

CHARACTERISTICS AND OXIDATIVE STABILITY OF BREAD FORTIFIED WITH ENCAPSULATED SHRIMP OIL

SIRIMA TAKEUNGWONGTRAKUL^a, SOOTTAWAT BENJAKUL^{a*} and ARAN H-KITTIKUN^b

^aDepartment of Food Technology, Faculty of Agro-Industry,
Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand

^bDepartment of Industrial Biotechnology, Faculty of Agro-Industry,
Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand

*Corresponding author: Tel. +66 74 286334, Fax +66 74 558866,
email: soottawat.b@psu.ac.th

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) (TAKEUNGWONGTRAKUL *et al.*, 2012), (TAKEUNGWONGTRAKUL *et al.*, 2014a). The recommended daily intakes for linoleic acid, linolenic acid and long-chain n-3 PUFA are 4.4-20 g/d; 1.35-2.2 g/d and 0.16-1.6 g/d, respectively (MEYER *et al.*, 2003). Nevertheless, oil from hepatopancreas is very susceptible to oxidation, leading to undesirable off-odor (TAKEUNGWONGTRAKUL *et al.*, 2012). Rancidity is the major drawback for application of shrimp oil. Encapsulation of oil under the appropriate condition can be a promising means to extend its shelf-life. Encapsulation has appeared as a key technology in delaying or inhibiting oxidation and masking undesirable odor and flavor in the final product. The process involves the conversion of the oil into a free flowing powder, which can be easily handled and used for food fortification. Encapsulation can be defined as a process, in which tiny droplets, namely core, are surrounded by wall materials (GALLARDO *et al.*, 2013). Proteins and carbohydrates are frequently used as matrices for micro-encapsulation of lipophilic compounds by spray drying (GHARSALLAOUI *et al.*, 2007). TAKEUNGWONGTRAKUL *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 Botanicessence (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). Glucose syrup (Dextrose equivalent; 40-43) was purchased from Charoenworrakit Co., Ltd. (Samut Prakan, 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 homogeneous 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 homogeniser (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 (Lab-Plant 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 × 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-KOZAK *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^* , b^* and ΔE^* , representing lightness, redness/greenness, yellowness/blueness and total difference of color respectively. ΔE^* was also calculated using the following equation:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

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, FEI, Eindhoven, Netherlands) at an acceleration voltage of 15 kV with magnification of 2000 \times .

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 $^{\circ}$ -30 $^{\circ}$ 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 SPME 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 μ m DVB/CarboxenTM/PDMS StableFlexTM) (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 $^{\circ}$ 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 \pm 0.25 mm ID, with film thickness of 0.25 μ m). The GC oven temperature program was: 35 $^{\circ}$ C for 3 min, followed by an increase of 3 $^{\circ}$ C/min to 70 $^{\circ}$ C, then an increase of 10 $^{\circ}$ C/min to 200 $^{\circ}$ C, and finally an increase of 15 $^{\circ}$ C/min to a final temperature of 250 $^{\circ}$ 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).

RESULTS AND DISCUSSION

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). GÖKMEN *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

Table 1 - Loaf volume and textural at properties of breads incorporated with MSO at different levels at day 0 and 3 of storage.

Storage time (day)	MSO (% w/w)	Loaf volume (mL)	Hardness (g)	Springiness (mm)	Cohesiveness (g.mm)	Gumminess	Chewiness	Resilience
0	Control	296.06±9.36bA	1362.22±80.23aB	0.90±0.02aA	0.59±0.02aA	759.25±50.08aB	739.70±38.73aB	0.22±0.01bA
	0 %	269.25±10.84cA	1020.99±54.21bB	0.91±0.03aA	0.61±0.02aA	670.14±57.86abB	712.87±51.31aB	0.24±0.01aA
	1 %	315.22±10.47abA	1022.73±46.70bB	0.91±0.01aA	0.59±0.02aA	622.09±46.49bB	554.13±38.71bB	0.22±0.01bA
	3 %	317.67±9.13aA	938.58±65.57bB	0.90±0.02aA	0.60±0.03aA	610.30±42.41bB	480.51±37.57bcB	0.22±0.01bA
	5 %	317.47±8.86aA	927.85±69.48bB	0.90±0.03aA	0.60±0.01aA	503.91±42.57cA	466.17±47.67cB	0.22±0.02bA
3	Control	290.83±8.96bA	2454.22±84.41aA	0.89±0.02aA	0.50±0.02aB	1146.05±54.24aA	1034.57±56.96aA	0.19±0.01aB
	0 %	267.22±11.34cA	2158.52±49.27bA	0.90±0.01aA	0.52±0.02aB	1097.85±39.01aA	979.92±70.76aA	0.19±0.02aB
	1 %	312.17±8.86aA	1532.61±58.13cA	0.90±0.02aA	0.52±0.03aB	838.77±44.37bA	759.42±64.20bA	0.19±0.01aB
	3 %	313.89±10.37aA	1257.53±77.38dA	0.90±0.01aA	0.53±0.01aB	720.33±40.92cA	622.02±59.62cA	0.19±0.01aB
	5 %	313.33±5.77aA	1225.42±34.58dA	0.90±0.01aA	0.52±0.02aB	672.64±54.40cA	617.68±60.45cA	0.17±0.01aB

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 ($p < 0.05$).

Table 2 - Color of breads incorporated with MSO at different levels at day 0 and 3 of storage.

Storage time (day)	MSO (% w/w)	Crust color				Crumb color			
		L*	a*	b*	ΔE*	L*	a*	b*	ΔE*
0	Control	56.16±3.89bA	15.20±2.37bA	36.30±1.36abA	54.28±4.32bA	71.08±1.12aA	0.13±0.02dA	11.67±0.89dA	25.17±1.47eA
	0 %	69.13±2.98aA	9.76±1.12cA	32.12±1.07cA	41.91±1.56cdA	67.66±1.22bB	0.12±0.02dA	11.89±0.60dA	28.35±1.19dA
	1 %	67.88±3.46aA	12.28±1.08bA	35.38±1.06bA	45.42±2.65cA	64.53±1.56cB	2.15±0.41cA	13.72±1.42cA	32.08±1.91cA
	3 %	64.95±2.55aA	13.75±1.17bA	35.73±0.43bA	47.77±1.77cA	63.90±2.07cdB	7.19±0.68bA	18.92±0.91bA	35.89±0.76bA
	5 %	47.27±1.99cA	20.08±0.52aA	38.67±1.28aA	63.47±1.56aA	60.77±1.48dB	11.45±0.51aB	22.59±0.61aB	41.46±0.83aA
3	Control	56.68±3.95bA	14.38±2.26bA	36.31±1.42aA	53.69±0.91bA	72.98±1.09aA	0.12±0.01dA	12.63±1.76dA	23.97±1.36dA
	0 %	67.58±2.16aA	9.67±0.99cA	32.69±0.92cA	42.77±1.88dA	71.99±1.33aA	0.11±0.04dA	12.20±0.76dA	24.61±1.12cdB
	1 %	66.72±2.20aA	11.59±2.17bcA	35.72±1.27bA	46.09±1.09cA	70.94±1.69aA	2.27±0.33cA	13.90±1.28cA	26.55±1.19cB
	3 %	64.10±3.29aA	13.19±2.04bA	35.96±1.10bA	48.28±1.37cA	66.17±2.02bA	7.58±0.36bA	20.13±0.86bA	34.80±1.39bA
	5 %	46.36±0.57cA	20.44±0.69aA	38.54±0.97aA	64.32±0.41aA	65.11±1.54bA	13.34±0.50aA	26.12±0.99aA	40.90±0.69aA

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).

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 addition of MSO or spray dried empty capsule 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. 1 A. 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-



Fig. 1 - The photographs of breads incorporated with MSO at different levels. A) Bread crust, B) Bread crumb.

curred. 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 ΔE^* - 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 ΔE^* - 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%

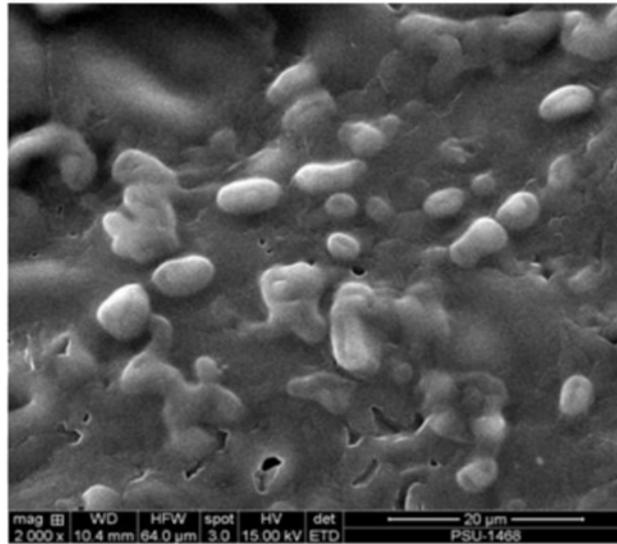
was incorporated, bread crumb had the lowest L^* - value but highest a^* -, b^* - and ΔE^* - values than others ($P < 0.05$). For control bread (with only spray dried empty capsule), a^* - and b^* - values of crumb were not different from those of bread without MSO ($P > 0.05$). Spray dried empty capsule 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^* -, b^* - and ΔE^* - values ($P > 0.05$). Nevertheless, crumb of bread incorporated with 5% MSO had the increases in a^* - and b^* - values. It was noted that those with 0 and 1% MSO had the decrease in ΔE^* - 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.

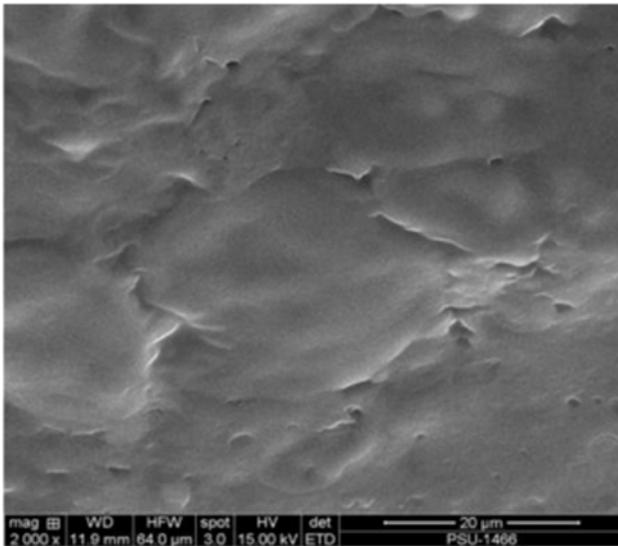
Microstructure

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-

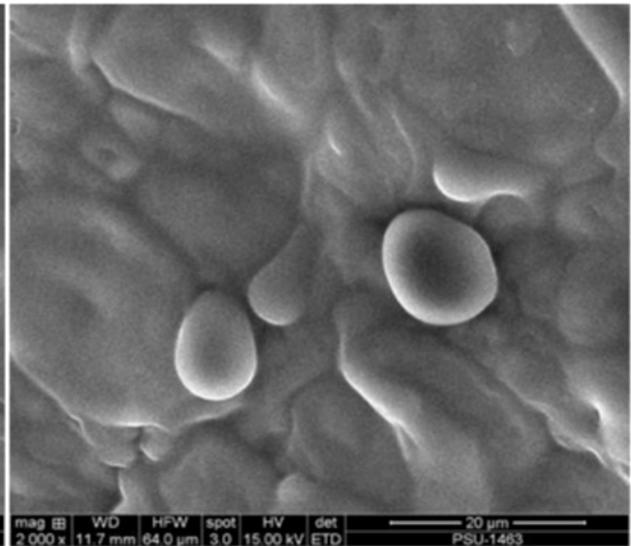
A) Control bread



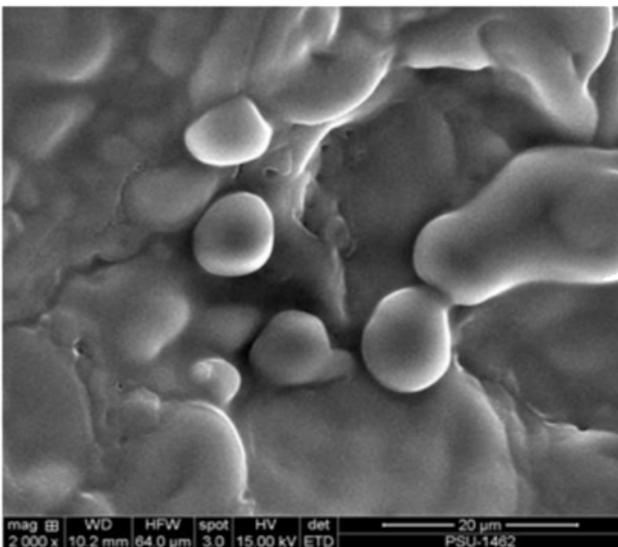
B) Bread with 0% (w/w) MSO



C) Bread with 1% (w/w) MSO



D) Bread with 3% (w/w) MSO



E) Bread with 5% (w/w) MSO

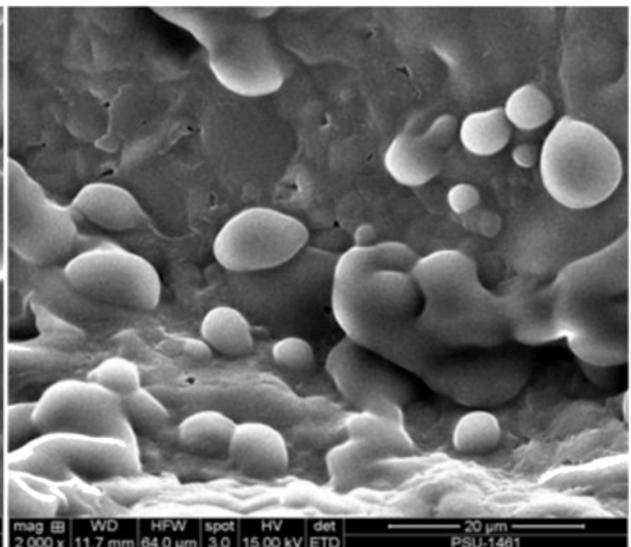


Fig. 2 - Surface morphology of breads incorporated with MSO at different levels. (Magnification: $\times 2000$).

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 (TAKEUNGWONGTRAKUL *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, crumb 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 pop-

ulation 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-al 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 (VARLET *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.

Storage time (day)	MSO (% w/w)	Crust color	Crumb color	Texture	Appearance	Odor	Overall likeness
0	Control	7.04±0.78aA	6.90±1.08aA	6.47±1.17aA	7.00±1.04aA	7.13±0.94aA	7.03±1.00aA
	0 %	6.92 ±0.65aA	6.75±1.16aA	6.70±0.76aA	6.79±0.92aA	7.00±0.94aA	6.90±0.82aA
	1 %	7.04±0.81aA	6.90±0.90aA	7.03±1.27aA	7.07±1.16aA	6.93±0.82aA	6.83±0.95aA
	3 %	7.31±0.89aA	7.10±0.90aA	7.00±0.98aA	6.93±1.00aA	6.97±0.88aA	6.80±0.77aA
	5 %	6.67±0.88aA	5.54±1.12bA	6.97±1.37aA	6.63±1.36aA	6.47±0.95aA	5.63±1.12bA
3	Control	6.94±0.89aA	6.90±1.24aA	6.04±0.91aA	7.00±0.89aA	6.52±1.23aA	6.44±0.93aA
	0 %	6.72±0.96aA	6.74±0.96aA	6.38±1.10aA	7.00±1.21aA	6.58±1.31aA	6.36±0.91aA
	1 %	6.83±1.33aA	6.87±1.41aA	6.74±0.90aA	7.06±1.21aA	6.56±1.01aA	6.28±0.96aA
	3 %	7.00±0.80aA	7.00±0.91aA	6.70±0.95aA	6.74±1.21aA	5.96±0.75aA	6.14±1.09aA
	5 %	6.66±1.14aA	5.43±1.07bA	6.60±1.22aA	6.39±1.36aA	5.16±0.94bA	5.36±0.99bA

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 ($p < 0.05$).

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

Compounds	Peak area (Abundance) × 10 ⁶				
	Control	0% MSO	1% MSO	3% MSO	5% MSO
2-methyl-1-propanol	ND	424 (309)	414	388	264
3-methyl- 1-butanol	1350	1383 (1406)	1293	1264	1255
2-pentyl-furan	ND	ND (128)	ND	ND	ND
3-hydroxy-2-butanone	180	ND	ND	ND	168
Heptenal	ND	ND (74)	ND	ND	118
1-hexanol	414	302 (253)	402	426	441
Nonanal	296	207 (122)	303	366	414
2-octen-1-al	ND	ND (33)	ND	ND	ND
1-octen-3-ol	102	89 (63)	133	176	226
Decanal	41	40 (ND)	26	78	83
Benzaldehyde	347	450 (354)	285	274	477
1-octanol	29	35 (33)	48	52	53
2-octen-1-ol	ND	ND	21	17	20
2-furanmethanol	ND	ND	ND	152	220
(E)-6-nonen-1-ol	89	ND	102	119	ND
(Z)- 3-decen-1-ol	58	ND	59	67	135
Benzenemethanol	22	ND	ND	ND	44
Benzeneethanol	795	716 (683)	728	856	1437
3-hydroxy-2-methyl-4H-pyran-4-one	ND	ND	ND	ND	50
Dihydro-5-pentyl-2(3H)-furanone	ND	19 (15)	23	30	20

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.

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 benze-

neethanol. 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. TAKEUNGWONGTRAKUL *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.

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.

ACKNOWLEDGEMENT

"This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission". The TRF Distinguished Research Professor Grant and Prince of Songkla University were also acknowledged.

REFERENCES

- Borneo R., Kocer D., Ghai G., Tepper B. and Karwe M. 2007. Stability and consumer acceptance of long-chain omega-3 fatty acids (eicosapentaenoic acid, 20: 5, n-3 and docosahexaenoic acid, 22: 6, n-3) in cream-filled sandwich cookies. *J. Food Sci.* 72: S049-S054.
- Carr B.T., Meilgaard M. and Civille G. 1999. Sensory evaluation techniques. CRC Press, Washington, DC.
- Chang H.J., Xu X.L., Zhou G.H., Li C.B. and Huang M. 2012. Effects of characteristics changes of collagen on meat physicochemical properties of beef semitendinosus muscle during ultrasonic processing. *Food Bioproc. Technol.* 5:285-297.
- Cleary L.J., Andersson R. and Brennan C.S. 2007. The behaviour and susceptibility to degradation of high and low molecular weight barley β -glucan in wheat bread during baking and *in vitro* digestion. *Food Chem.* 102:889-897.
- Conforti F.D. and Davis S.F. 2006. The effect of soya flour and flaxseed as a partial replacement for bread flour in yeast bread. *Int. J. Food Sci. Technol.* 41:95-101.
- Ezhilarasi P.N., Indrani D., Jena B.S. and Anandharamakrishnan C. 2014. Microencapsulation of Garcinia fruit extract by spray drying and its effect on bread quality. *J. Sci. Food Agric.* 94:1116-1123.
- Gallardo G., Guida L., Martinez V., López M.C., Bernhardt D., Blasco R., Pedroza-Islas R. and Hermida L.G. 2013. Microencapsulation of linseed oil by spray drying for functional food application. *Food Res Int.* 52:473-482.
- Gharsallaoui A., Roudaut G., Chambin O., Voilley A. and Saurel R. 2007. Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Res Int.* 40:1107-1121.
- Gökmen V., Mogol B.A., Lumaga R.B., Fogliano V., Kaplun Z. and Shimoni E. 2011. Development of functional bread containing nanoencapsulated omega-3 fatty acids. *J Food Eng.* 105:585-591.
- Hansen Å. and Hansen B. 1994. Influence of wheat flour type on the production of flavour compounds in wheat sourdoughs. *J. Cereal Sci.* 19:185-190.
- Henna Lu F.S. and Norziah M.H. 2011. Contribution of microencapsulated n-3 PUFA powder toward sensory and oxidative stability of bread. *J. Food Proc. Pres.* 35:596-604.
- Jiamyangyuen S., Srijesdaruk V. and Harper W.J. 2005. Extraction of rice bran protein concentrate and its application in bread. *Songklanakar J. Sci. Technol.* 27:55-64.
- Krupa-Kozak U., Altamirano-Fortoul R., Wronkowska M. and Rosell C.M. 2012. Breadmaking performance and technological characteristic of gluten-free bread with inulin supplemented with calcium salts. *Eur. Food Res. Technol.* 235:545-554.
- Lee J. and Min D.B. 2010. Analysis of volatile compounds from chlorophyll photosensitized linoleic acid by head-space solid-phase microextraction (HS-SPME). *Food Sci. Biotechnol.* 19:611-616.
- Maire M., Rega B., Cuvelier M.E., Soto P. and Giampaoli P. 2013. Lipid oxidation in baked products: Impact of formula and process on the generation of volatile compounds. *Food Chem.* 141:3510-3518.
- Meyer B.J., Mann N.J., Lewis J.L., Milligan G.C., Sinclair A.J. and Howe P.R. 2003. Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. *Lipid.* 38: 391-398.
- Nunes M., Ryan L.A.M. and Arendt E. 2009. Effect of low lactose dairy powder addition on the properties of gluten-free batters and bread quality. *Eur. Food Res. Technol.* 229:31-41.
- Purlis E. and Salvadori V.O. 2009. Modelling the browning of bread during baking. *Food Res. Inter.* 42:865-870.
- Ross C.F. and Smith D.M. 2006. Use of volatiles as indicators of lipid oxidation in muscle foods. *Compr. Rev. Food Sci. Food Saf.* 5:18-25.
- Shahidi F. and Pegg R.B. 1994. Hexanal as an indicator of the flavor deterioration of meat and meat products. In "Lipid oxidation in food". A.J. Angelo (Ed.), p 105-19. American Chemical Society., Washington, D.C.
- Shahidi F. and Wanasundara U.N. 1995. Oxidative stability of encapsulated seal blubber oil. In "Flavor Technology-Physical Chemistry, Modification, and Process". C.T. Ho, C.T. Tan and C.H. Tong (Eds.), pp. 139-151. American Chemical Society., Washington, DC.
- Steel, R. G. D. and Torrie, J. H. 1980. "Principles and Procedures of Statistics" 2nd ed. A Biometrical Approach, McGraw-Hill, NY.
- Takeungwongtrakul S. and Benjakul S. 2013. Oxidative stability of shrimp oil-in-water emulsions as affected by antioxidant incorporation. *Int. Aquat Res.* 5:1-12.
- Takeungwongtrakul S., Benjakul S. and H-kittikun A. 2012. Lipids from cephalothorax and hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*): Compositions and deterioration as affected by iced storage. *Food Chem.* 134:2066-2074.
- Takeungwongtrakul S., Benjakul S., Santoso J., Trilaksani W. and Nurilmala M. 2014a. Extraction and stability of carotenoid-containing lipids from hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*). *J. Food Proc. Pres.* 39:10-18.
- Takeungwongtrakul S., Benjakul S. and H-kittikun A. 2015. Wall materials and the presence of antioxidants influence encapsulation efficiency and oxidative stability of micro-encapsulated shrimp oil. *Eur. J. Lipid Sci. Technol.* 117: 450-459.
- Varlet V., Knockaert C., Prost C. and Serot T. 2006. Comparison of odor-active volatile compounds of fresh and smoked salmon. *J. Agric. Food Chem.* 54:3391-3401.
- Wehrle K., Grau H. and Arendt E.K. 1997. Effects of lactic acid, acetic acid, and table salt on fundamental rheological properties of wheat dough. *Cereal Chem. J.* 74:739-744.