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1 **IMPACT OF HIGH PRESSURE PROCESSING ON THE FUNCTIONAL ASPECTS OF**
2 **BEEF MUSCLE INJECTED WITH SALT AND/OR SODIUM PHOSPHATES**
3
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31 **Abstract:** This study aimed to determine the interactions among salt (NaCl), sodium phosphate
32 (SP) and mild HPP in brine-injected beef. Beef strip loin segments were injected to 10% over
33 initial weight with solutions containing water and various levels of salt (0, 2 or 4% of solution)
34 and/or SP (0 or 4% of solution). Pieces from the loin sections were exposed to varying pressure
35 levels (0.1, 152 or 303 MPa) and evaluated for selected quality and biochemical characteristics.
36 Use of SP and pressure application increased pH by ~0.2 units. *L** values were increased by
37 pressure and decreased by SP. Redness (*a**) increased at 303 MPa. Purge increases due to
38 pressure were mitigated by SP. Pressure application at 303 MPa reduced total and sarcoplasmic
39 protein solubility by 24 and 32%, respectively. There were no beneficial interactions among salt
40 or SP and HPP. However, results indicate SP may prevent yield loss due to HPP.
41
42 **Keywords:** High pressure processing, beef, salt, phosphate, brine injection
43
44 **Practical application**: This study has demonstrated that beneficial interactions between high
45 pressure processing (HPP) and salt are not achieved in a brine-injected whole muscle product
46 when salt levels are at or below 0.4% of final product weight. Use of sodium phosphates
47 prevented reduced yields and alleviated some of the color change incurred by HPP at mild
48 pressures.
49
50 ## INTRODUCTION
51
52 It is common practice to inject whole muscle, fresh meat cuts with a brine containing
53 sodium chloride (salt; NaCl) and sodium phosphates (SP), as they act synergistically through
54 several mechanisms to increase the water-binding ability of meat proteins. Salt causes
55 depolymerization of myosin as well as a downward shift in the protein's isoelectric point (Offer
56 and Knight 1988). Sodium phosphates dissociate the acto-myosin bond, further increasing
57 solubilization and relaxation of the protein structure, and increase meat pH (Offer and Knight
58 1988). These effects translate to a significantly heightened ability of salt/SP-injected meat to
59 retain injected fluid throughout storage, display and cooking (McGee *et al.* 2003; Lawrence *et al.*
60 2004; Baublits *et al.* 2006a). Sodium phosphates also improve color stability (Baublits *et al.*
61 2006b) and inhibit lipid oxidation (McGee *et al.* 2003).

High pressure processing (HPP) is a non-thermal, non-chemical treatment, which subjects materials to very high hydrostatic pressure (100-1000 MPa). Observations in comminuted products suggest the potential for HPP to be used as a way to enhance the functionality of salt and SP, possibly allowing for usage reductions of these sodium-heavy ingredients. Low-salt (1%) beef hot dogs had similar cook losses and improved texture characteristics compared to controls (2%) when subjected to 200 MPa for 2 min (Sikes *et al.* 2009). Water-holding capacity and protein solubility of 0.5% salt restructured turkey rolls were increased when subjected to 50 – 200 MPa HPP (Chan *et al.* 2011). However, whole muscle products are very different from comminuted products in their character: instead of an amorphous protein network there is the ordered structure of myofibers and connective tissue layers. Duranton *et al.* (2012) noted that making inferences on the effects of HPP on whole muscle products based on results from restructured or comminuted food matrices is ill-advised. In their work it was found that 1.5 or 3% salt injection increased tenderness and water holding capacity of HPP (500 MPa; 6 min) whole muscle hams compared to no salt. Beef loin muscle injected with 1% salt and HPP treated (650 MPa; 10 min) had lower expressible moisture than raw meat without salt with or without high pressure treatment (Fernández *et al.* 2007). However, these salt levels are much higher than what is commonly used in brine enhanced beef (<0.5%). Additionally, sodium phosphates, commonly used concurrently with salt, have not been investigated in whole muscle HPP-treated product. This study aims to establish what, if any, interactions exist between salt, phosphates and high pressure in brine-injected beef. Common indicators of protein functionality, like protein solubility, and quality aspects, like color and purge loss, were investigated.

METHODS AND MATERIALS

Raw materials and injection

Beef strip loins (IMPS 180; n=10) were purchased from a local processor. Loins were trimmed of all excess fat and connective tissue, cut into three segments and assigned to injection treatments. Loin segments were injected to 110% of initial weight with a solution containing 0, 2 or 4% salt (0, 0.2 or 0.4% final product weight) with or without 4% (0.4% final product weight) of a commercially available sodium phosphate blend (Brifisol® 85 Instant; BK Giulini Corp., Simi Valley, CA, USA) using a single-needle hand-operated injector (Koch, Kansas City, MO,

93 USA). The segments identified as 0% salt and 0% SP were injected with water targeting 110%
94 of initial weight. Segments were then weighed and allowed to rest for 20 min after injection
95 (equilibration period) before being cut into 2.5 cm x 2.5 cm x ~8 cm pieces, re-weighed, then
96 vacuum packaged in 15.5 x 22 cm oxygen impermeable bags.
97

98 **Pressure treatment**

99 Pressurization took place in a 22-L chamber (National Forge Company, Andover, MA,
100 USA) using a pressurization medium of soluble oil (Hydrolubric® 123-B, Houghton
101 International, Valley Forge, PA, USA) in water (5% w/w). Packaged beef pieces were subjected
102 to either 0.1 (atmospheric pressure), 152 or 303 MPa for 1 minute. The samples were submerged
103 in ice-water within a nylon bag placed in the chamber during pressurization to deter temperature-
104 induced denaturation. The pressure ramp-up rate was approximately 4 MPa/sec.
105 Depressurization time was ~30 sec regardless of final pressure.
106

107 **Processing and sampling**

108 After pressurization beef pieces were either frozen for further analyses or removed from
109 their packages, pat dry with a paper towel and reweighed to determine purge due to
110 pressurization. Subsequently, these pieces were allowed to bloom for 30 min and used for color
111 and pH analysis.
112

113 **pH and color**

114 A pH meter (pH 3210, WTW GmbH, Weilheim, Germany) equipped with a piercing
115 probe was used to determine pH. Instrumental color was determined using a Minolta CM – 600
116 (Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA) with a 10° observer and illuminant
117 A calibrated with a white tile. CIE L^* and a^* values (CIE 1978) were recorded with the spectral
118 component excluded. Measurements were taken perpendicular to muscle fiber direction, as if
119 they were taken on the sliced surface of a steak.
120

121 **Purge**

122 Multiple variables were used to detail the fluid loss from beef loin segments and pieces.
123 The calculations used to generate these variables are presented in Lowder *et al.* (2011, 2013).
124 They are described here briefly.

125 $Purge_{inj}$ represents the fluid lost during the equilibration period as a percentage of the
126 total loin segment weight. It was calculated by taking the difference between the loin segments
127 immediately after injection and after the 20-min rest period, dividing by the weight of the
128 injected segments and multiplying by 100.

129 $Brine\ Loss_{inj}$ reports the fluid lost during the equilibration period as a percentage of the
130 total fluid injected. To calculate, the difference between loin segment weight immediately after
131 injection and after equilibration is divided by the difference between segment weight
132 immediately after injection and initial segment weight. The resulting value is multiplied by 100
133 to get a percentage.

134 *Purge* represents the fluid lost from the beef piece during pressurization. It is calculated
135 using the following equation: $Purge\ (\%) = (S_0 - S_1)/S_0 \times 100$, where S_0 = the weight of the beef
136 piece at slicing and S_1 = the weight of the beef piece after pressurization.

137 $Purge_{total}$ represents the weight lost from injection through pressurization as a percentage
138 of the total weight of the piece.

139

140 **Protein solubility**

141 Sarcoplasmic (water-soluble) and total (salt- and water-soluble) protein solubility was
142 determined using the Bradford (1976) method with premixed reagents (Bio-Rad Laboratories,
143 Hercules, CA, USA). For sarcoplasmic, two g of sample were homogenized (Polytron PT10-35,
144 Kinematica, Inc., Bohemia, NY) at 10,000 rpm for 30 s in 10 volumes of a low ionic strength
145 buffer (30 mM sodium phosphate, pH 7.4) then incubated on a rocker on ice for 2 h. Samples
146 were kept on an ice bath or under refrigeration immediately before and after homogenization.
147 After centrifugation at 5000 x *g* and 4 °C for 20 min, supernatant was decanted, reacted with
148 Bradford reagent and read on a spectrophotometer (Shimadzu UV-2400, Shimadzu Scientific
149 Instruments, Inc, Columbia, MD) at 595 nm. Total solubility used the same procedures with a
150 high ionic strength buffer (0.6 M KCl, 50 mM sodium phosphate, pH 7.4). Myofibrillar
151 solubility was estimated by subtracting the water-soluble fraction from the total fraction for a
152 given sample. Protein solubility was reported as mg/g sample.

153

154 **Total and reactive sulfhydryls (SH)**

155 Total and reactive sulfhydryl groups were determined in the presence and absence of urea
156 by a modification of the procedures described by Hamada *et al.* (1994). Samples (1 mg
157 protein/ml) were retained from the protein solubility test. Adjusted sample (0.5 mL) was mixed
158 with 2 mL 8M urea in 0.2 M Tris (pH 7.0) and 50 µL of 10 mM 5,5'-dithiobis(2-nitrobenzoic
159 acid) (DTNB) with 0.1 M sodium phosphate and 0.2 mM ethylenediaminetetraacetic acid
160 (EDTA) (pH 7.2) and incubated in a 40 °C water bath for 15 min. Reacted samples were
161 measured on a spectrophotometer (UV 2401, Shimadzu Corporation, Kyoto, Japan) at 412 nm.
162 Total SH content was determined as µMol/mg protein using a molar extinction coefficient of
163 14,150 M^{-1} cm^{-1} (Riddles *et al.* 1979). The adjusted protein sample was incubated at 5 °C for 1
164 hr without urea to determine reactive SH content.

165

166 **Experimental design and statistical analysis**

167 The experiment was arranged as a split plot with the whole plot being a 3 x 2 factorial (3
168 salt levels; 2 SP levels) in a balanced incomplete block design with loin as the block. The whole
169 plot was replicated 5 times and each treatment appeared in a block twice with any other given
170 treatment. The split plot factor was pressure level (0.1, 152, 303 MPa) with a replication of 30.
171 Data were analyzed in PROC GLIMMIX of SAS Version 9.2 with block (loin) defined as
172 a random variable. The whole plot error term was defined as block x salt level x phosphates
173 level. Significance was pre-determined at $\alpha = 0.05$. Treatment effects were subjected to analysis
174 of variance (ANOVA) and, where applicable, means were separated using t-tests. In order to
175 protect experiment-wise error while maintaining power, mean separations within two-way
176 interactions were performed only across certain levels of the other main effect. The *L** data, due
177 to a non-normal distribution, were transformed using the procedure of Box and Cox (1964) as
178 implemented by SAS for ANOVA and mean comparisons. Because of a three-way interaction,
179 the *L** data were sliced by SP level and subjected to two-way ANOVA. Figures and reported
180 means for *L** are based on the reverse-transformed geometric means; standard errors are
181 approximations from those means.

182

183 **RESULTS AND DISCUSSION**

Brine retention in loins

Brine was injected into meat until initial weight was increased 10%. However, a significant amount of brine can be lost after injection (equilibration). Following the equilibration period, product weight was only 5.3 to 7.7% above initial weight. The fluid loss as a percentage of product weight ($Purge_{inj}$) and total fluid injected ($Brine\ Loss_{inj}$) is reported in Table 1. Curiously, $Purge_{inj}$ was not significantly affected ($P > 0.05$) by either salt or SP, though it was numerically reduced by the presence of each (Table 1). $Brine\ Loss_{inj}$ was reduced ($P < 0.05$) by 15.13 or 16.38% by using 4% salt or SP, respectively, in the brine. Fluid retention during the rest period was lower than that seen in previous studies using similar ingredients and raw materials (Lowder *et al.* 2011, 2013). Fluid loss may have been encouraged by segmenting the loins, which increased surface area relative to internal area of the muscle, or the use of a hand-operated, as opposed to automated, pump injection system.

pH and color

A pressure level*SP interaction was observed ($P = 0.013$) for pH (Fig. 1). As expected, phosphates increased ($P < 0.05$) pH regardless of pressure treatment. In addition, pressure treatment at 152 MPa further increased ($P = 0.014$) the pH of samples with SP but not those without it. Pressure at 303 MPa increased pH of samples not treated with SP. Both SP and pressure treatment as low as 200 MPa have been shown to raise the pH of muscle foods by 0.1 - 0.2 units (Lawrence *et al.* 2004; Ma and Ledward 2004). The two effects are seen here to be additive, with SP not only increasing the final pH of 303 MPa pressurized meat, but reducing the pressure needed to cause the increase to, at most, 152 MPa. The changes in pH seen here are similar to those seen by other researchers (Lawrence *et al.* 2004; Ma and Ledward 2004) even though the values of the non-pressurized meat are lower than those frequently observed (Lowder *et al.* 2011; Parsons *et al.* 2011). Increases in meat pH due to pressure have been attributed to increased ionization, which may sequester free hydrogen ions, and protein denaturation, which can bury acidic side groups (Macfarlane *et al.* 1980; Ma and Ledward 2004).

The L^* (lightness) values are shown visually in Fig. 2. Data were sliced by salt level and analyzed using the SP and pressure variables to explain the three-way interaction. This, unfortunately, limits the inferences that can be made on the impact of salt level. When salt was absent from the brine, SP inclusion reduced ($P < 0.01$) lightness at the 0.1 and 152 MPa pressure

215 levels, but not at 303 MPa (*P* = 0.98). Sodium phosphates reduced (P < 0.017) lightness at all
216 pressure levels at 2% salt but had no significant effect (P > 0.05) at 4% salt.
217 In addition to increasing water-binding ability, SP increases the pH of meat which increases
218 mitochondrial oxygen consumption rate, causing competition with myoglobin and resulting in
219 darker muscle appearance (Faustman and Cassens 1990). Absent salt, 152 MPa decreased (*P* =
220 0.021) lightness of beef without SP but did not affect (*P* = 0.28) beef with SP. Lightness was
221 decreased (*P* < 0.05) by 152 MPa regardless of SP presence at 2% salt, but at 4% salt it was the
222 SP treated beef that was darker (*P* < 0.01). At 303 MPa, all beef was significantly lighter (*P* <
223 0.05) than atmospheric pressure or 152 MPa except the salt and phosphate free treatment which
224 was almost significantly higher (*P* = 0.052). Previous studies (Carlez *et al.* 1993; Hong *et al.*
225 2005) on minced beef and pork loin found numerical but non-significant increases in *L** values
226 upon pressurization at 150 MPa, whereas the current data show a minor (1 – 4 units) decrease at
227 152 MPa. Higher pressures are more commonly examined and the current study is in agreement
228 with the majority of the literature. At pressures in the range of 200 – 400 MPa with as little as
229 15-s dwell time a major (5–15 units) increase in lightness is frequently observed in beef and pork
230 (Carlez *et al.* 1995; Cheftel and Culioli 1997; Hong *et al.* 2005; Souza *et al.* 2011). The increase
231 in lightness, believed to be caused by globin denaturation or protein coagulation, is often cited as
232 a negative effect of HPP on fresh beef and, in this case, the darkening effect of SP at 2% salt
233 should be considered a benefit (Carlez *et al.* 1995).
234 Incorporation of SP without salt increased (*P* = 0.005) redness by 2.3 units, but had no
235 effect (*P* > 0.515) on redness with salt at 2 or 4% of the brine (Table 2). Salt inclusion increased
236 redness at 4% without SP, but had no significant effect (*P* > 0.13) when SP was also present.
237 Increasing salt level is not typically associated with an increase in *a** values, as salt is pro-
238 oxidative and has been shown to contribute to myoglobin oxidation (Lawrence *et al.* 2004;
239 Baublits *et al.* 2006a). The pressure*SP interaction (Fig. 3) shows an increase (*P* < 0.001) in
240 redness upon pressurization to 303 MPa by 2 units over non-pressurized for both 0 and 4% SP.
241 An increase of redness (*P* = 0.044) at 303 MPa by SP was also observed. Much like salt, the
242 observed increases of *a** due to SP use are curious and not common in the literature, though it is
243 known to increase color stability over time by acting as an antioxidant (Baublits *et al.* 2006a). It
244 should be noted that color measurements were taken within several hours of injection and it is
245 unlikely there was sufficient time for oxidation to develop in the salt-treated samples. The

246 increase in redness by HPP at 303 MPa is supported by other work. Hong *et al.* (2005) noticed
247 an increase in redness of pork *longissimus dorsi* at 200 MPa and Jung *et al.* (2003) observed
248 increased redness between 130 and 350 MPa in beef *biceps femoris*. The mechanism of this
249 redness increase is not understood, but often attributed to the activation of enzyme systems with
250 metmyoglobin reducing activity (MRA; Jung *et al.* 2003). The observation by Cheah and
251 Ledward (1997) that this phenomenon is more apparent in the *longissimus dorsi*, which is
252 considered color stable with a surplus of MRA species, than in the *psoas major*, which lacks in
253 color stability, lends credence to this idea (Joseph *et al.* 2012).
254
255 **Purge**
256 *Purge*, the fluid loss during packaging and pressurization, increased ($P = 0.003$) in non-
257 SP treated beef by 0.80% when exposed to 152 MPa but was only numerically greater ($P =$
258 0.279) when exposed to 303 MPa (Fig. 4). In the beef with SP, *Purge* values ranged from 2.94
259 to 3.17%, but were unaffected ($P = 0.725$) by HPP and were 1.35 – 2.31% lower ($P < 0.001$)
260 than their non-SP counterparts. The loss of fluid from time of injection through pressurization,
261 $Purge_{total}$, produces a pattern of fluid loss that is exactly the same as that seen for *Purge* (Fig. 5);
262 152 MPa, but not 303 MPa increased ($P = 0.033$) fluid loss from 0% SP meat while having no
263 effect ($P = 0.487$) on SP meat. The salt*SP interaction showed a synergistic cooperation, with at
264 least 2% salt reducing ($P = 0.035$) $Purge_{total}$ of SP beef by an additional 1.5 – 2.5% over that
265 without salt. The cooperative effects of salt and SP on fluid retention properties of muscle foods
266 are well documented and understood (Offer and Knight 1988; Lawrence *et al.* 2004; Lowder *et*
267 *al.* 2013); observations of these actions here are expected. Pressurization has been previously
268 documented as negatively affecting fluid retention variables in whole muscle meat. 200 MPa of
269 pressure (5 min) reduced WHC of beef *semitendinosus* steaks (Kim *et al.* 2007). Hong *et al.*
270 (2005) and Marcos *et al.* (2010) investigated pressurization on pork loin muscle. The former
271 noted reduced WHC at 150 – 200 MPa when exposed for long periods of time (15 – 60 min)
272 while the latter observed increased expressible moisture at 400 MPa, but not 200 MPa. Pork
273 *biceps femoris* was reduced in water holding capacity (WHC) upon pressurization to 500 MPa
274 for 6 min (Duranton *et al.* 2012). Observations of cross-sectional microstructure through
275 electron microscopy revealed contracted myofibril structure and increased space in extracellular
276 channels, which are known to reduce water holding capacity by allowing diffusion of water out

277 of myofibrils (Kim *et al.* 2007; Liu *et al.* 2010; Duranton *et al.* 2012). Reduction in protein
278 solubility, which is correlated with water binding characteristics, was also reported concurrently
279 with rises in fluid loss in cases where it was investigated (Joo *et al.* 1999; Kim *et al.* 2007;
280 Marcos *et al.* 2010); it is likely that the two phenomena are related. Duranton *et al.* (2012) and
281 Fernandez *et al.* (2007) showed that salt injection of pork and beef, respectively, prevented the
282 detrimental effects of pressurization on fluid retention. These effects were not seen in the
283 present work, most likely because the salt level (0.4% final target) was much lower than the
284 other studies (1.5 and 1% final target, respectively). Use of SP in this study, however, which
285 was targeted (0.4%) near the legal limit of 0.5% final weight, was able to negate the water loss
286 induced by HPP.
287
288 **Protein solubility and sulfhydryls**
289 Protein solubility was only affected ($P < 0.002$) by pressure level (Table 3). In all cases,
290 solubility was decreased when HPP was applied at 303 but not 152 MPa. No tested factor
291 influenced reactive or total sulfhydryls (SH) or the reactive/total SH ratio. Pressure level showed
292 a tendency to increase ($P = 0.078$) reactive SH content, but, curiously, the reactive/total SH ratio
293 did not seem to be influenced by this ($P = 0.520$). Varying observations on protein solubility of
294 HPP meats have been reported. Lee *et al.* (2007) reported decreased solubility of beef
295 *semitendinosus* in 0.6 M KCl at pressures of 400 MPa or greater; using low ionic strength buffer
296 (0.1 M KCl); however, solubilized greater amounts of protein at 200 MPa. Solubility of beef
297 *biceps femoris* myofibrils was increased by pressurization at 300 – 600 MPa while in 0.1 M KCl
298 buffer (Jung *et al.* 2000), while that of chicken breast myofibrils was increased at 100 – 300 MPa
299 in a similar solution (Iwasaki *et al.* 2006). Marcos *et al.* (2010) noted stepwise decreases in
300 solubility of sarcoplasmic protein fractions (extracted under very low ionic strength) when
301 subjected to 200 and 400 MPa for 20 min. Total protein extraction (0.55 M KI) from minced
302 chicken breast containing 0 or 2.5% salt was decreased when 400 or 600 MPa pressure was
303 applied when compared to 200 MPa, but this decrease was almost completely counteracted when
304 0.3% sodium tripolyphosphate and 1% salt were used (Omana *et al.* 2011). The same study
305 reported decreased solubility of the sarcoplasmic fraction regardless of included non-meat
306 ingredients. Decreases in solubility, regardless of fraction (myofibrillar/sarcoplasmic) are
307 associated with protein denaturation and aggregation, both of which reduce functionality of

308 proteins important to meat quality (Joo *et al.* 1999). Denaturation of proteins, specifically a
309 folded to unfolded transition, with exposure of hydrophobic side groups, is expected when
310 proteins are subjected to high hydrostatic pressure (Mozhaev *et al.* 1996). Observations of
311 pressurized myofibrillar suspensions support this assertion (Chapleau *et al.* 2002, 2003). The
312 cited studies characterized pressure-induced changes to myofibrillar proteins, including a
313 maximum three-fold increase in surface hydrophobicity, indicative of denaturation, and
314 formation of protein aggregates. Greater exposure of sulfhydryl groups due to unfolding may
315 encourage this aggregation, as the ratio of reactive to total SH groups has been seen to increase
316 upon pressurization in myofibrillar suspensions (Chapleau *et al.* 2003) and in model meat
317 systems (Chan *et al.* 2011; Omana *et al.* 2011). While reactive SH group exposure showed some
318 amenability to pressure application in the current work, truly significant effects on SH
319 characteristics may have been difficult to discern from analysis of total extracted protein as
320 opposed to a more pure fraction. The overall lack of influence of salt and SP on solubility
321 suggests that, at the tested levels, they were unable to significantly alter how hydrostatic pressure
322 affects muscle protein. However, the fact still remains that SP use was able to completely
323 counteract the purge increase due to pressurization. Given the evidence from this study, we
324 assert that the additive pH increase from combined SP/pressure treatment played a primary role
325 in that phenomenon.
326
327 **CONCLUSIONS**
328
329 In the current study, no evidence of interactions between high hydrostatic pressure and
330 the ingredients salt or sodium phosphates were seen. It is likely that levels in this study, while
331 appropriate for a similar commercial product, where too low to elicit any changes due to
332 pressure. Additionally, the highly ordered character of intact muscle prevents the degree of
333 ingredient-protein interaction achievable in comminuted and restructured products. However, SP
334 addition was able to lessen the whitening effect to some degree and completely counteract the
335 purge losses incurred upon pressurization. Since protein solubility and SH content were not
336 affected and salt was unable to achieve the same result, the additive SP/pressure induced pH
337 increase is implicated in both cases rather than retention of protein functionality. This assertion
338 is not definitive, however, as only basic protein characterization was carried out in this study.

339 Current results suggest SP may play a role in producing acceptable fresh HPP-treated whole
340 muscle beef, but further experimentation at higher pressures with longer dwell times is needed to
341 confirm this. Independent of pressure application, the ability of salt and SP to retain fluid in
342 whole muscle injected beef seen here further supports the body of work already done on the
343 subject.
344

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346
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349

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TABLE 1. MAIN EFFECT LS MEANS FOR $PURGE_{INJ}$ AND $BRINE\ LOSS_{INJ}$ OF LOIN SEGMENTS INJECTED TO 10% OVER INITIAL WEIGHT WITH VARYING LEVELS OF SALT AND/OR SODIUM PHOSPHATES (SP)

Salt (%)[c]	$Purge_{inj}$	SEM[d]	$Brine\ Loss_{inj}$	SEM[d]
0	4.66	0.31	66.67[a]	3.75
2	3.55	0.31	56.59[ab]	3.92
4	3.77	0.27	51.54[b]	3.45
SP (%)[c]				
0	4.19	0.25	66.46[a]	3.19
4	3.79	0.23	50.08[b]	2.87

[a,b]Means within a column and main effect with differing superscripts are significantly different ($P < 0.05$)
[c]As a percentage of the brine
[d]Standard error of the mean

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TABLE 2. REDNESS (A* VALUES) OF BEEF PIECES FROM LOINS INJECTED TO 10% OVER INITIAL WEIGHT WITH VARYING LEVELS OF SALT AND/OR SODIUM PHOSPHATES (SP) AND SUBJECTED TO HIGH PRESSURE PROCESSING AT 0.1, 152 OR 303 MPA AS AFFECTED BY SALT/SP LEVELS

	Salt (%)[c]		
SP (%)[c]	0	2	4
0	20.72[b] ± 0.63	22.23[ab] ± 0.61	22.46[a] ± 0.56
4	23.07 ± 0.56	22.02 ± 0.61	22.01 ± 0.56
P value[d]	0.005	0.795	0.515

[a,b]Means (± standard error of the mean) within a row with differing superscripts are significantly different ($P < 0.05$)
[c]As a percentage of the brine
[d]*P* value of the comparison of SP levels at a given salt level

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TABLE 3. SARCOPLASMIC, MYOFIBRILLAR AND TOTAL PROTEIN SOLUBILITY AND REACTIVE, TOTAL AND REACTIVE/TOTAL SULFHYDRYL (SH) RATIO OF BEEF PIECES FROM LOINS INJECTED TO 10% OVER INITIAL WEIGHT WITH VARYING LEVELS OF SALT AND/OR SODIUM PHOSPHATES (SP) AS AFFECTED BY APPLIED HIGH PRESSURE PROCESSING (HPP)

Pressure (MPa)	Sarcoplasmic Solubility[c]	Myofibrillar Solubility[c]	Total Solubility[c]	Reactive SH[d]	Total SH[d]	Reactive/Total SH Ratio
0.1	$1.64^{a} \pm 0.07$	$1.64^{a} \pm 0.11$	$3.28^{a} \pm 0.15$	106 ± 4.7	150 ± 6.6	0.74 ± 0.03
152	$1.58^{a} \pm 0.07$	$1.69^{a} \pm 0.11$	$3.26^{a} \pm 0.15$	113 ± 4.7	155 ± 6.3	0.76 ± 0.03
303	$1.37^{a} \pm 0.07$	$1.11^{b} \pm 0.12$	$2.48^{b} \pm 0.15$	120 ± 4.6	156 ± 6.7	0.77 ± 0.03
P value[e]	0.002	<0.001	<0.001	0.078	0.576	0.52

[a,b]Means within a column with differing superscripts are significantly different ($P < 0.05$)
[c]Protein solubility means are expressed as mg protein/g sample ± standard error of the mean
[d]Sulfhydryl means are expressed as µMol SH/mg protein ± standard error of the mean
[e]*P* value for the main effect of pressure level

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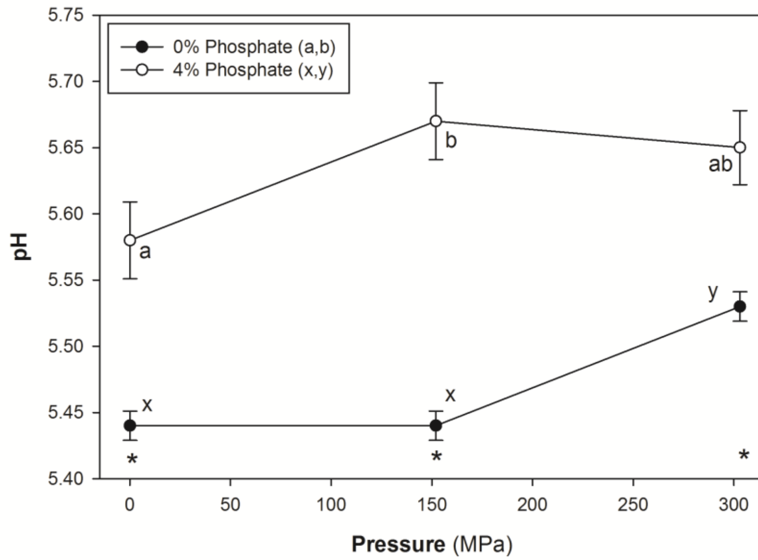

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636 FIG. 1. PRESSURE X SODIUM PHOSPHATE (SP) INTERACTION PH VALUES FOR BEEF
637 FROM STRIP LOINS INJECTED TO 110% OF INITIAL WEIGHT WITH SOLUTIONS
638 CONTAINING SALT (0, 2 OR 4% OF SOLUTION) AND/OR SP (0 OR 4% OF SOLUTION) AND
639 EXPOSED TO HIGH PRESSURE (0.1, 152 OR 303 MPa; 1 MIN AT AMBIENT
640 TEMPERATURE). DATA POINTS WITH DIFFERING LETTERS WITHIN AN SP LEVEL ARE
641 SIGNIFICANTLY DIFFERENT ($P < 0.05$); AN '*' DENOTES A SIGNIFICANT DIFFERENCE (P
642 < 0.05) BETWEEN SP LEVELS AT THAT PRESSURE.
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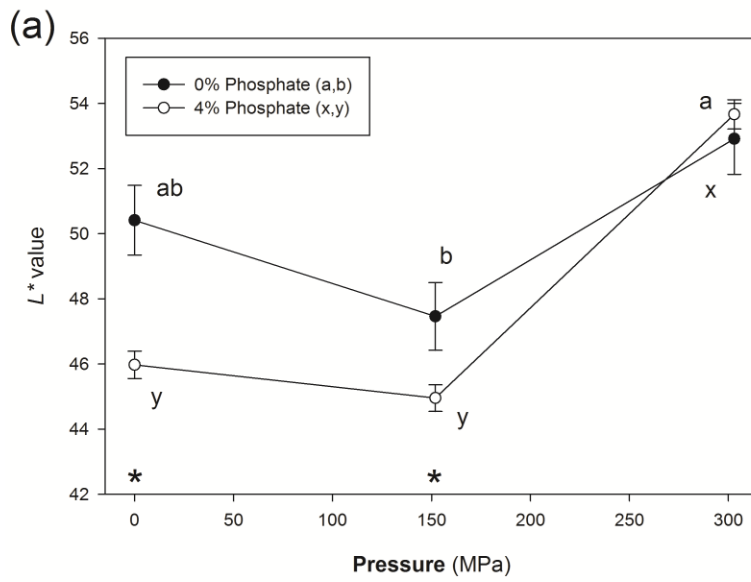
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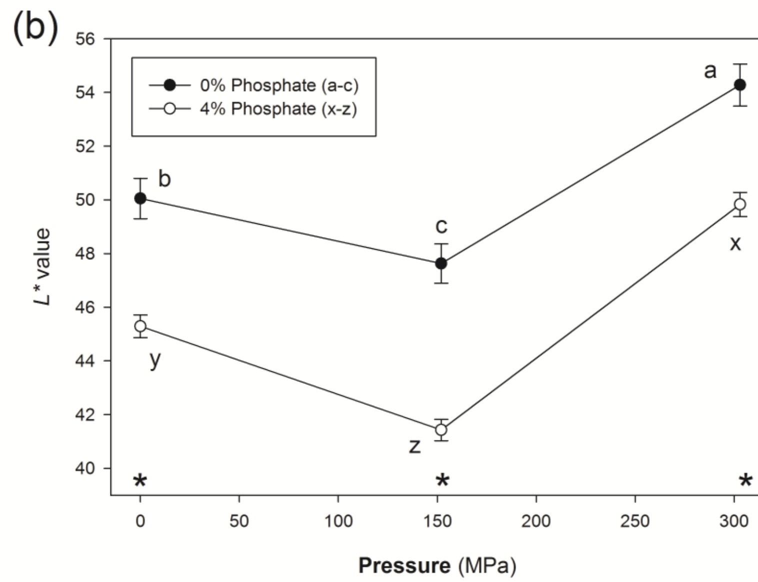
(b)
0% Phosphate (a-c)
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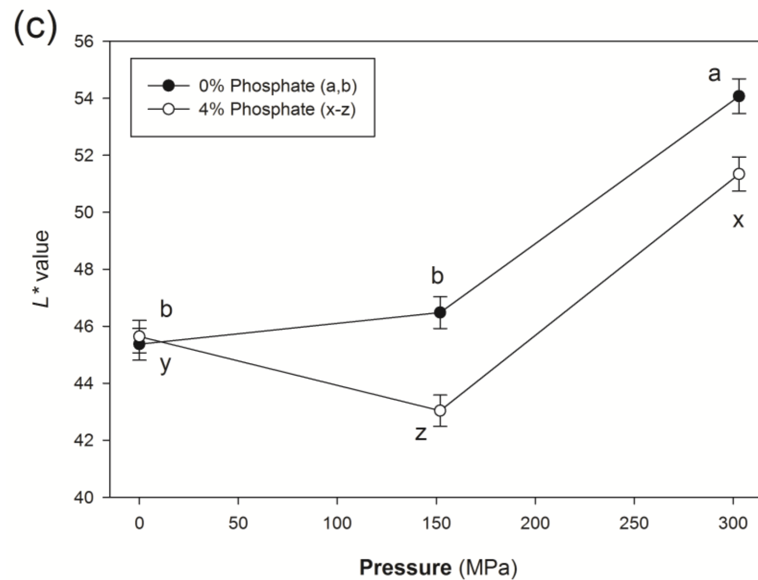
(c)
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4% Phosphate (x-z)
L* value
Pressure (MPa)
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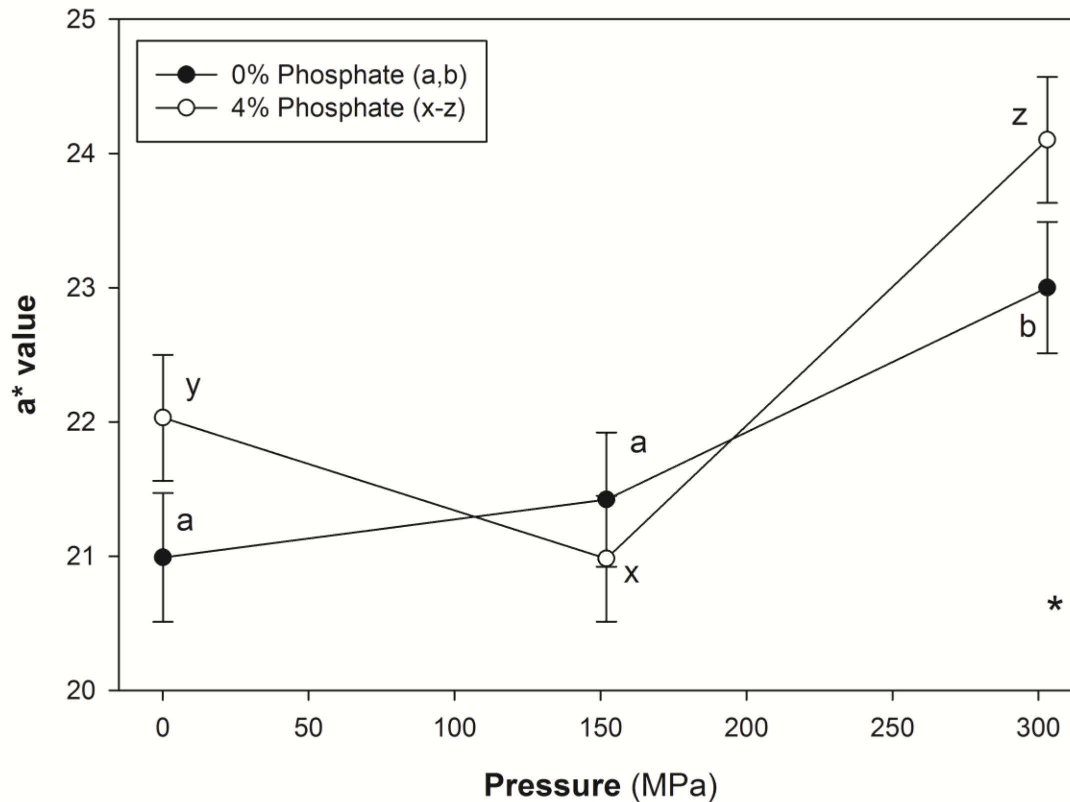
FIG. 2. *L** (LIGHTNESS) VALUES OF BEEF FROM STRIP LOINS INJECTED TO 110% OF INITIAL WEIGHT WITH SOLUTIONS CONTAINING SALT AT (a) 0, (b) 2 OR (c) 4% OF SOLUTION AND/OR SP (0 OR 4% OF SOLUTION) AND EXPOSED TO HIGH PRESSURE (0.1, 152 OR 303 MPA; 1 MIN AT AMBIENT TEMPERATURE). DATA POINTS WITH DIFFERING LETTERS WITHIN AN SP LEVEL ARE SIGNIFICANTLY DIFFERENT ($P < 0.05$); AN '*' DENOTES A SIGNIFICANT DIFFERENCE BETWEEN SP LEVELS AT THAT PRESSURE.



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665 FIG. 3. PRESSURE X SODIUM PHOSPHATE (SP) INTERACTION FOR *a** (REDNESS)
666 VALUES OF BEEF FROM STRIP LOINS INJECTED TO 110% OF INITIAL WEIGHT WITH
667 SOLUTIONS CONTAINING SALT (0, 2 OR 4% OF SOLUTION) AND/OR SP (0 OR 4% OF
668 SOLUTION) AND EXPOSED TO HIGH PRESSURE (0.1, 152 OR 303 MPa; 1 MIN AT
669 AMBIENT TEMPERATURE). DATA POINTS WITH DIFFERING LETTERS WITHIN AN SP
670 LEVEL ARE SIGNIFICANTLY DIFFERENT ($P < 0.05$); AN '*' DENOTES A SIGNIFICANT
671 DIFFERENCE ($P < 0.05$) BETWEEN SP LEVELS AT THAT PRESSURE.

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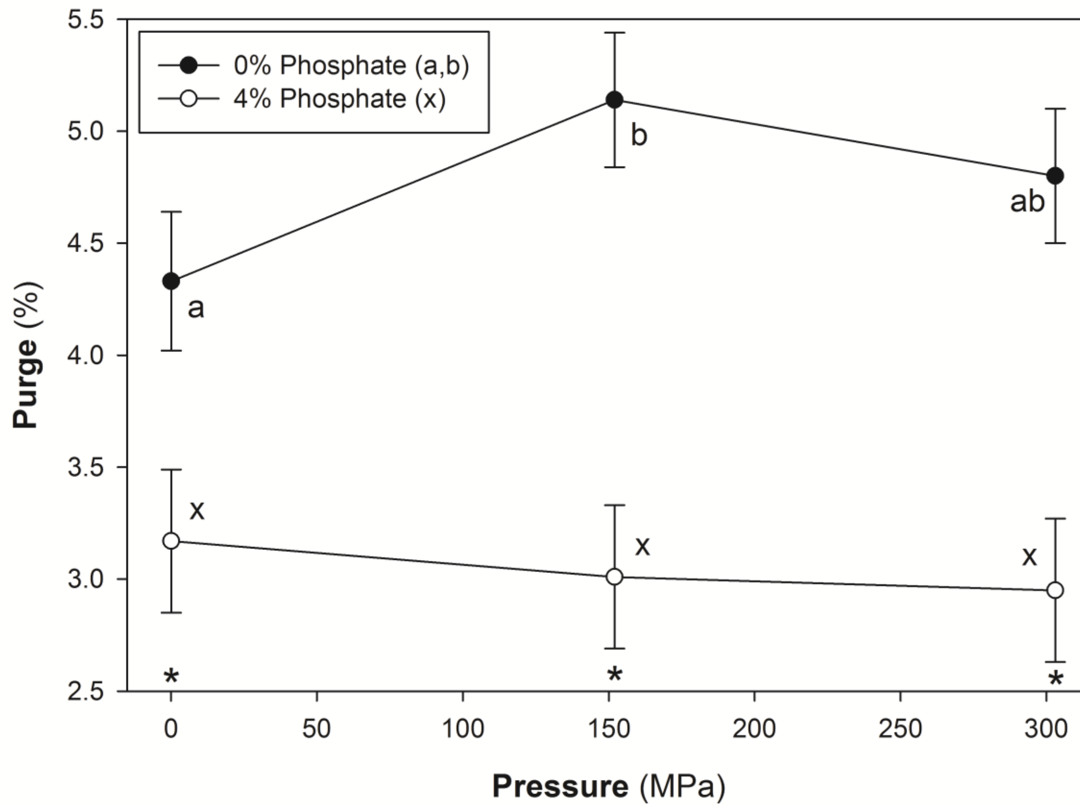

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686 FIG.4. PRESSURE X SODIUM PHOSPHATE (SP) INTERACTION FOR *PURGE* VALUES OF

687 BEEF FROM STRIP LOINS INJECTED TO 110% OF INITIAL WEIGHT WITH SOLUTIONS

688 CONTAINING SALT (0, 2 OR 4% OF SOLUTION) AND/OR SP (0 OR 4% OF SOLUTION) AND

689 EXPOSED TO HIGH PRESSURE (0.1, 152 OR 303 MPa; 1 MIN AT AMBIENT

690 TEMPERATURE). DATA POINTS WITH DIFFERING LETTERS WITHIN AN SP LEVEL ARE

691 SIGNIFICANTLY DIFFERENT ($P < 0.05$); AN '*' DENOTES A SIGNIFICANT DIFFERENCE (P

692 < 0.05) BETWEEN SP LEVELS AT THAT PRESSURE.

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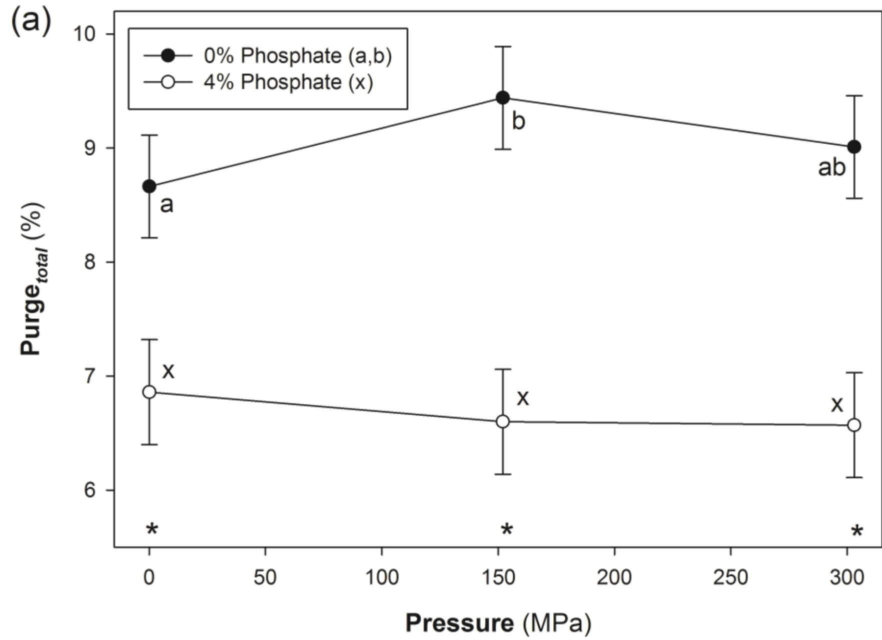

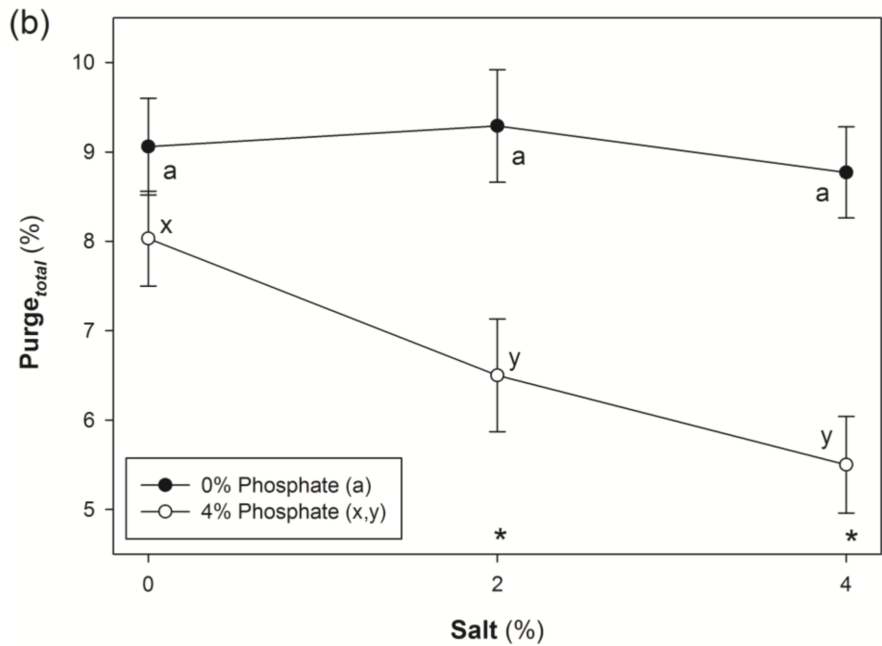

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702 FIG. 5. (A) PRESSURE X SODIUM PHOSPHATE (SP) AND (B) SALT X SP INTERACTIONS
703 FOR *PURGE*$_{TOTAL}$ VALUES OF BEEF FROM STRIP LOINS INJECTED TO 110% OF INITIAL
704 WEIGHT WITH SOLUTIONS CONTAINING SALT (0, 2 OR 4% OF SOLUTION) AND/OR SP (0
705 OR 4% OF SOLUTION) AND EXPOSED TO HIGH PRESSURE (0.1, 152 OR 303 MPA; 1 MIN
706 AT AMBIENT TEMPERATURE). DATA POINTS WITH DIFFERING LETTERS WITHIN AN SP

707 LEVEL ARE SIGNIFICANTLY DIFFERENT ($P < 0.05$); AN ‘*’ DENOTES A SIGNIFICANT
708 DIFFERENCE BETWEEN SP (A) OR SALT (B) LEVELS AT THAT PRESSURE.