International Journal of Nutrition Sciences

Journal Home Page: ijns.sums.ac.ir

ORIGINAL ARTICLE

# Stability of Whey Protein Nanoparticles at Various Protein Concentrations

**Azam Abbasi[1*], Zahra Emam-Djomeh[2], Mohammad Ebrahimzadeh Mousavi[2]**

1. Nutrition Research Center, Department of Food Hygiene and Quality Control, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Food Sciences, Technology and Engineering, Faculty of Agricultural Engineering and Technology, Agricultural Campus of the University of Tehran, Karaj, Iran

ARTICLE INFO

*Keywords:*
Nanoparticles
Whey protein
Stability

**Corresponding author:*
Azam Abbasi,
Nutrition Research Center, Department of Food Hygiene and Quality Control, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
**Tel:** +98-263-2248804
**Fax:** +98-263-2249453
**Email:** abbasi@sums.ac.ir
**Received:** February 19, 2017
**Revised:** July 24, 2017
**Accepted:** August 29, 2017

ABSTRACT

**Background:** Different pH, temperature, protein concentration and presence of ions such as calcium and sodium can easily affect functional properties and size of protein nanoparticles. This study determined the stability of whey protein nanoparticles at various protein concentrations.

**Methods:** Whey protein isolate (WPI) nanoparticles with controlled size were produced by pH cycling and different concentration of $CaCl_2$. After preparation, the samples were diluted 1 to 1 and 1 to 3 with water and stored at 4° C for 1, 15 and 28 days. The particle size, optical density of nanoparticle dispersions were investigated after one day. Stability of nanoparticles also had been evaluated during 28 days at 4° C.

**Results:** The size of nanoparticles can be controlled by adding different amount of $CaCl_2$ , and adjusting pH or aging time of WPI solution before ultrasonication. There was no significant difference between nanoparticles at protein concentrations of 0.5 and 1%, but at 2% protein concentration, the nanoparticles were larger.

**Conclusion:** Size of all of WPI nanoparticles was reduced one day after preparation; but, there was no noticeable change during 28 days.

Please cite this article as: Abbasi A, Emam-Djomeh Z, Ebrahimzadeh Mousavi M. Stability of Whey Protein Nanoparticles at Various Protein Concentrations. Int J Nutr Sci 2017;2(3):165-169.

## Introduction

Nanotechnology can create improvement in all aspects of food industry. Nano-packaging, nano-materials and nano-sensors have been used widely (1). Nanoparticles have high surface to volume ratio which increases the reactivity and improve the physical properties such as mechanical, electrical, and optical properties. They were shown to be used for modification of nutritional, physical and organoleptic characteristics of food materials. For example, nanocapsules have been used for the protection and controlled release of bioactive compounds such as antioxidants, probiotics and vitamins (2-5).

In last decade, scientists were interested in use of natural biopolymers for production of nanocapsules (6-8). They applied several type of pure or non-pure forms of carbohydrate, protein and fat for these

purposes. These food biopolymers did not reveal any risk of having artificial materials and can be used in food formulation with more confidence. Whey proteins isolate is an example of these biopolymers and have found widespread application in food formulation due to their excellent functional and nutritional properties (9,10).

Food materials are complex of many ingredients that are in contact with each other. The mentioned biopolymers structure change when they are added to food system. Different pH, temperature, protein concentration and presence of ions such as calcium and sodium can easily affect the functional properties of proteins (11-13). The objective of our research was production of WPI nanoparticles with distinct sizes using pH cycling and evaluation of their physical properties and stability in different condition regarding the pH, NaCl and protein concentrations. This study determined the stability of whey protein nanoparticles at various protein concentrations.

## Materials and Methods

WPI was obtained from Arla Foods (Videbaek, Denmark). The WPI powder composition was the dry content of 92±2% protein, 0.2% lactose, 0.2% fat, a maximum of 6.0% moisture, and a maximum of 4.0% ash. All other chemical reagents were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Girox method was used for the production of nanoparticles.[14] First, WPI solution (8% w/v) was prepared in deionized water and adjusted to pH=7 with the dropwise addition of 1 N NaOH. Sodium azide was used as antimicrobial agent. After 12 h, the mixture was warmed up to room temperature and degassed under vacuum (560 mmHg) for 20 min, to be then heated to 80ºC under stirring and stored in this temperature for 15 min.

After cooling, the mixture was diluted at the ratio of 1:3 with distilled water. Different volumes of 25 mM CaCl2 (0, 0.4 and 0.8 ml) were added to the diluted mixtures and the pH was adjusted to 5.5 (Table 1). They were later stored at 4º C for 0 and 22 h. The dispersion was allowed to warm up to room temperature and neutralized to pH=7. Then, ultrasound (Hielscher UP200S, power 200 W, frequency 24 kHz, Dr Hielscher Co., Teltow, Germany) was used as homogenizer at different durations and powers. When accomplished, a temperature-controlled water bath was used to prevent sonication-induced temperature rise. The obtained nanoparticles were stored for 1-28 days at 4º C and their stability was investigated.

The particle size was measured as a stability indicator of the produced nanoparticle in this study by DLS instrument (90Plus, Brookhaven Instruments Corp., Vienna, Austria). To investigate the effect of protein concentration, after homogenization, the samples were diluted 1 to 1 and 1 to 3 with water and stored at 4° C for 1, 15 and 28 days. Particle size and zeta potential of the solutions were measured by dynamic light scattering (DLS) instrument (90Plus, Brookhaven Instruments Corp., Vienna, Austria).

The optical density of the samples was measured by a UV/visible light spectrophotometer (BioQuest CE 2502, Cecil Ins., Cambridge, UK) at 500 nm using 1 cm path plastic curettes. Deionized water was used as a blank reference. The tests were performed in duplicate. All experiments were performed in duplicates. The effect of different conditions on nanoparticle stability was determined by analysis of variance, using statistical analyses with SPSS software (SPSS Inc., Chicago, IL, USA).

## Results

Regarding the effect of the different nanoparticulation conditions on nanoparticle size, the best result was obtained at 200 W for 5 min. In Table 1, the different nanoparticulation conditions were introduced. As shown in this table, the size of nanoparticles can be controlled by addition of different amounts of $CaCl_2$ , and adjusting pH or aging time of WPI solution before ultrasonication. Furthermore, the polydispersity index of nanoparticle dispersions were lower than 0.25 (Table 1), so it seems that nanoparticle obtained in this research had narrow size distribution or ultrasonication that can be a good procedure for nanoparticulation.

To evaluate the effect of protein concentration on the size of nanoparticles, solutions containing two, one and a half percent protein were prepared as shown in Figure 1-4. After 1 day of production of nanoparticles, there was no significant difference between nanoparticles at protein concentration of 0.5 and 1%, but at 2% protein concentration, the nanoparticles were larger. Considering optical density measurement, Figure 5 shows the effect of protein concentration on

**Table 1:** Nanoparticles produced at different condition of pH, aging time and $CaCl_2$ concentration

| Particle type | Cacl2 (mM) | Aggregation pH | Aging time (hr) | Particle size (nm) | Polydisperesity |
|---|---|---|---|---|---|
| A | 5 | 5.50 | 22 | 250±6.00 a | 0.25 |
| B | 2.50 | 5.50 | 22 | 190±5.00 b | 0.24 |
| E | 0 | 5.50 | 0 | 45±2.50 c | 0.28 |

Different capital letters reveal that there are significant differences between various nanoparticles

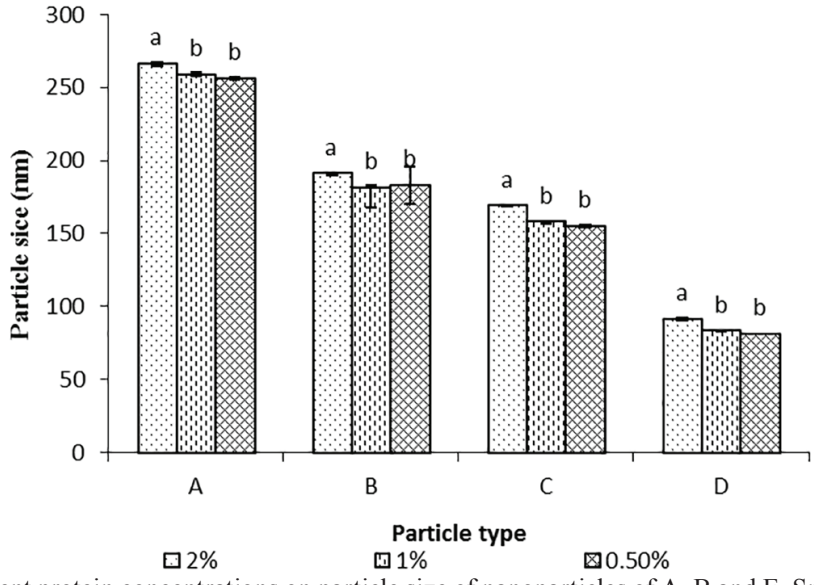


**Fig. 1:** Effect of different protein concentrations on particle size of nanoparticles of A, B and E. Small letters denote to significant differences ($p$<0.05) amongst various protein concentrations.

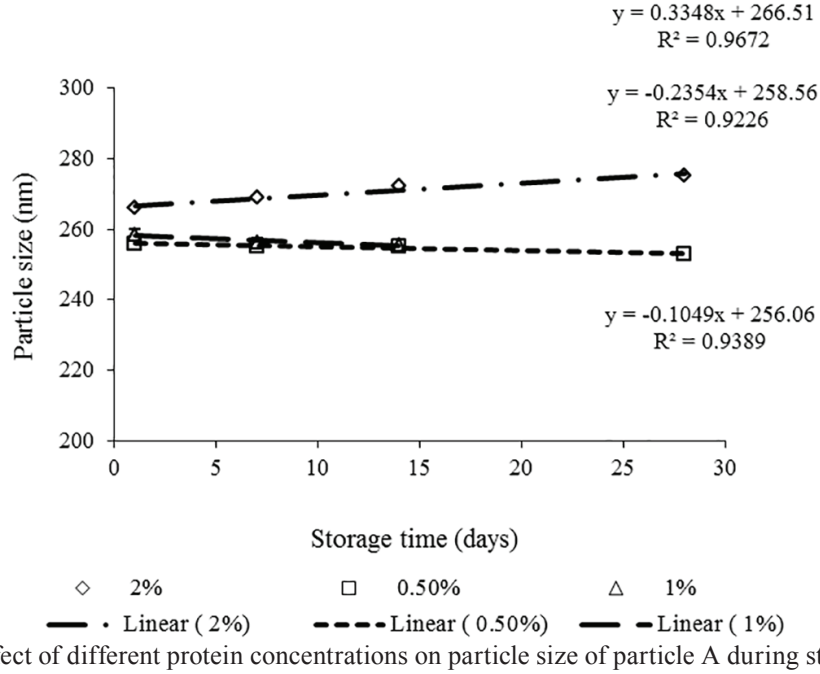


**Fig. 2:** Effect of different protein concentrations on particle size of particle A during storage time.

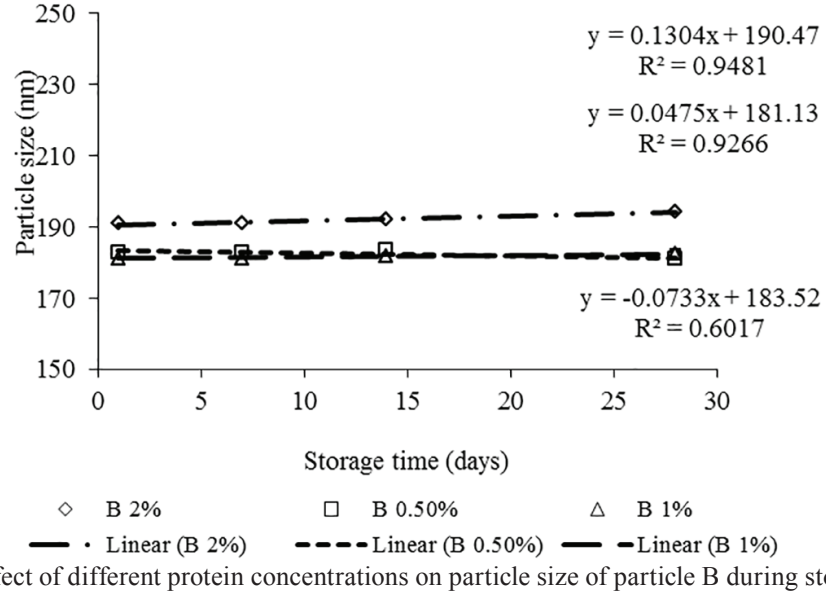


**Fig. 3:** Effect of different protein concentrations on particle size of particle B during storage time.

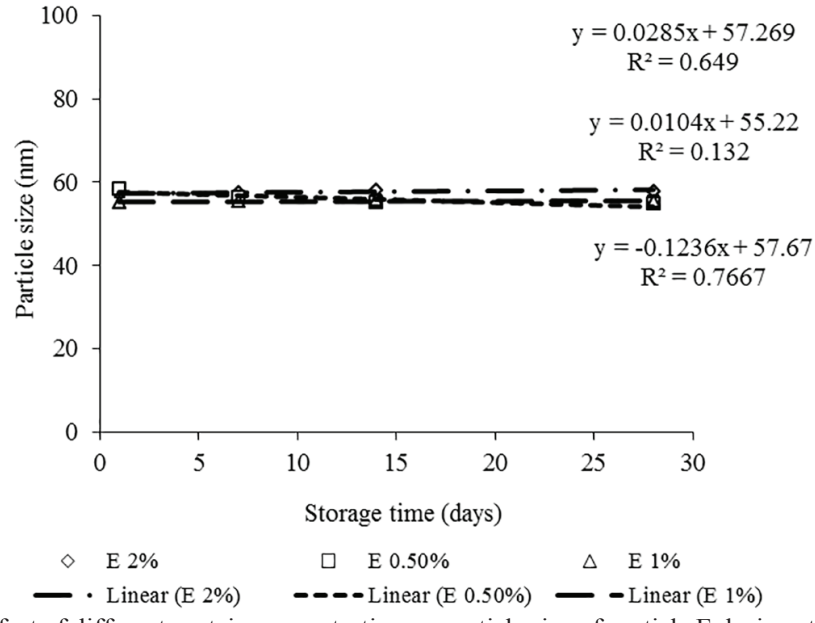


**Fig 4:** E.ffect of different protein concentration on particle size of particle E during storage time.

the turbidity of nanoparticle solutions.

## Discussion

Several studies have been carried out on the effect of protein concentration on the size of protein particles. The results obtained in this study are consistent with a previous study (15) produced alpha lactalbumin nanotubes using different concentrations of calcium chloride. The dilution lead to the opening of the nanotubes and their conversion into smaller units. The dilutions could eliminate the calcium ions from protein polymers and, as a result, could dissolve them (12).

With similar results, the factor of protein concentration in caseins or whey solutions as the most important determinant of particle size was produced deducing that each particle was consisted of smaller particles. If they were diluted, they were separated and converted into distinct particles (16). Also, the same results in relation to the effect of protein concentration were demonstrated introducing the increase in concentration as the main factor in the accumulation of molecules at pH of 3.5 and formation of a larger protein aggregate or formation of the gel at protein concentration of 8%. At concentration of half and one percent of protein, the size of all nanoparticles reduced after one day, but during the storage, their size remained almost constant, while a slight increase in particle size was observed at a concentration of two percent of the protein during storage. Considering optical density measurement, reduction in protein concentrations

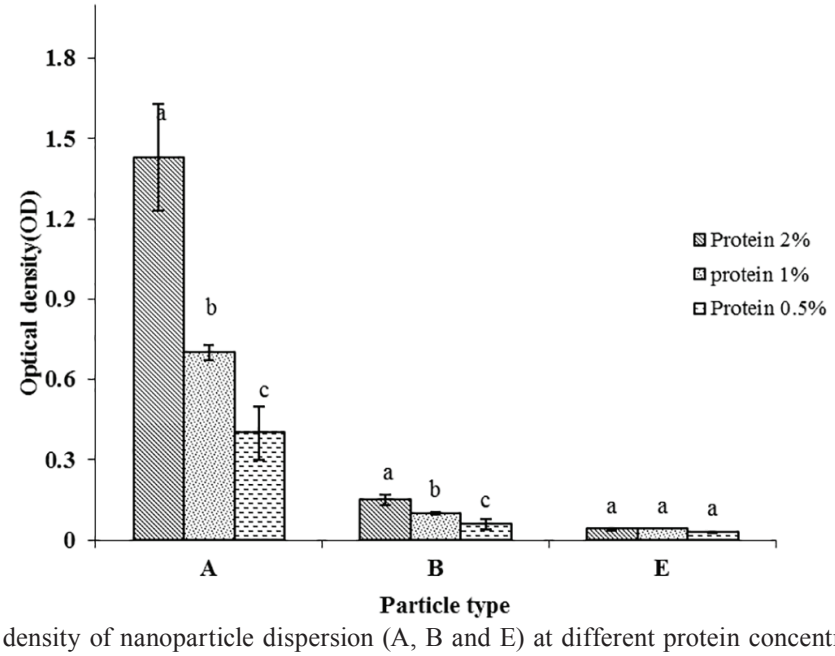


**Fig. 5:** The optical density of nanoparticle dispersion (A, B and E) at different protein concentrations. Small letters reveal significant differences ($p$<0.05) amongst various protein concentrations.

was shown to decrease the particle size and thus reduce the absorption of light at 500 nm (3).

The effect of protein and polysaccharide concentrations on the physical properties of the pectin-whey protein solution was assessed and was found that protein concentration had direct effect on the size of protein-polysaccharide aggregates and the opacity of these solutions. In addition, the results of others studies on the effect of protein concentration on the transparency of gels made from whey proteins confirmed this fact (12,16).

## Conclusion

Size of all of the produced WPI nanoparticles reduced one day after preparation; but, they did not have any noticeable change during 28 days. Furthermore, different nanoparticles had different optical densities; so, they are applicable in clear or non-clear beverage as nanocarrier of nutraceutical compounds.

## Acknowledgement

The authors thank Mr Banii for his collaboration in this research.

## Conflict of Interest

None declared.

## References

1 Neethirajan S, Jayas DS. Nanotechnology for the food and bioprocessing industries. *Food Bio Tech*. 2011;4:39-47. DOI:1007/s11947-010-0328-2.

2 Jafari M, Assadpoor E, Bhandari B, et al. Nano-particle encapsulation of fish oil by spray drying. *Food Res Int*. 2008;41:172–83. DOI:1016/j.foodres.2007.11.002 .

3 Ron N, Zimet P, Bargarum J, et al. Beta-lactoglobulin polysaccharide complexes as nanovehicles for hydrophobic nutraceuticals in non-fat foods and clear beverages. *Int Dairy J*. 2010;20:686-93. DOI:1016/j.idairyj.2010.04.001.

4 Pegoraro NS, Mattiazzi J, da Silveira EF, et al. Improved photostability and cytotoxic effect of coenzyme Q10 by its association with vitamin E acetate in polymeric nanocapsules. *Pharm Dev Technol*. 2017;7:1-7. DOI:1080/10837450.2017.1332641. PMID: 28521578.

5 Silva HD, Cerqueira MA, Vicente AA. Nanoemulsions for food applications: development and characterization. *Food Bio Tech*. 2012;5:854–67. DOI:1007/s11947-013-1094-8.

6 Jiang L, Li Y, Wang X, et al. Preparation and properties of nano-hydroxyapatite/chitosan/carboxymethyl cellulose composite scaffold. *Carbohydrate Poly*. 2008;74:680–4. DOI:1016/j.carbpol.2008.04.035 .

7 Mozafari MR, Flanagan J, Matia-Merino L, et al. Review recent trends in the lipid-based nanoencapsulation of antioxidants and their role in foods. *J Sci Food Agri*. 2006;86:2038–45. DOI:1002/jsfa.2576.

8 Weiss J, Takhistov P, McClements DJ. Functional materials in food nanotechnology. *J Food Sci*. 2006;71:R107-R116. DOI:1111/j.1750-3841.2006.00195.x .

9 Sadat L, Cakir-Kiefer C, N'Negue MA, et al. Isolation and identification of antioxidative peptides from bovine a-lactalbumin. *Int Dairy J*. 2011;21:214-21. DOI:1016/j.idairyj.2010.11.011.

10 Madureira AR, Pereira CI, Gomes AMP, et al. Bovine whey proteins Overview on their main biological properties. *Food Res Int*. 2007;40:1197-211. DOI:1016/j.foodres.2007.07.005 .

11 Boulet M, Britten M, Lamarche F. Voluminosity of some food proteins in various pH and ionic strengths. *Food Hydrocolloids*. 1998;12:433-41. DOI:1016/s0268-005x(98)00009-5.

12 Boulet M, Britten M, Lamarche F. Aggregation of some food proteins in aqueous dispersions: effects of concentration, pH and ionic strength. *Food Hydrocolloids*. 2000;14:135–44. DOI:1016/s0268-005x(99)000594.

13 Bryant CM, McClements DJ. Influence of NaCl and CaCl2 on cold-set gelation of heat-denatured whey protein. *J Food Sci*. 2000;65:801-4. DOI:1111/j.1365-2621.2000.tb13590.x .

14 Giroux HJ, Britten M. Encapsulation of hydrophobic aroma in wh protein nanoparticles. *J Microencapsulation*. 2010;28:337–43. DOI:3109/02652048.2011.569761. PMID:21736518.

15 Graveland-Bikker JF, Schaap IA, Schmidt CF, et al. Structural and mechanical study of a self-Assembling protein nanotube. *Nano Lett*. 2006;6:616-21. DOI:1021/nl052205h. PMID:16608254.

16 Mudgal P, Daubert CR, Foegeding EA. Cold-set thickening mechanism of b-lactoglobulin at low pH: Concentration effects. *Food Hydrocolloids*. 2009;23:1762–70. DOI:1016/j.foodhyd.2009.03.009 .