Response surface modeling to predict fluid loss from beef strip loins and steaks injected with salt and phosphate with or without a dehydrated beef protein water binding adjunct

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ABSTRACT

This study was conducted using response surface methodology to predict fluid loss from injected beef strip steaks as influenced by levels of salt and sodium phosphates (SP) in the injection brine. Also, a beef-based dehydrated beef protein (DBP) water binding ingredient was evaluated. Paired U.S. select beef strip loins were quartered before being injected to 110% of initial weight with a brine containing salt and SP (CON) or salt, SP and 5% DBP. Steaks were sliced, overwrapped and stored in the dark for 4 d. Purge values ranged from 0.6% to 4.6% for CON and 0.3% to 2.1% for DBP. Purge loss values when accounting for the fluid lost from injection to slicing were as high as 6.8% for CON brines, but only 2.8% for DBP brines. The equations generated here and the DBP product could help producers achieve acceptable purge while reducing sodium use.

Key Words: response surface, phosphate, beef

1. Introduction

Meat products are commonly injected with brines containing sodium chloride (NaCl; salt) and sodium phosphates (SP) in an effort to improve product quality and consistency. The combined use of salt and sodium phosphates alter protein functionality in such a way that the water holding capacity of the product is increased, causing greater retention of injected and natural fluids (Offer and Knight, 1988). This increases sensory attributes such as tenderness and juiciness (Vote, Platter, Tatum, Schmidt, Belk, Smith & Speer, 2000) and offsets the loss of fluids during storage and display (purge). As a result, the use of salt and sodium phosphate based brines has become economically important to processors.

Currently, however, the industry is looking to scale back the use of sodium in its products. Processed meats contribute an estimated 21% of the sodium in the typical U.S. diet (Engstrom, Tobelmann & Albertson, 1997). This is largely because of salt and sodium phosphates, as they are the most common ingredients added to brine injected meats besides water and contain a high amount of sodium (39.3 and 31.2%, respectively; Ruusunen & Puolanne, 2005). Since these ingredients are directly responsible for the increased functionality seen in brine injected meats, removing them entirely is not an attractive option. It is likely possible to make minor, or even significant, reductions in salt and sodium phosphates and still retain enough functionality to keep water holding capacity and fluid loss at a minimum. There is very little research in this area with respect to brine injected beef. Additionally, it is important to research the use of alternative ingredients that act as water binders and could reduce, or potentially replace, the use of salt or sodium phosphates in brine injected meats. In support of these objectives a study was conducted using response surface experimental methodology to model the changes in purge of beef strip loin steaks according to the amount of salt and phosphate included in the injection brine. A second model was also created which included the use of a dehydrated beef protein powder (DBP; Proliant Meat Ingredients, Inc.), a newly developed, beef collagen based, water binding non-meat ingredient.

2. Materials and Methods

2.1 Collection of select beef strip loins

Paried U.S. Select beef strip loins (IMPS 169a) were collected at a processing facility at the time of carcass fabrication. Carcasses were aged 48 hours prior to fabrication. Loins were vacuum packaged at the processing facility and placed on ice in a cooler for transportation. Upon arrival, loins were transferred to a 4°C cooler and stored overnight. All subsequent preparation of brines and raw materials was conducted in a processing facility with a constantly maintained temperature of 4°C.

2.2 Brines

Table 1 provides the concentration by weight of salt and sodium phosphates (SP; Brifisol® 85 Instant; BK Giulini Corporation, Simi Valley, CA) of each of the brines that were evaluated. For brines containing the dehydrated beef protein (DBP; Proliant Meat Ingredients, Inc., Ankeny, IA), the level utilized was 5%. All brines contained 1% Herbalox seasoning HT-S (Kalsec, Kalamazoo, MI). When DBP was used in the formulation it was first mixed with 9.07 Kg of water at 30°C. A separate ice water slurry was created using 13.61 Kg of ice water (1:1) solution containing required levels of salt and STPP. This ice slurry was then added to the DBP mixture. Herbalox was then added and followed by the balance of water at 4°C. The technique and amounts used for making the DBP containing brines was following the supplier's recommendations. Brines without DBP were formulated with 4°C water. All brines were injected at 4°C. Individual brine batches (45.45 Kg) were prepped immediately prior to injection.

2.3 Injection

Brine injection was conducted on three separate days. Paired loins were each quartered. A sample was removed for initial pH and proximate composition. The quarters from one side of each pair were randomly assigned to brines with DBP. The other side of the pair was assigned to brines without DBP. Strip loin quarters were weighed and then injected with brine at 4ºC using a 20 single needle (interior bore size of 25 mm, Model # 2 – 1 – 4 x 0.6 x 272 – H x 2 x 2.5 x 5, Fomaco Food Machinery Co., Copenhagen, Denmark) automatic brine injector (Fomaco Model FGM 20/20S, Fomaco Food Machinery Co., Copenhagen, Denmark) calibrated to inject at ~110% of the recorded initial meat weight. Needles penetrated meat to 0.64 cm above the bottom of the meat surface at a rate of 40 strokes/minute and a pressure of 26 psi. Needles were approximately 2.54 cm apart. Loins were re-weighed immediately after injection.

2.4 Equilibration, slicing, and packaging

Injected loin quarters were allowed to equilibrate 30 min on cutting tables and then re-weighed prior to slicing into 2.54 cm steaks using a standard 33.02 cm manual slicer (Model 3600P, Globe Food Equipment Co., Ohio, U.S.A.). Three steaks were collected from each quarter.

weighed and packaged by overwrapping. Overwrapped steaks were placed individually onto an absorbent pad (Pad-Loc Super Absorbent Pads (PLS), Sealed Air, Duncan, SC) in a 21.75 X 16.51 X 2.78 cm white tray (Cryovac 3 Processor Trays, Sealed Air, Duncan, SC). Trays were then overwrapped with oxygen permeable film (Oxygen transmission rate = 23250cc/m^2(24hrs), OmniFilm, Pliant Corp., Schaumburg, IL) and sealed using a film wrapper (Model WHSS-1, Win-HOLT Equipment Group, Syosset, NY). Overwrapped steaks were placed in 63.5 x 76.5 cm bags ("motherbags"; OTR: <0.1 cc per 645 cm^2/24 h @ 23°C and 0% RH), each containing 4 steaks. The air was evacuated and replaced with 35% CO_2, 0.4% CO and 64.6% N_2 gas using a MultiVac C500 (MultiVac, In., Wolfertschwenden, Germany). The final atmosphere in the motherbag was evaluated with a headspace analyzer (CheckMate 9900 O_2/CO_2, PBI Dansensor, Denmark). The motherbags were filled using the MultiVac C500 with a gas inlet pressure at 35 psi, gas flushing at 600 mbar and seal at 250 mbar.

2.4 Storage

Motherbags were stored for 4 days at 4°C in the dark to simulate transportation conditions. On d 5, the atmosphere in the motherbag was evaluated and then overwrapped packages were removed from the bag and steaks were evaluated.

2.5 Purge

Purge was measured and calculated as described previously by Lowder et al. (2011). Purge measurements included $Brine\ loss_{30min}$, $Brine\ Loss_{total}$, *Purge*, and $Purge_{total}$. Briefly, $Brine\ Loss_{30min}$ represents the injection fluid lost after the 30 min equilibration period. $Brine\ Loss_{total}$ represents the total amount of fluid lost from injection through storage. Both are calculated as a percentage of the total fluid injected. *Purge* represents the fluid lost from the steak during retail display and is calculated as percentage of initial steak weight. $Purge_{total}$ represents the fluid lost from the steak from injection through storage. It back calculates the theoretical weight of the steak if it had been cut immediately after injection instead of after the 30 min loin equilibration period.

<u>Measurements taken:</u>

A = initial weight of loin

B = weight of loin after injection

C = weight of loin 30 min after injection

D = initial weight of steak

E = weight of steak on day 5

B – A = brine added to the loin

B – C = fluid loss 30 min after injection

D – E = fluid loss from time steak was cut until day 5.

$\frac{D}{C}$ = proportion of steak from loin

<u>Calculations:</u>

$$\%\ Brine\ loss_{30min} = \frac{B-C}{B-A} \times 100$$

$$\%\ Brine\ Loss_{total} = \frac{\frac{D}{C} \times (B-C) + (D-E)}{(B-A) \times \frac{D}{C}} \times 100$$

$$\%\ Purge \quad = \frac{D-E}{D} \times 100$$

$$\%\ Purge_{total} = \frac{\frac{D}{C} \times (B-C) + (D-E)}{(\frac{D}{C} \times (B-C) + D} \times 100$$

2.6 Experimental design

In order to develop a response surface model, a central composite design was applied. Variables were concentration of sodium chloride (salt, NaCl) and concentration of sodium phosphates (SP) applied in the combinations shown in Table 1. The combinations were assigned to experimental units (loin quarter) in a randomized incomplete block design with animal (loin pairing) designated as the block. Four replications were analyzed for each combination except 9 (1.8% salt, 2.25% SP) which had six replications and was present within each block.

2.7 Statistical analysis

PROC GLIMMIX of SAS (Version 9.2, Cary, NC) was used to generate the least squares estimators that were used to create the response surfaces for each treatment (CON, DBP). The following saturated model was assumed for all response variables:

(1) $\hat{Y} = \beta_0 + \beta_1\text{NaCl} + \beta_2\text{SP} + \beta_{11}\text{NaCl*NaCl} + \beta_{22}\text{SP*SP} + \beta_{12}\text{NaCl*SP}$

Non-significant quadratic and interaction terms were removed when $P > 0.1$. NaCl and SP were left in the model regardless of significance.

Loin pairing was designated as a random effect. Separate equations were created for the control and DBP treatment for each response variable. *Purge* and $Purge_{total}$ were modeled using a normal distribution and a linear mixed model. $Brine\ Loss_{30\ min}$ and $Brine\ Loss_{total}$ were converted to proportions by dividing the values by 100, and analyzed using a generalized linear mixed model (GLMM) with a logit link function since responses followed a beta distribution. The following equation is used to invert obtained predicted values to proportions, which can then be multiplied by 100 to produce percentages:

(2) $\%\ Brine\ loss_x = ((\exp(logit\hat{Y}))/(1+\exp(logit\hat{Y})))*100$ where $\%\ Brine\ loss_x$ is the respective response variable and $logit\hat{Y}$ is the predicted value obtained from the GLMM analysis.

Data for these two variables were converted back to percentages for visual representations. Three dimensional response surface graphs and contour plots were generated by SigmaPlot for Windows (Version 12.0, Systat Software, Inc., Chicago, IL). Comparisons among brine combinations (Table 1) and treatments (CON, DBP) were made, where necessary, using Tukey's honestly significant difference method with a predetermined significance level of $p = 0.05$.

3. Results

3.1 Loin and Steak pH

The pH of loins measured prior to injection was between 5.39 and 5.7 (Table 2). There were differences in initial loin pH amongst loins ($p < 0.05$), however, there were no differences between loins within a pair. The pH of loins after injection with their respective brines was affected by both brine combination and treatment. Overall, DBP treatment loins had a slightly higher ($p = 0.0105$) pH than control loins (5.77 vs 5.71). The brines containing 0.66% SP or less had the lowest pH's. The maximum pH occurred with the brine containing 4.5% SP. On d 5, loins injected with brines with at least 3.84% SP had higher ($p < 0.05$) pH's than brines which contained 0.66% SP or less. The presence of DBP did not impact pH on d 5. Observed pH values for injected and non-injected steaks are similar to those reported by Baublits, Pohlman, Brown Jr. & Johnson (2006) and Knock, Seyfert, Hunt, Dikeman, Mancini, Unruh, Higgins & Monderen (2006).

3.2 Fluid loss

3.2.1 Brine loss$_{30\ min}$

The targeted injection level was 110% of initial meat weight; actual injections ranged from 106.6-116.6% with a mean of 111.4 ± 1.9%. After the 30 minute equilibration time, the injections ranged from 105.6-114% with a mean of 109.7 ± 1.9%. As expected, SP was predicted to have a greater impact in improving fluid retention during the equilibration period than salt (Table 3).

When brines did not contain DBP, maximum observed values for *Brine* $loss_{30min}$ (brine lost during the equilibration period, the time between injection and slicing) were in excess of 30%, while minimums were observed at slightly less than 5% (Table 1; Figure 1a). This is contrasted with the addition of DBP (Figure 1b) which significantly reduced ($P < 0.05$) *Brine* $loss_{30\ min}$ during the equilibration period in all but three of the brine combinations tested (Table 1). The decreased DBP effectiveness with respect to fluid retention was observed for brines containing high levels of SP (4.5%, 3.84%, and 2.25%) in combination with high levels of NaCl (1.8%, 3.07%, and 3.6%, respectively). The numeric reduction in *Brine* $loss_{30\ min}$ as a result of DBP addition ranged from 3.72% to 19.2%.

Adding DBP to the brine is predicted to effectively reduce *Brine* $loss_{30\ min}$ during equilibration, however, this impact is minimal at high SP and salt levels.

3.2.2 Purge and $Purge_{total}$

Contour plots for *Purge* (this represents brine loss from the steaks during storage) are shown in Figure 2. The plots were generated using the coefficients given in Table 3. Observed *Purge* values ranged from 0.6% to 4.6% for CON and 0.3% to 2.1% for DBP (Table 1). For both equations, the factors of salt level, SP level, quadratic SP level and the salt x SP interaction were significant. Predicted values suggest that, at the levels investigated, using SP alone would be more effective in reducing *Purge* values than salt alone. Predicted values for *Purge* from steaks demonstrate that if processors are willing to accept a fluid loss minimum at around 2.0% as opposed to <1%, both SP and salt can be reduced by ~50%. However, by including DBP in the brine a 50% reduction in SP and salt would result in a *Purge* of <1%. The *Purge* intercept (Table 3), which denotes the predicted *Purge* level if no salt or SP had been added, of 2.71% in the DBP equation as compared to 6.02% for the control equation reinforces this observation. The range of *Purge* values for the control steaks seen in this study are similar to those seen by

Lawrence, Dikeman, Hunt, Kastner & Johnson (2004) and Rowe, Pohlman, Brown, Johnson, Whiting & Galloway (2009) when using similar brines.

Contour plots are shown for the predicted $Purge_{total}$ of brines without and with DBP (Figure 3). Prediction equation coefficients for the control and DBP brines are given in Table 3. The $Purge_{total}$ calculation takes into account not only the fluid lost after the steak was cut, but it back calculates to account for the the fluid that was originally in the steak at the time of injection. Observed $Purge_{total}$ values were as high as 6.8% for CON brines, but only 2.8% for DBP brines. The contour plot for control brines is similar to the one seen for *Purge*, showing decreased values as the levels of both salt and SP in the brine increase. As expected, inclusion of phosphates reduced fluid loss. As with *Purge,* addition of DBP can significantly reduce $Purge_{total}$. There are a broad range of values of salt or SP or combinations of both ingredients that produce predicted $Purge_{total}$ values of less than 1.5% or less when DBP is present. Another interesting observation is the use of DBP with high levels of salt and SP in combination can be detrimental to fluid retention, as indicated by the much higher positive interaction coefficient in the DBP equation (0.3068) as compared to the control equation (0.059).

3.2.4 Brine loss$_{total}$

Response surfaces are shown for the predicted *Brine loss*$_{total}$ of control brines (Figure 4a) and DBP brines (Figure 4b). Without DBP in the brine, predicted *Brine loss*$_{total}$ from injection to d 5 of storage can be reduced to just under 20% of the total amount of injected fluid when both salt and SP are maximized. Reductions in salt and/or SP are predicted to increase that value, reaching near 80% at minimum levels of both ingredients. Adding DBP to the brine reduces the maximum amount of fluid loss to just under 40% given minimum levels of salt and SP. Using median to high levels of SP (>2.25%) with minimum levels of salt (<1.5%) with DBP is predicted to decrease total injected fluid loss to less than 10%.

Comment [A1]: Quotes usage?

Comment [CD2]: Not necessary

4. Discussion

The higher pH values seen in steaks injected with sodium phosphates is expected (Lawrence et al., 2004; Baublits et al., 2006; Cerruto-Noya, VanOverbeke & Mireles DeWitt, 2009). Increased pH values are often considered important in injected or marinated meat products because as the pH shifts away from the isolelectric point of muscle proteins, their ability to bind water increases (Oreskovich, Bechtel, McKeith, Novakofski, & Basgall, 1992). However, there are other factors to consider with regard to the water binding abilities of meat proteins. Trout and Schmidt (1986) observed increased cook yields and tensile strengths of beef rolls when increasing ionic strength at a constant pH. While sodium phosphates increase both pH and ionic strength, much of their functionality is due to their ability to dissociate the acto-myosin bond (Trout & Schmidt, 1986; Offer & Knight, 1988). Additionally, sodium chloride (NaCl) generally does not increase pH, but it is effective in increasing the water binding ability of meat by increasing ionic strength, dissolving myosin filaments and reducing the isoelectric point of muscle proteins (Offer & Knight, 1988).

Salt and SP have long been known to work synergistically to affect the functional attributes of meat proteins (Offer & Trinick, 1983; Trout & Schmidt, 1986). Since SP is restricted to no more than 0.5% in the final product, its effects at or near that level combined with various levels of salt have been thoroughly researched and are well known. The efficacy of salt/phosphate combinations using amounts of SP closer to the minimum is less clear, however. Also, since the use of salt is not restricted, amounts used in previous research of whole muscle injected beef can differ greatly. Salt levels targeted for the final injected product are typically 0.5% or lower (Vote, Platter, Tatum, Schmidt, Belk, Smith & Speer, 2000; Robbins, Jensen, Ryan, Homco-Ryan, McKeith & Brewer, 2002; McGee, Platter, Tatum, Schmidt, Belk, Smith & Speer, 2003; Lawrence et al, 2004; Wicklund, Homco-Ryan, Ryan, McKeith, McFarlane & Brewer, 2005; Cerruto-Noya et al., 2009; Rowe et al., 2009). The maximum level of salt used for this study was 0.36% in the final product and extrapolation beyond that amount is not statistically appropriate. While it is possible that further increases in salt concentration could have functional benefits, many of the surface maps and contour plots show a plateau near their maximum evaluated levels. This suggests that the functional value gained from further increases beyond this point would be greatly diminished, especially when DBP and/or SP are included in the brine. This plateau effect at higher levels of NaCl has been demonstrated previously with response surface modeling using posterior pork loin sections injected with salted marinade (Detienne and Wicker, 1999).

Detienne and Wicker (1999) performed a response surface study on pork loins, with level of salt (0 – 1.5%) and SP (0 – 0.45%) in the final product as the variables. The purge calculation described by the authors is similar to the $\textit{Brine loss}_{total}$ calculation performed in this study. The authors saw maximum predicted values of greater than 90% purge loss for tail loin sections and just over 70% for head sections of the loin when salt and SP were at their minimum levels and minimum predicted values near 0% for both sections when salt and SP were at their maximum. The predictive equations for Detienne and Wicker (1999) included the significant effects of salt concentration, quadratic salt concentration and the salt x phosphate interaction for the head section, whereas, similar to the present study, all effects were significant for the tail section.

The reduction of salt and SP can also be achieved by the addition of non-meat ingredients that act as alternative alkaline pH substitutes or water binding agents. The use of a high pH brine using 0.1% ammonium hydroxide (AH) as the alternative alkaline pH substitute and 3.6% salt was compared to a 4.5% SP, 3.6% salt brine in beef strip steaks injected to 110% green weight (Cerruto-Noya et al., 2009). The AH injected steaks had 3.16% higher ($P < 0.05$) purge values after 4 d of dark storage and 2.9% higher values ($P < 0.05$) purge values after an additional 7 d of retail display. Authors concluded higher levels of AH would be needed to completely replace phosphates in the brine and produce a comparable quality product. A follow-up study by Parsons et al., 2011a demonstrated that 1% AH could successfully replace

4.5% SP in a meat injection brine. Purge from beef loin steaks was reported as being an average of 1.31% from SP injected beef loins, while AH injected steaks had a purge of 2.07%. Data suggests that complete replacement of the SP by an alternative alkaline agent such as AH was predicted to reduce final sodium in the product by almost 50%. However, there are concerns with the use of an alternative alkaline agent, such as AH. Although this ingredient has been determined to be GRAS by USDA when used to increase meat brines to a final pH ≤11.6, their has been mis-guided consumer concern with this ingredient as the media has associated it with toxic levels of "ammonia". As a result, water binding agents such as the dehydrated beef protein described in this study may offer more acceptable alternatives from a consumer perspective as it is a same source ingredient as the meat it is being incorporated.

Studies evaluating same source meat binding ingredients have been previously reported. Lawrence et al. (2004) compared a commercial beef broth and carrageenan in their ability to prevent purge losses in strip steaks injected to a final concentration of 0.44% SP and 0.22% salt. After 5 d of retail display the authors saw no significant differences in purge values between the steaks injected with salt and SP alone, and steaks injected with 1 or 2% beef broth or carrageenan in addition to the salt and SP. A brine containing acid solubilized beef protein with 1.8% salt was evaluated compared to a 3.6% salt, 4.5% phosphate brine injected into strip loins at 110% (Vann & Mireles DeWitt, 2007). Protein injected steaks had 6.64% higher ($P < 0.05$) purge values than phosphate injected steaks after 5 d of storage. A pork collagen protein powder, similar to the one used in this study, was evaluated by Schilling, Mink, Gochenour, Marriott and Alvarado (2003) and Prabhu, Doerscher and Hull (2004). Schilling et al. (2003) found that restructured hams formulated with 3% collagen protein powder had lower expressible moisture than those formulated without it. Frankfurters formulated with at least 1.5% collagen and hams including 3% collagen experienced significantly less purge losses than those formulated without it (Prabhu et al., 2004). Lowder et al. (2011) evaluated quality attributes of steaks from brine injected beef loins in which the sodium phosphate in the brine was completely replaced with 5% dehydrated beef protein (DBP). They measured fluid loss, lipid oxidation, cooked yield, sensory, color, and microbial plate counts. Product was overwrapped and placed in motherbags for 4 d at 4 ºC prior to being placed in retail display. Steaks were evaluated over 6d. Results determined that DBP treated steaks were comparable to those containing sodium phosphate. There are several possible mechanisms that allow collagen protein powders to increase water holding capacity of raw and cooked meat, including: (1) hydration of hydrophilic moieties; (2) increase in viscosity of the brine dispersion due to thickening of collagenous proteins at low temperatures, (3) formation of a progressive gel network after injection into the meat product, (4) possible interactions with myofibrillar proteins (Schilling et al., 2003; Lowder et al., 2011). The calculated $Brine\ loss_{30\ min}$ value represents the percentage of fluid injected into the meat product that is lost between injection and slicing/packaging. This variable is typically not reported in scientific studies. However, as shown here, the losses during this short equilibration time can be significant. The ability to hold more injected fluid during this time period is important to processors because it represents an ability to increase the weight of packaged product and reduce plant generated waste. The $Brine\ loss_{30\ min}$ values seen during this study suggest the equilibration time is a major point of fluid loss for processors. As a collective, the eighteen brines tested in this study suffered ~48. % of their $Brine\ loss_{total}$ in the first thirty minutes after injection (data not shown). This time period seems to be when the DBP has the greatest effect in reducing fluid loss. The increase in viscosity and ability to form a gel network at low temperatures act to restrict free water within the muscle structure immediately after injection. With salt and SP, the benefits of ionic strength and pH increases may be seen right away, but effects of reorganizing the myofibrillar protein structure to increase water binding may take more time.

Finally, data demonstrates that minor compromises in the loss of brine from the product can produce significant reductions in the sodium content regardless of whether a water binding agent (DBP) is included or not. The prediction equations such as those generated by this study allow processors to predict the fluid loss that will occur when salt or phosphates are reduced or a binding agent such as DBP is added to beef strip loins. In the past, salt and phosphate incorporation has been focused on maximizing the quality attributes of injected products. The impact on nutritional quality of the product was thought to be minimal as levels of salt and phosphate incorporated in injected meat products are much lower than levels used in comminuted products. While the levels of sodium are much lower than what we find in products such as frankfurters, they are at least 4x higher than the levels in natural meat (Parsons and others, 2011b). Due to the increasing concern over the level of sodium consumption by consumers, the meat industry should be looking to carefully scrutinize and justify the level of sodium formulated into all products, not just comminuted meat products. Results from this study demonstrate that an increased understanding of the dynamic between salt and phosphate in injected beef products can produce opportunities for sodium reduction.

5. Conclusions

Use of salt and SP in injection brines decreased observed and predicted purge losses in beef strip loins and steaks. At the levels tested in this study, SP appears to have a stronger effect than salt on reducing purge losses when used alone. The use of DBP reduced observed and predicted purge losses at most levels of salt and SP. The functional benefits of adding DBP are predicted to be strongest when either salt or SP are eliminated or included at only minimal levels. The equations generated by these results could help processors to achieve acceptable purge losses while reducing sodium use.

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Table 1. Least squares means of Brine loss and Purge measurements of loins and steaks injected with a brine containing salt and sodium phosphate with or without a dehydrated beef protein

NaCl[i] %	SP[j] %	DBP[k] %	*Brine loss*$_{30min}$ %	*Purge* %	*Purge*$_{total}$ %	*Brine loss*$_{total}$ %
0	2.25	0	23.39^{ab}	3.60^{ab}	6.10^{a}	54.26^{bc}
0	2.25	5	4.47^{de}	0.79^{ef}	1.24^{e}	12.53^{fgh}
0.53	0.66	0	25.57^{ab}	4.40^{ab}	6.83^{a}	69.55^{ab}
0.53	0.66	5	7.93^{cde}	2.07^{cd}	2.82^{cd}	29.57^{def}
0.53	3.84	0	17.24^{bcd}	0.99^{def}	3.04^{cd}	25.53^{defg}
0.53	3.84	5	3.70^{e}	0.67^{ef}	1.07^{e}	9.57^{gh}
1.8	0	0	30.65^{a}	4.61^{a}	7.31^{a}	80.02^{a}
1.8	0	5	13.46^{bcde}	1.53^{de}	2.79^{cd}	38.10^{cde}
1.8	2.25	0	23.95^{ab}	1.54^{de}	3.84^{bc}	40.16^{cd}
1.8	2.25	5	7.90^{cde}	0.77^{ef}	1.51^{de}	16.94^{fgh}
1.8	4.5	0	15.73^{bcde}	0.60^{ef}	2.26^{cde}	21.42^{efgh}
1.8	4.5	5	8.35^{cde}	0.34^{f}	1.15^{e}	12.06^{gh}
3.07	0.66	0	22.52^{ab}	3.06^{bc}	5.74^{ab}	47.41^{c}
3.07	0.66	5	3.31^{e}	0.55^{ef}	0.95^{e}	8.19^{h}
3.07	3.84	0	18.20^{abc}	0.59^{ef}	2.32^{cde}	24.84^{defgh}
3.07	3.84	5	14.48^{bcde}	0.39^{f}	1.71^{de}	18.82^{fgh}
3.6	2.25	0	13.19^{bcde}	0.71^{ef}	2.08^{cde}	20.05^{fgh}
3.6	2.25	5	4.45^{de}	0.32^{f}	0.76^{e}	7.77^{h}
SEM[l]	-	-	4.45	0.29	0.51	4.73

[a-h]Means within a column with different superscripts are significantly different ($P < 0.05$)
[i]Sodium Chloride
[j]Sodium Phosphates (Brifisol® 85 Instant; BK Giulini Corp., Simi Valley, CA, USA)
[k]Dehydrated beef protein (Proliant Meat Ingredients, Ankeny, IA, USA)
[l]Standard error of the mean

Table 2. Least squares means of pH for the main effect of brine combination before and after injection and on d 5 of storage of loins and steaks injected with varying levels of salt and sodium phosphates with or without a dehydrated beef protein

		pH					
NaCl[e] %	SP[f] %	Before Injection	*SEM*[g]	After Injection	*SEM*[g]	5 d	*SEM*[g]
0	2.25	5.42^{bcd}	0.02	5.69^{c}	0.04	5.75^{abc}	0.04
0.53	0.66	5.39^{d}	0.02	5.41^{d}	0.04	5.57^{cd}	0.04
0.53	3.84	5.50^{abc}	0.02	5.89^{b}	0.04	5.92^{a}	0.04
1.8	0	5.40^{cd}	0.02	5.38^{d}	0.04	5.54^{d}	0.04
1.8	2.25	5.47^{bcd}	0.02	5.71^{c}	0.03	5.73^{bc}	0.04
1.8	4.5	5.59^{a}	0.02	6.17^{a}	0.04	5.84^{ab}	0.04
3.07	0.66	5.52^{ab}	0.02	5.58^{c}	0.04	5.68^{bcd}	0.04
3.07	3.84	5.58^{a}	0.02	5.99^{b}	0.04	5.82^{ab}	0.04
3.6	2.25	5.52^{ab}	0.03	5.87^{b}	0.04	5.72^{bcd}	0.04

[a-d]Means within a column with different superscripts are significantly different ($P < 0.05$)
[e]Sodium Chloride
[f]Sodium Phosphate (Brifisol® 85 Instant; BK Giulini Corp., Simi Valley, CA, USA)
[g]Standard error of the mean

Table 3. Coefficients for substitution into Eq. (1)[a] for *Purge and Brine loss measurements* of loins and steaks injected with a final concentration of up to 0.36% salt and up to 0.45% sodium phosphates with or without a dehydrated beef protein (DBP)[b]

Variable	Intercept (β_0)	NaCl (β_1)	SP (β_2)	$NaCl^2$ (β_{11})	SP^2 (β_{22})	NaCl*SP (β_{12})
Purge						
Control	6.0014	-0.8364	-1.5136	-	0.0880	0.1167
DBP	2.7088	-0.5874	-0.9262	-	0.0880	0.1534
$Purge_{total}$						
Control	8.9270	-0.8750	-1.9351	-	0.1559	0.0585
DBP	4.1695	-0.8750	-1.5148	-	0.1559	0.3068
Brine $loss_{30min}$[c]						
Control	-0.0925	0.1233	-0.2715	-0.1159	-	0.1087
DBP	-2.4414	0.1233	-0.2715	-0.1159	-	0.2111
Brine $loss_{total}$[c]						
Control	1.9341	-0.2016	-1.0717	-0.1152	0.0785	0.1177
DBP	0.1815	-0.2016	-1.0717	-0.1152	0.0785	0.2548

[a] $\hat{Y} = \beta_0 + \beta_1 NaCl + \beta_2 SP + \beta_{11} NaCl*NaCl + \beta_{22} SP*SP + \beta_{12} NaCl*SP$ where NaCl is the level of salt in the brine and SP is the level of sodium phosphate in the brine

[b]Proliant Meat Ingredients, Ankeny, Iowa, USA

[c]Variables were converted to proportions and fit to a beta distribution

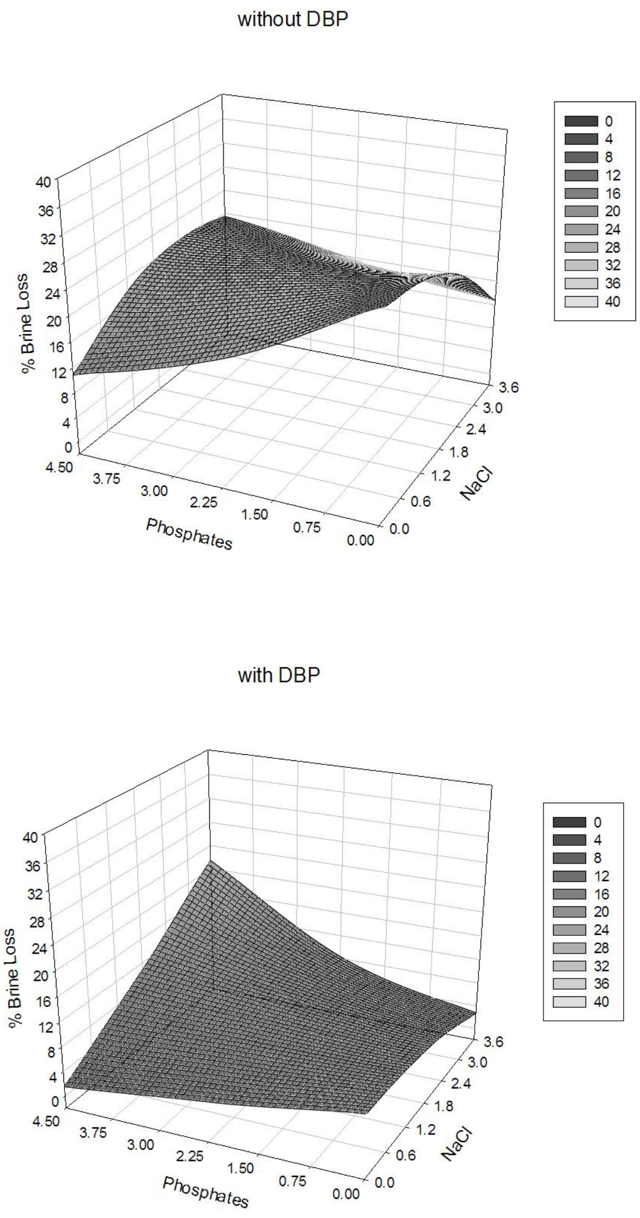


Figure 1. Response surfaces of predicted *Brine loss*$_{30\ min}$ (%) of overwrapped steaks as salt (NaCl) and sodium phosphate (SP) are varied in brines (a) without dehydrated beef protein (CON) and (b) with dehydrated beef protein (DBP).

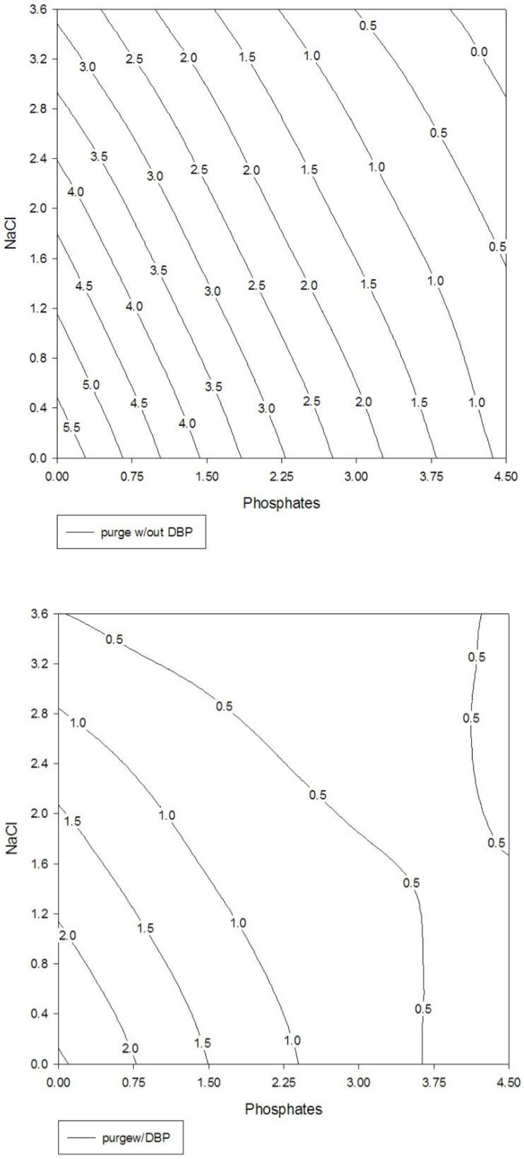
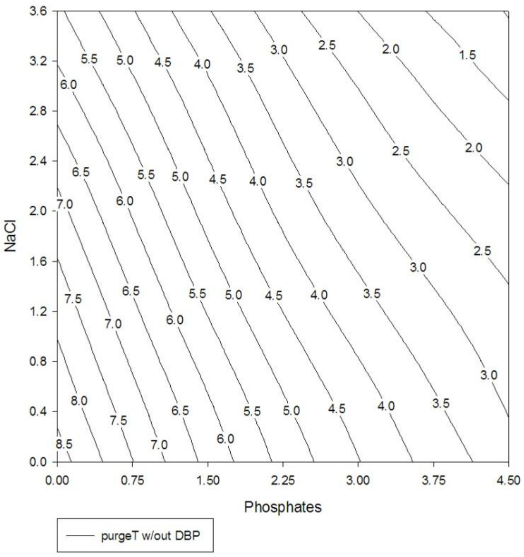


Figure 2. Contour plots of predicted *Purge* (%) of overwrapped steaks as salt (NaCl) and sodium phosphate (SP) are varied in brines (a) without dehydrated beef protein (CON) and (b) with dehydrated beef protein (DBP).



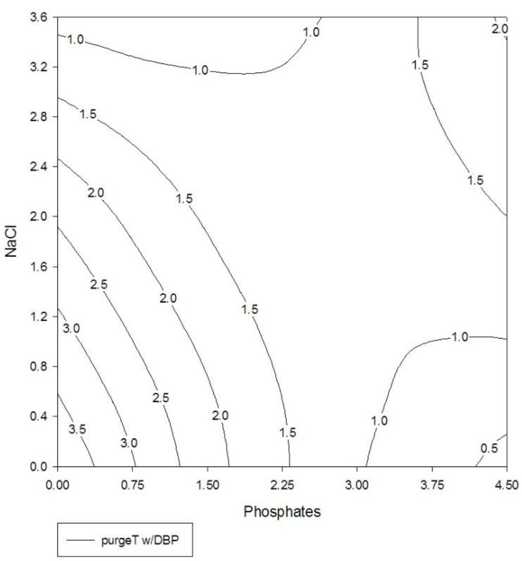
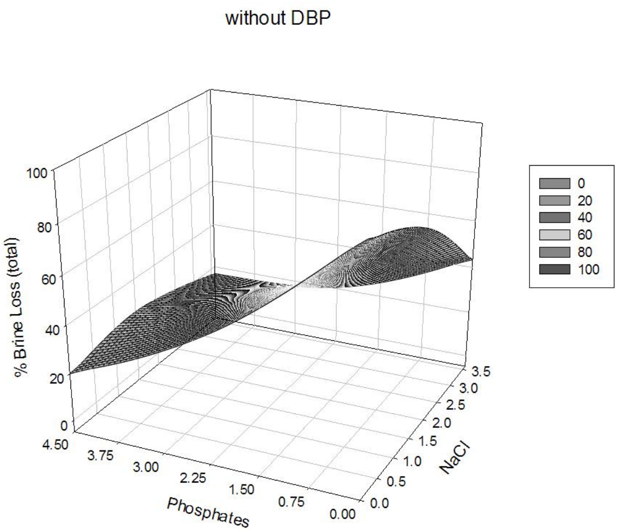


Figure 3. Contour plots of predicted *$Purge_{total}$* (%) of overwrapped steaks as salt (NaCl) and sodium phosphate (SP) are varied in brines (a) without dehydrated beef protein (CON) and (b) with dehydrated beef protein (DBP).


without DBP
% Brine Loss (total)
100
80
60
40
20
0
4.50
3.75
3.00
2.25
1.50
0.75
0.00
Phosphates
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
NaCl
0
20
40
60
80
100

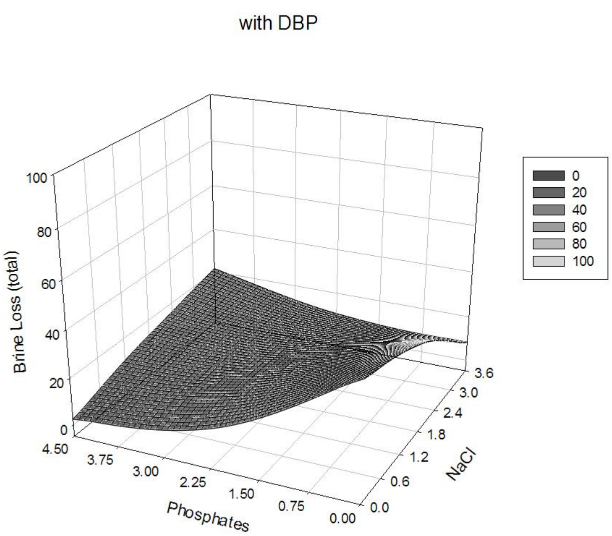

with DBP
Brine Loss (total)
100
80
60
40
20
0
4.50
3.75
3.00
2.25
1.50
0.75
0.00
Phosphates
0.0
0.6
1.2
1.8
2.4
3.0
3.6
NaCl
0
20
40
60
80
100

Figure 4. Response surfaces of predicted *Brine* $loss_{total}$ (%) of overwrapped steaks as salt (NaCl) and sodium phosphate (SP) are varied in brines (a) without dehydrated beef protein (CON) and (b) with dehydrated beef protein (DBP).