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RESEARCH ARTICLE

Stability of lutein encapsulated whey protein nano-emulsion during storage

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Abstract

Lutein is a hydrophobic carotenoid that has multiple health functions. However, the application of lutein is limited due to its poor solubility in water and instability under certain conditions during storage. Hereby we generated lutein loaded nano-emulsions using whey protein isolate (WPI) or polymerized whey protein isolate (PWP) with assistance of high intensity ultrasound and evaluate their stability during storage at different conditions. We measured the particle size, zeta-potential, physical stability and lutein content change. Results showed that the PWP based nano-emulsion system was not stable in the tested Oil/Water/Ethanol system indicated by the appearance of stratification within only one week. The WPI based nano-emulsion system showed stable physiochemical stability during the storage at 4˚C. The lutein content of the system was reduced by only 4% after four weeks storage at 4˚C. In conclusion, our whey protein based nano-emulsion system provides a promising strategy for encapsulation of lutein or other hydrophobic bioactive molecules to expand their applications.

Introduction

Lutein is a hydrophobic carotenoid abundantly present in dark green leafy vegetables. They are commercially extracted from the Marigold flowers (*Tagetes erecta*) for production of orange colorings for the food and beverage industries and dietary supplements. Carotenoids cannot be synthesized *de novo* in body [1, 2] and have to be obtained from the diet. Free or unesterified lutein obtained from the foods is transported and stored in the liver or in tegumentary sites as di-esterified lutein [3–5]. Supplementation with lutein can lead to an increment of lutein concentration in human serum and increase the optical density in the retina [6]. Because of the presence of lutein in the eye tissue [7], lutein supplementation can help reduce the risk of some age-related eye diseases including cataract and macular degeneration [8, 9]. In addition, high intake of lutein can reduce the risk of cardiovascular diseases [10, 11], cancers [12–14], as well as other diseases like atherosclerosis [15]. Since lutein is not rich in our common diet, adding certain carotenoids into our foods is therefore recommended [16].

Unfortunately, the application of lutein is limited due to its poor solubility in aqueous phase and instability under certain conditions like oxygens, heat, light or humidity [17–19]. Incorporation of lutein into a protective matrix is a promising means to address these problems, for example, micro-capsulation of lutein with porous starch and gelatin mixture using spray drying [20], encapsulation of lutein with hydroxypropylmethyl cellulose phthalate by supercritical antisolvent method [21], formation of lipid nanoparticles of lutein for dermal delivery by high pressure homogenization [22] and encapsulation of lutein into emulsions using different emulsifiers like polyvinylpyrrolidone [23], soy protein isolate [24], whey protein products [25], etc. Selecting proper packaging materials is important for the stability and bioaccessibility of the bioactive compounds.

The milk protein is an important additive in many products. The whey protein and casein are the most protein components in milk, both of which are good emulsifiers to prepare stable lutein-loaded emulsions [25, 26]. Whey protein isolate (WPI) is a commercially available protein product that contains over 90% of protein. WPI is less efficient than casein to stabilize lutein [27], but use of WPI is more efficiently to protect the lutein from color degradation during storage [28]. Additionally, whey protein is costly and can improve the nutritional values [29, 30]. The health benefits of whey protein have extended beyond its basic protein nutrition for adults including adiposity control, promotion of muscle protein synthesis, enhancement of immune function and anti-oxidant activity [31]. Frede *et. al.* generated β-lactoglobulin and whey protein hydrolysate based lutein loaded emulsions with the particle size in the range from 250 to 300 nm. This system is promising as it can protect lutein from degradation within 46 days tested [25]. Our recent research showed that the emulsifying ability of WPI was increased by thermal treatment, which resulted in formation of whey protein soluble aggregates called polymerized whey protein (PWP) [32]. However, the stability of the emulsion system with commercial WPI or PWP as the emulsifiers is not studied at different storage conditions.

High intensity ultrasound is considered to be a promising technique that is relatively economical and easy to operate. Several studies have shown that ultrasound treatment can change the gelation properties of different food proteins [33–36], including whey protein [37]. Therefore, the objective of the current research was to generate a WPI based lutein loaded nano-emulsion system using ultra intensity ultrasound and evaluate its stability at different storage conditions.

Materials and methods

Materials and reagents

Whey protein isolate (93.1% of protein: α-lactalbumin: β-lactoglobulin = 3.5:1, 0.36% of fat, 4.8% of moisture, 1.6% of ash and 0.7% of lactose) was purchased from Fonterra (Auckland, New Zealand). Lutein (99.0%) was from Kemai, China. All other reagents were of analytical grade.

Preparation of lutein-loaded emulsions

The lutein-whey protein nano-emulsion (20%, v/v; oil-in-water) was prepared using the method from Frede *et. al.* with slight modifications [25]. To form a pre-emulsion 1 ml of the oil phase and 4 ml of the aqueous phase were mixed and stirred. The pre-emulsion was homogenized using high intensity ultrasound for 5 min in an ice bath at the amplitude of 40%. The oil phase contained 2.5mM or other specified amount of lutein dispersed in Ethanol/MCT-oil (50/50, v/v). The aqueous phase was 5% or other specified amount of WPI solution

or polymerized WPI (PWP), which was prepared as previously reported [38]. The emulsions were stored at 4˚C, 25˚C and 37˚C for four weeks.

Particle size and zeta-potential measurements

The particle size and the zeta-potential (ζ, mV) was determined using the Zetasizer Nano ZS 90 (Malvern Instruments, UK) as previously reported [37, 39]. Polydispersity index (PDI) was also measured reflecting the width of particle size distributions.

ζ was calculated based on the Henry equation:

$$U_E = \frac{2\varepsilon \times \zeta \times f(\kappa\alpha)}{3^{\eta}} \quad (1)$$

Where U_E is the electrophoretic mobility, ε is the permittivity (Farad/m), η is the solution viscosity (mPa.s), κ is the Debye length (nm^{-1}) and α is the particle radius (nm). $f(\kappa\alpha)$ is equal to 1.5 based on the Smoluchowski approximation [40].

Centrifugal stability constant measurement

The stability of the emulsions was evaluated by centrifugal stability constant (Ke) based on the method as reported [41]. Briefly, a certain amount of emulsion was diluted by a factor of 500 with deionized water in a 5 mL tube, and its absorbance value (A) was measured at the wavelength of 490 nm after mixing, using a microplate reader. One 1 ml aliquot of the same emulsion was transferred into a 1.5 ml centrifuge tube and centrifuged at 1520 g for 15 min at 20˚C in a high-speed centrifuge. A certain amount of subnatant was diluted by a factor of 500 with deionized water in a 5 mL plastic tube and its absorbance value (A_0) was measured at the wavelength of 490 nm after mixing. The centrifugal stability constant (Ke) was calculated as follows:

$$K_e = \frac{(A - A_0) \times 100\%}{A_0} \quad (2)$$

Where A is the absorbance at 490 nm of the solution before centrifuge, while A_0 is the absorbance after centrifuge.

Analysis of lutein stability during storage at different temperatures

The content of lutein was represented by the absorbance measurements (450 nm) detected using a UV–visible spectrophotometer. Before spectrophotometric measurements, the emulsions were diluted 100 times in DMSO that dissolved lutein, oil, and protein to form transparent solutions suitable for UV–visible analyses.

Statistical analysis

Statistical analyses were carried out using the statistical program SPSS Version 17.0. Comparisons among data of different groups were performed with one-way or two-way ANOVA, where LSD method or Dunnett test were used on the basis of the homogeneity test. Student t test was applied where only two groups' data were compared. Results were presented as mean ± standard error (SEM) and considered to be significantly different when $p < 0.05$.

Table 1. Effect of different whey protein concentration on the particle size and distribution of emulsions.

concentration	WPI		PWP	
(w/v)	D_z(nm)	PDI	D_z(nm)	PDI
1%	219±18[a]	0.33±0.02[a]	209±3.3[a]	0.27±0.02[a]
3%	202±9.7[b]	0.29±0.02[b]	227±6.1[b]	0.30±0.02[a]
5%	208±2.7[ab]	0.26±0.02[b]	228±8.5[b]	0.30±0.02[a]
7%	203±2.5[b]	0.25±0.02[b]	224±7.1[b]	0.26±0.02[a]
10%	213±9.0[a]	0.32±0.03[a]	230±5.0[b]	0.22±0.02[b]

Note: Value = mean ± SEM, n = 3; only values with different lowercase letters were considered significantly different within the same column at $p<0.05$. Lutein content was 2.5 mM in tested emulsions.

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Results

Effect of whey protein concentration on the particle size and distribution of emulsions

The whey protein concentration affected the particle size of lutein emulsion particles. As for WPI, both low protein concentration (1%) and high protein concentration (10%) increased the particle size and the width of distribution, whereas over 3% of PWP had larger particle size. (see Table 1).

Effect of lutein content on the particle size and distribution of emulsions

Lutein content had little influence on the particle size but slightly changed the distribution in these two types of whey protein based emulsions. Specifically, 1 mM of lutein showed the narrowest width for both emulsions. The details were shown in Table 2.

Effect of pH on the zeta-potential of the emulsions

The isoelectric point (PI) of beta-lactoglobulin, the main component of whey protein is around 5.2. Consistently, when the pH was distant from the PI, the absolute value of zeta-potential was larger. There was no significant difference between WPI and PWP emulsions at the same pH condition (Table 3).

Physical stability during storage

After the nano-emulsions were prepared, the centrifuge stabilities of the emulsions were immediately evaluated based on the centrifuge constant coefficient. The PWP based nano-emulsion had significant lower centrifuge stability compared to the WPI based emulsions

Table 2. Effect of different lutein concentration on the particle size of emulsions.

Lutein	WPI		PWP	
(mM)	D_z(nm)	PDI	D_z(nm)	PDI
0	203±8.0	0. 36±0.03[a]	225±13	0.41±0.03[a]
1	204±2.8	0.30±0.02[b]	228±1.5	0.34±0.02[b]
2.5	209±9.1	0.37±0.03[a]	230±11	0.40±0.04[a]
5	211±8.6	0.35±0.03[a]	227±5.9	0.32±0.03[b]

Note: Value = mean ± SEM, n = 3; only values with different lowercase letters were considered significantly different within the same column at $p<0.05$. Lutein content was 2.5 mM in tested emulsions.

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Table 3. Effect of pH on the zeta-potential of the emulsions.

pH	WPI	PWP
2	57.7±3.4	53.6±3.2
3	29.1±2.5	28.3±1.9
4	18.1±0.7	22.8±1.8
6	-45.6±1.9	-43.3±1.5
7	-50.6±3.5	-48.6±2.3
8	-60.6±3.5	-60.7±3.4

Note: Note: Value = mean ± SEM, n = 3. Lutein content was 2.5 mM in tested emulsions. No significant difference was found between WPI and PWP groups.

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(Fig 1). Consistently, the PWP emulsion showed stratification just overnight. The WPI based nano-emulsion was stable at 4˚C, but showed appearance of stratification at 25˚C and 37˚C after four weeks' storage (Fig 2).

Particle size change of lutein-loaded emulsions during storage

As PWP emulsion was stratified in early stage, we only recorded the particle size change of the WPI based emulsions during four weeks' storage. The particle size of WPI emulsion began to significantly increase after three weeks' storage at 25˚C and 37˚C by approximately 4%, whereas the particle size of the emulsion at 4˚C was nearly constant during the experiment (Table 4).

Lutein retention of lutein-loaded emulsions during storage

Lutein content was slightly reduced indicated by the decrease of absorbance by approximately 5% after four weeks' storage, but no significant change of absorbance was observed at different temperatures (Fig 3).

Discussion

Whey protein is a type of nutritional protein with multiple functions including emulsifying property [42]. As a result, whey protein can be extensively applied in food products, for example, to form emulsions that can increase the stability and bioavailability of hydrophobic molecules [43]. Additionally, whey protein is little digestible by pepsin, but can be hydrolyzed rapidly by proteases such as chymotrypsin and trypsin in intestinal juice, making whey protein to be ideally suitable for controlled release for bioactive compounds [44]. We recently reported that PWP had better emulsifying property than that of WPI at proper ultrasound treatment [32]. Therefore, we tested both in a lutein loaded nano-emulsion system.

Nanotechnology is promising in increasing micronutrient bioavailability [45]. The droplet size of nano-emulsion usually falls typically in the range 20–200 nm [46]. The particle size of the emulsions was all around 200 nm—close to the threshold for toxicity [47], indicating that we successfully generated the food grade nano-emulsions with the assistance of high intensity ultrasound. The emulsions were shown to be stable at pH father than its isoelectric point. This was consistent with previous reports associated with emulsions containing milk protein-coated lipid droplets [26, 48]. Considering the hydrogen ion concentration in common food, we selected pH 7 for preparation of the whey protein solution. Generally, when the relative value of zeta-potential is greater than 30, the dispersion usually has good stability [49]. At pH 7, both nano-emulsions had proper zeta-potentials, indicating their potential to be stable during

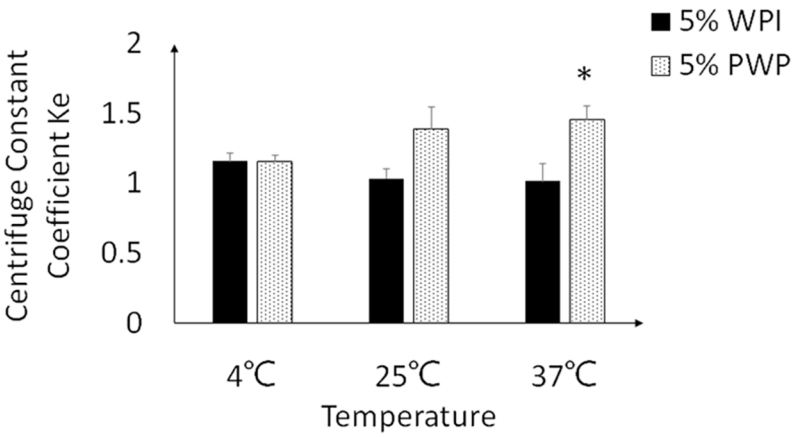

Fig 1. The centrifuge constant coefficient Ke values of the dispersions with WPI or PWP at different temperatures. The centrifuge constant coefficient Ke values of the dispersions with WPI or PWP were tested at temperatures of 4˚C, 25˚C and 37˚C. * means significant different between WPI and PWP based nano-emulsions at $p<0.05$. Value = mean ± SEM, n = 3.

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storage. However, the PWP nano-emulsion with good size and high emulsifying property showed quick appearance of stratification, which was partly predicted by its low centrifuge stability. It's possible that the inclusion of ethanol in the system interfered the reaction between whey protein and the lutein [50]. Optimization for the emulsion formulation process might solve this problem.

Temperature is an important factor for emulsion stability during storage. High temperature usually leads to increase of frequency in particle collision and promotes aggregation under certain conditions [51]. The WPI based nano-emulsion was stable within four weeks at 4˚C, but showed appearance of stratification at 25˚C and 37˚C. The result was a little different from the report of Frede *et. al.* [25]. Our result was better than theirs in particle size, which was probably attributable to the use of high intensity of ultrasound. However, they used β-lactoglobulin and whey protein hydrolysate instead, which was possibly the cause for the difference. Another possibility was the inclusion of ethanol in the system that affected the emulsions in a concentration dependent way [50].

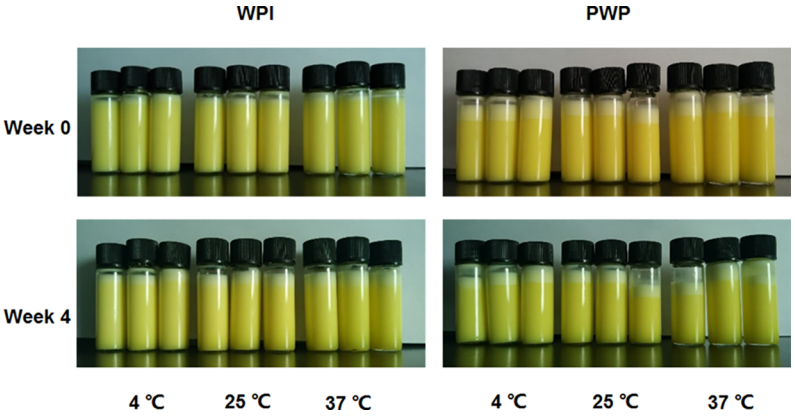

Fig 2. The stability of WPI or PWP based lutein emulsions at different temperatures during storage. PWP showed stratification within one week at all temperatures tested, whereas only WPI based lutein loaded emulsion was stable during four weeks' storage at 4˚C. The WPI emulsion showed a little of stratification at 25˚C and 37˚C.

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Table 4. Storage stability of lutein-loaded emulsions.

Emulsifier type	weeks	storage temperature (˚C)		
		4	25	37
WPI	0	209±9.1	209±9.1^{a}	209±9.1^{a}
	1	214±7.3	217±6.3^{ab}	212±6.9^{ab}
	2	209±3.6	216±4.1^{ab}	218±7.7^{ab}
	3	216±5.0	220±4.6^{b}	221±5.9^{b}
	4	208±1.1	224±6.2^{b}	229±7.8^{c}

Note: Value = mean ± SEM, n = 3; only values with different lowercase letters were considered significantly different at the same column at $p<0.05$. Lutein content was 2.5 mM in tested emulsions.

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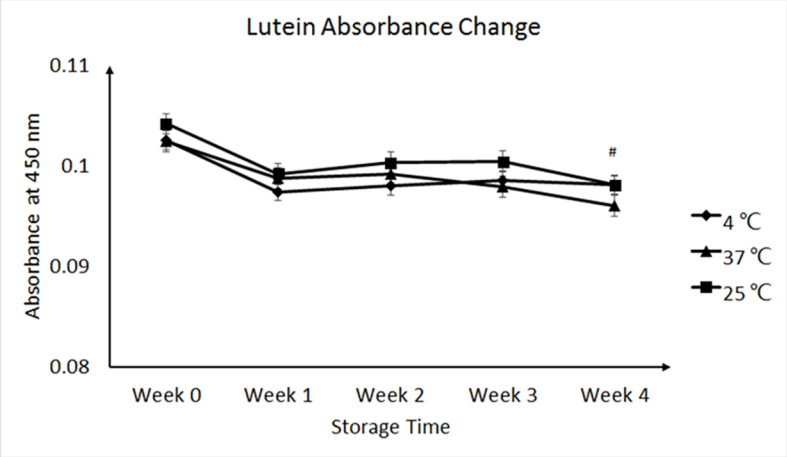

Fig 3. Absorbance change of the dispersions with whey proteins at different temperatures during storage. Absorbance change of the dispersions with whey proteins at temperatures of 4˚C, 25˚C and 37˚C were recorded weekly during storage. # Significantly different compared with the values at week 0 ($p<0.05$). No significant change was observed by temperature, though the dispersion showed lower absorbance at 37˚C. Value = mean ± SEM, n = 3.

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Lutein content was slightly reduced indicated by the decrease of UV absorbance by approximately 5% after four weeks' storage, which was probably because of the occasional light exposure—a factor that easily degrade lutein [52]. Lutein was abruptly reduced after one week and then kept at a constant level, which was probably attributed to loss of little amount of free lutein that failed to be effectively encapsulated.

From above, we conclude that the WPI based lutein loaded nano-emulsion as in the Oil/Water/Ethanol system prepared with assistance of high intensity of ultrasound has high potential of improving the stability of lutein during storage, which is promising for future use as a good carrier system for hydrophobic bioactive molecules.

Author Contributions

Conceptualization: Changhui Zhao.

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Formal analysis: Changhui Zhao, Xue Shen.

Funding acquisition: Changhui Zhao.

Investigation: Changhui Zhao.

Methodology: Changhui Zhao, Xue Shen.

Project administration: Changhui Zhao.

Resources: Changhui Zhao.

Supervision: Changhui Zhao, Mingruo Guo.

Validation: Changhui Zhao, Xue Shen.

Writing – original draft: Changhui Zhao.

Writing – review & editing: Changhui Zhao.

References

1. Alyssa H, Andrea B, Carlo P, Roberta P. Carotenoids and tocols of einkorn wheat (Triticum monococcum ssp.monococcum L.). Journal of Cereal Science. 2006; 44 182–93.
2. Breithaupt DE. Modern application of xanthophylls in animal feeding—a review. Trends in Food Science & Technology. 2007; 18 501–6.
3. Tyczkowski JK, Hamilton PB. Absorption, transport, and digestion in chickens of lutein diester, a carotenoid extracted from marigold (Tagetes erecta) petals. Poultry Science. 1986; 65:1526–31. PMID: 3588477
4. Tyczkowski JK, Hamilton PB. Lutein as a model dihydroxycarotenoid for the study of pigmentation in chickens. Poultry Science. 1986; 65:1141–5. PMID: 3737522
5. Hadden WL, Watkins RH, Levy LW, Regalado E, Rivadeneira DM, Breemen RBv, et al. Carotenoid composition of Marigold (Tagetes erecta) flower extract used as nutritional supplement. Journal of Agricultural and Food Chemistry. 1999; 47:4189–94. PMID: 10552789
6. Landrum JTL, Bone RAB, Kilburn MDK. The macular pigment: a possible role in protection from age-related macular degeneration. Advances in Pharmacology. 1997.; 38:537–56. PMID: 8895823
7. Bone RA, Landrum JT, Fernandez L, Tarsis SL. Analysis of the macular pigment by HPLC: Retina distribution and age study. Investigation Ophtalmology Visual Science. 1998; 29:843–9.
8. Mozaffarieh M, Sacu S, Wedrich A. The role of carotenoids, lutein and zeaxanthin, in protecting against age-related macular degeneration: A review based on controversial evidence. Nutrition Journal. 2003; 2:20–7. https://doi.org/10.1186/1475-2891-2-20 PMID: 14670087
9. Gale CR, Hall NF, Phillips DIW, Martyn CN. Lutein and zeaxanthin status and risk of age-related macular degeneration. Investigative Ophthalmology & Visual Science. 2003; 44(6):2461–5.
10. Neuman I, Nahum H, Ben-Amotz A. Prevention of exercise-induced asthma by a natural isomer mixture of betacarotene. Annual Allergy Ashtma Immunology. 1999; 82:549–53.
11. Halliwell B. Lipid peroxidation, antioxidants and cardiovascular disease: How should we move forward? Cardiovascular Research. 2000; 47(3):410–8. PMID: 10963714
12. De Stefani E, Boffetta P, Deneo-Pellegrini H, Mendilaharsu M, Carzoglio JC, Ronco A, et al. Dietary antioxidants and lung cancer risk: A case-control study in Uruguay Nutrition Cancer. 1999; 34:100–10.
13. Tavani A, Gallus S, La Vecchia C, Negri E, Montella M, Dal Maso L, et al. Risk factors for breast cancer in women under 40 years. European Journal of Cancer. 1999; 35:1361–7. PMID: 10658528
14. Reddy L, Odhav B, Bhoola KD. Natural products for cancer prevention: A global perspective. Pharmacology and Therapeutics. 2003; 99(1):1–13. PMID: 12804695
15. Cooper DA, Eldridge AL, Peters JC. Dietary carotenoids and certain cancers, heart disease, and age-related macular degeneration: A review of recent research. Nutrition Review. 1999; 57:201–14.
16. Shi XM, Chen F. Stability of lutein under various storage conditions. Food / Nahrung. 1997; 41(1):38–41.
17. Pratheesh VB, Benny N, Sujatha CH. Isolation, stabilization and characterization of xanthophyll. Modern Applied Science. 2009; 3(2):19–28.
18. Boon CS, D Julian M, Jochen W, Decker EA. Factors influencing the chemical stability of carotenoids in foods. Critical Reviews in Food Science & Nutrition. 2010; 50(6):515–32.
19. Qv XY, Zeng ZP, Jiang JG. Preparation of lutein microencapsulation by complex coacervation method and its physicochemical properties and stability. Food Hydrocolloids. 2011; 25(6):1596–603.

20. Wang Y, Ye H, Zhou C, Lv F, Bie X, Lu Z. Study on the spray-drying encapsulation of lutein in the porous starch and gelatin mixture. European Food Research & Technology. 2012; 234(1):157–63.
21. Jin H, Xia F, Jiang C, Zhao Y. Nanoencapsulation of lutein with hydroxypropylmethyl cellulose phthalate by supercritical antisolvent. Chinese Journal of Chemical Engineering (English Version). 2009; 17 (4):672–7.
22. Mitri K, Shegokar R, Gohla S, Anselmi C, Müller RH. Lipid nanocarriers for dermal delivery of lutein: Preparation, characterization, stability and performance. International Journal of Pharmaceutics. 2011; 414(s1–2):267–75.
23. Zhao C, Hui C, Jiang P, Yao Y, Jing H. Preparation of lutein-loaded particles for improving solubility and stability by Polyvinylpyrrolidone (PVP) as an emulsion-stabilizer. Food Chemistry. 2014; 156(11):123–8.
24. Chen C. Functional characterization of pH and ultrasound treated soy protein based nanoemulsions with lutein: University of Illinois at Urbana-Champaign; 2015.
25. Frede K, Henze A, Khalil M, Baldermann S, Schweigert FJ, Rawel H. Stability and cellular uptake of lutein-loaded emulsions. Journal of Functional Foods. 2014; 8(3):118–27.
26. Davidov-Pardo G, Gumus CE, Mcclements DJ. Lutein-enriched emulsion-based delivery systems: Influence of pH and temperature on physical and chemical stability. Food Chemistry. 2016; 196:821. https://doi.org/10.1016/j.foodchem.2015.10.018 PMID: 26593560
27. Yi J, Fan Y, Yokoyama W, Zhang Y, Zhao L. Characterization of milk proteins–lutein complexes and the impact on lutein chemical stability. Food Chemistry. 2016; 200:91–7. https://doi.org/10.1016/j.foodchem.2016.01.035 PMID: 26830565
28. Weigel F, Weiss J, Decker EA, Mcclements DJ. Lutein-enriched emulsion-based delivery systems: Influence of emulsifiers and antioxidants on physical and chemical stability. Food Chemistry. 2018:395–403. https://doi.org/10.1016/j.foodchem.2017.09.060 PMID: 29037706
29. Riggs LK, Beaty A, Mallon B. Milk proteins, nutritive value of whey powder protein. Journal of Agricultural and Food Chemistry. 1955;(4):333–7.
30. Gunnerud U, Holst JJ, Östman E, Björck I. The glycemic, insulinemic and plasma amino acid responses to equi-carbohydrate milk meals, a pilot- study of bovine and human milk. Nutrition Journal. 2012; 11 (7):1325–35.
31. Marshall K. Therapeutic applications of whey protein. Alternative Medicine Review A Journal of Clinical Therapeutic. 2004; 9(2):136–56. PMID: 15253675
32. Shen X, Fang T, Gao F, Guo M. Effects of ultrasound treatment on physicochemical and emulsifying properties of whey proteins pre- and post-thermal aggregation. Food Hydrocolloids. 2016; 63:668–76.
33. Madadlou A, Emam-Djomeh Z, Mousavi ME, Mohamadifar M, Ehsani M. Acid-induced gelation behavior of sonicated casein solutions. Ultrasonics Sonochemistry. 2009; 17(1):153–8. https://doi.org/10.1016/j.ultsonch.2009.06.009 PMID: 19592288
34. Hu H, Ecy LC, Wan L, Tian M, Pan S. The effect of high intensity ultrasonic pre-treatment on the properties of soybean protein isolate gel induced by calcium sulfate. Food Hydrocolloids. 2013; 32(2):303–11.
35. Hu H, Zhu X, Hu T, Cheung IWY, Pan S, Li-Chan ECY. Effect of ultrasound pre-treatment on formation of transglutaminase-catalysed soy protein hydrogel as a riboflavin vehicle for functional foods. Journal of Functional Foods. 2015; 19(1):182–93.
36. Zhang P, Hu T, Feng S, Xu Q, Zheng T, Zhou M, et al. Effect of high intensity ultrasound on transglutaminase-catalyzed soy protein isolate cold set gel. Ultrasonics Sonochemistry. 2016; 29:380. https://doi.org/10.1016/j.ultsonch.2015.10.014 PMID: 26585018
37. Shen X, Zhao C, Guo M. Effects of high intensity ultrasound on acid-induced gelation properties of whey protein gel. Ultrasonics Sonochemistry. 2017; 39:810. https://doi.org/10.1016/j.ultsonch.2017.05.039 PMID: 28733010
38. Liu D, Zhao C, Guo M. Sodium tripolyphosphate inhibits the formation of lysinoalanine in heat-treated whey protein. Journal of Food Processing & Preservation. 2017.
39. Shen X, Shao S, Guo M. Ultrasound-induced changes in physical and functional properties of whey proteins. International Journal of Food Science & Technology. 2016; 52.
40. Helgason T, Gislason J, McClements DJ, Kristbergsson K, Weiss J. Influence of molecular character of chitosan on the adsorption of chitosan to oil droplet interfaces in an in vitro digestion model. Food Hydrocolloids. 2009; 23(8):2243–53. https://doi.org/10.1016/j.foodhyd.2009.05.014 PubMed PMID: WOS:000270890200028.
41. Xie Y, Chen J, Zhang S, Fan K, Chen G, Zhuang Z, et al. The research about microscopic structure of emulsion membrane in O/W emulsion by NMR and its influence to emulsion stability. International Journal of Pharmaceutics. 2016; 500(1–2):110–9. https://doi.org/10.1016/j.ijpharm.2016.01.032 PMID: 26784978

42. Lam RSH, Nickerson MT. The effect of pH and temperature pre-treatments on the physicochemical and emulsifying properties of whey protein isolate. LWT—Food Science and Technology. 2015; 60(1):427–34.
43. Gunasekaran S, Ko S, Xiao L. Use of whey proteins for encapsulation and controlled delivery applications. Journal of Food Engineering. 2007; 83(1):31–40.
44. Yi J, Lam TI, Yokoyama W, Cheng LW, Zhong F. Controlled release of β-carotene in β-lactoglobulin-dextran-conjugated nanoparticles' in vitro digestion and transport with Caco-2 monolayers. Journal of Agricultural & Food Chemistry. 2014; 62(35):8900–7.
45. Joye IJ, Davidov-Pardo G, Mcclements DJ. Nanotechnology for increased micronutrient bioavailability. Trends in Food Science & Technology. 2014; 40(2):168–82.
46. Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: an advanced mode of drug delivery system. Biotech. 2015; 5(2):123–7.
47. Yu H, Huang Q. Investigation of the cytotoxicity of food-grade nanoemulsions in Caco-2 cell monolayers and HepG2 cells. Food Chemistry. 2013; 141(1):29–33. https://doi.org/10.1016/j.foodchem.2013.03.009 PMID: 23768322
48. Dickinson E. Flocculation of protein-stabilized oil-in-water emulsions. Colloids and Surfaces B: Biointerfaces. 2010; 81(1):130–40. https://doi.org/10.1016/j.colsurfb.2010.06.033 PMID: 20667698
49. Parlak M, Östürk Ö, Temel ÜN, Yapici K, editors. Heat transfer performance of water based nanofluids containing various types of metal oxide nanoparticles in an air-cooled microchannel heat exchanger. Thermal and Thermomechanical Phenomena in Electronic Systems; 2017.
50. Banks W, Muir DD. Effect of alcohol content on emulsion stability of cream liqueurs. Food Chemistry. 1985; 18(2):139–52.
51. Mcclements DJ. Food emulsions: principles, practices, and techniques. International Journal of Food Science & Technology. 2005; 36(2):223–4.
52. Li D, Liu ZL, Liu CQ. Study on degradation dynamics of lutein and lutein esters affected by light and heat (in Chinese). Jiangsu Journal of Agricultural Sciences. 2008; 24(1):97–8.