CHARACTERISTICS AND OXIDATIVE STABILITY OF BREAD FORTIFIED WITH ENCAPSULATED SHRIMP OIL

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ABSTRACT

Characteristics and oxidative stability of bread fortified with micro-encapsulated shrimp oil (MSO) were determined. The addition of MSO could improve the loaf volume of bread. Chewiness, gumminess and resilience of resulting bread were decreased. Bread crust and crumb showed higher redness and yellowness when MSO was incorporated (P<0.05). Microstructure study revealed that MSO remained intact with bread crumbs. The addition of MSO up to 3% had no adverse effect on bread quality and sensory acceptability. Oxidation took place in bread fortified with 5% MSO to a higher extent, compared with those with 1 or 3% MSO. Therefore, the bread could be fortified with MSO up to 3%.

- Keywords: shrimp oil, encapsulation, bread quality, lipid oxidation -
INTRODUCTION

Hepatopancreas, a byproduct generated from the manufacturing of hepatopancreas-free whole shrimp, is the excellent source of lipids with high polyunsaturated fatty acids (PUFA) (37.42 g/100g oil) and carotenoids (2.02 mg/g oil). Shrimp oil from hepatopancreas contained linoleic acid as the most abundant fatty acid, followed by oleic acid. Additionally, shrimp oil also contained PUFA including eicosapentaenoic acid (2.15 g/100 g oil) and docosahexaenoic acid (6.20 g/100 g oil) (Takeung Wongtrakul et al., 2012). Ethanolic precipitation and available equipment (Gharas et al., 2013). Proteins and carbohydrates are frequently used as matrices for micro-encapsulation of lipophilic compounds by spray drying (Gharsallaoui et al., 2007). Takeung Wongtrakul et al. (2014b) reported that the use of whey protein and Na-caseinate in combination with glucose syrup as the wall materials could improve encapsulation efficiency of micro-encapsulated shrimp oil more effectively than protein alone and protein in combination with gum arabic or maltodextrin. Amongst several encapsulation techniques, spray-drying is the most common micro-encapsulation technology used in food industry due to its low cost, continuous production, ease of industrialization and available equipment (Gharsallaoui et al., 2007). The world’s food market is currently focused on foods that provide nutritive values and health benefits to consumers. Functional foods are rapidly expanding and draw the great attention (Ezhilarasi et al., 2014). Fortification of highly nutritive ingredients such as polyunsaturated fatty acid rich oil, etc. is gaining the interest for food industry. The incorporation of micro-encapsulated oil into foods enables the development of new functional foods with minimal impact on the organoleptic properties of the food products (Ezhilarasi et al., 2014). Wall materials surrounding oil droplets can act as the shield, preventing the oil from oxidation.

Borneo et al. (2007) fortified micro-encapsulated n-3 fatty acids in cream-filled sandwich cookies without any adverse effect on sensory properties.

Bread has become popular, especially for the new generation (Cleary et al., 2007). The fortification of shrimp oil rich in PUFA and astaxanthin in the encapsulated form could increase the nutritive value of bread. As a consequence, the consumers can obtain the active nutrients with the health benefit from the bread. Nevertheless, no information regarding the fortification of micro-encapsulated shrimp oil in bread has been reported. The objective of this study was to investigate the effects of micro-encapsulated shrimp oil fortification on the characteristics and sensory property of bread.

MATERIALS AND METHODS

Chemicals

Ethylenediamine tetraacetic acid (EDTA) was obtained from Merck (Darmstadt, Germany). Tannic acid (99.5% purity) was purchased from Sigma (St. Louis, MO, USA). Essential oil (100% purity) from lemon was obtained from Botanicescence (Bangkok, Thailand). Sodium caseinate was procured from Vicchi enterprise Co., Ltd. (Bangkok, Thailand). Whey protein concentrate was obtained from I.P.S. International Co., Ltd. (Bangkok, Thailand). Wheat flour, sugar, salt, shortening, milk powder and yeast were procured from a local market in Hat Yai, Songkhla, Thailand.

Collection and preparation of hepatopancreas from Pacific white shrimp

Hepatopancreas of Pacific white shrimp (Litopenaeus vannamei) with the size of 50-60 shrimp/kg was obtained from the Sea wealth frozen food Co., Ltd., Songkhla province, Thailand during February and March, 2014. Pooled hepatopancreas (3-5 kg) was placed in a polyethylene bag. To maintain the quality of hepatopancreas during transportation, the bag was imbedded in a polystyrene box containing ice with a sample/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla within approximately 2 h. The sample was stored at -18°C until use, but the storage time was no longer than 1 month. Prior to oil extraction, hepatopancreas was thawed using running water (25°C) and ground in the presence of liquid nitrogen using a blender (Phillips, Guangzhou, China) for 30 sec.
Extraction of oils from hepatopancreas

Oil was extracted from hepatopancreas following the method of TAKEUNGWONGTRAKUL et al. (2014). The prepared hepatopancreas (20 g) was homogenized with 90 mL of cold solvent mixtures (isopropanol: hexane, 50: 50, v/v) (4°C) at the speed of 9500 rpm using an IKA Labortechnik homogenizer (Selangor, Malaysia) for 2 min at 4°C. The extract was filtered using a Whatman filter paper No.4 (Whatman International Ltd., Maidstone, England). The residue was extracted with cold solvent mixtures for another two times. The hexane fraction was pooled and repeatedly washed with an equal quantity of 1% NaCl in order to separate the phases and remove traces of polar solvents. Hexane fraction (approximately 135 mL) was then added with 2-5 g anhydrous sodium sulphate, shaken very well, and decanted into a round-bottom flask through a Whatman No. 4 filter paper. The solvent was evaporated at 40°C using an EYELA rotary evaporator N-1000 (Tokyo Rikakikai, Co. Ltd, Tokyo, Japan) and the residual solvent was removed by nitrogen flushing. The obtained oil with the yield of 19.04% (w/w) was used for micro-encapsulation.

Preparation of shrimp oil-in-water emulsion

Aqueous stock mixed solution of whey protein concentrate, sodium caseinate and glucose syrup at a ratio of 1: 1: 2 (w/w/w) in deionized water was prepared. The mixture was stirred overnight using a magnetic stirrer at room temperature (28°-30°C) to obtain the homogenous wall material solution as per the method of TAKEUNGWONGTRAKUL and BENJAKUL (2014b). Shrimp oil was added into the solution at a core/wall material ratio of 1:4 (v/v). The mixtures were homogenized at a speed of 10,000 rpm for 3 min using a homogenizer (Model T25 basic, IKA Labortechnik, Selangor, Malaysia). The obtained coarse emulsions were then passed through a Microfluidics homogenizer (Model HC-5000, Microfluidizer, Newton, MA, USA) at a pressure level of 4,000 psi for four passes. Emulsions were added with lemon essential oil + tannic acid + EDta. Prior to the incorporation, lemon essential oil (200 ppm) was dissolved in shrimp oil, whereas EDTA (50 ppm) and tannic acid (100 ppm) were dissolved in aqueous stock solution.

Preparation of micro-encapsulated shrimp oil (MSO)

The prepared emulsion was subjected to drying using a laboratory scale spray-dryer (LabPlant Ltd., LabPlant SD-06A, Huddersfield, UK) equipped with a 0.5 mm diameter nozzle. The emulsion was fed into the main chamber (215 mm diameter x 500 mm long) through a peristaltic pump. Feed flow rate was 8.08 mL/min; drying air flow rate was 4.3 m/s and compressor air pressure was 40.61 psi. Air inlet temperature was 180°±2°C. The outlet temperature was controlled at 90°±2°C. The obtained powder referred to as micro-encapsulated shrimp oil (MSO) was collected in the amber bottle and capped tightly. MSO was determined for the total oil content using the mixture of chloroform and methanol as per the method of SHAHIDI and WANASUNDARA (1995). MSO contained 18±1.34% shrimp oil and had 1.06±0.05% moisture content.

Fortification of MSO in bread

Bread was prepared with the following formulation: wheat flour (500 g), sugar (20 g), salt (8 g), shortening (20 g), milk powder (25 g) and yeast (7 g). Flour and other ingredients were mixed and kneaded uniformly, in which water (300 mL) was added during kneading. Thereafter, MSO was directly added to dough at different levels (0, 1, 3 and 5%, w/w). The dough was kneaded for another 10 min. Dough (150 g) was then subjected to bulk fermentation for approximately 1 h at 30°C and 75% relative humidity, followed by scaling, intermediate proving, moulding and second proving (for about 1-1.25 h). Finally, baking was carried out at 220°C for 20 min in baking oven (YXD-20, Guandzhou Xinnanfang electro-thermal equipment Co., Ltd., Guandzhou, China). After baking, bread samples were removed from mold and allowed to cool at room temperature. Bread incorporated with 5% (w/w) spray dried empty capsules (without the addition of shrimp oil) was used as the control bread. The bread samples were subjected to analyses.

Characterization of bread fortified with MSO

Loaf volume

The volume of bread was determined by sesame seed displacement method after the loaves were cooled to room temperature for approximately 2 h (JIAMYANGYUEN et al., 2005).

Texture profile analysis

Texture profile analysis (TPA) was performed using a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) with a cylindrical aluminum probe (35 mm diameter). The samples were sliced (each 2.0 cm thickness) and placed on the instrument’s base. The tests were performed with two compression cycles. Texture measurements were performed ten times for each sample and mean values were reported. Hardness, springiness,
cohesiveness, gumminess, chewiness and resilience were calculated from the force-time-curves generated for each sample (GÖKMEN et al., 2011). Hardness is expressed as the maximum force for the first compression, which relates to the strength of the samples under penetration. Gumminess is defined as the force required to disintegrate a semi-solid food before it is ready for swallowing. Springiness is a measure of how much the samples structure is broken down by the initial penetration and is calculated as the ratio of the time from the start of the second area up to the second probe reversal over the time between the start of the first area and the first probe reversal. Cohesiveness is a measure of the degree of difficulty in breaking down the internal structure of sample. Resilience reflects the reformation capacity of sample tissues after penetration (CHANG et al., 2012). Chewiness is related to the time required for masticating a bread piece prior to swallow, and the low chewing value means easy break of the bread in the mouth (KRUPA-KOZAR et al., 2012).

Color measurement

The color of crust and crumb samples were determined using a colorimeter (ColorFlex, Hunter Lab Reston, VA, USA) and reported in the CIE system, including L*, a* and b* and ΔE*, representing lightness, redness/greenness, yellowness/blueness and total difference of color respectively. ΔE* was also calculated using the following equation:

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

where ΔL*, Δa* and Δb* are the differences between the color parameter of the samples and the color parameters of the white standard ($L^* = 92.85$, $a^* = -1.20$, $b^* = 0.46$).

Scanning electron microscopy (SEM)

Bread morphology was evaluated by scanning electron microscopy (SEM). Fresh bread was sliced by a razor blade and mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West chester, PA, USA). The specimens were observed using a scanning electron microscope (Quanta 400, FEL, Eindhoven, Netherlands) at an acceleration voltage of 15 kV with magnification of 2000x.

Sensory evaluation

Sensory evaluation was performed by 30 untrained panelists with ages ranging from 20 to 35 years, who were familiar with the consumption of bread. Panelists were asked to evaluate for crust color, crumb color, odor, texture, appearance and overall likeness of bread samples using a nine-point hedonic scale, in which a score of 1 = not like very much, 5 = neither like nor dislike and 9 = like extremely, respectively. Panelists were asked to hand-feel the sample for texture. Freshly prepared bread was taken randomly for sensory evaluation at day 0 and 3. Each bread loaf was cut in half and slices were subsequently cut to a thickness of 2 cm. Bread was served in a closed odorless plastic container at room temperature. The samples were labeled with random three-digit codes. The order of presentation of the samples was randomized according to “balance order and carry-over effects design” (CARR et al., 1999).

Changes in volatile compounds in bread during storage

Bread was placed in polyethylene bag and sealed. Packaged bread samples with different treatments were stored at room temperature (28°-30°C). The samples were taken at day 0, 1, 2 and 3 for analyses. Volatile compounds in bread was analysed by headspace GC-MS using a solid-phase microextraction gas chromatography mass spectrometry (SPME GC-MS) following the method of GÖKMEN et al. (2011) with a slight modification.

Extraction of volatile compounds by SFME fiber

To extract volatile compounds, 1 gram of bread slice was placed in a headspace 20 mL-vial (Agilent Technologies, Palo Alto, CA, USA). The vial was tightly closed by means of a capper. A carboxen-polydimethylsiloxane solid phase micro-extraction fiber (50/30 µm DVB/Carboxen™/ PDMS StableFlex™) (Supelco, Bellefonte, PA, USA) was used to adsorb the volatile lipid oxidation compounds released from the sample. The fiber was inserted into the vial and equilibrated at 40°C for 30 min prior to GC-MS analysis.

GC-MS analysis

GC-MS analysis was performed in a HP 5890 series II gas chromatography (GC) coupled with HP 5972 mass-selective detector equipped with a splitless injector and coupled with a quadrupole mass detector (Hewlett Packard, Atlanta, GA, USA). Compounds were separated on a HP-Innowax capillary column (Hewlett Packard, Atlanta, GA, USA) (30 m×0.25 mm ID, with film thickness of 0.25 µm). The GC oven temperature program was: 35°C for 3 min, followed by an increase of 3°C/min to 70°C, then an increase of 10°C/min to 200°C, and finally an increase of 15°C/min to a final temperature of 250°C and holding for 10 min. Helium was employed as a carrier gas with a constant
flow of 1 mL/min. The injector was operated in the splitless mode and its temperature was set at 270°C. Transfer line temperature was maintained at 260°C. The quadrupole mass spectrometer was operated in the electron ionization (EI) mode and source temperature was set at 250°C. Initially, full-scan-mode data was acquired to determine appropriate masses for the later acquisition in scan mode under the following conditions: mass range: 25-500 amu and scan rate: 0.220 s/scan. All analyses were performed with ionization energy of 70 eV, filament emission current at 150 µA, and the electron multiplier voltage at 500 V.

Analyses of volatile compounds

Identification of the compounds was done by consulting ChemStation Library Search (Wiley 275.L). Identification of compounds was performed, based on the retention time and mass spectra in comparison with those of standards from ChemStation Library Search (Wiley 275.L). Quantification limits were calculated to a signal-to-noise (S/N) ratio of 10. Repeatability was evaluated by analysing 3 replicates of each sample. The identified volatile compounds, related to lipid oxidation, including aldehydes, alcohols, ketones, etc., were presented in the term of abundance of each identified compound.

Statistical analysis

All experiments were run in triplicate. All analyses were conducted in five replications. Statistical analysis was performed using one-way analysis of variance (ANOVA). Mean comparison was carried out using Duncan’s multiple range test (Steel and Torrie, 1960).

<table>
<thead>
<tr>
<th>Storage time (day)</th>
<th>MSO (%) w/w</th>
<th>Loaf volume (mL)</th>
<th>Hardness (g)</th>
<th>Springiness (mm)</th>
<th>Cohesiveness (g.mm)</th>
<th>Gumminess</th>
<th>Chewiness</th>
<th>Resilience</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control</td>
<td>296.06±9.36bA</td>
<td>1362.22±80.23aB</td>
<td>0.90±0.02aA</td>
<td>0.59±0.02aA</td>
<td>759.25±50.08aB</td>
<td>739.70±38.73bA</td>
<td>0.22±0.01bA</td>
</tr>
<tr>
<td>0 %</td>
<td></td>
<td>269.25±10.84cA</td>
<td>1020.99±54.21bB</td>
<td>0.91±0.03aA</td>
<td>0.61±0.02aA</td>
<td>670.14±57.86bB</td>
<td>712.87±51.31bB</td>
<td>0.24±0.01aA</td>
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<tr>
<td>1 %</td>
<td></td>
<td>315.22±10.47abA</td>
<td>938.58±65.57bB</td>
<td>0.90±0.02aA</td>
<td>0.60±0.03aA</td>
<td>610.30±42.10bB</td>
<td>480.51±57.97bB</td>
<td>0.22±0.01aA</td>
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<tr>
<td>3 %</td>
<td></td>
<td>317.67±9.13aA</td>
<td>927.95±69.48bB</td>
<td>0.90±0.03aA</td>
<td>0.60±0.01aA</td>
<td>503.91±42.57bB</td>
<td>460.17±57.76bB</td>
<td>0.22±0.02aA</td>
</tr>
<tr>
<td>5 %</td>
<td></td>
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<td>927.85±69.48bB</td>
<td>0.90±0.03aA</td>
<td>0.60±0.01aA</td>
<td>503.91±42.57bB</td>
<td>460.17±57.76bB</td>
<td>0.22±0.02aA</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>290.83±8.96bA</td>
<td>2454.22±84.41aA</td>
<td>0.89±0.02aA</td>
<td>0.50±0.02aB</td>
<td>1146.05±54.24aA</td>
<td>1034.57±56.96aB</td>
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<tr>
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<td>0.52±0.02aA</td>
<td>1097.85±39.01aA</td>
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<td>312.17±8.86aA</td>
<td>1532.81±58.13aA</td>
<td>0.90±0.02aA</td>
<td>0.52±0.03aA</td>
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<td>3 %</td>
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<tr>
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<td>0.52±0.02aA</td>
<td>672.64±54.40aA</td>
<td>6176.8±60.45aA</td>
<td>0.17±0.01aB</td>
</tr>
</tbody>
</table>

Control = Added with 5% (w/w) spray dried empty capsule without the addition of shrimp oil.
Data are expressed as mean±SD (n=3).
Lowercase letters in the same column within the same storage time indicate significant difference (p < 0.05).
Uppercase letters in the same column within the same sample indicate significant difference (p < 0.05).

**Characteristics of bread fortified with MSO**

**Loaf volume**

Loaf volume of bread fortified with MSO at different levels is shown in Table 1. Incorporation of MSO (1-5%) resulted in the increase in loaf volume (P< 0.05). However, similar loaf volume was obtained, regardless of amount of MSO added (P > 0.05). Loaf volume of bread incorporated with spray dried empty capsule (control bread) was not different from that of bread containing MSO at a level of 1% (w/w) (P > 0.05). Nevertheless, bread fortified with MSO at the levels of 3 and 5% (w/w) had higher loaf volume than the control bread (P< 0.05). Spray dried empty capsule, including whey protein concentrate, sodium caseinate and glucose syrup, could increase loaf volume of bread to some degree. Those proteins as well as glucose syrup might strengthen the loaf structure via interaction with wheat gluten, in which the bread matrix could hold gas more efficiently. Whey proteins demonstrated the ability to increase the loaf volume of the bread (Nunes et al., 2009). Gluten proteins of wheat flour create unique visco-elastic properties of dough, which allow dough to expand due to the formation of carbon dioxide during fermentation and retain most of this gas inside the dough texture (Wehrle et al., 1997). Gökmén et al. (2011) and EzhiLarasi et al. (2014) reported that increasing amount of micro-encapsulated oil or active compounds decreased loaf volume of bread. Encapsulated substances could decrease the concentration of gluten in the formulation and lower the retention of gases during the baking process. Therefore, bread fortified with MSO with the range of 3-5% (w/w) had
the higher loaf volume, in comparison with the control bread.

After storage of 3 days at room temperature, in which mold was not detected, no difference in loaf volume was noticeable in comparison with that found at day 0 (P > 0.05). Thus, bread structure was not collapsed within 3 days of storage. It was noted that the addition of MSO had no influence on the shelf-life of bread.

Textural properties

Textural properties of bread samples containing MSO at various levels are presented in Table 1. The addition of MSO generally had the effects on the texture profile of bread. However, MSO had no effect on hardness (P > 0.05), irrespective of amount used. It was noted that, the control bread had higher hardness value than others (P < 0.05). The proteins in spray dried empty capsules with smaller size might be distributed more uniformly and strengthened bread structure more efficiently. For gumminess, the addition of MSO decreased the value. The decrease was more pronounced as the level of MSO increased (P < 0.05). It was found that bread incorporated with 5% MSO showed the lowest gumminess, compared with others (P < 0.05). The proteins in spray dried empty capsules had no impact on springiness and cohesiveness (P > 0.05). Chewiness of bread decreased as the amount of MSO in bread increased (P < 0.05). Control bread showed similar chewiness to that without MSO (P > 0.05). For resilience, bread without MSO (0%) showed the higher value than others (P < 0.05).

After the storage at room temperature for 3 days, hardness, gumminess and chewiness increased, whilst the cohesiveness and resilience decreased (P < 0.05). Nevertheless, no changes in springiness were observed after the storage (P > 0.05). These results indicated that bread staling took place upon storage, probably due to retrogradation (HENNA-LU and NORZIAH, 2011). Therefore, MSO addition had the direct impact on textural property to different degrees, depending on the amount of MSO incorporated.

Color

Color of bread crust was affected by the amount of MSO added as shown in Table 2. The photographs of bread crust are shown in Fig. 1A. Bread crust had the decrease in L*-value, but the increases in a*- , b*- and ΔE*- values as the level of MSO increased (P < 0.05). Amongst all samples, that added with 5% MSO showed the lowest L*-value but highest a*- , b*- and ΔE*- values (P < 0.05). It was found that bread incorporated with spray dried empty capsule (control) had the lower L*- value than others, except for that added with 5% MSO. The color of bread crust is mostly attributed to non-enzymatic chemical reactions such as Maillard and caramelization reaction that produce colored compounds (formation of the golden yellow color) during bread baking (GÖKMEN et al., 2011). Proteins in spray dried empty capsule could serve as the reactant, especially for browning reaction, especially at crust region. GÖKMEN et al. (2011) reported that the particles in the crust region were partially destroyed due to more severe thermal conditions during baking. Furthermore, the wall materials also contained some sugars, which more likely underwent caramelization at high temperature. This could contribute to the brown color of bread crust. PURLIS and SALVADORI (2009) reported that bread had high browning reaction rates, particularly when caramelization oc-

![Table 2 - Color of breads incorporated with MSO at different levels at day 0 and 3 of storage.](image-url)

occurred. Therefore, browning reaction had a pronounced influence on bread color, particularly during bread baking.

For bread fortified with MSO, the increases in redness ($a^*$-value) were more likely due to the orange/red color of MSO. Shrimp oil contained a high amount of astaxanthin (TAKEUNGWONGTRAKUL et al., 2014). As a result, the bread crust turned to be more orange in color, when MSO was added, especially at higher levels. Thus, the color of bread crust might depend on both non-enzymatic browning reactions and astaxanthin partially released from MSO. However, the changes in color of crust were more likely caused by the releases of astaxanthin from MSO as evidenced by the more $a^*$-value (redness) in color of resulting bread. When comparing $L^*$-, $a^*$-, $b^*$- and $\Delta E^*$-values of all bread crust, all bread samples had no change in color after 3 days of storage ($P>0.05$). The result suggested that the pigments in MSO were stable after 3 days of storage as evidenced by the unchanged color of bread crust. It was presumed that wall material might protect the oxidation of astaxanthin to some degree during the storage.

The color of bread crumb was determined (Table 2). The levels of MSO incorporated in bread were coincidental with the color. The decrease in $L^*$- value and increases in $a^*$-, $b^*$- and $\Delta E^*$-values of bread crumb were found as the level of MSO increased ($P<0.05$). For color of bread crumb, crumb does not undergo Maillard reaction, but is affected by the ingredients in the formula (CONFORTI and DAVIS, 2006). Oils from shrimp hepatopancreas were reddish orange in color due to the presence of astaxanthin (TAKEUNGWONGTRAKUL et al., 2014). Additionally, surface oil and oil released to the surface of MSO during bread making could also contribute to color of bread crumb. When MSO at a level of 5% was incorporated, bread crumb had the lowest $L^*$- value but highest $a^*$-, $b^*$- and $\Delta E^*$- values than others ($P<0.05$). For control bread (with only spray dried empty capsule), $a^*$- and $b^*$-values of crumb were not different from those of bread without MSO ($P>0.05$). Spray dried empty capsule was visually white in color without red or yellow color. After 3 days of storage, the control bread had no change in $L^*$- value ($P>0.05$), whilst other bread had the increase in $L^*$-value ($P<0.05$). For $a^*$- and $b^*$- values, all bread crumb had no change in $a^*$- and $b^*$-values. It was noted that those with 0 and 1% MSO had the decrease in $\Delta E^*$- value after 3 days of storage ($P<0.05$).

The photographs of bread crumb are shown in Fig. 1B. During storage, the oil might be released from the wall to some degree. As a result, oil with high content of astaxanthin could contribute to the increase in $a^*$- and $b^*$-values to some extent. This was obvious for bread fortified with 5% MSO. Therefore, the addition of MSO directly affected the color of both crust and crumb of bread.

**Microstructure**

SEM microphotographs of all bread crumbs incorporated with the different levels of MSO are shown in Fig. 2. In general, MSO was embedded in the crumb of bread, which was constructed by gluten network. These results were consistent with GÖKMEN et al. (2011) who incorporated nano-encapsulated flax seed oil into bread. Powders added to dough remained intact in the bread crumb. For the control bread, the bead of spray dried empty capsule was observed throughout the crumb (Fig. 2A). How-
Fig. 2 - Surface morphology of breads incorporated with MSO at different levels. (Magnification: ×2000).
ever, there was no bead in the bread without MSO and spray dried empty capsule (Fig. 2B). It was clearly illustrated that the number of bead, representing MSO, increased as the level of MSO increased. In general, MSO were located uniformly in the crumb matrix. Those MSO could serve as the source of PUFA and astaxanthin rich shrimp oil.

**Sensory property**

Crust color, crumb color, texture, appearance, odor and overall likeness scores of all bread samples added with different amounts of MSO at day 0 and 3 of storage are shown in Table 3. There were no differences in all attributes amongst all bread samples (P> 0.05) at day 0 of storage, except for crumb color and overall likeness scores of bread incorporated with 5% MSO, which had the lower score (P< 0.05). The addition of 5% MSO to bread had negative effect on crumb color and overall likeness of bread. This was due to the marked increases in a* and b* values of bread crumb (Table 2). GÖKMEN et al. (2011) reported that the addition of micro-encapsulated n-3 fatty acids could increase functionality of bread. Shrimp oil is one of the important sources of n-3 fatty acids (TAREUNGWONGTRAKUL et al., 2012). Thus, MSO at 3% (w/w) could be added into bread to improve the nutritive values of bread without the negative effect on sensory property of bread.

After 3 days of storage, no differences in all attributes were observed amongst all bread samples (P> 0.05). Nevertheless, crust color, odor and overall likeness of bread added with 5% MSO were lower than others (P< 0.05). Wall materials could protect the entrapped core by providing a physical barrier against environmental conditions (GALLARDO et al., 2013). Food fortification is good way to induce the general population to consume components, such as n-3 fatty acids, and will add value to food product manufactured by the food industry (BORNEO et al., 2007). However, the addition of 5% MSO might result in the increased free oil, especially at the surface of MSO. This led to more free oil, which was susceptible to oxidation. As a consequence, the lower score of odor likeness was found. Therefore, MSO must be incorporated at the appropriate level to avoid the undesirable attributes of bread.

**Volatile compounds**

Volatile compounds in bread samples added with MSO at different levels after 3 days of storage are displayed in Table 4. Volatile compounds in bread without MSO (days 0) were also determined. Lipid oxidation generates a number of products, including volatile compounds, which are the major contributors to the rancid off-flavors and off-odors in the food product (ROSS and SMITH, 2006).

In general, all compounds present in bread without MSO at day 0 were lower in abundance than those found after 3 days of storage. Nevertheless, 3-methyl-1-butanol, 2-pentyl-furan, heptenal and 2-octen-1-ol were also lower in abundance after storage, plausibly due to the volatilization or decomposition. Several derivatives of aldehyde, ketone and alcohol can be formed by the oxidation of lipids (VARLETT et al., 2006). Aldehydes are the most prominent volatiles produced during lipid oxidation and have been used to successfully follow lipid oxidation in a number of foods (SHAHIDI and PEGG, 1994). After the storage, the bread without MSO contained new volatile compounds including decanal. The highest amount of lipid oxidation products such as 3-methyl-1-butanol, benzeneethanol, benzaldehyde and 2-methyl-1-propanol

### Table 3 - Likeness score of breads incorporated with MSO at different levels at day 0 and 3 of storage.

<table>
<thead>
<tr>
<th>Storage time (day)</th>
<th>MSO (%) w/w</th>
<th>Crust color</th>
<th>Crumb color</th>
<th>Texture</th>
<th>Appearance</th>
<th>Odor</th>
<th>Overall likeness</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Control</td>
<td>7.04±0.78aA</td>
<td>6.90±1.08aA</td>
<td>6.47±1.17aA</td>
<td>7.00±1.04aA</td>
<td>7.13±0.94aA</td>
<td>7.03±1.00aA</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>0</td>
<td>6.92±0.65aA</td>
<td>6.75±1.16aA</td>
<td>6.70±0.78aA</td>
<td>6.79±0.92aA</td>
<td>7.00±0.94aA</td>
<td>6.90±0.82aA</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7.04±0.81aA</td>
<td>6.90±0.90aA</td>
<td>7.03±1.27aA</td>
<td>7.07±1.16aA</td>
<td>6.93±0.82aA</td>
<td>6.83±0.95aA</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.31±0.89aA</td>
<td>7.10±0.90aA</td>
<td>7.00±0.98aA</td>
<td>6.93±1.00aA</td>
<td>6.97±0.88aA</td>
<td>6.80±0.77aA</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6.67±0.88aA</td>
<td>5.54±1.12aA</td>
<td>6.97±1.37aA</td>
<td>6.63±1.36aA</td>
<td>6.47±0.95aA</td>
<td>5.63±1.12aA</td>
</tr>
<tr>
<td>3 Control</td>
<td>6.94±0.89aA</td>
<td>6.90±1.24aA</td>
<td>6.04±0.91aA</td>
<td>7.00±0.89aA</td>
<td>6.52±1.23aA</td>
<td>6.44±0.93aA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>6.72±0.96aA</td>
<td>6.74±0.96aA</td>
<td>6.38±1.08aA</td>
<td>7.00±1.21aA</td>
<td>6.58±1.31aA</td>
<td>6.36±0.91aA</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6.85±1.33aA</td>
<td>6.87±1.41aA</td>
<td>6.74±0.93aA</td>
<td>7.06±1.21aA</td>
<td>6.56±1.01aA</td>
<td>6.28±0.96aA</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.00±0.80aA</td>
<td>7.00±0.91aA</td>
<td>6.70±0.95aA</td>
<td>6.74±1.21aA</td>
<td>5.96±0.75aA</td>
<td>6.14±1.09aA</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6.66±1.14aA</td>
<td>5.43±1.07aA</td>
<td>6.60±1.22aA</td>
<td>6.39±1.36aA</td>
<td>5.16±0.94aA</td>
<td>5.36±0.99aA</td>
</tr>
</tbody>
</table>

Control = Added with 5% (w/w) spray dried empty capsule without the addition of shrimp oil.

Data are expressed as mean±SD (n=3).

Lowercase letters in the same column within the same storage time indicate significant difference (p < 0.05).

Uppercase letters in the same column within the same sample indicate significant difference (p < 0.05).
was found in bread without MSO after 3 days. Among all the aldehydic compounds, benzaldehyde was found to be the major aldehyde in bread without MSO (0% MSO), followed by nonanal and decanal, respectively. Additionally, volatile ketones (dihydro-5-pentyl-2(3H)-furanone) and volatile alcohols (3-methyl-1-butanol, benzeneethanol, 2-methyl-1-propanol, 1-hexanol, 1-octen-3-ol and 1-octanol) were also found in bread without MSO. MAIRE et al. (2013) reported that flour appeared relatively rich in alcohols (3-methyl-1-butanol, 1-pentanol, 1-hexanol and 1-octen-3-ol). These compounds were also reported by HANSEN and HANSEN (1994) in flour with different millings, formed by either lipid oxidation or microorganism metabolism. 1-Octen-3-ol is a volatile generated from linoleic acid oxidation in the presence of singlet oxygen (LEE and MIN, 2010). This indicated that lipid oxidation took place in bread without MSO. MAIRE et al. (2013) reported that dough preparation seemed to be the crucial step toward lipid oxidation due to enzymes (lipoxygenase and lipase) as well as air inside the dough texture. Additionally, auto-oxidation could occur during baking by high temperatures, which promote an accelerated oxidation of ingredients in bread without MSO.

After storage, the formation of most volatile compounds in bread increased as the amount of MSO increased from none to 5%. Those compounds included 1-hexanol, nonanal, 1-octen-3-ol, 1-octanol, (Z)-3-decen-1-ol and benzeneethanol. However, 3-methyl-1-butanol decreased with increasing MSO. This could be due to volatilization. Benzaldehyde of sample added with 1 and 3% MSO showed the lower abundance than that without MSO. Benzaldehyde formed might bind with protein matrix of bread. Heptenal was also found in the sample added with 5% MSO. Higher abundance in nonanal and benzaldehyde was observed in the sample incorporated with 5% MSO, compared with others after 3 days of storage. Volatile compounds in bread added with 5% MSO were generally highest in abundance, compared with those added with others. TAKEUNGWONGTRA-KUL et al. (2014) reported that surface oil content of MSO prepared using whey protein concentrate: sodium caseinate: glucose syrup (1: 1: 2, w/w/w) as wall materials was 2.48% (w/w). Bread with higher level of MSO incorporated showed the higher amount of surface oil, which was more susceptible to oxidation. As a result, oxidation took place to a higher extent. Abundance of volatile compounds in all bread correlated well with the sensory property as shown in Table 3, in which bread added with 5% MSO had the lowest score of odor likeness. For bread added with 1% or 3% MSO and control bread, similar amount of volatile compounds was noticeable and no difference in sensory property of bread was observed (P > 0.05) (Table 3). Therefore, 3% MSO was the appropriate level to fortify in bread without negative effect on quality and acceptability.

### Table 4 - Volatile compounds in breads incorporated with MSO at different levels after storage of 3 days at 30°C.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Peak area (Abundance) × 10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>2-methyl-1-propanol</td>
<td>ND</td>
</tr>
<tr>
<td>3-methyl-1-butanol</td>
<td>1350</td>
</tr>
<tr>
<td>2-pentyl-furan</td>
<td>ND</td>
</tr>
<tr>
<td>3-hydroxy-2-butanoine</td>
<td>180</td>
</tr>
<tr>
<td>Heptanal</td>
<td>ND</td>
</tr>
<tr>
<td>1-hexanol</td>
<td>414</td>
</tr>
<tr>
<td>Nonanal</td>
<td>296</td>
</tr>
<tr>
<td>2-octen-1-ol</td>
<td>ND</td>
</tr>
<tr>
<td>1-octen-3-ol</td>
<td>102</td>
</tr>
<tr>
<td>Decanal</td>
<td>41</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>347</td>
</tr>
<tr>
<td>1-octanol</td>
<td>29</td>
</tr>
<tr>
<td>2-octen-1-ol</td>
<td>ND</td>
</tr>
<tr>
<td>2-furanmethanol</td>
<td>ND</td>
</tr>
<tr>
<td>(E)-6-nonen-1-ol</td>
<td>89</td>
</tr>
<tr>
<td>(Z)-3-decen-1-ol</td>
<td>58</td>
</tr>
<tr>
<td>Benzeneethanol</td>
<td>22</td>
</tr>
<tr>
<td>Benzeneethanol</td>
<td>795</td>
</tr>
<tr>
<td>3-hydroxy-2-methyl-4H-pyran-4-one</td>
<td>ND</td>
</tr>
<tr>
<td>Dihydro-5-pentyl-2(3H)-furanone</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: non-detectable

* Value in the parenthesis represents the abundance of compound in sample at 0 day.

Control: Added with 5% (w/w) spray dried empty capsule without the addition of shrimp oil.
CONCLUSION

MSO prepared using whey protein concentrate, sodium caseinate and glucose syrup (1:1:2, w/w/w) as wall materials could be fortified in bread product. Fortification of MSO had impact on the bread loaf volume, color and sensory properties. MSO up to 3% could be incorporated into bread to improve the nutritive value without affecting its sensorial properties. The fortified bread was quite stable up to 3 days of storage, in which no marked changes in color occurred and only slight increases in volatiles were obtained.

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