



## Evaluation of Clean Label Ingredients as Phosphate Alternatives in Enhanced Fresh Pork<sup>1</sup>

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**Abstract:** The effects of 5 clean label phosphate-free brines on the color, texture, and sensory characteristics of boneless pork loins were evaluated during 21 d of refrigerated storage. On a meat weight basis, a control brine (SPH) contained salt (0.2%), sodium tripolyphosphate (0.3%), and potassium lactate (2.35%), while the phosphate-free brines all contained sodium bicarbonate (0.25%), potassium chloride (0.25%), yeast extract (0.25%), dried vinegar powder (0.30%), and acerola cherry powder (0.43%), in addition to 1% rice bran extract (RICE), 0.35% plum concentrate (PLUM), or 0.85% Proteus® (a commercial functional meat protein; PR13; PR18). One brine was made with no additional ingredients (INCL), and an uninjected pork loin treatment (UNIN) was included as a negative control. Injected loins were injected to 13% of meat weight, except PR18, which was injected to 18%. Loins were sliced into chops, packaged, stored at 2°C and analyzed for pH, color (CIE  $L^*$ ,  $a^*$ ,  $b^*$ ), and texture (Warner-Bratzler shear force [WBSF]) on days 0, 7, 14, and 21, and by a trained sensory panel on days 7, 14, and 21. The sensory panel found SPH, RICE, PLUM, and PR18 chops to be juicier than UNIN ( $P = .003$ ), and RICE and PR18 chops to be more tender than UNIN ( $P = .005$ ), with overall tenderness being greater on day 21 ( $P = .09$ ). Pork flavor was greatest in RICE chops and lower in UNIN chops than RICE, PLUM, and PR18 chops ( $P = .001$ ). WBSF values were not different between treatments ( $P = .189$ ) but were lower on days 14 and 21 ( $P = .005$ ). RICE chops were darker than all others, while UNIN chops were lighter than those from RICE, PLUM, PR13 and PR18 treatments ( $P < .001$ ). INCL, RICE, PLUM, PR13, and PR18 chops were redder than SPH chops ( $P = .002$ ). These results suggest that rice bran extract has the most potential among the ingredients evaluated to serve as a supplemental phosphate alternative in enhanced fresh pork loins and chops, particularly in combination with other clean label ingredients.

**Key words:** enhanced pork, clean label ingredients, phosphate replacement

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## Introduction

Enhanced pork is fresh pork, whole or ground, typically processed by the addition of ingredients such as water, salt, phosphates, antioxidants, antimicrobials, and flavor enhancers. Salt, phosphate, and lactate have been utilized extensively for enhancement of fresh pork products because their inclusion typically

increases juiciness, tenderness, and flavor (Miller, 2006), factors crucial to the palatability of meat products (Moeller et al., 2010). Among these ingredients, phosphates play a key role (Miller, 2006).

Phosphates have the ability to alter pH, increase water binding, extend fresh meat color life, and reduce sodium from salt while making the product more tender and juicier. Phosphates, which are basic

compounds, achieve this by increasing meat pH and by partially removing the transverse myofibrillar proteins (Z line) that otherwise restrict myofibrillar swelling (Xiong, 2005), as shown in the classical work of Offer and Trinick (1983). Phosphates, when used in meat products, are limited by regulation to 5000 ppm, both in the United States (Directive 7120.1; USDA Food Safety and Inspection Service, 2024) and the European Union (Regulation [EC] 1333/2008; European Parliament and Council, 2008). Most meat processors formulate added phosphate concentrations for meat products at 3000–4000 ppm.

Recently, however, the use of phosphates in foods has come under scrutiny. There is a general consumer trend toward more natural and less processed foods with fewer food ingredients (the so-called “clean label” movement). It has been demonstrated that consumers are willing to pay a premium for “simple” ingredient lists (Holt et al., 2024), and this has given impetus to efforts to remove or reduce many food ingredients that are perceived as “chemical” or artificial, including phosphates. Because of the functional importance of phosphates in processed meats, there is considerable interest in replacing them with natural ingredients that have similar functionality (Aschemann-Witzel et al., 2019; Thangavelu et al., 2019). Some potential phosphate replacers that have been investigated recently include sodium bicarbonate, corn starch, yeast extract, rice bran extract, and plum concentrate (Miller, 2006; Jarvis et al., 2015; Petracci et al., 2012).

Sodium bicarbonate, yeast extract, rice bran extract, and plum concentrate each have various functions that can mimic some of the characteristics that phosphates give to enhanced meat products. Sodium bicarbonate, for example, is used to provide a high buffering capacity, raise meat pH, and increase ionic strength. When used in enhanced pork, sodium bicarbonate has been reported to reduce shear force, increase water holding capacity, and improve yield (Sheard and Tali, 2004; Petracci et al., 2012). Yeast extract is used to aid in savory/umami flavor development of enhanced meat products (Shan et al., 2022). Dried vinegar can be included in clean label applications to provide an antimicrobial contribution in place of lactate (King et al., 2015). Finally, acerola cherry is a natural ingredient that is high in ascorbic acid that serves as an antioxidant that can impact fresh meat color life (Xu et al., 2020).

Other more recently developed ingredients may offer supplemental effects to further improve the quality of enhanced pork products. Rice bran extract, for example, has recently become available for meat

applications. Rice bran has been shown to exhibit antioxidant properties along with moisture retention (Garofalo et al., 2021). Plum products have also been shown to have antioxidant and antimicrobial activity, improve color and textural properties, increase water holding capacity, and reduce cooking loss (Jarvis et al., 2015). Another potential supplemental ingredient is Proteus® (Kemin Industries, Inc.), a muscle protein extract manufactured using proprietary technology that expands the protein structure to expose natural water and protein binding sites (Kemin Industries, Inc., 2024). These ingredients all have the potential to be included in a clean label, phosphate-free enhanced meat product because they can contribute characteristics to meat that are similar to those of products currently being enhanced with salt, phosphate, and lactate. However, due to the specific functional properties each of these ingredients likely provide to enhanced pork, a combination of them is most likely to achieve product properties similar to or close to those of products enhanced with salt and phosphate.

The objective of this research was to determine the most effective combination of several clean label ingredients in enhanced pork loins for impact on the eating quality compared to pork loins enhanced with a phosphate/salt injection and to uninjected pork loins. It was hypothesized that the addition of rice bran extract, plum concentrate, or Proteus® in conjunction with other clean label ingredients would result in quality attributes (color, texture, flavor) of clean label, enhanced pork loins that are greater than or equal to those of traditionally enhanced pork loins over a 21-d storage period. If successful, these ingredients could provide the meat industry with clean label alternatives for phosphates while maintaining or improving sensory characteristics.

## Materials and Methods

### *Manufacturing materials*

Fourteen boneless pork loins were obtained from a commercial pork harvest facility on the day after slaughter. Selected loins varied in weight from 4.1–4.5 kg and in pH from 5.50–5.80. The loins were vacuum packaged and transported to the Iowa State Meat Laboratory within 90 min of packaging and stored at 2–3°C until used. Ingredients for this study included sodium tripolyphosphate (Innophos Canada, Inc., Lowbanks, Ontario, Canada), sodium bicarbonate (Church & Dwight, Ewing Township, NJ, USA),

potassium chloride (NuTek Natural Ingredients, Omaha, NE, USA), yeast extract (OHLY® STTF, Ohly, Hamburg, Germany), potassium lactate (PURASAL® Hi Pure P), dried vinegar powder (Verdad Powder N6, Corbion, Lenexa, KS, USA), acerola cherry powder (VegStable® 515), rice bran extract (VegStable® Plus 452, Florida Food Products, Inc., Eustis, FL, USA), plum concentrate (Sunsweet Growers Inc., Yuba City, CA, USA), and Proteus® functional meat protein extract (Kemin Industries, Inc., Des Moines, IA, USA).

## Formulations

The experiment consisted of 2 controls and 5 different treatments of enhanced raw pork loins, shown in Table 1. The control group consisted of loins injected with a combination of water, salt, sodium tripolyphosphate, and potassium lactate (SPH) to represent a common industry enhancement level and uninjected loins (UNIN) to serve as a negative control. The treatment group consisted of loins injected with water, sodium bicarbonate, potassium chloride, yeast extract, dried vinegar powder, and acerola cherry powder (INCL) to represent a base clean label formula and 4 treatments based on the INCL treatment but with an additional clean label ingredient. The RICE treatment included 1% rice bran extract, the PLUM treatment contained 0.35% plum concentrate, and PR 13 and PR18 brines both contained 0.85% Proteus®.

## Product manufacturing

All samples were manufactured in the Iowa State University Meat Laboratory in Ames, Iowa, and in the following order: UNIN, SPH, INCL, RICE, PLUM, PR13, PR18. Brines were prepared using a 53-cm Big Stik immersion blender (Waring Commercial, Torrington, CT, USA) to aid dispersion of ingredients into a solution, with the least soluble ingredients being added first. Once the brines were prepared, they were injected into pork loins using a multineedle injector (IMAX 300SL, Schröder Maschinenbau GmbH & Co. KG, Werther, Germany). The manufacturing sequence was chosen to separate the uninjected treatment and the phosphate treatment from the subsequent treatments by injecting the uninjected and phosphate treatments first. The injector was thoroughly rinsed between each of the treatment groups by running water through it several times between each of the batches. For the injected treatments, the target injection level was 13% of meat weight with the exception of PR18, which was injected to 18%, as recommended by the supplier. The PR13 treatment allowed comparison of the Proteus® ingredient at the same use level as all the others, while the PR18 treatment allowed evaluation of this ingredient according to the supplier's recommendation of an 18% injection level. Side port needles (4 mm) were used for all treatments except PR13 and PR18, which used hypodermic needles

**Table 1.** Brine formulations (values expressed as % of meat weight).

Ingredient	Treatment						
	UNIN	SPH	INCL	RICE	PLUM	PR13	PR18
Water	–	10.15	11.52	10.52	11.17	10.67	10.67
Salt (sodium chloride)	–	0.20	–	–	–	–	–
Sodium tripolyphosphate	–	0.30	–	–	–	–	–
Potassium lactate	–	2.35	–	–	–	–	–
Sodium bicarbonate	–	–	0.25	0.25	0.25	0.25	0.25
Potassium chloride	–	–	0.25	0.25	0.25	0.25	0.25
Yeast extract	–	–	0.25	0.25	0.25	0.25	0.25
Dried vinegar powder	–	–	0.30	0.30	0.30	0.30	0.30
Acerola cherry powder	–	–	0.43	0.43	0.43	0.43	0.43
Rice bran extract	–	–	–	1.00	–	–	–
Plum concentrate	–	–	–	–	0.35	–	–
Proteus®	–	–	–	–	–	0.85	0.85

UNIN, uninjected control.

SPH, salt and phosphate treatment at 13% injection.

INCL, injected clean label at 13% injection.

RICE, injected clean label with rice bran extract at 13% injection.

PLUM, injected clean label with plum concentrate at 13% injection.

PR13, injected clean label with Proteus® at 13% injection.

PR18, injected clean label with Proteus® at 18% injection.

(4 mm), due to the viscosity of the brine. The injection target amounts for all treatments were verified by reweighing each of the loins following the injection and calculating the percentage of brine injected. Two loins per treatment were injected, transferred to separate tumblers (DVTS R2-50, Daniels Food Equipment Inc., Parkers Prairie, MN, USA) with a dedicated tumbler used for each treatment, and tumbled under vacuum at approximately 16 rpm for 2 h. After tumbling, loins were sliced into ten 25.4-mm thick chops and two 101.6-mm thick roasts, as shown in Figure 1. Chops and roasts were then placed into vacuum bags (oxygen transmission rate of 3–6 cm<sup>3</sup>/m<sup>2</sup>/24 h at 23°C, 0% RH [relative humidity]; water vapor transmission rate of 7.8–9.3 g/m<sup>2</sup>/24 h at 38°C, 100% RH; Cryovac, Sealed Air Corporation, Duncan, SC, USA), sealed and stored in boxes at 2°C for 0, 7, 14, and 21 d. Chops and roasts from the first loin were used for day 0 and day 7 analyses, and those from the second loin were used for day 14 and day 21 analyses. Sections 1–6 and 7–12 from 1 loin were used for day 0 and day 7 analyses, respectively, and the same sections from the second loin were used for day 14 and day 21 analyses, respectively. Chops 1 and 2 were used for compositional assays, and chops 3, 4, and 5 were used for color, pH, cooking yield, and Warner-Bratzler shear force (WBSF) assays. For sensory analysis, section 7 from the first loin was used for day 7, and sections 6 and 7 from the second loin were used for days 14 and 21, respectively. Chops 8, 9, and 10 were used for color, pH, cooking yield, and WBSF assays on day 7. Chops 11 and 12 were used for composition, similar to chops 1 and 2. A second loin for each treatment was subdivided in the same way for analysis on days 14 and 21.

**Proximate composition analysis**

Proximate composition was determined for each of the treatments to verify product formulation and ensure raw material consistency. Chops 1 and 2 were first

homogenized in a food chopper (Express Chop, SharkNinja Operating LLC, Needham, MA, USA), and protein content was analyzed using the CEM Sprint Rapid Protein Analyzer (CEM Corporation Matthews, North Carolina, USA; AOAC method 2011.04, AOAC International, 2019). Moisture and fat content were measured in tandem according to AOAC method 2008.06 (AOAC International, 2019) using the SMART 6 system for moisture and the ORACLE system (CEM Corporation Matthews, North Carolina, USA) for fat content. All measurements were done in duplicate and averaged.

The pH of the chops was measured using a Mettler Toledo SevenMulti pH meter with a InLab solids PROISM electrode (Mettler Toledo, Columbus, OH, USA). Each chop was measured by inserting the electrode into 3 different locations.

**Color analysis**

Color was evaluated on days 0, 7, 14, and 21 post-packaging. The raw chops were removed from the package and the color was measured by a HunterLab MiniScan EZ 4500L colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA) using illuminant D65 (daylight at 6500 K), 10° observer angle, and a 2.54-cm aperture (King et al., 2023). The Commission Internationale de l’Eclairage (CIE) L\*a\*b\* color space was used to represent color. Color scans were taken in 3 different locations on each chop.

**Texture analysis and cooking yield**

Texture was analyzed on days 0, 7, 14, and 21 post-packaging. Each chop was weighed before and after cooking to calculate cooking yield (cooked weight divided by raw weight). The chops were cooked in a Ninja Foodi Grill (model AG301, SharkNinja Operating LLC, Needham, MA, USA) on the grill grate with the “grill” function set to 260°C. They were turned over after 5 min, cooked to an internal temperature of 71°C, and allowed to cool to room temperature. Three

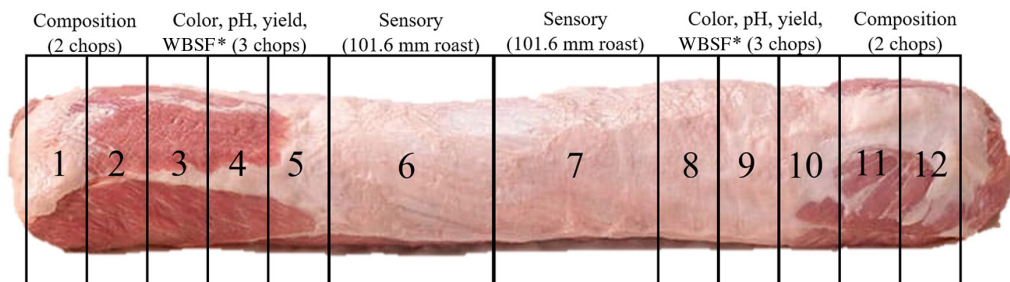


Figure 1. Pork loin sampling diagram. \*WBSF: Warner-Bratzler shear force.

separate 13-mm-diameter cores were cut and removed from each chop and sheared with a Warner-Bratzler knife with guillotine block fixture (TA-7, Stable Micro Systems, Surrey, UK) attached to a TA-XT2i texture analyzer equipped with a 30-kg load cell (Stable Micro Systems, Surrey, UK) at a test speed of 3.5 mm/s<sup>-1</sup>. Results of the 3 measurements were averaged.

### Sensory analysis

Sensory analysis was conducted on days 7, 14, and 21 postpackaging by a 5-member highly trained sensory panel. The panel was comprised of students, faculty, and staff of Iowa State University, all of whom had participated on multiple occasions in other trained panels involving similar products and were intimately familiar with the sensory protocol and vocabulary used in this study. For this particular study, one 60-min training session was held 1 wk before sample evaluation began, using untreated and enhanced pork chops. To prepare the sensory analysis samples, the roasts designated for this analysis were first cut into four 25.4-mm chops. The 2 middle chops were then placed in a Ninja Foodi Grill (model AG301, SharkNinja Operating LLC, Needham, MA, USA) on the grill grate with the “grill” function selected and set to 260°C and cooked an internal temperature of 63°C, turning them over 5 min into the cooking process. The chops were then cut into 25-mm cubes and mixed in a large bowl to randomize the sampling units. Cubes were then placed inside expanded polystyrene cups (1 cube per cup) labeled with 3-digit random numbers. The cups were covered with plastic lids, and the samples were served to the panel within 2 min of the end of cooking. The panelists were also provided with water and unsalted crackers as palate-cleansing agents. The panelists evaluated samples in separate examination booths under fluorescent lighting using a 15-cm unstructured line scale. The following sensory attributes were evaluated (scale anchor descriptors in parentheses): “juiciness” (dry/not juicy, juicy/not dry), “tenderness” (tough/not tender, tender/not tough), “chewiness” (not chewy, chewy), “pork flavor” (none, intense), and “other flavor” (none, intense). “Other flavor” was defined as any flavor that would be unusual for the product. The panelists were asked to describe the other flavor if detected. Data were collected using Compusense 5 sensory evaluation software (release 5.6, Compusense Inc., Guelph, ON, Canada). The sensory analysis protocol was reviewed and approved by the Iowa State University Institutional Review Board

**Table 2.** *P* values of fixed main factor and interaction effects.<sup>1</sup>

Dependent Variable	Treatment	Storage Time	Treatment × Storage Time
Fat, raw, %	<b>0.047</b>	–	–
Moisture, raw, %	<b>&lt;0.001</b>	–	–
Protein, raw, %	<b>0.017</b>	–	–
Salt, raw	<b>&lt;0.001</b>	–	–
pH, raw	<b>&lt;0.001</b>	<b>0.030</b>	–
Cooking yield, %	<b>&lt;0.001</b>	0.195	0.197
Color <i>L</i> *	<b>&lt;0.001</b>	0.496	0.450
Color <i>a</i> *	<b>0.002</b>	0.146	0.721
Color <i>b</i> *	<b>&lt;0.001</b>	0.138	0.880
Peak WBSF <sup>2</sup>	0.189	<b>0.005</b>	0.884
Sensory juiciness	<b>0.003</b>	0.122	0.418
Sensory tenderness	<b>0.005</b>	<b>0.009</b>	0.658
Sensory chewiness	<b>0.006</b>	0.157	0.654
Sensory pork flavor	<b>0.001</b>	0.239	0.486
Sensory other flavor	<b>0.001</b>	0.735	0.335

<sup>1</sup>Statistical significance established at *P* < .05 (highlighted in bold).

<sup>2</sup>WBSF: Warner-Bratzler shear force.

(IRB ID 21-458), and informed consent was obtained from all panelists prior to initiation of the study.

### Experimental design and statistical analysis

The experiment was designed as a randomized complete block and was replicated 3 times with separate manufacturing dates for each replication. The meat raw materials were from production days as described, and nonmeat ingredients were each obtained from a single production lot. For each replication, loins were assigned to treatments randomly. Data were analyzed statistically using JMP Pro 16 (SAS Institute, Cary, NC, USA) with treatment and day treated as fixed factors and replication, sensory evaluation sessions, and sensory panelists treated as random factors. Differences among treatments were determined using the Tukey-Kramer pairwise comparison method, with significance established at *P* < .05. *P* values for fixed main factor (treatment and storage time) effects and the interaction between them are shown in Table 2.

## Results and Discussion

### Proximate analysis, pH, and cooking yield

The proximate composition data are shown in Table 3. Fat content of UNIN chops was greater than

**Table 3.** Least-squares means for composition of raw enhanced pork loins.

Treatment	Fat (%)	Moisture (%)	Protein (%)	pH	Cooking Yield (%)
UNIN	4.17 <sup>a</sup>	72.77 <sup>bc</sup>	20.58 <sup>a</sup>	5.51 <sup>c</sup>	75.28 <sup>c</sup>
SPH	3.55 <sup>ab</sup>	72.44 <sup>c</sup>	19.26 <sup>ab</sup>	5.73 <sup>bc</sup>	80.97 <sup>ab</sup>
INCL	2.69 <sup>ab</sup>	74.82 <sup>a</sup>	19.84 <sup>ab</sup>	5.82 <sup>ab</sup>	77.90 <sup>bc</sup>
RICE	2.50 <sup>b</sup>	75.29 <sup>a</sup>	18.35 <sup>b</sup>	6.04 <sup>a</sup>	81.89 <sup>a</sup>
PLUM	3.06 <sup>ab</sup>	74.00 <sup>ab</sup>	20.02 <sup>ab</sup>	5.89 <sup>ab</sup>	78.95 <sup>ab</sup>
PR13	3.00 <sup>ab</sup>	74.35 <sup>a</sup>	19.86 <sup>ab</sup>	6.04 <sup>a</sup>	80.77 <sup>ab</sup>
PR18	2.85 <sup>ab</sup>	75.08 <sup>a</sup>	19.35 <sup>ab</sup>	5.96 <sup>ab</sup>	79.22 <sup>ab</sup>
SEM	0.36	0.34	0.37	0.12	1.29

UNIN, uninjected control.

SPH, salt and phosphate treatment at 13% injection.

INCL, injected clean label at 13% injection.

RICE, injected clean label with rice bran extract at 13% injection.

PLUM, injected clean label with plum concentrate at 13% injection.

PR13, injected clean label with Proteus® at 13% injection.

PR18, injected clean label with Proteus® at 18% injection.

SEM, standard error of mean.

<sup>a-c</sup>Means in the same column with different superscripts are significantly different ( $P < .05$ ).

$n = 14$  (1 loin  $\times$  7 treatments  $\times$  2 replications).

in RICE chops ( $P = .047$ ) due to no additional water, while INCL, RICE, PR13, and PR18 chops were higher in moisture than UNIN as a result of added brine. The SPH chops were also lower in moisture ( $P < .001$ ) than the other treatments, which may reflect the somewhat lower pH in this treatment. The UNIN treatment, which was not injected, might be expected to have greater fat and protein and lower moisture content due to the lack of additional ingredients, especially water, present in the injection brines of the other treatments. The RICE and PR13 treatments resulted in higher pH ( $P < .05$ ) than that of SPH and UNIN chops. Cooking yield was higher in RICE chops than in UNIN and INCL chops ( $P < .001$ ). The elevated pH of the RICE treatment would have contributed to a greater cooking yield.

### Instrumental color

Summarized instrumental color data over the 21-d shelf-life are shown in Table 4. There were no storage time or treatment  $\times$  storage time differences for  $L^*$ ,  $a^*$ , or  $b^*$  values ( $P > .050$ ). UNIN chops were lighter than RICE, PLUM, PR13, and PR18 chops ( $P = .002$ ) most likely due to its lower pH (Table 3). However, RICE chops were darker than all others, which may be attributed to the darker color of the rice bran extract. In a study by Min et al. (2009), researchers noted that

**Table 4.** Least-squares means for main effect of treatment on color of raw enhanced pork loins.

Treatment	$L^*$	$a^*$	$b^*$
UNIN	57.55 <sup>a</sup>	7.45 <sup>ab</sup>	15.74 <sup>cd</sup>
SPH	53.67 <sup>ab</sup>	5.88 <sup>b</sup>	13.99 <sup>d</sup>
INCL	53.72 <sup>ab</sup>	8.18 <sup>a</sup>	17.99 <sup>a</sup>
RICE	47.64 <sup>c</sup>	8.38 <sup>a</sup>	16.10 <sup>bc</sup>
PLUM	52.95 <sup>b</sup>	8.51 <sup>a</sup>	17.91 <sup>ab</sup>
PR13	51.67 <sup>b</sup>	8.30 <sup>a</sup>	18.50 <sup>a</sup>
PR18	52.31 <sup>b</sup>	8.24 <sup>a</sup>	18.54 <sup>a</sup>
SEM	1.60	0.55	0.82

UNIN, uninjected control.

SPH, salt and phosphate treatment at 13% injection.

INCL, injected clean label at 13% injection.

RICE, injected clean label with rice bran extract at 13% injection.

PLUM, injected clean label with plum concentrate at 13% injection.

PR13, injected clean label with Proteus® at 13% injection.

PR18, injected clean label with Proteus® at 18% injection.

SEM, standard error of mean.

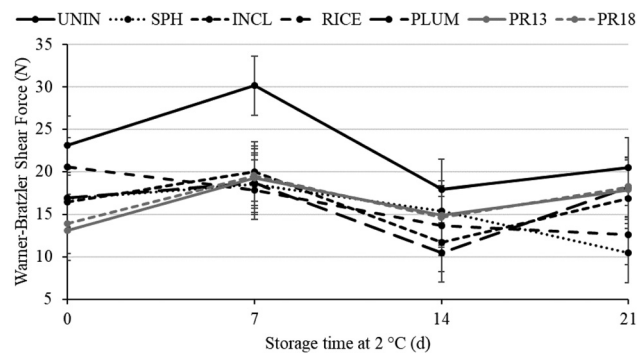
<sup>a-d</sup>Means in the same column with different superscripts are significantly different ( $P < .05$ ).

$n = 14$  (1 loin  $\times$  7 treatments  $\times$  2 replications).

treatment of catfish patties with rice bran also resulted in darker color than the controls. INCL, RICE, PLUM, PR13, and PR18 chops were redder (higher  $a^*$ ) than SPH chops ( $P = .002$ ), which may be attributed to the ascorbic acid content of the acerola cherry powder. The  $b^*$  values were higher in chops from the INCL, PR13, and PR18 treatments than in those from UNIN, SPH, and RICE treatments ( $P < .001$ ), which is consistent with the yellow hue of the Proteus® protein extract.

### Instrumental texture

WBSF results are shown in Figure 2. There were no treatment ( $P = .189$ ) or treatment  $\times$  day ( $P = .884$ ) differences. The only significant difference observed was at days 14 and 21, when chops had lower shear force values than at day 7 ( $P = .005$ ). This might be expected as a result of proteolysis during aging and storage (Warner, 2021; Johnson et al., 2023). During the postmortem period, proteins such as titin, nebulin, and troponin-T, as well as Z line-associated proteins such as desmin, filamin, dystrophin, and talin, are disrupted and degraded, leading to myofibrillar fragmentation, loss of muscle cell integrity, and, ultimately, a softer and more tender product (Huff-Lonergan et al., 2010). It has been reported that proteolysis can affect major changes and lead to increased tenderness within the first 7–14 d postmortem (Miller, 2002).



**Figure 2.** Least-squares means for effect of treatment on Warner-Bratzler shear peak force of enhanced pork loins over 21 d of refrigerated storage. Error bars indicate standard error of mean (SEM = 3.49).  $n = 14$  (1 loin  $\times$  7 treatments  $\times$  2 replications). UNIN, uninjected control; SPH, salt and phosphate treatment at 13% injection; INCL, injected clean label at 13% injection; RICE, injected clean label with rice bran extract at 13% injection; PLUM: injected clean label with plum concentrate at 13% injection; PR13: injected clean label with Proteus® at 13% injection; PR18: injected clean label with Proteus® at 18% injection.

Furthermore, Davis et al. (2004) demonstrated that protein hydrolysis in pork loins was not affected by injection brines, and protein degradation continued in the higher ionic strength environment in the loins following brine injection, an observation supported by Maddock et al. (2005) who showed that, while high ionic strength decreased activity of  $\mu$ - and m-calpain, it did not stop proteolysis completely.

### Sensory analysis

Sensory results are shown in Table 5. The main effect of treatment was significant for all attributes as was storage time for tenderness. Juiciness was greater in SPH, RICE, PLUM, and PR18 than in UNIN chops ( $P = .003$ ), which is consistent with our observation that moisture content and cooking yield for the SPH, RICE, PLUM, and PR18 treatments were greater than for UNIN ( $P < .001$ ; Table 3). Tenderness was higher in RICE and PR18 chops than in UNIN ( $P = .005$ ) and increased over time, being greater on day 21 ( $P = .009$ ) than on days 7 and 14. This time dependency was similar as that observed for instrumental texture, as discussed previously. UNIN chops were also higher in chewiness than RICE, PR13, and PR18 chops ( $P = .006$ ). RICE chops had greater pork flavor than those from all other treatments, while RICE, PLUM, and PR18 chops were all more flavorful than UNIN chops ( $P < .001$ ). Because UNIN chops did not have any added ingredients, less flavor may be expected. These results confirm the expected improvement of these sensory properties in pork chops with the use of enhancement brines for pork loins. However, the

**Table 5.** Least-squares means for main effects of treatment on sensory attributes of enhanced pork loins stored at 2°C.

Treatment	Tenderness	Juiciness	Chewiness	Pork Flavor	Other Flavor
UNIN	4.53 <sup>b</sup>	5.75 <sup>b</sup>	11.34 <sup>a</sup>	5.42 <sup>c</sup>	0.56 <sup>b</sup>
SPH	7.82 <sup>ab</sup>	8.52 <sup>a</sup>	7.71 <sup>ab</sup>	6.12 <sup>bc</sup>	1.97 <sup>a</sup>
INCL	7.80 <sup>ab</sup>	7.46 <sup>ab</sup>	8.44 <sup>ab</sup>	6.53 <sup>bc</sup>	0.72 <sup>b</sup>
RICE	11.03 <sup>a</sup>	9.61 <sup>a</sup>	5.05 <sup>b</sup>	8.42 <sup>a</sup>	0.89 <sup>b</sup>
PLUM	8.49 <sup>ab</sup>	8.65 <sup>a</sup>	7.55 <sup>ab</sup>	6.81 <sup>b</sup>	0.77 <sup>b</sup>
PR13	8.54 <sup>ab</sup>	7.78 <sup>ab</sup>	7.18 <sup>b</sup>	6.53 <sup>bc</sup>	0.65 <sup>b</sup>
PR18	9.57 <sup>a</sup>	8.49 <sup>a</sup>	6.68 <sup>b</sup>	7.01 <sup>b</sup>	0.98 <sup>b</sup>
SEM	0.97	0.84	0.95	1.11	0.38

UNIN, uninjected control.

SPH, salt and phosphate treatment at 13% injection.

INCL, injected clean label at 13% injection.

RICE, injected clean label with rice bran extract at 13% injection.

PLUM, injected clean label with plum concentrate at 13% injection.

PR13, injected clean label with Proteus® at 13% injection.

PR18, injected clean label with Proteus® at 18% injection.

SEM, standard error of mean.

<sup>a-c</sup>Means in the same column with different superscripts are significantly different ( $P < .05$ ).

$n = 14$  (1 loin  $\times$  7 treatments  $\times$  2 replications).

sensory panel noted that SPH was higher in “other flavor” than all other treatments ( $P = .001$ ). These flavors were described by the panelists predominantly as “sour,” “salty,” “metallic,” and may be due to the phosphates, which have been noted for contributing soapy, salty, sour, and metallic taste and flavor notes to food products (Chun et al., 2020; Ecarma and Nolden, 2021). The phosphate concentration (0.3%) used in this study is typical for commercial enhancement brines and is not normally expected to contribute to undesirable flavors. However, it is likely that the highly trained and experienced sensory panel members in this study were more perceptive of the “other flavors” noted.

### Limitations of the study

This study has some potential limitations that future studies should consider. The sample size was limited, the loins were injected in a nonrandom order, and 2 different types of injection needles were used. The sample size was smaller than it could be due to the availability of a single multineedle injector. To help minimize sample size effects, loins were hand selected directly from the processing line at the pork harvest facility based on weight (4.1–4.5 kg). They were processed and injected in a nonrandom order to prevent potential contamination of the no-phosphate treatments

with phosphate of the no-phosphate treatments due to the availability of a single multineedle injector. The use of hypodermic needles to inject the PR13 and PR18 treatments was unavoidable due to the viscosity of the brine. Any follow-up or confirmatory studies should consider the use of hypodermic needles for all treatments.

## Conclusions

The results of this study demonstrated that all the enhancement treatments studies (INCL, RICE, PLUM, PR13, and PR 18) maintained equivalent or better sensory quality to the traditional SPH treatment throughout the 21 d of refrigerated storage. The RICE treatments had consistently higher sensory scores for tenderness, juiciness, and flavor than the other treatments, though the differences were not statistically significant in all cases. These chops were also similar in juiciness, tenderness, and flavor to the SPH chops but without the noted off-flavors. Thus, rice bran extract appeared to have the most potential among the ingredients evaluated to serve as a supplemental phosphate alternative for use in injected fresh pork loins and chops combined with other “clean label” ingredients. Rice bran and rice bran extract have been noted for significant phosphorus content, primarily as phytic acid (Saunders, 1985). While a relatively inert compound, derivatives of phytic acid, including hexaphosphates and hexaphosphoric acid (Saunders, 1985; Makarenko et al., 2018), may be the source of the positive impact of rice bran extract in this study. At the same time, plum concentrate and Proteus® functional protein extract also have potential to serve as phosphate alternatives. Dried plum products have been previously reported as effective means of increasing the water binding properties of meat products, an effect attributed to the high sorbitol content of concentrated plum products (Jarvis et al., 2015). The Proteus® ingredient is a pork protein extract obtained by a proprietary process to expose a greater number of protein functional groups and increase water binding capacity when added to a meat mixture. Therefore, there are commercially available options for meat processors to use as clean label replacements of the traditional phosphates, salt, and lactate in enhanced fresh meat products without compromising product quality and consumer acceptability. Further research on rice bran extract and functional proteins in other processed meat applications, such as fresh sausage and emulsified products, should be conducted.

## Conflict of Interest

The authors declare no conflicts of interest.

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## Author Contribution

**Rachel I. Crowley:** Data curation, formal analysis, investigation, writing—original draft, writing—review and editing, and visualization. **Joseph G. Sebranek:** conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, writing—review and editing, visualization, supervision, and validation. **Kenneth H. Prusa:** data curation, investigation, methodology, and resources. **Nicholas J. Kelecich:** investigation. **Rodrigo Tarté:** conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, writing—review and editing, visualization, supervision, and validation.

## Literature Cited

- AOAC International. 2019. Official methods of analysis of AOAC International. 21st ed. AOAC International, Arlington, VA.
- Aschemann-Witzel, J., P. Varela, and A. O. Peschel. 2019. Consumers' categorization of food ingredients: do consumers perceive them as 'clean label' products expect? An exploration with projective mapping. *Food Qual. Prefer.* 71:117–128. <https://doi.org/10.1016/J.FOODQUAL.2018.06.003>.
- Chun, S., E. Chambers IV, and D. H. Chambers. 2020. Effects of shiitake (*Lentinus edodes* P.) mushroom powder and sodium tripolyphosphate on texture and flavor of pork patties. *Foods*. 9:611. <https://doi.org/10.3390/foods9050611>.
- Davis, K. J., J. G. Sebranek, E. J. Huff-Lonergan, and S. M. Lonergan. 2004. The effects of aging on moisture-enhanced pork loins. *Meat Sci.* 66:519–524. [https://doi.org/10.1016/S0309-1740\(03\)00154-2](https://doi.org/10.1016/S0309-1740(03)00154-2).
- Ecarma, M. J. Y., and A. A. Nolden. 2021. A review of the flavor profile of metal salts: understanding the complexity of metallic sensation. *Chem. Senses.* 46:bjab043. <https://doi.org/10.1093/chemse/bjab043>.
- European Parliament and Council. 2008. Regulation (EC) No. 1333/2008 of 16 December 2008 on food additives. *Official Journal of the European Union.* 354:16–33. <https://eur-lex.europa.eu/eli/reg/2008/1333/oj>. (Accessed 11 February 2025).
- Garofalo, S. F., T. Tommasi, and D. Fino. 2021. A short review of green extraction technologies for rice bran oil. *Biomass Conversion and Biorefinery.* 11:569–587. <https://doi.org/10.1007/s13399-020-00846-3>.

- Holt, D., P. Slade, and J. Hobbs. 2024. Do consumers care about clean labels? Willingness to pay for simple ingredient lists and front-of-package labels on beef and plant-based burgers. *Cand. J. Agr. Econ.* 72:5–21. <https://doi.org/10.1111/cjag.12346>.
- Huff-Lonergan, E., W. Zhang, and S. M. Lonergan. 2010. Biochemistry of postmortem muscle—lessons on mechanisms of meat tenderization. *Meat Sci.* 86:184–195. <https://doi.org/10.1016/j.meatsci.2010.05.004>.
- Jarvis, N., C. A. O'Bryan, S. C. Ricke, and P. G. Crandall. 2015. The functionality of plum ingredients in meat products: a review. *Meat Sci.* 102:41–48. <https://doi.org/10.1016/j.meatsci.2014.12.002>.
- Johnson, L. G., C. Zhai, E. M. Steadham, L. M. Reeve, K. J. Prusa, M. N. Nair, E. Huff-Lonergan, and S. M. Lonergan. 2023. Distinct myofibrillar sub-proteomic profiles are associated with the instrumental texture of aged pork loin. *J. Anim. Sci.* 101:skad327. <https://doi.org/10.1093/jas/skad327>.
- Kemin Industries, Inc. 2024. Proteus®—functional proteins. <https://kemin.com/na/en-us/markets/food/products/proteus>. (Accessed 25 November 2024).
- King, A. M., K. A. Glass, A. L. Milkowski, and J. J. Sindelar. 2015. Impact of clean-label antimicrobials and nitrite derived from natural sources on the outgrowth of *Clostridium perfringens* during cooling of deli-style turkey breast. *J. Food Prot.* 78:946–953. <https://doi.org/10.4315/0362-028X.JFP-14-503>.
- King, D. A., M. C. Hunt, S. Barbut, J. R. Claus, D. P. Cornforth, P. Joseph, Y. H. B. Kim, G. Lindahl, R. A. Mancini, M. N. Nair, K. J. Merok, A. Milkowski, A. Mohan, F. Pohlman, R. Ramanathan, C. R. Raines, M. Seyfert, O. Sørheim, S. P. Suman, and M. Weber. 2023. American Meat Science Association Guidelines for meat color measurement. *Meat and Muscle Biology.* 6:12473, 1–81. <https://doi.org/10.22175/mmb.12473>.
- Maddock, K. R., E. Huff-Lonergan, L. J. Rowe, and S. M. Lonergan. 2005. Effect of pH and ionic strength on  $\mu$ - and m-calpain inhibition by calpastatin. *J. Anim. Sci.* 83:1370–1376. <https://doi.org/10.2527/2005.8361370x>.
- Makarenko, N. V., L. A. Zemnuiklova, A. V. Nemtarev, A. V. Kovekhova, and O. D. Arefieva. 2018. Composition and structure of phytic acid derivatives from rice bran. *Bio Resources.* 13:3411–3419. <https://doi.org/10.15376/biores.13.2.3411-3419>.
- Miller, R. K. 2002. Factors affecting the quality of raw meat. In: J. Kerry, J. Kerry, and D. Ledward, editors, *Meat processing: improving quality*. Woodhead Publishing, Ltd., Cambridge, UK. p. 27–63. <https://doi.org/10.1533/9781855736665.1.27>.
- Miller, R. 2006. Fact sheet PIG 12-05-02: functionality of non-meat ingredients used in enhanced pork. Pork Information Gateway. U.S. Pork Center of Excellence, Clive, IA. <https://porkgateway.org/resource/functionality-of-non-meat-ingredients-used-in-enhanced-pork/>. (Accessed 25 November 2024).
- Min, B., M. H. Chen, and B. W. Green. 2009. Antioxidant activities of purple rice bran extract and its effect on the quality of low-NaCl, phosphate-free patties made from channel catfish (*Ictalurus punctatus*) belly flap meat. *J. Food Sci.* 74:C268–C277 <https://doi.org/10.1111/j.1750-3841.2009.01108.x>.
- Moeller, S. J., R. K. Miller, K. K. Edwards, H. N. Zerby, K. E. Logan, T. L. Aldredge, C. A. Stahl, M. Boggess, and J. M. Box-Steffensmeier. 2010. Consumer perceptions of pork eating quality as affected by pork quality attributes and end-point cooked temperature. *Meat Sci.* 84:14–22. <https://doi.org/10.1016/j.meatsci.2009.06.023>.
- Offer, G., and J. Trinick. 1983. On the mechanism of water holding in meat: the swelling and shrinking of myofibrils. *Meat Sci.* 8:245–281. [https://doi.org/10.1016/0309-1740\(83\)90013-X](https://doi.org/10.1016/0309-1740(83)90013-X).
- Petracci, M., L. Laghi, P. Rocculi, S. Rimini, V. Panarese, M. A. Cremonini, and C. Cavani. 2012. The use of sodium bicarbonate for marination of broiler breast meat. *Poultry Sci.* 91:526–534. <https://doi.org/10.3382/ps.2011-01753>.
- Saunders, R. M. 1985. Rice bran: composition and potential food uses. *Food Rev. Int.* 1:465–495. <https://doi.org/10.1080/87559128509540780>.
- Shan, Y., D. Pu, J. Zhang, L. Zhang, Y. Huang, P. Li, J. Xiong, K. Li, and Y. Zhang. 2022. Decoding the saltiness enhancement taste peptides from the yeast extract and molecular docking to the taste receptor T1R1/T1R3. *J. Agr. Food Chem.* 70:14898–14906. <https://doi.org/10.1021/acs.jafc.2c06237>.
- Sheard, P. R., and A. Tali. 2004. Injection of salt, tripolyphosphate and bicarbonate marinade solutions to improve the yield and tenderness of cooked pork loin. *Meat Sci.* 68:305–311. <https://doi.org/10.1016/j.meatsci.2004.03.012>.
- Thangavelu, K. P., J. P. Kerry, B. K. Tiwari, and C. K. McDonnell. 2019. Novel processing technologies and ingredient strategies for the reduction of phosphate additives in processed meat. *Trends Food Sci. Tech.* 94:43–53. <https://doi.org/10.1016/j.tifs.2019.10.001>.
- USDA Food Safety and Inspection Service. 2024. Safe and suitable ingredients used in the production of meat, poultry, and egg products—Revision 59. <https://www.fsis.usda.gov/policy/fsis-directives/7120.1>. (Accessed 11 February 2025).
- Warner, R., R. Miller, M. Ha, T. L. Wheeler, F. Dunshea, X. Li, R. Vascoska, and P. Purslow. 2021. Meat tenderness: underlying mechanisms, instrumental measurement, and sensory assessment. *Meat and Muscle Biology.* 4:17, 1–25. <https://doi.org/10.22175/mmb.10489>.
- Xiong, Y. L. 2005. Role of myofibrillar proteins in water-binding in brine-enhanced meats. *Food Res. Int.* 38:281–287. <http://dx.doi.org/10.1016/j.foodres.2004.03.013>.
- Xu, M., C. Shen, H. Zheng, Y. Xu, C. Xue, B. Zhu, and J. Hu. 2020. Metabolomic analysis of acerola cherry (*Malpighia emarginata*) fruit during ripening development via UPLC-Q-TOF and contribution to the antioxidant activity. *Food Res. Int.* 130:108915. <https://doi.org/10.1016/j.foodres.2019.108915>.