Use of ultrasonicated squid ovary powder as a replacer of egg white powder in cake

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Abstract Ultrasonicated squid ovary powder (USOP) was used to replace egg white powder (EWP) at different substitution levels (12.5–100%) and its effects on properties of batter and cake were investigated. High elastic modulus (G') and average bubble size of batter added with 100% USOP resulted in higher volume and lower baking loss, when compared to the control cake (100% EWP). For textural analysis, the lowest values of hardness, gumminess and chewiness were noticeable for cake containing 100% USOP. Crust showed the lower moisture content than crumb and bottom part of all the cakes. No difference in moisture content was observed for all parts of the cakes containing 25–100% USOP, while lower moisture content was obtained for cake added with 12.5% USOP and the control. The color difference (ΔE^*) between the control and cake added with USOP was increased with increasing USOP levels. When 100% of USOP was added into cake, higher likeness score was obtained for firmness and overall likeness. Microstructure study of cake added with 100% USOP revealed that oil phase was distributed in gluten matrix more uniformly, when compared to the control. Thus, replacement of EWP with 100% USOP resulted in the production of cake with superior quality and increased overall acceptance by consumers.

Keywords Squid ovary · Egg white powder · Cake · Baking · Ultrasonication

Introduction

Foam is a two-phase system formed by trapping pockets of gas in liquid or solid structure via the lamellar phase (Singh et al. 2018). In culinary, foam is a complex mixture of gases, solids, liquids and surfactants. Proteins such as liquid egg white (LEW), egg white powder (EWP), casein, whey protein, etc. are widely used as foaming agents (Cho et al. 2004; Song et al. 2009; Zayas 1997). However, some of them have limited applications due to the impaired functional properties such as solubility, etc. Therefore, proteins have been modified using chemical (enzymatic hydrolysis, deamidation, Maillard reaction, etc.) and physical methods (ultrasonication and irradiation etc.). Owing to the adverse effects of chemical modification, particularly related with toxicity, physical methods have been employed more widely (Singh et al. 2018).

Ultrasonication has lately gained substantial interest due to a wide range of applications including preservation, extraction and modification of proteins, carbohydrates, lipid, etc. High frequency ultrasonication is used for analytical assessment of physicochemical properties, whereas ultrasonication at low frequency has been employed for alteration of food structure by lowering the surface tension and increasing the rate of adsorption of proteins. Cavitation effect of ultrasonication causes the generation and collapse of the bubbles, which changes the protein conformation in the way that hydrophilic parts of amino acids from inside are opened and exposed to aqueous phase (Jambrak et al. 2008). This increases the solubility and other functional properties of proteins such as foaming, emulsifying properties, etc.

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Ultrasonication can cause hydrolysis or degradation of protein molecules via hydrodynamic forces, which were produced under high pressure generated due to cavitation effect (Antti et al. 2008; Jambrak et al. 2008). This might result in the reduction in molecular weight of proteins and the larger area of protein can be generated, which can enhance foaming ability (Jambrak et al. 2008).

Thailand and other Southeast Asian countries are the major exporters for the marine fisheries, specially squid and cuttlefish (Hoque et al. 2010). In Thailand, consumption of fresh and processed squid is 0.84 kg/capita/year (Needham and Funge-Smith 2014). Mostly mantle of squid has been consumed, whereas the other visceral parts along with ovary, accounting about 10 to 15% of total weight, are discarded by the squid processing industries (Singh et al. 2017). The squid ovary is rich in non-essential amino acids (52.26%) and polyunsaturated fatty acid (43.76%) and had high amount of docosahexaenoic acid (C22:6) (28.59%) (Singh et al. 2017). Therefore, squid ovary can be considered as the alternative ingredients with bioactivity and high nutritive value for food formulation. Additionally, Singh et al. (2017) documented that squid ovary possessed the excellent foaming properties, especially high foaming stability. Foaming properties of squid ovary were enhanced with the aid of partial enzymatic hydrolysis and ultrasonication (Singh and Benjakul 2018; Singh et al. 2018). However, squid ovary subjected to ultrasonication showed superior foaming properties to partially hydrolyzed squid ovary. Therefore, ultrasonicated squid ovary could serve as a novel foaming agent, especially for cake.

Generally, fresh egg white has been used for the production of cake. Owing to undesirable flavor and allergy of fresh egg white, various food proteins such as soy protein isolate, whey protein isolate (WPI), etc. have been used to replace egg white in various types of cakes (Jyotsna et al. 2007; Lin et al. 2017; Majzoobi et al. 2014). Egg white powder is another form, which can be used in bakery product because of the long shelf-life and the ease of transportation. Song et al. (2009) observed that the quality of angel cake was enhanced with the addition of irradiated EWP. No study has been reported on the utilization of squid ovary in the cake. Therefore, the objective of this work was to use ultrasonicated squid ovary powder (USOP) to replace EWP at various substitution levels and to investigate the impact of replacement on properties of batter and resulting cake.

Materials and methods

Ingredient and egg white powder

Commercial all-purpose flour (Imperial, KCG Corporation Co., Ltd, Bangkok, Thailand), unsalted butter (Orchid,

Indofood Sukses Makmur Tbk, Jawa Tengah, Indonesia), baking powder (sodium bicarbonate, 30%; disodium pyrophosphate, 24%; monocalcium phosphate, 16%; tapioca starch, 30%) (Best Foods, Unilever Thai Holdings Limited, Chachoengsao, Thailand), cake flour (Ribbin, Thai Flour Mill Industry Co., LTD, Bangkok, Thailand), fresh milk (4.1% fat content, CP-Meiji Co., Ltd. Saraburi, Thailand), sugar (Mitr Phol Sugar Corp. Ltd, Supanburi, Thailand) and salt (TRS, Co., Ltd, Nakhon Ratchasima, Thailand) were obtained from a local market in Hat Yai, Songkhla, Thailand. Egg white powder (EWP) was purchased from Mathawach Flavour Limited Partnership, Samut Sakhon, Thailand.

All-purpose and cake flour had protein content of 11.01 ± 1.19 and $3.11 \pm 0.19\%$ (w/w), respectively and both contained same amount of ash (0.1%). Gluten content and gluten index of all-purpose flour were 21.6 ± 1.36 and $87.9 \pm 5.12\%$, respectively, whereas, gluten content in cake flour was not detected. All the proximate compositions were determined by AOAC methods (AOAC 2000).

Preparation of ultrasonicated squid ovary powder (USOP)

The frozen squid (*Loligo formosana*) viscera were gifted from Sea Wealth Frozen Food, Songkhla, Thailand. The ovary was manually separated and chopped, followed by freeze-drying using a freeze-dryer at $-50\text{ }^{\circ}\text{C}$ for 4 days (CoolSafe 55, ScanLaf A/S, Lyngby, Denmark). The obtained powder was defined as ‘squid ovary powder’ or ‘SOP’. Dried ovary was ground and sieved using a screen (40 mesh). Sixteen grams of SOP was mixed with 400 mL of distilled water to obtain final concentration of 4% (w/v). The mixture was subjected to ultrasonication (Sonics, Model VC750, Sonic & Materials, Inc., Newtown, CT, USA) with operating frequency of $20\text{ kHz} \pm 50\text{ Hz}$ and high intensity power of 750 W at an amplitude of 70% for 30 min (Singh et al. 2018). The temperature of the mixture was maintained at $25\text{--}30\text{ }^{\circ}\text{C}$ during the whole operation. The obtained mixture was freeze-dried and the resultant powder was named as ‘ultrasonicated squid ovary’ or ‘USOP’.

Batter preparation

Cake batter (1000 g) was firstly prepared using the method of Levy (1981) with slight modifications. Based on the total volume of ingredient, cake recipe contained 17.48% of all-purpose flour, 16.26% of cake flour, 20.32% of sugar, 19.27% of fresh milk, 18.37% of unsalted butter, 4.3% of water, 0.57% of baking powder, 0.5% of salt, and one tea-spoon of vanilla flavor. EWP or USOP alone or the mixtures were added at 3.25%.

To study the replacement of EWP using USOP, EWP was substituted by USOP at levels of 0%, 12.5%, 25%, 50% and 100% (w/w). The batter was prepared in two steps; first, EWP or USOP or mixtures were whipped with water for 3 min along with the continuous addition of sugar. After foam was formed, the prepared mixture of melted butter, milk and vanilla flavor was added. The obtained mixture was manually mixed with a spatula for 3 min. Finally, dry ingredients, including all-purpose and cake flours, baking powder and salt were mixed with the whipped batter for another 3 min with a spatula. The obtained batter was subjected to following analysis.

Microstructure

Approximately 500 mg of batter was transferred on a microscope slide. Batter was pressed carefully with another slide to make a thin layer of batter without entrapment of air bubbles. Both slides were fixed with two paper clips to create a layer of batter of constant thickness between the slides. The samples were imaged using a fluorescence stereo microscope (Leica, DFC450 C, Wetzlar, Germany) at 40× magnification.

Rheological property

Viscoelastic properties of batters were determined using a rheometer (HAAKE RheoStress 1, Thermo Fisher Scientific, Karlsruhe, Germany) with cone and plate geometry (4° angle, 35 mm diameter) as per the method described by Hesso et al. (2015). The batter was carefully placed in the center of the lower plate. Afterward, the upper geometry was descended to a gap of 2 mm. The excessive batter was carefully removed using a plastic spatula. The linear viscoelastic region (LVR) was measured previously for an oscillation frequency of 1 Hz with 0.1% strain. Temperature sweep from 25 to 95 °C at a heating rate of 2.4 °C/min was performed at a frequency of 1 Hz and strain of 0.1%. Elastic (G') modulus were documented.

Cake preparation

The prepared batter (150 g) was poured into aluminum pans (250 mL) and baked at 150 °C for 60 min in a forced air oven (Mamaru MR-1214, Mamaru Co., Ltd., Bangkok, Thailand). The aluminum pans were greased with butter prior to the addition of batter. After baking, the cakes were taken out from the aluminum pan and cooled at room temperature (26–28 °C) for 3 h. The cakes were placed in a zip lock bag until used for the analysis.

Baking loss

Baking loss of the control and cakes added with USOP at various EWP substitutions (12.5–100%, w/w) was determined as described by Barcenilla et al. (2016). It was calculated by weighing (in gram) batter before baking (W_0) and after baking (W_f).

$$\text{Baking loss (\%, w/w)} = (W_0 - W_f/W_0) \times 100$$

Cake volume

Cake volume was measured by the sesame seed displacement method (Sinthusamran and Benjakul 2015)

Color measurement

The crust of cake was removed and cut into $0.5 \times 2 \times 2$ cm pieces and crumb was taken from the center of cake and cut into the pieces ($2 \times 2 \times 2$ cm). The L^* , a^* , and b^* of crust and crumb of cake were measured using a calibrated colorimeter having a standard illuminant of D65 as defined by the International Commission on Illumination (CIE) (Hunter Lab, Color Flex, Hunter Associates Laboratory, Reston, VA, USA). The colorimeter was standardized using white standard (L^* : 92.82; a^* : −12.24; and b^* : 0.50) and all the measurements were done at room temperature. L^* , a^* , and b^* represent the lightness, green–redness and blue–yellowness, respectively. The total difference in color (ΔE^*) was calculated as follows:

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

where ΔL^* , Δa^* and Δb^* are the difference between the color parameters of the samples and those of the control.

Textural profile

Textural profile analysis (TPA) of the cake samples was conducted using a texture analyzer (Model TA-XT2, Stable MicroSystems, Surrey, UK) according to the procedure of Sowmya et al. (2009).

Sensory evaluation

Sensory evaluation of cakes was evaluated by a panel of 80 non-trained panelists according to the procedure described by Sowmya et al. (2009). Panelist were asked to likeness score for various quality parameters, namely appearance, color, odor, firmness, flavor and taste using 9-point hedonic scale, where 9 was the highest quality score, 1 was the lowest, and 5 was an acceptable level.

Microstructure

The samples were cut into small pieces ($5 \times 5 \times 5$ mm) with a sharp blade followed by freeze-drying using a freeze drier. Cake samples were fixed on the sample holder using double sided scotch tape (O-BIZ, Samutprakarn, Thailand) and was exposed to gold sputtering (2 min, 2 mbar) (Sowmya et al. 2009). Microstructures of cakes were examined using a scanning electron microscope at magnification of 40 and $160\times$ (Quanta 400, FEI, Eindhoven, the Netherlands).

Statistical analysis

All experiments were performed in triplicate. Data were subjected to analysis of variance. Comparison of means was carried out by the Duncan's multiple range tests (Steel and Torrie 1986). Analysis was performed using a SPSS package (SPSS 22 for Windows, SPSS Inc., Chicago, IL, USA).

Results and discussion

Impact of USOP replacement of EWP on properties of cake batter

Microscopy of batter

The micrographs of batter added without and with USOP at several EWP substitution levels (12.5–100%, w/w) are shown in Fig. 1a. The micrograph of control batter (100% EWP), showed small air bubbles uniformly distributed in the matrix (Fig. 1a1). However, with increasing concentration of USOP in batter, a number of air bubbles with slightly larger size were noticeable (Fig. 1a2–5). Singh et al. (2018) reported that USOP foam had slightly larger sized air bubble surrounded by thick layer of proteins. Foam prepared from USOP had an excellent stability after 60 min at room temperature (Singh et al. 2018). Uniform distribution of air bubbles in the matrix more likely resulted in the formation of stable matrix (Zayas 1997). Generally, batter with small average air bubble size is required for good texture, volume, shape and grain of cake (Jia et al. 2014). However, batter with larger stabilized air bubble could entrap more air, which might result in the formation of cake with higher volume and softer texture.

Rheology of batter

Viscoelastic properties of batter added with and without USOP at various EWP substitution levels (12.5–100%, w/w) during heating from 25 to 95 °C are shown in Fig. 2. Elastic modulus (G') varied with increasing temperatures

for all batters. G' represents elastic behavior of the batter and it is defined as the amount of deformation energy stored in the sample during shearing (Shevkani et al. 2015). Firstly, G' was decreased for all the samples when heated from 25 to 40 °C. This was more likely due to the melting of fat associated with the reduction in liquid phase viscosity (Hesso et al. 2015). Carbon dioxide released during baking might be another possible reason to reduce the elasticity of batter (Meza et al. 2011; Schirmer et al. 2012). In general, batter added with 100% USOP showed the higher G' when heated up to 78 °C. It was noted that G' of the sample was quite constant at 45–80 °C. For the other samples, G' was increased with increasing temperatures. Thus, both EWP and USOP has the different impact on viscoelastic property of batter more likely due to various compositions. This might affect interaction of proteins with various ingredient, especially water. Protein isolates from cowpeas, kidney bean, field pea and amaranth increased the viscosity of gluten-free rice batter by enhancing water absorption, which resulted in lower movement of particle in the batter (Shevkani and Singh 2014; Shevkani et al. 2015). Shevkani and Singh (2014) stated that batter viscosity plays an important role in baking as appropriately viscous batter can trap air bubbles during mixing and retain them during baking. However, too high or too low batter viscosity may result in cake with low volume. The higher value of G' for batter containing USOP until 78 °C was more likely related with the presence of high amount carbohydrate in USOP (Singh et al. 2017, 2018), which might increase the viscosity of batter. The higher viscosity might provide elastic structure to the batter during baking. In addition, ultrasonication resulted in the exposure of buried hydrophobic domains of USOP, which might increase hydrophobic–hydrophobic interaction. This interaction could formed thick viscoelastic film around the air bubbles (Singh et al. 2018). Generally, 55 to 78 °C region indicated fluidization of batter while heating (Hesso et al. 2015). At the end, there was an increase in G' in the region of 78 to 88 °C, which is characteristics of protein denaturation, starch gelatinization, and structure fixation (Lee et al. 2004). However, during gelatinization, structure fixation plausibly resulted in similar value of G' for all batters. Thus, addition of USOP improved the viscoelastic properties of cake batter, which more likely strengthened the cake structure during baking.

Effect of USOP replacement of EWP on properties of cake

Baking loss and cake volume

Baking loss and volume of cake added without and with USOP at several EWP replacement levels (12.5–100%) are

Fig. 1 Microscopic image of cake batter (a), front (b) and cross-sectional (c) views of the control (100% EWP) and cakes added with USOP at various EWP substitution levels

Fig. 2 Elastic modulus (G') of the control (100% EWP) and cakes added with USOP at various EWP substitution levels during heating from 25 to 95 °C

shown in Table 1. Gas formation during baking caused an increase in vapor pressure, which was likely due to the expansion of liquids when heat was applied to the batter. The loss of gas during baking is called ‘baking loss’ (Lee 2015). Baking losses of all the samples were in the range of 8.41–8.88%. The highest baking loss was noted for cake added with 12.5% USOP and the control (100% EWP) ($p < 0.05$), whereas, cake added with 50 and 100% USOP showed slightly lower baking loss ($p < 0.05$). USOP addition could therefore strengthen the batter matrix via formation of strong and viscoelastic films surrounding the air bubbles. This might help entrap and hold more gas

during the expansion of cake matrix throughout the baking process.

Volume of cake indicated the ability of batter to expand and incorporate gas during the baking process. The volume of cake was increased when the levels of USOP was increased from 25 to 100% EWP substitution ($p < 0.05$), whereas the lowest volume was obtained for cake added with 12.5% USOP followed by the control ($p < 0.05$). Generally, the presence of initial water generates the sufficient vapor, which increases volume of cake and provides a moist texture (Choi et al. 2007). Figure 1c also depicts the increases in porosity and volume of crumb of cake, when USOP levels were increased from 12.5 to 100%. Disappearance of dome of cake was observed on the addition of USOP, particularly at high level. Presence of dome indicated inconsistency of batter, which resulted in the fast baking at surface of cake and a dome was formed (Fig. 1b-1). Presence of carbohydrates in USOP might increase water holding capacity of cake and caused higher volume. Additionally, batter containing 100% EWP showed smaller bubbles as compared to that added with 100% USOP. This might cause higher volume of cake from the latter than the former. Furthermore, EWP had high foaming capacity but lower foaming stability, as compared to USOP (data not shown). In the complex system of cake, EWP might be unable to form a stable foam in the presence of sugar, flour, lipid etc. This resulted in the baking loss and lower volume of cake added with EWP.

Table 1 Baking loss, cake volume and textural properties of the control (100% EWP) and cakes added with USOP at various EWP substitution levels

USOP (%)	Baking loss (%)	Volume (cm ³)	Hardness (g)	Springiness (cm)	Cohesiveness	Gumminess (g)	Chewiness (g x cm)
0	8.82 ± 0.19 ^a	157.5 ± 2.88 ^d	4605.30 ± 57.10 ^a	0.94 ± 0.01 ^a	0.62 ± 0.01 ^a	4243.94 ± 86.54 ^a	3221.06 ± 70.29 ^a
12.5	8.88 ± 0.20 ^a	153.75 ± 2.5 ^e	4538.56 ± 91.01 ^b	0.94 ± 0.00 ^a	0.63 ± 0.01 ^a	4129.39 ± 71.93 ^b	3085.97 ± 40.79 ^b
25	8.79 ± 0.14 ^a	163.75 ± 3.88 ^c	4070.40 ± 46.53 ^c	0.93 ± 0.01 ^b	0.62 ± 0.01 ^a	3876.41 ± 91.83 ^b	2909.28 ± 47.75 ^c
50	8.55 ± 0.17 ^b	167.00 ± 2.74 ^b	3872.48 ± 55.94 ^d	0.92 ± 0.01 ^b	0.61 ± 0.01 ^a	3641.89 ± 68.82 ^d	2722.80 ± 89.15 ^d
100	8.40 ± 0.20 ^b	173.57 ± 5.56 ^a	3779.80 ± 47.00 ^e	0.90 ± 0.00 ^c	0.61 ± 0.01 ^a	3494.03 ± 64.21 ^e	2408.04 ± 87.39 ^e

Values are expressed as mean ± SD (n = 3). Different lowercase superscripts in the same column indicate significant differences ($p < 0.05$)

Textural profile

Textural profile of cake in the presence and absence of USOP at different EWP substitution levels (12.5–100%, w/w) is shown in Table 1. Hardness is the applied force to compress sample to attain a certain deformation. The highest hardness was obtained for the control cake and the cake added with 12.5% USOP, as compared to other cakes ($p < 0.05$). When USOP level was increased up to 100%, hardness was continuously decreased ($p < 0.05$). The result was in agreement with the higher volume of cake added with 100% USOP (Table 1). The higher volume indicated more entrapment of gas during baking, which provided softer texture and lower hardness. Cohesiveness, an ability to deform the internal structure of food, showed no difference at all the levels (0–100%) of USOP incorporated into cake. Springiness, elastic recovery after the compressive force is removed, was decreased to the minimum value when 100% USOP was added to the cake ($p < 0.05$). No difference was observed in springiness of cake added with 25–50% USOP ($p \geq 0.05$). Also, similar springiness was noted between cake added with 12.5% USOP and the control ($p \geq 0.05$). Gumminess and chewiness reflect energy needed to breakdown and masticate the food to the point needed for swallowing, respectively. Both parameters were decreased with increasing levels of USOP ($p < 0.05$). Shevkani and Singh (2014) also reported the increased in volume of muffins with incorporation of different protein isolates (kidney bean, field pea and amaranth) which resulted in improved springiness and cohesiveness. Thus, USOP could be used for the preparation of cake with acceptable textural properties. Song et al. (2009) reported that irradiation improved the foaming capacity and stability of EWP. This resulted in enhanced textural properties and quality of angel cake added with modified EWP as compared to the control (Song et al. 2009). Therefore, inferior quality of cake added with EWP might be due to its impaired functional properties of EWP, as compared to USOP. However,

Arunepanlop et al. (1996) reported that replacement of EWP with whey protein more than 25% resulted in deterioration of textural property of angel cake. This was due to the inferior foaming properties of cake WPI (Arunepanlop et al. 1996). The result suggested that modification of squid ovary powder with the help of ultrasonication might improve the foaming properties which resulted in cake with high quality and better texture. Hence, USOP could be act as a novel foaming agent in the bakery industry.

Moisture content (MC)

MC of different parts of the control and cake added with USOP at various EWP substitution level is shown in Table 2. The lowest MC was observed at the top of cake, especially crust as compared to the other parts (center and bottom) of the cake ($p < 0.05$). No difference in MC of center and bottom parts was noted for the cake added with 25–100% USOP ($p \geq 0.05$). The lowest MC of crust was more likely due to the substantial decrease in surface water content during baking process (Lostie et al. 2002). For the control cake and cake added with 12.5% USOP, the lower MC was noticeable for all parts as compared to the remaining samples ($p < 0.05$). With increasing USOP levels, the higher MC was obtained for all parts (crust, crumb and bottom) of the cake ($p < 0.05$). However, the addition of USOP at 25–100% did not show any difference in MC of all parts of cake. The results suggested that USOP might help in holding water in the cake matrix. This result was in agreement with higher volume and lower hardness of cake containing USOP (Table 1). The higher MC was plausibly due to the interaction between of water molecules with hydrophilic amino acids of squid ovary exposed by ultrasonication. Additionally, the presence of large amount of carbohydrates might help hold water in cake matrix. MC play an important role in textural properties of cake. Sani et al. (2014) stated that typical cake has MC between 15 and 30%. In present study, the MC was in the

Table 2 Moisture content and color of different parts of the control (100% EWP) and cakes added with USOP at various EWP substitution levels

USOP (%)	0	12.5	25	50	100
Moisture content					
Top	16.86 ± 0.11 ^{bc}	17.09 ± 0.59 ^{bc}	18.83 ± .94 ^{aB}	18.74 ± 0.41 ^{aB}	19.03 ± 0.31 ^{aB}
Centre	29.46 ± 0.36 ^{bA}	30.11 ± 0.57 ^{abA}	31.16 ± 0.82 ^{aA}	31.51 ± 0.20 ^{aA}	31.39 ± .70 ^{aA}
Bottom	26.78 ± 0.42 ^{bB}	27.47 ± 0.52 ^{bB}	30.74 ± 0.33 ^{aA}	30.83 ± 0.98 ^{aA}	31.48 ± 0.50 ^{aA}
Crust color					
<i>L</i> *	68.68 ± 0.46 ^a	66.18 ± 0.71 ^b	64.71 ± 0.84 ^c	63.78 ± 2.58 ^{cd}	63.12 ± 0.24 ^d
<i>a</i> *	6.98 ± 0.49 ^b	11.39 ± 1.02 ^a	11.87 ± 0.70 ^a	11.69 ± 1.31 ^a	12.30 ± 0.95 ^a
<i>b</i> *	42.33 ± 0.43 ^a	42.53 ± 1.16 ^a	40.51 ± 1.33 ^b	40.90 ± 0.90 ^b	39.46 ± 1.16 ^c
ΔE^*	–	5.49 ± 0.82 ^c	6.78 ± 0.3 ^b	6.89 ± 0.47 ^b	7.76 ± 0.61 ^a
Crumb color					
<i>L</i> *	76.54 ± 0.99 ^a	76.17 ± 0.47 ^{ab}	75.42 ± 0.21 ^{bc}	74.89 ± 0.36 ^{cd}	74.35 ± 0.54 ^d
<i>a</i> *	0.59 ± 0.01 ^d	0.73 ± 0.05 ^c	0.85 ± 0.02 ^b	0.90 ± 0.4 ^b	1.48 ± 0.12 ^a
<i>b</i> *	23.32 ± 0.92 ^a	23.24 ± 0.61 ^a	23.82 ± 0.27 ^a	23.95 ± 0.87 ^a	24.29 ± 1.28 ^a
ΔE^*	–	0.77 ± 0.01 ^d	1.27 ± 0.03 ^c	1.96 ± 0.07 ^b	2.76 ± 0.19 ^a

Values are expressed as mean ± SD (n = 3). Different lowercase superscripts in the same row indicate significant differences ($p < 0.05$). Different uppercase superscripts in the same column indicate significant differences ($p < 0.05$)

recommended range. Thus, USOP had the potential to hold water during baking, which could increase the quality of cake.

Color

Color of crust and crumb of the control and cake added with USOP at various levels is presented in Table 2. The *L** and *b** values of crust were decreased with increasing levels of USOP, whereas *a** was increased with the addition USOP at all levels ($p < 0.05$). The color of crust is mainly governed by the Maillard reaction and caramelization during baking (Lin et al. 2017). The major constituents of USOP were protein and carbohydrates accounting for 67 and 27%, respectively (Singh et al. 2017), which might enhance the Maillard reactions, especially during baking at high temperature. This caused more redness and lower yellowness of the crust. Figure 1b shows the increase in the redness of crust with increasing concentration of USOP. For crumb, the lower value of *L** was observed for the cake added with 100% USOP ($p < 0.05$). The *a** of crumb was increased with increasing levels of USOP from 12.5 to 100% ($p < 0.05$). No difference in *b** value was observed for the control and cake added with USOP at all concentrations ($p \geq 0.05$). ΔE^* indicated the color difference between the control (cake containing 100% EWP) and cake added with USOP at various concentrations. It was noted that color difference was increased with increasing levels of USOP for both crust and crumb of cakes ($p < 0.05$). Generally, crumb color of the cakes is affected by the ingredient used and its formulation (Lin et al. 2017). The difference in chemical

composition of EWP and USOP might provide different colors of the cake.

Likeness score

Likeness scores of the control and cake incorporated with USOP at various EWP substitution levels (12.5–100%) are shown in Table 3. All the samples had no difference in likeness score for appearance, color, odor attributes ($p \geq 0.05$). The highest likeness score for firmness was obtained for cake containing 100% USOP ($p < 0.05$). Cake added without and with 12.5% USOP showed no difference in likeness score for firmness and the lowest value was obtained for the control ($p < 0.05$). This result was in agreement with hardness of cake (Table 1). The higher likeness score was noted for flavor and taste of cake added with 50 or 100% USOP ($p < 0.05$), however no difference in likeness score was obtained for other cakes ($p \geq 0.05$). The maximum likeness score was generally obtained for cake containing 100% USOP, followed by the 50% USOP ($p < 0.05$), but no difference was obtained among the other samples ($p < 0.05$). In another study, EWP was replaced by 25% WPI resulted in angle cake with similar high likeness score to that added with 100% EWP, thereafter with further replacement the likeness score was decreased drastically (Arunepanlop et al. 1996). The major factor contributing to the acceptance of cake was firmness. Other sensory attributes such as flavor, taste and odor might be masked or dominated by the presence of other ingredient such as butter, flavoring agent etc. Therefore, USOP could be incorporated to replace EWP completely into cake with

Table 3 Likeness score of the control (100% EWP) and cakes added with USOP at various EWP substitution levels

USOP (%)	Appearance	Color	Odor	Firmness	Flavour	Taste	Overall
0	7.46 ± 0.88 ^a	7.54 ± 0.88 ^a	6.08 ± 0.52 ^b	5.92 ± 0.65 ^c	6.87 ± 0.76 ^b	6.69 ± 0.86 ^b	6.92 ± 0.36 ^c
12.5	7.68 ± 0.64 ^a	7.85 ± 1.14 ^a	6.55 ± 0.50 ^b	6.38 ± 0.66 ^{bc}	6.92 ± 0.85 ^b	6.92 ± 0.67 ^b	6.92 ± 0.25 ^c
25	7.62 ± 0.96 ^a	7.77 ± 0.83 ^a	7.31 ± 0.48 ^a	6.69 ± 0.55 ^b	7.23 ± 0.83 ^{ab}	7.16 ± 0.77 ^{ab}	7.08 ± 0.23 ^c
50	7.69 ± 0.63 ^a	7.62 ± 0.51 ^a	7.31 ± 0.95 ^a	6.92 ± 0.34 ^b	7.62 ± 0.52 ^a	7.23 ± 0.83 ^{ab}	7.33 ± 0.23 ^b
100	7.58 ± 0.96 ^a	7.77 ± 0.83 ^a	7.53 ± 0.59 ^a	7.43 ± 0.43 ^a	7.63 ± 0.69 ^a	7.45 ± 0.73 ^a	7.63 ± 0.12 ^a

Values are expressed as mean ± SD (n = 3). Different lowercase superscripts in the same column indicate significant differences ($p < 0.05$)

consumer acceptability and without negative effect on sensory properties.

Microstructure of cake

Microstructures of the control cake (100% EWP) and cake added with 100% USOP at different magnifications (40 and 160×) are shown in Fig. 3. Cake added with 100% USOP was more porous than the control cake (Fig. 3a, b). The pores of varying sizes were uniformly distributed throughout the gluten matrix of cake added with 100% USOP (Fig. 3b). Whereas, control cake showed less pores

with non-uniformity (Fig. 3a). The result was in line with the higher number of air bubbles of batter containing 100% EWP (Fig. 1a5). At the high magnification, micrograph of the control cake containing 100% EWP showed continuous gluten matrix with starch granules (SG) embedded in the matrix. The shape of the starch granules was changed due to the gelatinization during the baking. Sowmya et al. (2009) stated the proteinaceous components of flour used for baking might act as matrix covering the starch granules. Cake added with 100% USOP showed scattered gluten matrix embedded with starch granules. This was more likely due to the different chemical composition between

Fig. 3 Scanning electron microscopic images of the control (100% EWP) and cake added with 100% USOP. Magnification: 160 ×. SG starch granules, PM protein matrix, OP oil phase

both EWP and USOP. Micrograph of cake added with USOP showed more oil phase as indicated by smooth dark area in the matrix as compared to the control. USOP treated with ultrasound had the exposed buried hydrophobic domains. Hydrophobic amino acids might interact with the oil in the matrix, which might stabilize the oil phase in the cake. Oil is one of the important factors providing the softness to cake. The matrix of the cake is controlled by various factors associated with nature and properties of ingredients used. Therefore, USOP could help stabilize the cake via interaction with other ingredients of cake.

Conclusion

EWP was replaced with USOP in cake at different levels ranging from 12.5 to 100%. Cake batter added with USOP had better viscoelastic properties than that of cake containing EWP. USOP at 100% yielded the cake with slightly superior properties to EWP. The addition of USOP alone yielded the cake with lower baking loss, higher volume and softer texture. Moreover, USOP rendered the cake with slightly higher moisture and darker color. Overall, USOP had no negative impact on the sensory attributes. Thus, USOP could be used as a novel surface-active agent in various types of cake. Apart from this, USOP might act as emulsifying agent, which could be used in mayonnaise or other emulsion-based products.

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