論 文 の 内 容 の 要 旨

Thesis Summary

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論文題目

Formulation and characterization of micro/nanoemulsion systems encapsulating vitamins and phytochemicals using conventional and microchannel emulsification

（機械的乳化及びマイクロチャネル乳化を利用したビタミン・ファイトケミカルを内包したマイクロ・ナノエマルションの作製及び特性評価）

The deficiency of micronutrients regarded as “hidden hunger” affects more than one-third of the world’s population, resulting devastating consequences for public health, social development and future of country. Vitamins and phytochemicals have a role in health beyond basic nutrition. These functional ingredients are very sensitive towards environmental stresses like heat, light and oxidation. Moreover, numerous physiological factors limit their usage in food and pharmaceutical products. The research focuses on the encapsulation of vitamins and phytochemicals in different emulsions and microspheres using microchannel and conventional emulsification systems. Moreover, the research also evaluates the physical and chemical stability of encapsulated vitamins and phytochemicals.

The first part of thesis deals with the encapsulation of L-ascorbic acid (L-AA) in different systems. The study in chapters 2 and 3 aimed to formulate monodisperse food-grade water-in-oil-in-water (W/O/W)

emulsions and aqueous microspheres containing a high concentration of L-AA. The W/O/W emulsions were prepared using homogenization and subsequent microchannel emulsification (MCE). The asymmetric straight-through microchannel (MC) array constitutes numerous 10×100 μm microslots with a 30 μm depth, each connected to a 10 μm-diameter circular MC with a 70 μm depth. Monodisperse W/O/W emulsions containing W/O droplets with average diameters (d_{av}) of 26.0 to 31.5 μm and coefficient of variations (CVs) below 10% were successfully formulated via an asymmetric straight-through MC array at a low hydrophobic emulsifier concentration, regardless of L-AA concentration. The d_{av} represents the average of the diameters of all the droplets in the emulsion sample. The W/O droplets dispersed in these monodisperse W/O/W emulsions were physically stable in variation of d_{av} and CV for more than 10 d of storage at 4 ºC. The monodisperse W/O/W emulsions also exhibited L-AA encapsulation efficiency ($EE_{L\text{-}AA}$) exceeding 80% during storage. Stable monodisperse aqueous microspheres containing high concentrations of L-AA (up to 30% (w/w)) with different concentrations of sodium alginate and $MgSO_4$ were generated using MCE. The aqueous microspheres generated from the MCs under optimized conditions had a d_{av} of 14 to 16 μm and a CV of less than 8% at the disperse phase pressures of 5 to 15 kPa. The L-AA loaded microspheres were physically stable in terms of their d_{av} and CV for >10 d of storage at 40 ºC. The aqueous microspheres exhibited $EE_{L\text{-}AA}$ exceeding 70% during the evaluated storage period.

Chapters 4 and 5 aimed to conduct comparative studies with conventional emulsification devices to encapsulate L-AA in the dispersed phase of W/O and W/O/W emulsions. W/O emulsions with $EE_{L\text{-}AA}$ greater than 95% were prepared using rotor-stator homogenizer at 7000 rpm for 5 min. The prepared W/O emulsions under this operating conditions had d_{av} of 2.0 to 3.0 μm and CV of 13% to 22%. All the W/O emulsions were stable for more than 30 d at 4 ºC or 25 ºC with slight increase in d_{av} and without phase separation. Their $EE_{L\text{-}AA}$ was 50% at 4 ºC and 30% at 25 ºC after 30 d of storage. Two-step homogenization was conducted to prepare W/O/W emulsions containing L-AA. First-step homogenization prepared W/O emulsions with a d_{av} of 2.0 to 3.0 μm. Second-step homogenization prepared W/O/W emulsions with an average W/O droplet diameter of 14 to 18 μm and CVs of 18% to 25%. The results indicated that stable W/O/W emulsions containing a high concentration of L-AA were obtained by adding gelatin and $MgSO_4$ in the inner aqueous phase and glucose in both aqueous phases. $EE_{L\text{-}AA}$ in the W/O/W emulsions was 40% on day 30 and followed first-order kinetics.

The second part of study encapsulates different forms of vitamin D. Chapter 6 deals with formulation of food grade O/W emulsions loaded with ergocalciferol (VD_2) by MCE. The primary characterization was performed with grooved type MCE, while storage stability and vitamin D encapsulation efficiency was

performed with straight through MCE. The grooved type MC array plate used has channel depth of around 5 μm and channel width of about 18 μm, while asymmetric straight-through MC array consists of numerous 10×80 μm microslots each connected to a 10 μm diameter circular MC. Monodisperse O/W emulsions with Sauter mean diameter ($d_{3,2}$) of 33.9 μm with relative span (RSF) width less than 0.20 (CV in between 5-10%) were successfully formulated via an asymmetric straight-through MC array. The $d_{3,2}$ represents the diameter of a droplet having the same area per unit volume as that of the total collection of droplets in emulsions. The O/W droplets were physically stable for more than 15 d of storage at 4 ^{o}C without any significant increase in $d_{3,2}$. The monodisperse O/W emulsions also exhibited VD_2 encapsulation efficiency (EE_{VD2}) of more than 85% during storage period. Chapter 7 deals with encapsulation of both VD_2 and cholecalciferol (VD_3) in food-grade O/W emulsions using asymmetric straight-through MCE. 1% (w/w) sodium cholate or Tween 20 in water was used as the continuous phase, while 0.5% (w/w) of each VD_2 and VD_3 in different oils served as the dispersed phase. Monodisperse O/W emulsions with $d_{3,2}$ of 28 to 32 μm and RSF widths below 0.3 were formulated via an asymmetric straight-through MC array under appropriate operating conditions. The monodisperse O/W emulsions stabilized with Tween 20 remained stable for >30 d with EE_{VD2} and EE_{VD3} of above 70% at 4 and 25 ^{o}C. In contrast, those stabilized with sodium cholate had stability of >30 d with EE_{VD2} and EE_{VD3} of over 70% only at 25 ^{o}C.

Chapter 8 deals with cross comparative studies with conventional homogenization techniques to encapsulate vitamin D. Both VD_2 and VD_3 at 0.5% (w/w) was encapsulated in refined soybean oil, olive oil and MCT. The phosphate buffer (pH 7) containing 1% (w/w) Tween 20 served as a continuous phase. The two liquid phases were emulsified with a homogenizer at 5,000 to 20,000 rpm for 5 min or the homogenizer at 7,000 rpm for 5 min plus a microfluidizer in a single pass at 100 MPa. The microfluidization produced the O/W emulsions with a volume mean diameter ($d_{4,3}$) of 0.41 μm and a RSF width of about 1.9. The $d_{4,3}$ represents the diameter of a droplet having the same volume in total weight of emulsions. The emulsions prepared at the high homogenization speeds and by microfluidization maintain liquid consistency and whitish color after 30 d of storage period. In contrast, those prepared at the low homogenization speeds were quickly destabilized after 1 d of storage. During the storage, there was no significant difference in $d_{4,3}$ of the O/W emulsions prepared with the microfluidizer in comparison to $d_{4,3}$ of the O/W emulsions prepared with the homogenizer. There was little effect of homogenization speed on release profile of the vitamin D-loaded O/W emulsions. The EE of both VD_2 and VD_3 became less than 70% after 10 d of storage and less than 10% after 30 d of storage at 4 ^{o}C.

The third part of research is to encapsulate phytochemicals and include quercetin, γ-oryzanol and β-sitosterol. Chapter 9 encapsulates quercetin in different food-grade O/W emulsions stabilized by different emulsifiers. 1% (w/w) Tween 20, sodium cholate, decaglycerol monolaurate, polyglyerol-5-laurate or bovine serum albumin in Milli-Q water was used as the continuous phase, while 0.4 mg mL^{-1} in different oils served as dispersed phase. Successful emulsification was conducted with all emulsifiers. The produced monodisperse droplets have $d_{3,2}$ of 28 to 34 μm with span width below 0.25. The $d_{3,2}$ slightly decreased at 25 °C, while increased $d_{3,2}$ was observed in emulsions stabilized by sodium cholate at 4 °C. The emulsions stabilized with 1% Tween 20 have quercetin EE of 80% and 70% at 4 and 25 °C respectively. Chapter 10 encapsulates a slight high concentration of β-sitosterol and γ-oryzanol in different food-grade oil O/W emulsions using MCE. A 24 × 24-mm MCE chip (WMS 1-4) containing 23,348 straight-through MCs was used for this study. 1% (w/w) Tween 20 or decaglycerol monolaurate (ML-750) in Milli-Q water was used as the continuous phase, while 0.5-4% (w/w) β-sitosterol and γ-oryzanol in MCT served as dispersed phase. Successful emulsification was conducted with different concentrations of β-sitosterol and γ-oryzanol. The $d_{3,2}$ of 1% (w/w) β-sitosterol and γ-oryzanol loaded emulsions ranged between 26 to 28 μm with span width below 0.21 at Q_d of 2 mL h^{-1} after 30 d of storage at 4 and 25°C. The O/W emulsions stabilized with Tween 20 have β-sitosterol and γ-oryzanol EEs above 80% at 4 and 25°C, in comparison to the emulsions stabilized with ML-750 have EE of β-sitosterol over 80% and γ-oryzanol EE above 50% at 4 and 25°C.

The research indicate that MCE is a promising technique for encapsulating vitamins and phytochemicals, with superior control of processing parameters and various other physical and chemical conditions. The forthcoming scaling up of MCE devices is expected to further improve the quality of different emulsions and make practical their production on industrial scales.