Meat Science 90 (2012) 701–710

Contents lists available at SciVerse ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Effect of the addition of conventional additives and whey proteins concentrates on technological parameters, physicochemical properties, microstructure and sensory attributes of *sous vide* cooked beef muscles

N. Szerman [a,b,*], C.B. Gonzalez [a,b,c], A.M. Sancho [a,d], G. Grigioni [a,b,d], F. Carduza [a], S.R. Vaudagna [a,b,d]

[a] Instituto Tecnología de Alimentos, Centro de Investigación de Agroindustria, Instituto Nacional de Tecnología Agropecuaria (INTA), CC 77 (B1708WAB) Morón, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[c] Universidad Nacional de Gral. San Martín, Argentina
[d] Fac. Agronomía y Cs. Agroalimentarias, Universidad de Morón, Argentina

ARTICLE INFO

Article history:
Received 8 September 2010
Received in revised form 24 July 2011
Accepted 10 August 2011

Keywords:
Sous vide system
Whey protein concentrate
Yield
Sodium chloride
Beef
Microstructure

ABSTRACT

Beef muscles submitted to four enhancement treatments (1.88% whey protein concentrate (WPC) + 1.25% sodium chloride (NaCl); 1.88% modified whey protein concentrate (MWPC) + 1.25%NaCl; 0.25% sodium tripolyphosphate (STPP) + 1.25%NaCl; 1.25%NaCl) and a control treatment (non-injected muscles) were *sous vide* cooked. Muscles with STPP + NaCl presented a significantly higher total yield (106.5%) in comparison to those with WPC/MWPC + NaCl (94.7% and 92.9%, respectively), NaCl alone (84.8%) or controls (72.1%). Muscles with STPP + NaCl presented significantly lower shear force values than control ones; also, WPC/MWPC + NaCl added muscles presented similar values than those from the other treatments. After cooking, muscles with STPP + NaCl or WPC/MWPC + NaCl depicted compacted and uniform microstructures. Muscles with STPP + NaCl showed a pink colour, meanwhile other treatment muscles presented colours between pinkish-grey and grey-brown. STPP + NaCl added samples presented the highest values of global tenderness and juiciness. The addition of STPP + NaCl had a better performance than WPC/MWPC + NaCl. However, the addition of WPC/MWPC + NaCl improved total yield in comparison to NaCl added or control ones.

1. Introduction

In the last years, the increment of the demand of ready-to-eat products has encouraged the application of new food processing technologies; among them the *sous vide* cooking system. This system is based on the application of a controlled cooking-pasteurization process to raw material (or raw material with intermediate foods) vacuum packaged in a heat-stable pouch or container (Hauben, 1999). After the thermal treatment, the product must be rapidly cooled at temperatures around 0–3 °C, and under this condition it could be stored for 3 to 5 weeks before reheating and consumption (Nyati, 2000; Vaudagna et al., 2002). This technology has emerged as an interesting alternative to expand the current beef-based products market (Garcia-Segovia, Andrés-Bello, & Martínez-Monzo, 2007; Resurreccion, 2003), and its commercial success depends on the quality and the shelf-life of the manufactured goods. However, the most important disadvantage of this technology, when it is applied to meat products, is the retention — inside the package — of the juices released during the thermal treatment (Church & Parsons, 2000; Szerman et al., 2007). As a consequence, the product has an unpleasant appearance and the economical profit is reduced. Thus, with the intention of increasing water holding capacity (WHC) of meat, and consequently, reducing cooking weight loss, additives such as sodium chloride and alkaline phosphates are frequently used for meat products manufacturing (Baublits, Pohlman, Brown, Yancey, & Johnson, 2006; Pietrasik & Shand, 2004, 2005; Vaudagna et al., 2008). Nevertheless, the consumption of high quantities of these additives could cause health problems in sensitive population. For instance, excessive intake of sodium has been linked to hypertension and consequently to an increased risk of stroke and premature death from cardiovascular diseases (Ruusunen & Puolanne, 2005). Alkaline phosphates are associated with the increased risk of bone diseases (Shahidi & Synowiecki, 1997). Several studies were carried out in order to reduce or replace those additives in meat products (Ruusunen & Puolanne, 2005). Most of them have evaluated the addition of natural functional ingredients, as whey and soy protein isolate and concentrate, polysaccharide gums, starches and blood plasma to different types of meat and products (Chen & Trout, 1991; El-Magoli, Laroia, & Hansen, 1996; Ensor, Mandigo, Calkins, & Quint, 1987; Hayes, Desmond, Troy, Buckley, & Mehra, 2006; Hughes, Mullen, & Troy, 1998; Kerry, Long, & Buckley, 2001; Lin & Keeton, 1998; Parks & Carpenter, 1987; Shand, Sofos, &

* Corresponding author at: Instituto Tecnología de Alimentos, Centro de Investigación de Agroindustria, Instituto Nacional de Tecnología Agropecuaria (INTA), CC 77 (B1708WAB) Morón, Argentina. Tel.: +54 11 4621 0446; fax: +54 11 4621 2012.
E-mail address: nszerman@cnia.inta.gov.ar (N. Szerman).

0309-1740/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2011.08.013

Schmidt, 1993; Szerman et al., 2007, Thomsen, 1996). Particularly, the addition of WPC to meat induces an increment of WHC, with the consequent reduction of the cooking weight loss. This additive also improves sensory quality and enhances nutritional values of meat products (Thomsen, 1996).

The addition of brines formulated with WPC to whole beef pieces presents some difficulties, especially in products prepared with low injection rates (10–30%) and high WPC levels [*i.e.* 3.5% (w/w) on product basis]. In those cases, the high viscosity of the brine induces an uneven distribution into the meat pieces. In previous studies of our research group, the most adequate injection and tumbling procedures were selected for the incorporation and distribution of brines containing WPC (Szerman, 2009; Szerman et al., 2007). Also, WPC and NaCl concentrations and cooking temperature were optimized for manufacturing of whole *sous vide* cooked beef muscles (Szerman et al., 2008). In our previous studies we concluded that the addition of WPC was effective to partially replace the sodium chloride in whole beef muscles, producing comparable yields and improving sensory attributes (Szerman et al., 2008). However, the addition of WPC had a lesser influence in the total yield when the NaCl concentration was higher than 1.6% (w/w) when the *sous vide* cooking-pasteurization protocol was 70 °C–2 min at the slowest heating point of muscles (Szerman et al., 2008). Moreover, considering the sensory analysis results of that study, it was suggested the maximum concentrations of NaCl and WPC of 1.9% and 2.6%, respectively, for the same cooking protocol. Regarding MWPC, there are few studies wherein its performance for the manufacturing of cooked meat products was evaluated (Thomsen, 1996). In addition, studies concerning the effect of MWPC on WHC, in comparison to the effect of native WPC, are scarce.

The objective of the present research was to compare the effect of the incorporation of the different additives [NaCl alone or in combination with WPC or MWPC or STPP] on technological parameters, physicochemical properties, microstructure and sensory attributes of *Semitendinosus* beef muscles cooked by *sous vide* system.

2. Materials and methods

2.1. Materials

Forty *Semitendinosus* muscles were dissected from British breed steer carcasses 48 h post slaughter, trimmed free of fat, vacuum packaged (Cryovac BB4L, permeability to: O_2 30 $cm^3.m^{-2}$, 24 $h^{-1}.bar^{-1}$; CO_2 150 $cm^3.m^{-2}.24\ h^{-1}.bar^{-1}$; vapour 20 $g.24\ h^{-1}.m^{-2}$, Sealed Air Co., Buenos Aires, Argentina), and stored for 72 h at 1.5 ± 0.5 °C until their processing. The unfatted raw muscles had an average weight of 1623.4 ± 157.6 g and an average pH of 5.50 ± 0.08. Total viable count mean value of raw muscles was 2.1 log CFU cm^2. Brines were formulated with the following ingredients: native whey protein concentrate [WPC, Lacprodan® 80, Arla Food Ingredients S.A., Buenos Aires, Argentina; protein content as is 85.9 ± 2.1% (w/w)], modified whey protein concentrate [MWPC, Nutrilac® SA7505, Arla Food Ingredients S.A., Buenos Aires, Argentina; protein content as is 86.1 ± 1.6% (w/w)], sodium tripolyphosphate (STPP, N 15–16, Chemische Fabrik Budenheim R.A Oetker, Budenheim) and sodium chloride (NaCl, Dos Anclas, Buenos Aires, Argentina). Also, ARYSA D20 antifoam [21% dimethylpolysiloxane (active compound), Arysa Argentina S.A., Buenos Aires, Argentina] was added to the brines.

2.2. Experimental design

A completely randomized block design with five fixed treatments (Table 1) and two randomized blocks was applied in this study. In each processing batch (corresponding to a block), four experimental units (whole muscle) were used for each treatment, using a total of twenty (20) muscles. In the complete design a total of forty (40) *Semitendinosus* muscles were used.

Table 1
Treatments applied on *Semitendinosus* beef muscles. Additive concentrations are expressed on the basis of muscle just injected (%w/w).

Treatments	Additives (%)[a]	
T1	NaCl: 1.25	WPC: 1.88
T2	NaCl: 1.25	MWPC: 1.88
T3	NaCl: 1.25	STPP: 0.25
T4	NaCl: 1.25	
T5		Control (non-injected)

NaCl, sodium chloride; WPC, whey protein concentrate; MWPC, modified whey protein concentrate; STTP, sodium trypoliphosphate.
[a] Expressed on injected raw muscle weight basis.

2.3. Tumbling and injection procedures

Firstly, all muscles (20) corresponding to a processing batch (block), were vacuum packaged in Cryovac CN510 bags (permeability to O_2 35 $cm^3.m^{-2}$, 24 $h^{-1}.bar^{-1}$, Sealed Air Co., Buenos Aires, Argentina), then submitted to a pre-injection tumbling (PreIT) treatment. This treatment consisted in the application of a continuous tumbling at 8.5 rpm for 0.5 h, at 1.5 ± 0.5 °C in a Lance Industries tumbler (model LT-15, Allenton, USA) using a drum load of 45 kg (half of the maximum working capacity) under constant vacuum (2 kPa). After the application of PreIT, the muscles were stored overnight at 1.5 ± 0.5 °C. The conditions applied in the PreIT procedure were based on previously reported studies (Szerman, Vaudagna, & Gonzalez, 2006; Szerman et al., 2007). Afterwards, muscles (except the ones from T5) were injected using an automatic multi-needle injector (36 needles, FRICOR, Buenos Aires, Argentina). Brines were formulated to reach the concentrations of additives established by the experimental design (Table 1). Those concentrations were selected based on previous studies (Szerman et al., 2008; Vaudagna et al., 2008). The antifoam was added to the brines at 0.15% (w/v). The temperature of brines was 1.5 ± 0.5 °C. The brine pH was different according to its formulation: 6.91 ± 0.06 for MWPC + NaCl, 6.92 ± 0.05 for WPC + NaCl, 8.07 ± 0.14 for NaCl and 8.13 ± 0.07 for STPP + NaCl. Muscles, at a temperature of 3.0 ± 1.0 °C, were injected to 130% over original weight (measured before the PreIT). In order to achieve this injection level, muscles were injected once, and then turned over and re-injected. The injection pressure was between 100 and 200 kPa, depending on the viscosity of the brine, and the number of strokes was 30 per minute.

Immediately after the injection procedure, muscles were vacuum packaged in Cryovac CN510 bags. Then, they were submitted to a post-injection tumbling (PostIT) treatment, which consisted in the application of a continuous tumbling at 8.5 rpm for 5 h at 1.5 ± 0.5 °C (drum load of 45 kg; vacuum at 2 kPa) in the equipment previously described. Thereafter, muscles were weighed and vacuum packaged into cook-in bags (Cryovac CN510) and finally submitted to *sous vide* cooking. As it was described for the PreIT, the conditions selected for the application of PostIT were based on previously reported studies (Szerman et al., 2006; Szerman et al., 2007). Control muscles (non-injected, T5) were submitted to the same PreIT and PostIT treatments as injected ones.

2.4. Sous vide treatment

All muscles (20) corresponding to a processing batch (block) were cooked in a water cascading retort (Steriflow Barriquand, model Microflow Roanne, France), operated in static basket mode. Time–temperature evolutions at the slowest heating point (SHP) of three muscles and in the retort chamber were measured using T type thermocouples and recorded with a digital multimeter Hydra 2625A

data logger (John Fluke Mfg. Co., Inc., Everett, USA). Temperature readings were taken at intervals of 30 s (scanning time) and the accuracy of temperature measurement was ±0.1 °C. In the present study, a temperature–time combination of 70 °C–2 min was applied at the muscle SHP. This treatment was previously reported by Gaze, Brown, Gaskell, and Banks (1989) to achieve a 6D reduction of *Listeria monocytogenes*. The suggested temperature–time combination was then applied by Hansen, KnØchel, Juncher, and Bertelsen (1995) and Szerman et al. (2007, 2008) for the *sous vide* processing of beef pieces. Immediately after cooking, samples were immersed in an ice-water bath until the temperature at SHP reached 26.0 ± 1.0 °C (Vaudagna et al., 2002) and finally stored at 1.5 ± 0.5 °C for 18 h until testing.

2.5. Sample analysis

2.5.1. pH measurement

The pH value was measured in the same muscle (raw and cooked). For raw samples, 1.0 cm-slice was separated from the end part of the muscle; and for cooked ones, 1.0 cm-slice was excised from the middle part. Slurries (5 g of sample: 25 ml of distilled water standardized at pH 7) were prepared with a laboratory blender (Stomacher™, Colworth, UK). The pH measurement was performed in duplicate with a pH-meter (Thermo Orion 710A+, Beverly MA, USA) equipped with a combination pH electrode (Thermo Orion Model 8102BN ROSS Electrode, Beverly MA, USA) and a ATC-Probe (Thermo Orion, Beverly MA, USA).

The pH variation (pH_{var}) was calculated as the difference between the cooked and the raw meat pH.

2.5.2. Technological parameters

The technological parameters measured on each muscle were: post-injection tumbling weight loss (P_1), cooking weight loss (P_2) and total processing yield (TY).

Each weight loss was determined using the relationship:

$$P_i = \frac{100 \times \left(m_i - m_f\right)}{m_{urm}}$$

where, for P_1, m_i is the mass of the injected muscle and m_f is the mass of the muscle after PostIT treatment; while for P_2, m_i is the mass of muscle after PostIT treatment (prior to *sous vide* treatment) and m_f is the mass of the muscle after thermal treatment. These percentages were calculated based on the mass of the unfatted raw muscle, m_{urm}.

TY was determined using the relationship:

$$TY = \frac{100 \times m_f}{m_{urm}}$$

where m_f is the mass of the muscle after thermal treatment and m_{urm} is the mass of the unfatted raw muscle.

2.5.3. Expressible moisture

Cooked meat samples of 1.5 ± 0.2 g were placed in a 50 ml centrifuge tubes containing a thimble, which consists in a filter paper Munktell 1003 (6 μm particle retention) folded around with a second filter paper Munktell 1 F (3 μm particle retention). Then, tubes were centrifuged at 4800×*g* for 20 min at 4 °C in a Sorvall (Model RC3C, Sorvall Instruments) centrifuge. All samples were run in triplicate. The expressible moisture (EM) was expressed as:

$$EM = \frac{100 \times (m_b - m_a)}{m_b}$$

where m_b and m_a are the mass of the sample before and after centrifugation, respectively.

2.5.4. Shear force

Warner Bratzler shear force (WBSF) was determined on eight cores (2.0 cm length; 1.27 cm diameter) obtained from a 2.0 cm-thick slice separated from the central portion of each cooked muscle. The procedure was performed following the general guidelines established by AMSA (1995). For this purpose, a Warner–Bratzler meat shear device (Chatllon, New York, USA) with a triangular shear was used.

2.5.5. Microstructure analysis

For scanning electron microscopy (SEM) analysis, pieces of 2×2×10 mm were excised from the central area of a slice separated from the medial portion of raw and cooked muscles. Samples were immediately fixed in glutaraldehyde solution (2% v/v). Then, three successive washes were made with sodium phosphate buffer 0.1 M pH 7, followed by dehydration in ethanol solution (15 min each) at increased concentrations (50% to 100% v/v). Afterwards, samples were immersed in ethanol:acetone solution (50:50), and acetone. The specimens were mounted on holders and coated with gold (Polaron model E5100). The chosen areas were viewed at 1000–10000 times magnification. Microscopic evaluation was performed using a Leitz-AMR Model 1200 electron microscope and micrograph pictures were taken with a 35 mm camera.

2.5.6. Colour parameters

Colour parameters of cooked muscles were measured with a BYK Gardner Spectrometer model ColorView 9000 (Silver Spring, USA) with a large view area using illuminant D65 and 10° observer. Results were expressed as L* (lightness), a*(redness/greenness) and b* (yellowness/blueness) in the CIELab system. Each colour parameter was measured on 4 different points on the surface of a 2.5 cm-thick slice, excised from the central portion of each cooked muscle, and its average value was used for statistical analysis.

2.5.7. Visual appearance analysis

The appearance of cooked beef was evaluated on a 2.0 cm-slice separated from the central portion of the muscle, and was assessed by 10 trained panellists in a Veri-Vide CAC120 Gabinet box (dimensions 129×75.5×62 cm) illuminated by a Veri-Vide Artificial Daylight F-40 T12/D65 (colour temperature 6500 K, under tolerances established in BS 950). The vacuum packed samples, prior to evaluation, were unwrapped and kept at room temperature for 45 min. All observers had been screened for normal colour vision using the Ishihara Colour Vision Test (Ishihara, 1998). Each cycle of viewing took between 30 and 45 min. During training, the group of panellists received a set of samples as colour references. These references were prepared by conversion of a portion of *Longissimus dorsi* beef muscle to fully deoxy, oxy and metmyoglobin following procedures described in AMSA (1995). Panellists were requested to judge four visual attributes of the samples following the specification of AMSA (1991) modified as described in Szerman et al. (2007): cooked beef colour (1: Very red, 2: Medium red, 3: Pink, 4: Slightly pink, 5: Pinkish-grey, 6: Grey-brown, 7: Brown); colour uniformity (1: No variation, 2: Slight amount of variation, 3: Small amount of variation, 4: Moderate amount of variation, 5: Extreme amount of variation); intensity of defects (1: None, 2: Slight, 3: Small, 4: Moderate, 5: Extreme), and amount of defects [expressed as percentage of total slice area; 1: Non (0%), 2: Slight (1–19%), 3: Small (20–39%), 4: Modest (40–59%) 5: Moderate (60–79%), 6: Extensive (80–99%), 7: Total (100%)].

2.5.8. Sensory analysis

Eight trained panellists, selected based on their experience in sensory analysis of meat, evaluated the eating quality of cooked muscles. The panellists were trained following the directions of Cross, Moen, & Stanfield, 1978. Samples (cubes, 1.5 cm length) were obtained from

the central portion of the muscles, corresponding to each treatment, and warmed previous to sensory evaluation. In each analysis session, panellist evaluated — at random — samples from different treatments, being sessions replicated twice. Attributes (cooked beef flavour and odour, myofibrillar and overall tenderness, juiciness and connective tissue amount) were evaluated on a nine point scale (1: extremely non-beef like, extremely though, extremely dry, abundant; 9: extremely beef like, extremely tender, extremely juicy, none). Additionally, panellist were asked to assess the intensity of recognized off-flavours ("liver", "sour", "metal", "stale", "salty" and "milky") and off-odours ("milky", "cheesy"), by representing the perceived stimulus on a 10 cm linear scale. The left end of the line corresponds to "no off-flavour/off-odour" while the right end of the line represented "extreme off-flavour/off-odour". All tests were conducted in individual booths under colour neutralizing lights.

2.6. Statistical analysis

For statistical analysis, a GLM (general linear model) was applied. For all the parameters evaluated in the present study, the block effect was non-significant ($p > 0.05$). For each parameter, significant differences between treatments were determined using the Bonferroni test ($p = 0.05$). Data were analysed using SAS software (version 8, SAS Institute Inc., 2004, Cary, NC).

3. Results and discussion

3.1. pH variation

The pH variation (pH_{var}) values obtained for all treatments are presented in Table 2.

Thermal treatment *per se* induced an increase of the pH value, as can be noticed in the difference of 0.31 ± 0.05 units between cooked and raw control muscles (T5). In a previous study, it was observed a similar pH difference between cooked (70 °C) and raw *Semitendinosus* beef muscles (Szerman et al., 2007). This increase would have been caused by the thermal denaturation of meat proteins and consequent exposure and ionization of the buried groups that took place during cooking (Ledward, 1979).

Besides, pH_{var} values of muscles with different additives were significantly higher ($p < 0.05$) than control one (T5). However, these increments were dependent on the additive used. Muscles with STPP + NaCl (T3) presented a significantly higher ($p < 0.05$) pH_{var} value than the ones with WPC/MWPC + NaCl (T1 and T2), which were similar between them. Muscles injected with brines only formulated with NaCl (T4) presented pH_{var} values significantly lower ($p < 0.05$) in comparison to muscles treated with the other additives. The mean values of cooked muscle pH were 6.03, 6.00, 6.19, 5.91 and 5.83 for T1, T2, T3, T4 and T5, respectively. The aforementioned pH increases were consequence of both, the thermal treatment and the addition of brines with higher pH than the raw beef (5.4–5.7). Brine pHs ranged from 6.8, when they were prepared with WPC/MWPC + NaCl, to 8.1 for the ones formulated with STPP + NaCl.

In previous studies of our group, it was observed a similar pH increase due to WPC + NaCl addition, also it was demonstrated that the pH increase was dependent on the WPC concentration (Szerman et al., 2007, 2008). Additionally, a similar behaviour was observed with the addition of STPP; when the amount of this salt increased from 0 to 0.25% (w/w) the pH increased almost linearly in *sous vide* cooked muscles (Vaudagna et al., 2008).

The pH value of muscles is considered an important factor since this parameter has a significant effect on the swelling of the fibres, and consequently, on the WHC of the tissue. The minimum WHC is reached when the pH is equal to the isoelectric point of meat proteins (pH 5.3–5.5) (Shults, Russell, & Wierbicki, 1972). For that reason, pH increases achieved in muscles injected with the different additives, particularly STPP, have a direct effect on the weight losses obtained during muscles processing.

Table 2
Effects of the incorporation of different additives to *Semitendinosus* beef muscles on pH variation (pH_{var}), post-injection *tumbling* weight loss (P_1), cooking weight loss (P_2) and total yield (TY).

Treatments[1]	Technological parameters			
	pH^2_{var}	P_1 (%)	P_2 (%)	TY (%)
T1	0.52 ± 0.05 b	7.4 ± 1.4 b	28.2 ± 3.3 bc	94.7 ± 6.2 b
T2	0.51 ± 0.06 b	7.5 ± 1.6 b	29.2 ± 3.3 b	92.9 ± 3.6 b
T3	0.70 ± 0.06 a	3.3 ± 0.8 c	20.6 ± 3.4 d	106.5 ± 3.9 a
T4	0.42 ± 0.03 c	9.6 ± 1.5 a	35.6 ± 3.3 a	84.8 ± 4.3 c
T5	0.31 ± 0.05 d	1.2 ± 0.4 d	25.7 ± 1.9 c	72.1 ± 2.1 d

a–d Means values with different letters in the same column are significantly different ($p < 0.05$).
[1]Concentrations in muscles after injection: T1: WPC 1.88% + NaCl 1.25%; T2: MWPC 1.88% + NaCl 1.25%; T3: STPP 0.25% + NaCl 1.25%; T4: NaCl 1.25%; T5: Control (non-injected).
[2]pH_{var} = cooked beef pH − raw beef pH.

3.2. Technological parameters

The effect of the addition of different additives on PostIT weight loss (P_1), cooking weight loss (P_2) and total yield (TY) of *Semitendinosus* beef muscles are presented in Table 2.

Regarding P_1, injected muscles presented significantly higher ($p < 0.05$) values than control ones (Table 2). Before cooking, injected muscles had higher water content in comparison to non-injected muscles (T5); consequently, the last were only able to lose part of the free physiological water of the tissue. Muscles injected only with NaCl (T4) presented the highest P_1 values among those treated with additives. The addition of WPC/MWPC + NaCl (T1 and T2, respectively) significantly reduced P_1 values ($p < 0.05$) in comparison to NaCl alone (T4). It is important to note that the addition of the brine with the highest pH value (STPP + NaCl) produced the lowest P_1 value.

The swelling of fibres is the most accepted mechanism to explain the increase of WHC with the addition of NaCl to fresh meat (Offer & Knight, 1988). In the present study, muscles with NaCl retained approximately a 66% of the incorporated brine ($P_1 = 9.6\%$; injection level 30%). However, when STPP + NaCl were incorporated, the water retention increased and P_1 was reduced up to 3.3%, which represents approximately a 90% of brine retention. The effect of the STPP on WHC was extensively reported (Offer & Knight, 1988; Ruusunen & Puolanne, 2005; Trout & Smith, 1986). Enhancement by STPP is observed even at low NaCl concentrations (0.4 M; Ruusunen & Puolanne, 2005) but not without NaCl, as a result of the synergic action of both salts in the depolymerisation of the thick filaments (Offer & Knight, 1988).

Muscles with WPC/MWPC + NaCl may retain water by a different mechanism than muscles with STPP + NaCl or NaCl alone. Thus, the P_1 values obtained for T1 and T2 treatments were similar between them; higher than for T3 (STPP + NaCl) and lower than for T4 treatment (NaCl alone). In this way, whey proteins might partially retain the water added with the brines due to their hydration properties (protein–water interactions) or to the formation of protein aggregates (Huffman, 1996). Moreover, it should be considered that brines formulated with WPC/MWPC + NaCl had a lesser amount of water than those formulated with salts (STPP + NaCl or NaCl alone).

For all treatments, except T1, cooking weight loss (P_2) was significantly different ($p < 0.05$) than the control (Table 2). Muscles with NaCl alone (T4) had the highest P_2 value (35.6%). The incorporation of STPP to the brine (T3) reduced significantly this parameter ($p < 0.05$), presenting the lowest value (20.6%). Similar P_2 values were obtained in muscles with WPC/MWPC + NaCl (T1 and T2, respectively), which were significantly lower ($p < 0.05$) than T4 but higher ($p < 0.05$) than T3.

Thermal treatment of muscles injected with salts (NaCl; STPP) induces the formation of a gel-like structure. The formation of this type of gels requires, as a first step, the salt-induced extraction and solubilisation of myofibrillar proteins and then, the application of heat (Katsaras & Budras, 1993; Offer & Knight, 1988; Vaudagna et al., 2008). The network formed helps to maintain the structural organization of the cells by minimizing fibres contraction and to retain the water incorporated with the brine through capillarity forces (Katsaras & Budras, 1993). In the present study, the addition of STPP + NaCl to muscles increased the pH and ionic strength values, consequently enhancing the absorption of the brine inside the fibres and the extraction and solubilisation of myofibrillar proteins. From these proteins, the subsequent heating would have induced the formation of a three-dimensional network capable of retaining water inside.

The addition of WPC/MWPC + NaCl to the muscles decreased cooking weight losses in comparison to muscles with NaCl alone. During thermal treatment, whey proteins are partially unfolded and consequently sites for water binding were available, favouring water retention and formation of aggregates (Huffman, 1996). The ionic strength reached with the addition of brine formulated with NaCl alone, would not be enough to achieve an important swelling of the myofibrils and a large protein extraction and, in consequence, the gel-like structure obtained would be weaker (Vaudagna et al., 2008). The P_2 values obtained for control samples were similar to the ones observed in previous studies (Szerman et al., 2007).

Regarding total yield (TY), muscles injected with additives (T1, T2, T3, and T4) presented significantly higher values than the control (T5, Table 2). The maximum yield was obtained for muscles with STPP + NaCl (T3), which was approximately a 34% above that attained for control muscles (106.5% *vs.* 72.1%). The addition of WPC/MWPC + NaCl (T1 and T2) also improved TY compared to the control muscles. The increase of this parameter was approximately 22% (94.7–92.9% *vs.* 72.1%). It is important to remark that both whey concentrates used (native or modified), produced similar TY values. In a previous study, it was observed that when *Semitendinosus* beef muscles were injected with WPC + NaCl similar yields were obtained when the concentration of one ingredient was diminished while the other one was increased. However, this effect was observed up to 1.9% NaCl, above that level further increments of WPC concentrations did not enhance TY (Szerman et al., 2008).

3.3. Expressible moisture

The effect of the incorporation of different additives on the expressible moisture (EM) of *sous vide* cooked *Semitendinosus* beef muscles is shown in Fig. 1.

Muscles with STPP + NaCl and WPC/MWPC + NaCl had similar EM values, which were significantly ($p<0.05$) higher than the control. EM value from T4 samples was also significantly ($p<0.05$) higher than the control, but similar to WPC/MWPC + NaCl added muscles.

Muscles which presented the higher TY values lost the major amount of water during centrifugation, indicating a positive relationship between TY and EM. Similar behaviour was observed by Szerman et al. (2007). In addition, Boles and Shand (2001) found that EM had a positive correlation with the cooking yield achieved in different enhanced beef muscles (1.8% NaCl, 1.0% sugar and 0.3% STPP) cooked to an endpoint temperature of 73 °C.

3.4. Shear force

The Warner–Bratzler shear force values (WBSF) obtained for the different treatments are depicted in Fig. 2. All WBSF values showed a tendency to be reduced by the incorporation of additives. However, only the muscles with STPP + NaCl (T3) presented significantly ($p<0.05$) lower WBSF values than control ones (T5).

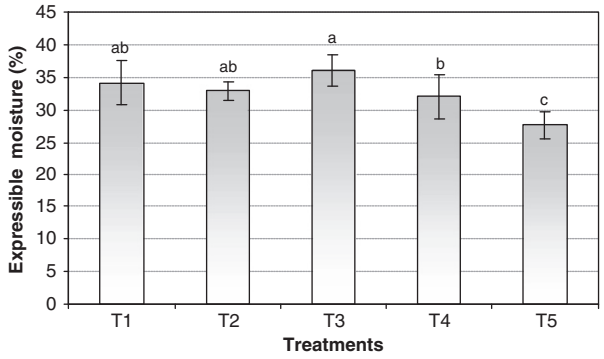


Fig. 1. Effect of the incorporation of different additives to *Semitendinosus* beef muscles *sous vide* cooked on expressible moisture (EM) values. T1: WPC 1.88% + NaCl 1.25%, T2: MWPC 1.88% + NaCl 1.25%, T3: STPP 0.25% + NaCl 1.25%, T4: NaCl 1.25%, T5: Control (all the concentrations are expressed on the basis of muscle just injected). a–c mean values are significantly different ($p<0.05$).

Previous reports support these findings, thus Baublits et al. (2006) reported that *Biceps femoris* beef muscles added with 0.4% STPP and 1.5% NaCl (cooked at 70 °C) showed significantly lower WBSF values when they were compared to non-injected muscles. Vote et al. (2000) achieved lower values of shear force for loin beef pieces containing 0.5% NaCl, 2.5% sodium lactate and 0.25% STPP (cooked at 66 °C and 77 °C) compared to control non-injected pieces. Sheard and Tali (2004) have studied the incorporation of different additives to pork loin cooked at 79 °C, establishing that the addition of 0.5% NaCl and/or 0.5% STPP reduced WBSF values in relation to the control muscles (non-added). Moreover, Pietrasik and Shand (2004, 2005) proposed a relationship between the reduction of WBSF values and the increment of the WHC of meat. Baublits et al. (2006) have suggested that the effect of salts addition on the shear force of beef may be related to the increase of protein solubilisation and the water retention enhancement, due to the formation of a protein–water matrix that requires less force to shear than non-injected beef pieces. In the present study, the described relationship between TY and WBSF was only observed for samples from T3.

3.5. Microstructure analysis

The study of the microstructure of raw and *sous vide* cooked meat tissue, provided further information to the results of technological and physical parameters and allowed a more complete interpretation of the effects produced by the different treatments.

Micrographs obtained of transverse sections of raw muscles injected with different additives and control muscles all subjected to tumbling treatments are presented in Fig. 3. Control muscles (T5)

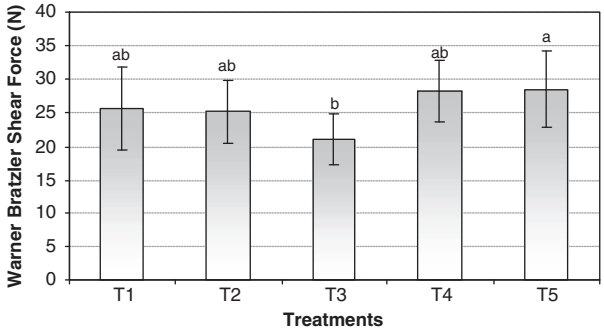


Fig. 2. Effect of the incorporation of different additives to *Semitendinosus* beef muscles *sous vide* cooked on Warner–Bratzler shear force (WBSF) values. T1: WPC 1.88% + NaCl 1.25%, T2: MWPC 1.88% + NaCl 1.25%, T3: STPP 0.25% + NaCl 1.25%, T4: NaCl 1.25%, T5: Control (all the concentrations are expressed on the basis of muscle just injected). a–b mean values are significantly different ($p<0.05$).

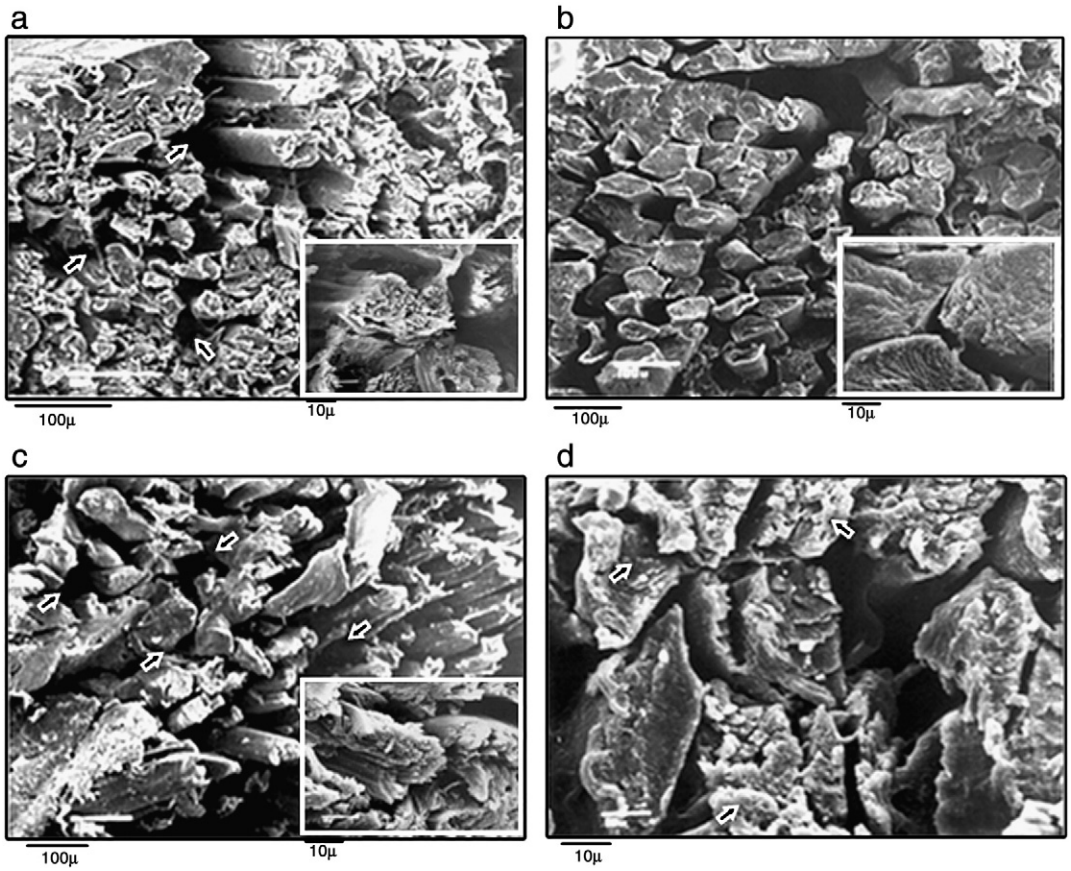


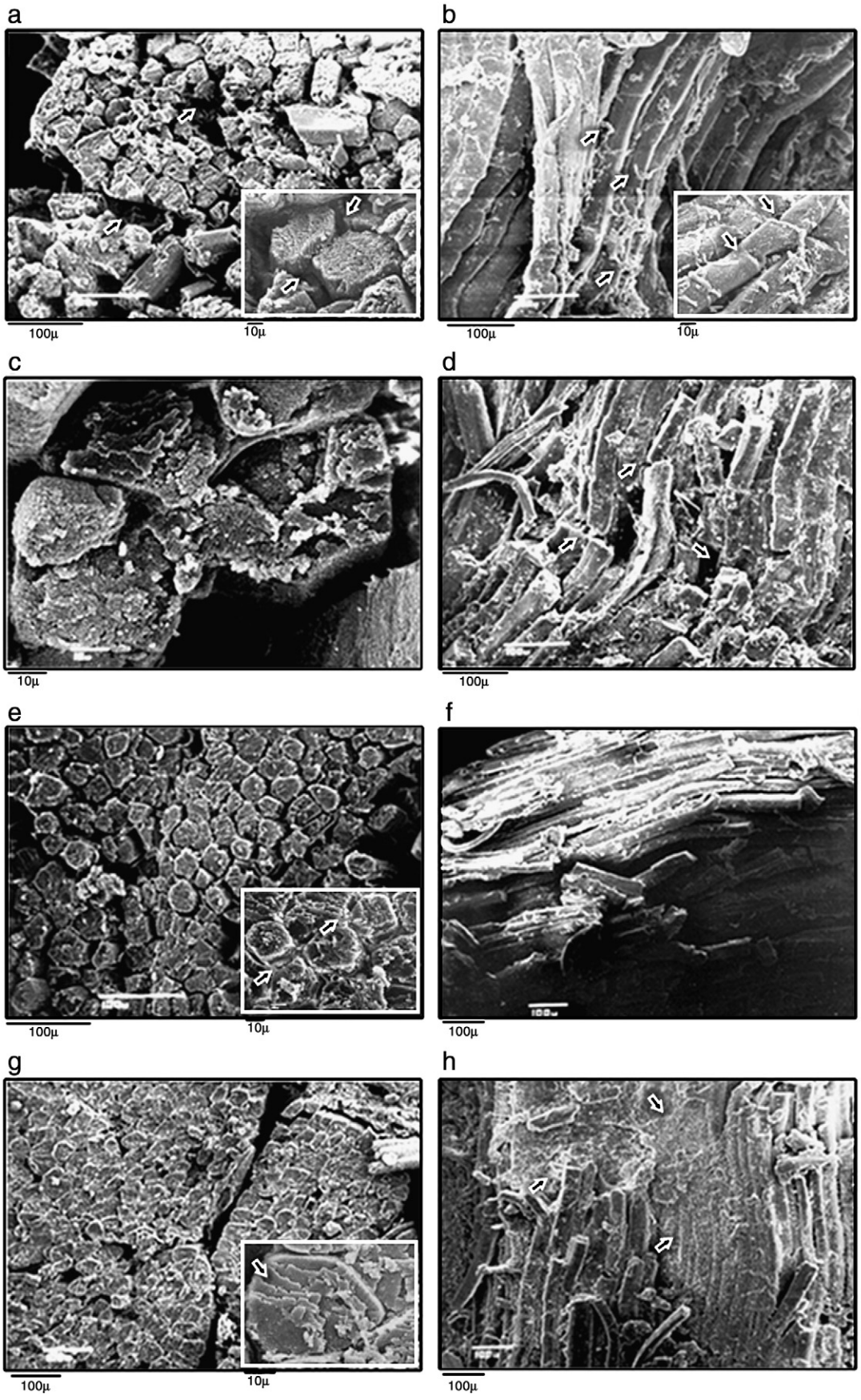
Fig. 3. Scanning electron micrographs of *Semitendinosus* beef muscles raw, 120 h post-mortem, submitted to pre-injection tumbling 8.5 rpm–0.5 h, injected to 130% (over original weight) and then submitted to post-injection tumbling 8.5 rpm–5 h. Transversal views: a) T5 (control), arrows indicate gaps among fibre bundles and between fibres; b) T4 (NaCl 1.25%); c) T2 (MWPC 1.88% + NaCl 1.25%), arrows indicate gaps among fibre bundles and between fibres; d) T3 (STPP 0.25% + NaCl 1.25%), arrows indicate the presence of material onto the fibres.

showed a disorganized structure, with appreciable gaps among fibre bundles and between fibres (Fig. 3a). In addition, the myofibrils showed a dehydrated and separated appearance even though they did not show an important fragmentation (inset in Fig. 3a). The NaCl added samples (T4, Fig. 3b) showed some differences with regard to the control (T5, Fig. 3a). The structure was also disorganized although it was more compacted than those from control samples. Thus, micrograph depicts fibres nearer than the fibres of control samples, due to the swelling of the myofibrils (inset in Fig. 3b). Muscles with MWPC + NaCl (Fig. 3c) and WPC + NaCl (not shown) presented similar micrographs. The structure appeared as disorganized as the control, showing gaps among fibre bundles and between fibres. In addition, myofibrils seemed to be dehydrated, separated and partially fragmented (inset in Fig. 3c). In the presence of whey proteins, NaCl did not produce the same structural changes that it did when it was added alone, particularly in relation to the hydration of myofibrils. Microstructure of muscles with STPP + NaCl (T3, Fig. 3d) presented more hydrated fibres in comparison to samples from treatments T5 and T2 (Fig. 3a and c). It was also seen as the presence of materials onto fibres, which could be composed of fragmented myofibrils and extracted myofibrillar proteins.

A general view of all micrographs from raw muscles indicated that cell microstructure was mainly modified by the mechanical treatment applied (tumbling). This effect was expected, because the application of tumbling treatment produces the separation of fibres and myofibrils and the disruption of the membranes (Feiner, 2006; Xargayó, 2010). Also it was found that tissue microstructure changes were dependent on the incorporated additive. Thus, muscles with NaCl alone or STPP + NaCl presented organized microstructures with compacted fibres and hydrated myofibrils. On the contrary, in samples with WPC/MWPC + NaCl, the myofibrils were dehydrated, separated and partially fragmented. Regarding these microstructural changes, it could be stated that the minimum P_1 value observed in muscles with STPP + NaCl was a consequence of a higher myofibrillar swelling and improved extraction of myofibrillar proteins (Offer & Knight, 1988). Also muscles with WPC/MWPC + NaCl hold more brine than those with NaCl alone (higher P_1); however nonperceptible myofibrillar hydration was observed in the muscles with WPC/MWPC + NaCl. Thus, it appeared that the WHC enhancement took place by a different mechanism than myofibrillar swelling. This could be associated, as it was aforementioned, to the capacity of hydration of whey proteins and/or to the possibility of forming aggregates capable of retaining water.

The analysis of the micrographs obtained from *sous vide* cooked muscles contributes to the understanding of the effect of the evaluated additives on the cooking weight loss (P_2). Micrographs from control cooked muscles show a dehydrated aspect, with a disorganized structure (Fig. 4a,b), which was more compacted in comparison to

Fig. 4. Scanning electron micrographs of *Semitendinosus* beef muscles, 120 h post-mortem, submitted to pre-injection tumbling 8.5 rpm–0.5 h, injected to 130%, over original weight, then submitted to post-injection tumbling 8.5 rpm–5 h, and *sous vide* cooked. a) Transversal view, T5 (control), arrows indicate gaps among fibre bundles; arrows in the inset figure indicate gaps between fibres; b) longitudinal view, T5, arrows indicate coagulated endomisial and perimisial collagen and granulated sarcolemma, arrows in the inset figure indicate partial fragmentation of the fibres; c) transversal view, T4 (NaCl 1.25%); d) longitudinal view, T4, arrows indicate rupture of the fibres; e) transversal view, T3 (STPP 0.25% + NaCl 1.25%), arrows in the inset figure indicate the presence of coagulated material and fragmented myofibrils; f) longitudinal view, T3; g) transversal view, T2 (MWPC 1.88% + NaCl 1.25%), arrow in the inset figure indicates fractures of myofibrils along the Z-line level; h) longitudinal view, T2, arrows indicate the presence of coagulated material.


a
100µ
10µ
b
100µ
10µ
c
10µ
d
100µ
e
100µ
10µ
f
100µ
g
100µ
10µ
h
100µ

raw muscle one (Fig. 3a). In addition, gaps among fibre bundles, and fragmentation and contraction of the fibres were observed (inset in Fig. 4a). Despite the fact that myofibrils were dehydrated, they were organized. In the longitudinal view, it can be seen as the partial rupture of fibres that could be caused by the mechanical treatment (inset in Fig. 4b). The fibres also had on their surface coagulated endomisial and perimisial collagen and granulated sarcolemma. Cooked muscles with NaCl (T4) presented a disorganized structure with rupture of the fibres and a great separation between fibre bundles (Fig. 4c,d). However, the fibres showed a compact aspect and a gel-like structure. In the longitudinal view (Fig. 4d), the presence of coagulated endomisial and perimisial collagen and granulated sarcolemma can be observed. The addition of STPP + NaCl induced important changes in the microstructure of cooked muscles (Fig. 4e,f). Firstly, the microstructure was more conserved and compacted than in T4 and T5 samples (Fig. 4a–d). Fibres had a well rounded shape, and fragmented myofibrils and coagulated material were observed in the interfibre spaces (inset in Fig. 4e). Muscles with MWPC + NaCl presented, as with STPP + NaCl, a more uniform microstructure and a lower separation between fibres (Fig. 4g,h) than samples from T4 and T5. Besides, fibres were more compacted and the presence of abundant coagulated material between and onto the fibres was observed, which could be composed of fragmented myofibrils and added whey protein. Whey protein addition would have induced the agglutination and fragmentation of myofibrils at heating. Thus, the terraced appearance observed in the inset of Fig. 4g would be the result of the fractures of the myofibrils along the fibre at Z-line level (Rowe, 1989). Samples of cooked muscles with WPC + NaCl presented similar microstructures (micrographs not shown).

In general, cooked muscles with STPP + NaCl or WPC/MWPC + NaCl presented a more conserved and compacted microstructure, with less separation between fibres than muscles with NaCl alone and than controls. However, microstructures of muscles from T1, T2 and T3, presented some differences. Thus, samples from T1 and T2 depicted a major rupture of the sarcomere, with the presence of fragmented material among fibres. Instead, T3 samples presented a gel-like material among the fibres. These differences could indicate that the additives improved water retention at heating by different mechanisms. Thus, the addition of STPP + NaCl increased the extraction and solubilisation of myofibrillar proteins resulting in the formation of a gel-like material by heating, responsible for the water retention. On the other hand, the addition of WPC/MWPC + NaCl would induce a lower extraction and solubilisation of myofibrillar proteins. However, the incorporation of these additives and the subsequent thermal treatment would have caused an important agglutination and fragmentation of myofibrils, resulting in a reduction of water loss at heating.

3.6. Colour parameters

The effect of the incorporation of different additives on chromatic parameters, L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) of *sous vide* cooked *Semitendinosus* beef muscles is presented in Table 3.

With respect to the L* parameter, control muscles (T5) and those treated with STPP + NaCl (T3) presented the lowest values, consequently they have a darker appearance, with no significant differences between them ($p > 0.05$, Table 3). Muscles from T1 and T4 presented the highest lightness values, which were significantly ($p < 0.05$) different from T5 and T3. Muscles from T2 showed no significant different L* values in comparison to muscles submitted to the other treatments.

Muscles with NaCl (T4) presented significantly ($p < 0.05$) lower a* values in comparison with muscles form T3 and T5. In association with this result, it has been reported that NaCl affects the thermal stability of myoglobin and promotes its oxidation (Lytras, Geileskey, King, & Ledward, 1999; Trout, 1989), phenomena that contributes to the reduction of the redness. Apparently, the incorporation of whey proteins (T1 and T2) minimizes the redness reduction, and the a* values of muscles from these treatments were similar to the control (T5) one. With regard to this issue, it has been proposed that whey protein concentrates might have antioxidant properties (Peña-Ramos & Xiong, 2003), diminishing the NaCl effect on oxidation. On the other hand, muscles treated with STPP + NaCl (T3) presented the highest a* value, which was similar to the control (T5). The incorporation of STPP could be responsible for the reduction of the myoglobin denaturation, by increasing pH and/or diminishing pigment oxidation because of its antioxidant capacity (Trout, 1989; Vaudagna et al., 2008).

Table 3
Effect of the incorporation of different additives to *Semitendinosus* beef muscles *sous vide* cooked on chromatic parameters.

Treatment[1]	Chromatic parameters		
	L*	a*	b*
T1	61.64 ± 1.54 a	6.06 ± 0.87 ab	17.24 ± 0.78
T2	60.40 ± 1.27 ab	5.80 ± 0.79 ab	17.61 ± 0.61
T3	58.96 ± 1.30 b	6.79 ± 0.77 a	17.48 ± 1.05
T4	61.41 ± 1.61 a	4.94 ± 0.62 b	17.19 ± 1.26
T5	58.48 ± 1.59 b	6.19 ± 0.56 a	17.40 ± 0.57

a–b Means values with different letters in the same column are significantly different ($p < 0.05$).
[1]Concentrations in muscles after injection: T1: WPC 1.88% + NaCl 1.25%; T2: MWPC 1.88% + NaCl 1.25%; T3: STPP 0.25% + NaCl 1.25%; T4: NaCl 1.25%; T5: Control (non-injected).

3.7. Visual appearance and sensory analysis

Table 4 shows the scores of the visual appearance attributes ("cooked beef colour", "colour uniformity", "intensity of defects" and "amount of defects") of *sous vide* cooked muscles. The addition of NaCl alone (T4) or in combination with WPC or MWPC (T1 and T2, respectively) did not modify cooked beef colour in relation to control samples (T5). All samples depicted a colour between "pinkish-grey" and "grey-brown". Conversely, muscles with STPP + NaCl (T3) had a significantly ($p < 0.05$) different colour in comparison to the other treatments, being more reddish ("pink" colour). This finding supports that one obtained for chromatic parameters, having muscles with STPP + NaCl the highest a* value (Table 3). Regarding colour uniformity, there were no significant differences ($p > 0.05$) among all studied treatments. Concerning the intensity of defects, muscles with STPP + NaCl (T3) presented values similar to T5, and both were significantly lower ($p < 0.05$) than samples submitted to the

Table 4
Effect of the incorporation of different additives to *Semitendinosus* beef muscles *sous vide* cooked on visual appearance attributes.

Treatment[1]	Cooked beef colour	Colour uniformity	Intensity of defects	Amount of defects
T1	5.25 ± 0.19 a	2.18 ± 0.22	4.28 ± 0.74 a	5.00 ± 0.59 a
T2	5.38 ± 0.43 a	2.73 ± 0.72	4.08 ± 0.19 a	3.48 ± 0.47 b
T3	3.78 ± 0.60 b	2.75 ± 0.39	1.78 ± 0.53 b	1.65 ± 0.44 c
T4	5.25 ± 0.42 a	2.63 ± 0.46	3.93 ± 0.52 a	3.03 ± 0.39 bc
T5	5.58 ± 0.15 a	2.73 ± 0.37	2.70 ± 0.83 b	4.00 ± 0.63 ab

a–c Means values with different letters in the same column are significantly different ($p < 0.05$).
[1]Concentrations in muscles after injection: T1: WPC 1.88% + NaCl 1.25%; T2: MWPC 1.88% + NaCl 1.25%; T3: STPP 0.25% + NaCl 1.25%; T4: NaCl 1.25%; T5: Control (non-injected).
Scales: Cooked beef colour (1: *Very red*, 7: *Brown*), colour uniformity (1: *No variation*, 5: *Extreme amount of variation*), Intensity of defects (1: *None*, 5: *Extreme*) and Amount of defects [1: *Non (0%)*, 7: *Total (100%)*].

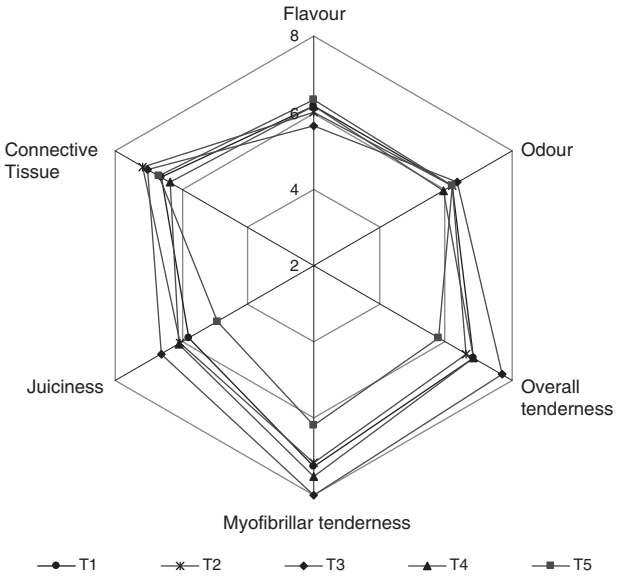


Fig. 5. Mean values obtained for sensory attributes for *Semitendinosus* beef muscles *sous vide* cooked. T1: WPC 1.88% + NaCl 1.25%, T2: MWPC 1.88% + NaCl 1.25%, T3: STPP 0.25% + NaCl 1.25%, T4: NaCl 1.25%, T5: Control (all the concentrations are expressed on the basis of muscle just injected).

other treatments (T1, T2 and T4; Table 4). Moreover, the amount of defects of these muscles (T3) was significantly lower ($p<0.05$) than the scores obtained for T1, T2 and T5, but similar to T4. The intensity and amount of defects described for the injected samples are related to the tissue disruption induced by the injector needles. However, for T3 samples (also injected), both defects were minimized. The explanation for that could be the higher protein extraction induced by the combination of NaCl and STPP and the consequent gel-like structure formed during cooking (showed in Fig. 4e). Conversely, the incorporation of whey proteins did not produce this mending effect on tissue structure.

Results of the sensory evaluation are presented in Fig. 5. All cooked samples were characterized as meat with a "slightly intense" flavour and odour. Regarding myofibrillar and overall tenderness, muscles with STPP + NaCl had higher scores than the control (T5), being classified as "very tender" and "slightly tender", respectively. Samples from T1, T2 and T4 treatments presented intermediate scores, and were classified as "tender". Regarding juiciness attribute, samples from T1, T2 and T4 treatments had intermediate scores corresponding to "slightly juicy" meats, whereas T3 samples were classified as "juicy" and T5 as "neither dry nor juicy" meat. Considering connective tissue amount attribute, all samples were assessed as meats with "practically nothing" connective tissue. The exceptions were samples with NaCl alone (T4), which were valuated as containing "some amount" of connective tissue.

4. Conclusions

Regarding all additives evaluated in this study, the combination of STPP and NaCl had the best performance, reducing weight losses and shear force values, and improving total yield and visual and sensory attributes of *sous vide* cooked beef muscles. The addition of whey proteins and NaCl had a lower performance than STPP + NaCl, although those additives improved total yield in comparison to NaCl alone or control samples. Native and modified whey proteins had similar effects on technological parameters and physical and sensory properties of *sous vide* cooked beef muscles.

Acknowledgement

We thank Mrs. Catalina B. Taibo from the Microscopy Lab. (CICVyA, INTA) and Natalio De Vincenzo from Universidad de Morón for their technical assistance in microscopy studies; and Juan Pablo Costa and Cristian Ourille for their helpful support in the development of the assays. This work has been funded by Instituto Nacional de Tecnología Agropecuaria, INTA (Subsidio PE AETA 1671 "Tecnologías de Procesamiento Mínimo") and Facultad de Agronomía y Ciencias Agroalimentarias, Universidad de Morón (Subsidio A-06-007-06).

References

American Meat Science Association (AMSA) (1991). *Proceedings 44th annual reciprocal meat conference (pp. 9–17), 9–12 June 1991, Kansas, USA*.

American Meat Science Association (AMSA) (1995). *Research guidelines for cookery, sensory evaluation, and instrumental measurement of fresh meat.* Chicago, IL: National Livestock and Meat Board.

Baublits, R. T., Pohlman, F. W., Jr., Brown, A. H., Yancey, E. J., & Johnson, Z. B. (2006). Impact of muscle type and sodium chloride concentration on the quality, sensory and instrumental colour characteristics of solution enhanced whole-muscle beef. *Meat Science*, *72*(4), 704–712.

Boles, J. A., & Shand, P. J. (2001). Meat cut and injection levels affect the tenderness and cook yield of processed roast beef. *Meat Science*, *59*(3), 259–265.

Chen, C. M., & Trout, G. R. (1991). Sensory, instrumental texture profile and cooking properties of restructured beef steaks made with various binders. *Journal of Food Science*, *56*(6), 1457–1460.

Church, I. J., & Parsons, A. L. (2000). The sensory quality of chicken and potato products prepared using cook-chill and *sous vide* methods. *International Journal of Food Science and Technology*, *35*, 361–368.

Cross, H. R., Moen, R., & Stanfield, M. S. (1978). Training and testing of judges for sensory analysis of meat quality. *Food Techonology*, *32*(7), 48–54.

El-Magoli, S., Laroia, S., & Hansen, M. T. (1996). Flavor and texture characteristics of low fat ground beef patties formulated with whey protein concentrate. *Meat Science*, *42*(2), 179–193.

Ensor, S. A., Mandigo, R. W., Calkins, C. R., & Quint, L. N. (1987). Comparative evaluation of whey protein concentrate, soy protein isolate and calcium-reduced non-fat dry milk as binders in an emulsion-type sausage. *Journal of Food Science*, *52*(5), 1155–1158.

Feiner, G. (2006). Chapter 8: Whole-muscle brine-injected products. *Meat products handbook: Practical science and technology*. Abington, England: Woodhead Publishing Limited.

Garcia-Segovia, P., Andrés-Bello, A., & Martínez-Monzo, J. (2007). Effect of cooking method on mechanical properties, color and structure of beef muscle (*M. pectoralis*). *Journal of Food Engineering*, *80*, 813–821.

Gaze, J. E., Brown, G. D., Gaskell, D. E., & Banks, J. G. (1989). Heat resistance of *Listeria monocytogenes* in homogenates of chicken, beef steak and carrot. *Food Microbiology*, *6*(4), 251–259.

Hansen, T. B., KnØchel, S., Juncher, D., & Bertelsen, G. (1995). Storage characteristics of *sous vide* cooked roast beef. *International Journal of Food Science and Technology*, *30*, 365–378.

Hauben, K. (1999, March 25–26). *Sous vide* cooking: State of art. *Proceedings of the third European symposium on sous vide* (pp. 11–27). Belgium: Leuven.

Hayes, J. E., Desmond, E. M., Troy, D. J., Buckley, D. J., & Mehra, R. (2006). The effect of enhancement with salt, phosphate and milk proteins on the physical and sensory properties of pork loin. *Meat Science*, *72*(3), 380–386.

Huffman, D. L. (1996, February). Processing whey protein for use as a food ingredient. *Food Technology*, 49–52.

Hughes, E., Mullen, A. M., & Troy, D. J. (1998). Effects of fat level, tapioca starch and whey protein on frankfurters formulated with 5% and 12% fat. *Meat Science*, *48*(1–2), 169–180.

Ishihara, S. (1998). *The series of plates designed as a test for colour deficiency* (24 plates ed., pp. 1–9). Tokyo, Japan: Kanchara & Co Ltd.

Katsaras, K., & Budras, K. (1993). The relationship of the microstructure of cooked ham to its properties and quality. *Lebensmittel-Wissenschaft und Technologie*, *26*(3), 229–234.

Kerry, J. F., Long, D., & Buckley, D. J. (2001, August 26–31). The effects of added novel protein and carbohydrate ingredients on the quality characteristics of low fat reformed meat. *Proceedings of the 47th International Congress of Meat Science and Technology*, *Vol. II.* (pp. 152–153)Poland: Kraków.

Ledward, D. A. (1979). Chapter 5: Meat. In R. J. Priestley (Ed.), *Effect of heating on foodstuffs* (pp. 121–157). London: Applied Science Publishers.

Lin, K., & Keeton, J. T. (1998). Textural and physicochemical properties of low-fat, precooked ground beef patties containing carrageenan and sodium alginate. *Journal of Food Science*, *63*(4), 571–574.

Lytras, G. N., Geileskey, A., King, R. D., & Ledward, D. A. (1999). Effect of muscle type, salt and pH on cooked meat haemoprotein formation in lamb and beef. *Meat Science*, *52*(2), 189–194.

Nyati, H. (2000). An evaluation of the effect of storage and processing temperatures on the microbiological status of *sous vide* extended shelf-life products. *Food Control*, *11*, 471–476.

Offer, G., & Knight, P. (1988). The structural basis of water-holding in meat. Part 1: General principles and water uptake in meat processing. In R. Lawrie (Ed.), *Developments in Meat Science*, *Vol. 4*. (pp. 63–170)London: Elsevier Science.

Parks, L. L., & Carpenter, J. A. (1987). Functionality of six non-meat proteins in meat emulsion systems. *Journal of Food Science*, *52*(2), 271–274 (278).

Peña-Ramos, E. A., & Xiong, Y. L. (2003). Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Science*, *64*(3), 259–263.

Pietrasik, Z., & Shand, P. J. (2004). Effect of blade tenderization and tumbling time on the processing characteristics and tenderness of injected cooked roast beef. *Meat Science*, *66*(4), 871–879.

Pietrasik, Z., & Shand, P. J. (2005). Effects of mechanical treatments and moisture enhancement on the processing characteristics and tenderness of beef *semimembranosus* roast. *Meat Science*, *71*(3), 498–505.

Resurreccion, A. V. A. (2003). Sensory aspects of consumer choices for meat and meat products. *Meat Science*, *66*(1), 11–20.

Rowe, R. W. D. (1989). Electron microscopy of bovine muscle. I — The native state of post rigor sarcolemma and endomysium. *Meat Science*, *26*, 271–279.

Ruusunen, M., & Puolanne, E. (2005). Review. Reducing sodium intake from meat products. *Meat Science*, *70*(3), 531–541.

Shahidi, F., & Synowiecki, J. (1997). Protein hydrolyzates from seal meat as phosphate alternatives in food processing applications. *Food Chemistry*, *60*(1), 29–32.

Shand, P. J., Sofos, J. N., & Schmidt, G. R. (1993). Properties of algium/calcium and salt/phosphate structured beef rolls with added gums. *Journal of Food Science*, *58*(6), 1224–1230.

Sheard, P. R., & Tali, A. (2004). Injection of salt, tripolyphosphate and bicarbonate marinade solutions to improve the yield and tenderness of cooked pork loin. *Meat Science*, *68*(2), 305–311.

Shults, G. W., Russell, D. R., & Wierbicki, E. (1972). Effect of condensed phosphates on pH, swelling and water-holding capacity of beef. *Journal of Food Science*, *37*(6), 860–864.

Szerman (2009). Estudio de la incorporación de aditivos funcionales de origen natural a músculos bovinos sometidos a cocción-pasteurización mediante el sistema *sous-vide*. PhD. Thesis. Universidad Nacional de La Plata. Buenos Aires. Argentina.

Szerman, N., Gonzalez, C. B., Sancho, A. M., Grigioni, G., Carduza, F., & Vaudagna, S. R. (2007). Effect of whey protein concentrate and sodium chloride addition plus tumbling procedures on technological parameters, physical properties and visual appearance of *sous vide* cooked beef. *Meat Science*, *76*(3), 463–473.

Szerman, N., Gonzalez, C. B., Sancho, A. M., Grigioni, G., Carduza, F., & Vaudagna, S. R. (2008). Optimization of whey protein concentrate and sodium chloride concentrations and cooking temperature of *sous vide* cooked whole-muscle beef from Argentina. *Meat Science*, *79*, 557–567.

Szerman, N., Vaudagna, S. R., & Gonzalez, C. B. (2006, November 15–17). Efecto de diferentes tratamientos de masajeado sobre la microestructura de músculos *Semitendinosus* bovino adicionados con proteínas de suero lácteo y cloruro de sodio, cocidos mediante el sistema *sous vide*. *Proceedings of the Congreso Internacional de Ciencia y Tecnología de los Alimentos* (pp. 123–124). Córdoba, Argentina: Agencia Córdoba Ciencia S.E.

Thomsen, B. (1996). Milk proteins in meat products. *Meat International*, *6*(1), 32–33 (35).

Trout, G. R. (1989). Variation in myoglobin denaturation and colour of cooked beef, pork, and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate, and cooking temperature. *Journal of Food Science*, *54*(3), 536–540 (544).

Trout, G. R., & Smith, G. M. (1986). Effect of phosphate on the functional properties of restructured beef rolls: The role of pH, ionic strength and phosphate type. *Journal of Food Science*, *51*(6), 1416–1423.

Vaudagna, S. R., Pazos, A. A., Guidi, S. M., Sanchez, G., Carp, D. J., & Gonzalez, C. B. (2008). Effects of salt addition on *sous vide* cooked whole beef muscles from Argentina. *Meat Science*, *79*(3), 470–482.

Vaudagna, S. R., Sánchez, G., Neira, M. S., Insani, E. M., Picallo, A. B., Gallinger, M. M., & Lasta, J. A. (2002). *Sous vide* cooked beef muscles: Effects of low temperature–long time (LT–LT) treatments on their quality characteristics and storage stability. *International Journal of Food Science and Technology*, *37*, 425–441.

Vote, D. J., Platter, W. J., Tatum, J. D., Schmidt, G. R., Belk, K. E., Smith, G. C., & Speer, N. C. (2000). Injection of beef strip loins with solutions containing sodium tripolyphosphate, sodium lactate, and sodium chloride to enhance palatability. *Journal of Animal Science*, *78*, 952–957.

Xargayó, M. (2010). Proceso de fabricación de productos cárnicos cocidos de musculo entero III: Masaje. *Metalquimia, artículos tecnológicos*. España: Metalquimia S.A. Girona Available at. www.metalquimia.com