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# Use of Microbial Transglutaminase and Nonmeat Proteins to Improve Functional Properties of Low NaCl, Phosphate-Free Patties Made from Channel Catfish (*Ictalurus punctatus*) Belly Flap Meat

B. MIN AND B.W. GREEN

**ABSTRACT:** This study was aimed at developing value-added low sodium chloride (NaCl), phosphate-free restructured patties using minced channel catfish (*Ictalurus punctatus*) belly flap meat. The effect of microbial transglutaminase (MTGase) and nonmeat proteins (isolated soy protein, ISP, and whey protein concentrate, WPC; 1.7%, respectively) alone and in combination were evaluated to improve cooking yield and textural properties in patties with reduced NaCl and no phosphate. The concentration effect of MTGase (0.05% to 0.7%) was also studied. The addition of MTGase increased textural properties such as binding strength, hardness, cohesiveness, chewiness, and springiness, but decreased cooking yield of the patties ( $P < 0.05$ ). Isolated soy protein increased cooking yield ( $P < 0.05$ ), but did not affect textural properties. Inclusion of WPC did not increase cooking yield or impact textural properties of patties. The combination of MTGase and ISP significantly increased both the cooking yield and textural properties of patties. As the concentration of MTGase increased at constant ISP, the textural properties of cooked patties significantly increased, but cooking yield decreased ( $P < 0.05$ ). In conclusion, we suggest that the combination of 0.05% to 0.1% of MTGase with 1.7% ISP is optimal for development of a low NaCl, phosphate-free patty using minced catfish belly flap meat.

**Keywords:** channel catfish belly flap meat, cooking yield, isolated soy protein, microbial transglutaminase, texture profile analysis

## Introduction

Channel catfish (*Ictalurus punctatus*) is the primary fish species of the aquaculture industry in the United States, and its production and consumption have increased dramatically over the past decade (Tucker and others 2004). A recent survey (House and others 2003) indicated that the main reasons for eating catfish are flavor, health and nutrition, and addition of variety to diets. Two factors that would stimulate catfish consumption are (1) continuous availability of a quality product and (2) broader availability of various ready-to-eat products (House and others 2003). Therefore, the development of value-added catfish products is one approach to increase the consumption of catfish products in the United States.

Most catfish products processed in the United States are sold as fresh or frozen fillets and whole dressed fish. The shank fillet of channel catfish is a primary product form produced during processing and is formed by trimming the belly flap or nugget section from a boneless fillet. The production of belly flap meat is around 20% of the fillet weight (Silva and Dean 2001). Belly flap meat has a high fat content that is responsible for its limited shelf life and undesirable sensory qualities. It contains around 15% fat,

compared to 9% in the fillet (Sathivel and others 2002). In addition, the black-pigmented peritoneal membrane present on the surface of the belly flap meat degrades its appearance and possibly darkens the color of minced meat. Wiles and others (2004) reported that washing minced belly flap meat reduced its fat content by approximately 50% and enhanced whiteness of minced products. Therefore, postharvest handling and processing methods such as washing to reduce fat content and depigment the adhered peritoneal membrane should improve the sensory qualities and shelf life of further-processed products made from channel catfish belly flap meat.

Sodium chloride (NaCl) has long been recognized as an essential ingredient for meat products to improve flavor, water holding capacity, cooking yield, textural properties, and consumer acceptance (Sofos 1983a, 1983b). In addition, NaCl is added to restructured products to enhance binding ability among meat particles (Huffman and others 1981). NaCl extracts salt-soluble proteins such as myofibrillar proteins that serve as a binding agent among meat particles (Aberle and others 2001); however, a high sodium diet is correlated with an increased risk of cardiovascular disease, and reduced dietary intake of NaCl is recommended (He and others 1999; Chobanian and Hill 2000).

In addition to NaCl, phosphate is an important additive for meat products that is used to improve water holding capacity and cooking yield (Knipe and others 1985; Aberle and others 2001); however, excessive dietary intake of phosphate may affect calcium, iron, and magnesium balances in human body and increase the risk of bone diseases (Shahidi and Synowiecki 1997; Kärkkäinen 1998).

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Therefore, the adverse effect of NaCl and phosphate on human health has stimulated research on the development of meat products with reduced NaCl and no-added phosphate (Ruusunen and others 2003; Dimitrakopoulou and others 2005).

The reduction of NaCl and removal of phosphate from meat products, however, adversely affect water and fat binding capacity, leading to degradation of textural properties and decrease in cooking yield (Girard and others 1990). Various additives such as microbial transglutaminase (MTGase) and nonmeat proteins have been used in low NaCl, phosphate-free meat products to improve water holding capacity and textural properties (Muguruma and others 2003; Ruusunen and others 2003; Dimitrakopoulou and others 2005; Pietrasik and others 2007). Microbial transglutaminase (protein-glutamine:amine  $\gamma$ -glutamyltransferase, EC 2.3.2.13) is the enzyme that catalyzes protein cross-linking through acyl-transfer reaction between  $\gamma$ -carbonyamide group of  $\epsilon$ -glutamine residues and the amino group of lysines in proteins, leading to inter- or intramolecular cross-linking (De Jong and Koppelman 2002). Binding strength and gel strength in restructured and other types of meat and fish products are improved with MTGase use (Kuraishi and others 1997; Ramírez and others 2000; Tseng and others 2000; Dimitrakopoulou and others 2005; Ahmed and others 2007). Common substrates for MTGase include meat, whey, casein, and soy protein (Schorsch and others 2000; De Jong and Koppelman 2002; Truong and others 2004; Tang and others 2006).

Nonmeat proteins such as isolated soy protein (ISP) and whey protein concentrate (WPC) possess important functional properties such as water and fat holding capacities and can aggregate with other solubilized ingredients in a protein matrix during heat treatment, which improves water holding capacity and gel strength in meat products (Rhee 1994; Hoogenkamp 2001). Additionally, these nonmeat proteins serve as bioavailable protein supplements (Rhee 1994). For example, ISP and WPC are reported to increase cooking yield in frankfurters (Atughonu and others 1998).

Many studies have been conducted to develop meat and fish products with low salt and/or without phosphate by using MTGase and/or nonmeat proteins (Muguruma and others 2003; Ruusunen and others 2003; Dimitrakopoulou and others 2005; Pietrasik and others 2007; Ramírez and others 2007a, 2007b); however, no study has been conducted using catfish belly flap meat to develop a low-salt, phosphate-free patty. The objective of this study was to investigate the effect of MTGase and nonmeat proteins, ISP and WPC alone, or in combinations, on the functional properties of a low-salt, phosphate-free patty made from minced channel catfish belly flap meat. In addition, the effect of various levels of MTGase combined with a single level of ISP was also evaluated.

### Materials and Methods

#### Meat preparation

Fresh channel catfish belly flap meat was purchased from an Arkansas processor. Upon receipt, the meat was washed using a pressure washer (Troy-Bilt Model 020295, Briggs & Stratton Power Products Group, LLC, Jefferson, Wis., U.S.A.) for 5 min to remove excessive fat and depigment the adhered peritoneal membrane. The washed meat was drained for about 2 h in the refrigerator before storing in vacuum-packaging bags (Cryovac® HP2700 vacuum bag, Sealed Air, Duncan, S.C., U.S.A.). The vacuum-packaged meat was stored at -20 °C until use. Samples of the belly flap meat before and after washing were taken before freezing for analysis of pH, color parameters, and proximate analysis. The frozen meat was used within 2 wk of freezing.

#### Experimental design

Two experiments were conducted to improve physical properties of a low NaCl (1.0%), phosphate-free channel catfish belly flap patty. In experiment I, the effect of MTGase (Activa® TI, Aginomoto Food Ingredients, Chicago, Ill., U.S.A.) and 2 nonmeat protein ingredients, ISP (Supro® 500E, Solae LLC, St. Louis, Mo., U.S.A.) and WPC (WPC 80%, Davisco Foods Intl., Le Sueur, Minn., U.S.A.), on the functional properties of low NaCl, phosphate-free patties was tested. Eight types of the patties in the 1st experiment were prepared with (1) 1% NaCl only (1Na), (2) 1% NaCl and 0.4% polyphosphate (Nutrifos® B-90, Astaris LLC, St. Louis, Mo., U.S.A.) (1NaP), (3) 2% NaCl and 0.4% polyphosphate (2NaP), (4) 1% NaCl and 0.7% MTGase (T), (5) 1% NaCl and 1.7% ISP (ISP), (6) 1% NaCl and 1.7% WPC (WPC), (7) 1% NaCl, 0.7% MTGase, and 1.7% ISP (TISP), and (8) 1% NaCl, 0.7% MTGase, and 1.7% WPC (TWPC). In experiment II, the concentration effect of MTGase at a constant ISP concentration was assessed. Eight types of the patties were prepared with (1) 1Na, (2) 2NaP, (3) ISP, and (4 to 8) 1% NaCl, 1.7% ISP, and 5 different levels of MTGase (0.05%, 0.1%, 0.2%, 0.4%, and 0.7% used in treatments T0.05, T0.1, T0.2, T0.4, and T0.7, respectively). In experiments I and II, patties with 1% NaCl (1Na) were considered as the control. Patties with additional NaCl and phosphate (2NaP) were prepared to bracket the range of concentrations typically used in the industry. Patties with ISP only were prepared as the ISP-reference in experiment II. Eight replicate batches (around 350 g per batch) were prepared for each treatment in each experiment. The treatment denominations and formulations for the 1st and 2nd experiments are shown in Table 1.

#### Production of patties

Belly flap meat was thawed overnight in a refrigerator and ground separately twice using a grinder (Model 722, Biro Manufacturing Co. Ltd., Marblehead, Ohio, U.S.A.) fitted with a plate with six 18-mm-dia openings. The ground meat was mixed with sodium

**Table 1 – Formulation of patties using channel catfish belly flap meat with treatments in the experiment I and II.**

Treatment	Ingredients (g)						
	Meat	Water	NaCl	Phosphate	MTGase <sup>a</sup>	ISP <sup>a</sup>	WPC <sup>a</sup>
Experiment I <sup>b</sup>							
1Na	300.0	34.0	3.4	—	—	—	—
2NaP	300.0	34.0	6.8	1.2	—	—	—
1NaP	300.0	34.0	3.4	1.2	—	—	—
T	300.0	34.0	3.4	—	2.4	—	—
ISP	300.0	34.0	3.4	—	—	6.0	—
WPC	300.0	34.0	3.4	—	—	—	6.0
TISP	300.0	34.0	3.4	—	2.4	6.0	—
TWPC	300.0	34.0	3.4	—	2.4	—	6.0
Experiment II <sup>c</sup>							
1Na	300.0	34.0	3.4	—	—	—	—
2NaP	300.0	34.0	6.8	1.2	—	—	—
ISP	300.0	34.0	3.4	—	—	6.0	—
T0.05	300.0	34.0	3.4	—	0.17	6.0	—
T0.1	300.0	34.0	3.4	—	0.34	6.0	—
T0.2	300.0	34.0	3.4	—	0.68	6.0	—
T0.4	300.0	34.0	3.4	—	1.36	6.0	—
T0.7	300.0	34.0	3.4	—	2.40	6.0	—

<sup>a</sup>MTGase = microbial transglutaminase; ISP = isolated soy protein; WPC = whey protein concentrate.

<sup>b</sup>Experiment I treatment designations: 1Na, 1% NaCl only; 2NaP, 2% NaCl and 0.4% polyphosphate; 1NaP, 1% NaCl and 0.4% polyphosphate; T, 0.7% MTGase; ISP, 1.7% ISP; WPC, 1.7% WPC; TISP, 0.7% MTGase and 1.7% ISP; TWPC, 0.7% MTGase and 1.7% WPC. Treatments T, ISP, WPC, TISP, and TWPC contained 1% NaCl.

<sup>c</sup>Experiment II treatment designations: treatments 2NaP, 1Na, and ISP were the same as in Experiment I. Treatments T0.05 to 0.7 indicate that the added concentrations of MTGase (0.05% to 0.7%) plus 1% NaCl and 1.7% ISP.

chloride, polyphosphate, and water as appropriate for 2 min in a mixer (Model KSM150PSOB, KitchenAid, St. Joseph, Mich., U.S.A.). Microbial transglutaminase was dissolved in water (1:3, w/w). ISP (1:4, w/w) and WPC (1:2, w/w) were mixed with water in a mixer. These mixtures were subsequently added to the mince and mixed for 2 min. Total mixing time was standardized for 4 min, and the final temperature of the meat mixture was kept below 12 °C throughout the mixing process for all treatments. Patties (100 g; 100-mm diameter) were formed using a hamburger press (Model 1404, Univex, Salem, N.H., U.S.A.) and stored overnight at 4 °C in a refrigerator for the reaction of MTGase. Patties were cooked in a superheated steam oven (Model AX700R, Sharp Electronics, Mahwah, N.J., U.S.A.) at 227 °C to an internal temperature of 80 °C. Cooked patties were cooled and stored at 4 °C in a refrigerator until further analysis.

### Proximate analysis and pH measurement

Proximate analysis and pH measurement were conducted on the raw unwashed and washed belly flap mince and the raw and cooked patties made in the experiment I only. All determinations were performed in quadruplicate. Moisture, protein, and ash contents were determined according to the AOAC official methods (AOAC 2005). Total fat was determined by the method of Ahn and others (1995). The pH of a homogenate of 10-g sample in 90 mL distilled water was determined by using a pH meter (Accumet XL25, Waltham, Mass., U.S.A.).

### Color measurement

Commission Internationale de l'Eclairage (CIE)  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) for the raw mince before and after washing and for raw and cooked patties were determined using a HunterLab Miniscan XE plus colorimeter (Model 4500L, Hunter Associate Laboratory Inc., Reston, Va., U.S.A.) with 25-mm aperture, CIE D65 illuminant, and 10° standard observer. The colorimeter was calibrated against black and white reference tiles. Color values were measured on the surface of 2 patties per batch and 6 measurements per patty (three on each side of the patty). Whiteness (Park 2000), chroma, and hue angle were calculated by the following equations:

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$$

$$\text{Hue angle} = \tan^{-1}(b^*/a^*)$$

### Cooking yield

Each of 2 patties per batch was weighed before and after cooking. The cooked patties were gently patted with a paper towel to remove excess moisture and fat on the surface, and stored at 4 °C in a refrigerator overnight for temperature equilibration before being weighed. Cooking yield was calculated as the ratio of cooked weight to raw weight expressed as a percentage.

### Binding strength and texture profile analysis

Binding strength (BS) was determined and texture profile analysis (TPA) was performed on cooked patties using an Instron universal testing machine (Model 3342, Instron Co., Norwood, Mass., U.S.A.) equipped with a 500-N load cell. Binding strength was determined according to Field and others (1984) with some modifications: a core (60 mm in diameter and 18 mm in thickness) was excised from the center of the cooked patties using a cookie cutter and centered over a 38-mm-dia center hole on a base plate custom designed for the Instron machine. A 19-mm-dia ball welded

to a shaft was lowered through the patty at a crosshead speed of 100 mm/min, and the peak force (N) required to break through the sample was recorded as a BS.

Texture profile analysis was performed using a 90-mm-dia aluminum plunger according to the methods of Bourne (2002). Each patty (30 mm in diameter and 18 mm in thickness) was compressed through 2 repeated cycles with 80% compression of the initial height at a crosshead speed of 60 mm/min. There was a 5-s rest period between the 2 cycles to allow the sample to recover its height. Hardness, cohesiveness, chewiness, and springiness were calculated from the TPA curve. Hardness (N) was defined as the peak force during the 1st compression cycle, cohesiveness as the ratio of the positive force area under the 2nd compression to under the 1st compression, chewiness (N\*mm) as the energy required to compress the sample and the product of hardness × cohesiveness × springiness, and springiness (%) as the ratio of the height recovered during the rest period between the 1st and 2nd cycles to the original height to the sample (Bourne 2002).

### Statistical analysis

All data were analyzed by 1-way analysis of variance using the SAS program (version 9.1 for Windows, SAS Inst. Inc., Cary, N.C., U.S.A.) and reported as means and standard error of means (SEM) in tables and means and standard deviation (SD) in figures. Eight batches for each treatment were produced for surface color, BS, and TPA measurements ( $n = 8$ ). For proximate analysis and pH measurement, 4 batches from each treatment were randomly selected and analyzed ( $n = 4$ ). Student–Newman–Keuls' multiple range test ( $P < 0.05$ ) was used to compare the mean values of treatments (Kuehl 2000).

**Table 2—pH and proximate analysis of raw mince and patties made from channel catfish belly flap meat supplemented with NaCl (Na), phosphate (P), microbial transglutaminase (MTGase; T), and/or nonmeat proteins (isolated soy protein, ISP; whey protein concentrate, WPC).<sup>A</sup>**

Treatment <sup>B</sup>	pH	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Raw mince					
Unwashed	6.61 <sup>b</sup>	75.61 <sup>b</sup>	14.95 <sup>a</sup>	10.14 <sup>a</sup>	0.96 <sup>a</sup>
Washed	6.65 <sup>a</sup>	79.27 <sup>a</sup>	14.25 <sup>b</sup>	5.74 <sup>b</sup>	0.75 <sup>b</sup>
SEM	0.01	0.91	0.38	1.86	0.02
Raw patty					
1Na	6.65 <sup>b</sup>	79.69 <sup>a</sup>	12.60 <sup>b</sup>	5.04	1.54 <sup>c</sup>
2NaP	6.82 <sup>a</sup>	79.18 <sup>ab</sup>	12.59 <sup>b</sup>	5.30	2.93 <sup>a</sup>
1NaP	6.84 <sup>a</sup>	80.09 <sup>a</sup>	12.83 <sup>b</sup>	4.66	1.85 <sup>b</sup>
T	6.67 <sup>b</sup>	78.71 <sup>abc</sup>	12.39 <sup>b</sup>	4.97	1.55 <sup>c</sup>
ISP	6.68 <sup>b</sup>	77.90 <sup>bc</sup>	14.03 <sup>a</sup>	6.06	1.55 <sup>c</sup>
WPC	6.60 <sup>c</sup>	77.24 <sup>c</sup>	13.62 <sup>a</sup>	5.06	1.51 <sup>c</sup>
TISP	6.67 <sup>b</sup>	77.25 <sup>c</sup>	14.10 <sup>a</sup>	5.47	1.58 <sup>c</sup>
TWPC	6.60 <sup>c</sup>	77.55 <sup>bc</sup>	13.71 <sup>a</sup>	6.14	1.55 <sup>c</sup>
SEM	0.02	0.84	0.38	0.83	0.06
Cooked patty					
1Na	—	71.92 <sup>c</sup>	17.92 <sup>c</sup>	7.26 <sup>bc</sup>	1.77 <sup>c</sup>
2NaP	—	75.69 <sup>a</sup>	14.88 <sup>f</sup>	5.69 <sup>e</sup>	3.08 <sup>a</sup>
1NaP	—	75.91 <sup>a</sup>	15.55 <sup>e</sup>	5.14 <sup>f</sup>	2.09 <sup>b</sup>
T	—	70.15 <sup>d</sup>	19.33 <sup>b</sup>	7.75 <sup>ab</sup>	1.68 <sup>c</sup>
ISP	—	73.84 <sup>b</sup>	16.56 <sup>d</sup>	6.11 <sup>de</sup>	1.79 <sup>c</sup>
WPC	—	71.25 <sup>c</sup>	20.01 <sup>a</sup>	6.59 <sup>cd</sup>	1.69 <sup>c</sup>
TISP	—	71.97 <sup>c</sup>	19.01 <sup>b</sup>	7.14 <sup>bc</sup>	1.74 <sup>c</sup>
TWPC	—	68.58 <sup>e</sup>	20.10 <sup>a</sup>	8.25 <sup>a</sup>	1.67 <sup>c</sup>
SEM	—	0.70	0.43	0.40	0.07

<sup>A</sup>Means with different letters (a to f) within the same column are significantly different ( $P < 0.05$ ). SEM = standard error of the means.  $n = 8$  or pH and  $n = 4$  for proximate analyses.

<sup>B</sup>See Table 1 for treatment descriptions.

## Results and Discussion

### Effect of washing on raw meat quality

Washing significantly reduced fat, protein, and ash contents in raw meat (Table 2). The washing process effectively removed adi-

**Table 3 – Commission Internationale de l’Eclairage (CIE) color values, whiteness, chroma, and hue angle of raw mince and patties made from channel catfish belly flap meat supplemented with NaCl (Na), phosphate (P), microbial transglutaminase (MTGase; T), and/or nonmeat proteins (isolated soy protein, ISP; whey protein concentrate, WPC).<sup>A</sup>**

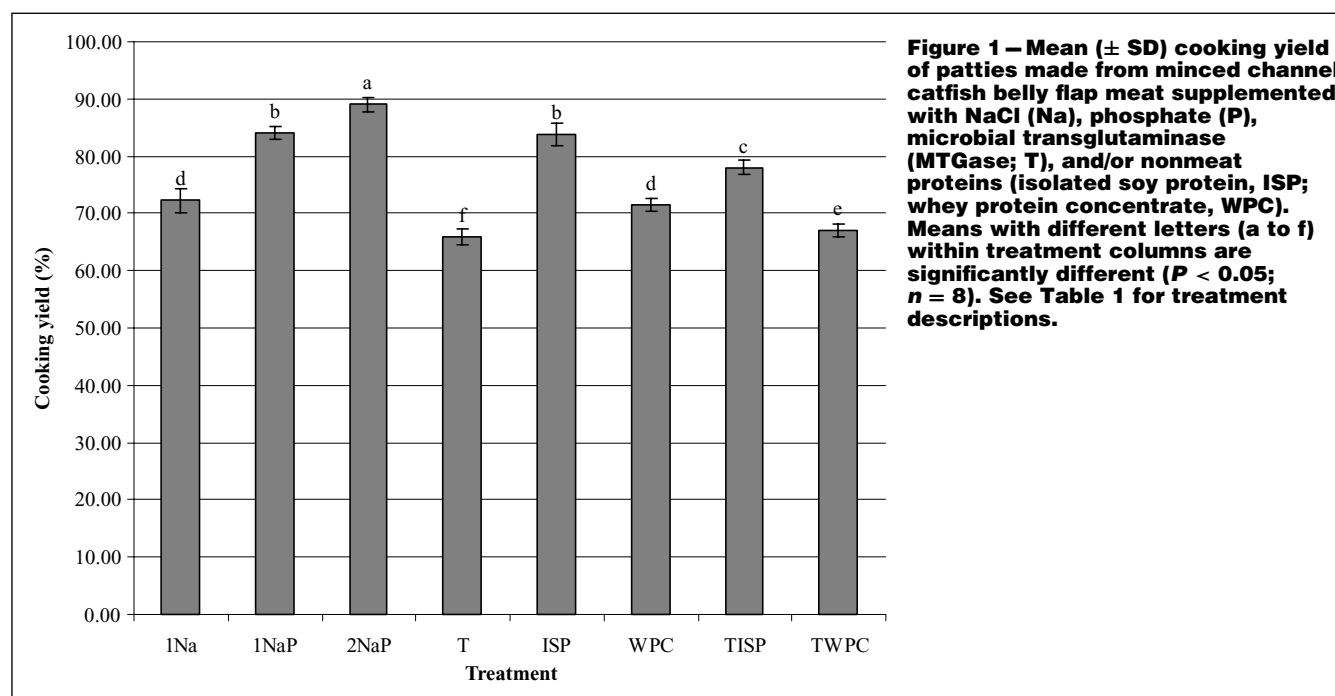
Treatment <sup>B</sup>	L*	a*	b*	Whiteness	Chroma	Hue
Raw mince						
Unwashed	65.77 <sup>b</sup>	2.63 <sup>b</sup>	10.21 <sup>b</sup>	64.11 <sup>b</sup>	10.56 <sup>b</sup>	75.70 <sup>a</sup>
Washed	72.62 <sup>a</sup>	3.81 <sup>a</sup>	12.62 <sup>a</sup>	69.64 <sup>a</sup>	13.21 <sup>a</sup>	73.31 <sup>b</sup>
SEM	0.70	0.44	0.47	0.77	0.55	1.56
Raw patty						
1Na	73.69	2.21 <sup>ab</sup>	10.63 <sup>d</sup>	71.52 <sup>a</sup>	10.87 <sup>d</sup>	78.32 <sup>b</sup>
2NaP	73.63	2.46 <sup>a</sup>	11.80 <sup>c</sup>	70.99 <sup>ab</sup>	12.07 <sup>c</sup>	77.53 <sup>b</sup>
1NaP	72.71	2.08 <sup>ab</sup>	10.26 <sup>d</sup>	70.75 <sup>ab</sup>	10.48 <sup>d</sup>	78.59 <sup>b</sup>
T	73.72	2.51 <sup>a</sup>	12.10 <sup>c</sup>	70.94 <sup>ab</sup>	12.36 <sup>bc</sup>	78.27 <sup>b</sup>
ISP	72.70	1.95 <sup>b</sup>	12.82 <sup>b</sup>	69.75 <sup>b</sup>	12.98 <sup>b</sup>	81.34 <sup>a</sup>
WPC	73.76	2.46 <sup>a</sup>	12.29 <sup>c</sup>	70.90 <sup>ab</sup>	12.55 <sup>bc</sup>	78.80 <sup>b</sup>
TISP	73.14	2.23 <sup>ab</sup>	13.66 <sup>a</sup>	69.75 <sup>b</sup>	13.85 <sup>a</sup>	80.74 <sup>a</sup>
TWPC	74.11	2.41 <sup>a</sup>	12.08 <sup>c</sup>	71.31 <sup>a</sup>	12.33 <sup>bc</sup>	78.29 <sup>b</sup>
SEM	0.97	0.31	0.52	0.92	0.53	1.36
Cooked patty						
1Na	75.74 <sup>b</sup>	-0.21 <sup>c</sup>	15.51 <sup>abc</sup>	71.19 <sup>b</sup>	15.51 <sup>abc</sup>	88.75 <sup>abc</sup>
2NaP	76.42 <sup>ab</sup>	-0.39 <sup>c</sup>	14.99 <sup>cd</sup>	72.04 <sup>ab</sup>	15.00 <sup>d</sup>	88.49 <sup>bc</sup>
1NaP	76.75 <sup>ab</sup>	-0.37 <sup>c</sup>	15.20 <sup>bcd</sup>	72.20 <sup>ab</sup>	15.21 <sup>bcd</sup>	88.54 <sup>dc</sup>
T	75.91 <sup>b</sup>	-0.01 <sup>b</sup>	15.86 <sup>ab</sup>	71.15 <sup>b</sup>	15.86 <sup>ab</sup>	89.10 <sup>a</sup>
ISP	75.92 <sup>b</sup>	-0.00 <sup>b</sup>	15.98 <sup>ab</sup>	71.09 <sup>b</sup>	15.98 <sup>ab</sup>	89.17 <sup>a</sup>
WPC	77.21 <sup>a</sup>	-0.34 <sup>c</sup>	14.61 <sup>d</sup>	72.92 <sup>a</sup>	14.61 <sup>cd</sup>	88.38 <sup>c</sup>
TISP	75.92 <sup>b</sup>	0.33 <sup>a</sup>	16.08 <sup>a</sup>	71.03 <sup>b</sup>	16.09 <sup>a</sup>	88.80 <sup>abc</sup>
TWPC	76.33 <sup>ab</sup>	-0.20 <sup>c</sup>	15.21 <sup>bcd</sup>	71.85 <sup>ab</sup>	15.21 <sup>bcd</sup>	88.94 <sup>ab</sup>
SEM	0.74	0.14	0.58	0.86	0.58	0.36

<sup>A</sup>Means with different letters (a to e) within the same column are significantly different ( $P < 0.05$ ). SEM = standard error of the means.  $n = 8$ .  
<sup>B</sup>See Table 1 for treatment descriptions.

pose tissues and water-soluble substances such as sarcoplasmic proteins, some metal ions, and, probably, metal-ion containing proteins such as hemoglobin, which are known to be major catalysts of lipid oxidation (Min and Ahn 2005). Lipid oxidation is one of the primary causes of quality deterioration in meat and meat products. Sathivel and others (2002) reported that 70.1% of total fatty acid in channel catfish belly flap meat was unsaturated fatty acids of which 25.1% were polyunsaturated fatty acids. The rate of lipid oxidation in meat is proportional to the unsaturation level of fatty acids. In addition, washing process increased all color parameters of the raw meat, including  $L^*$  (lightness),  $a^*$  (redness),  $b^*$  (yellowness), and whiteness (Table 3). Although increased moisture content in the raw meat after washing may affect color attributes (Park 2000), the removal of the black pigmentation from the peritoneal membrane by washing appeared to be the main factor for the increased lightness and whiteness of the washed raw meat. Thus, the reduction of belly flap meat fat content and black pigmentation by washing improved the oxidative stability and the functional, sensory, and nutritional quality of the further-processed products using the channel catfish belly flap meat.

### Effect of MTGase and nonmeat proteins

Moisture, protein, and ash contents of raw patties were affected significantly by the addition of additives compared to the control (patty 1Na), but not fat content (Table 2). Moisture, protein, and ash contents ranged from 77.24% to 80.09%, 12.39% to 14.10%, and 1.51% to 2.93%, respectively. The addition of nonmeat proteins such as ISP and WPC significantly increased the protein content and significantly decreased moisture content in the raw patties (patty ISP, WPC, TISP, and TWPC) compared to the control. Inclusion of additional NaCl and phosphate (patty 2NaP and 1NaP) significantly increased ash contents compared to the control because of increased sodium and phosphorous concentrations. The addition of phosphate significantly increased pH of raw patties. Phosphate has been well known to enhance water holding capacity and cooking yield in meat products because phosphate can



**Figure 1 – Mean (± SD) cooking yield of patties made from minced channel catfish belly flap meat supplemented with NaCl (Na), phosphate (P), microbial transglutaminase (MTGase; T), and/or nonmeat proteins (isolated soy protein, ISP; whey protein concentrate, WPC). Means with different letters (a to f) within treatment columns are significantly different ( $P < 0.05$ ;  $n = 8$ ). See Table 1 for treatment descriptions.**

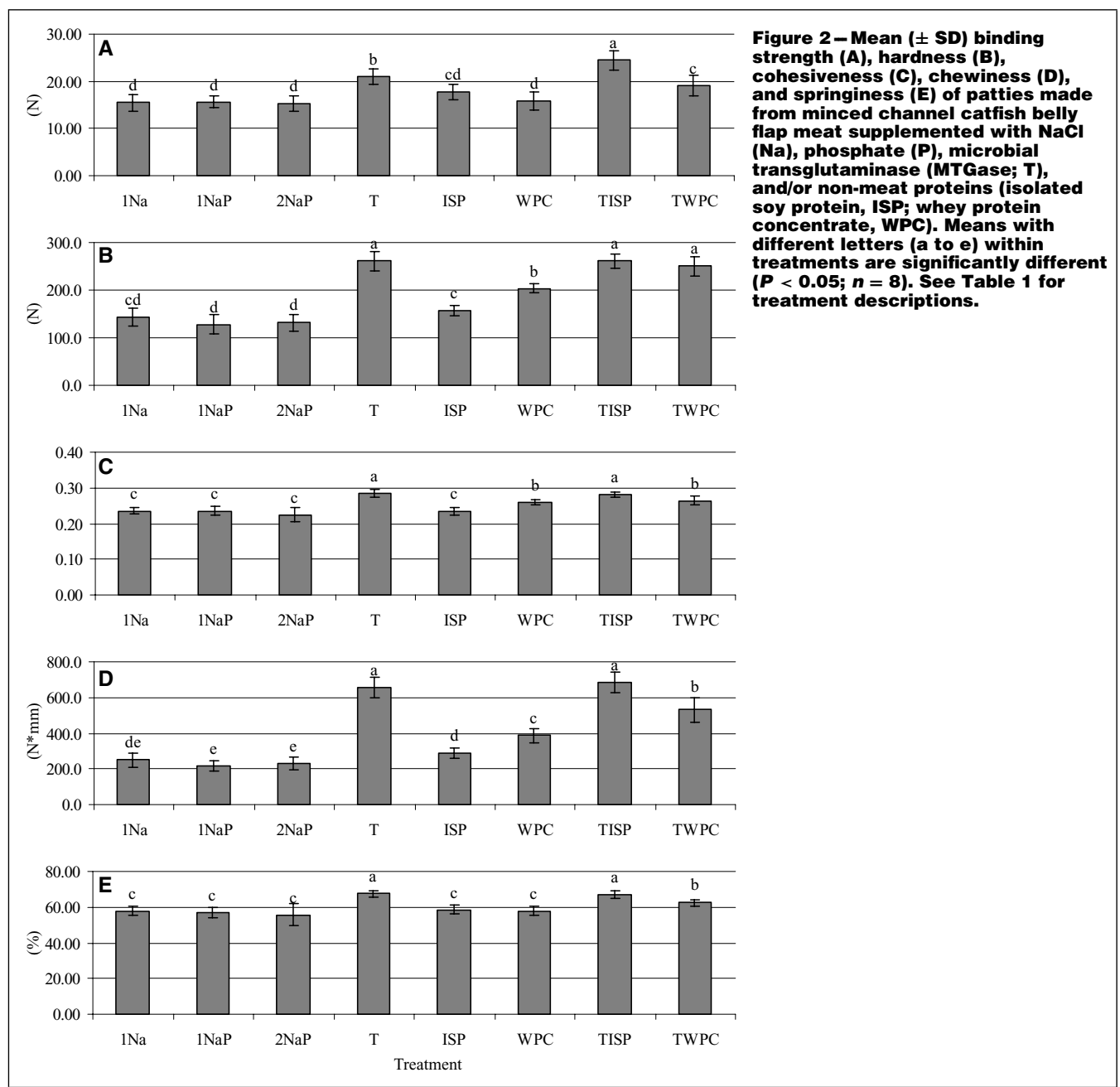
increase the pH and ionic strength of meat (Shahidi and Synowiecki 1997).

In cooked patties, all additives significantly affected moisture, protein, fat, and ash contents, compared to the control (Table 2). The moisture content of cooked patties ranged from 68.58% to 75.91%, significantly lower than that of raw patties, because of moisture loss during cooking. Phosphate-supplemented (patty 2NaP and 1NaP) and ISP patties had significantly higher moisture content than the control (Table 2). The lower protein content in 2NaP and 1NaP patties likely resulted in significant increases in moisture content. The protein, fat, and ash contents of cooked patties were significantly higher than those of raw patties because of moisture loss during cooking. Treatment differences for cooked patties were related to moisture content.

No significant differences were observed among treatments for raw patty  $L^*$  values (Table 3). The values of  $a^*$  and  $b^*$  in raw pat-

ties appeared to be affected by ISP to a greater extent than any of the other ingredients. The addition of ISP also significantly decreased whiteness but increased hue value of raw patties, indicating a change from a reddish hue to a slightly more yellowish hue. This result probably was due to the yellowness of ISP powder, which led to an increase in patty  $b^*$  value. Chroma values varied from 10.48 to 13.85 and showed a similar behavior to  $b^*$  value, indicating that the chroma value in this study was associated with  $b^*$  value rather than  $a^*$  value. The ISP-supplemented patties had the highest chroma value among treatments because of their high  $b^*$  value. The additive-induced color changes observed in raw patties in the present study are consistent with the results reported for another fish species (Ramírez and others 2007b).

Cooking affected all color parameters. In the cooked patties,  $L^*$ ,  $b^*$ , whiteness, chroma, and hue values increased but  $a^*$  value approached to zero, indicating the disappearance of



**Figure 2 – Mean (± SD) binding strength (A), hardness (B), cohesiveness (C), chewiness (D), and springiness (E) of patties made from minced channel catfish belly flap meat supplemented with NaCl (Na), phosphate (P), microbial transglutaminase (MTGase; T), and/or non-meat proteins (isolated soy protein, ISP; whey protein concentrate, WPC). Means with different letters (a to e) within treatments are significantly different ( $P < 0.05$ ;  $n = 8$ ). See Table 1 for treatment descriptions.**

redness (Table 3). Addition of WPC affected color parameters of cooked patties to a great extent. Although the additives significantly affected color parameters in the raw patties, compared to the control, their effects appeared to be attenuated by cooking.

Inclusion of the additives significantly affected the cooking yield of the patties (Figure 1). As expected, the cooking yield of the control patties was significantly lower than that of patties supplemented with phosphate and/or additional NaCl. Sofos (1983a, 1983b) indicated that the reduction of NaCl content to less than 2.0% without phosphate reduced cooking yield and degraded textural properties in meat products such as frankfurter and that functional ingredients might be needed to ensure the good quality of the products. Consequently, 2.0% to 2.5% NaCl appeared to be necessary for commercial frankfurter production in the absence of ingredients such as phosphate and other proteins that may supplement the effects of NaCl (Sofos 1983a, 1983b). Cooking yield depends on water holding capacity of restructured patty and is highly related to the production cost (Pietrasik 1999). Therefore, while 1% NaCl is surely not enough to produce the meat products with acceptable quality, it does serve as a baseline against which we were able to compare the impacts of the functional ingredients tested. Other ingredients such as MTGase and nonmeat proteins that can improve water holding capacity and other functional properties in the reduced NaCl, phosphate-free patty are required.

The addition of ISP significantly increased the cooking yield compared to the control patty (Figure 1). The cooking yield of the ISP patty was similar to that of the 1NaP patty, indicating that 1.7% ISP can compensate for the removal of phosphate in a low NaCl patty. Isolated soy protein interacts with meat proteins on the surface of meat particles to improve cooking yield and other textural properties (Tsao and others 2002). On the other hand, WPC patties had cooking yields similar to the control patties but lower than the ISP patty. Karmas and Turk (1976) reported that ISP showed superior water holding capacity and swelling properties to WPC in fish products.

The cooking yield of T patties was significantly lower than that of the control. Furthermore, differences were observed in the cooking yield between nonmeat protein-containing patties with and without MTGase. Use of MTGase reduced water holding capacity of patties. This result is consistent with that of O'Kennedy and others (2000), who reported that the use of MTGase in conjunction with sodium caseinate increased cooking loss (reduced water holding capacity) of restructured pig meat. Protein-protein interactions increased and protein-water interactions decreased as MTGase concentration increased, resulting in decreased water holding capacity (Ramírez and others 2002).

According to the suggested mechanism by which NaCl improves the water holding capacity in meat products (Hamm 1960; Offer and Knight 1983), chloride ions penetrate into the myofilaments and associate with positive charges on meat proteins, which increase the net negative charge on meat proteins. This leads to increased repulsion among meat proteins, which allows more space for water penetration into the network, and increases charged groups for water holding. Sodium ions form an ion "cloud" around the myofilaments, which increases osmotic pressure within myofibrils and space within the filament lattice. The suggested mechanism by which phosphate enhances the water holding capacity in meat is similar to that of chloride ions (Aberle and others 2001). Therefore, the space within the protein network in meat products is the most important factor for water holding capacity. Meat,

soy, and whey proteins are good substrates for MTGase (De Jong and Koppelman 2002). The high concentration of MTGase may cause excessive protein aggregation and a very tight protein network, which reduces the space within the network and results in more moisture loss during cooking. In contrast, Pietrasik and others (2007) showed that the addition of MTG decreased cooking loss in a pork gel. They reported that the meat protein content in their raw pork gel was adjusted to 8% by adding water and shredded ice. Protein content of the raw patty in the present study was 12.6% (Table 1). Ramírez and others (2002) suggested that water holding capacity decreased as MTGase concentration increased because protein-protein interactions increased and protein-water interactions decreased. Protein-protein interactions were expected to increase as protein content increased and to result in a tighter matrix in the product. Given the relationship between water holding capacity and matrix volume, it was likely that the higher protein content of the patty in the present study was responsible for the difference in cooking loss compared to that reported by Pietrasik and others (2007).

Binding strength and texture profile analysis of cooked patties with various additives are shown in Figure 2. The control patty showed the lowest binding strength, hardness, cohesiveness, chewiness, and springiness. The level of NaCl and the addition of phosphate did not affect any textural properties examined in this study. The moisture content in the control was significantly lower than that in patty 2NaP and 1NaP but its solid content was higher, leading to drier, firmer texture of the control. In frankfurters, decreased NaCl concentration in the formulation decreased hardness (Sofos 1983a). The texture properties of ISP patties also did not differ from those of the control patties. The addition of ISP did not affect textural profiles in low fat bologna and restructured beef products (Chen and Trout 1991; Chin and others 2000). The WPC patties had higher hardness and chewiness than the control patties. McCord and others (1998), in their study using the gel formed from the mixture of salt-soluble muscle proteins (SSP) with nonmeat proteins, reported that gel from the mixture of SSP with ISP showed lower gel rigidity than that from SSP with WPC. Addition of MTGase significantly increased all textural properties of patties. When combined with ISP or WPC, the effect of MTGase on hardness and chewiness was dominant. These results are consistent with other reports where MTGase is used to improve textural properties of low-salt meat products (Serrano and others 2004; Dimitrakopoulou and others 2005; Ramírez and others 2007a, 2007b). The binding strength among meat particles in the TISP patties was significantly higher than that in the T patties, indicating that the addition of ISP provided good protein substrates for MTGase, resulting in more protein-protein bonding between meat and soy proteins.

### Effect of the concentration of MTGase

The TISP patties had significantly higher hardness and chewiness but significantly lower cooking yield than ISP and 1NaP patties because of the high concentration of MTGase (0.7%). The concentration of MTGase should be adjusted to the level where both cooking yield and textural properties are acceptable.

As shown in the 1st experiment, the addition of ISP affected color parameters of raw and cooked patties, but not MTGase (Table 4). In raw patties, the addition of ISP increased  $b^*$ , chroma, and hue values but decreased whiteness compared to the control patty. Higher  $b^*$  and hue values indicated more yellowish surface of patties; however, the concentration of MTGase did not affect the color

parameters in raw patties. Cooking increased  $L^*$ ,  $b^*$ , chroma, and hue values, but reduced  $a^*$  value to nearly zero, indicating no redness observed in the cooked meat. As the concentration of MTGase in the cooked patties with ISP increased, the  $b^*$ , chroma, and hue values increased significantly, indicating that the cooked patties turned more yellowish.

Cooking yields of the ISP patty (85.15%) and the patties treated with all levels of MTGase and ISP [T0.05 (84.51%), T0.1 (83.49%), T0.2 (82.06%), T0.4 (80.56%), and T0.7 (73.77%)] were significantly greater than that of the control patties (71.10%), but were sig-

nificantly lower than that of 2NaP patties (88.46%) (Figure 3). As the level of MTGase increased, the cooking yield decreased. The higher concentration of MTGase may cause more protein aggregation and tighter protein network, which should have reduced the space within the network, leading to greater loss of moisture during cooking; however, the addition of 0.05% of MTGase with ISP did not decrease the cooking yield compared to ISP patties.

Binding strength, hardness, and chewiness were significantly higher in patties with MTGase compared to other treatments (Figure 4). Each of these textural attributes increased linearly with the increase of MTGase concentration over the range evaluated. These results are consistent with results reported for restructured fish meat (Cardoso and others 2007) and shrimp gel (Tammatinna and others 2007). Protein-protein bonding increased with increased MTGase concentration, which led to a stronger protein network and higher binding strength among patty meat particles. No differences between patties with 0.05% and 0.1% MTGase were observed for binding strength and other textural properties, although the patties with 0.05% MTGase had a significantly higher cooking yield than those with 0.1% MTGase.

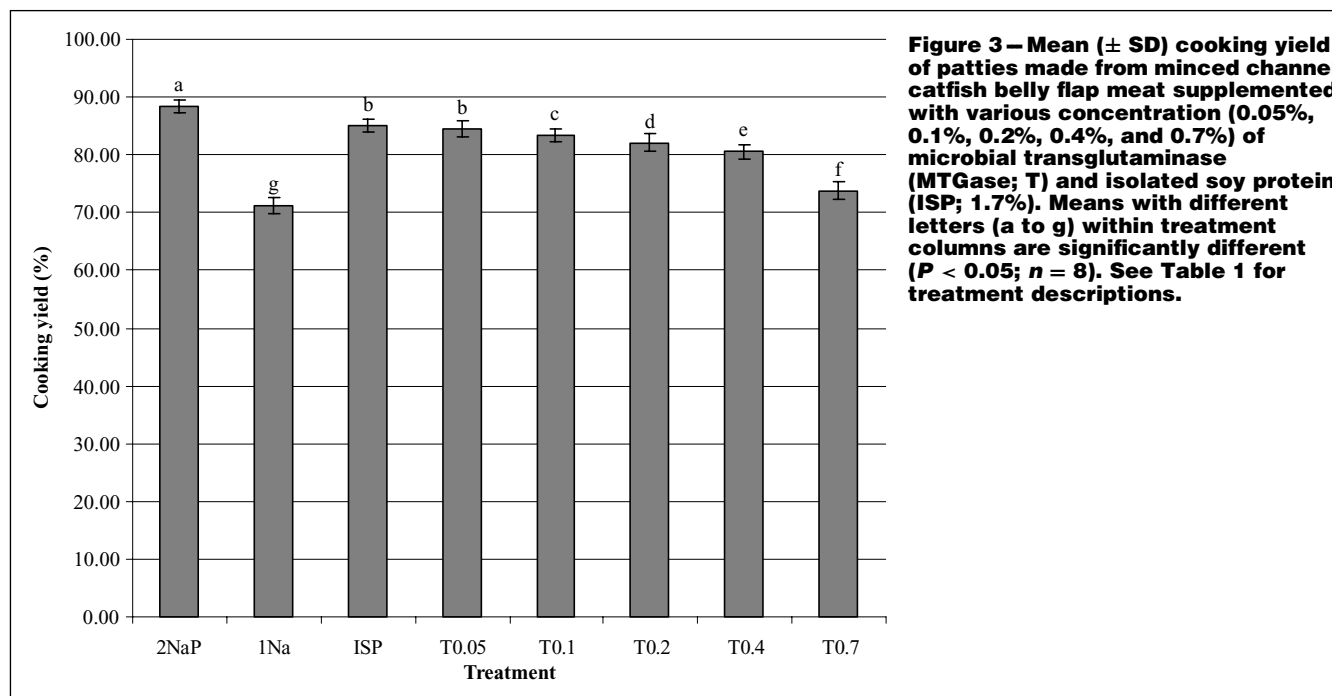
**Table 4 – Commission Internationale de l’Eclairage (CIE) color values, whiteness, chroma, and hue angle of patties made from minced channel catfish belly flap meat supplemented with various concentration (0.05%, 0.1%, 0.2%, 0.4%, and 0.7%) of microbial transglutaminase (MTGase; T) and isolated soy protein (ISP; 1.7%).<sup>A</sup>**

Treatment <sup>B</sup>	$L^*$	$a^*$	$b^*$	Whiteness	Chroma	Hue
Raw patty						
1Na	74.30	1.98	10.58 <sup>c</sup>	72.11 <sup>a</sup>	10.77 <sup>c</sup>	79.36 <sup>b</sup>
2NaP	74.63	2.03	11.59 <sup>b</sup>	72.01 <sup>a</sup>	11.77 <sup>b</sup>	80.01 <sup>b</sup>
ISP	74.06	1.80	13.42 <sup>a</sup>	70.72 <sup>c</sup>	13.55 <sup>a</sup>	82.41 <sup>a</sup>
T0.05	73.66	1.99	13.28 <sup>a</sup>	70.40 <sup>c</sup>	13.43 <sup>a</sup>	81.47 <sup>a</sup>
T0.1	73.65	2.01	13.52 <sup>a</sup>	70.28 <sup>c</sup>	13.68 <sup>a</sup>	81.59 <sup>a</sup>
T0.2	73.82	1.86	13.32 <sup>a</sup>	70.55 <sup>c</sup>	13.45 <sup>a</sup>	82.08 <sup>a</sup>
T0.4	74.21	1.87	13.37 <sup>a</sup>	70.87 <sup>c</sup>	13.50 <sup>a</sup>	82.07 <sup>a</sup>
T0.7	73.99	1.99	13.54 <sup>a</sup>	70.59 <sup>c</sup>	13.69 <sup>a</sup>	81.65 <sup>a</sup>
SEM	1.01	0.25	0.71	0.77	0.71	1.08
Cooked patty						
1Na	75.67 <sup>b</sup>	-0.45 <sup>d</sup>	14.83 <sup>d</sup>	71.48 <sup>b</sup>	14.84 <sup>d</sup>	88.19 <sup>b</sup>
2NaP	76.51 <sup>a</sup>	-0.62 <sup>e</sup>	14.66 <sup>d</sup>	72.29 <sup>a</sup>	14.67 <sup>d</sup>	87.51 <sup>c</sup>
ISP	75.87 <sup>ab</sup>	-0.47 <sup>d</sup>	15.27 <sup>c</sup>	71.41 <sup>b</sup>	15.28 <sup>c</sup>	88.08 <sup>b</sup>
T0.05	75.50 <sup>b</sup>	-0.16 <sup>c</sup>	15.98 <sup>b</sup>	70.73 <sup>c</sup>	15.98 <sup>b</sup>	89.21 <sup>a</sup>
T0.1	75.21 <sup>b</sup>	-0.07 <sup>bc</sup>	16.41 <sup>ab</sup>	70.25 <sup>c</sup>	16.41 <sup>ab</sup>	89.17 <sup>a</sup>
T0.2	75.13 <sup>b</sup>	-0.14 <sup>c</sup>	16.12 <sup>b</sup>	70.35 <sup>c</sup>	16.12 <sup>b</sup>	89.27 <sup>a</sup>
T0.4	74.95 <sup>b</sup>	0.00 <sup>ab</sup>	16.46 <sup>ab</sup>	70.00 <sup>c</sup>	16.46 <sup>ab</sup>	89.46 <sup>a</sup>
T0.7	74.89 <sup>b</sup>	-0.09 <sup>a</sup>	16.74 <sup>a</sup>	70.81 <sup>c</sup>	16.74 <sup>a</sup>	89.49 <sup>a</sup>
SEM	0.96	0.14	0.60	0.79	0.60	0.49

<sup>A</sup>Means with different letters (a to e) within the same column are significantly different ( $P < 0.05$ ). SEM = standard error of the means.  $n = 8$ .  
<sup>B</sup>See Table 1 for treatment descriptions.

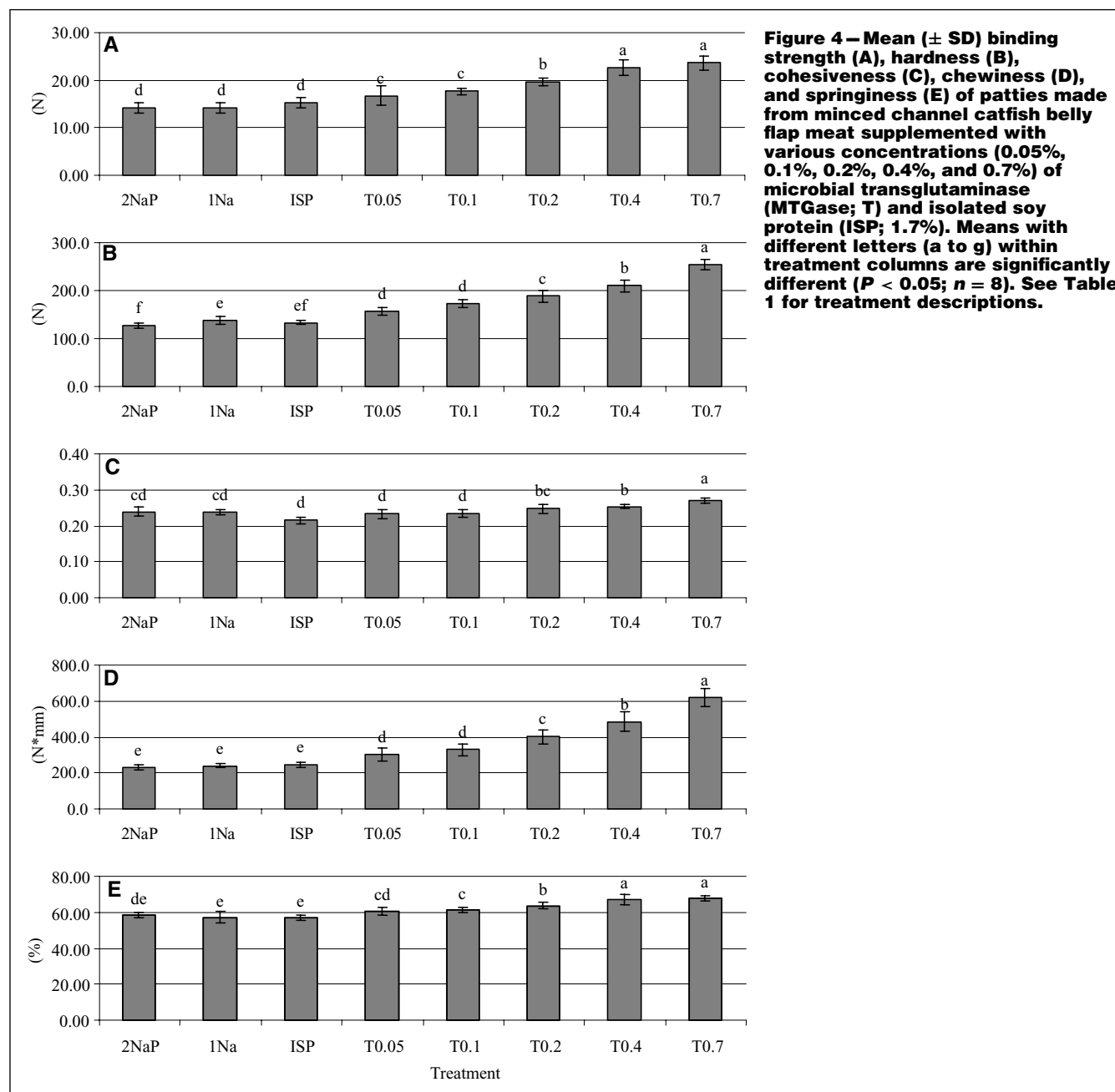
### Conclusions

MTGase improved the textural properties of patties without changing the color parameters, but lowered the cooking yield. ISP enhanced the cooking yield, but did not affect the textural properties of patties. The combination of ISP and MTGase improved the cooking yield and textural properties. The cooking yield and textural properties were not improved by addition of WPC. The concentration of MTGase was directly proportional to the textural properties, but inversely proportional to the cooking yield. The combination of MTGase (0.05% to 0.1%) with ISP (1.7%) was the best combination to improve both cooking yield and textural properties in low NaCl, phosphate-free patties made from minced channel catfish belly flap meat.



**Figure 3 – Mean (± SD) cooking yield of patties made from minced channel catfish belly flap meat supplemented with various concentration (0.05%, 0.1%, 0.2%, 0.4%, and 0.7%) of microbial transglutaminase (MTGase; T) and isolated soy protein (ISP; 1.7%). Means with different letters (a to g) within treatment columns are significantly different ( $P < 0.05$ ;  $n = 8$ ). See Table 1 for treatment descriptions.**





E: Food Engineering & Physical Properties

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