SOUS VIDE COOKED BEEF MUSCLE. EFFECT OF SALT ADDITION ON BIOCHEMICAL PARAMETERS

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Background

Semitendinosus beef muscles injected with salt solutions -sodium chloride (SC) and sodium tripolyphosphate (STPP)- and cooked by the *sous vide* system (research carried out by our group), showed that both salts -alone or in combination- successfully reduced cooking weight losses (Sanchez, *et al*. 2004). The best results were obtained with the following salts combinations (0.25 g STPP + 1.20 g SC, and 0.25 g STPP + 0.70 g SC, per 100 g tissue), reducing cooking weight losses to almost 0%. Salt incorporation modified both, pH and Hunter Lab *a* parameter. Sample pH increased as STTP concentration increased. Hunter Lab *a* parameter was affected by both, salts and temperature. The optimum cooking temperature (CT) range was 60-65 ºC, this temperature range modified structural components of the muscle inducing disintegration of filaments and lateral and longitudinal shrinkage of myofibrils. The magnitude of the alterations depends on CT. The combination of STPP and SC salts and a CT of 65ºC produced the best gel-like structure that preserves cellular structures and increases water holding capacity (Gonzalez *et al.* 2004). Even though the micro and ultra structural studies described certain mechanisms involved in salts and CT effects on these muscles, it was important to evaluate biochemical parameters in order to confirm or further explain these effects on the final quality of the product.

Objectives

To determine tissue salts concentrations and evaluate its distribution and analyse collagen, salt soluble and total protein content, and protein patterns in cooking juice after the application of both, salts injection and *sous vide* cooking, on *Semitendinosus* muscles. To evaluate myofibrillar protein patterns in non-cooked samples.

Materials and methods

Beef *Semitendinosus* muscles were excised from steers carcasses and collected at a beef packaging plant 48 h after slaughter. Fat free muscles (pH average: 5.49 ± 0.26) were injected (10%w/w, hand operated brine pump) with appropriate salt solutions to obtain the final tissue concentrations of STPP and SC indicated in tables I and II. Injected and non-injected samples were then submitted to *sous vide* cooking procedure, temperatures applied were 55, 58, 65, 72 and 75 ºC (Sanchez *et al*. 2004). Tissue phosphate extraction was carried out as in Sanchez, *et al*. (2004) and the determination was accomplished utilizing a Sigma Diagnostics Kit based on the Fiske-Soubarrow reaction. Sodium Chloride quantification was performed following the directions of Ockerman (1985). Total collagen was evaluated following the procedure reported by Hill (1966). Salt soluble protein extraction was carried out as in Saffle & Galbreath (1981), and protein quantification was completed by the Bradford methodology (1976). Total protein content was achieved by the Kjeldahl method (AOAC 1994). Purified myofibrils were isolated following the method described by Molina & Toldrá (1992), and the SDS-PAGE was performed following the procedure of Laemmli (1970). Molecular weight (MW) and relative quantity of protein bands in the gels were analysed with a Bio Rad GS-800 Calibrated Densitometer.

Results and discussion

Table I shows the average and standard deviation (SD) for each salt actual value (experimentally obtained values) expressed as g/ 100 g dried tissue, and the comparison to the nominal one (theoretically injected value). It can be seen that until 0.25% STPP, the nominal and actual values were close similar, and at higher STPP concentrations lower amount of the salt was retained inside the muscle. Instead, SC was almost totally

retained at all concentrations utilized. This result made evident that the procedure selected to incorporate the salts was appropriate.

Figure 1 depicts the slice sampling performed to study salts distribution. Table II illustrates the significant differences of STPP and SC actual values for each slice sampling. It can be seen that the higher the STPP concentration is, the higher is the difference between values of the centre (site of the injection) and the peripheral positions, indicating a restricted diffusion of this large molecule inside the muscular tissue. However, at the same STPP concentration injected, the presence of SC improved STPP distribution, probably accelerating its diffusion by establishing a competence between both ions (increases effective diffusion coefficient). In the case of SC, this table shows that at the lower SC concentration the salt is evenly distributed nevertheless when the salt concentration is increased to ≥ 0.7 (g/100 g tissue) the distribution became less homogeneous. However, when STPP concentration increased it improved SC distribution as was previously described. The overall result indicated that salts distribution was not as homogeneous as we expected. Consequently further investigations related to the present issue were conducted applying multi-needle injection and tumbling (massaging) procedures to improve salts distribution.

There were not significant differences ($p>0.05$) in total collagen concentration due to the different salt treatments and CTs including 72 ºC (data not shown), indicating that this parameter was not affected by the incorporation of the salts and/or by changes in CT between 55-72 ºC. On the other hand at 75ºC, the total amount of collagen in the tissue consistently diminished (no significant differences were found among salt treatments), probably due to the solubilization/gelatinization of the collagen that occurs at temperatures between 75-85 ºC, with the potential loss of this protein in the cooking-juices.

Fig. 2 shows the salt soluble protein content for each salt treatment at the different CTs grouped as Low CT (55 ºC to 58 ºC), Medium CT (65ºC) and High CT (72ºC to 75ºC) expressed as mg/100 g of dried tissue. Non significant differences were found among salt treatments for each temperature range ($p> 0.05$). Instead, when CT increased the salt soluble protein concentrations in the tissue decreased ($p < 0.05$). This tissue reduction was probably due to a greater protein solubilization induced by the higher temperatures, and the consequent loss of the solubilised protein in the cooking-juices.

Non changes in the total protein content of the tissue (data not shown) were found among salt treatments or comparing the different CTs, it seems that the small but significant changes detected in the tissue concentration of the salt soluble proteins were not evidenced in the total protein content of the tissue.

Supernatants (final wash) of the myofibril extraction of non-cooked samples (figs. 3 & 4), and of cooking-juices (65 ºC, data not shown) coming from salt-treated and control samples, were analysed for type and quantity of proteins. These figures depict the protein patterns of the 10% and 7.5% SDS-PAGE of the same sample. It can be seen that three major bands in the range of 43-45 kD, 105 kD and 200 kD appeared in the 5th line (0.25% STPP + 0.70% SC), which 2 to 3 times higher concentration compared to the other salt treatments and the control. Densitometry analysis showed that the MW of these major bands are in accordance to the actine (42-43 kD), α-actinine (100-103 kD), and Heavy Chain Myosin (200 kD) bands. It appeared that these proteins were much more solubilized by this combination of salts. However, when higher SC concentration (0.25% STPP + 1.2%SC) was applied, it seems that a salting out effect reduced protein solubilization. In the protein pattern of the cooking-juices (data not shown), it was also detected an increment in proteins concentration, mainly in those of lower MW, corroborating partially previous results. Again, it appears that 0.7% SC is the more suitable concentration to increase protein solubilization, and the combination of both salts improved this solubilization. The differences found between supernatant (non-cooked) and cooking-juice was probably due to the CT applied, which also increased protein solubilization disregarding of the salt concentration used. As well, the appearance of smaller MW bands could be related to the temperature-induced degradation. The presence of these solubilised proteins could be responsible for the gel-like structure described previously by this group (Sanchez, *et al.* 2004, Gonzalez, *et al*. 2004), where the strongest gel-like structure formed and the lowest cooking weight loss was obtained with the salt combination of 0.25% STPP + 0.70% SC and 65 ºC cooking temperature.

Conclusions

Salts incorporated were not evenly distributed in the muscle suggesting the use of another methodologies to improve incorporation and distribution.

Salt addition (particularly 0.25% STPP + 0.70% SC) induced protein solubilization in the fresh tissue, this result supports the formation of a gel-like structure at 65 ºC that reduce cooking weight loss reported in previous work of the group.

No other biochemical changes were observed with the different salt combinations.

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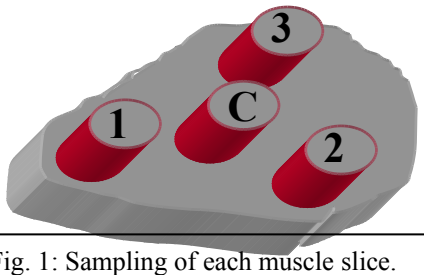


Fig. 1: Sampling of each muscle slice.

Table I: Nominal and Actual value of STPP and SC for each salt treatment

% STPP		% SC	
Nominal Value	**Actual Value ± SD**	**Nominal Value**	**Actual Value ± SD**
***	***	0.70	0.57±0.15
0.10	0.11±0.01	0.20	0.15±0.04
0.10	0.12±0.01	1.20	1.00±0.37
0.25	0.24±0.05	***	***
0.25	0.23±0.03	0.70	0.74±0.11
0.25	0.26±0.05	1.40	1.30±0.18
0.40	0.32±0.05	0.20	0.21±0.07
0.40	0.35±0.05	1.20	1.35±0.35
0.50	0.35±0.05	0.70	0.71±0.15

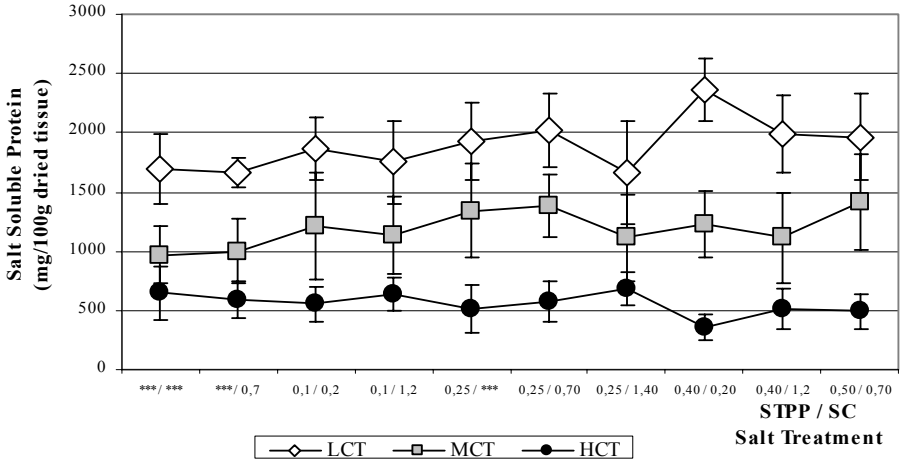
SD: Standard Deviation

Table II: Significant differences for STPP and SC actual values

Nominal Value (%) STPP / SC	STPP				SC			
	1	2	3	C	1	2	3	C
*** / 0.70	*	*	*	*	b	b	b	a
0.10 / 0.20	a	b	ab	ab	a	a	a	a
0.10 / 1.20	a	a	a	a	b	b	b	a
0.25 / ***	a	a	a	b	*	*	*	*
0.25 / 0.70	a	a	a	b	ab	b	b	a
0.25 / 1.40	a	a	a	a	b	ab	b	a
0.40 / 0.20	abc	a	ab	c	a	a	a	a
0.40 / 1.20	ab	a	ab	b	b	ab	ab	a
0.50 / 0.70	a	a	a	b	a	a	a	a

Different letters in the same row (within each salt), indicate significant differences among the positions for each salt treatment

Fig. 2: Salt Soluble Protein (mg/100g dried tissue) for the different salt treatments at each grouped CT



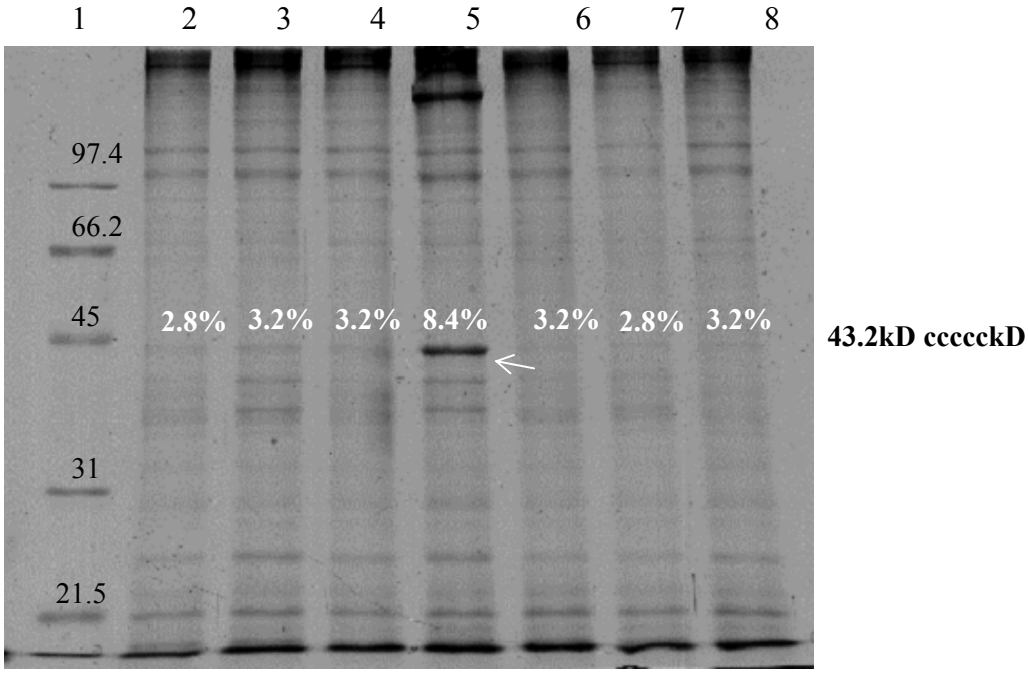


Fig. 3: 10% SDS-PAGE of myofibril supernatants (final wash) Lines: 1= MW standard (kD), 2= SC 0.7%, 3= SC 1.2%, 4= STPP 0.25%, 5= SC 0.7% + STPP 0.25%, 6= SC 1.2% + STPP 0.25%, 7= water injected, 8= control (non injected)

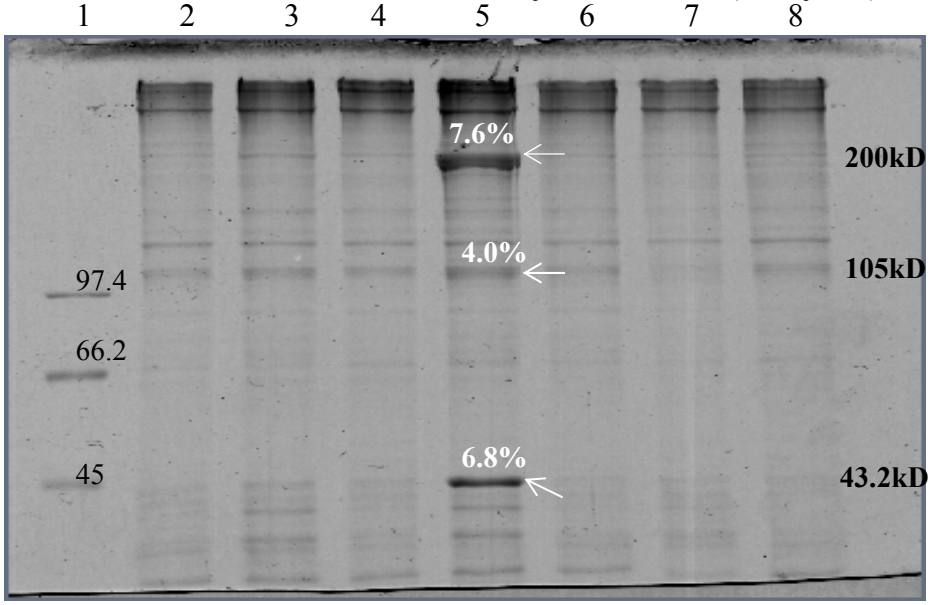


Fig. 4: 7.5% SDS-PAGE of myofibril supernatants (final wash). Lines: 1= MW standard (kD), 2= SC 0.7%, 3= SC 1.2%, 4= STPP 0.25%, 5= SC 0.7% + STPP 0.25%, 6= SC 1.2% + STPP 0.25%, 7= water injected, 8= control (non injected)

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