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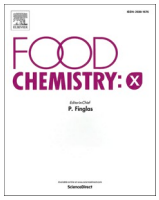

Excipient emulsion prepared with pectin and sodium caseinate to improve the bioaccessibility of carotenoids in mandarin juice: The effect of emulsifier and polymer concentration

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ABSTRACT

Excipient emulsions were prepared using different emulsifiers (pectin and sodium caseinate, individually or compositely) to study the emulsifying properties and their co-digested effects on the retention and bioaccessibility of carotenoids in mandarin juice, which is a good source of carotenoids in people's diet. Results showed that both pectin (PC) and pectin-sodium caseinate (PC-SC) emulsion significantly increased the carotenoids retention and bioaccessibility of mandarin juice, with the effects depending on both emulsifiers and polymer concentration. Whether for PC or PC-SC emulsion, lower pectin content accompanied with lower viscosity showed higher carotenoids bioaccessibility. And for the complexed emulsions, appropriate sodium caseinate addition could be more beneficial in improving carotenoids bioaccessibility. It had been found that the viscosity comparing with particle size seemed to play a more important role in affecting carotenoid bioaccessibility during the co-digestion. This study could provide a basis for improving the carotenoids bioaccessibility in the real system of fruits and vegetables with excipient emulsions.

1. Introduction

Carotenoids, as the natural pigment widely presenting in fruits and vegetables, play an important role in the growth, metabolism, and immune activities of the human body, and have the benefits of antioxidation, anti-aging, and cancer prevention (Boonlao, Ruktanonchai, & Anal, 2022). However, carotenoids cannot be synthesized in the human body and need to be ingested through diet to meet nutrient requirements for people. Citrus, currently the largest fruit crop in the world and with Mandarin (*Citrus reticulata*) ranking first in total citrus production in China (67 %), is rich in various carotenoids (Li et al., 2021). Thus, their products (such as mandarin juice) are increasingly popular as important sources of carotenoids in people's diets. However, it is worth noting that, carotenoids, as lipophilic compounds, are usually having a quite low bioaccessibility and bioavailability in the human body when ingested alone in fruit and vegetable products (Saini, Keum, Daglia, & Rengasamy, 2020). One approach to overcome this situation is to add oily substances for co-ingesting with fruit and vegetable products to improve the solubility and bioaccessibility of carotenoids (Gonzalez-Casado, Martin-Belloso, Elez-Martinez, & Soliva-Fortuny, 2018).

The usage of excipient emulsions (O/W or W/O) as lipid sources is one approach to improve the bioaccessibility of fat-soluble nutrients in fruit and vegetables (Boonlao et al., 2022; Huang et al., 2021). Although excipient emulsion may not have biological activity itself, it can improve the bioaccessibility of fat-soluble nutrients by introducing oil to dissolve, and affecting its transport to small intestinal epithelial cells and uptake (McClements et al., 2016). Chen et al. (2018) enhanced the solubility, stability, and bioaccessibility of quercetin during in vitro digestion with a protein-based excipient emulsion. Luo et al. (2022) found that the co-consumption of vegetable salad with canola oil emulsion or black pepper significantly increased the bioavailability of carotenoids (2–6 times). Moreover, Yuan, Xiao, Liu, McClements, Cao, and Xiao (2019) reported that the effects of excipient emulsions was significantly depended on emulsion polymer component, and different excipient emulsions

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prepared with protein, polysaccharides showed different strengthening effects on the bioavailability of carotenoids in spinach. With the increasing demand for food safety, people may prefer green and healthy polymer emulsion systems, and it is a general trend to use natural macromolecular materials as excipient emulsion carriers.

Natural food homologous molecules such as polysaccharides and protein have attracted wide attentions due to their outstanding biological functional properties and biodegradability (Cai, Pan, Li, Xu, Pan, & Liu, 2022; Hou et al., 2022; Li, Zhixuan, Ran, Siyu, Fengxia, & Siyi, 2021). Pectin, a natural plant polysaccharide widely presenting in fruits and vegetables, has been shown to have good emulsifying properties, which was affected by its source and structural characteristics (such as degrees of esterification and acetylation, protein content) (Li et al., 2021; Miaomiao et al., 2016; Wan, Chen, Huang, Liu, & Pan, 2019), and has showed good biological functions such as hypoglycemia, probiotic effects (Li et al., 2021). Citrus processing by-products (such as citrus peel) is one of the main sources of commercial pectin today, and preliminary studies had shown that mandarin peel pectin had a better emulsifying ability than commercial citrus pectin (Duan, Yang, Yang, Liu, Xu, & Pan, 2021; Duan, Zhu, Shu, Gao, Liu, & Pan, 2022). The use of plant-derived pectin (prepared from the mandarin peel) as the aqueous phase of excipient emulsion to improve the bioaccessibility of carotenoids in fruits and vegetables may be more in line with people's pursuit of healthy, natural, and green food. Studies have shown that the interaction or complexation of polysaccharide-protein is more conducive to improving the stability and delivery performance of the prepared system, such as emulsion or emulsion gel (Li, Wang, Hu, Wu, & Van der Meeren, 2022). Alavi and Chen (2022) showed that the complexes prepared by nano-fibrilated egg albumin and pectin have better emulsion stability. Liao, Elaissari, Ghnimi, Dumas, and Gharsallaoui (2022) showed that the addition of pectin in sodium caseinate-pectin emulsion improved environmental resistance and stability. And novel plant sterol delivery systems based on sodium caseinate-pectin-soluble complexes have been shown to improve its stability and bioavailability (Gan, Liu, Zhang, Shi, He, & Jia, 2022). However, the present studies had focused more on the use of pectin-based emulsions embedding or loading carotenoids to investigate their effects on the bioaccessibility or bioavailability of carotenoids (Verkempinck, Salvia-Trujillo, Denis, Van Loey, Hendrickx, & Grauwet, 2018; Verkempinck, Salvia-Trujillo, Moens, et al., 2018). There were few types of research that had explored the effect of homologous pectin-based or protein–polysaccharides based excipient emulsions on the carotenoids bioaccessibility in real fruit and vegetable food systems.

Therefore, in this paper, excipient emulsions prepared with mandarin peel pectin (PC) and sodium caseinate (SC) individually or compositely were characterized using different polymer concentrations and ratios, and the basic characteristics of emulsions were determined to explore their possible effects on the digestion process. The in vitro simulated digestion model was used to investigate the co-digestion properties of excipient emulsion based on PC or PC-SC with carotenoids-rich mandarin juice and their effects on carotenoid bioaccessibility. Based on this, this study may provide a new way on how to improve the bioaccessibility of fat-soluble nutrients (carotenoids) in people's daily diet.

2. Materials and methods

2.1. Materials and chemicals

Soybean oil was purchased from Yihai Kerry Arowana Cereals, Oils & Foodstuffs Co., Ltd. (Shanghai, China). Sodium caseinate (SC), bile salts from pig (Cholic acid content = 60.4 %/mg, S30895), and Nile red were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Butylated hydroxytoluene (BHT), pepsin from porcine gastric mucosa (664 units/mg, P7000), lipase from porcine pancreas (650 units/mg, Type II, L3126), and pancreatin from porcine pancreas (8 USP/mg, P7545) were purchased from Sigma Aldrich Chemical Company (St. Louis, MO, USA). Ethanol, *n*-hexane, acetone, etc. were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Chromatographic grade methanol and methyl tert-butyl ether (MTBE) were purchased from Thermo Fisher Scientific (San Jose, CA, USA) and American Mreda (USA), respectively.

Fresh mature mandarin fruit was purchased from Yichang (Hubei, China). The samples were peeled and crushed with a juicer (SP503, Zhejiang Supor Co., Ltd., Hangzhou, China) for 40 s to obtain fresh mandarin juice. Moreover, the mandarin peel was freeze-dried, crushed, milled, and the homologous pectin was extracted from the mandarin peel powder used citric acid solution (pH 1.4/85 ℃/70 min) with a feed-liquid ratio of 1:30, according to our previous study (Duan et al., 2021).

2.2. PC and PC-SC emulsion preparation

The emulsion preparation method was referred from the previous literature (Duan et al., 2021; Han et al., 2023). PC or SC solutions with different concentrations were dissolved in citric acid-sodium citrate buffer solution (final pH 6.5) overnight for complete hydration. For PC emulsions, 2 g of soybean oil (10 % oil phase) was mixed with 18 g pectin solution (90 % water phase) to a final pectin concentration of 0.30 %, 0.45 %, 0.60 %, and 0.90 %, respectively. For the preparation of PC-SC emulsion, PC and SC solution were first mixed to make them in different concentration ratios (1:1, 1:2, 2:1, 0:1, respectively), with all in the same total polymer concentration (0.90 %), and then 18 g of the mixture were added with 2 g of soybean oil respectively. The above samples were first homogenized using an Ultra-Turrax (IKA-25, Staufen, Germany) at 12000 rpm for 3 min to prepare the coarse emulsion. Thereafter, the coarse emulsion was treated by a JY92-2D ultrasonic processor (Ningbo Sentz Biotechnology Co., Ltd., Ningbo, China), at 300 W for 10 min to obtain the final emulsion.

2.3. Characterization of PC and PC-SC emulsion

2.3.1. Particle size and its distribution

The measurement of particle size and its distribution in the emulsions was carried out with a laser particle sizer of Malvern master sizer 2000 (Malvern Instruments Ltd. UK), referred to Duan et al. (2021). The pump speed was set to 2000 rpm/min, and water was set as the measurement background. The laser intensity was greater than 78 %, with the range from 0.04 μm to 2000 μm, and the refractive indexes of water and oil were set to 1.33 and 1.47, respectively. The particle size distribution, the volume average particle size $D_{[4,3]}$, and the area average particle size value $D_{[3,2]}$ were recorded.

2.3.2. Zeta potential

The zeta potential value of the prepared emulsions was measured by a Malvern Nano ZS instrument (Malvern Instruments Ltd. UK), according to Wan, Wang, et al. (2019). Soybean oil was set as the dispersing phase, and water was the continuous phase. To avoid the phenomenon of multiple scattering in the emulsion, the fresh emulsion was diluted 300 times with ultrapure water for zeta potential measurement.

2.3.3. Rheological properties

The Haake Rheostress 6000 rheometer (Thermo Scientific, New Castle, DE, USA) was used to determine the rheological properties of emulsions, according to the study of Wan, Wan et al. (2019). The entire test was measured using a 40 mm aluminum plate at 25 °C in a stable shear state mode in the range of 0.01 s^{-1}-100 s^{-1}. The temperature control system was a Peltier system. Apparent viscosity was obtained from the data analysis software.

2.3.4. Microstructure observation

Confocal scanning laser microscopy (CLSM, FV3000, Olympus, Japan) was used to observe emulsion structure, and the method was

referred to Yang et al. (2022). The oil phase was stained with Nile red (1 mg/mL, in ethanol) before confocal microscopy, and samples were observed at 100 × magnification. The excitation and emission spectra of Nile red were 488 nm and 580 nm, respectively, and microscopic images for confocal microscopy were taken and analyzed using image analysis software.

2.4. In vitro simulated co-digestion of excipient emulsions and mandarin juice

The gastrointestinal tract (GIT) digestion model was prepared according to Brodkorb et al. (2019). Before digestion, the mandarin juice and the prepared emulsion were mixed evenly in a ratio of 1:1 (w/w), which represented as the initial system. Each sample passed through a three-step simulated (GIT) model consisting of an oral stage, a gastric stage, and a small intestine stage. And all electrolytes, including simulated saliva fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) were incubated at 37 °C for 15 min before use.

For the simulated oral stage, 4 mL SSF mixed with 5 g initial sample were supplemented with 50 μL $CaCl_2$ (0.3 mol/L) and ultrapure water to 10 mL. Then the mixture was incubated in a constant temperature shaker (37 °C, 10 min) to simulate oral chewing. After that, 8 mL SGF and 10 μL $CaCl_2$ was added to the oral stage digestive mixture, with pH adjusted to 3.0 using HCl (1.0 mol/L). Then pepsin and ultrapure water were added to make the final enzyme concentration in the digestive being 2000 U/mL and the final volume being 20 mL. After that, the mixture was incubated in a constant temperature shaker (37 °C, 2 h) to simulate the peristalsis of stomach for the gastric stage. Thereafter, 16 mL SIF was added to the gastric stage digestive mixture and supplemented with 80 μL $CaCl_2$. NaOH (1.0 mol/L) was added to adjust pH to 7.0. Bile salt was added to make the final concentration reach to 10 mmol/L, and pancreatin and pancreatic lipase were added to make the enzyme activities reached to 100 U/mL and 2000 U/mL, respectively. Then water was supplemented to make the final volume of digestive reach to 40 mL. During digestion in the small intestine, the pH-stat was used to monitor at 37 °C for 2 h and maintained pH at 7.0.

2.5. Determination of zeta potential during digestion

The digestion solution was mixed evenly, and samples of different digestion stages were taken to measure the zeta potential, according to the method described in 2.3.2.

2.6. Microstructure observation during digestion

The microstructural changes of samples before and after exposure to various GIT phases were characterized using confocal fluorescence microscopy. And the method was the same as described in 2.3.4.

2.7. Free fatty acids (FFAs) release during digestion

FFAs were released from lipids in the small intestine due to the action of lipase and pancreatic enzymes, and then formed micelles with carotenoids. However, the release of FFAs would cause the pH of the system to decrease, so the automatic titrator (pH-stat) was used to monitor the pH at 37 °C for 2 h and maintained the pH at 7.0 (by titrating 0.25 mol/L NaOH into the reaction vessel). The amount of FFAs released was calculated from the titration curve (assuming 2 FFAs per 1 triacylglycerol molecule).

$$FFA\% = 100 \times \frac{V_{NaOH} \times m_{NaOH} \times M_{lipid}}{w_{lipid} \times 2} \quad (1)$$

where V_{NaOH} was the volume of sodium hydroxide (mL) required to neutralize the resulting FFAs, m_{NaOH} was the molar concentration (mol/L) of NaOH used, w_{lipid} was the total weight of oil initially present in the reaction vessel (g), and M_{lipid} was the molecular weight of the oil (for soybean oil is 880 g/mol).

2.8. Carotenoid bioaccessibility in mandarin juice co-digested with different excipient emulsions

2.8.1. Carotenoid extraction and analysis

Carotenoid extraction was referred to Zhu et al. (2022) and modified slightly. Ten gram of mandarin juice was fully extracted by adding 40 mL extract solution, which consisting of *n*-hexane, acetone and ethanol ((2:1:1, v/v/v), BHT (0.1 %, w/v), and NaCl (0.01 %, w/v)), stirred for 30 min and stood still for 5 min. The supernatant (organic phase) was collected and the above step was repeated once for fully carotenoids extracting. Then, the organic phase was combined and 10 mL NaCl (10 %, w/w) was added to the sample extract, mixed well, and stood for stratification. The aqueous phase was removed and this step was repeated twice for washing away impurities such as ethanol. Then the organic phase was collected and concentrated to the volume about 10 mL by nitrogen blowing. Thereafter, double volume of 20 % (w/v) KOH methanol solution was added to saponification for 1 h, and then washed with double volume of NaCl (10 %, w/w) and pure water (twice) in turn. Finally, the organic phase was collected, nitrogen-blown dried and placed at −20 ℃ until analysis.

Carotenoid analysis was referred to Lu, Huang, Lv, and Pan (2017). The dried exact sample was reconstituted into 1 mL by the solution (methanol: MTBE = 1:2), passed through an organic phase fitter (0.22 μm), and then analyzed by a HPLC system (2695 system, Waters Corp., MA, USA) equipped with the HPLC-DAD-APCI-MS/MS system (Agilent1100, Palo Alto, CA, USA), and an ion-trap mass spectrometer using an APCI ionization source (Esquire 4000, Bruker Daltonics, Bremen, Germany) operated in both negative and positive modes. A C30 reversed-phase column (250 × 4.6 mm, 5 μm; YMC. Inc. Wilmington, NC, USA) was used to separate carotenoids. Eluent A was composed of methanol/ MTBE /distilled water (81:15:4, v/v/v), and eluent B was composed of MTBE/methanol (90:10, v/v). The column temperature was maintained at 25 ℃, and 20 μL of sample was injected for analysis. For HPLC, the flow rate was set at 1 mL/min, and the UV–Vis spectrum was acquired from 210 to 600 nm with detection wavelengths at 450, 286, and 350 nm. The mobile phase gradient was programmed as following: 0–25 min: 100–75 % A; 25–80 min: 75–15 % A; 80–82 min: 15–100 % A. And for mass spectrometry analysis, nebulizer pressure of 60 psi, dry gas flow of 5 L/h, source temperature of 320 ℃, corona current of 4000nA, and HV capillary of 3500 V were used. The calculation of individual carotenoid was referred to Lu et al. (2017), with the concentrations of β-carotene and violaxanthin calculated according to the external calibration curve of their corresponding commercial standards. Other carotenoids were quantified by representative standards with similar UV–Vis spectral characteristics, such as zeaxanthin, β-cryptoxanthin, etc. All isomers of carotenoids are quantified according to their corresponding curves of all-trans commercial standards (Li, Deng, Liu, Loewen, & Tsao, 2012; Xu, Fraser, Wang, & Bramley, 2006). All the result were expressed as μg/g fresh sample.

And for the small intestine and micelles, carotenoid extraction analysis was carried out according to Liu et al. (2019), and the total carotenoid content (TCC) was calculated as the following formula (2). The micelles separation was referred to Yao et al. (2021), the intestinal digestate was first centrifuged (10,000 rpm/20 min/4 ℃), and the supernatant was collected and passed through a 0.45 μm hydrophilic filter to obtain the micelle phase for carotenoid extraction and analysis.

$$TCC\left(\frac{\mu g}{g}\right) = \frac{A \times volume \times 10^4}{E_{1cm}^{1\%} \times W} \quad (2)$$

where A was the sample absorbance at 450 nm, volume was the total volume of extract (mL), W was the weight of intestinal or micelle fluid (g), $E_{1cm}^{1\%}$ was the extinction coefficient (2560) for β-carotene in hexane,

TCC was expressed as μg/g of digest.

2.8.2. Carotenoid retention and bioaccessibility

Carotenoids are easily affected by environmental factors, such as light, heat, and pH affecting their stability during digestion. After the carotenoids in mandarin juice were digested by the gastrointestinal tract, their content may change significantly due to acid, heat, etc. The carotenoid retention was calculated as formula (3). Moreover, the prerequisite for carotenoids to be absorbed by the body was that carotenoids must be dissolved in the micellar layer composed of FFAs, phospholipids, and bile salts in the small intestine. The ratio of carotenoids transferred to the micellar layer indicated its bioaccessibility, as shown in formula (4).

$$\text{Carotenoid retention}\ (\%) = \frac{C_{\text{intestinal fluid}}}{C_{\text{initial}}} \times 100 \quad (3)$$

$$\text{Total carotenoid bioaccessibility}\ (\text{TCB}\%) = \frac{C_{\text{micelles}}}{C_{\text{intestinal luid}}} \times 100 \quad (4)$$

where $C_{initial}$, $C_{micelles}$, and $C_{intestinal\ fluid}$ were the total carotenoids content in the initial system, micelle and intestinal fluid (μg/g), respectively.

2.9. Statistical analysis

All experiments were performed at least in triplicate and the data were subjected to one-way analysis of variance (ANOVA) and least significant difference (LSD) test. The significant level was $p < 0.05$ throughout the study. All the data were reported as mean values ± standard deviation (SD), analyzed and graphed with origin 9.0.

3. Result and discussion

3.1. The species and content of carotenoids in mandarin juice

The carotenoid in mandarin juice was identified and analyzed based on the peak time, UV–Vis spectra and MS information (shown in Table S1), and with reference to studies of red navel Cara (Lu et al., 2017), citrus (Multari, Licciardello, Caruso, & Martens, 2020) and red citrus (Citrus reticulata) (Zhu et al., 2022). As shown in Table 1, fourteen carotenoids were identified and quantitated in mandarin juice, mainly including β-cryptoxanthin, violaxanthin, β-carotene, and their isomers. β-cryptoxanthin and its isomers accounted for 41.81 %, and violaxanthin and its isomers accounted for 48.99 % of the total carotenoids in mandarin juice. The total carotenoids content in mandarin juice was 93.08 ± 3.21 μg/g fresh, which was significantly higher than those reported by Yoo and Moon (2016) in mature citrus, with Yuza (*C. junos* Sieb ex Tabaka) being 79.8 ± 0.7 μg/g fresh, Kjool (*C. unshiu* Marcow) being 60.1 ± 0.2 μg/g fresh, and Dangyooja (*C. grandis* Osbeck) being 69.5 ± 0.5 μg/g fresh. The result showed that mandarin could be a good carotenoids source in people's daily diet, especially for β-cryptoxanthin and violaxanthin. It had been found that β-cryptoxanthin with vitamin A activity could reduce the risk of various types of cancer (Kaur & Kaur, 2015), and violaxanthin had good antioxidant and antiproliferative activity (e.g. breast cancer cells) (Manochkumar, Doss, Efferth, & Ramamoorthy, 2022). However, carotenoids are difficult to be absorbed and utilized by the human body due to their fat-soluble properties, resulting in low bioaccessibility. Based on this, the excipient emulsion was prepared in this study to co-digest with mandarin, trying to improve the absorption and utilization degree of carotenoids.

Table 1
The carotenoids content in mandarin juice.

Carotenoid	Content (μg/g fresh)
9-*cis*-violaxanthin	0.21 ± 0.01
9-*cis*-antheraxanthin	0.43 ± 0.04
9-*cis*-zeaxanthin	0.23 ± 0.02
13- or 15-*cis*-β-cryptoxanthin	1.35 ± 0.11
α-cryptoxanthin	0.49 ± 0.03
β-cryptoxanthin	25.08 ± 1.14
β-carotene	6.57 ± 0.48
9-*cis*-violaxanthin-C12:0- C12:0	0.57 ± 0.06
9-*cis*-violaxanthin- C12:0-C14:0	2.37 ± 0.02
9-*cis*-violaxanthin-C12:0-C18:1	15.27 ± 0.14
9-*cis*-violaxanthin-C14:0-C14:0	5.08 ± 0.12
Mixture of 9-*cis*-violaxanthin-C12:0-C16:0and 9-*cis*-violaxanthin-C14:0-C18:1	21.12 ± 1.38
Mixture of Violaxanthin-C14:0-C16:0 andViolaxanthin-C16:0-C18:1	1.33 ± 0.04
β-cryptoxanthin-C18:0	13.00 ± 1.41
Total Carotenoid	93.08 ± 3.21

3.2. Characterization of PC and PC-SC emulsions

3.2.1. Particle size distribution and microstructure observation

The microstructure and particle size distribution of the prepared emulsions are shown in Fig. 1-A, and the two results were mutually corroborative. For PC emulsions, with the pectin concentration increased, its peak gradually shifted to the left and narrowed in width, indicating that the particle size distribution was more uniform and finer. Pi, Liu, Guo, Guo, and Meng (2019) also showed that the particle size distribution of emulsion was dependent on pectin concentration, and the higher pectin concentration could promote the formation of smaller droplets. Liu et al. (2022) reported that emulsion with a narrow, unimodal droplet size distribution, which representing the droplets were more uniform in size, may contribute to resisting the Ostwald ripening. For PC-SC emulsions, under the same total polymer concentration (0.90 %), the addition of sodium caseinate could reduce the particle size of the emulsion to a certain extent. When the PC: SC ratio came to 1:2 (with sodium caseinate concentration being 0.60 %), the particle size showed bimodal distribution but still with obvious shifting to the left, indicating that the particle size tended to decrease.

At the same pectin concentration, the addition of sodium caseinate was beneficial to the left shift of particle size distribution. And the aggregation appeared to occur when the pectin concentration was at 0.60 %, but not at low concentrations (0.30 %, 0.45 %), observing from the microstructure. The same phenomenon was observed by Liao et al. (2022) that, the aggregation phenomenon of pectin and sodium caseinate emulsions was not pronounced at low pectin concentrations (0.00 %-0.10 %), but at high concentrations (0.25–0.50 %). This phenomenon was reversible and could be disrupted by mild stirring or dilution.

3.2.2. Average particle size

For PC emulsions, both $D_{[3,2]}$ and $D_{[4,3]}$ values gradually decreased with pectin concentration increasing, as shown in Fig. 1-B-(a). Similar result was obtained, that with the increase of hawthorn pectin concentration (0.50 %-1.50 %), the particle size of emulsion gradually decreased (Cuevas-Bernardino, Lobato-Calleros, Román-Guerrero, Alvarez-Ramirez, & Vernon-Carter, 2016). These results may be attributed to the amount of emulsifier available to form a monolayer adsorbed on the surface of the emulsion droplets, that a higher emulsifier concentration was required to form smaller droplets with higher surface area. The emulsifier covered the emulsified oil droplets in the emulsion as much as possible would favor its emulsion stability (Ozturk & McClements, 2016).

For PC-SC emulsions, the addition of sodium caseinate could reduce the particle size of emulsions as shown in Fig. 2-B-(b). At the same pectin concentration, the average particle size of PC-SC emulsions was significantly smaller than that of PC emulsions, for both $D_{[3,2]}$ and $D_{[4,3]}$. At the same total polymer concentration (0.90 %), as the ratio of sodium caseinate increased, the particle size decreased to a lower level. This

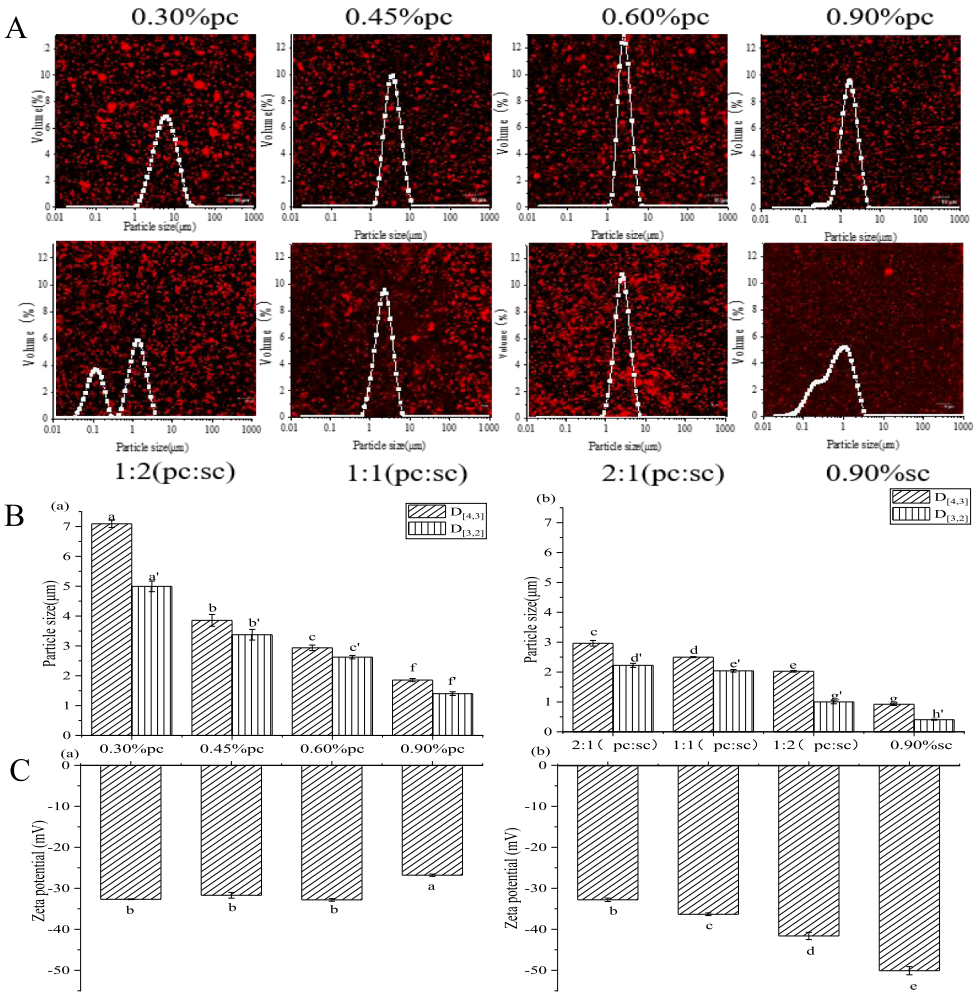

Fig. 1. Particle size, microstructure, and zeta potential value of different excipient emulsions (A, particle size distribution and microstructure observation; B, average particle size; C, zeta potential. (a), PC emulsion; (b), PC-SC emulsion).

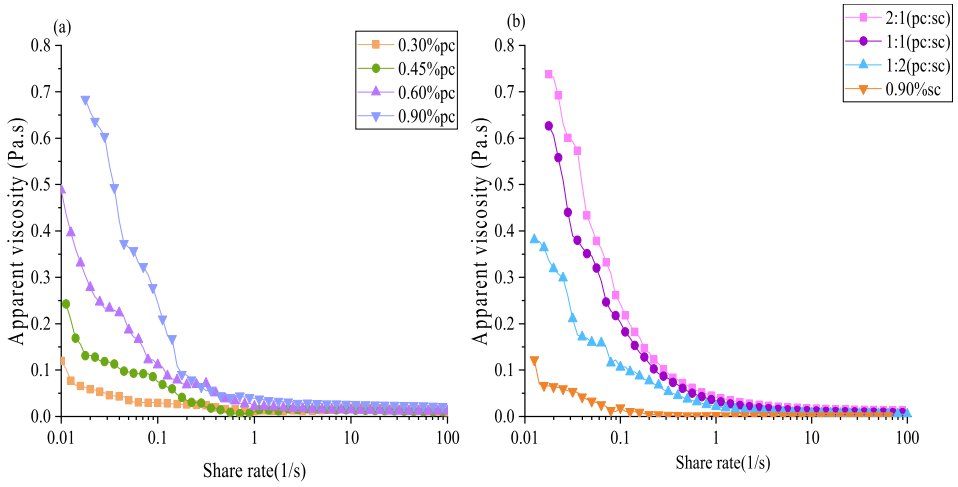

Fig. 2. Apparent viscosity of PC emulsion (a) and PC-SC emulsion (b).

may be because the proportion of pectin in the emulsion was decreased, which increased the adsorption of polysaccharides, reducing flocculation, and reduced the droplet size (Liao et al., 2022). However, the average particle size ($D_{[4,3]}$ and $D_{[3,2]}$) of the formulated emulsion was significantly larger than that of single PC (0.90 %) or single SC (0.90 %) emulsion. This may be caused by a certain degree of aggregation of sodium caseinate and pectin after compounding.

3.2.3. *Zeta potential*

All the prepared excipient emulsions were showing negative values,

mainly because both pectin and sodium casein were negatively charged under near-neutral conditions. For PC emulsion, no significant differences were found on zeta potential for samples from pectin concentration 0.30–0.60 % (Fig. 1-C-(a)), while significantly decrease in zeta potential absolute value was found for sample from pectin concentration of 0.90 %. Niu, Chen, Luo, Chen, and Fu (2022) also reported that the absolute value of zeta potential was decreased with beet pectin concentration (0.50–2.00 %) increasing, which may be attributed to the large number of micelles formed by high colloidal molecules concentration in the aqueous solution.

For PC-SC emulsion, it could be clearly observed that its absolute value of zeta potential increased significantly with the increase of sodium caseinate ratio (Fig. 1-C-(b)). And at higher pectin concentrations (0.45 %, 0.60 %), the addition of sodium caseinate seemed to increase the absolute value of the emulsion zeta potential. The reason may be attributed that one was because the absolute zeta potential value of sodium caseinate was greater, and as the proportion increased, it increased the absolute potential value of the emulsion, second, pectin and sodium caseinate had negative electricity, the mutual exclusion of the two may also affect the potential value of emulsion. However, it has been reported in previous studies that the addition of sodium caseinate into pectin emulsion showed no influence on zeta potential (Liao et al., 2022). Zeta potential reflected the stability of the emulsion system to a certain extent, assessing the interaction between emulsified oil droplets and components present in the surrounding medium, such as pectin (Verkempinck, Kyomugasho, et al., 2018). And it also characterized the degree of electrostatic attraction or repulsion between adjacent particles in an emulsion system, which was also a key indicator of emulsion stability. It had been reported that electrostatic interactions between proteins and polysaccharide molecules were dependent on the charge density and distribution of polymers at a specific pH (Gu, Decker, & McClements, 2005). Some studies had shown that the larger the absolute value of zeta potential, the more stable the system was.

3.2.4. Apparent viscosity

Rheological properties had significant impacts on the stability of emulsion products. Apparent viscosity would affect the fluidity of droplets and lipase, thereby affecting the adsorption of lipase at the oil–water interface and its digestive properties (Wooster et al., 2014). It could be observed that all emulsions exhibited shear thinning behavior (Fig. 2), and the decrease in physical interactions between adjoining polymer chains or the structural breakdown under the shear force maybe the cause. For PC emulsion (Fig. 2-(a)), its apparent viscosity increased with the pectin concentration increasing. Higher pectin concentration would lead to stronger intermolecular aggregation, and the strong electrostatic repulsion would make the pectin molecules extend along the chain direction, which would help the cross-linking between pectin molecules (Jiang, Xu, Li, Li, & Huang, 2020). Similar results were observed for PC-SC emulsion with the same total polymer concentration (Fig. 2-B), where the viscosity increased as the amount of pectin increasing, which may be due to the depletion flocculation of both. The same result that higher pectin concentration led to higher emulsion viscosity was obtained in the report of Liao et al. (2022). At the same pectin concentration, the apparent viscosity of the emulsion increased with the addition of sodium caseinate, which may be mainly because the total polymer concentration was higher than that of a single pectin emulsion.

There were many factors affecting the stability of the emulsion, such as particle size, potential, viscosity, interfacial adsorption, interfacial rheology, etc., (Niu et al., 2023), and the stability of the emulsion prepared by pectin and sodium caseinate was evaluated from particle size, zeta potential and apparent viscosity. It could infer that the stability of excipient emulsions expressed by indicators such as particle size, zeta potential or viscosity, was related to emulsifier type and concentration. Differences in these metrics could lead to different effects on the system during digestion. Salvia-Trujillo, Verkempinck, Sun, Van Loey, Grauwet, and Hendrickx (2017) had reported that lipid digestion, micelle formation and carotenoid bioavailability in emulsions were affected by emulsion droplet size. Thus, the functional properties of the above emulsions or the possible utilization of the emulsions as excipients were needed to be further explored.

3.3. Effects of PC and PC-SC emulsion on the co-digestive behavior and carotenoid bioaccessibility of mandarin juice

3.3.1. Zeta-potential changes during digestion

Compared with the zeta potential value of different excipient emulsions as shown in Fig. 1-C, it seemed that there had little effect on the zeta potential of PC emulsions co-digestive system for the addition of mandarin juice as shown in Fig. 3-(a). However, for PC-SC emulsion co-digestive system, due to the addition of mandarin juice, the absolute value of zeta potential for the system decreased significantly with the proportion of sodium caseinate increasing (Fig. 3-(b)). The change was especially significant in 0.90 % SC emulsion (from −50 mV to –22 mV). These results indicated that the presence of pectin seemed to slow down the tendency of the absolute value of zeta potential to decrease which may be attributed to the dissociation of pectin to equilibrate the zeta potential change of the system. And, the decrease of zeta potential absolute values may be attributed to that the isoelectric point of sodium caseinate was 4.6, and the addition of mandarin juice (pH 3.8) changed the biological environment of emulsion, thereby affecting its structure and stability, while the presence of pectin could help to stabilize the stability of sodium caseinate in an acidic environment. Differences in zeta potential values of co-digestion system may also affect the behavior of carotenoids during the digestion phase and their bioaccessibility.

After oral digestion, the zeta potential changed for PC and PC-SC emulsion co-digestive systems were not pronounced, suggesting that the addition of SSF had little effect on the emulsion (Fig. 3). However, for the0.90 % SC emulsion co-digestive system, the zeta potential absolute value decreased significantly, which may be because the SC emulsion was destroyed after the full mixing of the mandarin juice and emulsion system when simulating oral digestion, thus affecting the stability of the system. After gastric digestion, the absolute value of zeta potential in all co-digestion systems decreased due to the addition of SGF, which may be due to the electrostatic shielding effect caused by the low pH and high ionic strength of the gastric solution. The zeta potential difference between different samples was not significant, but not suitable for a single SC co-digestive system, which may be attributed to the destruction of this system in a peracid environment. After digestion in the small intestine, the zeta potential absolute value increased significantly in all systems. Although the higher absolute value of zeta potential indicated that the system was more stable, this may not be applicable for the digestive system, there were presented various types of colloidal particles of anionic components such as bile salts, phosphoric acid, and FFAs released from lipid digestion in the small intestinal digest (Mutsokoti et al., 2017; Verkempinck, Salvia-Trujillo, Denis, et al., 2018).

During the co-digestion of mandarin juice and excipient emulsions, the absolute value of zeta potential changed greatly at different digestion stage, but varied little under different co-digestion systems. This may be because that the zeta potential change was mainly due to the change of salt ions in the digest. However, for the SC emulsion co-digestive system, the absolute value of zeta potential was lower as compared to those of other co-digestion system in different digestion stages, it may be that sodium caseinate was enzymatically hydrolyzed into small molecules during digestion, which may be neutralized with the electrolytes added, thus reducing the absolute value of the zeta potential in the system.

3.3.2. Microstructural changes during digestion

The changes of oil droplets during digestion were observed by confocal laser (Fig. 4). In the initial state, for PC emulsion co-digestive

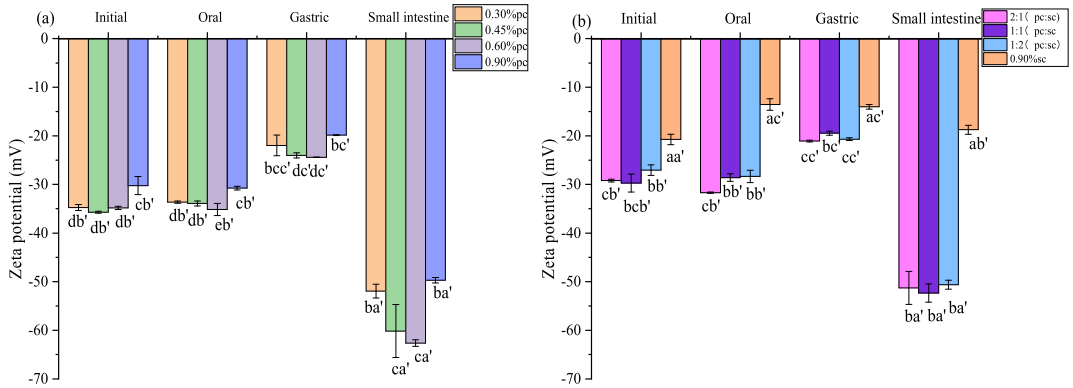

Fig. 3. Zeta potential changes at different digestion stages for co-digestion of mandarin juice and excipient emulsion ((a), pectin emulsion; (b), pectin-sodium caseinate emulsion; a-e represented the potential significance of different emulsion systems at the same stage; a'-c' represented the potential significance of different digestion stages for the same emulsion system).

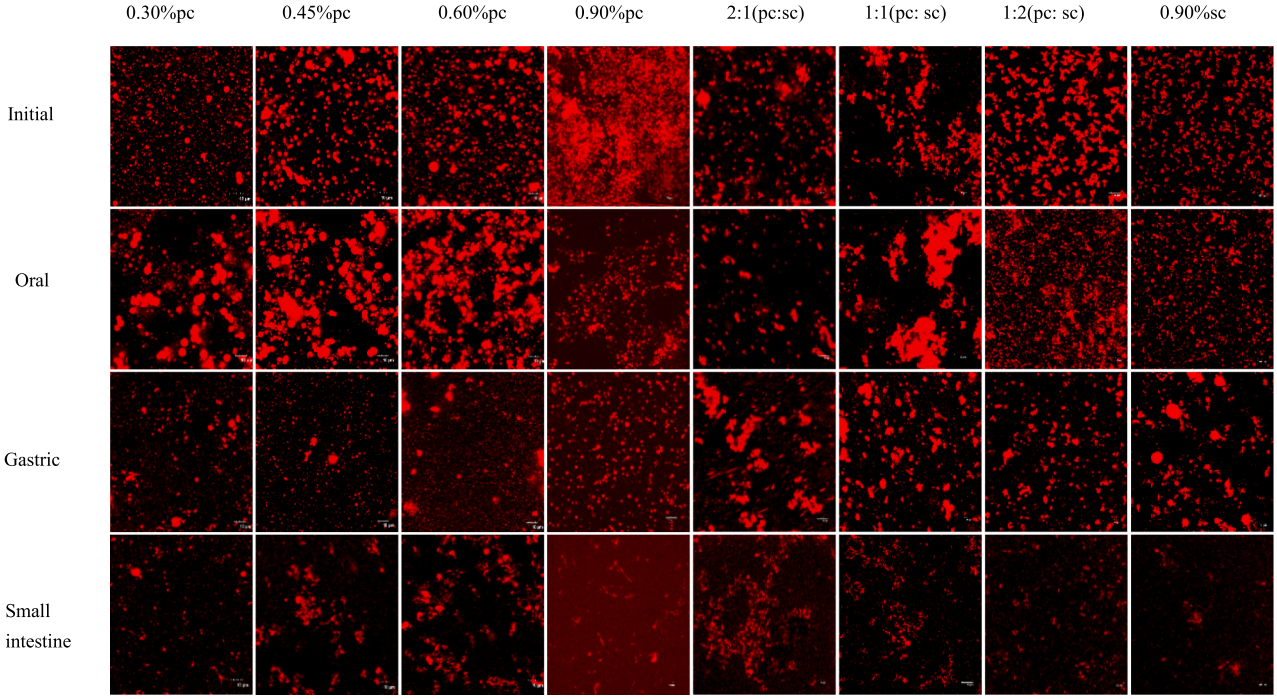

Fig. 4. Microstructure changes for co-digestion of excipient emulsion and mandarin juice at different digestion stages.

system, the oil droplets in the mixture of mandarin juice and emulsion became larger as the pectin concentration increased. For PC-SC emulsion co-digestive system, the addition of mandarin juice seemed to further agglomerate the oil droplets, but the distribution was not uniform, and the shape of the oil droplets was non-circular. At the same pectin concentration, the addition of sodium caseinate made the aggregation of oil droplets more obvious. After oral digestion, the oil droplets became significantly larger for samples with PC emulsion co-digestive system from lower pectin concentration (0.30–0.60 %). But for samples with PC-SC emulsions co-digestive systems, significant aggregation of oil droplet occurred at the gastric stage. This may be because the sodium caseinate was easily hydrolyzed in the stomach, which destroyed the emulsion system and accumulated the oil droplets. After simulating small intestinal digestion, both for PC and PC-SC emulsion co-digestive system, it can be clearly observed that the released lipids were further lipolysis for a certain degree. And the lipolysis degree appeared to be more obvious with the increase proportion of sodium caseinate and decrease of pectin concentration. The release of lipids may affect the possibility of forming micelles with lipid-soluble substances (such as carotenoids) during digestion and the degree of improved bioaccessibility. And more FFAs released from soybean oil which were long-chain polyunsaturated fatty acids, mixing with bile salts and carotenoids to form micelles, would improve carotenoid bioaccessibility.

3.3.3. *Lipid digestion and FFA release*

The FFAs release during the co-digestion of excipient emulsions and mandarin juice in the small intestine is shown in Fig. 5. It could be observed that regardless of PC or PC-SC emulsion co-digestive system, the release of FFAs increased rapidly within the first 20 min for lipid digestion and were produced more slowly over longer periods until comparable levels were reached. The fast digestion rate at the initial stage could be due to the presence of small droplet sizes in excipient emulsions, which provided a large oil–water interface for lipase. Thus, the lipids were rapidly hydrolyzed by lipase (de Figueiredo Furtado, Guedes Silva, de Andrade, & Cunha, 2018). With the hydrolysis process continuing, a lot of FFAs and monoglycerides were generated (especially generating for long-chain polyunsaturated fatty acids), tending to form

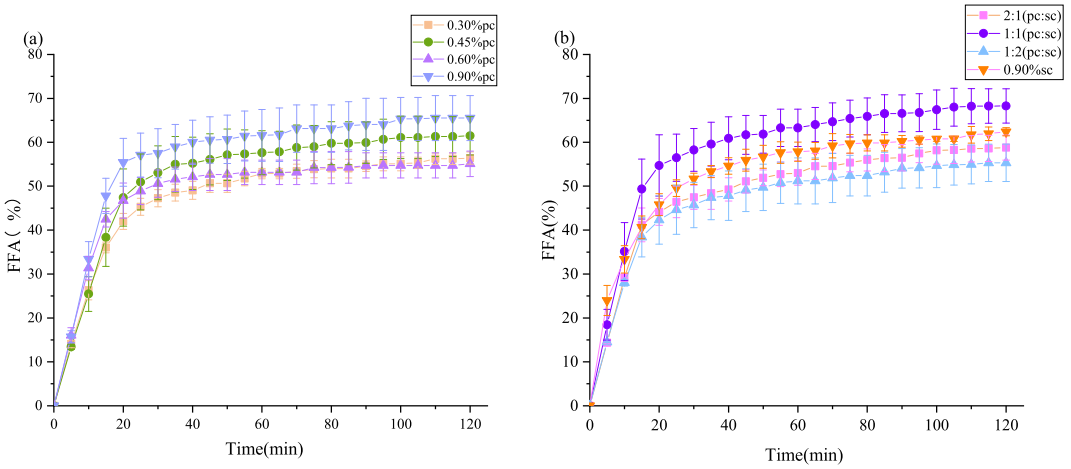

Fig. 5. FFAs release from co-digestion of excipient emulsions and mandarin juice in the small intestine stage ((a), pectin emulsion; (b), pectin-sodium caseinate emulsion).

micelles and vesicles under the action of bile salts, or crystallize on the surface of undigested oil droplets, thus slowing down the digestion rate of remaining lipids (Ozturk, Argin, Ozilgen, & McClements, 2015). The final release degree of FFAs in all systems was about 55 %-68 %, and showed no significant difference for different excipient emulsions, which could attribute to the complexity and uncontrollability of the digestive system. Yuan et al. (2019) also found that when co-digested with spinach, the release of FFAs from different excipient emulsions based on sodium caseinate, Tween 20, and octenyl succinic anhydride (OSA)-modified starch showed no significant difference. And it was also found that for the $W_1/O/W_2$ (W_1, sodium caseinate; W_2, pectin) emulsion prepared from different pectin concentrations, there was no significant difference in FFAs release degree at low pectin concentrations (0.00–1.50 %) (Teixé-Roig, Oms-Oliu, Ballesté-Muñoz, Odriozola-Serrano, & Martín-Belloso, 2022). Moreover, the effect of different pectin concentrations (0.00–2.00 %) on emulsion digestion was studied by adding soybean lecithin stabilized emulsion, and the results showed that the pectin concentration had no effect on the FFAs release degree under the gastric pH at 3.0 (Lin & Wright, 2018). While for pectin, it could affect lipid digestion in many ways, such as increasing the viscosity or gelation of the aqueous phase, thereby reducing mixing and diffusion processes, or may alter floc formation and structure of flocs, thus affecting the entry of lipase onto the surface of lipid droplets (Zhang, Zhang, Zhang, Decker, & McClements, 2015). It also found that the addition of pectin (0.50 %) appeared to have a promoting effect on lipid initial digestion of sodium caseinate, a result consistent with our results, for SC emulsions alone, when the ratio was 1:1 seemed to promote lipid digestion and release. The reason may be attributed to the ability of pectin to inhibit roughly dense floc formation (Zhang et al., 2015).

Indeed, the release of oil would affect the bioaccessibility of carotenoids, and the FFAs released by oil formed mixed micelles with carotenoids and bile salts, which were then absorbed and transported by the small intestine. However, micellar formation may be also influenced by other substances in the system such as phospholipids and cholesterol (Geng et al., 2023; Simin et al., 2017). At the same time, the composition and viscosity of micelles affected the degree to which carotenoids could be absorbed and transformed by human bodies (Cervantes-Paz et al., 2016). For different system compositions, FFAs and carotenoid bioavailability or bioaccessibility may show different results. Huang et al. (2022) prepared the $W_1/O/W_2$ emulsion (W_1, type A gelatin; W_2, gelatin-EGCG-pectin ternary complex) to deliver vitamin C, and found that the W_1/O had higher FFA release than $W_1/O/W_2$, but lower vitamin C bioavailability during digestion.

3.2.4. *Carotenoid retention and bioaccessibility during digestion*

It was obvious that the addition of different excipient emulsions could significantly improve the carotenoids retention and bioaccessibility of mandarin juice in the real food system, regardless of PC or PC-SC emulsions co-digestive system (Fig. 6). All excipient emulsions improved the retention of mandarin juice carotenoids significantly during digestion, indicating that they could better protect mandarin juice carotenoids from being affected by the digestive environment. And the effect of different excipient emulsions on carotenoid bioaccessibility was related to emulsifier type and polymer concentration. For PC emulsions co-digestive system (Fig. 6-(a)), carotenoid bioaccessibility was inversely proportional to pectin concentration, which was consistent with the result of Cervantes-Paz et al. (2016). Ruojie et al. (2016) found that when the excipient emulsion prepared based on soybean protein isolate was co-digested with carrot, the particle size of emulsion was inversely proportional to the bioaccessibility of carotenoids. However, it had been found that PC emulsion with higher pectin concentration showed significantly smaller particle size, while with lower carotenoids bioaccessibility. One possible reason was that pectin would increase the viscosity of digested matter, making it difficult for carotenoids to interact with oil decomposers, bile salts, etc., as shown in Fig. 4 that more oil was not released into lipolysis. And second, the change in pectin concentration made it possible to form some polysaccharide structures during the digestion process, in which carotenoids and oils were encapsulated so that the oil did not lipolyze and the two could not interact (Salvia-Trujillo & McClements, 2016). It was consistent with the above results that PC emulsion of higher pectin concentration did show larger viscosity, which may be more likely to affect carotenoid bioaccessibility than other factors (particle size) in pectin emulsions.

For PC-SC emulsion co-digestive system, it also showed a good ability to increase the bioaccessibility of carotenoids. With the same total polymer concentration (0.90 %), its ability to improve the bioaccessibility of carotenoid was proportional to the amount of sodium caseinate added, while inversely proportional to the pectin concentration. This result was consistent with the reduction in particle size and viscosity as shown in Fig. 1-B and Fig. 2, and the increased oil droplets was shown in Fig. 4. However, for the emulsions at the same pectin concentration, it appeared that the addition of sodium caseinate did not significantly improve or even reduce the carotenoids bioaccessibility of the mandarin juice. These results may be due to the junction and flocculation of pectin and sodium caseinate during digestion, affecting the release and absorption of carotenoids. Therefore, in the daily diet, appropriate plant-derived excipient emulsion can be selected for co-ingesting, which was more conducive to improving the degree of carotenoid absorption and utilization.

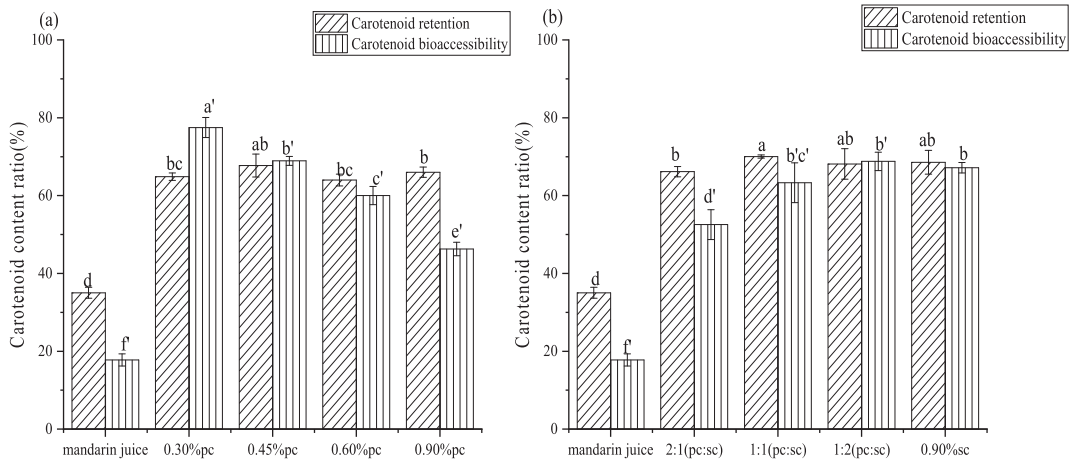

Fig. 6. Carotenoid retention and bioaccessibility of mandarin juice co-digested with different excipient emulsions ((a), PC emulsion; (b), PC-SC emulsion).

4. Conclusion

Mandarin was a good source of carotenoids in daily diet, rich in 14 kinds of carotenoids, with β-cryptoxanthin and violaxanthin as the main carotenoid varieties. To increase its carotenoids bioaccessibility, excipient emulsions were prepared with homologous pectin (PC) and sodium caseinate (SC) individually or compounded with different ratios. Whether PC or PC-SC emulsion co-digesting with mandarin juice, lower pectin concentration accompanied with lower viscosity showed higher carotenoids bioaccessibility. The reason may be that the pectin with lower concentration favored to the release of oil and decreased the viscosity of the intestinal digest, thereby affecting the formation of micelles with carotenoids. When the total polymer concentration was 0.90 %, appropriate sodium caseinate addition appeared to be more beneficial in improving the carotenoids bioaccessibility, which could attribute to a combination of low viscosity and particle size.

As the result showing, the carotenoid bioaccessibility was highly improved when the mandarin juice was co-ingested and co-digested with excipient emulsions, and the different effects could be attributed to the emulsifier type and polymer concentration. The addition of homologous pectin from the mandarin peel to corresponding mandarin juice was more in line with the current pursuit of green and healthy. This study could provide a basis for the development of high carotenoid bioaccessibility products such as compound plant-based milk products in people's daily diet, and further study on the stability of mixed excipient emulsion with them as well as their in vitro or vivo digestion could provide a more comprehensive basis for this application. Moreover, this research focused more on the total carotenoids bioaccessibility, the effects and mechanism of the designated excipient emulsions on the absorption and utilization of certain carotenoid also need to be further revealed in the following study.

CRediT authorship contribution statement

Jinyan Yang: Writing – original draft, Methodology, Data curation. **Hekai Fan:** Methodology, Conceptualization. **Bing Jiang:** Investigation, Writing – review & editing. **Ruoxuan Li:** Visualization, Investigation. **Jiangtao Fan:** Visualization, Investigation. **Bowen Li:** Visualization, Investigation. **Jinjiang Ge:** Investigation. **Siyi Pan:** Supervision. **Fengxia Liu:** Conceptualization, Supervision, Writing – review & editing, Resources.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100909.

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