Porous Food Microparticles as Carriers of Lipophilic Bioactive Compounds to Improve Functionalities

A Dissertation Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Inseob Choi

December 2021

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my major academic advisor, Dr. Qixin Zhong. He provided me with this valuable research opportunity although I was a fresh graduate without strong lab experience. He has always benevolently guided me from conceptualizing research objectives to publishing scientific results in peer-reviewed journals. Whenever I stuck in my research progress, he was standing at the same place, giving me constructive feedback and advice. Dr. Zhong, it has been an honor to work with you as one of your students.

I am thankful to my doctoral committee members, Dr. Mark Morgan, Dr. Vermont Dia, and Dr. Guoxun Chen for their time to give me invaluable questions and help me develop the direction of my research. Dr. Morgan, it was awesome to take my first Food Engineering course from the coolest professor in our department. I remember that I had to take one-day responsibility of teaching undergrads. It was one of the most exciting moments as a graduate student, and I thank you for providing that opportunity. Dr. Dia, I cannot forget the moment when you were receiving the 2017 Undergraduate Faculty Research Mentor of the Year Award during your Food Chemistry lecture. Thanks so much for kindly guiding me from my master's thesis to PhD dissertation. Dr. Chen, I remember your complimentary comments on my proposal for comprehensive exam. That means a lot to me and gave me motivation. I really appreciate your feedback and insight outside of food science that have helped me shape and improve my research.

I thank all my peers: Anyi Wang, Lianger Dong, Shan Hong, Mary Ross Lamsen, Tao Wang, Kai Huang, Wenfei Xiong, Cuixia Sun, Melody Fagan, Jennifer Vuia-Riser, Nan Li, Andrea Nieto Veloza, Hao Zhang, Chao Chen, and Hyejung Lee. It was a great pleasure to tread this academic journey with these awesome, smart, and fun people. Nan, I appreciate your support and thorough feedback on my experimental design.

I am very thankful to my girlfriend, Ashley Miller. She has always believed me, encouraged me, and supported me.

I would like to express my appreciation to my mom, dad, and brother. Although they are far away from Knoxville, I can feel their support and kind heart nearby.

ABSTRACT

Lipophilic bioactive compounds (LBCs) have been gaining attention due to their antioxidation and health-promotion properties. Although many delivery systems have been studied to encapsulate LBCs to increase stability and bioaccessibility of the LBCs, most of delivery systems lack of practicality for application in the food industry due to energy-extensive encapsulation processes and high-cost carrier materials used. Additionally, while a variety of encapsulating systems exist in nanoscale to achieve efficient encapsulation of LBCs, nanoscale systems with encapsulated LBCs may be subjected to reassessment of toxicity. In this dissertation, freeze-dried potato microparticles (FDPMs) and freeze-dried mushroom microparticles (FDMMs) were studied as carrier materials to load three LBCs: resveratrol (RSV), curcumin (CCM), and quercetin (QCT). The porous structure of both FDPMs and FDMMs provided the physical basis for use as novel carrier materials to load high concentrations of LBCs. The solutions with high amounts of RSV, CCM, and QCT were prepared in the binary solvent of ethanol and polyethylene glycol 400 (40:60 v/v) for diffusion in the porous matrix. After evaporation of ethanol, the crystalline structure of the studied pristine LBCs became amorphous after loading in FDPMs and FDMMs. Loading LBCs in FDPMs and FDMMs reduced the degradation during UV irradiation, and the amorphous state of loaded LBCs improved their solubility significant to their functionalities. The antioxidant capacity was observed for the increased ferric reducing power of RSV loaded in FDPMs and the increased DPPH· scavenging activity of CCM and QCT loaded in FDPMs. The

enhanced bioaccessibility than pristine RSV was observed for RSV loaded in FDPMs. The synergistic antioxidant activity was observed for CCM and QCT co-loaded in FDMMs. When CCM and QCT co-loaded in FDMMs were incorporated in beef patties, more significant inhibition of lipid oxidation than treatments with pristine ingredients was observed after cooking and refrigerated storage. Findings from the present dissertation may be significant to manufacturing functional foods to enhance the health benefits of LBCs and their utilization as natural antioxidants to improve food quality.

Key words: porous food matrix; lipophilic bioactive compounds; delivery system; bioaccessibility; lipid oxidation

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1 Introduction and literature review

1.1 Abstract

Lipophilic bioactive compounds (LBCs) such as polyphenols and carotenoids are found in plants. Due to their potential health benefits, LBCs have attracted great attention by food and pharmaceutical scientists. However, low water-solubility, distinct flavor, and susceptibility to the degradation during storage and processing of LBCs make them difficult to be applied in food systems. Therefore, the encapsulation of LBCs in various delivery systems has been proposed to increase bioaccessibility of LBCs by enhancing water solubility, dispersing LBCs in matrices, and protecting LBCs from degradation. In this chapter, currently available delivery systems and encapsulation technologies for LBCs are discussed, and then plant matrix and diffusion are introduced as alternative carrier materials and encapsulating technologies, respectively.

1.2 Introduction

These days, more and more consumers are recognizing the consumption of plant-based foods as a way to prevent diseases (Aschemann-Witzel et al., 2020). This is mostly due to the awareness of the health benefits provided by nutrients and LBCs in plant-based foods (Kim et al., 2019; McMacken & Shah, 2017). Particularly, LBCs are of great interest due to their health-promoting effects and antioxidant capacity (Fraga et al., 2019; Nabi et al., 2020). Several categories of LBCs include carotenoids, oil-soluble vitamins, sterols, and polyphenols. The evidence has been reported that antioxidant properties of LBCs attribute their ability to prevent cancer, cardiovascular disease, obesity, and neurodegenerative diseases (Abdul Rahman et al., 2017; Gan et al., 2010; Grigalius & Petrikaite, 2017; Roleira et al., 2010). Although LBCs are omnipresent in plant-based foods, the health-promoting benefits of LBCs depend on many factors, such as their chemical stability (Campos-Vega et al., 2015), plant matrix in which LBCs

are located (Reginio et al., 2020), and digestion parameters (Lucas-González et al., 2018). The incorporation of LBCs into food products has been limited due to the propensity to form insoluble crystals and poor *in vivo* results, such as low physiological stability during digestion and low bioaccessibility (McClements et al., 2007; Nagarajan et al., 2020). Therefore, there has been a strong need to develop delivery systems to load, protect, and release desirable LBCs.

Numerous delivery systems in food systems have been studied to protect LBCs from degradation and to release LBCs in target organs, mostly intestines, thereby increasing bioaccessibility of encapsulated LBCs. Comprehensively, the bioaccessibility of LBCs is crucial to their health-promoting effects because LBCs must be released from the food matrix to the gastrointestinal (GI) tract during digestion and become available for absorption. When bioaccessibility is high, more LBCs can be absorbed into the bloodstream in a biochemically active form, leading to enhanced bioavailability of LBCs (Martínez-Las Heras et al., 2017). In food science, the bioaccessibility is usually evaluated *in vitro* by measuring the concentration of solubilized LBCs treated with simulated digestive fluids (Ketnawa et al., 2021). The positive correlation between enhanced bioaccessibility and *in vivo* bioavailability of encapsulated LBCs has been reported (Lamsen et al., 2020; Ozkan et al., 2020). Because the health benefits of LBCs are directly related to the effective dose and bioavailability of LBCs, most delivery systems in food science have focused on achieving reasonable loading capacity and high bioaccessibility of LBCs (Zou et al., 2016). However, the use of toxic solvents and surfactants, severe processing conditions (e.g., high temperature, high pressure, and extreme pH), and the instability of delivery system during storage are several factors that should be improved in developing delivery systems (Ozkan et al., 2019).

In this review, antioxidant properties and bioaccessibility of LBCs are discussed and three studied LBCs (resveratrol (RSV), curcumin (CCM), and quercetin (QCT)) are introduced. It is critical to choose appropriate delivery systems to load LBCs of interest and accomplish desired goals, such as high loading capacity and improved bioaccessibility of LBCs. Therefore, common delivery systems and encapsulation technologies that are used for delivery application are reviewed for their advantages and drawbacks. The last section reviews porous matrices of plants as potential carrier materials for LBCs and the diffusion technologies available for plant matrix-based delivery systems.

1.3 Lipophilic bioactive compounds

1.3.1 Antioxidant activity of lipophilic bioactive compounds

LBCs in plants are usually produced as second metabolites, which display health-promoting effects on human body (Zhao et al., 2015). Many LBCs have been identified as having antioxidant properties, encompassing carotenoids, oil-soluble vitamins, and phytosterols (Yao et al., 2014). In human body, carotenoids are not synthesized de novo but after being consumed and bioconverted (e.g., two molecules of retinal from one molecule of β-carotene), carotenoid derivatives become constituents of antioxidant defense system (Stahl & Sies, 2003). Among a variety of defense mechanisms, carotenoids are usually engaged with scavenging singlet molecular oxygen and peroxyl radicals (Young & Lowe, 2001). Similarly, oil-soluble vitamins, such as vitamin E exhibit antioxidant activity mainly through their radical scavenging activity and lead to the reduction of oxidative stress in organisms or lipid oxidation in food products (Pfluger et al., 2004). Antioxidant properties of phytosterols have been demonstrated by their ability to inhibit the oxidation of low-density lipoprotein (LDL) cholesterol (Homma et al.,

2003). However, the correlation of antioxidant activity of phytosterols and their health beneficial effects should be further studied (Trautwein & Demonty, 2007).

Among LBCs, the most studied and important category is polyphenols (PPs) due to their strong antioxidative properties and abundance in nature (Vladimir-Knežević et al., 2012). PPs are naturally occurring organic compounds with multiple phenol units and found in colored berries, herbs and spices, nuts, and some vegetables (Pérez-Jiménez et al., 2010). Many studies have reported that the antioxidant activity of plant-based foods is proportional to the concentration of PPs present within the foods (Ketnawa et al., 2020; Koehnlein et al., 2016). The regulation of enzyme activity is the main antioxidative mechanism of PPs (Ji et al., 2020). The enzymes related to antioxidant activity can be categorized into antioxidant enzymes and oxidases. PPs improve the activity of endogenous antioxidative enzymes usually by inducing synthesis of the antioxidative enzymes. For example, superoxide dismutase (SOD) is the enzyme that can break superoxide anion ($O2^-$) into O_2 and H_2O_2, and the addition of CCM into hypoxia-induced adipocytes increased the concentration of SOD (Priyanka et al., 2014). QCT also increased the expression levels of many antioxidant enzymes including SOD, glutathione peroxidase, and catalase, resulting in significant neuroprotective effects on pyramidal neurons of rats against ischemic injury (Chen et al., 2017). Also, PPs exhibit inhibitory effects on oxidases such as lipoxygenase, nicotinamide adenine dinucleotide phosphate oxidase (NOX), and monoamine oxidase. NOX leads to the formation of reactive oxygen species (ROS) in vascular system and causes atherosclerosis; however, RSV effectively suppressed NOX activity and protected endothelial cells from oxidative damages (Chow et al., 2007).

The antioxidant activities of PPs are mainly involved with the disposition of hydroxyl group (–OH) and the variation in phenolic rings (Rice-Evans et al., 1997). For example, increased

antioxidant activity was observed when hydroxyl group is located at the ortho (σ-) or para (ρ-) position in a phenolic ring (Shahidi & Ambigaipalan, 2015). Not only can these PPs reduce oxidation by trapping singlet oxygen but also can control first-chain initiation by scavenging initial radicals (e.g., OH·) and retard oxidation through enzymatic induction or binding metal ion prooxidants (Cu^{2+} and Fe^{2+}) (Ketnawa et al., 2021; Shahidi & Ambigaipalan, 2015). Additionally, enhanced antioxidative potency of PPs has been reported due to additive or synergistic effect of PPs (Guo et al., 2021). Numerous *in vitro* and *in vivo* studies have revealed that the antioxidant activity of PPs plays important roles in preventing the development of oxidative stress-mediated diseases, including cancer, cardiovascular diseases, and neurodegeneration (Gupta et al., 2014; Mitjavila & Moreno, 2012; Uddin et al., 2020).

1.3.2 Bioaccessibility of lipophilic bioactive compounds

Bioaccessibility is defined as the amount of a nutrient, which is released from ingested food matrix and therefore is available for the absorption in intestine after digestion (Hedrén et al., 2002). The bioaccessibility of LBCs in foods is influenced by the structure of food matrix, but little is known about the exact impact of food matrix on the bioaccessibility of LBCs entrapped in foods. The complexity of food matrix may reduce bioaccessibility of LBCs due to less release of LBCs. For example, only 22.0% of initial concentration of isoflavones was present in the supernatant after *in vitro* digestion of cookies and centrifugation, whereas it was 90.0% for fruit juice (de Pascual-Teresa et al., 2006). The result suggests that the complex structure of cookie matrix composed of sugar, protein, and fat may have inhibited the release of entrapped isoflavones. The morphology of food matrix also affects the bioaccessibility of LBCs (Parada & Aguilera, 2007). Crystalline substructure of chromoplasts where carotenoids are stored, led to the

lowest bioaccessibility of β-carotene among tubular, globular, and crystalline chromoplasts (Sitte et al., 1980).

The structure of LBCs itself is also critical to bioaccessibility. Typically, insoluble crystalline form of many LBCs leads to poor solubility of LBCs in water. When LBCs are consumed orally, only a limited portion of LBCs is dissolved and becomes bioaccessible in the GI tract (Rein et al., 2013). Therefore, it is important to transform crystalline structure of LBCs to enhance bioaccessibility. For example, higher bioaccessibility of β-carotene was achieved when crystalline β-carotene changed its structure to amorphous form being dissolved in oil-in-water nanoemulsion than when its crystalline form was physically mixed with phosphate buffer saline (PBS) (Xia et al., 2015). Reduced crystallinity of LBCs encapsulated in various delivery systems has been widely reported (Ahmad et al., 2019; Lin et al., 2012). However, it should be highlighted that LBCs in delivery systems do not always exist in amorphous form. For example, crystalline structure of CCM was observed in the emulsions prepared with 10.0 mM PBS, corn oil, and tween 80 (Zou et al., 2015). CCM in crystalline state can be encapsulated into food matrix, but the release of CCM may not be encouraged if CCM in carrier materials remains in the crystalline form, which leads to poor solubility of CCM in GI fluids and low bioaccessibility in intestines (Araiza-Calahorra et al., 2018). Also, the selection of suitable material for delivery systems is critical to enhance the bioaccessibility of encapsulated LBCs. In a study, carotenoids (Lycium *barbarum* pigments) were loaded into the emulsion prepared with medium-chain triglycerides, PBS buffer (pH 7.0, 10 mM), and soy protein isolate (Zhang et al., 2015). The chitosan coating onto the emulsion droplets reduced bioaccessibility of carotenoids because pancreatic lipase cannot migrate into chitosan aggregates formed at pH 7.0, which is higher than the pKa of chitosan (pH 6.5), in which chitosan is half-charged.

1.3.3 Resveratrol

RSV (3,5,4`- trihydroxy-stilbene) is a naturally produced polyphenol phytoalexin in plants, such as grapes, peanuts, and soybeans (Burns et al., 2002). RSV is produced as a response to environmental stress factors, including fungal infection, ultraviolet (UV) irradiation, and injury by insects and animals (Ndiaye et al., 2011; Neves et al., 2012). These stress factors promote the release of stilbene synthase enzyme, which is responsible for synthesizing RSV by acting on the precursor of RSV, i.e., phenylalanine (López-Hernández et al., 2007). RSV is hydrophobic with an octanol/water partition coefficient (K_{ow}) of 3.1, containing two benzene rings with three hydroxyl groups attached. Therefore, aqueous solubility of RSV is very low (< 0.03 mg/mL), while it is slightly dissolved in DMSO (16 mg/mL) and ethanol (50 mg/mL) (Soto‐Valdez et al., 2011). Although there are two geometric isomer forms (i.e., *trans* and *cis*) of RSV, *trans*-RSV is more abundant and predominantly associated with therapeutic properties (Augustin et al., 2013). In general, the conversion of *trans*-RSV into *cis*-RSV occurs when exposed to UV light. For example, UV irradiation at 366 nm for 2 h induced 91% of isomerization from *trans*-RSV to *cis*-form (Trela & Waterhouse, 1996). Recent studies observed positive correlation between light intensity and the rate of *cis*-*trans* RSV isomerization until the ratio of *cis*-RSV and *trans*-RSV reached equilibrium after 2 h of UV irradiation (Montsko et al., 2008; Silva et al., 2013). However, *trans*-RSV was stable without being converted into *cis*-form for more than 4 weeks when *trans*-RSV was stored in the mixture of 50 mM phosphoric acid buffer and ethanol (1:1 ratio) at pH 1-7 without light treatment (Trela & Waterhouse, 1996).

Therapeutic potential of RSV has attracted great interest from researchers over the last decade as studies have revealed medicinal effects of RSV, including anticancer, anti-inflammatory, antioxidant, and cardioprotective properties (Abdelgawad et al., 2019; Das & Das,

2007; Hsieh & Wu, 2010). In an *in vivo* study on Wistar rats, the supplementation of RSV (8 mg/kg) helped the restoration of DNA damage caused by 1,2-dimethylhydrazine-induced oxidative stress, showing the role of RSV in quenching ROS (Sengottuvelan et al., 2009). This ROS-quenching ability of RSV is ascribable to its phenolic groups that can scavenge free radicals, such as hydroxy radical, superoxide anion, and lipid peroxyl radicals (Murias et al., 2005). Chronic RSV supplementation also has shown that cellular antioxidant activity is increased due to elevated levels of superoxide dismutase, various antioxidant enzymes, and other co-factors, such as β-carotene, glutathione, and vitamin E (Sengottuvelan et al., 2009).

The dosage of RSV should be considered to achieve targeted health benefits. At the dose range of 50-100 mg/kg, RSV protected spinal cord injuries in rats by preventing lipid peroxidation and enhancing energy metabolism (Yang & Piao, 2003). Similar studies reported that rabbit spinal cord was protected from injury due to inhibited lipid peroxidation and improved nitric oxide production at 1 mg/kg or 10 mg/kg dose (Kiziltepe et al., 2004; Olas et al., 2008). Muscle disuse induces atrophy and oxidative stress; however RSV administration of 12.5 mg/kg for 21 days decreased lipid peroxidation and hydrogen peroxide in muscle of rats (Jackson et al., 2010). In senescence-accelerated mice, the administration of RSV at 25, 50, or 100 mg/kg for 8 weeks improved learning and memory ability (Liu et al., 2012). The RSV dose of 25 mg/kg did not improve activities of glutathione peroxidase, which protects organisms from oxidative damages, but doses of 50 and 100 mg/kg were effective at reducing lipid oxidation and preventing cerebral mitochondrial DNA deletion significantly. Regarding cardiovascular system of rabbits, the RSV dose of 4 mg/kg significantly decreased ADP-induced platelet aggregation, demonstrating the potential of RSV to prevent coronary heart disease (Wang et al., 2002).

1.3.4 Curcumin

CCM, also known as diferuloylmethane, is a yellowish symmetric molecule with crystal structure found in the rhizome of *Curcuma longa* (i.e., turmeric) (Aggarwal et al., 2003; Araiza-Calahorra et al., 2018). Due to its relatively hydrophobic nature, CCM is practically insoluble in aqueous solution with the partition coefficient (K_{ow}) of 4.12 in the mixture of water and soy oil (Zhou et al., 2021). Although CCM shows membrane permeability due to its considerable lipophilic nature, CCM is also readily soluble in polar solvents, such as DMSO, methanol, and acetone (Araiza-Calahorra et al., 2018; Hussain et al., 2017). CCM is sensitive to various environmental factors, such as light, alkaline pH, elevated temperature, and oxygen (Kharat et al., 2017). When exposed to these conditions, three reactive functional sites (i.e., one diketone moiety and two phenolic groups) are readily oxidized by hydrogen abstraction (Priyadarsini, 2013). This leads to the formation of phenoxyl radicals, which are less reactive than peroxyl radicals, consequently resulting in reduced antioxidant property of CCM (Priyadarsini, 2014). Generally, the degradation of CCM in aqueous solution produces *trans*-6-(4’-hydroxy-3’methoxyphenyl)-2,4-dioxo-5-hexenal as a major product and ferulic acid, feruloylmethane, and vanillin as minor products (Schneider et al., 2015).

The health benefits of CCM are little suspect as various therapeutic effects of CCM have been demonstrated through *in vitro* and clinical studies (Ma et al., 2020; Noorafshan & Ashkani-Esfahani, 2013; Voulgaropoulou et al., 2019). Due to functional properties and pharmaceutical values of CCM, it has been widely used as a natural colorant, a flavoring substance, and food preservative and antioxidant in many food and beverage products (Eybl et al., 2006; Wakte et al., 2011). Antioxidant property of CCM is a primary mechanism that explains most of therapeutic effects of CCM on various health diseases. It has been shown that oxidative stress indices in

young male adults are significantly attenuated after oral supplementation of CCM due to its antioxidant capacity (Roohi et al., 2017). CCM exerts its antioxidant capacity on free radicals via different mechanisms. First, CCM shows "superb" scavenging activity for free radicals, such as hydrogen peroxide via proton donation from phenolic group (Ak & Gülçin, 2008). Also, CCM can promote the activation of glutathione peroxidase and superoxide dismutase, which are enzymes responsible for neutralizing free radicals (Marchiani et al., 2014). Lastly, CCM inhibits ROS-producing enzymes, such as cyclooxygenase and lipoxygenase (Lin et al., 2007).

1.3.5 Quercetin

QCT (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one) is a dietary flavonol found in many plant-based foods, including berries, citrus fruits, lettuce, onion, and tomato (Bhagwat et al., 2014). QCT is usually bound to ethers, sugars, or other phenolic acids in plants (Wang et al., 2016). The various forms of QCT derivatives affect the rate of QCT absorption in a human body. Typical western diet provides 15-40 mg QCT/day, while it has been reported that the diet supplementation with 150 mg of QCT is associated with lowered systolic blood pressure and reduced oxidized LDL in plasma (Egert et al., 2009). Various QCT supplements with suggested QCT doses of 250-1500 mg are readily available, being marketed as alternative therapies for allergies, hypertension, bacterial infection, bone defects, and neurodegenerative disorders backed by data from *in vitro* and *in vivo* studies (Elumalai & Lakshmi, 2016; Mlcek et al., 2016; Shoskes et al., 1999; Wong & Rabie, 2008). Particularly, the therapeutic effect of QCT on hypertension has been thoroughly studied and is evidenced by multiple human clinical trials (Larson et al., 2012).

QCT is susceptible to substantial chemical degradation during processing and storage. The chemical stability of QCT is greatly influenced by pH. For example, at pH below 5, QCT exists

as a positive ion or neutral molecule (Momić et al., 2007). At higher pH, however, –OH groups of QCT are dissociated and this promotes the autoxidation of QCT (Dangles, Dufour, et al., 1999). When pH is above 8.0, QCT undergoes autoxidation both in aqueous and organic solvents (Dangles, Fargeix, et al., 1999). The accelerated rate of oxidation product formation was observed when QCT was prepared in Britton-Robinson buffer at pH 10.0 compared to at pH 5.0 and 7.5 (Momić et al., 2007). Also, complete degradation of QCT in aqueous media was observed after 180 min at pH 8 (Buchner et al., 2006) and 120 min at pH 10 (Moon et al., 2008). This autoxidation of QCT should be prevented by encapsulating QCT in a delivery system because antioxidant capacity of QCT is strongly dependent on its –OH groups (Brett & Ghica, 2003). Additionally, the encapsulation of QCT is useful in that undesirable sensory bitter flavor of QCT can be masked through encapsulation (Sun-Waterhouse et al., 2013).

1.4 An overview of delivery systems

1.4.1 Emulsions

1.4.1.1 Oil-in-water emulsions

In general, oil-in-water (O/W) emulsions are composed of two immiscible liquid phases: oil as a dispersed phase and water as a continuous phase (i.e., dispersion medium). Usually, oil droplets are surrounded by an interfacial layer stabilized by emulsifier molecules, such as surfactants, proteins, and phospholipids (Maldonado-Valderrama & Patino, 2010). The size of most oil droplets is within the range of 10 nm – 100 μm (Langevin, 1988; McClements et al., 2007). Due to high affinity to oils, many LBCs have been encapsulated in O/W emulsions through various methods (Lu et al., 2016).

Homogenization is often used to transform separated oil and water into a homogenized emulsion. Because characteristics of homogenized emulsion are influenced by homogenization parameters, pressure, temperature, and cycle of homogenization should be carefully controlled to achieve desired oil droplet size and the viscosity of an emulsion (Yuan et al., 2008). Microchannel emulsification (ME) is another method to encapsulate LBCs in O/W emulsions. In ME, droplets are spontaneously produced at an O/W interface (Sugiura et al., 2001). Briefly, dispersed phase passes through a narrow channel and comes out of the channel through an outlet to spacious terrace where continuous phase is present (Khalid et al., 2018). The dispersed phase expands slowly because internal pressure of the dispersed phase toward the outlet is higher than inwards. This expansion occurs until the difference in pressure becomes equal to Laplace pressure difference between dispersed and continuous phase. Then a neck is formed once pressure balance becomes opposite due to fast flow of dispersed phase, leading to the detachment of dispersed phase and the formation of oil droplets. The advantage of ME over homogenization is that ME does not require external forces to produce oil droplets, preventing the degradation of labile LBCs (Maan et al., 2015). The droplet size distribution from ME is also narrower (< 1%) than from homogenization (Boom & Schroën, 2011). However, only droplets of micro-scale size (1-550 μm) can be prepared using ME (Khalid et al., 2018; Kobayashi et al., 2010). Also, ME is difficult to scale up in comparison to homogenization (Neves et al., 2015).

In a recent study, RSV was encapsulated into O/W emulsion using the mixture of miglyol and orange oil as a continuous phase (Matos et al., 2018). The authors argued that creaming was significantly delayed for RSV-containing emulsion stabilized by modified quinoa starch particles, whereas it took less than 1 h for the emulsion stabilized by a non-ionic surfactant, Tween 20. CCM was encapsulated into the nanoemulsion that consists of the dispersed phase of

palm olein and curcumin dissolved in ethanol and the continuous phase of water (Raviadaran et al., 2018). The resultant oil droplet size (250-1100 nm) was controlled by adjusting the combination of pressure and the number of cycles of a microfluidizer operation. However, the concentration of Tween 80 used (0.25-1% w/w) may be considered high. For example, only 0.1% w/w of Tween 80 can be used as an emulsifier in finished frozen desserts and no more than 360 mg/day of Tween 80 should be present in special dietary foods (FDA, 2020a). A group of researchers loaded CCM and QCT individually into O/W emulsions stabilized by a modified starch (HI-Cap-100) (Iqbal et al., 2020). The mean droplet size was 81.2 nm for CCM and 110 nm for QCT. Encapsulated CCM and QCT showed a high retention rate (83-88%) of CCM and QCT during 5 months of storage at pH 7 and 25 °C. However, the retention rate decreased to 68-80% at pH 2 and 65-74% at 1M of ionic strength during same storage period. This shows the importance of storage conditions for O/W emulsions.

The main disadvantage of O/W emulsions is that they are inherently thermodynamically unstable losing easily their integrity during storage via various physical mechanisms, such as coalescence, flocculation, Ostwald ripening, and gravitational separation (Dickinson & McClements, 1995). Therefore, environmental stresses, such as heating, chilling, and pH change should be carefully controlled. Also, oxidation is a common reason for chemical instability of an O/W emulsion (McClements & Decker, 2000). These physical and chemical instabilities pose break-down of oil droplets, resulting in the failure to protect loaded LBCs. Additionally, it is difficult to control the release of encapsulated LBCs to target sites because the diffusion of the LBCs from interior of oil droplet to outside occurs in a short time (McClements et al., 2007).

1.4.1.2 Water-in-oil-in-water emulsions

Water-in-oil-in-water (W/O/W) multiple emulsions are similar to O/W emulsions, but smaller water droplets are contained within larger dispersed oil droplets in W/O/W emulsions. W/O/W multiple emulsions are ideal to co-encapsulate hydrophilic and hydrophobic compounds. Therefore, many studies loaded hydrophilic catechin and hydrophobic CCM (Aditya et al., 2015) or QCT (Chen et al., 2018) simultaneously in W/O/W emulsions, expecting higher loading capacity of bioactive compounds or synergistic beneficial effects between hydrophilic and hydrophobic compounds (Hu et al., 2012). However, several studies on W/O/W emulsions encapsulated only one individual LBC to achieve a higher loading capacity of the LBC. For example, a study prepared W/O/W emulsions by optimizing the ratio of oil and internal water phase (80:20 v/v), the concentration of hydrophilic (5% w/w) and lipophilic emulsifiers (10% w/w), and homogeneous pressures for first (30 MPa) and second (10 MPa) homogenization step (Wang et al., 2017). As a result, RSV was encapsulated within both internal water and oil phase with the zeta-potential less than -30 mV, which is effective value to stabilize multiple emulsions (Tamnak et al., 2016). However, the loading capacity of RSV in whole W/O/W system was only 0.056 % w/w, which may not be enough to deliver beneficial health effects. The goal of another study was to enhance the loading capacity of RSV in W/O/W emulsions for practical application (Díaz-Ruiz et al., 2020). While maintaining the ratio of internal water and internal oil phase as 30:70 or 40:60 (v/v), the ratio of internal phase and continuous phase was studied in the range from 20:80 to 80:20 (v/v). High encapsulating efficiency (~ 90%) was observed as the volume occupied by internal phase was increased from 20% to 80%, but the maximum concentration of loaded RSV was 10.8 mg/L for 30:70 (W_1/O, v/v) and 14.4 mg/L for 40:60 (W_1/O, v/v), suggesting significant room to be improved regarding loading capacity.

The release of loaded LBCs within droplets in W/O/W emulsions to target sites can be delayed due to more complex structure of W/O/W emulsions than that of O/W emulsions (Garti & Bisperink, 1998). Another benefit of using W/O/W emulsions is that total fat contents can be reduced compared to O/W emulsions by loading internal water phase into oil droplets; however, the oil droplets in W/O/W emulsions can have volume fraction and droplet size comparable to those in O/W emulsions (McClements et al., 2007). Despite these advantages, W/O/W emulsions still share same drawbacks with conventional O/W emulsions: vulnerability to environmental stresses. Therefore, storage conditions such as temperature, pH, and ionic strength should be carefully monitored and controlled to prevent coalescence, creaming, and Ostwald ripening. Additionally, LBCs encapsulated in the inner water phase within oil droplets can sometimes gradually diffuse into oil phase or outermost water phase, failing to achieve planned target delivery of LBCs (Lu et al., 2016).

1.4.2 Liposomes

Liposomes refer to spherical vesicles with concentric lipid bilayers (Le Bihan et al., 2009). Because liposomes comprise aqueous core surrounded by phospholipid bilayers, hydrophilic bioactive compounds can be carried within the aqueous core while LBCs can be entrapped in phospholipid bilayers (Thompson et al., 2009).

Liposomes are among the first concepts for encapsulating RSV (Summerlin et al., 2015). Many studies have utilized liposome-based delivery systems to encapsulate RSV using different materials, such as soy lecithin, oleic acid, and egg yolk phosphatidylcholine (Caddeo et al., 2013; Huang et al., 2019; Pando et al., 2013). The diameter of liposomes varied (70-470 nm) depending on the preparation method of liposomes, while generally high encapsulation efficiency of RSV was achieved around 80-90% (Balanč et al., 2016; Huang et al., 2019).

Principally, liposomes are prepared using phosphatidylcholine with extrusion or sonication but various extra materials, such as cholesterol may be added to liposomal bilayers to achieve better stability of the vesicles (Lee et al., 2005). For example, low-methoxyl pectin was used to coat RSV-encapsulating liposomes, resulting in superior system stability (Feng et al., 2020). Enhanced antioxidant property of CCM was also reported when CCM-encapsulating liposomes were coated with Eudragit S100, which is a pH-sensitive polymer (De Leo et al., 2018). In addition, the physical stabilization of liposomes was achieved after co-encapsulation of RSV and CCM due to the orientation of encapsulated materials, where CCM was placed in hydrophobic core formed by bilayers, whereas RSV positioned themselves toward polar head groups of liposomes (Huang et al., 2019). Similarly, hydrophobic QCT and hydrophilic epigallocatechin-3-gallate encapsulated within a liposome showed synergistic antioxidant effect probably due to their physical location in the liposome (Chen et al., 2019).

However, low loading capacity of LBCs in liposomes is the limiting factor because overloading causes the loss of structural integrity of liposomes due to limited available volume to encapsulate LBCs within phospholipid bilayers (J. Chen et al., 2014). Additionally, unstable physicochemical properties and unexpected release of loaded LBCs during the storage of liposomes have been reported (Sarabandi et al., 2019).

1.4.3 Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are colloidal carriers, in which lipid phase is fully solidified in nanodimension scale (10-1000 nm) (Mardhiah Adib et al., 2016). The concept of SLNs was initially developed in 1991 with the aim of providing physicochemical stability of, improving biocompatibility of, and protecting encapsulated materials (Geszke-Moritz & Moritz, 2016). SLNs consist of lipid core enclosed by a surfactant, and as a result, have the hydrophilic

outermost layer with hydrophobic core. This structure enables SLNs to encapsulate LBCs, such as RSV into hydrophobic interior, although hydrophilic bioactive compounds can also be encapsulated into the particles (Souto & Müller, 2010). SLNs are usually prepared by fabricating O/W emulsions with high-shear homogenization, ultrasonication, solvent evaporation, and microemulsion-based methods (Lingayat et al., 2017). Main advantage of SLNs over other delivery systems is that it is easier to scale-up (Mukherjee et al., 2009). Also, the use of organic solvents can be avoided, consequently mitigating possible toxicological risks associated with organic solvents (Parveen & Sahoo, 2008). In general, encapsulated LBCs are protected from chemical degradation as molecular diffusion is impeded by solidified lipid phase (Weiss et al., 2008). To achieve this, it is desired that LBCs are encapsulated within solid matrix once lipid crystallization is completed. Sometimes, ongoing lipid crystallization often causes the expulsion of encapsulated LBCs (Mukherjee et al., 2009). The stability and loading capacity of LBCs within formed SLNs are mainly dependent on the type of lipids used as well as the characteristics of emulsifiers added to stabilize oil droplets (McClements et al., 2007). When SLNs are made of similar lipid molecules, such as tristearin, the molecules are capable of forming almost perfect crystals with few imperfections (Mukherjee et al., 2009). However, LBCs are usually encapsulated between fatty acid chains and exist within crystal imperfections, therefore, highly ordered crystalline state of lipids does not provide enough space for large amounts of loaded LBCs (Salminen et al., 2013). Accordingly, the use of lipids that have complex structure is recommended to attain high loading capacity of LBCs.

Most studies about RSV in SLNs have been done for non-food application (e.g., pharmaceutical production), encapsulating RSV in a drug form to target specific diseases (Gumireddy et al., 2019; Mohseni et al., 2019). Therefore, further studies on RSV-loaded SLNs

are necessary for food application. A few researchers studied SLNs to encapsulate CCM regarding food application (Ramalingam et al., 2016; Xue et al., 2018). The SLNs coated with food-grade chitosan were prepared using high shear homogenization and ultrasonication (Ramalingam et al., 2016). The study reported that both oral bioavailability and chemical stability of CCM were increased significantly compared to those of non-encapsulated CCM. Also, the combination of high-shear homogenization and ultrasonication showed the feasibility of large-scale production of SLNs for CCM delivery (Ramalingam et al., 2016).

As aforementioned, however, crystalline structure of SLNs leads to have low loading capacity of LBCs. For example, the maximum loading for RSV in glyceryl behenate-based SLNs was no more than 3% (Jose et al., 2014). Additionally, specific range of storage temperature for SLNs is required due to possible expulsion of LBCs resulted from crystallization process or polymorphic changes during storage (Yoon et al., 2013), limiting wide application of SLNs in food industry.

1.4.4 Biopolymer-based particles

Biopolymers such as proteins and polysaccharides have been gaining attention due to their biocompatibility and biodegradability (Gopi et al., 2016). Biopolymers can be fabricated using different modification methods to encapsulate LBCs and to produce biopolymer-based particles (BPs) with desired physicochemical properties (McClements, 2019). BPs are often prepared by self-association of biopolymers in aqueous solutions.

When globular proteins are used to produce BPs, the temperature above thermal denaturation is commonly applied to globular protein solutions to induce self-association of protein molecules (Jones et al., 2009). The dimension and surface charge of these BPs are controlled by adjusting initial globular protein concentration, temperature and duration of heat

treatment, and pH and ionic strength of the solutions (Jones et al., 2010). For example, whey protein isolate solution was heated at 60 °C for 30 min and then adjusted to pH 9.0 using 2 M NaOH to obtain smaller date palm pit extract-encapsulating particles with the diameter of ~104 nm than at other pH (Bagheri et al., 2013).

Alternatively, desolvation is used to obtain BPs. Desolvation entails the self-assembly of biopolymers to form BPs (Giri, 2016). In desolvation process, alcohol or acetone is often used as a desolvating agent being added to aqueous biopolymer solutions to change the solvent quality and therefore to induce spontaneous self-aggregation of biopolymers in the solution (Donev, 2015). The ratio of desolvating agent and aqueous phase can be carefully selected to obtain desirable behavior of biopolymers, such as casein and xanthan gum (Brunchi et al., 2021; Konnova et al., 2013). In a recent study, RSV was loaded into kafirin/casein nanoparticles by diluting binary solution of kafirin/RSV in water and ethanol (20:80 v/v) with aqueous solution of casein, resulting in the precipitation of nanoparticles (Khan et al., 2019). The smallest particle size was observed with 0.2% w/v of casein, whereas 5% w/v casein led to most stabilized particles with the lowest zeta-potential among the studied concentration range of casein (0-6% w/v). However, loaded RSV exhibited reduced $ABTS^{\cdot+}$ scavenging activity.

Desolvation methods have several limitations. First, it has been reported that protein-based BPs prepared from desolvation have usually low loading capacity (Chavoshpour-Natanzi & Sahihi, 2019). Second, toxicity of desolvation agent (e.g., acetone) arouses the safety concern of using desolvation for food application (Ezpeleta et al., 1996). Finally, BPs prepared from desolvation often show poor stability, requiring specific pH and ionic strength for storage.

Cross-linking can be used to enhance physical stability and delivery potentials of BPs. A variety of cross-linkers are available such as glutaraldehyde and genipin (Matalanis et al., 2011).

Each cross-linker can crosslink biopolymers through either physical or chemical interaction so that BPs can be stable under environmental stresses. Physical cross-linking involves non-covalent interactions, including hydrophobic interaction and hydrogen bonding, while chemical cross-linking is formed through covalent bonds between biopolymers (Echalier et al., 2019). However, most chemical cross-linkers are toxic. For example, genetic toxicity of glutaraldehyde has been widely studied (Takigawa & Endo, 2006; Zeiger et al., 2005). Excessive use of photo-based crosslinkers also aroused safety concerns due to chemical toxicity (C. Li et al., 2020). High cost of cross-linkers is another factor that limits their application in food system (Keasler et al., 2017; Muzzarelli et al., 2015).

1.4.5 Safety and regulatory issues of nanoencapsulation systems

The emergence of nanotechnology has allowed food researchers to adopt the application of nanotechnology to develop delivery system for LBCs (Abbas et al., 2009). As a result, most of aforementioned delivery systems have focused on producing nano-sized carriers due to the advantages, such as improved stability and increased oral bioavailability of LBCs. Despite a plethora of advantages of using nanoencapsulation, it has been facing safety, environmental, and therefore ethical concerns over the last few years owing to possible consequences of using nano-scale carriers on human health and environment (Shishir et al., 2018). Therefore, the viewpoint on nanoencapsulation has been changing, concerning the potential risk to human health caused by consuming nanoparticles (NPs) (Katouzian et al., 2017). Notwithstanding, details on the safety of nanoencapsulation have yet been explored, requiring extensive risk assessment and long-term toxicity in particular (He & Hwang, 2016).

Generally, NPs can enter human body via skin permeation or oral consumption. As NPs are orally consumed, they may penetrate into various organs through connective tissues (Katouzian

& Esfanjani, 2017). Detrimental effects, such as inflammatory bowel disease have been reported when NPs were accumulated in intestinal cells (Tetley, 2007). Also, the translocation of NPs aggregated in the intestine of rat and fish to other organs was observed, resulting in inflammation responses (Jani et al., 1994; Zhang et al., 2007). The repercussion of inflammation response includes increased absorption rate of NPs. This may pose problems because the presence of NPs in intraepithelial zone inhibits the intake of foods with regular dimension owing to large surface area and exceptional reactivity of NPs (Fröhlich & Roblegg, 2012). Furthermore, when NPs enter the cells, NPs can modify the DNA in the cells and eventually manipulate normal functions of the cells (Katouzian & Esfanjani, 2017). The accumulation of NPs in cells and organs in the long term may pose significant cytotoxicity and immunological reactions (Petrarca et al., 2020). However, few studies have been conducted for toxicity and absorption profile of food-based NPs, calling for encapsulation methods that are not solely based on nanotechnology.

Surface properties of NPs often matter regarding cytotoxicity. A study showed that negatively charged or neutral NPs possess less toxicity compared to positively charged ones (El Badawy et al., 2011). Therefore, modifying surface chemistry by adding different functional groups on or coating surface of NPs can be used to alter cell viability in animal and human bodies (He & Hwang, 2016; T. Silva et al., 2014). For example, cytotoxicity profiles of NPs were studied by MTT assay after the addition of NPs to human fibroblasts, and the result showed that polyethylene glycol-coated iron oxide NPs improve cell viability compared to the NPs without coating (Gupta & Wells, 2004).

Another issue regarding the application of NPs is the regulation. Currently, no legislation exists that is globally agreed on the use of NPs in food and pharmaceutical sectors. While most countries do not provide regulation guidelines on the risk evaluation of nano-encapsulated

materials, European Commission clearly defined nanomaterials as intentionally engineered materials with one or more dimensions of the order of 100 nm or less, including structures or aggregates that may have the dimension above the order of 100 nm but maintain characteristics of nanoscale materials (Rauscher et al., 2014). According to the regulation (EU) no. 10/2011, NPs may possess toxicological properties, therefore, should be evaluated on a case-by-case basis (EFSA Panel on Food Contact Materials et al., 2020). Additionally, NPs must not be included in functional barrier because the migration of non-authorized substances from multi-layer may occur (Amenta et al., 2015). The FDA does not postulate elaborate guideline on NPs within food and drug matrix; however, it published "Draft Guidance for Industry", which encompasses general safety and regulatory guidance on novel technologies in food industry (Duvall, 2012). The regulatory organizations in other countries also started to suggest that safety and hazard investigation should be conducted before the commercialization of food and beverages that contain NPs (Katouzian et al., 2017).

1.5 Encapsulation technologies

1.5.1 Coacervation

Coacervation is based on the spontaneous phase separation of homogenous colloidal system into coacervate (solute-rich phase) and equilibrium phase (solute-depleted phase). In simple coacervation, a water-soluble macromolecule is mixed with water and alcohol of various volume ratios. Then, the separation of the macromolecule occurs between two phases of water and alcohol of certain ratio; super dense phase with high macromolecule concentration and another phase with high concentration of water (Eghbal & Choudhary, 2018). Complex coacervation involves oppositely charged macromolecules in a single solvent that is separated into two or

more phases with distinct concentrations of macromolecules. In complex coacervation, intermolecular associate interactions established by charged macromolecules lead to the loss of solvation and therefore phase separation of a single solvent (Tiwari et al., 2009). In the delivery system prepared by coacervation, suspended or emulsified LBCs become core materials and macromolecules act as layers. The encapsulation that utilizes complex coacervation has four defined steps: 1) emulsification, 2) coacervation, 3) gelation, and 4) hardening (Salaün, 2016). The structure and size of nano- and micro- coacervate capsules are significantly affected by processing conditions, such as homogenization rate (Dong et al., 2011).

Natural polymers, such as polysaccharides and proteins, are usually used to prepare coacervate capsules loading LBCs. RSV was loaded into casein using coacervation process by mixing casein dissolved in water and RSV dissolved in ethanol (Peñalva et al., 2018). Prepared RSV-loaded NPs exhibited the mean size of 200 nm and the loading capacity of 3% (w/w). The release of RSV was increased after encapsulation in casein NPs and was not influenced by pH condition (*in vitro*). Oral bioavailability of loaded RSV was 10 times higher than that of pristine RSV, establishing high correlation between *in vitro* and *in vivo* data. Similarly, coacervation was used to produce CCM-encapsulating NPs prepared with ovalbumin (OVA) and κ-carrageenan (CAR) (Xie et al., 2019). The DPPH· scavenging activity was not very high at pH 7.0, i.e., ~33% for OVA NPs and ~23% for CAR NPs, probably due to low loading capacity of CCM in the NPs (1.82% for OVA and 0.14% for CAR). However, the photostability against natural light for 25 days was significantly improved for both NPs. Embedding QCT in okra polysaccharides by coacervation resulted in porous sponge-like particles of mean particle diameter of 334 nm (J. Li et al., 2020). The resultant particles showed pH-responsive release rate, releasing less QCT at lower pH (gastric digestion) but more QCT at higher pH (intestinal digestion). These results

suggest that particles produced by coacervation can be used to increase stability and bioaccessibility of encapsulated LBCs.

The major drawback of using coacervation is that coacervate capsules are only stable within a very limited range of pH and ionic strength (Timilsena et al., 2019; Zhang et al., 2009). This restricts the type of macromolecules (i.e., carrier materials) that can be used to protect LBCs. Furthermore, coacervation is an expensive technology to encapsulate food ingredients, being limited to deliver expensive drugs or labile compounds (Fang & Bhandari, 2010).

1.5.2 Spray-drying

Spray-drying is an operating method to produce dry powder by atomizing a liquid system through a hot gas current (Vehring, 2008). The initial liquid feeding system to be dried may be a solution, colloidal suspension, or emulsion (Gharsallaoui et al., 2007). The encapsulation process using spray-drying comprises four steps: 1) preparation of initial liquid feeding system, 2) homogenization of the feeding liquid, 3) atomization of the liquid sample, and 4) dehydration of atomized particles (Shahidi & Han, 1993). The liquid system to be spray-dried is usually prepared by dispersing core materials in the solution of carrier materials. The core materials are often hydrophobic while gum, maltodextrin, and modified starch are commonly used as carrier materials (Fang & Bhandari, 2010). After being homogenized, the mixture is fed into a spray-dryer, then solvent is evaporated, and dried capsules are collected once they gravitate to the bottom of a dryer chamber (F. Gibbs, 1999). The collected dried powders vary in their size with the range of 10-100 μm and typically have spherical shape (Fang & Bhandari, 2010).

Spray-drying has been widely used to encapsulate LBCs because of its continuous and economical operation, resulting in dry and stable LBCs protected by wall materials. RSV emulsion was prepared by mixing RSV ethanol solution with individual sodium caseinate,

maltodextrin, and glucose syrup powder (1:1:1 w/w/w) in water and palm oil, and then spray-dried to obtain microparticles (~ 10.2 μm) (Consoli et al., 2020). High encapsulation efficiency of 97.7% was achieved, but loading capacity was only 0.08% w/w. CCM was also loaded into skim milk powder by spray-drying CCM-containing skim milk concentrates; the stability of encapsulated CCM during storage was improved, but the stability of pristine CCM was not monitored for comparison (Neves et al., 2019). High encapsulation efficiency of QCT in *vinal* or Arabic gum was reported (Busch et al., 2017); it may be due to strong interactions of flavonoid structure of QCT with galactomannan present in *vinal* gum (Busch et al., 2015) and arabinogalactan of Arabic gum (Zuidam & Nedovic, 2010). However, due to its poor thermal stability, QCT has not been widely encapsulated through spray-drying. For instance, the encapsulation efficiency for vanillin was within the range of 36.6-53.3 % (w/w) depending on the type of carrier materials used (e.g., alginate, hydroxypropyl methylcellulose, inulin, and methyl β-cyclodextrin), whereas the encapsulation efficiency for QCT was much lower (8.90-18.5%) (Sun-Waterhouse et al., 2013).

Spray-drying is one of the most widely used processing technologies in food industry due to its rapid, inexpensive, and simple operation (Shishir et al., 2018). However, spray-drying as an encapsulation technology has several disadvantages. For example, only a few food polymers can be considered as carrier materials because they must be dissolved in solvent at desired concentration (Desai & Jin Park, 2005). Also, some core materials, including probiotic strains, cannot maintain their integrity because of thermal denaturation by hot air during atomization (Petrović et al., 2007). This requires the need for the addition of thermo-protectants into the initial liquid feed system (Timilsena et al., 2020). Additional steps required to process fine

microcapsules (e.g., agglomeration) have been also reported as a disadvantage of spray-drying (Gharsallaoui et al., 2007).

1.5.3 Layer-by-layer deposition

Layer-by-layer (LbL) deposition technique utilizes the adsorption of charged polymers on oppositely charged particles, resulting in the reversal of the particle surface charge (Thanasukarn et al., 2006). By sequentially depositing positively and negatively charged biopolymers, multilayered interfacial membranes can be created, and interfacial properties, such as thickness, permeability, and stability against environmental stresses can be controlled (Ogawa et al., 2003; Shi & Caruso, 2001). Although a majority of particles from LbL deposition are produced with synthetic biopolymers, natural biopolymers should be used for food applications due to their biocompatibility (Ochs et al., 2008).

A study reported the effectiveness of LbL deposition to encapsulate CCM. The authors prepared kafirin-based hollow NPs (hNPs) using LbL deposition and solid NPs (sNPs) using desolvation (Li et al., 2019). The particle size of hNPs (56-68 nm) was significantly smaller than that of sNPs (119-133 nm). They argued that sodium carbonate crystals used as a template for LbL deposition functioned as heterogenous nucleation point and prevented the growth of hNPs during LbL deposition. The release of CCM from NPs was slower for hNPs than for sNPs because most of CCM was entrapped in the core for hNPs but adsorbed on the surface for sNPs. QCT was also encapsulated into lecithin/chitosan NPs coated by κ-carrageenan with a total of 5 layers (3 κ-carrageenan and 2 QCT-NPs) using LbL deposition (Souza et al., 2018). The authors reported DPPH· radical scavenging activity and ferric reducing power of multilayered NPs but did not compare the results with control sample.

The difficulty of producing LbL deposited particles in high concentration is a downside of LbL deposition. Also, lengthy process of preparing delivery system through LbL deposition and challenging scale-up process at industry level limit the application of LbL deposition in wide areas (Guzmán et al., 2017).

1.5.4 Molecular inclusion complexes

Molecular inclusion complexation is the technique, in which "guest" molecule is accommodated into the cavity of "host" molecule using physical forces, such as van der Waals bonding (Nunes & Mercadante, 2007). Geometric compatibility and intermolecular interactions between host and guest molecules are important factors for the efficacy of inclusion process. The helical structures of amylose form a hydrophobic cavity to attract nonpolar guest molecules as complexes. Therefore, inclusion complexes of amylose and unsaturated fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid have been studied to protect them from oxidation (Park et al., 2018). The physical properties of amylose-lipid inclusion complexes are influenced by the length of amylose chain. For example, the desirable degree of polymerization (DP) of amylose is between 20 and 60. When amylose chains are too short (DP < 20), forming a complex with a guest molecule is not possible (Godet et al., 1995), whereas DP higher than 60 results in conformational disorder in crystalline complexes (Gelders et al., 2004).

Cyclodextrins (CDs) are the most commonly used host molecules (Astray et al., 2009; Li & Yan, 2002). CDs are cyclic oligosaccharides that can be produced from starch by enzymatic modification (Kfoury et al., 2016). They consist of six (α-CDs), seven (β-CDs), or eight (γ-CDs) glucopyranose units, being β-CDs widely used for food application. These three types of CDs are found in the US Food and Drug Administration (FDA) "generally recognized as safe" (GRAS) list as food additives (FDA, 2020c). The hydrophobic inner cavity of CDs allows the entrapment

of LBCs forming “inclusion complex” while hydrophilic exterior enables the complex to be dissolved in aqueous solutions (Astray et al., 2009; Brewster & Loftsson, 2007). The thermodynamic parameters for the inclusion process of bioactive compounds can be obtained from the following equation:

$$\Delta G = -RTlnK$$

where ΔG is the change of Gibbs free energy, R is the gas constant (J/mol·K), T is the absolute temperature (K), and K is the binding constant (mol^{-1}).

The ΔG values from inclusion complexation were -19.14 $kJ \cdot mol^{-1}$ for RSV and -27.04 $kJ \cdot mol^{-1}$ for CCM at 303 K, indicating that the inclusion process for both RSV and CCM with CDs is spontaneous (Lu et al., 2009; Prabu & Mohamad, 2020). The water solubility of CCM and RSV was greatly increased via inclusion complex formation with CDs, achieving around 10^4 times higher water solubility for CCM and even up to 7×10^5 folds higher for RSV (Ansari et al., 2011; F. Silva et al., 2014; Tønnesen et al., 2002). However, the decrease in the photostability of CCM was observed when CCM was complexed with CDs (Karpkird et al., 2018; Tønnesen et al., 2002), whereas introducing RSV into CDs enhanced its photostability (Allan et al., 2009; Cheng et al., 2018). The instability of encapsulated compounds as well as high production cost and inability to target-controlled release limit the wide application of inclusion complexes (Choi et al., 2009; Sambasevam et al., 2013).

1.5.5 Electro-spinning and electro-spraying

Electro-spinning (e-spinning) and electro-spraying (e-spraying) are relatively new techniques as alternatives for other encapsulating techniques due to simplicity and cost-effectiveness (Coelho et al., 2020). Both techniques are characterized by one-step process to

produce encapsulation structures using electrohydrodynamic fabrication without the assistance of thermal treatment or potentially harmful solvents (Chakraborty et al., 2009). In e-spinning, a polymer solution is being charged by electrical forces as the solution flows out of a nozzle (Bhardwaj & Kundu, 2010). When the charged jet is dispensed from the nozzle toward a collector, a solvent is evaporated, and the jet becomes elongated, producing a continuous fiber. During e-spraying, similar principle is applied; however, the jet forms fine droplets, leading to spherical micro- or nano- particles (Gómez-Mascaraque et al., 2015). The advantages of e-spinning and e-spraying include easy operation, uniform distribution of LBCs in carrier materials, and high encapsulation efficiency (Bock et al., 2012; Chakraborty et al., 2009).

E-spinning was used to encapsulate RSV using whey protein isolate and pullulan as wall materials (Seethu et al., 2020). The nanofiber of a mean diameter of 63-208 nm was produced, and loaded RSV had comparable antioxidant activity to pristine RSV but showed more controlled *in vitro* release because pullulan prevents burst release of loaded LBCs (Borel & Sabliov, 2014). In another study, QCT-loaded zein NPs were prepared by e-spraying (Rodrí guez‐Félix et al., 2019). Viscosity is a critical factor to produce small, spherical, and monodispersed NPs. Therefore, viscosity was optimized by adjusting the concentration of zein (5% w/v) and ethanol (80% v/v). The QCT loaded within zein NPs showed higher *in vitro* bioavailability (5.9%) than pristine QCT (1.9%). Most recently, nanofibers with multilayers were prepared using sequential e-spinning (Wang et al., 2020). In this study, CCM-loaded inner gelatin nanofiber was covered by outer layer of ethyl cellulose nanofibers. Due to hydrogen bonding between adjacent layers, multilayered nanofibers had better thermal stability than gelatin-only nanofiber. However, mechanical properties, such as tensile strength were significantly lower for multilayered fibers than for gelatin-only fibers.

E-spinning and e-spraying equipped with multi nozzles have been used to enhance the yield of nanofibers and NPs production; however, scaling up e-spinning and e-spraying is challenging (Prabu & Dhurai, 2020). Another obstacle for industrial application is the presence of strict standards to follow (Molnar & Nagy, 2016). Also, the shrinkage and poor mechanical properties of an end product are identified as a few drawbacks to be overcome (Senthamizhan et al., 2017).

1.6 Plant matrix as a natural alternative carrier material

1.6.1 Plant matrix and other natural porous materials

Studies on the plant tissues at micrometer level have revealed that plant tissues have porous structures (Cloetens et al., 2006; Pieczywek & Zdunek, 2012). The porous matrix of plants allows the transport of external liquid to the matrix through either simple or force diffusion (Vatankhah & Ramaswamy, 2017). Mature plant tissues are classified as parenchyma, vascular, protecting, and supporting tissues while edible portions of plants are mainly parenchyma cells (Alzamora et al., 2005). These parenchyma cells make up cell wall membrane and allow solvent molecules to pass through the membrane easily (Torreggiani, 1993). It was reported that the size of pores in the walls of parenchyma is around 50 Å, allowing the crossing of small molecules, such as sugars and amino acids as well as small-sized proteins and polysaccharides (Carpita et al., 1979). However, not only wall pores but also intracellular spaces are also often referred as pores. Generally, intracellular spaces exist in parenchyma tissues and account for about 20% of total volume of apple, 40% of mushroom, and 1% of potato (Alzamora et al., 2005). Changing intercellular spaces may allow easier passage of large molecules into matrix of plants. For example, senescence of apple enabled intracellular spaces to become enlarged, which allowed the passage of large molecules and even microbes (Ben-Arie et al., 1979). Unlike synthetic

porous materials, plant matrices have complex geometries and inhomogeneous pore distributions, making it difficult to predict liquid profiles in plant matrices during diffusion (Chen, 2007).

Some natural porous materials can be used to encapsulate LBCs. Ferritin is a natural porous protein that are present in almost all living organisms and has the pore size of 0.3-0.5 nm (Li et al., 2007). Ferritin has been studied to encapsulate LBCs, such as anthocyanidin, β-carotene, and CCM (L. Chen et al., 2014; Zhang et al., 2014), but to introduce co-assembly of protein-LBCs, ferritin requires urea or guanidine hydrochloride (Liu et al., 2019) that has irritant effects on human body or falls into toxic compound category, respectively (Ertell, 2006). Additionally, protein-based natural porous materials, including ferritin can be significantly hydrolyzed by proteases before they reach targeted organ, be disintegrated, and release entrapped LBCs (Lee et al., 2012). Porous hydrogels made of natural materials, such as agar, calcium-alginate, and α-carrageenan can also be used to encapsulate microorganisms for fermentation (Udenni Gunathilake et al., 2017). However, these natural porous hydrogels are not suitable for food system due to physical and chemical instability (Verbelen et al., 2006). These call for the necessity of natural porous carrier materials that are physicochemically stable and not solely based on proteins.

Porous starch is another natural ingredient with non-toxicity and biocompatibility, having the potential as an encapsulating material due to high adsorption of molecules of interest and slow release of them (Chen et al., 2020; Ma et al., 2015). Porous starch is prepared from physical hole-forming methods, such as microwave and mechanical extrusion, to increase specific surface areas and volume of pores in native starch (Chen et al., 2020). After pore-formation process, many pores extend from the exterior surface to the core of starch, providing the starch with

efficient absorbent property (Cheng et al., 2015). A typical absorption process consists of three steps (Chen et al., 2020). First, the molecule of interest, dissolved in a solution, diffuse toward the surface of porous starch. Then, the molecule adsorbs onto the surface and further diffuses into the interior of porous starch. Eventually, the diffusion of molecule into porous starch reaches dynamic equilibrium.

Porous starch has been combined with alginate or β-CD to prepare microemulsion gels (Li et al., 2021) or microspheres (Zheng et al., 2021) to load CCM; however, loading capacity of CCM in the microemulsion gels was low (1.49% w/w), while the ultrasonication-facilitated crosslinking of β-CD and porous starch may be difficult to scale up. Meclofenamic sodium salt, a moderately water-soluble (15 mg/mL) anti-inflammatory drug, was loaded into the porous starch by blending starch and ethylene vinyl alcohol (60:40 mol/mol) and processing in a microwave oven with hydrogen peroxide as a blowing agent (Malafaya et al., 2001). However, this approach is not suitable to load LBCs such as RSV, CCM, and QCT. Another study reported that residual acetone concentration in porous starch was 0.015-0.024% (150-240 ppm) after loading an anticancer drug (paclitaxel) (Wang et al., 2019), while 30 ppm is the maximal residual concentration for acetone used for spice extraction (FDA, 2020b). Additionally, the conformation of porous starch is susceptible to the change in environmental factors, e.g., moisture level and humidity (Chen et al., 2020). However, there are no studies on improving stability of porous starch; therefore, porous starch as a loading vehicle for food application is still to be studied.

1.6.2 Solution diffusion into plant matrix

Vacuum impregnation (VI) and osmotic dehydration (OD) are two major food processing technologies to incorporate solutes into plant tissues for higher nutritional property of plant

matrix, being based on diffusion without disrupting initial plant matrix (Fito et al., 2001). Although these two technologies are often confused with each other, they are completely different. For VI, mechanically-induced difference in pressures is the driving force of the transfer of solutes that are dissolved or dispersed in a liquid phase into the porous structure of food system, while the transfer of water molecules in OD is caused by the difference in concentrations between the solution with solutes (e.g., ions and vitamins) and the intracellular liquid in plant matrix (Rydzak et al., 2017). OD occurs by immersing plant tissues in a hypertonic solution at atmospheric pressure (Shi & Le Maguer, 2002). As a consequence, water is partially removed from the plant tissue, travelling in the direction of concentration gradient (Raoult-Wack, 1994). However, leaching of nutrients from plant tissues occurs occasionally, resulting in altered composition and nutritional profile of plants (Ahmed et al., 2016). Furthermore, sugar or salt in hypertonic solutions may change organoleptic or functional properties of food matrix (Radziejewska-Kubzdela et al., 2014). The main goal of VI, however, is to transfer the external solution into the inside of food system with the assistance of vacuum, allowing the use of isotonic solution (Cháfer et al., 2003). The incorporation of external solution introduces desired solutes into intracellular spaces of plant matrix. This process notably changes or improves various properties of foods, such as enhanced nutritional value (e.g., by enriching with bioactive compounds, micronutrients, and probiotics), extended shelf life (e.g., by decreasing pH), or modified sensory characteristics (e.g., by introducing sugar) (Allali et al., 2010; Derossi et al., 2013).

1.6.3 Porosity of plant matrix

It is assumed that the degree of food matrix impregnation considerably depends on porosity of food matrix (Fito et al., 1996). In addition, porosity directly affects other physical properties

of food, such as mass diffusion coefficient and thermal diffusivity (Joardder et al., 2013). Therefore, modifying porosity of plant matrix may be necessary to achieve desired impact of diffusion on the quality of final product. Most moisture in plant matrix exists in pores and capillaries (Ahmed et al., 2011). Therefore, drying can be used to enhance the porosity of plants (Joardder et al., 2017). The glass transition theory has been introduced to expound the mechanism of collapse and shrinkage of matrix during the drying of plants. Based on this theory, shrinkage is negligible in food matrix if the food matrix is dried below its glass transition temperature (T_g), whereas significant shrinkage takes place when there is a significant difference in drying temperature and T_g of the food (Rahman, 2001). Freeze-drying is usually performed at the temperature below T_g, in which materials are in glassy state (Krokida et al., 1998). Therefore, the shrinkage of plants during drying is insignificant, resulting in high porosity of dried product. However, plants are in rubbery state during conventional drying (e.g., hot air drying and fluidized bed drying) with drying temperature above T_g, and considerable shrinkage of plants is observed. Therefore, dense and shriveled plants are produced after conventional drying (Achanta & Okos, 1996).

In a previous study, the porosity of apple, banana, carrot, and potato was compared after the drying with five different methods (hot air, vacuum, microwave, freeze, and osmotic drying) (Krokida & Maroulis, 1997). The result was apparent that freeze-drying developed more porous structures (90% in porosity) of potato, than other drying methods, such as 70% for microwave drying, 30% for vacuum drying, and 20% for hot air drying. The difference in drying temperature (i.e., -35 °C for freeze-drying and 70 °C for vacuum and hot air drying) partially explains resultant difference in the porosity of potato as T_g of potato starch is within 40-80 °C (Thiewes & Steeneken, 1997). The low porosity of plants after vacuum drying is not desired for

encapsulating LBCs because less-developed porous structure does not provide enough volumetric spaces for incorporating external solution into matrix (Sam Saguy et al., 2005). Also, extreme drying conditions, including high temperature, promote rapid surface drying of moistures in plants and induce the formation of crust on the surface (Troncoso & Pedreschi, 2007). The difficulty of encapsulating bioactive compounds is increased as this impermeable crust forms. In a study, the formation of impermeable layer of banana chips was accelerated when the proportion of hot air drying was increased in the combination drying of hot air and microwave (Mui et al., 2002). This indicates the importance of selecting an appropriate drying method and drying conditions to obtain high porosity of plants without crust formation and to achieve better diffusion and incorporation of external LBCs solution.

1.7 Lipid oxidation

1.7.1 Lipid oxidation in meat products

Lipids contained within both intra- and extra-cellular matrix of meats are susceptible to lipid oxidation. Lipid oxidation results in the quality deterioration of meats, including decrease in nutritional value, development of off-flavor, possible production of toxic compounds, and reduced shelf-life (Chaijan, 2008). Typically, lipid oxidation in meat products consists of three phases of radical chain reactions: initiation, propagation, and termination. Alkyl radicals (R·) are generated at the initiation phase, being subsequently reacted with oxygen to produce peroxyl radicals (ROO·) at the propagation phase (Figure 1.1). Then peroxyl radicals interact with unsaturated fatty acids and produce hydroperoxides (ROOH), which decompose and generate volatile aromatic compounds that provide meat products with rancid odor and off-flavor. Also, non-radical products such as aldehydes and conjugated dienes are produced by reactions between

alkyl and peroxyl radicals (Wasowicz et al., 2004). It has been identified that aldehydes directly contribute to quality deterioration of meats, such as discoloration and compromised functionality and stability of proteins in meats (Min & Ahn, 2005). Additionally, the accumulation of aldehydes in human body is involved with occurrence of atherosclerosis, cancer, and putative mutagens (Duthie et al., 2013). Therefore, it is desirable to inhibit lipid oxidation in meat products by incorporating antioxidants.

1.7.2 Synthetic and natural antioxidants in meat products

To delay lipid oxidation in meat products, a variety of antioxidants have been studied and used in the meat industry (Kumar et al., 2015). Antioxidants can be directly added into meat products or coated on packing materials to reduce lipid oxidation in meat products. Antioxidants can be classified into two categories: synthetic and natural antioxidants. Synthetic antioxidants commonly added to meat products are tertiary butyl hydroquinone (TBHQ), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG) (Fasseas et al., 2008), while compounds that show antioxidative effect and are obtained from natural resources, such as vegetables, fruits, oilseeds, and herbs, and spices, are considered as natural antioxidants (Shahidi & Zhong, 2010). Due to high chemical stability, superior antioxidative potential, and no adverse effect at a low concentration, synthetic antioxidants have been widely added in meat products to retard lipid oxidation. For example, TBHQ was more effective than BHA and BHT at 200 ppm in reducing peroxide values during storage of herring (Kamil et al., 2002). In another study, antioxidant activity of various synthetic and natural antioxidants for sheep meats was evaluated by measuring the concentration of 2-thiobarbituric acid reactive substance (TBARS) during 6-day storage (Jayathilakan et al., 2007). After 6 days, highest antioxidant activity of reducing TBARS production was observed for TBHQ (91.0% reduction) among other synthetic

and natural antioxidants: BHA (42.9%), PG (40.6%), ascorbic acid (67.4%), cloves (68.2%), and cinnamon (37.2%). However, carcinogenic and toxicological properties of synthetic antioxidants have been reported (Faine et al., 2006; Sarafian et al., 2002). Therefore, the concentration of synthetic antioxidants is regulated due to the toxicity caused by long-term consumption (Taghvaei & Jafari, 2015). As the safety concern for synthetic antioxidants increases, the food industry and consumers are demanding more natural antioxidants, avoiding the use and consumption of synthetic antioxidants in meat products.

1.7.3 TBARS test

Malondialdehyde (MDA) is a prime marker of lipid oxidation and is generated during the secondary lipid oxidation in meat products. MDA is also the cause of rancidity even present at a low concentration in meats (Banerjee et al., 2017). Therefore, the evaluation of MDA has been proposed as the indicator of lipid oxidation and quality deterioration of meat products. 2-Thiobarbituric acid (TBA) can be used to quantify MDA because TBA and MDA form pink color complexes that can be measured at 532 nm (Sørensen & Jørgensen, 1996). However, TBA can react with not only MDA but also other oxidation products, collectively called TBARS (Nollet & Toldrá, 2008). MDA must be extracted from meat products to react with TBA. To perform extraction of MDA, distillation method and acid extraction method have been suggested (Sørensen & Jørgensen, 1996). For the acid extraction, meats are homogenized with trichloroacetic acid (TCA) and centrifuged to acquire the extracts. However, TCA may extract other colored components as well as MDA, leading to possible overestimation of TBARS value (Shahidi, 1994). The interfering substances obtained during the acid extraction are not contained in the extracts from distillation method. In distillation method, volatile compounds are separated from meats using an appropriate solvent, and extracts are obtained after the distillation step.

However, the overestimation of TBARS values is still a potential pitfall because distillation at a high temperature generally degrades preexisting hydroperoxides and produces new TBARS (Nollet & Toldrá, 2008). After extraction, the reaction between one mole of MDA and two moles of TBA is promoted by heating extracts at a temperature higher than 70 °C (Sørensen & Jørgensen, 1996). The pink complexes formed by the reaction during heat treatment are usually measured at 532 nm using a UV-Vis spectrophotometer. Due to simplicity and the correlation with reduced sensory quality of meats, TBARS test is the most widely conducted method to determine lipid oxidation in meat products (De Leon & Borges, 2020).

1.8 Hypotheses and overview of dissertation research

This dissertation contains two hypotheses. First, porous matrix of freeze-dried plants can be utilized to load LBCs by diffusing the solution of LBCs into the matrix. Second, the porous plant matrix can protect the loaded LBCs against chemical degradation and, together with possible transformation of crystalline LBCs to the amorphous state, maintain and possibly improve the antioxidant properties and bioavailability of the loaded LBCs.

To test the hypotheses, in Chapter 2, freeze-dried potato microparticles (FDPMs) and RSV were studied as a loading vehicle and an LBC, respectively. The loading solution was prepared by dissolving RSV in the mixture of ethanol/polyethylene glycol 400, and FDPMs were added into the solution for the diffusion of RSV into FDPMs. The loading of RSV within FDPMs was evaluated by quantifying loaded RSV using HPLC. The effect of loading RSV into FDPMs on the bioaccessibility of RSV was evaluated.

The following Chapter 3 covered the co-loading of CCM and QCT into freeze-dried mushroom microparticles (FDMMs) with the expectation of synergistic antioxidant effect of the LBCs. The spatial distribution of the loaded LBCs was captured through confocal laser scanning

microscopy. To determine the impact of CCM-QCT loaded FDMMs on the lipid oxidation in muscle foods, the concentration of lipid oxidation byproducts was measured after the incorporation of CCM-QCT loaded FDMMs in cooked beef patties.

References

Abbas, K., Saleh, A., Mohamed, A., & MohdAzhan, N. (2009). The recent advances in the nanotechnology and its applications in food processing: A review. *J Food Agric Environ*, *7*(3-4), 14-17.

Abdelgawad, I. Y., Grant, M. K., & Zordoky, B. N. (2019). Leveraging the cardio-protective and anticancer properties of resveratrol in cardio-oncology. *Nutrients*, *11*(3), 627.

Abdul Rahman, H., Saari, N., Abas, F., Ismail, A., Mumtaz, M. W., & Abdul Hamid, A. (2017). Anti-obesity and antioxidant activities of selected medicinal plants and phytochemical profiling of bioactive compounds. *International Journal of Food Properties*, *20*(11), 2616-2629.

Achanta, S., & Okos, M. R. (1996). Predicting the quality of dehydrated foods and biopolymers—research needs and opportunities. *Drying Technology*, *14*(6), 1329-1368.

Aditya, N. P., Aditya, S., Yang, H., Kim, H. W., Park, S. O., & Ko, S. (2015). Co-delivery of hydrophobic curcumin and hydrophilic catechin by a water-in-oil-in-water double emulsion. *Food Chemistry*, *173*, 7-13. https://doi.org/https://doi.org/10.1016/j.foodchem.2014.09.131

Aggarwal, B. B., Kumar, A., & Bharti, A. C. (2003). Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer research*, *23*(1/A), 363-398.

Ahmad, M., Mudgil, P., Gani, A., Hamed, F., Masoodi, F. A., & Maqsood, S. (2019). Nano-encapsulation of catechin in starch nanoparticles: Characterization, release behavior and bioactivity retention during simulated in-vitro digestion. *Food Chemistry*, *270*, 95-104. https://doi.org/https://doi.org/10.1016/j.foodchem.2018.07.024

Ahmed, I., Qazi, I. M., & Jamal, S. (2016). Developments in osmotic dehydration technique for the preservation of fruits and vegetables. *Innovative Food Science & Emerging Technologies*, *34*, 29-43.

Ahmed, J., Sinha, N., & Hui, Y. (2011). Drying of vegetables: principles and dryer design. *Handbook of vegetables and vegetable processing*, 279-298.

Ak, T., & Gülçin, İ. (2008). Antioxidant and radical scavenging properties of curcumin. *Chemico-Biological Interactions*, *174*(1), 27-37. https://doi.org/https://doi.org/10.1016/j.cbi.2008.05.003

Allali, H., Marchal, L., & Vorobiev, E. (2010). Effects of vacuum impregnation and ohmic heating with citric acid on the behaviour of osmotic dehydration and structural changes of apple fruit. *biosystems engineering*, *106*(1), 6-13.

Allan, K. E., Lenehan, C. E., & Ellis, A. V. (2009). UV light stability of α-cyclodextrin/resveratrol host–guest complexes and isomer stability at varying pH. *Australian journal of chemistry*, *62*(8), 921-926.

Alzamora, S. M., Salvatori, D., Tapia, M. S., López-Malo, A., Welti-Chanes, J., & Fito, P. (2005). Novel functional foods from vegetable matrices impregnated with biologically active compounds. *Journal of Food Engineering*, *67*(1-2), 205-214.

Amenta, V., Aschberger, K., Arena, M., Bouwmeester, H., Botelho Moniz, F., Brandhoff, P., Gottardo, S., Marvin, H. J. P., Mech, A., Quiros Pesudo, L., Rauscher, H., Schoonjans, R., Vettori, M. V., Weigel, S., & Peters, R. J. (2015). Regulatory aspects of nanotechnology in the agri/feed/food sector in EU and non-EU countries. *Regulatory Toxicology and Pharmacology*, *73*(1), 463-476. https://doi.org/https://doi.org/10.1016/j.yrtph.2015.06.016

Ansari, K. A., Vavia, P. R., Trotta, F., & Cavalli, R. (2011). Cyclodextrin-based nanosponges for delivery of resveratrol: in vitro characterisation, stability, cytotoxicity and permeation study. *Aaps Pharmscitech*, *12*(1), 279-286.

Araiza-Calahorra, A., Akhtar, M., & Sarkar, A. (2018). Recent advances in emulsion-based delivery approaches for curcumin: From encapsulation to bioaccessibility. *Trends in Food Science & Technology*, *71*, 155-169. https://doi.org/https://doi.org/10.1016/j.tifs.2017.11.009

Aschemann-Witzel, J., Gantriis, R. F., Fraga, P., & Perez-Cueto, F. J. (2020). Plant-based food and protein trend from a business perspective: markets, consumers, and the challenges and opportunities in the future. *Critical Reviews in Food Science and Nutrition*, 1-10.

Astray, G., Gonzalez-Barreiro, C., Mejuto, J. C., Rial-Otero, R., & Simal-Gándara, J. (2009). A review on the use of cyclodextrins in foods. *Food Hydrocolloids*, *23*(7), 1631-1640. https://doi.org/https://doi.org/10.1016/j.foodhyd.2009.01.001

Augustin, M. A., Sanguansri, L., & Lockett, T. (2013). Nano- and micro-encapsulated systems for enhancing the delivery of resveratrol. *Annals of the New York Academy of Sciences*, *1290*(1), 107-112. https://doi.org/https://doi.org/10.1111/nyas.12130

Bagheri, L., Madadlou, A., Yarmand, M., & Mousavi, M. E. (2013). Nanoencapsulation of date palm pit extract in whey protein particles generated via desolvation method. *Food Research International*, *51*(2), 866-871. https://doi.org/https://doi.org/10.1016/j.foodres.2013.01.058

Balanč, B., Trifković, K., Đorđević, V., Marković, S., Pjanović, R., Nedović, V., & Bugarski, B. (2016). Novel resveratrol delivery systems based on alginate-sucrose and alginate-chitosan microbeads containing liposomes. *Food Hydrocolloids*, *61*, 832-842.

Banerjee, R., Verma, A. K., & Siddiqui, M. W. (2017). *Natural antioxidants: Applications in foods of animal origin*. CRC Press.

Ben-Arie, R., Kislev, N., & Frenkel, C. (1979). Ultrastructural changes in the cell walls of ripening apple and pear fruit. *Plant Physiology*, *64*(2), 197-202.

Bhagwat, S., Haytowitz, D. B., & Holden, J. M. (2014). USDA database for the flavonoid content of selected foods, Release 3.1. *US Department of Agriculture: Beltsville, MD, USA*.

Bhardwaj, N., & Kundu, S. C. (2010). Electrospinning: a fascinating fiber fabrication technique. *Biotechnology advances*, *28*(3), 325-347.

Bock, N., Dargaville, T. R., & Woodruff, M. A. (2012). Electrospraying of polymers with therapeutic molecules: state of the art. *Progress in polymer science*, *37*(11), 1510-1551.

Boom, R., & Schroën, C. (2011). Emulsification with micro-structured membranes and micro-engineered systems. In *Bubble and Drop Interfaces Volume 2* (pp. 481-501). Brill.

Borel, T., & Sabliov, C. (2014). Nanodelivery of bioactive components for food applications: types of delivery systems, properties, and their effect on ADME profiles and toxicity of nanoparticles. *Annual review of food science and technology*, *5*, 197-213.

Brett, A. M. O., & Ghica, M. E. (2003). Electrochemical oxidation of quercetin. *Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis*, *15*(22), 1745-1750.

Brewster, M. E., & Loftsson, T. (2007). Cyclodextrins as pharmaceutical solubilizers. *Advanced Drug Delivery Reviews*, *59*(7), 645-666. https://doi.org/https://doi.org/10.1016/j.addr.2007.05.012

Brunchi, C.-E., Morariu, S., & Bercea, M. (2021). Impact of ethanol addition on the behaviour of xanthan gum in aqueous media. *Food Hydrocolloids*, *120*, 106928. https://doi.org/https://doi.org/10.1016/j.foodhyd.2021.106928

Buchner, N., Krumbein, A., Rohn, S., & Kroh, L. W. (2006). Effect of thermal processing on the flavonols rutin and quercetin. *Rapid Communications in Mass Spectrometry*, *20*(21), 3229-3235. https://doi.org/https://doi.org/10.1002/rcm.2720

Burns, J., Yokota, T., Ashihara, H., Lean, M. E. J., & Crozier, A. (2002). Plant Foods and Herbal Sources of Resveratrol. *Journal of agricultural and food chemistry*, *50*(11), 3337-3340. https://doi.org/10.1021/jf0112973

Busch, V. M., Kolender, A. A., Santagapita, P. R., & Buera, M. P. (2015). Vinal gum, a galactomannan from Prosopis ruscifolia seeds: Physicochemical characterization. *Food Hydrocolloids*, *51*, 495-502.

Busch, V. M., Pereyra-Gonzalez, A., Šegatin, N., Santagapita, P. R., Poklar Ulrih, N., & Buera, M. P. (2017). Propolis encapsulation by spray drying: Characterization and stability. *LWT*, *75*, 227-235. https://doi.org/https://doi.org/10.1016/j.lwt.2016.08.055

Caddeo, C., Manconi, M., Fadda, A. M., Lai, F., Lampis, S., Diez-Sales, O., & Sinico, C. (2013). Nanocarriers for antioxidant resveratrol: formulation approach, vesicle self-assembly and stability evaluation. *Colloids and Surfaces B: Biointerfaces*, *111*, 327-332.

Campos-Vega, R., Vázquez-Sánchez, K., López-Barrera, D., Loarca-Piña, G., Mendoza-Díaz, S., & Oomah, B. D. (2015). Simulated gastrointestinal digestion and in vitro colonic fermentation of spent coffee (Coffea arabica L.): Bioaccessibility and intestinal permeability. *Food Research International*, *77*, 156-161.

Carpita, N., Sabularse, D., Montezinos, D., & Delmer, D. P. (1979). Determination of the pore size of cell walls of living plant cells. *Science*, *205*(4411), 1144-1147.

Cháfer, M., González-Martínez, C., Chiralt, A., & Fito, P. (2003). Microstructure and vacuum impregnation response of citrus peels. *Food Research International*, *36*(1), 35-41.

Chaijan, M. (2008). Lipid and myoglobin oxidations in muscle foods. *Songklanakarin Journal of Science & Technology*, *30*(1).

Chakraborty, S., Liao, I.-C., Adler, A., & Leong, K. W. (2009). Electrohydrodynamics: A facile technique to fabricate drug delivery systems. *Advanced drug delivery reviews*, *61*(12), 1043-1054.

Chavoshpour-Natanzi, Z., & Sahihi, M. (2019). Encapsulation of quercetin-loaded β-lactoglobulin for drug delivery using modified anti-solvent method. *Food Hydrocolloids*, *96*, 493-502. https://doi.org/https://doi.org/10.1016/j.foodhyd.2019.05.051

Chen, B. H., Park, J. H., Ahn, J. H., Cho, J. H., Kim, I. H., Lee, J. C., Won, M.-H., Lee, C.-H., Hwang, I. K., Kim, J.-D., Kang, I. J., Cho, J. H., Shin, B. N., Kim, Y. H., Lee, Y. L., & Park, S. M. (2017). Pretreated quercetin protects gerbil hippocampal CA1 pyramidal neurons from transient cerebral ischemic injury by increasing the expression of antioxidant enzymes. *Neural regeneration research*, *12*(2), 220-227. https://doi.org/10.4103/1673-5374.200805

Chen, J., Lu, W.-L., Gu, W., Lu, S.-S., Chen, Z.-P., Cai, B.-C., & Yang, X.-X. (2014). Drug-in-cyclodextrin-in-liposomes: a promising delivery system for hydrophobic drugs. *Expert opinion on drug delivery*, *11*(4), 565-577.

Chen, J., Wang, Y., Liu, J., & Xu, X. (2020). Preparation, characterization, physicochemical property and potential application of porous starch: A review. *International Journal of Biological Macromolecules*, *148*, 1169-1181.

Chen, L., Bai, G., Yang, R., Zang, J., Zhou, T., & Zhao, G. (2014). Encapsulation of β-carotene within ferritin nanocages greatly increases its water-solubility and thermal stability. *Food Chemistry, 149*, 307-312.

Chen, W., Zou, M., Ma, X., Lv, R., Ding, T., & Liu, D. (2019). Co‐encapsulation of EGCG and quercetin in liposomes for optimum antioxidant activity. *Journal of food science*, *84*(1), 111-120.

Chen, X., McClements, D. J., Wang, J., Zou, L., Deng, S., Liu, W., Yan, C., Zhu, Y., Cheng, C., & Liu, C. (2018). Coencapsulation of (−)-Epigallocatechin-3-gallate and quercetin in particle-stabilized W/O/W emulsion gels: Controlled release and bioaccessibility. *Journal of agricultural and food chemistry*, *66*(14), 3691-3699.

Chen, X. D. (2007). Moisture diffusivity in food and biological materials. *Drying technology*, *25*(7-8), 1203-1213.

Cheng, J. G., Tian, B. R., Huang, Q., Ge, H. R., & Wang, Z. Z. (2018). Resveratrol functionalized carboxymethyl-β-cyclodextrin: synthesis, characterization, and photostability. *Journal of Chemistry*, *2018*.

Cheng, R., Cheng, X., Xiang, B., Ou, S., & Li, Y. (2015). Fabrication of modified porous starch for the removal of vanadate from aqueous solutions. *Desalination and Water Treatment*, *53*(8), 2100-2105. https://doi.org/10.1080/19443994.2013.860403

Choi, M.-J., Soottitantawat, A., Nuchuchua, O., Min, S.-G., & Ruktanonchai, U. (2009). Physical and light oxidative properties of eugenol encapsulated by molecular inclusion and emulsion–diffusion method. *Food Research International*, *42*(1), 148-156. https://doi.org/https://doi.org/10.1016/j.foodres.2008.09.011

Chow, S.-E., Hshu, Y.-C., Wang, J.-S., & Chen, J.-K. (2007). Resveratrol attenuates oxLDL-stimulated NADPH oxidase activity and protects endothelial cells from oxidative functional damages. *Journal of Applied Physiology*, *102*(4), 1520-1527. https://doi.org/10.1152/japplphysiol.00881.2006

Cloetens, P., Mache, R., Schlenker, M., & Lerbs-Mache, S. (2006). Quantitative phase tomography of Arabidopsis seeds reveals intercellular void network. *Proceedings of the National Academy of Sciences*, *103*(39), 14626-14630.

Coelho, S. C., Estevinho, B. N., & Rocha, F. (2020). Encapsulation in food industry with emerging electrohydrodynamic techniques: Electrospinning and Electrospraying-A review. *Food Chemistry*, 127850.

Consoli, L., Hubinger, M. D., & Dragosavac, M. M. (2020). Encapsulation of resveratrol using Maillard conjugates and membrane emulsification. *Food Research International*, *137*, 109359. https://doi.org/https://doi.org/10.1016/j.foodres.2020.109359

Dangles, O., Dufour, C., & Bret, S. (1999). Flavonol–serum albumin complexation. Two-electron oxidation of flavonols and their complexes with serum albumin. *Journal of the Chemical Society, Perkin Transactions 2*(4), 737-744.

Dangles, O., Fargeix, G., & Dufour, C. (1999). One-electron oxidation of quercetin and quercetin derivatives in protic and non protic media. *Journal of the Chemical Society, Perkin Transactions 2*(7), 1387-1396.

Das, S., & Das, D. K. (2007). Anti-inflammatory responses of resveratrol. *Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy)*, *6*(3), 168-173.

De Leo, V., Milano, F., Mancini, E., Comparelli, R., Giotta, L., Nacci, A., Longobardi, F., Garbetta, A., Agostiano, A., & Catucci, L. (2018). Encapsulation of curcumin-loaded

liposomes for colonic drug delivery in a pH-responsive polymer cluster using a pH-driven and organic solvent-free process. *Molecules*, *23*(4), 739.
De Leon, J. A. D., & Borges, C. R. (2020). Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. *JoVE (Journal of Visualized Experiments)*(159), e61122.
de Pascual-Teresa, S., Hallund, J., Talbot, D., Schroot, J., Williams, C. M., Bugel, S., & Cassidy, A. (2006). Absorption of isoflavones in humans: effects of food matrix and processing. *The Journal of nutritional biochemistry*, *17*(4), 257-264.
Derossi, A., De Pilli, T., & Severini, C. (2013). Application of vacuum impregnation techniques to improve the pH reduction of vegetables: study on carrots and eggplants. *Food and Bioprocess Technology*, *6*(11), 3217-3226.
Desai, K. G. H., & Jin Park, H. (2005). Recent developments in microencapsulation of food ingredients. *Drying technology*, *23*(7), 1361-1394.
Díaz-Ruiz, R., Martínez-Rey, L., Laca, A., Álvarez, J. R., Gutiérrez, G., & Matos, M. (2020). Enhancing trans-Resveratrol loading capacity by forcing W1/O/W2 emulsions up to its colloidal stability limit. *Colloids and Surfaces B: Biointerfaces*, *193*, 111130. https://doi.org/https://doi.org/10.1016/j.colsurfb.2020.111130
Dickinson, E., & McClements, D. J. (1995). *Advances in food colloids*. Springer Science & Business Media.
Donev, R. (2015). *Protein and Peptide Nanoparticles for Drug Delivery*. Academic Press.
Dong, Z., Ma, Y., Hayat, K., Jia, C., Xia, S., & Zhang, X. (2011). Morphology and release profile of microcapsules encapsulating peppermint oil by complex coacervation. *Journal of Food Engineering*, *104*(3), 455-460. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2011.01.011
Duthie, G., Campbell, F., Bestwick, C., Stephen, S., & Russell, W. (2013). Antioxidant effectiveness of vegetable powders on the lipid and protein oxidative stability of cooked turkey meat patties: Implications for health. *Nutrients*, *5*(4), 1241-1252.
Duvall, M. N. (2012). FDA regulation of nanotechnology. *Washington, DC, USA: Beveridge & Diamond, PG*.
Echalier, C., Valot, L., Martinez, J., Mehdi, A., & Subra, G. (2019). Chemical cross-linking methods for cell encapsulation in hydrogels. *Materials Today Communications*, *20*, 100536. https://doi.org/https://doi.org/10.1016/j.mtcomm.2019.05.012
EFSA Panel on Food Contact Materials, E., Aids, P., Silano, V., Barat Baviera, J. M., Bolognesi, C., Chesson, A., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., & Lambré, C. (2020). Review and priority setting for substances that are listed without a specific migration limit in Table 1 of Annex 1 of Regulation 10/2011 on plastic materials and articles intended to come into contact with food. *EFSA Journal*, *18*(6), e06124.
Egert, S., Bosy-Westphal, A., Seiberl, J., Kürbitz, C., Settler, U., Plachta-Danielzik, S., Wagner, A. E., Frank, J., Schrezenmeir, J., & Rimbach, G. (2009). Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. *British Journal of Nutrition*, *102*(7), 1065-1074.
Eghbal, N., & Choudhary, R. (2018). Complex coacervation: Encapsulation and controlled release of active agents in food systems. *LWT*, *90*, 254-264. https://doi.org/https://doi.org/10.1016/j.lwt.2017.12.036

El Badawy, A. M., Silva, R. G., Morris, B., Scheckel, K. G., Suidan, M. T., & Tolaymat, T. M. (2011). Surface Charge-Dependent Toxicity of Silver Nanoparticles. *Environmental Science & Technology*, *45*(1), 283-287. https://doi.org/10.1021/es1034188

Elumalai, P., & Lakshmi, S. (2016). Role of quercetin benefits in neurodegeneration. In *The Benefits of Natural Products for Neurodegenerative Diseases* (pp. 229-245). Springer.

Ertell, K. G. (2006). A Review of Toxicity and Use and Handling Considerations for Guanidine, Guanidine Hydrochloride, and Urea.

Eybl, V., Kotyzova, D., & Koutensky, J. (2006). Comparative study of natural antioxidants – curcumin, resveratrol and melatonin – in cadmium-induced oxidative damage in mice. *Toxicology*, *225*(2), 150-156. https://doi.org/https://doi.org/10.1016/j.tox.2006.05.011

Ezpeleta, I., Irache, J. M., Stainmesse, S., Chabenat, C., Gueguen, J., Popineau, Y., & Orecchioni, A.-M. (1996). Gliadin nanoparticles for the controlled release of all-trans-retinoic acid. *International Journal of Pharmaceutics*, *131*(2), 191-200. https://doi.org/https://doi.org/10.1016/0378-5173(95)04338-1

F. Gibbs, S. K., Inteaz Alli, Catherine N. Mulligan, Bernard. (1999). Encapsulation in the food industry: a review. *International Journal of Food Sciences and Nutrition*, *50*(3), 213-224.

Faine, L., Rodrigues, H., Galhardi, C., Ebaid, G., Diniz, Y., Fernandes, A., & Novelli, E. (2006). Butyl hydroxytoluene (BHT)-induced oxidative stress: effects on serum lipids and cardiac energy metabolism in rats. *Experimental and Toxicologic Pathology*, *57*(3), 221-226.

Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols–a review. *Trends in Food Science & Technology*, *21*(10), 510-523.

Fasseas, M., Mountzouris, K., Tarantilis, P., Polissiou, M., & Zervas, G. (2008). Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chemistry*, *106*(3), 1188-1194.

FDA. (2020a). *21CFR172.840*.

FDA. (2020b). *Code of Federal Regulations Title 21. 173.210 Acetone.*

FDA. (2020c). *GRAS Notice Inventory*. Retrieved Nov 17 from https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices

Feng, S., Sun, Y., Wang, P., Sun, P., Ritzoulis, C., & Shao, P. (2020). Co‐encapsulation of resveratrol and epigallocatechin gallate in low methoxyl pectin‐coated liposomes with great stability in orange juice. *International Journal of Food Science & Technology*, *55*(5), 1872-1880.

Fito, P., Andrés, A., Chiralt, A., & Pardo, P. (1996). Coupling of hydrodynamic mechanism and deformation-relaxation phenomena during vacuum treatments in solid porous food-liquid systems. *Journal of Food Engineering*, *27*(3), 229-240.

Fito, P., Chiralt, A., Betoret, N., Gras, M., Cháfer, M., Martínez-Monzó, J., Andrés, A., & Vidal, D. (2001). Vacuum impregnation and osmotic dehydration in matrix engineering: Application in functional fresh food development. *Journal of Food Engineering*, *49*(2-3), 175-183.

Fraga, C. G., Croft, K. D., Kennedy, D. O., & Tomás-Barberán, F. A. (2019). The effects of polyphenols and other bioactives on human health. *Food & function*, *10*(2), 514-528.

Fröhlich, E., & Roblegg, E. (2012). Models for oral uptake of nanoparticles in consumer products. *Toxicology*, *291*(1), 10-17. https://doi.org/https://doi.org/10.1016/j.tox.2011.11.004

Gan, R.-Y., Xu, X.-R., Song, F.-L., Kuang, L., & Li, H.-B. (2010). Antioxidant activity and total phenolic content of medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases. *Journal of Medicinal Plants Research*, *4*(22), 2438-2444.

Garti, N., & Bisperink, C. (1998). Double emulsions: Progress and applications. *Current Opinion in Colloid & Interface Science*, *3*(6), 657-667. https://doi.org/https://doi.org/10.1016/S1359-0294(98)80096-4

Gavahian, M., Chu, Y.-H., Mousavi Khaneghah, A., Barba, F. J., & Misra, N. N. (2018). A critical analysis of the cold plasma induced lipid oxidation in foods. *Trends in Food Science & Technology*, *77*, 32-41. https://doi.org/https://doi.org/10.1016/j.tifs.2018.04.009

Gelders, G., Vanderstukken, T., Goesaert, H., & Delcour, J. (2004). Amylose–lipid complexation: a new fractionation method. *Carbohydrate Polymers*, *56*(4), 447-458.

Geszke-Moritz, M., & Moritz, M. (2016). Solid lipid nanoparticles as attractive drug vehicles: Composition, properties and therapeutic strategies. *Materials Science and Engineering: C*, *68*, 982-994. https://doi.org/https://doi.org/10.1016/j.msec.2016.05.119

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. https://doi.org/https://doi.org/10.1016/j.foodres.2007.07.004

Giri, T. (2016). Nanoarchitectured Polysaccharide-Based Drug Carrier for Ocular. *Nanoarchitectonics for smart delivery and drug targeting*, 119.

Godet, M., Bizot, H., & Buleon, A. (1995). Crystallization of amylose—fatty acid complexes prepared with different amylose chain lengths. *Carbohydrate Polymers*, *27*(1), 47-52.

Gómez-Mascaraque, L. G., Lagarón, J. M., & López-Rubio, A. (2015). Electrosprayed gelatin submicroparticles as edible carriers for the encapsulation of polyphenols of interest in functional foods. *Food Hydrocolloids*, *49*, 42-52. https://doi.org/https://doi.org/10.1016/j.foodhyd.2015.03.006

Gopi, S., Pius, A., & Thomas, S. (2016). Biodegradable Green Composites. In *Biodegradable Green Composites* (pp. 1-17). https://doi.org/https://doi.org/10.1002/9781118911068.ch1

Grigalius, I., & Petrikaite, V. (2017). Relationship between antioxidant and anticancer activity of trihydroxyflavones. *Molecules*, *22*(12), 2169.

Gumireddy, A., Christman, R., Kumari, D., Tiwari, A., North, E. J., & Chauhan, H. (2019). Preparation, characterization, and in vitro evaluation of Curcumin-and resveratrol-loaded solid lipid nanoparticles. *AAPS PharmSciTech*, *20*(4), 145.

Guo, Y., Baschieri, A., Amorati, R., & Valgimigli, L. (2021). Synergic antioxidant activity of γ-terpinene with phenols and polyphenols enabled by hydroperoxyl radicals. *Food Chemistry*, *345*, 128468. https://doi.org/https://doi.org/10.1016/j.foodchem.2020.128468

Gupta, A. K., & Wells, S. (2004). Surface-Modified Superparamagnetic Nanoparticles for Drug Delivery: Preparation, Characterization, and Cytotoxicity Studies [Article]. *IEEE Transactions on Nanobioscience*, *3*(1), 66-73. https://doi.org/10.1109/TNB.2003.820277

Gupta, R. K., Patel, A. K., Shah, N., Choudhary, A. K., Jha, U. K., Yadav, U. C., Gupta, P. K., & Pakuwal, U. (2014). Oxidative stress and antioxidants in disease and cancer: a review. *Asian Pacific Journal of Cancer Prevention*, *15*(11), 4405-4409.

Guzmán, E., Mateos-Maroto, A., Ruano, M., Ortega, F., & Rubio, R. G. (2017). Layer-by-Layer polyelectrolyte assemblies for encapsulation and release of active compounds. *Advances*

in Colloid and Interface Science, *249*, 290-307. https://doi.org/https://doi.org/10.1016/j.cis.2017.04.009

He, X., & Hwang, H.-M. (2016). Nanotechnology in food science: Functionality, applicability, and safety assessment. *journal of food and drug analysis*, *24*(4), 671-681.

Hedrén, E., Mulokozi, G., & Svanberg, U. (2002). In vitro accessibility of carotenes from green leafy vegetables cooked with sunflower oil or red palm oil. *International Journal of Food Sciences and Nutrition*, *53*(6), 445-453.

Homma, Y., Ikeda, I., Ishikawa, T., Tateno, M., Sugano, M., & Nakamura, H. (2003). Decrease in plasma low-density lipoprotein cholesterol, apolipoprotein B, cholesteryl ester transfer protein, and oxidized low-density lipoprotein by plant stanol ester-containing spread:: A randomized, placebo-controlled trial. *Nutrition*, *19*(4), 369-374.

Hsieh, T. c., & Wu, J. M. (2010). Resveratrol: Biological and pharmaceutical properties as anticancer molecule. *Biofactors*, *36*(5), 360-369.

Hu, S.-H., Chen, S.-Y., & Gao, X. (2012). Multifunctional nanocapsules for simultaneous encapsulation of hydrophilic and hydrophobic compounds and on-demand release. *ACS nano*, *6*(3), 2558-2565.

Huang, M., Liang, C., Tan, C., Huang, S., Ying, R., Wang, Y., Wang, Z., & Zhang, Y. (2019). Liposome co-encapsulation as a strategy for the delivery of curcumin and resveratrol. *Food & function*, *10*(10), 6447-6458.

Hussain, Z., Thu, H. E., Amjad, M. W., Hussain, F., Ahmed, T. A., & Khan, S. (2017). Exploring recent developments to improve antioxidant, anti-inflammatory and antimicrobial efficacy of curcumin: A review of new trends and future perspectives. *Materials Science and Engineering: C*, *77*, 1316-1326. https://doi.org/https://doi.org/10.1016/j.msec.2017.03.226

Iqbal, R., Mehmood, Z., Baig, A., & Khalid, N. (2020). Formulation and characterization of food grade O/W nanoemulsions encapsulating quercetin and curcumin: Insights on enhancing solubility characteristics. *Food and Bioproducts Processing*, *123*, 304-311. https://doi.org/https://doi.org/10.1016/j.fbp.2020.07.013

Jackson, J. R., Ryan, M. J., Hao, Y., & Alway, S. E. (2010). Mediation of endogenous antioxidant enzymes and apoptotic signaling by resveratrol following muscle disuse in the gastrocnemius muscles of young and old rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *299*(6), R1572-R1581. https://doi.org/10.1152/ajpregu.00489.2010

Jani, P. U., McCarthy, D. E., & Florence, A. T. (1994). Titanium dioxide (rutile) particle uptake from the rat GI tract and translocation to systemic organs after oral administration. *International Journal of Pharmaceutics*, *105*(2), 157-168. https://doi.org/https://doi.org/10.1016/0378-5173(94)90461-8

Jayathilakan, K., Sharma, G. K., Radhakrishna, K., & Bawa, A. S. (2007). Antioxidant potential of synthetic and natural antioxidants and its effect on warmed-over-flavour in different species of meat. *Food Chemistry*, *105*(3), 908-916. https://doi.org/https://doi.org/10.1016/j.foodchem.2007.04.068

Ji, M., Gong, X., Li, X., Wang, C., & Li, M. (2020). Advanced Research on the Antioxidant Activity and Mechanism of Polyphenols from Hippophae Species-A Review. *Molecules (Basel, Switzerland)*, *25*(4), 917. https://doi.org/10.3390/molecules25040917

Joardder, M. U., Kumar, C., & Karim, M. (2017). Food structure: Its formation and relationships with other properties. *Critical reviews in food science and nutrition*, *57*(6), 1190-1205.

Joardder, M. U. H., Karim, A., & Kumar, C. (2013). Effect of moisture and temperature distribution on dried food microstructure and porosity. Proceedings of from model foods to food models: the DREAM project international conference,

Jones, O. G., Decker, E. A., & McClements, D. J. (2009). Formation of biopolymer particles by thermal treatment of β-lactoglobulin–pectin complexes. *Food Hydrocolloids*, *23*(5), 1312-1321.

Jones, O. G., Decker, E. A., & McClements, D. J. (2010). Comparison of protein–polysaccharide nanoparticle fabrication methods: Impact of biopolymer complexation before or after particle formation. *Journal of Colloid and Interface Science*, *344*(1), 21-29.

Jose, S., Anju, S. S., Cinu, T. A., Aleykutty, N. A., Thomas, S., & Souto, E. B. (2014). In vivo pharmacokinetics and biodistribution of resveratrol-loaded solid lipid nanoparticles for brain delivery. *International journal of pharmaceutics*, *474*(1), 6-13. https://doi.org/https://doi.org/10.1016/j.ijpharm.2014.08.003

Kamil, J. Y. V. A., Jeon, Y.-J., & Shahidi, F. (2002). Antioxidative activity of chitosans of different viscosity in cooked comminuted flesh of herring (Clupea harengus). *Food Chemistry*, *79*(1), 69-77. https://doi.org/https://doi.org/10.1016/S0308-8146(02)00180-2

Karpkird, T., Khunsakorn, R., Noptheeranuphap, C., & Midpanon, S. (2018). Inclusion complexes and photostability of UV filters and curcumin with beta-cyclodextrin polymers: effect on cross-linkers. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *91*(1-2), 37-45.

Katouzian, I., & Esfanjani, A. (2017). Release, Characterization and Safety of Nanoencapsulated Food Ingredients. In (pp. 401-453). https://doi.org/10.1016/B978-0-12-809740-3.00010-6

Katouzian, I., Faridi Esfanjani, A., Jafari, S. M., & Akhavan, S. (2017). Formulation and application of a new generation of lipid nano-carriers for the food bioactive ingredients. *Trends in Food Science & Technology*, *68*, 14-25. https://doi.org/https://doi.org/10.1016/j.tifs.2017.07.017

Keasler, V., De Paula, R. M., Nilsen, G., Grunwald, L., & Tidwell, T. J. (2017). 23 - Biocides overview and applications in petroleum microbiology. In A. M. El-Sherik (Ed.), *Trends in Oil and Gas Corrosion Research and Technologies* (pp. 539-562). Woodhead Publishing. https://doi.org/https://doi.org/10.1016/B978-0-08-101105-8.00023-1

Ketnawa, S., Reginio Jr, F. C., Thuengtung, S., & Ogawa, Y. (2021). Changes in bioactive compounds and antioxidant activity of plant-based foods by gastrointestinal digestion: A review. *Critical Reviews in Food Science and Nutrition*, 1-22.

Ketnawa, S., Suwannachot, J., & Ogawa, Y. (2020). In vitro gastrointestinal digestion of crisphead lettuce: Changes in bioactive compounds and antioxidant potential. *Food Chemistry*, *311*, 125885. https://doi.org/https://doi.org/10.1016/j.foodchem.2019.125885

Kfoury, M., Hădărugă, N. G., Hădărugă, D. I., & Fourmentin, S. (2016). 4 - Cyclodextrins as encapsulation material for flavors and aroma. In A. M. Grumezescu (Ed.), *Encapsulations* (pp. 127-192). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-804307-3.00004-1

Khalid, N., Kobayashi, I., Neves, M. A., Uemura, K., & Nakajima, M. (2018). Microchannel emulsification: A promising technique towards encapsulation of functional compounds. *Critical Reviews in Food Science and Nutrition*, *58*(14), 2364-2385.

Khan, M. A., Fang, Z., Cheng, H., Gao, Y., Deng, Z., & Liang, L. (2019). Encapsulation and protection of resveratrol in kafirin and milk protein nanoparticles. *International Journal of Food Science & Technology*, *54*(11), 2998-3007.

Kharat, M., Du, Z., Zhang, G., & McClements, D. J. (2017). Physical and chemical stability of curcumin in aqueous solutions and emulsions: impact of pH, temperature, and molecular environment. *Journal of agricultural and food chemistry*, *65*(8), 1525-1532.

Kim, H., Caulfield, L. E., Garcia-Larsen, V., Steffen, L. M., Grams, M. E., Coresh, J., & Rebholz, C. M. (2019). Plant-based diets and incident CKD and kidney function. *Clinical Journal of the American Society of Nephrology*, *14*(5), 682-691.

Kiziltepe, U., Turan, N. N. D., Han, U., Ulus, A. T., & Akar, F. (2004). Resveratrol, a red wine polyphenol, protects spinal cord from ischemia-reperfusion injury. *Journal of vascular surgery*, *40*(1), 138-145.

Kobayashi, I., Wada, Y., Uemura, K., & Nakajima, M. (2010). Microchannel emulsification for mass production of uniform fine droplets: integration of microchannel arrays on a chip. *Microfluidics and Nanofluidics*, *8*(2), 255-262.

Koehnlein, E. A., Koehnlein, É. M., Corrêa, R. C. G., Nishida, V. S., Correa, V. G., Bracht, A., & Peralta, R. M. (2016). Analysis of a whole diet in terms of phenolic content and antioxidant capacity: effects of a simulated gastrointestinal digestion. *International Journal of Food Sciences and Nutrition*, *67*(6), 614-623. https://doi.org/10.1080/09637486.2016.1186156

Konnova, T., Faizullin, D., Haertlé, T., & Zuev, Y. F. (2013). [beta]-casein micelle formation in water-ethanol solutions. Doklady. Biochemistry and biophysics,

Krokida, M., Karathanos, V., & Maroulis, Z. (1998). Effect of freeze-drying conditions on shrinkage and porosity of dehydrated agricultural products. *Journal of Food Engineering*, *35*(4), 369-380.

Krokida, M., & Maroulis, Z. (1997). Effect of drying method on shrinkage and porosity. *Drying Technology*, *15*(10), 2441-2458.

Kumar, Y., Yadav, D. N., Ahmad, T., & Narsaiah, K. (2015). Recent trends in the use of natural antioxidants for meat and meat products. *Comprehensive Reviews in Food Science and Food Safety*, *14*(6), 796-812.

Lamsen, M. R. L., Wang, T., D'Souza, D., Dia, V., Chen, G., & Zhong, Q. (2020). Encapsulation of vitamin D3 in gum arabic to enhance bioavailability and stability for beverage applications. *Journal of food science*, *85*(8), 2368-2379. https://doi.org/https://doi.org/10.1111/1750-3841.15340

Langevin, D. (1988). Microemulsions. *Accounts of Chemical Research*, *21*(7), 255-260.

Larson, A. J., Symons, J. D., & Jalili, T. (2012). Therapeutic potential of quercetin to decrease blood pressure: review of efficacy and mechanisms. *Advances in nutrition*, *3*(1), 39-46.

Le Bihan, O., Bonnafous, P., Marak, L., Bickel, T., Trépout, S., Mornet, S., De Haas, F., Talbot, H., Taveau, J.-C., & Lambert, O. (2009). Cryo-electron tomography of nanoparticle transmigration into liposome. *Journal of structural biology*, *168*(3), 419-425.

Lee, P. S., Yim, S. G., Choi, Y., Ha, T. V. A., & Ko, S. (2012). Physiochemical properties and prolonged release behaviours of chitosan-denatured β-lactoglobulin microcapsules for potential food applications. *Food Chemistry*, *134*(2), 992-998.

Lee, S. C., Lee, K. E., Kim, J. J., & Lim, S. H. (2005). The effect of cholesterol in the liposome bilayer on the stabilization of incorporated retinol [Article]. *Journal of Liposome Research*, *15*(3-4), 157-166. https://doi.org/10.1080/08982100500364131

Li, C., Sheng, L., Sun, G., & Wang, L. (2020). The application of ultraviolet-induced photo-crosslinking in edible film preparation and its implication in food safety. *LWT*, *131*, 109791. https://doi.org/https://doi.org/10.1016/j.lwt.2020.109791

Li, J., & Yan, D. (2002). Preparation and Characterization of the Crystalline Inclusion Complex between β‐Cyclodextrin and Poly (neopentyl glycol). *Macromolecular Chemistry and Physics*, *203*(1), 155-158.

Li, J., Yang, X., Li, X., Zhang, Z., Wei, Z., Xing, Z., Deng, S., & Duan, F. (2020). Okra polysaccharides/gelatin complex coacervate as pH-responsive and intestine-targeting delivery protects isoquercitin bioactivity. *International Journal of Biological Macromolecules*, *159*, 487-496. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2020.05.067

Li, M., Viravaidya, C., & Mann, S. (2007). Polymer‐Mediated Synthesis of Ferritin‐Encapsulated Inorganic Nanoparticles. *Small*, *3*(9), 1477-1481.

Li, X., Maldonado, L., Malmr, M., Rouf, T. B., Hua, Y., & Kokini, J. (2019). Development of hollow kafirin-based nanoparticles fabricated through layer-by-layer assembly as delivery vehicles for curcumin. *Food Hydrocolloids*, *96*, 93-101. https://doi.org/https://doi.org/10.1016/j.foodhyd.2019.04.042

Li, Y.-H., Wang, Y.-S., Zhao, J.-S., Li, Z.-Y., & Chen, H.-H. (2021). A pH-sensitive curcumin loaded microemulsion-filled alginate and porous starch composite gels: Characterization, in vitro release kinetics and biological activity. *International Journal of Biological Macromolecules*, *182*, 1863-1873. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2021.05.174

Lin, H.-L., Lin, S.-Y., Lin, C.-C., Hsu, C.-H., Wu, T.-K., & Huang, Y.-T. (2012). Mechanical grinding effect on thermodynamics and inclusion efficiency of loratadine–cyclodextrin inclusion complex formation. *Carbohydrate Polymers*, *87*(1), 512-517. https://doi.org/https://doi.org/10.1016/j.carbpol.2011.08.010

Lin, Y. G., Kunnumakkara, A. B., Nair, A., Merritt, W. M., Han, L. Y., Armaiz-Pena, G. N., Kamat, A. A., Spannuth, W. A., Gershenson, D. M., & Lutgendorf, S. K. (2007). Curcumin inhibits tumor growth and angiogenesis in ovarian carcinoma by targeting the nuclear factor-κB pathway. *Clinical Cancer Research*, *13*(11), 3423-3430.

Lingayat, V. J., Zarekar, N. S., & Shendge, R. S. (2017). Solid lipid nanoparticles: a review. *Nanoscience and Nanotechnology Research*, *2*, 67-72.

Liu, G.-S., Zhang, Z.-S., Yang, B., & He, W. (2012). Resveratrol attenuates oxidative damage and ameliorates cognitive impairment in the brain of senescence-accelerated mice. *Life Sciences*, *91*(17), 872-877. https://doi.org/https://doi.org/10.1016/j.lfs.2012.08.033

Liu, Y., Yang, R., Liu, J., Meng, D., Zhou, Z., Zhang, Y., & Blanchard, C. (2019). Fabrication, structure, and function evaluation of the ferritin based nano-carrier for food bioactive compounds. *Food Chemistry*, *299*, 125097. https://doi.org/https://doi.org/10.1016/j.foodchem.2019.125097

López-Hernández, J., Paseiro-Losada, P., Sanches-Silva, A. T., & Lage-Yusty, M. A. (2007). Study of the changes of trans-resveratrol caused by ultraviolet light and determination of trans- and cis-resveratrol in Spanish white wines. *European Food Research and Technology*, *225*(5), 789-796. https://doi.org/10.1007/s00217-006-0483-x

Lu, W., Kelly, A. L., & Miao, S. (2016). Emulsion-based encapsulation and delivery systems for polyphenols. *Trends in Food Science & Technology*, *47*, 1-9.

Lu, Z., Chen, R., Liu, H., Hu, Y., Cheng, B., & Zou, G. (2009). Study of the complexation of resveratrol with cyclodextrins by spectroscopy and molecular modeling. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *63*(3-4), 295-300.

Lucas-González, R., Viuda-Martos, M., Pérez-Alvarez, J. A., & Fernández-López, J. (2018). In vitro digestion models suitable for foods: Opportunities for new fields of application and challenges. *Food Research International*, *107*, 423-436.

Ma, C., Zhuang, Z., Su, Q., He, J., & Li, H. (2020). Curcumin Has Anti-Proliferative and Pro-Apoptotic Effects on Tongue Cancer in vitro: A Study with Bioinformatics Analysis and in vitro Experiments. *Drug Design, Development and Therapy*, *14*, 509.

Ma, X., Liu, X., Anderson, D. P., & Chang, P. R. (2015). Modification of porous starch for the adsorption of heavy metal ions from aqueous solution. *Food Chemistry*, *181*, 133-139. https://doi.org/https://doi.org/10.1016/j.foodchem.2015.02.089

Maan, A. A., Nazir, A., Khan, M. K. I., Boom, R., & Schroën, K. (2015). Microfluidic emulsification in food processing. *Journal of Food Engineering*, *147*, 1-7.

Malafaya, P. B., Elvira, C., Gallardo, A., San Román, J., & Reis, R. L. (2001). Porous starch-based drug delivery systems processed by a microwave route. *Journal of Biomaterials Science, Polymer Edition*, *12*(11), 1227-1241. https://doi.org/10.1163/156856201753395761

Maldonado-Valderrama, J., & Patino, J. M. R. (2010). Interfacial rheology of protein–surfactant mixtures. *Current Opinion in Colloid & Interface Science*, *15*(4), 271-282.

Marchiani, A., Rozzo, C., Fadda, A., Delogu, G., & Ruzza, P. (2014). Curcumin and curcumin-like molecules: from spice to drugs. *Current medicinal chemistry*, *21*(2), 204-222.

Mardhiah Adib, Z., Ghanbarzadeh, S., Kouhsoltani, M., Yari Khosroshahi, A., & Hamishehkar, H. (2016). The Effect of Particle Size on the Deposition of Solid Lipid Nanoparticles in Different Skin Layers: A Histological Study. *Adv Pharm Bull*, *6*(1), 31-36. https://doi.org/10.15171/apb.2016.06

Martínez-Las Heras, R., Pinazo, A., Heredia, A., & Andrés, A. (2017). Evaluation studies of persimmon plant (Diospyros kaki) for physiological benefits and bioaccessibility of antioxidants by in vitro simulated gastrointestinal digestion. *Food Chemistry*, *214*, 478-485. https://doi.org/https://doi.org/10.1016/j.foodchem.2016.07.104

Matalanis, A., Jones, O. G., & McClements, D. J. (2011). Structured biopolymer-based delivery systems for encapsulation, protection, and release of lipophilic compounds. *Food Hydrocolloids*, *25*(8), 1865-1880.

Matos, M., Laca, A., Rea, F., Iglesias, O., Rayner, M., & Gutiérrez, G. (2018). O/W emulsions stabilized by OSA-modified starch granules versus non-ionic surfactant: Stability, rheological behaviour and resveratrol encapsulation. *Journal of Food Engineering*, *222*, 207-217. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2017.11.009

McClements, D. J. (2019). *Nanoparticle-and microparticle-based delivery systems: Encapsulation, protection and release of active compounds*. CRC press.

McClements, D. J., & Decker, E. A. (2000). Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems. *Journal of food science*, *65*(8), 1270-1282. https://doi.org/https://doi.org/10.1111/j.1365-2621.2000.tb10596.x

McClements, D. J., Decker, E. A., & Weiss, J. (2007). Emulsion‐based delivery systems for lipophilic bioactive components. *Journal of food science*, *72*(8), R109-R124.

McMacken, M., & Shah, S. (2017). A plant-based diet for the prevention and treatment of type 2 diabetes. *Journal of geriatric cardiology: JGC*, *14*(5), 342.

Min, B., & Ahn, D. (2005). Mechanism of lipid peroxidation in meat and meat products-A review. *Food Science and Biotechnology*, *14*(1), 152-163.

Mitjavila, M., & Moreno, J. (2012). The effects of polyphenols on oxidative stress and the arachidonic acid cascade. Implications for the prevention/treatment of high prevalence diseases. *Biochemical Pharmacology*, *84*(9), 1113-1122.

Mlcek, J., Jurikova, T., Skrovankova, S., & Sochor, J. (2016). Quercetin and its anti-allergic immune response. *Molecules*, *21*(5), 623.

Mohseni, R., ArabSadeghabadi, Z., Ziamajidi, N., Abbasalipourkabir, R., & RezaeiFarimani, A. (2019). Oral administration of resveratrol-loaded solid lipid nanoparticle improves insulin resistance through targeting expression of SNARE proteins in adipose and muscle tissue in rats with type 2 diabetes. *Nanoscale research letters*, *14*(1), 227.

Molnar, K., & Nagy, Z. K. (2016). Corona-electrospinning: Needleless method for high-throughput continuous nanofiber production. *European Polymer Journal*, *74*, 279-286. https://doi.org/https://doi.org/10.1016/j.eurpolymj.2015.11.028

Momić, T., Savić, J., Černigoj, U., Trebše, P., & Vasić, V. (2007). Protolytic equilibria and photodegradation of quercetin in aqueous solution. *Collection of Czechoslovak Chemical Communications*, *72*(11), 1447-1460.

Montsko, G., Nikfardjam, M. S. P., Szabo, Z., Boddi, K., Lorand, T., Ohmacht, R., & Mark, L. (2008). Determination of products derived from trans-resveratrol UV photoisomerisation by means of HPLC–APCI-MS. *Journal of Photochemistry and Photobiology A: Chemistry*, *196*(1), 44-50. https://doi.org/https://doi.org/10.1016/j.jphotochem.2007.11.011

Moon, Y. J., Wang, L., DiCenzo, R., & Morris, M. E. (2008). Quercetin pharmacokinetics in humans. *Biopharmaceutics & Drug Disposition*, *29*(4), 205-217. https://doi.org/https://doi.org/10.1002/bdd.605

Mui, W. W., Durance, T. D., & Scaman, C. H. (2002). Flavor and texture of banana chips dried by combinations of hot air, vacuum, and microwave processing. *Journal of agricultural and food chemistry*, *50*(7), 1883-1889.

Mukherjee, S., Ray, S., & Thakur, R. (2009). Solid lipid nanoparticles: a modern formulation approach in drug delivery system. *Indian journal of pharmaceutical sciences*, *71*(4), 349.

Murias, M., Jäger, W., Handler, N., Erker, T., Horvath, Z., Szekeres, T., Nohl, H., & Gille, L. (2005). Antioxidant, prooxidant and cytotoxic activity of hydroxylated resveratrol analogues: structure–activity relationship. *Biochemical Pharmacology*, *69*(6), 903-912. https://doi.org/https://doi.org/10.1016/j.bcp.2004.12.001

Muzzarelli, R. A., El Mehtedi, M., Bottegoni, C., Aquili, A., & Gigante, A. (2015). Genipin-crosslinked chitosan gels and scaffolds for tissue engineering and regeneration of cartilage and bone. *Marine drugs*, *13*(12), 7314-7338.

Nabi, F., Arain, M. A., Rajput, N., Alagawany, M., Soomro, J., Umer, M., Soomro, F., Wang, Z., Ye, R., & Liu, J. (2020). Health benefits of carotenoids and potential application in poultry industry: A review. *Journal of animal physiology and animal nutrition*, *104*(6), 1809-1818.

Nagarajan, S., Nagarajan, R., Kumar, J., Salemme, A., Togna, A. R., Saso, L., & Bruno, F. (2020). Antioxidant activity of synthetic polymers of phenolic compounds. *Polymers*, *12*(8), 1646.

Ndiaye, M., Kumar, R., & Ahmad, N. (2011). Resveratrol in cancer management: where are we and where we go from here? *Annals of the New York Academy of Sciences*, *1215*(1), 144-149. https://doi.org/https://doi.org/10.1111/j.1749-6632.2010.05851.x

Neves, A. R., Lúcio, M., Lima, J. L. C., & Reis, S. (2012). Resveratrol in medicinal chemistry: A critical review of its pharmacokinetics, drug-delivery, and membrane interactions [Review]. *Current Medicinal Chemistry*, *19*(11), 1663-1681. https://doi.org/10.2174/092986712799945085

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.

Neves, M. I. L., Desobry-Banon, S., Perrone, I. T., Desobry, S., & Petit, J. (2019). Encapsulation of curcumin in milk powders by spray-drying: Physicochemistry, rehydration properties, and stability during storage. *Powder Technology*, *345*, 601-607. https://doi.org/https://doi.org/10.1016/j.powtec.2019.01.049

Nollet, L. M., & Toldrá, F. (2008). *Handbook of muscle foods analysis*. CRC press.

Noorafshan, A., & Ashkani-Esfahani, S. (2013). A review of therapeutic effects of curcumin. *Current pharmaceutical design*, *19*(11), 2032-2046.

Nunes, I. L., & Mercadante, A. Z. (2007). Encapsulation of lycopene using spray-drying and molecular inclusion processes. *Brazilian Archives of Biology and Technology*, *50*(5), 893-900.

Ochs, C. J., Such, G. K., Städler, B., & Caruso, F. (2008). Low-fouling, biofunctionalized, and biodegradable click capsules. *Biomacromolecules*, *9*(12), 3389-3396.

Ogawa, S., Decker, E. A., & McClements, D. J. (2003). Production and characterization of O/W emulsions containing cationic droplets stabilized by lecithin− chitosan membranes. *Journal of agricultural and food chemistry*, *51*(9), 2806-2812.

Olas, B., Wachowicz, B., Nowak, P., Stochmal, A., Oleszek, W., Glowacki, R., & Bald, E. (2008). Comparative studies of the antioxidant effects of a naturally occurring resveratrol analogue‐trans-3, 3′, 5, 5′-tetrahydroxy-4′-methoxystilbene and resveratrol–against oxidation and nitration of biomolecules in blood platelets. *Cell Biology and Toxicology*, *24*(4), 331-340.

Ozkan, G., Franco, P., De Marco, I., Xiao, J., & Capanoglu, E. (2019). A review of microencapsulation methods for food antioxidants: Principles, advantages, drawbacks and applications. *Food Chemistry*, *272*, 494-506. https://doi.org/https://doi.org/10.1016/j.foodchem.2018.07.205

Ozkan, G., Kostka, T., Esatbeyoglu, T., & Capanoglu, E. (2020). Effects of Lipid-Based Encapsulation on the Bioaccessibility and Bioavailability of Phenolic Compounds. *Molecules*, *25*(23), 5545.

Pando, D., Caddeo, C., Manconi, M., Fadda, A. M., & Pazos, C. (2013). Nanodesign of olein vesicles for the topical delivery of the antioxidant resveratrol. *Journal of Pharmacy and Pharmacology*, *65*(8), 1158-1167.

Parada, J., & Aguilera, J. (2007). Food microstructure affects the bioavailability of several nutrients. *Journal of food science*, *72*(2), R21-R32.

Park, E. Y., Choi, S. M., Lim, S.-T., & Kim, J.-Y. (2018). Effects of dextrinization and octenylsuccinylation of high amylose starch on complex formation with ω-3 fatty acids (EPA/DHA). *Food Hydrocolloids*, *77*, 357-362.

Parveen, S., & Sahoo, S. K. (2008). Polymeric nanoparticles for cancer therapy. *Journal of Drug Targeting*, *16*(2), 108-123. https://doi.org/10.1080/10611860701794353

Peñalva, R., Morales, J., González-Navarro, C. J., Larrañeta, E., Quincoces, G., Peñuelas, I., & Irache, J. M. (2018). Increased oral bioavailability of resveratrol by its encapsulation in casein nanoparticles. *International journal of molecular sciences*, *19*(9), 2816.

Pérez-Jiménez, J., Neveu, V., Vos, F., & Scalbert, A. (2010). Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. *European journal of clinical nutrition*, *64*(3), S112-S120.

Petrarca, C., Mangifesta, R., & Di Giampaolo, L. (2020). Immunotoxicity of Nanoparticles. In *Allergy and Immunotoxicology in Occupational Health-The Next Step* (pp. 75-94). Springer.

Petrović, T., Nedović, V., Dimitrijević-Branković, S., Bugarski, B., & Lacroix, C. (2007). Protection of probiotic microorganisms by microencapsulation. *Chemical Industry and Chemical Engineering Quarterly*, *13*(3), 169-174.

Pfluger, P., Kluth, D., Landes, N., Bumke-Vogt, C., & Brigelius-Flohé, R. (2004). Vitamin E: underestimated as an antioxidant. *Redox report*, *9*(5), 249-254.

Pieczywek, P. M., & Zdunek, A. (2012). Automatic classification of cells and intercellular spaces of apple tissue. *Computers and electronics in agriculture*, *81*, 72-78.

Prabu, G. T. V., & Dhurai, B. (2020). A Novel Profiled Multi-Pin Electrospinning System for Nanofiber Production and Encapsulation of Nanoparticles into Nanofibers. *Scientific reports*, *10*(1), 4302. https://doi.org/10.1038/s41598-020-60752-6

Prabu, S., & Mohamad, S. (2020). Curcumin/beta-cyclodextrin inclusion complex as a new "turn-off" fluorescent sensor system for sensitive recognition of mercury ion. *Journal of Molecular Structure*, *1204*, 127528.

Priyadarsini, K. I. (2013). Chemical and structural features influencing the biological activity of curcumin. *Current pharmaceutical design*, *19*(11), 2093-2100.

Priyadarsini, K. I. (2014). The chemistry of curcumin: from extraction to therapeutic agent. *Molecules*, *19*(12), 20091-20112.

Priyanka, A., Anusree, S. S., Nisha, V. M., & Raghu, K. G. (2014). Curcumin improves hypoxia induced dysfunctions in 3T3‐L1 adipocytes by protecting mitochondria and down regulating inflammation. *Biofactors*, *40*(5), 513-523.

Radziejewska-Kubzdela, E., Biegańska-Marecik, R., & Kidoń, M. (2014). Applicability of vacuum impregnation to modify physico-chemical, sensory and nutritive characteristics of plant origin products—a review. *International journal of molecular sciences*, *15*(9), 16577-16610.

Rahman, M. S. (2001). Toward prediction of porosity in foods during drying: a brief review. *Drying Technology*, *19*(1), 1-13.

Ramalingam, P., Yoo, S. W., & Ko, Y. T. (2016). Nanodelivery systems based on mucoadhesive polymer coated solid lipid nanoparticles to improve the oral intake of food curcumin. *Food Research International*, *84*, 113-119. https://doi.org/https://doi.org/10.1016/j.foodres.2016.03.031

Raoult-Wack, A.-L. (1994). Recent advances in the osmotic dehydration of foods. *Trends in Food Science & Technology*, *5*(8), 255-260.

Rauscher, H., Roebben, G., Sanfeliu, A. B., Emons, H., Gibson, N., Koeber, R., Linsinger, T., Rasmussen, K., Sintes, J. R., & Sokull-Klüttgen, B. (2014). Towards a review of the EC Recommendation for a definition of the term "nanomaterial". *Eur Union. doi*, *10*, 36237.

Raviadaran, R., Chandran, D., Shin, L. H., & Manickam, S. (2018). Optimization of palm oil in water nano-emulsion with curcumin using microfluidizer and response surface methodology. *LWT*, *96*, 58-65. https://doi.org/https://doi.org/10.1016/j.lwt.2018.05.022

Reginio, F. C., Qin, W., Ketnawa, S., & Ogawa, Y. (2020). Bio-properties of Saba banana (Musa 'saba', ABB Group): Influence of maturity and changes during simulated in vitro gastrointestinal digestion. *Scientific reports*, *10*(1), 1-11.

Rein, M. J., Renouf, M., Cruz‐Hernandez, C., Actis‐Goretta, L., Thakkar, S. K., & da Silva Pinto, M. (2013). Bioavailability of bioactive food compounds: a challenging journey to bioefficacy. *British journal of clinical pharmacology*, *75*(3), 588-602.

Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in plant science*, *2*(4), 152-159.

Rodríguez‐Félix, F., Del‐Toro‐Sánchez, C. L., Javier Cinco‐Moroyoqui, F., Juárez, J., Ruiz‐Cruz, S., López‐Ahumada, G. A., Carvajal‐Millan, E., Castro‐Enríquez, D. D., Barreras‐Urbina, C. G., & Tapia‐Hernández, J. A. (2019). Preparation and Characterization of Quercetin‐Loaded Zein Nanoparticles by Electrospraying and Study of In Vitro Bioavailability. *Journal of food science*, *84*(10), 2883-2897.

Roleira, F. M., Siquet, C., Orrù, E., Garrido, E. M., Garrido, J., Milhazes, N., Podda, G., Paiva-Martins, F., Reis, S., & Carvalho, R. A. (2010). Lipophilic phenolic antioxidants: Correlation between antioxidant profile, partition coefficients and redox properties. *Bioorganic & Medicinal Chemistry*, *18*(16), 5816-5825.

Roohi, B. N., Moradlou, A. N., & Bolboli, L. (2017). Influence of curcumin supplementation on exercise-induced oxidative stress. *Asian J Sports Med*, *8*, e35776.

Rydzak, L., Sagan, A., Andrejko, D., Starek, A., Ślaska-Grzywna, B., Blicharz-Kania, A., Kobus, Z., & Natoniewski, M. (2017). Changes in Particle Size Distribution of Bran and Flour Obtained from Vacuum Impregnated and Infra-red Heated Wheat Grain. *Food and Bioprocess Technology*, *10*(11), 1997-2004. https://doi.org/10.1007/s11947-017-1949-5

Salaün, F. (2016). 9 - Microencapsulation technology for smart textile coatings. In J. Hu (Ed.), *Active Coatings for Smart Textiles* (pp. 179-220). Woodhead Publishing. https://doi.org/https://doi.org/10.1016/B978-0-08-100263-6.00009-5

Salminen, H., Helgason, T., Kristinsson, B., Kristbergsson, K., & Weiss, J. (2013). Formation of solid shell nanoparticles with liquid ω-3 fatty acid core. *Food chemistry*, *141*(3), 2934-2943.

Sam Saguy, I., Marabi, A., & Wallach, R. (2005). New approach to model rehydration of dry food particulates utilizing principles of liquid transport in porous media. *Trends in Food Science & Technology*, *16*(11), 495-506. https://doi.org/https://doi.org/10.1016/j.tifs.2005.07.006

Sambasevam, K. P., Mohamad, S., Sarih, N. M., & Ismail, N. A. (2013). Synthesis and characterization of the inclusion complex of β-cyclodextrin and azomethine. *International journal of molecular sciences*, *14*(2), 3671-3682.

Sarabandi, K., Jafari, S. M., Mahoonak, A. S., & Mohammadi, A. (2019). Application of gum Arabic and maltodextrin for encapsulation of eggplant peel extract as a natural antioxidant and color source. *International Journal of Biological Macromolecules*, *140*, 59-68. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2019.08.133

Sarafian, T. A., Kouyoumjian, S., Tashkin, D., & Roth, M. D. (2002). Synergistic cytotoxicity of Δ9-tetrahydrocannabinol and butylated hydroxyanisole. *Toxicology letters*, *133*(2-3), 171-179.

Schneider, C., Gordon, O. N., Edwards, R. L., & Luis, P. B. (2015). Degradation of curcumin: from mechanism to biological implications. *Journal of agricultural and food chemistry*, *63*(35), 7606-7614.

Seethu, B., Pushpadass, H. A., Emerald, F. M. E., Nath, B. S., Naik, N. L., & Subramanian, K. (2020). Electrohydrodynamic encapsulation of resveratrol using food-grade nanofibres: Process optimization, characterization and fortification. *Food and Bioprocess Technology*, *13*(2), 341-354.

Sengottuvelan, M., Deeptha, K., & Nalini, N. (2009). Resveratrol ameliorates DNA damage, prooxidant and antioxidant imbalance in 1,2-dimethylhydrazine induced rat colon carcinogenesis. *Chemico-Biological Interactions*, *181*(2), 193-201. https://doi.org/https://doi.org/10.1016/j.cbi.2009.06.004

Senthamizhan, A., Balusamy, B., & Uyar, T. (2017). 1 - Electrospinning: A versatile processing technology for producing nanofibrous materials for biomedical and tissue-engineering applications. In T. Uyar & E. Kny (Eds.), *Electrospun Materials for Tissue Engineering and Biomedical Applications* (pp. 3-41). Woodhead Publishing. https://doi.org/https://doi.org/10.1016/B978-0-08-101022-8.00001-6

Shahidi, F. (1994). Assessment of lipid oxidation and off-flavour development in meat and meat products. In *Flavor of meat and meat products* (pp. 247-266). Springer.

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of functional foods*, *18*, 820-897.

Shahidi, F., & Han, X. Q. (1993). Encapsulation of food ingredients. *Critical Reviews in Food Science & Nutrition*, *33*(6), 501-547.

Shahidi, F., & Zhong, Y. (2010). Novel antioxidants in food quality preservation and health promotion. *European Journal of Lipid Science and Technology*, *112*(9), 930-940. https://doi.org/https://doi.org/10.1002/ejlt.201000044

Shi, J., & Le Maguer, M. (2002). Osmotic dehydration of foods: mass transfer and modeling aspects. *Food Reviews International*, *18*(4), 305-335.

Shi, X., & Caruso, F. (2001). Release behavior of thin-walled microcapsules composed of polyelectrolyte multilayers. *Langmuir*, *17*(6), 2036-2042.

Shishir, M. R. I., Xie, L., Sun, C., Zheng, X., & Chen, W. (2018). Advances in micro and nano-encapsulation of bioactive compounds using biopolymer and lipid-based transporters. *Trends in Food Science & Technology*, *78*, 34-60.

Shoskes, D. A., Zeitlin, S. I., Shahed, A., & Rajfer, J. (1999). Quercetin in men with category III chronic prostatitis: a preliminary prospective, double-blind, placebo-controlled trial. *Urology*, *54*(6), 960-963.

Silva, C. G., Monteiro, J., Marques, R. R., Silva, A. M., Martínez, C., Canle, M., & Faria, J. L. (2013). Photochemical and photocatalytic degradation of trans-resveratrol. *Photochemical & Photobiological Sciences*, *12*(4), 638-644.

Silva, F., Figueiras, A., Gallardo, E., Nerín, C., & Domingues, F. C. (2014). Strategies to improve the solubility and stability of stilbene antioxidants: A comparative study between cyclodextrins and bile acids. *Food Chemistry*, *145*, 115-125. https://doi.org/https://doi.org/10.1016/j.foodchem.2013.08.034

Silva, T., Pokhrel, L. R., Dubey, B., Tolaymat, T. M., Maier, K. J., & Liu, X. (2014). Particle size, surface charge and concentration dependent ecotoxicity of three organo-coated silver nanoparticles: Comparison between general linear model-predicted and observed toxicity. *Science of The Total Environment*, *468-469*, 968-976. https://doi.org/https://doi.org/10.1016/j.scitotenv.2013.09.006

Sitte, P., Falk, H., & Liedvogel, B. (1980). Chromoplasts. *Pigments in plants*, 117-148.

Sørensen, G., & Jørgensen, S. S. (1996). A critical examination of some experimental variables in the 2-thiobarbituric acid (TBA) test for lipid oxidation in meat products. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *202*(3), 205-210.

Soto‐Valdez, H., Auras, R., & Peralta, E. (2011). Fabrication of poly (lactic acid) films with resveratrol and the diffusion of resveratrol into ethanol. *Journal of Applied Polymer Science*, *121*(2), 970-978.

Souto, E. B., & Müller, R. H. (2010). Lipid nanoparticles: effect on bioavailability and pharmacokinetic changes. In *Drug delivery* (pp. 115-141). Springer.

Souza, M. P., Vaz, A. F., Costa, T. B., Cerqueira, M. A., De Castro, C. M., Vicente, A. A., & Carneiro-da-Cunha, M. G. (2018). Construction of a biocompatible and antioxidant multilayer coating by layer-by-layer assembly of κ-carrageenan and quercetin nanoparticles. *Food and Bioprocess Technology*, *11*(5), 1050-1060.

Stahl, W., & Sies, H. (2003). Antioxidant activity of carotenoids. *Molecular Aspects of Medicine*, *24*(6), 345-351. https://doi.org/https://doi.org/10.1016/S0098-2997(03)00030-X

Sugiura, S., Nakajima, M., Iwamoto, S., & Seki, M. (2001). Interfacial tension driven monodispersed droplet formation from microfabricated channel array. *Langmuir*, *17*(18), 5562-5566.

Summerlin, N., Soo, E., Thakur, S., Qu, Z., Jambhrunkar, S., & Popat, A. (2015). Resveratrol nanoformulations: challenges and opportunities. *International journal of pharmaceutics*, *479*(2), 282-290.

Sun-Waterhouse, D., Wadhwa, S. S., & Waterhouse, G. I. (2013). Spray-drying microencapsulation of polyphenol bioactives: a comparative study using different natural fibre polymers as encapsulants. *Food and Bioprocess Technology*, *6*(9), 2376-2388.

Taghvaei, M., & Jafari, S. M. (2015). Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. *Journal of food science and technology*, *52*(3), 1272-1282. https://doi.org/10.1007/s13197-013-1080-1

Takigawa, T., & Endo, Y. (2006). Effects of glutaraldehyde exposure on human health. *Journal of occupational health*, *48*(2), 75-87.

Tamnak, S., Mirhosseini, H., Tan, C. P., Amid, B. T., Kazemi, M., & Hedayatnia, S. (2016). Encapsulation properties, release behavior and physicochemical characteristics of water-in-oil-in-water (W/O/W) emulsion stabilized with pectin–pea protein isolate conjugate and Tween 80. *Food Hydrocolloids*, *61*, 599-608.

Tetley, T. D. (2007). Health effects of nanomaterials [Conference Paper]. *Biochemical Society Transactions*, *35*(3), 527-531. https://doi.org/10.1042/BST0350527

Thanasukarn, P., Pongsawatmanit, R., & McClements, D. J. (2006). Utilization of layer-by-layer interfacial deposition technique to improve freeze–thaw stability of oil-in-water emulsions. *Food Research International*, *39*(6), 721-729. https://doi.org/https://doi.org/10.1016/j.foodres.2006.01.010

Thiewes, H. J., & Steeneken, P. A. M. (1997). The glass transition and the sub-Tg endotherm of amorphous and native potato starch at low moisture content. *Carbohydrate Polymers*, *32*(2), 123-130. https://doi.org/https://doi.org/10.1016/S0144-8617(96)00133-6

Thompson, A. K., Couchoud, A., & Singh, H. (2009). Comparison of hydrophobic and hydrophilic encapsulation using liposomes prepared from milk fat globule-derived phospholipids and soya phospholipids. *Dairy science & technology*, *89*(1), 99-113.

Timilsena, Y. P., Haque, M. A., & Adhikari, B. (2020). Encapsulation in the Food Industry: A Brief Historical Overview to Recent Developments. *Food and Nutrition Sciences*, *11*(06), 481.

Timilsena, Y. P., Vongsvivut, J., Tobin, M. J., Adhikari, R., Barrow, C., & Adhikari, B. (2019). Investigation of oil distribution in spray-dried chia seed oil microcapsules using synchrotron-FTIR microspectroscopy. *Food Chemistry*, *275*, 457-466.

Tiwari, A., Bindal, S., & Bohidar, H. B. (2009). Kinetics of Protein−Protein Complex Coacervation and Biphasic Release of Salbutamol Sulfate from Coacervate Matrix. *Biomacromolecules*, *10*(1), 184-189. https://doi.org/10.1021/bm801160s

Tønnesen, H. H., Másson, M., & Loftsson, T. (2002). Studies of curcumin and curcuminoids. XXVII. Cyclodextrin complexation: solubility, chemical and photochemical stability. *International Journal of Pharmaceutics*, *244*(1), 127-135. https://doi.org/https://doi.org/10.1016/S0378-5173(02)00323-X

Torreggiani, D. (1993). Osmotic dehydration in fruit and vegetable processing. *Food Research International*, *26*(1), 59-68.

Trautwein, E. A., & Demonty, I. (2007). Phytosterols: natural compounds with established and emerging health benefits. *Oléagineux, Corps Gras, Lipides*, *14*(5), 259-266.

Trela, B. C., & Waterhouse, A. L. (1996). Resveratrol: isomeric molar absorptivities and stability. *Journal of agricultural and food chemistry*, *44*(5), 1253-1257.

Troncoso, E., & Pedreschi, F. (2007). Modeling of textural changes during drying of potato slices. *Journal of Food Engineering*, *82*(4), 577-584.

Uddin, M. S., Al Mamun, A., Kabir, M. T., Ahmad, J., Jeandet, P., Sarwar, M. S., Ashraf, G. M., & Aleya, L. (2020). Neuroprotective role of polyphenols against oxidative stress-mediated neurodegeneration. *European Journal of Pharmacology*, 173412.

Udenni Gunathilake, T., Ching, Y. C., Ching, K. Y., Chuah, C. H., & Abdullah, L. C. (2017). Biomedical and microbiological applications of bio-based porous materials: A review. *Polymers*, *9*(5), 160.

Vatankhah, H., & Ramaswamy, H. S. (2017). Dynamics of fluid migration into porous solid matrix during high pressure treatment. *Food and Bioproducts Processing*, *103*, 122-130. https://doi.org/https://doi.org/10.1016/j.fbp.2017.03.007

Vehring, R. (2008). Pharmaceutical particle engineering via spray drying. *Pharmaceutical research*, *25*(5), 999-1022.

Verbelen, P. J., De Schutter, D. P., Delvaux, F., Verstrepen, K. J., & Delvaux, F. R. (2006). Immobilized yeast cell systems for continuous fermentation applications. *Biotechnology Letters*, *28*(19), 1515-1525. https://doi.org/10.1007/s10529-006-9132-5

Vladimir-Knežević, S., Blažeković, B., Štefan, M. B., & Babac, M. (2012). *Plant polyphenols as antioxidants influencing the human health*. Intechopen.

Voulgaropoulou, S., van Amelsvoort, T., Prickaerts, J., & Vingerhoets, C. (2019). The effect of curcumin on cognition in Alzheimer's disease and healthy aging: A systematic review of pre-clinical and clinical studies. *Brain Research*, *1725*, 146476.

Wakte, P. S., Sachin, B. S., Patil, A. A., Mohato, D. M., Band, T. H., & Shinde, D. B. (2011). Optimization of microwave, ultra-sonic and supercritical carbon dioxide assisted extraction techniques for curcumin from Curcuma longa. *Separation and Purification Technology*, *79*(1), 50-55. https://doi.org/https://doi.org/10.1016/j.seppur.2011.03.010

Wang, J., Shi, A., Agyei, D., & Wang, Q. (2017). Formulation of water-in-oil-in-water (W/O/W) emulsions containing trans-resveratrol. *RSC advances*, *7*(57), 35917-35927.

Wang, L., Zhao, X., Yang, F., Wu, W., Wu, M., Li, Y., & Zhang, X. (2019). Loading paclitaxel into porous starch in the form of nanoparticles to improve its dissolution and bioavailability. *International Journal of Biological Macromolecules*, *138*, 207-214. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2019.07.083

Wang, P., Li, Y., Zhang, C., Feng, F., & Zhang, H. (2020). Sequential electrospinning of multilayer ethylcellulose/gelatin/ethylcellulose nanofibrous film for sustained release of curcumin. *Food Chemistry*, *308*, 125599. https://doi.org/https://doi.org/10.1016/j.foodchem.2019.125599

Wang, W., Sun, C., Mao, L., Ma, P., Liu, F., Yang, J., & Gao, Y. (2016). The biological activities, chemical stability, metabolism and delivery systems of quercetin: A review. *Trends in Food Science & Technology*, *56*, 21-38.

Wang, Z., Zou, J., Huang, Y., Cao, K., Xu, Y., & Wu, J. M. (2002). Effect of resveratrol on platelet aggregation in vivo and in vitro. *Chinese medical journal*, *115*(3), 378-380. http://europepmc.org/abstract/MED/11940369

http://www.cmj.org/information/full.asp?pmid=20023378

Wasowicz, E., Gramza, A., Hes, M., Jelen, H., Korczak, J., Malecka, M., Mildner-Szkudlarz, S., Rudzinska, M., Samotyja, U., & Zawirska-Wojtasiak, R. (2004). Oxidation of lipids in food. *Polish journal of food and nutrition sciences*, *13*(Spec. Issue 1).

Weiss, J., Decker, E. A., McClements, D. J., Kristbergsson, K., Helgason, T., & Awad, T. (2008). Solid Lipid Nanoparticles as Delivery Systems for Bioactive Food Components. *Food Biophysics*, *3*(2), 146-154. https://doi.org/10.1007/s11483-008-9065-8

Wong, R. W. K., & Rabie, A. B. M. (2008). Effect of quercetin on preosteoblasts and bone defects. *The open orthopaedics journal*, *2*, 27-32. https://doi.org/10.2174/1874325000802010027

Xia, Z., McClements, D. J., & Xiao, H. (2015). Influence of physical state of β-carotene (crystallized versus solubilized) on bioaccessibility. *Journal of agricultural and food chemistry*, *63*(3), 990-997.

Xie, H., Xiang, C., Li, Y., Wang, L., Zhang, Y., Song, Z., Ma, X., Lu, X., Lei, Q., & Fang, W. (2019). Fabrication of ovalbumin/κ-carrageenan complex nanoparticles as a novel carrier for curcumin delivery. *Food Hydrocolloids*, *89*, 111-121. https://doi.org/https://doi.org/10.1016/j.foodhyd.2018.10.027

Xue, J., Wang, T., Hu, Q., Zhou, M., & Luo, Y. (2018). Insight into natural biopolymer-emulsified solid lipid nanoparticles for encapsulation of curcumin: Effect of loading methods. *Food Hydrocolloids*, *79*, 110-116.

Yang, Y.-B., & Piao, Y.-J. (2003). Effects of resveratrol on secondary damages after acute spinal cord injury in rats. *Acta Pharmacologica Sinica*, *24*(7), 703-710.

Yao, M., Xiao, H., & McClements, D. J. (2014). Delivery of Lipophilic Bioactives: Assembly, Disassembly, and Reassembly of Lipid Nanoparticles. *Annual review of food science and technology*, *5*(1), 53-81. https://doi.org/10.1146/annurev-food-072913-100350

Yoon, G., Park, J. W., & Yoon, I.-S. (2013). Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs): recent advances in drug delivery. *Journal of Pharmaceutical Investigation*, *43*(5), 353-362.

Young, A. J., & Lowe, G. M. (2001). Antioxidant and prooxidant properties of carotenoids. *Archives of Biochemistry and biophysics*, *385*(1), 20-27.

Yuan, Y., Gao, Y., Zhao, J., & Mao, L. (2008). Characterization and stability evaluation of β-carotene nanoemulsions prepared by high pressure homogenization under various

emulsifying conditions. *Food Research International*, *41*(1), 61-68. https://doi.org/https://doi.org/10.1016/j.foodres.2007.09.006
Zeiger, E., Gollapudi, B., & Spencer, P. (2005). Genetic toxicity and carcinogenicity studies of glutaraldehyde—a review. *Mutation Research/Reviews in Mutation Research*, *589*(2), 136-151.
Zhang, C., Xu, W., Jin, W., Shah, B. R., Li, Y., & Li, B. (2015). Influence of anionic alginate and cationic chitosan on physicochemical stability and carotenoids bioaccessibility of soy protein isolate-stabilized emulsions. *Food Research International*, *77*, 419-425. https://doi.org/https://doi.org/10.1016/j.foodres.2015.09.020
Zhang, T., Lv, C., Chen, L., Bai, G., Zhao, G., & Xu, C. (2014). Encapsulation of anthocyanin molecules within a ferritin nanocage increases their stability and cell uptake efficiency. *Food Research International*, *62*, 183-192.
Zhang, W., Yan, C., May, J., & Barrow, C. (2009). Whey protein and gum Arabic encapsulated Omega-3 lipids. The effect of material properties on coacervation. *Agro food industry hi-tech*, *20*(4), 20-23.
Zhang, X., Sun, H., Zhang, Z., Niu, Q., Chen, Y., & Crittenden, J. C. (2007). Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles. *Chemosphere*, *67*(1), 160-166. https://doi.org/https://doi.org/10.1016/j.chemosphere.2006.09.003
Zhao, Y., Wu, Y., & Wang, M. (2015). Bioactive Substances of Plant Origin 30. *Handbook of food chemistry*, *967*.
Zheng, X., Qiu, C., Long, J., Jiao, A., Xu, X., Jin, Z., & Wang, J. (2021). Preparation and characterization of porous starch/β-cyclodextrin microsphere for loading curcumin: Equilibrium, kinetics and mechanism of adsorption. *Food Bioscience*, *41*, 101081. https://doi.org/https://doi.org/10.1016/j.fbio.2021.101081
Zhou, H., Zheng, B., & McClements, D. J. (2021). In Vitro Gastrointestinal Stability of Lipophilic Polyphenols is Dependent on their Oil–Water Partitioning in Emulsions: Studies on Curcumin, Resveratrol, and Quercetin. *Journal of agricultural and food chemistry*, *69*(11), 3340-3350. https://doi.org/10.1021/acs.jafc.0c07578
Zou, L., Zheng, B., Liu, W., Liu, C., Xiao, H., & McClements, D. J. (2015). Enhancing nutraceutical bioavailability using excipient emulsions: Influence of lipid droplet size on solubility and bioaccessibility of powdered curcumin. *Journal of functional foods*, *15*, 72-83. https://doi.org/https://doi.org/10.1016/j.jff.2015.02.044
Zou, L., Zheng, B., Zhang, R., Zhang, Z., Liu, W., Liu, C., Xiao, H., & McClements, D. J. (2016). Enhancing the bioaccessibility of hydrophobic bioactive agents using mixed colloidal dispersions: Curcumin-loaded zein nanoparticles plus digestible lipid nanoparticles. *Food Research International*, *81*, 74-82.
Zuidam, N. J., & Nedovic, V. (2010). Encapsulation technologies for active food ingredients and food processing.

Appendix

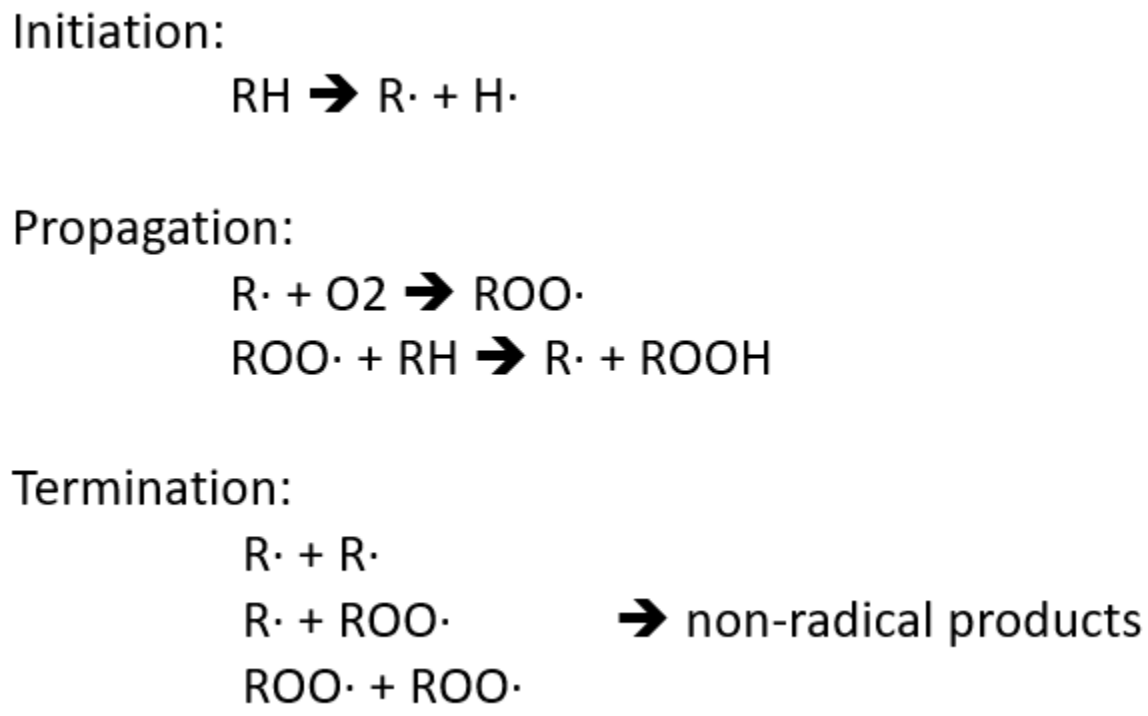


Figure 1.1 Radical chain reactions of lipid oxidation(Gavahian et al., 2018).

Chapter 2 Freeze-dried potato microparticles as a natural matrix to load resveratrol for enhanced bioaccessibility

2.1 Abstract

Resveratrol (RSV) is a natural polyphenol with many potential health benefits. However, RSV is lipophilic and susceptible to degradation, and strategies are to be studied to enable its distribution in food matrices, prevent its degradation during storage, and increase its bioaccessibility during digestion. In this study, the porous matrix of milled freeze-dried potato microparticles (FDPMs) was studied as a natural absorbent to load RSV. The binary solvent of ethanol and polyethylene glycol 400 (40:60 v/v) was used to dissolve 30% w/v RSV for diffusion into FDPMs. After evaporation of ethanol, the loading capacity was 112 mg RSV/g FDPMs and was maintained at 92.9% after 110-day ambient storage. The RSV stability under UV irradiation at 253 nm was improved, and the ferric reducing power was higher than the pristine RSV. The release of RSV in FDPMs was significantly higher than pristine RSV during simulated gastric and intestinal digestions. The increased reducing power and bioaccessibility were supported by the amorphous state of RSV in FDPMs. The present study illustrates the potential of porous vegetable microparticles as natural matrices to load lipophilic bioactive compounds.

2.2 Introduction

Resveratrol (RSV, 3,6,5'-trihydroxystilbene) is a polyphenol phytoalexin naturally produced in plants such as grapes (R Neves et al., 2012). RSV has attracted much interest as many studies have revealed its medicinal effects, including anticancer, anti-inflammatory, antioxidant, and cardioprotective properties (Abdelgawad et al., 2019; Das & Das, 2007; Hsieh & Wu, 2010). Although there are two geometric isomers, *trans* and *cis*, *trans*-RSV contributes predominantly

to therapeutic properties of RSV (Augustin et al., 2013). However, *trans*-RSV is susceptible to degradation under various environmental factors, such as light and extreme temperature. For example, the conversion of *trans*-RSV to *cis*-RSV occurs when exposed to UV irradiation (Trela & Waterhouse, 1996). Additionally, RSV, containing two benzene rings with a total of three hydroxyl groups attached, has a very low water solubility (<0.03 mg/mL), and is moderately dissolved in dimethyl sulfoxide (16 mg/mL) and ethanol (50 mg/mL) (Soto‐Valdez et al., 2011). Although the oral administration of RSV is preferred as a preventive treatment, the low aqueous solubility and the vulnerability to degradation necessitate delivery systems to increase its stability and bioavailability.

Various delivery systems have been studied for RSV. Liposome-based encapsulating systems for RSV have been studied utilizing different materials, such as soy lecithin, oleic acid, and egg yolk phosphatidylcholine (Caddeo et al., 2013; M. Huang et al., 2019; Pando et al., 2013). The diameter of liposomes varied (70-470 nm) depending on the preparation of liposomes, while high encapsulation efficiency of RSV (80-90%) was achieved (Balanč et al., 2016; M. Huang et al., 2019). Molecular inclusion complexation in cyclodextrins is another commonly studied method that results in an increase in water solubility by as much as 10^4 times (Prabu & Mohamad, 2020; Silva et al., 2014). Although these methods have displayed successful encapsulation of RSV, the instability of encapsulated RSV, the high cost of production, and the inability to control the targeted release are some of the drawbacks limiting their practical applications (Celli et al., 2015). Furthermore, most delivery systems have focused on developing nanoscale carriers, but most of these systems have not been studied for the toxicity (He & Hwang, 2016).

To tackle the aforementioned issues, freeze-dried potato microparticles (FDPMs) were chosen as a natural food matrix to load RSV in this study. Potato, as one of the major staples in the world, may provide a novel low-cost and safe delivery option compared to nanoscale materials that are confronted with unanswered toxicity issues (Borel & Sabliov, 2014). Because vegetable matrices have the tissues with internal pores wherein the biological fluid is contained, various external fluids containing probiotics or minerals have been introduced into different vegetables using diffusion without disrupting the original matrix (Alzamora et al., 2005; Fito et al., 2001). To load RSV within potato, the porosity of potato is a critical factor because it affects the diffusion and loading of molecules through potato cells (Li et al., 2021). Porosity refers to the ratio of void volume inside a material to the volume of the entire material (Aprajeeta et al., 2015). Higher porosity of vegetables after various drying techniques has been reported as water within pores is vaporized (Palzer et al., 2012; Ramos et al., 2003). The development of porous structures of potato was observed after vacuum, microwave, and freeze drying, resulting in the porosity of 31%, 78%, and 89%, respectively (Krokida & Maroulis, 1997). Freeze-drying also avoids the phenomenon of case-hardening, which is the excessive drying of outer layers to often create undesirable textures (Gulati & Datta, 2015).

The overall objective of this study was to develop and characterize FDPMs as a novel delivery system for RSV. The first task was to identify a solvent enabling dissolution of a high concentration of RSV, studied for a binary mixture with different volume fractions of ethanol and polyethylene glycol 400 (PEG400). After diffusion of the RSV solution in FDPMs and evaporation of ethanol, the structure and physicochemical properties of RSV-loaded FDPMs were characterized for the potential as a delivery system. FDPMs were prepared by milling

freeze-dried potato cubes and had open pores. RSV-loaded FDPMs therefore may not have closed boundaries as in conventional encapsulation studies. Nevertheless, encapsulation and microcapsules are used here for simplicity of expression.

2.3 Materials and methods

2.3.1 Materials

RSV (98% purity) was obtained from Accela Chembino (San Diego, CA). Freeze-dried potato cubes of a dimension of 1 cm × 1 cm × 1 cm were a product of North Bay Trading (Brue, WI). Ethanol was purchased from Decon Labs (King of Prussia, PA). PEG400 and ferric chloride were supplied by Acros Organics (Fair Lawn, NJ). Bile salt, α-amylase from human saliva, pepsin, pancreatin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and trichloroacetic acid were the products of Sigma-Aldrich Corp. (St Louis, MO). Potassium ferricyanide and sodium phosphate (monobasic and dibasic) were purchased from MP Biomedicals, LLC (Santa Ana, CA). Methanol was obtained from Thermo Fisher Scientific Inc. (Pittsburgh, PA).

2.3.2 Preparation and characterization of freeze-dried potato microparticles

The freeze-dried potato cubes were milled using a 3383-L10 Wiley Mini Mill (Thomas Scientific, Swedesboro, NJ) equipped with a sieve of 30 mesh (0.595 mm). The porosity of FDPMs was determined to be 79.3 % based on mercury intrusion porosimetry (AutoPore V 9600, Micrometrics Instrument Corp., Norcross, GA) and had an average pore diameter of 2.94 μm.

2.3.3 Encapsulation of resveratrol

To obtain a high concentration of RSV in a solvent, the solubility of RSV in the mixture of ethanol and PEG400 with different volume ratios was first determined based on the visual inspection of solution clarity and stability as RSV concentration increased. RSV was dissolved at ~5% w/v in ethanol alone and ~25% w/v in PEG 400. The binary mixture with ethanol and PEG 400 at a volume ratio of 40:60 was able to dissolve 30% w/v RSV and was thus used to prepare the RSV solution for encapsulation. To encapsulate RSV, 0.5 g of FDPMs was immersed in 10 mL of the RSV solution and mixed on a magnetic stirrer (990 rpm) overnight for the diffusion of RSV solution in FDPMs. Then, FDPMs were separated from the suspension using a polyvinylidene fluoride (PVDF) membrane filter with a pore size of 0.45 μm (MilliporeSigma, Burlington, MA) and dried in a vacuum oven at 4,000 Pa under pressure to evaporate ethanol at 50 °C for 18 h. The dried FDPMs with RSV was stored in amber glass vials at 22 °C until before each experiment.

2.3.4 Particle size of milled freeze-dried potato microparticles before and after encapsulation of resveratrol

The size of milled FDPMs was determined using a laser diffraction particle size analyzer (PSA 1190, Anton Paar, Graz, Austria). Measurements were performed with the vibrator duty cycle of 56%, vibrator frequency of 44 Hz, and air pressure of 40,000 Pa at 22 °C.

2.3.5 Loading capacity of resveratrol

The dried RSV-loaded FDPMs were mixed with 10 mL of methanol for 4 h on a magnetic stirrer and centrifuged at 14,100 *g* for 30 min (Minispin Plus, Eppendorff, Hamburg, Germany). The supernatant was collected, diluted ten-folds using methanol, and filtered into amber vials using PVDF syringe filters with the pore size of 0.45 µm (MilliporeSigma, Burlington, MA) for HPLC analysis (Agilent 1100 Series). The mobile phase was the mixture of water and methanol (50:50 v/v) supplemented with 0.25% (v/v) acetic acid (Zupančič et al., 2015). Each sample was eluted in an Eclipse Plus C18 column (4.6 × 250 mm, 5 µm, Agilent, Santa Clara, CA), and RSV was detected at 306 nm using a UV-VIS detector. The injection volume was 20.0 µL, flow rate was 0.80 mL/min, and run time was 20.0 min. The area under the curve of the *trans*-RSV peak was used to determine RSV concentration. The standard curve was prepared from RSV solutions (10-50 µg/mL in methanol, $R^2 = 0.9985$). The loading capacity was calculated as follows:

$$\text{Loading capacity (\%)} = \frac{amount\ of\ RSV\ in\ FDPMs\ (g)}{amount\ of\ FDPMs\ (g)} \times 100 \qquad (1)$$

2.3.6 X-ray diffraction (XRD)

The XRD patterns of FDPMs, FDPMs after treatment at encapsulation conditions without RSV, pristine RSV, the physical mixture of FDPMs and RSV, and RSV-loaded FDPMs were determined. The Panalytical Empyrean diffractometer (Malvern Panalytical, Worcestershire, UK) was equipped with a Cu Kα (1.5406 Å) X-ray laser. The 2θ angle range was from 5° to 60° with a step size of 0.02626° and a scan speed of 0.1117°/s.

2.3.7 Scanning electron microscopy (SEM)

The morphology of powder samples was characterized using SEM. The samples were glued on a specimen stub and sputter-coated with palladium for 1 min. Images of samples were captured using a LEO 1525 SEM microscope (FIB/SEM Zeiss Auriga, Oberkochen, Germany).

2.3.8 Differential scanning calorimetry (DSC)

The thermal stability of samples was analyzed using a DSC system (DSC 250 Discovery, TA Instruments, New Castle, DE). Each sample (~ 5 mg) was weighed on an aluminum pan and then the pan was sealed with a hermetic pinhole lid. The samples were heated from 25 °C to 320 °C at a rate of 10 °C/min, with an empty pan being used as a reference.

2.3.9 Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR)

FTIR spectroscopy was used to analyze the chemical structure of samples. The ATR-FTIR spectra were generated with 64 scans in the range of 4000-400 cm^{-1} using a Spectrum Two FTIR instrument (PerkinElmer, Waltham, MA) at a wavenumber resolution of 4 cm^{-1}.

2.3.10 DPPH· radical scavenging activity

The DPPH· assay was performed according to the reported procedure (Molyneux, 2004). Samples were mixed in methanol for 4 h at RSV concentrations of 5, 10, 50, and 100 µg/mL. The supernatant (2 mL) was collected after centrifugation at 15,000 *g* for 30 min at 21 °C (Sorvall Legend Micro 21R, Thermo Fisher Scientific, Waltham, MA) and added to 2 mL of a DPPH· solution (0.1 mM in methanol). After vortex-mixing for 10 s, samples were incubated for

30 min at 21 °C. Absorbance was read at 517 nm using a UV-vis spectrophotometer (Thermo Scientific, Waltham, MA), and the radical scavenging activity (i.e., reduction of the DPPH·, *Q*) was calculated using the following equation:

$$Q\ (\%) = 100\ (A_0 - A_C)/A_0 \qquad (2)$$

where A_0 represents the absorbance of the control (methanol substituting for the test sample) and A_C is the absorbance of a test sample.

2.3.11 Ferric reducing power

The ferric reducing potential of RSV samples was evaluated using a previously reported method with slight modification (X. Huang et al., 2019). Standard ascorbic acid solutions were prepared with a concentration range of 0-154.4 mM. RSV sample (0.16 mg/mL) was mixed with a phosphate buffered saline (PBS, 2.5 mL, 0.2 M, pH 7.1) and a potassium ferricyanide solution (2.5 mL, 1% w/v). The solution mixtures were incubated at 50 °C for 20 min in a water bath. After cooling down in an iced water bath for 10 s, samples were acidified using trichloroacetic acid (2.5 mL, 10% w/v) and then centrifuged for 10 min at 3,000 *g* and 21 °C. The supernatant (0.1 mL) was mixed with deionized water (0.9 mL) and reacted with a ferric chloride solution (0.1 mL, 0.1% w/v) for 10 min. Absorbance was measured at 700 nm using the UV-vis spectrophotometer mentioned above. The reducing power of RSV samples was expressed in ascorbic acid equivalent (mM).

2.3.12 Photostability test

To study the photostability of RSV, samples were prepared by suspending 10 mg of RSV-loaded FDPMs in 10 mL of PBS (pH 7.0, 50 mM). The control was pristine RSV at a

concentration comparable to that in FDPMs in the methanol-PBS mixture (1:99 v/v). The light-shielding effect of FDPMs was analyzed by comparing the extent of UV-induced isomerization of two sample sets. Samples were exposed for up to 1 h with the distance of 35 cm from a UV lamp (30 W, 253 nm). The samples were taken at certain time intervals (0, 5, 10, 30, and 60 min) and mixed with 10 mL methanol for 4 h at 22 °C. After centrifugation at 14,100 *g* for 30 min, the supernatant was filtered using the PVDF filter membrane of 0.45 μm pore size for HPLC analysis with the conditions described above.

2.3.13 Thermal stability test

Samples prepared in the same way as in the photostability test were contained within amber vials. The vials were placed in a water bath conditioned at 90 °C and were cooled immediately in an iced water bath after 0, 10, 20, 30, and 60 min of heating. Then, samples were mixed with 10 mL of methanol for 4 h before HPLC analysis following the above procedures.

2.3.14 Release of resveratrol during simulated digestions

Pristine RSV, the physical mixture of pristine RSV and FDPMs, and RSV-loaded FDPMs were mixed in 15 mL of PBS (5 mM, pH 7.0) at the RSV concentration of 1 mg/mL. Simulated digestions were performed following the previously described method (X. Huang et al., 2019).

Oral phase. Because potato contains starch, oral phase was included in the *in vitro* digestion. Three units of α-amylase per mg of potato was added to a simulated saliva solution (PBS with 5 mM NaCl) that was used to suspend powder treatments corresponding to an RSV concentration of 1 mg/mL. Samples were incubated in a model C76 water bath shaker (New Brunswick Scientific, Edison, NJ) at 100 rpm and 37 °C for 5 min.

Gastric phase. After the oral phase digestion, 15 mL of a simulated gastric fluid (50 mM NaCl and 3.2 mg/mL pepsin, pH 1.2) was added to each sample. The mixture pH was adjusted to 2.7 using an NaOH solution (1.0 M), and samples were incubated in the above water bath shaker at 100 rpm and 37 °C for 120 min.

Intestinal phase. The pH of mixtures after the gastric phase digestion was adjusted to 7.0 using an NaOH solution (1.0 M). Then, 4 mL of a bile salt solution (12.5 mg/mL in PBS) and 2.5 mL of a pancreatic solution (8.0 mg/mL in PBS) were added, and samples were maintained in the 37 °C water bath shaker while being shaken at 130 rpm for 240 min.

Samples collected at pre-determined time points during simulated digestions were heated at 90 °C for 5 min to stop the reaction and then centrifuged at 14,100 *g* for 30 min. The supernatant was collected, diluted ten-folds using methanol, and filtered using 0.45 μm PVDF filter membranes for HPLC analysis as described above.

2.3.15 Statistical analysis

Mean values and standard deviations from at least two replicates were presented for antioxidant capacity, photostability, thermal stability, and bioaccessibility results. Statistical differences between mean values were determined from analysis of variance (ANOVA) with Tukey's post-hoc test ($\alpha = 0.05$) using SAS enterprise guide 7.1 (SAS Institute Inc, Cary, NC).

2.4 Results and discussion

2.4.1 Particle size, loading capacity, and storage stability

The size distribution of FDPMs before and after loading RSV is shown in Fig. 2.1. The volume-weighted mean particle diameter ($D_{4,3}$) of milled FDPMs was 227.9 μm before loading RSV and significantly ($p < 0.05$) increased to 315.7 μm after loading RSV, probably due to the swelling effect of PEG400 on porous particles (Guo et al., 2004). The loading capacity of RSV in FDPMs was 11.2±0.3% in freshly prepared samples and was 10.4±0.1% (92.9% retention) after 110-day of ambient storage in amber vials. Previous studies reported various loading capacity of RSV using different delivery systems. The RSV content in nanoparticles made of zein and pectin using antisolvent precipitation and electrostatic deposition was 6.2 – 10.2% (w/w) depending on the initial amount of RSV used (Huang et al., 2017). Relatively low loading capacity of RSV was reported for spray drying studies, e.g., ~1.6% (w/w) using sodium alginate and carboxymethyl cellulose (Maria Leena et al., 2020) and 4.1% (w/w) using gum Arabic as the wall material (Cardoso et al., 2019). The loading capacity was 3.1% after complexing RSV with cyclodextrin (Soo et al., 2016), whereas, the loading capacity was 56% for sonicated liposomes (Isailović et al., 2013). The loading capacity of RSV in the present study is comparable to or better than studies utilizing scalable processes.

2.4.2 Physical state of resveratrol in freeze-dried potato microparticles

XRD pattern was analyzed to evaluate the reduction in crystallinity of RSV after encapsulation (Fig. 2.2). No distinct diffraction peaks were observed for both FDPMs and the FDPMs after treatment at encapsulation conditions, suggesting that the ethanol/PEG400 mixture

solvent did not affect the crystallinity of FDPMs. The overall broad peak of FDPMs suggests that amorphous structures are the dominant structures in FDPMs (Zhang et al., 2020), except for a minor peak at 2θ of around 16° that indicates the presence of minor crystalline regions in FDPMs. According to the vendor, freeze-dried potato cubes were blanched, which may explain the lack of distinct diffraction peaks expected for starch granules with amylopectin lamellar structures (Keetels et al., 1996). Pristine RSV displayed several noticeable peaks at 2θ values of 6.6°, 16.3°, 19.1°, and 28.3°, which are similar to previously reported values (Khan et al., 2019), indicating the crystalline structure of pristine RSV. These distinct characteristic peaks of RSV still appeared on the smooth XRD curve of FDPMs for the physical mixture of FDPMs and pristine RSV at the same ratio used in encapsulation. For FDPMs loaded with RSV, no sharp peak was observed, showing a pattern comparable to that of control FDPMs. The results suggest that RSV in FDPMs is in the amorphous state. This is notable because the crystallinity of lipophilic bioactive compounds results in low water solubility and aggregation during digestion (McClements, 2015); whereas, amorphous structures might increase the bioaccessibility of RSV (Shin et al., 2016).

2.4.3 Morphology of resveratrol and freeze-dried potato microparticles before and after encapsulation

The SEM images illustrated the porous external surface of FDPMs (Fig. 2.3A), even after the treatment with the ethanol/PEG400 binary solvent (Fig. 2.3B). However, pores on the outer surface of FDPMs were not easily identified after the encapsulation (Fig. 2.3E). In general, solutions can enter into a void of matrix more easily when the pore diameter is sufficiently big

(Pérez-Esteve et al., 2016), but the large pore size of encapsulating materials also promotes the leakage of entrapped compounds (Zhang et al., 2016). The disappearance of apparent pores on FDPMs after encapsulation, therefore, may contribute to the protection of encapsulated RSV during storage and digestion. The pristine RSV had the rod-like crystal structure (Fig. 2.3C) that was also observed in its physical mixture with FDPMs (Fig. 2.3D). In contrast, no crystal structure of RSV was observed after encapsulation in FDPMs (Fig. 2.3E).

2.4.4 DSC analysis

DSC thermal profiles of RSV in its pristine and encapsulated forms are compared in Fig. 4. In thermograms of samples containing FDPMs, the first minor endothermic peak was noticed at around 53 °C and the second major endothermic peak was observed at around 130 °C. Two endothermic peaks were also observed for thermoplastic potato starch prepared by heating a mixture with potato starch, glycerol, and water at a mass ratio of 5:3:2 (Altayan et al., 2017). The first peak can be attributed to the gelatinization of starch (Altayan et al., 2017), and the small peak area can be due to a small amount of moisture within FDPMs causing negligible gelatinization. The second major peak can be attributed to remaining starch granules and recrystallized starch after processes used to prepare freeze-dried potato cubes (Altayan et al., 2017; Larsson & Eliasson, 1991). The thermogram of RSV (Fig. 2.4C) displayed a single endothermic peak with high intensity around 270 °C corresponding to the melting point of RSV (Duarte et al., 2015). The sharp melting point in the thermogram is a typical characteristic of anhydrous crystalline substances. Therefore, the crystalline status of pristine RSV observed in DSC agrees with the XRD results (Fig. 2.2C). There was no shifting of this peak for the physical mixture of FDPMs and pristine RSV (Fig. 2.4D). However, this peak was absent in the

thermogram of RSV-loaded FDPMs. Therefore, RSV became amorphous after being entrapped within FDPMs (Karoyo et al., 2013).

2.4.5 ATR-FTIR analysis

The interaction between RSV and FDPMs was analyzed using FTIR in the range of 4000-400 cm^{-1} (Fig. 2.5). The IR spectra between 3800 and 3000 cm^{-1} indicate O-H stretching (Kaczmarska et al., 2018). For FDPMs, the treatment by the ethanol/PEG400 mixture solvent increased the intensity of the peaks in the range of 3800 and 3000 cm^{-1}. The peak centered around 1080 cm^{-1} represents C-H bending, and the intensity of this peak also increased after FDPMs were treated by the mixture solvent; whereas, the physical mixture of FDPMs and pristine RSV did not show significant changes in the peak intensity. The increased intensity at 2869 cm^{-1} is due to vibration of the -CH_2- group, resulting from the presence of PEG400 in FDPMs (Tan et al., 2016). The pristine RSV displayed several characteristic peaks, including C-C aromatic double bond stretching at 1605 cm^{-1}, C-C olefinic stretching at 1584 cm^{-1}, C-C ring stretching vibration at 1381 cm^{-1}, and *trans* olefinic band at 964 cm^{-1} (Popova et al., 2014). These peaks were still observed for the physical mixture of FDPMs and the pristine RSV. However, most of these characteristic peaks of the pristine RSV shifted or disappeared after encapsulation, suggesting not only physical absorption but possible new interaction between RSV with PEG400 or FDPM components.

2.4.6 Antioxidant activity analysis

Resveratrol has an excellent activity scavenging various radicals (R Neves et al., 2012). Therefore, the scavenging ability of encapsulated RSV was studied using the DPPH· and ferric

reducing power assays. DPPH· is a nitrogen free radical with strong purple color, and its reduction by an antioxidant can be measured spectrophotometrically (Holtz, 2009). The results of the DPPH· assay are presented in Fig. 2.6A. The FDPMs did not exhibit any antioxidant activity, and the same was observed for FDPMs treated with the ethanol/PEG400 mixture solvent. RSV showed a DPPH· scavenging capacity, which is congruent with previously reported data (Duarte et al., 2015). In the studied concentration of RSV, 5-100 μg/mL, the reduced amount of DPPH· increased with an increase in the RSV concentration. No significant difference in the DPPH· scavenging capacity was observed among the pristine RSV, the pristine RSV in the physical mixture with FDPMs, and the encapsulated RSV. This can be due to the dissolution of RSV in the medium (methanol) used in the DPPH· assay. The results in the present study are in agreement with a study after binding RSV with methyl-β-cyclodextrin (Duarte et al., 2015), indicating that encapsulation had an insignificant impact on the DPPH· scavenging capacity of RSV.

The phenolic hydroxyl groups of RSV can donate hydrogen atoms to other substances, providing RSV with reducing power. As the direct correlation between reducing power and the antioxidant capacity of compounds has been established (Subramanian et al., 2013), the capability of reducing ferric ions by RSV in various forms was determined, shown in Fig. 2.6B. FDPMs alone did not show any reducing power, corresponding to ~0 mM ascorbic acid equivalent. The pristine RSV alone and that in the mixture with FDPMs had a comparable ferric reducing power of around 120 mM ascorbic acid equivalent. In comparison, RSV encapsulated in FDPMs displayed a significantly higher ferric reducing power. The reducing power of RSV after encapsulation in zein/pectin nanoparticles was slightly higher than that of the pristine RSV

(Huang et al., 2017). When quantified for solubility in PBS, the solubility of encapsulated RSV was approximately 2.5 times that of pristine RSV, corresponding to the increased reducing power of RSV after encapsulation.

2.4.7 Chemical stability of resveratrol

The UV irradiation initiates partial geometric isomerization of *trans*-RSV to *cis*-RSV, causing reductions in the biological activity of RSV (Chen et al., 2007). Improving the UV stability of RSV, therefore, is important for practical applications (Sessa et al., 2011). The residual amount of *trans*-RSV after UV irradiation is presented in Fig. 2.7A. The exposure to UV for a relatively short time, i.e., 5 min, was sufficient to significantly decrease the amount of *trans*-RSV in the pristine RSV solution; whereas, there was no decrease of *trans*-RSV content in the suspension with encapsulated RSV. The difference of the residual amount of *trans*-RSV between the two samples increased as the length of UV exposure was extended to 60 min. Compared with the pristine RSV, the encapsulated RSV showed an appreciably higher photostability, with no significant difference during the first 30 min of UV irradiation. After 60 min of UV exposure, the residual% of *trans*-RSV encapsulated in FDPMs decreased to 90.5%, while this number was 68.4% for the pristine RSV solution. The observed gradual isomerization of *trans*-RSV in the solution upon UV 253 nm irradiation agrees with a study quantifying isomerization at UV 260 nm (Figueiras et al., 2011). The high UV stability of encapsulated *trans*-RSV suggests that RSV is present within FDPMs, and FDPMs effectively absorbs UV radiation to protect *trans*-RSV from isomerization. The residual *trans*-RSV in the solution after 60 min of UV irradiation in this study (68.4%) was higher than a reported value of ~30% after irradiation at 366 nm (Xiong et al., 2018). The residual content of *trans*-RSV after irradiation at

366 nm for 100 min (9.4%) has been reported to be lower than that after 10 h of irradiation at 254 nm (37%) (Trela & Waterhouse, 1996).

The thermal stability of RSV was evaluated after heat treatment at 90 °C, which is a high enough temperature to induce thermal degradation of RSV (Bancuta et al., 2018). Unlike the results from photostability test, thermal stability was similar for the pristine RSV and encapsulated RSV (Fig. 2.7B). This indicates that the encapsulation of RSV within FDPMs did not protect the thermal degradation of RSV. The possible explanation may be the release of encapsulated RSV, resulting from the structural changes of FDPMs during the thermal treatment. Heating FDPMs in water promotes swelling and possible destruction of potato starch granules, which in turn increases the number of parenchyma cells with high permeability (Dadmohammadi et al., 2020), and therefore the release of encapsulated RSV (Wang et al., 2020). Our results contrast with the improved thermal stability of RSV after nanocomplexation with α-lactalbumin (Liu et al., 2018).

2.4.8 In vitro release of resveratrol during simulated gastrointestinal digestion

To exert the biological effects of RSV, it is important to ensure that the encapsulated RSV is released in the intestines after oral consumption. Therefore, the release kinetics of RSV was characterized during simulated digestions by comparing the pristine RSV, a physical mixture of the pristine RSV and FDPMs, and RSV-loaded FDPMs. After the oral phase, there was no significant difference among the three treatments (Fig. 2.8). In both model gastric and intestinal fluids, the release% of RSV was the highest for the encapsulation treatment, which can be attributed to the increased RSV solubility after encapsulation, as mentioned previously. The improved solubility and bioaccessibility of RSV after encapsulation may result from the

structural change of RSV from the crystalline to amorphous form (Kanaujia et al., 2015). The increased surface area of amorphous RSV can also increase the solubility in mixed bile surfactant micelles, resulting in more complete release and increased bioaccessibility (Davidov-Pardo et al., 2015; Maldonado-Valderrama et al., 2011; Peng et al., 2018). Furthermore, binding of RSV to hydrophobic patches of peptides may occur during digestion (Yao et al., 2018). Because the proteases in GI fluids digest potato proteins (X. Huang et al., 2019; Mäkinen et al., 2016), it is plausible that the formed peptides bind with RSV to increase its solubility, resulting in slightly higher bioaccessibility of RSV in the physical mixture of FDPMs and pristine RSV than the pristine RSV treatment.

2.5 Conclusion

To sum up, our study demonstrated that FDPMs can be used to load RSV via diffusion of the RSV solution with the binary solvent of ethanol/PEG400. The RSV encapsulated within FDPMs showed high stability with a decrease in retention by only 7% during the ambient storage for 110-day. Encapsulation in FDPMs improved the photostability of RSV but not the thermal stability. The XRD and DSC analyses demonstrated that RSV encapsulated in FDPMs had the amorphous structure. The amorphous structure of encapsulated RSV increased its water solubility, the ferric reducing power, and release% during *in vitro* digestions. Findings in the present study suggest the novel delivery system based on FDPMs may be used as safe and inexpensive ingredients to utilize lipophilic antioxidants such as RSV to improve food quality and bioaccessibility.

2.6 Acknowledgments

The authors are grateful to Dr. John Dunlap, Dr. Michael Koehler, Dr. Tong Wang, and Kendhl Seabright for allowing the use of instruments and assisting with experiments. Funding of this work was provided by the University of Tennessee Institute of Agriculture and USDA National Institute of Food and Agriculture hatch project TEN00568. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s).

References

Abdelgawad, I. Y., Grant, M. K., & Zordoky, B. N. (2019). Leveraging the cardio-protective and anticancer properties of resveratrol in cardio-oncology. *Nutrients*, *11*(3), 627.

Altayan, M. M., Al Darouich, T., & Karabet, F. (2017). On the Plasticization Process of Potato Starch: Preparation and Characterization. *Food Biophysics*, *12*(4), 397-403. https://doi.org/10.1007/s11483-017-9495-2

Alzamora, S. M., Salvatori, D., Tapia, M. S., López-Malo, A., Welti-Chanes, J., & Fito, P. (2005). Novel functional foods from vegetable matrices impregnated with biologically active compounds. *Journal of Food Engineering*, *67*(1-2), 205-214.

Aprajeeta, J., Gopirajah, R., & Anandharamakrishnan, C. (2015). Shrinkage and porosity effects on heat and mass transfer during potato drying. *Journal of Food Engineering*, *144*, 119-128. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2014.08.004

Augustin, M. A., Sanguansri, L., & Lockett, T. (2013). Nano‐ and micro‐encapsulated systems for enhancing the delivery of resveratrol. *Annals of the New York Academy of Sciences*, *1290*(1), 107-112.

Balanč, B., Trifković, K., Đorđević, V., Marković, S., Pjanović, R., Nedović, V., & Bugarski, B. (2016). Novel resveratrol delivery systems based on alginate-sucrose and alginate-chitosan microbeads containing liposomes. *Food Hydrocolloids*, *61*, 832-842.

Bancuta, O. R., Chilian, A., Bancuta, I., Setnescu, R., Setnescu, T., & Ion, R. M. (2018). Thermal characterization of resveratrol. *Revista De Chimie*, *69*(6), 1346-1351.

Borel, T., & Sabliov, C. (2014). Nanodelivery of bioactive components for food applications: types of delivery systems, properties, and their effect on ADME profiles and toxicity of nanoparticles. *Annual review of food science and technology*, *5*, 197-213.

Caddeo, C., Manconi, M., Fadda, A. M., Lai, F., Lampis, S., Diez-Sales, O., & Sinico, C. (2013). Nanocarriers for antioxidant resveratrol: formulation approach, vesicle self-assembly and stability evaluation. *Colloids and Surfaces B: Biointerfaces*, *111*, 327-332.

Cardoso, T., Gonçalves, A., Estevinho, B. N., & Rocha, F. (2019). Potential food application of resveratrol microparticles: Characterization and controlled release studies. *Powder Technology*, *355*, 593-601. https://doi.org/https://doi.org/10.1016/j.powtec.2019.07.079

Celli, G. B., Ghanem, A., & Brooks, M. S.-L. (2015). Bioactive encapsulated powders for functional foods—a review of methods and current limitations. *Food and Bioprocess Technology*, *8*(9), 1825-1837.

Chen, X., He, H., Wang, G., Yang, B., Ren, W., Ma, L., & Yu, Q. (2007). Stereospecific determination of cis‐ and trans‐resveratrol in rat plasma by HPLC: application to pharmacokinetic studies. *Biomedical Chromatography*, *21*(3), 257-265.

Dadmohammadi, Y., Kantzas, A., Yu, X., & Datta, A. K. (2020). Estimating permeability and porosity of plant tissues: Evolution from raw to the processed states of potato. *Journal of Food Engineering*, *277*, 109912. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2020.109912

Das, S., & Das, D. K. (2007). Anti-inflammatory responses of resveratrol. *Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy)(Discontinued)*, *6*(3), 168-173.

Davidov-Pardo, G., Pérez-Ciordia, S., Marín-Arroyo, M. a. R., & McClements, D. J. (2015). Improving Resveratrol Bioaccessibility Using Biopolymer Nanoparticles and Complexes: Impact of Protein–Carbohydrate Maillard Conjugation. *Journal of Agricultural and Food Chemistry*, *63*(15), 3915-3923. https://doi.org/10.1021/acs.jafc.5b00777

Duarte, A., Martinho, A., Luís, Â., Figueiras, A., Oleastro, M., Domingues, F. C., & Silva, F. (2015). Resveratrol encapsulation with methyl-β-cyclodextrin for antibacterial and antioxidant delivery applications. *LWT - Food Science and Technology*, *63*(2), 1254-1260. https://doi.org/https://doi.org/10.1016/j.lwt.2015.04.004

Figueiras, T. S., Neves-Petersen, M. T., & Petersen, S. B. (2011). Activation Energy of Light Induced Isomerization of Resveratrol. *Journal of Fluorescence*, *21*(5), 1897-1906. https://doi.org/10.1007/s10895-011-0886-3

Fito, P., Chiralt, A., Betoret, N., Gras, M., Cháfer, M., Martínez-Monzó, J., Andrés, A., & Vidal, D. (2001). Vacuum impregnation and osmotic dehydration in matrix engineering: Application in functional fresh food development. *Journal of Food Engineering*, *49*(2-3), 175-183.

Gulati, T., & Datta, A. K. (2015). Mechanistic understanding of case-hardening and texture development during drying of food materials. *Journal of Food Engineering*, *166*, 119-138.

Guo, W., Luo, G. S., & Wang, Y. J. (2004). A new emulsion method to synthesize well-defined mesoporous particles. *Journal of Colloid and Interface Science*, *271*(2), 400-406. https://doi.org/https://doi.org/10.1016/j.jcis.2003.08.056

He, X., & Hwang, H.-M. (2016). Nanotechnology in food science: Functionality, applicability, and safety assessment. *journal of food and drug analysis*, *24*(4), 671-681.

Holtz, R. W. (2009). Chapter 13 - In Vitro Methods to Screen Materials for Anti-aging Effects. In N. Dayan (Ed.), *Skin Aging Handbook* (pp. 329-362). William Andrew Publishing. https://doi.org/https://doi.org/10.1016/B978-0-8155-1584-5.50017-X

Hsieh, T. c., & Wu, J. M. (2010). Resveratrol: Biological and pharmaceutical properties as anticancer molecule. *Biofactors*, *36*(5), 360-369.

Huang, M., Liang, C., Tan, C., Huang, S., Ying, R., Wang, Y., Wang, Z., & Zhang, Y. (2019). Liposome co-encapsulation as a strategy for the delivery of curcumin and resveratrol. *Food & function*, *10*(10), 6447-6458.

Huang, X., Dai, Y., Cai, J., Zhong, N., Xiao, H., McClements, D. J., & Hu, K. (2017). Resveratrol encapsulation in core-shell biopolymer nanoparticles: Impact on antioxidant and anticancer activities. *Food Hydrocolloids*, *64*, 157-165. https://doi.org/https://doi.org/10.1016/j.foodhyd.2016.10.029

Huang, X., Liu, Y., Zou, Y., Liang, X., Peng, Y., McClements, D. J., & Hu, K. (2019). Encapsulation of resveratrol in zein/pectin core-shell nanoparticles: Stability, bioaccessibility, and antioxidant capacity after simulated gastrointestinal digestion. *Food Hydrocolloids*, *93*, 261-269. https://doi.org/https://doi.org/10.1016/j.foodhyd.2019.02.039

Isailović, B. D., Kostić, I. T., Zvonar, A., Đorđević, V. B., Gašperlin, M., Nedović, V. A., & Bugarski, B. M. (2013). Resveratrol loaded liposomes produced by different techniques. *Innovative Food Science & Emerging Technologies*, *19*, 181-189. https://doi.org/https://doi.org/10.1016/j.ifset.2013.03.006

Kaczmarska, K., Grabowska, B., Spychaj, T., Zdanowicz, M., Sitarz, M., Bobrowski, A., & Cukrowicz, S. (2018). Effect of microwave treatment on structure of binders based on sodium carboxymethyl starch: FT-IR, FT-Raman and XRD investigations. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *199*, 387-393.

Kanaujia, P., Poovizhi, P., Ng, W. K., & Tan, R. B. H. (2015). Amorphous formulations for dissolution and bioavailability enhancement of poorly soluble APIs. *Powder Technology*, *285*, 2-15. https://doi.org/https://doi.org/10.1016/j.powtec.2015.05.012

Karoyo, A. H., Sidhu, P., Wilson, L. D., & Hazendonk, P. (2013). Characterization and dynamic properties for the solid inclusion complexes of β-cyclodextrin and perfluorooctanoic acid. *The Journal of Physical Chemistry B*, *117*(27), 8269-8282.

Keetels, C., Oostergetel, G., & Van Vliet, T. (1996). Recrystallization of amylopectin in concentrated starch gels. *Carbohydrate Polymers*, *30*(1), 61-64.

Khan, M. A., Yue, C., Fang, Z., Hu, S., Cheng, H., Bakry, A. M., & Liang, L. (2019). Alginate/chitosan-coated zein nanoparticles for the delivery of resveratrol. *Journal of Food Engineering*, *258*, 45-53. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2019.04.010

Krokida, M., & Maroulis, Z. (1997). Effect of drying method on shrinkage and porosity. *Drying Technology*, *15*(10), 2441-2458.

Larsson, I., & Eliasson, A. C. (1991). Annealing of starch at an intermediate water content. *Starch - Stärke*, *43*(6), 227-231.

Li, H.-T., Chen, S.-Q., Bui, A. T., Xu, B., & Dhital, S. (2021). Natural 'capsule' in food plants: cell wall porosity controls starch digestion and fermentation. *Food Hydrocolloids*, 106657. https://doi.org/https://doi.org/10.1016/j.foodhyd.2021.106657

Liu, Y., Fan, Y., Gao, L., Zhang, Y., & Yi, J. (2018). Enhanced pH and thermal stability, solubility and antioxidant activity of resveratrol by nanocomplexation with α-lactalbumin. *Food & function*, *9*(9), 4781-4790.

Mäkinen, S., Streng, T., Larsen, L. B., Laine, A., & Pihlanto, A. (2016). Angiotensin I-converting enzyme inhibitory and antihypertensive properties of potato and rapeseed protein-derived peptides. *Journal of Functional Foods*, *25*, 160-173. https://doi.org/https://doi.org/10.1016/j.jff.2016.05.016

Maldonado-Valderrama, J., Wilde, P., Macierzanka, A., & Mackie, A. (2011). The role of bile salts in digestion. *Advances in Colloid and Interface Science*, *165*(1), 36-46. https://doi.org/https://doi.org/10.1016/j.cis.2010.12.002

Maria Leena, M., Gover Antoniraj, M., Moses, J. A., & Anandharamakrishnan, C. (2020). Three fluid nozzle spray drying for co-encapsulation and controlled release of curcumin and resveratrol. *Journal of Drug Delivery Science and Technology*, *57*, 101678. https://doi.org/https://doi.org/10.1016/j.jddst.2020.101678

McClements, D. J. (2015). Nanoscale nutrient delivery systems for food applications: improving bioactive dispersibility, stability, and bioavailability. *Journal of Food Science*, *80*(7), N1602-N1611.

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. sci. technol*, *26*(2), 211-219.

Palzer, S., Dubois, C., & Gianfrancesco, A. (2012). Generation of product structures during drying of food products. *Drying Technology*, *30*(1), 97-105.

Pando, D., Caddeo, C., Manconi, M., Fadda, A. M., & Pazos, C. (2013). Nanodesign of olein vesicles for the topical delivery of the antioxidant resveratrol. *Journal of Pharmacy and Pharmacology*, *65*(8), 1158-1167.

Peng, S., Li, Z., Zou, L., Liu, W., Liu, C., & McClements, D. J. (2018). Improving curcumin solubility and bioavailability by encapsulation in saponin-coated curcumin nanoparticles prepared using a simple pH-driven loading method. *Food & function*, *9*(3), 1829-1839.

Pérez-Esteve, É., Ruiz-Rico, M., de la Torre, C., Villaescusa, L. A., Sancenón, F., Marcos, M. D., Amorós, P., Martínez-Máñez, R., & Barat, J. M. (2016). Encapsulation of folic acid in different silica porous supports: A comparative study. *Food Chemistry*, *196*, 66-75. https://doi.org/https://doi.org/10.1016/j.foodchem.2015.09.017

Popova, M., Szegedi, A., Mavrodinova, V., Tušar, N. N., Mihály, J., Klébert, S., Benbassat, N., & Yoncheva, K. (2014). Preparation of resveratrol-loaded nanoporous silica materials with different structures. *Journal of Solid State Chemistry*, *219*, 37-42.

Prabu, S., & Mohamad, S. (2020). Curcumin/beta-cyclodextrin inclusion complex as a new "turn-off" fluorescent sensor system for sensitive recognition of mercury ion. *Journal of Molecular Structure*, *1204*, 127528.

R Neves, A., Lucio, M., LC Lima, J., & Reis, S. (2012). Resveratrol in medicinal chemistry: a critical review of its pharmacokinetics, drug-delivery, and membrane interactions. *Current medicinal chemistry*, *19*(11), 1663-1681.

Ramos, I., Brandão, T. R., & Silva, C. (2003). Structural changes during air drying of fruits and vegetables. *Food Science and Technology International*, *9*(3), 201-206.

Sessa, M., Tsao, R., Liu, R., Ferrari, G., & Donsì, F. (2011). Evaluation of the Stability and Antioxidant Activity of Nanoencapsulated Resveratrol during in Vitro Digestion. *Journal of Agricultural and Food Chemistry*, *59*(23), 12352-12360. https://doi.org/10.1021/jf2031346

Shin, G. H., Li, J., Cho, J. H., Kim, J. T., & Park, H. J. (2016). Enhancement of Curcumin Solubility by Phase Change from Crystalline to Amorphous in Cur-TPGS Nanosuspension. *Journal of Food Science*, *81*(2), N494-N501. https://doi.org/https://doi.org/10.1111/1750-3841.13208

Silva, F., Figueiras, A., Gallardo, E., Nerín, C., & Domingues, F. C. (2014). Strategies to improve the solubility and stability of stilbene antioxidants: A comparative study between cyclodextrins and bile acids. *Food Chemistry*, *145*, 115-125. https://doi.org/https://doi.org/10.1016/j.foodchem.2013.08.034

Soo, E., Thakur, S., Qu, Z., Jambhrunkar, S., Parekh, H. S., & Popat, A. (2016). Enhancing delivery and cytotoxicity of resveratrol through a dual nanoencapsulation approach. *Journal of Colloid and Interface Science*, *462*, 368-374. https://doi.org/https://doi.org/10.1016/j.jcis.2015.10.022

Soto‐Valdez, H., Auras, R., & Peralta, E. (2011). Fabrication of poly (lactic acid) films with resveratrol and the diffusion of resveratrol into ethanol. *Journal of Applied Polymer Science*, *121*(2), 970-978.

Subramanian, R., Subbramaniyan, P., & Raj, V. (2013). Antioxidant activity of the stem bark of Shorea roxburghii and its silver reducing power. *SpringerPlus*, *2*(1), 1-11.

Tan, B., Huang, Z., Yin, Z., Min, X., Liu, Y. g., Wu, X., & Fang, M. (2016). Preparation and thermal properties of shape-stabilized composite phase change materials based on

polyethylene glycol and porous carbon prepared from potato. *RSC advances*, *6*(19), 15821-15830.

Trela, B. C., & Waterhouse, A. L. (1996). Resveratrol: Isomeric Molar Absorptivities and Stability. *Journal of Agricultural and Food Chemistry*, *44*(5), 1253-1257. https://doi.org/10.1021/jf9504576

Wang, S., Hu, X., Wang, Z., Bao, Q., Zhou, B., Li, T., & Li, S. (2020). Preparation and characterization of highly lipophilic modified potato starch by ultrasound and freeze-thaw treatments. *Ultrasonics Sonochemistry*, *64*, 105054. https://doi.org/https://doi.org/10.1016/j.ultsonch.2020.105054

Xiong, W., Ren, C., Li, J., & Li, B. (2018). Enhancing the photostability and bioaccessibility of resveratrol using ovalbumin–carboxymethylcellulose nanocomplexes and nanoparticles. *Food & function*, *9*(7), 3788-3797.

Yao, K., Chen, W., Song, F., McClements, D. J., & Hu, K. (2018). Tailoring zein nanoparticle functionality using biopolymer coatings: Impact on curcumin bioaccessibility and antioxidant capacity under simulated gastrointestinal conditions. *Food Hydrocolloids*, *79*, 262-272. https://doi.org/https://doi.org/10.1016/j.foodhyd.2017.12.029

Zhang, K., Tian, Y., Liu, C., & Xue, W. (2020). Effects of temperature and shear on the structural, thermal and pasting properties of different potato flour. *BMC Chemistry*, *14*(1), 20. https://doi.org/10.1186/s13065-020-00670-w

Zhang, Z., Zhang, R., Chen, L., & McClements, D. J. (2016). Encapsulation of lactase (β-galactosidase) into κ-carrageenan-based hydrogel beads: Impact of environmental conditions on enzyme activity. *Food Chemistry*, *200*, 69-75. https://doi.org/https://doi.org/10.1016/j.foodchem.2016.01.014

Zupančič, Š., Lavrič, Z., & Kristl, J. (2015). Stability and solubility of trans-resveratrol are strongly influenced by pH and temperature. *European Journal of Pharmaceutics and Biopharmaceutics*, *93*, 196-204.

Appendix

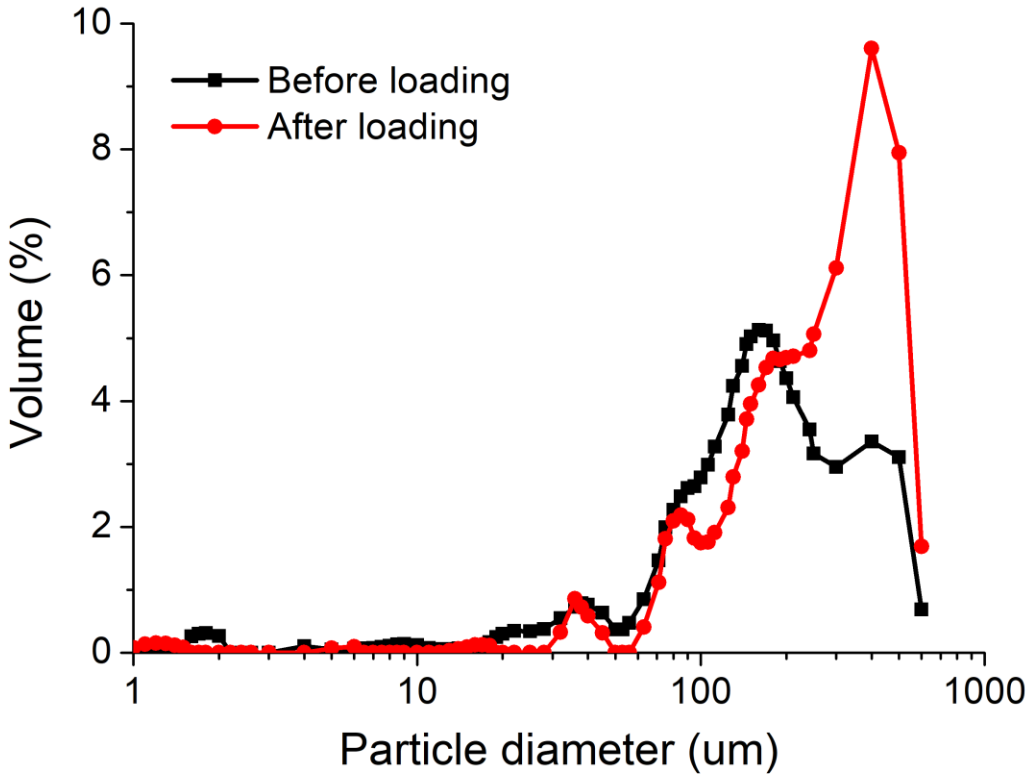


Figure 2.1 The diameter distribution of freeze-dried potato microparticles before and after loading resveratrol.

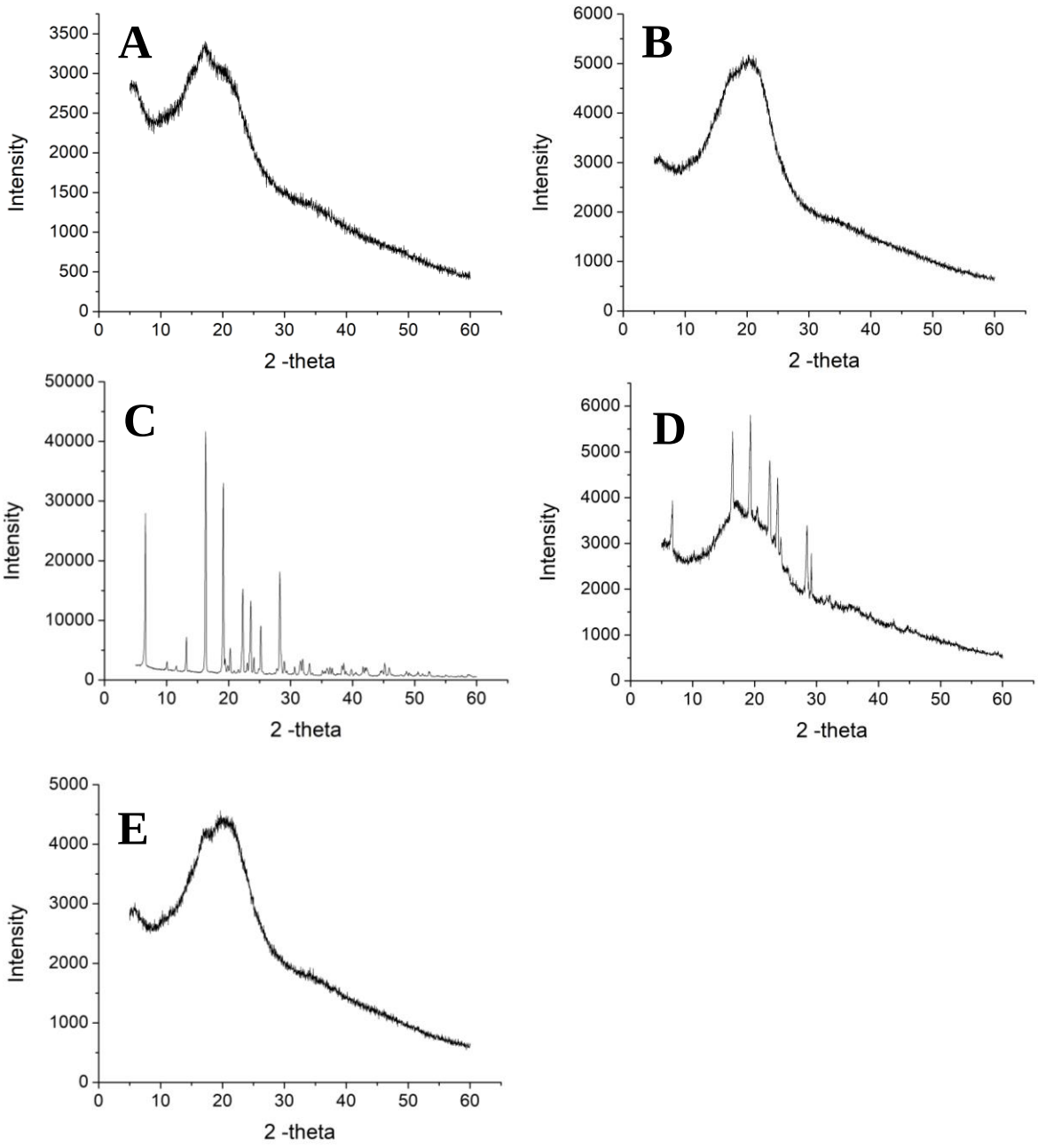
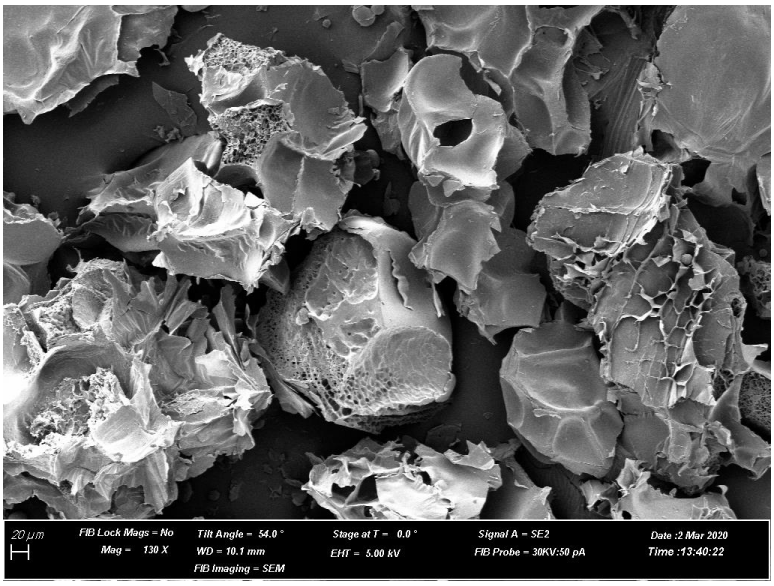


Figure 2.2 XRD patterns of freeze-dried potato microparticles (FDPMs, A), FDPMs treated at encapsulation conditions (B), pristine resveratrol (RSV) (C), physical mixture of FDPMs and pristine RSV (D), and RSV-loaded FDPMs (E).

A



B

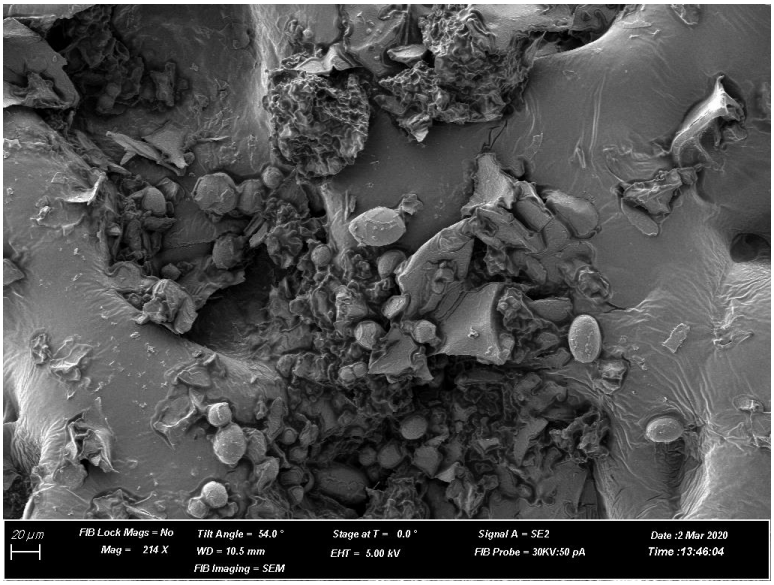
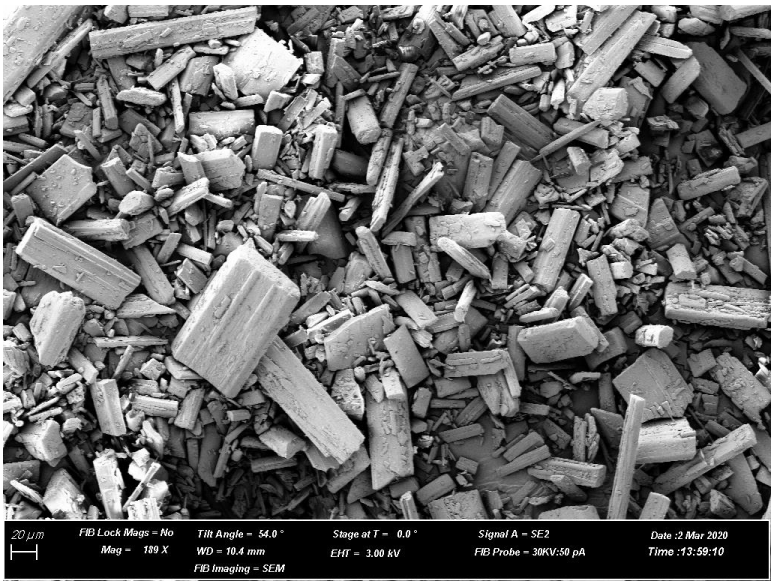


Figure 2.3 SEM images of freeze-dried potato microparticles (FDPMs, A), FDPMs treated at encapsulation conditions (B), pristine resveratrol (RSV) (C), physical mixture of FDPMs and pristine RSV (D), and RSV-loaded FDPMs (E).

C



D

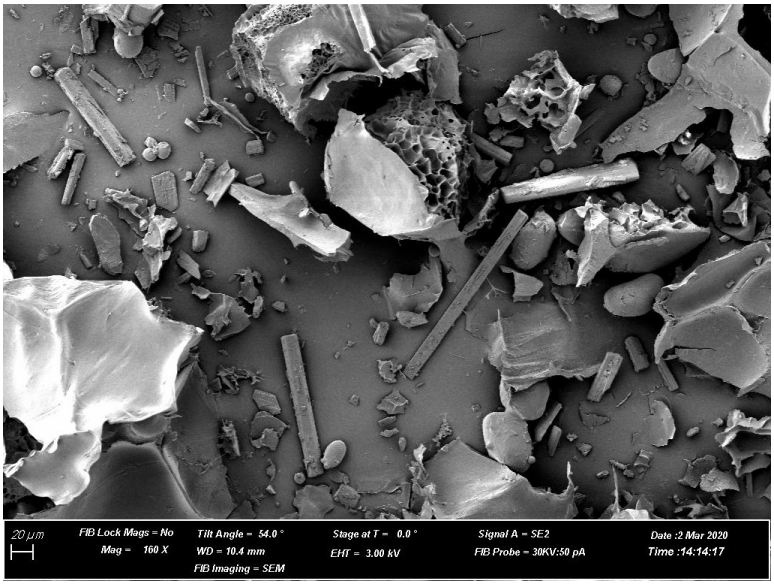


Figure 2.3 continued

E

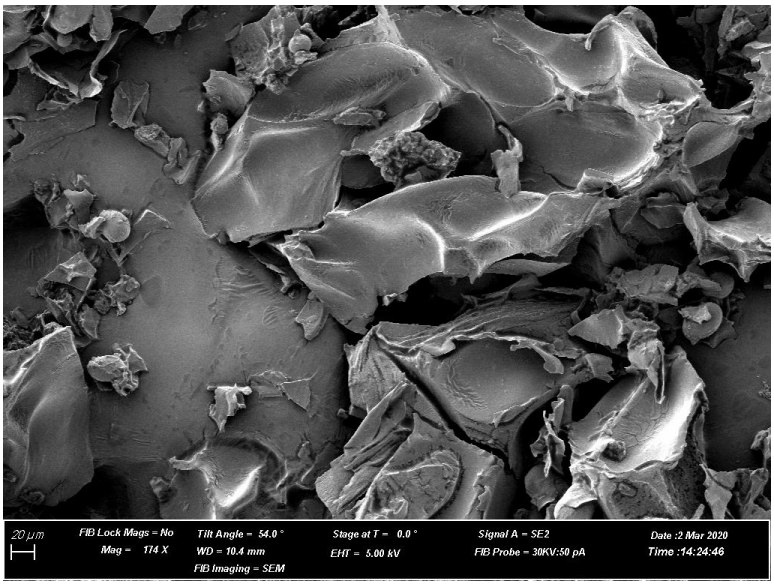


Figure 2.3 continued

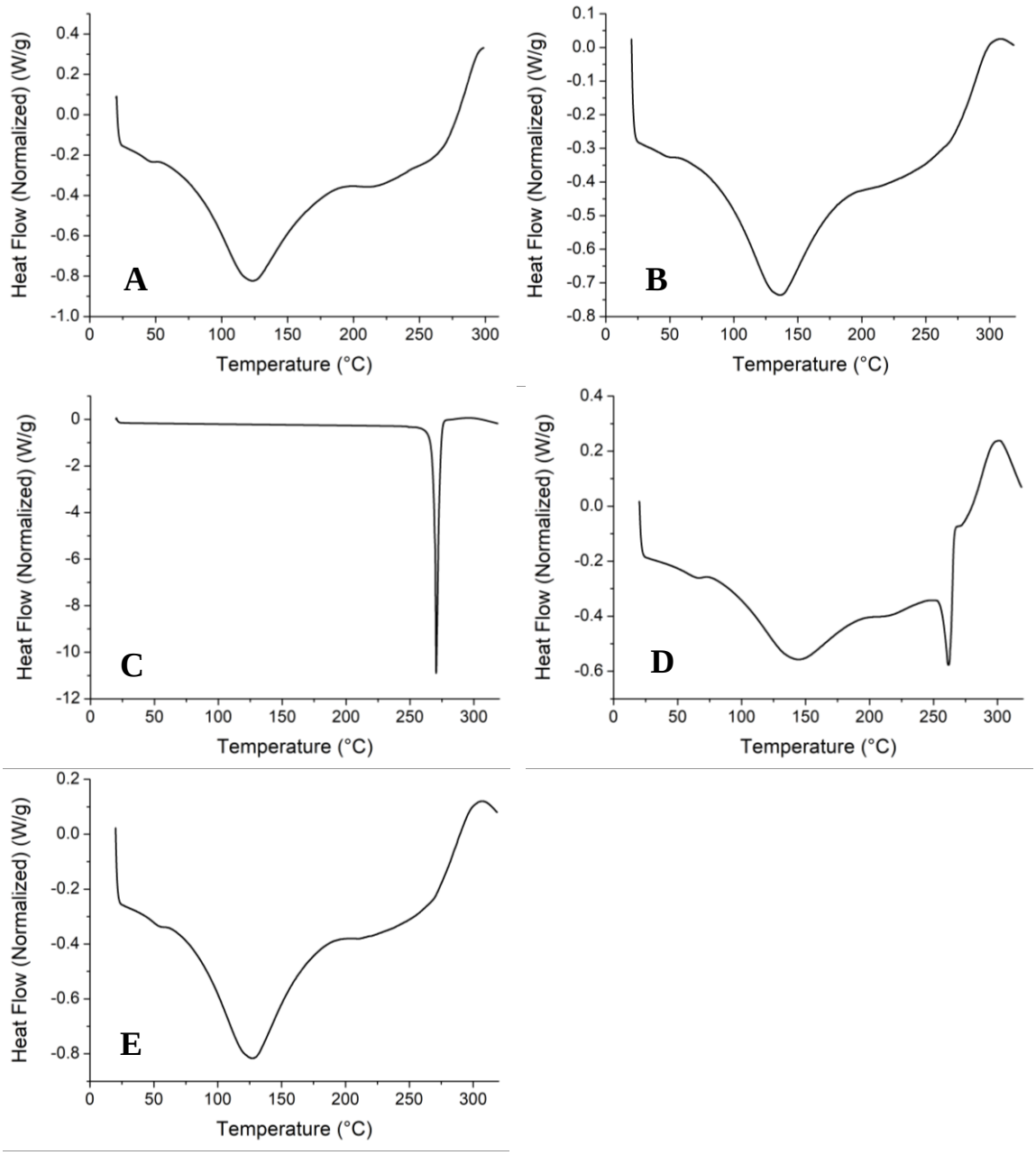


Figure 2.4 DSC thermographs of freeze-dried potato microparticles (FDPMs, A), FDPMs treated at encapsulation conditions (B), pristine resveratrol (RSV) (C), physical mixture of FDPMs and pristine RSV (D), and RSV-loaded FDPMs (E).

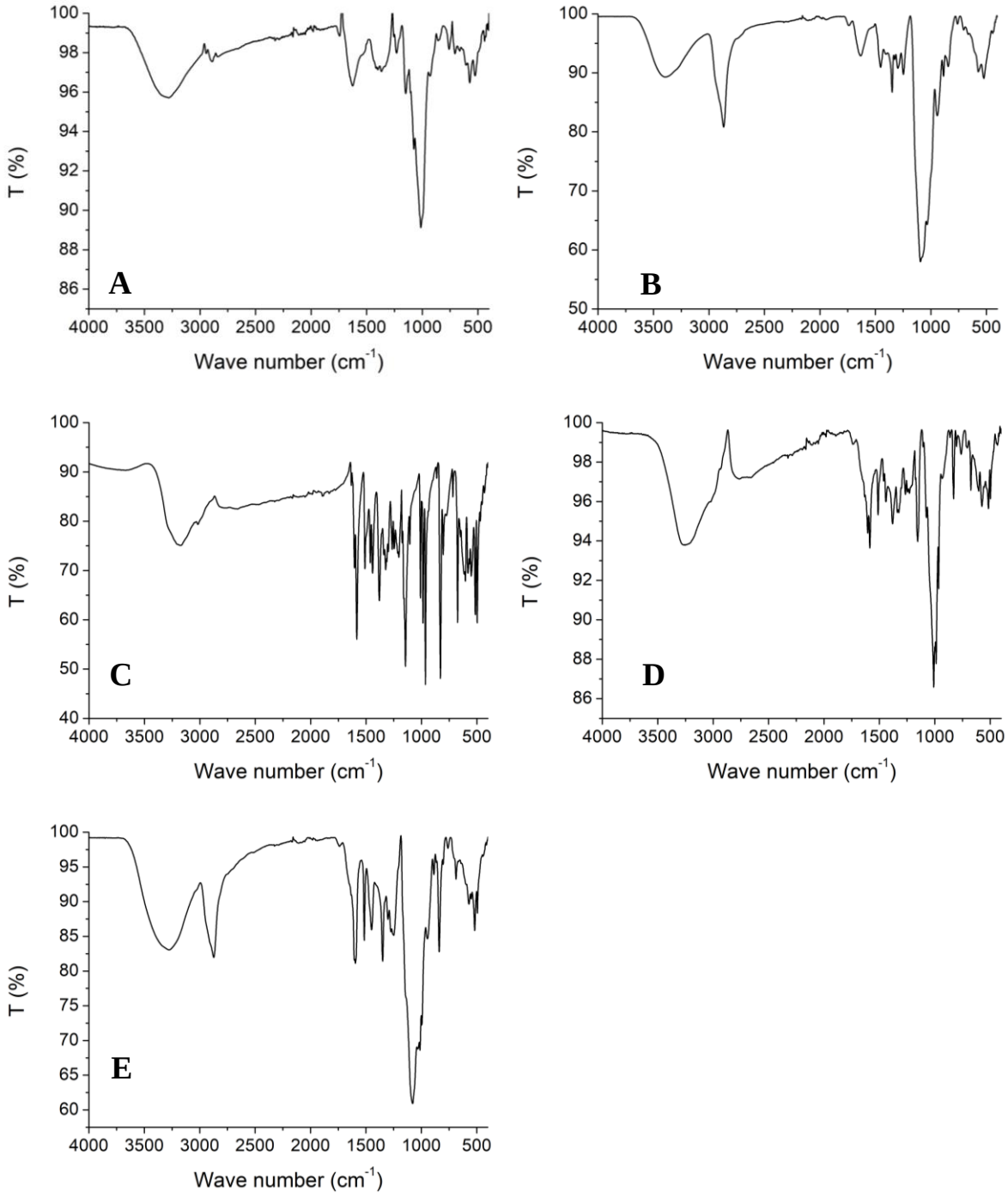


Figure 2.5 FTIR spectra of freeze-dried potato microparticles (FDPMs, A), FDPMs treated at encapsulation conditions (B), pristine resveratrol (RSV) (C), physical mixture of FDPMs and pristine RSV (D), and RSV-loaded FDPMs (E).

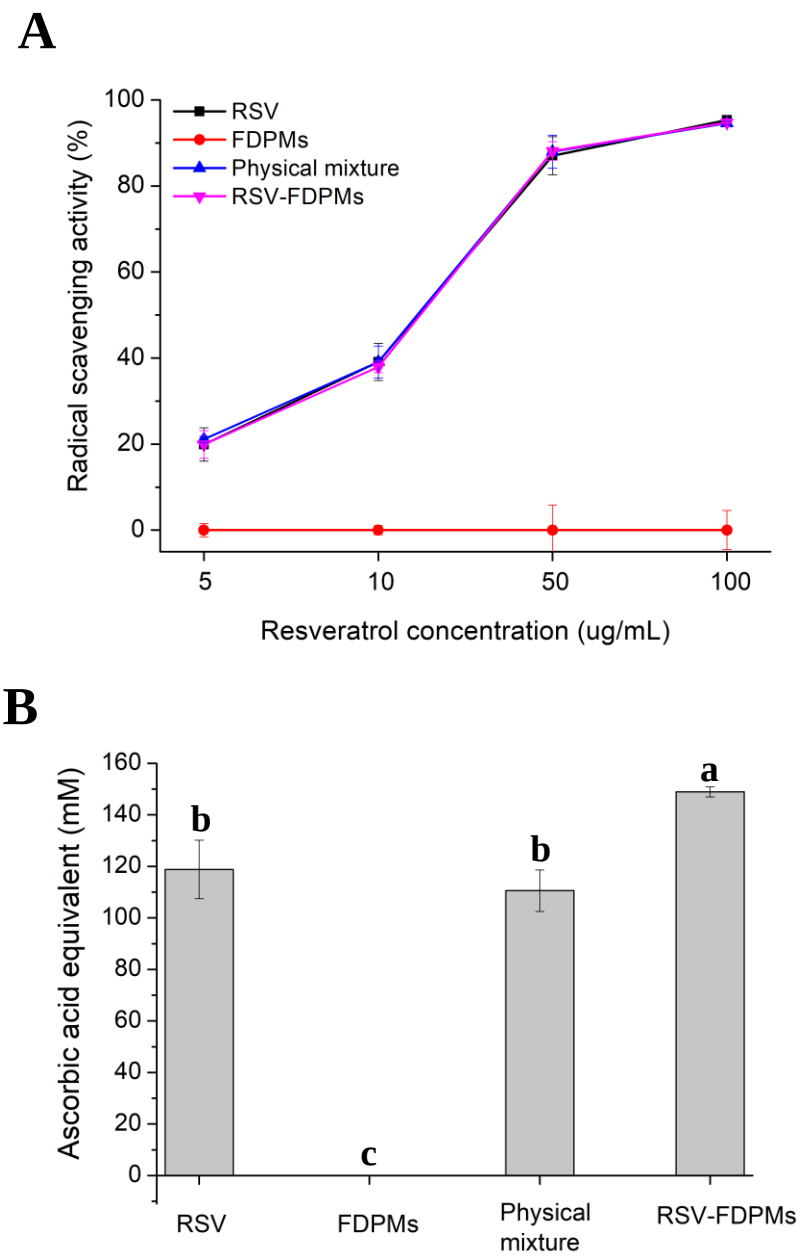


Figure 2.6 DPPH· radical scavenging activity (A) and ferric reducing power (B) of resveratrol (RSV), freeze-dried potato microparticles (FDPMs), physical mixture of RSV and FDPMs, and FDPMs loaded with RSV (RSV-FDPMs). Error bars are SD (n = 2). Different letters above columns in (B) indicate significant difference between mean values ($p < 0.05$).

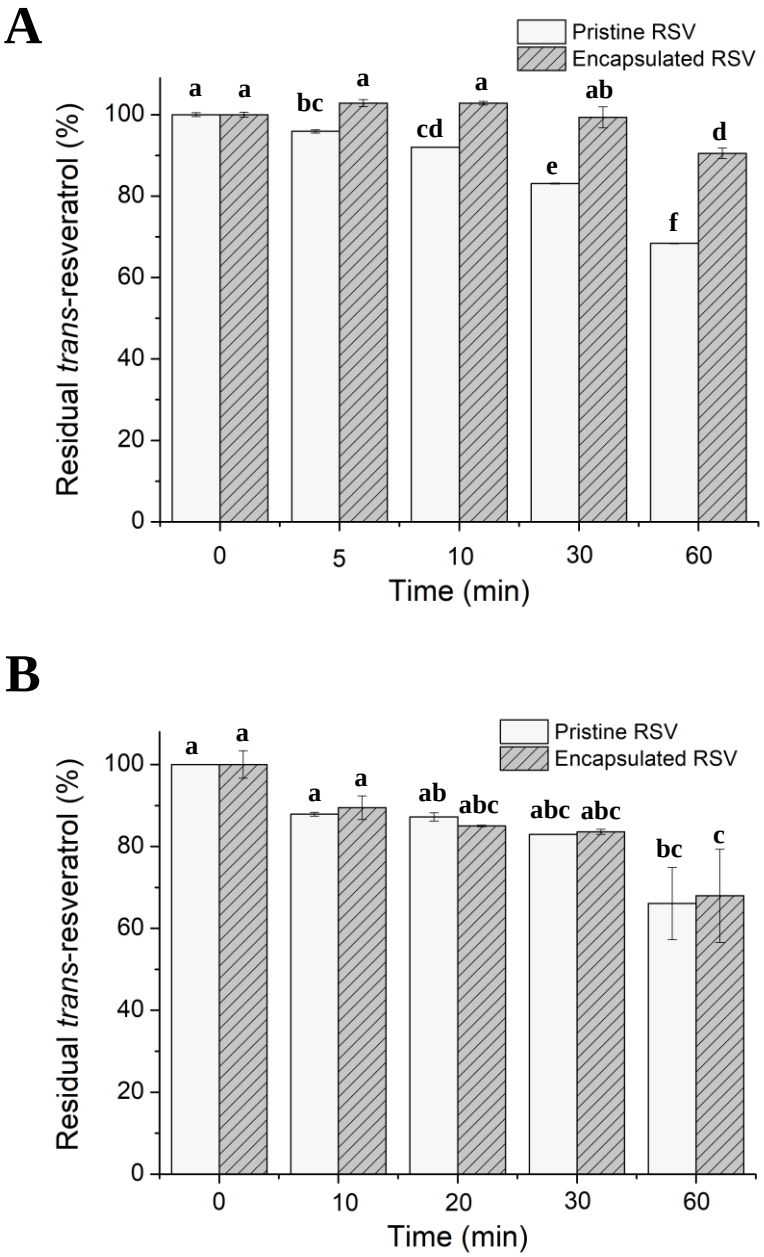


Figure 2.7 Percentage of residual *trans*-resveratrol (RSV) in the pristine or encapsulated form during UV 253 nm irradiation for up to 60 min (A) or heating at 90 °C for up to 60 min (B). Error bars are SD (n = 2). Different letters above columns indicate significant difference between mean values in the same plot ($p < 0.05$).

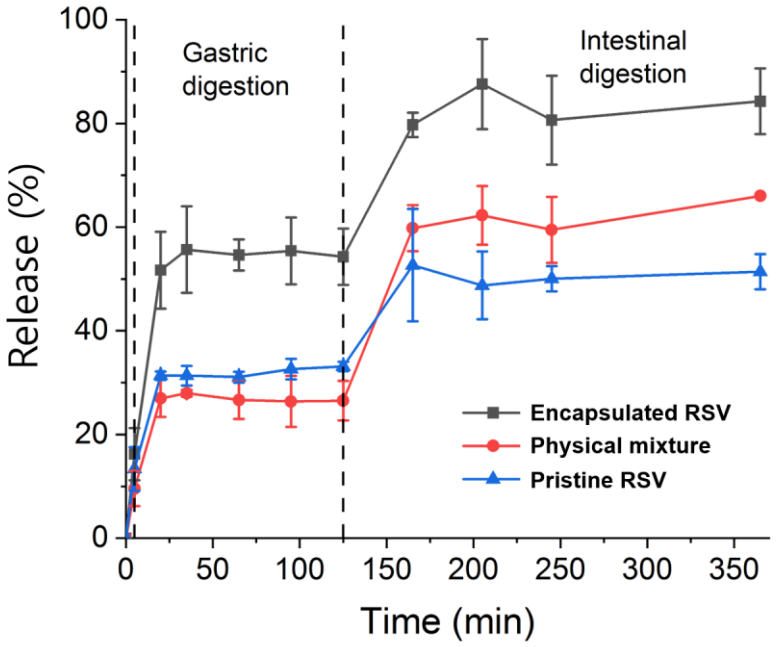


Figure 2.8 The release profiles of pristine resveratrol (RSV), the physical mixture of pristine RSV and freeze-dried potato microparticles, and RSV loaded in freeze-dried potato microparticles during *in vitro* oral, gastric, and small intestinal digestions. Error bars are SD (n = 2).

Chapter 3 Co-loading curcumin and quercetin in freeze-dried mushroom microparticles to inhibit lipid oxidation in beef patties

3.1 Abstract

Curcumin (CCM) and quercetin (QCT) are natural polyphenols with antioxidative properties. In this study, the two antioxidants (5:1 w/w) were loaded into freeze-dried mushroom microparticles (FDMMs) to achieve synergistic antioxidative effect, and CCM-QCT-loaded FDMMs were incorporated into cooked beef patties to inhibit lipid oxidation during storage. The loading was done by diffusing a solution with CCM-QCT dissolved in ethanol and polyethylene glycol 400 (40:60 v/v) into FDMMs and evaporating the solvent in a vacuum oven. The loading capacity was 4.3% and 1.3% (w/w) for CCM and QCT, respectively. Crystalline CCM and QCT became amorphous after loading into FDMMs according to differential scanning calorimetry, X-ray diffraction, and scanning electron microscopy. Confocal laser scanning microscopy confirmed the diffusion of CCM and QCT into the intracellular matrix of FDMMs. Both CCM and QCT were effectively preserved within FDMMs during UV irradiation at 253 nm. While the cooked beef patty controls exhibited significant increase in 2-thiobarbituric acid reactive substances (TBARS) values during 12-day storage at 4 °C, the minimum TBARS values were observed for the patties with CCM-QCT-loaded FDMMs. Our results demonstrate the potential of porous vegetable matrices as carrier materials to utilize lipophilic antioxidants to improve food quality.

Key words: freeze-dried mushroom, diffusion, synergistic antioxidants, lipid oxidation, beef patties

3.2 Introduction

Lipid oxidation is a major culprit of deterioration in the quality of lipid-containing foods, resulting in rancidity, decrease in nutritional value (e.g., loss of vitamins and essential fatty acids), color loss, possible production of toxic compounds, and reduced shelf-life (Ahmed et al., 2016). Muscle foods are one of the major foods that experience quality deterioration during preparation and processing. After meat and fish are slaughtered, lipid oxidation in muscle foods promptly starts and continues progressively during processing and storage (Mariutti & Bragagnolo, 2017). For example, muscle membrane system is disrupted during grinding, resulting in the liberation of lipid components (Sato & Hegarty, 1971). The released lipid components are exposed to oxygen and oxidative catalysts, and cooking can accelerate lipid oxidation by inactivating endogenous antioxidants and breaking down pre-existent lipid hydroperoxide (Decker & Hultin, 1992; Erickson, 2008). The rate and intensity of lipid oxidation in muscle foods are influenced by several parameters, such as composition of muscle foods, cooking conditions, storage temperature, and the use of antioxidants (Erickson, 2008).

To reduce lipid oxidation, synthetic antioxidants have been added into muscle foods for a long time but the meat industry and consumers are turning their attention to natural antioxidants due to controversy over negative health effects of synthetic antioxidants (Domínguez et al., 2019). Although many studies have added natural antioxidants into foods to retard the rate and extent of lipid oxidation (Alamed et al., 2009), traditional cooking causes the reduction of antioxidant activity (16-38%) of natural antioxidants (Koç et al., 2017), whereas some synthetic antioxidants, such as tert-butylhydroquinone (TBHQ), show high thermal stability (Allam & Mohamed, 2002). Also, many natural antioxidants are insoluble in water and have not been

studied for potential toxicity at high concentrations, while their low antioxidant activity at a low concentration (~0.01%) has been identified as a disadvantage. This has limited the application of natural antioxidants in muscle foods (Bouayed & Bohn, 2010; Lorenzo et al., 2018).

Curcumin (CCM) and quercetin (QCT) are two major natural lipophilic antioxidants (NLAs) with biological activities and are classified as polyphenols due to the presence of multiple phenolic hydroxyl groups. Both CCM and QCT have been drawing great attention from researchers due to their remarkable antioxidant properties (Barclay et al., 2000; Zhang et al., 2011). Particularly, CCM and QCT showed synergistic effect in antioxidant activity, suggesting their potential as effective antioxidants in muscle food system (Aftab & Vieira, 2010). As different NLAs can contribute to the inhibition of oxidation process by different mechanisms (Yan et al., 2020), the mixture of different NLAs may have a greater antioxidant activity than a single NLA (Schmidt et al., 2008). However, both NLAs are susceptible to photo-degradation. For example, pristine CCM in water-ethanol mixture (95:5 v/v) was completely degraded after 4 h of sunlight exposure (Suwannateep et al., 2012). QCT also has poor UV stability when dissolved in a polar organic solvent or alkaline aqueous solutions (Dall'Acqua et al., 2012). Since UV-induced polyphenol degradation decreases the antioxidant activity (Volf et al., 2014), it is desirable to obtain synergistic antioxidant activity of CCM and QCT and maintain their structure by protecting them within carrier materials to preserve their antioxidant activity in foods. Inhibition of lipid oxidation in chicken breast was reported after the injection of a nanodispersion with CCM encapsulated in myofibrillar protein (Wu et al., 2019). However, no studies have been conducted to impede lipid oxidation of muscle foods by incorporating powdered ingredients loaded with antioxidants.

To shield CCM and QCT from UV irradiation, freeze-dried mushroom microparticles (FDMMs) were used in this study as a natural plant-based encapsulating material for loading CCM and QCT. Freeze drying is advantageous to the final dried product with little shrinkage and high porosity (Koç et al., 2008). In this study, mushroom was used because it displays higher porosity than many vegetables and fruits after freeze drying (Oikonomopoulou et al., 2011). The overall objectives of this study were to co-load CCM and QCT into FDMMs for synergistic antioxidant activity and evaluate the effectiveness of CCM-QCT-loaded FDMMs in inhibiting oxidation of beef patties as a model muscle food system.

3.3 Materials and methods

3.3.1 Materials

Freeze-dried white mushroom (*Agaricus bisporus*) slices (~0.2 cm of thickness) were obtained from North Bay Trading (Brue, WI). CCM with the purity of 95% was the product of Alfa Aesar (Haverhill, MA). QCT hydrate (95% purity), polyethylene glycol 400 (PEG400), and ferric chloride were purchased from Acros Organics (Fair Lawn, NJ). Ethanol (200 proof) was supplied by Decon Labs (King of Prussia, PA). Trichloroacetic acid (TCA), 1,1,3,3-tetraethoxypropane (TEP), 2-thiobarbituric acid (TBA), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich Corp. (St Louis, MO). Mono- and di-sodium phosphate and potassium ferricyanide were the product of MP Biomedicals, LLC (Santa Ana, CA). Acetonitrile, methanol, and phosphoric acid (85%) were from Fisher Scientific (Pittsburgh, PA). Fresh ground beef with 80% lean and 20% fat was purchased from a local supermarket.

3.3.2 Preparation of freeze-dried mushroom microparticles and determination of porosity

FDMMs were prepared by milling freeze-dried mushroom slices through a sieve with a mesh size of 0.4 mm using a bench top mill (3383-L10 Wiley Mini Mill, Thomas Scientific, Swedesboro, NJ). The porosity of FDMMs was 82.7% and the average pore diameter was 4.62 μm as determined using mercury intrusion porosimetry (AutoPore V 9600, Micrometrics Instrument Corp., Norcross, GA).

3.3.3 Loading of curcumin and quercetin into freeze-dried mushroom microparticles

CCM (8% w/v) and QCT (1.6% w/v) were dissolved in a binary solvent of ethanol and PEG400 (40:60 v/v) and stirred for 4 h on a magnetic stirrer at 990 rpm. The concentration of CCM was the maximal concentration that showed no precipitation in the binary solvent from the solubility test. The QCT concentration was chosen based on the CCM-QCT ratio that exhibited the highest synergistic antioxidant activity from preliminary experiments. FDMMs (0.100 g) were added into 10 mL of CCM-QCT solution, stirred overnight for diffusion, and separated from the solution by filtrating the solution through a 0.45 μm polyvinylidene fluoride (PVDF) membrane (MilliporeSigma, Burlington, MA). The obtained FDMMs were dried at 50 °C in a vacuum oven with an under-pressure of 4,000 Pa for 20 h to evaporate ethanol. The dried FDMMs loaded with CCM and QCT were placed in an amber glass container and kept at 22 °C until each testing was conducted.

3.3.4 Co-detection of curcumin and quercetin

CCM-QCT-loaded FDMMs (10.0 mg) were suspended in 10 mL of methanol and stirred at 22 °C for 4 h. After centrifugation at 14,100 g for 30 min (Minispin Plus, Eppendorff, Hamburg, Germany), the supernatant was filtered through a polytetrafluoroethylene (PTFE) syringe filter with a 0.45 μm pore size (Agilent Technologies, Santa Clara, CA). Chromatographic separation was performed using a C18 column (4.6 × 250 mm, 5 μm, Agilent Technologies, Santa Clara, CA) and an HPLC system (Agilent 1100 Series) equipped with a UV-Vis detector. To detect CCM and QCT simultaneously, the mobile phase of acetonitrile and 1% v/v phosphoric acid (50:50 v/v) was operated in the isocratic mode at a flow rate of 1.0 mL/min for 20 min at 22 °C. The detection wavelength was set at 425 nm for CCM and at 370 nm for QCT. The loading capacity was calculated according to the following equation:

$$\text{Loading capacity (\%)} = \frac{amount\ of\ CCM\ or\ QCT\ in\ \ FDMMs\ (g)}{amount\ of\ FDMMs\ (g)} \times 100 \qquad (1)$$

3.3.5 Particle size of freeze-dried mushroom microparticles before and after loading antioxidants

The size of FDMMs before and after loading curcumin and quercetin was compared by using laser diffraction technique with a particle size analyzer (PSA 1190, Anton Paar, Graz, Austria). The parameters for the dry powder were 56% for vibrator duty cycle, 44 Hz for vibrator frequency, and 40,000 Pa for air pressure. After 10-s measurement at 22 °C, particle size distribution was obtained based on Fraunhofer theory (Naining & Hongjian, 1986).

3.3.6 Scanning electron microscopy (SEM)

The morphology of FDMMs, CCM, and QCT before and after loading NLAs was studied using SEM. Samples in the powder form were attached on an aluminum specimen stub by a carbon tape and gold-coated for 1 min with a sputter coater. Then, samples were observed with a LEO 1525 SEM microscope (FIB/SEM Zeiss Auriga, Oberkochen, Germany).

3.3.7 Confocal laser scanning microscopy (CLSM)

The distribution of CCM and QCT loaded in FDMMs was studied using a confocal laser scanning microscope (Leica TCS SP8, Leica Microsystems, Wetzlar, Germany) equipped with diode UV laser and argon ion laser. Due to the natural fluorescence of CCM, QCT, and some compounds of FDMMs, no labelling was needed. To visualize FDMMs, CCM, and QCT, the excitation wavelength was set as 405 nm, 488 nm, and 458 nm,(Bourbon et al., 2016; Pawlikowska-Pawlęga et al., 2007) respectively. Images were obtained at 10-fold magnification using LAS X software (Leica Microsystems, Wetzlar, Germany).

3.3.8 X-ray diffraction (XRD)

The XRD patterns of various samples were obtained using an X-ray diffractometer (The Panalytical Empyrean, Malvern Panalytical, Worcestershire, UK) equipped with a Cu Kα source at a wavelength of 1.5406 Å. Measurements were operated with a scan speed of 0.112°/s and a step size of 0.026°. The 2θ angle range was set from 5° to 60°.

3.3.9 Differential scanning calorimetry (DSC)

The thermal stability of powder samples was characterized using a DSC system (DSC 250 Discovery, TA Instruments, New Castle, DE). Approximately 5 mg of powder was weighed and transferred to an aluminum pan, and an empty pan was used as a reference. After the pan was tightly sealed using a hermetic lid with a pinhole, samples were heated from 30 °C to 340 °C at a rate of 10 °C/min. The flow rate of the transfer nitrogen gas was 30 mL/min.

3.3.10 Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra of FDMMs, CCM, and QCT before and after encapsulation were obtained from 64 scans using a Spectrum Two FTIR instrument (PerkinElmer, Waltham, MA). The scanning region was from 4000 to 400 cm^{-1}, and the wavenumber resolution was set at 4 cm^{-1}.

3.3.11 Photostability test

The chemical stability of CCM and QCT against UV irradiation was studied. Briefly, CCM-QCT-loaded FDMMs (10.0 mg) were suspended in 10 mL of PBS (pH 7.0, 50 mM). Pristine CCM (0.43 mg) and QCT (0.13 mg) samples were suspended at concentrations comparable to CCM-QCT-loaded FDMMs in methanol/PBS mixture (20:80, v:v). A UV lamp (30 W) with a wavelength of 253 nm was used for irradiation. Samples were irradiated while being stirred on a magnetic stirrer for 1 h at the distance of 35 cm from the lamp. Each 10-mL sample was withdrawn at predetermined time intervals (0, 5, 10, 30, and 60 min) and mixed with methanol (10 mL) for 4 h at 22 °C. The supernatant after centrifugation of samples at 14,100 *g* for 30 min

was filtered through a PTFE syringe filter (0.45 μm pore size) and analyzed for residual CCM and QCT concentrations using the HPLC method described above.

3.3.12 DPPH· assay for synergistic antioxidant activity

The synergistic antioxidant effect of CCM and QCT was evaluated using the DPPH· assay following the principle described previously (Molyneux, 2004). FDMMs (0.22 mg), pristine CCM (10 μg), pristine QCT (3 μg), and their mixtures at same mass, or FDMMs loaded with NLAs (0.233 mg) were prepared in methanol. The ratio of FDMMs to CCM/QCT was the same for the physical mixture and capsules. Each sample (2 mL) was mixed with the same volume of methanol-based 0.1 mM DPPH solution for 10 s and incubated for 1 h in amber vials at 22 °C. The absorbance of the top clear serum was measured at 517 nm using a model Evolution 201 UV-vis spectrophotometer (Thermo Scientific, Waltham, MA). The reduction of the DPPH·, *Q*% (i.e., DPPH· scavenging activity) was calculated using the following equation:

$$Q\% = 100\ (A_0 - A_C)/A_0 \qquad (2)$$

where A_0 and A_C are the absorbance of the control (methanol without test sample) and a test sample, respectively.

The synergistic effect (*SE*) can be defined as the ratio between the experimental antioxidant activity and the theoretical antioxidant activity (Zanfini et al., 2010). The *SE* of CCM and QCT on DPPH· scavenging activity was calculated using eq. 3. CCM and QCT display synergistic action if SE is greater than 1.

$$SE = \frac{Q}{Q_{theoretical}} \qquad (3)$$

Where, $Q_{theoretical}$ is $Q_{CCM} + Q_{QCT} - \frac{Q_{CCM} \times Q_{QCT}}{100}$ (Zanfini et al., 2010).

3.3.13 Ferric reducing antioxidant power (FRAP) test

The FRAP was conducted with slight modification of a previously reported method (Huang et al., 2019). FDMMs (77.0 mg), CCM (3.3 mg), or QCT (1.0 mg) were mixed with 2.5 mL of PBS (0.2 M, pH 7.0), and then potassium ferricyanide solution (2.5 mL, 1% w/v) was added. After being heated for 20 min in a water bath at 50 °C, the solution mixtures were cooled down in an iced water bath for 10 s and acidified with 2.5 mL of a 10% w/v TCA solution. The supernatant (0.1 mL) was collected after centrifugation for 10 min at 3,000 *g* and 21 °C and mixed with deionized water (0.9 mL) and ferric chloride solution (0.1 mL, 0.1% w/v) for 10 min. The absorbance was read at 700 nm using the UV-vis spectrophotometer mentioned above. The standard curve was prepared using ascorbic acid solutions at the concentration range of 0-70 µg/mL. The ferric reducing power of samples was converted to ascorbic acid equivalent (µg/mL).

3.3.14 Preparation of hamburger patties

Hamburger patties with a total mass of 200 g were prepared using 80% lean ground beef according to the formulations presented in Table 1. The ingredients were mixed using the grind function of a commercial blender (model 58148A, Hamilton Beach, Glen Allen, VA) for 1 min and then divided into duplicate samples (~ 90 g). Patties were flattened to approximately 1 cm of thickness and cooked on an electric skillet at 180 °C until the internal temperature of patties reached 75 °C. After cooling down to 22 °C in styrene foam tray, the cooked patties were stored in Ziploc bags for 12 days in a refrigerator with a temperature of ~ 4 °C.

3.3.15 Lipid oxidation test

The lipid oxidation of the patties was evaluated for the TBA reactive substances (TBARS) (Sørensen & Jørgensen, 1996). Patties were cut into small chunks and homogenized with 20% TCA solution using a commercial blender (model 51131, Hamilton Beach, Glen Allen, VA) for 1 min and a microprocessor homogenizer (a model Cyclone I.Q., VirTis, Gardiner, NY) for 1 min at 30,000 rpm. The homogenized samples were filtered through a PTFE filter (0.45 μm), and the filtrate (0.7 mL) was mixed with the same volume of 0.01 M TBA solution. After being heated for 30 min at 70 °C, samples were measured for the absorbance at 532 nm using the above UV-VIS spectrophotometer. The absorbance was used to quantify TBARS values that were expressed as mg of malondialdehyde (MDA) per kg of beef. To prepare MDA stock solution, 73.37 mg of TEP was dissolved in 10 mL of 0.1 M HCl and heated in a water bath for 5 min at 95 °C to convert TEP to MDA. The standard curve of MDA was established in the concentration range of 2.4 – 40 μg/mL.

3.3.16 Statistical analysis

Each measurement was done with at least two replicates. The mean and standard deviation (SD) were calculated for loading capacity, DPPH· scavenging capacity, FRAP, and photostability. Statistical analysis was conducted using SAS enterprise guide 7.1 (SAS Institute Inc, Cary, NC). Fisher's least significant-difference (LSD) test with the analysis of variance (ANOVA) was used to determine the significant differences of mean values of treatment samples at the significance level of 0.05.

3.4 Results and discussion

3.4.1 Particle size and loading capacity

The particle size distribution of FDMMs (Fig. 3.1) showed that the number of FDMMs smaller than 50 μm slightly decreased after loading CCM and QCT, whereas more FDMMs in the range of 50 - 105 μm were observed after the loading. However, the De Brouckere mean diameter ($D_{4,3}$) of FDMMs from the particle size distribution was 161.9 ± 2.5 and 153.9 ± 6.5 μm before and after loading CCM and QCT, respectively, showing no significant difference ($p > 0.05$). This suggests that the encapsulation of CCM and QCT can be achieved without affecting the size of FDMMs.

The loading capacity was 4.3% for CCM and 1.3% for QCT. The loading capacity of NLAs greatly depends on the composition of encapsulation materials and methods. For example, when protein nanoparticles were used to encapsulate CCM, the loading capacity was 11.7%, whereas the loading capacity was only 0.4% when lipid nanoparticles were used (Zou et al., 2016). QCT is often dissolved in an organic solvent before being encapsulated. When QCT was dissolved in 100% ethanol for nanoformulation with cellulose nanofibers, the loading capacity was 24.3%; however, the loading capacity was greatly enhanced to 74.2% by dissolving QCT in the mixture of water and ethanol (50:50 v/v) (X. Li et al., 2019). A study reported the loading capacity of QCT in three different delivery systems: solid lipid nanoparticles, nanostructured lipid carriers, and lipid nanoemulsions being 0.6%, 0.9%, and 0.9%, respectively (Aditya et al., 2014). The results demonstrated that the simple and scalable method used in the present study to load CCM and QCT in FDMMs can obtain a loading capacity comparable to literature studies.

3.4.2 Morphology of curcumin, quercetin, and freeze-dried mushroom microparticles

The SEM surface morphology of CCM, QCT, and FDMMs before and after encapsulation is displayed in Fig. 3. Before encapsulation, the FDMMs had irregular structures with a highly porous surface (Fig. 3.2A and E), which probably promoted the absorption of CCM and QCT dissolved in the ethanol/PEG400 solution. A recent study reported similar flaky structures of pulverized mushroom powder with a porous surface (Kim et al., 2021). Micrometer scale pores were also observed for the mushroom powder studied as absorbents (Banga et al., 2015). As shown in Fig. 3.2B, pristine CCM had irregular crystalline structures with a length of 2-30 μm, which was similar to the observation in a previous study (Kakran et al., 2012). On the other hand, crystalline QCT displayed more needle-like structures (Fig. 3.2C). This distinction of crystalline structures between CCM and QCT was captured in the physical mixture (Fig. 3.2D and E). By loading CCM and QCT in FDMMs, the original crystalline structures of both compounds disappeared (Fig. 3.2F), which indicates structural transformation of both CCM and QCT. In addition, CCM-QCT-loaded FDMMs had a smooth surface, probably due to the pore-reducing effect of PEG (Kumar et al., 2020).

3.4.3 Distribution of curcumin and quercetin loaded within freeze-dried mushroom microparticles

CLSM imaging was used to evaluate the distribution of CCM and QCT loaded within FDMMs (Fig. 3.3). Endogenous auto-fluorescent compounds of FDMMs exhibited fluorescent intensity at the excitation wavelength of 405 nm regardless of the loading of CCM and QCT (Fig. 3.3A-1 and B-1). Strong fluorescent intensity from CCM at 488 nm and from QCT at 458

nm was observed along the contour of FDMMs auto-fluorescent compounds, illustrating the uniform distribution of loaded CCM and QCT. The observance of fluorescence from z-stack image at 150 μm indicates that diffusion of NLA solution occurred effectively, delivering CCM and QCT into not only the surface pores but also the intra-cellular tissues of FDMMs. This indicates that the binding of loaded CCM and QCT to the cytoplasm and organelles of FDMMs occurred (Rai et al., 2021). Embedding CCM and QCT in FDMMs may be responsible to the improvement in UV stability observed for QCT in Fig. 3.7.

3.4.4 Crystalline structures

All the samples exhibited their characteristic peaks in XRD curves, shown in Fig. 3.4. FDMMs showed distinguished peaks at 9.6° and 21.1°. The cellular wall of mushroom has a high amount of chitin present in different crystalline forms depending on the source of raw materials: α-, β-, and γ-chitin (Ospina et al., 2015). The α-chitin has sharp peaks around 9.6° and 21.1° (Jang et al., 2004), which were observed for FDMMs. The FDMMs treated at loading conditions without or with CCM and QCT still exhibited characteristic peaks of α-chitin, but with the reduced intensity. In a previous study, the reduced peak intensity from crystalline chitosan was also observed when chitosan was blended with PEG (Parker et al., 2016). Narrow crystalline peaks at 2θ of 8.9°, 14.5°, 17.3°, 18.2°, 24.6°, and 25.6° were observed for pristine CCM, and 10.8°, 12.5°, and 27.4° for QCT, which are comparable to previous studies (Li et al., 2016; Sahoo et al., 2011). The sharp peaks of pristine CCM and QCT were maintained in the CCM-QCT and CCM-QCT-FDMMs mixtures, revealing the same crystalline state of the NLAs in the physical mixture form. However, CCM-QCT-loaded FDMMs did not exhibit diffraction

peaks corresponding to pristine CCM and QCT, indicating that the NLAs existed in the amorphous state rather than the crystalline state.

3.4.5 DSC thermal properties

The endothermic peak resulting from glass transition or melting was identified for each sample in DSC (Fig. 3.5). A minor broad peak was observed around 110 °C for FDMMs both before and after treatment at loading conditions without CCM and QCT. Due to the amorphous structure of FDMMs as observed in SEM (Fig. 3.2A), FDMMs did not show well-resolved sharp peaks. Another study also reported a smooth peak at around 125 °C for mushroom concentrate powder (Umaña et al., 2021). The wide and flat peak of FDMMs is partially attributed to their heterogenous composition. CCM and QCT showed an endothermic peak at its melting point of 180 °C and 327 °C, respectively. The curve of QCT had another peak at around 135 °C due to dehydration (Pool et al., 2012). Similar curves were observed for the physical mixture of pristine CCM and QCT with and without FDMMs, maintaining the endothermic peaks around 130 °C and 180 °C. However, a slight change in the peak position of QCT dehydration and the disappearance of QCT melting peak around 327 °C suggest that QCT might interact with CCM during heating. CCM-QCT-loaded FDMMs presented no typical thermal transition observed in pristine CCM and QCT, suggesting that both CCM and QCT were successfully loaded in FDMMs.

3.4.6 ATR-FTIR analysis

FTIR spectra of FDMMs, CCM, and QCT in various forms are presented in Fig. 3.6. The control FDMMs without any treatment displayed several absorption peaks at 3265, 2935, 1635,

1548, 1305, 1081, 1022, 930, 890, and 622 cm^{-1}. Mushrooms of different origins may display varied FTIR spectrum patterns due to the difference in chemical compositions. The peaks observed from our commercial freeze-dried mushroom with unknown species were similar to those of Boletaceae mushrooms (Yao et al., 2018). Two sharp peaks at 1081 cm^{-1} and 1022 cm^{-1} are a result of C–O–C and C–O–H stretching vibration of glycosidic linkages (W. Li et al., 2019). The similarity of peaks from FDMMs with previously reported spectrum of mushrooms and chemical standards reveals that characteristic peaks from our observation are mainly assigned to protein (e.g., 1635 cm^{-1} from amide I vibration of proteins (Barth, 2007)) and polysaccharides (e.g., 1081, 1022, and 890 cm^{-1}), especially β-D-glucan which is the major compound in mushroom (Choong et al., 2014).

The recorded peaks of CCM at 3509, 1601, 1508, 1273, 1025, and 962 cm^{-1} were comparable to the observation from a study that thoroughly analyzed vibrational spectra of CCM molecules (Kolev et al., 2005). The peaks at 3509 cm^{-1} and 1601 cm^{-1} are from the stretching vibration of O–H and C=C from the phenolic ring, respectively (Bich et al., 2009). Another sharp peak at 1508 cm^{-1} is attributed to mixing of C–O and C=C vibrations while the one at 1273 cm^{-1} is from C–O stretching of ether group (Roy & Rhim, 2020). Characteristic bands of C–H and C–O were also observed at 1025 cm^{-1} and 962 cm^{-1}, respectively.

QCT presented strong intensities at 3398, 1663, 1608, 1520, 1259, 1166, 674, and 638 cm^{-1}. The characteristic peaks of CCM and QCM were maintained in the FTIR spectra of the mixture of CCM and QCT as well as the mixture of CCM, QCT, and FDMMs, with the reduced intensity due to dilution with other components. CCM-, QCT-, and CCM-QCT-loaded FDMMs exhibited characteristic peaks of FDMMs without any new peaks generated. This indicates that there were

no chemical interactions but only physical interactions at the studied conditions. Shifts in characteristic peaks were observed from 1501 to 1514 cm^{-1} and 1273 to 1285 cm^{-1} for CCM-loaded FDMMs and from 1663 to 1652 cm^{-1} for QCT-loaded FDMMs but not in solvent-treated FDMMs. The results suggest that the shifts in peaks resulted from the binding of CCM and QCT to FDMMs.

3.4.7 UV-stability of curcumin and quercetin loaded in freeze-dried mushroom microparticles

Photochemical stability of CCM and QCT after irradiation at 253 nm is shown in Fig. 3.7. During 1 h of UV irradiation, residual CCM barely changed (98.9% for pristine CCM and 97.7% for CCM loaded in FDMMs), while the residual QCT concentration did not decrease for the encapsulated sample after UV irradiation (97.7%, $p > 0.05$) but was only 73.7% for the pristine QCT. CCM degradation during UV irradiation is remarkably affected by the pH of solvent. For example, after 2 h of UV irradiation at 254 nm, the residual CCM was about 25% in the PBS at pH 8.0 but was close to 75% in the PBS at pH 7.0 (Lee et al., 2013). The little change of residual CCM in present study may be due to the use of neutral pH (7.0) of the PBS and short-time treatment (1 h) of UV irradiation. QCT has five hydroxyl groups (–OH) on its C3, 3′, 4, 5, and 7 positions (Dall'Acqua et al., 2012). The higher number of hydroxyl groups in QCT makes it more degradable than CCM when QCT is dissolved in nucleophilic solvents such as PBS. This is because nucleophilic molecules such as water promote intermolecular hydrogen bonding between nucleophiles and hydroxyl group of flavonoids, changing molecular geometry of QCT (Borghetti et al., 2012). For example, when CCM and QCT were separately dissolved in PBS-based solution for 3 h, 46% of residual CCM was detected but no QCT was observed (Vaz et al., 2020). The degradation of hydroxyl groups in QCT mainly occurs at C3, 3′ and 4 positions,

which are responsible for the antioxidant activity of QCT (Dall'Acqua et al., 2012). A study reported that 42% of QCT was degraded in the mixture of water and ethanol but only ~14% of QCT degradation was observed in nanoemulsions (Kaur et al., 2016). Our results suggest that FDMMs can effectively protect QCT from photodegradation in nucleophilic solvents.

3.4.8 Synergistic antioxidant effect of curcumin and quercetin

Pristine CCM and QCT at the concentrations corresponding to those loaded in FDMMs showed comparable *Q* values, and FDMMs barely had a *Q* value of only 2.5% (Table 2). Both pristine CCM and QCT presented antioxidant activity due to their phenolic hydroxyl groups (–OH), which are directly related to their free radical scavenging ability (Chen et al., 2020). FDMMs loaded with one antioxidant, CCM or QCT, had a similar *Q* value as that of pristine CCM and QCT ($p > 0.05$). This trend was also observed when CCM and QCT were loaded together in FDMMs. This indicates that the antioxidant activity of CCM and QCT was still retained after being loaded in FDMMs, demonstrating the potential of FDMMs as a carrier material to maintain antioxidant properties of the NLAs. Another study also reported the unaffected antioxidant activity of CCM after encapsulation in millet protein (Wang et al., 2018).

The *SE* (eq. 3) of CCM and QCT in physical mixture and encapsulated forms was derived (Table 2). The combination of CCM and QCT presented the *SE* value greater than 1, in both free and encapsulated forms, suggesting their synergistic antioxidant activity. The higher *SE* was found for CCM and QCT loaded in FDMMs than the physically mixed CCM and QCT. However, interaction mechanisms that elucidate the synergism in antioxidant activity of different NLAs are very little known because the exact antioxidant mechanism of each compound is needed to prove the synergy (Guleria, 2017). Some NLAs coordinate antioxidant activity

depending on their reaction rates or the concentration of antioxidants at the oxidation location, and the NLA with a slower reaction rate tends to regenerate the other NLA by transferring hydrogen to the radical from the reacted antioxidant (Cuvelier et al., 2000; Guo et al., 2021). QCT usually has a higher oxidation inhibition rate than CCM, (Fujisawa & Kadoma, 2006) and CCM may help the regeneration of QCT radicals, resulting in the synergy of the two NLAs.

3.4.9 Inhibition of lipid oxidation of beef patties

TBARS values of beef patties prepared with different formulations (Table 1) during refrigerated storage (4 °C) are presented in Fig. 3.8. On the first day of storage, the treatment with CCM-QCT-loaded FDMMs had the lowest TBARS value, while the highest TBARS value was observed from the treatment with physical mixture of QCT and FDMMs. The TBARS value of the control patties increased during storage and was higher than other samples from day 3 ($p < 0.05$). Patties with FDMMs did not present an increase in TBARS value ($p > 0.05$) after 12-day storage, with the CCM-QCT-loaded FDMMs treatment showing a lower level than any other treatments ($p < 0.05$). This suggests that lipid oxidation in cooked patties was significantly inhibited with the incorporation of FDMMs, CCM, and QCT, most effectively with the addition of CCM-QCT-loaded FDMMs.

A study reported that TBARS production was inhibited in pork patties with 0.05 wt% clove, cassia bark, or rosemary extracts, and the inhibition by these extracts was similar and was comparable to the treatment with 0.05 wt% butylated hydroxyanisole (Kong et al., 2010). In our study, CCM-QCT-loaded FDMMs inhibited TBARS increases better than the physical mixture with same amounts of CCM, QCT, and FDMMs. One reason may be that the antioxidant activity of CCM and QCT in beef patties after cooking was retained better when loaded in FDMMs. For

example, the increased heat stability was reported for CCM loaded in soluble polysaccharides nanocomplexes (Chen et al., 2017) and QCT within niosomes (Elmi et al., 2021). Another reason is that the synergistic antioxidant activity of CCM and QCT (Table 2) is better fulfilled when the two NLAs are in the amorphous structure improving the water solubility and are in proximity. Results in Fig. 3.8 also suggest the strong antioxidative properties FDMMs, which was not observed in the DPPH· scavenging assay (Table 2). When quantified for FRAP, the FRAP of FDMMs (291.2 ± 5.3 μg/mL) was comparable to that of CCM (336.0 ± 8.6 μg/mL) and QCT (331.1 ± 3.0 μg/mL). Therefore, it is possible that FDMMs can weaken the prooxidant role of multivalent cations in beef patties rich in iron.

3.5 Conclusion

Successful loading of CCM and QCT in FDMMs demonstrated the potential application of porous vegetable matrices as carriers of various NLAs. Diffusion of the NLA solution in FDMMs, as confirmed in CLSM, resulted in a loading capacity comparable to previous reports. Inclusion of the NLAs in FDMMs improved the stability during UV irradiation, more significant for QCT. Inclusion of the NLAs in FDMMs also transformed the crystalline to amorphous state of CCM and QCT, as observed for the disappearance of characteristics peaks in XRD spectra and melting peak in DSC thermograms. The presence of the two NLAs with synergistic antioxidant activity in FDMMs and likely their amorphous state resulted in the improved ability to effectively reduce lipid oxidation in cooked beef patties during storage. The findings from our study may expand the use of vegetable microparticles for incorporating a variety of NLAs to improve food quality.

3.6 Acknowledgment

The authors are thankful to Dr. Tong Wang, Dr. John Dunlap, Dr. Michael Koehler, Kendhl Seabright, and Jaydeep Kolape for providing trainings on the operation of equipment and offering technical assistance. The present study was funded by the University of Tennessee Institute of Agriculture and USDA National Institute of Food and Agriculture hatch project TEN00568. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s).

References

Aditya, N. P., Macedo, A. S., Doktorovova, S., Souto, E. B., Kim, S., Chang, P.-S., & Ko, S. (2014). Development and evaluation of lipid nanocarriers for quercetin delivery: A comparative study of solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and lipid nanoemulsions (LNE). *LWT - Food Science and Technology*, *59*(1), 115-121. https://doi.org/https://doi.org/10.1016/j.lwt.2014.04.058

Aftab, N., & Vieira, A. (2010). Antioxidant activities of curcumin and combinations of this curcuminoid with other phytochemicals. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *24*(4), 500-502.

Ahmed, M., Pickova, J., Ahmad, T., Liaquat, M., Farid, A., & Jahangir, M. (2016). Oxidation of Lipids in Foods. *Sarhad Journal of Agriculture*, *32*(3).

Alamed, J., Chaiyasit, W., McClements, D. J., & Decker, E. A. (2009). Relationships between free radical scavenging and antioxidant activity in foods. *Journal of agricultural and food chemistry*, *57*(7), 2969-2976.

Allam, S. S. M., & Mohamed, H. M. A. (2002). Thermal stability of some commercial natural and synthetic antioxidants and their mixtures. *Journal of Food Lipids*, *9*(4), 277-293.

Banga, S., Varshney, P. K., & Kumar, N. (2015). Purification of Jatropha curcas based biodiesel by dry washing, using banana peel and mushroom powder as natural adsorbents. *Biofuels*, *6*(5-6), 261-267.

Barclay, L. R. C., Vinqvist, M. R., Mukai, K., Goto, H., Hashimoto, Y., Tokunaga, A., & Uno, H. (2000). On the antioxidant mechanism of curcumin: classical methods are needed to determine antioxidant mechanism and activity. *Organic letters*, *2*(18), 2841-2843.

Barth, A. (2007). Infrared spectroscopy of proteins. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1767*(9), 1073-1101. https://doi.org/https://doi.org/10.1016/j.bbabio.2007.06.004

Bich, V. T., Thuy, N. T., Binh, N. T., Huong, N. T. M., Yen, P. N. D., & Luong, T. T. (2009). Structural and spectral properties of curcumin and metal-curcumin complex derived from turmeric (Curcuma longa). In *Physics and engineering of new materials* (pp. 271-278). Springer.

Borghetti, G., Carini, J., Honorato, S., Ayala, A., Moreira, J., & Bassani, V. (2012). Physicochemical properties and thermal stability of quercetin hydrates in the solid state. *Thermochimica acta*, *539*, 109-114.

Bouayed, J., & Bohn, T. (2010). Exogenous antioxidants—double-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxidative medicine and cellular longevity*, *3*(4), 228-237.

Bourbon, A. I., Cerqueira, M. A., & Vicente, A. A. (2016). Encapsulation and controlled release of bioactive compounds in lactoferrin-glycomacropeptide nanohydrogels: Curcumin and caffeine as model compounds. *Journal of Food Engineering*, *180*, 110-119.

Chen, F.-P., Ou, S.-Y., Chen, Z., & Tang, C.-H. (2017). Soy soluble polysaccharide as a nanocarrier for curcumin. *Journal of agricultural and food chemistry*, *65*(8), 1707-1714.

Chen, J., Yang, J., Ma, L., Li, J., Shahzad, N., & Kim, C. K. (2020). Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of

phenolic acids. *Scientific Reports*, *10*(1), 2611. https://doi.org/10.1038/s41598-020-59451-z

Choong, Y.-K., Xu, C.-H., Lan, J., Chen, X.-D., & Jamal, J. A. (2014). Identification of geographical origin of Lignosus samples using Fourier transform infrared and two-dimensional infrared correlation spectroscopy. *Journal of Molecular Structure*, *1069*, 188-195. https://doi.org/https://doi.org/10.1016/j.molstruc.2014.04.001

Cuvelier, M.-E., Bondet, V., & Berset, C. (2000). Behavior of phenolic antioxidants in a partitioned medium: structure—Activity relationship. *Journal of the American Oil Chemists' Society*, *77*(8), 819-824.

Dall'Acqua, S., Miolo, G., Innocenti, G., & Caffieri, S. (2012). The Photodegradation of Quercetin: Relation to Oxidation. *Molecules*, *17*(8), 8898-8907. https://www.mdpi.com/1420-3049/17/8/8898

Decker, E. A., & Hultin, H. O. (1992). Lipid oxidation in muscle foods via redox iron.

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants*, *8*(10), 429.

Elmi, N., Ghanbarzadeh, B., Ayaseh, A., Sahraee, S., Heshmati, M. K., Hoseini, M., & Pezeshki, A. (2021). Physical properties and stability of quercetin loaded niosomes: Stabilizing effects of phytosterol and polyethylene glycol in orange juice model. *Journal of Food Engineering*, *296*, 110463. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2020.110463

Erickson, M. C. (2008). *Lipid oxidation of muscle foods*. CRS Press, Boca Ratón (EEUU).

Fujisawa, S., & Kadoma, Y. (2006). Anti-and pro-oxidant effects of oxidized quercetin, curcumin or curcumin-related compounds with thiols or ascorbate as measured by the induction period method. *in vivo*, *20*(1), 39-44.

Guleria, K. S. S. a. S. (2017). Synergistic Antioxidant Activity of Natural Products. *Annals of Pharmacology and Pharmaceutics*, *2*(16), 1086-1091.

Guo, Y., Baschieri, A., Amorati, R., & Valgimigli, L. (2021). Synergic antioxidant activity of γ-terpinene with phenols and polyphenols enabled by hydroperoxyl radicals. *Food Chemistry*, *345*, 128468. https://doi.org/https://doi.org/10.1016/j.foodchem.2020.128468

Huang, X., Liu, Y., Zou, Y., Liang, X., Peng, Y., McClements, D. J., & Hu, K. (2019). Encapsulation of resveratrol in zein/pectin core-shell nanoparticles: Stability, bioaccessibility, and antioxidant capacity after simulated gastrointestinal digestion. *Food Hydrocolloids*, *93*, 261-269. https://doi.org/https://doi.org/10.1016/j.foodhyd.2019.02.039

Jang, M. K., Kong, B. G., Jeong, Y. I., Lee, C. H., & Nah, J. W. (2004). Physicochemical characterization of α - chitin, β - chitin, and γ - chitin separated from natural resources. *Journal of Polymer Science Part A: Polymer Chemistry*, *42*(14), 3423-3432.

Kakran, M., Sahoo, N. G., Tan, I. L., & Li, L. (2012). Preparation of nanoparticles of poorly water-soluble antioxidant curcumin by antisolvent precipitation methods. *Journal of Nanoparticle Research*, *14*(3), 757. https://doi.org/10.1007/s11051-012-0757-0

Kaur, K., Kumar, R., & Mehta, S. K. (2016). Formulation of saponin stabilized nanoemulsion by ultrasonic method and its role to protect the degradation of quercitin from UV light. *Ultrasonics Sonochemistry*, *31*, 29-38. https://doi.org/https://doi.org/10.1016/j.ultsonch.2015.11.017

Kim, H., Kang, S., Li, K., Jung, D., Park, K., & Lee, J. (2021). Preparation and characterization of various chitin-glucan complexes derived from white button mushroom using a deep eutectic solvent-based ecofriendly method. *International Journal of Biological Macromolecules*, *169*, 122-129. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2020.12.081

Koç, B., Eren, İ., & Kaymak Ertekin, F. (2008). Modelling bulk density, porosity and shrinkage of quince during drying: The effect of drying method. *Journal of Food Engineering*, *85*(3), 340-349. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2007.07.030

Koç, M., Baysan, U., Devseren, E., Okut, D., Atak, Z., Karataş, H., & Kaymak-Ertekin, F. (2017). Effects of different cooking methods on the chemical and physical properties of carrots and green peas. *Innovative Food Science & Emerging Technologies*, *42*, 109-119. https://doi.org/https://doi.org/10.1016/j.ifset.2017.06.010

Kolev, T. M., Velcheva, E. A., Stamboliyska, B. A., & Spiteller, M. (2005). DFT and experimental studies of the structure and vibrational spectra of curcumin. *International Journal of Quantum Chemistry*, *102*(6), 1069-1079.

Kong, B., Zhang, H., & Xiong, Y. L. (2010). Antioxidant activity of spice extracts in a liposome system and in cooked pork patties and the possible mode of action. *Meat science*, *85*(4), 772-778.

Kumar, P., Saini, M., Kumar, V., Dehiya, B. S., Sindhu, A., Fouad, H., Ahmad, N., Mahmood, A., & Hashem, M. (2020). Polyethylene Glycol (PEG) Modified Porous Ca5(PO4)2SiO4 Bioceramics: Structural, Morphologic and Bioactivity Analysis. *Coatings*, *10*(6), 538. https://www.mdpi.com/2079-6412/10/6/538

Lee, B. H., Choi, H. A., Kim, M.-R., & Hong, J. (2013). Changes in chemical stability and bioactivities of curcumin by ultraviolet radiation. *Food Science and Biotechnology*, *22*(1), 279-282.

Li, J., Shin, G. H., Lee, I. W., Chen, X., & Park, H. J. (2016). Soluble starch formulated nanocomposite increases water solubility and stability of curcumin. *Food Hydrocolloids*, *56*, 41-49. https://doi.org/https://doi.org/10.1016/j.foodhyd.2015.11.024

Li, W., Song, K., Wang, S., Zhang, C., Zhuang, M., Wang, Y., & Liu, T. (2019). Anti-tumor potential of astragalus polysaccharides on breast cancer cell line mediated by macrophage activation. *Materials Science and Engineering: C*, *98*, 685-695. https://doi.org/https://doi.org/10.1016/j.msec.2019.01.025

Li, X., Liu, Y., Yu, Y., Chen, W., Liu, Y., & Yu, H. (2019). Nanoformulations of quercetin and cellulose nanofibers as healthcare supplements with sustained antioxidant activity. *Carbohydrate Polymers*, *207*, 160-168. https://doi.org/https://doi.org/10.1016/j.carbpol.2018.11.084

Lorenzo, J. M., Pateiro, M., Domínguez, R., Barba, F. J., Putnik, P., Kovačević, D. B., Shpigelman, A., Granato, D., & Franco, D. (2018). Berries extracts as natural antioxidants in meat products: A review. *Food Research International*, *106*, 1095-1104. https://doi.org/https://doi.org/10.1016/j.foodres.2017.12.005

Mariutti, L. R. B., & Bragagnolo, N. (2017). Influence of salt on lipid oxidation in meat and seafood products: A review. *Food Research International*, *94*, 90-100. https://doi.org/https://doi.org/10.1016/j.foodres.2017.02.003

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. sci. technol*, *26*(2), 211-219.

Naining, W., & Hongjian, Z. (1986). A STUDY OF THE ACCURACY OF OPTICAL FRAUNHOFER DIFFRACTION SIZE ANALYZER. *Particulate Science and Technology*, *4*(4), 403-408. https://doi.org/10.1080/02726358608906471

Oikonomopoulou, V. P., Krokida, M. K., & Karathanos, V. T. (2011). The influence of freeze drying conditions on microstructural changes of food products. *Procedia food science*, *1*, 647-654.

Ospina, N. M., Alvarez, S. P. O., Sierra, D. M. E., Vahos, D. F. R., Ocampo, P. A. Z., & Orozco, C. P. O. (2015). Isolation of chitosan from Ganoderma lucidum mushroom for biomedical applications. *Journal of Materials Science: Materials in Medicine*, *26*(3), 135.

Parker, A. C., Rhodes, C., Jennings, J. A., Hittle, L., Shirtliff, M., Bumgardner, J. D., & Haggard, W. O. (2016). Preliminary evaluation of local drug delivery of amphotericin B and in vivo degradation of chitosan and polyethylene glycol blended sponges. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *104*(1), 78-87.

Pawlikowska-Pawlęga, B., Gruszecki, W. I., Misiak, L., Paduch, R., Piersiak, T., Zarzyka, B., Pawelec, J., & Gawron, A. (2007). Modification of membranes by quercetin, a naturally occurring flavonoid, via its incorporation in the polar head group. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1768*(9), 2195-2204.

Pool, H., Quintanar, D., Figueroa, J. d. D., H. Bechara, J. E., McClements, D. J., & Mendoza, S. (2012). Polymeric Nanoparticles as Oral Delivery Systems for Encapsulation and Release of Polyphenolic Compounds: Impact on Quercetin Antioxidant Activity & Bioaccessibility. *Food Biophysics*, *7*(3), 276-288. https://doi.org/10.1007/s11483-012-9266-z

Rai, R., Merrell, C., Yokoyama, W., & Nitin, N. (2021). Infusion of trans-resveratrol in micron-scale grape skin powder for enhanced stability and bioaccessibility. *Food Chemistry*, *340*, 127894.

Roy, S., & Rhim, J.-W. (2020). Preparation of antimicrobial and antioxidant gelatin/curcumin composite films for active food packaging application. *Colloids and Surfaces B: Biointerfaces*, *188*, 110761. https://doi.org/https://doi.org/10.1016/j.colsurfb.2019.110761

Sahoo, N., Kakran, M., Shaal, L., Li, L., Müller, R., Pal, M., & Tan, L. (2011). Preparation and characterization of quercetin nanocrystals. *Journal of pharmaceutical sciences*, *100*(6), 2379-2390.

Sato, K., & Hegarty, G. R. (1971). Warmed‐over flavor in cooked meats. *Journal of food science*, *36*(7), 1098-1102.

Schmidt, B., Ribnicky, D. M., Poulev, A., Logendra, S., Cefalu, W. T., & Raskin, I. (2008). A natural history of botanical therapeutics. *Metabolism*, *57*, S3-S9.

Sørensen, G., & Jørgensen, S. S. (1996). A critical examination of some experimental variables in the 2-thiobarbituric acid (TBA) test for lipid oxidation in meat products. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *202*(3), 205-210.

Suwannateep, N., Wanichwecharungruang, S., Haag, S., Devahastin, S., Groth, N., Fluhr, J., Lademann, J., & Meinke, M. (2012). Encapsulated curcumin results in prolonged curcumin activity in vitro and radical scavenging activity ex vivo on skin after UVB-irradiation. *European Journal of Pharmaceutics and Biopharmaceutics*, *82*(3), 485-490.

Umaña, M., Turchiuli, C., Rosselló, C., & Simal, S. (2021). Addition of a mushroom by-product in oil-in-water emulsions for the microencapsulation of sunflower oil by spray drying. *Food Chemistry*, *343*, 128429. https://doi.org/https://doi.org/10.1016/j.foodchem.2020.128429

Vaz, G. R., Clementino, A., Bidone, J., Villetti, M. A., Falkembach, M., Batista, M., Barros, P., Sonvico, F., & Dora, C. (2020). Curcumin and Quercetin-Loaded Nanoemulsions: Physicochemical Compatibility Study and Validation of a Simultaneous Quantification Method. *Nanomaterials*, *10*(9), 1650.

Volf, I., Ignat, I., Neamtu, M., & Popa, V. I. (2014). Thermal stability, antioxidant activity, and photo-oxidation of natural polyphenols. *Chemical Papers*, *68*(1), 121-129.

Wang, L., Gulati, P., Santra, D., Rose, D., & Zhang, Y. (2018). Nanoparticles prepared by proso millet protein as novel curcumin delivery system. *Food Chemistry*, *240*, 1039-1046. https://doi.org/https://doi.org/10.1016/j.foodchem.2017.08.036

Wu, C., Li, L., Zhong, Q., Cai, R., Wang, P., Xu, X., Zhou, G., Han, M., Liu, Q., Hu, T., & Yin, T. (2019). Myofibrillar protein–curcumin nanocomplexes prepared at different ionic strengths to improve oxidative stability of marinated chicken meat products. *LWT*, *99*, 69-76. https://doi.org/https://doi.org/10.1016/j.lwt.2018.09.024

Yan, Z., Zhong, Y., Duan, Y., Chen, Q., & Li, F. (2020). Antioxidant mechanism of tea polyphenols and its impact on health benefits. *Animal Nutrition*, *6*(2), 115-123. https://doi.org/https://doi.org/10.1016/j.aninu.2020.01.001

Yao, S., Li, T., Liu, H., Li, J., & Wang, Y. (2018). Traceability of Boletaceae mushrooms using data fusion of UV–visible and FTIR combined with chemometrics methods. *Journal of the Science of Food and Agriculture*, *98*(6), 2215-2222.

Zanfini, A., Corbini, G., La Rosa, C., & Dreassi, E. (2010). Antioxidant activity of tomato lipophilic extracts and interactions between carotenoids and α-tocopherol in synthetic mixtures. *LWT - Food Science and Technology*, *43*(1), 67-72. https://doi.org/https://doi.org/10.1016/j.lwt.2009.06.011

Zhang, M., Swarts, S. G., Yin, L., Liu, C., Tian, Y., Cao, Y., Swarts, M., Yang, S., Zhang, S. B., & Zhang, K. (2011). Antioxidant properties of quercetin. In *Oxygen transport to tissue XXXII* (pp. 283-289). Springer.

Zou, L., Zheng, B., Zhang, R., Zhang, Z., Liu, W., Liu, C., Xiao, H., & McClements, D. J. (2016). Food-grade nanoparticles for encapsulation, protection and delivery of curcumin: comparison of lipid, protein, and phospholipid nanoparticles under simulated gastrointestinal conditions. *Rsc Advances*, *6*(4), 3126-3136.

Appendix

Table 3.1 Formulations of 200 g hamburger patties composed of various amounts of ground beef, freeze-dried mushroom microparticles (FDMMs), pristine curcumin (CCM), pristine quercetin (QCT), and FDMMs loaded with CCM and QCT.*

Sample	Sodium chloride (g)	FDMMs (g)	CCM (mg)	QCT (mg)
Control	2	0	0	0
FDMMs	2	3.5	0	0
FDMMs+CCM	2	3.5	140	0
FDMMs+QCT	2	3.5	0	44
FDMMs+CCM+QCT	2	3.5	140	44
FDMMs loaded with CCM+QCT	2	3.5	140	44

*The remainder is ground beef mass.

Table 3.2 The DPPH· scavenging activity (*Q*%) and synergistic effect (*SE*) determined for freeze-dried mushroom microparticles (FDMMs), curcumin (CCM), quercetin (QCT), and their mixtures, as well as FDMMs loaded with one or two antioxidants.

Sample	$Q\%$	$Q_{theoretical}\%^{*}$	$SE^{\#}$
FDMMs	2.5 ± 0.4 ^{e}		
CCM	22.7 ± 2.4 ^{cd}		
QCT	16.8 ± 1.8 ^{d}		
CCM+QCT	43.0 ±0.8 ^{ab}	35.7 ± 3.3	1.20
CCM+QCT+FDMMs	43.4 ± 0.6 ^{ab}		
CCM-loaded FDMMs	35.2 ± 1.8 ^{b}		
QCT-loaded FDMMs	26.4 ± 3.6 ^{c}		
CCM-QCT-loaded FDMMs	50.9 ± 8.8 ^{a}	37.3 ± 3.0	1.37

$^{*}Q_{\text{theoretical}} = Q_{CCM} + Q_{QCT} - \frac{Q_{CCM} \times Q_{QCT}}{100}$

$^{\#}SE = Q/Q_{\text{theoretical}}$

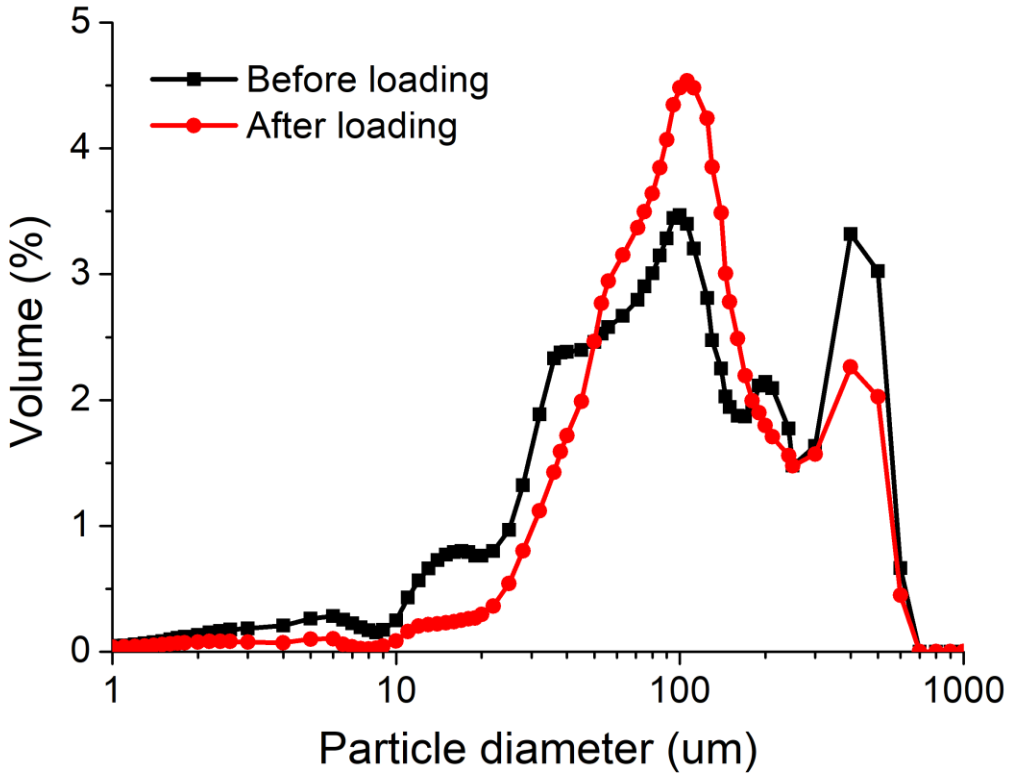
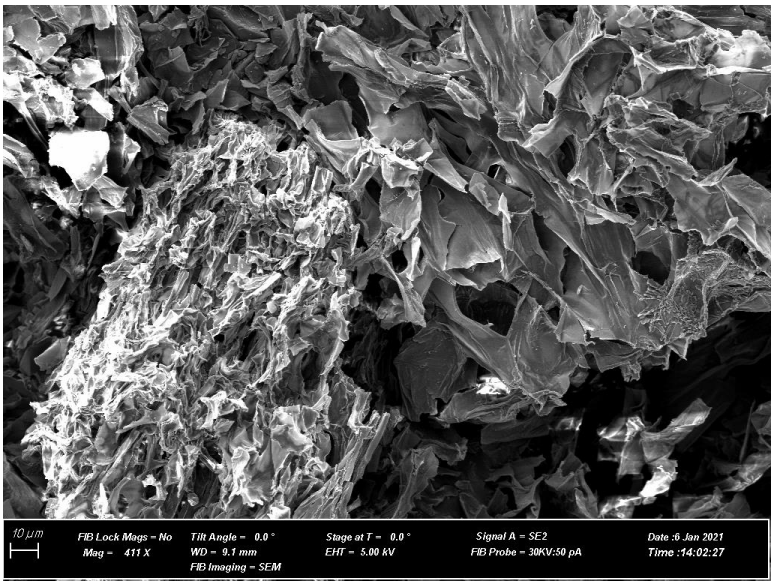


Figure 3.1 The diameter distribution of freeze-dried mushroom microparticles before and after loading curcumin and quercetin.

A



B

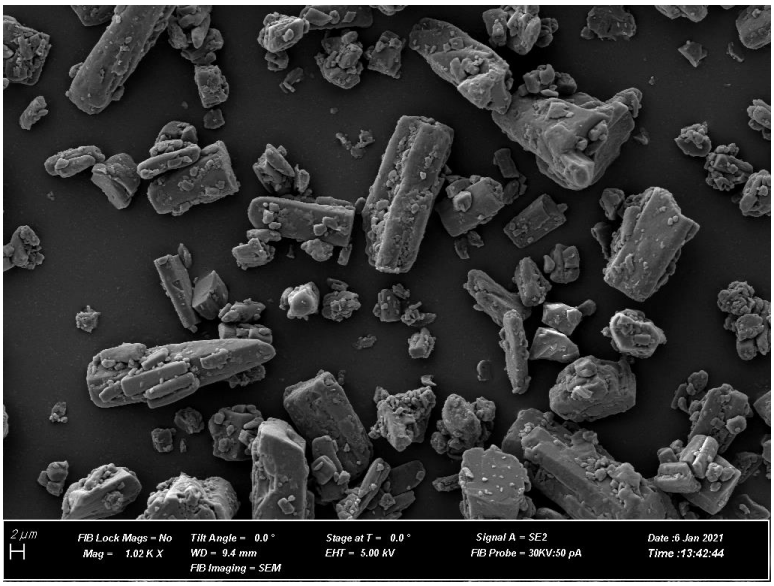
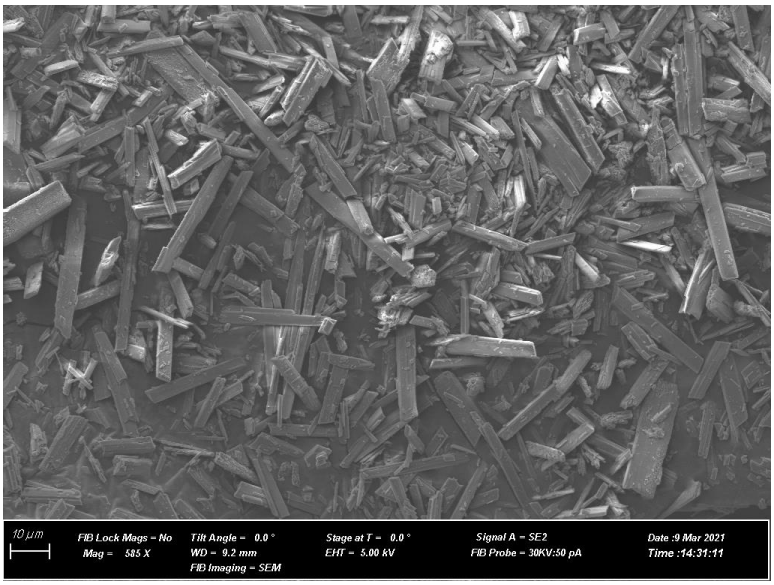


Figure 3.2 SEM images of freeze-dried mushroom microparticles (FDMMs, A), pristine curcumin (CCM, B), pristine quercetin (QCT, C), physical mixture of CCM and QCT (D), physical mixture of FDMMs, CCM, and QCT (E), and CCM-QCT-loaded FDMMs (F).

C



D

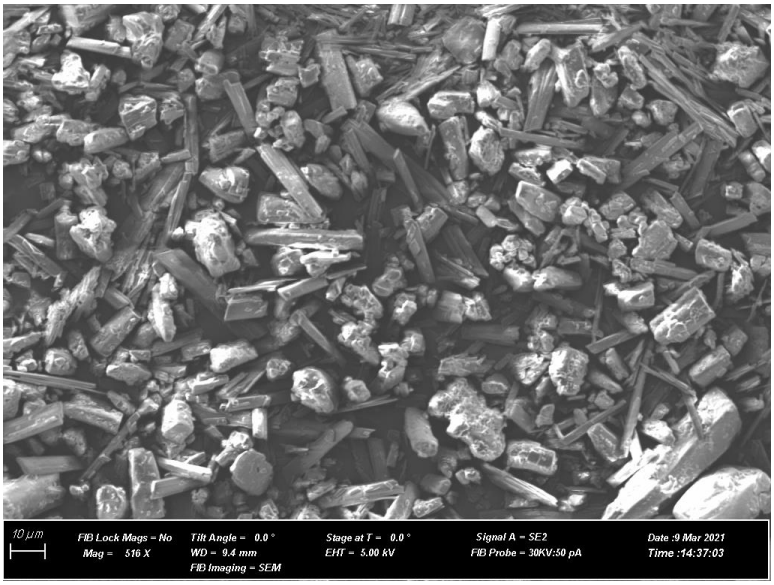
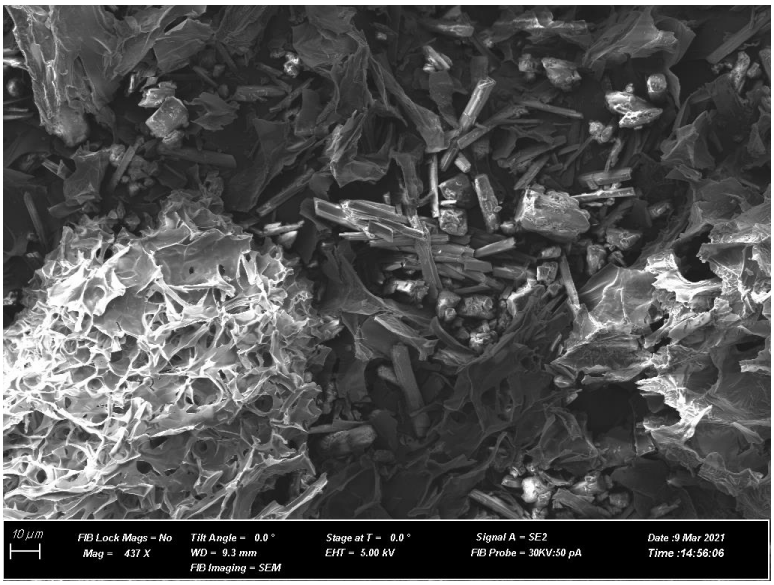


Figure 3.2 continued

E



F

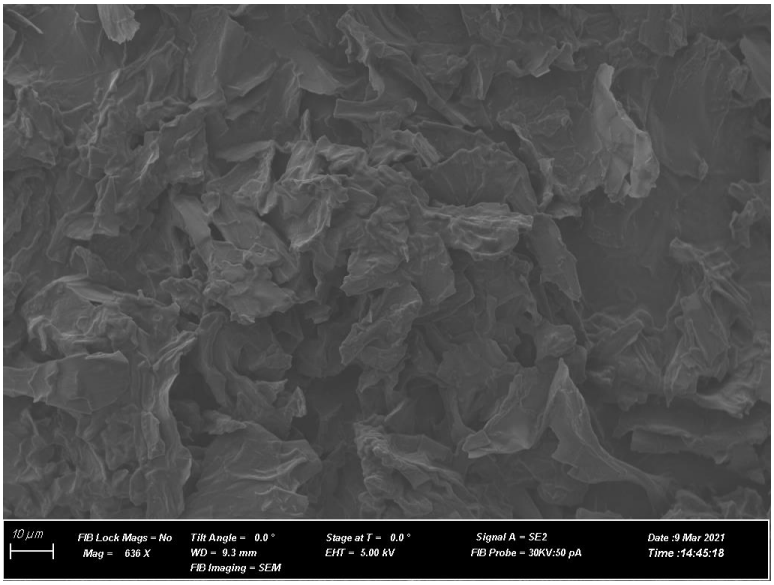


Figure 3.2 continued

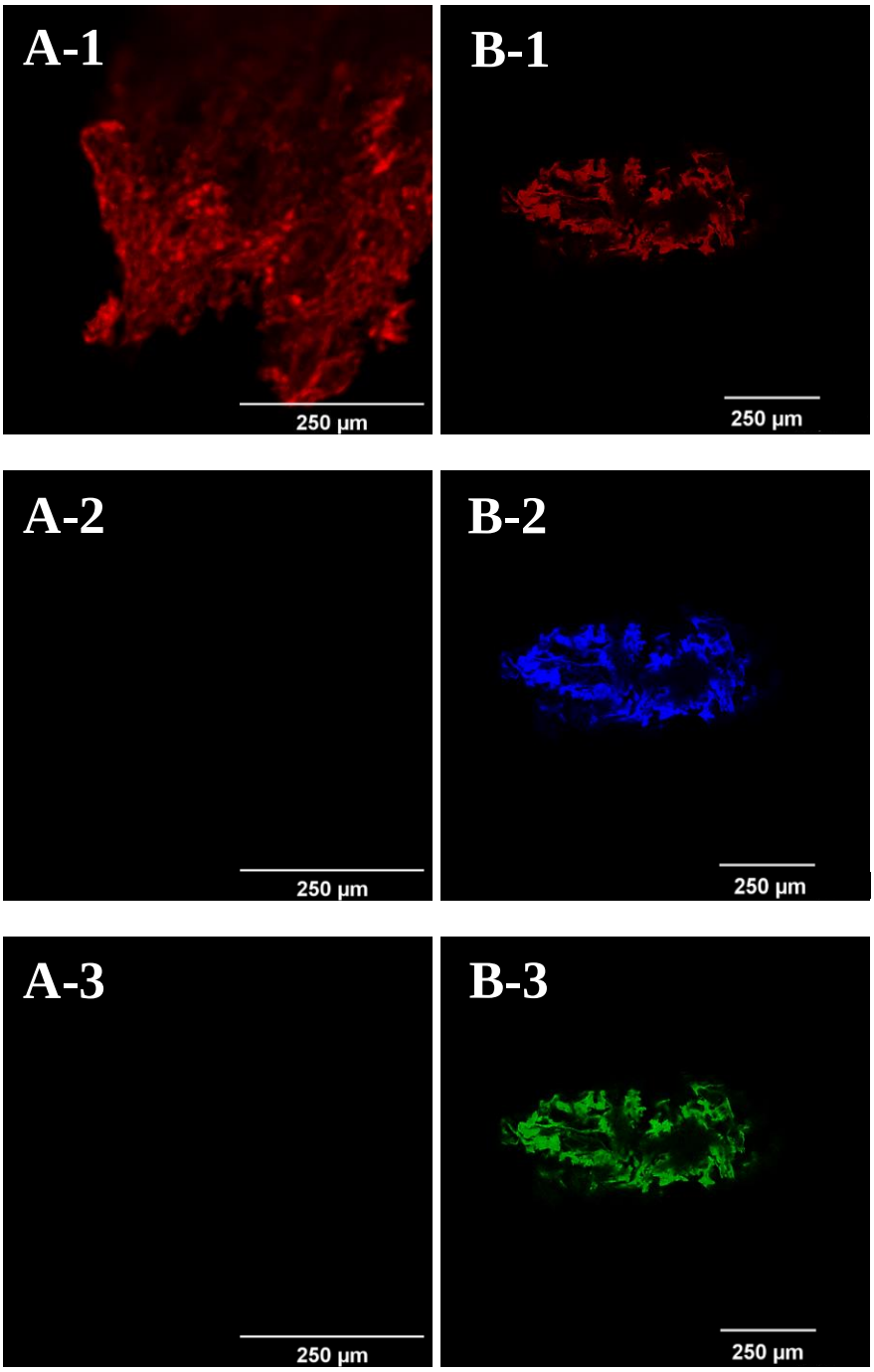


Figure 3.3 CLSM images at the z-stack of 150 µm of freeze-dried mushroom microparticles (FDMMs) before (A) and after (B) the loading of curcumin (CCM) and quercetin (QCT) with the excitation wavelength at 405 nm for FDMMs (1), 458 nm for QCT (2), and 488 nm for CCM (3).

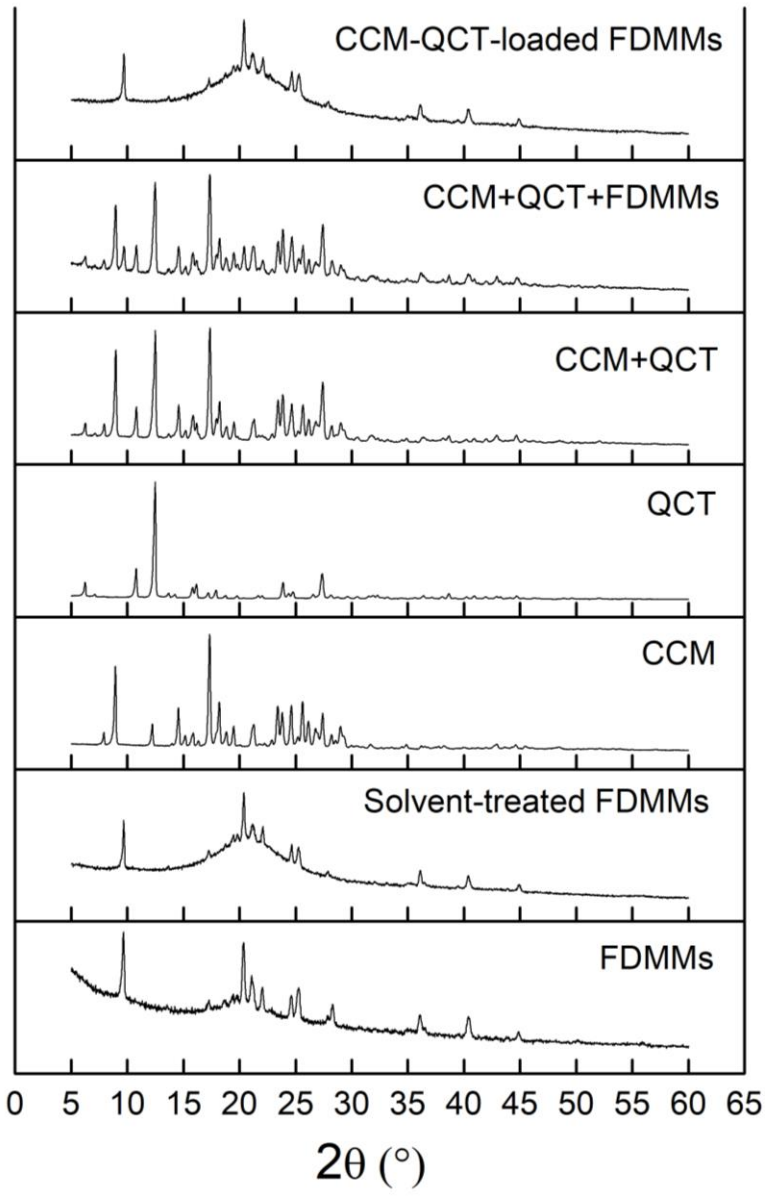


Figure 3.4 XRD patterns of freeze-dried mushroom microparticles (FDMMs), FDMMs treated at encapsulation conditions without CCM and QCT, pristine curcumin (CCM), pristine quercetin (QCT), physical mixture of CCM and QCT, physical mixture of CCM, QCT, and FDMMs, and CCM-QCT-loaded FDMMs.

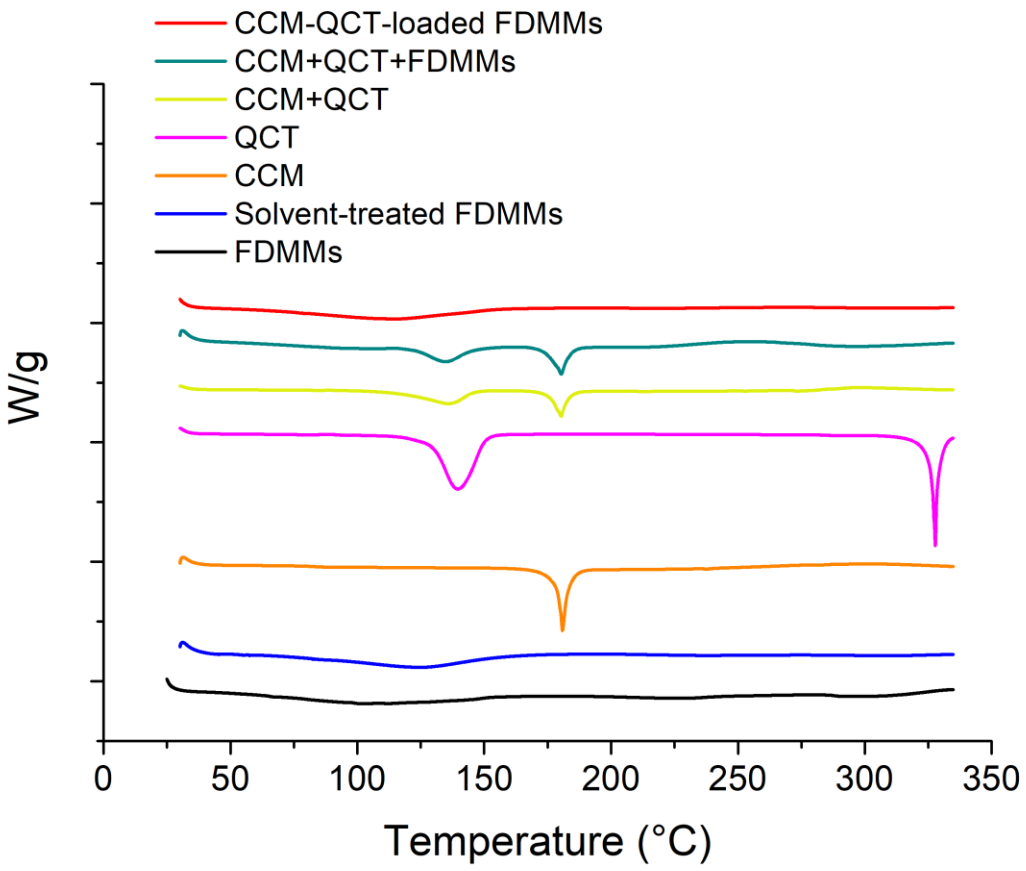


Figure 3.5 DSC thermographs of freeze-dried mushroom microparticles (FDMMs), FDMMs treated at encapsulation conditions without CCM and QCT, pristine curcumin (CCM), pristine quercetin (QCT), physical mixture of CCM and QCT, physical mixture of CCM, QCT and FDMMs, and CCM-QCT-loaded FDMMs.

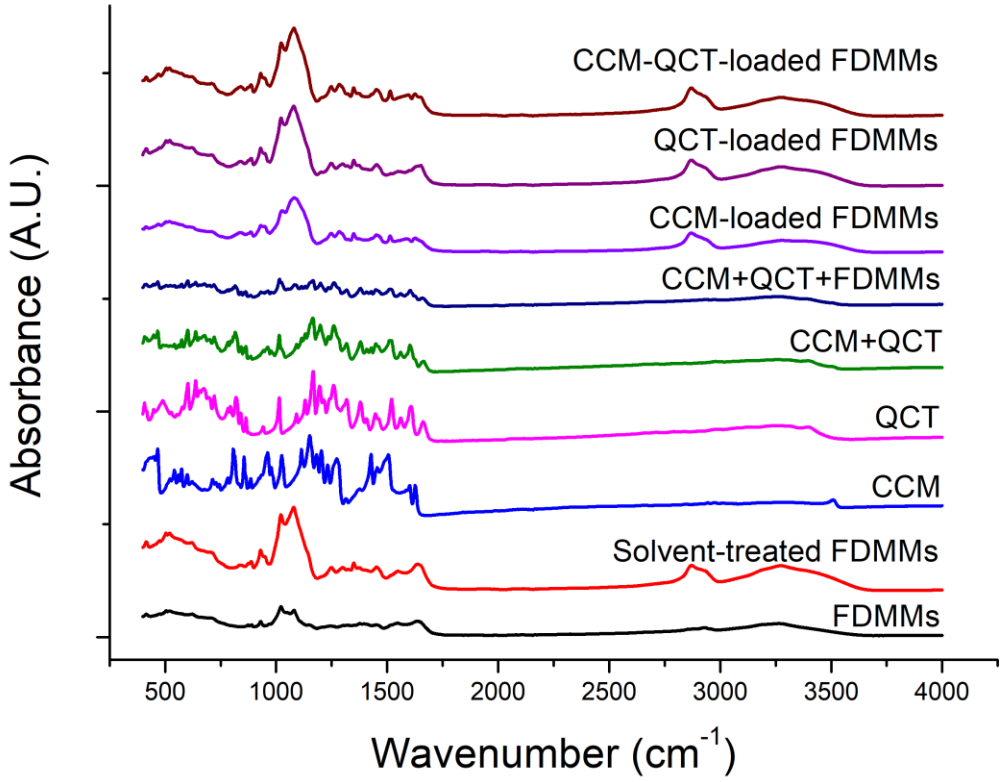


Figure 3.6 FTIR spectra of freeze-dried mushroom microparticles (FDMMs), FDMMs treated at encapsulation conditions without CCM and QCT, pristine curcumin (CCM), pristine quercetin (QCT), physical mixture of CCM and QCT, physical mixture of CCM, QCT, and FDMMs, CCM-loaded FDMMs, QCT-loaded FDMMs, and CCM-QCT-loaded FDMMs.

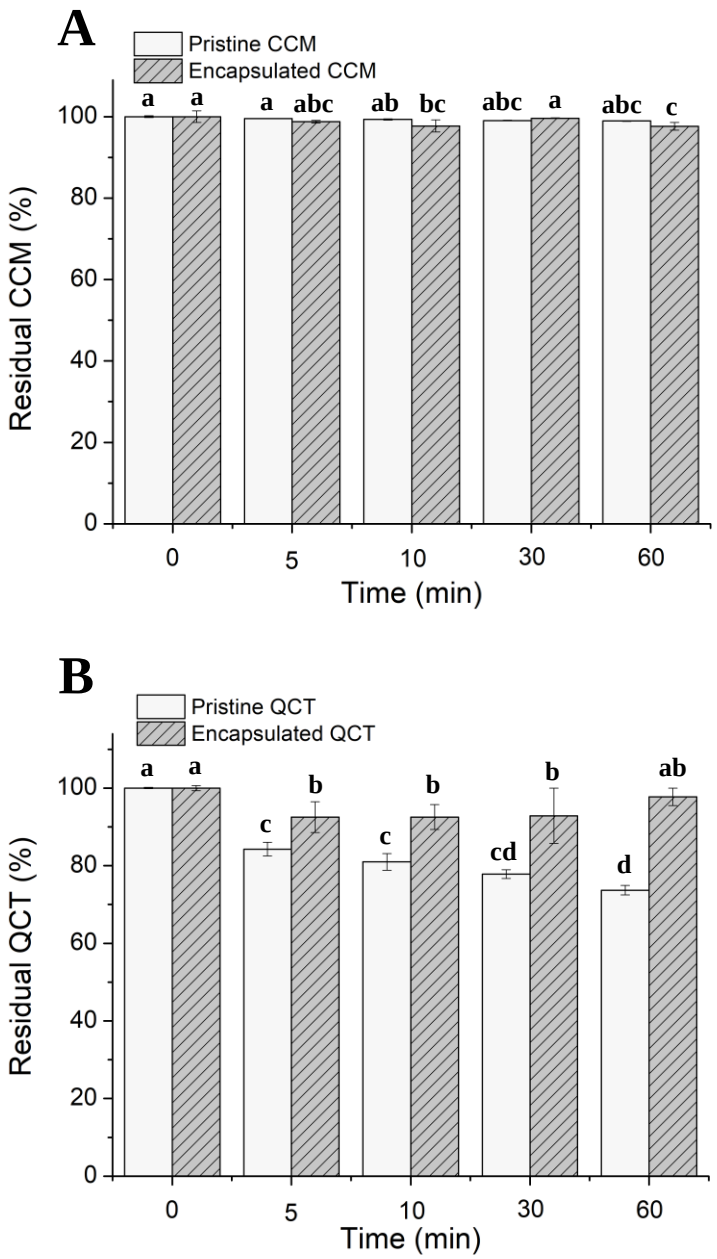


Figure 3.7 Percentage of residual curcumin (CCM, A) and quercetin (QCT, B) in pristine or encapsulated form during UV irradiation 253 nm for 60 min. Error bars are SD (n = 2). Different letters above columns indicate significant difference between mean values in the same plot ($p < 0.05$).

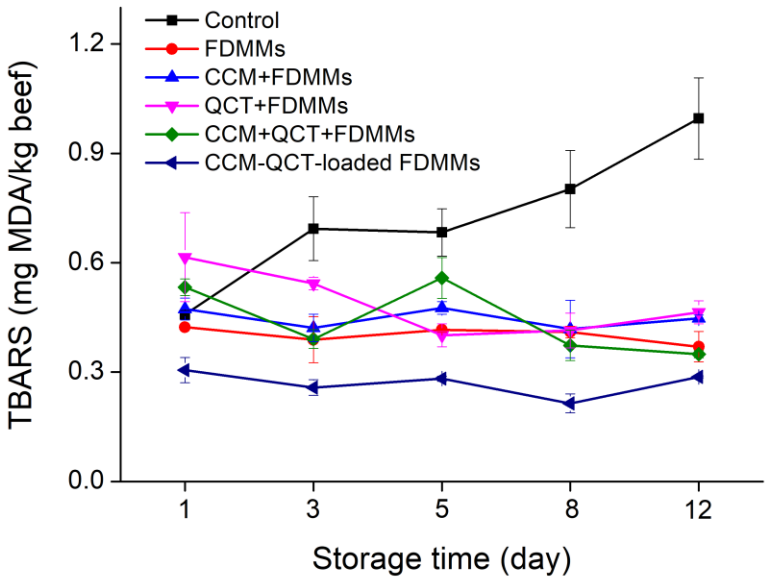


Figure 3.8 The effects of adding freeze-dried mushroom microparticles (FDMMs), curcumin (CCM), and quercetin (QCT) in pristine or encapsulated form on TBARS of cooked beef patties during 12-day storage at 4 °C. Error bars are SD (n = 2).

Chapter 4 Concluding remarks and future work

4.1 Conclusions

In this dissertation, freeze-dried porous matrices of a plant and a fungus were used to load various LBCs. This suggests that other plant materials have the potential as carrier materials and may be selected based on the functionalities of interest. The binary mixture of ethanol and PEG400 enabled the dissolution of RSV and CCM at high concentrations and the diffusion of high amounts of the LBCs into porous microparticles. To load other target LBCs, different mixtures of solvents may be considered to dissolve LBCs at high concentrations.

4.2 Future work and application perspectives

4.2.1. Future work

Findings in this dissertation showed that the bioaccessibility and the antioxidant activity of LBCs can be significantly improved by loading into porous plant or fungus microparticles. Although it has been observed that *in vitro* bioaccessibility and *in vivo* bioavailability of loaded LBCs are positively correlated (Ribas-Agustí et al., 2018; Ting et al., 2015), it requires *in vivo* animal studies to claim that loading LBCs into porous plant microparticles can lead to higher bioavailability of LBCs compared to pristine LBCs. Also, the diffusion kinetics of LBCs solution in the pore network of porous plant microparticles can be studied to predict and optimize the loading process of LBCs into porous plant microparticles. Finally, the exact antioxidative mechanism of individual LBC and the mechanism of antioxidant synergism are worth exploring to achieve more effective antioxidant activities of LBCs loaded within porous plant microparticles.

4.2.2. Perspectives for food applications

There are many opportunities in the functional food market for ingredients that contain LBCs with enhanced bioaccessibility (Xavier & Mercadante, 2019). Natural porous matrices as vehicles of LBCs have the advantage of label friendliness and applicability in unique market sectors. The findings from this study may be adopted for functional food applications that require plant or fungus-based materials. The improved bioaccessibility (~60% increase) and high stability of RSV loaded within FDPMs (92.9% retention after more than 3 months) show the potential of RSV-loaded FDPMs as functional foods ingredients with unique features. For example, potato powder can substitute wheat flour up to 40% (w/w) and improve rheological and textural properties of instant noodles (Nawaz et al., 2019). FDPMs enriched with RSV may also be used to enhance sensory qualities and consumer acceptance of milk dessert (Chakraborty & Bandyopadhyay, 2017). Additionally, the application of CCM-QCT-loaded FDPMs can be expanded from increasing antioxidant activity of pasta (Wang et al., 2020) to decreasing lipid oxidation in different types of muscle foods, such as beef, fish, and goat meats (Banerjee et al., 2020; Bao et al., 2008). Lastly, simple diffusion used in this study can be easily scaled-up (Cullen & O'donnell, 2009) and does not require high energy input. Therefore, findings from this study may be significant to the food industry.

References

Banerjee, D. K., Das, A. K., Banerjee, R., Pateiro, M., Nanda, P. K., Gadekar, Y. P., Biswas, S., McClements, D. J., & Lorenzo, J. M. (2020). Application of Enoki Mushroom (Flammulina Velutipes) Stem Wastes as Functional Ingredients in Goat Meat Nuggets. *Foods*, *9*(4), 432. https://www.mdpi.com/2304-8158/9/4/432

Bao, H. N. D., Ushio, H., & Ohshima, T. (2008). Antioxidative Activity and Antidiscoloration Efficacy of Ergothioneine in Mushroom (Flammulina velutipes) Extract Added to Beef and Fish Meats. *Journal of agricultural and food chemistry*, *56*(21), 10032-10040. https://doi.org/10.1021/jf8017063

Chakraborty, C., & Bandyopadhyay, K. (2017). Textural Analysis of Spongy Indian Milk Dessert (Rasogolla) Fortified with Potato Powder. *Int. J Curr. Microbiol. App. Sci*, *6*(4), 2414-2420.

Cullen, P., & O'donnell, C. P. (2009). Mixing in the food industry: trends and challenges. *Food mixing: principles and applications*.

Nawaz, A., Xiong, Z., Li, Q., Xiong, H., Liu, J., Chen, L., Wang, P., Walayat, N., Irshad, S., & Regenstein, J. M. (2019). Effect of wheat flour replacement with potato powder on dough rheology, physiochemical and microstructural properties of instant noodles. *Journal of food processing and preservation*, *43*(7), e13995.

Ribas-Agustí, A., Martín-Belloso, O., Soliva-Fortuny, R., & Elez-Martínez, P. (2018). Food processing strategies to enhance phenolic compounds bioaccessibility and bioavailability in plant-based foods. *Critical Reviews in Food Science and Nutrition*, *58*(15), 2531-2548.

Ting, Y., Jiang, Y., Lan, Y., Xia, C., Lin, Z., Rogers, M. A., & Huang, Q. (2015). Viscoelastic emulsion improved the bioaccessibility and oral bioavailability of crystalline compound: A mechanistic study using in vitro and in vivo models. *Molecular pharmaceutics*, *12*(7), 2229-2236.

Wang, L., Brennan, M. A., & Brennan, C. S. (2020). Improving antioxidant capacity of foods: adding mushroom powder to pasta. In *Pathology* (pp. 289-296). Elsevier.

Xavier, A. A. O., & Mercadante, A. Z. (2019). The bioaccessibility of carotenoids impacts the design of functional foods. *Current Opinion in Food Science*, *26*, 1-8. https://doi.org/https://doi.org/10.1016/j.cofs.2019.02.015

Vita

Inseob Choi was born in Bucheon in South Korea, on March 10, 1990. He grew up and lived in the same city until he moved to Knoxville, Tennessee. During his first international travel in Europe in 2012, the idea of studying abroad came to his mind. While he enjoyed both of his undergrad majors, “Food Science” and “Economics”, the curiosity to science attracted him and led him to study Food Science in graduate program. He decided to study at the University of Tennessee, Knoxville where he fortunately met his academic advisor, Dr. Qixin Zhong, and earned his Master of Science degree in Food Science.