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Viscoelastic behavior and physico-chemical characteristics of heated *swai*-fish (*Pangasius hypophthalmus*) based emulsion containing fermented soybeans

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ABSTRACT

Various proportions of fermented soybeans i.e. *thua nao* and rice-*koji miso* were added into the *swai*-fish emulsion to improve the health potential of the products. Accordingly, the physico-chemical, microbiological properties and sensory attributes of the fish-based emulsions as well as the main ingredients were determined. The results showed that fatty acid profile of the *swai*-fish fillet consisted mainly of saturated and monounsaturated fatty acids, while isoflavone profiles of the fermented soybeans illustrated that *thua nao* contained more aglycones than rice-*koji miso*. For physical properties, the profiles of storage and loss moduli, creep parameters and gel strength depicted that samples added *thua nao* gave rise to stronger gel structure than those added rice-*koji miso* or the whole fish emulsion. Microbiological examination indicated that the fish emulsion with *thua nao* addition showed high total plate or spore counts.

1. Introduction

Swai-fish (*Pangasius hypophthalmus*) is one of the most popular oily fresh-waterfish sold in Thailand. It has white and tender flesh, low cholesterol (21–39 mg100 g^{-1}), less fishy odor and good flavor when cooked (Na-Nakorn & Moeikum, 2009; Orban et al., 2008). Because of its low price compared to other fish, *swai*-fish is frequently available on the local markets for being supplied to the health conscious consumers. To develop health comminuted-fish emulsion, high quality products of fermented soy protein such as *thua nao* (*natto*) or *miso* were incorporated in this study. *Thua nao* (*natto*) is well-known for its highly essential amino acids and phenolic compounds especially isoflavone in the forms of aglycones e.g. daidzein, glycitein and genistein, or glucosides e.g. daidzin, glycitin and genistin, which are largely produced during

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fermentation (Dajanta, Chukeatirote, Apichartsrangkoon, & Frazier, 2009). It is believed that these phytoestrogens could improve health protective effects such as reducing the risk of cardiovascular disease, lower breast and colon cancers and promoting bone health (Murphy, Barua, & Hauck, 2002). Therefore, including these phytochemicals in food would definitely add value to the products. Despite this alkaline fermented soybean provides high nutrients, it is unlikely that common consumers would accept its strong unique odor. *Miso* giving an umami flavor is frequently fermented by *Aspergillus oryzae*, *Aspergillus sojae* or, *Aspergillus awamori* for the preparation of rice-*koji* (Giri, Osako, Okamoto, Okazaki, & Ohshima, 2011). *Miso* is another fermented soy protein which contains several hydrolyzed amino acids and isoflavones. Yamabe, Kobayashi-Hattori, Kaneko, Endo, and Takita (2007) fermented rice-*koji* miso by *A. oryzae* for 6 months and found that the glycoside contents decreased from 86.4 to 44.9%, whereas aglycones increased from 9.6 to 53.3%.

Rheology is extensively used to characterize the physical structure of food biopolymers which can form either viscoelastic true or weak gels (coagulant type gel). Various gels have weak viscoelastic characteristic. For instance, gluten heated at 90 °C for 30 min upto 6 h were characterized as weak viscoelastic gel, since their storage modulus (G′) essentially dominated the loss modulus (G″) and both modulus-profiles were slightly frequency dependent (Apichartsrangkoon, 2002). Another example of weak viscoelastic characterization was the pressurized gels of ostrich-meat sausages which G′ was larger than G″ with small tanδ values (0.23). The difference between G′ and G″ plots was essentially 1 log cycle, depicting that these gels exhibited strong elasticity with solid-like behavior (Chattong & Apichartsrangkoon, 2009). Apart from the oscillatory measurement, other rheological techniques such as creep and relaxation tests were also frequently used to characterize the structure of gels. Chattong, Apichartsrangkoon, and Bell (2007) compared the measuring procedures between oscillatory and creep tests by using pressurized ostrich-meat gel containing xanthan gum as the specimens and found that creep testing better demonstrated the textural changes than oscillatory testing. However, this wasn't with the case of pressurized ostrich-meat sausages without gum additive, since the oscillatory results were well coincident with the creep parameters (Chattong & Apichartsrangkoon, 2009).

The aim of this study was to develop *swai*-fish emulsion with the addition of *thua nao* and rice-*koji miso* to improve the health-promoting effect. Accordingly, their rheological, physical, chemical and microbiological qualities were assessed.

2. Materials and methods

2.1. Experimental materials

Swai-fish (*P. hypophthalmus*) fillet was purchased from a local market in Chiang Mai province, Thailand. *Thua nao* was produced following a modified method of Dajanta, Apichartsrangkoon, Chukeatirote, and Frazier (2011) using soybean cultivar CM60 and *Bacillus subtilis* TISTR 001 (Thailand Institute of Science and Technological Research, Thailand) as a starter culture. Rice-*koji miso* (Ken Co. Ltd., Japan) was purchased from a local market in Chiang Mai, Thailand.

2.2. Preparation of swai-fish based emulsions containing fermented soybeans

The *swai*-fish fillet was chopped with *thua nao* and rice-*koji miso* with various proportion according to the formula in Table 1. The mixture of each formula was then blended for 6 min with 10 g kg^{-1} sodium chloride and 3 g kg^{-1} sodium tripolyphosphate by a household blender. The final temperature of the batter was maintained at around 10 °C. The batters were then stuffed in collagen casing 2.3 cm in diameter (Food EQ Ltd., Thailand) and heated at 72 °C for 30 min in a water bath (Techarang & Apichartsrangkoon, 2015).

Table 1
Formulation of *swai*-fish based batters containing fermented soybeans.

Major raw materials	Control	Treatments				
		1	2	3	4	5
Swai-fish fillet (g kg^{-1} batter)	1000	900	900	900	900	900
Thua nao (g kg^{-1} batter)	–	100	–	70	50	30
Rice-*koji miso* (g kg^{-1} batter)	–	–	100	30	50	70

2.3. Proximate analysis

The *swai*-fish based emulsion and the ingredients such as *swai*-fish fillet, *thua nao* and rice-*koji miso* were analyzed for crude protein, crude fat and moisture content according to the standard AOAC methods (AOAC, 2000). The pH was also measured using a pH meter (Sartorius PB-20, Germany). The analysis was done in triplication (n = 6).

2.4. Color measurement

A colorimeter (Minolta Chroma Meter, CR-300, Japan) was used to measure the color parameters (L, a^* and b^*) of *swai*-fish fillet, *thua nao*, rice-*koji miso* and the finished products. The parameters in term of L, a^* and b^* were used to calculate the browning index (BI) following Equation (1) (Ferrari, Maresca, & Ciccarone, 2010).

$$BI = [100(x - 0.31)]/0.172 \quad (1)$$

where $x = (a^* + 1.75L)/(5.645L + a^* - 3.012b^*)$.
The analysis was done in triplication (n = 9).

2.5. Determination of isoflavones in fermented soybean

Thua nao or rice-*koji miso* was extracted separately following a modified method of Murphy et al. (2002). One gram of each freeze-dried samples included 110 μL flavones (Sigma-Aldtich Co. Ltd., UK) as an internal standard were extracted with 5 mL acetonitrile, 10 mL of 0.1 mol L^{-1}HCl and 5 mL distilled water. The supernatant was centrifuged at 1677 × g for 5 min, then filtered through 0.45 μm membrane filter for HPLC analysis.

HPLC analysis was performed following a modified method of Klejdus et al. (2005). The Shimadzu HPLC systems were equipped with Inersil ODS-3 C18 column (4.6 × 250 mm, 5.0 μm) and SPD-M20A photodiode array detector. The mobile phase consisted of 0.1 mL 100 mL^{-1} acetic acid in filtered MilliQ water (solvent A) and 80 mL 100 mL^{-1} methanol (solvent B). The injection volume was 20 μL with gradient elution. Flow rate of the mobile phase was 1 mL min^{-1} and temperature of the column was 40 °C. The eluted isoflavones were detected at λ_{max} 255 nm. Concentration of each component was achieved from the corresponding calibration curves after adjustment with the internal standard. Typical chromatogram of the detected isoflavones are shown in Fig. 1a and b. The analysis was done in triplication (n = 6).

2.6. Determination of fatty acid in swai-fish fillet

Swai-fish fillet was the major proportion (900 g kg^{-1}) of the formulas, hence only fatty acids of this ingredient were determined. The fatty acid profile of *swai*-fish fillet was determined following a

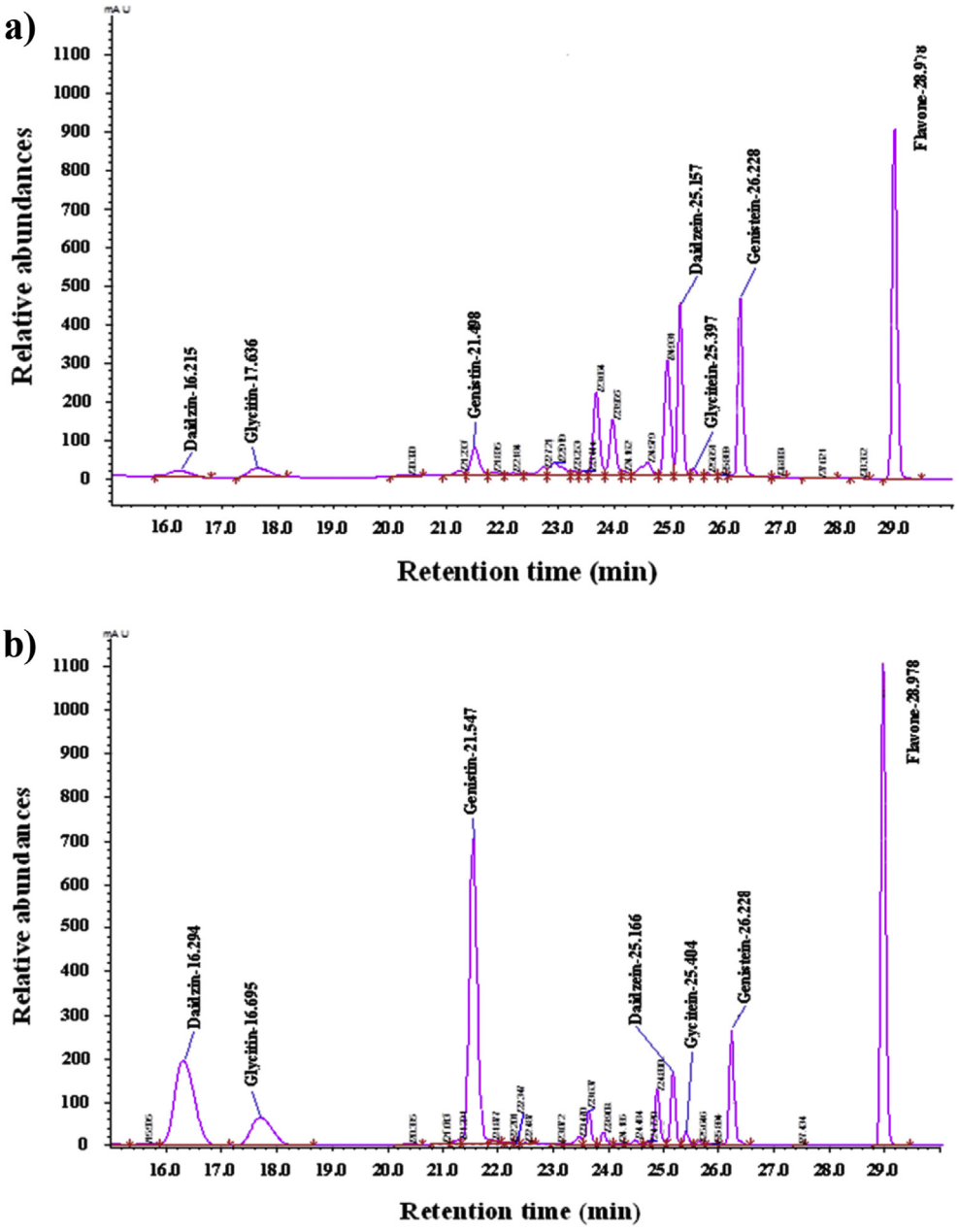

Fig. 1. Chromatograms of isoflavones found in *thua nao* (a) and rice-*koji miso* (b).

modified method of AOAC 996.06 (2005) as follows.

The fat was extracted by adding 100 mg pygallic acid, 10.0 mL of 8.3 mol L^{-1} HCl and 2 mL ethanol into 2 g of ground *swai*-fish fillet. After hydrolysis, the fat portion was separated, and mixed with 3 mL chloroform and 3 mL diethylether. Subsequently, 2 mL of 7 g boron trifluoride in 100 g methanol and 1 mL toluene were added into the remained fat, then heated at 100 °C for 45 min. A volume of 5 mL distilled water, 1 mL hexane, and 1 g sodium sulfate were added into the mixture. Top layer of fatty acid methyl esters (FAMEs) was filtered through a membrane filter for GC experiment. Gas chromatographic system using an Agilent Technologies 6890NGC (Santa Clara, CA, USA) equipped with a FID detector and a fused-silica capillary column with 100 m × 0.25 mm diameter, 0.2 μm film thickness (SupelcoSP-2560). Helium with a flow rate of 0.75 mL min^{-1} was used as a carrier gas. One μL injection of the FAME mixture was set for a split ratio of 200:1. The temperatures of the injector and the detector were 250 and 285 °C, respectively. The program of oven temperature was commencing at 140 °C, held for 5 min, then set the temperature program for 3 °C min^{-1} to 250 °C and held for 17 min. The total running time was 55 min. Concentration of each component was achieved from the calibration curves of corresponding standard fatty acids. Typical chromatogram of total fatty acid is shown in Fig. 2. The analysis was done in triplication (n = 6).

2.7. Determination of gel strength

Gel strength of the fish emulsions was determined using a Texture Analyser TA-XT Plus (Stable Micro Systems Ltd., Surrey, UK) with a 50 kg load cell. The samples were cut into pieces of 23 mm diameter × 20 mm height. During measurement, a Warner Blatzler blade was cut through a piece of sample with sharing cross-head

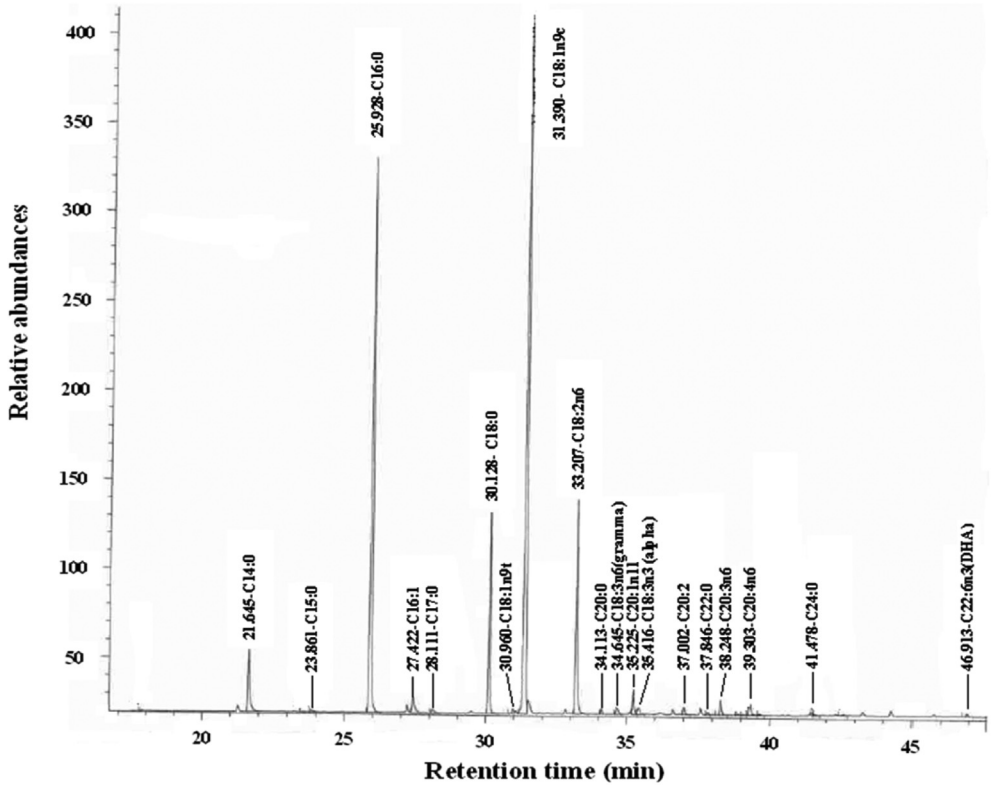

Fig. 2. Chromatogram of fatty acids of *swai*-fish fillet.

speed of 10 mm/s Peaks of the shear force were recorded and the gel strength was then calculated by multiplying shear force (N) with the distance of shearing (m) (Techarang & Apichartsrangkoon, 2015).

The measurement was performed in triplication (n = 15).

2.8. Determination of water holding capacity

Percentage of water holding capacity which is the ability of emulsion to hold the entire water in the system can be assessed from the released water plus expressible water subtracted from 1 (×100) (Techarang & Apichartsrangkoon, 2015). Released water is the weight of sample left after blotting water from the surface, while expressible water is the weight of the sample left after the water in the sample being discharged under compression (Funami, Yada, & Nakao, 1998). The measurement was performed in triplication (n = 9).

2.9. Dynamic viscoelastic characterization

The viscoelastic behavior of the fish based emulsions was determined following a procedure of Techarang and Apichartsrangkoon (2015). A controlled stress rheometer (AR2000, TA Instruments-Waters LLC, New Castle, USA) equipped with a parallel plate of 25 mm diameter and a gap width of 1 mm was used. This is the most appropriate gap for avoiding slipperiness. Initially stress-amplitude sweeps were performed at a frequency of 1 Hz to search for a linear viscoelastic region (Fig. 3). Accordingly, a stress amplitude of 20 Pa was chosen for further frequency-sweep and creep tests. Oscillatory frequency-sweeps were carried out at 25 °C scanned across a frequency range of 0.1–10 Hz. Subsequently, storage (G′), loss (G″) moduli and loss tangent (tan δ) were recorded. Creep measurement was performed

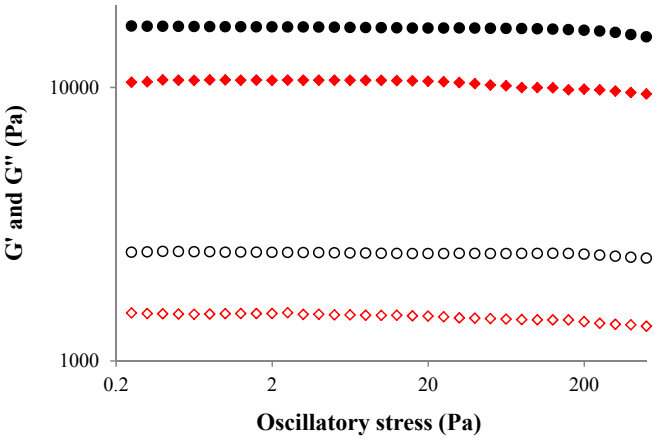

Fig. 3. Stress amplitude sweep (0.1–500 Pa) at a frequency of 1 Hz for heated *swai*-fish emulsion (◆, ◇ control) and heated *swai*-fish emulsion containing 10% *thua nao* (●, ○ treatment 1) G′ = filled symbols and G″ = unfilled symbols.

for 300 s and the recovery testing was instantaneously undertaken for 900 s. The mathematical models of the creep curves were calculated following Equation (2). A four-element "Burgers" model (Steffe, 1996) was then fitted as shown in Fig. 4.

$$J(t) = J_0 + J_1[1 - \exp(-t/\lambda_{ret})] + t/\mu_0 \quad (2)$$

where J_0 (the instantaneous elastic compliance, Pa^{-1}).
J_1 (retarded compliance for Kelvin–Voigt model, Pa^{-1}).
λ_{ret} (retardation time for Kelvin–Voigt model, s).
μ_0 (Newtonian viscosity, Pa s) and t (time, s).
The measurement was performed in triplication (n = 9).

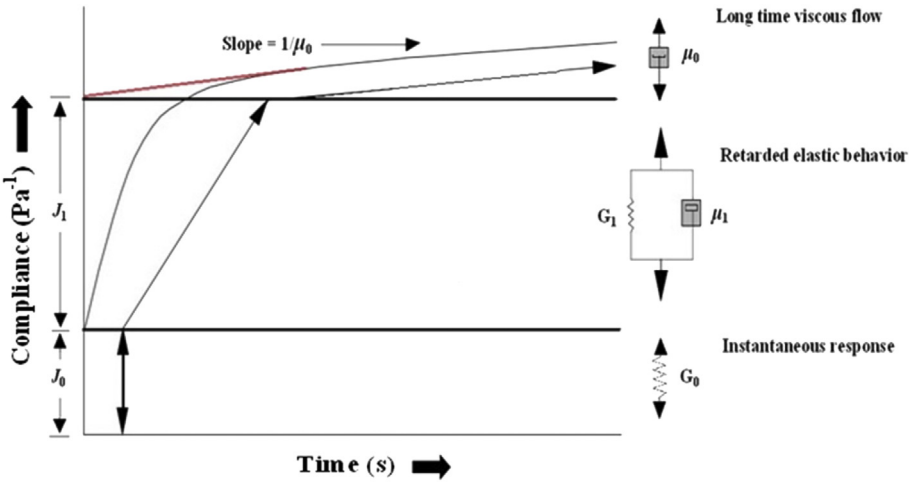

Fig. 4. Standard creep curve of the compliance versus deformation time with an indication of the four-element Burger's model.

2.10. Microbiological assessment

A standard plate count of *swai*-fish fillet, *thua nao*, rice-*koji miso* and the fish-based emulsions was carried out following the AOAC method (AOAC, 2000). Purposely, 1 g of the sample was added into 9 mL of 0.1 g 100 mL^{-1} peptone water for the preparation of series of dilution. A volume of 1 mL dilutions was then transferred to the plate count agar, and incubated at 37 °C for 2 days. The spore count was applied the same procedure as standard plate count, excepting that series of peptone dilution were heated at 80 °C for 10 min prior to transferring to the plate count agar (Siemer, Toepfl, & Heinz, 2014). Viable cell and spore quantification were expressed as colony-forming units per g (CFU/g). The determination was performed in triplication (n = 6).

2.11. Sensory evaluation

For sample presentation, 5 formulas of fish-based emulsions and the control with size of 23 mm diameter × 1 cm height were served to the panelists by completely randomized order (Macfie, Bratchell, Greenhoff, & Vallis, 1989). Fifty untrained panelists evaluated for color, flavor, odor, firmness, air cells and overall acceptability and provided judgments according to 5-point hedonic scale (where, 1 = extremely dislike; 5 = extremely like). The assessment was performed in duplication.

2.12. Statistical analysis

The treatments and the control in this study were carried out with triplication. Analysis of variance (ANOVA) was done by using SPSS Version 22 (SPSS Inc., Chicago, USA), and determination of significant differences among treatment means was applied Duncan's multiple range tests ($P \leq 0.05$).

3. Results and discussion

3.1. Physical, chemical and microbiological qualities of main ingredients

3.1.1. Color parameters

The main ingredients used in this study comprised *swai*-fish fillet, *thua nao* which was fermented by *B. subtilis* TISTR 001 and rice-*koji miso*. Their physical and chemical qualities are shown in Table 2. Color parameters in Table 2 illustrated that *swai*-fish fillet and rice-*koji miso* had the equivalent lightness, while *thua nao* displayed darker color reflected by the *L* parameter, which might be due to significantly highest intensity ($P \leq 0.05$) of redness and yellowish of *thua nao*. In overall, *swai*-fish fillet was the lightest ingredient followed by rice-*koji miso* and *thua nao*, respectively depicted by the browning index. A study by Dajanta, Chukeatirote, and Apichartsrangkoon (2012) determined color of different Thai commercial *thua nao* supported that the products had lightness (*L*) in the range of 38.63–47.18, while a^* and b^* were 6.91–9.10 and 14.79–23.12, respectively.

3.1.2. Chemical properties

Thua nao is an alkaline fermented soybean having high pH in turn, whereas rice-*koji miso* has lower pH. However, both ingredients contain various isoflavones which are phytoestrogens in the forms of glucosides and aglycones. Table 2 and Fig. 1a and b showed the detected isoflavones in the fermented soybeans consisting of daidzin, glycitin, genistin, daidzein, glycitein and genistein. It is interesting that *thua nao* contained much more aglycones than glucosides, whereas rice-*koji miso* had more glucosides than aglycones. In *thua nao*, the aglycones: daidzein, genistein and glycitein were the major components, while daidzin, genistin and glycitin were the predominant glucosides in rice-*koji miso*. This result was also coincident with the study of Xu, Du, and Xu (2015) revealed that the isoflavones in *natto* were very much higher than those in rice-*koji miso*. Obviously, the aglycone form provides better health potential than the glucoside form. Since the glucoside form conjugating with glucose moiety has greater molecular weight and higher hydrophilicity than the free aglycone form. These characteristics of glucoside triggered off poor absorption into small intestine (Izumi et al., 2000; Kano, Takayanagi, Harada, Sawada, & Ishikawa, 2006). However, this bioactivity of glucoside could be improved by an enzyme such as β-glucosidase transforming the glucoside to aglycone (Malashree, Mudgil, Dagar, Kumar, & Puniya, 2012; Uzzan & Abuza, 2004).

Despite, *swai*-fish fillet had lower protein and fat contents than *thua nao* and rice-*koji miso*, the characteristic of fat (97 g kg^{-1}) in *swai*-fish fillet would reflect the overall product quality. Because *swai*-fish fillet is the main ingredient (900 g kg^{-1}) in the formula, other fermented soybeans (100 g kg^{-1}) were the trivial contribution. Table 3 and Fig. 2 show that the principle fatty acids in the fat of *swai*-fish fillet comprised of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs). The majorities of such fatty acids were palmitic acid (C16:0), strearic acid (C18:0) and cis-9-

Table 2
Physico-chemical and microbiological properties of *swai*-fish fillet, *thua nao* and rice-*koji miso*.

Properties	Major raw materials		
	Swai-fish fillet	*Thua nao*	Rice *koji-miso*
Color parameters			
L	53.16 ± 1.10^{a}	42.91 ± 2.39^{b}	52.92 ± 2.19^{a}
a^*	0.73 ± 0.07^{c}	5.69 ± 0.45^{a}	4.24 ± 0.35^{b}
b^*	2.95 ± 0.53^{b}	14.45 ± 0.99^{a}	14.66 ± 0.74^{a}
Browning index	6.49 ± 0.85^{c}	49.55 ± 1.21^{a}	37.33 ± 0.95^{b}
Chemical compositions			
Moisture (g 100 g^{-1})	75.50 ± 1.02^{a}	46.63 ± 2.26^{b}	45.21 ± 1.65^{b}
Protein (g 100 g^{-1})	15.88 ± 1.23^{c}	39.75 ± 2.27^{a}	27.04 ± 1.66^{b}
Fat (g 100 g^{-1})	9.69 ± 0.85^{c}	16.68 ± 0.42^{a}	11.07 ± 0.32^{b}
pH	7.38 ± 0.04^{b}	8.43 ± 0.22^{a}	6.74 ± 0.15^{c}
Isoflavones			
Total-glucosides (μg g^{-1})	–	214.72 ± 19.98^{b}	1778.96 ± 82.40^{a}
Daidzin (μg g^{-1})	–	51.78 ± 8.67^{bC}	703.59 ± 27.98^{aB}
Glycitin (μg g^{-1})	–	96.00 ± 8.24^{bA}	120.95 ± 13.77^{aC}
Genistin (μg g^{-1})	–	66.94 ± 3.07^{bB}	954.42 ± 40.65^{aA}
Total-aglycones (μg g^{-1})	–	729.33 ± 53.37^{a}	348.40 ± 16.92^{b}
Diadzein (μg g^{-1})		295.08 ± 19.70^{aA}	152.52 ± 8.24^{bA}
Glycitein (μg g^{-1})	–	178.20 ± 16.65^{aC}	43.67 ± 2.57^{bB}
Genistein (μg g^{-1})	–	256.05 ± 17.02^{aB}	152.21 ± 6.11^{bA}
Microbiological qualities			
Standard plate count (CFU g^{-1})	$2.14 \pm 0.19 \times 10^{3c}$	$5.08 \pm 0.95 \times 10^{8a}$	$5.19 \pm 0.59 \times 10^{3b}$
Spore count (CFU g^{-1})	$<10^{b}$	$4.22 \pm 0.83 \times 10^{8a}$	$<10^{b}$

Means followed the same small or capital letters in each row or in each column are not significantly different ($P > 0.05$).

Table 3
Fatty acid profile of *swai*-fish fillet

Type of fatty acids	Contents (g kg^{-1})
Myristic acid (C14:0)	3.49 ± 0.014
Pentadecanoic acid (C15:0)	0.21 ± 0.023
Palmitic acid (C16:0)	28.31 ± 0.041
Heptadecanoic acid (C17:0)	0.28 ± 0.010
Stearic acid (C18:0)	8.80 ± 0.014
Arachidic acid (C20:0)	0.32 ± 0.025
Behenic acid (C22:0)	0.09 ± 0.014
Lignoceric acid (C24:0)	0.12 ± 0.013
Total saturated fatty acids	**41.62 ± 0.054**
Palmitoleic acid (C16:1n7)	1.20 ± 0.112
trans-9-Elaidic acid (C18:1n9t)	0.23 ± 0.013
cis-9-Oleic acid (C18:1n9c)	39.56 ± 0.140
cis-11-Eicosenoic acid (C20:1n11)	1.24 ± 0.010
Total monounsaturated fatty acids	**42.23 ± 0.133**
cis-9,12-Linoleic acid (C18:2n6)	10.43 ± 0.131
γ-Linolenic acid (C18:3n6)	0.47 ± 0.011
α-Linolenic acid (C18:3n3)	0.46 ± 0.014
cis-11,14-Eicosadienoic acid (C20:2)	0.40 ± 0.103
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.80 ± 0.015
Arachidonic acid (C20:4n6)	0.76 ± 0.102
4,7,10,13,16,19-Docosahexaenoic acid (DHA) (C22:6n3)	0.22 ± 0.013
Total polyunsaturated fatty acids	**13.54 ± 0.113**
trans fats	**0.22 ± 0.013**
Omega-3	**0.95 ± 0.024**
Omega-6	**12.53 ± 0.102**
Omega-9	**39.81 ± 0.133**

The bold numbers are the high quantities.

oleic acid (C18:1n9c). On the other hands, cis-9,12-linoleic acid (C18:2n6) was the major polyunsaturated fatty acid (PUFA) present with the lesser extent. Fortunately, only little trans fats were found in *swai*-fish fillet, while omega-9 (η-9) fatty acids existed with high concentration, followed by omega-6 (η-6) and omega-3(η-3) fatty acids, respectively. Therefore, 97 g fat kg^{-1} flesh in *swai*-fish fillet could provide some extent of health potential to such fish fillet, besides the beneficial fish protein. Other studies, for instance, Maqsood and Benjakul (2010) reported that total 92 g fat kg^{-1} flesh of Thai striped catfish (*P. hypophthalmus*) contained 42.60 g MUFAs 100 g^{-1} fat, followed by 33.87 g SFAs 100 g^{-1} fat and 21.95 g PUFAs 100 g^{-1} fat. Earlier, Orban et al. (2008) found 44.77 g SFAs 100 g^{-1} fat in frozen Vietnamese *sutchi* catfish (*P. hypophthalmus*) fillets, followed by 34.68 g MUFAs 100 g^{-1} fat and 15.55 g PUFAs 100 g^{-1} fat with the ratio of η-3/η-6 being 0.40.

3.1.3. Microbiological qualities

The microbiological qualities in Table 2 depicted that *thua nao* exhibited highest standard plate and spore counts. Since *thua nao* contained vegetative cells and spores of *B. subtilis* TISTR 001 which were fairly tolerant to heat and could be present in the standard plate and spore counts. On the other hand, the standard plate count of *swai*-fish fillet was roughly 3 log CFU g^{-1} which was considered as an acceptable benchmark (ICMSF, 1986). The standard plate count for rice-*koji miso* also exhibited around 3 log CFU g^{-1} which could be the remained lactic-acid bacteria from pasteurization as mentioned earlier.

3.2. Physical, chemical, microbiological properties and sensory scores of heated swai-fish based emulsions containing thua nao and rice-koji miso

3.2.1. Color parameters

Table 4 shows the physical properties of *swai*-fish based emulsion with various treatment conditions as shown in Table 1. Usually, heated fish emulsions had white opaque color, when incorporating fermented soybeans, color of the treated samples would change to light yellowish. Accordingly, *L* parameter for lightness indicated that the control was significantly lightest ($P \leq 0.05$), followed sequentially by treatments 1, 3, 4, 2 and 5. For a^* parameter, treatments 1 and 3 had the positive a^*, while other treatments and the control showed negative a^*. Because of treatments 1 and 3 containing higher amount of *thua nao*, slight red color from this ingredient would trigger off a^* parameter to appear in positive direction. In overall, the control had significantly lower b* parameter (yellowish) and browning index ($P \leq 0.05$) than other treatments.

Table 4
Physico-chemical, microbiological properties and sensory attributes of heated *swai*-fish emulsions containing *thua nao* and rice-*koji miso*.

Characteristics	Control	Treatments				
		1	2	3	4	5
Physical properties						
Color parameters						
L	86.20 ± 1.09^{a}	80.37 ± 0.61^{b}	78.49 ± 0.54^{c}	79.96 ± 0.61^{b}	79.93 ± 0.37^{b}	77.90 ± 1.93^{bc}
a^*	−2.14 ± 0.13^{d}	0.34 ± 0.13^{a}	−0.38 ± 0.04^{c}	0.31 ± 0.14^{a}	−0.19 ± 0.04^{b}	−0.32 ± 0.05^{c}
b^*	5.84 ± 0.52^{b}	20.14 ± 0.67^{a}	20.81 ± 0.43^{a}	20.23 ± 0.58^{a}	20.37 ± 0.51^{a}	20.83 ± 0.64^{a}
Browning index	4.95 ± 0.67^{b}	28.16 ± 1.14^{a}	29.33 ± 0.61^{a}	28.43 ± 0.75^{a}	28.20 ± 0.82^{a}	29.68 ± 0.47^{a}
Water holding capacity (g 100 g^{-1})	93.06 ± 0.47^{a}	91.83 ± 0.34^{b}	92.81 ± 0.25^{a}	92.42 ± 0.54^{ab}	92.55 ± 0.41^{ab}	92.74 ± 0.47^{a}
Gel strength (N m)	0.57 ± 0.05^{b}	0.79 ± 0.04^{a}	0.64 ± 0.04^{b}	0.75 ± 0.07^{ab}	0.69 ± 0.07^{ab}	0.65 ± 0.03^{b}
Chemical properties						
Moisture content (g 100 g^{-1})	86.05 ± 0.50^{a}	83.04 ± 0.65^{c}	84.70 ± 0.49^{b}	83.38 ± 0.39^{c}	83.61 ± 0.27^{c}	84.52 ± 0.34^{b}
pH	7.30 ± 0.04^{d}	7.80 ± 0.05^{a}	7.02 ± 0.02^{e}	7.70 ± 0.02^{b}	7.49 ± 0.02^{c}	7.23 ± 0.03^{d}
Rheological parameters						
Loss tangent (G″/G′) at 1 Hz	0.13 ± 0.01^{b}	0.19 ± 0.02^{a}	0.14 ± 0.02^{b}	0.19 ± 0.02^{a}	0.18 ± 0.01^{a}	0.17 ± 0.03^{ab}
$J_0 \times 10^{-4}$ (Pa^{-1})	2.70 ± 0.22^{a}	2.18 ± 0.15^{b}	2.58 ± 0.11^{a}	2.32 ± 0.10^{b}	2.40 ± 0.12^{ab}	2.44 ± 0.15^{ab}
$J_1 \times 10^{-4}$ (Pa^{-1})	5.98 ± 0.26^{a}	1.45 ± 0.10^{e}	4.56 ± 0.18^{b}	2.19 ± 0.17^{d}	3.03 ± 0.28^{c}	4.18 ± 0.33^{b}
λ_{ret} (s)	50.58 ± 2.48^{a}	33.24 ± 1.53^{e}	45.36 ± 2.06^{b}	38.04 ± 1.60^{d}	41.53 ± 1.76^{c}	43.60 ± 2.97^{bc}
$\eta_0 \times 10^{5}$ (Pa s)	4.75 ± 0.19^{e}	16.65 ± 1.32^{a}	5.54 ± 0.36^{d}	13.83 ± 1.02^{b}	8.28 ± 0.29^{c}	5.60 ± 0.33^{d}
Microbiological qualities						
Total plate count (CFU g^{-1})	<10^{f}	1.58 ± 0.23 × 10^{4a}	4.39 ± 1.02 × 10^{2e}	7.23 ± 0.65 × 10^{3b}	4.88 ± 0.37 × 10^{3c}	2.08 ± 0.23 × 10^{3d}
Spore count (CFU g^{-1})	<10^{e}	1.21 ± 0.10 × 10^{4a}	<10^{e}	6.67 ± 0.72 × 10^{3b}	4.01 ± 0.59 × 10^{3c}	1.78 ± 0.28 × 10^{3d}
Sensory attributes						
Color	2.21 ± 0.20^{d}	3.19 ± 0.16^{c}	3.58 ± 0.13^{b}	**3.86 ± 0.15**^{a}	3.54 ± 0.17^{b}	**3.70 ± 0.15**^{ab}
Flavor	2.69 ± 0.18^{c}	2.97 ± 0.19^{bc}	3.16 ± 0.16^{b}	**3.56 ± 0.17**^{a}	**3.52 ± 0.19**^{a}	3.21 ± 0.20^{ab}
Odor	2.59 ± 0.19^{b}	**2.88 ± 0.22**^{ab}	**3.13 ± 0.17**^{a}	**3.21 ± 0.16**^{a}	**3.17 ± 0.20**^{a}	**3.10 ± 0.20**^{a}
Firmness	**3.44 ± 0.18**^{a}	3.08 ± 0.16^{b}	3.10 ± 0.16^{b}	**3.35 ± 0.14**^{ab}	**3.21 ± 0.19**^{ab}	2.84 ± 0.16^{b}
Air cells	3.42 ± 0.19^{ab}	3.32 ± 0.18^{b}	3.30 ± 0.19^{b}	**3.74 ± 0.16**^{a}	**3.73 ± 0.17**^{a}	3.24 ± 0.21^{b}
Overall acceptability	2.81 ± 0.20^{c}	3.05 ± 0.15^{bc}	3.30 ± 0.16^{b}	**3.64 ± 0.13**^{a}	**3.42 ± 0.16**^{ab}	3.17 ± 0.16^{b}

Means followed the same letter in each row are not significantly different ($P > 0.05$). The bold numbers in sensory attributes were the most acceptable scores.

3.2.2. Water holding capacity, gel strength, moisture content and pH

Water holding capacity of the emulsions was insignificantly different ($P > 0.05$) among treatments and the control (Table 4) which could be the effect of adding salt and phosphate in the emulsified formula masking this characteristic. Therefore, the water holding capacities were not treatment sensitive in the presence of these additives. Usually, salt enhances more soluble myofibrillar proteins which then migrate to the fat globule surface, concentrate and form protein matrix at the fat/water interface (Youssef & Barbut, 2010). Apart from salt, mechanical emulsification of protein and fat in the batter could affect water holding capacity of the product. When the protein is improperly emulsified from mechanical overheating during blending process and the fat separates to the product surface, these could lead to lowering emulsifying property of the batter, consequently, lowering water holding capacity of the product (Liu, Callahan,& Solomon, 2009).

Gel strength of the treatments was significantly higher ($P \leq 0.05$) than that of the control, presumably, due to lower moisture content of the treated samples than the control. In addition, Table 4 shows that treatments 1 and 3 which contained higher amount of *thua nao* (an alkaline fermented soybean) than other treatments had significantly highest pH and gel strength ($P \leq 0.05$). Since the slimy mucilage in *thua nao* which was composed of levan (β-2,6-fructan) and polyglutamic acid had binding potential, resulting firmer product structure was achieved (Donot, Fontana, Baccou, & Schorr-Galindo, 2012; Zhang et al., 2014).

3.2.3. Rheological behavior

3.2.3.1. Geometrically oscillatory measurement. Dynamic oscillatory measurement is a popular physical characterization of bio polymeric gels. In this context, the profiles of storage (G′) and loss (G″) moduli of the control and the fish-based emulsions were illustrated in Fig. 5. The plots of G′ and G″ as a function of frequency for treatment 1 were depicted for the strongest sample, while those of the controls were the weakest, due to the former displaying highest G′ and G″ and vice versa for the weak samples. It is noteworthy that these two viscoelastic geometries increased with the increase of *thua nao* addition which could be associated with the reduction of moisture contents and the effect of the slimy mucilage in *thua nao* as mentioned earlier. Moreover, the gel strength (Table 4) also agreed with the viscoelastic behaviors. In overall, G′ was bigger than G″ across the frequency range with small loss tangent (G″/G′ = 0.13–0.19) as shown in Table 4 and the difference between G′ and G″ of each plot was around one log cycle. This was a mirror of weak viscoelastic or coagulant gel types with some crosslink density (Chattong & Apichartsrangkoon, 2009). Moreover, these results were also complied with the study of Apichartsrangkoon (2002) demonstrating that G′ and G″ plots of the heated gluten and soy mixed-gels were little frequency dependence and G′ was bigger than G″. This reflected of solid-like characteristic with high crosslink density.

3.2.3.2. Creep and recovery testing. The creep curves for the control and the treated emulsions were best fitted by the four-element Burgers model which comprised Kelvin-Voigt model connected in series to a spring and a dashpot elements as shown in Fig. 4. Other finding of the viscoelastic behavior of O/W model-system meat emulsions and chicken meat frankfurters was also characterized using the Burgers model (Dzadzl, Markowski, Sadowski, Jakóbczak, & Janulin, 2015; Yilmaz, Karaman, Dogan, Yetim, & Kayacier, 2012).

Fig. 6 illustrated the creep and recovery curves of the fish based emulsions and the control. It is apparent that the creep curve of the treatment 1 laid on the lowest position or having lowest instantaneous elastic compliance (J_0), while the control curve laid on the highest position suggesting that treatment 1 was the strongest gel and vice versa for the control. In addition, the creep parameters in Table 4 such as J_0, J_1 and λ_{ret} increased with the reduction of gel rigidity, accordingly, treatment 1 had the highest gel rigidity,

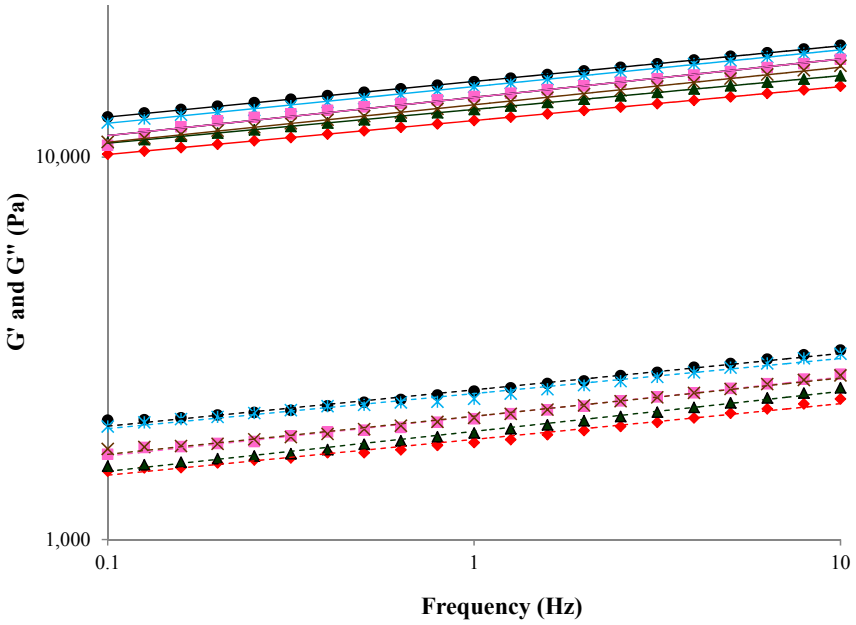

Fig. 5. Plots of storage (G′) and loss (G″) moduli versus frequency (0.1–10 Hz) of the heated control (*swai*-fish emulsion) and the heated *swai*-fish emulsions containing *thua nao* or rice-*koji miso* (G′ = straight lines and G″ = dotted lines; ◆ = control, ● = treatment 1, ▲ = treatment 2, ✳ = treatment 3, ■ = treatment 4 and × = treatment 5).

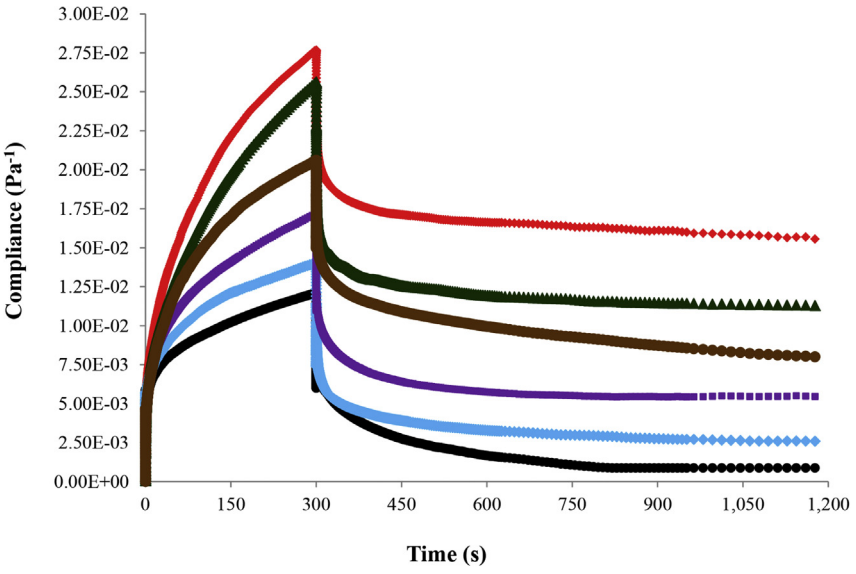

Fig. 6. Creep-recovery curves of the heated control (*swai*-fish emulsion) and the heated *swai*-fish emulsions containing *thua nao* or rice-*koji miso* (◆ = control, ● = treatment 1, ▲ = treatment 2, ◆ = treatment 3, ■ = treatment 4 and ● = treatment 5).

sequentially followed by treatment 3, 4, 5, 2 and the control, respectively. These results were also well complied with the oscillatory profiles (Fig. 5) and the gel strength (Table 4). The viscosity or μ_0 referred to the final steady-state of a Newtonian flow has a reversed meaning from J_0, J_1 and λ_{ret} (Chattong & Apichartsrangkoon, 2009; Chattong et al., 2007).

3.2.4. Microbiological qualities

Table 4 illustrated that every treatment exhibited total plate count in the range of 2–4 log CFU g^{-1} and spore count in the range of 3–4 log CFU g^{-1} except treatment 2 (spore count) and the control without adding *thua nao*. It is obvious that the spore of *B. subtilis* TISTR 001 was fairly resistant to heat treatment (72 °C/30 min), thus some of this bacteria and spore could survive after heating and were present in the total plate and spore counts. Since the total plate and spore counts of the control were less than 1 log CFU g^{-1}, this might imply that general microbes in the corresponding treatments were also satisfactorily inhibited. Other investigations, for instance, Scheldeman, Herman, Foster, and Hendrickx (2006) noted that *Bacillus* species such as *Bacillus cereus*, *Bacillus coagulans*, *B. subtilis*, and *Bacillus sporothermodurans* were likely to form highly heat resistant spores which could survive from normal heat treatment. Kort et al. (2005) also stated that a temperature of more than 100 °C was needed to inhibit *Bacillus* pores to1 log CFU g^{-1}. Therefore, a temperature of 70 °C in this study was certainly insufficient to inactivate the spore of *B. subtilis*.

3.2.5. Sensory evaluation

Sensory evaluation was performed by 50 untrained panelists to judge six essential attributes following 5-point hedonic scale. The mean scores of each attribute of 5 treatments and the control are shown in Table 4. It was found that treatment 3 received significantly highest scores ($P \leq 0.05$) for all attributes, consecutively followed by treatments 4, 2, 5, 1 and the control, respectively. It is noticeable that the control had less flavor, while treatment 1 had strong odor as well as treatments 2 and 5 with high amount of additional rice-*koji miso* were rather salty. Therefore, these treatments were scored for lower overall acceptability, only treatments 3 and 4 gained high sensorial scores.

4. Conclusions

Fatty acid profile of the *swai*-fish fillet was composed mainly of saturated and monounsaturated fatty acids with the ratio of η-3/η-6 being 0.08, while isoflavone profiles of the fermented soybeans revealed that *thua nao* contained more aglycones such as diadzein, glycitein and genistein than rice-*koji miso*, which could provide better health supplements. The physico-chemical properties of fish based emulsions varied 5 treatments condition could be clarified as follows. Browning indices of all treatments were higher than those of the control. In addition, the oscillatory and creep testing illustrated that treatment 1 was the strongest gel followed by treatments 3, 4, 5, 2 and the control, while the gel strengths increased with the increase of *thua nao* in the formula. For microbiological quality, total plate or spore counts showed that fish based emulsions with *thua nao* addition retained high viable cells and spores of *B. subtilis*. In overall, treatments 3 and 4 received the highest sensorial scores.

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