



Pickering emulsions from rice protein-xanthan gum nanoparticles at different oil content: emulsion properties and using producing cake as a fat replacer

Farklı yağ içeriklerinde pirinç proteini-ksantan zamkı nanopartiküllerinden Pickering emülsiyonları: emülsiyon özellikleri ve yağ ikame maddesi olarak kek üretiminde kullanımı

Elif Meltem İŞÇİMEN^{1*} 

¹ Erciyes University, Engineering Faculty, Food Engineering Department, 38039-Kayseri, Türkiye

¹<https://orcid.org/0000-0002-9849-6352>

To cite this article:

İşçimen, E. M. (2025). Pickering emulsions from rice protein-xanthan gum nanoparticles at different oil content: emulsion properties and using producing cake as a fat replacer. Harran Tarım ve Gıda Bilimleri Dergisi, 29(1): 162-176

DOI: 10.29050/harranziraat.1568345

*Address for Correspondence:

Elif Meltem İŞÇİMEN
e-mail:
eliferen@erciyes.edu.tr

Received Date:

16.10.2024

Accepted Date:

20.02.2025

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at www.dergipark.gov.tr/harranziraat



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ABSTRACT

In the present study, Pickering emulsions with different oil content (15%(PE15),30 (PE30), 45(PE45), and 60 (PE60)) were created with nanoparticles produced from rice protein isolate (RPI) and xanthan gum (XG). The aim was to produce cakes with reduced oil content with these emulsions. For this purpose, firstly the emulsion properties were evaluated. Emulsion activity (EAI)-stability indexes (ESI), ζ -potentials, and nanoparticle structures of the emulsions were investigated. The EAI value was determined as $54.14 \pm 3.19 \text{ m}^2/\text{g}$ and $54.15 \pm 0.95 \text{ m}^2/\text{g}$ for the emulsions containing 15% and 30 (w/w) oil, respectively, while the lowest EAI value was determined as $30.12 \pm 0.89 \text{ m}^2/\text{g}$ for the emulsion containing 60% oil. While the ζ -potential value decreased with increasing oil concentration, oil globule diameters increased. Pickering emulsions with 15%, 30, 45, and 60 oil (C-PE15, C-PE30, C-PE45, and C-PE60) and a control sample were produced with oil. The features of cakes made using emulsions with different oil contents were examined, including measuring the viscosity of batter, baking loss, symmetry index, moisture content, ash content, and sensory analysis. When viscosity values are examined, it can be said that the lowest value was generally recorded in the cake batter prepared with PE15. The pH values decreased as the oil content in the cake batter increased. Baking loss and symmetry index did not significantly differ ($p > 0.05$) between cakes made with emulsion and control. The decrease in the oil ratio in the emulsion and the increase in the RPI-XG nanoparticle solution ratio increased the moisture. As a result, RPI-XG nanoparticles are a suitable material for producing Pickering emulsion. Additionally, cakes can be made with the emulsions that are formed. For product compositions with minimal oil content, the usage of PE15 emulsion can be suggested.

Key Words: rice protein; xanthan gum; nanoparticle; Pickering emulsion; reduced fat cake

ÖZ

Pirinç protein izolatı (RPI) ve ksantan gum (XG) kullanılarak üretilen nanopartiküller ile farklı yağ oranlarına sahip (%15(PE15),30 (PE30), 45(PE45) ve60 (PE60)) Pickering emülsiyonlar oluşturulmuştur. Üretilen bu emülsiyonlar ile yağ oranı düşürülmüş kekler üretilmesi hedeflenmiştir. Bu amaçla ilk olarak emülsiyon özellikleri değerlendirilmiştir. Emülsiyonların emülsiyon aktivite-stabilite indeksleri (EAI-ESI), Zeta(ζ)-potansiyelleri ve partikül yapıları incelenmiştir. EAI değeri %15 va 30 (w/w) yağ içeren PE15 ve PE30

emülsiyonları için sırasıyla $54.14 \pm 3.19 \text{ m}^2/\text{g}$ ve $54.15 \pm 0.95 \text{ m}^2/\text{g}$ olarak belirlenirken en düşük EAI değeri $30.12 \pm 0.89 \text{ m}^2/\text{g}$ olarak %60 yağ içeren PE60 emülsiyon için belirlenmiştir. (ζ)-potansiyel değeri artan yağ konsantrasyonu ile birlikte azalırken yağ parçacık çapları artmıştır. Üretilen Pickering emülsiyonları, yağ yerine kek formülasyonunda kullanılmıştır. Bu amaçla, %15, 30, 45 ve 60 yağ içeren Pickering emülsiyonları (C-kontrol, C-PE15, C-PE30, C-PE45 ve C-PE60) kek üretmek için kullanılmıştır ve kontrol olarak sadece yağ içeren kek örneği hazırlanmıştır. Farklı yağ içeriklerine sahip emülsiyonlar kullanılarak yapılan keklerin özellikleri, hamurun viskozitesi, pişirme kaybı, simetri indeksi, nem içeriği ve kül içeriğinin ölçülmesi incelenmiştir. Viskozite değerleri incelendiğinde genel olarak en düşük değerlerin %15 yağ içeren emülsiyon ile hazırlanan kek hamurunda olduğu söylenebilir. Kek hamuru içerisinde ki yağ oranı arttıkça pH değerlerinin düştüğü görülmüştür. Pişirme kaybı ve simetri indeksi açısından kontrol formülasyonu ile emülsiyon bazlı kekler arasında kayda değer bir fark ($p > 0,05$) olmamıştır. Emülsiyondaki yağ oranının azalması ve RPI-XG nanopartikül çözelti oranının artması nem değerlerini artırmıştır. Sonuç olarak, RPI-XG nanopartikülleri Pickering emülsiyonu üretmek için uygun bir malzemedir. Ek olarak, üretilen emülsiyonlar yağ azaltılmış kek üretimi için uygundur. Minimum yağ içeriğine sahip ürün kompozisyonları için PE15 emülsiyonunun kullanımı önerilebilir.

Anahtar Kelimeler: pirinç proteini; ksantan zımkı; nanopartikül; Pickering emülsiyonu; yağ azaltılmış kek

Introduction

A solid-particle-stabilized emulsion rather than conventional organic surfactants is known as a Pickering emulsion. Pickering stabilizers made of natural and food-grade polymers are the subject of current research because of their food compatibility and improved resistance to coalescence and separation (Tu, Zhang et al. 2023). Since proteins have unique nutritional value and desired techno-functional qualities, they are used to create stable food-grade Pickering emulsions. Plant protein nanoparticles attracted far more attention than animal protein because of their plentiful and reasonably priced source (Shi, Feng et al. 2020). However, the stabilizing capacity of protein-based particles is limited by their propensity to agglomerate at the interface. The emulsification ability of polysaccharide-based particles is limited due to their high hydrophilia, which is caused by the abundance of hydroxyl groups on their surface (Wu, Tang et al. 2022). Compared with single polysaccharide particles or single protein particles, protein-polysaccharide-formed particles have favorable surface activity, and strong spatial stabilization capacity at the same time (Xu, Li et al. 2023, Li, Wu et al. 2024). Considering this situation, protein-polysaccharide coacervates were formed in the present study to increase the effectiveness of Pickering emulsion. In previous studies, Pickering emulsions were created by obtaining rice protein gum arabic coesarvate

(Igartúa, Dichano et al. 2024) and also by obtaining protein-polysaccharide-phenol complex from rice bran (Li, Wu et al. 2024). On the other hand, there are studies examining the effect of protein xanthan gum interaction on Pickering emulsions (Xu, Liu et al. 2023, Li, Wang et al. 2024). However, to the best of the authors' knowledge, there is no study in the literature on emulsions produced with RPI and XG coacervate.

One of the staple foods that is most commonly consumed worldwide is rice (*Oryza sativa* L.). Approximately 750 million tons of rice are produced worldwide, and 18,000 known kinds are farmed in more than 100 countries (Amagliani, O'Regan et al. 2017, Peanparkdee and Iwamoto 2019). Rice, which is considered the main source of protein, especially in developing and underdeveloped countries, contains approximately 7-9% protein in its endosperm part (Roy, Singh et al. 2023). In the food industry, plant proteins are widely used as emulsifiers in place of animal proteins (Xie, Ouyang et al. 2023). Rice protein is a viable source for the food industry due to its excellent physical-functional qualities, large availability, low production cost, and good quality (Moirangthem, Jenkins et al. 2020). Rice protein, produced from rice and rice by-products low in allergens and rich in nutrients, can be used to create emulsions (Xie, Huang et al. 2021).

The bacteria *Xanthomonas campestris* ferments carbohydrates aerobically to create XG, an anionic heteropolysaccharide (Krstonošić,

Dokić et al. 2015). Because it created a double helix structure. It works well as a stabilizer for emulsions and suspensions to increase system viscosity and stability (Xing, Chitrakar et al. 2022).

It was aimed to in the current study, form nanoparticles by utilizing the interaction between RPI and XG and to produce Pickering emulsions from the newly created solution. Pickering emulsions were produced at different oil concentrations and EAI, ESI, zeta potential values were measured and particle diameters were determined from optical microscope images of the emulsions. In addition, Pickering emulsions containing different oil content were added to the cake formulations in order to produce cakes with reduced oil content, and their effects on the physical properties of the cake batter and cake were investigated.

Material and methods

The rice protein (RPI) was purchased from Türkiye (Vegrano) and xanthan gum was purchased from Sigma Aldrich (St. Louis, MO). Protein isolate is 80% protein by weight. For the analyses, every chemical and chemical reagent used was of analytical grade. Sterilized whole milk

was used in the production of cake products. Milk, sunflower oil and corn starch were purchased from a local grocery store.

Preparation of RPI and XG solution

RPI solutions were prepared in accordance with earlier research (Wu, Tang et al. 2022) with some modifications. To create 2% (w/v) RPI dispersions, RPI was first dissolved in distilled water while being constantly stirred at 25 °C for 30 minutes. Then, the protein solution heating were used to cause protein unfolding for 30 minutes of 90 °C. The solutions were then cooled to 25 °C.

The XG solutions were prepared according to previous literature (Matsuyama, Kazuhiro et al. 2021) with some modifications. 0.3% (w/v) XG solutions were heated to 80 °C while being continuously stirred until the solution completely dissolved. To guarantee proper mixing and hydration, the RPI solutions and the XG solutions were constantly mixed at a ratio of 1:1 (v/v) for three hours.

The average particle diameter of the prepared solution was measured using a Zetasizer (ZS90, Malvern Instruments, UK). The particle diameter of the produced solution was found to be 842.2 nm (Figure 1).

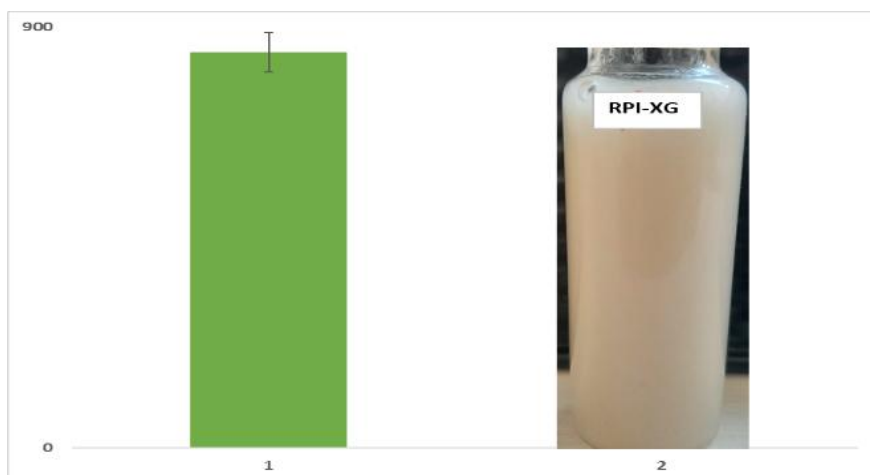


Figure 1. The particle size of the RPI-XG solution

Preparation of Pickering emulsion

The emulsion was prepared according to Xie, Lei et al. (2021) and Abbaszadeh, Aalami et al. (2023) with some modifications. The RPI-XG solutions were mixed with sunflower oil. To investigate the effect of sunflower oil volume

fraction in emulsions on emulsifying behavior, oil was added at different ratios (15%, 30, 45, and 60 w/w) to make a total weight of 40 g. The sunflower oil content was determined based on preliminary experiments and literature (Xie, Lei et al. 2021). The mixture was homogenized at 11000

rpm/min for 3 minutes using a homogenizer (IKA-T18, Staufen, Germany). Emulsions are named PE15, PE30, PE45, and PE60 according to their oil content.

Determination of emulsifying properties

Emulsifying Activity Index (EAI) and Emulsion Stability Index (ESI) of emulsions were measured according to Sui, Bi et al. (2017) and Tang, Yang et al. (2024) with slight modifications. The freshly produced emulsions (20 µL) and then 4.98 mL of sodium dodecyl sulfate (1% w/v) was mixed. The absorbance value of the mixture was measured at 500 nm. Equation following was used to calculate the EAI and ESI based on these measurements:

$$EAI \left(\frac{m^2}{g} \right) = \frac{2 \times 2.303 \times A_0 \times N}{c \times \Phi \times L \times 10^4} \quad (1)$$

$$ESI (h) = \frac{A_0 \times t}{(A_0 - A_t)} \quad (2)$$

N stands for the dilution factor of emulsions and c (grams per milliliter) for the protein content in the protein aqueous solution. Φ , denotes the oil volume fraction in the original emulsions. L, is the thickness of the cuvette. A_0 , absorbance immediately after forming the emulsion and A_t =absorbance 6 h after the emulsion was formed.

ζ -potential

A Zetasizer (ZS90, Malvern Instruments, UK) connected to dynamic light scattering and electrophoresis apparatus was used to assess the

ζ -potential of emulsions. The materials were diluted with distilled water (pH 7) before analysis.

Determination of microstructure

The morphological features of the emulsions were characterized using optical microscopy (Leica, DM500, USA) at a 10x magnification, enabling the examination of their structural characteristics on the first day. Next, using Image J software, the sizes of the emulsion droplets were measured in photos. The average droplet diameters and diameter distribution of emulsions were calculated using the Origin program (Thorne, Simkovic et al. 2019).

Cake batter preparation

The pound cake recipe used as reference batter was produced according to (Bedoya-Perales and Steel 2014) (Table 1). A Bosch mixer was used to produce the cake batter. The cakes produced with emulsions containing 15%, 30, 45 and 60 percent oil were named as C-PE15, C-PE30, C-PE45, and, C-PE60, respectively. The formulation is the same in all cakes except for the oil content (Table 2). Also, C-Control produced with only sunflower oil. In the first stage of batter production, eggs, and sugar were mixed at the highest speed of the mixer for 3 minutes in order to cream them and ensure sufficient aeration. In the 2nd stage, other ingredients (milk, emulsion/oil, wheat flour, corn starch, salt, and baking powder) were added and mixed for 2 minutes at the lowest speed. In the 3rd stage, the cakes were baked in the oven at 200 °C for 30 minutes and then cooled.

Table 1. Formulation used in the preparation of cake batter

Ingredients	Grams
Wheat flour	950
Corn starch	50
Whole milk (3% oil)	450
Liquid egg	500
Sugar	787,5
Fat	400
(Sunflower oil, PE15, PE30, PE45, and PE45)	
Baking powder	25
Salt	5

PE15, PE30, PE45, and, PE60 are Pickering emulsions produced with RPI-XG nanoparticles and 15%, 30%, 45%, and 60% sunflower oil respectively.

Table 2. Sample code and PE-oil content

	control	C-PE15	C-PE30	C-PE45	C-PE60
RP-XG (g)		340	280	220	160
Sunflower oil (g)	400	60	120	180	240

PE15, PE30, PE45, and, PE60 are Pickering emulsions produced with RPI-XG nanoparticles and 15%, 30, 45, and 60 sunflower oil respectively.

Determination of viscosity of cake batter

The viscosity of the cake batter was determined at room temperature (25°C) at varying rpm speeds with a viscometer (Thermo Scientific, HAAKE Viscometer-C, USA) with an L-04 type probe in the C-Control batter and in batters containing Pickering emulsions prepared with different oil ratios (Baltacıoğlu, Temzisoy et al. 2020).

Baking loss

The following equation was used to obtain the baking loss (%) throughout the baking process;

$$\text{Baking loss (\%)} = \frac{W_i - W_f}{W_i} \times 100 \quad (3)$$

where W_f is the weight of the baked cake after it has cooled to room temperature and W_i is the weight of the batter before baking (Grossi Bovi Karatay, Rebellato et al. 2022).

pH

A pH meter (Hanna Instruments, Italy) was used to measure the pH of the cake. 10 g of cake batter was homogenized in 100 mL of distilled water at 25 °C in order to measure the pH (Bedoya-Perales and Steel 2014, Baltacıoğlu and Uyar 2017).

Symmetry index

The cake symmetry index was computed according to Mustafa, He et al. (2018) with some modifications. To put it briefly, three slices that were positioned at one-quarter (B), one-half (C), and three-quarters (D) of the cake length each had their center height determined. The symmetry index was then determined on the day of production using Eq.4.

$$2xC - B - D \quad (4)$$

Moisture Content

About 3 g of the differently formulated cakes were placed in an oven (IN 160Plus Memmert, Germany) and dried at 105 °C for 3h (Grossi Bovi Karatay, Rebellato et al. 2022).

Sensory Analysis

Thirty semi-trained panelists, including faculty and students from Erciyes University's Food Engineering department, participated in the sensory evaluation of the cakes. White plates containing samples at room temperature were supplied. Each sample's colour, odor, texture, taste, and overall acceptability were assessed by the panelists. A five-point was used to rate the samples: 1 strong dislike, 2 dislike, 3 neither a like nor a dislike, 4 like and 5 represented a strong like (Azadfar, Elhami Rad et al. 2023).

Statistical analysis

Using Minitab software (Minitab Ltd., Coventry, England), the ANOVA multiple comparison approach was used to statistically analyze the data collected for this study. Two replicates were studied and Tukey test was used for multiple comparisons of means.

Result and discussion

Emulsifying properties

It is usual practice to use the emulsifying activity index (EAI) and emulsion stability index (ESI) to assess ability to create and maintain an emulsion of protein (Tang, Yang et al. 2024). As seen in Figure 2, as the oil content of the emulsions increased, the EAI value decreased. The difference between the EAI values of PE15 (54.14±3.19 m²/g) and PE30 (54.15±0.95 m²/g) samples was found to be statistically insignificant (p>0.05). Similarly, there was no significant

difference between PE45 ($36.16 \pm 3.34 \text{ m}^2/\text{g}$) and PE60 ($30.12 \pm 0.89 \text{ m}^2/\text{g}$) samples ($p > 0.05$). The highest value was recorded for PE15 and PE30. It's possible that the capacity to expand and disperse of proteins enhances the emulsification

process by increasing spatial repulsion and preventing droplet agglomeration and emulsion precipitation (Tang, Yang et al. 2024). Accordingly, it can be said that high protein content improves the emulsion structure.

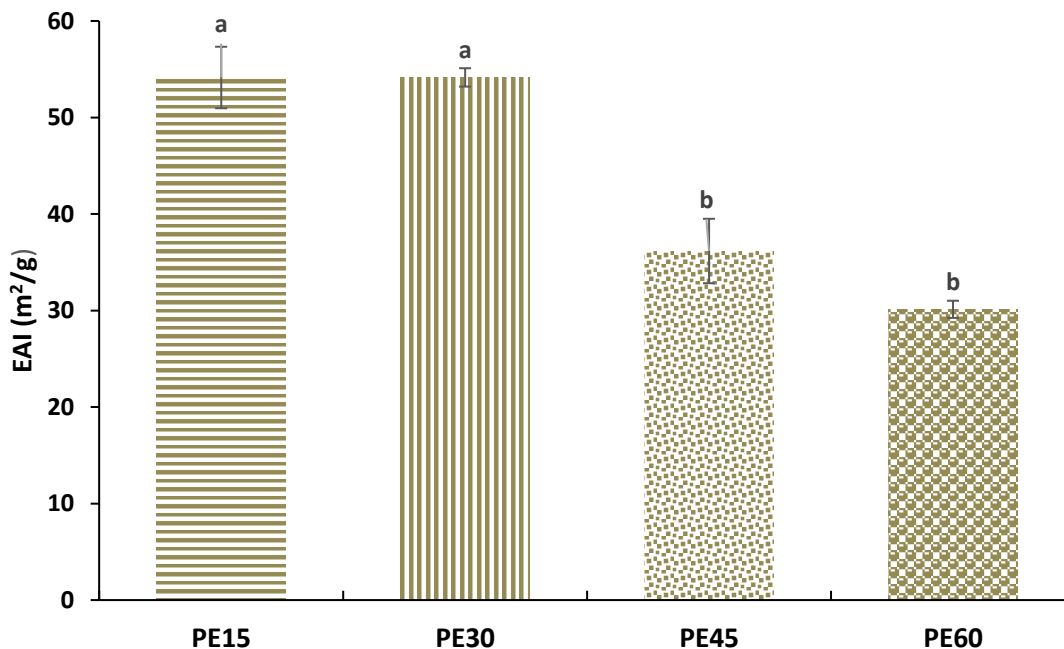


Figure 2. Emulsion activity index (EAI) of Pickering emulsion prepared with RPI-XG nanoparticles. Different letters indicate significant differences ($p \leq 0.05$) between means. The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %.

ESI value is given in Figure 3. The highest stability index was measured as $31.71 \pm 0.87 \text{ h}$ for PE60. While no difference ($p > 0.05$) was observed between the ESI values of PE15 and PE45 samples, the lowest ESI was determined for PE30. The ESI value was found as 24.88 ± 0.23 , $16.06 \pm 0.58 \text{ h}$, $26.21 \pm 0.02 \text{ h}$, and $31.71 \pm 0.87 \text{ h}$ for PE15, PE30, PE45, and PE60. The substantial rise in the ESI at 60% oil phase fraction may have resulted from an increase in the rate at which oil

droplets accumulated, raising the viscosity of the emulsion and lowering the rate of fat lifting (Sun and Gunasekaran 2009). These findings suggest that the creaming of emulsions is primarily caused by an increase in the oil phase. Therefore, as the oil phase fraction increased, the packing percentage of oil droplets increased as well, improving emulsion stability. (Dickinson and Golding 1997).

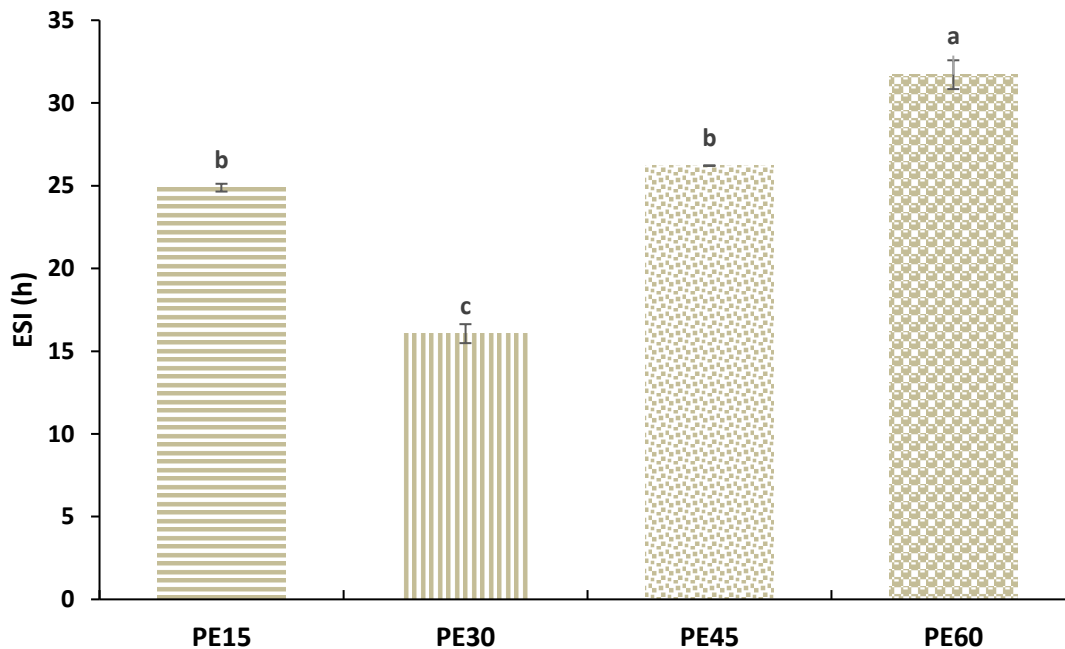


Figure 3. The emulsion stability index (ESI) of Pickering emulsion prepared with RPI-XG nanoparticles. Different letters indicate significant differences ($p \leq 0.05$) between means. The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %.

ζ -potential

The electrostatic stability of particles is typically described using the ζ -potential, which represents the net charges on the particle surface and has a direct impact on the functional characteristics of proteins (López-Monterrubio, Lobato-Calleros et al. 2020). Since the pH of the emulsion was greater than the isoelectric point of the RPI, the ζ -potential of every emulsion was negative, allowing the protein to adsorb OH^- (Zhao, Wei et al. 2015). The highest ζ -potential value was recorded for PE15, the sample with the highest protein/oil ratio as -34.10 ± 0.90 mV. A higher oil phase volume fraction necessitates more protein to reduce the surface tension

because negatively charged protein can adsorb onto droplet surfaces and the effect of oil phase volume on surface charge is dependent on protein concentration (Sun and Gunasekaran 2009). Accordingly, while the amount of protein remained constant, the increase in the amount of oil decreased the ζ -potential. While the difference between the ζ -potential values of PE15 (34.10 ± 0.90 mV), PE30 (32.88 ± 0.92 mV), and PE45 (31.83 ± 0.99 mV) samples was not significant, the PE60 (27.55 ± 0.33 mV) emulsion was found to be statistically different ($p \leq 0.05$) from them.

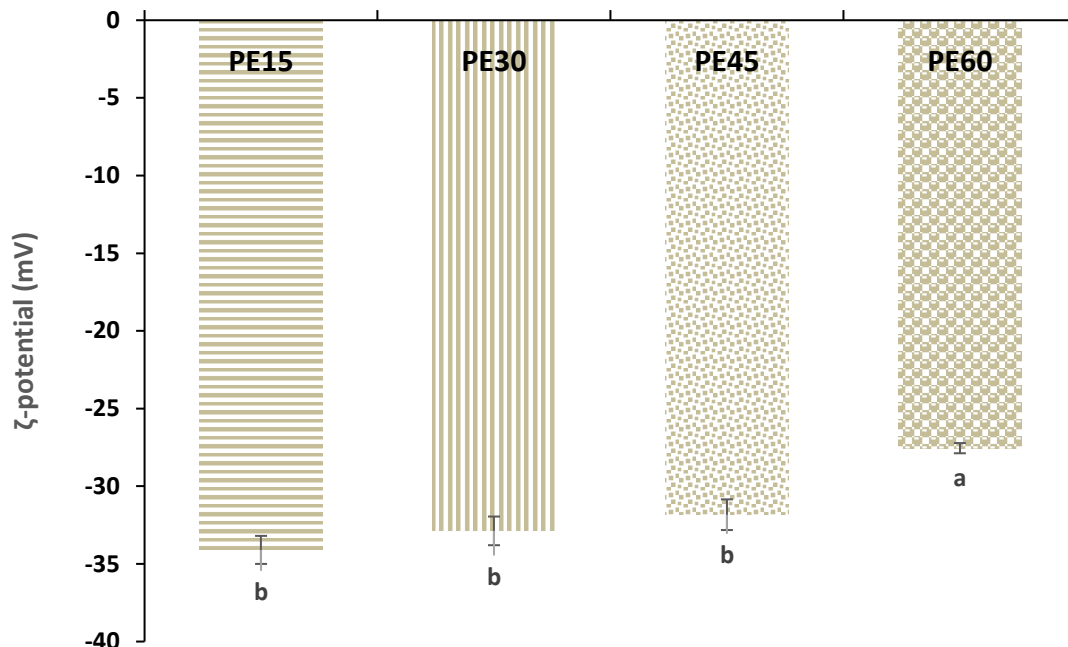


Figure 4. ζ -potential value of the Pickering emulsion prepared with RPI-XG nanoparticles.

Different letters indicate significant differences ($p \leq 0.05$) between means.

The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %.

Determination of microstructure

Digital photos and optical micrographs of Pickering emulsions stabilized by RPI-XG nanoparticles with varying oil phase volume percentages are displayed in Figure 5. The emulsion droplets had a spherical shape and were typically smaller than 30 μm . All of the emulsions displayed an unimodal droplet size distribution, and the droplet size progressively grew as the oil phase volume percentage increased. As the percentage of sunflower oil rose from 15% to 60%, the size distribution grew. Similar results have been reported by (Xie, Lei et al. 2021). The distribution of globule diameters and the average particle size determined by taking the average of measurements from two different images with the image J program were determined with the origin program. Accordingly, the average particle diameters of PE15, PE30, PE45 and PE60 were found to be 22.63 ± 1.13 , 23.18 ± 1.89 , 23.41 ± 2.99 ,

and 29.16 ± 1.57 μm , respectively. As a result, it can be said that the size distribution of the emulsion is affected by the sunflower oil ratio. These findings demonstrated that, at a very low and consistent particle concentration, the Pickering emulsion system made with RPI-XG nanoparticles generated a greater oil-water interface as the oil fraction increased. Consequently, there were fewer particles accessible per unit of the oil-water interface, which led to the coalescence of oil droplets and an increase in droplet size (Song, Pei et al. 2015, Tan, Han et al. 2021). It is anticipated that as the oil phase volume fraction rises, the frequency of emulsion droplet collisions would rise as well, leading to faster flocculation (Soleimanpour, Koocheki et al. 2013).

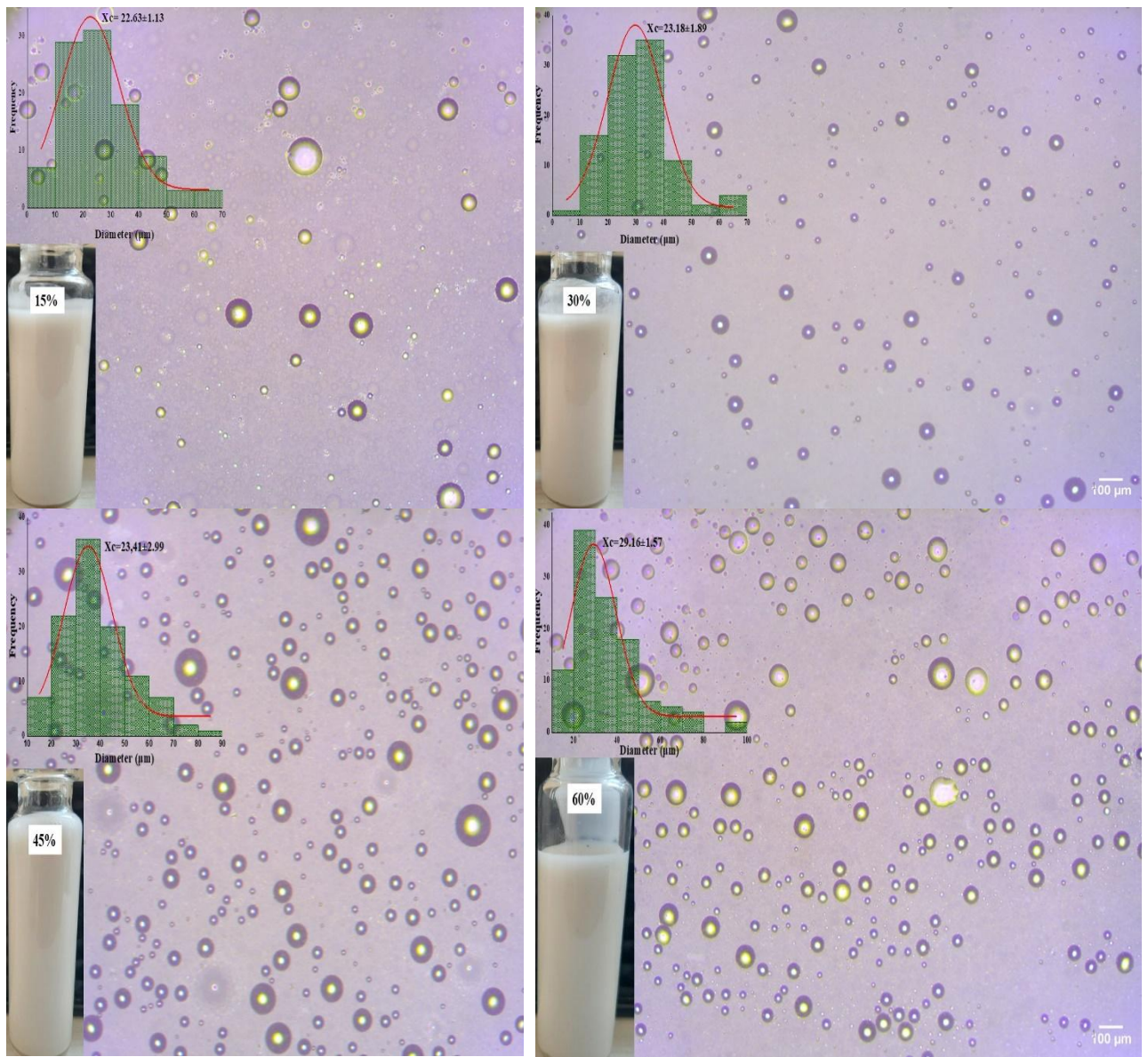


Figure 5. Micrographs of the emulsions stabilized with RPI-XG nanoparticle.

The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60%. Inset: digital photos of the corresponding emulsions and the droplet size distribution of fresh emulsions with different oil phase volume fractions.

Cake batter viscosity

Since viscosity affects bubble production and the movement of bubbles upward owing to stability is inversely proportional to viscosity, it is known that the viscosity of the cake batter influences the final cake volume (Handleman, Conn et al. 1961). Viscosity values of cake batters prepared with Pickering emulsions containing different amounts of oil were measured at different rpm (Figure 6). In general, the lowest viscosity values were recorded in the C-PE15 cake batter prepared with PE15 emulsion. It was observed that the viscosity value increased as the amount of oil in the Pickering emulsion increased. Except for the results obtained at 5 rpm, the highest viscosity value was recorded in the

control sample (C-control) batter produced using only sunflower oil. Lakshminarayan, Rathinam et al. (2006) reported that when the fat in the formulation was reduced, there was a decrease in batter viscosity and therefore cake volume. Bath, Shelke et al. (1992) said that the retention of leavening gas during baking was related to cake batter viscosity. Incorporating more air bubbles into the batter and preventing them from rising to the top are made possible by higher viscosities in cake batter, which gives the cake greater stability (Kumari, Jeyarani et al. 2011). The results showed that the dough containing 100% sunflower oil was more stable and there was a decrease in dough viscosity with decreasing oil content.

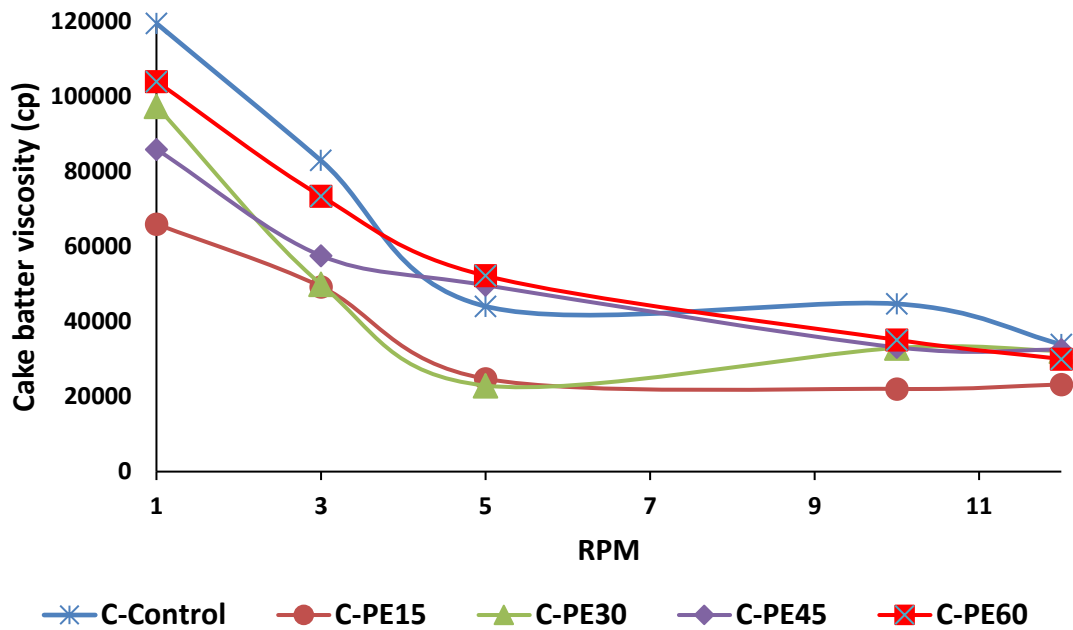


Figure 6. Viscosity of reduced fat cake batter with Pickering emulsions

C-control: the cake produced sunflower oil; C-PE15: the cake produced with PE15; C-PE30: the cake produced with PE30; C-PE45: the cake produced with PE45; C-PE60: the cake produced with PE60. The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %

Cake Properties

Photos taken from the side, top, and middle of cakes made with Pickering emulsions with different fat content are shown in Figure 7. It can be said that the surface color of the control sample is darker, but the bubble distribution is more homogeneous when looking at the internal structure. It was found that when the oil content of the Pickering emulsions added to the cakes increased, the pH value decreased, and the lowest pH value was recorded as 6.62 in the

control sample (Table 3). This situation can be attributed to the high pH value of RPI-XG used in emulsion production. As the oil content in the mixture increased, the pH also decreased. In previous studies, it has been reported that cake pH values vary between 6 and 8 (Baik, Marcotte et al. 2000, Masoodi, Sharma et al. 2002). pH values 6.50 to 7.70 are considered good for cake batters because cake texture and color are correlated with pH (Bedoya-Perales and Steel 2014).

Table 3. Physical properties of reduced fat cakes produced with Pickering emulsion produced with RPI-XG nanoparticles

Sample	pH	Baking Loss (%)	Symmetry Index	Moisture (%)	Ash (%)
C-Control	6.62±0.01 ^e	15.12±1.00 ^a	7.0±1.00 ^a	18.23±0.82 ^b	1.30±0.00 ^b
C-PE15	6.86±0.01 ^b	12.40±2.78 ^a	7.5±0.50 ^a	25.08±2.36 ^a	1.51±0.05 ^a
C-PE30	6.89±0.00 ^a	15.53±3.02 ^a	7.5±0.50 ^a	26.09±3.30 ^a	1.42±0.00 ^a
C-PE45	6.83±0.01 ^c	15.53±3.02 ^a	6.5±0.05 ^a	18.33±1.36 ^b	1.42±0.01 ^a
C-PE60	6.78±0.01 ^d	13.93±2.91 ^a	7.0±1.50 ^a	19.39±0.88 ^b	1.29±0.06 ^b

Different letters indicate significant differences ($p \leq 0.05$) between means.

The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %. Inset: digital photos of the corresponding emulsions and the droplet size distribution of fresh emulsions with different oil phase volume fractions.

Baking loss, symmetry index, moisture, and ash parameters of cakes made with emulsions containing different oil content (Table 3) were determined. In terms of baking loss and symmetry index, there was no discernible difference ($p > 0.05$) between the control formulation and emulsion-based cakes. When ash and moisture values are examined, it can be said that C-PE15

and C-PE30 cake samples are different from the control, C-PE45, and C-PE60. The decrease in the oil ratio in the emulsion and the increase in the RPI-XG nanoparticle solution ratio increased the moisture and ash values. The strong capacity of the hydrocolloids and the protein in the mixture to bind and retain water may be the cause of this (Azmoon, Saberi et al. 2021). Hydrocolloids and

proteins are thought to have a strong tendency to create hydrogen bonds with water molecules because they contain a large number of hydroxyl

groups. As a result, more water molecules would be engaged (Kohajdová, Karovičová et al. 2009).

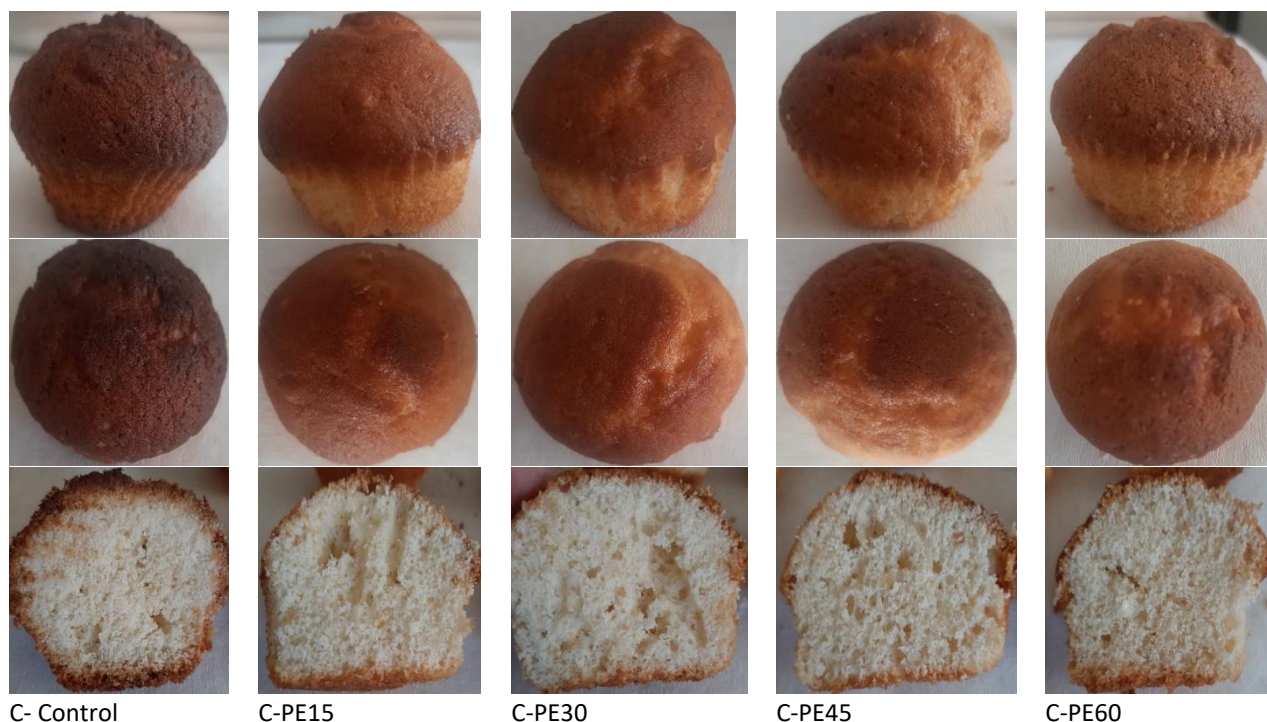


Figure 7. Photos of reduced-fat cake samples

C-control: the cake produced sunflower oil; C-PE15: the cake produced with PE15; C-PE30:the cake produced with PE30; C-PE45:the cake produced with PE45; C-PE60:the cake produced with PE60. The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %

Sensory analysis

Sensory acceptability and/or consumer preferences are crucial in assessing success of a product (Zarzycki, Wirkijowska et al. 2022). Figure 8a shows the average sensory evaluations of the control samples and the prepared cakes made by panelists with varying levels of training. Reducing the oil in the PE emulsion affected the taste and overall quality scores due to the overall oily taste of the cake. One of the most important factors affecting first appeal of baked goods is color. C-PE15 cakes enriched with 15% PE had the lowest color scores, while the control sample had the greatest values. As the oil content in the cake samples increased, the color scores also increased. However, the scores of the samples containing PE are very close but lower than the control sample. The C-PE15 cake samples had the lowest odor value. It can be said that there is no difference in odor score between the C-PE30, C-

PE45 and C-PE60 samples. Similar situations are valid for texture and taste. The C-PE60 sample had the scores that were closest to the control sample when we looked at general acceptability. Again, C-PE15 received the lowest rating. Semi-trained panelists conducted evaluations on cake samples for overall acceptability (Figure 8b). The C-Control sample received a score of 3.97 ± 1.00 , while C-PE60, C-PE45, C-PE30, and C-PE15 samples received scores of 3.73 ± 1.01 , 3.87 ± 0.87 , 3.8 ± 0.81 , and 3.3 ± 1.02 , respectively. In terms of general appreciation, the acceptability values of the samples containing PE are close to each other. In general, it is noteworthy that there were no negative ratings in samples in which the oil content was reduced by creating PE, indicating that PE can be a good fat substitute in cakes because it can preserve the sensory properties of these baked goods even when the fat replacer is high.

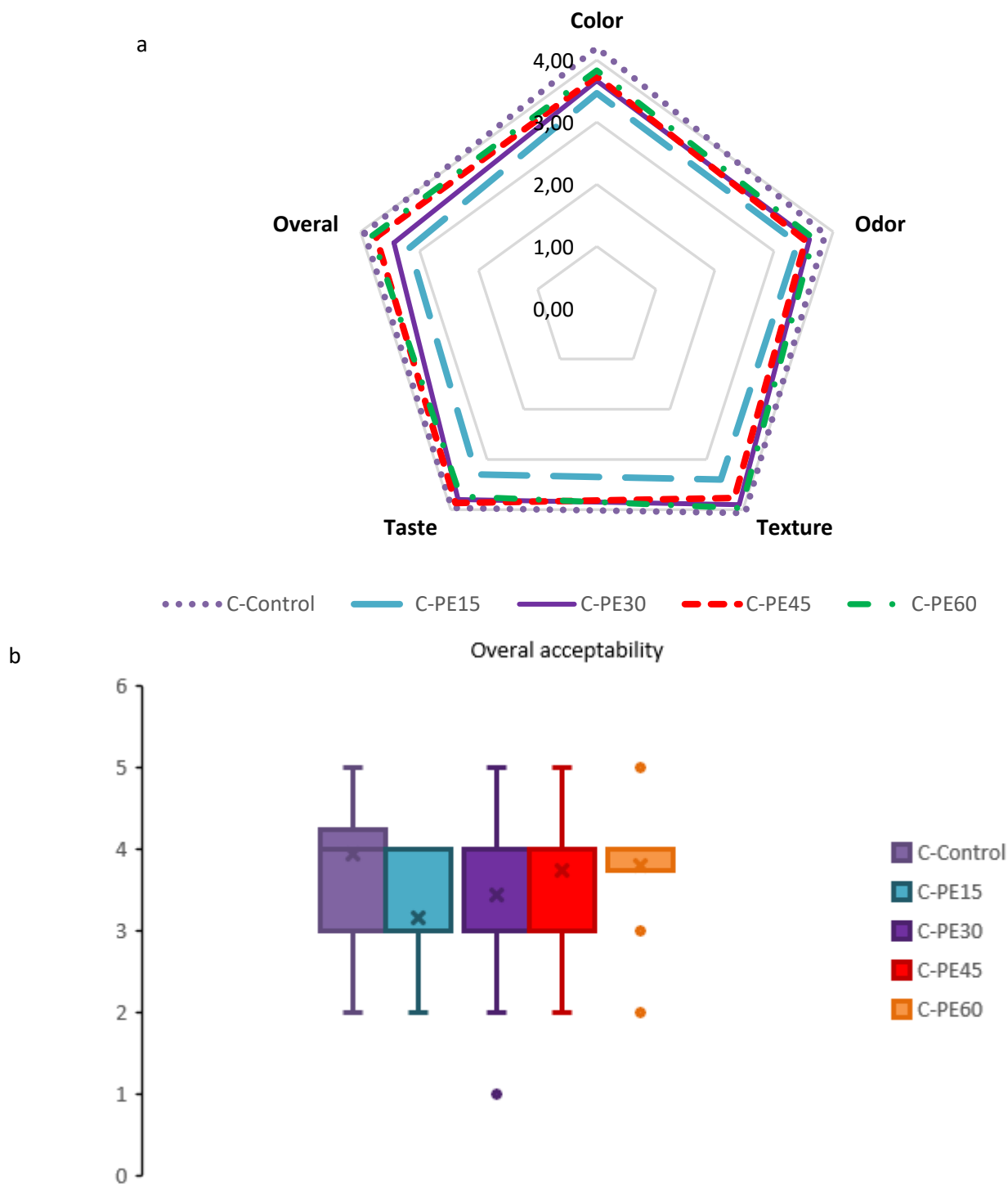


Figure 8. Sensory evaluations and overall acceptability of reduced-fat cake samples
 C-control: the cake produced sunflower oil; C-PE15: the cake produced with PE15; C-PE30:the cake produced with PE30; C-PE45:the cake produced with PE45; C-PE60:the cake produced with PE60. The oil phase volume fraction of Pickering emulsion was (PE15) 15%, (PE30) 30%, (PE45) 45%, and (PE60) 60 %

Conclusion

In the present study, it was aimed to produce cake with reduced fat content using emulsions having different oil ratios (15%, 30, 45, and 60) produced from nanoparticles produced with RPI and XG. Firstly, the properties of emulsions produced with different oil ratios were evaluated. For this purpose, EAI-ESI and zeta potential were determined. The particle size of oil globules was

measured by examining the microstructure of emulsions with an optical microscope. The highest value was recorded as $54.14 \pm 3.19 \text{ m}^2/\text{g}$ for PE15 for EAI. As the oil content in the emulsion increased, a decrease in the EAI value was observed. ESI value increased with increasing oil phase ratio due to the increase in the packing ratio of oil droplets. The ζ -potential values of the samples were recorded for PE15, PE30, PE45, and PE60 as $34.10 \pm 0.90 \text{ mV}$, $32.88 \pm 0.92 \text{ mV}$,

31.83±0.99 mV, and 27.55±0.33 mV. As the oil content in Pickering emulsions increased, the potential value decreased. On the other hand, In the images taken with an optical microscope, the globule sizes of all emulsions were measured below 30 µm. In general, it was observed that the PE sample with the lowest oil content was good in terms of emulsion properties.

The Pickering emulsions produced were replaced with oil in the cake formulation. Therefore, cake production was carried out with a control sample containing 100% oil and Pickering emulsions containing 15%, 30, 45, and 60 oil (C-control, C-PE15, C-PE30, C-PE45, and C-PE60). Firstly, the viscosity of the doughs was measured at different rpm and it was observed that the viscosity generally increased with the increase in the fat content. Baking loss, symmetry index, moisture content, and ash characteristics of cakes prepared using emulsions with varying oil contents were investigated. In general, no negative effect of fat reduction on physical properties of cake was observed. In the present study, fat-reduced cake was produced and in addition, fat was replaced with a solution containing valuable nutritional value such as protein. RPI-XG nanoparticles are a suitable example for producing Pickering emulsion. Also, the produced emulsions are suitable for cake production. The use of PE15 emulsion can be recommended for product formulations with low oil content. In future studies, studies can be conducted to evaluate cake shelf life.

Conflict of interest: Concerning the publishing of this work, the authors disclosed no potential conflicts of interest.

Authors' Contribution: Elif Meltem İŞÇİMEN were in charge of choosing the research subject, carrying out the experiments, gathering and analyzing the data, and composing and reviewing the manuscript.

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