



## Penetration of hydrolysed soy protein-added brine and its effect on yield and pH of beef steaks from the *biceps femoris* muscle

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### ARTICLE INFO

#### Article history:

Received 3 November 2009

Received in revised form

2 June 2010

Accepted 3 June 2010

#### Keywords:

Slice

Marination

Tumbling

Water absorption

### ABSTRACT

This study was aimed to evaluate the penetration behaviour of different brines with tumbled beef steaks from the *biceps femoris* muscle, specifically their interactions with pH and effects on yield. Six muscles from different animals, divided into origin (OP) and insertion (IP) portions, were cut into 60 steaks of 2.5 cm thickness and tumbled for 30 or 60 min. The steaks were tumbled with two brines, with (WTB/HSP) or without (WTB) hydrolysed soy protein (HSP), and steaks that were not tumbled with brine or water were used as controls. Brine penetration was verified by measuring the amount of dye-containing brine (absorbance at 627 nm) recovered from homogenates of four thin (2 mm) slices from the surface of the beef steaks after tumbling. The WTB/HSP steaks exhibited greater ( $P < 0.05$ ) brine penetration when tumbled for 60 min than for 30 min. The OP steaks showed greater yield and lower pH ( $P < 0.05$ ) than IP steaks. HSP-added brine increased the water absorption and retention in the first slices of the steaks, and its efficiency was increased with a longer tumbling time. The portion of the *biceps femoris* muscle used influenced brine absorption and retention, impacting meat yield.

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### 1. Introduction

Meat marination is a practice that uses a wide variety of brine compositions in order to improve the tenderness, juiciness and yield of meat cuts (Christensen, Torngren, Karlsson, & Erthjerg, 2007; Szerman et al., 2007). Yield improvements are of particular interest to the industry because they may increase profit margins (Smith & Young, 2007; Xiong, 2005).

Meat yield improvement depends on the effectiveness of the marination process, which is defined by brine penetration and the properties of the additives. Brine penetration is positively associated with protein extractability in saline solution, which is greater in muscle composed predominantly of white fibres. This property underlies the results seen for poultry meat yield after tumbling (Lawrie, 2005, 384 pp.; Richardson & Jones, 1987). The measurement of brine penetration into tumbled chicken cuts was reported by Sanders (1969), Xiong and Kupski (1999a, 1999b) and Alvarado and Sams (2004). In beef, which is characterised by a greater proportion of red-type fibres, tumbling seems to allow more uniform distribution of the brine after injection (Cheng et al., 2007). In this meat, FD&C blue dye plus brine solution has been used as a good indicator of brine penetration (Uttaro & Aalhus, 2007).

The most common brine additives, alkaline phosphates and sodium chloride, have been very efficient in increasing the yield of tumbled meat cuts. Phosphates raise the meat pH, immobilising the added water (Xiong, 2004). Sodium chloride enhances this effect by solubilising the myosin in the muscle, forming a water-binding matrix (Hamm, 1960; Offer & Trinick, 1983).

The effects of adding hydrolysed soy protein to the brine of meat cuts are not known, although this protein is commonly used in meat products such as sausages and hamburgers in United States. It has been reported to increase the availability and solubility of 11S and 7S protein components, which is related to water holding capacity (WHC) in meat products (Kinsella, 1979; Xiong, 2005).

Therefore, the objective of this study was to evaluate the penetration of hydrolysed soy protein-added brine, its interaction with pH and its effect on yield of tumbled steaks from beef *biceps femoris* muscles using a dye-tracing method.

### 2. Materials and methods

#### 2.1. Muscle sampling and portions

Six beef *biceps femoris* muscles (~5.8 kg) were collected from the right sides of carcasses from castrated male Nellore cattle (*Bos indicus*). The cattle were between 31 and 35 months of age and of moderate fat thickness (3–6 mm).

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The *biceps femoris* muscles were divided into two portions: the OP, or origin portion of the muscle, and the IP, or insertion portion of the muscle. The portion of these muscles located near the *Semitendinosus* muscle and limited by a fascia (Fig. 1) was not considered in these analyses. Each portion (OP and IP) was divided into five steaks of approximately 2.5 cm in thickness, four of which were tumbled with two different brines (2 steaks/brine, compositions described below) and one of which was used as a control (no tumbling with brine or water) (Fig. 2). Thus, 10 steaks were obtained from each muscle for a total of 60 steaks.

## 2.2. Brine composition and tumbling times

The amount of brine (ml) used for the marination by tumbling corresponded to 20 g of brine per 100 g steak (weight obtained before the tumbling). The two brines used in the tumbling process

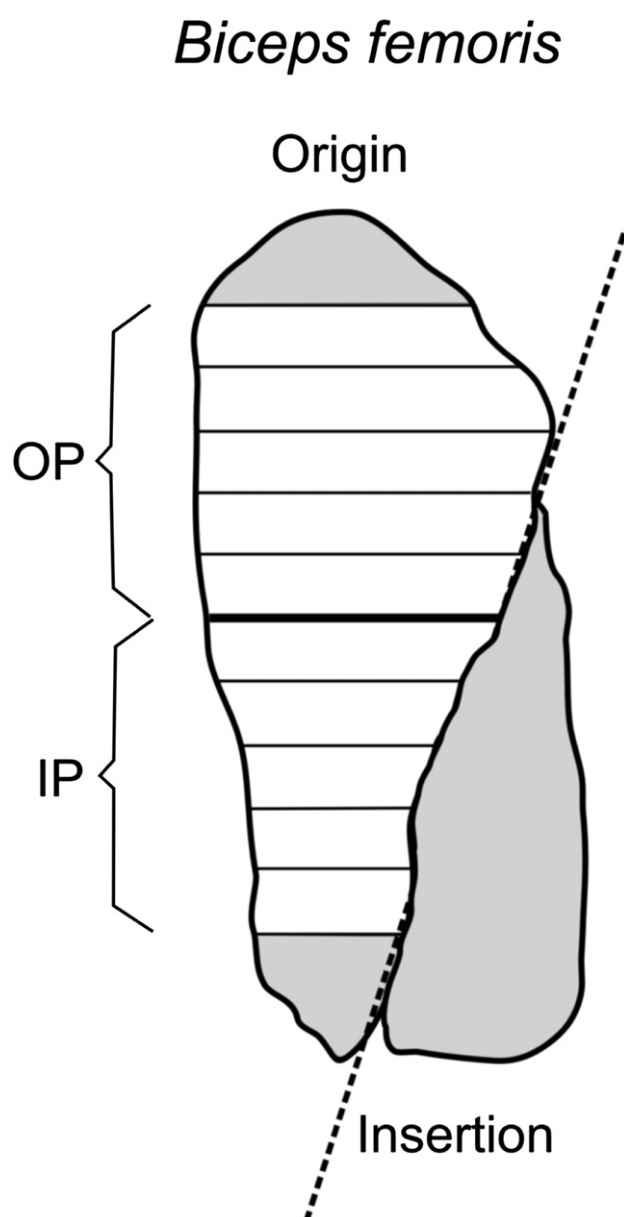


Fig. 1. Design of the sampling plan of the *biceps femoris* muscle, where OP is the portion near the origin of the muscle and IP is the portion near the insertion of the muscle. The regions in gray color of the figure were not used.

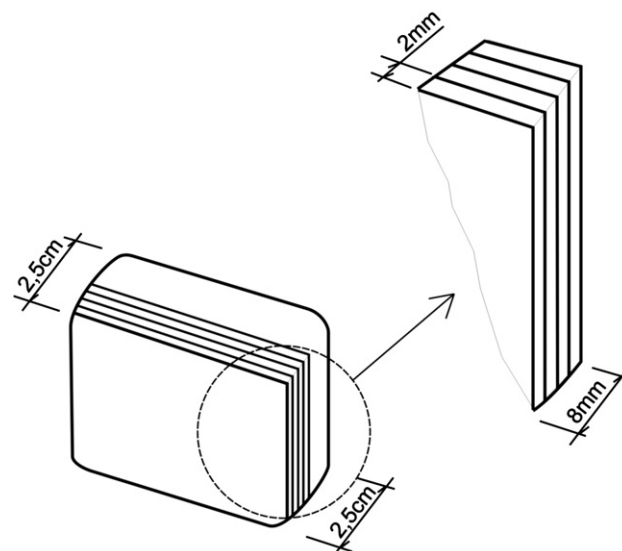


Fig. 2. Schematic demonstration of the sampling of *biceps femoris* steak. The slices were collected from the superficial to the core portion of the steak.

of steaks were referred to as WTB (with tumbling and addition of brine) and WTB/HSP (with tumbling and addition of brine plus hydrolysed soy protein). The brines were prepared by dissolving specific ingredients (Table 1) and FD&C Blue No. 1 dye (0.15 g/100 g final concentration of brine) in water. The pH of both brines (WTB and WTB/HSP) was measured before the tumbling of steaks and found to be 7.77 and 7.58, respectively. Control steaks were not tumbled with brine or water.

The WTB and WTB/HSP steaks were tumbled for 30 or 60 min in a vacuum tumbler (TF-30VE, Frigomaq, Chapecó, SC, Brazil) with intermittent rotation (15 min on, 1 min off) at 30 RPM. Immediately after tumbling, the steaks were packaged in low density (UNIPAC/UNIVAC B320) barrier pouches, using a vacuum sealer (300B, Selovac, São Paulo, SP, Brazil) and the absolute pressure of the vacuum was 98.7 kPa. The pouches were 90  $\mu\text{m}$  thick with an oxygen permeability of  $40 \text{ cm}^3 \text{ m}^{-2} \text{ d}^{-1} \text{ atm}^{-1}$  at 77% relative humidity (RH), 23 °C. After packaging, the steaks were placed in the appropriate boxes and left in a cooling chamber for 36 h at  $0 \pm 2$  °C to allow the brine to equilibrate in the steaks. After equalisation, the steaks were removed from the packages, weighed and submitted for analysis of brine penetration, meat yield and pH.

## 2.3. Hydrolysed soy protein

Soy protein was hydrolysed with the Alcalase enzyme to a maximum hydrolysis level of 4% as described by Feng and Xiong

Table 1  
Additives in the final product and the WTB and WTB/HSP brines (g/100 g).

Ingredients	WTB brine		WTB/HSP brine	
	Final product (g/100 g)	Brine (g/100 g)	Final product (g/100 g)	Brine (g/100 g)
Salt	1.800	10.800	1.800	10.800
Monosodium glutamate	0.150	0.900	0.150	0.900
Sugar	0.100	0.600	0.100	0.600
Soy hydrolysed protein	—	—	0.333	2.000
Triphosphosphate	0.300	1.800	0.300	1.800
Sodium nitrite	0.015	0.090	0.015	0.090
Sodium erythorbate	0.050	0.300	0.050	0.300
Cold water	14.250	85.510	13.920	83.510
	16.670	100.000	16.670	100.000

WTB — Brine used in the tumbling of the steaks. WTB/HSP — Brine added of hydrolysed soy protein used in the tumbling of the steaks.

(2003). This product is marketed by Solae Brazil, which supplied the samples for this work.

#### 2.4. Monitoring brine penetration

Brine penetration was measured according to the procedure described by Xiong and Kupski (1999a), with some modifications. Briefly, the penetration of brine solutions into the steaks was monitored by tracing the amount of FD&C Blue No. 1 dye that diffused into different slices of the steaks. Four slices of 2 mm thickness were obtained from the surface of each steak sampled (Fig. 2) using a meat slicer (GP30V, Hobart, Troy, OH, USA).

To extract the dye, the S0 (0–2 mm), S1 (2–4 mm), S2 (4–6 mm) and S3 (6–8 mm) slices were homogenised in an analytical mill (Q-298A21, Ika, Campinas, SP, Brazil) for approximately 12 s. The homogenates were conditioned in plastic bags and chilled at  $1 \pm 2^\circ\text{C}$  for approximately 24 h. Then, 2.5 g of each homogenate was collected, added diluted 1/10 (w:v) in distilled water and submitted to homogenisation using a high rotation blender (HGBWTS3, Waring, Torrington, CT, USA). The final homogenate was transferred to labelled tubes and centrifuged for 10 min at  $10,000 \times g$  in a refrigerated centrifuge (RC-5B, Sorvall, Wilmington, DE, USA). The supernatant was decanted, and the pellet was discarded. Absorbance was measured by reading an aliquot of the supernatant at 627 nm in a Shimadzu UV mini 1240 spectrophotometer (Chiyoda-ku, Tokyo, Japan). The absorbance values were normalised to the weights of the slice according to the method described by Xiong and Kupski (1999a), i.e., the absorbance values were multiplied by 2 and then divided by the weight of the each slice (grams).

#### 2.5. Yield of the steaks

The steaks were weighed with a semi-analytical scale (BG-2000, GEHAKA, São Paulo, SP, Brazil) before the tumbling process, immediately after the tumbling process and after 36 h of brine equalisation. The yield immediately after tumbling (YIAT, g/100 g) was calculated as  $(W_a - W_b)/W_b \times 100$ , where  $W_a$  is the weight of the steak after tumbling and  $W_b$  is the weight of the steak before tumbling. Percent yield was obtained at 36 h after tumbling (Y36AT, g/100 g), the formula used was  $(W_e - W_b)/W_b \times 100$ , where  $W_e$  is the weight of the steak after equalisation and  $W_b$  is the weight of the steak before tumbling. The relationship between YIAT and Y36AT was calculated by formula  $(W_e - W_a)/W_a \times 100$ , where  $W_e$  is the weight of the steak after equalisation and  $W_a$  is the weight of the steak after tumbling.

#### 2.6. pH values

pH measurements were taken at the four corners of the steaks before tumbling (initial pH) and 36 h after tumbling (final pH) by inserting a pH meter (pH 300, Oakton, Vernon Hills, IL, USA) into a small incision.

#### 2.7. Statistical analysis

The experimental design used completely randomised blocks in an incomplete factorial arrangement of 2 muscle portions  $\times$  3 brine compositions  $\times$  2 tumbling times. Six *biceps femoris* muscles from different animals represented the blocks (replicates). Instead of 12 total treatments, however, only 10 treatments were applied; the control steaks were not tumbled with brine or water at different tumbling times. Differences between muscle portions, brine compositions, tumbling times and slices were analysed as repeated measurements with the PROC MIXED program (SAS Inst. Inc., Cary,

NC). The level of significance was set at  $P < 0.05$ . The appropriate covariate structure for the model for each response variable was determined using model-fitting statistics generated in SAS. The initial weight of the steaks was used as a covariate for the variables mentioned below. The dependent variables in the general linear model were brine penetration monitoring by tracing, pH value and meat yield. Data are presented as least square means with SEM. When a significant ( $P < 0.05$ ) effect was observed, differences between means were determined using a Tukey–Kramer test. A probability level of  $P < 0.05$  was considered significant.

### 3. Results and discussion

#### 3.1. Monitoring brine penetration into meat

The steaks tumbled with the WTB and WTB/HSP brines exhibited greater absorbance values ( $P < 0.05$ ) than did control steaks not tumbled with brine or water (Table 2), demonstrating that brine penetration and retention was increased in tumbled steaks. The strong mechanical impact of the tumbler walls and the presence of phosphate and sodium chloride within the brines might have favoured greater brine penetration and retention. Disruption of peripheral connective tissue during tumbling is associated with increased brine penetration and proteins extraction (Xiong & Kupski, 1999a). Furthermore, the phosphates and NaCl promote protein unfolding, opening more binding sites for  $\text{H}_2\text{O}$ , increasing the amount of bound or immobilised  $\text{H}_2\text{O}$  in the muscle structure. The phosphates increase negative charges and pH, causing electrostatic repulsion, opening space in the muscle allowing water to enter (Offer & Trinick, 1983).

The most superficial slice from the steaks (S0) exhibited greater ( $P < 0.05$ ) brine penetration than did deeper slices (S1, S2 and S3), regardless ( $P > 0.05$ ) of brine composition (WTB or WTB/HSP) or tumbling time (30 or 60 min; Fig. 3). This result suggests that the brine penetration by the tumbling is most effective at the surface of the steaks (S0) because this slice is exposed to direct contact with the brine and receives a greater mechanical impact from the tumbler walls. The latter effect makes the outer slice more susceptible to structural disruption, facilitating brine penetration. On the other hand, internal slices from beef steaks have a thicker perimysium, which could impede brine penetration.

Decreasing brine penetration was observed as muscle depth increased (0–6 mm) for all brine compositions and tumbling times ( $P < 0.05$ ), possibly due to the low permeability of the muscle cell membranes. Similar results were observed at a depth of 5 mm in chicken fillet tumbled with brine containing different types of phosphates (Xiong, 2005). However, for the WTB/HSP at a tumbling time of 30 min, S1 presented similar ( $P > 0.05$ ) absorbance values as S2 and S3 (Table 2). This demonstrated that the presence of hydrolysed soy protein (HSP) favoured increased absorption, since the WTB/HSP 30 min samples showed greater absorbances ( $P < 0.05$ ) than did the WTB 30 min samples. The values were also similar ( $P > 0.05$ ) to those of the WTB 60 min samples for slices S2 and S3. The contribution of protein hydrolysates to water-retention capacity is attributed to the strong hydrophilicity of soy peptides (Adler-Nissen & Olsen, 1979). It is also possible that their synergistic interactions with muscle proteins might form a gel matrix capable of immobilising extraneous water (Feng & Xiong, 2002, 2003). This may have occurred because of the protein (11S) present in HSP, which is very soluble and has great water-retaining potential (Zayas, 1997).

With respect to slice S3, the steaks tumbled with WTB brine for 60 min and the steaks tumbled with WTB/HSP brine for both 30 and 60 min showed similar levels of brine penetration ( $P > 0.05$ ); these were better ( $P < 0.05$ ) than the WTB 30 min treatment. The

**Table 2**Penetration of different brines in *biceps femoris* for different tumbling times and steak depth (slices), through absorbance reading.

Slices	Treatments				
	CONTROL		WTB		WTB/HSP
	Time 0 <sup>f</sup>		Time 30 <sup>f</sup>		Time 60 <sup>f</sup>
	Absorbance (627 nm) <sup>e</sup>				
S0	0.17(0.17) <sup>b,A</sup>		0.98 (0.06) <sup>a,A</sup>	1.07 (0.06) <sup>a,A</sup>	0.93 (0.06) <sup>a,A</sup>
S1	0.16 (0.05) <sup>c,A</sup>		0.46 (0.05) <sup>b,B</sup>	0.47 (0.05) <sup>b,B</sup>	0.38 (0.05) <sup>b,B,C</sup>
S2	0.15 (0.03) <sup>d,A</sup>		0.27 (0.03) <sup>c,C</sup>	0.31 (0.03) <sup>c,b,C</sup>	0.37 (0.03) <sup>b,B</sup>
S3	0.15 (0.02) <sup>c,A</sup>		0.24 (0.02) <sup>b,C</sup>	0.30 (0.02) <sup>a,C</sup>	0.31 (0.02) <sup>a,C</sup>

<sup>a,b,c,d</sup> Different lowercase letters in the same row differ statistically  $P < 0.05$ .<sup>A,B,C,D</sup> Different uppercase letters in the same column differ statistically  $P < 0.05$ .

CONTROL – Steaks that were not tumbled with brine or water. WTB – Steaks tumbled with brine. WTB/HSP – Steaks tumbled with brine added of hydrolysed soy protein. Slice (steak depth) – S0 (0–2 mm), S1 (2–4 mm), S2 (4–6 mm), S3 (6–8 mm).

<sup>e</sup> Mean value, ( ) Standard error. Higher value means greater absorbance and more dye presence.<sup>f</sup> Tumbling times are expressed in minutes.

similarity between the WTB/HSP 30 min and WTB 60 min treatments shows that the use of HSP reduced the tumbling time necessary to obtain the same brine absorption and retention. This is significant given that minimisation of tumbling time length is an important goal in industrial food processes. The high solubility properties and WHC attributed to hydrolysed soy protein (Kinsella, 1979; Zayas, 1997) likely contributed to the greater efficiency of the WTB/HSP brine in a shorter tumbling time.

The deeper slice (S3) from tumbled steaks showed greater ( $P < 0.05$ ) brine penetration than did control steaks, although the penetration was limited (Table 2). In this work, brine was traced to a depth of 8 mm of the steak but did not reach the midpoint of its thickness (approximately 12.5 mm deep). Therefore, a full assessment of brine penetration behaviour along the steak was not possible. Future research should examine the effects of brines on deeper slices of the steak.

In general, brine containing hydrolysed soy protein (WTB/HSP) exhibited improved penetration and retention in steaks of 2.5 cm of thickness tumbled for 60 min, with greater ( $P < 0.05$ ) absorbance values in S1 and S2 slices (Table 2). The high HSP solubility allows an increase in the protein/water ratio, the effect of which is enhanced by sodium chloride and phosphate, causing a rise in pH and an increase in the ionic strength of the medium (Baublits, Pohlman, Brown, & Johnson, 2005; Kinsella, 1979). Thus, complex networks are formed by the association of soy and meat proteins,

increasing the WHC, and decreasing the brine outflow (Xiong, 2005). Finally, a longer tumbling time (60 min) in conjunction with the properties of HSP allows a greater disruption of structure and solubilisation of protein, particularly myosin (Huang, Chou, Chen, Tseng, & Lin, 2007).

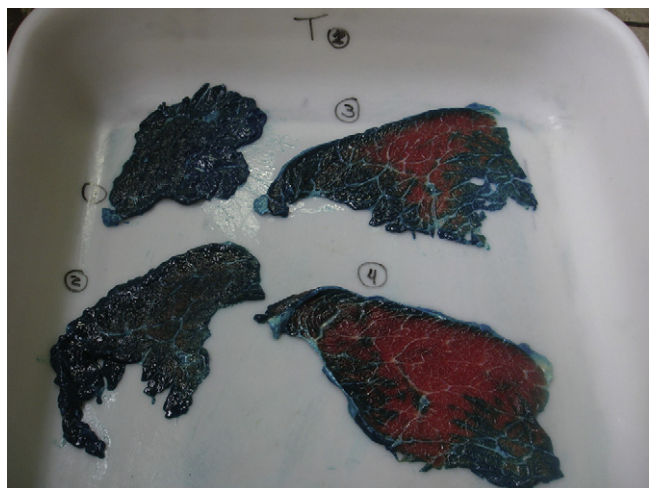
### 3.2. Meat yield and pH

The yield immediately after tumbling (YIAT) of steaks submitted to different brines was influenced by the tumbling time ( $P < 0.01$ ) and muscle portion ( $P < 0.01$ ) used (Table 3). Greater YIAT was observed ( $P < 0.05$ ) for steaks tumbled for 60 min compared to those tumbled for 30 min. The longer tumbling time likely increased the disruption of muscle fibres and membranes, which has been correlated with tissue fragility and susceptibility to brine absorption as well as protein solubilisation (Barbut, 2002). These effects would explain the greater brine penetration, and consequently greater YIAT, in steaks tumbled for 60 min. Positive effects of longer tumbling times on brine absorption have also been observed in chicken fillets (Xiong & Kupski, 1999b).

The origin portions (OP; 11.96 g/100 g) had greater YIAT ( $P < 0.05$ ) compared to the insertion portions (IP; 9.16 g/100 g). Differences in fibre compositions between these two portions, which may be due to differences in myofibrillar protein isoforms, could explain these results. It has been reported that myofibrillar protein isoforms from different muscle fibre types present different solubility in saline solution (Xiong, 2004). Different fibre diameters along the *biceps femoris* muscle found in mice (Goldspink, 1962) might also influence the YIAT responses in the different muscle portions after tumbling with brine.

There were significant effects for brine compositions ( $P < 0.05$ ), tumbling times ( $P < 0.01$ ) and muscle portions ( $P < 0.01$ ) on the yield 36 h after tumbling (Y36AT) (Table 3). The control steaks lost weight (−2.16 g/100 g) and had lower ( $P < 0.05$ ) Y36AT compared to WTB (7.11 g/100 g) and WTB/HSP (7.38 g/100 g) steaks, which were similar to one another ( $P > 0.05$ ). The Y36AT of WTB/HSP steaks was likely not greater than those of WTB steaks as expected, because the weights used to calculate the Y36AT were obtained from raw steaks. All the previous studies in which HSP was reported to form gelatinous matrix complexes, resulting in increased WHC and yield, were conducted in cooked meat products (Feng & Xiong, 2002; Feng, Xiong, & Mikel, 2003; Xiong, 2005).

Y36AT values were similarly influenced by tumbling times and muscle portions but were consistently smaller than YIAT values (Table 3). Steaks tumbled for 60 min showed greater Y36AT ( $P < 0.05$ ) than did those tumbled for 30 min. The properties underlying the difference in YIAT between tumbling times likely



**Fig. 3.** Photograph of the four slices S0 (upper left), S1 (lower left), S2 (upper right) and S3 (lower right) collected from *biceps femoris* muscle steak after tumbling for 60 min with the addition of the FD&C blue dye to brine.



**Table 3**

Steak weight differential (yield) immediately after tumbling and at 36 h after tumbling for different tumbling times, muscle portions and brine compositions.

Yield immediately after tumbling (YIAT, g/100 g) <sup>d,e</sup>			
Tumbling time	30 min	60 min	
	9.08 (0.89) <sup>c,b</sup>	12.01 (0.89) <sup>a</sup>	
Portion	OP	IP	
	11.96 (0.98) <sup>a</sup>	9.16 (0.85) <sup>b</sup>	
Yield 36 h after tumbling (Y36AT, g/100 g) <sup>d,f</sup>			
Brine composition	CONTROL	WTB	WTB/HSP
	−2.16 (0.79) <sup>b</sup>	7.11 (0.72) <sup>a</sup>	7.38 (0.72) <sup>a</sup>
Tumbling time	30 min	60 min	
	6.30 (0.72) <sup>b</sup>	8.19 (0.72) <sup>a</sup>	
Portion	CONTROL	WTB and WTB/HSP	
OP	−1.39 (1.00) <sup>A</sup>	8.34 (0.75) <sup>A</sup>	
IP	−2.93 (0.99) <sup>B</sup>	6.15 (0.75) <sup>B</sup>	
Y36AT – YIAT (g/100 g) <sup>g</sup>			
Brine composition	WTB	WTB/HSP	
	−3.09 (0.08) <sup>b</sup>	−2.75 (0.08) <sup>a</sup>	

<sup>a,b</sup> Different lowercase letters in the same row differ statistically  $P < 0.05$ .

<sup>A,B</sup> Different uppercase letters in the same column differ statistically  $P < 0.05$ .

<sup>c</sup> Mean value, ( ) Standard error.

<sup>d</sup>  $n = 60$  steaks from the *biceps femoris* muscle.

<sup>e</sup> YIAT was calculated as  $(W_a - W_b)/W_b \times 100$ , where  $W_a$  is the weight of the steak after tumbling and  $W_b$  is the weight of the steak before tumbling.

<sup>f</sup> Y36AT was calculated as  $(W_e - W_b)/W_b \times 100$ , where  $W_e$  is the weight of the steak after equalisation and  $W_b$  is the weight of the steak before tumbling.

<sup>g</sup> Y36AT – YIAT was calculated as  $(W_e - W_a)/W_a \times 100$ , where  $W_e$  is the weight of the steak after equalisation and  $W_a$  is the weight of the steak after tumbling.

also explain the changes in Y36AT. Tumbled OP steaks (WTB and WTB/HSP) had greater Y36AT ( $P < 0.05$ ) than did tumbled IP steaks. Also, the control OP steaks lost less ( $P < 0.05$ ) weight than did control IP steaks (Table 3). The *biceps femoris* muscle is quite large, and there is a strong possibility that the origin portion (near the sirloin) and the insertion portion of the muscle have distinct characteristics that respond differently to maturation processes (Kim et al., 2007; Lawrie, 2005; Reuter, Wulf, & Maddock, 2002). The OP may have absorbed more water and resulted in greater yields due to the predominance of white fibre, which is known to be more soluble in salt (Gotoh, 2003; Xiong, 1994).

Data obtained from the relationship between YIAT and Y36AT in steaks tumbled with WTB and WTB/HSP brines demonstrated the significant effect ( $P < 0.01$ ) of brine composition (Table 3). It was observed that WTB/HSP steaks lost less ( $P < 0.05$ ) weight during the equalisation process compared to WTB steaks, showing the effect of hydrolysed soy protein on water retention in muscle. This may be attributed to the ability of HSP to absorb water and the interaction between muscle proteins and soy proteins through electrostatic repulsion (Feng & Xiong, 2003; Kinsella, 1979; Zayas, 1997).

In general, YIAT was greater than Y36AT. The lower Y36AT could be explained by changes in the water balance in muscle. The increase in solute concentration in the muscle, especially sodium chloride, due to the addition of brine would make the muscle hypertonic, which would allow water to escape from the muscle cell (Smith, 1999). This water loss mechanism tends to stabilise after equalisation. Thus, the YIAT obtained in the first minutes after tumbling would only reflect brine absorption. For this reason, measurements taken immediately after tumbling are considered an indication of the percentage of brine initially absorbed, not necessarily the amount retained in the muscle (Xiong & Kupski, 1999b).

There was a significant effect of muscle portion ( $P < 0.05$ ) on initial pH, and an interaction between the effects of brine compositions and tumbling times ( $P < 0.01$ ) on final pH was observed (Table 4).

**Table 4**

Effect of muscle portion on initial pH and effect of brine composition and tumbling time on final pH.

Initial pH <sup>e</sup>		
Portion	OP	IP
	5.59(0.03) <sup>c,b</sup>	5.62(0.03) <sup>a</sup>
Final pH <sup>f</sup>		
Brine composition × time <sup>d</sup>	30 min	60 min
CONTROL	5.52(0.02) <sup>a,C</sup>	5.52(0.02) <sup>a,C</sup>
WTB	5.71(0.02) <sup>a,A</sup>	5.66(0.02) <sup>a,B</sup>
WTB/HSP	5.64(0.02) <sup>b,B</sup>	5.74(0.02) <sup>a,A</sup>

<sup>a,b</sup> Different lowercase letters in the same row differ statistically  $P < 0.05$ .

<sup>A,B,C</sup> Different uppercase letters in the same column differ statistically  $P < 0.05$ .

OP – Portion near the origin of the muscle. IP – Portion near the insertion of the muscle. CONTROL – Steaks that were not tumbled with brine or water. WTB – Steaks tumbled with brine. WTB/HSP – Steaks tumbled with brine added of hydrolysed soy protein.

<sup>c</sup> Mean value, ( ) Standard error.

<sup>d</sup> Tumbling time expressed in minutes.

<sup>e</sup> pH measurements of the steaks taken before tumbling.

<sup>f</sup> pH measurements of the steaks taken 36 h after tumbling.

The OP and IP portions of the muscle had different initial pH values ( $P < 0.05$ ), with the former showing a lower pH ( $5.59 \pm 0.03$ ) than the latter ( $5.62 \pm 0.03$ ). These results could indicate metabolic differences between the two muscle portions, which would cause different initial pHs. A sharp decrease in pH is characteristic of muscles where there is a predominance of fast and glycolytic fibres (Grann & Merkel, 1978; Ouali et al., 1983), such as the OP. Although pH differences between muscle portions have been observed, the final pH of the muscles did not differ ( $P > 0.05$ ) after marination with WTB and WTB/HSP brine, exhibiting values of  $5.68 (\pm 0.02)$  and  $5.70 (\pm 0.02)$  for OP and IP, respectively. These results indicate that the tumbling and brine treatments promoted greater pH homogeneity for the muscle as a whole.

Brine compositions and tumbling times ( $P < 0.01$ ) were found to have interacting effects on final pH. Final pH values were similar ( $P < 0.05$ ) in steaks tumbled with WTB brine for either 30 or 60 min. On the other hand, in steaks tumbled with WTB/HSP, the pH of steaks was greater ( $P < 0.05$ ) after 60 min of tumbling than after 30 min, with average values of  $5.74 (0.02)$  and  $5.64 (0.02)$ , respectively. Greater final pH values in WTB/HSP steaks tumbled for 60 min could be attributed to further solubilisation of HSP and consequently greater extraction of proteins. High final pH values and increased WHC would allow greater yields for WTB/HSP steaks tumbled for 60 min, as observed in Table 3.

Overall, it was observed that steaks submitted to tumbling with WTB and WTB/HSP brines had greater pHs ( $P < 0.05$ ) than control steaks. This probably occurred due to the presence of alkaline phosphate in the brines, which raised the pH of meat from 5.7 to 6.0. This elevation in pH could have increased bound water and decreased free water in the steaks (Smith, 2001; Xiong, 2004). Corroborating our results, pH elevation of the *biceps femoris* muscle after injection with phosphate and sodium chloride has been reported (Baublits, Pohlman, Brown, & Johnson, 2006; Harada, 2004). Similarly, an increase in the percentage of bound water (from 14.5 to 40.6%) in muscles marinated with 18% tripolyphosphate in brine has been observed (Baublits et al., 2006).

#### 4. Conclusions

Brine penetration in steaks from beef *biceps femoris* muscle is not linearly related to tumbling time. Tumbling time and muscle portion are important variables that influence meat yield. The inclusion of HSP and the length of tumbling time interact to dictate

changes in brine penetration and meat pH. Final pH values are increased in steaks submitted to the marination process.

## Acknowledgements

We thank the “Fundação de Amparo a Pesquisa do Estado de São Paulo – FAPESP” for providing the scholarship to the first author and for the financial support to the project (Number 2007/57337-6). The authors also acknowledge Solae Brazil® for donating the hydrolysed soy protein.

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