

Final Report

Comparative effects of curcumin nanoemulsions and nanoparticles in use for food applications: as a colorant and antioxidant agent

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Abstract

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Abstract:

Recently, various compositions and properties of curcumin nano-formulations have been proposed for medicinal and food uses. Most studies focused on optimizing synthesis of nanocarriers with improving physiological characteristics, enhancing curcumin stability under storage conditions, and improved its antioxidant activity. The comparative effects of nanocarriers to prevent deterioration of curcumin under processing and storage conditions, particularly during on-shelf storage within food matrix, which causes physical and chemical stability changes of nanoparticles and their functionality, is addressed in this study. Nanoemulsions (NE) and nanoparticles (NP) were synthesized at the same level of surfactant concentration relative to nanosuspensions (NS) used as a control. For commercial applications, all systems were subjected to processing conditions: pH, ionic strength and thermal. The effect of nanoparticles had better on pH and thermal treatments with the highest retention amount, but the particles precipitated in the presence of salt, while the nanoemulsion systems were more stable but the surface charge was dramatically decreased under thermal conditions. The effect of nanoparticles was less pronounced after 15 days of storage compared with nanoemulsion systems and the release of entrapped curcumin under PBS was sustained for 72 hours for both emulsified and nanodelivered systems. When added to milk, nanoemulsions and nanoparticles had the potential to inhibit lipid oxidation with no significantly affected to the color changes of the fortified milk after stored for 5 days. This study provides insights which can help choose the most suitable nanocarrier for delivering curcumin as a food additive in different commercial food products, depending on the conditions of use.

Keywords : Curcumin, Nanoemulsions, Nanoparticles, Stability, Functionality

บทคัดย่อ

รหัสโครงการ : MRG6080132

ชื่อโครงการ : เปรียบเทียบประสิทธิภาพของ nanoemulsions และ nanoparticles สำหรับนำส่ง curcumin เพื่อใช้ในอาหาร

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บทคัดย่อ :

ปัจจุบันมีงานวิจัยมากมายที่ศึกษาเกี่ยวกับองค์ประกอบและคุณสมบัติของตัวนำส่งเคอร์คูมิน (curcumin) ที่มีขนาดอนุภาคระดับนาโนเพื่อใช้ในทางการแพทย์และอาหาร งานวิจัยส่วนใหญ่มี วัตถุประสงค์เพื่อสังเคราะห์ตัวนำส่งให้มีคุณภาพดีทั้งทางด้านปรับปรุงคุณลักษณะทางกายภาพ ปรับปรุงความคงตัวของตัวนำส่งภายใต้สภาวะการเก็บรักษา รวมทั้งปรับปรุงฤทธิ์ในการต้านอนุมูลอิสระ การเปรียบเทียบประสิทธิภาพของตัวนำส่งเพื่อปกป้องการเสื่อมสลายของ curcumin ภายใต้สภาวะการแปรรูปและเก็บรักษา โดยเฉพาะอย่างยิ่งในสภาวะ on-shelf เมื่อผสมรวมกับอาหาร สภาวะเหล่านี้ส่งผลให้ curcumin เกิดการเปลี่ยนแปลงทั้งทางด้านกายภาพและเคมีและ หน้าที่การใช้งานของตัวนำส่ง ในงานวิจัยนี้ได้ทำการสังเคราะห์ nanoemulsions และ nanoparticles ขึ้นและใช้สารเพิ่มความเสถียรที่ระดับความเข้มข้นเท่ากับทั้งสองระบบ ส่วน nanosuspensions ที่สังเคราะห์ขึ้นมาใช้เป็นตัวควบคุม การนำไปใช้งานทางด้านการค้า ระบบนำส่ง curcumin ทั้งสามแบบถูกนำไปทดสอบภายใต้กระบวนการแปรรูป (pH, ionic strength และ ความร้อน) ประสิทธิภาพของตัวนำส่งแบบ nanoparticles ยังคงรักษาสภาพได้ดีที่สภาวะ pH และการทดสอบด้วยความร้อน ซึ่งพบว่าปริมาณที่เหลือของ curcumin หลังผ่านความร้อนมีค่าสูงที่สุดเมื่อเทียบกับระบบนำส่งอื่นๆ แต่เมื่อนำไปทดสอบในสภาวะเกลือที่ระดับความเข้มข้นต่างๆ พบว่า nanoparticles เกิดการตกตะกอน ขณะเดียวกันระบบนำส่งแบบ nanoemulsions มีความเสถียรมากกว่า แต่ประจุที่ผิวกลับลดลงมากกว่าระบบอื่น ซึ่งส่งผลให้ตัวนำส่งเสียสภาพได้เมื่อผ่านความร้อน การทดสอบความมีเสถียรภาพเมื่อเก็บรักษาไว้เป็นเวลา 15 วัน พบว่า nanoparticles มีประสิทธิภาพต่ำกว่า nanoemulsions แต่ทั้งสองสามารถควบคุมการปลดปล่อย curcumin ได้ดีเป็นระยะเวลา 72 ชั่วโมง การทดสอบเปรียบเทียบฤทธิ์ในการต้านอนุมูลอิสระเมื่อนำระบบทั้งสามนี้เติมลงในนม พบว่าทั้ง nanoemulsions และ nanoparticles มีฤทธิ์ในการต้านการเกิดปฏิกิริยา lipid oxidation และไม่ส่งผลกระทบต่อสีของนมหลังจากการเก็บรักษาไว้ 5 วัน งานวิจัยนี้สามารถให้ข้อมูลเพื่อการเลือกใช้ระบบนำส่งสำหรับ curcumin เพื่อใช้เป็นสารแต่งสีและสารต้านอนุมูลอิสระในผลิตภัณฑ์อาหารที่แตกต่างกันได้

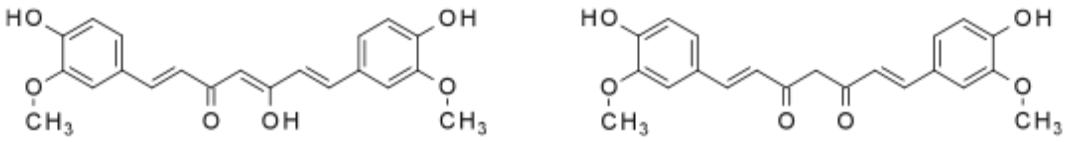
คำสำคัญ : เคอร์คูมิน, Nanoemulsions, Nanoparticles, ความเสถียร, หน้าที่ใช้งาน

Executive Summary

Several nanoforms with different compositions and properties to encapsulate curcumin have been recently developed for improving the solubility, chemical stability, and bioavailability of curcumin in use for medicinal and food applications. Nanosuspension focused on the increased solubility of curcumin, while nanoemulsions and nanoparticles are the most promising approaches used as smart delivery systems to deliver curcumin and protect its stability. However, these systems have particular advantages and disadvantages for particular applications. Curcumin nanosuspension stabilized by surfactant had irregular shape like mesh and showed the lowest stability for all treatments. Nanoemulsion and nanoparticle systems with entrapped curcumin expressed stable characteristics but nanoemulsions had the highest encapsulate efficiency. After subjected to processing conditions: pH, thermal, and ionic strength, the nanoparticles were more stable in different pHs and thermal conditions confirmed by retention rate, but the particles precipitated in the presence of salt, while the nanoemulsion systems were stable but the surface charge was dramatically decreased under thermal conditions. The effect of nanoparticles was less pronounced on the stability compared with nanoemulsion systems after 15 day of storage, but it had a better sustained release profile. Curcumin could have the potency to inhibit lipid oxidation, this may benefit to dairy product. At the same level of surfactant concentration, nanoemulsion and nanoparticles had the antioxidant potency to inhibit MDA formation, which implied on the protection of the functionality of its entrapped curcumin and enhanced nutritional property of milk. The fortified milk with all curcumin nanocarries had the color insignificantly changes from the control milk after stored for 5 days. Thus, the differences observed between these different delivery systems in terms of physical stability, release, chemical stability, and antioxidant activity of curcumin substantiate the need to validate each delivery systems separately and to choose the optimum one to protect the entrapped curcumin under specific conditions for the benefit of utilizing curcumin in commercial food products.

1. Introduction

Curcuminoids consists of three types of phenolic compounds: curcumin, bisdemethoxycurcumin, and demethoxycurcumin. Curcumin is the main bioactive component isolated from turmeric rhizomes and it serves as a food coloring. The antioxidant potency of curcumin is the highest compared to other two components, which posses various biological functions of anti-cancer activity, anti-inflammatory and lowering blood pressure (Menon & Sudheer, 2007). It also has the ability to inhibit lipid peroxidation (Carvalho, Takeuchi, Geraldine, Moura, & Torres, 2015). As the food additive, curcumin is widely used as a colorant, preservative, antimicrobial and antioxidant agent (Jayaprakasha, Jaganmohan Rao, & Sakariah, 2006), however the stability of curcumin is pH and temperature-dependent that limits its applications in the food industry.



(A) Enolic form (B) Keto form

Figure 1: chemical structures of curcumin (A) enolic form and (B) keto form

Curcumin, a diarylheptanoid belonging to the group of curcuminoids, is a natural phenol responsible for antioxidant activity. As seen in Figure 1, its chemical structures known as keto-enol tautomerism is highly thermodynamically driven and favors the formation of the keto form at room temperature. It exists chemically equilibrium in enolic form in organic solvents and more stable as a keto form in water.

Jin, Lu, and Jiang (2016) stated that color of curcumin is faded when pH values of surrounding environment changed from 7.5 to 9 and is completely degraded after 40 hours under alkaline condition. It was consistent with the result of Wang et al. (1997), when incubated curcumin in 0.1 M phosphate buffer with pH 7.2, it decomposed 90% within 30 min at 37°C. Moreover, pure curcumin is highly unstable to chemical degradation in alkaline aqueous solutions (pH ≥ 7.0) and it tended to crystallize out of aqueous acidic solutions (pH < 7), resulting curcumin crystals formed relatively growth to 10-50 μm, which made them likely to rapid sedimentation (M. Kharat, Du, Zhang, & McClements, 2017). Besides, curcumin undergoes rapid hydrolytic degradation under thermal processing conditions (Suresh, Manjunatha, & Srinivasan, 2007) (Suresh, Gurudutt, & Srinivasan, 2009) (Giménez, Fernández-López, Angosto, & Obón, 2015). The thermal loss of curcumin content in turmeric by boiling for 10-20 minutes was up to 53%. Giménez et al. (2015) tracked the effect of heat treatment on visual color of food

coloring isolated from plants. As temperature increased, the colorants especially curcumin degraded rapidly, demonstrating its instability at elevated temperatures up to 90°C. These findings: basic pH (including digestive system) and high thermal processing, are susceptible to retard the stability of curcumin, comprising of the poor aqueous solubility, potential degradation, rapid metabolism and elimination led to low bioavailability under physiological conditions, all limit the applications of curcumin in the pharmaceutical and food industries.

To protect the stability of curcumin, encapsulation techniques in several forms of nanocarriers have been proposed to overcome those limitations. In pharmaceutical aspects, the formulation of curcumin loaded mixed micelles in human lung cancer cells improved the oral bioavailability and maintained anticancer activity of curcumin (Patil, Choudhary, Rathore, Roy, & Mahadik, 2015). The study of Tsai, Chien, Lin, and Tsai (2011) concluded that curcumin loaded PLGA nanoparticles had the ability to cross the blood-brain barrier (BBB) that considered as neuro-protective and anticancer agents. Moreover, curcumin loaded in the form of nanoparticles could enhance the anti-microbial ability against Gram-positive bacteria (Basniwal, Buttar, Jain, & Jain, 2011). These studies of curcumin as remedial tools in several nano-devices exhibited a great potential providing curcumin the improved aqueous solubility and enhanced bioavailability. For food applications, curcumin is widely used as a colorant and antioxidant agent due to its ability to inhibit lipid peroxidation and scavenge superoxide anion, singlet oxygen, nitric oxide, and hydroxyl radicals (Carvalho et al., 2015). Spherical phospholipid bilayer vesicles, liposomes are commonly used to encapsulate essential oils, flavors, colorants, antioxidant and antimicrobial agents, including curcumin. The traditional liposome used lecithin obtained from natural lipid soybean or egg yolk could benefit to low toxicity compared with polymeric materials. However, the efficiency to encapsulate bioactive of liposome is theoretically low (approximately 60%), which depends on the part of phospholipid layer and its stability under various conditions (pH, light, temperature) were not good enough compared with synthetic materials (Jin et al., 2016). Another approach to overcome the instability of curcumin is emulsions, which allow the solubility of hydrophobic material in an aqueous solution.

Nanotechnology has led to numerous innovative solutions to deliver bioactive compounds and protect their physical-chemical stability. Nanodelivery systems such as nanosuspensions, nanoemulsions, nanoparticles etc. have been recently demonstrated possessing several functional advantages including biodegradability, biocompatibility, solubility of poorly soluble compounds, and sustained-releasing capability (Chuacharoen & Sabliov, 2017). Effect of nanoencapsulation on stability and functionality of entrapped compounds has also been pronounced because it enhances the solubility of the poorly soluble compounds trapped inside the system and protects them during food

processing, including when food matrix components employed (Chuacharoen & Sabliov, 2016a). Thus, several developments of formulations of nanocarriers such as nano-liposomes, nanosuspension, nanoemulsions, nanoparticles etc. to encapsulate curcumin have been made to protect curcumin against unfavorable conditions and satisfy with the increased stability as a food additive.

Lipid bilayers as a delivery system for encapsulating curcumin was studied by Jin, Lu, and Jiang (2015). They synthesized curcumin nanoliposomes using milk fat globule membrane (MFGM) phospholipids as a membrane material. The results indicated that MFGM liposome had a good potential and encapsulation efficiency was low (~70%) which was considered to be low compared with entrapment ability of zein nanoparticles (Chuacharoen & Sabliov, 2016b). However, amount of encapsulated curcumin loaded in liposome depended on capacity of lipid membrane layer, when the ratio of layer to curcumin increased, the encapsulation efficiency also relatively increased. A possible solution to avoid the excessive use of surfactant would be an alternative to development formulations of curcumin in the forms of nanosuspension, nanoemulsion and nanoparticles with improved stability (Figure 2).

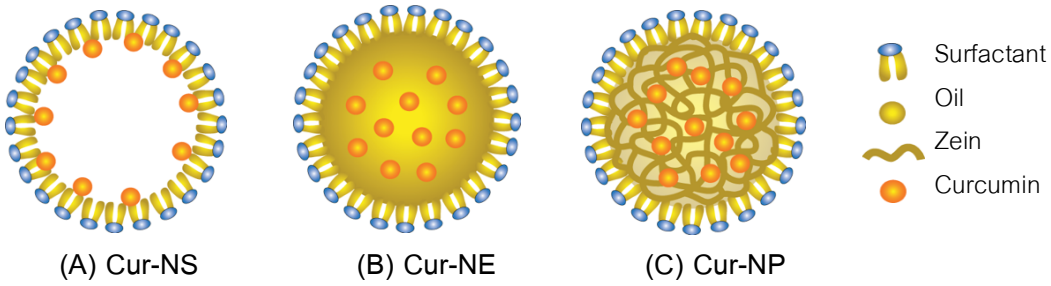


Figure 2: proposed structures of (A) curcumin nanosuspension (Cur-NS), (B) curcumin loaded nanoemulsion (Cur-NE) and (C) curcumin-loaded zein nanoparticle (Cur-NP): interaction between surfactant, core materials, and curcumin compounds

Nanoscale drug delivery carriers with a size range from 10 to 1000 nm attribute a large surface area in contact with the solvent, resulting in the increased water-solubility. Nanosuspensions enhance the dissolution of poorly water-soluble compounds, which keep bioactive compounds at the submicron levels in a liquid phase stabilized by added stabilizers (Muthuirulappan & Francis, 2013). Many researchers have studied on enhancing the aqueous solubility and bioavailability of curcumin employed nanosuspension stabilized with surfactants. Carvalho et al. (2015) hypothesized when the size reduced, the solubility of curcumin nanoparticles increased relative to surface area of solvent contacting. Tween 80 was used as a surfactant to stabilize curcumin nanosuspension by preventing particle re-aggregation, which increased the aqueous

solubility of curcumin and the antioxidant activity. They also proved that the preparation process of the curcumin nanosuspension by injecting pure curcumin solution in water with Tween 80 did not degrade the curcumin compounds, but its degradation against enzymatic digestion and under processing conditions did not state. The study of Zhao et al. (2015) using supercritical carbon dioxide (SEDS) to fabricate nanocurcumin stated that the particles possessed higher solubility and dissolution rate than original curcumin. The increased antioxidant activity of nanocurcumin confirmed by the Kakran, Sahoo, Tan, and Li (2012) that when the size of curcumin down to nanosize without adding surfactants, they have higher capability to eliminate DPPH free radicals than that of the original curcumin due to its solubility of nanoparticles increased. These studies showed that nanosuspensions were more soluble than free curcumin; however, the reduced particle size with stabilized surfactant did not show any improvement in the stability under undesirable conditions.

Due to the benefit of food-grade nano-colloidal systems to encapsulate active ingredients, nanoemulsions have offered several advantages such as their higher optical clarity for beverage uses, better physical stability to particles aggregation, and novel rheological properties compared to conventional emulsions (Saifullah, Ahsan, & Shishir, 2016). The factors that affect stability of nanoemulsions were oil type, surfactant types and composition ratio (oil/water/surfactant), and preparation method. Study of Joung et al. (2016) developed stable curcumin nanoemulsions, the formation of nanoemulsions was over 50% of aqueous phase, less than 33% of oil phase, and less than 32% of surfactant content. They also studied the stability and antioxidant activity of curcumin nanoemulsions stabilized by Tween 20 that were fortified to commercial milk. The entrapment efficiency of curcumin was 91-98%, which is theoretically higher than the loading capacity of liposome. The physical stability of Cur-NEs was good for 15 days at room temperature and up to 3 months at a refrigerated storage, and thus nanodroplets showed better effective oxygen scavenging activity, when interacted to milk components, the lipid oxidation was less pronounced comparing to unfortified milk.

Another study of curcumin loaded nanoemulsions used medium chain triglyceride (MCT), Tween 80 and lecithin was reported by Li, Hwang, Chen, and Park (2016). Entrapment efficiency was 95% and chitosan was used as a coating material to prevent the droplets from phase separation. Nanoemulsions were stable at room temperature and inhibited curcumin degradation during thermal and UV irradiation treatments, and also interfered lipolysis of nanoemulsions during *in vitro* digestion. The impact of pH and storage temperature on physical stability of curcumin in nanoemulsion was also examined by Mahesh Kharat, Du, Zhang, and McClements (2016). Curcumin nanoemulsions found unstable and entrapped curcumin degraded through an autoxidation process under alkaline conditions.

Solubility of curcumin in MCT oil showed higher amount compared with other commonly used oils such as coconut oil, corn oil, and olive oil (Joung et al., 2016). Meanwhile, the MCT oil fabricated NE can increase the digestibility and bio-accessibility of hydrophobic bioactive compound (Ahmed, Li, McClements, & Xiao, 2012). Tween 80 was utilized as surfactant to stabilize nanoemulsions due to its sterical effect. Lecithin was applied as co-surfactant to stabilize and confer the prepared nanoemulsions with negative charge at the same time. They proposed the weight ratio of MCT, lecithin, Tween 80 and distilled water was at 10:6:4:80. The average size of nanoemulsions was 113.93 nm with 0.23 PDI. It had high negative zeta potential value at -36.23 mV due to the effect of lecithin. The prepared nanoemulsions encapsulated curcumin at the concentration of 0.548 mg/ml had loading efficiency of 95.10%. The dispersibility was higher than free curcumin up to 1400 times. However, the encapsulation efficiency depending on the amount of surfactant used, and the instability of unmodified nanoemulsions under processing may cause undesirable characteristics in foods and may not meet the requirement to deliver bioactives (Chuacharoen & Sabliov, 2017).

Nanoparticles are alternative to deliver curcumin in which its controled release rate. Functional food ingredients such as nutrients, vitamins, or essential oils can successfully be entrapped in biopolymeric nanoparticles with the same goal of protecting the bioactives from degradation and of controlling release to improve their bioavailability and functionality. Moreover, entrapment of hydrophobic bioactives in nanoparticles increased their stability during thermal processing, such as pasteurization, sterilization, spray-drying (Weiss et al., 2008). Pathak, Kanwal, and Agrawal (2015) formed curcumin lipid-biopolymer nanoparticles by electrostatic interaction of two oppositely charged lipid and polymer using self-assembly method. The entrapment efficiency was up to 92% depended on the initial amount of curcumin. The particles were stable in pH ranges from 2 to 6 and presented higher antioxidant activity than curcumin powder in aqueous media. Recently, zein nanoparticles have gained many attentions as a novel nanodelivery system (Chuacharoen & Sabliov, 2016a; Gomez-Estaca, Balaguer, Gavara, & Hernandez-Munoz, 2012). It is a naturally obtained, FDA-approved material for foods. Gomez-Estaca et al. (2012) used the electrohydro-dynamic atomization synthesized zein nanoparticles and the particles showed a spherical structure and narrow size distribution with the encapsulation efficiency around 85-90% and curcumin homogeneously distributed in the zein matrix. The particles were stable in size, shape and curcumin content after storage. The coloring capacity indicated a good dispersion of curcumin in semi-skimmed milk. Our previous study also worked on carotene-loaded zein nanoparticles stabilized by pluronic F127 and lecithin as surfactants. A combination of lecithin and pluronic F127 used in the study was kept as 0.045:0.090% (w/v), which was much lower than the amount of surfactants used for the synthesis of

nanoemulsions based on the same initial amount of entrapped compounds.

The knowledge of entrapment mechanism of compound interacting to the core material may be useful for the stability of nanocarriers. In nanoemulsion system, two simultaneous steps are involved in the release of entrapped molecules: diffusion in continuous phase and passing of the diffusing molecules through the interfacial membrane of the dispersed phase, which is considered as a limiting step (Calderó, Patti, Llinàs, & García-Celma, 2012). The concentration of surfactant as an interfacial membrane could play an important role on the release rate of entrapped compounds out of nanoemulsions. Unlike nanoparticles, the release mechanism is mainly controlled by the diffusion and desorption of bioactives and degradation of the nanoparticle matrix (Chuacharoen & Sabliov, 2017). Thus it would be of great interest to study a comparative effects of the nanocarrier systems: interaction force between the core materials and its entrapped compound by neglect surfactant (interfacial membrane), which influence on the stability of nanodelivery systems. This would elicit the important way to select the type of curcumin-loaded nanocarriers for food applications.

The study of Masalova, Foudazi, and Malkin (2011) showed that an increase in surfactant concentration results in decreased rheological parameters. Most studies (Joung et al., 2016; Sari et al., 2015) synthesized nanoemulsions using the concentration of surfactants at least 30 times more than the synthesis of zein nanoparticles stabilized by lecithin reported by (Chuacharoen & Sabliov, 2016b). The natural lecithin extracted from soybean or egg yolk is widely used as a food supplement and it had the benefit of low toxicity, biocompatibility and inexpensive compared with polymeric materials, these cause lecithin an excellent surfactant in nanoemulsion system for food applications. (Haidar, Harding, Bowater, Eldridge, & Charman, 2017). The degradation of lecithin, particularly its pH dependence affects the lipid part, which degraded the entire emulsion. So it is important to study the stability of nanocarriers with surfactant used and its concentration. Surfactant such as Tween 80 can be cooperated to stabilize particles for preventing the system from aggregation. Tween 80 known as polysorbate 80 – the monooleate of ethoxylated sorbitan 80 – is a nonionic emulsifier widely used in nanoparticle works as a surfactant. The concern is that what type of nanodelivery systems can increase the efficacy of curcumin by ensuring its bioavailability and minimizing its loss over the insolubility. For fair comparison among the systems, the presence of surfactant types, concentration used, and synthesize method would be controlled in the same level and conditions for preparing curcumin based nanocarries with remaining stable for long period of time under various stress environments. Based on the studies of Joung et al. (2016) and Li et al. (2016), MCT was used as carrier lipid to fabricate curcumin loaded nanoemulsions and zein nanoparticles was selected regarding to our previous studies (Chuacharoen & Sabliov,

2016a, 2016b). Consequently, both systems were stabilized with the same concentration of a combined lecithin and Tween 80.

Nanoemulsions and nanoparticles are the most promising approaches to deliver curcumin exhibiting great potentials as smart delivery systems. These systems have individual advantages and disadvantages for particular applications. The comparison effects between nanoemulsion and nanoparticle systems would be useful to choose the nanocurcumin-based delivery system as a coloring and antioxidant agent to incorporate to several foods, supplements, and commercial food products (such as pasteurized milk, food meals, and fruit juices) which might have the potential to extend shelf-life by inhibiting lipid oxidation and to maintain the perception quality of products fortified with nano-curcumin.

Thus, it was hypothesized that the effects of the nanocarriers would maintain physical stability and enhance the functionality of entrapped curcumin compared to the original free curcumin and nanoparticles would provide a better protection to the entrapped curcumin against manufacturing conditions and will inhibit lipid oxidation better than nanoemulsion when food matrix components employed.

2. Objective

The objectives of this study were to synthesize entrapped curcumin nanosuspensions (Cur-NS), nanoemulsions (Cur-NE), and nanoparticles (Cur-NP) with a combination of lecithin and Tween 80 (same concentration). The systems were examined to validate a detailed comparison of nanocarriers (NS, NE, and NP), which impact their physicochemical stability (size, PDI, surface charge, and retention rate) of curcumin nanocarriers under processing (pH, ionic strength, and thermal) and storage conditions (4°C and room temperature). Subsequently the functionality (lipid oxidation inhibition) of entrapped curcumin and color changes when added to milk as a food matrix model were investigated. Thus this would be benefit for curcumin to be used as a colorant and antioxidant agent for food applications.

3. Materials and methods

3.1 Materials

Curcumin (mixture of demethoxycurcumin and bisdemethoxycurcumin, ≥ 98%) was purchased from Acros Organic (NJ, USA). Soybean lecithin was purchased for Fisher Scienctific (NJ, USA), MCT oil was purchased from Med Lab supply (FL, USA). Ethanol, methanol, and DMSO were purchased from Sigma-Aldrich (MO, USA). All other reagents were of analytical grade.

3.2 Preparation and characterization of curcumin nanosuspensions (Cur-NS), curcumin nanoemulsions (Cur-NE), and curcumin nanoparticles (Cur-NP)

Curcumin nanoemulsion was prepared in two stages using the method described by Joung et al. (2016) and Li et al. (2016) with slight modifications. Briefly, 70 mg of curcumin was completely dispersed into 10 ml MCT oil with heating and stirring overnight. The undissolved curcumin crystals were removed by syringe filtration (0.45μm). Tween80 and lecithin were dissolved into distilled water which was added to the mixture of MCT oil. The coarse emulsion was then prepared by injecting the mixture into a nanopure water containing Tween 80. The weight ratio of MCT oil, lecithin, Tween 80 and distilled water was 10:6:4:80 based on our preliminary data. Then, fine emulsion was prepared by microfluidizing the coarse emulsion using a high-pressure homogenizer (M-110P Microfluidizer, Microfluidics, Newton, Mass., U.S.A.) at pressure of 30,000 psi for 3 cycles. Subsequently, the sample was placed in dialysis membrane to remove free surfactants for 24 hours. After complete dialysis, the samples were kept in the dark, without humidity for further analysis.

Curcumin-loaded zein nanoparticles stabilized by lecithin and Tween 80 were synthesized using the liquid-liquid dispersion method with slight modifications (Chuacharoen & Sabliov, 2016b; Gomez-Estaca et al., 2012). Briefly, curcumin solution (1mg/ml) was prepared in ethanol and added into zein solution (10mg/ml dissolved in 70% ethanol-aqueous solution). The mixture solution was injected to aqueous phase containing lecithin (0.045% w/v) and Tween 80 (0.03% w/v), and then homogenized by using a high-pressure homogenizer (M-110P Microfluidizer, Microfluidics, Newton, Mass., U.S.A.) at pressure of 30,000 psi for 3 cycles. Subsequently, the sample was evaporated to remove ethanol under vacuum (at approximately 500-600 mmHg) and nitrogen injection (80 mmHg) in a rotovapor (Buchi R-124, Buchi Analytical Inc., DE, USA). After complete evaporation of ethanol, the curcumin-loaded zein nanoparticles produced was placed in dialysis membrane to remove free surfactants for 24 hours. After complete dialysis, the samples were kept in the dark, without humidity for further analysis. Curcumin nanosuspension (Cur-NS) was prepared in parallel using the same method, without adding zein to serve as a control.

3.3 Size, PDI, zeta potential, and morphology of Cur-NS, Cur-NE and Cur-NP

Freshly prepared samples of Cur-NS, Cur-NE and Cur-NP were determined the average particle size, size distribution, and surface charge characteristic using dynamic light scattering (DLS) instrument at 25°C. Transmission electron microscope (TEM) was used to image morphology of entrapped curcumin-nanocarrier structures.

3.4 Determination of encapsulation efficiency (%EE)

Freshly prepared curcumin-loaded nanoforms were centrifuged at 17,217 g for 30 min to remove unstable particles and excess curcumin. The precipitate was

separated and extracted by 1ml pure ethanol with vortexing for 5 minutes. The concentration of curcumin (μg/ml) was calculated according to a standard curve generated by reading at 419 nm of several concentrations of standard curcumin dissolved ethanol solutions using a UV-Vis spectrophotometer (Evolution 201, Thermo Scientific, Waltham, MA, USA). Curcumin concentration was calculated according to a calibration equation (y = 0.148x – 0.0146, R^2 = 0.999), where Y is the absorption at 419 nm and X is the concentration of curcumin (mg/ml). The entrapment efficiency was calculated by the weight ratio of curcumin determined in NS NE and NP solutions and curcumin initially added using the following equations:

$$\text{EE}\ (\%) = \frac{\text{entrapped curcumin amount}}{\text{Initial curcumin amount}} \text{x}100$$

3.5 Determination of curcumin retention rate (RR)

In terms of stability under various conditions, curcumin entrapped with nanocarriers were subjected to heat and storage conditions, which led to the destruction and mass loss of curcumin. The retention rate (RR) of entrapped curcumin is an effective index for the assessment of chemical stability of curcumin nanocarriers. The measured solution was extracted with pure ethanol and then vortexed for 5 minutes. The extracted curcumin was determined for the retention rate (RR) by measuring the absorbance at 419 nm using a UV/Vis spectrophotometer. The %RR was obtained by the following equation:

$$\text{RR}\ (\%) = \left(\frac{\text{Extrated curcumin}}{\text{Initial curcumin}}\right) \times 100$$

Where Cur_0 and Cur are the amount of curcumin existing in samples at initial and after a time of treatments, respectively.

3.6 Validate a stability comparison of Cur-NS, Cur-NE and Cur-NP under processing conditions (pH, thermal, and ionic strength)

Cur-NS, Cur-NE and Cur-NP samples were tested under pH conditions (pH 2-9) and thermal processing (63°C for 30 minutes and 95°C for 10 minutes) and ionic strength (0.1, 0.5, and 1.0M NaCl) for their commercial utilization. The average-particle size, distribution and zeta potential were measured after exposing the curcumin nanoforms to each treatment. To study the effect of pH on the carriers, the stability of the samples were checked in the pH range of 2 to 9 adjusting the lower pH with 0.1M HCl solution and higher pH with 0.1M NaOH solution. After adjustment of the pH, the samples were left in a screw-capped vial and kept at room temperature for 24 hours

and subsequently characterized with respect to their size diameter, polydispersity index (PDI), and zeta potential. For the effect of thermal processing, the samples were exposed to different processing conditions: 63°C for 30 minutes and 95°C for 10 minutes, subsequently characterized with respect to their size diameter, polydispersity index (PDI), and zeta potential. Similar to the effect of ionic strength, the samples were exposed to different ionic strength conditions: 0.1, 0.5 and 1.0 M NaCl conditions for 24 hours and subsequently characterized with respect to their size diameter, polydispersity index (PDI), and zeta potential.

3.7 Validate a stability comparison of Cur-NS, Cur-NE and Cur-NP under storage conditions (4°C and 25°C for 15 days)

The physicochemical attributes of Cur-NS, Cur-NE and Cur-NP were assessed by keeping the samples in a screw-capped vials in dark at 4°C and 25°C for the period of 15 days. The physicochemical attributes were checked with respect to average size, zeta potential, polydispersity index (PDI), morphology, and curcumin retention.

3.8 *In vitro* release of curcumin from Cur-NS, Cur-NE and Cur-NP

Similar to what was reported by Chuacharoen and Sabliov (2016b) with slight modification, the release of the entrapped curcumin from nanoemulsion and zein nanoparticles was studied in phosphate-buffered saline (PBS) solution (pH 7.4 at 37°C) with 0.5% of Tween 80 was added to PBS to improve the solubility of curcumin released. Briefly, 10 ml of freshly prepared nanoparticles were thoroughly mixed with 20 ml of PBS solution. The mixture was divided and placed into 1 ml centrifuge tubes, placed into a shaking incubator at 37°C. At pre-defined time intervals, a centrifuge tube was sampled and centrifuged at 17,217 g for 5 minutes. The removed supernatant was extracted and vortexed with ethanol for 5 minutes. The extracted curcumin was determined by measuring the absorbance at 419 nm using a UV/Vis spectrophotometer as described under entrapment efficiency section.

3.9 Chemical functionality of entrapped curcumin when interacted with food matrix components

For food matrix conditions, considering the potential of curcumin nanocarriers applicable for foods, their fortification into food matrix may possess the ability as an antioxidant agent, which prolong the shelf life of some foods. Semi-skimmed milk was selected as an aqueous food matrix. The coloring capacity and lipid oxidation property of curcumin loaded in NS, NE and NP systems added to the milk were investigated at day 0, 5 and 15 days of refrigerated storage at 4°C in the dark to mimic on-shelf condition with respect to color change and antioxidant activity tested by TBARS (thiobarbituric acid) assay (Jrad et al., 2014; King, 1962; M. & D., 1991)

3.10 Color measurement

Color of milk samples was determined at room temperature using a chromameter (Konica Minolta CM-5, Tokyo, Japan) and recorded in CIE- L* a* b* system (*L** - lightness, *a**-color axis ranging from greenness (-*a**) to redness (+*a**), *b**-color axis ranging from blueness (-*b**) to yellowness (+*b**)). Sample was placed in a glass cuvette, inserted into a black chamber (provided by Konica Minolta), and connected to the chromameter. Color measurement was taken in triplicate for each sample and average values were used. To better describe the values of chroma, *C** and hue angle, *h**, they were calculated from *a** and *b** values using the following equations:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad \text{and} \quad h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

3.11 Data Statistical Analysis

All of the experiments were performed in triplicate and the results were reported as the mean ± standard error (n=3) and statistical analysis was performed in SAS (version 9.4, SAS Institute Inc., NC, USA). The analysis of variance (ANOVA) was used to determine significant differences between the systems. The significance level (*P value*) was set at 0.05.

4. Results and discussion

4.1 Preparation of curcumin-loaded nanocarriers

The purpose of this study was to compare the effect of nanocarriers with the same concentration of surfactant on their physicochemical properties in terms of average diameter size, size distribution, zeta potential and %EE, including the investigation of curcumin retention (%RR) as the function of temperature processing and storage conditions. Hence, curcumin-loaded nanocarriers: nanosuspension (Cur-NS), nanoemulsions (Cur-NE), and nanoparticles (Cur-NP), were synthesized based on the same surfactant concentration used and their compositions (core and shell materials) were presented in Table 1. The initial curcumin amount was controlled to be 0.83 mg/ml for all systems. An anionic lecithin was utilized to stabilize nanosystems and Tween 80, a non-ionic surfactant, used in foods was applied as a co-surfactant with its sterical effect to prevent the particles from aggregation. The concentration of surfactant was kept constant for all systems as well. However, Cur-NE10 and Cur-NE30 systems represented 10 and 30 times of concentration of surfactants, respectively, which were synthesized for comparison purpose.

Table 1: Compositions of curcumin nanosuspension (Cur-NS), curcumin-loaded nanoemulsions (Cur-NE), and curcumin-loaded zein nanoparticles (Cur-NP)

Components	**Nano-carriers**		
	Cur-NS	**Cur-NE**	**Cur-NP**
Core	1ml of 70% ethanol solution	10ml of MCT oil	10mg of zein dissolved in 1ml of 70% ethanol solution
	1mg of curcumin dissolved in 1ml of 100% ethanol solution	10mg of curcumin dissolved in 80ml of distilled water	1mg of curcumin dissolved in 1ml of 100% ethanol solution
Shell	7.5ml of lecithin/Tween80 solution (0.045% lecithin+ 0.030% Tween80)	180mg of lecithin + 120mg of Tween80 (**Cur-NE1**)	7.5ml of lecithin/Tween80 solution (0.045% lecithin + 0.030% Tween80)
		1800mg of lecithin + 1200mg of Tween80 (**Cur-NE10**)	
		5400mg of lecithin + 3600mg of Tween80 (**Cur-NE30**)	

As seen in Figure 3, the compositions of oil phase, aqueous phase and surfactant were varied to develop stable curcumin nanoemulsions (Cur-NE). MCT oil was used as the oil phase to solubilize curcumin because Joung et al. (2016) proposed the higher solubility of curcumin was found in MCT oil compared with other common oil phases. This oil could also enhance the digestibility and bioaccessibility of hydrophobic bioactive compound as demonstrated in previous study (Ahmed et al., 2012).

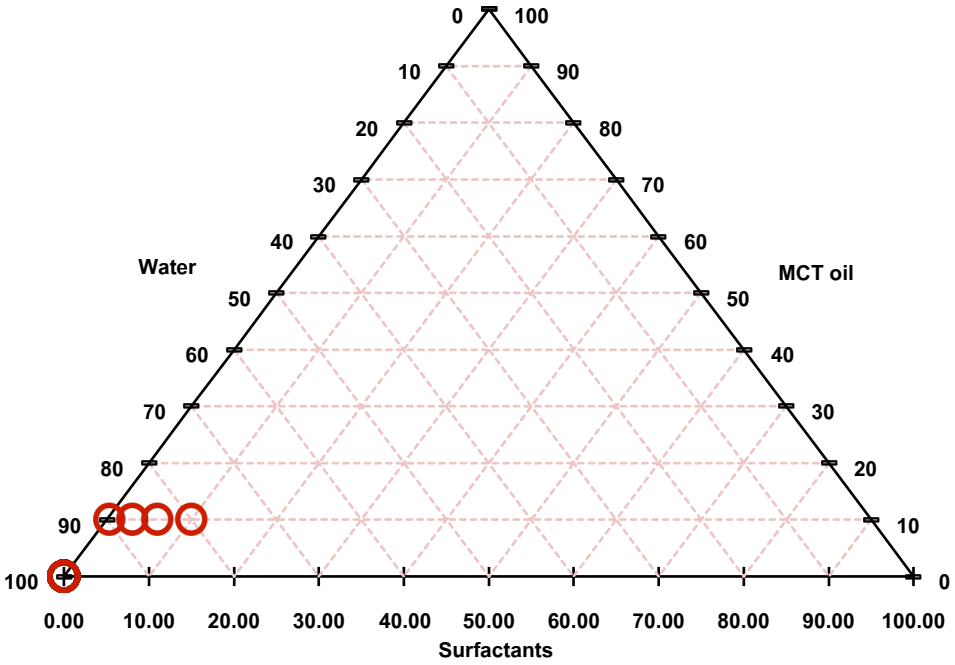


Figure 3. Ternary phase diagram of curcumin-nanoemulsion systems: MCT as an oil phase stabilized with a combined lecithin and Tween 80 as a combined surfactant

The Cur-NE30 was the highest amount of surfactants and this level was commonly used as the concentration of surfactants for nanoemulsions in several studies (Joung et al., 2016; Sari et al., 2015). For example, Joung et al. (2016) used 50% of aqueous phase, less than 33% of oil phase, and less than 32% of surfactant content to fabricate stable nanoemulsions. Sari et al. (2015) proposed most stable nanoemulsion obtained with 2% of MCT, 2% of Tween 80, and 0.5% of emulsifier. The concentration of surfactant in these formulations were similar to Cur-NE30 in this study. The composition and physical properties of curcumin nanoemulsions were shown in Table 2.

Table 2: Composition weight ratio, particle size, polydispersity index (PDI), and zeta potential of Cur-NE systems

Samples	Composition weight ratio (%)				Size (nm)	PDI *	Zeta (mV)	EE (%) *
	MCT	Lecithin	Tween80	Water				
NE1	10	0.18	0.12	89.7	193.93±12.63 [a]	0.18±0.09	-62.93±3.22 [a]	92.86±0.64
NE10	10	1.8	1.2	87	86.68±9.58 [b]	0.19±0.07	-54.27±3.90 [b]	95.38±1.56
NE30	10	5.4	3.6	81	44.39±3.11 [c]	0.23±0.02	-48.00±2.83 [c]	99.51±0.75

NE1, NE10, and NE30 represent 1, 10, and 30 folds of the concentration of surfactant used to form nanoemulsions, results are presented as mean ± stand error (n=3), superscripts indicate significant difference ($p<0.05$) in the column.

Table 3: Size, PDI, and zeta potential of Cur-NS, Cur-NE, and Cur-NP after synthesized

Sample	Size (nm)	PDI	Zeta (mV)	%EE
Cur-NS	667.07±39.22 [a]	0.54±0.04 [a]	-48.50±6.21 [a]	55.27±1.44 [a]
Cur-NE1	193.93±12.63 [b]	0.18±0.09 [b]	-62.93±3.22 [b]	92.86±0.64 [b]
Cur-NE10	86.68±9.58 [c]	0.19±0.07 [b]	-54.27±3.90 [c]	95.38±1.56 [b]
Cur-NE30	44.39±3.11 [d]	0.21±0.04 [b]	-48.00±2.83 [a]	99.51±0.75 [b]
Cur-NP	96.87±5.40 [c]	0.16±0.01 [b]	-49.27±1.71 [a]	87.69±1.49 [c]

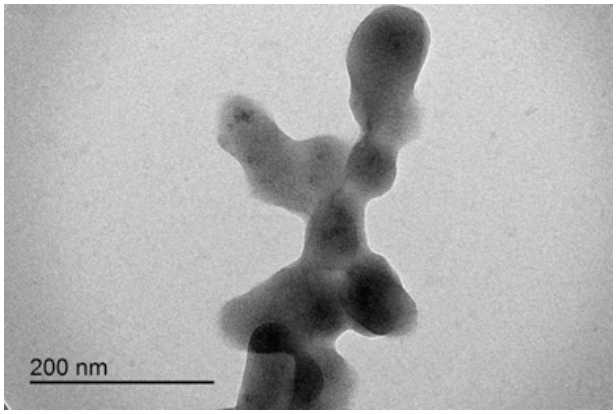
Results are presented as mean ± stand error (n=3), superscripts indicate significant difference ($p<0.05$) in the column.

Of nanoemulsion systems, nanoemulsions with the lowest surfactant concentration (Cur-NE1) had the biggest size of 193.93 nm with the smallest PDI value of 0.18 and the highest negative zeta potential value of -62.93 mV. When multiplied the concentration of surfactants to 10 and 30 folds, the size significantly decreased to 86.68 and 44.39 nm with increased PDI values of 0.19 and 0.23, respectively and the charge values were less negative (-54.27 and -48 mV) relative to higher amount of lecithin (Sari et al., 2015). The average size of nanoemulsions decreased with increased surfactant concentration that was described by more surfactant molecules make a larger oil-water interface (Joung et al., 2016). As expected, encapsulation efficiency of the systems was very high with a range of 92.86-99.51%, the increased surfactant applied; the capability to encapsulate curcumin was higher. It was obvious that the droplet size, surface charge and %EE of Cur-NEs significantly depended on the ratio of surfactant and all systems (Cur-NE1, NE10, and NE30) were stable. These results were supported by Guttoff, Saberi, and McClements (2015) that at a low surfactant and oil ratio, it could form stable nanoemulsions by using high pressure homogenization. Thus due to all formulations resulted in stable nanoemulsions and neglect the effect of surfactant, the curcumin nanoemulsions with the lowest surfactant concentration (Cur-NE1) was selected to investigate for the comparative effect on the stability between nanoemulsion and nanoparticle systems, which had the same concentration of surfactant.

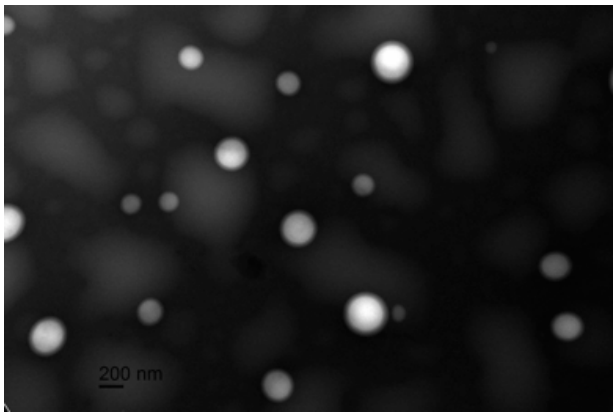
Curcumin nanosuspensions had the largest diameter of 667.07 nm indicating unstable with very high PDI value of 0.54. At the same level of surfactants, there were no significantly difference of zeta potential values of Cur-NS (-48.50 mV) and Cur-NP (-49.27 mV), which were influenced by surfactant lecithin, but the highest negative charge was found for Cur-NE1 (-62.93 mV). However, the negatively charge was reduced when increased surfactant ratio (-54.27 mV of Cur-NE10 and -48.00 mV of Cur-NE30) influencing by the anion lecithin. The average size of curcumin nanoparticles was 96.87 nm with 0.16 PDI value that indicated a stable system and the entrapment efficiency was 87.69%, so that the values were consistent with those of zein nanoparticles with entrapped hydrophobic bioactive compounds proposed in our previous study (Chuacharoen & Sabliov, 2016b). The entrapment efficiency of curcumin nanosuspensions surrounded by lecithin and Tween80 was very low for 55.27%. It was supported according to the description of Kaewnopparat et al. (2009). The pure curcumin suspension demonstrated low rate of dissolution in water solution due to its hydrophobicity causing flowing powder in the surface and reducing the contact area with the solvent. At the lowest surfactant concentration caused curcumin dissolved not well led to low encapsulation amount reported from Cur-NS. The capability of NE systems to entrap curcumin increased when added more surfactants with no significant difference (Table 3). Thus, it concluded that the effect of curcumin dissolubility in oil might depict more effective over the effect of the increased surfactant concentration.

4.2 Morphology of curcumin-loaded nanocarriers

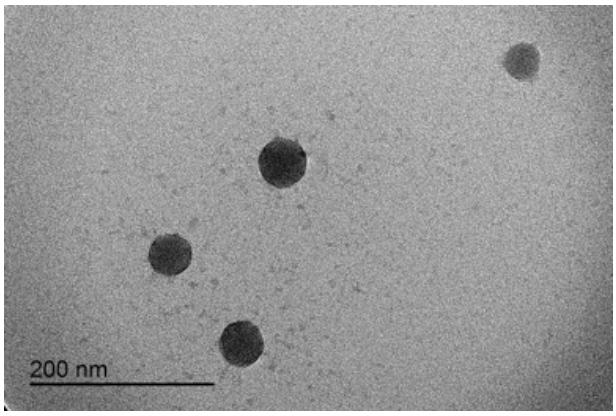
Surface morphological properties of curcumin-loaded nanosystems revealed by TEM analysis were depicted in Figure 4. All systems were fabricated under the same concentration of surfactants. The morphology of Cur-NS had no prefect sphere with particles connected in a surfactant mesh (Figure 4A) and was distinctly different from Cur-NE and Cur-NP (Figure 4B and 4C). Zhao et al., 2015 mentioned original free curcumin powder had irregular shape with a mean size of approximately 3.58 μm, which was approximately 5 times larger than the size of nanosuspension particles (667.07nm). The TEM photograph of curcumin nanoemulsions exhibited a smooth surface and spherical shape with a mean particle size approximately 193.93 nm. The surface morphology of the nanoparticles was similar but slightly half-smaller than Cur-NE that were consistent with the DLS results (Table 3).



(A) Cur-NS



(B) Cur-NE1



(C) Cur-NP

Figure 4: TEM images of (A) curcumin nanosuspension (Cur-NS), (B) curcumin-loaded nanoemulsions (Cur-NE1), and (C) curcumin-loaded zein nanoparticles (Cur-NP) after synthesized, bar represents 200 nm

It concluded that curcumin-encapsulated inside nanocarriers, fabricated under the same surfactant and synthesis conditions, possessed smaller particle sizes, and stable spherical shapes as seen in nanoemulsions and nanoparticles, compared with conventional curcumin shape. Therefore, the comparative effect of surfactant and processing condition to fabricate all three nanocarriers on the stability of nanocarriers depends on surfactant types and concentration, core material, and interaction-bond for entrapment.

A combination of lecithin and Tween 80 was used as a stabilized system for curcumin-entrapped nanocarriers. The hydrophilic head of lecithin connects with a non-ionic Tween 80 creating aqueous particles, whereas its hydrophobic polypropylene possibly connects with hydrophobic parts, which are free curcumin in nanosuspensions, oil in nanoemulsions, and zein in nanoparticles (Figure 2). For nanoemulsion systems, the droplets covered by higher lecithin concentration showed stronger anionic surface charge, which induce the decreased negative charge range from -62.93 to -48.00 mV relative to electrostatic interactions with negatively charged compounds. For nanoparticle system, hydrophilic head of lecithin connects with Tween 80 whereas hydrophobic polypropylene possibly connects with hydrophobic part of zein matrix. As a result, the hydrophilic zein nanoparticle can be loaded with hydrophobic curcumin inside by electrostatic interaction, useful for the compound dispersibility to aqueous phase and protect it from degradation (Chuacharoen & Sabliov, 2016b).

By controlling the fabricate process parameters, curcumin nanocarriers with the same surfactant and processing condition can be successfully prepared and used to compare their stabilities in the physical and chemical aspects.

4.3 Physical stability of curcumin-loaded nanocarriers under processing conditions

Physical stability in terms of size, PDI, and surface charge of Cur-NS, Cur-NE and Cur-NP was investigated under processing conditions (pH, thermal, and ionic strength). The results were shown in Table 4, 5, and 6.

Table 4: Effect of pH on particle size, PDI, and zeta potential of Cur-NS, Cur-NE, and Cur-NP

Sample	Size (nm)			Polydispersity index, PDI			Zeta potential (mV)		
	pH 2	pH 7	pH 9	pH 2	pH 7	pH 9	pH 2	pH 7	pH 9
Cur-NS	393.4±2.5	406.7±9.6	415.1±8.7	0.47±0.02	0.50±0.03	0.48±0.10	-2.3±1.4[a]	-32.5±5.0[b]	-31.9±0.8[b]
Cur-NE1	197.3±0.9	191.9±5.8	204.4±4.2	0.19±0.00	0.20±0.00	0.13±0.01	-7.5±0.1[a]	-24.9±4.6[b]	-51.9±1.4[b]
Cur-NE10	91.2±2.1	90.9±2.7	90.6±2.3	0.16±0.02	0.16±0.00	0.17±0.01	-37.2±3.0	-51.6±1.1	-50.2±4.4
Cur-NE30	49.7±0.3	46.9±0.8	43.9±2.5	0.29±0.01	0.25±0.02	0.28±0.00	-23.8±1.6	-43.6±0.1	-35.5±6.8
Cur-NP	117.5±10.1	97.4±10.3	102.5±11.9	0.19±0.06[a]	0.28±0.06[a]	0.41±0.05[b]	-28.3±0.8	-39.1±0.2	-40.9±6.7

Results are presented as mean ± stand error (n=3), superscripts indicate significant difference ($p<0.05$) among pH treatments.

Table 5: Effect of ionic strength on particle size, PDI, and zeta potential of Cur-NS, Cur-NE, and Cur-NP

Sample	Size (nm)			Polydispersity index, PDI			Zeta potential (mV)		
	0.1M NaCl	0.5M NaCl	1.0M NaCl	0.1M NaCl	0.5M NaCl	1.0M NaCl	0.1M NaCl	0.5M NaCl	1.0M NaCl
Cur-NS	33.0±0.5	38.3±2.3	48.7±0.9	0.24±0.05	0.22±0.01	0.29±0.01	-4.6±0.5[a]	-6.5±2.6[a]	-11.2±0.7[b]
Cur-NE1	282.8±6.9	292.5±5.3	220.2±7.8	0.22±0.02	0.23±0.03	0.20±0.00	-2.1±0.3[a]	-5.1±0.2[a]	-21.3±1.2[b]
Cur-NE10	88.7±1.7	86.9±0.4	83.7±2.9	0.16±0.02	0.13±0.01	0.14±0.00	-8.7±0.2[a]	-14.2±1.0[a]	-25.2±0.1[b]
Cur-NE30	45.7±1.7	44.4±2.7	43.9±2.0	0.18±0.01	0.18±0.03	0.22±0.02	-6.38±0.5[a]	-9.4±0.9[a]	-18.9±2.8[b]
Cur-NP	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Results are presented as mean ± stand error (n=3), N/A means the particles precipitated; superscripts indicate significant difference ($p<0.05$) among ionic strength treatments.

The physical stability of Cur-NS, Cur-NE, and Cur-NP prepared with the same surfactants and initial curcumin loaded, was subjected to different pH treatments (pH 2, 7, and 9) for 24 hours, which was applicable for their commercial utilization. The results were expressed in Table 4.

In alkaline environment, free curcumin was theoretically degraded due to the destructured conjugated diene by its color fading when the pH was higher than 7, and degraded completely after 40 hours (Jin et al., 2016). Average size and distribution had no significant changed at any pH for all nanocarriers, except for PDI of Cur-NP at pH 9 showed significantly higher, that imply to unstable particles occurred. However, the values of average diameter size, particle distribution, and surface charge of Cur-NS showed unstable with reduced size from the initial diameter of 667.07 nm due to degraded surfactant mesh at any pH values. Zeta potential is a function of the surface charge that commonly used to predict stability of the formulation. It is a good index of the magnitude of the electrostatic repulsive interaction between particles. The zeta values of all systems have less negative when subject to pH treatments, relatively stable at pH 9 compared to the values after synthesized.

Surface charges were nearly zero for Cur-NS and Cur-NE1 when added to high acidic environment, which should be attributed by surfactants. For Cur-NE systems, it could be due to surface charge of oil phase and surfactants shifts to positive at pH below the isoelectric point (<6). The same direction was mentioned by Rao and McClements (2011). They studied the stability of nanoemulsions impacted by various stress and stated that at neutral, nanoemulsions were stable, but at lower pHs, the droplets were extensively growth. This could be due to the effect of electrical charge reduces the electrostatic repulsion among particles, which cause aggregation at high acidic environment (Rao & McClements, 2012). However, when the surfactant concentration increased, the charges of Cur-NE10 and NE30 were stable. Likewise, the charge of Cur-NP was insignificant changed even pH value changed. Theoretically, at pH 7, zein will precipitate at the isoelectric point (pH 6-7) due to zero net charge. But the results showed no precipitation (no size change). It suggested that at the lowest level of surfactant used, the stability of Cur-NP showed better, compared with Cur-NS and Cur-NE1.

Table 6: Effect of thermal processing on particle size, PDI, and zeta potential of Cur-NS, Cur-NE, and Cur-NP

Sample	Size (nm)		Polydispersity Index (PDI)		Zeta potential (mV)	
	63°C (30 min)	95°C (10 min)	63°C (30 min)	95°C (10 min)	63°C (30 min)	95°C (10 min)
Cur-NS	95.5±5.7[a]	35.4±4.2[b]	0.14±0.01[a]	0.64±0.03[b]	-50.5±3.9	-41.6±5.3
Cur-NE1	197.3±0.9	191.9±5.9	0.19±0.00	0.20±0.00	-17.5±0.1	-14.9±4.6
Cur-NE10	81.1±4.7	80.9±5.5	0.21±0.00	0.22±0.02	-48.4±1.1	-50.3±0.1
Cur-NE30	49.2±0.9	46.9±0.8	0.39±0.01	0.42±0.02	-43.8±1.6	-43.6±0.1
Cur-NP	95.5±5.7	97.0±3.9	0.14±0.01	0.14±0.03	-42.0±3.6	-39.4±3.1

Results are presented as mean ± stand error (n=3); superscripts indicate significant difference ($p<0.05$) between thermal treatments.

Salts affect functional properties of nanocarriers when fortified in foods and digested in human gastrointestinal tract. The effect of salt was examined at 0.1M, 0.5M, and 1.0M NaCl and Cur-NP precipitated in the present of salts at any concentrations.

Average size and size distribution showed no significant change at any concentrations for all nanocarriers. At the lowest level of surfactants, the size of Cur-NS significantly decreased (initial size was 667.07 nm) to 33-48 nm due to the degradation effect. For nanoemulsion systems, they all have no significant changes in size and PDI. The zeta potential of all nanocarriers shifted toward zero due to the decreased electrostatic repulsion between the particles and consequently the negatively charges were negatively low (<-30 mV), which indicate unstable condition of the solutions. Thus the addition of salt ions has less effect to Cur-NE systems. The precipitation of the Cur-NP can be attributed to the electrostatic repulsion between the zein nanoparticles stabilized by surfactant, when surrounding by the salt ions, it is not sufficiently strong enough to prevent the attractive forces leading to particles aggregation. This was supported by de Folter, van Ruijven, and Velikov (2012). Surfactant-free zein colloidal particles were subjected to different NaCl concentrations (1mM, 0.01M, 0.1M, and 1M) with a constant pH to study the effect of ionic strength, which has a strong effect on colloidal stability through influencing the contribution of repulsive electrostatic forces interacting between the particles. At a higher ionic strength (>0.1M), zein particles started to aggregate indicating no stable was formed, while for low to moderate ionic strength it was stable at both pH above and below the isoelectric point of zein (de Folter et al., 2012).

Thermal processing in the food industry affects stability of colloidal delivery systems in terms of size distribution, charge, and degradation. For practical applications, processing temperatures (63°C for 30 minutes as pasteurization and 95°C for 10 minutes as sterilization) were employed to investigate physical and chemical stabilities of Cur-NS, Cur-NE, and Cur-NP (Table 6 and Figure 5).

The effect of thermal processing on the physical properties of curcumin nanocarriers was evaluated at 63°C for 30 minutes (as pasteurization) and 95°C for 10 minutes (as boiling) and the results are shown in Table 6. The average size of the Cur-NS particles dramatically significant changed (initial size after synthesized was 667.07nm) for both thermal treatments due to the denaturation of surfactant mesh, while after pasteurization, PDI was still stable, but at boiling point, it significantly increased to 0.64, which indicate unstable particles. All Cur-NE systems showed significantly size and PDI stable during thermal treatments even the system that had lowest surfactants (Cur-NE1). The thermal degradation of surfactants caused the change in the surface charge of particles and the magnitude of the charge decreased relative to temperature increasing. After pasteurization and boiling, nanoemulsion and nanoparticle systems had relatively low net charges, but Cur-NE1 had the magnitude of zeta potential dramatically dropped (initial was -62.98mV) compared with other systems. The results were consistent with Qian et al. (2012), they investigated influence of temperature on the physical and chemical stability of β-carotene enriched nanoemulsions and concluded that the nanoemulsions were prone to droplet aggregation due to low magnitude charge at elevated temperatures (>37°C). Also the study of Guttoff et al. (2015) suggested that under thermal treatments (>80°C), this effect could be attributed to progressive dehydration of the non-ionic Tween 80 head groups causing the packing parameter of the surfactant, resulting in the decreased interfacial tension, which promote droplet coalescence. On the other hand, in the presence of zein interaction bond with surfactant this effect was suppressed increasing the repulsive interaction between particles, which reduced the tendency for particles to aggregation. Thus at lowest level of surfactant used to stabilize particles, only Cur-NP showed significantly stable with no changes in size, PDI, and zeta potential values against thermal processing. It concluded that the influence of the nanoparticles promoted the stability compared with nanoemulsion systems. In this case, zein matrix has a good potential to stabilize the particles against high thermal processing, while curcumin covered only surfactants was unstable at elevated temperature.

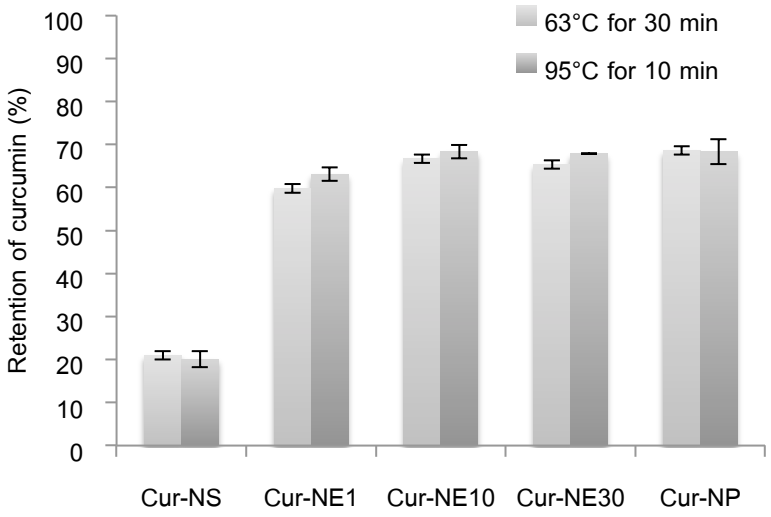


Figure 5: Retention rate of curcumin in Cur-NS, Cur-NE1, Cur-NE10, Cur-NE30, and Cur-NP during thermal treatments (63°C for 30 min and 95°C for 10 min)

After subjected to thermal treatments, retention rate of curcumin in NS were 20.96% and 20.08%, respectively. When curcumin was loaded in nanoemulsion systems with different concentrations of surfactants, the retention rates of curcumin were between 59.77% and 68%, which were increased when surfactants increased. The retention rate of curcumin in zein nanoparticles after thermal treatments was 68.65% and 68.30%, respectively. Therefore, the protective effect of the NP was more significantly pronounced than that of NEs. Thus at the same concentration of surfactants, the retention rate of curcumin was found the highest in nanoparticles for both temperatures (63°C for 30 minutes and 95°C for 10 minutes). The results confirmed that the structure of nanoparticles not only maintained its stability but also provided a better protection of entrapped curcumin during thermal treatments compared with NS and NE forms.

4.4 Physicochemical stability of curcumin-loaded nanocarriers under storage conditions

Storage stability of curcumin nanocarriers was studied as a function of temperature at 4°C and 25°C for the period of 15 days to meet the application for the commercial use. The attributes of Cur-NS, Cur-NE and Cur-NP were investigated in terms of average size, polydispersity index (PDI), and zeta potential (Table 7). The retention rate (RR) of curcumin in each sample was detected and shown in Figure 6.

Table 7: Average size, polydispersity index (PDI), zeta potential after 15 days storage at 4°C and room temperature (RT).

Sample	Size (nm)		Polydispersity Index (PDI)		Zeta potential (mV)	
	4°C	RT °C	4°C	RT °C	4°C	RT °C
Cur-NS	182.9±8.3[a]	237.5±19.7[b]	0.60±0.07[a]	0.92±0.04[b]	-49.6±5.1	-44.4±3.1
Cur-NE1	197.3±0.9	191.9±5.9	0.19±0.00	0.20±0.00	-17.5±0.1[a]	-24.9±4.6[b]
Cur-NE10	81.1±4.7	80.9±5.5	0.21±0.00	0.22±0.02	-48.4±1.1	-50.3±0.1
Cur-NE30	49.2±0.9	46.9±0.8	0.39±0.01	0.42±0.02	-43.8±1.6	-43.6±0.1
Cur-NP	95.5±5.7	97.0±3.9	0.14±0.01	0.14±0.03	-42.0±3.6	-39.2±3.1

Results are presented as mean ± stand error (n=3); superscripts indicate significant difference ($p<0.05$) between storage times.

Cur-NS was not formed spherical particles perfectly and dramatically decreased in both conditions when stored for 15 days. The size distribution was slightly increased for refrigerator and nearly 1.0 when kept at room temperature. The magnitude of zeta potential did not significantly change at 4°C and slightly increased at room temperature. The average diameter size and size distribution of Cur-NE systems were not significant changed, while the zeta values were significantly decreased after storage 15 days compared to those values of after synthesized (Table 3). The higher concentration of surfactant used, the stability of nanoemulsions pronounced. However, at 30 times concentration of surfactant, the PDI values increased for both storage conditions (0.39 and 0.42, respectively), compared to initial value (0.21). For Cur-NP, the particles showed relatively stable in size, PDI and zeta values for both conditions after 15 days of storage, which indicated a good stability of zein nanoparticles.

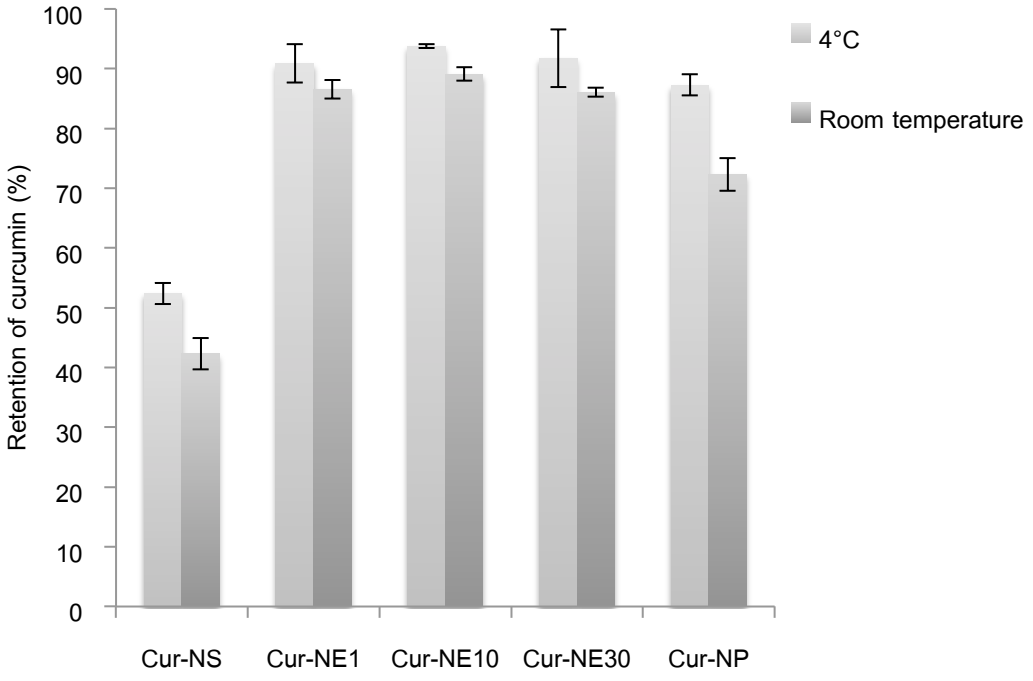


Figure 6: Retention rate of entrapped curcumin loaded in nanocarriers of Cur-NS, Cur-NE1, Cur-NE10, Cur-NE30, and Cur-NP exposed to refrigerator (4°C) and room temperature conditions for 15 days

After 15 days of storage, the retention rate of curcumin in NS were 52.38% for 4°C and less remained to 42.31% at room temperature, respectively. When curcumin was loaded in nanoemulsion systems, the curcumin were highly protected with the retention range of 90-93% for 4°C and 86-89% for room temperature, respectively. The effect of zein nanoparticles to protect curcumin from degradation found slightly less than NE systems, the retention rates were 87% at 4°C and decreased to 72% when kept at

room temperature. Therefore, the nanoemulsion systems had better protective effect on curcumin degradation at the same concentration of surfactants.

4.5 *In vitro* release profiles of curcumin from Cur-NS, Cur-NE and Cur-NP

For the comparative effect of curcumin nanocarriers on release mechanism, nanosuspensions was not included. Phosphate buffered saline (PBS) is a water-based salt buffer solution (pH 7.4) widely used in testing bioactive release. The release profiles of curcumin from nanoemulsion systems and zein nanoparticles in the PBS solution were shown (Figure 7).

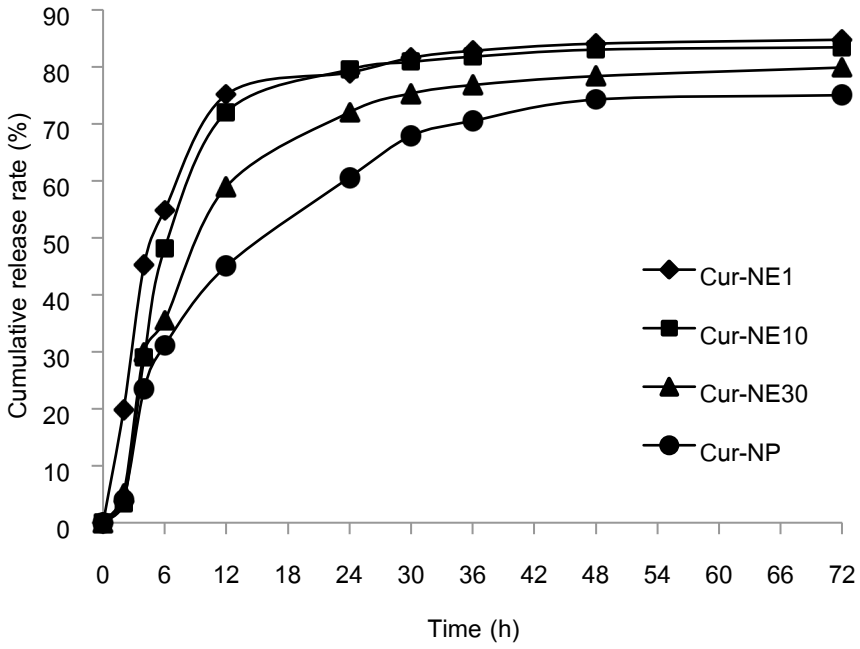


Figure 7: *In vitro* release profiles of Cur-NS, Cur-NE and Cur-NP in PBS solution at 37°C for 72 hours

Release profiles of Cur-NE1, Cur-NE10 and Cur-NE30 apparently showed a two-phase pattern: an initial-burst release at 12 hours (75.09, 72.01 and 58.94%, respectively) and later had slowly release profiles. The release mechanism of curcumin loaded in nanoemulsions was limited by shell membrane, which was related to the concentration of surfactant. Cur-NE30 had an effect to retard the release of curcumin, whereas zein nanoparticles had gradually released the entrapped curcumin at slower rate. It was not surprise that zein matrix associated with curcumin was expected to be released slowly due to zein degradation, compared with the diffusion of curcumin from oily-nanodroplets. Moreover, hydrophobic interaction between lecithin, Tween 80 and curcumin was expected to inhibit the hydrolytic degradation of zein responsible for a more sustained release of curcumin (Chuacharoen & Sabliov, 2016b).

4.6 Lipid oxidation inhibition of curcumin-loaded nanocarriers fortified with milk (TBARS)

In this point, functionality of curcumin nanocarriers and their effect on visual quality in real food matrix were evaluated. Curcumin nanocarriers were added to commercial milk and investigated its ability to inhibit lipid oxidation using TBARS assay. TBARS was used to determine the secondary product formed from lipid peroxidation, particularly in milk system (King, 1962; Spanier & Traylor, 1991). The assay detects the formation of MDA (malondialdehyde), a secondary metabolite of oxidation formed as a result of the polyunsaturated fatty acid degradation, from lipid oxidative deterioration in fat-containing foods. The MDA is reacted with two molecules of thiobarbituric acid (TBA) to form a pink complex (TBARS) detected by spectrophotometric quantitation at 532 nm of maximum absorption. The TBARS value defines as mg of MDA equivalent per liter of milk.

Table 8: TBARS values (mg/L) of entrapped curcumin loaded in nanocarriers (Cur-NS, Cur-NE1, Cur-NE10, Cur-NE30, and Cur-NP) in the presence of milk at 0, 5, and 15 days.

Sample	TBARS (mg/L)		
	Day 0	Day 5	Day 15
Milk (control)	1.369±0.020 ^{aA}	0.978±0.014 ^{aB}	0.642±0.027 ^{aC}
Cur-NS	0.316±0.016 ^{bA}	0.232±0.019 ^{bB}	0.136±0.016 ^{bC}
Cur-NE1	0.297±0.042 ^{bA}	0.101±0.029 ^{cB}	0.069±0.019 ^{cC}
Cur-NE10	0.209±0.041 ^{bA}	0.087±0.019 ^{cB}	0.028±0.002 ^{dC}
Cur-NE30	0.095±0.035 ^{cA}	0.029±0.039 ^{dB}	0.013±0.001 ^{dC}
Cur-NP	0.801±0.024 ^{dA}	0.090±0.021 ^{cB}	0.027±0.013 ^{dC}

Results are presented as mean ± stand error (n=3); a-d and A-C superscripts indicate significant difference ($p<0.05$) due to curcumin nanocarriers and storage times (0, 5, and 15 days), respectively

At the beginning, milk substrates were oxidized with added AAPH. Curcumin compound as an antioxidant inhibited the oxidation and the mechanism was detected by adding TBA substrate to form TBARS which was colored-diminished depending on the ability of curcumin. The interference of excessive milk substances during measurement was eliminated by protein precipitation and centrifugation (King, 1962). Milk was used as a control, and its total antioxidant capacity was initially 1.37 mg/L and decreased to 0.98 mg/L and 0.64 mg/L after stored in refrigerator for 5 and 15 days. The initial TBARS value was closed to the range of 1.02 to 1.31 mg/L of MDA concentration measured from 4 commercial milk powders reported by Fenaille et al. (Fenaille, Mottier, Turesky, Ali, & Guy, 2001).

After adding curcumin nanocarriers to milk and kept it in the refrigerator for 15 days, the samples were measured at 0, 5, and 15 days. The results were significantly different as a function of time. Cur-NS produced the TBARS value not significant difference from Cur-NE1 and Cur-NE10, but it significantly decreased with the highest surfactant of nanoemulsions (Figure 8).

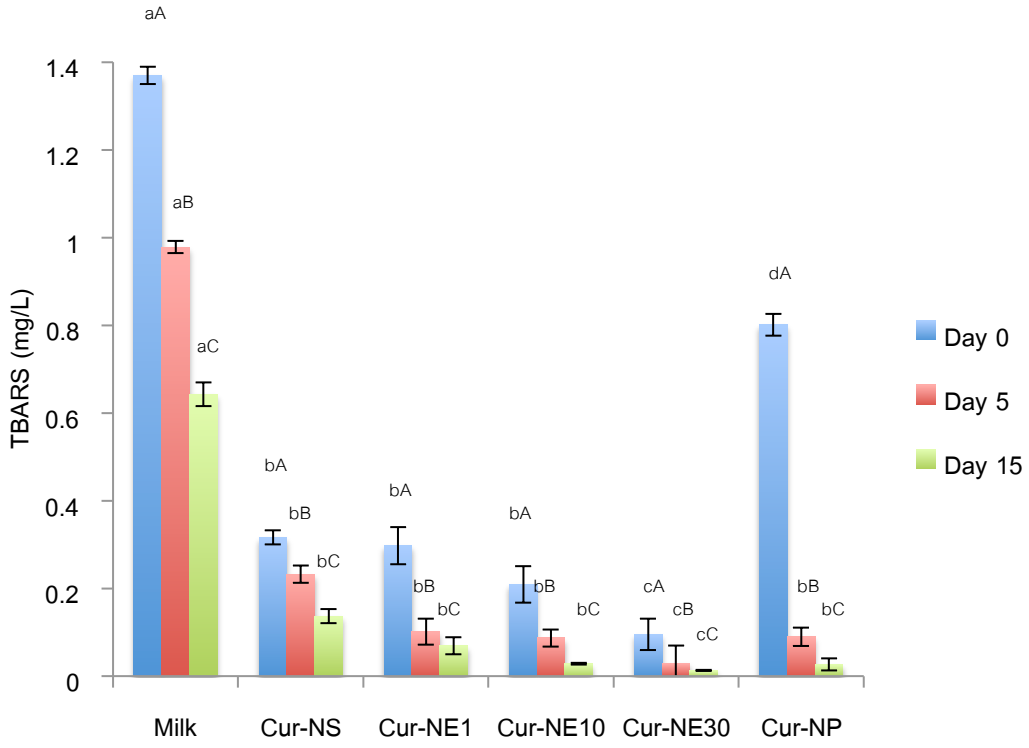


Figure 8: TBARS values (mg/L) of entrapped curcumin loaded in nanocarriers (Cur-NS, Cur-NE1, Cur-NE10, Cur-NE30, and Cur-NP) in the presence of milk at different exposure times (days). Bars represent mean±standard error (n=3). a-d and A-C show statistically significant difference due to curcumin nanocarriers and storage times (0, 5, and 15 days), respectively.

The particle system produced the highest TBARS compared to suspension and emulsified forms. It was due to the release mechanism of the device, which curcumin was entrapped inside the zein nanoparticles, and gradually released as a function of time, resulting in less potential to diminish the TBARS formation initially. After 5 days, the TBARS values of all curcumin nanocarriers were reduced to the range of 0.232 to 0.087 mg/L of MDA concentration, except for Cur-NE30, which had the significant lowest value (0.029 mg/L). After 15 days, all values were significantly decreased and the trend did not differ from those of Day 5 for all systems. It indicated that at the same level of surfactant concentration, Cur-NE1 and Cur-NP had the most antioxidant capacitity to inhibit MDA formation implied on the protection of the functionality of its entrapped curcumin and the enhanced nutritional property of milk.

4.7 Color change of fortified milk

Curcumin is one of a natural food colorant, encapsulating curcumin into several nanocarriers relatively affects its color resulting in the color change of foods when fortified. The colors of the Cur-NS, Cur-NEs and Cur-NP were pictured for visual monitoring (Figure 9).

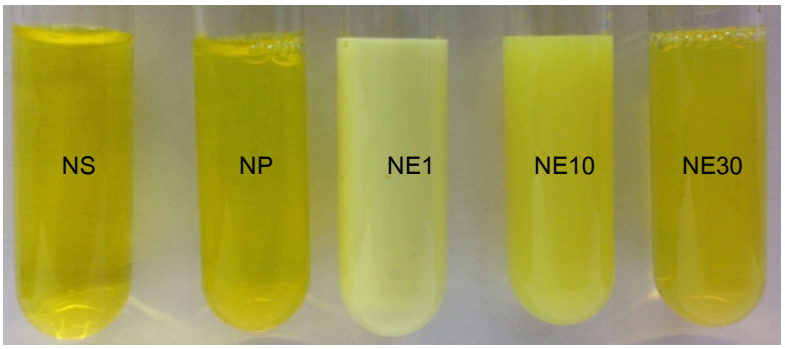


Figure 9: images of Cur-NS, Cur-NEs, and Cur-NP after synthesized

All samples were apparently yellowish solutions. Cur-NE1 solution had a milky character with light yellowish, it became more transparent when stabilized by the higher concentration of surfactant (Cur-NE10 and Cur-NE30). After stored in the refrigerator for 15 days, there was no any change found for all the samples (picture not shown).

When added the curcumin nanocarriers to milk, color parameters of the fortified milk were measured using a spectrophotometer (Konica Minolta Co. Ltd., Tokyo, Japan) immediately at 0, 5 and 15 days and milk was used as a control. The chromatic properties of fortified milk in terms of lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*), and hue angle (h^*) were given in Table 9. The values found for control milk were similar from those reported by Popov-RaljiĆ, LakiĆ, LaliČiĆ-PetronijeviĆ, BaraĆ, and SikimiĆ (2008) (L^*=89.88, a^*=-3.26, b^*=9.27) and slightly less values found from Kneifel, Ulberth, and Schaffer (1992) (L^*=86.2, a^*=-2.1, b^*=7.8). After immediately

added Cur-NS, Cur-NEs, and Cur-NP to milk, the yellow color of the solutions gave the values of lightness (L^* = 89.62, 89.65, 89.66, 89.70, and 89.64, respectively), which were higher than that of untreated milk (L^* = 89.60). When stored the samples at the refrigerator for 5 and 15 days, the lightness parameter of all samples were decreased. The a* and b* values also had the same tend to the lightness.

For chroma C^*, which is mainly dependent on the yellowness b^*. It was noted that milk with encapsulated curcumin in several nanoforms showed insignificantly higher chroma and yellowness than the control. The highest yellowness and chroma were found in milk added with Cur-NE30 at all storage times, while milk with Cur-NS that had no significant difference than the control. Adding the yellowish solution had an effect to increase the yellowness color of milk. Being degraded as a function of time of the entrapped curcumin, the yellowness color of the fortified milk was significantly decreased compared with that of the untreated milk after stored for 15 days. The high value of h^* was probably associated with the low value of greenness ($-a^*$).

In sum, all the samples had no significant difference from the control after stored for 5 days, which the highest color change was found in Cur-NE30. Thus using excessive concentration of surfactant to stabilize the nanoemulsion system could affect the appearance of milk.

Table 9: Color parameters of fortified milk with added curcumin nanocarriers (Cur-NS, Cur-NE1, Cur-NE10, Cur-NE30, and Cur-NP)

Sample		Color parameters (CIE *L*, a*, b** system)				
		L*	***a****	***b****	***Chroma, C****	***Hue, h****
Milk	Day 0	89.60±0.17 ^{aA}	-3.02±0.01 ^{aA}	10.22±0.03 ^{aA}	10.66±0.03 ^{aA}	106.46±0.16 ^{aA}
	Day 5	89.57±0.01 ^{aB}	-3.06±0.03 ^{aA}	10.18±0.02 ^{aA}	10.63 ±0.02 ^{aA}	106.73±0.30 ^{aA}
	Day 15	89.32±0.02 ^{aC}	-3.22±0.09 ^{aB}	9.16±0.43 ^{aB}	9.71±0.40 ^{aB}	109.37±0.54 ^{aB}
Cur-NS	Day 0	89.62±0.02 ^{aA}	-3.02±0.04 ^{aA}	10.35±0.03 ^{aA}	10.78±0.03 ^{aA}	106.27±0.35 ^{aA}
	Day 5	89.54±0.02 ^{aB}	-3.05±0.03 ^{aA}	10.27±0.04 ^{aA}	10.71±0.04 ^{aA}	106.54±0.40 ^{aA}
	Day 15	89.48±0.02 ^{aB}	-3.31±0.02 ^{bB}	9.57±0.02 ^{bB}	10.13±0.02 ^{aB}	109.08±0.20 ^{aB}
Cur-NE1	Day 0	89.65±0.01 ^{aA}	-3.03±0.04 ^{aA}	10.54±0.03 ^{bA}	10.97±0.03 ^{aA}	106.04±0.36 ^{aA}
	Day 5	89.55±0.02 ^{aB}	-3.06±0.02 ^{aA}	10.37±0.03 ^{aA}	10.81±0.03 ^{aA}	106.44±0.23 ^{aA}
	Day 15	89.52±0.03 ^{bB}	-3.23±0.01 ^{aB}	10.04±0.02 ^{bB}	10.55±0.02 ^{bB}	107.83±0.16 ^{aA}
Cur-NE10	Day 0	89.66±0.01 ^{aA}	-2.99±0.02 ^{aA}	10.57±0.01 ^{bA}	10.98±0.01 ^{aA}	105.79±0.13 ^{aA}
	Day 5	89.55±0.02 ^{aB}	-3.04±0.02 ^{aA}	10.36±0.05 ^{aA}	10.80±0.05 ^{aA}	106.35±0.40 ^{aB}
	Day 15	89.53±0.01 ^{bB}	-3.21±0.03 ^{aB}	10.05±0.02 ^{bB}	10.55±0.02 ^{bB}	107.71±0.22 ^{aB}
Cur-NE30	Day 0	89.70±0.01 ^{aA}	-2.95±0.01 ^{aA}	10.63±0.03 ^{cA}	11.03±0.03 ^{aA}	105.51±0.30 ^{aA}
	Day 5	89.58±0.01 ^{aB}	-3.04±0.02 ^{aA}	10.42±0.04 ^{bA}	10.85±0.04 ^{aA}	106.26±0.24 ^{aB}
	Day 15	89.56±0.03 ^{bB}	-3.19±0.02 ^{aB}	10.09±0.02 ^{bB}	10.58±0.02 ^{bB}	107.54±0.12 ^{aB}
Cur-NP	Day 0	89.64±0.04 ^{aA}	-3.03±0.02 ^{aA}	10.52±0.03 ^{bA}	10.95±0.03 ^{aA}	106.07±0.30 ^{aA}
	Day 5	89.54±0.02 ^{aB}	-3.06±0.03 ^{aA}	10.36±0.09 ^{aA}	10.80±0.06 ^{aA}	106.46±0.40 ^{aA}
	Day 15	89.52±0.02 ^{bB}	-3.23±0.03 ^{aB}	10.01±0.06 ^{bB}	10.52±0.05 ^{bB}	107.88±0.45 ^{aA}

Results are presented as mean ± SD (n=3), a-b and A-B subscripted show statistically significant difference ($p<0.05$) among curcumin nanocarriers and storage times (0, 5, and 15 days), respectively.

5. Conclusions

Several nanoforms with different compositions and properties to encapsulate curcumin have been recently developed for improving the solubility, chemical stability, and bioavailability of curcumin in use for medicinal and food applications. Nanosuspension focused on the increased solubility of curcumin, while nanoemulsions and nanoparticles are the most promising approaches used as smart delivery systems to deliver curcumin and protect its stability. However, these systems have particular advantages and disadvantages for particular applications. Curcumin nanosuspension stabilized by surfactant had irregular shape like mesh and showed the lowest stability for all treatments. Nanoemulsion and nanoparticle systems with entrapped curcumin expressed stable characteristics but nanoemulsions had the highest encapsulate efficiency. After subjected to processing conditions: pH, thermal, and ionic strength, the nanoparticles were more stable in different pHs and thermal conditions confirmed by retention rate, but the particles precipitated in the presence of salt, while the nanoemulsion systems were stable but the surface charge was dramatically decreased under thermal conditions. The effect of nanoparticles was less pronounced on the stability compared with nanoemulsion systems after 15 day of storage, but it had a better sustained release profile. Curcumin could have the potency to inhibit lipid oxidation, this may benefit to dairy product. At the same level of surfactant concentration, nanoemulsion and nanoparticles had the antioxidant potency to inhibit MDA formation, which implied on the protection of the functionality of its entrapped curcumin and enhanced nutritional property of milk. The fortified milk with all curcumin nanocarries had the color insignificantly changes from the control milk after stored for 5 days. Thus, the differences observed between these different delivery systems in terms of physical stability, release, chemical stability, and antioxidant activity of curcumin substantiate the need to validate each delivery systems separately and to choose the optimum one to protect the entrapped curcumin under specific conditions for the benefit of utilizing curcumin in commercial food products.

6. Recommendation for Future Work

The comparative effects of nanocarriers as curcumin delivery systems in this study may be useful for utilization in functional food and beverage products, which have various properties in pH, ionic composition, ingredient interactions, storage conditions, and preparation procedures, or even in agricultural applications such as fruit coating. Thus, it is very important to validate the stability of the curcumin nanocarriers to protect the entrapped curcumin under the precise conditions. This must be benefit for utilizing the curcumin delivery systems in commercial food products.

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Output (Acknowledge the Thailand Research Fund)

1. International Journal Publication

- **Thanida Chuacharoen** and Cristina M. Sabliov. Comparative effects of curcumin when delivered in a nanoemulsion or nanoparticle form as a colorant and antioxidant agent for food applications. [In preparation for submitting at LWT-food science and technology]

2. Application

- Since our research is the basic research and mainly focus on knowledge of comparative observed between the three different delivery systems in terms of physical stability, release, chemical stability, and antioxidant activity of curcumin. Our finding will be useful for future work with the applications of curcumin delivery systems in nanoemulsions and/or zein nanoparticles.

3. Others e.g. national journal publication, proceeding, international conference, book chapter, patent

- **Thanida Chuacharoen** and Cristina M. Sabliov. Stability of Curcumin Loaded Nanoparticles Compared to Nanoemulsions under Thermal Processing and Storage Conditions. The 6th Thailand International Nanotechnology Conference, December 12-14, 2018 at Bangkok, Thailand. [Poster presentation; accepted]

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