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ORIGINAL ARTICLE

Biscuits fortified with micro-encapsulated shrimp oil: characteristics and storage stability

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Abstract Characteristics and storage stability of biscuits fortified with micro-encapsulated shrimp oil (MSO) were determined. The addition of MSO increased spread ratio, whilst decreased the thickness of biscuit. The highest hardness of biscuit was obtained with addition of 9 or 12% MSO. Biscuit surface showed higher redness and yellowness when MSO was incorporated ($p < 0.05$). The addition of MSO up to 6% had no adverse effect on biscuit quality and acceptability. When biscuits added with 6% MSO were stored under different illumination conditions (light and dark), lipid oxidation in all samples increased throughout the storage of 12 days. Light accelerated lipid oxidation of biscuits as evidenced by the increases in both peroxide values and abundance of volatile compounds. No marked change in EPA, DHA and astaxanthin contents were noticeable in biscuit fortified with MSO after 12 days of storage. Therefore, the biscuit could be fortified with MSO up to 6% and must be stored in dark to assure its oxidative stability.

Keywords Shrimp oil · Micro-encapsulation · Biscuit · Lipid oxidation · Storage

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Introduction

Food byproducts are considered as a cheap source of valuable components. The existent technologies allow the recovery of target compounds and the development of new products with a market value (Galanakis 2012). With increasing demand for health-oriented products with high fibre, antioxidant as well as other active components, a number of new products supplemented with health promoted ingredients have been developed. High fibre sponge cakes added with cabbage leaf powder was developed by Prokopov et al. (2015).

Lipids from hepatopancreas, a byproduct generated from the manufacturing of hepatopancreas-free whole shrimp, are rich in polyunsaturated fatty acids (PUFAs) and astaxanthin, known as health benefit. Shrimp oil from hepatopancreas contained linoleic acid as the most abundant fatty acid, followed by oleic acid. Additionally, shrimp oil also contained eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Takeungwongtrakul et al. 2012). Despite high nutritive value, the use of shrimp oil in food products is limited owing to the high susceptibility of PUFAs to oxidation. As a consequence, off-flavour compounds as well as toxic products (Ayala et al. 2014) are formed. To prevent such a deterioration, micro-encapsulation can be as a key technology in delaying or inhibiting oxidation and masking undesirable odours and flavours in the final product (Tonon et al. 2011). In addition, it is a useful tool to prevent non-functional interactions with food matrix during their utilization as additives and improve their delivery into foods (Galanakis 2012). Encapsulated oil, a free flowing powder, can be easily handled and used for food fortification. Fortification of highly nutritive ingredients such as PUFAs rich oil, etc. is gaining the interest for food industry. The bakery industry is one of the

largest organized food industries all over the world. Due to the competition, the incorporation of different nutritive ingredients can yield the diversification of bakery products. Biscuits are the most popular bakery products because of their convenience, ready to eat nature, affordable cost, availability in different tastes and long shelf-life (Gandhi et al. 2001). The fortification of micro-encapsulated shrimp oil (MSO) rich in PUFAs and astaxanthin could increase the nutritive value of biscuits. However, the biscuit added with MSO might be susceptible to oxidation associated with off-odours and off-flavours. To extend the shelf-life, an appropriate storage condition is required to retard the deterioration of biscuits during storage. Factors promoting oxidation such as prooxidant, light, etc. must be excluded. Nevertheless, no information regarding the fortification of MSO in biscuit has been reported. The objectives of this study were to investigate the effects of MSO fortification on the characteristics and sensory property and to study the impact of illumination on quality of biscuit during storage.

Materials and methods

Chemicals

Astaxanthin was obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium caseinate was procured from Vicchi enterprise Co., Ltd. (Bangkok, Thailand). Glucose syrup (Dextrose equivalent; 40–43) was purchased from Charoenworrakit Co., Ltd. (Samut Prakan, Thailand). Fish skin gelatin (GEL) with bloom strength of 230–250 g was obtained from Lapi Gelatine S.p.A. (Milano, Italy). Wheat flour, sugar, salt, unsalt butter, milk and baking power were procured from a local market in Hat Yai, Songkhla, Thailand.

Preparation of micro-encapsulated shrimp oil (MSO)

Hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*) obtained from the Sea Wealth frozen food Co., Ltd., Songkhla, Thailand was used for oil extraction. Isopropanol/hexane mixture was used as a solvent following the method of Takeungwongtrakul et al. (2015b).

Aqueous stock mixed solution of sodium caseinate, fish gelatin and glucose syrup at a ratio of 1: 1: 4 (w/w/w) in deionised water was prepared and used as "wall materials". The mixtures were homogenised, followed by being passed through a microfluizdics homogenizer (Model HC-5000, Microfluidizer, Newton, MA, USA) at a pressure level of 4,000 psi for four passes (Takeungwongtrakul et al. 2015a).

The prepared emulsions were 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 × 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 MSO was collected in the amber bottle and capped tightly. MSO contained 17.89 ± 1.05% shrimp oil.

Fortification of MSO in biscuits

Biscuits were prepared with the following formulation: wheat flour (95 g), sugar (8.5 g), salt (1.5 g), unsalt butter (57 g), milk (84 g) and baking powder (4 g). Dry ingredients were mixed uniformly. Milk was added and kneaded gently using a hand to form dough. Thereafter, MSO was directly added to dough at different levels (0, 3, 6, 9 and 12%, w/w). Additionally, shrimp oil with the amount equivalent to that found in 12% MSO was added to dough. Biscuit incorporated with 12% (w/w) spray dried wall material (without the addition of shrimp oil) was used as the control. The dough was kneaded for another 10 min. Dough was sheeted using a rolling pin to a thickness of 3.0 mm. Biscuits were shaped with a cutter of 51-mm diameter and baked on aluminum trays at 205 °C for 20 min in a baking oven (YXD-20, Guandzhou Xinnanfang electro-thermal equipment Co., Ltd., Guandzhou, China). Biscuits were cooled for 30 min at room temperature. Different samples (40 g) were packed in polypropylene bag and heat-sealed. The biscuit samples were subjected to analyses.

Characterization of biscuits fortified with MSO

Determination of moisture content

Moisture content was determined by oven method (AOAC 2000).

Determination of diameter, thickness and spread ratio

Diameter and thickness of biscuits were measured with a vernier caliper and reported in centimeters. Spread ratio was calculated by dividing the average value of diameter by average value of thickness of biscuits (Akubor and Ukwuru 2003).

Measurement of hardness

Hardness of biscuits was determined by a texture analyser (Stable Micro Systems, Godalming, Surrey, UK) using a

test speed of 0.5 mm/s and a distance of 2 mm with a load cell of 5 kg. The maximum force required to break biscuits individually was calculated for each sample.

Colour measurement

The colour of surface of samples was determined using a colourimeter (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 colour respectively. ΔE* was also calculated as described by Takeungwongtrakul et al. (2015a).

Sensory evaluation

Sensory evaluation was performed by 50 untrained panellists with ages ranging from 20 to 35 years, who were familiar with the consumption of biscuits. Panellists were asked to evaluate for appearance, colour, odour, texture, taste and overall likeness 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. The samples were labeled with random three-digit codes. Panellists were instructed to rinse their mouth with water after each sample evaluation. The order of presentation of the samples was randomized according to "balance order and carry-over effects design" (Meilgaard et al. 2006).

Oxidative stability of biscuits during storage as affected by illumination

The biscuits fortified without and with 6% MSO were packed in the polypropylene bag and heat-sealed. Packaged biscuit samples were placed under different illumination conditions (dark and light, 600 lx/cm^2) at room temperature (28–30 °C). The samples were taken at day 0, 3, 6, 9 and 12 for analyses.

Peroxide value (PV)

PV was determined in oil extracted from the biscuits using the Bligh and Dyer method (Bligh and Dyer 1959). PV was determined using the ferric thiocyanate method (Chaijan et al. 2006). A standard curve was prepared using cumene hydroperoxide with the concentration range of 0.5–2 ppm. PV was expressed as mg cumene hydroperoxide/kg oils.

Volatile compounds

The volatile compounds in the biscuit samples were determined at day 0 and 12 of storage using a solid-phase microextraction gas chromatography mass spectrometry (SPME GC–MS) following the method of Intarasirisawat et al. (2015). Volatiles were expressed as the abundance (peak area).

Fatty acid profile

Fatty acid profile in oil extracted from the biscuits was determined at day 0 and 12 of storage as fatty acid methyl esters (FAMEs), which were prepared according to the method of AOAC (2000). FAMEs were injected to the gas chromatography (Shimadzu, Kyoto, Japan) equipped with the flame ionisation detector (FID) at a split ratio of 1:20. A fused silica capillary column (30 m × 0.25 mm), coated with bonded polyglycol liquid phase, was used. The analytical conditions were: injection port temperature of 250 °C and detector temperature of 270 °C. The oven was programmed from 170 to 225 °C at a rate of 1 °C/min (no initial or final hold). Retention times of FAME standards were used to identify chromatographic peaks of the samples. Fatty acid content was calculated, based on the peak area ratio and expressed as g fatty acid/100 g lipid.

Astaxanthin content

Astaxanthin was determined by high performance liquid chromatography (LC-20A, Shimadzu, Kyoto, Japan) equipped with a 250 × 4.6 mm, 5 μm Microsorb-MV C_{18} reversed-phase column, using photodiode array detector at 474 nm. The isocratic eluent consisted of 100% methanol with a flow rate of 0.8 mL/min. Astaxanthin (0.06–0.0003 mg/mL) was prepared in chloroform and used as the external standard.

Statistical analysis

Experiments were run in triplicate using three different lots of samples. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range test. Statistical analysis was performed using the statistical package for social science (SPSS for windows, SPSS Inc, Chicago, IL, USA).

Results and discussion

Characteristics of biscuits fortified with MSO

Moisture content

Moisture contents of all samples were in the range of 3.12–5.59% (w/w) (Table 1), which was within the typical

Table 1 Moisture content, diameter, thickness, spread ratio and hardness of biscuits incorporated with MSO at different levels

MSO (%w/w)	Moisture content (w/w)	Diameter (cm)	Thickness (cm)	Spread ratio	Hardness (kg force)
Shrimp oil	5.59 ± 0.61a	4.32 ± 0.17a	0.39 ± 0.06b	11.08 ± 0.65a	1.01 ± 0.11d
Control	3.71 ± 0.10bc	4.33 ± 0.25a	0.39 ± 0.03b	11.10 ± 0.71a	2.72 ± 0.49a
0%	3.12 ± 0.08c	4.34 ± 0.30a	0.52 ± 0.10a	8.35 ± 1.46b	1.76 ± 0.26c
3%	3.54 ± 0.16bc	4.39 ± 0.25a	0.50 ± 0.07a	8.78 ± 0.20b	1.79 ± 0.45c
6%	3.53 ± 0.14bc	4.39 ± 0.18a	0.48 ± 0.06a	9.15 ± 0.23b	1.83 ± 0.46c
9%	4.16 ± 0.29b	4.38 ± 0.24a	0.40 ± 0.03b	10.95 ± 0.41a	2.04 ± 0.46bc
12%	5.31 ± 0.38a	4.38 ± 0.20a	0.40 ± 0.03b	10.95 ± 0.36a	2.21 ± 0.26b

Shrimp oil = Added with shrimp oil at amount equivalent to that found in 12% MSO

Control = Added with 12% (w/w) wall material powder without the addition of MSO

Data are expressed as mean ± SD (n = 3)

Lowercase letters in the same column indicate significant difference ($p < 0.05$)

range (1–5%) of freshly baked biscuits (Robertson 2012). Biscuits have very low moisture content and the majority of water lies in a thin lamella of material near the centre. The surface and the outer periphery of biscuits are nearly dry (Mamat et al. 2010). Moisture content of biscuits incorporated with spray dried wall material powder (control biscuit) was not different from that of biscuits containing MSO at level of 0–9% (w/w) ($p > 0.05$). Nevertheless, higher moisture content was found in biscuits added with 12% MSO or with shrimp oil, compared with others ($p < 0.05$). With increasing amount of MSO added, the wall materials, especially glucose syrup, which are hygroscopic in nature, could absorb more water. This was indicated by higher moisture content of the obtained biscuit, particularly that added with 12% MSO. Furthermore, oil in free form or encapsulated form might act as the barrier for water migration during baking. The remaining water has been reported to determine storage stability of biscuits. Hydrolytic and enzymatic reactions could occur in biscuit at very low moisture content (Romani et al. 2015).

Diameter, thickness and spread ratio

Diameter, thickness and spread ratio of biscuits are presented in Table 1. There were no differences in the diameter amongst all biscuit samples ($p > 0.05$). Biscuits added with MSO at high levels, 9 and 12% (w/w), had lower thickness than those containing MSO at lower levels (0–6%; $p < 0.05$). Sudha et al. (2014) reported that biscuit thickness decreased when the levels of encapsulated oil containing sodium caseinate as wall material increased. For the control biscuit, the decrease in thickness was also noticeable. The result suggested that sodium caseinate, fish gelatin and glucose syrup used as wall material had the influence on thickness of biscuits. Spread ratio value increased with increasing levels of MSO ($p < 0.05$). Additionally, biscuits incorporated with shrimp oil and spray dried wall material powder showed the higher spread ratio than that incorporated with 0–6% MSO ($p < 0.05$). Sudha et al. (2014) reported that the thickness and spread ratio of the biscuits varied with native oil or encapsulated oil. Spread ratio is affected by the competition of ingredients for the available water and flour or any other ingredient, which absorbs water during dough mixing (Fuhr 1962). MSO, oil or dried wall material might reduce the cohesion between ingredients in dough, as indicated by the increased spread ratio. When oil content in the biscuits increased, spread ratio of biscuits increased, whereas thickness decreased (Ahmad and Ahmad 2014). The presence of oil in the dough shows an advantage in biscuit-making because it prevents the dough from shrinking after sheeting. Increasing oil content of biscuit generally leads to higher spread ratio, which has a positive effect on the final biscuit quality (Sudha et al. 2007).

Hardness

Biscuits fortified with MSO at 0–9% (w/w) had no difference in hardness value ($p > 0.05$) (Table 1). However, biscuits incorporated with 9% MSO showed similar hardness to that containing 12% MSO ($p > 0.05$). The control biscuit had the highest hardness value, whilst that containing shrimp oil showed the lowest value ($p < 0.05$). The result indicated that the addition of spray dried wall material powder or MSO at high level increased the hardness of biscuits. The proteins in spray dried wall material powder might be distributed more uniformly and strengthened the structure of biscuit more efficiently. Umesha et al. (2015) reported that the hardness value of biscuits might depend on the porous nature of the biscuits. When shrimp oil was added directly, the marked decrease in hardness was found. High moisture content was related with the decrease hardness. Furthermore, oil droplets might lower interaction of biscuit dough, thereby lowering the hardness.

Colour

Colour of biscuit surface was affected by the amount of MSO added as shown in Table 2. Biscuit surface had the decrease in L^*-value as the level of MSO was above 6% ($p < 0.05$). The lowest L^*-value was found in sample added with 12% MSO and the control sample ($p < 0.05$). For a^*-value, biscuit fortified with shrimp oil or 12% MSO had the highest values ($p < 0.05$). Shrimp oil was reddish orange in colour due to the presence of astaxanthin (Takeungwongtrakul et al. 2015b). As a result, the biscuit surface turned to be more orange in colour, when either shrimp oil or 12% MSO was added. The increases in b^*- and ΔE^*-values of biscuit were also found as the amount of MSO increased ($p < 0.05$). Surface oil and oil released to the surface of MSO during biscuit making might contribute to the increases in b^*- and ΔE^*-values. When comparing b^*- and ΔE^*-values, the sample added with shrimp oil had the higher value than that containing 12% MSO ($p < 0.05$). Wall of MSO might mask the colour of shrimp oil to some degree. Control biscuit had the higher a^*-value than biscuits incorporated with 0 and 3% MSO ($p < 0.05$). b^*- and ΔE^*-values of control biscuit were not different from those of biscuit added with up to 6% MSO ($p > 0.05$). The development of colour in baked goods is the result of two simultaneously occurring processes, the Maillard reaction where sugars interact with amino acids and caramelisation which is a direct degradation of sugars. Parate et al. (2011) reported that the colour of biscuits is mostly attributed to Maillard reaction that produces coloured compounds during baking. Proteins and reducing compounds in syrup in spray dried wall material powder could serve as the reactants, especially for browning reaction. Takeungwongtrakul et al. (2015a) reported that the colour of bread crust might depend on non-enzymatic chemical reactions and astaxanthin released from MSO during baking.

Sensory property

Appearance, colour, odour, texture, taste and overall likeness scores of all biscuit samples added with different amounts of MSO are shown in Table 3. There were no differences in appearance and colour likeness scores amongst all biscuit samples ($p > 0.05$). Odour likeness of biscuits added with 12% MSO or shrimp oil was lower than others ($p < 0.05$). The result indicated that biscuits added with MSO up to 9% (w/w) had no change in odour likeness. However, the addition of 12% MSO might result in the increased free oil, especially at the surface of MSO. This led to more free oil associated with off-odour. Biscuit incorporated with 0, 3 and 6% MSO had the highest texture score ($p < 0.05$). Biscuit fortified with MSO at the level higher than 6% (w/w) had the decreases in texture likeness score. It was noted that texture likeness scores of biscuit added with 9 and 12% MSO were not different from those of biscuits containing wall material powder or shrimp oil ($p > 0.05$). Texture likeness was more likely related with hardness value (Table 1), in which the addition of shrimp oil softened the biscuit. For taste likeness, MSO and wall material powder had no effect on taste likeness of biscuits ($p > 0.05$). Biscuit added with shrimp oil had slightly lower taste likeness score than others ($p < 0.05$). This was possibly due to typically fishy taste of shrimp oil. However, there was no difference in taste likeness score of biscuit incorporated with 9–12% MSO and that of sample added with shrimp oil ($p > 0.05$). For overall likeness scores, biscuits added with MSO up to 6% showed the similar score to the biscuit without addition of MSO or shrimp oil ($p < 0.05$). Biscuit incorporated with MSO above 6% showed the decreases in overall likeness score ($p < 0.05$). Biscuit fortified with shrimp oil and wall material powder had the lower overall likeness score than others ($p < 0.05$), more likely related with poor texture and odour. MSO up to 6% (w/w) could be added into biscuit to improve the

Table 2 Colour of biscuits incorporated with MSO at different levels

MSO (%w/w)	L^*	a^*	b^*	ΔE^*
Shrimp oil	73.56 ± 1.03b	19.47 ± 0.48a	39.76 ± 0.75a	49.10 ± 0.80a
Control	71.42 ± 0.96d	12.73 ± 0.52d	34.88 ± 0.40d	43.25 ± 0.84d
0%	74.49 ± 1.07a	10.32 ± 0.75f	33.94 ± 0.66f	40.18 ± 1.00f
3%	75.11 ± 1.52a	12.11 ± 0.79e	34.73 ± 0.52e	41.16 ± 1.33e
6%	74.51 ± 0.93a	13.39 ± 0.37c	36.00 ± 0.92d	42.87 ± 1.24d
9%	72.82 ± 1.29c	15.86 ± 0.76b	37.40 ± 0.71c	45.68 ± 1.24c
12%	71.60 ± 0.66d	19.03 ± 0.54a	39.15 ± 0.61b	48.28 ± 0.68b

Shrimp oil = Added with shrimp oil at amount equivalent to that found in 12% MSO

Control = Added with 12% (w/w) wall material powder without the addition of MSO

Data are expressed as mean ± SD (n = 3)

Lowercase letters in the same column indicate significant difference ($p < 0.05$)

Table 3 Likeness score of biscuits incorporated with MSO at different levels

MSO (%w/w)	Appearance	Colour	Odour	Texture	Taste	Overall likeness
Shrimp oil	7.19 ± 1.26a	7.03 ± 1.23a	6.03 ± 1.64b	5.03 ± 2.09bc	5.38 ± 1.53b	5.12 ± 1.42d
Control	6.19 ± 1.65a	6.59 ± 1.48a	7.03 ± 1.22a	5.19 ± 2.02bc	6.34 ± 1.81a	5.52 ± 1.42d
0%	6.13 ± 1.64a	6.13 ± 1.70a	7.23 ± 1.15a	6.81 ± 1.82a	7.22 ± 0.94a	7.20 ± 1.04a
3%	6.27 ± 1.68a	6.59 ± 1.58a	7.16 ± 1.00a	6.47 ± 1.63a	7.03 ± 0.90a	7.12 ± 0.88ab
6%	6.35 ± 1.64a	6.84 ± 1.25a	6.71 ± 1.01a	6.63 ± 1.41a	6.45 ± 1.86a	6.68 ± 1.31abc
9%	6.28 ± 1.57a	6.75 ± 1.05a	6.71 ± 1.04a	5.63 ± 1.98b	6.43 ± 0.99ab	6.56 ± 0.71bc
12%	7.06 ± 1.32a	7.00 ± 1.41a	5.71 ± 1.62b	4.50 ± 2.34c	5.42 ± 1.54ab	6.24 ± 1.23c

Shrimp oil = Added with shrimp oil at amount equivalent to that found in 12% MSO

Control = Added with 12% (w/w) wall material powder without the addition of MSO

Data are expressed as mean ± SD (n = 3)

Lowercase letters in the same column indicate significant difference ($p < 0.05$)

nutritive values of biscuit without the negative effect on sensory property.

Oxidative stability of biscuits incorporated with MSO during storage

Peroxide value (PV)

PV of biscuits fortified with 0 and 6% MSO under different illumination conditions (dark and light) during 12 days of storage is presented in Fig. 1. At day 0 of storage, biscuits fortified with 0% MSO (without MSO) had the lower PV than those fortified with 6% MSO, regardless of illumination conditions ($p < 0.05$). Primary oxidation products in shrimp oil might contribute to higher PV. Wall materials of MSO might be broken during kneading and sheeting of biscuits, thus releasing shrimp oil into biscuit dough. Additionally, high temperature during baking could accelerate lipid oxidation, especially in biscuit containing MSO. Caponio et al. (2008) reported that kneading and baking stage caused a decrease in PUFAs and a concomitant increase in oxidised triglyceride. During the storage, biscuits added with 6% MSO had higher PV than that without MSO for both illumination conditions ($p < 0.05$). Biscuit without MSO addition had slight increase in PV and there were no differences in PV between samples stored in dark and under light throughout the storage ($p > 0.05$). The results indicated that samples without MSO exhibited a slower rate of hydroperoxide formation. Biscuit added with 6% MSO had the higher PV, compared with those without MSO addition during storage of 12 days. The higher increase in PV was observed when the sample was stored under light throughout 12 days of storage ($p < 0.05$). During the storage, shrimp oil could be released to the surface of MSO. Shrimp oil is one of the important sources of PUFAs and astaxanthin

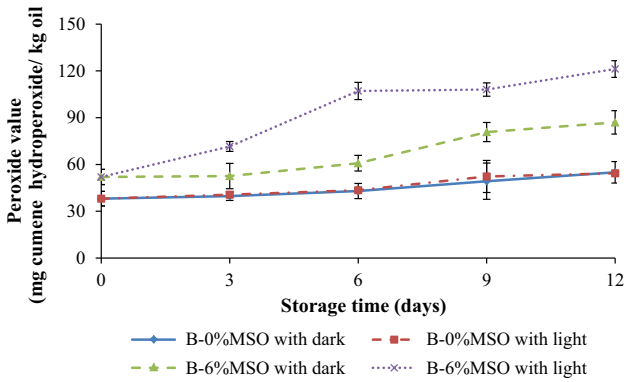

Fig. 1 Peroxide values of oil extracted from biscuits incorporated without and with 6% MSO stored under different illumination conditions during the storage of 12 days at 30 °C. *Bars* represent SD (n = 3)

(Takeungwongtrakul et al. 2012). PUFAs and astaxanthin could be prone to oxidation. The increase in PV of all samples indicated that the samples were in propagation stage of lipid oxidation with a lower rate of decomposition of hydroperoxides formed. Lipid hydroperoxides are formed by various pathways including the reaction of singlet oxygen with unsaturated fatty acids or the lipoxygenase-catalysed oxidation of PUFAs (Nawar 1996). The oxidation of oil in biscuits creates a variety of compounds including free radicals and hydroperoxides. Schaich et al. (2013) reported that visible light lacks the energy to produce radicals directly, but it can initiate lipid oxidation indirectly through photosensitisers that absorb low-level light energy, and transform it to chemical energy sufficient to drive reactions. Astaxanthin is sensitive to photodegradation (Niamnuy et al. 2008). The radicals generated more likely induced lipid oxidation in biscuit to a higher extent in the presence of light. The result indicated that the absence of light could increase the oxidative stability of biscuits fortified with MSO.

Fatty acid profile

Fatty acid profiles of oils extracted from biscuits without MSO (B-0%MSO) and added with 6% MSO (B-6%MSO) under different illumination conditions at day 0 and 12 of storage are shown in Table 4. At 0 day, oils from B-0%MSO and B-6%MSO contained lauric acid (C12:0) palmitic acid (C16:0) and myristic acid (C14:0) as the most abundant saturated fatty acids. Cis-9-octadecenoic acid (C18:1(n−9)) was the major monounsaturated fatty acid. Oil from B-0%MSO had the higher contents of lauric acid, palmitic acid and myristic acid, but possessed the lower contents of cis-9-octadecenoic acid than oil from B-6%MSO. Trans-elaodic acid (C:18:1(n−9t)), cis-9,12-octadecatrienoic acid (C:18:2(n−6)), cis-11,14-eicosadienoic acid (C20:2(n−6)), cis-5,8,11,14-eicasatetraenic acid (arachidonic acid, ARA; C20:4(n−6)), EPA (C20:5(n−3)), behenic acid (C22:0) and DHA (C22:6(n−3)) were found in oil from B-6%MSO. B-6%MSO generally contained more unsaturated fatty acids than B-0%MSO. B-6%MSO contained 0.03%DHA and 0.01%EPA. Both EPA and DHA were mainly from MSO. Chaijan et al. (2006) reported that DHA is usually more abundant than EPA (up to 2–3 times) in marine lipids. The n−3 fatty acids in biscuit could be enriched by incorporation of MSO.

When B-0%MSO and B-6%MSO samples were stored in dark and under light for 12 days, some changes in fatty acid profiles were found. Some fatty acids decreased after the storage, more likely due to the oxidation. This was related with the increased PV value (Fig. 1). Nevertheless, there was no loss or change in EPA and DHA contents after storage under both illumination conditions ($p > 0.05$). This was possibly due to the preventive effect of mixed wall material surrounding shrimp oil droplets containing DHA and EPA towards oxidation. Umesha et al. (2015) reported that the oxidation rate of alpha-linolenic acid (ALA) was high in biscuits added with oil compared to biscuits fortified with micro-encapsulated oil, indicating that the encapsulation was able to prevent oxidation of ALA in biscuits. Borneo et al. (2007) reported that no loss of EPA and DHA in cookies fortified with encapsulated EPA and DHA at different temperatures (18 and 35 °C) packed under atmospheric and vacuum conditions. Thus, MSO could be incorporated/supplemented to increase EPA and DHA levels in biscuits.

Astaxanthin content

Astaxanthin contents of B-0%MSO and B-6%MSO under different illumination conditions at day 0 and 12 of storage are shown in Table 4. No astaxanthin was detected in B-0%MSO. B-6%MSO had astaxanthin content of 0.13 mg/100 g at day 0. After storage of 12 days, astaxanthin content in B-6%MSO sample slightly decreased ($p < 0.05$). However, there were no differences in astaxanthin content between B-6%MSO samples stored in dark and under light after the storage ($p > 0.05$). Astaxanthin contain a high number of conjugated double bonds, which are susceptible to oxidation (Choubert and Baccaunaud 2006). It was noted that the illumination conditions had no marked effect on astaxanthin in biscuits fortified with MSO.

Volatile compounds

Volatile compounds in B-0%MSO and B-6%MSO samples under different illumination conditions at day 0 and after 12 days of storage are displayed in Table 5. At day 0 of storage, the major volatile compound found in all biscuits was 2-furanmethanol, which more likely severs as the volatile marker of baking process (Mildner-Szkudlarz et al. 2009). Volatile compounds in B-0%MSO were generally lower in abundance, compared with those of B-6%MSO. Nonenal, octadecanal, ethanone, 1-(2-furanyl)-, 2-hexyl-1-octanol, 1-octanol, 2-butyl-, ethanol, 2-butoxy, 2-nonen-1-ol, cyclohexanol, 2-methyl- were found only in B-6%MSO at day 0 of storage. Several derivatives of aldehyde, ketone and alcohol can be formed by the oxidation of lipids. The results suggested that lipid oxidation might take place in biscuits to some extent during biscuit making, particularly for B-6%MSO rich in PUFAs. This was in agreement with higher PV of this sample in comparison with the sample without MSO (Fig. 1).

All volatile compounds present in B-0%MSO and B-6%MSO at day 0 were lower in abundance than those found after 12 days of storage for both illumination conditions. Nevertheless, 2-undecanone in B-0%MSO kept in dark was lower in abundance after storage in comparison with those found at day 0. For B-0%MSO kept under light, 3-undecanone was also lower in abundance after storage. No 2-heptenal, 3-nonanone, 3-undecanone, 2(3H)-furanone, 5-methyl-, 2-tridecanone, ethanone, 1-(1H-pyrrol-2-yl)-, 1-pentadecanol, 1-tetradecanol and 1-octen-3-ol were found in B-0%MSO after storage, regardless of illumination condition. For B-6%MSO, no octadecanal, 1-tetradecanol, 2-hexyl-1-octanol, 1-octanol, 2-butyl-, ethanol, 2-butoxy, 2-nonen-1-ol and cyclohexanol, 2-methyl- were found after storage. This was possibly due to the volatilisation or decomposition of those compounds. The lower abundance of volatile compounds during storage is probably due to the further oxidative changes on lipids or reactions of volatile compounds with other substances (Andrés et al. 2004).

Additionally, tetradecanal, propanal, octadecanal, hexadecanal, 6-dodecanone, 1-octanol, 2-butyl- and 2-ethyl

Table 4 Fatty acid profile and astaxanthin content of oils extracted from biscuits incorporated without MSO (B-0%MSO) and with 6% MSO (B-6%MSO) stored under different illumination conditions at day 0 and 12 of storage

Fatty acids (g/100 g)		B-0%MSO			B-6%MSO		
		0 day	12 day		0 day	12 day	
		Dark/light	Dark	Light	Dark/light	Dark	Light
Caproic acid	C6:0	0.03 ± 0.01[a]A[c]	0.03 ± 0.00b[b]B	0.12 ± 0.03aA	0.04 ± 0.00A	0.10 ± 0.01aA	0.12 ± 0.01aA
Caprylic acid	C8:0	0.23 ± 0.02A	0.23 ± 0.11aA	0.31 ± 0.02aA	0.24 ± 0.04A	0.22 ± 0.01bA	0.26 ± 0.01aB
Capric acid	C10:0	0.36 ± 0.00A	0.38 ± 0.09aA	0.44 ± 0.04aA	0.36 ± 0.01A	0.35 ± 0.05aA	0.37 ± 0.09aA
Undecanoic acid	C11:0	0.01 ± 0.00A	0.01 ± 0.00aA	0.01 ± 0.00aA	0.01 ± 0.00A	0.01 ± 0.00aA	0.01 ± 0.00aA
Lauric acid	C12:0	4.78 ± 0.01A	5.00 ± 0.05aA	5.06 ± 0.16aA	4.66 ± 0.08B	4.57 ± 0.08bB	4.78 ± 0.05aB
Tridecanoic acid	C13:0	0.01 ± 0.00A	0.01 ± 0.00aA	0.01 ± 0.00aA	0.01 ± 0.00A	0.01 ± 0.00aA	0.01 ± 0.00aA
Myristic acid	C14:0	2.43 ± 0.00A	2.43 ± 0.01aA	2.33 ± 0.03bA	2.34 ± 0.02B	2.31 ± 0.05aB	2.3 ± 0.01aA
Myristoleic acid	C14:1	0.03 ± 0.01A	0.03 ± 0.01aA	0.03 ± 0.01aA	0.03 ± 0.01A	0.03 ± 0.00aA	0.03 ± 0.00aA
Pentadecanoic acid	C15:0	0.06 ± 0.00A	0.06 ± 0.00aA	0.05 ± 0.01aA	0.06 ± 0.00A	0.06 ± 0.00aA	0.06 ± 0.00aA
Palmitic acid	C16:0	4.13 ± 0.01A	4.04 ± 0.04aA	3.68 ± 0.02bA	4.07 ± 0.08A	4.00 ± 0.13aA	3.92 ± 0.30aA
Palmitelaidic methyl ester	(C16:1t9)	0.02 ± 0.02A	0.01 ± 0.01bA	0.01 ± 0.02aA	0.02 ± 0.02A	0.01 ± 0.00aA	0.01 ± 0.00aB
Palmitoleic acid	C16:1 n−7	0.06 ± 0.02A	0.06 ± 0.01A	ND	0.07 ± 0.02A	0.07 ± 0.00aA	0.07 ± 0.01aA
Heptadecanoic acid	C17:0	0.04 ± 0.00A	0.04 ± 0.00aA	0.03 ± 0.01aA	0.04 ± 0.01A	0.04 ± 0.00aA	0.04 ± 0.00aA
Stearic acid	C18:0	1.77 ± 0.00A	1.68 ± 0.1aA	1.52 ± 0.03bA	1.71 ± 0.03B	1.69 ± 0.05aA	1.65 ± 0.19aA
Trans-elaodic acid	C18:1n9t	ND	0.53 ± 0.05bA	0.61 ± 0.02aA	0.58 ± 0.01	0.47 ± 0.04aA	0.34 ± 0.04bB
Cis-9-octadecenoic acid	C18:1 n−9	3.35 ± 0.09B	3.13 ± 0.09aB	3.01 ± 0.30aA	3.67 ± 0.07A	3.47 ± 0.02aA	3.20 ± 0.04bA
Cis-9,12-octadecatrienoic acid	C18:2 n−6	ND	0.91 ± 0.08aB	0.81 ± 0.07aB	1.01 ± 0.14	1.05 ± 0.03aA	0.95 ± 0.01bA
Cis-6,9,12-octadecatrienoic acid	C18:3 n−6	0.88 ± 0.24	ND	ND	ND	ND	ND
Trans-octadecadienoic acid	(C18:2t9t12)	0.70 ± 0.00A	0.08 ± 0.00bA	0.04 ± 0.00aA	0.04 ± 0.06B	0.02 ± 0.01aB	0.04 ± 0.01aB
Cis-9,12,15-octadecatrienoic acid	C18:3 n−3	0.04 ± 0.02A	0.04 ± 0.01aA	0.04 ± 0.00aA	0.04 ± 0.02A	0.05 ± 0.00aA	0.04 ± 0.00bA
Arachidic acid	C20:0	0.03 ± 0.00A	0.03 ± 0.01aA	0.03 ± 0.00aA	0.03 ± 0.00A	0.03 ± 0.00aA	0.03 ± 0.00aA
Cis-11-eicosenoic acid	C20:1 n−9	0.01 ± 0.00B	0.01 ± 0.00aB	0.01 ± 0.00aB	0.02 ± 0.00A	0.02 ± 0.00aA	0.02 ± 0.00aA
Cis-11,14-eicosadienoic acid	C20:2 n−6	ND	ND	ND	0.01 ± 0.00	0.01 ± 0.00a	0.01 ± 0.00a
Cis-5,8,11,14-eicasatetraenic acid	C20:4 n−6 (ARA)	ND	ND	ND	0.01 ± 0.01	0.01 ± 0.00a	0.01 ± 0.00a
Cis-5,8,11,14,17-eicasapentaenoic acid	C20:5 n−3 (EPA)	ND	ND	ND	0.01 ± 0.01	0.01 ± 0.00a	0.01 ± 0.00a
Behenic acid	C22:0	ND	ND	0.01 ± 0.00A	0.01 ± 0.00	0.01 ± 0.00a	0.01 ± 0.00aA
Cis-4,7,10,13,16,19-docosahexaenoic acid	C22:6 n−3 (DHA)	ND	ND	ND	0.03 ± 0.00	0.03 ± 0.00a	0.02 ± 0.00b
Lignoceric acid	C24:0	0.01 ± 0.00B	0.01 ± 0.00aB	0.01 ± 0.00aA	0.02 ± 0.00A	0.02 ± 0.00aA	0.01 ± 0.00bA
Astaxanthin content (mg/100 g)		ND	ND	ND	0.13 ± 0.01	0.11 ± 0.01a	0.11 ± 0.00a

ND Non-detectable

[a] Values are given as mean ± SD from triplicate determinations

[b] Different lowercase letters in the same row within the same sample at the same storage time indicate significant differences ($p < 0.05$)

[c] Different uppercase letters in the same row within the same storage condition at the same storage time indicate significant differences ($p < 0.05$)

Table 5 Volatile compounds in biscuits incorporated without and with 6% MSO stored under different illumination conditions at day 0 and 12 of storage

Compounds	Peak area (Abundance)× 10^6					
	0% MSO			6% MSO		
	Day 0	Dark	Light	Day 0	Dark	Light
Aldehyde						
2-Heptenal	14	ND	ND	46	58	433
Nonanal	46	53	78	57	65	80
2-Octenal	ND	ND	ND	ND	11	69
2-Furancarboxaldehyde	77	137	140	148	170	420
Benzaldehyde	41	75	95	ND	46	50
Nonenal	ND	ND	ND	9	13	20
2-Nonenal	ND	ND	21	ND	ND	62
Dodecanal	ND	ND	37	ND	ND	ND
Tetradecanal	ND	39	40	ND	ND	ND
Propanal	ND	106	ND	ND	ND	ND
Octadecanal	ND	33	51	8	ND	ND
Hexadecanal	ND	24	91	ND	ND	13
Ketone						
6-Dodecanone	ND	102	ND	ND	ND	ND
6,7-Dodecanedione	ND	ND	ND	ND	ND	14
3-Nonanone	16	ND	ND	ND	26	84
2-Nonanone	28	52	67	34	47	218
3-Undecanone	140	ND	23	ND	8	50
Ethanone, 1-(2-furanyl)-	ND	ND	41	31	54	93
2-Undecanone	49	34	ND	74	ND	342
2(3*H*)-Furanone, dihydro-5-pentyl-	13	ND	ND	ND	ND	ND
2(5*H*)-furanone	15	ND	37	ND	41	158
2-Tridecanone	8	ND	ND	ND	9	54
4*H*-Pyran-4-one, 2-hydroxy-3-methyl-	ND	ND	ND	ND	30	282
Ethanone, 1-(1*H*-pyrrol-2-yl)-	6	ND	ND	13	24	95
Alcohol						
1-Pentadecanol	40	ND	ND	ND	ND	ND
1-Dodecanol, 2-methyl-	ND	ND	45	ND	ND	ND
1-Pentanol	ND	ND	70	ND	ND	56
1-Tetradecanol	64	ND	ND	82	ND	ND
1-Hexadecanol, 2-methyl-	9	40	ND	ND	ND	ND
2-Hexyl-1-octanol	ND	ND	20	13	ND	ND
1-Octanol, 2-butyl-	ND	28	64	28	ND	ND
Ethanol, 2-butoxy	ND	ND	ND	9	ND	ND
2-Nonen-1-ol	ND	ND	ND	8	ND	ND
Cyclohexanol, 2-methyl-	ND	ND	ND	7	ND	ND
4-Undecanol, 7-ethyl-2-methyl-	ND	ND	16	ND	ND	ND
1-Octen-3-ol	6	ND	ND	13	15	73
2-Ethyl hexanol	ND	129	ND	ND	ND	ND
1-Octanol	ND	ND	ND	ND	10	45
1,2-Propanediol	ND	ND	118	ND	ND	ND
2-Octen-1-ol	ND	ND	ND	ND	6	30
2-Furanmethanol	429	530	630	568	685	1223
1-Hexadecanol	ND	ND	16.4	ND	ND	ND

ND Non-detectable

[a] Value in the parenthesis represents the abundance of compound in sample at 0 day

hexanol were identified as new volatiles in B-0%MSO after storage in dark. New volatile compounds in B-0% MSO kept under light included 2-nonenal, dodecanal, tetradecanal, octadecanal, hexadecanal, ethanone, 1-(2-furanyl)-, 1-dodecanol, 2-methyl-, 1-pentanol, 2-hexyl-1-octanol, 1-octanol, 2-butyl-, 4-undecanol, 7-ethyl-2-methyl-, 1-hexadecanol, 1,2-propanediol and 1-hexadecanol. B-6%MSO contained several new volatile compounds including 2-octenal benzaldehyde, 3-nonanone, 3-undecanone, 2-undecanone, 2(5*H*)-furanone, 2-tridecanone, 4*H*-pyran-4-one, 2-hydroxy-3-methyl-, 1-octanol and 2-octen-1-ol after storage, irrespective of illumination. Moreover, 2-nonenal, hexadecanal, 6,7-dodecanedione, 1-pentanol were identified as new volatiles in B-6%MSO stored under light.

After 12 days of storage, the most predominant volatiles in B-0%MSO kept in dark were 2-furanmethanol, 2-furancarboxaldehyde, 2-ethyl hexanol, propanal and 6-dodecanone. 2-Furanmethanol, 2-furancarboxaldehyde, 1,2-propanediol, benzaldehyde and hexadecanal were major products from the oxidation of B-0%MSO stored under light after storage. The highest amount of lipid oxidation products such as 2-furanmethanol, 2-furancarboxaldehyde, 2-undecanone, nonanal and 2-heptenal were found in B-6%MSO after 12 days in dark. 2-Furanmethanol, 2-heptenal, 2-furancarboxaldehyde, 2-undecanone, 4*H*-pyran-4-one and 2-hydroxy-3-methyl- were found as dominant volatile compounds in B-6%MSO after 12 days of storage under light.

Amongst all the aldehydic compounds, 2-furancarboxaldehyde was found to be the major aldehyde in all biscuits. 2-Furancarboxaldehyde is a Maillard type component, formed in reactions between amino acids, peptides, or proteins and reducing sugars. Maillard reaction products are important aroma components in baked products (Mottram 2007). Propanal is typical oxidation product due to oxidation of linolenic acid during heating (Chan et al. 1997). The presence of nonanal could be related to oil rich in oleic acid, whilst benzaldehyde and 2-nonenal could be formed from the decomposition of linoleic acid (Domínguez et al. 2014). 2-Heptenal and 2-octenal are produced by decomposition of the most abundant linoleic acid hydroperoxides (García-Martínez et al. 2009). Lipids in all biscuits contained unsaturated fatty acids, in which oleic acid (C18:1(n−9)) was the dominant fatty acid (Table 4). Additionally, EPA and DHA were also found in B-6%MSO sample (Table 4). In general, ketones are amongst the main contributors to flavour and their concentration is linked to lipid oxidation (Mottram 1998). Amongst ketones, 3-nonanone, 2-nonanone, ethanone, 1-(2-furanyl)-, 2-undecanone, 2(5*H*)-furanone, 2-tridecanone, 4*H*-pyran-4-one, 2-hydroxy-3-methyl- and ethanone, 1-(1*H*-pyrrol-2-yl)- were found at the high abundance in B-6%MSO, especially that stored under light. Yanagimoto et al. (2002) found that 2(5*H*)-furanone and 2(3*H*)-furanone, 5-methyl- were identified as oxidised products from heterocyclic compounds when reacted with hydrogen peroxide. 4*H*-Pyran-4-one, 2-hydroxy-3-methyl- actively contribute to the "toasty caramel" aroma in pathway of the Maillard reaction (Cutzach et al. 1997). Aliphatic alcohols, particularly unsaturated alcohols, were also alternatively involved in off-flavours due to their lower threshold values than those of the saturated ones (Song et al. 2011). 1-Octen-3-ol is a volatile generated from linoleic acid oxidation in the presence of singlet oxygen. 1-octen-3-ol originates from n−6 fatty acid autoxidation (Lee and Min 2010).

Overall, the types and abundance of volatile compounds detected in samples varied when different levels of MSO and storage condition were used. These compounds indicated that lipid oxidation took place in the biscuits. Autoxidation could occur during baking at high temperatures, which promote the oxidation of ingredients in all samples. Higher formation of volatile lipid oxidation products in B-6%MSO stored under light correlated well with the higher PV value and lower likeness score as shown in Fig. 1 and Table 3, respectively. Therefore, biscuits should be stored in dark to prevent the formation of volatile lipid oxidation compounds related with the rancidity in biscuits.

Conclusion

MSO prepared using sodium caseinate, fish gelatin and glucose syrup at a ratio of 1:1:4 (w/w/w) as wall materials could be fortified in biscuit product. Fortification of MSO had impact on the biscuit quality and sensory properties. MSO up to 6% could be incorporated into biscuit to improve the nutritive value without negative impact on sensorial properties. Additionally, the biscuit should be stored in dark condition to retard lipid oxidation taken place during the storage.

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