Solubilization of Capsaicin and Its Nanoemulsion Formation in the Sonication and Self-Assembly Methods

A. J. Choi[*], C. J. Kim[*], Y. J. Cho[*], J. K. Hwang[**] and C. T. Kim[*]
[*]Nano-Bio Research Group, Korea Food Research Institute, Kyeonggido, Korea, ctkim@kfri.re.kr
[**]Department of Biotechnology, Yonsei University, Seoul, Korea,
jkhwang@yonsei.ac.kr

ABSTRACT

In this research, we investigated the optimum condition for the preparation of O/W nanoemulsion containing surfactants and oleoresin capsicum(OC) in four-component systems, and characterized the structure and stability of nanoemulsions by using the ternary phase diagrams of systems. Various types of nanoemulsion including single-layer, double-layers and triple-layers nanoemulsions could be produced depending on the polyelectrolytes such as alginate and chitosan. O/W nanoemulsions of OC could be prepared by the ultra-sonication process at the ration of mixture of OC:Tween 80(1:0.7) and by self-assembly method at the ratio of mixture of OC:Tween 80(1:3) with a particle size of 20-100nm and having a good stability during storage. The ultra-sonication method may be more powerful tool to prepare the nanoemulsion than the self-assembly process, it might be due to the formation capacity of nanoemulsion phase.

Keywords: nanoemulsion, capsaicin, solubilization, bioactive ingredient, self-assembly, stability

INTRODUCTION

Recently, a bioactive lipids such as phytosterols, carotenoids, and ω-3 fatty acids have generated interest in the functional food as they providing specific health benefits to humans. However, these bioactive lipids still have many problems to use as nutraceutical or functional foods because of their physicochemical and physiological properties, including solubility, stability, and bioavailability [1]. Nanoemulsions have excellent longterm thermodynamic stability, and they are capable of solubilizing considerable amounts of water-soluble and oil-soluble compounds. Nanoemulsions have also potential advantages over macroemulsions offering sustained controlled release, improved bioavailability and high stability for bioactive ingredients [2]. Capsaicin has been used for many years in food additives to prevent the development of arteriosclerosis, reduce high blood pressure and to improve prothrombin, thrombin and partial thromboplastin times [3]. It is effective to the growth of human leukemic cells, gastric, and hepatic carcinoma cells in vitro [4]. And also recently it has a profound antiproliferative effect on prostate cancer cells, inducing the apoptosis of both androgen receptor positive and -negative prostate cancer cell lines [5]. However, up to now, known bioactive substances imparting functionality to human body lack in solubilization, stability, and bioavailability, which results in unsuccessful quality enhancement and commercialization.

MATERIALS AND METHODS

1. Materials

NSTI-Nanotech 2008, www.nsti.org, ISBN 978-1-4200-8503-7 Vol. 1

Oleoresin capsicum (OC, SHU 100,000) was purchased from G&F Co., Seoul, Korea. Chitosan (Mw 330,000, 93% of deacetylation) solution was prepared by dispersing 0.05 wt% in distilled water and stirring for 2 hr at room temperature.

2. Preparation of nanoemulsion

The composition of three-component nanoemulsion system included oleoresin capsicum(OC) , Tween 80 and water. An oil-in-water emulsion was prepared by sonication or self-assembly method. Chitsan and alginate solution were used to form the double- or triple-layers nanoemulsions bsed on the single nanoemulsions [6].

3. Ternary phase diagram

Existence of a clear, one-phase nanoemulsion region in a three-component mixture was determined by the construction of ternary phase diagrams. Ternary mixtures with varying compositions of OC, Tween 80 and water were prepared. OC concentration was varied from 1.9 to 55.6% (w/w), Tween 80 concentration was varied from 1.3 to 42.9% (w/w) and water concentration was varied from 27.8 to 42.9% (w/w).

4. Particle size measurements

The mean droplet size and size distribution were determined by laser light scattering (Nanotrac ™250, Microtrac Inc., PA, U.S.A) at 25°C.

5. ζ-potential measurements

3 ml of nano-emulsions were injected into the measurement chamber of of a particle electrophoresis instrument (Zetasizer Nanoseries ZS, Malvern Instrument, UK), and the ζ-potential was determined by measuring the direction and velocity that the droplets moved in the applied electric field.

RESULTS AND DISCUSSIONS

1. Phase diagram of nanoemulsions

The phase diagrams indicating the behavior of the systems composed of OC, Tween 80 and water, respectively, and area of nanoemulsion existence are shown Fig. 1. Area enclosed within the solid line represents the extent of nanoemulsion formation, which was a clear and one-phase region. In place of an extensive nanoemulsion region, 2-phase and large liquid crystal regions were formed at high surfactant (60-84 wt %), low OC concentrations, together with very large, cloudy multiphase regions. It is clear that the nanoemulsions could be obtained from OC (1.92-27 %, w/w), Tween 80 (1.93-27.3%, w/w) and water (54.1-96.2 %, w/w) within range of 15-150 nm.

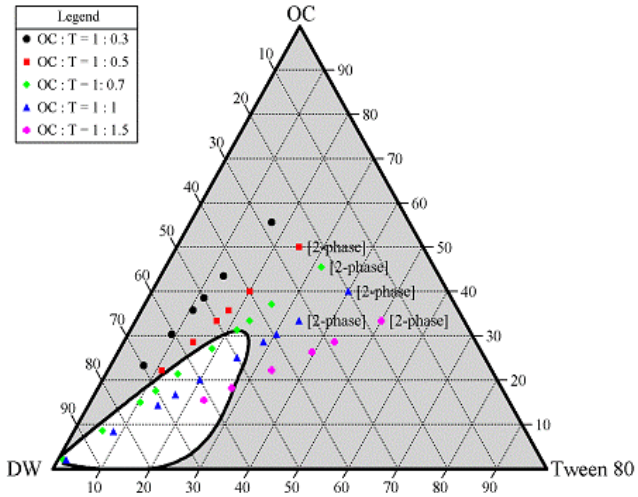

Figure 1 : Ternary phase diagram of nanoemulsion formed by the ultra-sonication on the system of OC/Tween 80/water.

2. Electrical charge of nanoemulsions

The electrical charge (ζ-potential) on the emulsion droplets of double- and triple layers nanoemulsions which layered with chitosan, alginate and the combination of both them by self-assembly, exhibited a positive or negative as the pH increased (Fig. 2). The ζ-potential reached a maximum value −50.3, −39.6, and −31.3 mV at pH 8 for AN, CN, and CAN respectively. The fact that the AN had more higher negative ζ-potential than CA can be attributed to the fact that the anionic charge of sodium alginate. Since most foods or biopolymers can be digested easily in the colon whereas pH value is in the range of 6.5–7.0, it is expected that this type of nanoemulsions seems to be a good candidate for effective functional food delivery system. It is reported that the stable multilayer emulsions could be produced by adsorbing chitosan or alginate onto oil droplets stabilized by a nonionic surfactant [7].

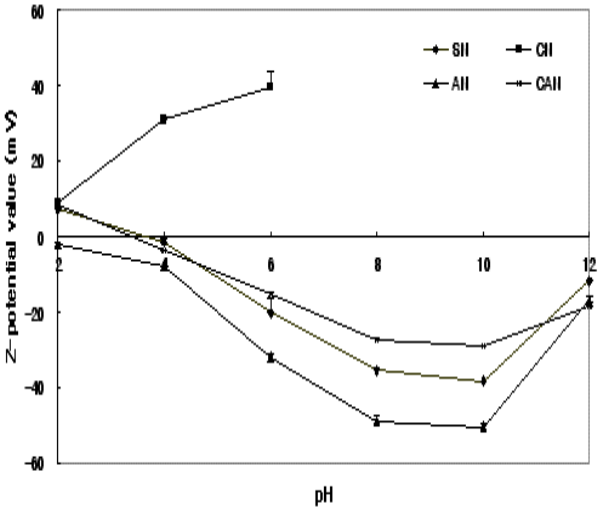

Figure 2 : ζ-potential value of nanoemulsions. SN: single-layer nanoemulsion, CN:double-layers nanoemulsion with chitosan, AN:double-layers nanoemulsion with alginate, CAN:triple-layers nanoemulsion with alginate/chitosan.

3. AFM observation of nanoemulsion

Atomic force microscpy (AFM) observations of the double-layers nanoemulsion with alginate (AN) was made immediately after the emulsion was prepared and after storage for four weeks at 25°C, and the resultant micrographs are presented in Fig. 3. In general, the particles were slightly smaller than 80 nm in diameter, which supported the results of particle sizing by the dynamic light scattering method. Most of particles appear spherical or irregular in shape, which were likely due to the agglomeration of smaller particles. The images showed little differences between the samples analyzed immediately after the emulsion was prepared and after four weeks storage, suggesting that the nanoemulsion had a good stability during storage.

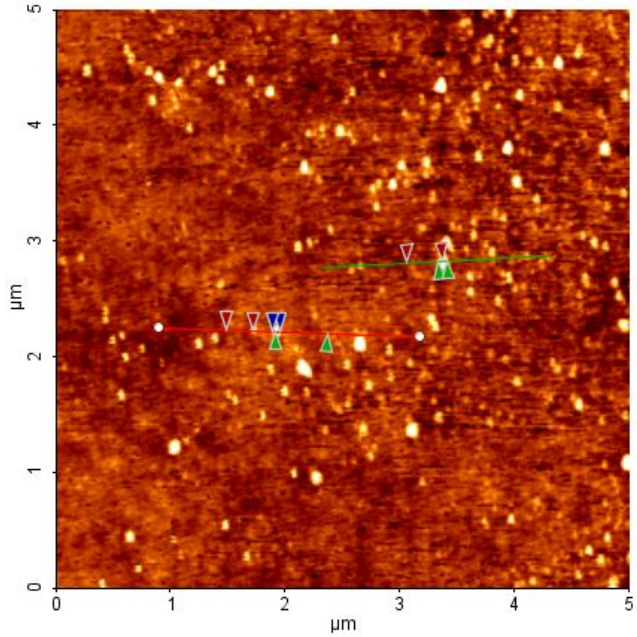

Figure 3 : AFM image of double-layers nanoemulsion with alginate (AN).

NSTI-Nanotech 2008, www.nsti.org, ISBN 978-1-4200-8503-7 Vol. 1

CONCLUSIONS

1. O/W nanoemulsions containing bioactive ingredient including oleresin capsicum and surfactant such as Tween 80, glycol and sucrose fatty acid ester could be produced by using ultra-sonication and self-assembly method.
2. Optimal conditions for the preparation of food nanoemulsions by ultra-sonication were confirmed to be mixture of OC : Tween 80 : water = 1 : 0.7 : 2 on the ternary phase system, and the droplet diameter was in the range of 50 to 100 nm.
3. The nanoemulsion could be formed by self-assembly when the mixture ratio of OC : Tween 80 : water = 1 : 3 : 5 on the ternary phase system, and the droplet diameter was in the range of 10 to 20 nm.
4. Double- and triple-layers nanoemulsions prepared with biopolymers such as alginate and/or chitosan by self-assembly, showed a particle size of below 20nm.
5. In conclusion, the double-layers nanoemulsions incorporated with alginate and chitosan can be expected to improve the stability and bioavailability of bioactive ingredient, therefore it may be apply for the production of functional foods containing nutraceutical ingredients.

ACKNOWLEDGMENT

This work supported by the Food Nanotechnology Development Project (2008), Ministry of Education, Science Technology, Korea.

REFERENCES

[1] D. J. McClements, E. A. Decker and J. Weiss, Emulsion-based delivery systems for lipophilic bioactive components, J. Food Sci., 72, R109, 2007.

[2] J. Flangan and H. Singh, Microemulsions: A potential system for bioactives in food, Crit. Rev. Food Sci. & Nutr., 46, 221, 2006.

[3] R. K. Kempaiah, H. Manjunatha and K. Srinivasan, Protective effect of dietary capsaicin on induced oxidation of low-density lipoprotein in rats, Molecular and Cellular Biochem., 275, 7, 2005.

[4] K. Ito, T. Nakazato and K. Yamato, Induction of apoptosis in leukemic cells by homovanillic acid derivative, capsaicin, through oxidative stress: Implication of phosphorylation of p53 at ser-15 residue by reactive oxygen species. Cancer Res., 64, 1071, 2004.

[5] A. Mori, S. Lehmann, J. O'Kelly, T. Kumagai, J. C. Desmond, M. Pervan, W. H. McBride, M. Kizaki and H. P. Koeffler, Capsaicin, a component of red peppers, Inhibits the growth of androgen-independent, p53 mutant prostate cancer cells, Cancer Res., 66, 3222, 2006.

[6] C. T. Kim, Y. J. Cho, C. J. Kim, I. H. Kim, Y. H. Kim and A. J. Choi, Research report of Korea Food Research Institute, No. E071000-07085. Development of process technology for functional food nanocomposite. In: Report of the Development of Food Nanotechnology, December 31, Kyeonggi, Korea, 123-266, 2007.

[7] S. Mun, E. A. Decker and D. J. McClements, Influence of droplet characteristics on the formation of oil-in-waater emulsions stabilized by surfactant-chitosan layers, Langmuir, 21, 6228, 2005.