



## Rice Bran Oil Emulsion Organogels as Fat Baking for Brownies

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### Abstract

Rice bran oil emulsion organogels with different organogelators including triglyceride and policosanol are prepared and utilized as a bakery fat for making brownies. Texture profiles, percent of height gain, percent of weight loss, color, fatty acid composition, and sensory properties of the organogel brownies are investigated and compared with those of brownies made with rice bran oil and commercial margarine. The organogel brownies exhibit similar percent weight loss, percent height gain, hardness, gumminess and chewiness when compared to the commercial margarine brownies. Hardness, gumminess and chewiness in the organogel brownies are lower than rice bran oil brownie. The fatty acid compositions of both organogel brownies are similar to the rice bran oil brownie. Brownies prepared with the organogels exhibit considerable reduction in saturated fatty acid contents from 50.47% to 30.25% when compared with brownie prepared with commercial margarine. Policosanol and triglyceride organogels are potentially effective for producing nutritionally superior brownies with comparable quality attributes when compared to the margarine brownies.

### Introduction

Solid fats containing high saturated fats have been extensively utilized in the bakery industry. Solid fats play an important roles in quality attributes of baked goods for improving palatability, air-incorporation, moisture barrier, shelf-life, and the tender texture and mouthfeel of the final product by preventing the cohesion of gluten strands (Cheong et al., 2011; Pylar & Gorton, 2008). However, consumption of oils rich in saturated fats could also cause health problems. Saturated fats are known to associate with increasing risk of heart disease. Reducing

fat intake, particularly saturated and trans-fat, led to a dramatic decline in coronary heart disease mortality (Andy, 2004; Aranceta & Pérez-Rodrigo, 2012; Mozaffarian & Ludwig, 2015). More importantly, consumption of unsaturated fatty acids is highly recommended due to their health benefits (Micha & Mozaffarian, 2010; Rodriguez-Leyva et al., 2010).

In recent years, different attempts have been carried out to find alternative ways to produce solid fat with a low amount of saturated fatty acid (SFA). One of the effective techniques is using organogelation. Liquid oil can be entrapped in a thermo-reversible gel network

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with an assistance of various organogelators, resulting in organogels with semi-solid properties. These organogels did not alter the fatty acid composition of the entrapped liquid oil and no trans fats were generated (Marangoni, 2012; Stortz et al., 2012). The organogels are interesting products which are recently being used in structuring vegetable oils for emulsion-based products, margarine, and shortening-like products. Especially, the organogels have been utilized in substitution to solid fats in bakery products to reduce saturated fats. Various edible oils and organogelators have been evaluated for different bakery products. Cookies and biscuits prepared with organogels had similar quality parameters with those prepared with shortening (Devi & Khatkar, 2016; Hwang et al., 2016; Onacik-Gür & Żbikowska, 2019). Cakes and muffin produced with organogels had lower levels of saturated fatty acids without quality loss when they were replaced with shortening (Amoah et al., 2017; Pehlivanoglu et al., 2018; Willett & Akoh, 2019; Zhou et al., 2011). Baking fat affects dough structure and the desired final product attributes. Therefore, the replacement of conventional shortening or margarine poses tremendous challenge in the bakery production (Demirkesen & Mert, 2020).

In this study, rice bran oil emulsion with two organogelators, i.e. triglyceride and policosanol, are employed to produce solid-like organogels. Effects of the organogels on the quality attributes of the organogel brownies are then compared with commercial margarine and rice bran oil brownies in terms of texture profile, color, percent weight lost and height gain, fatty acid composition and sensory characteristics.

## Materials and methods

### 1. Materials

Rice bran oil, triglyceride (Palsgaard 6111®), Palsgaard, Morris Plains, NJ, USA) policosanol, butter flavor,  $\beta$ -carotene, and citric acid were used to make the organogels margarines. Rice bran oil, commercial margarine, commercial all-purpose wheat flour, white sugar, sodium bicarbonate, sea salt, and cacao powder were purchased from a local grocery store.

### 2. Organogel preparation

Triglyceride organogel (organogel-1) and policosanol organogels (organogel-2) were prepared by the reported method with some modifications (Hwang et al., 2013). The water in oil emulsion organogels were prepared by dissolving 4.0% (w/w) organogelators in rice bran oil at 75°C. Water phase was prepared by mixing water with

1% (w/w) salt, 0.5% (w/w) butter flavor, 0.05% (w/w)  $\beta$ -carotene, and 0.03% (w/w) citric acid. The water phase was subsequently poured into the oil phase while homogenizing (T-25 basic Ultra Turrax®, Janke and Kunkel IKA, Germany) for 5 minutes. The water-in-oil emulsion was then cooled to 4°C for 1 hour. The formation of organogels was performed by placing the emulsion at -18°C for 4 hours. Once the gelation was completed, the samples were stored in a refrigerator at 4°C before baking the brownies.

### 3. Brownie preparation

The brownie samples were prepared by the reported method of Uruakpa & Fleischer (2016). Firstly, solid fats were melted using a double boiler method and cooled down to room temperature. Then, rice bran oil or melted fat, eggs, white sugar, and vanilla extract were mixed in a stainless-steel bowl. The mixture was then combined with the cooled chocolate mixture. All-purpose wheat flour, cacao powder, salt, and baking powder were then added into the mixture. The final mixture was placed in a baking tin (10 cm  $\times$  10 cm  $\times$  4 cm) and was oven-baked at 175°C for 15 minutes. After baking, the sample was removed from the baking tin and left to cool for 1 hour at room temperature. Cooled brownies were sealed in plastic wraps and placed in zip-lock plastic bag and stored at 4°C prior to further chemical analysis. Physical properties and sensory analysis were performed on cooled brownies. Four formulation of brownies presented in Table 1.

**Table 1** Brownies formulation

Ingredients	Brownies formulation		
	Rice bran oil	Organogels	Conventional margarine
Sugar (g)	158.00	158.00	158.00
Salt (g)	1.78	1.78	1.78
Vanilla extract (g)	1.78	1.78	1.78
Eggs (g)	126	126	126
Wheat flour (g)	106.00	106.00	106.00
Cocoa powder (g)	43.00	43.00	43.00
Baking powder (g)	3.55	3.55	3.55
Rice bran oil (g)	64.00	0	0
Organogels (g)	0	64.00	0
Conventional margarine (g)	0	0	64.00

### 4. Texture profile analysis

Texture profiles of cooled brownies with dimensions of 3 cm  $\times$  3 cm  $\times$  3 cm were analyzed after 24 hours of baking with a texture analyzer (TA. XT2i Texture Analyzer, Texture Technologies Corp, Ltd. Hamilton, MA) under a two-cycle compression. A 75-mm

compression plate (P/75) probe was used. The strain 50 % at 10 mm with a force of 5.0 g were employed. Hardness, cohesiveness, springiness, chewiness, and gumminess of each brownie were averaged from 10 replicates.

#### 5. Weight loss and height gain of brownies

Heights of the brownies were measured using a Vernier caliper (Model No. CD-800CSX, Mitutoyo Corp., Kawasaki, Japan). Height was measured from the highest point of the brownie to the bottom. Brownies were weighed and measured 5 times, that is before and after baking and cooling. The difference in weight and height was calculated as weight loss and height gain.

#### 6. Color of brownies

Color of all the brownie samples were evaluated by a colorimeter (Chroma Meter CR-410, Konica Minolta, Inc., Osaka, Japan). The measuring head was placed at the center of each brownie. Color values were measured using CIE scale in 3 replicates and average values were reported as  $L^*$  = lightness (0 = black, 100 = white),  $a^*$  ( $-a^*$  = greenness,  $+a^*$  = redness) and  $b^*$  ( $-b^*$  = blueness,  $+b^*$  = yellowness).

#### 7. Fatty acid composition analysis

Fatty acid composition of all brownies sample was analysis using Gas chromatography technique in 3 replicates. Oil extracted from brownies with a mass of 10 mg were mixed with 3 mL of toluene in a screw-capped glass tube. Then, 1 mL of 5% (w/v) methanolic NaOH was added. The mixture was vortexed for 3 minutes and 1 mL of glacial acetic acid was added. The toluene phase was washed several times with distilled water and dried over anhydrous sodium sulfate before analysis with gas chromatography (Kaewkool et al., 2009).

Gas chromatography analysis were performed with a model 2010 gas chromatograph equipped with a flame ionization detector (Shimadzu, Tokyo, Japan), electronic pneumatic control, and a split/splitless injector. The detector time constant was set at 100 ms. A 1- $\mu$ L sample was injected with a split ratio of 50:1 by an autoinjector. The injector switch was set at split throughout the analysis. Data acquisition and analysis were performed with a CBM 102 data processor. The injector and detector temperatures were set at 250°C. Helium was used as the carrier gas at a flow rate of 0.5–1.0 mL/min. The nitrogen makeup gas flow rate was 30 mL/min (Kaewkool et al., 2009).

#### 8. Sensory evaluation

Hedonic test was utilized to determine the degree of overall preference for brownies. Fifty panelists were given with four random samples (2 cm  $\times$  2 cm  $\times$  2 cm)

from the midsection of the brownies that had been held at room temperature for 24 hours. The brownies were evaluated for preference of appearance, color, texture, flavor and overall acceptability based on a nine-point hedonic scale (1 = extremely dislike, 9 = extremely like). All samples labeled with 3-digit random code numbers were randomly served to panelists. Samples were presented according to a Latin Square. The sensory test was performed in separate booths at room temperature. The mouth was rinsed with water to minimize any residual effects between each sample. Sensory evaluation was performed after the approval from the Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand (Reference number: 31/2561).

#### 9. Statistical analysis

The Completely Randomized Design or CRD was applied to create the different formulations of brownies for physicochemical properties study. The Randomized complete block design (RCBD) was applied for sensory evaluation. Three replications were performed for each sample. The mean values recorded for each test were compared using analysis of variance (ANOVA). Tukey's test was applied to detect the differences among the samples ( $P < 0.05$ ).

### Results and discussion

#### 1. Texture profiles of brownies

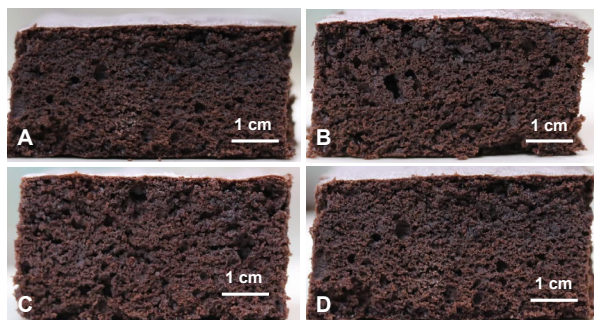
The texture profiles of the brownies prepared with organogels, rice bran oil, and commercial margarine were compared as shown in Table 2. Hardness of the rice bran oil brownie (4681.72 $\pm$ 12.31) was significantly higher than the organogel-1 brownie (2843.20 $\pm$ 15.78), the organogel-2 brownie (2822.13 $\pm$ 10.48), and commercial margarine brownie (2666.67 $\pm$ 16.04). The highest hardness was observed in the rice bran oil brownies. The brownies prepared with organogels and margarine exhibited a softer texture. The reason for this observation could be attributed to the type of baking fat. Commonly, margarine plays an important role in the stabilization of air bubbles during creaming process. Air cells are incorporated into the fat phase during the mixing stage and then released into the aqueous phase when the shortening is melted during baking, giving rise to a foam structure or a homogenous crumb structure (Campbell & Mougeot, 1999). Utilization of rice bran oil led to less incorporation of air cells into the batters (Fig. 1). Furthermore, the decreasing hardness observed in margarine and both organogel brownies might be due to

**Table 2** Texture profile of brownies

Formulation	Parameters				
	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness
Rice bran oil	4681.72±12.31 <sup>a</sup>	0.57±0.01 <sup>a</sup>	0.28±0.01 <sup>b</sup>	1318.57±17.22 <sup>a</sup>	525.61±26.69 <sup>a</sup>
Organogel 1	2843.20±15.78 <sup>b</sup>	0.59±0.05 <sup>a</sup>	0.34±0.03 <sup>a</sup>	805.79±35.37 <sup>c</sup>	476.21±29.92 <sup>a</sup>
Organogel 2	2822.13±10.48 <sup>b</sup>	0.58±0.03 <sup>a</sup>	0.34±0.01 <sup>a</sup>	943.65±14.39 <sup>b</sup>	552.13±13.23 <sup>a</sup>
Conventional margarine	2666.67±16.04 <sup>c</sup>	0.45±0.05 <sup>b</sup>	0.28±0.02 <sup>b</sup>	744.00±15.45 <sup>c</sup>	332.76±26.40 <sup>b</sup>

**Remark:** Different letters in the same column are significantly different ( $P < 0.05$ )

the presence of emulsifiers playing a critical role in the stabilization of air bubbles during creaming process. Pylar & Gorton (2008) reported that baking fat contain emulsifier help in the dispersion of the fat in the batter system resulted bakery product have tender and softer textures as compared to rice bran oil. Visual observation of the internal texture revealed that brownies made of commercial margarine had well-distributed air cells as compared to other samples. Rice bran oil brownies contained less air cells that are not homogenously distributed (Fig. 1). This might be due to rice bran oil are non-emulsifiers baking fat dispersed upon mixing throughout the batter in the form of globules that are less effective in their shortening and aerating actions (Hartnett & Thalheimer, 1979).



**Fig. 1** Photographs of brownies prepared with rice bran oil (A), organogel with 4% of triglyceride (B), organogel with 4% of policosanol (C) and commercial margarine (D)

The springiness of organogel-1 brownies ( $0.59 \pm 0.05$ ) organogel-2 brownies ( $0.58 \pm 0.03$ ) and rice bran oil brownies ( $0.57 \pm 0.01$ ) were significantly higher than those of commercial margarine brownies ( $0.45 \pm 0.05$ ). The higher springiness values of the organogels compared to the margarine brownies due to increasing elasticity. The elasticity of the organogel brownies is reflected in the higher values of chewiness as a result of the higher level of protein crosslinking in the batter (Patel et al., 2014).

Cohesiveness relates to crumbliness or perceptions of denseness. Cohesiveness of organogels-1 brownies

( $0.34 \pm 0.03$ ) and organogel-2 brownies ( $0.34 \pm 0.01$ ) exhibited the highest values, followed by commercial margarine brownies ( $0.28 \pm 0.02$ ) and rice bran oil brownies ( $0.28 \pm 0.01$ ). There were significant differences in the cohesiveness of brownies with organogels and rice bran oil. This might be due to the compact and dense cell structure of the brownies led to chewy in texture of the organogels brownies.

Gumminess was calculated by hardness and cohesiveness value of food product, whereas chewiness, defined as the energy required to chew solid food to a state of readiness for swallowing (Karaoglu & Kotancilar 2009). Chewiness and gumminess values in brownies were similar trend with the hardness values. The lowest gumminess and chewiness were observed in the commercial margarine brownie.

## 2. Weight loss and height gain of brownies

Percent weight loss and height gain of various brownies are shown in Table 3. Percent height gains for the brownies prepared with rice bran oil, organogel-1, and organogels-2 were 75.22, 75.81, and 76.38%, respectively. Brownie prepared with commercial margarine had the maximum height gain of 79.23. Percent height gain of brownies prepared with organogels and rice bran oil were lower than those of margarine brownies ( $P > 0.05$ ). The reason for this observation could be attributed to the number of air bubbles (Patel et al., 2014). Commercial margarine had the most gained height of brownies due to during creaming process, dispersed gas could move freely to coalesce into bubbles and float rapidly to the surface and air that can be retained into the batter during the baking process (Matsakidou et al., 2010).

**Table 3** Percent of height gain and weight loss of brownies after baking

Formulation	Weight loss	Height gain
Rice bran oil	5.87±0.01 <sup>a</sup>	75.22±0.11 <sup>b</sup>
Organogel 1	5.80±0.02 <sup>a</sup>	75.81±0.11 <sup>b</sup>
Organogel 2	5.52±0.03 <sup>a</sup>	76.38±0.11 <sup>b</sup>
Commercial margarine	3.73±0.01 <sup>b</sup>	79.23±0.10 <sup>a</sup>

**Remark:** Different letters in the same column are significantly different ( $P < 0.05$ )



Percent weight loss for the brownies prepared with rice bran oil, organogel-1, and organogel-2 were 5.87, 5.80 and 5.52%, respectively. The lowest weight loss was observed in the commercial margarine brownies (3.73%). Brownies contained organogels and rice bran oil displayed a nonsignificant weight loss after baking ( $P > 0.05$ ). The high weight loss during baking could be related to the fact that there was not a developed structure that would retain its shape and its components to any external change. Therefore, the lack of structural integrity in rice bran oil could allow easier water evaporation during baking, even if water were already hydrating the matrix (Rodríguez-García et al., 2013). Organogel brownies have performed inferior weight loss and height gain when compare to the commercial margarine brownies.

### 3. Color

Effects of different fats on the colors of baked brownies are presented in Table 4. The lightness values, i.e.  $L^*$ , of the crumb and crust exhibited the lowest value when organogel-1 and commercial margarine were used, indicating a darker color. Brownies with rice bran oil and organogel-2 displayed the highest  $L^*$  values. The rice bran oil and organogels brownies exhibited higher  $b^*$  values. The crumb and crust color became yellowness for brownies prepared with organogels. The values of redness, i.e.  $a^*$ , became lower for commercial margarine (crust), suggesting that the brownies contained organogels gave lower redness values. The result indicated that brownies showed significant differences in color measurement. However, dark brown colored brownies were visually seen as having very similar. This could be observed in the visual appearance of brownies in Fig. 1.

### 4. Fatty acid composition

The fatty acid compositions of brownies made with various baking fats are presented in Table 5. Commercial margarine brownie contained the highest amount of total SFA (50.47 %) and the lowest amounts of poly unsaturated fatty acid (PUFA) (9.51 %) compared to other brownies. Percentage of monounsaturated fatty

acid (MUFA) was highest in margarine brownies (36.6 %) followed by rice bran oil (40–41 %) and organogels brownies (40–41 %). Organogels and rice bran oil brownies had similar amount of SFA (30–33 %) and had high content of unsaturated fatty acid (USFA). The major SFA in all of brownies sample were palmitic acid (C16:0) and stearic acid (C:18). The major MUFA and PUFA in the brownie samples was oleic (C18:1c9) and linoleic acid (C18:2), respectively. Utilization organogels for baking fat resulted in 27 % increase in USFA content of brownies. The total PUFA content in the organogels brownies increased by 64% when compared to the commercial margarine brownie MUFA s. Furthermore, the total SFA content in the organogels brownies decreased by 40% when compared to the commercial margarine brownies. Replacing the baking fat with organogels could decrease in SFA content, while increasing PUFA content, i.e. linoleic acid, in brownie products.

**Table 5** Fatty acid composition of brownies

Fatty acid composition	Formulations			
	Rice bran oil	Organogel 1	Organogel 2	Commercial margarine
Lauric acid (C12:0)	1.45	0.47	0.46	0.79
Myristic acid (C14:0)	0.90	0.50	0.50	1.16
Palmitic acid (C16:0)	23.29	21.08	21.49	41.26
Stearic acid (C18:0)	4.85	6.85	5.90	6.87
Arachidic acid (C20:0)	0.76	1.15	0.90	0.39
Behenic acid (C22:0)	0.23	2.44	0.91	-
Lignoceric acid (C24:0)	0.33	0.34	0.34	-
Cerotic acid (C26:0)	-	-	-	-
<b>Total SFA</b>	<b>31.81</b>	<b>32.83</b>	<b>30.50</b>	<b>50.47</b>
Palmitoleic acid (C16:1)	0.33	0.39	0.40	0.37
Eicosenoic acid (C20:1)	0.45	0.42	0.45	-
Oleic acid (C18:1c9)	38.98	39.55	39.90	38.86
Oleic acid (C18:1c11)	0.80	0.17	0.92	0.80
<b>Total MUFA</b>	<b>40.56</b>	<b>40.53</b>	<b>41.67</b>	<b>40.03</b>
Linoleic acid (C18:2)	26.79	25.82	26.96	9.51
Linolenic acid (C18:3)	0.85	0.82	0.86	-
<b>Total PUFA</b>	<b>27.64</b>	<b>26.64</b>	<b>27.82</b>	<b>9.51</b>
<b>Total USFA</b>	<b>68.20</b>	<b>67.17</b>	<b>69.49</b>	<b>49.54</b>

**Remark:** SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, poly unsaturated fatty acid; USFA, unsaturated fatty acid

**Table 4** Color of brownies

Formulation	Lightness ( $L^*$ )		Redness ( $a^*$ )		Yellowness ( $b^*$ )	
	Crumb	Crust	Crumb	Crust	Crumb	Crust
Rice bran oil	26.39±0.58 <sup>a</sup>	19.58±0.71 <sup>a</sup>	4.28±0.36 <sup>a</sup>	3.78±0.48 <sup>a</sup>	12.09±0.42 <sup>b</sup>	14.88±0.33 <sup>ab</sup>
Organogel 1	25.56±0.60 <sup>b</sup>	18.58±0.52 <sup>b</sup>	3.38±0.60 <sup>b</sup>	2.63±0.74 <sup>b</sup>	12.19±0.60 <sup>a</sup>	15.03±0.56 <sup>a</sup>
Organogel 2	26.31±0.63 <sup>ab</sup>	18.11±0.75 <sup>b</sup>	3.81±0.64 <sup>ab</sup>	2.45±0.45 <sup>bc</sup>	12.14±0.72 <sup>a</sup>	14.62±0.42 <sup>b</sup>
Commercial margarine	25.68±0.97 <sup>b</sup>	18.07±0.59 <sup>b</sup>	3.68±0.92 <sup>b</sup>	2.22±0.92 <sup>c</sup>	12.16±0.67 <sup>ab</sup>	14.22±0.45 <sup>c</sup>

**Remark:** Different letters in the same column are significantly different ( $P < 0.05$ )

High amount of SFA and low PUFA of were found in the margarine brownies because commercial margarine is prepared by palm oil. Rice bran oil and organogels brownies had similarly fatty acid composition because organogelation did not alter their fatty acid composition (Stortz et al., 2012). Brownies prepared with organogels instead of commercial bakery fats potentially showed the nutritional superiority to the margarine brownie. The organogels and rice bran oil brownies had higher levels of unsaturated fatty acids compared to the commercial margarine. Therefore, they could be utilized as healthier alternatives for bakery fats. Moreover, organogel-2 was prepared with policosanol, long chain alcohols extracted from rice bran wax. It is used as a dietary supplement for lowering blood cholesterol (Weerawatanakorn et al., 2019). Therefore, policosanol organogel brownie not only had lower SFA but also presence healthy functional compound (4% by weight).

### 5. Sensory characteristics

Sensory properties of brownies including appearance, odor, texture, taste and the overall acceptability are important factors determining consumer acceptability. The preference mean sensory scores of various brownies are shown in Table 6. No significant differences were observed in the preference of the evaluated appearance and odor in all the brownie samples. Preference texture scores of rice bran oil, organogel-1, organogel-2 and commercial margarine brownies were 6.72±0.99, 7.08±0.90, 7.36±0.22, and 7.72±0.33, respectively. The brownies using organogel-2 showed high preferences in texture followed by commercial margarine and organogel-1 brownie. Rice bran oil brownies had the lowest scores ( $P < 0.05$ ). Mean scores of texture preference for organogel-1, organogel-2 and commercial margarine brownies were insignificantly different. In the taste evaluation, there was insignificant differences between organogel-2 and commercial margarine brownies. Mean score of organogel-1 and rice bran oil brownies had significantly lower than other sample for taste preference. Rice bran oil, organogel-1, organogel-2 and commercial margarine brownies had overall acceptability scores

of 6.04±0.61, 7.80±0.62, 7.26±0.87, and 7.14±0.28, respectively. No significant differences were observed between organogel-1 and organogel-2 brownies. These results indicated that both of organogel brownies and commercial margarine brownies were comparable in overall acceptability characteristic.

### Conclusion

Rice bran oil was structured with policosanol and triglyceride to form organogels with semi-solid properties. The organogels were utilized for brownie preparation. Organogel brownies with performed similarly to the commercial margarine brownies in terms of texture, while providing the good sensory properties. Reduction in the SFA content of the baked product by completely replacing commercial margarine with organogels were the most effective in producing nutritionally superior brownies with comparable quality attributes to the margarine brownies.

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**Table 6** Sensory characteristic of brownies

Brownies formulation	Sensory characteristic				
	Appearances	Odor	Texture	Taste	Overall acceptability
Rice bran oil	7.92±0.81 <sup>a</sup>	8.60±0.62 <sup>a</sup>	6.72±0.99 <sup>b</sup>	6.64±0.77 <sup>c</sup>	6.04±0.61 <sup>b</sup>
Organogel 1	7.76±0.46 <sup>a</sup>	8.64±0.80 <sup>a</sup>	7.08±0.90 <sup>ab</sup>	7.30±0.69 <sup>b</sup>	7.80±0.62 <sup>a</sup>
Organogel 2	7.64±0.77 <sup>a</sup>	8.78±0.98 <sup>a</sup>	7.36±0.22 <sup>a</sup>	7.94±0.79 <sup>a</sup>	7.26±0.87 <sup>a</sup>
Commercial margarine	7.30±0.97 <sup>a</sup>	8.82±0.08 <sup>a</sup>	7.72±0.33 <sup>a</sup>	7.96±0.84 <sup>a</sup>	7.14±0.28 <sup>a</sup>

**Remark:** Different letters in the same column are significantly different ( $P < 0.05$ )

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