Encapsulation of Carotenoids in Emulsion-based Delivery Systems: Enhancement of β -carotene Water-Dispersibility and Chemical Stability

Jingjing Chen^{1,2}, Fang Li², Zhengze Li², David Julian McClements², Hang Xiao^{2*}

Abstract

β -carotene is a lipophilic micronutrient that is important for maintaining human health and wellbeing. However, the utilization of β -carotene in functional foods and dietary supplements is currently limited due to its poor water-dispersibility and chemical stability. A β -carotene-enriched delivery system was therefore developed to overcome these problems using a microfluidizer to produce an oil-in-water emulsion. The fabrication process was optimized to enhance the water-dispersibility and chemical stability of the encapsulated β -carotene. A sonication-assisted method was more effective ($1.73 \pm 0.27\%$, w/w) in dissolving β -carotene in the lipid phase than commonly used heating method ($1.21 \pm 0.16\%$, w/w). Powdered versions of the β -carotene-enriched emulsion were produced using either spray or freeze-drying. Higher β -carotene retention was obtained in freeze-dried powders than spray dried ones (95% vs 15%-28%). Moreover, the freeze-dried powder had better water-dispersibility and lower moisture content. In conclusion, sonication-assisted dissolving method together with freeze drying might be an effective method to prepare β -carotene-enriched functional foods and dietary supplements.

Keywords: β -carotene, emulsion, degradation, freeze-dry, spray dry, functional foods

1. Introduction

β -carotene is an orange colored compound belonging to the carotenoid family that can be found in many fruits and vegetables, including pumpkins, mangoes, tomatoes, carrots, and spinach (Durante, Lenucci, D'Amico, Piro, & Mita, 2014; Gul, Tak, Singh, Singh, Yousuf, & Wani, 2015). Consumption of this carotenoid is important for maintaining human health and wellbeing because it is enzymatically converted to retinol (vitamin A) in the human intestine by the β -carotene 15,15'-monooxygenase thus constituting the most common and safe source of vitamin A (Álvarez, Vaz, Gronemeyer, & de Lera, 2014; Haskell, 2012). Besides its pro-vitamin A function, β -carotene has many other activities that may contribute to human health, such as the ability to exhibit antioxidant activity (Kasperczyk, Dobrakowski, Kasperczyk, Ostalska, Zalejska-Fiolka, & Birkner, 2014), reduce the risk of type 2 diabetes (Sluijs, Cadier, Beulens, van der A, Spijkerman, & van der Schouw), lower metabolic syndrome in middle-aged adults (J. Liu, Shi, Cao, He, Guan, Ling, et al., 2014), enhance immune system performance, and reduce the risk of cardiovascular diseases (Tanaka, Shnimizu, & Moriwaki, 2012). However, the application of β -carotene in many functional foods is currently limited due to its poor water-dispersibility, chemical stability, and bioavailability (Chen & Zhong, 2015).

One approach to overcome the challenges associated with β -carotene utilization as an ingredient for the preparation of functional foods and beverages is to use nanoemulsion-based delivery systems (Donhowe & Kong, 2014; Mao & Miao, 2015; Neves, Hashemi, & Prentice, 2015). β -Carotene has better solubility in food-grade oils than in water (Qian,

Decker, Xiao, & McClements, 2012d), and therefore it can be dissolved in these oils and then dispersed in an aqueous phase in the form of small lipid droplets using homogenization processes (McClements & Rao, 2011). Studies have reported that the incorporation of β -carotene in nanoemulsions can enhance its functional attributes. The bioaccessibility of β -carotene has been reported to be improved when it is incorporated into oil-in-water emulsions that contain small lipid droplets (Qian, Decker, Xiao, & McClements, 2012c; Salvia-Trujillo, Qian, Martin-Belloso, & McClements, 2013a, 2013b). The physical and chemical stability of β -carotene-enriched nanoemulsions can be improved by careful selection of emulsifiers, by adding antioxidants to the oil or aqueous phases (Guan, Wu, & Zhong, 2016; Qian, Decker, Xiao, & McClements, 2012a, 2012e; Xu, Wang, Jiang, Yuan, Decker, & Gao, 2013), or by coating the lipid droplets with antioxidant emulsifiers (F. Liu, Wang, Xu, Sun, & Gao, 2016; Wei & Gao, 2016).

For commercial applications, it is often important to convert a liquid form of a nutraceutical delivery system into a powdered form, in order to reduce storage and transportation costs, facilitate handling and utilization, and extend shelf life (Maher, Roos, & Fenelon, 2014). Spray-drying and freeze-drying are two of the most widely used technologies for converting liquids into powders (Abdelwahed, Degobert, Stainmesse, & Fessi, 2006; Dias, Ferreira, & Barreiro, 2015). Spray drying is commonly used in the food and pharmaceutical industries because it is suitable for continuous large-scale production of powders, and because the short drying time reduces potential damage to heat-sensitive components (Dias, Ferreira, & Barreiro, 2015; Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). The higher costs and longer processing times

associated with freeze-drying make the process less economical than spray-drying for large-scale production of powders, but it typically produces higher quality powders with better physicochemical characteristics (Abdelwahed, Degobert, Stainmesse, & Fessi, 2006; Ratti, 2001). The successful formulation of powdered delivery systems requires the optimization of product formulation and processing conditions. The incorporation of wall materials, such as maltodextrin, into an emulsion before dehydration is particularly important because they increase the glass transition temperature of the solid matrix surrounding the oil droplets, thereby improving the chemical stability of any encapsulated substances (Mosquera, Moraga, & Martínez-Navarrete, 2012). The inclusion of wall materials also improves the flow characteristics and water-dispersibility of the powder, which is important for many commercial applications (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). The nature of the emulsifier used to coat the oil droplets in an emulsion or nanoemulsion also plays a critical role in determining the physicochemical properties of the powders produced after dehydration. Previous studies have reported that β -carotene-loaded oil droplets stabilized by various kinds of emulsifiers can be converted into powders, including gum arabic (Lim, Burdikova, Sheehan, & Roos, 2016), almond gum (Mahfoudhi & Hamdi, 2014), modified starch (Liang, Huang, Ma, Shoemaker, & Zhong, 2013), hydroxypropyl cellulose (Claudia P. Coronel-Aguilera & San Martín-González, 2015), sodium caseinate (C. P. Coronel-Aguilera & Martin-Gonzalez, 2015), soy protein (Deng, Chen, Huang, Fu, & Tang, 2014), soy protein-gum arabic complex (Nakagawa & Fujii, 2015), and cyclodextrin (Durante, Lenucci, Marrese, Rizzi, De Caroli, Piro, et al., 2016). Most of these studies used spray dry to prepare

nanoemulsion powder and were aimed to determine the physiochemical properties of dried powder, or to increase carotenoid stability by manipulating the composition. However, there's still a lack of knowledge on how to increase the loading capacity and stability of β -carotene in the delivery system using simple processing procedure.

The objective of the current study was to develop a powdered β -carotene-enriched delivery system with high carotenoid load, good water-dispersibility, and minimum carotenoid degradation. First, a β -carotene-enriched oil phase was prepared using a cold-sonication-assisted method. Second, a β -carotene-enriched emulsion was prepared by microfluidization of this oil phase with an aqueous phase containing casein as an emulsifier. Third, the emulsions were mixed with maltodextrin (wall material), and then converted into a powder using two different microencapsulation methods: spray-drying and freeze-drying. The β -carotene loading, physiochemical properties, and chemical stability of the powders were then characterized. The formulation and processing conditions were optimized to produce high carotenoids content with low levels of chemical degradation.

2. Material and Methods

2.1. Material

β -carotene (with purity $\geq 93\%$), casein, butylated hydroxytoluene (BHT), *tert*-Butylhydroquinone (TBHQ), and sodium azide were purchased from the Sigma-Aldrich Corporation (St.Louis, MO). Wesson vegetable oil (ConAgra Foods Inc., Omaha, NE) was purchased from a local supermarket. Maltodextrin (Maltrin M180) was obtained

from the Grain Processing Corporation (Muscatine, IA). HPLC grade ethanol, methanol, isopropyl alcohol, acetonitrile, and chloroform were purchased from Fischer Scientific (Thermo Scientific, Waltham, MA).

2.2. Dissolution of β -carotene

2.2.1. Temperature-assisted dissolution

β -carotene (10-40 mg) was placed in a 1.5 mL centrifuge tube, and then 1 g of vegetable oil was added. Samples were thoroughly mixed and then placed on a water bath (50 °C) for 5 min. Another set of samples were prepared with the same method but with BHT or TBHQ (0.01% or 0.05%, w/w) dissolved in the oil phase. The antioxidants were dissolved in oil in sealed glass bottles flushed with nitrogen gas. Samples were then vortexed for 3 min on a vortex mixer. Samples were then centrifuged at 20,800 g for 30 min and the supernatant was diluted with mobile phase before being analyzed for β -carotene content using HPLC.

2.2.2. Cold-sonication-assisted dissolution

β -carotene was placed in a 1.5 mL centrifuge tube, and then 1 g of vegetable oil was added. The sample was thoroughly mixed on a vortex mixer, and then placed in an ice bath and sonicated for 30 s, 1 min or 3 min using an ultrasonic probe (model FB 505, Fisher Scientific, PA). Samples were centrifuged at 20,800 g for 30 min and supernatant was diluted with mobile phase for HPLC analysis to determine β -carotene content.

2.3. Emulsion preparation

Vegetable oil containing dissolved β -carotene obtained by cold-sonication was used as the oil phase to prepare the emulsions. Ten grams of vegetable oil containing β -carotene dissolved by cold-sonication were added to 100 g of aqueous phase containing 1% (w/w) of casein as the emulsifier. Then the sample was mixed with a hand mixer (BioSpec 10810, Biospec Products, Bartlesville, OK) for 1 min. The emulsion was produced with a M110 microfluidizer (Microfluidics, Newton, MA) at 12,000 psi for 3 passes. The β -carotene was shielded from light exposure throughout the preparation of the oil phase and emulsion by keeping it in opaque containers. An aliquot of fresh emulsion was stored at -20 °C until analysis of β -carotene content.

2.4. Stability of β -carotene emulsion at elevated temperature

The freshly prepared β -carotene emulsion was mixed with sodium azide (0.02 %, w/w) and kept at 50 °C for a month in the dark. An aliquot of 200 μ L solution was withdrawn from the test tube every other day. The samples were kept in a -20°C freezer before being analyzed using a HPLC system for β -carotene content.

2.5. Spray drying

Freshly prepared β -carotene emulsions were mixed with 50 % maltodextrin aqueous solutions (w/w) at a mass ratio of 4:1. The resultant mixture was stirred on a magnetic-stirrer for 10 min to ensure homogeneity. Both β -carotene emulsion and β -carotene emulsion-maltodextrin mixture were spray dried using a laboratory spray dryer (B290 mini, Buchi Corporation, Switzerland). Air inlet temperature was set at 100°C, 110°C, or

120°C, outlet temperature was set at 60°C, and the feed rate was 1mL/ min. Samples were collected and stored in airtight glass bottles in a -20 °C freezer before further analysis.

2.6. Freeze-drying

Samples from the emulsions used to prepare powders by spray drying were also used to prepare powders by freeze-drying. Aliquots of the emulsions were also powdered by freeze-drying either with or without the addition of 50 % maltodextrin solution (w/w) at a mass ratio of 4:1 before freeze-drying. Samples were dried in a pilot-plant freeze dryer (25EL Virtis, SP Scientific, PA) for two days. The freeze-dried powder was stored in airtight glass bottles in a -20°C freezer before further analysis.

2.7. Characterization of wet and powdered emulsions

2.7.1. Particle size and ζ - potential

The particle size and ζ -potential of the emulsions were analyzed using a combined dynamic light scattering and electrophoresis instrument (Zetasizer Nano ZS, Malvern Instruments, UK). Freshly prepared emulsions were diluted 50 times using PBS buffer (10 mM, pH 7) prior to analysis. Spray-dried and freeze-dried emulsion powders were reconstituted to the original oil concentration using distilled water and then diluted 50 times using PBS buffer (10 mM, pH 7) before analysis. The particle size was reported as the intensity-weighted mean particle diameter (Z-average).

2.7.2.Determination of β -carotene content

The β -carotene content was determined using a HPLC system equipped with a UV detector using a CDC Laboratory Procedure Manual (Vitamin A, Vitamin E, Gamma Tocopherol, Retinyl Palmitate, and Retinyl Stearate in Serum NHANES 1999-2000) with some modifications. The mobile phase (1L) consisted of 972 mL acetonitrile, 148 ml tetrahydrofuran and 60 ml of isopropanol alcohol and methanol mixture (1:1, v/v).

For fresh emulsions, 10 mg of sample was added to a 1.5 ml centrifuge tube, then 500 μ L of mobile phase was added, and the tube was vortexed for 1 min. Then 500 μ L of chloroform was added, the tube was vortexed for another minute, and then centrifuged at 20,800g for 20 min. After centrifugation, there were three layers: a colorless aqueous phase on top; a yellowish organic phase at the bottom; and a thin protein layer between these two phases. The solution at the bottom (which contained the carotenoids) was carefully collected, while the remaining sample was extracted again using the same method another two times. The extracting solvent phases were then combined and diluted with mobile phase for HPLC analysis. Similarly, 10 mg of spray dried or freeze-dried powder was reconstituted into a liquid emulsion using double distilled water and then extracted with 500 μ L of mobile phase and 500 μ L of chloroform. The extracting solutions were combined and diluted for HPLC analysis of β -carotene content.

2.7.3.Determination of moisture content

The moisture content of spray dried and freeze-dried powders was determined using an oven drying method (Nielsen, 1998, 2010). The test powder (1g) was accurately

weighed and placed in a drying pan with a cover. Then the sample was put in a forced draft oven for 24 h at 102 °C. Finally, the sample was cooled down in a desiccator before being weighed again. Three replicas were tested for each sample.

2.8. Statistical analysis

All data are expressed as means $\pm$ standard deviations. **Three replicates were tested for each experiment.** The Student's t-test was used to determine the significance of difference between two groups. ANOVA was used to analyze the significance of difference for more than two groups. A significance level of 5% was used for all statistical analysis.

3. Results and Discussion

3.1. Comparison of heat-assisted and cold-sonication-assisted dissolution

β -carotene is prone to chemical degradation and isomerization when exposed to heat, light, and oxygen (Chen & Zhong, 2015; Knockaert, Pulissery, Lemmens, Van Buggenhout, Hendrickx, & Van Loey, 2012). Therefore, it is important to determine any chemical changes in β -carotene that occur as a result of the different processing steps used to **prepare** the delivery systems. The dissolution of crystalline β -carotene in the oil phase is the first step involved in preparing the delivery systems. In this study, we compared the commonly used heat-assisted method of dissolving β -carotene in the oil phase with a cold-sonication-assisted method. We hypothesized that the cold-sonication method would produce less chemical degradation of this heat labile carotenoid.

For oily carotenoid solutions prepared using the traditional heat-assisted method, a peak was observed in the HPLC chromatogram that corresponded to degraded β -carotene (Fig. 1b). This peak accounted for about 19 % of the total β -carotene peak area. The appearance of this thermal degradation product may have been due to isomerization and/or oxidation of the β -carotene at the elevated temperatures used during the dissolution process. The potential impact of oxidation on β -carotene degradation was assessed by incorporating two commonly used antioxidants into the oil phase prior to heating: butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ). The addition of the antioxidants only had a marginal effect on decreasing the level of β -carotene degradation products generated (Fig. 2): BHT decreased degradation by about 3-5%, while TBHQ reduced the degradation by about 1-3%. These results suggest that the thermal degradation of β -carotene observed in our study may have been mostly due to other mechanisms.

β -carotene is known to undergo isomerization and degradation reactions at elevated temperatures that produce compounds such as all-*E*-5, 8-exoxy- β -carotene (Zeb & Murkovic, 2013), 13-*Z*- β -carotene and 15-*Z*- β -carotene (Knockaert, Pulissery, Lemmens, Van Buggenhout, Hendrickx, & Van Loey, 2012). Considerably less degradation occurred when β -carotene was dissolved in oil using the cold-sonication method for 1 minute (around 4%, Fig. 1c) than using the heat-assisted method (around 19%, Fig. 1a). However, sonication for longer times (3 min) led to a significant increase in the amount of β -carotene degradation product formed (Fig. 1d). Prolonged sonication might have promoted carotenoid degradation through two mechanisms: (i) the temperature of the

samples increased at longer times; (Nakagawa & Fujii) free radicals were produced by sonication. In the following experiments, cold-sonication with a treatment time of 1 min was used for dissolving the β -carotene in the oil phase.

Another potential advantage of using the cold-sonication dissolution method is that more β -carotene could be solubilized in the oil. As high as 1.73 ± 0.27 % (w/w) of β -carotene could be dissolved in the oil phase using the cold-sonication method, while only 1.21 ± 0.16 % (w/w) could be solubilized using the heat-assisted method.

In summary, the cold-sonication method had a number of advantages over the more traditional heat-assisted method: a shorter preparation time; a higher carotenoid load; and less carotenoid degradation.

3.2. Characterization of emulsions and emulsion powders

3.2.1. Structural and physicochemical properties

The mean particle diameters, ζ -potentials and appearances of the original and reconstituted emulsions were measured (Table 1, Fig. 3). The freshly prepared β -carotene emulsion had a mean particle diameter around 231 nm and a ζ -potential around -64.5 mV. These results show that the homogenization conditions and emulsifier used were effective at preparing oil-in-water emulsions containing small droplets. The fact that the oil droplets had a high electrical potential suggests that they would have good stability against aggregation due to the strong electrostatic repulsion acting between them (Zou, Zhang, Zhang, Liu, Liu, Xiao, et al., 2016).

The spray-dried samples all contained maltodextrin but they were prepared at three different operating temperatures (100, 110, and 120 °C). All the spray-dried samples were free flowing yellowish powders that could simply be dispersed in water (Figures 3a, b, and c). Nevertheless, there were some differences in the properties of the reconstituted emulsions. After reconstitution, there was a thin layer of orange-yellow oil on top of the sample **from** the powder that was obtained by spray drying at 120 °C (Fig. 3c). This may have been due to some coalescence of the oil droplets during spray drying at elevated temperatures (Anton, Jakhmola, & Vandamme, 2012). Performing spray-drying at lower operating temperatures reduced the amount of oil present at the top of the reconstituted emulsions (Figure 3a, b and c): only a very thin layer of surface oil was observed after spray drying at 110 °C, and no surface oil was observed after spray drying at 100 °C. The reconstituted spray-dried emulsion powder (prepared at 100 °C) had a mean particle diameter around 277 nm and a ζ -potential around -49.3 mV (Table 1). This result suggests that a small amount of irreversible particle aggregation occurred during spray-drying, as well as some change in interfacial composition, which may have been due to the exposure of the oil droplets to elevated temperatures within the spray drier.

All the freeze-dried samples were prepared using **identical** freezing and drying conditions, but with different amounts of maltodextrin. In the presence of 20 or 50% maltodextrin, the dried samples formed were initially crispy yellow solid lumps (Figure 3d and e), but they could be converted into powders by physical crushing. In the absence of maltodextrin, the dried samples formed a yellowish paste-like material (Figure 3f). Despite these differences, all the freeze-dried powders were easier to disperse in water

than the spray-dried powders, *i.e.*, they dispersed more quickly and completely without forming an oil layer at the surface. The reconstituted freeze-dried emulsion had a mean particle diameter around 230 nm, which was similar to that of the original emulsions (Table 1), suggesting that little irreversible droplet aggregation occurred. Nevertheless, there was an appreciable decrease in the magnitude of the ζ -potential on the droplets to around -54.1 mV, which suggests that there may have been some change in interfacial composition.

In conclusion, these results demonstrated that spray-drying (at 100 °C) or freeze-drying could be used to prepare microencapsulated β -carotene emulsions that could easily be reconstituted in water.

3.2.2. Moisture content of emulsion powders

The moisture content of spray-dried and freeze-dried β -carotene emulsion powders were measured (Table 2). The powders obtained by spray-drying at 100 and 120 °C had water contents around 3.2 and 4.2% (w/w), respectively, whereas the powder obtained by freeze-drying had a water content around 1.6% (w/w). Thus, the spray-dried powders had higher moisture contents than freeze-dried powder, which may have been because the relatively short drying time and low drying temperature used during spray drying were insufficient to remove all of the moisture (Wang, Kho, Cheow, & Hadinoto, 2012).

However, the powder obtained by freeze-drying the β -carotene emulsion without maltodextrin contained a negligible amount of moisture (There was no change in powder weight before or after drying). This may suggest that the water binding activity of maltodextrin might contributed to the retention of moisture in the dried powder

(Klinkesorn, Sophanodora, Chinachoti, & McClements, 2004). In conclusion, both spray-drying and freeze-drying could be used to produce powdered β -carotene emulsions with relatively low moisture contents, but freeze-drying was more effective.

3.3. β -carotene degradation

3.3.1. β -carotene degradation in emulsions during storage

The β -carotene content in the emulsion decreased rapidly during the accelerated storage test at 50 °C (Fig. 4). Indeed, there was almost no β -carotene left in the emulsion after storage at 50 °C for 30 days. There was a corresponding decrease in the intensity of the yellow color of the emulsions during storage, but no visible phase separation due to creaming was observed, which suggested that the emulsions were physically stable. This result was in accordance with previous published study (Qian, Decker, Xiao, & McClements, 2012b).

3.3.2. β -carotene degradation during spray-drying and freeze-drying

Although cold-sonication reduced the amount of β -carotene degradation that occurred during dissolution of the β -carotene in the oil phase, the processes of homogenization, spray-drying and freeze-drying may still have induced some β -carotene degradation. Therefore, it was important to monitor β -carotene degradation after each processing step. The fraction of β -carotene remaining in the samples after each processing operation was calculated using the initial β -carotene content in the oil phase as a reference. The initial β -carotene content in the oil phase used to prepare the emulsions was 1.73 ± 0.27 % (w/w). The measured β -carotene content in the initial emulsions was

0.19 ± 0.04 % (w/w), which is close to the theoretical content (0.17%). This suggested that there was minimum degradation of the β -carotene due to the microfluidization process. The microfluidizer channels were immersed in an ice bath during homogenization to maintain the emulsion at a low temperature, and so there may have been minimum thermal degradation or isomerization of the β -carotene due to this process. However, a significant amount of β -carotene was lost during the spray-drying process, with the extent of degradation increasing as the spray drying temperature increased (Table 3). For example, the β -carotene loss was about 72%, 79%, and 85% at operating temperatures of 100, 110, and 120 °C, respectively. Although the spray-drying of the individual droplets was rapid, the spray dried powders actually remained in the receiving chamber for an appreciably longer time, where they were exposed to elevated temperatures. The result was in accordance with trehalose-WPI stabilized carotenoid emulsion (Lim, Burdikova, Sheehan, & Roos, 2016). This phenomenon might be the major reason for the observed degradation of the β -carotene during spray drying. In contrast, about 95% of the β -carotene remained after freeze-drying (Table 3), which was mainly attributed to the fact that the carotenoids were not exposed to elevated temperatures that could promote thermal degradation.

The degradation products of β -carotene were also quantified after the emulsions were exposed to the various processing steps used to produce the powders (Fig. 5). The level of β -carotene degradation products present in the initial oil phase produced using the cold-sonication method was relatively low (3.6 ± 0.3%). The utilization of microfluidization to produce the emulsions from the oil phase generated about 2.4±0.3%

of β -carotene degradation products. Spray drying resulted in a $5.3 \pm 0.4\%$ increase in degradation products, whereas freeze-drying only resulted in a $1.35 \pm 0.01\%$ increase. These results supported our findings that freeze-drying caused less chemical degradation of β -carotene than spray-drying. It is noteworthy that the amount of degradation products of β -carotene that were detectable by our HPLC method was considerably lower than the total loss of β -carotene detected (Table 3). This suggested that not all of the degradation products of β -carotene formed could be detected using the HPLC method used.

4. Conclusion

In conclusion, a β -carotene-enriched delivery system with high loading capacity and minimum degradation during processing has been developed. Using a cold-sonication method to facilitate dissolution of the carotenoids in the oil phase led to less β -carotene degradation than the traditional heating method. This effect was attributed to the fact that thermal degradation and isomerization of β -carotene was reduced at lower preparation temperatures. Spray-drying led to an appreciable loss of β -carotene, which increased with increasing operating temperature. In contrast, freeze-drying resulted in high stability of β -carotene. Furthermore, freeze-drying offered other advantages such as better powder dispersibility, smaller droplet sizes, and better emulsion stability after reconstitution. The strategies developed in this study provide will be useful for optimizing the formation of β -carotene delivery systems for application in both wet and dry functional foods.

Acknowledgement

This material is based upon work supported by the United State Department of Agriculture NRI Grants (2011-67021, 2014-67021, 2011-03539 and 2013-03795) and Independent Projects for Young Scholars at Jiangnan University (2016 3K 383).

References

- Abdelwahed, W., Degobert, G., Stainmesse, S., & Fessi, H. (2006). Freeze-drying of nanoparticles: Formulation, process and storage considerations. *Advanced Drug Delivery Reviews*, 58(15), 1688-1713.
- Álvarez, R., Vaz, B., Gronemeyer, H., & de Lera, Á. R. (2014). Functions, Therapeutic Applications, and Synthesis of Retinoids and Carotenoids. *Chemical Reviews*, 114(1), 1-125.
- Anton, N., Jakhmola, A., & Vandamme, T. F. (2012). Trojan microparticles for drug delivery. *Pharmaceutics*, 4(1), 1-25.
- Chen, H., & Zhong, Q. (2015). Thermal and UV stability of β -carotene dissolved in peppermint oil microemulsified by sunflower lecithin and Tween 20 blend. *Food Chemistry*, 174(0), 630-636.
- Coronel-Aguilera, C. P., & Martin-Gonzalez, M. F. S. (2015). Encapsulation of spray dried beta-carotene emulsion by fluidized bed coating technology. *Lwt-Food Science and Technology*, 62(1), 187-193.
- Coronel-Aguilera, C. P., & San Martín-González, M. F. (2015). Encapsulation of spray dried β -carotene emulsion by fluidized bed coating technology. *LWT - Food Science and Technology*, 62(1, Part 1), 187-193.
- Deng, X. X., Chen, Z., Huang, Q., Fu, X., & Tang, C. H. (2014). Spray-Drying Microencapsulation of beta-Carotene by Soy Protein Isolate and/or OSA-Modified Starch. *Journal of Applied Polymer Science*, 131(12).
- Dias, M. I., Ferreira, I., & Barreiro, M. F. (2015). Microencapsulation of bioactives for food applications. *Food & Function*, 6(4), 1035-1052.
- Donhowe, E. G., & Kong, F. (2014). Beta-carotene: Digestion, Microencapsulation, and In Vitro Bioavailability. *Food and Bioprocess Technology*, 7(2), 338-354.
- Durante, M., Lenucci, M. S., D'Amico, L., Piro, G., & Mita, G. (2014). Effect of drying and co-matrix addition on the yield and quality of supercritical CO₂ extracted pumpkin (*Cucurbita moschata* Duch.) oil. *Food Chemistry*, 148, 314-320.
- Durante, M., Lenucci, M. S., Marrese, P. P., Rizzi, V., De Caroli, M., Piro, G., Fini, P., Russo, G. L., & Mita, G. (2016). α -Cyclodextrin encapsulation of supercritical CO₂ extracted oleoresins from different plant matrices: A stability study. *Food Chemistry*, 199, 684-693.

- Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International*, 40(9), 1107-1121.
- Guan, Y., Wu, J., & Zhong, Q. (2016). Eugenol improves physical and chemical stabilities of nanoemulsions loaded with β -carotene. *Food Chemistry*, 194, 787-796.
- Gul, K., Tak, A., Singh, A. K., Singh, P., Yousuf, B., & Wani, A. A. (2015). Chemistry, encapsulation, and health benefits of β -carotene - A review. *Cogent Food & Agriculture*, 1(1), 1018696.
- Haskell, M. J. (2012). The challenge to reach nutritional adequacy for vitamin A: beta-carotene bioavailability and conversion-evidence in humans. *American Journal of Clinical Nutrition*, 96(5), 1193S-1203S.
- Kasperczyk, S., Dobrakowski, M., Kasperczyk, J., Ostalska, A., Zalejska-Fiolka, J., & Birkner, E. (2014). Beta-carotene reduces oxidative stress, improves glutathione metabolism and modifies antioxidant defense systems in lead-exposed workers. *Toxicology and Applied Pharmacology*, 280(1), 36-41.
- Klinkesorn, U., Sophanodora, P., Chinachoti, P., & McClements, D. J. (2004). Stability and rheology of corn oil-in-water emulsions containing maltodextrin. *Food Research International*, 37(9), 851-859.
- Knockaert, G., Pulissey, S. K., Lemmens, L., Van Buggenhout, S., Hendrickx, M., & Van Loey, A. (2012). Carrot β -Carotene Degradation and Isomerization Kinetics during Thermal Processing in the Presence of Oil. *Journal of Agricultural and Food Chemistry*, 60(41), 10312-10319.
- Liang, R., Huang, Q., Ma, J., Shoemaker, C. F., & Zhong, F. (2013). Effect of relative humidity on the store stability of spray-dried beta-carotene nanoemulsions. *Food Hydrocolloids*, 33(2), 225-233.
- Lim, A. S. L., Burdikova, Z., Sheehan, J. J., & Roos, Y. H. (2016). Carotenoid stability in high total solid spray dried emulsions with gum Arabic layered interface and trehalose-WPI composites as wall materials. *Innovative Food Science & Emerging Technologies*, 34, 310-319.
- Liu, F., Wang, D., Xu, H., Sun, C., & Gao, Y. (2016). Physicochemical properties of β -carotene emulsions stabilized by chlorogenic acid-lactoferrin-glucose/polydextrose conjugates. *Food Chemistry*, 196, 338-346.
- Liu, J., Shi, W. Q., Cao, Y., He, L. P., Guan, K., Ling, W. H., & Chen, Y. M. (2014). Higher serum carotenoid concentrations associated with a lower prevalence of the metabolic syndrome in middle-aged and elderly Chinese adults. *Br J Nutr*, 112(12), 2041-2048.
- Maher, P. G., Roos, Y. H., & Fenelon, M. A. (2014). Physicochemical properties of spray dried nanoemulsions with varying final water and sugar contents. *Journal of Food Engineering*, 126, 113-119.
- Mahfoudhi, N., & Hamdi, S. (2014). Kinetic degradation and storage stability of beta-carotene encapsulated by spray drying using almond gum and gum arabic as wall materials. *Journal of Polymer Engineering*, 34(8), 683-693.

- Mao, L., & Miao, S. (2015). Structuring Food Emulsions to Improve Nutrient Delivery During Digestion. *Food Engineering Reviews*, 7(4), 439-451.
- McClements, D. J., & Rao, J. (2011). Food-Grade Nanoemulsions: Formulation, Fabrication, Properties, Performance, Biological Fate, and Potential Toxicity. *Critical Reviews in Food Science and Nutrition*, 51(4), 285-330.
- Mosquera, L. H., Moraga, G., & Martínez-Navarrete, N. (2012). Critical water activity and critical water content of freeze-dried strawberry powder as affected by maltodextrin and arabic gum. *Food Research International*, 47(2), 201-206.
- Nakagawa, K., & Fujii, Y. (2015). Protein-Based Microencapsulation with Freeze Pretreatment: Spray-Dried Oil in Water Emulsion Stabilized by the Soy Protein Isolate-Gum Acacia Complex. *Drying Technology*, 33(13), 1541-1549.
- Neves, M. A., Hashemi, J., & Prentice, C. (2015). Development of novel bioactives delivery systems by micro/nanotechnology. *Current Opinion in Food Science*, 1, 7-12.
- Nielsen, S. S. (1998). *Food analysis*. Gaithersburg, MD: Aspen Publishers.
- Nielsen, S. S. (2010). Food analysis laboratory manual. In). New York: Springer.
- Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2012a). Inhibition of beta-carotene degradation in oil-in-water nanoemulsions: Influence of oil-soluble and water-soluble antioxidants. *Food Chemistry*, 135(3), 1036-1043.
- Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2012b). Inhibition of β -carotene degradation in oil-in-water nanoemulsions: Influence of oil-soluble and water-soluble antioxidants. *Food Chemistry*, 135(3), 1036-1043.
- Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2012c). Nanoemulsion delivery systems: Influence of carrier oil on beta-carotene bioaccessibility. *Food Chemistry*, 135(3), 1440-1447.
- Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2012d). Nanoemulsion delivery systems: Influence of carrier oil on β -carotene bioaccessibility. *Food Chemistry*, 135(3), 1440-1447.
- Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2012e). Physical and chemical stability of beta-carotene-enriched nanoemulsions: Influence of pH, ionic strength, temperature, and emulsifier type. *Food Chemistry*, 132(3), 1221-1229.
- Ratti, C. (2001). Hot air and freeze-drying of high-value foods: a review. *Journal of Food Engineering*, 49(4), 311-319.
- Salvia-Trujillo, L., Qian, C., Martin-Belloso, O., & McClements, D. J. (2013a). Influence of particle size on lipid digestion and beta-carotene bioaccessibility in emulsions and nanoemulsions. *Food Chemistry*, 141(2), 1472-1480.
- Salvia-Trujillo, L., Qian, C., Martin-Belloso, O., & McClements, D. J. (2013b). Modulating beta-carotene bioaccessibility by controlling oil composition and concentration in edible nanoemulsions. *Food Chemistry*, 139(1-4), 878-884.
- Sluijs, I., Cadier, E., Beulens, J. W. J., van der A, D. L., Spijkerman, A. M. W., & van der Schouw, Y. T. Dietary intake of carotenoids and risk of type 2 diabetes. *Nutrition, Metabolism and Cardiovascular Diseases*(0).

- Tanaka, T., Shnimizu, M., & Moriwaki, H. (2012). Cancer Chemoprevention by Carotenoids. *Molecules*, 17(3), 3202-3242.
- Wang, Y., Kho, K., Cheow, W. S., & Hadinoto, K. (2012). A comparison between spray drying and spray freeze drying for dry powder inhaler formulation of drug-loaded lipid-polymer hybrid nanoparticles. *International Journal of Pharmaceutics*, 424(1-2), 98-106.
- Wei, Z., & Gao, Y. (2016). Physicochemical properties of β -carotene bilayer emulsions coated by milk proteins and chitosan-EGCG conjugates. *Food Hydrocolloids*, 52, 590-599.
- Xu, D., Wang, X., Jiang, J., Yuan, F., Decker, E. A., & Gao, Y. (2013). Influence of pH, EDTA, alpha-tocopherol, and WPI oxidation on the degradation of beta-carotene in WPI-stabilized oil-in-water emulsions. *Lwt-Food Science and Technology*, 54(1), 236-241.
- Zeb, A., & Murkovic, M. (2013). Determination of thermal oxidation and oxidation products of β -carotene in corn oil triacylglycerols. *Food Research International*, 50(2), 534-544.
- Zou, L., Zhang, Z., Zhang, R., Liu, W., Liu, C., Xiao, H., & McClements, D. J. (2016). Encapsulation of protein nanoparticles within alginate microparticles: Impact of pH and ionic strength on functional performance. *Journal of Food Engineering*, 178, 81-89.

Figure Captions

Figure 1. HPLC profile of β -carotene dissolved by different methods. (Abdelwahed, Degobert, Stainmesse, & Fessi) β -carotene standard, (b) heat-assisted dissolution, (c) sonication-assisted dissolution with sonication for 1 min, (d) sonication-assisted dissolution with sonication for 3 min. Representative HPLC profiles were shown. (N=3).

Figure 2. Influence of antioxidants on the relative abundance of degradation product of β -carotene. Antioxidants were dissolved in the oil phase in the absence of oxygen (*, $p < 0.05$, $n = 3$).

Figure 3. Appearances of spray-dried and freeze-dried β -carotene emulsion powders and the reconstituted liquids formed after they were dispersed in distilled water. Spray dried powders were prepared using three operating temperatures: (a) 100 °C, (b) 110 °C and (c) 120 °C. Freeze dried emulsion powders were prepared using different maltodextrin contents: (d) 20% (w/w) maltodextrin, (e) 50 % (w/w) maltodextrin, and (f) no maltodextrin.

Figure 4. Stability of β -carotene in emulsion stored at 50°C ($n = 3$).

Figure 5. Percentage of degraded products in different samples produced during different operation steps. The data was presented as the percentage of degraded β -carotene over

total β -carotene. Different letters between groups indicated statistical significance,
 $p < 0.05$, $n = 3$.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Table 1. Particle size (mean droplet diameter $d_{3,2}$, nm) and z-potential (mV) of fresh emulsion and reconstituted emulsion.

	Fresh	Spray dried	Freeze dried
Particle diameter (nm)	231 ± 2^a	277 ± 13^b	230 ± 8^a
z-potential (mV)	-64.5 ± 5.8^a	-49.3 ± 1.27^b	-54.05 ± 0.07^b

*Different letters between groups indicates statistical significance, $p < 0.05$, $n = 3$.

Table 2. Moisture content of spray dried and freeze-dried β -carotene emulsion powder.

	SD 100 °C	SD 120 °C	FD
Moisture content (%)	3.21 ± 0.94^a	4.17 ± 0.39^a	1.6 ± 0.59^b

*SD: Spray dry; FD: Freeze dry. Different letters between groups indicates statistical significance, $p < 0.05$, $n = 3$.

Table 3. β -carotene recovery rate in emulsion powder produced by different methods.

Both spray dried and freeze-dried samples had the same maltodextrin content (10%, w/w).

	β-carotene recovery rate (%)
SD 100 °C	28.23 ± 0.77^a
SD 110 °C	21.18 ± 0.01^b
SD 120 °C	15.29 ± 0.04^c
FD sample	95.29 ± 0.08^d

* SD: Spray dry; FD: Freeze dry. Different letters between groups indicated statistical significance, $p < 0.05$, $n = 3$.

**β -carotene
+
Lipids**

Cold sonication dissolution

Microfluidization

Nanoemulsion

Spray-dried

Freeze-dried

**Degraded
 β -carotene**

Heat dissolution