# PHYSICOCHEMICAL AND GELLING PROPERTIES OF CHICKEN PROTEIN ISOLATE AS INFLUENCED BY FREEZE-THAW CYCLE

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**Abstract – Freeze-thaw cycles affected the physicochemical and gelling properties of chicken protein isolate. When the number of freeze-thaw cycles increased, the moisture content decreased with the increase in pH ($p<0.05$). Discoloration of chicken protein isolate was observed with increasing freeze-thaw cycles as indicated by the increases in a* and b* values as well as the decreases in L* value and whiteness ($p<0.05$). The freeze-thaw cycles played an essential role in the oxidation acceleration as shown by a sharp increase in thiobarbituric acid-reactive substances (TBARS) with increasing freeze-thaw cycles. Additionally, repeated freeze-thawing strongly influenced the gelling properties of chicken protein isolate. Gel hardening and discoloration occurred with increasing freeze-thaw cycles as shown by the increase in breaking force and the reduction of whiteness.**

**Key Words – protein isolate, chicken meat, freeze-thaw, quality**

## I. INTRODUCTION

Acid- and alkaline-aided solubilization or pH shift method is a technology that efficiently recovers functional and nutritious protein isolates from muscle foods [1]. The extraction mechanism of the two processes is to solubilize the muscle proteins at low and high pH to separate soluble proteins, bone, skin, connective tissue, cellular membranes and neutral storage lipids through the centrifugation. The solubilized proteins are collected and recovered by isoelectric precipitation to give a highly functional and stable protein isolate [2]. Recently, the pH shift processing has been introduced to recover functional proteins from poultry meats and their by-products [3] Panpipat and Chaijan [3] reported that acid-aided process recovered more protein with less total heme pigments resulting in a greater breaking force and whiteness of the isolate gel compared to alkaline counterpart.

Frozen storage is an important preservation method for muscle food product. It can effectively retard quality changes of meat and meat products. After shipping and distribution of frozen products, thawing is a common means to be implemented prior to the display of the products. The repeated freeze-thawing is therefore a practice in retail shop, restaurant or home [4]. This freeze-thaw process can impair the quality of meat products. Thus, the objective of this work was to evaluate the effect of freeze-thaw cycle on physicochemical and gelling properties of acid-made chicken protein isolate.

## II. MATERIALS AND METHODS

### Chicken meat sample

Breast and thigh meat of broiler chicken were obtained from local market in Thasala, Nakhon Si Thammarat. The broiler meat were kept in ice, using the meat/ice ratio of 1:2 (w/w), and transported to the laboratory within 30 min. Upon arrival, the broiler meat were fabricated where the skin, bone and visible connective tissues were removed. In this study, the breast (white meat) and thigh (dark meat) meat were mixed at a ratio of 1:1 representing the whole broiler meat. Thereafter, the meat was manually cut and subsequently minced to uniformity using a meat grinder (a 4 mm hole diameter; Panasonic MK-G20MR, Japan). The muscles were kept on ice during preparation. Minced broiler meat was vacuum packed and kept at -18°C until used. The initial pH value of minced broiler meat was 6.65±0.03.

Protein recovery from broiler meat by acid aided process

Recovery of protein from broiler meat by acid pH shift method was performed according to the method of Marmon and Undeland [2]. Minced broiler meat was thawed under running cold water. The mince, typically 100 g, was mixed with 9 times ice-cold distilled water and homogenized for 2×30 s using an IKA Labortechnik homogenizer (Selangor, Malaysia). The pH of the homogenate was adjusted to 2.5 using 2 M HCl during constant manual stirring until the pH was stable. The pH was monitored with a calibrated pH meter (Cyberscan 500, Singapore). The pH-adjusted homogenates were centrifuged at 8,000×*g* in a RC-5B plus centrifuge (Sorvall, Norwalk, CT, U.S.A.) at 4°C for 20 min. The solubilized proteins in the supernatant were collected and separated from the pellet and the floating fat layer by filtering through three layers of cotton sheet. Thereafter, the pH of supernatant was adjusted to 5.5 by using 2 M NaOH. A second centrifugation was performed, and the pellet, referred to as chicken protein isolate, was collected.

Freeze-thawing of chicken protein isolate

Chicken protein isolates were packed in polyethylene bags (250 g per bag), heat-sealed and frozen at -20°C using an air-blast freezer for 24 h. These frozen chicken protein isolate were thawed using running tap water (27-28°C) for 1 h. The core temperature of the sample after thawing was approximately 0-2°C. Thawed samples were again frozen for 24 h. Freeze-thawing was repeated up to 3 cycles. Chicken protein isolate without freeze-thawing was used as the control. All samples obtained were subjected to measurement of moisture content, pH, color and TBARS. To prepare the gel, chicken protein isolate was mixed with 2.5% (w/w) of dry NaCl and chopped for 5 min to obtain the homogeneous sol. The sol was then stuffed into polyvinylidine casing with a diameter of 2.5 cm and both ends of the casing were sealed tightly. After thermal gelation (setting at 40°C for 30 min prior to heating at 90°C for 20 min) [3], gel properties including breaking force, deformation, whiteness and expressible drip were determined.

Determination of moisture content and pH

Moisture content of chicken protein isolate was determined according to the methods of AOAC [5]. The pH was determined using a slurry method in which 5 g of the sample was homogenized in 20 ml of distilled water using a homogenizer at 13,600 rpm for 30 s and the pH of homogenate was measured using a pH meter calibrated at pH 4.0 and 7.0 equipped with a pH electrode [6].

Color measurement

Colorimetric values of the protein isolate and its gel were obtained by using a portable Hunterlab Miniscan/EX instrument (10° standard observers, illuminant D65, Hunter Assoc. Laboratory; VA, U.S.A.). The tristimulus L* (lightness), a* (redness/greenness), and b* (yellowness/blueness) measurement mode was used as it relates to the human eye response to color. The whiteness was calculated as described by Chaijan *et al*. [6] as follows:

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

Determination of TBARS

TBARS assay was performed as described by Buege and Aust [7]. Ground protein isolate (0.5 g) was homogenized with 2.5 ml of a solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 N HCl, using an IKA Labortechnik homogenizer. The mixture was heated in a boiling water bath (95-100°C) for 10 min to develop a pink color, cooled with running tap water and centrifuged at 3,600×*g* at 25°C for 20 min. The absorbance of the supernatant was measured at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane at concentrations ranging from 0 to 10 ppm. TBARS was calculated and expressed as mg malonaldehyde (MDA) equivalent/kg sample.

Determination of breaking force and deformation

Texture analysis of the gels was performed using a TA-XT2 texture analyzer (Stable Micro Systems,

Godalming, Surrey, U.K.) equipped with a spherical plunger (diameter 5 mm; depression speed 60 mm.min$^{-1}$) as described by Chaijan *et al*. [6]. Breaking force (gel strength) and deformation (elasticity/deformability) were recorded.

Expressible drip

Expressible drip of gel was measured according to the method of Ng [8]. A sample with a thickness of 0.5 cm was weighed and placed between two pieces of Whatman filter paper No. 1 at the top and three pieces of the same type of filter paper at the bottom. The standard weight (5 kg) was placed on the top of the sample and maintained for 2 min. The sample was then removed and weighed again. Expressible drip was calculated and expressed as percentage of sample weight.

Statistical analysis

A completely randomized design (n = 3) was used in this study. All parameters studied were determined in triplicate. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test. Statistical analysis was performed using the Statistical Package for Social Science (SPSS 8.0 for windows, SPSS Inc., Chicago, IL).

## III. RESULTS AND DISCUSSION

### Effect of multiple freeze-thaw cycles on physicochemical changes of chicken protein isolate

Changes in some physicochemical properties of chicken protein isolate as influenced by freeze-thaw cycles are shown in Table 1. Moisture content decreased with increasing freeze-thaw cycles ($p<0.05$) suggesting the increase in drip loss. The pH tended to increase with increasing freeze-thaw cycle. The increase in pH was probably related to the leakage of electrolytes or organic acids from the protein isolate as drip [9]. Freeze-thaw cycle strongly influenced the color of chicken protein isolate. Chicken protein isolate underwent yellow discoloration with increasing freeze-thaw cycles as indicated by a sharp increase in b* with a gradual increase in a* value as well as a marked decrease in L* value ($p<0.05$). Such changes led to the significant decrease in whiteness of the protein isolate ($p<0.05$). Additionally, lipid oxidation as measured by TBARS increased with increasing freeze-thaw cycles ($p<0.05$). Release of non-heme iron due to the degradation of muscle proteins during freeze-thaw process can be a major factor causing the lipid oxidation of the protein isolate. It has been reported that the freeze-thawing caused some loss of integrity in the muscle system. The ice crystals formed could injure the cell and cause the release of pro-oxidants for lipid oxidation, especially free iron [9]. Furthermore, the decrease in whiteness was correlated well with the increase in TBARS ($R^2$ =0.9344). It has been reported that aldehyde lipid oxidation products can form Maillard reaction with amines in muscle leading to the formation of yellow-brown pigments and hence discoloration of the meat product [10].

Table 1. Changes in moisture, pH, color and TBARS of chicken protein isolate subjected to multiple freeze-thaw cycles

| Physicochemical properties | Freeze-thaw cycle | | |
|---|---|---|---|
| | 0 | 1 | 3 |
| Moisture (%) | 88.19±0.43c[#] | 80.34±0.29b | 77.41±0.51a |
| pH | 4.88±0.03a | 4.92±0.05ab | 4.97±0.02b |
| Color | | | |
| L* | 79.31±0.01c | 72.33±0.07b | 67.92±0.46a |
| a* | -0.44±0.03a | -0.13±0.14b | -0.03±0.08b |
| b* | 12.65±0.02a | 15.72±0.04b | 17.96±0.10c |
| Whiteness | 75.74±0.02c | 68.18±0.08b | 63.24±0.45a |
| TBARS (mg MDA equivalent/kg sample) | 3.49±0.37a | 30.48±0.93b | 35.42±1.84c |

Values are given as means±SD from triplicate determinations.
[#]Different letters in the same row indicate significant differences ($p<0.05$).

### Gelling properties of chicken protein isolate subjected to multiple freeze-thaw cycles

Effect of multiple freeze-thaw cycles on gel-forming ability of chicken protein isolate is presented in Table 2. Generally, breaking force of

gel increased significantly with increasing freeze-thaw cycle ($p<0.05$) suggesting the toughening of gel. This was probably due to the the loss of moisture upon repeated freeze-thawing (Table 1) which can cause the imbalance between protein-protein and protein-water interactions. Protein-protein interaction would more predominant over the protein-water interaction with increasing freeze-thaw cycles and thus the protein aggregates can be formed to a higher extent. However, deformation was not affected by freeze-thaw cycle ($p>0.05$). The whiteness of the gel decreased with increasing freeze-thaw cycle ($p<0.05$) suggesting the discoloration of gel. Expressible drip of gel decreased with increasing freeze-thaw cycle ($p<0.05$). This was probably due to the fact that the residual moisture of the protein isolate with repeated freeze-thawing was lower than control (Table 1).

Table 2. Changes in gelling properties of chicken protein isolate subjected to multiple freeze-thaw cycles

| Gelling properties | Freeze-thaw cycle | | |
|---|---|---|---|
| | 0 | 1 | 3 |
| Breaking force (g) | 1,708±87a[#] | 1,922±37b | 2,224±115c |
| Deformation (mm) | 7.50±0.16a | 7.45±0.11a | 7.52±0.29a |
| Whiteness | 75.40±0.08c | 73.57±0.12b | 72.61±0.40a |
| Expressible drip (%) | 79.31±0.01c | 72.33±0.07b | 67.92±0.46a |

Values are given as means±SD from triplicate determinations.
[#]Different letters in the same row indicate significant differences ($p<0.05$).

## IV. CONCLUSION

Freeze-thaw cycles directly affected the physicochemical and gelling properties of chicken protein isolate. The freeze-thaw process caused the destabilization of the muscle structure, leading to acceleration of lipid oxidation and discoloration of chicken protein isolate. Also, repeated freeze-thawing resulted in a decrease in the gel-forming ability of chicken protein isolate.

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