tumbling type: the friction massage with a high work-to-rest ratio was found to be more effective. Moreover, the presence of not denatured actin and myosin in the final exudate is essential for the development of product texture after cooking.

ACKNOWLEDGEMENTS

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