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ARTICLE

Changes in Quality Characteristics of Pork Patties Containing Multilayered Fish Oil Emulsion during Refrigerated Storage

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Abstract

This study was performed to determine the effect of multilayered fish oil (FO) emulsion without or with *trans*-cinnamaldehyde on pork patties. Multilayered FO (-primary, -secondary, -tertiary) emulsions were prepared using a layer-by-layer deposition technique with Tween 20, chitosan, and low methoxyl pectin, and were added to pork patties at the same concentration. Pork patties were then stored for 20 d in a refrigerator (5°C) to study changes in quality. The results showed that the pH value of all samples significantly decreased but cooking loss increased during storage ($p<0.05$). However, water-holding capacity and moisture content showed no remarkable difference between treatments and storage periods ($p>0.05$). All pork patties containing multilayered FO (treated samples) showed higher values for lightness and significantly lower values for yellowness compared to control pork patties (untreated sample). Lipid oxidation was higher in treated pork patties than in control pork patties during storage. In addition, lipid oxidation and total viable bacterial count in pork patties decreased as the number of coating layers increased. However, hardness, cohesiveness, and springiness of all samples showed no significant change during storage ($p>0.05$) as compared to fresh pork patties. Furthermore, these did not remarkable change with addition of *trans*-cinnamaldehyde in all pork patties. From our results, we suggest that FO emulsion did not affect the texture characteristics of fresh pork patties, indicating that it could be used to improve the quality of pork patties by contributing high-quality fat such as unsaturated fatty acids.

Key words: fish oil, multilayered emulsions, lipid oxidation, texture, pork patties

Introduction

Emulsion-type meat products are commercially produced from poultry, pork, or beef emulsified with back fat. Although meat products are an important source of protein, vitamins, and minerals, they contain large quantities of fat, saturated fatty acids, salt, and cholesterol (Jiménez-Colmenero *et al.*, 2001). High fat intake causes hypertension, obesity, cardiovascular disease, and coronary heart disease (Colmenero, 1996). Many studies have reported that high intake of fat is related to risk of obesity and cardiovascular disease resulting from low-density lipoprotein-cholesterol (LDL-cholesterol) production. Therefore, several attempts are being made to reduce animal fat contents in meat products.

Josquin *et al.* (2012) replaced animal fat in emulsified sausages with olive to enhance the polyunsaturated fatty acid (PUFA) content. They also attempted to increase PUFA content by adding linseed oil, rapeseed oil, and fish oil to meat products. Therefore, reduction of fat and addition of high-quality fatty acid in meat products is desirable as they contain fewer calories. This positive effect could be further enhanced by substituting animal fat (Lurueña-Martýinez *et al.*, 2004).

In general, it is reported that the addition of fish oil (FO) in meat product formulations prevents and modulates diseases such as coronary heart disease, cancer, hypertension, and arthritis (Shahidi and Miraliakbari, 2004; Uauy and Valenzuela, 2000). However, problems associated with addition of FO to meat products include a strong fishy flavor and oxidative susceptibility resulting in protein oxidation (McClements and Decker, 2000; Panpipat and Yongsawatdigul, 2008). Many studies have reported the incorporation of FO or PUFA in various foods, including bread, milk, meat, and ice cream (Muguerza *et al.*, 2004; Valencia *et al.*, 2006).

Oil-in-water (O/W) emulsions are efficient systems to

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disperse PUFA and thus help their incorporation into meat products. However, conventional O/W emulsions show low resistance to oxidation stress due to a single layer and generate strong flavors in fish products, thus making it difficult to apply this system to the food industry. To prevent the drawback of oxidation, the FO emulsion system should be made multilayered, by including hydrocolloids such as chitosan and low methoxyl pectin in the preparation of the emulsion (Chun *et al.*, 2013).

To mask the fishy flavor, it would be effective to add flavoring agents such as cinnamon oil. Based on previous research, *trans*-cinnamaldehyde (cinnamon oil; CO) suppressed the fishy flavor of Thai fish sauce when added to the fish oil emulsion system (Choi *et al.*, 2010). Alternate potential advantages of CO are its antimicrobial and antioxidative properties, which are applied to food packaging procedures (Becerril *et al.*, 2007; Jo *et al.*, 2015). It was hypothesized that CO would provide better storage stability in ground meat patties and reduce the fishy flavor caused by FO. Therefore, in this study, we aimed to evaluate the effect of multilayered FO emulsion systems containing cinnamon oil on the physicochemical properties and microbial stability of pork patties.

Materials and Methods

Materials

For multilayered FO preparation, FO and CO (98%) were purchased from Sigma-Aldrich Chemical Company (USA). Polyoxyethylene sorbitan monolaurate (Tween 20), used as the emulsifier, was obtained from Amresco Inc. (USA). Chitosan from shrimp shells (>75% deacetylated according to the manufacturer's specification) and pectin from citrus peel (low methoxyl pectin) with galacturonic acid over 74% (dried basis) were chosen as biopolymers and were purchased from Sigma-Aldrich Chemical Company (USA). Fresh, lean pork meat and pork back fat were obtained from a local meat market to prepare the pork patties. All chemical products were of analytical grade.

Preparation of multilayered FO

Multilayered FO was formulated by the layer-by-layer (LbL) electrostatic deposition method described by Jo *et al.* (2012) and Chun *et al.* (2013), as depicted in Fig. 1. The composition of coating materials to prepare a multilayered FO (1.0%, w/w) without or with CO (0.05%, w/w) included 1.25% (w/w) Tween 20 (primary layer), 0.1% (w/w) chitosan (secondary layer), and 0.2% (w/w) low methoxyl pectin (tertiary layer).

Pork patty preparation

Fresh, lean pork meat and pork back fat were initially ground through an 8-mm plate. Seven batches were prepared differing in composition with respect to the type of multilayered emulsion and quantity added (Table 1). For each batch of patties, pork meat (75%), pork back fat (15%), cold water (2% to 10%), multilayered emulsions (2% to 8%), and sodium chloride (NaCl, 1.5%) were emulsified using a homomixer (5K5SS; USA) for 30 min.

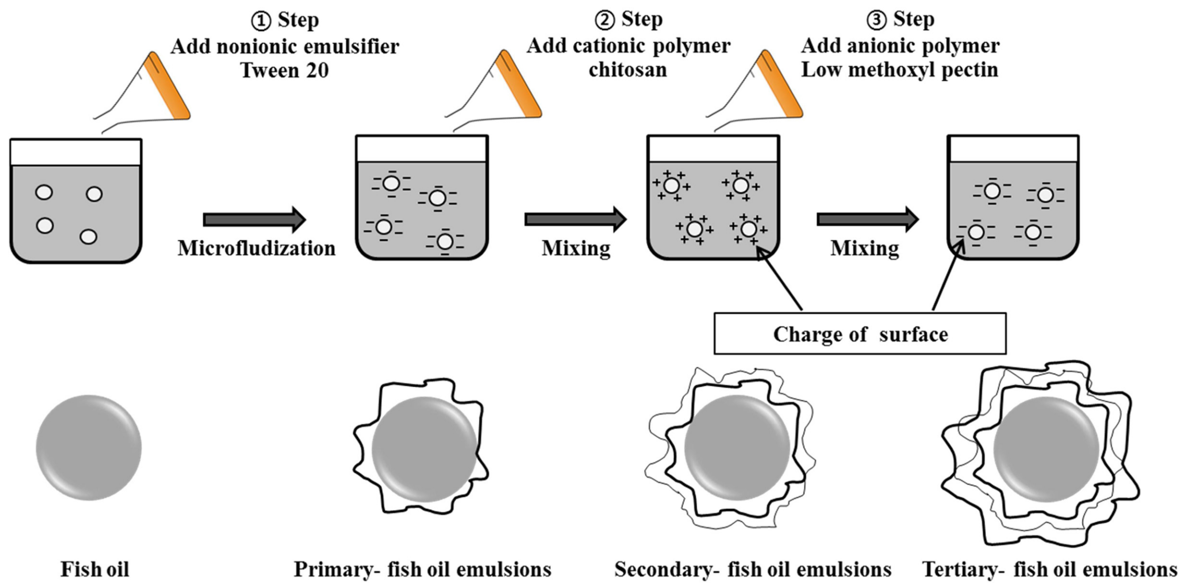

Fig. 1. Schematic of method of formulation of multilayered fish oil emulsions.

Table 1. Formulation of pork patties with various levels of multilayered fish oil emulsions without or with cinnamon oil

Ingredients (%, w/w)	Control	Treatments[1]					
		F1	F2	F3	FC1	FC2	FC3
Pork meat	75	75	75	75	75	75	75
Pork fat	15	15	15	15	15	15	15
Cold water	10	8	6	2	8	6	2
Fish oil emulsions	0	2	4	8	2	4	8
Total	100	100	100	100	100	100	100
salt	1.5	1.5	1.5	1.5	1.5	1.5	1.5

[1]Control, pork patties without fish oil emulsions; F1, pork patties with primary-fish oil (1%) emulsion; F2, pork patties with secondary-fish oil (0.5%) emulsion; F3, pork patties with tertiary-fish oil (0.25%) emulsion; FC1, pork patties with primary-fish oil (1%) emulsion added to cinnamon oil (0.05%); FC2, pork patties with secondary-fish oil (0.5%) emulsion added to cinnamon oil (0.025%); FC3, pork patties with tertiary-fish oil (0.25%) emulsion added to cinnamon oil (0.0125%).

After emulsification, the samples weighed approximately 80 g and were placed in a Petri dish (100-mm-diameter, 15-mm-thick). The pork patties were packaged in thin polyethylene film with high oxygen permeability (equal to aerobic packaging) and then stored at 4°C until use. This procedure was performed in triplicate for each meat patty with multilayered FO. The quality and storage stability were determined after 0, 5, 10, 15, and 20 d at 5°C.

pH value

A 5 g sample from each pork patty was taken, 50 mL distilled water was added and placed in a bag-filter (Interscience; France). The sample was blended using a stomacher (WS400, Shanghai Zhisun Equipment Co., Ltd.; China) for 3 min. The pH of the filtrated solution was determined using a pH meter (Model S220, Mettler Toledo GmbH; Switzerland).

Color evaluation

The color of pork patties was determined using a colorimeter (Minolta Chroma meter CR-210; Japan) calibrated with a white standard (CIE L*=+97.83, CIE a*= -0.43, CIE b*=+1.96). The color measurement of pork patties was performed on the surface and measured 5 times in each pack at different locations. The color values of L^*, a^*, and b^* were determined as indicators of lightness, redness, and yellowness, respectively. The total color difference (ΔE) between the control (at 0 d) and pork patties containing multilayered FO was numerically calculated with the following equation:

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Cooking loss

A 5-cm-diameter and 1-cm-height sample (weight 20± 0.5 g) reconstructed from pork patties was placed into a polypropylene bag, cooked for 15 min at 75°C in a water bath, and cooled to room temperature. The cooking loss of cooked pork patties was determined by calculating the difference in weight before and after cooking as follows:

Cooking loss (%)= [weight of patty before cooking (g)] − [weight of patty after cooking (g) / weight of patty before cooking (g)] × 100

Texture profile analysis (TPA)

TPA was performed using a texture analyzer (CT3, Brookfield Engineering Labs Inc.; USA) equipped with a TA43 sphere 25.4-mm D (Brookfield Engineering Labs Inc.; USA). As mentioned above, pork patties were reconstructed to a 5-cm-diameter and 1-cm-height shape and cooked for 15 min at 75°C in a water bath. Before analysis, the cooked pork patties were kept to equilibrate to room temperature. The conditions of TPA were as follows: pre-test speed; 2.0 mm/s, post-test speed; 5.0 mm/s, test speed; 1.0 mm/s, maximum load; 1 kg, distance; 8.0 mm, force; 750 g. Value for hardness (kg), springiness (ratio), cohesiveness, gumminess (kg), and chewiness (kg) were determined.

Thiobarbituric acid reactive substances (TBARS)

The TBARS in pork patties were measured by the method of Hoyland and Taylor (1991). A 5 g sample was homogenized with 50 mL distilled water in a filter stomacher bag using a stomacher. A 0.75 mL aliquot of the filtrate was mixed with 2.5 mL benzene and 2.5 mL 2-thiobarbituric acid (TBA) reagent (0.69% TBA solution mixed with equal amount of acetic acid) and centrifuged at 1500 *g* for 10 min. After phase separation, the lower phase was filtered using a 0.45-μm syringe filter (DISMIC, Advantec MFS, Inc.; Japan) and boiled for 30 min in a water bath at 95°C. After cooling at ambient temperature, absorbance of the sample was measured at 530 nm. The results were calculated as milligrams of malonaldehyde per kilogram of sample.

Total viable count (TVC)

For TVC analysis, a 5-g sample from each pork patty was aseptically homogenized in 45 mL sterile saline solution (0.85% NaCl) in a filter stomacher bag using a stomacher for 3 min. A 1-mL aliquot of the filtrate was appropriately diluted using sterilized 0.85% NaCl and plated on plate count agar (Sigma-Aldrich Co.; USA). After incubation at 37°C for 2 d, plates with 30 to 300 visual colonies were selected and colonies were counted. Results are expressed as the logarithm of the number of colony-forming units (Log CFU/g).

Statistical analysis

One-way analysis of variance (ANOVA) was performed using a SAS statistical program, ver. 9.2 (SAS Institute; USA); the means were separated by Duncan's multiple range test ($p<0.05$), and correlations between independent variables and measured values were calculated using Pearson's correlation coefficients. All measurements were performed on at least 3 samples and are rep-

Table 2. Change in pH, cooking loss, water-holding capacity, and moisture content of pork patties with various levels of multi-layered fish oil emulsions during refrigerated storage

Treatments[1)]	Storage periods (d)				
	0	5	10	15	20
pH					
Control	5.54±0.01^{Ba*}	5.46±0.02^{Ab}	5.17±0.02^{Cc}	5.05±0.02^{Ce}	5.10±0.03^{Cd}
F1	5.53±0.04^{Ba}	5.45±0.02^{Ab}	5.14±0.01^{Cc}	5.05±0.02^{Cd}	5.14±0.02^{Dd}
F2	5.49±0.01^{Ca}	5.30±0.01^{Db}	5.16±0.01^{Cc}	5.08±0.03^{Cd}	5.18±0.06^{Dcd}
F3	5.58±0.01^{Aa}	5.40±0.01^{Bb}	5.25±0.01^{Ac}	5.15±0.03^{Bd}	5.19±0.03^{ABd}
FC1	5.60±1.09^{Aa}	5.40±0.01^{Bb}	5.21±0.00^{Bc}	5.15±0.02^{Bd}	5.23±0.08^{Bd}
FC2	5.46±0.01^{Da}	5.28±0.01^{Eb}	5.08±0.01^{Dc}	5.00±0.01^{Dd}	5.01±0.04^{Ed}
FC3	5.54±0.01^{Ba}	5.36±0.02^{Cb}	5.22±0.03^{ABc}	5.19±0.03^{Ac}	5.11±0.02^{Ac}
Cooking loss (%)					
Control	15.88±1.93^{Bc}	19.48±1.36^{Cb}	17.95±0.29^{Cbc}	25.83±2.57^{Aa}	25.00±0.5^{ABa}
F1	17.22±0.83^{ABc}	18.71±0.17^{Cbc}	19.68±2.29^{ABb}	24.67±1.26^{Aa}	25.17±0.29^{ABa}
F2	19.19±1.01^{ACc}	22.06±0.68^{ABb}	23.28±1.48^{Aab}	25.17±1.04^{Aa}	25.17±1.04^{ABa}
F3	18.75±1.18^{Ad}	21.03±0.35^{ABc}	19.93±0.24^{BCDc}	26.17±0.76^{Aa}	23.17±1.04^{Bb}
FC1	18.16±1.46^{ACc}	19.53±1.08^{Cbc}	21.41±0.6^{ABbc}	25.17±1.26^{Aa}	24.50±0.5^{ABa}
FC2	18.93±0.61^{Ab}	20.93±0.69^{Bb}	19.67±0.89^{BCb}	26.33±3.4^{Aa}	25.67±1.04^{Aa}
FC3	18.47±0.92^{Ad}	22.40±0.25^{Ac}	21.99±0.21^{ABc}	25.83±1.26^{Aa}	24.33±0.29^{ABb}
Water holding capacity (%)					
Control	79.79±3.01^{Aab}	83.48±5.6^{Aa}	81.48±0.63^{BCab}	76.04±5.33^{Ab}	74.91±0.83^{Db}
F1	80.57±2.97^{Abc}	86.10±3.63^{Aa}	82.93±2.47^{BCab}	76.23±0.83^{Acd}	74.51±2.33^{Dd}
F2	81.90±3.51^{Aab}	85.02±2.7^{Aa}	81.17±3.6^{BCab}	77.39±2.09^{Ab}	78.41±1.31^{BCb}
F3	78.82±5.67^{Ab}	88.57±2.48^{Aa}	87.00±1.06^{Aa}	85.12±4.28^{Aab}	84.98±0.52^{Aab}
FC1	81.50±3.51^{Ab}	89.20±1.92^{Aa}	84.75±2.32^{ABab}	81.46±1.57^{Aab}	83.16±2.54^{Aab}
FC2	77.24±4.37^{Aa}	85.75±2.28^{Aa}	80.53±0.75^{Ca}	78.15±12.15^{Aa}	76.80±0.45^{CDa}
FC3	81.86±1.31^{Abc}	84.95±2.48^{Aa}	82.76±0.23^{BCab}	75.51±1.44^{Ac}	79.73±0.71^{Bc}
Moisture contents (%)					
Control	64.58±2.87^{Aa}	67.40±0.35^{Aa}	67.10±0.49^{Aa}	66.35±1.15^{Aa}	64.21±2.47^{Aa}
F1	65.42±2.14^{Aab}	66.03±0.9A^{Bab}	66.40±0.27^{ABa}	65.85±0.08^{Aab}	63.96±1.21^{Ab}
F2	65.23±1.22^{Ac}	67.45±0.11^{Aa}	67.08±0.49^{Aab}	65.73±0.26^{Abc}	65.20±1.11^{Ac}
F3	65.22±2.25^{Aa}	66.23±0.4A^{Ba}	66.08±1.32^{ABCa}	66.19±0.72^{Aa}	64.62±2.88^{Aa}
FC1	65.47±1.31^{Aa}	64.90±1.01^{Ba}	65.53±0.74^{ABC}	65.74±0.41^{Aa}	65.29±0.97^{Aa}
FC2	64.34±1.72^{Aa}	65.72±1.58^{Ba}	64.50±1.68^{Ca}	65.74±1.59^{Aa}	66.03±0.69^{Aa}
FC3	64.50±1.48^{Aa}	65.33±0.83^{Ba}	65.24±0.71^{BCa}	64.89±0.53^{Aa}	65.62±0.59^{Aa}

[1)]Control, pork patties without fish oil emulsions; F1, pork patties with primary-fish oil (1%) emulsion; F2, pork patties with secondary-fish oil (0.5%) emulsion; F3, pork patties with tertiary-fish oil (0.25%) emulsion; FC1, pork patties with primary-fish oil (1%) emulsion added to cinnamon oil (0.05%); FC2, pork patties with secondary-fish oil (0.5%) emulsion added to cinnamon oil (0.025%); FC3, pork patties with tertiary-fish oil (0.25%) emulsion added to cinnamon oil (0.0125%). *Mean±standard deviation of triplicate determinations (n=3). $^{A-G}$Means with different superscripts within the same column are significantly different ($p<0.05$). $^{a-e}$Means with different superscripts within the same row are significantly different ($p<0.05$).

orted as means with standard deviations (SD).

Results and Discussion

Color

Differences in values for lightness (L* value), redness (a* value), and yellowness (b*value) of pork patties containing multilayered FO were significantly affected by the type of emulsion added (Table 2). Lightness and yellowness in the control showed the lowest values. During storage, lightness values of all samples significantly increased, but yellowness values decreased ($p<0.05$). However, the redness values of all samples were unchanged over the storage period ($p>0.05$). Similar trends in redness values were previously shown by Serrano *et al.* (2006), where storage had no significant effect on the redness value of final meat products, nor were such values variable over the storage period. Discoloration is a major problem for final meat products due to the negative impact on consumer acceptance (Chen and Trout, 1991). The initial values for total color difference (ΔE) indices of all treated samples ranged from 5.34 to 6.67, which showed no significant difference ($p>0.05$) between treatments. However, ΔE of all samples dynamically increased during refrigerated storage ($p<0.05$); moreover, the ΔE of samples containing multilayered FO were signifi-

Table 3. Change in CIE color of pork patties with various levels of multilayered fish oil emulsions during refrigerated storage

Treatments[1)]	Storage periods (d)				
	0	5	10	15	20
CIE L*					
Control	$59.48\pm0.84^{Dd*}$	59.61 ± 0.754^{Dd}	63.17 ± 0.792^{Dc}	64.59 ± 0.854^{Db}	66.53 ± 0.278^{Ba}
F1	65.87 ± 1.149^{Ab}	64.06 ± 0.565^{Cc}	67.15 ± 1.013^{BCab}	67.90 ± 0.726^{Bca}	68.30 ± 0.465^{Aa}
F2	64.27 ± 0.653^{BCd}	65.89 ± 0.426^{Bc}	67.60 ± 0.65^{BCb}	67.34 ± 0.19^{Cb}	69.02 ± 0.652^{Aa}
F3	65.65 ± 0.656^{Abd}	66.69 ± 0.431^{Abc}	69.21 ± 0.639^{Ab}	69.26 ± 0.442^{Ab}	68.52 ± 0.399^{Aa}
FC1	64.58 ± 0.295^{ABCc}	65.91 ± 0.262^{Bb}	66.33 ± 0.838^{Cb}	68.23 ± 0.098^{ABCa}	68.08 ± 0.715^{Aa}
FC2	65.39 ± 0.623^{ABCc}	67.27 ± 0.103^{Ab}	67.30 ± 0.835^{BCb}	68.58 ± 0.662^{ABa}	69.14 ± 0.798^{Aa}
FC3	64.22 ± 0.641^{Cb}	66.36 ± 0.44^{Bc}	68.59 ± 0.711^{ABa}	68.40 ± 0.407^{ABa}	68.50 ± 1.055^{Aa}
CIE a*					
Control	7.44 ± 0.298^{Bb}	8.60 ± 0.198^{Aa}	7.11 ± 0.395^{Cb}	7.83 ± 0.913^{ABab}	7.44 ± 0.298^{Abb}
F1	5.69 ± 0.603^{Db}	7.73 ± 0.591^{Bca}	7.67 ± 0.213^{Aba}	7.85 ± 0.148^{Aa}	7.49 ± 0.322^{Aa}
F2	6.55 ± 0.23^{Cc}	7.36 ± 0.099^{Cb}	7.97 ± 0.216^{Aa}	7.74 ± 0.088^{ABab}	6.70 ± 0.347^{Bc}
F3	7.50 ± 0.038^{Ba}	7.39 ± 0.384^{Cab}	7.36 ± 0.115^{BCab}	6.82 ± 0.372^{Cc}	6.98 ± 0.086^{ABbc}
FC1	6.40 ± 0.07^{Cc}	7.33 ± 0.195^{Cab}	7.54 ± 0.22^{ABCa}	7.08 ± 0.437^{ABCab}	6.95 ± 0.352^{ABb}
FC2	6.96 ± 0.173^{BCb}	8.01 ± 0.115^{Ba}	7.99 ± 0.367^{Aa}	7.61 ± 0.516^{ABCa}	6.68 ± 0.268^{Bb}
FC3	8.32 ± 0.591^{Aa}	6.40 ± 0.204^{Dc}	7.24 ± 0.127^{BCb}	6.94 ± 0.255^{BCbc}	7.00 ± 0.484^{Abbc}
CIE b*					
Control	8.14 ± 0.038^{Ba}	8.18 ± 0.251^{Ca}	5.95 ± 0.229^{Cc}	7.10 ± 0.163^{Cb}	6.13 ± 0.378^{Cc}
F1	8.76 ± 0.455^{Ba}	$8.64\pm0.66B^{Ca}$	7.94 ± 0.601^{Ba}	8.66 ± 0.527^{Aa}	8.43 ± 0.362^{Aa}
F2	10.28 ± 0.128^{Aa}	9.46 ± 0.411^{Abb}	8.14 ± 0.605^{Bc}	8.29 ± 0.132^{Abc}	7.90 ± 0.074^{Abc}
F3	9.93 ± 0.416^{Aa}	8.37 ± 0.297^{Cb}	8.18 ± 0.386^{Bbc}	7.72 ± 0.267^{BCc}	7.60 ± 0.146^{Bbc}
FC1	9.86 ± 0.198^{Aa}	8.29 ± 0.238^{Cab}	8.51 ± 0.151^{Abb}	7.56 ± 0.279^{Cac}	7.28 ± 0.19^{Bc}
FC2	10.30 ± 0.427^{Aa}	9.26 ± 0.168^{ABb}	9.02 ± 0.464^{Ab}	8.87 ± 0.557^{Ab}	7.74 ± 0.426^{Bc}
FC3	10.21 ± 0.821^{Aa}	9.76 ± 0.811^{Aa}	8.20 ± 0.076^{Bbc}	8.65 ± 0.332^{Ab}	7.25 ± 0.596^{Bc}
Total color difference (ΔE)					
Control		1.34 ± 0.05^{Fc}	4.34 ± 0.71^{Db}	5.30 ± 0.73^{Db}	7.35 ± 0.36^{Ba}
F1	6.67 ± 1.27^{Ab}	4.68 ± 0.46^{Ec}	7.70 ± 0.99^{BCab}	8.46 ± 0.75^{BCa}	8.83 ± 0.45^{Aa}
F2	5.34 ± 0.59^{Ad}	6.55 ± 0.49^{CDc}	8.16 ± 0.64^{BCb}	7.88 ± 0.19^{Cb}	9.58 ± 0.68^{Aa}
F3	6.44 ± 0.71^{Ab}	7.23 ± 0.42^{Bb}	9.74 ± 0.64^{Aa}	9.82 ± 0.45^{Aa}	9.08 ± 0.40^{Aa}
FC1	5.49 ± 0.29^{Ac}	6.45 ± 0.26^{Db}	6.87 ± 0.83^{Cb}	8.79 ± 0.10^{BCa}	8.67 ± 0.68^{Aa}
FC2	6.34 ± 0.42^{Ac}	7.90 ± 0.09^{Ab}	7.91 ± 0.82^{BCb}	9.15 ± 0.64^{ABa}	9.71 ± 0.82^{Aa}
FC3	5.29 ± 0.87^{Ac}	7.18 ± 0.48^{BCb}	9.12 ± 0.71^{ABa}	8.96 ± 0.38^{ABa}	9.10 ± 1.03^{Aa}

[1)]Control, pork patties without fish oil emulsions; F1, pork patties with primary-fish oil (1%) emulsion; F2, pork patties with secondary-fish oil (0.5%) emulsion; F3, pork patties with tertiary-fish oil (0.25%) emulsion; FC1, pork patties with primary-fish oil (1%) emulsion added to cinnamon oil (0.05%); FC2, pork patties with secondary-fish oil (0.5%) emulsion added to cinnamon oil (0.025%); FC3, pork patties with tertiary-fish oil (0.25%) emulsion added to cinnamon oil (0.0125%). *Mean±standard deviation of triplicate determinations (n=3). $^{A-G}$Means with different superscripts within the same column are significantly different ($p<0.05$). $^{a-e}$Means with different superscripts within the same row are significantly different ($p<0.05$).

cantly higher than that of control samples during storage. Therefore, we concluded that the color of samples showed no significant difference with layered FO, whereas the color changed during storage.

pH, cooking loss, water-holding capacity, moisture content

Table 3 shows the results for pH values of pork patties containing multilayered FO during storage. The pH values of all samples significantly decreased from 5.60 to 5.00 during storage. F2 and FC2 had lower pH values than the control, F1, F3, FC1, and FC3. During storage, the pH consistently decreased during storage by 20 d, similar to pH trends previously observed by Choi *et al.* (2011) when different amounts of lotus leaf powder were added to chicken patties during refrigerated storage over 28 d. According to Kim *et al.* (2007), the pH value of ground pork meat containing paprika significantly decreased.

Cooking loss in pork patties containing multilayered FO during storage is presented in Table 3. The fresh pork patties had the lowest cooking loss owing to low water and fat content. During storage, cooking loss consistently increased for all samples ($p<0.05$). On the other hand, water-holding capacity and moisture content were unchanged. Razminowicz *et al.* (2006) reported that cooking loss of meat was related to water-holding capacity, defined as the ability of meat to retain water during exposure to external forces such as cutting, heating, grinding, and pressing, and is an important factor to determining the juiciness of meat (Zhang *et al.*, 2005). Furthermore, Hutchings (1999) reported that moisture content may be relevant to the lightness value of color in food. However, in our study, moisture content was not affected although lightness of color in the pork patties increased during storage. Therefore, we suggest that multilayered FO can be applied to pork patties without loss of meat quality.

TPA

Texture properties of the pork patty samples containing multilayered FO are presented in Table 4. No significant

Table 4. Change in hardness, cohesiveness, and springiness of pork patties with various levels of multilayered fish oil emulsions during refrigerated storage

Treatments[1)]	Storage periods (d)				
	0	5	10	15	20
Hardness (g)					
Control	1532±29[b*]	1747±117[a]	1466±202[ab]	1643±135[ab]	2081±197[ab]
F1	1467±134[b]	1753±100[ab]	1611±139[ab]	1555±91[ab]	1686±110[a]
F2	1334±89[b]	1540±121[ab]	1581±213[b]	1625±121[ab]	1828±238[ab]
F3	1418±180[a]	1669±50[a]	1467±190[a]	1638±116[a]	1894±40[a]
FC1	1579±245[a]	1621±152[a]	1550±82[a]	1770±77[a]	1831±69[a]
FC2	1290±166[b]	1699±165[a]	1377±149[b]	1751±355[a]	1722±348[a]
FC3	1445±159[a]	1869±79[a]	1483±166[a]	1828±79[a]	1988±260[a]
Cohesiveness					
Control	0.60±0.012[a]	0.59±0.015[a]	0.54±0.038[b]	0.55±0.038[b]	0.56±0.023[b]
F1	0.58±0.017[a]	0.58±0[a]	0.53±0.017[a]	0.51±0.046[a]	0.50±0.02[a]
F2	0.55±0.053[a]	0.59±0.089[ab]	0.52±0.015[b]	0.49±0.049[ab]	0.53±0.055[ab]
F3	0.58±0.025[a]	0.55±0.049[a]	0.50±0.075[b]	0.57±0.006[b]	0.56±0.026[ab]
FC1	0.58±0.012[a]	0.61±0.025[a]	0.51±0.061[a]	0.51±0.025[a]	0.56±0.026[a]
FC2	0.55±0.026[a]	0.59±0.015[ab]	0.35±0.309[bc]	0.53±0.053[bc]	0.51±0.055[c]
FC3	0.60±0.006[a]	0.58±0.015[a]	0.53±0.035[b]	0.52±0.021[b]	0.51±0.064[b]
Springiness (mm)					
Control	3.45±0.112[ab]	3.74±0.047[ab]	3.32±0.295[b]	3.67±0.091[ab]	3.66±0.101[ab]
F1	3.33±0.143[b]	3.62±0.029[a]	3.47±0.074[ab]	3.49±0.068[ab]	3.50±0.139[ab]
F2	3.46±0.268[a]	3.61±0.186[a]	3.48±0.092[a]	3.54±0.081[a]	3.53±0.105[a]
F3	3.52±0.121[a]	3.55±0.084[a]	3.50±0.24[a]	3.62±0.2[a]	3.67±0.096[a]
FC1	3.55±0.121[bc]	3.74±0.1[a]	3.55±0.071[bc]	3.49±0.056[c]	3.69±0.056[ab]
FC2	3.44±0.118[b]	3.75±0.045[a]	3.39±0.136[b]	3.57±0.185[ab]	3.55±0.159[ab]
FC3	3.43±0.127ab	3.64±0.055a	3.36±0.127b	3.69±0.139a	3.50±0.2ab

[1)]Control, pork patties without fish oil emulsions; F1, pork patties with primary-fish oil (1%) emulsion; F2, pork patties with secondary-fish oil (0.5%) emulsion; F3, pork patties with tertiary-fish oil (0.25%) emulsion; FC1, pork patties with primary-fish oil (1%) emulsion added to cinnamon oil (0.05%); FC2, pork patties with secondary-fish oil (0.5%) emulsion added to cinnamon oil (0.025%); FC3, pork patties with tertiary-fish oil (0.25%) emulsion added to cinnamon oil (0.0125%). *Mean±standard deviation of triplicate determinations (n=3). [A-G]Means with different superscripts within the same column are significantly different ($p<0.05$). [a-e]Means with different superscripts within the same row are significantly different ($p<0.05$).

change in hardness, cohesiveness, and springiness were observed between the control and pork patties containing multilayered FO during refrigerated storage. Ziegler *et al.* (1987) reported that texture characteristics of sausages were related to moisture level; for example, increase in moisture content causes decrease in hardness and increase in springiness of sausages. Moreover, they suggested that the moisture content had no effect on cohesiveness. The results of this study suggest that multilayered FO did not significantly affect the texture characteristics of the pork patties.

Lipid oxidation

Fig. 2 shows the TBARS values of pork patties containing multilayered FO during storage. The TBARS values of pork patties containing multilayered FO were significantly higher than those of the control ($p<0.05$), while the TBARS values of all samples generally increased during refrigerated storage. At 20 d of storage, TBARS values of the control, F1, F2, F3, FC1, FC2, and FC3 were 2.14, 6.53, 5.80, 4.54, 5.08, 4.85, and 4.44 mg malonaldehyde/kg, respectively. In our study, the TBARS value was remarkably decreased as the coating layer number increased ($p<0.05$). It was thought that with fewer coating layers, free oil (FO, CO) would be released more quickly in emulsions. In addition, little antioxidant activity was observed in pork patties containing multilayered FO with CO (Fig. 2B). Namely, F1, F2, and F3 showed higher oxidation than FC1, FC2, and FC3 owing to addition of CO. According to López-López *et al.* (2010), lower antioxidant activity was required from seaweed due to the low level of lipid oxidation in the product. Other researchers reported that when olive oil was partially replaced by pork back fat no oxidation problems were encountered in fermented pork sausage.

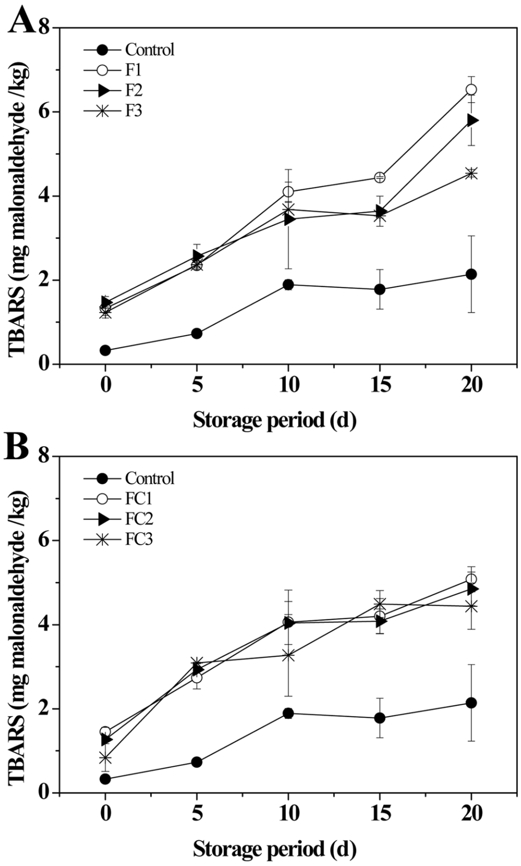

Fig. 2. Change in TBARS of pork patties with various levels of multilayered fish oil emulsions during refrigerated storage. Control, pork patties without fish oil emulsions; F1, pork patties with primary fish oil (1%) emulsion; F2, pork patties with secondary fish oil (0.5%) emulsion; F3, pork patties with tertiary fish oil (0.25%) emulsion; FC1, pork patties with primary fish oil (1%) emulsion added to cinnamon oil (0.05%); FC2, pork patties with secondary fish oil (0.5%) emulsion added to cinnamon oil (0.025%); FC3, pork patties with tertiary fish oil (0.25%) emulsion added to cinnamon oil (0.0125%).

TVC

Results of the TVC analysis of the pork patties containing multilayered FO during refrigerated storage are presented in Fig. 3. TVC of fresh patties significantly increased from 5.00 Log CFU/g to 6.46 Log CFU/g during storage, and pork patties containing multilayered FO were also constantly increased. At 20 d of storage, the TVC of control samples was 6.46 Log CFU/g and that of treated samples F1, F2, F3, FC1, FC2, and FC3 was 6.43, 6.42, 5.88, 6.18, 6.48, and 5.96 Log CFU/g, respectively. In general, when the total aerobic bacteria counts in meat reached 7.0 Log CFU/g, corruption and off-odor occurred (Egan *et al*., 1980). Analyzing the stored pork patties after 20 d, the TVC of F3 (5.88 Log CFU/g) and FC3 (5.96 Log CFU/g) was significantly lower than those of F1, FC1, F2 and FC2. Namely, deterioration of F3 and FC3 was occurred less than that of other samples. Because the F3 and FC3 showed inhibition of microbial proliferation, these were safe from bacteria during storage. In addition, these did not remarkable change with addition of CO, probably since only a small amount of CO was used in this study. López-López *et al.* (2010) studied the characteristics of beef patties with low fat by adding olive oil to water emulsions. They reported that microbial populations generally increased with addition of the seaweed Wakame, and decreased with addition of olive oil. Therefore, the multilayered FO could be extended the storage

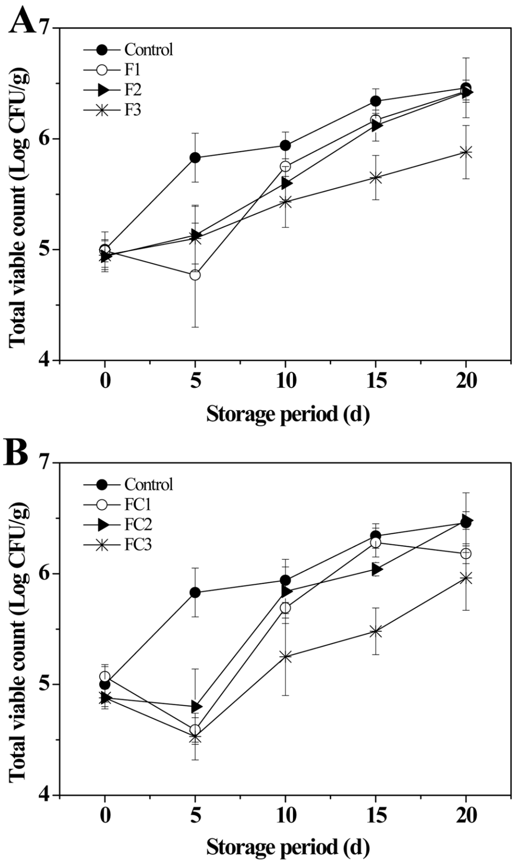

Fig. 3. Change in total viable count of pork patties with various levels of multilayered fish oil emulsions during refrigerated storage. Control, pork patties without fish oil emulsions; F1, pork patties with primary fish oil (1%) emulsion; F2, pork patties with secondary fish oil (0.5%) emulsion; F3, pork patties with tertiary fish oil (0.25%) emulsion; FC1, pork patties with primary fish oil (1%) emulsion added to cinnamon oil (0.05%); FC2, pork patties with secondary fish oil (0.5%) emulsion added to cinnamon oil (0.025%); FC3, pork patties with tertiary fish oil (0.25%) emulsion added to cinnamon oil (0.0125%).

periods by inhibiting deterioration of meat products by microorganisms.

Conclusion

In this study, the addition of multilayered FO to pork patties was shown to provide high-quality fat and at the same time did not influence physical and textural changes during storage. However, the pork patties made with addition of multilayered FO had significantly higher TBARS values as compared to the control. Moreover, the effects of CO had no remarkable difference in pork patties. Thus, in order to improve the shelf-life and commercial application in the meat industry, further study is required to reduce oxidation of fats and oils in meat and meat products. To maintain the organoleptic properties of meat products, a sensory test is also required.

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