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Food-grade olive oil Pickering emulsions stabilized by starch/β-cyclodextrin complex nanoparticles: Improved storage stability and regulatory effects on gut microbiota

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ABSTRACT

The beneficial effects of olive oil consumption make it be one of the leading healthy edible oils worldwide. However, the water insolubility and oxidation of olive oil hinder its applications. In this study, food-grade oil-in-water olive oil Pickering emulsions (OOPEs) stabilized by hybrid starch/β-cyclodextrin nanoparticles (starch/β-CD NPs) was developed and characterized. The starch/β-CD NPs concentration and oil phase fraction had a significant influence on the droplet size, zeta potential and storage stability of emulsions. Confocal laser scanning microscopic observation revealed that the starch/β-CD NPs mainly located at the interface of oil droplets, forming a network structure to stabilize emulsions. Rheological analysis indicated the shear-thinning behavior and gel-like structure of starch/β-CD NPs stabilized OOPEs. Additionally, the obtained OOPEs remarkably improved the oxidative stability and storage stability by inhibiting the lipid oxidation compared to pure olive oil. Furthermore, animal experiments indicated that OOPEs modulated the gut microbiota in mice with an enhanced richness and diversity, resulting in an increased abundance of health-promoting bacteria. Notably, high-dosage OOPEs significantly decreased the ratio of phyla Firmicutes/Bacteroidetes. These findings could facilitate the development of olive oil Pickering emulsion with excellent stability and health effects on gut microbiota for applications in foods.

1. Introduction

Olive oil is extracted from olives by non-chemically induced mechanical means, and thus retains more original flavor and beneficial components, including fatty acid, terpenes, chlorophylls, carotenoids, and (poly)phenols such as tyrosol, hydroxytyrosol, and oleocanthal (Bonvino et al., 2018). Olive oil is credited as one of the key healthy ingredients in the Mediterranean diet, and is becoming an appreciated dietary fat worldwide. Consumption of olive oil is proven to be associated with several health promoting attributes such as reducing the risk of cardiovascular disease, cancer, obesity, diabetes, hypercholesterolemia, and inflammation disorders (Gavahian et al., 2019). In addition, the bioactive components present in olive oil also have potential to confer intestinal health via modulation of the gut microbiota (Gavahian et al., 2019). Specifically, the polyphenols of olive oil inhibit the growth of foodborne pathogens, while stimulate a higher biodiversity of beneficial gut bacteria in mice and enhance their balance (Hidalgo et al., 2014; Martinez et al., 2019).

Olive oil has a relatively longer shelf life than other oils largely attributed to its high content of polyphenols and other antioxidants. Nevertheless, the denaturation of antioxidants in olive oil occurs during the first three months, thereby affecting the nutritional value and resulting in rancidity (Krichene, Desamparados Salvador, & Fregapane, 2015). Olive oil is mainly consumed by daily intake via cooking, whereas heating may deactivate the beneficial ingredients in olive oil and lose the optimal aroma. Meanwhile, the lack of olive oil product is partly owing to its incompatibility in aqueous media, which limits the application of olive oil. Therefore, it is imperative to develop a technique for olive oil to be applied in aqueous systems and to prolong the shelf life. Emulsification is an effective way to incorporate easily

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degradable bioactive compounds, and to protect them against oxidation and increase their bioavailability (Katsouli, Polychniatou, & Tzia, 2018). In addition, encapsulating olive oil as emulsions could be a novel food product and act as an animal fats alternative.

Many food products are typical emulsion-based systems such as milk, salad, creams, mayonnaise and sauces. Nevertheless, nowadays most of the emulsifiers used in food are synthetic surfactants, which is contrary to consumers' perspective on the natural foods with clean label. In the past decade, the emulsions stabilized by solid particles, known as Pickering emulsions, have attracted significant attention owing to their surfactant-free character and good application prospects in improving storage stability and sustainably releasing bioactive substances (Mwangi, Lim, Low, Tey, & Chan, 2020). Compared to the conventional surfactant-stabilized emulsions, Pickering emulsions have low toxicity, high stability against coalescence, and environmental friendliness (Geng et al., 2021). Present studies have reported the superior performance of Pickering emulsions in terms of substance delivery (Meng et al., 2020), storage stability (Hu et al., 2020), and quality improvement (Huang, Liu, Zhang, & Guan, 2021). In particular, soybean oil (Hu et al., 2020; Qi, Song, Zeng, & Liao, 2021), corn germ oil (Jiang et al., 2019), and sunflower oil (Dai et al., 2021) have been successfully formulated as food-grade Pickering emulsions using different nanoparticles, and all the emulsions exhibit excellent storage stability.

As an abundant, inexpensive, and the biocompatible and biodegradable properties, starch have received growing attention for emulsion stabilization material. However, starch nanoparticles are prone to aggregation before emulsification due to the inherent polarity and hydrogen bond interaction, which restricts their application in stabilizing Pickering emulsions (Hu et al., 2020). On the other hand, β-cyclodextrin (β-CD) bearing hydrophobic cavity and hydrophilic outer surface can easily adsorb onto the oil-water interface by encapsulation of oil molecules (Shimada, Kawano, Ishii, & Nakamura, 1992). Interestingly, the hybrid materials of β-CD and starch display both advantages of β-CD and starch nanoparticles with good water dispersity and emulsifying property, thereby hindering the natural aggregation of starch nanoparticles (Lin, Huang, & Dufresne, 2012). A recent research has demonstrated that starch/β-CD complex nanoparticles showed superior performance in Pickering emulsion preparation to enhance the storage stability of plant oil (Hu et al., 2020). Therefore, in this study, olive oil Pickering emulsions (OOPEs) were prepared using starch/β-CD nanoparticles (starch/β-CD NPs) as stabilizer. The characteristics, storage stability and oxidation stability of the OOPEs were systematically investigated. Furthermore, we explored the regulatory effects of the OOPEs on the intestinal microbiota of mice *in vivo*.

2. Materials and methods

2.1. Materials

Virgin olive oil and rap oil were provided by Sichuan Dingrunyuan Biological Technology Co., Ltd. (Sichuan province, China). β-Cyclodextrin (≥99%) and pullulanase (EC 3.2.1.41) were purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Waxy maize starch (approximately 2% amylose and 98% amylopectin), Nile Red and Nile Blue A were obtained from Aladdin Regent Co. (Shanghai, China). All other chemicals were of analytical grade.

2.2. Preparation of starch/β-CD NPs

Starch/β-CD NPs were prepared according to a previously reported method with minor modifications (Hu et al., 2020). Briefly, short linear glucose was obtained by hydrolyzing waxy maize starch with pullulanase (134 U/g starch) at 58 °C for 6 h. After centrifugation, the supernatant was recrystallized and freeze-dried to obtain starch nanoparticles. The starch nanoparticles (1 g) were dispersed in 100 mL of water and stirred at 100 °C for 30 min. Subsequently, 2 g β-CD was then added into the solution and thoroughly dissolved, followed by addition of 400 mL of ethanol with further agitation for 1 h. Finally, the mixture was centrifuged at 4000 rpm for 10 min, rinsed with ethanol and freeze-dried to obtain starch/β-CD complex nanoparticles.

2.3. Characterization of starch/β-CD NPs

The starch/β-CD NPs were diluted to 1 mg/mL with ultrapure water, then the particle size and zeta potential of starch/β-CD NPs were measured by Malvern Zetasizer Nano (Malvern Panalytical, Ltd., Malvern, UK). The water contact angle of starch/β-CD NPs was measured by using a DSA25 optical contact angle meter (Kruss, Hamburg, German) at room temperature under atmospheric conditions. Sample was prepared as pellet of 10 mm diameter and 2 mm thickness, then a drop of ultrapure water (2 μL) was deposited on the surface of the pallet using a high-precision injector. After 10 s, the drop image was recorded using the high-speed video camera originally assembled by the contact angle meter.

2.4. Preparation of olive oil Pickering emulsions

Olive oil Pickering emulsion were prepared using starch/β-CD NPs as stabilizer by a high-speed homogenizer (Ultra Turrax Digital D-500, Wiggens, Germany). Briefly, different amounts of starch/β-CD NPs were dispersed in water and incubated at 60 °C for 30 min. Olive oil was then added and homogenized for 20 s each time and 10 s intermittently with a total of 6 times to obtain Pickering emulsions.

2.5. Characterization of Pickering emulsions

2.5.1. Visual appearance and creaming index analysis

Creaming index was used to characterize the stability of starch/β-CD NPs stabilized Pickering emulsions as described previously with some modifications (Ge et al., 2017). Freshly prepared emulsions were poured into 30 mL glass tubes and sealed, then stored at room temperature for 6 months. The creaming stability was calculated as Eq. (1):

$$\text{Creaming index } (\%) = H_s/H_e \times 100\% \quad (1)$$

where H_s is the height of the subnatant serum layer and H_e is the total height of the emulsion after 6-month storage.

2.5.2. Droplet size and zeta potential measurements

A Malvern Zetasizer Nano (Malvern Panalytical, Ltd., Malvern, UK.) was utilized to measure the average size and zeta potential of starch/β-CD NPs stabilized Pickering emulsions. Diluted samples (1%) using neutral ultra-pure water were measured at room temperature.

2.5.3. Morphology observation

Morphology of the prepared Pickering emulsions was visually observed. Briefly, an aliquot (1 mL) of Pickering emulsion was stained with 20 μL of a mixed staining solution containing 1 mg/mL Nile red and 1 mg/mL Nile blue A. The dyed emulsions were placed on slides and covered with coverslips. The fluorescence image from the samples was viewed with a Leica SP8 confocal laser scanning microscope (CLSM, Leica Microsystems, IL, USA) equipped with an argon ion laser. The excitation wavelength was set at 488 nm for Nile red and 633 nm for Nile blue A.

2.5.4. Rheology analysis

The rheological properties of the emulsions were characterized by using a Physica MCR 302 Rotational rheometer (Anton Paar, Graz, Austria) at room temperature. A parallel plate (PP50) was used with a diameter of 40 mm and gap of 0.10 mm. The apparent viscosities of the emulsions were carried out in the shear rate range of 0.01–100 s^{-1}. In order to determine the linear viscoelastic region, the strain sweep

experiment was first executed at the frequency of 10 rad/s in the strain range of 0.001–100%. When the strain was fixed at 0.01%, which was in the linear viscoelastic region, the storage modulus (G′) and loss modulus (G″) as a function of frequency were obtained.

2.6. Oxidative stability measurements

2.6.1. Peroxide value (PV) measurement

Pickering emulsions were collected in test tubes and stored in an electrothermal incubator at 55 °C for 14 d to accelerate oxidation. The lipid hydroperoxides (primary lipid reaction products) of the emulsions were measured periodically according to the previous method with some modifications (Shao & Tang, 2014). In brief, 1.5 mL of a mixture of isooctane and isopropanol (3:1, *v/v*) was mixed with 0.1 g emulsions under continuous shaking for 10 s. After centrifugation, the upper layer was collected (200 μL) and reacted with a solution containing 2.8 mL of methanol/butanol mixture (2:1, *v/v*), 15 μL of potassium thiocyanate (3.94 M), and 15 μL of divalent iron ion solution (0.132 M $BaCl_2$ and 0.144 M $FeSO_4$ mixed in a 1:1 ratio). After 20 min of reaction in the dark, the absorbance of the mixture was measured at 510 nm on a UV spectrophotometer (UV-2600, Shimadzu, Japan). Lipid hydroperoxides were quantified by an external standard curve of benzene hydroperoxide.

2.6.2. Thiobarbituric acid-reactive substances (TBARS) measurement

The TBARS (secondary lipid reaction products) of the Pickering emulsions during storage was determined based on the previously reported method with a few modifications (Yang, Yang, Qiu, Xu, & Wang, 2021). Briefly, 0.1 g emulsion was mixed with 1.9 mL of water first, and then 4 mL of thiobarbituric acid (TBA) test solution (15 g trichloroacetic acid and 0.375 g TBA dissolved in 100 mL of 4 M HCl) was add to the mixture. The mixture was boiled in water bath for 15 min and cooled to ambient temperature. After centrifugation, TBARS in the supernatant was quantified by recording at 532 nm on the UV spectrophotometer referred above according to a standard curve with 1,1,3, 3-tetraethoxypropane.

2.6.3. OXITEST analysis

The oxidation tests of olive oil and the prepared OOPEs were conducted by using an OXITEST reactor (Velp Scientifica, Milan, Italy) according to a previous study (Verardo et al., 2013). The sample was placed in a chamber and then sealed, heated to 90 °C and injected oxygen pressure to 6 bar. The pressure change in the chamber was monitored throughout the procedure, and the induction periods (IP) was automatically calculated by using two-tangent method and OXISoft™ program of the instrument.

2.7. Animal treatment procedures

Specific pathogen-free (SPF) male BALB/c mice (weighing 19 ± 1 g, 5-week-old) were purchased from Hua Fukang Biotechnology Co., Ltd. (Beijing, China) and housed under controlled surroundings (23 ± 2 °C, 12/12 h light/dark cycles, 60–70% humidity) with free access to chow diet and water. All experimental procedures were approved by the Animal Care and Use Committee, Sichuan University (protocol number 2021SCU12093-P001). After a one-week acclimation, the mice were randomly divided into six groups (n = 6 per group): (1) control group (8 mL/kg of distilled water), (2) RO group (8 mL/kg of rap oil), (3) OO group (8 mL/kg of olive oil), (4) OOEH group (16 mL/kg of olive oil Pickering emulsion), (5) OOEM group (8 mL/kg of olive oil Pickering emulsion), (6) OOEL group (4 mL/kg of olive oil Pickering emulsion). The mice were received rap oil, olive oil or olive oil Pickering emulsions by gastric gavage once a day for three weeks consecutively. The dose of the three emulsion groups was designed according to the olive oil content in the emulsion, and the gavage dosages in OOEH group, OOEM group, and OOEL group were equivalent to 8, 4, and 2 mL/kg of olive oil, respectively.

2.8. Bacterial DNA extraction, 16S rRNA sequencing and bioinformatic analysis

The feces samples of mice were collected within 1 h and stored at −80 °C until DNA extraction. Bacterial total genomic DNA was extracted using a DNA Extraction Kit (BioTeke, Beijing, China) following the manufacturer's instructions. The V3–V4 region of the bacterial 16S rRNA gene was amplified using the forward primer 343F (5′-TACGGRAGGCAGCAG-3′) and reverse primer 798R (5′-AGGGTATCTAATCCT-3′). The PCR products were purified with Agencourt AMPure XP beads (Beckman Coulter Co., Chaska, MN, USA) and quantified using Qubit dsDNA assay kit (Invitrogen Life Technologies, Carlsbad, CA, USA). Sequencing was performed on an Illumina NovaSeq6000 (Illumina Inc., San Diego, CA, USA) with two paired-end read cycles of 250 bases each by OE Biotech Company (Shanghai, China). Paired-end reads were preprocessed using Trimmomatic software to cut off ambiguous bases, and assembled with FLASH software. After quality filtration, the reads with chimera were further removed using VSEARCH. Sequences with ≥97% similarity were assigned to the same operational taxonomic units (OTUs). The representative read of each OTU was selected using QIIME package and annotated and blasted against Silva database (Version 132) using RDP classifier. The microbial diversity in feces samples were calculated by alpha diversity and beta diversity with QIIME.

2.9. Statistical analysis

The experiments were performed in triplicate and data were expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was applied to statistical analysis using IBM SPSS 25.0 package (SPSS Inc., Evanston, IL, USA). Significant differences between mean values were determined using Duncan's test ($P < 0.05$).

3. Results and discussion

3.1. Characterization of starch/β-CD NPs

The prepared starch/β-CD NPs presented uniform white powder after freeze drying (Fig. 1A). The particle size distribution of starch/β-CD NPs ranged from 396 to 1106 nm with a unimodal size distribution around 825 nm (Fig. 1B). The single peak in size distribution curve revealed that particle swarm distribution was relatively concentrated. The average diameter of starch/β-CD NPs was determined to be 753 nm. Conventional Pickering stabilizers require a suitable contact angle, generally around 90° to ensure suitable wettability, and when contact angle is smaller than 90°, an oil-in-water Pickering emulsion is formed (Shi, Feng, Wang, & Adhikari, 2020). As shown in Fig. 1C, our results suggested the high hydrophilicity of starch/β-CD NPs with a contact angle of 27.5°, which seems to be not suitable to prepare Pickering emulsion. However, in practice, starch/β-CD NPs with high wettability promoted the formation of Pickering emulsion by a unique stabilization mechanism at the oil-water interface (Hu et al., 2020). Meanwhile, our results showed that the zeta potential values of starch/β-CD NPs and β-CD were −40.7 ± 3.8 and −17.5 ± 1.5 mV, respectively. According to a previous study, the high absolute value of zeta potential can enhance the properties of emulsifier particles (Luo et al., 2012). Thus, it was suggested that the higher absolute zeta potential of the starch/β-CD NPs enabled the potential as Pickering stabilizer.

3.2. Effect of starch/β-CD NPs concentration, oil phase fraction and homogeneous speed on Pickering emulsions

The preparation process of Olive oil Pickering emulsions stabilized by starch/β-CD NPs was illustrated in Scheme 1. The obtained emulsion is a light yellow semi-solid paste, with uniform and delicate texture and strong fragrance of olive oil. All prepared emulsions were dispersed

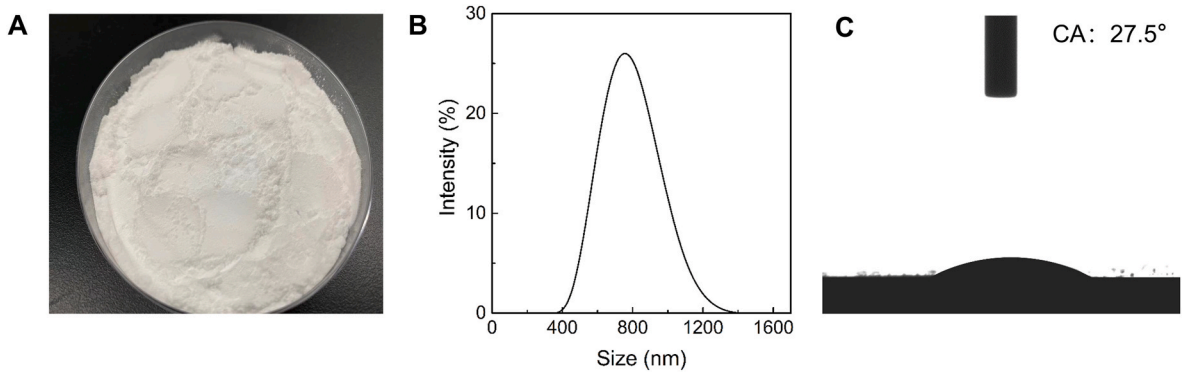
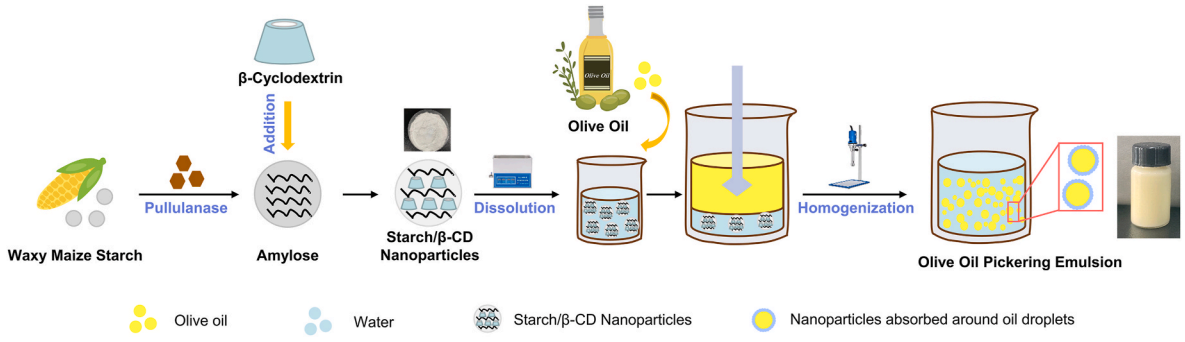


Fig. 1. The photograph (A), size distribution (B), and contact angle (C) of the prepared starch/β-CD hybrid nanoparticles.



Scheme 1. Schematic illustration of preparation of olive oil Pickering emulsion. Starch/β-CD hybrid nanoparticles are prepared with waxy maize starch and β-CD. Olive oil Pickering emulsions are stabilized by the starch/β-CD nanoparticles, which absorbed around the oil droplets and formed an ordered interfacial layer.

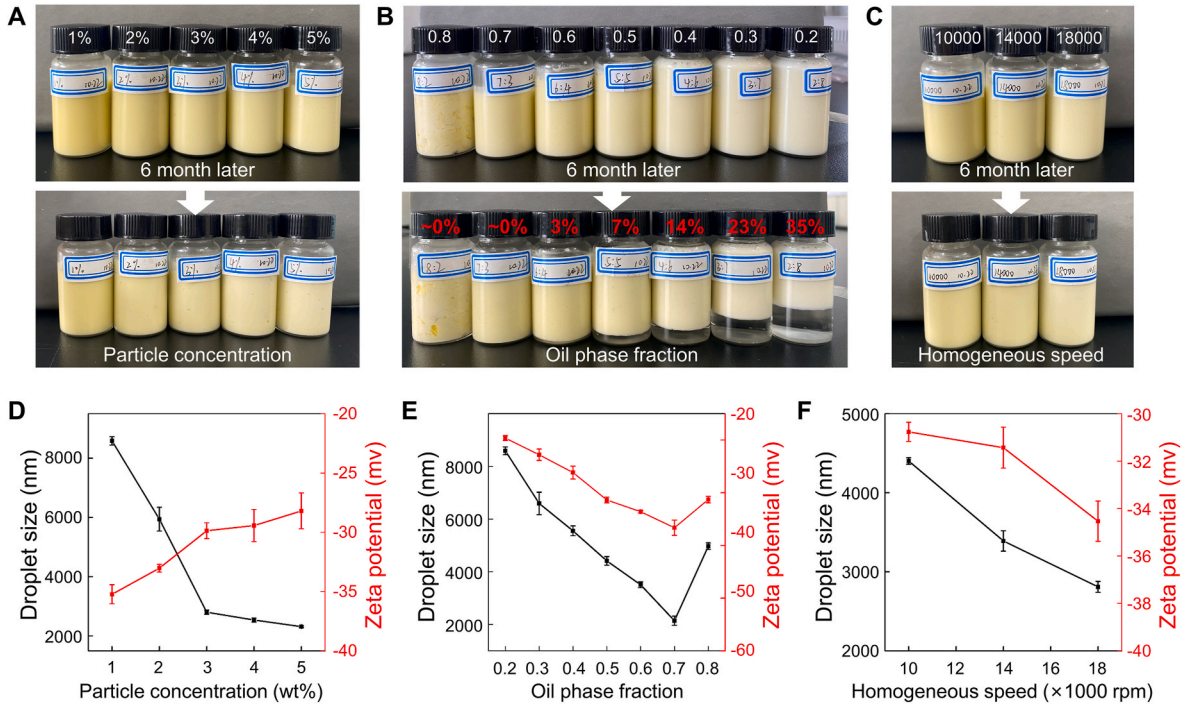


Fig. 2. Photographs of olive oil Pickering emulsions prepared with starch/β-CD NPs at different particle concentrations (1 wt%, 2 wt%, 3 wt%, 4 wt%, and 5 wt%) with a homogeneous speed of 10,000 rpm and oil phase fraction of 0.7 (A); and different oil phase fractions (0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8) with a particle concentration of 3 wt% and homogeneous speed of 10,000 rpm (B); and different homogeneous speed (10,000, 14,000, and 18,000 rpm) with a particle concentration of 3 wt% and oil phase fraction of 0.7 (C). The creaming index values are listed in red. The average droplet size and zeta potential of Pickering emulsions prepared with starch/β-CD NPs at different particle concentrations (D), oil phase fractions (E), and homogeneous speed (F). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

rapidly in water and aggregated in corn oil (data not shown), indicating that starch/β-CD NPs stabilized olive oil Pickering emulsions prepared in this study belonged to oil-in-water type emulsions.

3.2.1. Effect of starch/β-CD NPs concentration on Pickering emulsions

The concentration of a particle-based emulsifier used is known to determine the size of the droplets formed in Pickering emulsions, which in turn affects their stability characteristics (Aveyard, Binks, & Clint, 2003). For this reason, the impacts of starch/β-CD NPs concentration on visual appearance, droplet size and zeta potential were investigated at a fixed oil volume fraction (0.7) and homogeneous speed (14,000 rpm) (Fig. 2A and D). The visual appearance and creaming index values of all the OOPEs kept unchanged even after 6 months of storage, indicating that the emulsions had excellent long-term stable ability. For freshly prepared emulsions, the droplet size decreased from 8584 to 2316 nm with increasing the starch/β-CD NPs concentration from 1 to 5 wt%. The reduction in droplet size observed at higher nanoparticle concentrations might be attributed to the ability to cover a larger interfacial area of the oil droplets, which could form a dense three-dimensional network to stop the emulsion droplets against coalescence or creaming (Mwangi, Ho, Tey, & Chan, 2016). On the other hand, when the particle concentration increased from 1 to 5 wt%, the zeta potential values of OOPEs ranged from −35.2 to −28.2 mV, which is lower than the minimum absolute zeta potential (30 mV) required for physical stable Pickering emulsions (Kibici & Kahveci, 2019). The results suggested that the zeta potential had a negligible effect on the emulsion stability in our case, which has also been observed in a previous research on Pickering emulsions stabilized by particles involved in β-CD (Hu et al., 2020). In fact, it has been demonstrated that CD-based Pickering emulsion droplets with absolute zeta potential values below 30 mV exhibit no obvious difference for stabilities (Li et al., 2014). Hence, it was implied that the unique stabilization mechanism of OOPEs might be mainly because the swelling property of starch fractions in the starch/β-CD NPs provided strong steric hindrance among droplets, preventing the coagulation or flocculation in emulsions. This in turn explained that more stable emulsions with higher particle concentration was partially ascribed to the stronger steric hindrance caused by the swelled starch molecules.

3.2.2. Effect of oil phase fraction on Pickering emulsion

The impacts of oil volume fraction on visual appearance, droplet size and zeta potential were investigated at a fixed starch/β-CD NPs concentration (3 wt%) and homogeneous speed (14,000 rpm). Obviously, a clear boundary between a top cream layer and a bottom serum layer were observed in the emulsions with low oil phase fraction ($\leq$0.5) after stored for 6 months, and the creaming extent increased with decreasing of oil phase fractions (Fig. 2B). In contrast, paste-like emulsion was prepared when the oil phase fraction increased to 0.6 and above, and did not cream throughout the entire storage period of 6 months. Additionally, as shown in Fig. 2E, the droplet size decreased from 8590 to 2142 nm with increasing oil phase fraction from 0.2 to 0.7, whereas it inversely increased to 4969 nm when oil phase fraction at 0.8. Similarly, the zeta potential first decreased with an increase of oil phase fraction, however, when oil phase fraction exceeded 0.7, it raised slightly due to the emulsifying saturated. The results were agreement with a previous study which reported that relatively higher oil fractions within the critical value could lead to more stable Pickering emulsions (Ge et al., 2017). The remarkable influence of oil phase fraction on the droplet size is possibly because oil volume could change the amounts of particles available to adsorb at the oil-water interface.

3.2.3. Effect of homogeneous speed on Pickering emulsions

As shown in Fig. 2C and F, the impacts of homogeneous speed on visual appearance, droplet size and zeta potential were investigated at a fixed starch/β-CD NPs concentration (3 wt%) and oil phase fraction (0.7). With the homogeneous speed increased from 10,000 to 18,000 rpm, the particle size decreased from 4402 to 2809 nm, and the absolute zeta potential values raised from 30.8 to 34.5 mV. However, when the homogeneous speed exceeded 18,000 rpm, emulsification occurred first and then was immediately demulsified (data not shown). The visual appearance showed that Pickering emulsions were uniformly emulsified, which exhibited extraordinary storage stability over a period of 6 months. Storage stability is a key parameter of any formulation because it determines to some degree if a product is suitable for its intended use (Hu et al., 2016). Therefore, the results implied that the prepared food-grade OOPEs might be applied as a potential novel olive oil product in the food industry.

3.3. Microscopy analysis of Pickering emulsions

The interfacial microstructure of the emulsions and the location of starch/β-CD NPs at the two-phase interface was analyzed by CLSM, and the results are displayed in Fig. 3. The starch/β-CD NPs dispersions emit red fluorescence after stained with Nile Blue A, while the olive oil droplets present green fluorescence after stained with Nile Red. As clearly observed from the CLSM images, the red circles of starch/β-CD NPs dispersed continuously around the bright green fluorescence of spherical oil droplets, further confirming that the prepared OOPEs were typical oil-in-water emulsions. The emulsions stabilized with more starch/β-CD NPs showed a relatively small droplets size with a more compact red area like other research (Winuprasith & Suphantharika, 2015), which was also accordance with our results of droplet size measurements, suggesting an effective surface adsorption and oil droplet coverage by starch/β-CD NPs. Moreover, when oil phase fraction <0.5 (Fig. 3F–I), the droplets were scarce and vast aggregates of particles occurred, whereas the reduction of droplets size was observed with the oil phase fraction increased to 0.7 (Fig. 3J-L). CLSM observations also verified that homogeneous speed was a favorable factor for droplet reduction. Overall, starch/β-CD NPs particles in OOPEs (3 wt% starch/β-CD NPs with more than 0.5 oil fraction) were located at the edges of the oil droplets as an ordered interfacial layer. Meanwhile, most of the oil droplets were tightly packed with each other and hard to move, representing a gel-like network structure. This robust interface architecture would prevent the coalescence of oil droplets and confer an excellent physical stability of OOPEs.

3.4. Rheological properties of Pickering emulsions

Rheological measurements can provide a better understanding about the stability and microstructure of emulsions. As illustrated in Fig. 4A and B, the apparent viscosity of the prepared emulsions was increased significantly with the increasing contents of starch/β-CD NPs and the oil phase fraction, at the same shear rate. However, the effect of homogeneous speed on apparent viscosity was not obvious (Fig. 4C). The increased viscosity with the increase of starch/β-CD NPs might be due to the accumulation of particle layer and the formation of stronger network (Zhao, Gu, Hong, Liu, & Li, 2021). The emulsions showed a constant decrease in viscosity with the increasing of the shear rate, indicating that they exhibited shear-thinning behavior and belonged to pseudo-plastic fluids (Jiang et al., 2019). Similar results were also observed for Pickering emulsions stabilized by protein-based nanoparticles (Zhao et al., 2021), which might be explained as the disruption of droplet or droplet clusters result from the high shear rate. In addition, for all OOPEs, the storage modulus (G′) was always higher than the loss modulus (G″) with no crossover throughout the entire frequency sweeps periods (Fig. 4D–F), revealing that the OOPEs exhibited predominantly typical elastic gel-like behavior. Moreover, the increasing trend of G′ and G″ with increased particle concentrations, oil phase fractions and homogeneous speed over the entire frequency range implied the enhanced gel strength, which could be owing to the reduction in droplets size and formation of denser packed oil droplets (Ge et al., 2017).

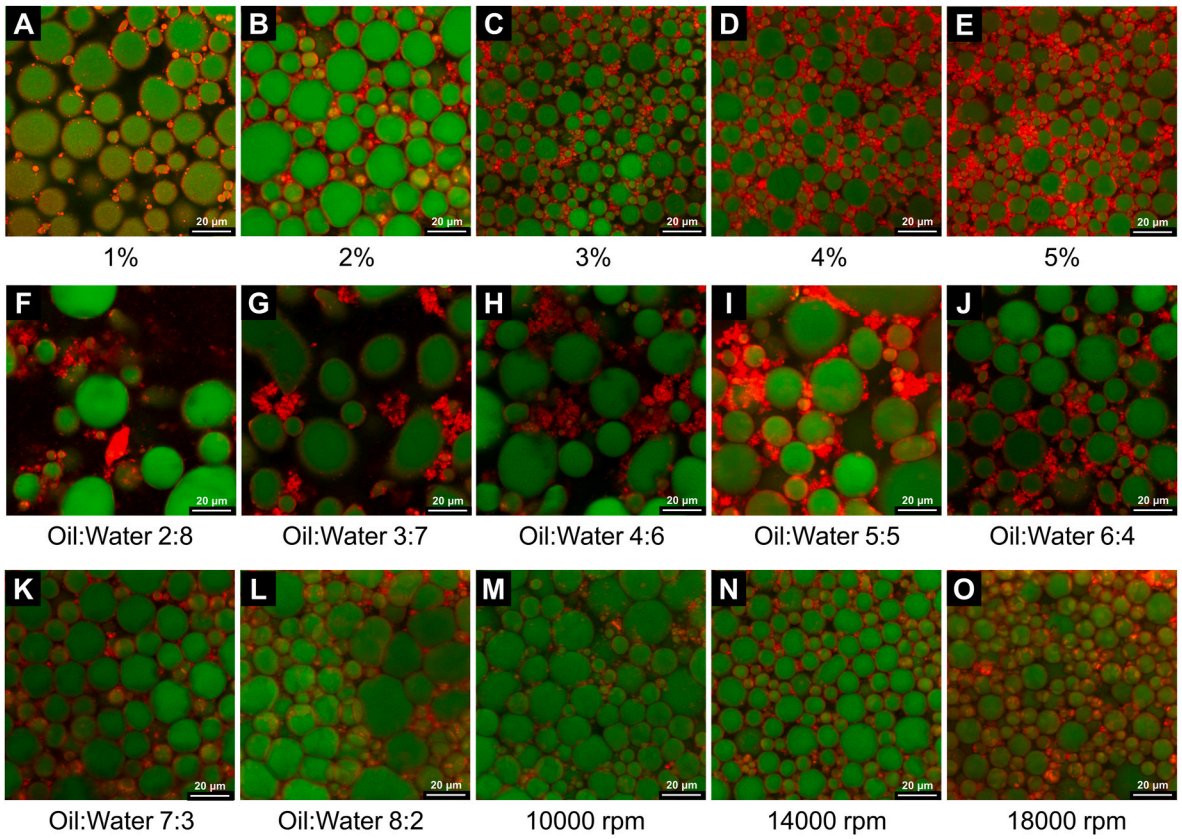


Fig. 3. The confocal laser scanning microscope images of olive oil Pickering emulsions stabilized by starch/β-CD NPs at different particle concentrations (A–E), oil phase fractions (F–L) and homogeneous speed (M–O).

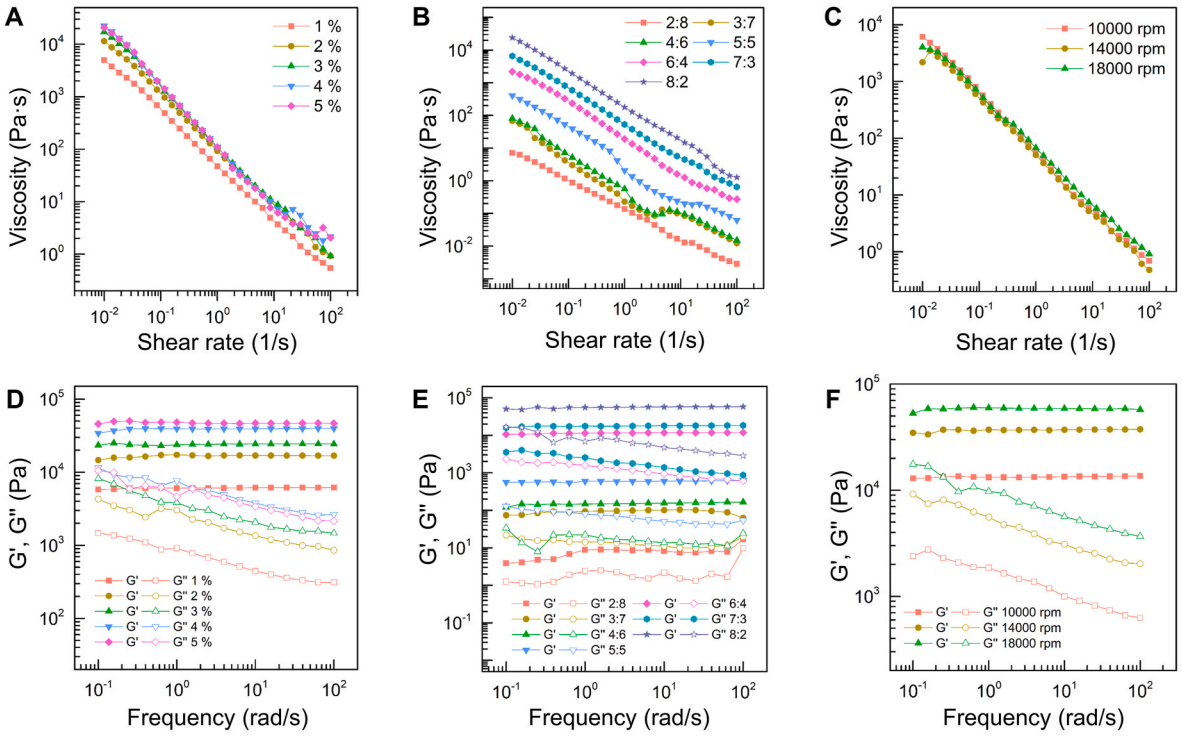


Fig. 4. The apparent viscosity (A–C), storage modulus (G′) and loss modulus (G″) (D–F) of olive oil Pickering emulsions stabilized by starch/β-CD NPs at different particle concentrations (A, D), oil phase factions (B, E), and homogeneous speed (C, F).

3.5. Oxidative stability of Pickering emulsions

3.5.1. Oxidative stability by PV and TBARS measurements

The lipid oxidative stability of the OOPEs was evaluated by determining the formation of primary (PV) and secondary (TBARS) lipid oxidation products, along with measurement of pure olive oil for comparison. As shown in Fig. 5, the PV and TBARS values of OOPEs and pure olive oil increased steadily during storage, however, the OOPEs showed significantly lower PV and TBARS values compared to pure olive oil after 14 d storage ($P < 0.05$). Interestingly, the lipid oxidation further decreased with an increased amount of starch/β-CD NPs, which was consistent with a previous study that reported an enhanced lipid oxidative stability of emulsions with an increase in stabilized particles concentration (Yang & Xiong, 2015). Additionally, the lipid oxidation accelerated with more oil phase fraction. When oil phase fraction over 0.7, the PV and TBARS values were increased remarkably due to the incomplete emulsification as discussed before. However, weak influence on lipid oxidation was observed for the homogeneous speed. In food emulsions, the composition and structure of oil-water interface has critical impact on the rate and extent of lipid oxidation, which is a major cause of quality deterioration in food (Waraho, McClements, & Decker, 2011). In our case, the encapsulated lipid droplets within the starch/β-CD NPs effectively restricted the contact of olive oil with pro-oxidants, thereby delaying the lipid oxidation.

3.5.2. Oxidative stability by OXITEST method

The OXITEST method is an effective method to evaluate the antioxidant capacity of a given substrate, and can directly measure the whole foods without any separation (Verardo et al., 2013). IP was expressed as the final results of OXITEST, which is the "stability time" before lipid oxidation. Higher IP value represents the higher stability of the lipid contained in a sample. As shown in Fig. 5G, the gas pressure inside the test chamber first remained steady and then reduced rapidly, regardless of pure olive oil or OOPEs, indicating the occurrence of lipid oxidation. The IP value of OOPEs determined by OXITEST apparatus was 68.45 h, which was 1.88 times that of pure olive oil (36.42 h). Obviously, emulsification can significantly extend the IP value of olive oil, consequently prolonging the storage time. These results suggested that the prepared OOPEs showed an excellent oxidative stability, which further provided favorable evidence for its application in foods.

3.6. Modulation of gut microbiota by Pickering emulsions

To explore the overall differences in gut microbiota of mice fed with different oil diets, principal component analysis (PCA) was performed. The results showed that mice clustered into relatively distinct groups despite partially overlapping (Fig. 6A). Olive oil and emulsion diets resulted in quite distinguishable gut microbiota composition compared with control group, while rap oil group was close to control group. Moreover, alpha diversity analysis indicated that the community richness and diversity were increased in all treatment group, as evidenced by the elevation of the Chao1 and Shannon index values compared to control group (Fig. 6B and C). However, it is worth noting that only RO and OOEM groups caused a significant increase in Chao1 and Shannon index ($P < 0.05$).

Furthermore, analysis at phylum level revealed that the gut microbiota in all groups was dominated by Bacteroidetes and Firmicutes, accounting for more than 90% (Fig. 6D), which play an important role in regulating absorption, energy conversion, and glucose metabolism (Koliada et al., 2017). Other phyla included Proteobacteria, Epsilonbacteraeota, and Deferribacteres were also detected. Mice fed with oil and emulsion were associated with a reduction in Firmicutes, Proteobacteria and Epsilonbacteraeota, and an increase in Bacteroidetes and Deferribacteres compared with the control group, and Bacteroidetes of OOEH group significantly increased ($P < 0.05$). Generally, the predominance of Firmicutes and decreased level of Bacteroidetes was positively associated with obesity, irritable bowel syndrome, and nonalcoholic steatohepatitis (Boursier & Diehl, 2015; Ley et al., 2005). A high Firmicutes/Bacteroidetes (F/B) ratio is an indicator of microbial imbalance and relates to metabolic disorders and various health risks in the host (Kim, Kim, Kim, Lee, & Jang, 2021). Our taxonomic profiling data showed that treatment with oil and emulsion led to a decrease in F/B ratio in mice, and OOEH group decreased significantly ($P < 0.05$, Fig. 6F). Similar reduction of F/B ratio has also been reported in mice fed with phenol compounds-enriched *Penthorum chinense* Pursh, which was thought to be a beneficial factor against dysbiosis of intestinal flora (Yin et al., 2020). Moreover, it is noteworthy that an obvious decrease in the abundance of the phylum Proteobacteria, a marker for dysbiosis in gut

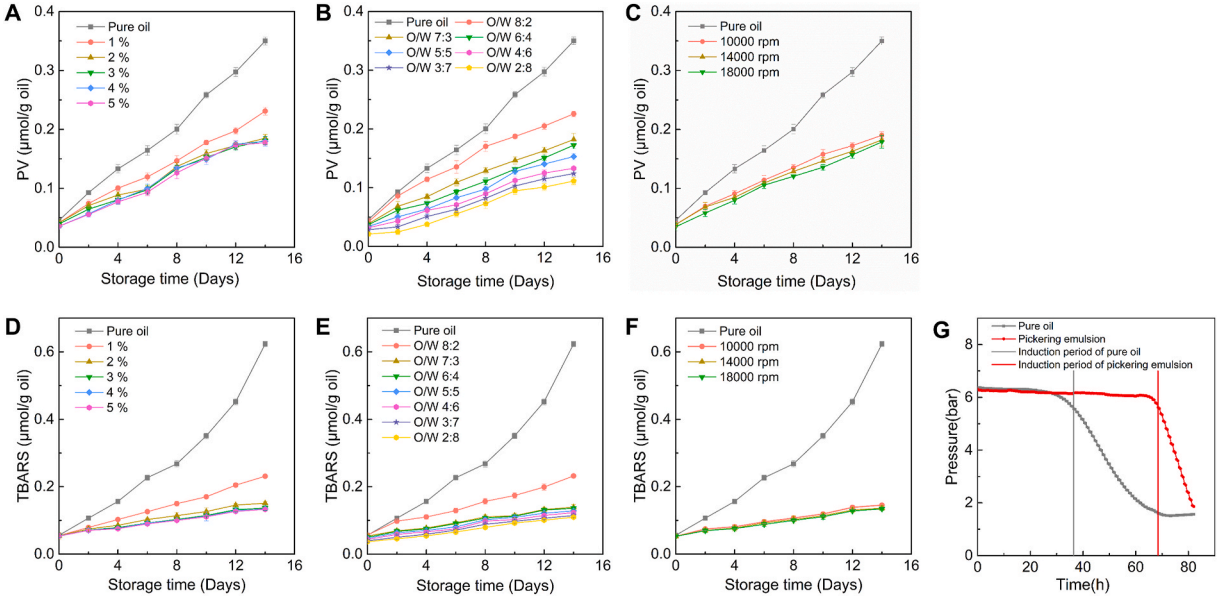


Fig. 5. Oxidative stability of olive oil Pickering emulsions stabilized by starch/β-CD NPs. PV (A–C) and TBARS (D–F) values of emulsions at different particle concentrations (A, D), oil phase fractions (B, E), and homogeneous speed (C, F) under temperature of 55 °C storage for 14 days. The auto oxidation curve of olive oil and Pickering emulsions measured by OXITEST automatic oxidation analyzer (G).

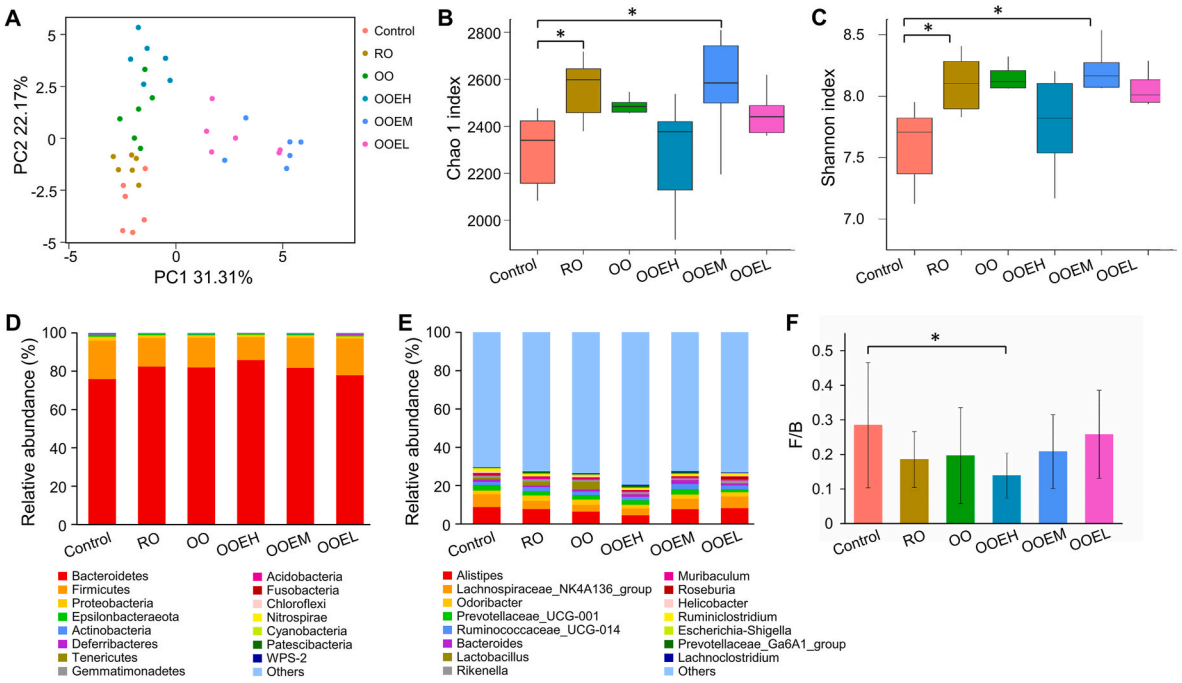


Fig. 6. The structural modulation effect of olive oil Pickering emulsions on the gut microbiota in mice. Principal component analysis (PCA) of the gut microbial composition (A). Boxplots of the alpha diversity as determined by Chao1 (B) and Shannon index (C). Relative abundance of the top 15 phylum level (D) and genus level (E). The average ratio of Firmicutes to Bacteroidetes (F). Data are expressed as mean ± SD, n = 6 mice per group. *$P < 0.05$.

microbiota and a potential diagnostic criterion for disease (Shin, Whon, & Bae, 2015), was observed in all treatment group.

At the genus level, the abundance of *Oscillibacter* tended to be lower in all treatment groups, whereas the genus *Odoribacter* was more abundant than that in the control group. The results are interesting because *Oscillibacter* is closely associated with weight gain and plays role in diet-induced metabolic dysfunctions (Lam et al., 2012). However, *Odoribacter* has been demonstrated to produce butyrate that is important for maturation of intestine and regulation of host inflammation (Yin et al., 2020). In addition, compared with the control group, the mice feed with oils or emulsions exhibited a reduction in the genus *Mucispirillum* except for OOEL group. *Mucispirillum* is known to have a potential capacity to degrade mucin in colon, leading to the pro-inflammation with an alteration in intestinal permeability (Li et al., 2019). More importantly, an increase in the abundance of genus *Bifidobacterium* was observed in treatment groups except for OOEL group. *Bifidobacterium* is one of the most important probiotics, which promotes host health by stimulating the immune system and inhibiting pathogen growth (Lee, Tung, Wu, Tu, & Yen, 2018). Meanwhile, *Bifidobacterium* also has been demonstrated to be negatively associated with the development of metabolic syndrome (Chen et al., 2018).

Taken together, these results revealed that the prepared emulsions could modulate the composition of gut microbiota in mice, with an enhanced richness and diversity, and resulting in an increased abundance of health-promoting bacteria. The health beneficial components in olive oil such as polyphenol, mono and polyunsaturated fatty acids, might have direct impact on intestinal microbiota and produce health benefits. However, the detailed mechanism of which component in olive oil regulated the gut microbiota was worth investigating in future research.

4. Conclusion

A novel food-grade olive oil Pickering emulsion stabilized by starch/β-CD NPs was successfully prepared. The Pickering emulsions exhibited extraordinary long-term storage stability with constant creaming index value even after 6 months. The physical properties and microstructure of the emulsions were significantly influenced by the starch/β-CD NPs concentration and oil phase fraction, while the homogeneous speed was a favorable factor for droplet reduction. The confocal microscopic observation confirmed that starch/β-CD NPs absorbed around the oil droplets and formed an ordered interfacial layer to stabilize the Pickering emulsions. The rheological analysis revealed that the emulsions showed a shear-thinning behavior with a gel-like structure. Notably, emulsification of olive oil with starch/β-CD NPs efficiently improved the oxidative stability by preventing lipid oxidation. In addition, OOPEs had a potential healthy effect on the regulation of gut microbiota in mice and increased the richness and diversity, with a reduction in the ratio of Firmicutes/Bacteroidetes. This study provided a new strategy for food-grade olive oil emulsions with excellent stability and health-beneficial effects, which could be applied as a potential animal fats substitute or as a semi-finished product to be further developed into different food products.

CRediT authorship contribution statement

Qiaolei Li: Investigation, Data curation, Formal analysis, Methodology, Software, Writing – original draft. **Yanni Huang:** Methodology, Data curation. **Yaliyi Du:** Investigation, Data curation. **You Chen:** Investigation, Data curation. **Yanping Wu:** Writing – review & editing, Supervision, Revision, Funding acquisition. **Kai Zhong:** Methodology, Software. **Yina Huang:** Methodology, Writing – review & editing. **Hong Gao:** Writing – review & editing, Supervision, Project administration, Revision. All authors read and approved the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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