Microbiological and physicochemical characteristics of fresh meat and meat ball as affected by edible soy protein isolated films and coatings containing lactic acid-induced egg white powder

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Abstract—**This study investigates the microbiological and physicochemical characteristics of fresh meat and meat ball as affected by edible soy protein isolated (SPI) films and coatings containing 60mg, 120mg and 180mg concentrations of lactic acid-induced gelled egg white powder during refrigerated 1, 3, 5 and 7 day for 4°C frozen 1, 15, 30, 60 and 90 day for - 18°C storage time. The microbiological evaluation results showed that APC and *Coliform* of meat products were significantly inhibited by edible SPI films and coatings containing lactic acid-induced gelled egg white powder. The volatile basic nitrogen (VBN) of meat products treated with edible SPI films and coatings could retard the increase of VBN value during storage time. The thiobarbituric acid value (TBA) of meat products included treated and control groups were increased with increasing storage time, while the TBA value of fresh meat wrapped with SPI films containing with lactic acid-induced egg white powder were maintained below the 0.5mg MDA/kg during storage time. The metmyoglobin (MetMb) content, weight loss percentages and redness loss of fresh meat wrapped with edible SPI films containing lactic acid-induced egg white powder were increased with added amount of lactic acid-induced egg white powder, but the coated meat ball was not significantly changed (*p*>0.05) during storage time. This study revealed that the edible SPI films and coatings containing various amounts of lactic acid-induced gelled egg white powder could be used as bacteriostatic agent, and to maintain physicochemical characteristics of fresh meat and meat ball products during storage time.**

Keywords—**Soy protein isolate (SPI), Bacteriostatic ability, Lactic acid-induced gelled egg white powder**

1. INTRODUCTION

Active packaging refers to the incorporation of certain additives into packaging system with the aim of maintaining or extending product quality and shelf-life [1]. Cutter [2] mentioned that numerous researchers have used antimicrobial compounds such as organic acids (lactic, acetic acid), enzymes (lysozyme), proteins (SPI, conalbumin), bacteriocins (nisin), spice and plant extracts, EDTA could be added to edible films to reduce bacteria in solution, on culture media, or on a variety of meat products. Egg white could offer the potential use as protein matrix for package film. Our previous studies show that the lactic acid-induced gelled egg white powder has possessed bacteriostatic functions and emulsifying capacity [3], and the physicochemical properties of modified lysozyme from lactic acid-induced gelled egg white powder show stability under a wide variety of treatment conditions, such as pH, temperature, and concentrations of salt, and were effective against *B. cereus* (BCRC15324), *E. coli* K12 and *S. typhimurium* TA98 [4]. Chen *et al*. [5] and Wang *et al*. [6] have conducted on applying different concentrations of lactic acid-induced gelled egg white powder as a protein additive or a natural bacteriostatic agent in sausage and hamburger products, which result showed that lactic acid-induced gelled egg white powder provides good sensory quality and bacteriostatic ability in ground meat products. Lactic acid also is generally recognized as a safe (GRAS) substance in multiple purpose food substance catalogs. Cagri *et al*. [7] mentioned that lactic acid can be used as an acidulant in chitosan and collagen films and can be used to modify both the tensile strength and antimicrobial properties of collagen casings. Inherent bacteriostatic properties of lactic acid-induced gelled egg white powder could be the primary driving force in the development of new application for edible antimicrobial film. In this study, soy protein isolate was used as a edible film polymeric matrix to incorporate lactic acid-induced gelled egg white powder. Our objective was to develop edible antimicrobial films with lactic acid-induced gelled egg white powder and also to evaluate the quality characteristic of fresh meat and meat ball products.

I. MATERIALS AND METHODS

A. MATERIALS

1. Preparation of lactic acid-induced gelled egg white powder:

The lactic acid-induced gelled egg white powder was prepared by the method of Chen *et al*. [5]. The eggs were purchased from Chiayi University farm, and then separated into egg yolk and egg white after washing the eggs. The kept egg white was then homogenized by homogenizer (Type DS70S. DC. SERVO MQTOR, Taiwan) for 4-5min. Homogenized egg white was filled in cellulosic casing (caliber 3.5cm) and soaked into different concentration of lactic acid solution (6%, 8%, and 10%), then gelled by shaking (model 902, Hitech Instruments Corp., Taiwan) for 24hrs. The different concentration acid-induced gelled egg white gel were made into powder by vacuum freezing drying machine (FD-1 EYELA, Tokyo Rikakikai), and then stored at 0-4□.

2. Preparation of antimicrobial films and solutions:

The 6%, 8% and 10% of lactic acid-induced gelled egg white powder were used as the antimicrobial agent to prepare antimicrobial SPI films and solutions. The antimicrobial-incorporated SPI film was prepared according to the method described by Padgett *et al.* [8] with modifications. SPI solutions were prepared by dissolving 3.0 g of glycerol and 5.0 g of SPI sample in 95mL of sterile distilled water, which was heated on a hot plate magnetic stirrer to 85□for 30min, then kept at room temperature for 30min to allow bubbling to dissipate prior to pouring. The 60mg, 120mg and 180mg lactic acid-induced gelled egg white powder was mixed with the SPI solution, which was as antimicrobial solutions. Another, a portion of 3mL antimicrobial-SPI solutions was transferred into a Petri dish (diameter of 9 cm) to make the films and dried in the incubator 37□for 72hrs. SPI films without different concentrations of lactic acid-induced gelled egg white powder were also prepared and used for comparison. The pH value of each groups film solutions were measured by pH meter.

3. Prepared of fresh meat slices and meat ball products:

The experimental treatment involved eleven groups at fresh meat slices and meat ball products, respectively.

3.1 Preparation of antimicrobial incorporated SPI wrapped fresh meat slices: The chilled loin meat (approx. 2.5×2.5×1 cm) samples were wrapped with 6%, 8% and 10% antimicrobial films containing 60mg, 120mg and 180mg of lactic acid-induced gelled egg white powder. Each wrapped loin meat was weighed, placed individually in a sterile plastic bag (PLASTIBRAND®, Germany) and stored at 4□for further analysis during 1, 3, 5 and 7 day.

3.2 Preparation of antimicrobial incorporated SPI coated meat balls: The antimicrobial-incorporated SPI film was prepared according to the method described by Padgett *et al.* [8]with modifications. The meat batter was shaped into balls (approximately 20 g) cooked in a water bath at 85℃ for 30 min to an internal temperature 71℃. The meat balls were submerged into the antimicrobial SPI solution for 20s with gentle rolling by chopsticks to ensure thorough covering of the antimicrobial-SPI solution. Then, the meat balls were individually stuck on a platform consisting of bamboo toothpicks and a thick foamed polystyrene board for about 30min to allow dried. Each coated meat ball was weighed, placed individually in a sterile plastic bag and stored at -15℃~-18℃ for further analysis during 1, 15, 30, 60 and 90 day.

B. METHODS

1. The quality properties of fresh meat slices and meat ball product

1.1 VBN: Volatile basic nitrogen was determined by the Conway micropipette diffusion method and was expressed as mg VBN/100 g of sample.

1.2 TBA: by Salih *et al*. [9] with modifications.

1.3 MetMb: The percent MetMb was determined using the procedure of Chu *et al*. [10] and Trout [11], and then calculated using the formula by Krzywicki [12]. $\text{MetMb}(\%)=1.395-[(A572-A700)/(A525-A700)]\times 100$.

1.4 Microbiological analysis: Microbiological analysis was determined using the procedure of FDA (Bacteriological Analytical Manual for Foods, BAM 1996). The plate count agar (Merck), Coliform Agar (Merck) and SS Agar (Merck) were used for enumeration of aerobic plate count (APC), *Coliforms* and *Salmonella*, respectively.

2. Statistical analysis

Whole experiments were replicated three times with three observations per each replication. Statistical analysis was performed with the SAS program for Windows V9.1 (SAS Institute, Cary, NC, USA). ANOVA with Tukey's multiple range tests was carried out to analyze the significant differences among the treatments ($P < 0.05$).

C. DISUSSION AND RESULTS

The MetMb content (Fig. 1), weight loss percentages and redness loss (Table 1) of fresh meat wrapped with edible SPI films containing lactic acid-induced egg white powder were increased with added amount of lactic acid-induced egg white powder, but the coated meat ball was not significantly changed (p>0.05) during storage time (data not shown).The TBA value of meat products included treated and control groups were increased with increasing storage time, while the TBA value of fresh meat wrapped with SPI films containing with lactic acid-induced egg white powder were maintained below the 0.5mg MDA/kg during storage time (Fig. 2). The VBN of meat products treated with edible SPI films and coatings could retard the increase of VBN value during storage time (Fig. 3). The microbiological evaluation results showed that APC and *Coliform* of meat products were significantly inhibited by edible SPI films and coatings containing lactic acid-induced gelled egg white powder (Fig. 4 and Fig. 5). However, the *Salmonella* was not detected both fresh meat slices and meat ball products in this experiment (date not show).

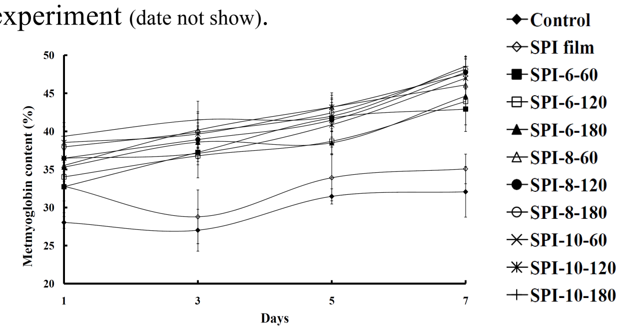

Fig. 1 Change in MetMb content of fresh meat slices wrapped with soy protein isolate films containing different concentrations of lactic acid-induced gelled egg white powder during refrigerated storage (0-4).

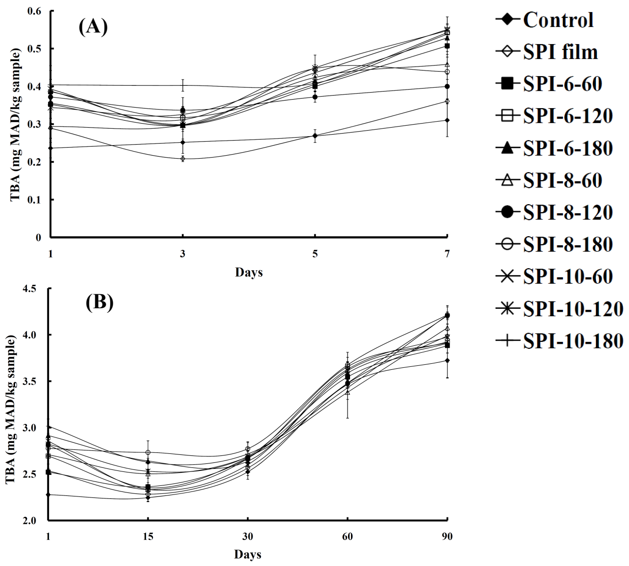

Fig. 2 Change in TBA values of fresh meat slices wrapped (A) and meat ball coated (B) with soy protein isolate films containing different concentrations of lactic acid-induced gelled egg white powder during refrigerated (0-4) and frozen (-18) storage.

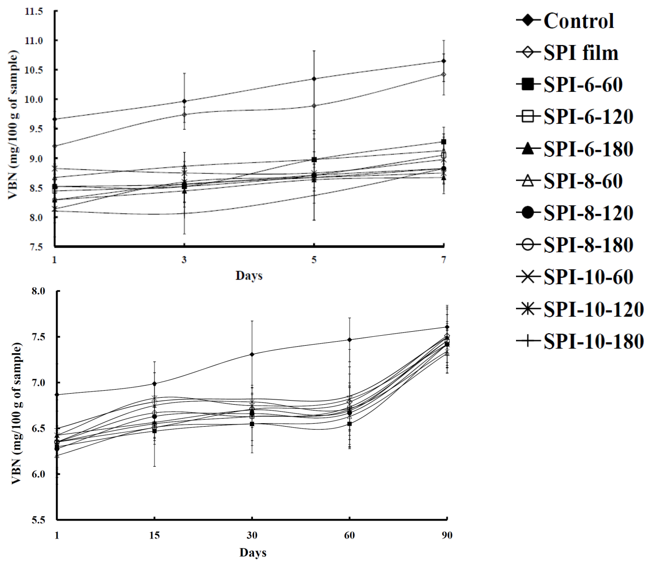

Fig. 3 Change in VBN values of fresh meat slices wrapped (A) and meat ball coated (B) with soy protein isolate films containing different concentrations of lactic acid-induced gelled egg white powder during refrigerated (0-4) and frozen (-18) storage.

 Control; Soy protein isolate film; SPI film incorporated 60 mg of 6% lactic acid-induced gelled egg white powder; SPI film incorporated 120 mg of 6% lactic acid-induced gelled egg white powder; ▲ SPI film incorporated 180 mg of 6% lactic acid-induced gelled egg white powder; SPI film incorporated 60 mg of 8% lactic acid-induced gelled egg white powder; SPI film incorporated 120 mg of 8% lactic acid-induced gelled egg white powder; SPI film incorporated 180 mg of 8% lactic acid-induced gelled egg white powder; × SPI film incorporated 60 mg of 10% lactic acid-induced gelled egg white powder; SPI film incorporated 120 mg of 10% lactic acid-induced gelled egg white powder; SPI film incorporated 180 mg of 10% lactic acid-induced gelled egg white powder

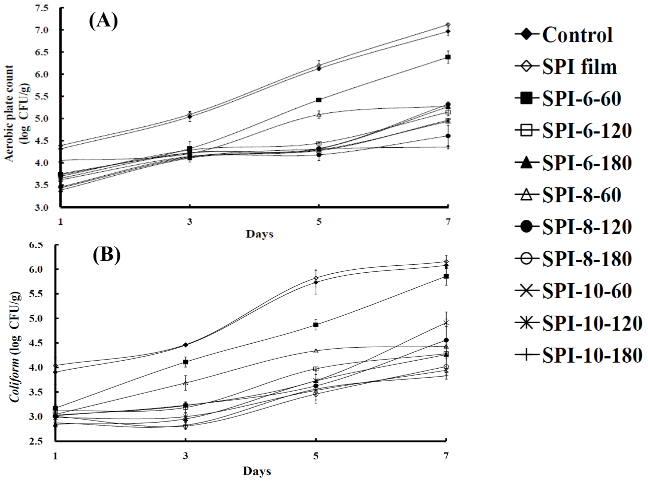

Fig. 4 Inhibition of APC (A) and *Coliform* (B) on fresh meat slices with SPI films containing different concentrations of lactic acid-induced gelled egg white powder during refrigerated storage (0-4).

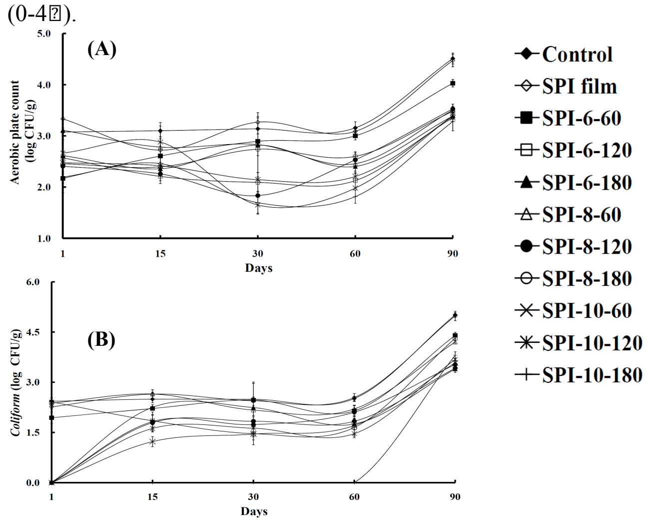

Fig. 5 Inhibition of APC (A) and *Coliform* (B) on meat ball products with SPI films containing different concentrations of lactic acid-induced gelled egg white powder during frozen storage (-18).

Table 1 Change in a value and weight loss percentages of fresh meat slices wrapped with soy protein isolate films containing different concentrations of lactic acid-induced egg white powder during refrigerated storage

Treatment[1]	Concentration (mg/mL)	Refrigerated storage time (day)			
		1	3	5	7
a value					
Control	0	$6.98\pm0.05^{ab,wx}$	$8.38\pm0.81^{a,w}$	$7.13\pm0.05^{ab,xy}$	$5.81\pm0.36^{b,x}$
SPI	0	$9.00\pm0.28^{a,v}$	$7.33\pm0.72^{ab,wxy}$	$6.70\pm0.22^{ab,xy}$	$5.97\pm0.34^{b,x}$
6%	60	$6.39\pm0.65^{a,wx}$	$7.72\pm0.09^{ab,wx}$	$7.96\pm0.15^{b,x}$	$4.40\pm1.23^{b,xyz}$
	120	$4.74\pm0.30^{a,z}$	$5.62\pm0.21^{a,xy}$	$5.55\pm0.24^{a,yz}$	$4.42\pm0.33^{a,xyz}$
	180	$5.10\pm0.99^{b,yz}$	$7.73\pm0.01^{a,wx}$	$5.77\pm1.23^{ab,xyz}$	$4.43\pm1.01^{b,xyz}$
8%	60	$6.26\pm0.01^{ab,x}$	$8.19\pm0.19^{a,yz}$	$5.55\pm1.23^{ab,yz}$	$2.90\pm0.53^{b,yz}$
	120	$7.33\pm0.08^{a,w}$	$6.98\pm0.11^{ab,wxy}$	$6.08\pm1.54^{ab,xyz}$	$4.99\pm1.17^{b,xy}$
	180	$4.75\pm0.17^{a,z}$	$6.42\pm1.41^{a,xyz}$	$5.88\pm1.60^{a,xyz}$	$5.44\pm1.61^{a,x}$
10%	60	$6.07\pm0.02^{ab,xy}$	$7.03\pm0.20^{a,wxyz}$	$6.28\pm1.11^{ab,xyz}$	$4.34\pm0.36^{b,xyz}$
	120	$7.27\pm0.04^{a,w}$	$6.41\pm1.54^{a,xyz}$	$5.23\pm0.60^{ab,yz}$	$4.85\pm0.60^{b,xyz}$
	180	$6.05\pm0.38^{a,xy}$	$5.32\pm0.75^{a,z}$	$4.23\pm0.02^{ab,z}$	$2.79\pm0.88^{b,z}$
Weight loss (%)					
Control	0	$0.23\pm0.05^{b,z}$	$1.07\pm0.05^{ab,z}$	$1.85\pm0.46^{ab,z}$	$2.13\pm0.42^{a,z}$
SPI	0	$0.93\pm0.06^{b,xy}$	$1.30\pm0.18^{b,yz}$	$2.48\pm0.04^{ab,yz}$	$3.02\pm0.05^{a,yz}$
6%	60	$0.75\pm0.13^{b,y}$	$1.37\pm0.01^{b,yz}$	$2.78\pm0.96^{ab,xyz}$	$4.24\pm0.05^{a,xy}$
	120	$0.75\pm0.26^{b,y}$	$2.03\pm0.24^{b,wx}$	$4.46\pm0.58^{a,wxy}$	$5.46\pm0.65^{a,wx}$
	180	$0.77\pm0.05^{b,y}$	$2.05\pm0.05^{b,wx}$	$5.29\pm0.14^{a,w}$	$6.06\pm0.14^{a,wx}$
8%	60	$0.77\pm0.08^{b,y}$	$1.86\pm0.21^{b,xy}$	$4.47\pm0.51^{a,wx}$	$4.98\pm0.57^{a,wx}$
	120	$0.82\pm0.03^{b,xy}$	$2.08\pm0.29^{b,wx}$	$5.06\pm1.18^{a,w}$	$5.79\pm1.61^{a,wx}$
	180	$1.09\pm0.02^{b,x}$	$2.16\pm0.27^{b,wx}$	$4.65\pm0.60^{a,wx}$	$6.14\pm0.28^{a,wx}$
10%	60	$0.94\pm0.04^{b,xy}$	$2.13\pm0.09^{b,wx}$	$5.23\pm0.14^{a,w}$	$6.02\pm0.27^{a,wx}$
	120	$0.78\pm0.10^{c,y}$	$2.46\pm0.37^{b,wx}$	$5.74\pm1.09^{a,w}$	$6.27\pm0.87^{a,w}$
	180	$1.04\pm0.09^{b,xy}$	$2.56\pm0.17^{b,w}$	$5.81\pm0.53^{a,w}$	$6.70\pm0.62^{a,w}$

Means ± SD within the row having unlike letters (a-c) are significantly different ($p < 0.05$).
Means ± SD within the column for the same test having unlike letters (w-z) are significantly different ($p < 0.05$).
[1] Treatments: 6% lactic acid induced egg white powder; 8% lactic acid induced egg white powder; 10% lactic acid induced egg white powder. SPI: Soy protein isolate film.

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