THE EFFECT OF STORAGE TEMPERATURE ON THE PROPERTIES OF ENCAPSULATED CATECHIN IN PEA PROTEIN/ALGINATE BEADS

BY

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ABSTRACT

Phenolic compounds are natural antioxidants present in many fruits and vegetables. They hold many health-promoting properties such as antimutagenic, anti-obesity, anti-inflammatory, and antiviral properties. However, their stability is affected by many environmental factors, such as oxygen, light, high temperature, and pH. Encapsulation of phenolic compounds can protect them from degradation during storage. Because of their biocompatibility, non-toxicity, and renewability, biopolymers like proteins and polysaccharides have been of interest and investigated as starting materials in the design of encapsulation matrices. There are many methods to encapsulate phenolic compounds, such as emulsion, spray drying, extrusion, electrospinning, coacervation etc. Among these methods, extrusion is one of the most common methods used to encapsulate bioactive components, due to its mild process and suitability for encapsulating both hydrophilic and hydrophobic compounds.

In this thesis, pea protein/alginate beads were synthesized by extrusion. To study the changes in the properties of the beads during storage, the freeze dried beads were stored at three temperatures (room temperature, 50 ^{o}C, and 100 ^{o}C) for 12 days. The chemical composition and the thermal stability of the beads were studied by the Fourier transform infrared spectrometer (FTIR) and thermogravimetric analysis (TGA), respectively. The results showed that these properties did not change during storage for all temperatures tested. The morphology of the beads was investigated by scanning electron microscopy (SEM). The images generated by SEM showed that there were different shapes and surface characteristics of the beads after freeze drying treatment. These surface characteristics were retained during storage. The mechanical properties of the beads including the hardness and the resilience were measured by a texture analyzer. The average hardness values of the beads stored at 100 ^{o}C showed a decreasing trend over storage time while their average resilience values remained relatively stable during storage for all temperatures tested. The hardness and the resilience of the beads stored at all temperatures was not significantly different ($P>0.05$) during the storage period.

To evaluate the protective effect of pea protein/alginate beads on phenolic compounds, (+)-catechin hydrate was encapsulated within the composite beads, referred to as encapsulated

catechin beads. The encapsulated catechin beads were stored at three temperatures (room temperature, 50 ^oC, and 100 ^oC) for 12 days. The chemical composition, the thermal stability, the morphology, and the mechanical properties of the encapsulated catechin beads showed similar results as the blank beads. In addition, the antioxidant activity of the encapsulated catechin beads during storage was measured by the ferric reducing ability of plasma (FRAP) assay. The antioxidant activity values of encapsulated catechin beads showed a decreasing trend during storage for all temperatures tested. However, and surprisingly, the antioxidant activity values of non-encapsulated (+)-catechin hydrate powder showed lower decrease in the percentage of the antioxidant activity than the encapsulated catechin beads for all storage temperatures tested. The antioxidant activity of the encapsulated catechin beads stored at room temperature was not significantly different ($P>0.05$) during the storage period, while for those stored at 50 ^oC and 100 ^oC was significantly different ($P<0.05$).

Since the physical properties of beads were relatively stable for all temperatures tested, the beads could serve as encapsulating matrices. However, the beads were not effective in maintaining the antioxidant activity of (+)-catechin hydrate as compared to the un-encapsulated catechin powder. This could be due because catechin could have bind to the encapsulating matrices. This hypothesis should be verified in future work.

RÉSUMÉ

Les composés phénoliques sont des antioxydants naturels présents dans de nombreux fruits et légumes. Ils possèdent de nombreuses propriétés favorisant la santé telles que les propriétés antimutagènes, anti-obésité, anti-inflammatoires et antivirales. Cependant, leur stabilité dépend de nombreux facteurs environnementaux, tels que l'oxygène, la lumière, les températures élevées et le pH. L'encapsulation des composés phénoliques peut protéger contre la dégradation pendant l'entreposage. En raison de leur biocompatibilité et leur caractère non-toxiques, les biopolymères tels les protéines et les polysaccharides sont étudiés comme matières premières dans la conception des matrices d'encapsulation. Il existe de nombreuses méthodes pour encapsuler des composés phénoliques, tels que l'émulsion, le séchage, l'extrusion, l'électrofilage, ainsi que la coacervation et la pulvérisation. Parmi ces méthodes, l'extrusion est une des méthodes plus courantes utilisées pour encapsuler les composants bioactifs.

Dans le cadre de cette étude, des hydrogels micro-sphériques réticulés physiquement à base d'isolats de protéines de pois et d'alginate furent synthétisés par la méthode d'extrusion dans le but d'encapsuler des composés phénoliques. Afin d'étudier les changements dans les propriétés des hydrogels pendant l'entreposage, les hydrogels furent entreposés à trois températures (température ambiante, 50 °C et 100 °C) pendant une période de 12 jours. La composition chimique et la stabilité thermique des hydrogels ont été étudiés par le spectromètre infrarouge à transformée de Fourier (FTIR) et l'analyse thermogravimétrique (TGA), de façon respective. Les résultats ont montré que ces propriétés n'ont pas changées au cours de l'entreposage pour toutes les températures testées. La morphologie des hydrogels a été étudiée par microscopie électronique (SEM). Les valeurs de dureté moyenne des hydrogels stockés à 100 °C ont montré une tendance à la baisse au fil du temps de stockage alors que leurs valeurs de résilience moyenne sont demeurées relativement stables au cours du stockage pour toutes les températures testées. Il n'y avait pas de différence significative ($P > 0,05$) pour les résultats des tests de dureté et de résilience des hydrogels au cours de l'entreposage.

Afin d'évaluer l'effet protecteur des hydrogels sur les composés phénoliques, de la catéchine fut encapsulée dans les hydrogels. La catéchine encapsulée fut entreposée à trois températures

(température ambiante, 50 °C et 100 °C) pendant 12 jours. Les résultats de la composition chimique, la stabilité thermique, la morphologie et des propriétés mécaniques de la catéchine encapsulée étaient similaires aux hydrogels ne contenant pas de catéchine. De plus, l'activité antioxydante de la catéchine encapsulée au cours de la période d'entreposage fut mesurée par la capacité de réduction ferrique de dosage plasmatique (FRAP). Les valeurs de l'activité antioxydante de la catéchine encapsulée ont montré une tendance à la baisse au cours de l'entreposage pour toutes les températures testées. Étonnamment, les valeurs de l'activité antioxydante de la catéchine non-encapsulée ont montré une diminution plus faible de l'activité antioxydante que la catéchine encapsulé pour toutes les températures testées. L'activité antioxydante de la catéchine encapsulée stockée à température ambiante n'a pas été significativement différente ($P > 0,05$) au cours de l'entreposage, alors que des températures de stockage de 50 ^{o}C et de 100 ^{o}C, les résultats étaient significativement différents ($P < 0,05$).

Comme les propriétés physiques des hydrogels étaient relativement stables pour toutes les températures testées, les hydrogels pourraient servir de matrices d'encapsulation. Cependant, les hydrogels n'étaient pas efficaces pour maintenir l'activité antioxydante de l'hydrate de (+)-catéchine par rapport à la poudre de catéchine non encapsulée. Cela pourrait être dû au fait que la catéchine pourrait se lier aux matrices d'encapsulation. Cette hypothèse devrait être vérifiée lors d'une prochaine étdude.

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Catechins are phenolic compounds classified under the flavonoid family. These compounds are naturally present in plants. High concentrations of catechins have been found in fresh tea leaves, red wine, broad beans, black grapes, apricots and strawberries [1]. Although they are not essential nutrients, they help to prevent various diseases such as coronary heart disease and breast cancer [1]. However, a number of factors influence the stability of catechins, which include the oxygen concentration, the light exposure, the temperature, and the pH [1, 2]. Among these factors, oxygen and light have the most destructive parameters [1]. In solution, catechins are prone to undergo autoxidation. As autoxidation occurs, catechins are oxidized with O_2 to form quinones, which undergo polymerization [3]. A promising solution to maintain the stability and to enhance the bioavailability of catechins is encapsulation. Encapsulation is a strategy to protect the unstable molecules from undesirable environmental conditions during storage and food processing [4]. Nevertheless, the encapsulation of catechins is a challenging process and requires further progress [5].

The matrix material for encapsulation of food products must be food grade. Alginate is nontoxic, cheap [6], and listed as generally recognized as safe (GRAS) [7]. Besides, alginate has abundant sources. It is the second most abundant biopolymer, only after cellulose, in the world [8]. Alginate generally refers to a family of water soluble polyanionic polysaccharides naturally present in the cell walls and the intracellular spaces of brown seaweed (*Phaeophyceae*) [8, 9]. It can also be produced by bacteria such as *Azotobacter* and *Pseudomonas* [9]. In the presence of divalent cations, such as Ca^{2+}, St^{2+}, and Ba^{2+}, alginate can be easily cross-linked and form a gel [10]. In practice, the bioactive compound to be encapsulated is first dissolved or dispersed in an alginate solution, followed by the injection of the alginate solution into a gelling solution containing divalent cations [7]. Spherical hydrogel beads are formed when these divalent cations diffuse into the droplet [11]. This technique is called extrusion which can be performed using simple tools like a pipette or a syringe [6].

Alginate hydrogels are one of the commonly used materials for the encapsulation of functional ingredients for the food, cosmetic, and pharmaceutical industries [10]. These hydrogels hold many advantages: 1) the interior of the hydrogel beads is chemically inert [10]; 2) the hydrogels are non-toxic, abundant, cheap, biocompatible, and thermally and chemically stable [9, 12]; 3) the formation of hydrogel beads is conducted under very mild conditions without the requirements of high temperatures or chemical crosslinking agents [11]; 4) the hydrogel beads can be converted to the sol state by adding chelating agents, such as Na^+ and EDTA [11]; and 5) the porosity of the alginate beads can be modified through changes in the pH, since their porosity decreases in acidic conditions and increases in alkaline conditions. This property makes it an ideal coating material to control the release of bioactive compounds [10].

Proteins from both animal and vegetable sources have been widely used for the encapsulation of bioactive substances. Proteins are natural polymers which have several advantages such as: biocompatibility, biodegradability, and amphiphilic properties [13]. The applications of animal proteins have been regulated in some food markets, due to religious concerns, moral beliefs, and dietary preferences. In recent years, consumers became increasingly concerned about the safety of the animal-derived products, the breeding and the slaughter conditions of animals [4]. Therefore, plant proteins have been considered as a replacement for animal proteins in various applications in the food industry. Besides, the use of vegetable proteins in place of animal proteins reflects a "green" trend within the food industry [4]. Compared to animal proteins, plant proteins are cheaper, less allergenic, and possess better emulsification and solubility functionalities along with a lower environmental footprint [4, 14].

Legumes are considered as the second most important source of human food after cereals [15]. Peas are the dried seeds of *Pisum sativum* L. They are also known as dry, smooth or field peas, and are categorized within the yellow or green cotyledon varieties [16]. The cultivation of peas started approximately 9000 years ago, which suggests it is one of the oldest crops in the world [17]. Peas are the second most important leguminous crop and are grown over 25 million acres worldwide [15] for human and animal consumption [16]. In 2014, 11.3 million tonnes of dry peas were produced worldwide with Canada being the most important producer accounting for

3.4 million tonnes [18]. Compared to other pulses, peas are more commonly used in commercial products since the hull can easily be removed [19]. Like other pulses and grains, peas are relatively inexpensive and contain a high nutrient density [17]. Peas provide a cheap and readily available source of proteins, complex carbohydrates, vitamins, and minerals [16]. In addition, field peas contain 5-20% less trypsin inhibitors than soybeans [15]. This property of peas is nutritionally beneficial, since the trypsin inhibitors irreversibly bind to trypsin in the human digestive tract, which interferes with digestion [17]. In addition, peas have higher levels of essential amino acids lysine and tryptophan than cereal grains [15]. The consumption of pea or pea constituents could also provide some health benefits, such as reducing postprandial blood glucose [20], and improving bowel movement frequency [21].

Pea proteins represent 20-30% fraction of pea's chemical content where globulins account for 65–80% of the total pea proteins [13]. There are three forms of commercial pea protein products: pea flour, pea protein concentrates and pea isolate. Pea protein isolate is produced by wet processing, involving alkali or acid solubilization, followed by isoelectric precipitation or ultrafiltration. The generated product has a protein content of 85-95% [19]. In the food industry, pea proteins can easily be incorporated into other food products due to their varied functionalities, such as fat- and water-binding capabilities, emulsifying, foaming, gelation, and texture properties [19, 22]. Such products include bakery products, protein bars, soup mixes, breakfast cereals, processed meats, gluten-free confectionery, pastas, and mayonnaise [17, 22]. Pea proteins have demonstrated health benefits such as reducing the risk of cardiovascular disease [23] and high blood pressure [24].

1.2 Study Objectives

Alginate beads formed by the extrusion method are commonly used to encapsulate bioactive compounds. The addition of proteins to the alginate beads has gained interests in recent years. Pea proteins could be a promising ingredient for the development of protein/alginate beads since these proteins are inexpensive and abundant. Therefore, the general objectives of this thesis were to synthesize and characterize pea protein/alginate beads and to encapsulate a bioactive compound, (+)-catechin hydrate, using these composite beads. The detailed objectives of this thesis are listed below:

I. To review the techniques used to encapsulate phenolic compounds using proteins as wall materials and to discuss previous studies related to encapsulation of phenolic compounds (Chapter 2).

II. To synthesize pea-protein/alginate beads using the extrusion technique and to characterize the properties of these composite beads during a 12-day storage period at different temperatures (Chapter 3).

III. To encapsulate a model chemical, (+)-catechin hydrate, using pea protein/alginate beads and to investigate the antioxidant activity and other physical and chemical properties of the encapsulated composite beads during a 12-day storage period at different temperatures (Chapter 4).

CHAPTER 2

LITERATURE REIVEW

Encapsulation of Phenolic Compounds Present in Plants Using Protein Matrices

2.1 Introduction

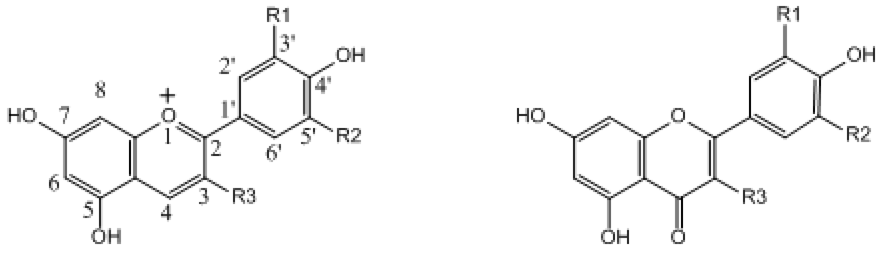
Plants contain many bioactive phenolic compounds, such as anthocyanins, flavonols, flavan-3-ols, proanthocyanidins, phenolic acid derivatives, and curcumin. These phenolic compounds have antioxidant and antimicrobial properties and therefore, fruits like cranberries have many health benefits as they can help preventing some diseases such as urinary tract infections [25], stomach ulcers and cancers [26], and dental diseases [27]. However, these phenolic compounds are chemically unstable in most environments, such as at neutral pH and when exposed to oxygen [28]. To improve the bioavailability of phenolic compounds in the human body, encapsulation seems to be an ideal method. There are many encapsulation techniques available to encapsulate these compounds, such as emulsion, spray drying, extrusion, electrospinning, and coacervation.

Among the wall materials, proteins are ideal because they are generally regarded as safe (GRAS) and contain a high nutritional value [29]. Most proteins can be easily digested by the human gastrointestinal tract [30]. Furthermore, proteins are insoluble in acidic conditions as the isoelectric point (pI) of most proteins ranges between pH 3 and pH 5. Proteins matrices are usually dissolved at alkaline pH to encapsulate the compounds of interest and the solution is thereafter acidified to form particles [13]. Both animal and plant proteins have been used as encapsulating matrices. The most common animal proteins used include gelatin, caseins and whey proteins (mainly β-lactoglobulin). Plant-derived protein matrices include zein, soy protein, etc. [13, 30].

In recent years, the encapsulation of phenolic compounds has been researched increasingly. Excellent reviews discussing the encapsulation of phenolic compounds are found in the literature [31-33]. However, to the best of the authors' knowledge, there is no review article discussing the encapsulation of phenolic compounds using proteins. Therefore, the objectives of this review are to summarize the phenolic compounds found in plants and to critically discuss the encapsulation of these compounds using proteins as starting materials for the synthesis of encapsulating matrices.

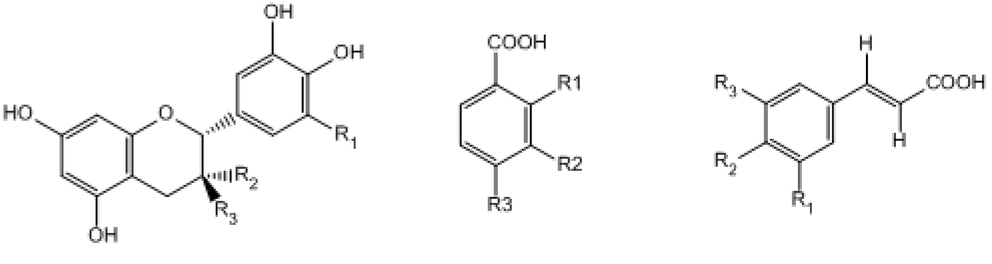
2.2 Phenolic Compounds in Plants

The main categories of bioactive compounds found in plants like cranberries are anthocyanins, flavonols, flavan-3-ols (catechin monomers), proanthocyanidins, and phenolic acid derivatives [34]. Moreover, flavanones and nonflavonoid polyphenols are present in lower amount [26]. The chemical structures of the major phenolic compounds present in plants are shown in Fig. 2.1.



a) Anthocyanins

b) Flavonols



c) Flavan-3-ols

d) Hydroxybenzoic acid

e) Hydroxycinnamic acid

Fig. 2.1. The chemical structures of the main phenolic compounds present in cranberries: a) anthocyanins; b) flavonols; c) flavan-3-ols; d) hydroxybenzoic acid; e) hydroxycinnamic acid

2.2.1 Anthocyanins

The chemical structure of anthocyanins is shown in Fig. 2.1(a). The R1 and R2 groups of anthocyanins can be a methoxy group, a hydroxyl group or a hydrogen atom. Anthocyanins are water-soluble bioactive compounds which are widely present in flowers, fruits, and vegetables [35]. The high amount of anthocyanins contributes to the color of the plants [25]. Their color depends on the pH: at pH below 3, anthocyanins exist as red-colored flavylium cations, which are the stable form; at pH ranging from 6 to 8, they convert to quinoidal base (blue color), which is an unstable state and therefore, the bioavailability of anthocyanins is decreased [36]. In plants, the pH of the cells is acidic therefore, anthocyanins are stable. However after extraction, anthocyanins are prone to degradation, as they tend to be unstable at high pH and in the presence of oxygen [28, 35]. Heat treatment, UV light exposure, presence of specific enzymes or some metallic ions also can degrade anthocyanins [28, 37]. After digestion, anthocyanins are absorbed by the stomach and the intestine [38]. However, the environmental conditions in the gastrointestinal tract trigger the degradation of anthocyanins, which is why the bioavailability of anthocyanins is very low [39].

Berry fruits, such as bilberries, blackberries, blueberries, and cranberries are rich in anthocyanins [40]. However, the concentration of anthocyanins in berry fruits varies at different stages. For example, the average content of anthocyanins in cranberries is 95 mg/100 g at harvest [41]. The concentration can increase in response to climatic factors, such as light and temperature [41]. The most common anthocyanin molecules are cyanidin, delphinidin, and peonidin, which bind to one or more sugar molecules. The glycosylation almost always occurs at the C3 position of the anthocyanidin molecule [41].

2.2.2 Flavonols

Flavonols are mainly concentrated in the skin of fruits [41]. They are present as yellow undertones which only contribute slightly to the color of berries [26]. The chemical structure of flavonols is shown in Fig. 2.1(b). Like anthocyanins, flavonols are also bound to sugars at the C3 position [41]. The content of flavonols in cranberries is higher than in blueberries and blackberries [26]. On a weight basis, the average flavonol content in cranberries is 20–30 mg/100 g [42], while in bilberries, blackberries, and blueberries, it ranges from 0.32 to 1.7 mg/100 g [25].

Unlike anthocyanins and proanthocyanidins, the concentration of flavonols is quite constant during the different maturity stages of cranberries [26].

Quercetin, also known as 3,3',4',5'-7-pentahydroxyflavone, belongs to flavonols [43]. The quercetin molecule is found in high quantities in apples, onions, teas and red wines; at which it exists as a glucoside [44]. However, it is prone to degradation under heat treatment, exposure to oxygen and light [44]. Moreover, it has low water solubility and poor stability during food processing, which makes it difficult to be incorporated in food products [43, 44]. The bioavailability of quercetin is very low because of its poor stability in the gastrointestinal tract [43].

2.2.3 Flavan-3-ols and Proanthocyanidins (PACs)

Flavan-3-ols, also known as catechin monomers, occur naturally in plant food as aglycons of catechin and epicatechin without glycosylation [26]. The chemical structure of flavan-3-ol is shown in Fig. 2.1(c). Flavan-3-ols can esterify with gallic acid and ellagic acid, forming hydrolysable tannins. The R2 or R3 group can be gallic acid or ellagic acid that binds to the C3 position of the flavan-3-ol molecule [41]. Flavan-3-ols are abundant in tea. The concentration of flavan-3-ols in unfermented tea ranges from 35 to 115 mg/g of tea leaves, with (-)-epigallocatechin gallate (EGCG) being the most abundant [45]. The stability of EGCG is affected by the pH, oxygen, and the temperature [46]. At neutral and basic pH, EGCG is easily oxidized and forms a dimer, which is irreversible [47]. At high temperature, it undergoes epimerization [48]. After ingestion, EGCG is stable in the stomach due to the low pH. While in the intestine, the pH rises to a neutral pH which promotes EGCG degradation [49]. Therefore, the bioavailability of EGCG is very low. The concentration of EGCG in the tissues and blood is about 0.0003-0.45% of the initial intake amount after 60 minutes [50].

Flavan-3-ols can form oligomeric and copolymeric compounds PACs (also known as non-hydrolyzable tannins) through O- and C-glycosyl linkages [41]. PACs are plants' secondary metabolites, which are oligomers of (-)-epicatechin and (+)-catechin [51]. There are two types of PACs: B-type and A-type. The B-type PACs are formed by a single C-C bond between the C4 position of the upper flavan-3-ol monomer and the C8 or the C6 position of the lower unit (C4→C6 or C4→C8 linkage). The A-type PACs are formed by an ether bond between the C2 position of the upper unit and the hydroxyl group at the C7 position of the lower unit (C2→O→C7 linkage) [25]. PACs have health benefits. For example, A-type procyanidins are effective in preventing urinary tract infections [51]. Berry fruits are rich in PACs. Among the berry fruits, chokeberry contains the highest amount of PACs [40]. In cranberries, PACs constitute around 85% of the total flavan-3-ols on a weight basis [25, 52]. However, like many other polyphenols, they are prone to degradation at high temperature, when exposed to oxygen, and also in the gastrointestinal environment due to the low pH and the presence of other compounds [53]. Besides, PACs can interact with denatured proteins, which account for the astringent or bitter taste of berry fruits [54].

2.2.4 Phenolic Acid Derivatives

Phenolic acid derivatives include derivatives of hydroxybenzoic acid (HBA) and hydroxycinnamic acid (HCA). The chemical structures of these two compounds are shown in Fig. 2.1(d) and (e), respectively. Phenolic acid derivatives are rich in berry fruits [40]. They are often bound to sugars by glycosidic linkage and therefore rarely present in the free form [41]. Like HCA derivatives, HBA derivatives (ellagic, gallic and vanillic acid) are also bound with sugars. Ellagic acid can esterify with flavan-3-ols to form ellagitannins [41]. In cranberries, HBAs with very high content of benzoic acid (474-557 mg/100 g on a fresh weight (FW) basis) are present [25]. Phenolic acids present in cranberries are correlated to their characteristic flavor as well as their potent antioxidant and antimicrobial properties [26].

2.3 Common Methods Used to Encapsulate Polyphenolics

There are two types of encapsulation strategies: top-down and bottom-up. The advantages and disadvantages of these strategies are listed in Table 2.1. The top-down approaches such as extrusion and emulsion consist of breaking down large structures, typically bulk liquids or solids, into smaller particles by external mechanical disruptive forces, such as shear, impact, and compression [30]. These approaches require sophisticated equipment such as homogenizer, which generate a heavy weight on cost due to maintenance and running. Moreover, it is quite difficult to create particles with a desired structure [30].

Table 2.1. Advantages and disadvantages of commonly used encapsulation methods

Methods	Approach Type	Advantages	Disadvantages
Emulsion	Top-down	Suitable for both hydrophilic and hydrophobic compounds [55]	Require sophisticated equipment [30]
Spray drying	Bottom-up	Simple, rapid, and relatively low cost [13]	Low yield [13] Not suitable for heat-sensitive compounds [13]
Extrusion	Top-down	Mild process [56] Suitable for both hydrophilic and hydrophobic compounds [56] Long shelf life [56]	Large particle size [56] Suitable for limited types of matrix materials [56] Scaling up is difficult and expensive [56]
Electro-spinning	Bottom-up	Simple [57] Low cost [58] Suitable for heat-sensitive compounds [57]	The fibers are porous [59]
Coacer-vation	Bottom-up	High encapsulation efficiency [56, 60]	Unstable in aqueous solution [30] High production cost [32, 33] Process is sensitive to many factors, such as pH [55]

In contrast, bottom-up approaches involve the association of molecules or small particles into larger particles by self-assembly or self-organization of molecules. The formation of microcapsules can be achieved by mixing the compounds to be encapsulated and the matrix materials in solution. It may require further treatments such as changes in pH, temperature, concentration, and ionic strength [61]. Compared to top-down approaches, bottom-up approaches require less energy and the methods allow for a better control of the size and the morphology of the microcapsules [30]. Typically, bottom-up approaches include coacervation, spray drying, electrospinning, anti-solvent precipitation, and binding [30, 61].

2.3.1 Emulsions

An emulsion contains a mixture of two immiscible liquids, where one liquid is dispersed into another (e.g. oil-in-water (O/W) or water-in-oil (W/O)). An emulsifier, composed of amphiphilic structures, is required to stabilize the emulsion [62]. After the emulsion is formed, the proteins are acting as the internal phase and are gelled by heating or by addition of gelling agents, where multivalent cations such as Ca^{2+}, Mg^{2+}, Ba^{2+}, Cu^{2+}, Zn^{2+} and Al^{3+} are commonly used [30].

Nanoemulsions are colloidal dispersions containing two immiscible liquids, with a diameter of less than 100 nm [55]. Given that nanoemulsions are non-equilibrium systems, that is, they cannot be formed spontaneously; they require a large amount of emulsifier and a high-energy input to be generated [60]. At the laboratory scale, sonicators and homogenizers are used to generate nanoemulsions, while on a large scale, high-pressure homogenizers and microfluidizers are required [62]. Nanoemulsions can be used directly in the liquid state however, to improve their stability under storage, they are usually dried to powder by spray drying or freeze drying after emulsification [60].

Double emulsions are another form of emulsion. They can be generated by single-step or two-step emulsification. For the one-step process, a mixture of emulsifiers undergoes a phase inversion under heating, resulting in the formation of a double emulsion [63]. While a two-step process consists of a first step where a primary emulsion is formed using a surfactant. In the case of W/O/W emulsion, it requires a low hydrophilic-lipophilic balance (HLB) surfactant, while a high HLB surfactant is used in O/W/O emulsion. The second step involves dispersing the W/O emulsion in an aqueous phase by adding a HLB surfactant to form a W/O/W emulsion [63]. Fig. 2.2 shows the two-step process of the double emulsion.

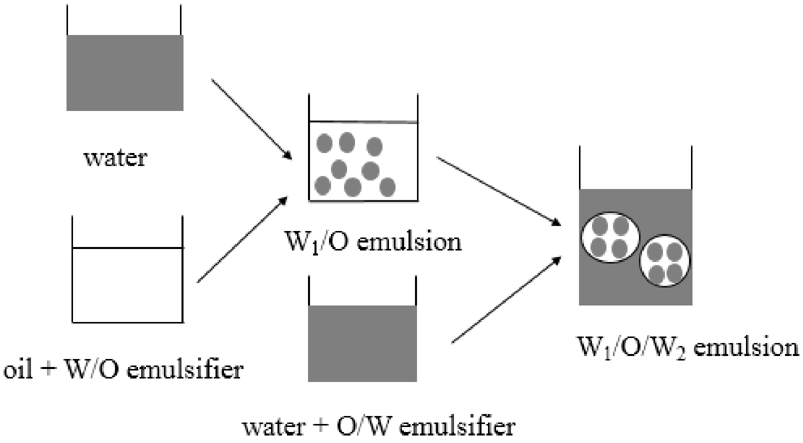


Fig. 2.2. Two-step process of double emulsions (adapted from Frank et al. [37])

2.3.2 Spray Drying

The spray drying process converts a liquid sample into a solid powder. Before drying, the compounds to be encapsulated are homogenized with the matrix materials. The homogenized liquid samples can be a solution, an emulsion or a suspension [13]. The liquid mixture is loaded into a spray dryer and then atomized by a nozzle or a spinning wheel. Drying happens as the atomized liquid is sprayed into hot air. The solvent, which is mainly water, is evaporated and a powder is formed. The powder is collected at the bottom of the drier. Typically, the shape of the particles is spherical and the mean size is in the range of 10-100 μm [32]. To achieve a successful microencapsulation by spray-drying, the important requisites are the high solubility of the matrix materials in the solvent and the low viscosity of the solution [13]. This process is simple, rapid, and relatively low-cost. However, the yield of the product is low because of the adhesion of the powder to the spray-dryer. Sensitive products may be degraded at high drying temperatures [13].

2.3.3 Extrusion

Extrusion is a process where a biopolymer solution, containing the compounds of interest and the matrix materials, is pushed through a nozzle into a gelling solution [30]. These steps are conducted at low temperature. On a laboratory scale, extrusion is conducted in a syringe, where the biopolymer solution is loaded and extruded through the needle [30]. The device can be modified by applying a direct-current (DC) supply [64]. The most widely used extrusion system consists of extruding a solution containing sodium alginate into a calcium chloride solution [33]. Recent studies have shown that proteins can be mixed with sodium alginate acting as the matrix materials [65, 66]. The size of the formed spherical gel particles depends mainly on the diameter of the needle and the flow rate of the biopolymer solution [30]. When a DC supply is applied, the size of the particles is also affected by the voltage [64]. Beside the syringe, a spinning disk and a double capillary can also be used [55]. The advantages of extrusion are the following: 1. the process is mild and very easy to conduct in a laboratory; 2. any compounds, hydrophobic or hydrophilic, can be encapsulated; and 3. the gel particles have a very long shelf life [56].

However, extrusion also has many drawbacks: 1. the particles are generally quite large and porous which makes it easy for encapsulated compounds to diffuse; 2. the options for the matrix materials are limited; and 3. the scaling up of the process is difficult and the cost is high [56].

2.3.4 Electrospinning and Electrospraying

Electrospinning is widely used in microencapsulation because of its simplicity and low cost. It utilizes electric fields to spin fibers, which involves drawing the polymer solution from a syringe into a collector [59]. The electrospinning apparatus consists of (i) a capillary that pumps the polymer solution at a constant rate; (ii) a high voltage source (1-30 kV) which induces charges in the polymer solution; and (iii) a grounded collector which is connected to the counter electrode (Fig. 2.3) [67]. The capillary pumps the polymer solution to form a droplet at the tip. A high voltage is applied to the droplet or to the needle, which results in the accumulation of charges on the surface of the droplet. The droplet elongates and forms a conical shaped Taylor cone, as shown in Fig. 2.3. The elongation of the droplet is the result of the repulsion of the charges in the droplet and the attraction between the oppositely charged droplet and the collector [67]. When the repulsion is greater than the surface tension of the droplet, a thread of polymer solution is ejected [68, 69]. After ejection, the polymer fiber becomes thinner due to the evaporation of the solvent. Finally, a randomly oriented, non-woven mat is placed on the collector [69, 70]. The formed nanofibers have a diameter in the range from 10 to hundreds of nanometers [57]. Nanofibers control the release of the encapsulated compounds due to their nanosize and their large surface-to-volume ratio. Moreover, heat treatment is not involved in electrospinning, which makes it ideal for encapsulating heat-sensitive bioactive compounds [57].

Electrospraying, which is considered as a “sister” technology of electrospinning, is a process where a liquid is atomized into droplets. The electrospraying apparatus is the same as for electrospinning. The difference between these techniques is the formation of nanofibers or nanodroplets, which is determined by the solution intrinsic viscosity (η) and the biopolymer concentration (c). The Berry’s number is equal to the product of η and c. When the Berry’s number is greater than a certain value, fibers are produced. Otherwise, droplets are formed if the Berry’s number of a solution is lower than the critical value [67]. After the formation of the nanodroplets, solvent evaporation takes place which results in the formation of solid polymer particles deposited on the collector [70]. Because of the electrical repulsions between the charged droplets, the agglomeration and coagulation of the droplets are prevented during their trajectory towards the collector [68, 71].

A single fluid is usually employed for the electrospinning process however; two different fluids can also be employed. Coaxial electrospinning is an example. In this process, one fluid referred as core fluid and another referred as sheath fluid are filled into two syringe pumps respectively. The core/sheath structures are formed by a concentric spinneret [58].

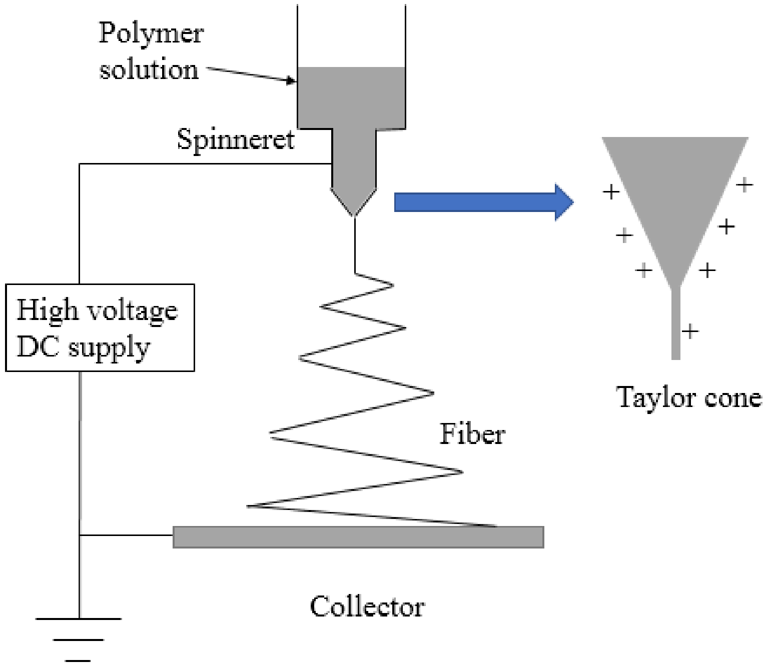


Fig. 2.3. Diagram of an electrospinning process (adapted from Ghorani & Tucker [68])

2.3.5 Coacervation

Coacervation is a commonly used method to generate capsules. The steps of formation of coacervates are shown in Fig. 2.4. The coacervate phase is formed by the phase separation of one or a mixture of polyelectrolytes in a solution. Then, the formed coacervate phase deposits around the compound to be encapsulated [56, 60]. When the coacervate phase is composed of a polymer, it is called simple coacervation; while in the case of a mixture of polymers, it is called a complex coacervation. The formed shell can be crosslinked by a chemical or enzymatic crosslinker to enhance its robustness [32, 56, 60]. The electrostatic interactions are the driving forces in forming coacervates, as they should be strong enough to induce interactions. However, the interactions cannot be too large to avoid precipitation [33, 55]. Hydrophobic interactions and hydrogen bonds also account for the interactions [30, 55].

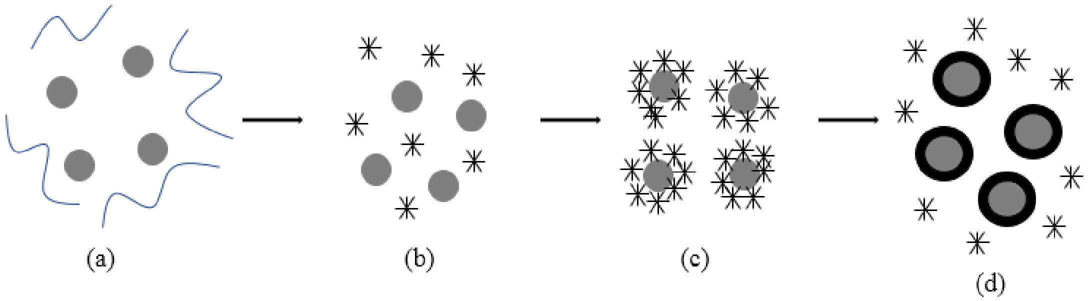


Fig. 2.4. Formulation of coacervate: (a) dispersion of compounds in the system containing the matrix; (b) coacervates formed in the dispersion; (c) coacervates formed at the surface of encapsulated compounds; (d) formation of the shell (adapted from Augustin & Hemar, [55])

Protein-polysaccharide complex is widely-used as the coacervate phase. This complex can be a cationic protein and an anionic polysaccharide or vice and versa. For a cationic protein and an anionic polysaccharide in solution, when the pH is higher than the pI of the protein, both the protein and the polysaccharide are negatively charged and an electrostatic repulsive force is created between them. When the pH approaches the pI, the cationic region of the protein interacts with the polysaccharide, but the interaction is weak. When the pH decreases to the point where the net charge is neutral, the interactions between the protein and the polysaccharide maximize and the complex is phase separated from the solution, which is referred as the coacervate phase [30].

The coacervation is a promising process because it achieves high encapsulation efficiency (up to 99 %) and possibly controls the release of encapsulated compounds. Based on the drying techniques, such as vacuum drying and freeze drying, the particles have a size in the range of 100-600 nm [60]. However, particles formed by coacervation are not perfectly spherical, tend to agglomerate, and are not stable in aqueous solution [30]. Moreover, the process is expensive, sensitive to pH and to ionic strength [32, 33, 55]. Therefore, it requires a good control of the experimental parameters [13].

2.3.6 Other Methods

Encapsulation can be achieved by the gelation of protein matrices to entrap phenolic compounds. The gelation of proteins is based on the amphiphilic nature of proteins. The gelation and aggregation of proteins trigger the formation of networks [55]. Heat treatment is a common gelling strategy, which includes cold-set gelation and heat-set gelation. Protein cold-set gelation is performed by heating the proteins in the solution, followed by a cooling step, and finally by adding the compounds of interest for encapsulation [61]. While in heat-set gelation, the steps are opposite: the proteins interact with the compounds of interest first and then undergo heat treatment [72]. After heat treatment, the unfolded polypeptide chains expose their hydrophobic amino acid residues which entrap the phenolic compounds, forming a three-dimensional network [29]. The acid gelation is achieved by first adjusting the pH of the solution above the isoelectric point (pI) of the proteins to prevent their gelation. The pH of the solution is then adjusted to a value close to the pI. At that pH, it enhances the electrostatic interactions within the protein molecules, forming particulate gels [55]. The ionic gelation is another method based on the electrostatic interactions between the carboxylic groups of the proteins and cations. Calcium ions are commonly used to promote the gelation of proteins such as casein [55]. In some applications, heat treatment, acid gelation, and ionic gelation were utilized in forming protein gels, such as whey protein nanoparticles [73].

The Layer-by-Layer method (LbL) is a self-assembly process where layers of opposite charge are being deposited onto the primary layer. The secondary layer enhances the robustness of the microcapsules and controls the release of the encapsulated compounds [33, 55]. Proteins like casein or whey proteins usually act as the outside layer by interacting with anionic or cationic biopolymers at specific pH [74].

Anti-solvent precipitation is a promising method which does not require any specific equipment therefore, facilitating the scale-up operations [30]. For this process, the compounds to be encapsulated are dissolved in a binary solvent, usually water and an organic solvent. After addition of one solvent, the solubility of the solute is decreased and the solution is supersaturated, leading to the formation of nanoparticles [30]. The liquid-liquid dispersion method has a similar principle to form particles. In this method, the compounds to be encapsulated are dissolved in a solvent. The solvent is mixed with another solvent at specific ratio to form particles [75].

2.4 Encapsulation of Polyphenolic Compounds

2.4.1 Anthocyanins

Studies related to the encapsulation of anthocyanins are listed in Table 2.2. In most researches, anthocyanins being encapsulated are plant extracts which contain a mixture of anthocyanin molecules. However, there has been a research performed to encapsulate a pure anthocyanin compound, cyanidin-3-O-glucoside (C3G), within a ferritin matrix [38]. Soybean ferritin was used as a matrix material since it dissociates at pH 2.0 and reconstitutes at pH 7.5. Therefore, C3G was mixed with ferritin at pH 2.0 and then formed microencapsulates at pH 7.5. The encapsulation increased the stability of the C3G under thermal treatment at 50 ^{o}C. This was because ferritin was not denatured at that temperature, which provided a protection effect against thermal treatment [38].

Emulsion is a common method to encapsulate anthocyanins. Bilberry anthocyanins were encapsulated by emulsion/heat gelation as follow: bilberry extracts were dissolved in whey protein solution and the pH was adjusted to 1.5 or 3 [72]. The mixture was centrifuged and the supernatant was poured into sunflower oil, stirred and heated at 80 ^{o}C to denature the proteins. After the centrifugation of the protein/oil mixture, the microcapsules were collected from the sediment. The average diameter of microcapsules generated by emulsion/heat gelation ranged from 0.5 mm to 2.5 mm, while with the addition of the emulsifier, the average diameter was reduced to 70 μm [72]. However, the addition of the emulsifier promoted the transition of anthocyanins from the protein solution to the oil phase. It was found that whey protein hydrogels were suitable matrices to protect anthocyanins under intestinal conditions (pH 6.8) since an *in vitro* release test performed at pH 6.8 showed that the microcapsules delayed the release of anthocyanins in the simulated intestinal fluids (SIF) [28]. In a study, anthocyanins containing bilberry extracts were encapsulated in 3 different matrices respectively: pectinate capsules formed by extrusion, whey protein isolate microcapsules formed by emulsion/heat gelation, and shellac coated polysaccharide powders formed by spray drying [36]. In simulated gastric fluids (SGF), the released anthocyanins from the microcapsules were stable while under SIF, anthocyanins degraded rapidly due to an increase in pH. However, all of the three encapsulation systems did not prevent an early release of the anthocyanins in SGF. Anthocyanins were also encapsulated in $W_1/O/W_2$ double emulsions by a two-step process [37]. The double emulsions were investigated under SGF *in vitro*. It was found that the outer aqueous phase (W_2) composed of whey protein isolates maintained the structural stability of the double emulsion in gastric conditions as compared with polysaccharide based W_2 layer. Polysaccharides tend to aggregate in gastric fluids [76].

Whey protein isolates were used as raw material to encapsulate blueberry anthocyanins by spray drying [77]. The results of the mass spectra suggested that the structures of anthocyanins were modified as the result of encapsulation. The spray dried powder retained a large amount of monomeric anthocyanins during digestion [78]. However, the amount of monomeric anthocyanins was decreased at higher storage temperature (i.e. 45 ^oC) [35]. Protein matrices were compared with carbohydrate matrices in spray drying. Pomegranate extracts containing anthocyanins were encapsulated in soybean protein isolates (SPI) and maltodextrin (MD) matrix respectively by spray drying [79]. The results of the storage test performed at 60 ^oC without light exposure suggested that the degradation rate of anthocyanins for MD matrix was lower as compared to SPI matrix, which indicated that the MD matrix had a good protection effect. In another study, whey protein isolates and MD were used to encapsulate bayberry juice by spray drying [80]. It was found that whey protein isolates were more effective than MD in spray drying, as it required less proteins to spray dry the bayberry juice than MD.

There are other methods to encapsulate anthocyanins. Bilberry extracts, rich in anthocyanins, were encapsulated in alginate/poly-L-lysine capsules by extrusion [81]. The results showed that the release was high at the beginning, but a high concentration of anthocyanins was retained within the capsules. Roselle (*Hibiscus sabdariffa* L.) calyx extract, abundant in polyphenolics, was encapsulated in gelatin beads coated with alginate [82]. The release profile fitted a first-order Weibull distribution, and the release was controlled by changing the number of alginate layers and the immersion time in calcium chloride solution. Anthocyanin-rich extracts were encapsulated by the coacervation method in whey protein isolates and beet pectin nanoparticles [83]. The nanoparticles had a diameter of less than 200 nm. The loading efficiency of anthocyanins was 55%, and the nanoparticles displayed enhanced heat stability.

Table 2.2. Summary of the methods to encapsulate anthocyanins

Encapsulation methods	Encapsulated compound	Matrix	Observations	References
Emulsion	Bilberry anthocyanins	Whey protein (heat gelation)	- The average diameter was reduced with the help of an emulsifier. - The addition of the emulsifier lowered the encapsulation efficiency.	[72]
			- The heat treatment decreased significantly the antioxidant activity of encapsulated microcapsules. - The microcapsules delayed the release of anthocyanins in the simulated intestinal fluids (pH 6.8).	[28, 36, 84]
			- The encapsulated bilberry extracts inhibited the growth of HT29 colon carcinoma cells.	[85]
			- The oxidative stress of colon cancer cells was decreased by the encapsulated bilberry extract.	[86]
	Anthocyanins	Whey protein isolate (double emulsion)	- Whey protein isolate matrix maintained the stability of the double emulsion in gastric conditions.	[76]
Spray drying	Blueberry anthocyanins	Whey protein isolate	- Whey protein microcapsules showed sustained release of anthocyanins.	[77]
			- The microcapsules retained a large amount of monomeric anthocyanins during digestion.	[78]
	Blueberry pomace	Whey protein isolate	- The amount of monomeric anthocyanins decreased during storage at higher temperature.	[35]
	Bayberry juice	Whey protein isolate maltodextrin	- Whey protein isolate was more efficient than maltodextrin in spray drying, as it required less proteins to spray dry the bayberry juice than maltodextrin.	[80]
	Pomegranate juice	Soybean protein isolate maltodextrin	- The maltodextrin matrix had a good protection effect for anthocyanins as compared to soybean protein matrix. The stability of anthocyanins was maintained in yogurt.	[79]
Extrusion	Bilberry extracts	Alginate/poly-L-lysine	- The release of anthocyanins at pH 1.0 and 4.5 was high, but a high content of anthocyanins was retained in the capsules.	[81]
Layer-by-Layer	Roselle calyx extracts	Gelatin/ alginate	- The release of total polyphenols could be controlled by increasing the number of alginate layers and the immersion time in calcium chloride solution.	[82]
Gelation	Cyanidin-3-O-glucoside (C3G)	Soybean ferritin	- The encapsulation increased the thermal stability and transport of C3G.	[38]
Coacervation	Anthocyanin rich extracts	Whey protein isolate/beet pectin	- The loading efficiency of anthocyanin was 55% and the heat stability of anthocynin was enhanced.	[83]

2.4.2 Quercetin

The studies related to the encapsulation of quercetin are listed in Table 2.3. Both liquid-liquid dispersion and anti-solvent precipitation methods have been proposed to encapsulate quercetin. For the liquid-liquid dispersion strategy, quercetins were dissolved in dimethyl sulfoxide (DMSO) and bovine serum albumin was dissolved in water. Both solutions were mixed. Finally, DMSO was removed by freeze drying and the nanoparticles were generated [87]. The formed nanoparticles had a diameter of less than 70 nm. The antioxidant activity of quercetin was protected within the nanoparticles [88]. Under SIF conditions, the stability of quercetin was maintained by the nanoparticles [87]. For the anti-solvent precipitation strategy, quercetin and zein were dissolved in an ethanol/water solution. The ethanol/water solution was added to a sodium caseinate aqueous solution. Finally, the solvent was removed by vacuum evaporation and the particles were separated by centrifugation [89]. The generated particles had a size ranging from 130 to 161 nm. Results showed that the morphology of the particles could be needle-like or spherical depending on the zein concentration. The encapsulated quercetin molecules were chemically stable when exposed to alkaline pH and UV light [89]. Chitosan oligosaccharide substituted with linoleic acid combined with β-lactoglobulin (CSO-LA/β-Lg) was used as matrix material to encapsulate quercetin [43]. CSO-LA/β-Lg nanoparticles were prepared by modified ionic gelation where the mixture of quercetin, CSO-LA, and β-Lg were treated at sub-ambient temperature followed by tripolyphosphate treatment. The results showed that as the charged amount of LA increased and the sub-ambient temperature decreased, both the association efficiency of CSO and β-Lg and the encapsulation efficiency of quercetin increased. While when both the charged amount of LA and the sub-ambient temperature increased, the size of the nanoparticles increased. In another study, quercetin was encapsulated within amaranth protein isolates/pullulan ultrathin fibers by electrospinning [44]. It was shown that quercetin molecules were evenly distributed along the smooth fibers. The results of the *in-vitro* digestion showed that the encapsulated compounds had a better antioxidant capacity than the un-encapsulated compounds.

Table 2.3. Summary of the methods to encapsulate quercetin

Encapsulation methods	Encapsulated compounds	Matrix	Observations	References
Liquid-liquid dispersion	Quercetin	Bovine serum albumin, lysozyme, or myoglobin	The antioxidant activity of quercetin was protected within the nanoparticles.	[88]
			The stability of quercetin was protected by the nanoparticles in simulated intestinal fluid.	[87]
		Zein/ caseinate	The morphology of the particles could be needle-like or spherical depending on the zein proportions. The encapsulated quercetin had chemical stability against alkaline pH and UV light.	[89]
Gelation	Quercetin	Chitosan oligosaccharide substituted with linoleic acid combined with β-lactoglobulin	When both the charged amount of linoleic acid and the sub-ambient temperature increased, the size of the nanoparticles increased.	[43]
Electrospinning	Quercetin and ferulic acid	Amaranth protein isolate/pullulan ultrathin	The encapsulated antioxidants had better antioxidant capacity than free antioxidants *in-vitro*.	[44]

2.4.3 Catechins (Flavan-3-ols)

Microencapsulation is an effective method to prevent catechin degradation in an aqueous environment. The studies performed on the subject are listed in Table 2.4. Among the catechins, epigallocatechin-3-gallate (EGCG) has been widely studied in microencapsulation. Catechins have been encapsulated in β-lactoglobulin (β-Lg) matrices by gelation [46]. To synthesize such nanoparticles, an EGCG solution was added to a pre-heated β-Lg solution to form co-assemblies (cold-set gelation process). The size of the nanoparticles was less than 50 nm and the nanoparticles were transparent in color, indicating potential applications in beverages. The nanoparticles showed activities against oxidation and degradation. Moreover, these particles had good encapsulation efficiency (60 - 70%) for EGCG. Because of the limited release of EGCG under SGF digestion, the nanoparticles could be a potential matrix for the protection of EGCG in the stomach and the release of EGCG in the intestine [47]. In another study, EGCG was encapsulated in heat-denatured β-Lg by adding an EGCG solution to a denatured β-Lg solution followed by fast cooling [48]. Four factors including pH, heating temperature of β-Lg, molar ratio of β-Lg to EGCG, and β-Lg concentration, could affect the particle size, ζ-potential, and the entrapment efficiency of EGCG. Another study was performed by encapsulating EGCG in native, heated, and desolvated β-Lg matrix respectively [90]. The desolvated β-Lg was prepared by desolvating β-Lg solution in ethanol. The encapsulation efficiencies of all three nanoparticles were approximately 90%. However, compared to the native β-Lg nanoparticles, both heated and desolvated β-Lg nanoparticles had a larger size. This was due because heating caused the aggregation of β-Lg, while the addition of ethanol to a β-Lg solution increased the α-helix content of β-Lg, which triggered the aggregation of the protein monomers. Other protein matrices have also been utilized to encapsulate catechins. For example, EGCG-encapsulated glycosylated casein nanocomplex had little aggregation during storage [91]. Results of an *in vitro* release tests showed that this nanocomplex had a slow and sustained release in SIF. This suggested that this complex could protect EGCG from degradation in the intestine.

Coacervation is another commonly used strategy to encapsulate catechins. In a study, EGCG aqueous solution was added to caseinophosphopeptide solution, followed by the addition of s chitosan solution [92]. It formed nanoparticles spontaneously by ionic gelation mechanism. The encapsulation efficiency of EGCG in the nanoparticles was 70.5-81.7%, the particle size was 150 ± 4.3 nm, and the polydispersity index of the particles ranged from 0.05 to 0.14, which indicated a homogeneous dispersion [93]. Moreover, the nanoparticles had a spherical shape [94]. These nanoparticles were biocompatible and stable at a pH ranging from 2.5 to 7.0 [93]. Cellular uptake of EGCG-loaded nanoparticles into the human hepatocellular caricinoma (HepG2) cells was also observed [92]. In another study, tea polyphenols were encapsulated in complex coacervate core micelles (C3Ms) with gelatin-dextran conjugates as matrix materials [95]. The gelatin-dextran conjugates were synthesized by Maillard reaction. The C3M had an average diameter of 86 nm and a spherical shape, where the core consisted of gelatin with tea polyphenols and the shell was made of dextran. The encapsulation capacity of tea polyphenols was high. Green tea polyphenols extracts (GTPE) were encapsulated in caseinate beads [96]. The storage test was performed in the dark at 21 ^{o}C and 44% RH and showed that the antioxidant activity of GTPE was stable during 42 days of storage.

There are other methods to encapsulate catechins. Catechins-loaded O/W emulsions were prepared by adding an oil phase drop by drop to a mixture of catechins, BSA or ovalbumin, and emulsifier Tween-20. The microemulsion showed good stability and strong antioxidant activity [97]. In another study, EGCG-encapsulated O/W emulsions were prepared by high-pressure homogenization [50]. The study showed that successful encapsulation occurred when the droplet size was about 400 nm and that the EGCG concentration was less than 0.5%. Zein, dissolved in ethanol, was used to form protein fibers by electrospinning at 15 and 20 kV to encapsulate EGCG [57]. EGCG was incorporated in fiber-forming solution, and the formed fibers had a diameter ranging from 150 to 600 nm. EGCG-loaded gelatin particles were also generated by electrospraying [98]. EGCG was added to a gelatin solution, which was dissolved in 20% acetic acid, followed by electrospraying. The antioxidant activity of EGCG was retained by encapsulation in aqueous solution at pH 7.4.

Table 2.4. Summary of the methods to encapsulate catechins

Encapsulation methods	Encapsulated Compounds	Matrix	Observations	References
Gelation	EGCG	β-lactoglobulin (β-Lg)	The co-assemblies showed good properties against oxidation and degradation as compared to un-encapsulated EGCG.	[46]
			The bitterness and astringency of EGCG was masked by the nanoparticles. The release of EGCG under simulated gastric digestion was limited.	[47]
			Four factors including pH, heating temperature of β-Lg, molar ratio of β-Lg to EGCG, and β-Lg concentration, could affect the particle size, ζ-potential, and the entrapment efficiency of EGCG.	[48]
			The nanoparticles could decrease cellular proliferation. The bio-efficacy of EGCG was not affected by encapsulation.	[90]
		Casein micelles	With the presence of EGCG, the gelation properties of the casein micelles changed.	[99]
			The anticancer properties of EGCG against colon cancer cells were not diminished by encapsulation.	[49]
		Glycosylated casein	The nanocomplex had little aggregation during storage and demonstrated a slow and sustained release in simulated intestinal fluid.	[91]
		Gelatin	The nanoparticles demonstrated antioxidant activities after 3 weeks of storage.	[100]
		Transferrin	The EGCG released relatively rapidly from the vesicles therefore, it suppressed the growth of tumor cells and enhanced the cellular uptake of EGCG.	[101]
Emulsion	EGCG	ι-carrageenan and β-lactoglobulin	When the droplet size was about 400 nm and EGCG concentration was less than 0.5%, EGCG was successfully encapsulated and the droplet size was negligibly changed within 14 days.	[50]
	Catechins	Bovine serum albumin or ovalbumin	The microemulsion demonstrated good stability and strong antioxidant activity.	[97]
Electrospinning	EGCG	Zein	The formed fibers had a diameter ranging from 150 to 600 nm.	[57]
Electrospraying	EGCG	Gelatin	The encapsulation efficiency of EGCG was close to 100%.	[98]
Coacervation	EGCG	Chitosan/caseino-phosphopeptide	The release of EGCG from nanoparticles was controllable and the nanoparticles demonstrated strong antioxidant activities against free radicals.	[92]
			The nanoparticles could enhance the intestinal absorption of EGCG significantly.	[94]
		Fuctose-chitosan/gelatin	The *in vitro* release results showed that the release of EGCG from nanoparticles was controllable. Compared to EGCG solution, these nanoparticles could more effectively reduced *H. pylori* gastric inflammation.	[102]
	Catechin	Chitosan-whey protein	The *in vitro* release properties were related to the degree of crosslinking.	[103]
	Green tea polyphenols extracts	Caseinate	The antioxidant activity of beads was stable during 42 days of storage, and the antioxidant activity of GTPE loaded beads was significantly ($P<0.05$) higher than that of non-GTPE beads.	[96]
	Tea polyphenols	Gelatin-dextran	The complex coacervate core micelle had stronger antitumor activity against human breast carcinoma MCF-7 cells than free tea polyphenols.	[95]

2.4.4 Proanthocyanidins (PACs) and Hydrolysable Tannins

As mentioned in section 2.3, PACs are prone to degrade under oxygen and high temperature. Besides, PACs impart bitterness and astringency, which affect their sensory attributes [53]. Therefore, encapsulation of PACs is of interest. The studies performed on encapsulation of PACs and hydrolysable tannins are summarized in Table 2.5. Sorghum condensed tannins (SCT) were encapsulated in kafirin matrix by coacervation [104]. The microparticles were synthesized by mixing a kafirin ethanolic solution with a SCT ethanolic solution followed by adding water to form coacervates. The encapsulation efficiency of SCT was around 48% and only a limited amount of SCT was digested under simulated gastrointestinal fluid conditions. In another study, SCT-encapsulated kafirin microparticles were synthesized by a different method: SCT extracts were mixed with pre-prepared kefirin microparticles followed by drying at ambient conditions [105]. The formed microparticles had a porous surface, an irregular shape and controlled-release properties. Cranberry procyanidins (CPs) were encapsulated in zein matrix by dissolving CPs and zein in an ethanol/water binary solvent followed by water addition leading to the formation of nanoparticles [51]. The formed spherical particles had a size ranging from 392 to 447 nm. The loading efficiency of CPs decreased with increased CPs-to-zein ratio. Pomegranate ellagitannins (PPE), containing mainly punicalagin, were encapsulated in gelatin particles by gelation [106]. The formed particles had a size of less than 200 nm and displayed a high loading efficiency. The anticancer activity of encapsulated ellagitannins and procyanidins has been observed [51, 106]. Another study showed the limited procyanidin release of double emulsions during a 14-days storage period [53]. The emulsion retained procyanidins because of its resistance to dehydration [107]. The $W_1/O/W_2$ double emulsions were made by a two-step premix membrane emulsification. The W_1/O phase was emulsified in the W_2 phase. The coarse emulsions were forced through a membrane. The formed double emulsions had a size of about 10 μm with a narrow size distribution [53].

Table 2.5. Summary of the methods to encapsulate PACs and hydrolysable tannins

Encapsulation methods	Encapsulated compounds	Matrix	Observations	References
Coacervation	Sorghum condensed tannins (SCT)	Kafirin	The encapsulation efficiency of SCT was around 48% and only a limited amount of SCT was digested under the condition of simulated gastrointestinal fluids.	[104]
	Catechin and sorghum condensed tannins	Kafirin	The microparticles had a porous surface, an irregular shape and controlled-release properties.	[105]
Gelation	Pomegranate ellagitannins (PPE), mainly punicalagin	Gelatin	The nanoparticle had similar apoptotic effect as PPE on human promyelocytic leukemia cells HL-60.	[106]
Emulsion	Procyanidin	Whey protein-chitosan, or whey protein-gum Arabic (double emulsion)	The double emulsions had limited procyanidin release during a 14 days storage period. The emulsion could retain procyanidins because of its resistance to dehydration.	[53, 107]
Liquid-liquid dispersion	Cranberry procyanidins (CP)	Zein	The cytotoxicity of CPs-zein nanoparticles against human promyelocytic leukemia HL-60 cancer cells was lower than that of CPs solution.	[51]

2.4.5 Phenolic Acids

Because of the antioxidant activity of phenolic acids, some studies have been performed to study their encapsulation as summarized in Table 2.6. Ferulic acid was encapsulated in zein composite fibers by coaxial electrospinning with the addition of unspinnable acetic acid [58]. The electrospinning process ran smoothly without clogging. The formed fibers had a reduced diameter and were of higher quality than those formed by single-fluid electrospinning process. Moreover, the fibers generated by coaxial electrospinning demonstrated sustained release of ferulic acid *in vitro*. Gallic acid was also encapsulated in zein fibers by electrospinning [59]. The generated ultra-fine fibers had a diameter ranging from 327 to 387 nm. The antioxidant activity of gallic acid was retained in the fibers. Besides electrospinning, caffeic acid was encapsulated in an O/W emulsion at different pH [108]. With the presence of BSA, the antioxidant activity of caffeic acid was enhanced. The ideal pH to stabilize caffeic acid ranged from 3 to 5. The storage test showed that the stability of the antioxidants improved with the presence of BSA. During storage, protein-antioxidant adducts were formed [109]. Ellagic acid was encapsulated within synthesized threonine based peptide microtubes [110]. The microtubes were formed for weeks and the ellagic acid was loaded by incubation at ambient conditions. The release of ellagic acid was influenced by the pH. The ellagic acid loaded microtubes had activity against *Escherichia coli* and *Staphylococcus aureus*. Some natural polyphenols, including EGCG, tannic acid, and theaflavin, were respectively encapsulated in gelatin nanoparticles coated with a LbL shell of polyelectrolytes consisting mainly of a mixture of proteins and polysaccharides. The LbL shell was synthesized by adding polyanions and polycations to a solution containing gelatin nanoparticles. The nanoparticles retained the biological activity of the polyphenols [111].

Table 2.6. Summary of the methods to encapsulate phenolic acids

Encapsulation methods	Encapsulated compounds	Matrix	Observations	References
Electrospinning	Ferulic acid	Zein	The fibers showed sustained release of ferulic acid *in vitro*.	[58]
	Gallic acid	Zein	The antioxidant activity of gallic acid was retained in the fibers.	[59]
Gelation	Ellagic acid	Threonine based peptide	The release of ellagic acid was influenced by the pH. The ellagic acid loaded microtubes had activity against *Escherichia coli* and *Staphylococcus aureus*.	[110]
Layer-by-Layer	EGCG, tannic acid, theaflavin	Gelatin	The nanoparticles demonstrated a similar biological activity as free EGCG.	[111]
Emulsion	Caffeic acid	Bovine serum albumin (BSA)	The storage test showed that the stability of the antioxidants improved with the presence of BSA. During storage, protein-antioxidant adducts were formed.	[109]
			The ideal pH to stabilize caffeic acid ranged from 3 to 5.	[108]

2.5 Conclusion

Plants contain many phenolic compounds, such as anthocyanins, flavonols, flavan-3-ols, proanthocyanidins, and phenolic acid derivatives. These phenolic compounds have antioxidant properties and many health benefits. However, these compounds are prone to degradation when not protected by an encapsulation system. Protein matrices are used in food applications due to their nontoxicity and nutritional properties. Unlike polysaccharide matrices, the utilization of protein matrices to encapsulate phenolic compounds is not common. Combined protein/polysaccharide matrices have also been used especially in emulsion and coacervation methods.

Encapsulation methods using protein matrices to encapsulate phenolic compounds present in plants have been discussed. These methods are classified as top-down and bottom-up strategies, including gelation, emulsion, spray drying, extrusion, electrospinning, coacervation, among others. Emulsion, spray drying, and coacervation have been widely used mainly because they are suitable for a wide spectrum of compounds. However, the high energy consumption required for encapsulation via emulsion, the degradation of heat-sensitive compounds when spray drying and the instability of coacervates, limit their applications. Although there are not many studies reporting on extrusion, anti-solvent precipitation, and electrospinning for the encapsulation of phenolic compounds with protein matrices, these techniques may gain in popularity due to their numerous advantages.

The encapsulation efficiency of phenolic compounds varies, ranging from 50% to more than 90%, depending on the encapsulation method and matrix materials. In most cases, the biological activity of the phenolic compounds is retained in the encapsulation systems. Some examples include inhibiting the growth of cancer cells, decreasing cellular proliferation, reducing microbial growth, and exhibiting antioxidant activity. With encapsulation, the release of phenolic compounds is controllable. The absorption of phenolic compounds in the intestine is also improved. This review showed that encapsulation enhances the antioxidant activity and chemical stability of phenolic compounds in different storage conditions.

Connecting Statement

The utilization of proteins as wall material for the encapsulation of phenolic compounds was discussed in Chapter 2. In Chapter 3, composite beads synthesized with pea proteins and alginate were synthesized by extrusion. The resulting hydrogel beads were freeze dried and stored at three different temperatures (room temperature, 50 ^oC, and 100 ^oC) for 12 days. The changes in the chemical composition, the thermal stability, the morphology, and the mechanical properties of the beads over a storage period of 12 days were studied.

CHAPTER 3

PHYSICAL AND CHEMICAL PROPERTIES OF PEA PROTEIN/ALGINATE BEADS DURING STORAGE AT DIFFERENT TEMPERATURES

3.1 Abstract

The objectives of this work were to generate freeze dried pea protein/alginate beads and to study the changes in their physical properties during storage at different temperatures (room temperature, 50 ^{o}C, and 100 ^{o}C). The properties studied included the chemical composition, the thermal stability, the morphology, and the mechanical properties. These properties were measured and tested using a Fourier transform infrared spectrometer (FTIR), a thermogravimetric analyzer (TGA), a scanning electron microscope (SEM), and a texture analyzer, respectively. The results showed that the chemical composition, the thermal stability, and the morphology of the composite beads did not change during the storage period for all temperatures tested. The mechanical properties of the beads included measurements of the hardness and the resilience. The average resilience values of the beads were relatively stable for all storage conditions. The average hardness values of the beads stored at 100 ^{o}C showed a decreasing trend during storage, while the values of the beads stored at room temperature and 50 ^{o}C did not show an obvious decreasing trend. However, the statistical analysis showed that both the hardness and the resilience of the beads stored at all temperatures were not significantly different ($P>0.05$) during storage. Based on the results, as the stability of the beads was maintained during storage, the beads could be suitable as encapsulating matrices in food applications.

3.2 Introduction

In the design of encapsulating matrices destined for oral delivery, the polymers used must be food grade. Alginate is considered as a suitable coating material because of its good biocompatibility, stability in gastric fluid, and degradation in intestinal fluid [112]. Alginate has been approved by the United States Food and Drug Administration and the European Food Safety Authority as a food-grade coating material [12].

Alginate is an anionic polymer consisting of alternating blocks of 1,4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues. These uronic acid units are distributed along the polymer chain in a sequence of M-blocks, G-blocks, and MG-blocks. The composition and the sequence of the G and M residues depend on their origin [113]. Alginate can chelate with divalent cations to form hydrogels. Ca^{2+} is commonly used to induce the sol-to-gel transformation [113, 114]. A common method to induce gelation is through the extrusion technique, where the Na^+-alginate solution is dropped into a cross-linking solution, such as a $CaCl_2$ solution. Divalent cations can replace Na^+ in the polyguluronate segments of the alginate molecule, forming a cross-linked “egg-box” model, where alginate entraps cations by the ionic interaction [114]. The "egg-box" model is shown in Fig. 3.1. In addition, the van der Waal forces between the alginate segments lead to the formation of a three-dimensional gel network [114]. The size and the shape of the beads depend on the diameter of the dripping tip, the collecting distance, as well as the stirring rate and the curing time in the cross-linking solution during the process [115].

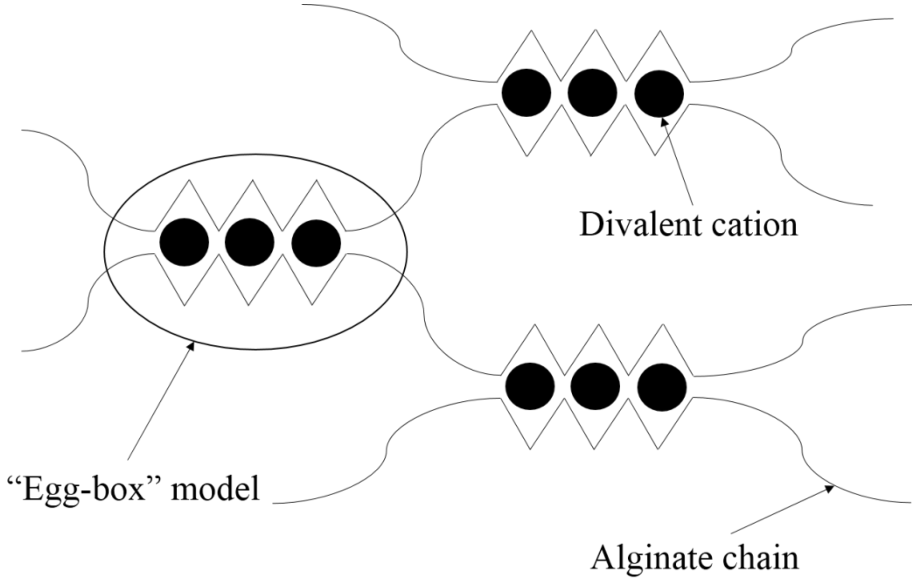


Fig. 3.1. "Egg-box" model of an alginate hydrogel through chelation with divalent cations (adapted from Burey et al. [116])

The formation of the alginate beads by the extrusion technique has some limitations. First, the alginate solution has a high viscosity even at low concentrations. Therefore, in order to form the Ca^{2+}-alginate beads the maximum concentration of alginate solution should be 2–4%. As a consequence, the resulting gel network exhibits a low density [117]. Second, the low density network triggers a rapid *in vitro* release of the encapsulated substance [118]. The dehydration of the alginate beads is usually conducted to extend the shelf life however, the mechanical integrity of the alginate structure is disrupted during the dehydration process [12].

To overcome these limitations, the addition of proteins to the alginate solution could help solving some of these issues [9]. As such, a study showed that the addition of proteins to an alginate solution increased the particle size and the hardness of the hydrogel beads [118]. It also increased the encapsulation efficiency of the encapsulated phenolic compounds. However, the increase or decrease of the viscosity of the alginate solution depended on the type of proteins being added [118]. In another study, the swelling behavior and the degradation of whey protein/alginate beads were evaluated. At a pH of 1.2, both the water uptake and the weight loss of the beads were very low due to the shrinkage of alginate. At a pH of 6.8, the beads were partly swelled and degraded due to the degradation of the alginate and the stability of the whey protein [119]. Therefore, from the knowledge to date, it was concluded that these encapsulating matrices could increase the intestinal delivery and the bioavailability of insulin [120].

The applications of pea protein isolate as the wall material for encapsulation are quite limited. Some bioactive compounds and ingredients, such as ascorbic acid [121], conjugated linoleic acid [4], and flaxseed oil [22], have been encapsulated within pea protein isolate powders by spray drying. Besides, pea protein/alginate beads have been used to encapsulate probiotics (e.g. *Bifidobacterium adolescentis* [122] and *Lactobacillus casei* ATCC 393 [66]) and prebiotics (e.g. fructooligosaccharides [122]). The encapsulated probiotic *B. adolescentis* was protected under gastric conditions and it was released under an intestinal environment [122].

Even though the chemical composition, the thermal stability, and the morphology, of pea protein/alginate beads have been investigated [65], the change in their physical properties in function of the storage time and temperature should also be considered. In this study, pea protein/alginate beads were prepared by the extrusion technique. The composite beads were stored at different temperatures (room temperature, 50 ºC, and 100 ºC) for 12 days and the changes in their chemical composition, thermal stability, morphology, and mechanical properties were investigated.

3.3 Materials and Methods

3.3.1 Materials

Pea protein isolate (Propulse N™, 81.73% protein, <10.3% sugars, <0.7% starch, 3.40% moisture, <0.5% fat and <4.0% ash) was obtained from Nutri-Pea Ltd. (Portage la Prairie, MB, Canada). Alginic acid sodium salt from brown algae (medium viscosity, Lot# SLBM3663V) and calcium chloride dihydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide was obtained from EMD (Darmstadt, Germany). Concentrated hydrochloric acid (37%) was provided by Fisher Scientific (Fair Lawn, NJ, USA). Anhydrous ethanol was obtained from Greenfield Specialty Alcohols Inc., (Brampton, ON, Canada).

3.3.2 Preparation of Pea Protein/Alginate Beads

The method to synthesize pea protein/alginate beads was modified from the methodology described by Xu & Dumont [65]. 1.8 g of pea protein isolate was dissolved in a 50 mL of 0.1M NaOH solution. The solution was heated at 80 °C in a water bath while stirring for 30 min to denature the protein. The protein solution was then cooled down to room temperature, followed by the pH adjustment to 7.0 with 1% HCl solution, prepared from concentrated hydrochloric acid. 0.45 g of sodium alginate was added to the solution. The solution was heated at 80 °C in a water bath while stirring for 60 min. The pea protein/alginate solution was then cooled down to room temperature. 5 mL of anhydrous ethanol was added to the solution and stirred. The solution was sonicated in a water bath for 5 min. Then, 10 mL of distilled water was added to the mixture and stirred. The solution was finally extruded through a 26 G needle, connected with a syringe, into a 150 mL of 0.1 M $CaCl_2$ solution under stirring at 300 rpm. The composite beads were formed in the $CaCl_2$ solution. The formed beads were kept in the gelation solution for 30 min. The liquid was filtrated by vacuum filtration and the beads were then washed with 2 portions of 20 mL of distilled water to remove the excess salt. The beads were transferred to centrifuge tubes and the excess moisture was carefully drained off. The beads were immersed in liquid nitrogen and freeze dried with a vacuum freeze-dryer (Gamma 1-16 LSCplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) at shelf temperature of 25 ^oC and 0.85 mbar for at least 48 h.

3.3.3 Storage Conditions of Pea Protein/Alginate Beads

Samples of the freeze dried pea protein/alginate beads were stored in sealed glass bottles at different temperatures (room temperature, 50 ^oC, 100 ^oC) for 12 days. Every 3 days, starting from day 0, bead samples were taken out to determine their physical and chemical properties.

3.3.4 Chemical Composition

The FTIR analyses of sodium alginate, pea protein isolate, and freeze dried pea protein/alginate beads were conducted using a Nicolet iS5 FTIR spectrometer (Thermo Fisher Scientific, Madison, WI, USA). The composite beads were grinded before analysis. Each sample was analyzed in duplicate. For each spectrum, a total of 32 scans were performed at 4 cm^{-1} resolution. The measurements were recorded between 500-4000 cm^{-1}. The spectra were analyzed using the OMNIC software (version 8.2, Thermo Fisher Scientific, Madison, WI, USA).

3.3.5 Thermal Stability

The thermal stability of sodium alginate, pea protein isolate, and the freeze dried pea protein/alginate beads was determined by TGA (Q50, TA Instrument, Inc., New Castle, DE, USA). Around 10 mg of bead sample from each storage temperature and storage day was grinded and loaded into a platinum pan. The samples were heated from room temperature to 600 °C at a constant rate of 10 °C/min. The analyses were performed under nitrogen gas at a constant flow rate of 60 mL/min. All samples were run in duplicate.

3.3.6 Morphology

The morphology of the freeze dried beads was examined with a Hitachi Tabletop SEM Microscope (TM3000, Hitachi High-Technologies Co., Tokyo, Japan). At least three beads were placed on a carbon conductive double-sided tape on the specimen stub. The samples were examined at accelerated voltages of 5 kV under vacuum.

3.3.7 Mechanical Properties

The hardness and the resilience of the freeze dried pea protein/alginate beads were evaluated. A texture analyzer (TA.HD*Plus*, Stable Microsystems, Surrey, UK) connected with a cylinder probe (TA-25,7 mm diameter) was used to measure the applied force over time. The probe was connected to a 5 kg load cell. Each individual bead having a diameter ranging from 0.9 to 1.2 mm was compressed to 30% strain at a speed of 2 mm/s. Fifteen (N=15) measurements were performed. The compression measurements were performed in the "Compression Return to Start" mode. The pre-test, test, and post-test speeds were all set at 2 mm/s, with a trigger force of 0.5 g. The data were recorded by Texture Exponent Software (Version 6,1,9,0). The hardness (g) was measured from the maximum compression force (anchor 2 in Fig. 3.2). The resilience (%) was calculated by the percentage of the ratio between the area under the curve (AUC) of anchors 2 and 3 ($AUC_{2–3}$) and AUC of anchors 1 and 2 ($AUC_{1–2}$) from a force–time graph (Fig. 3.2).

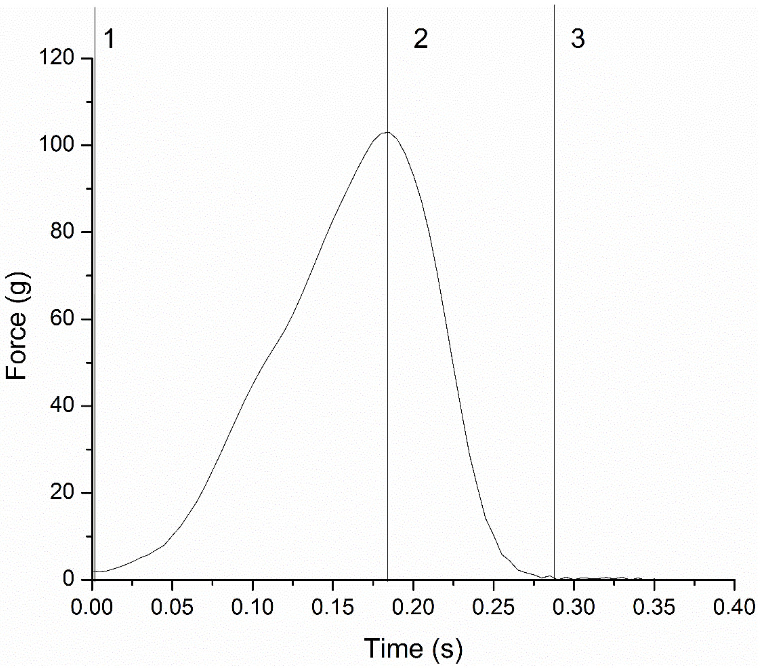


Fig. 3.2. A typical force-time graph of freeze dried beads studying hardness and resilience

3.3.8 Statistical Analysis

Analysis of variance (ANOVA), performed by SPSS® (Version 23, IBM®, Armonk, NY, USA), was used to test if the hardness and the resilience of the beads were affected by the storage time.

Shapiro-Wilk test and Levene's test were performed to test the normality and homogeneity of variances of the data, respectively. In the assumption that the normality or homogeneity of variances was violated, non-parametric Kruskal-Wallis one-way analysis of variance was used instead of ANOVA. All the comparisons were performed at a significant level of 5%. The error bars in the plot represent the standard deviation of the data.

3.4 Results and Discussion

3.4.1 Chemical Composition

The FTIR spectrum of sodium alginate is presented in Fig. 3.3. The bands at 1601 and 1409 cm^{-1} correspond to the symmetric stretching vibration and the asymmetric stretching vibration of the carboxyl group (-COO^{-}), respectively [65, 123]. The broad band at 3358 cm^{-1} is due to the OH stretch, and the peak at 2930 cm^{-1} is attributed to the CH stretch [124]. The FTIR spectrum of sodium alginate is consistent with the absorption pattern of sodium alginate reported in a previous study [124]. For pea protein isolate (Fig. 3.3), the peaks at 3291 cm^{-1}, 1651 cm^{-1}, 1538 cm^{-1}, and 1239 cm^{-1} correspond to the amide-A (NH- stretching coupled with hydrogen bonding), the amide-I (C=O stretching/hydrogen bonding coupled with -COO), the amide-II (bending vibration of N–H groups and stretching vibrations of C-N groups), the amide-III (vibrations in plane of C-N and N-H groups of bound amide), respectively [125]. In a previous study, it was found that the secondary structures of pea protein isolate were mainly β-sheets, α-helix, and β-turns as shown in the amide I region of the FTIR spectrum [15].

The FTIR spectrum of the composite beads shows an overlap of the pea protein isolate and the sodium alginate spectrum (Fig. 3.3). The absorbance peak at 3293 cm^{-1} corresponds to the amide-A peak of pea proteins and the OH stretch of sodium alginate. The absorbance peak at 1634 cm^{-1} corresponds to the C=O stretching, which is present in both the pea proteins and sodium alginate spectra. The absorbance peak of the amide-II (1538 cm^{-1}) is also present in the spectrum of the composite beads. Finally, an absorbance peak at 1417 cm^{-1} corresponds to the asymmetric stretching vibration of the carboxyl group of the sodium alginate spectrum.

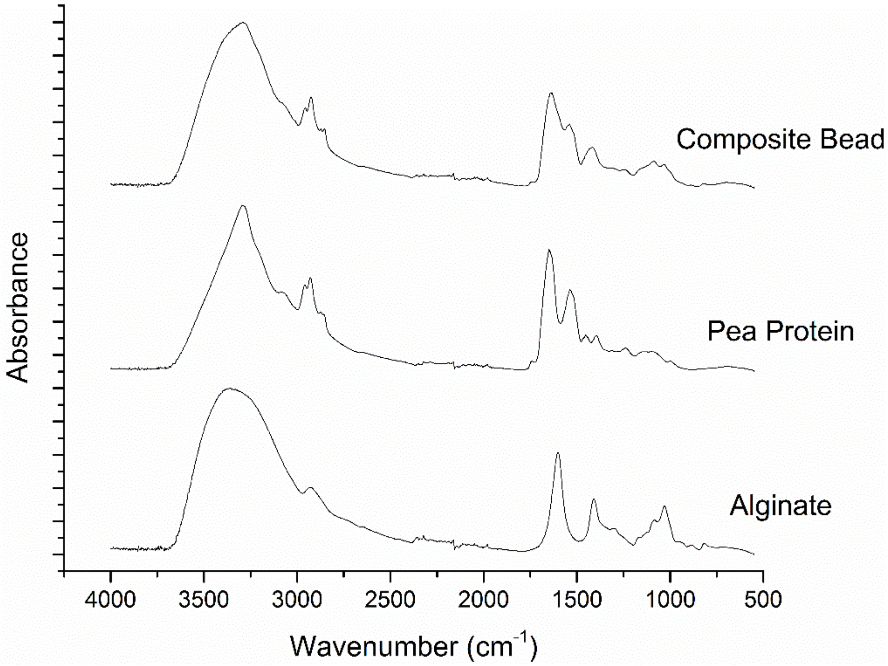


Fig. 3.3. FTIR spectra of sodium alginate, pea protein, and composite bead

During the storage periods, the FTIR spectra of the composite beads did not change for all temperatures tested (Fig. 3.4). It suggests that the chemical composition of the composite beads is very stable under increasing temperature (up to 100 °C) treatment.

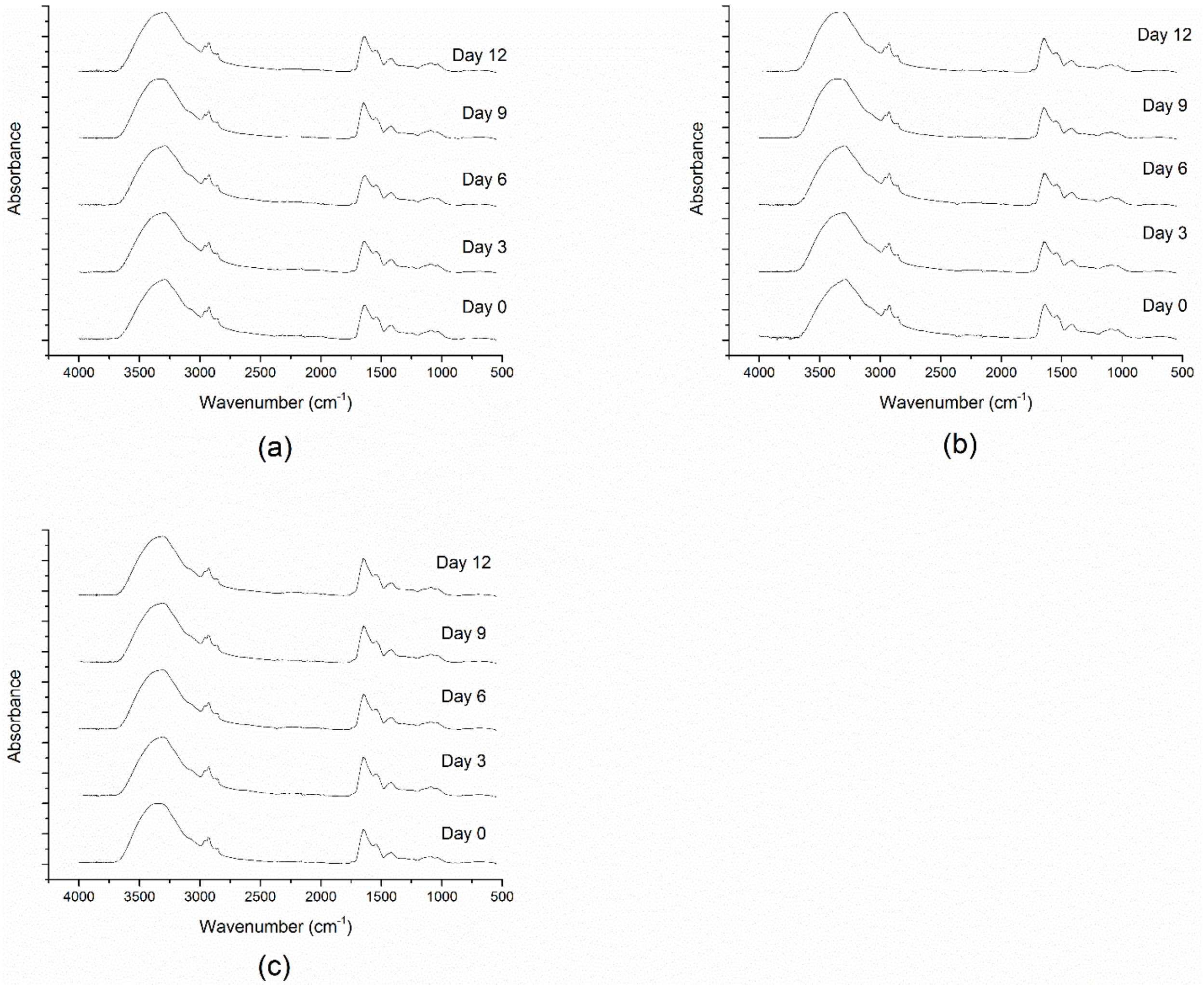


Fig. 3.4. FTIR spectra of composite beads stored at (a) room temperature; (b) 50 ^{o}C; (c) 100 ^{o}C

3.4.2 Thermal Stability

The TGA curves of pea proteins, sodium alginate and the composite beads are presented in Fig. 3.5. From that figure, the sodium alginate and pea protein isolate demonstrate different patterns of weight loss before decomposition, with the pea protein isolate having higher degradation temperature onset than sodium alginate. The degradation temperature (Td) 5% and Td 10% values of sodium alginate were 57 ^{o}C and 100 ^{o}C, while the values of pea protein were 58 ^{o}C and 215 ^{o}C. However, sodium alginate shows a lower degradation weight loss than pea protein isolate. The Td 5% and Td 10% values of the beads were 68 ^{o}C and 134 ^{o}C. The beads show a lower degradation weight loss than pea protein isolate, but higher degradation weight loss than sodium alginate. The difference in the degradation weight loss suggests the presence of both pea protein isolate and sodium alginate in the beads.

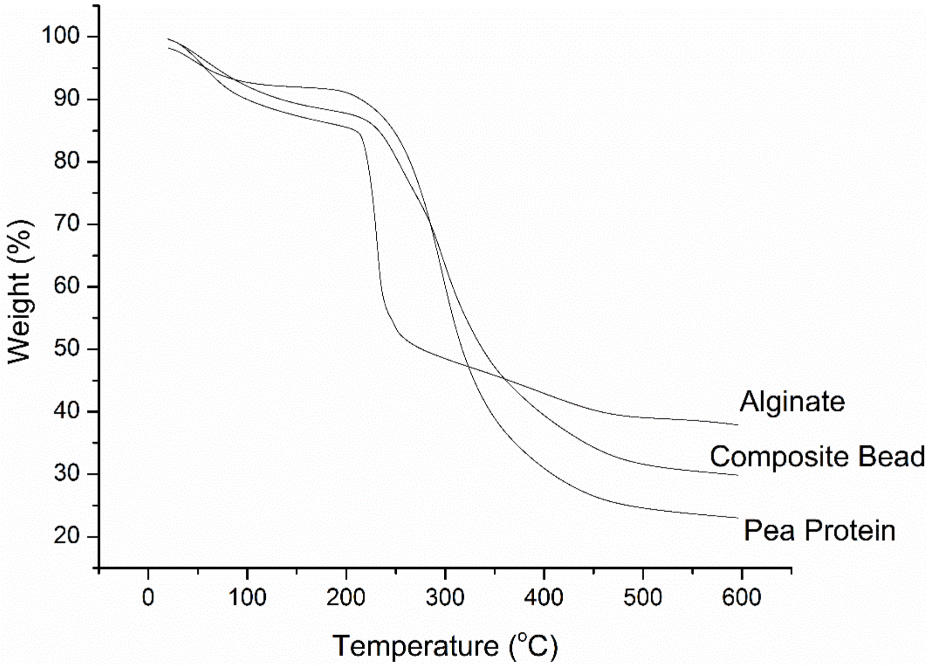


Fig. 3.5. TGA curves of sodium alginate, pea protein, and composite beads

The derivatives of the TGA (DTGA) curves are presented in Fig. 3.6. From that figure, sodium alginate shows decomposition peaks at around 55 ^{o}C and 230 ^{o}C. The fist peak corresponds to the evaporation of water [126, 127]. The second peak is related to the decomposition of the alginate macromolecular structure [126, 127]. Like sodium alginate, the DTGA curve of pea protein isolate has a degradation peak at 50 ^{o}C and a broad degradation peak at 296 ^{o}C (Fig. 3.6). The peak at 50 ^{o}C corresponds to the dehydration of water. The degradation peak at 296 ^{o}C is associated with the protein chain rupture and peptide bonds breakage [98]. Compared to sodium alginate, the degradation peak of pea protein isolate is very broad. It is because the chemical composition of sodium alginate was relatively pure, while pea protein isolate contain sugars, starch, and fat. The DTGA curve of the composite beads (Fig. 3.6) shows three degradation peaks at 45-55 ^{o}C, 245-255 ^{o}C, and 290-300 ^{o}C. The first peak corresponds to the evaporation of water, the second peak is due to the degradation of sodium alginate and pea protein isolate, and the third peak shows the degradation of the pea protein isolate. The DTGA curve of the beads (Fig. 3.6) shows that the height of the third degradation peak is higher than for the second degradation peak. It could be due to the fact that the mass ratio of pea protein isolate to sodium alginate is 4:1 in the formulation.

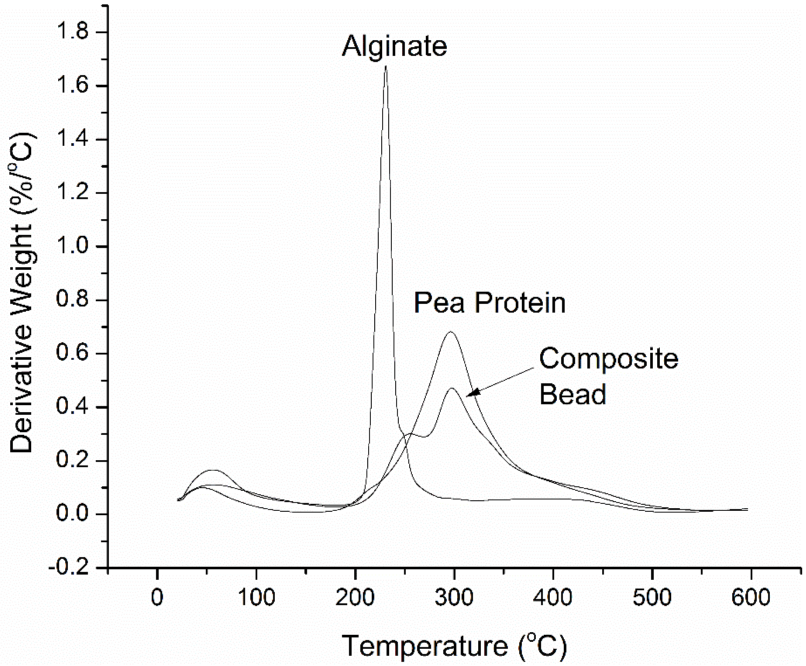


Fig. 3.6. DTGA curves of sodium alginate, pea protein, and composite beads

During the storage performed at 3 different temperatures, the DTGA curve patterns did not change (Fig. 3.7). It indicates that the thermal stability of the beads was not affected by the storage temperature.

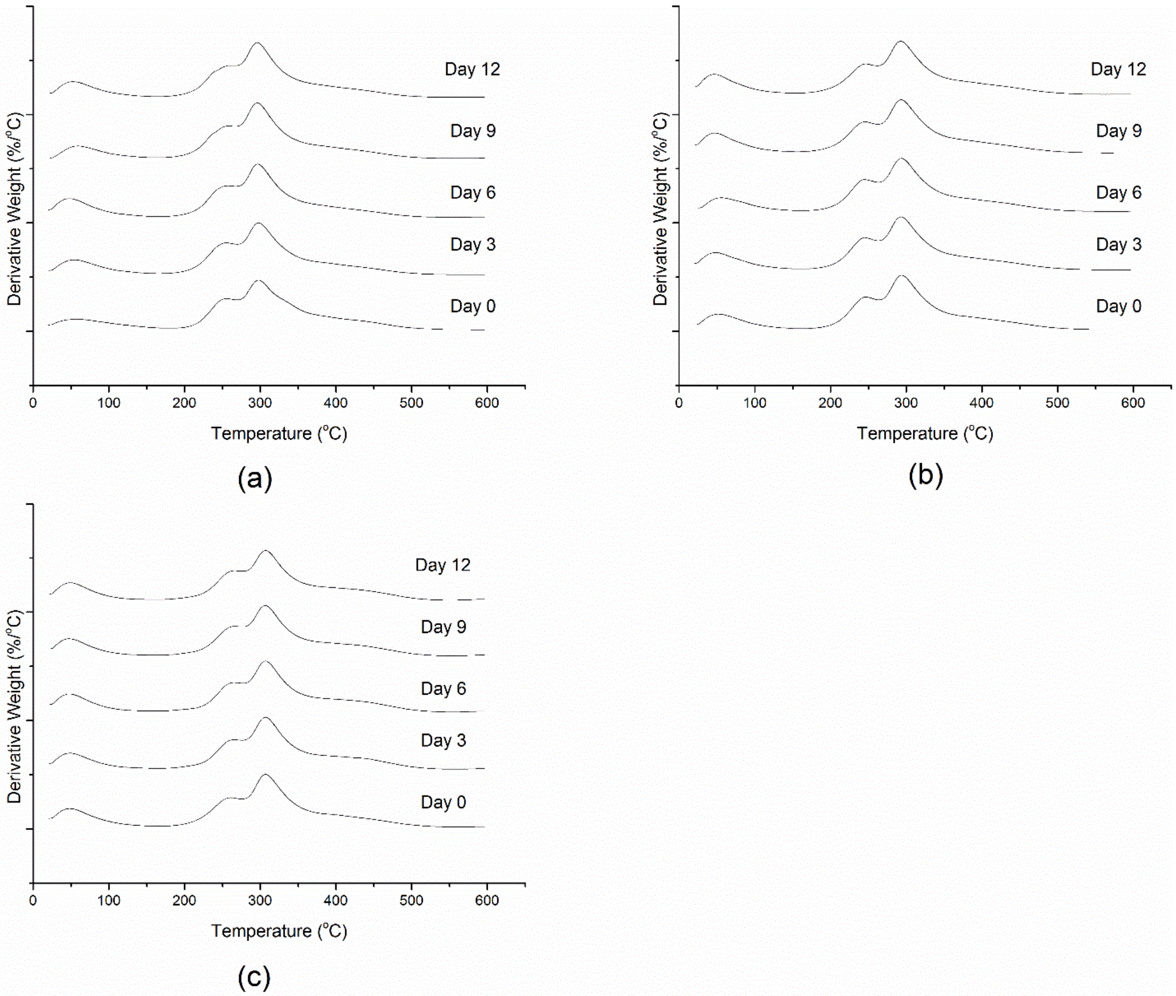


Fig. 3.7. DTGA curves of composite beads stored at (a) room temperature; (b) 50 °C; (c) 100 °C

3.4.3 Morphology

The SEM images of the composite beads are shown in Fig. 3.8. The shape of the freeze dried beads varied from being spherical (Fig. 3.8 a), having cavities (Fig. 3.8 b) and having an oval shape (Fig. 3.8 c). Before freeze drying, most beads had a spherical shape. The shape of some beads was not spherical because they were affected by vacuum filtration and sample handling. After freeze drying, different shapes of the beads were formed. The cavities (Fig. 3.8 b) and furrows (Fig. 3.8 d) at the surface of the beads are likely due to the combined effects of the expansion of ice crystals and the contraction of the matrix during the immersion in liquid nitrogen and the freeze drying step, which triggered the non-uniform volume change of the beads [12].

During the storage at toom temperature, 50 ^{o}C and 100 ^{o}C, the morphology of the beads remained the same (Fig. 3.9 - 3.11). It suggests that the morphology of the composite beads was quite stable during storage at different temperatures up to 100 ^{o}C.

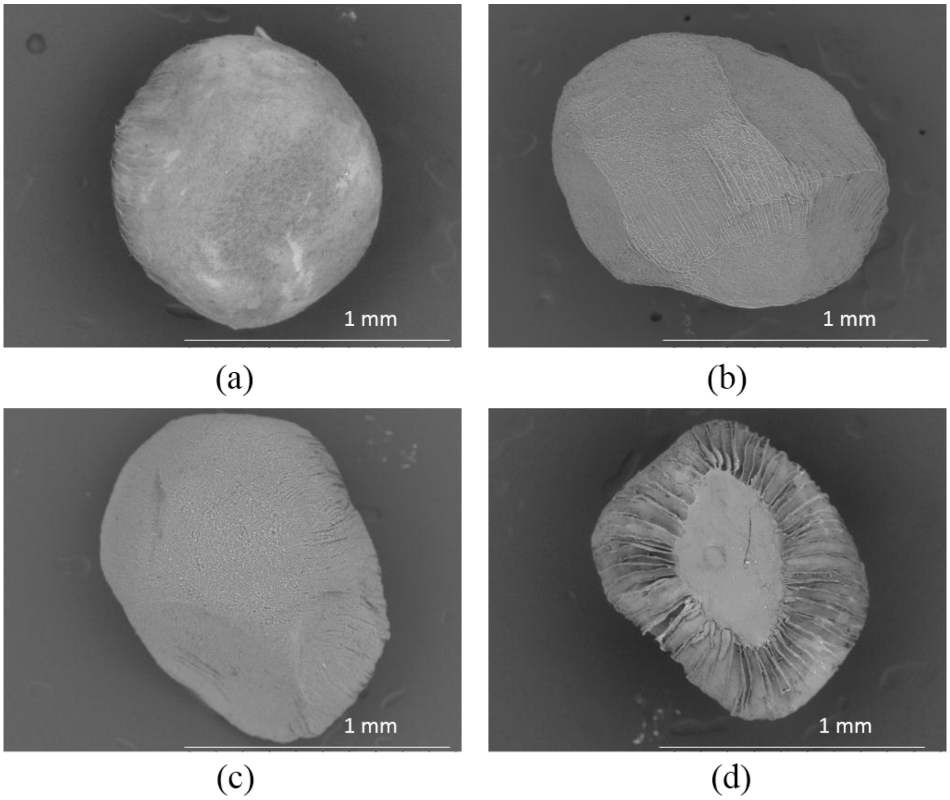
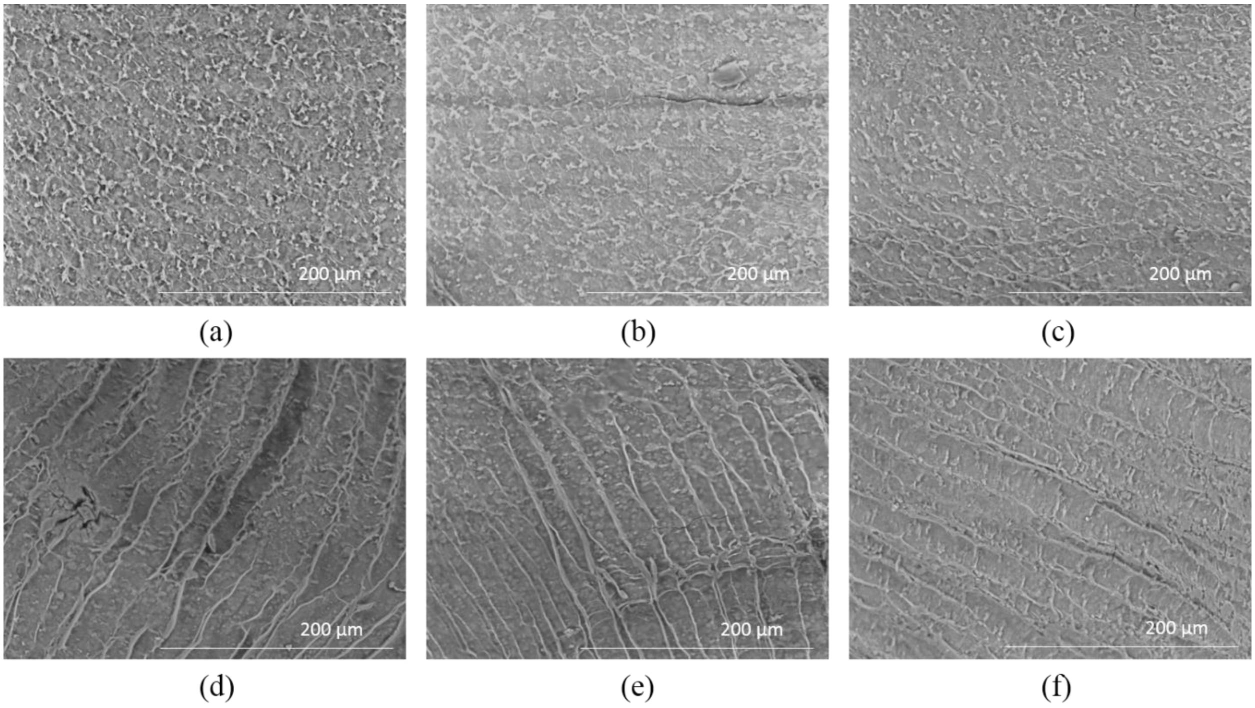


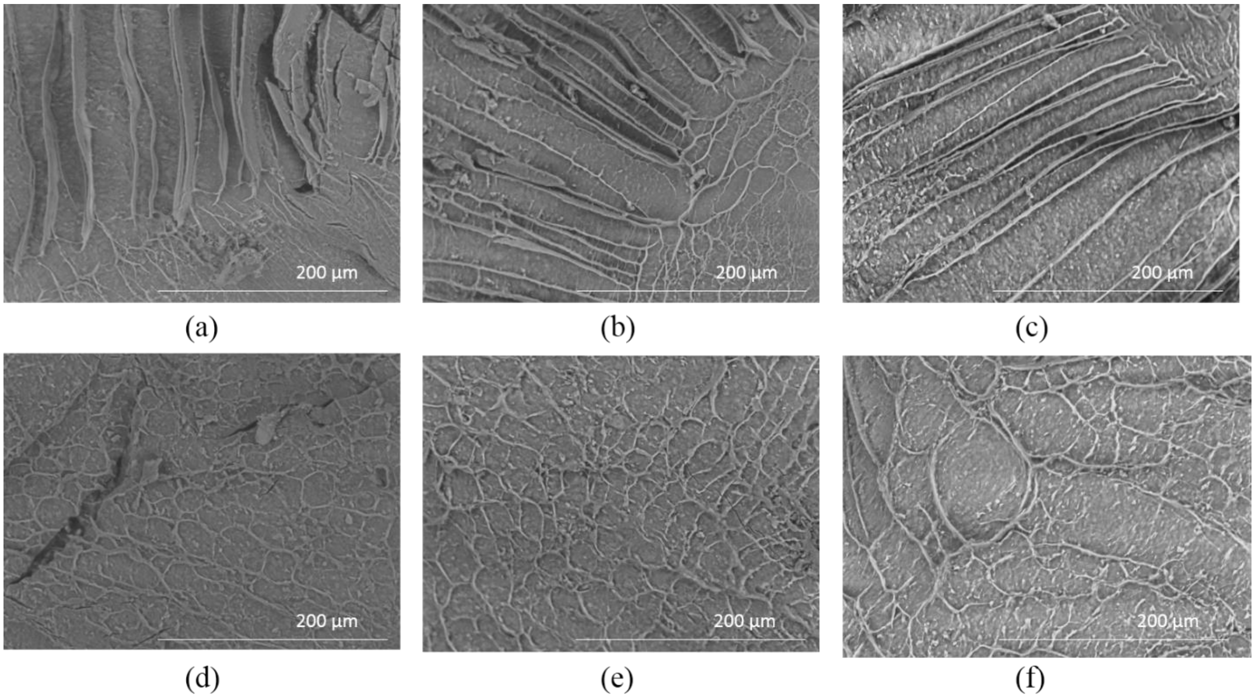
Fig. 3.8. SEM images of the whole composite beads of different shapes: a) spherical; c) oval; and different surface characteristics: beads with b) cavities; d) furrows at × 100



(a) (b) (c)

(d) (e) (f)

Fig.3.9. SEM images of different surface characteristics of the composite beads (× 500) during 12-day storage at room temperature: day 0 (a, d); day 6 (b, e); day 12 (c, f)



(a) (b) (c)

(d) (e) (f)

Fig.3.10. SEM images of different surface characteristics of the composite beads (× 500) during 12-day storage at 50 ^{o}C: day 0 (a, d); day 6 (b, e); day 12 (c, f)

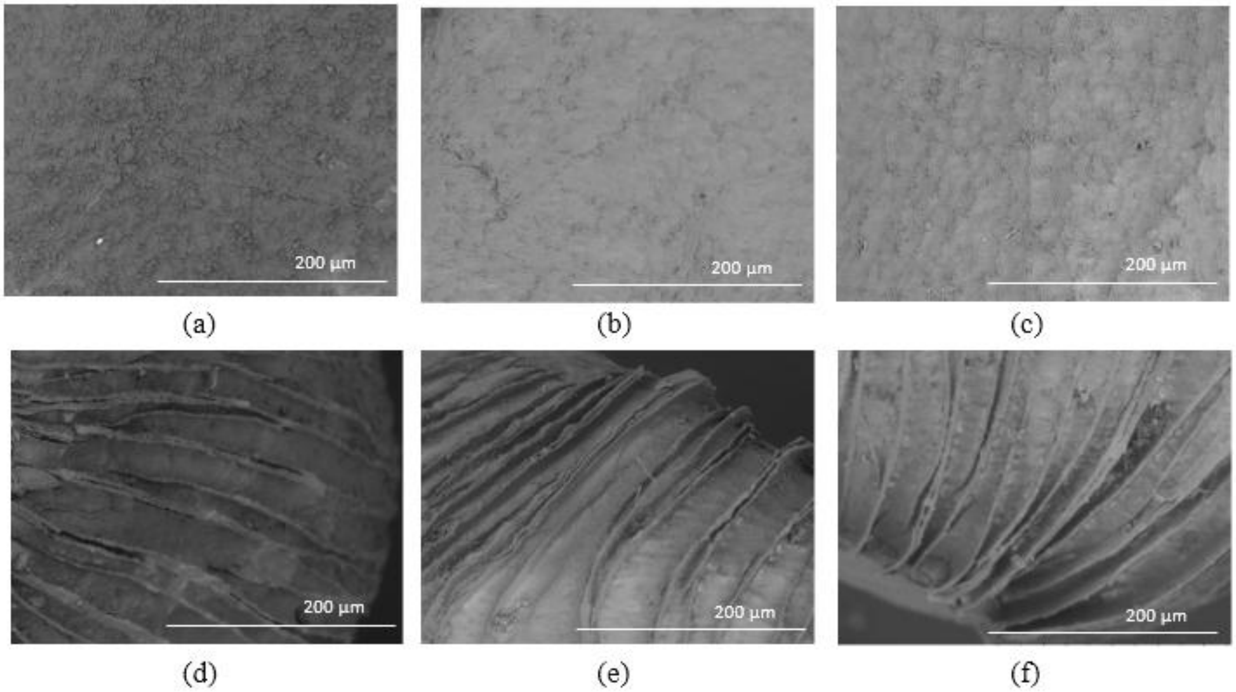


Fig.3.11. SEM images of different surface characteristics of the composite beads (× 500) during 12-day storage at 100 ^{o}C: day 0 (a, d); day 6 (b, e); day 12 (c, f)

3.4.4 Mechanical Properties

Compression testing is a commonly used method to investigate the mechanical properties of bio-based polymer materials, such as bio-based beads [128], wafers [129], pellets [130], scaffolds [131], etc. The hardness and resilience are two important parameters generated from the compression test. The hardness is an indication of the resistance of the matrix against deformation [132]. On the other hand, the resilience refers to the capacity of the matrix to recover to its original size and shape after a compression force is applied [132]. The resilience is also an indication of the viscoelasticity and porosity of the materials [128, 129]. Generally speaking, the lower value of resilience corresponds to lower viscoelasticity [130] and higher porosity of the matrix [129].

The effects of storage temperature and storage time on the hardness of the composite beads are shown in Fig. 3.12. The average hardness values of the beads stored at 100 ^{o}C showed a decreasing trend during storage. However, the average hardness values of the beads stored at room temperature and 50 °C did not show an obvious decreasing trend. The statistical results showed that the hardness of the beads stored at all temperatures, including 100 °C, was not significantly different ($P>0.05$) during storage period. For the resilience, the average values were relatively stable during storage for all temperatures tested (Fig. 3.13). The range of the average resilience values of the beads stored at each temperature during 12 days was mostly within 5%, with an exception that the average value of beads stored at room temperature at day 6 was unusually high. The statistical results also suggested that the resilience of the beads was relatively stable, since the resilience of the beads stored at all temperatures was not significantly different ($P>0.05$) during the storage period. In addition, both the results of the hardness and the resilience showed large standard deviation values. As described in section 3.4.3 Morphology, the beads showed different shapes and surface characteristics. The variation in the shape of the beads could account for the large standard deviation values displayed in Fid 3.12 and 3.13.

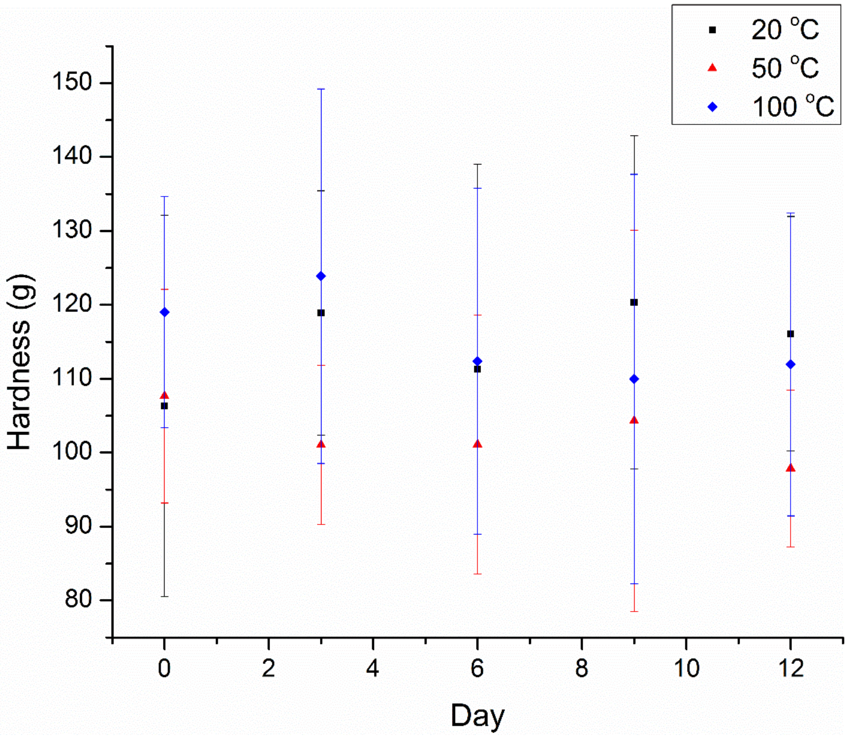


Fig. 3.12. Hardness changes of composite beads during a 12-day storage period at room temperature (square), 50 °C (triangle), and 100 °C (diamond)

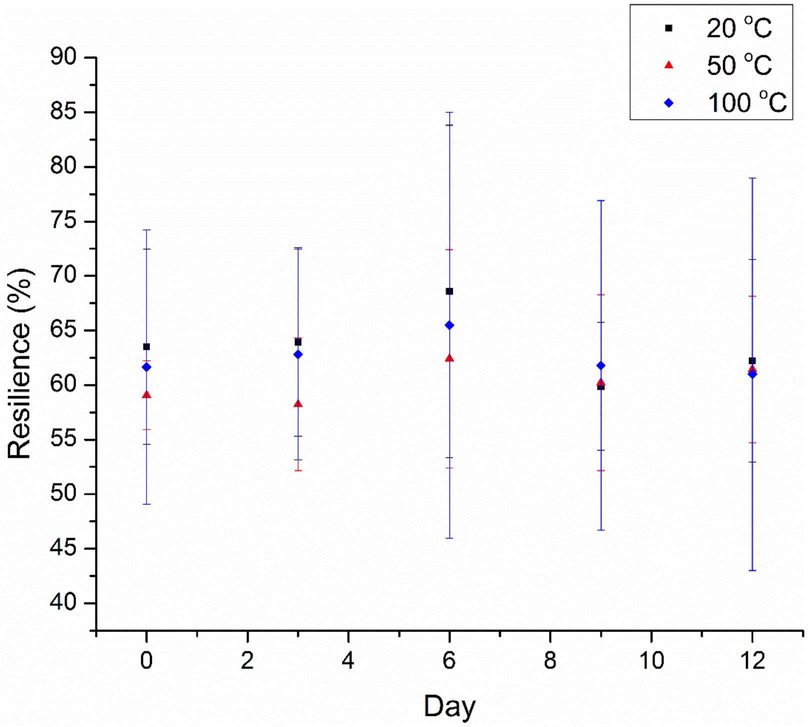


Fig. 3.13. Resilience changes of composite beads during a 12-day storage period at room temperature (square), 50 °C (triangle), and 100 °C (diamond)

3.5 Conclusion

Pea protein/alginate beads were successfully synthesized by extrusion and the effects of the temperature on the properties of the freeze dried beads during storage were investigated. The presence of both pea protein and alginate in the composite beads were shown in both DTGA curves and FTIR graphs. During storage, the thermal stability and the chemical composition of the composite beads did not change. The SEM images showed that the freeze dried beads have different shape and surface characteristics. These surface characteristics were retained during storage. The average hardness values of the beads stored at 100 °C showed a decreasing trend during storage, whereas the hardness of the beads stored at room temperature and 50 °C did not show an obvious decreasing trend. The average resilience values of the beads were relatively stable for all temperatures tested. Both the hardness and the resilience of the beads stored at all temperatures were not significantly different ($P>0.05$) during storage time. These results suggest that the pea protein/alginate beads were relatively stable during storage for all temperatures tested. Given that the properties of the pea protein/alginate beads were not greatly affected by the storage conditions, the beads could be suitable as encapsulating matrices.

Connecting Statement

The changes in the properties of pea protein/alginate beads during storage at different temperatures were examined in Chapter 3. It was found that the chemical composition and the thermal stability of the beads were stable during storage, which suggests that the beads have the potential to protect phenolic compounds from degradation at high temperature and during a storage period of up to 12 days. In Chapter 4, a phenolic compound, (+)-catechin hydrate, was encapsulated within the pea protein/alginate beads. The properties of these encapsulated catechin beads and their antioxidant activity during a 12-day storage period were investigated. The antioxidant activity of encapsulated catechin beads and pure catechin powder were compared.

CHAPTER 4

THE EFFECT OF STORAGE TEMPERATURE ON THE PROPERTIES OF ENCAPSULATED CATECHIN IN PEA PROTEIN/ALGINATE BEADS

4.1 Abstract

Catechins are chemically unstable and their stability is affected by different environmental conditions. The objectives of this work were to study the changes in their physical properties during storage at different temperatures, since heat treatment is required in many operations during food processing. This work reports the encapsulation of (+)-catechin hydrate within the pea protein/alginate beads, which were synthesized by extrusion. The freeze dried beads, referred to as the encapsulated catechin beads, were stored at three different temperatures (room temperature, 50 ^{o}C, 100 ^{o}C) for 12 days. The thermal stability and the chemical composition of the encapsulated catechin beads were investigated by a thermogravimetric analyzer (TGA) and a Fourier transform infrared spectrometer (FTIR), respectively. The results showed that these properties did not change during storage for all temperatures tested. The scanning electron microscope (SEM) images of the surface of the encapsulated catechin beads showed that some surface characteristics of the encapsulated catechin beads were retained during storage. The average hardness values of the beads stored at 100 ^{o}C showed an obvious decreasing trend during storage, while the average values of the beads stored at room temperature and 50 ^{o}C did not show an obvious decreasing or increasing trend. However, no significant difference ($P>0.05$) was observed in the hardness of the beads during storage for all temperatures tested. For the resilience, no significant difference ($P>0.05$) was observed for the beads during storage at room temperature and 100 ^{o}C. However, there is a significant difference ($P<0.05$) for the beads during storage at 50 ^{o}C. The antioxidant activity values of the encapsulated catechin beads showed a decreasing trend and the values after storage at room temperature, 50 ^{o}C, and 100 ^{o}C were 87.9 ± 4.6%, 83.4 ± 1.7%, and 65.2 ± 2.2% of the original antioxidant activity, respectively. However, as comparison, the antioxidant activity of (+)-catechin hydrate powder showed a lower decrease in the percentage of antioxidant activity than encapsulated catechin beads. The antioxidant activity values of (+)-catechin hydrate powder after storage at room temperature, 50 ^{o}C, and 100 ^{o}C were 88.9 ± 1.5%, 90.3 ± 0.9%, and 87.6 ± 1.7%, respectively. The antioxidant activity of the

encapsulated catechin beads stored at room temperature was not significantly different ($P>0.05$) during storage time, while it was significantly different ($P<0.05$) for those stored at 50 ^{o}C and 100 ^{o}C. In summary, the physical properties of beads were relatively stable for all temperatures tested. However, the beads were not effective in maintaining the antioxidant activity of (+)-catechin hydrate.

4.2 Introduction

Catechins, also known as flavan-3-ols, are phenolic compounds containing two aromatic rings and several hydroxyl groups [1]. Catechins have gained much interest in scientific research, because they hold multiple health-promoting properties, such as antioxidant, antimutagenic, anti-obesity, anti-inflammatory, and antiviral properties among others [1, 133]. Unfortunately, they are unstable. In solution, catechins undergo chemical transformations through epimerization [1] and autoxidation [3]. Particularly, the epi-structure of catechin is easily transformed into the non epi-structure at high pH and under heat treatment [1].

Therefore, strategies to enhance the bioavailability of catechins must be developed. The purpose of encapsulation is to protect bioactive compounds from deleterious environmental conditions [1, 134]. Several encapsulation techniques have been applied to encapsulate catechins. Some of the commonly used techniques include spray drying, fluidized bed coating, spray-chilling/cooling, freeze-drying, etc. [1]. Among the coating materials, starch derivatives (e.g. dextrins, maltodextrins, etc.) and plant exudates (e.g. pectins, gum arabic, etc.) are commonly used [1]. Some research have been performed to encapsulate catechins using food-grade materials, such as calcium pectinate beads [5], chitosan–tripolyphosphate nanoparticles [135], bovine serum albumin nanoparticles [136], calcium alginate microparticles [137], etc.

The incorporation of catechins in food such as bakery products [138], cheese [139], muffins [140], apple products [141], bread [142], and wheat biscuits [143] is common. However, the stability of catechin in food systems depends on many factors, such as the pH, the temperature, the presence of oxygen, metal ions, and other active ingredients (e.g. sucrose, citric acid and ascorbic acid) [138]. Therefore, in practice, it is important to understand the stability of catechins in foods during processing and storage in order to maintain their activities [138]. Among the factors that influence the stability of catechins, the temperature is an important factor to be considered, since heat treatment is required in many operations during food process. Some examples of these operations include mild heating (38 °C) in cheese making [139], proofing (40 °C), baking (185 °C), and steaming in bread processing [142]. In this study, (+)-catechin hydrate was encapsulated within pea protein/alginate hydrogel beads, referred to as encapsulated catechin beads. The chemical structure of (+)-catechin is shown in Fig 4.1. The objectives of this work were to study the changes in the properties of encapsulated catechin beads during a 12-day storage period at different temperatures (room temperature, 50 °C, and 100 °C). The antioxidant activity of the encapsulated catechin beads during storage was investigated. The chemical composition, the thermal stability, the morphology, and the mechanical properties of the encapsulated catechin beads during storage were also studied.

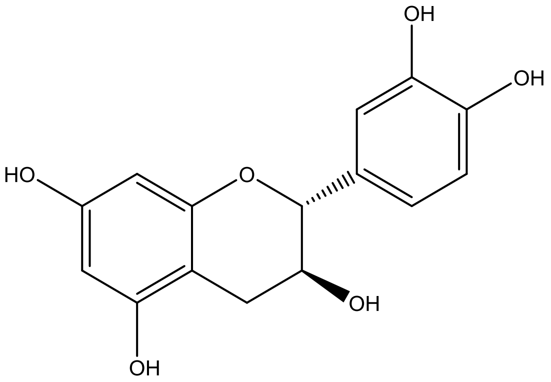


Fig. 4.1. Chemical structure of (+)-catechin [144]

4.3 Materials and Methods

4.3.1 Materials

Pea protein isolate (Propulse N™, 81.73% protein, <10.3% sugars, <0.7% starch, 3.40% moisture, <0.5% fat and <4.0% ash) was obtained from Nutri-Pea Ltd. (Portage la Prairie, MB, Canada). Alginic acid sodium salt from brown algae (medium viscosity, Lot# SLBM3663V), calcium chloride dihydrate, (+)-catechin hydrate powder (≥98%, HPLC grade), sodium citrate tribasic dihydrate (≥99%), trichloroacetic acid (≥99.0%), and TPTZ (2,4,6-tris(2-pyridyl)-s-trizaine, ≥98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Concentrated hydrochloric acid (37%) and ascorbic acid were provided from Fisher Scientific (Fair Lawn, NJ, USA). Sodium hydroxide was obtained from EMD (Darmstadt, Germany). Anhydrous ethanol was provided from Greenfield Specialty Alcohols Inc. (Brampton, ON, Canada). Acetic acid (glacial) was obtained from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Anhydrous sodium acetate was purchased from MP Biomedicals, LLC. (Solon, OH, USA). Iron (III) chloride hexahydrate were provided from BOH Inc. (Toronto, ON, Canada).

4.3.2 Preparation of Encapsulated Catechin Beads

A pea protein/alginate solution was prepared as described in section 3.3.2 Preparation of Pea Protein/Alginate Beads. Once the solution cooled down to room temperature, 0.15 g of (+)-catechin hydrate powder was dissolved in 5 mL anhydrous ethanol. The alcoholic solution was added to the pea protein/alginate solution, and then an ethanol layer formed at the top of the solution. The solution was stirred to solubilize the ethanol layer. The solution was sonicated in a water bath for 5 min. Then, 10 mL of distilled water was added to the solution and stirred. The beads were prepared by extrusion and freeze dried according to the method described in section 3.3.2 Preparation of Pea Protein/Alginate Beads. The formed beads are referred to as the encapsulated catechin beads.

4.3.3 Storage Conditions of Encapsulated Catechin Beads

The freeze dried encapsulated catechin beads were stored in sealed glass bottles at different temperatures (room temperature, 50 ^{o}C, 100 ^{o}C) for 12 days. Every 3 days, starting from day 0, bead samples were taken out to monitor their chemical composition, thermal properties, morphology, mechanical properties, and antioxidant activity.

4.3.4 Chemical Composition

The FTIR analyses of (+)-catechin hydrate and encapsulated catechin beads were conducted using a Nicolet iS5 FTIR spectrometer (Thermo Fisher Scientific, Madison, WI, USA). The beads were grinded before analysis. Each sample was analyzed in duplicate. For each spectrum, a total of 32 scans were performed at 4 cm^{-1} resolution. The measurements were recorded between 500-4000 cm^{-1}. The spectra were analyzed using the OMNIC software (version 8.2, Thermo Fisher Scientific, Madison, WI, USA).

4.3.5 Thermal Stability

The thermal stability of the freeze dried encapsulated catechin beads and (+)-catechin hydrate was determined by TGA (Q50, TA Instrument, Inc., New Castle, DE, USA). Around 10 mg of bead sample from each storage temperature and storage day was grinded and loaded into a platinum pan. The samples were heated from room temperature to 600 °C at a constant rate of 10 °C/min. The analysis was performed under a nitrogen atmosphere (constant flow rate of 60 mL/min). Each sample was tested in duplicate.

4.3.6 Morphology

The morphology of the freeze dried beads was examined with a Hitachi Tabletop SEM Microscope (TM3000, Hitachi High-Technologies Co., Tokyo, Japan). At least three beads were placed on a carbon conductive double-sided tape on the specimen stub. The samples were examined at accelerated voltages of 5 kV under vacuum.

4.3.7 Mechanical Properties

The hardness and the resilience of the encapsulated catechin beads were evaluated with a texture analyzer (TA.HDPlus, Stable Microsystems, Surrey, UK). Each individual bead having a diameter ranging from 0.9 to 1.2 mm was compressed to 30% strain at a speed of 2 mm/s. Fifteen (N=15) measurements were performed. The compression measurements were performed in the “Compression Return to Start” mode. The pre-test, test, and post-test speeds were all set at 2 mm/s, with a trigger force of 0.5 g. The data were captured by Texture Exponent Software (Version 6,1,9,0). The hardness (g) and the resilience (%) were measured and calculated as described in section 3.3.7 Mechanical Properties.

4.3.8 Antioxidant Activity of Encapsulated Catechin Beads

50 mg of encapsulated catechin beads was degraded in 4 mL of 2% (w/v) sodium citrate tribasic solution under vortex, according to the methodology described by Belscak-Cvitanovic et al. [118]. All the bead samples were prepared in triplicates. The blank beads followed the same treatment. Sodium citrate can destabilize the calcium alginate gel structure by exchanging the Ca^{2+} of the beads with the Na^{+} of the citrate [145]. After the beads were degraded completely, 1 mL of anhydrous ethanol was added and mixed well. Given that the solubility of (+)-catechin in water is very low, the addition of ethanol to the solution helped to increase the solubility of (+)-catechin [146]. Then, 1 mL of 20% (w/v) trichloroacetic acid (TCA) was added to 1 mL of the solution. The mixture was kept in an ice bath for 15 min. Then, the solution was centrifuged at 4 °C and 10 000 rpm for 15 min, according to the methodology described by Dehkharghanian et al. [96]. The ferric reducing ability of plasma (FRAP) assay test was performed to measure the antioxidant activity of the supernatant [147]. 0.4 mL of the supernatant from the encapsulated catechin beads or the blank bead samples was mixed with 2.4 mL FRAP reagent. The solutions were kept in the dark for 30 min. The absorbance of the solutions from the encapsulated catechin beads was measured. The solution from the blank beads was used as the reference cell. The measurements were performed using a spectrophotometer (Ultrospec 2100 *pro*, Biochrom Ltd, Cambridge, UK) in a wavescan mode in a range between 590 and 635 nm. The absorption peak was located at 622 nm. The FRAP reagent consists of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ prepared in 40 mM HCl, and 20 mM ferric chloride in a volume proportion of 10:1:1. Fresh FRAP reagent was prepared every day. A standard curve was generated using different concentrations of ascorbic acid standard at 50, 100, 200, 300, 400 and 500 µM, prepared in a solution of 2% sodium citrate (w/v) solution, anhydrous ethanol, and 20% (w/v) trichloroacetic acid (4:1:5 v/v/v). 0.4 mL of the ascorbic acid solution was reacted with 2.4 mL FRAP reagent in the dark for 30 min. The standard curve had a slope of 0.0041 and an intercept at 0.1073, with a R^2 of 0.9932. The result of the antioxidant activity of catechin-encapsulated beads was calculated as µmol ascorbic acid equivalent (AAE)/g of beads.

For comparison, (+)-catechin hydrate powder was stored in sealed glass bottles at different temperatures (room temperature, 50 ºC, 100 ºC) for 12 days. Every 3 days, starting from day 0, the powder was taken out to measure its antioxidant activity. 50 mg of the powder was dissolved in 10 mL of anhydrous ethanol, followed by addition of 40 mL of 2% sodium citrate (w/v) solution in a 50 mL volumetric flask. All the samples were prepared in triplicates. The 50 mL of catechin solution was referred to as the bulk solution. 1 mL of the bulk solution was diluted to 5 mL. 1 mL of the blank solution, which constituted of 2% sodium citrate (w/v) solution, anhydrous ethanol, and 20% (w/v) trichloroacetic acid (4:1:5 v/v/v), was also prepared. 1 mL of the diluted solution or blank solution was mixed with 1 mL of 20% (w/v) TCA solution. 0.4 mL of the sample solution or blank solution was reacted with 2.4 mL FRAP reagent and kept in the dark for 30 min. The absorbance of the sample solution was measured using a spectrophotometer with the absorption peak at 622 nm. The solution from the blank was used as the reference cell. The result of the antioxidant activity of (+)-catechin hydrate powder was calculated as mmol AAE/g of beads.

4.3.9 Statistical Analysis

Analysis of variance (ANOVA) was used to test if the hardness and the resilience of the beads were affected by storage time. Shapiro-Wilk test and Levene's test were performed to test for the normality and homogeneity of variances of the data, respectively. In the assumption that the normality or homogeneity of variances was violated, non-parametric Kruskal-Wallis one-way analysis of variance was used instead of ANOVA. To determine if the antioxidant activity of the encapsulated catechin beads and (+)-catechin hydrate powder were affected by the storage time, non-parametric Kruskal-Wallis one-way analysis of variance was performed. Statistical analyses were performed by SPSS® (Version 23, IBM®, Armonk, NY, USA). All the comparisons were performed at a significant level of 5%. The error bars in the plot represent the standard deviation of the data.

4.4 Results and Discussion

4.4.1 Appearance of the Freeze-dried Blank Beads and Encapsulated Catechin Beads

In appearance, the color of blank beads was white, while the encapsulated catechin beads was reddish-brown in color (Fig. 4.2). This color is due to the presence of catechin which showed a reddish-brown color in solution.

Fig. 4.2. Freeze dried encapsulated catechin beads (left) and blank beads (right)

4.4.2 Chemical Composition

The FTIR spectrum of (+)-catechin hydrate is presented in Fig. 4.3. The broad peak at 3323 cm^{-1} corresponds to the OH group stretch. The peaks at 1607 cm^{-1}, 1455 cm^{-1}, and 1282 cm^{-1} correspond to C=O, C=C, and C-O groups, respectively. The results are consistent with a previous study [148]. As Fig. 4.3 shows, the FTIR spectrum of the encapsulated catechin beads have absorbance peaks at 3293, 1634, 1538, and 1417 cm^{-1}, which are also present in the blank bead's spectrum. It indicates that no unique absorbance peak of (+)-catechin hydrate are shown in the FTIR spectrum of the encapsulated catechin beads. This is due because the absorbance peaks of (+)-catechin hydrate overlap with the bead.

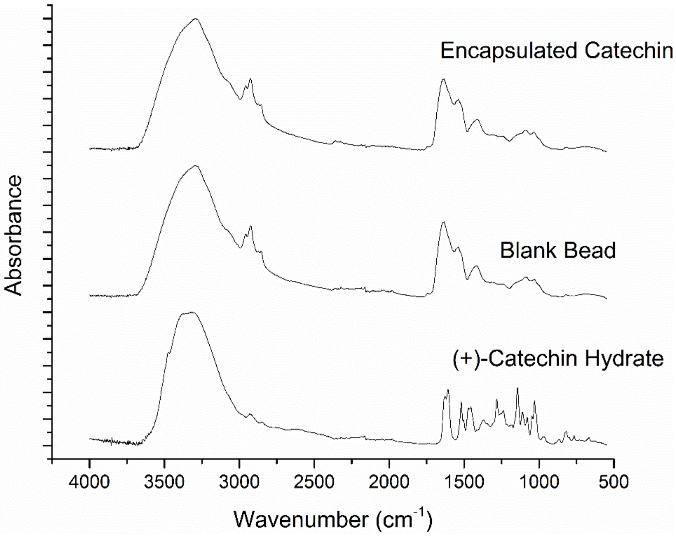


Fig. 4.3. FTIR spectra of (+)-catechin hydrate, blank beads, and encapsulated catechin beads

During storage for all temperatures, the FTIR spectra of the encapsulated catechin beads did not change (Fig. 4.4), which indicates that the chemical composition of the beads was stable at temperatures up to 100 ^{o}C. The results are consistent with the FTIR results of the blank beads shown in section 3.4.1 Chemical Composition.

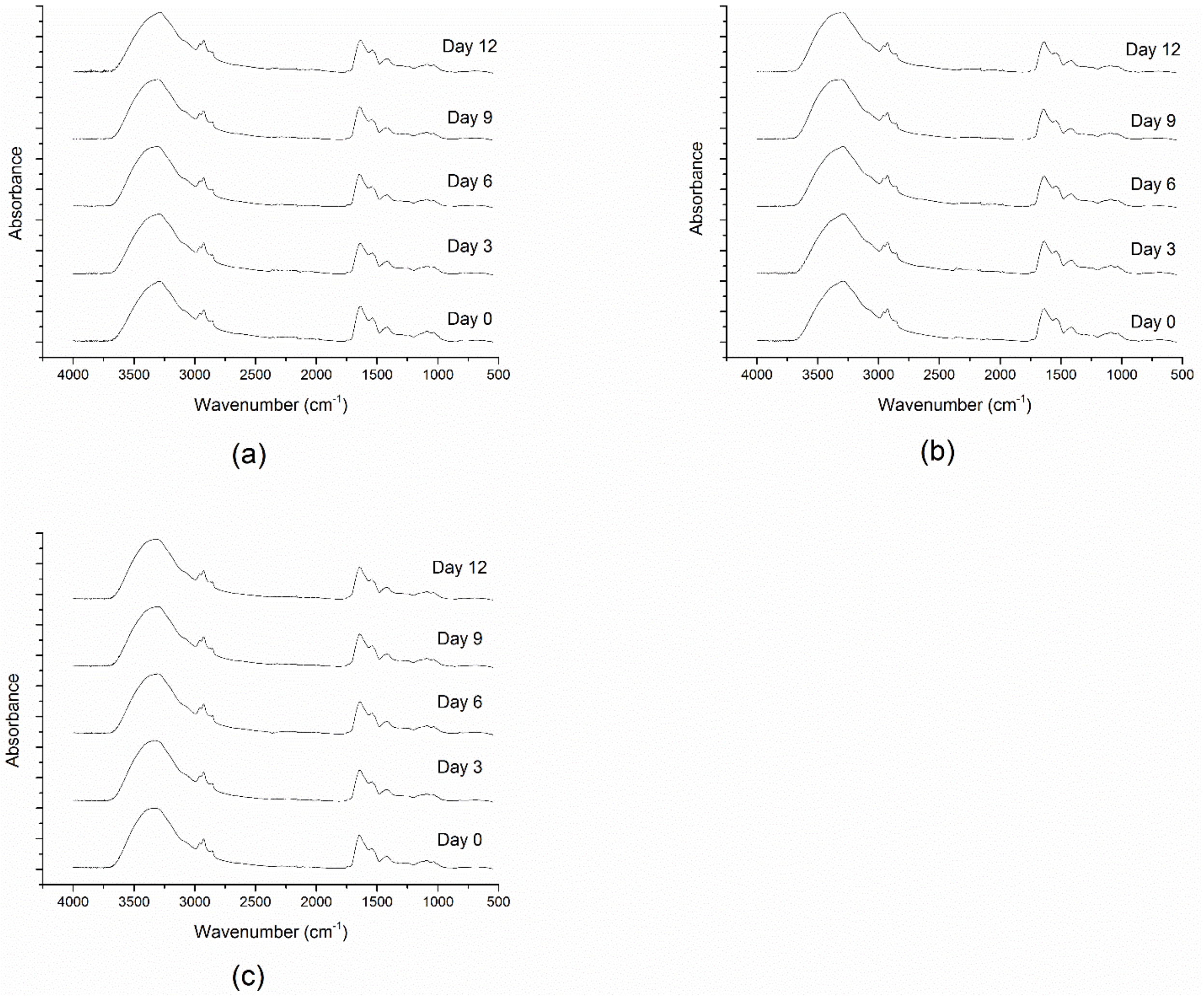


Fig. 4.4. FTIR spectra of the encapsulated catechin beads stored at (a) room temperature; (b) 50 °C; (c) 100 °C

4.4.3 Thermal Stability

The TGA curves of (+)-catechin hydrate and the encapsulated catechin beads are presented in Fig. 4.5. The Td 5% and Td 10% values of the encapsulated catechin beads were 69 °C and 135 °C. The TGA curve of the encapsulated catechin beads is consistent with the TGA curve of the blank beads as shown in section 3.4.2 Thermal Stability, which suggests that there is no difference in their thermal stability. A previous study, where ascorbic acid was encapsulated in gelatin/alginate beads, showed that the TGA curves of both blank beads and encapsulated beads had a similar decomposition profile [149]. The pattern of the weight loss of (+)-catechin hydrate is different from when encapsulated. The Td 5% and Td 10% values of (+)-catechin hydrate were 198 °C and 244 °C, respectively. The remaining weight of the encapsulated catechin beads after thermal degradation was 30%. However, nearly 50% of the weight remained in (+)-catechin hydrate after degradation at 600 °C. It suggests that the remained (+)-catechin hydrate could be degraded at higher temperatures. A previous study shows that (+)-catechin would further decompose at 835 °C [150]. The TGA curve of (+)-catechin hydrate is consistent with the pattern of the curves in previous studies [151, 152].

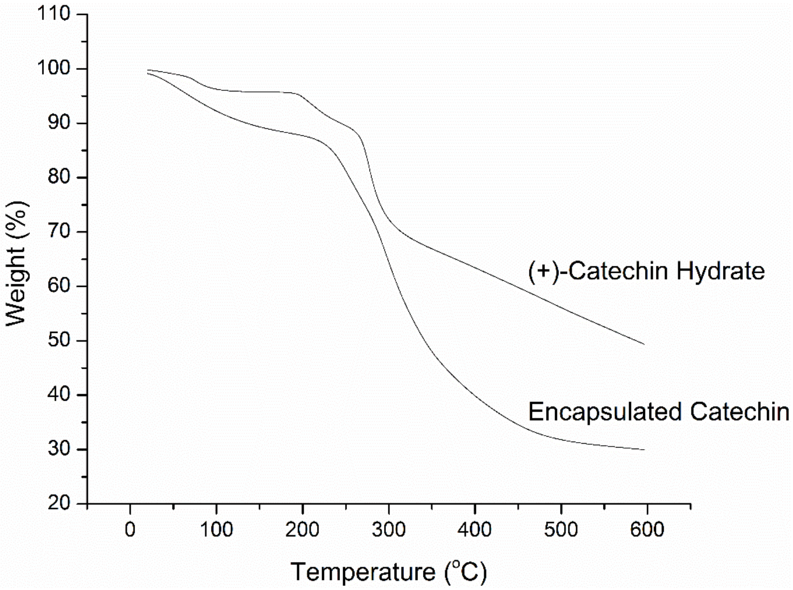


Fig. 4.5. TGA curves of (+)-catechin hydrate and encapsulated catechin beads

The DTGA curves of (+)-catechin hydrate and the encapsulated catechin beads are presented in Fig. 4.6. Unlike sodium alginate and pea protein isolate, the DTGA curve of (+)-catechin hydrate presents 3 decomposition peaks (Fig. 4.4). The first peak corresponds to the loss of water. A small peak at 209 °C and a large peak at 277 °C suggest that (+)-catechin hydrate underwent two thermal degradation stages. The DTGA curve of (+)-catechin hydrate is consistent with a previous study [151]. The DTGA curve of the encapsulated catechin beads shows three peaks at 45-55 °C, 245-255 °C, and 290-300 °C, which are similar to the weight loss pattern of the blank beads (Fig. 3.5). No peak attributed to the degradation of (+)-catechin hydrate is present in the DTGA curve of the encapsulated catechin beads (Fig. 4.6). Previous studies where (-)-epigallocatechin gallate (EGCG) was encapsulated in gelatin submicroparticles also showed that the degradation peak of the EGCG was not detected in the DTGA curve of the encapsulated submicroparticles [98]. A possible explanation could be that the degradation peak of (+)-catechin hydrate in the DTGA curve overlapped with the pea protein isolate.

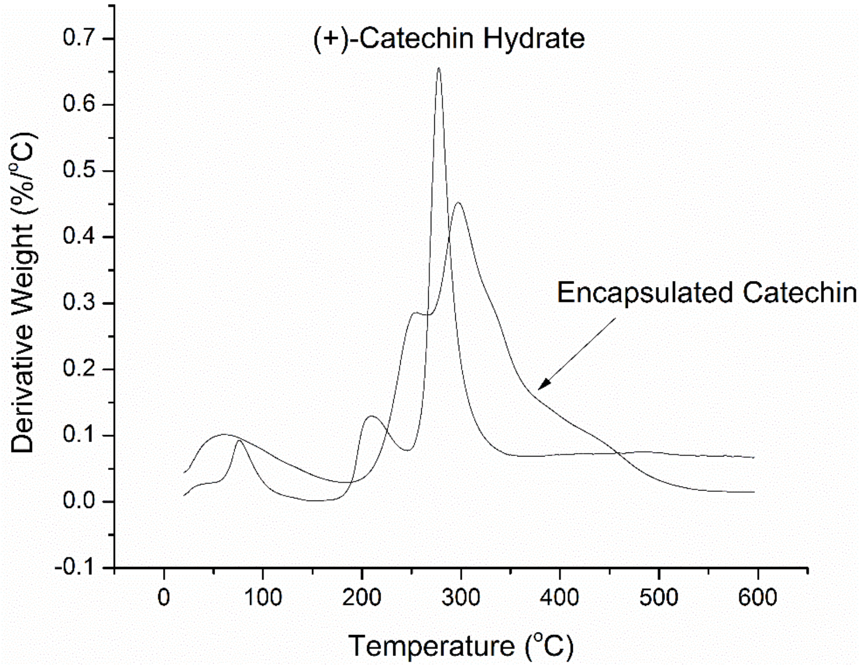


Fig. 4.6. DTGA curves of (+)-catechin hydrate and encapsulated catechin beads

During storage at different temperature conditions, the DTGA curve patterns of the encapsulated catechin beads did not change. It suggests that the thermal stability of the encapsulated catechin beads was not affected by the storage conditions. The results are consistent with the blank beads as described in section 3.4.2 Thermal Stability. The results are also consistent with a previous study where betalain extracts were encapsulated with albumin/alginate. After 25 days of storage at 25 °C and 50 °C, the TGA profiles of the beads after storage were similar to the beads before storage [153].

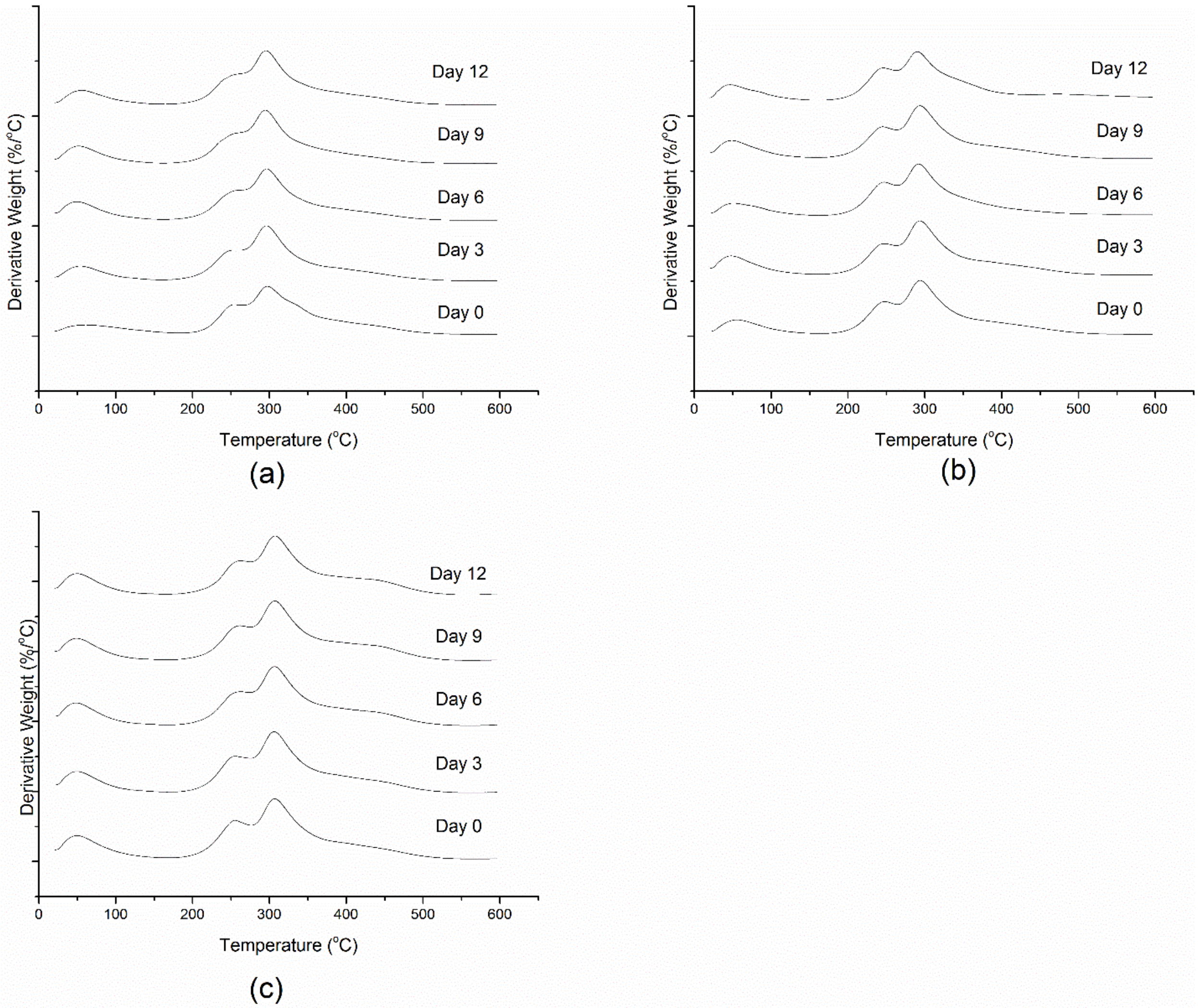


Fig. 4.7. DTGA curves of the encapsulated catechin beads stored at (a) room temperature; (b) 50 °C; (c) 100 °C

4.4.4 Morphology

The SEM images of the encapsulated catechin beads are shown in Fig. 4.8. Like the beads shown in section 3.4.3 Morphology, the shape of the freeze dried encapsulated catechin beads shows various patterns as some beads are spherical (Fig. 4.8 a), some have an irregular shape (Fig. 4.8 b), and an oval shape (Fig. 4.8 c). As mentioned in section 3.4.3 Morphology, the non-uniformity of the encapsulation matrices is due to the combined effects of the expansion of ice crystals and the contraction of the matrix during the immersion in liquid nitrogen and the freeze drying step. During the 12-day storage period for all temperatures, different surface characteristics of the beads were retained (Fig. 4.9 – 4.11). It suggests that the morphology of the beads was relatively stable.

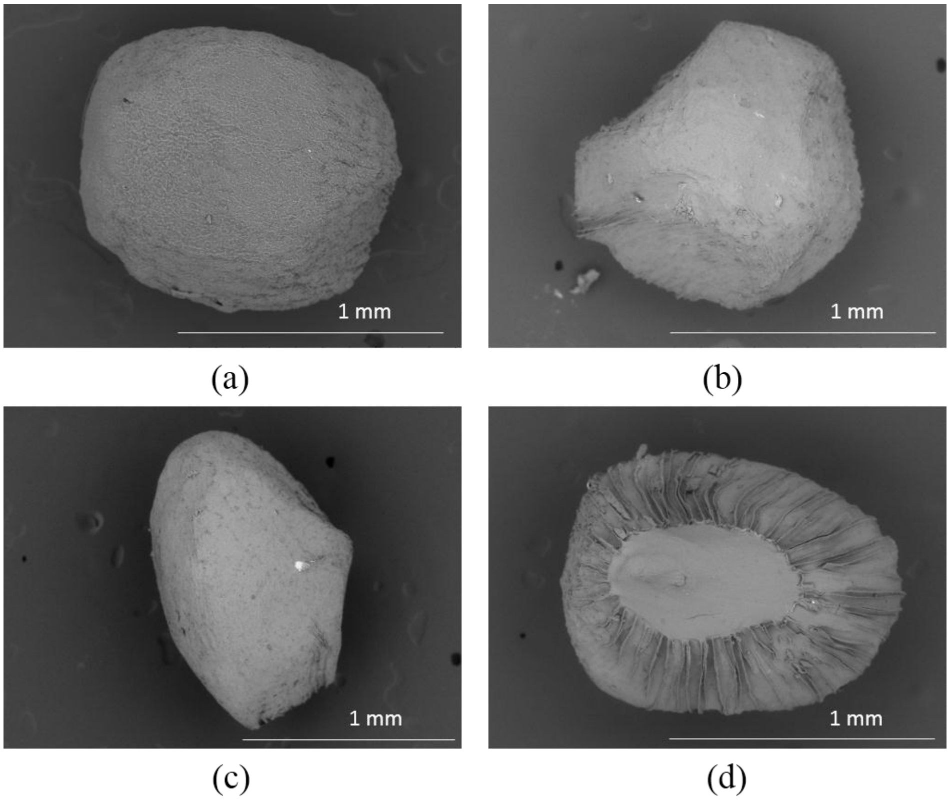


Fig. 4.8. SEM images of the whole encapsulated catechin beads: (a), (b), (d) at × 100; and (c) at × 80

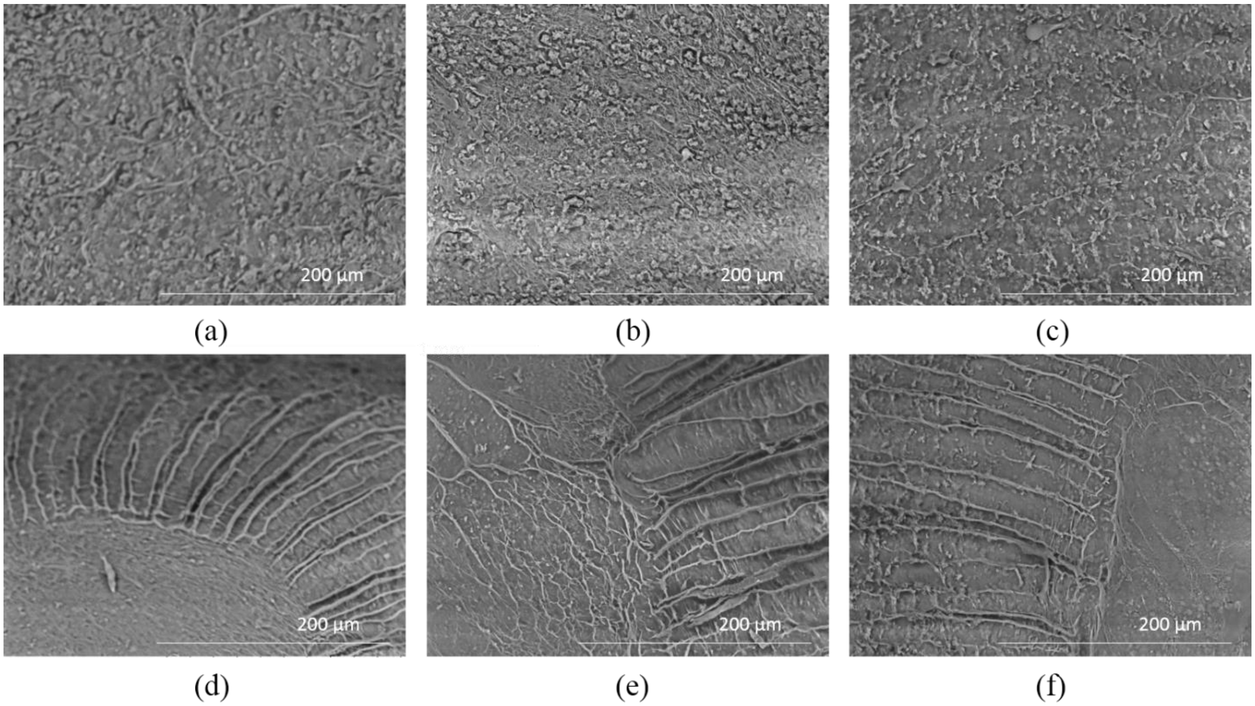


Fig. 4.9. SEM images of different surface characteristics of the encapsulated catechin beads (× 500) during 12-day storage at room temperature: day 0 (a, d); day 6 (b); day 9 (e); day 12 (c, f)

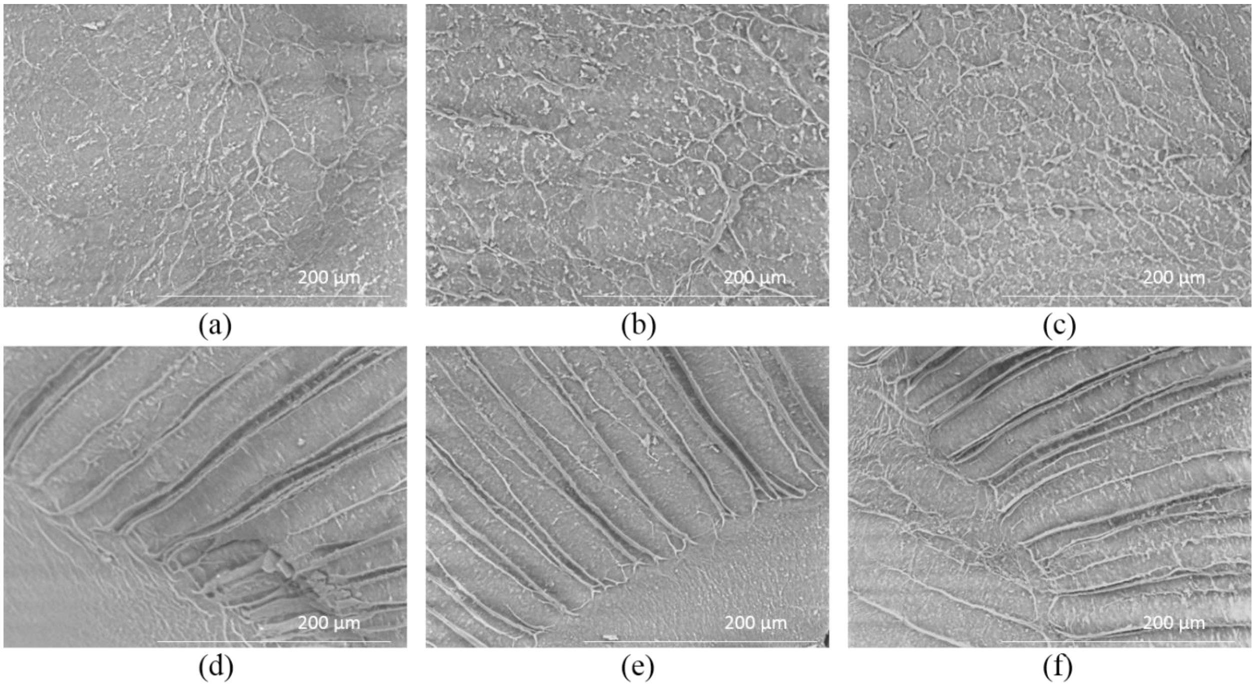


Fig. 4.10. SEM images of different surface characteristics of the encapsulated catechin beads (× 500) during 12-day storage at 50 ºC: day 0 (a, d); day 3 (e); day 6 (b); day 12 (c, f)

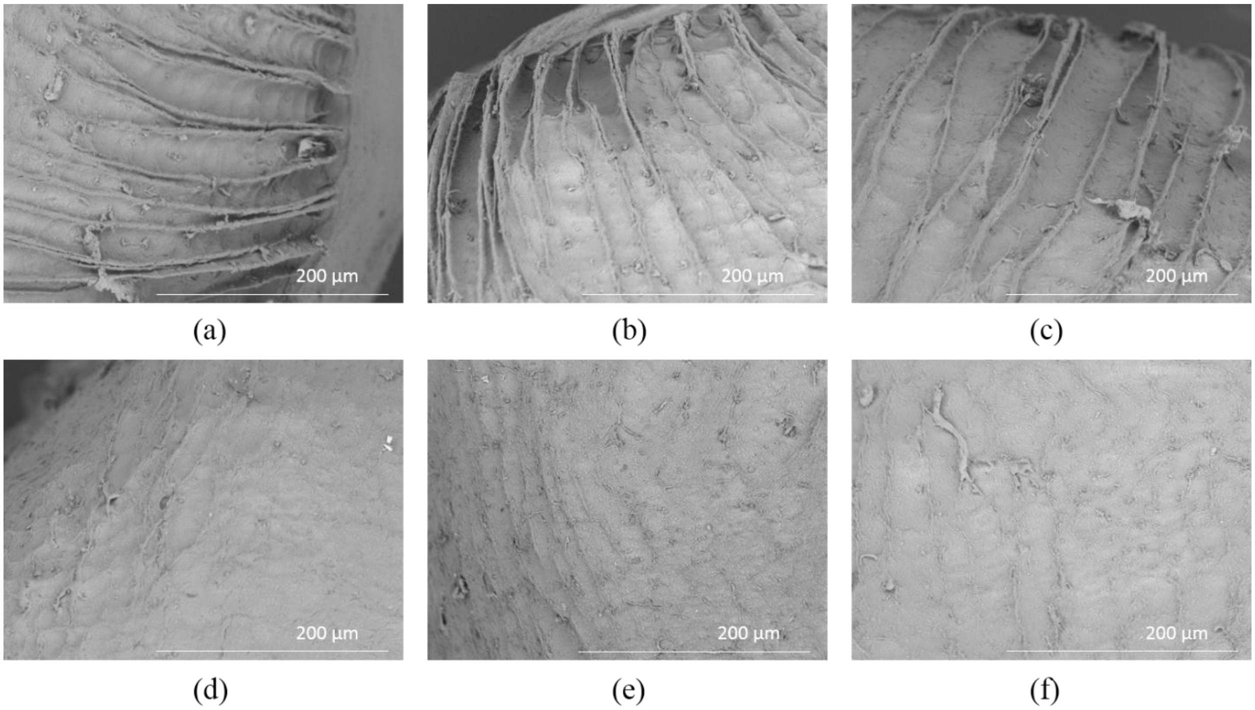


Fig. 4.11. SEM images of different surface characteristics of the encapsulated catechin beads (× 500) during 12-day storage at 100 ^{o}C: day 0 (a); day 3 (d); day 6 (b); day 9 (e); day 12 (c, f)

4.4.5 Mechanical Properties

The effects of storage temperature and storage time on the hardness and the resilience of the encapsulated catechin beads are shown in Fig. 4.12 and Fig. 4.13, respectively. The average hardness values of the beads stored at 100 ^{o}C demonstrated an obvious decreasing trend during storage. However, the average hardness values of the beads stored at room temperature and 50 ^{o}C did not show an obvious decreasing or increasing trend. The statistical results showed that the hardness of the beads stored at all temperatures was not significantly different ($P>0.05$) during storage. The average resilience values of the beads were relatively stable during storage for all temperatures tested, with their range for the beads stored at each temperature during 12 days was mostly within 7% (Fig. 4.13). However, the average values of the beads stored at 100 ^{o}C at day 6 and day 12 were beyond that range. The reason was that there were few extremely high individual values that increased the average values. The resilience of the beads stored at room temperature and 100 ^{o}C was not significantly different ($P>0.05$) during storage time. However, the resilience of the beads stored at 50 ^{o}C was significantly different ($P<0.05$) during storage.

This is probably due because the resilience of the beads stored at 50 °C at day 6 was relatively high and the standard deviation of the resilience of the beads stored at 50 °C for the storage period was relatively small, which accounted for the significant difference. The standard deviation values for both the hardness and the resilience were large. The variation in the shape of the beads and the non-uniform volume of the beads due to the effect of freeze drying could contribute to the large standard deviation values and the extremely high or low individual values. The results of the hardness and the resilience of the encapsulated catechin beads are almost consistent with the blank beads as described in section 3.4.4 Mechanical Properties.

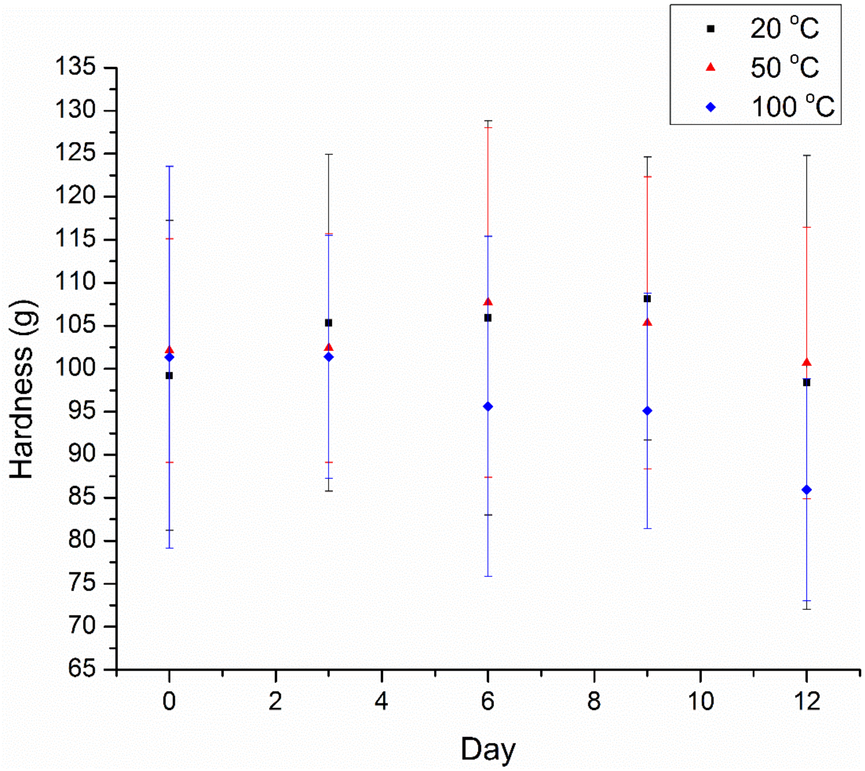


Fig. 4.12. Hardness changes of encapsulated catechin beads during a 12-day storage period at room temperature (square), 50 °C (triangle), and 100 °C (diamond)

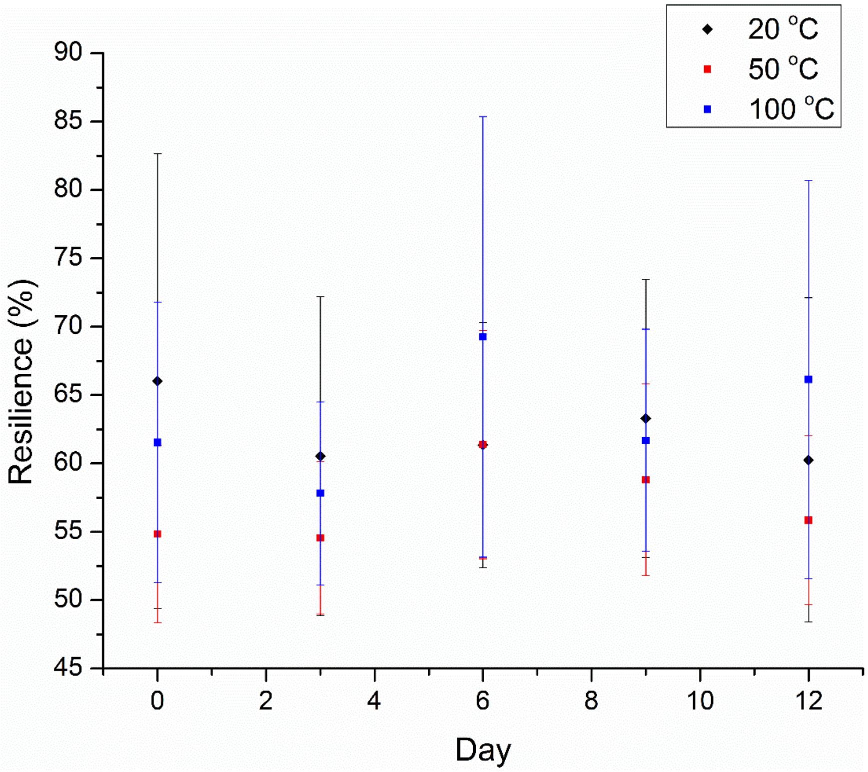


Fig. 4.13. Resilience changes of encapsulated catechin beads during a 12-day storage period at room temperature (square), 50 ^oC (triangle), and 100 ^oC (diamond)

4.4.6 Antioxidant Activity of Encapsulated Catechin Beads during Storage

The changes in the antioxidant activity of encapsulated catechin beads and (+)-catechin hydrate powder during storage are shown in Fig. 4.14 and Fig. 4.15, respectively. The antioxidant activity values of the encapsulated catechin beads showed a decreasing trend during storage for all temperatures tested (Fig. 4.14). The decreasing trend was more obvious for the beads stored at higher temperatures. The antioxidant activity values after 12-day storage were 87.9 ± 4.6%, 83.4 ± 1.7%, and 65.2 ± 2.2% for the samples stored at room temperature, 50 ^oC, and 100 ^oC, respectively. The statistical results also suggested the decreasing trend. The antioxidant activity of the encapsulated catechin beads stored at room temperature was not significantly different ($P>0.05$), while those stored at 50 ^oC and 100 ^oC was significantly different ($P<0.05$) during the storage period. The antioxidant activity values of (+)-catechin hydrate powder also showed a decreasing trend during storage for all temperatures tested (Fig. 4.15). However, the decrease in the percentage of antioxidant activity of (+)-catechin hydrate powder was lower than encapsulated catechin beads. The antioxidant activity values were 88.9 ± 1.5%, 90.3 ± 0.9%, and 87.6 ± 1.7% for the samples stored at room temperature, 50 °C, and 100 °C, respectively. The statistical results showed that the antioxidant activity of (+)-catechin hydrate powder stored at all temperatures was significantly different ($P<0.05$) during the storage period.

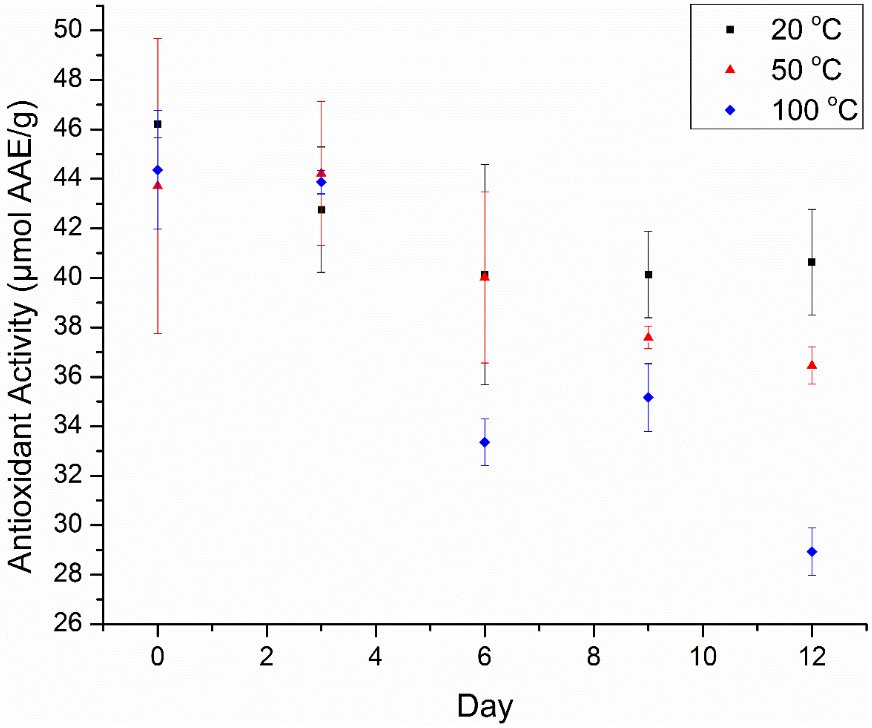


Fig. 4.14. Antioxidant activity of (µmol/g AAE) of encapsulated catechin beads during a 12-day storage period at room temperature (square), 50 °C (triangle), and 100 °C (diamond)

The antioxidant activity and the amount of the catechin powder and the encapsulated catechin beads during storage have been studied before. In a previous study, caseinate beads containing tea polyphenols and unencapsulated tea polyphenols were stored at 21 °C and 44% relative humidity in the dark for 42 days. The results showed that the free radical scavenging capacity of both encapsulated and unencapsulated tea polyphenols retained 90 ± 2% of their free radical scavenging capacity after storage [96]. However, another study where polyphenols encapsulated by alginate microbeads were stored for 6 months showed that the amount of (+)-catechin stored at room temperature in darkness decreased by more than 40% during the storage period [154].

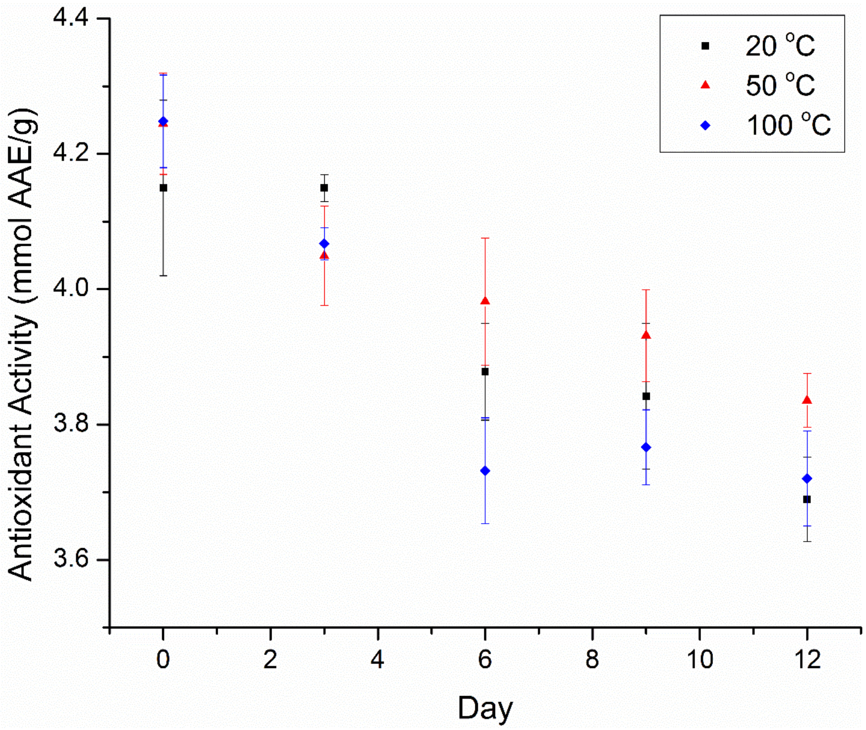


Fig. 4.15. Antioxidant activity of (mmol/g AAE) of (+)-catechin hydrate powder during a 12-day storage period at room temperature (square), 50 °C (triangle), and 100 °C (diamond)

4.5 Conclusion

In this study, (+)-catechin hydrate was encapsulated within pea protein/alginate beads by the extrusion technique. These matrices were stored at different temperatures (room temperature, 50 °C, 100 °C). The results of the chemical composition and thermal stability experiments showed that both properties did not change under these conditions tested. The SEM images of the surface of the encapsulated catechin beads showed that some surface characteristics were retained during storage. The average resilience values of the beads were relatively stable under all storage conditions. However, the resilience of the beads stored at 50 °C was significantly different ($P<0.05$) during the storage period, while the beads stored at room temperature and 100 °C was not significantly different ($P>0.05$). The average hardness values of the beads stored at 100 °C showed an obvious decreasing trend during storage, whereas it did not show an obvious decreasing or increasing trend for beads stored at room temperature and 50 °C. However, the statistical results showed that the hardness of the beads stored at all temperatures, including 100

^{o}C, was not significantly different ($P>0.05$) during storage time. The antioxidant activity values of the encapsulated catechin beads remained after 12-day storage were 87.9 ± 4.6%, 83.4 ± 1.7%, and 65.2 ± 2.2% of the original antioxidant activity for samples stored at room temperature, 50 ^{o}C, and 100 ^{o}C, respectively. However, in comparison, the antioxidant activity values of (+)-catechin hydrate powder showed a lower decrease in the percentage of antioxidant activity than encapsulated catechin, with the values after storage being 88.9 ± 1.5%, 90.3 ± 0.9%, and 87.6 ± 1.7% of the original antioxidant activity for samples stored at room temperature, 50 ^{o}C, and 100 ^{o}C, respectively. The statistical results showed that the antioxidant activity of the encapsulated catechin beads stored at room temperature was not significantly different ($P>0.05$) during the storage period, while for those stored at 50 ^{o}C and 100 ^{o}C, it was significantly different ($P<0.05$). For (+)-catechin hydrate powder, the antioxidant activity of the powder stored at all temperatures was significantly different ($P<0.05$) during storage time. These findings indicate that the physical properties of the encapsulated catechin beads were relatively stable for all temperatures tested, which was consistent with the results of the blank pea protein/alginate beads. However, the beads were not effective in maintaining the antioxidant activity of (+)-catechin hydrate as compared to pure powder.

CHAPTER 5

GENERAL CONCLUSION AND RECOMMENDATION

5.1 General Conclusion and Summary

The main objectives of this study were to encapsulate (+)-catechin hydrate within pea protein/alginate composite beads. The changes in the physical and chemical properties of both the encapsulated catechin beads and the blank beads during storage were monitored at three different temperatures (room temperature, 50 ^{o}C, 100 ^{o}C). Detailed conclusions of this study are listed below:

The thermal stability, the chemical composition, and the morphology of both the encapsulated catechin beads and the blank beads were relatively stable during storage for all temperature conditions. Their thermal stability and chemical composition were investigated by a thermogravimetric analyzer (TGA) and a Fourier transform infrared spectrometer (FTIR), respectively. The presence of alginate and pea protein in the beads was confirmed by both TGA and FTIR tests. However, these tests did not show the presence of catechin within the beads.

The average resilience values of the encapsulated catechin beads and the blank beads were relatively stable under all storage conditions. However, the average hardness values of both beads stored at 100 ^{o}C showed a decreasing trend during storage, whereas it did not show an obvious decreasing or increasing trend for beads stored at room temperature and 50 ^{o}C. The antioxidant activity values of encapsulated catechin beads showed a decreasing trend during storage for all temperatures tested. However, in comparison, the antioxidant activity values of (+)-catechin hydrate powder showed a decreasing trend with a lower decrease in the percentage of antioxidant activity than for the encapsulated catechin beads.

5.2 Recommendation for Future Studies

This study investigated some physical and chemical properties of composite beads during a 12-day storage period. Many other aspects of this work should be studied.

1) The stability and the antioxidant activity of catechin decrease in alkaline conditions. Therefore, the protective effect and the release properties of the beads should be studied in simulated intestinal fluids.

2) The changes in the binding capacity of catechin to the encapsulating matrices during storage should be studied. Moreover, the effect of storage conditions on the swelling properties of the beads should also be investigated.

REFERENCES

1. Gadkari, P.V. and M. Balaraman, *Catechins: Sources, extraction and encapsulation: A review.* Food and Bioproducts Processing, 2015. **93**: p. 122-138.
2. Jia, Z., M.-J. Dumont, and V. Orsat, *Encapsulation of phenolic compounds present in plants using protein matrices.* Food Bioscience, 2016. **15**: p. 87-104.
3. Mochizuki, M., et al., *Kinetic analysis and mechanistic aspects of autoxidation of catechins.* Biochimica Et Biophysica Acta-General Subjects, 2002. **1569**(1-3): p. 35-44.
4. Costa, A.M.M., et al., *Effective stabilization of CLA by microencapsulation in pea protein.* Food Chemistry, 2015. **168**: p. 157-166.
5. Lee, J.S., et al., *Characteristics and antioxidant activity of catechin-loaded calcium pectinate gel beads prepared by internal gelation.* Colloids and Surfaces B-Biointerfaces, 2009. **74**(1): p. 17-22.
6. Lupo, B., et al., *Characterization of alginate beads with encapsulated cocoa extract to prepare functional food: Comparison of two gelation mechanisms.* Food Hydrocolloids, 2015. **49**: p. 25-34.
7. Zam, W., et al., *Alginate-pomegranate peels' polyphenols beads: effects of formulation parameters on loading efficiency.* Brazilian Journal of Pharmaceutical Sciences, 2014. **50**(4): p. 741-748.
8. Rijo, P., et al., *Optimization of medicinal plant extraction methods and their encapsulation through extrusion technology.* Measurement, 2014. **58**: p. 249-255.
9. Ben Messaoud, G., et al., *Alginate/sodium caseinate aqueous-core capsules: A pH-responsive matrix.* Journal of Colloid and Interface Science, 2015. **440**: p. 1-8.
10. Zeeb, B., et al., *Retention and release of oil-in-water emulsions from filled hydrogel beads composed of calcium alginate: impact of emulsifier type and pH.* Soft Matter, 2015. **11**(11): p. 2228-2236.
11. Gu, F., B. Amsden, and R. Neufeld, *Sustained delivery of vascular endothelial growth factor with alginate beads.* Journal of Controlled Release, 2004. **96**(3): p. 463-472.
12. Stojanovic, R., et al., *Encapsulation of thyme (Thymus serpyllum L.) aqueous extract in calcium alginate beads.* Journal of the Science of Food and Agriculture, 2012. **92**(3): p. 685-696.
13. Nesterenko, A., et al., *Vegetable proteins in microencapsulation: A review of recent interventions and their effectiveness.* Industrial Crops and Products, 2013. **42**: p. 469-479.
14. Wang, J.P., et al., *Encapsulation of Bifidobacterium adolescentis cells with legume proteins and survival under stimulated gastric conditions and during storage in commercial fruit juices.* Food Science and Biotechnology, 2015. **24**(2): p. 383-391.
15. Shevkani, K., et al., *Structural and functional characterization of kidney bean and field pea protein isolates: A comparative study.* Food Hydrocolloids, 2015. **43**: p. 679-689.
16. Dahl, W.J., L.M. Foster, and R.T. Tyler, *Review of the health benefits of peas (Pisum sativum L.).* British Journal of Nutrition, 2012. **108**: p. S3-S10.
17. Roy, F., J.I. Boye, and B.K. Simpson, *Bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil.* Food Research International, 2010. **43**(2): p. 432-442.
18. FAOSTAT. *Food and Agriculture Organization.* [cited 2016 Aug 18]; Available from: http://faostat.fao.org.

19. Day, L., *Proteins from land plants - Potential resources for human nutrition and food security.* Trends in Food Science & Technology, 2013. **32**(1): p. 25-42.
20. Lunde, M.S., et al., *Variations in postprandial blood glucose responses and satiety after intake of three types of bread.* Journal of nutrition and metabolism, 2011. **2011**.
21. Dahl, W.J., et al., *Increased stool frequency occurs when finely processed pea hull fiber is added to usual foods consumed by elderly residents in long-term care.* Journal of the American Dietetic Association, 2003. **103**(9): p. 1199-1202.
22. Bajaj, P.R., J.M. Tang, and S.S. Sablani, *Pea Protein Isolates: Novel Wall Materials for Microencapsulating Flaxseed Oil.* Food and Bioprocess Technology, 2015. **8**(12): p. 2418-2428.
23. Rigamonti, E., et al., *Hypolipidemic effect of dietary pea proteins: Impact on genes regulating hepatic lipid metabolism.* Molecular Nutrition & Food Research, 2010. **54**: p. S24-S30.
24. Li, H., et al., *Blood Pressure Lowering Effect of a Pea Protein Hydrolysate in Hypertensive Rats and Humans.* Journal of Agricultural and Food Chemistry, 2011. **59**(18): p. 9854-9860.
25. Blumberg, J.B., et al., *Cranberries and Their Bioactive Constituents in Human Health.* Advances in Nutrition, 2013. **4**(6): p. 618-632.
26. Pappas, E. and K.M. Schaich, *Phytochemicals of Cranberries and Cranberry Products: Characterization, Potential Health Effects, and Processing Stability.* Critical Reviews in Food Science and Nutrition, 2009. **49**(9): p. 741-781.
27. Weiss, E.I., *Inhibiting interspecies coaggregation of plaque bacteria with a cranberry juice constituent (vol 130, pg 1719, 1999).* Journal of the American Dental Association, 1999. **130**(2): p. 178-178.
28. Betz, M., et al., *Antioxidant capacity of bilberry extract microencapsulated in whey protein hydrogels.* Food Research International, 2012. **47**(1): p. 51-57.
29. Chen, L.Y., G.E. Remondetto, and M. Subirade, *Food protein-based materials as nutraceutical delivery systems.* Trends in Food Science & Technology, 2006. **17**(5): p. 272-283.
30. Joye, I.J. and D.J. McClements, *Biopolymer-based nanoparticles and microparticles: Fabrication, characterization, and application.* Current Opinion in Colloid & Interface Science, 2014. **19**(5): p. 417-427.
31. Cavalcanti, R.N., D.T. Santos, and M.A.A. Meireles, *Non-thermal stabilization mechanisms of anthocyanins in model and food systems-An overview.* Food Research International, 2011. **44**(2): p. 499-509.
32. Fang, Z.X. and B. Bhandari, *Encapsulation of polyphenols - a review.* Trends in Food Science & Technology, 2010. **21**(10): p. 510-523.
33. Munin, A. and F. Edwards-Lévy, *Encapsulation of natural polyphenolic compounds; a review.* Pharmaceutics, 2011. **3**(4): p. 793-829.
34. Cote, J., et al., *Analyzing Cranberry Bioactive Compounds.* Critical Reviews in Food Science and Nutrition, 2010. **50**(9): p. 872-888.
35. Flores, F.P., R.K. Singh, and F.B. Kong, *Physical and storage properties of spray-dried blueberry pomace extract with whey protein isolate as wall material.* Journal of Food Engineering, 2014. **137**: p. 1-6.

36. Oidtmann, J., et al., *Preparation and Comparative Release Characteristics of Three Anthocyanin Encapsulation Systems.* Journal of Agricultural and Food Chemistry, 2012. **60**(3): p. 844-851.
37. Frank, K., K. Kohler, and H.P. Schuchmann, *Formulation of Labile Hydrophilic Ingredients in Multiple Emulsions: Influence of the Formulation's Composition on the Emulsion's Stability and on the Stability of Entrapped Bioactives.* Journal of Dispersion Science and Technology, 2011. **32**(12): p. 1753-1758.
38. Zhang, T., et al., *Encapsulation of anthocyanin molecules within a ferritin nanocage increases their stability and cell uptake efficiency.* Food Research International, 2014. **62**: p. 183-192.
39. Betz, M. and U. Kulozik, *Whey protein gels for the entrapment of bioactive anthocyanins from bilberry extract.* International Dairy Journal, 2011. **21**(9): p. 703-710.
40. Szajdek, A. and E.J. Borowska, *Bioactive Compounds and Health-Promoting Properties of Berry Fruits: A Review.* Plant Foods for Human Nutrition, 2008. **63**(4): p. 147-156.
41. Cote, J., et al., *Bioactive Compounds in Cranberries and their Biological Properties.* Critical Reviews in Food Science and Nutrition, 2010. **50**(7): p. 666-679.
42. Neto, C.C., *Cranberry and its phytochemicals: A review of in vitro anticancer studies.* Journal of Nutrition, 2007. **137**(1): p. 186S-193S.
43. Ha, H.K., et al., *Formation and characterization of quercetin-loaded chitosan oligosaccharide/beta-lactoglobulin nanoparticle.* Food Research International, 2013. **52**(1): p. 82-90.
44. Aceituno-Medina, M., et al., *Improved antioxidant capacity of quercetin and ferulic acid during in-vitro digestion through encapsulation within food-grade electrospun fibers.* Journal of Functional Foods, 2015. **12**: p. 332-341.
45. Socha, R., et al., *Determination of free radicals and flavan-3-ols content in fermented and unfermented teas and properties of their infusions.* European Food Research and Technology, 2013. **237**(2): p. 167-177.
46. Shpigelman, A., G. Israeli, and Y.D. Livney, *Thermally-induced protein–polyphenol co-assemblies: beta lactoglobulin-based nanocomplexes as protective nanovehicles for EGCG.* Food Hydrocolloids, 2010. **24**(8): p. 735-743.
47. Shpigelman, A., Y. Cohen, and Y.D. Livney, *Thermally-induced β-lactoglobulin–EGCG nanovehicles: Loading, stability, sensory and digestive-release study.* Food Hydrocolloids, 2012. **29**(1): p. 57-67.
48. Li, B., et al., *Preservation of (-)-Epigallocatechin-3-gallate Antioxidant Properties Loaded in Heat Treated beta-Lactoglobulin Nanoparticles.* Journal of Agricultural and Food Chemistry, 2012. **60**(13): p. 3477-3484.
49. Haratifar, S., K.A. Meckling, and M. Corredig, *Bioefficacy of tea catechins encapsulated in casein micelles tested on a normal mouse cell line (4D/WT) and its cancerous counterpart (D/v-src) before and after in vitro digestion.* Food & Function, 2014. **5**(6): p. 1160-1166.
50. Ru, Q.M., H.L. Yu, and Q.R. Huang, *Encapsulation of Epigallocatechin-3-gallate (EGCG) Using Oil-in-Water (O/W) Submicrometer Emulsions Stabilized by iota-Carrageenan and beta-Lactoglobulin.* Journal of Agricultural and Food Chemistry, 2010. **58**(19): p. 10373-10381.
51. Zou, T., et al., *Fabrication, characterization, and cytotoxicity evaluation of cranberry procyanidins-zein nanoparticles.* Food Hydrocolloids, 2012. **27**(2): p. 293-300.

52. Gu, L.W., et al., *Concentrations of proanthocyanidins in common foods and estimations of normal consumption.* Journal of Nutrition, 2004. **134**(3): p. 613-617.
53. Berendsen, R., C. Guell, and M. Ferrando, *A procyanidin-rich extract encapsulated in water-in-oil-in-water emulsions produced by premix membrane emulsification.* Food Hydrocolloids, 2015. **43**: p. 636-648.
54. Santos-Buelga, C. and A. Scalbert, *Proanthocyanidins and tannin-like compounds - nature, occurrence, dietary intake and effects on nutrition and health.* Journal of the Science of Food and Agriculture, 2000. **80**(7): p. 1094-1117.
55. Augustin, M.A. and Y. Hemar, *Nano- and micro-structured assemblies for encapsulation of food ingredients.* Chemical Society Reviews, 2009. **38**(4): p. 902-912.
56. Gouin, S., *Microencapsulation: industrial appraisal of existing technologies and trends.* Trends in Food Science & Technology, 2004. **15**(7-8): p. 330-347.
57. Li, Y., L.T. Lim, and Y. Kakuda, *Electrospun Zein Fibers as Carriers to Stabilize (-)-Epigallocatechin Gallate.* Journal of Food Science, 2009. **74**(3): p. C233-C240.
58. Yang, J.M., et al., *Coaxial electrospinning with acetic acid for preparing ferulic acid/zein composite fibers with improved drug release profiles.* Colloids and Surfaces B-Biointerfaces, 2013. **102**: p. 737-743.
59. Neo, Y.P., et al., *Encapsulation of food grade antioxidant in natural biopolymer by electrospinning technique: A physicochemical study based on zein-gallic acid system.* Food Chemistry, 2013. **136**(2): p. 1013-1021.
60. Ezhilarasi, P.N., et al., *Nanoencapsulation Techniques for Food Bioactive Components: A Review.* Food and Bioprocess Technology, 2013. **6**(3): p. 628-647.
61. Tavares, G.M., et al., *Milk proteins as encapsulation devices and delivery vehicles: Applications and trends.* Trends in Food Science & Technology, 2014. **37**(1): p. 5-20.
62. Wang, S., et al., *Application of nanotechnology in improving bioavailability and bioactivity of diet-derived phytochemicals.* Journal of Nutritional Biochemistry, 2014. **25**(4): p. 363-376.
63. Lamba, H., K. Sathish, and L. Sabikhi, *Double Emulsions: Emerging Delivery System for Plant Bioactives.* Food and Bioprocess Technology, 2015. **8**(4): p. 709-728.
64. Li, J., et al., *Calcium-alginate beads loaded with gallic acid: Preparation and characterization.* Lwt-Food Science and Technology, 2016. **68**: p. 667-673.
65. Xu, M. and M.J. Dumont, *Evaluation of the stability of pea and canola protein-based hydrogels in simulated gastrointestinal fluids.* Journal of Food Engineering, 2015. **165**: p. 52-59.
66. Xu, M., et al., *Encapsulation of Lactobacillus casei ATCC 393 cells and evaluation of their survival after freeze-drying, storage and under gastrointestinal conditions.* Journal of Food Engineering, 2016. **168**: p. 52-59.
67. Fathi, M., A. Martin, and D.J. McClements, *Nanoencapsulation of food ingredients using carbohydrate based delivery systems.* Trends in Food Science & Technology, 2014. **39**(1): p. 18-39.
68. Ghorani, B. and N. Tucker, *Fundamentals of electrospinning as a novel delivery vehicle for bioactive compounds in food nanotechnology.* Food Hydrocolloids, 2015. **51**: p. 227-240.
69. Zamani, M., M.P. Prabhakaran, and S. Ramakrishna, *Advances in drug delivery via electrospun and electrosprayed nanomaterials.* International Journal of Nanomedicine, 2013. **8**: p. 2997-3017.

70. Bhushani, J.A. and C. Anandharamakrishnan, *Electrospinning and electrospraying techniques: Potential food based applications.* Trends in Food Science & Technology, 2014. **38**(1): p. 21-33.

71. Bock, N., T.R. Dargaville, and M.A. Woodruff, *Electrospraying of polymers with therapeutic molecules: State of the art.* Progress in Polymer Science, 2012. **37**(11): p. 1510-1551.

72. Betz, M. and U. Kulozik, *Microencapsulation of bioactive bilberry anthocyanins by means of whey protein gels.* 11th International Congress on Engineering and Food (Icef11), 2011. **1**: p. 2047-2056.

73. Sadeghi, S., A. Madadlou, and M. Yarmand, *Microemulsification-cold gelation of whey proteins for nanoencapsulation of date palm pit extract.* Food Hydrocolloids, 2014. **35**: p. 590-596.

74. Livney, Y.D., *Milk proteins as vehicles for bioactives.* Current Opinion in Colloid & Interface Science, 2010. **15**(1-2): p. 73-83.

75. Pan, K. and Q.X. Zhong, *Low energy, organic solvent-free co-assembly of zein and caseinate to prepare stable dispersions.* Food Hydrocolloids, 2016. **52**: p. 600-606.

76. Frank, K., et al., *Stability of Anthocyanin-Rich W/O/W-Emulsions Designed for Intestinal Release in Gastrointestinal Environment.* Journal of Food Science, 2012. **77**(12): p. N50-N57.

77. Flores, F.P., et al., *Total phenolics content and antioxidant capacities of microencapsulated blueberry anthocyanins during in vitro digestion.* Food Chemistry, 2014. **153**: p. 272-278.

78. Flores, F.P., et al., *In vitro release properties of encapsulated blueberry (Vaccinium ashei) extracts.* Food Chemistry, 2015. **168**: p. 225-232.

79. Robert, P., et al., *Encapsulation of polyphenols and anthocyanins from pomegranate (Punica granatum) by spray drying.* International Journal of Food Science and Technology, 2010. **45**(7): p. 1386-1394.

80. Fang, Z.X. and B. Bhandari, *Comparing the efficiency of protein and maltodextrin on spray drying of bayberry juice.* Food Research International, 2012. **48**(2): p. 478-483.

81. Leick, S., A. Kemper, and H. Rehage, *Alginate/poly-L-lysine capsules: mechanical properties and drug release characteristics.* Soft Matter, 2011. **7**(14): p. 6684-6694.

82. Diaz-Bandera, D., et al., *Release kinetics of antioxidant compounds from Hibiscus sabdariffa L. encapsulated in gelatin beads and coated with sodium alginate.* International Journal of Food Science and Technology, 2013. **48**(10): p. 2150-2158.

83. Arroyo-Maya, I.J. and D.J. McClements, *Biopolymer nanoparticles as potential delivery systems for anthocyanins: Fabrication and properties.* Food Research International, 2015. **69**: p. 1-8.

84. Schantz, M., et al., *Triggered Gastrointestinal Release of Anthocyanins from Bilberries (Vaccinium myrtillus L.).* X International Symposium on Vaccinium and Other Superfruits, 2014. **1017**: p. 381-386.

85. Kropat, C., et al., *Effect of Microformulation on the Bioactivity of an Anthocyanin-rich Bilberry Pomace Extract (Vaccinium myrtillus L.) in Vitro.* Journal of Agricultural and Food Chemistry, 2013. **61**(20): p. 4873-4881.

86. Baum, M., et al., *Is the antioxidative effectiveness of a bilberry extract influenced by encapsulation?* Journal of the Science of Food and Agriculture, 2014. **94**(11): p. 2301-2307.

87. Fang, R., et al., *Bovine Serum Albumin Nanoparticle Promotes the Stability of Quercetin in Simulated Intestinal Fluid.* Journal of Agricultural and Food Chemistry, 2011. **59**(11): p. 6292-6298.
88. Fang, R., et al., *Design and characterization of protein-quercetin bioactive nanoparticles.* Journal of Nanobiotechnology, 2011. **9**.
89. Patel, A.R., et al., *Quercetin loaded biopolymeric colloidal particles prepared by simultaneous precipitation of quercetin with hydrophobic protein in aqueous medium.* Food Chemistry, 2012. **133**(2): p. 423-429.
90. Lestringant, P., et al., *Effect of Processing on Physicochemical Characteristics and Bioefficacy of beta-Lactoglobulin-Epigallocatechin-3-gallate Complexes.* Journal of Agricultural and Food Chemistry, 2014. **62**(33): p. 8357-8364.
91. Xue, J., et al., *Fabrication of Epigallocatechin-3-gallate Nanocarrier Based on Glycosylated Casein: Stability and Interaction Mechanism.* Journal of Agricultural and Food Chemistry, 2014. **62**(20): p. 4677-4684.
92. Hu, B., et al., *Bioactive Peptides/Chitosan Nanoparticles Enhance Cellular Antioxidant Activity of (-)-Epigallocatechin-3-gallate.* Journal of Agricultural and Food Chemistry, 2013. **61**(4): p. 875-881.
93. Hu, B., et al., *Nanochemoprevention by encapsulation of (-)-epigallocatechin-3-gallate with bioactive peptides/chitosan nanoparticles for enhancement of its bioavailability.* Chemical Communications, 2012. **48**(18): p. 2421-2423.
94. Hu, B., et al., *Cellular uptake and cytotoxicity of chitosan-caseinophosphopeptides nanocomplexes loaded with epigallocatechin gallate.* Carbohydrate Polymers, 2012. **89**(2): p. 362-370.
95. Zhou, H.H., et al., *Fabrication of Biopolymeric Complex Coacervation Core Micelles for Efficient Tea Polyphenol Delivery via a Green Process.* Langmuir, 2012. **28**(41): p. 14553-14561.
96. Dehkharghanian, M., M. Lacroix, and M.A. Vijayalakshmi, *Antioxidant properties of green tea polyphenols encapsulated in caseinate beads.* Dairy Science & Technology, 2009. **89**(5): p. 485-499.
97. Almajano, M.P., M.E. Delgado, and M.H. Gordon, *Albumin causes a synergistic increase in the antioxidant activity of green tea catechins in oil-in-water emulsions.* Food Chemistry, 2007. **102**(4): p. 1375-1382.
98. Gomez-Mascaraque, L.G., J.M. Lagaron, and A. Lopez-Rubio, *Electrosprayed gelatin submicroparticles as edible carriers for the encapsulation of polyphenols of interest in functional foods.* Food Hydrocolloids, 2015. **49**: p. 42-52.
99. Haratifar, S. and M. Corredig, *Interactions between tea catechins and casein micelles and their impact on renneting functionality.* Food Chemistry, 2014. **143**: p. 27-32.
100. Chen, Y.C., et al., *Novel Technology for the Preparation of Self-Assembled Catechin/Gelatin Nanoparticles and Their Characterization.* Journal of Agricultural and Food Chemistry, 2010. **58**(11): p. 6728-6734.
101. Lemarie, F., et al., *Antitumor activity of the tea polyphenol epigallocatechin-3-gallate encapsulated in targeted vesicles after intravenous administration.* Nanomedicine, 2013. **8**(2): p. 181-192.
102. Lin, Y.H., et al., *Preparation of epigallocatechin gallate-loaded nanoparticles and characterization of their inhibitory effects on Helicobacter pylori growth in vitro and in vivo.* Science and Technology of Advanced Materials, 2014. **15**(4).

103. Zhang, L.Y., et al., *Macromolecular conjugate based particulates: Preparation, characterisation and evaluation of controlled release properties.* European Polymer Journal, 2009. **45**(7): p. 1960-1969.
104. Links, M.R., et al., *Sorghum condensed tannins encapsulated in kafirin microparticles as a nutraceutical for inhibition of amylases during digestion to attenuate hyperglycaemia.* Journal of Functional Foods, 2015. **12**: p. 55-63.
105. Taylor, J., et al., *Kafirin Microparticle Encapsulation of Catechin and Sorghum Condensed Tannins.* Journal of Agricultural and Food Chemistry, 2009. **57**(16): p. 7523-7528.
106. Li, Z., et al., *Fabrication of nanoparticles using partially purified pomegranate ellagitannins and gelatin and their apoptotic effects.* Molecular Nutrition & Food Research, 2011. **55**(7): p. 1096-1103.
107. Berendsen, R., C. Guell, and M. Ferrando, *Spray dried double emulsions containing procyanidin-rich extracts produced by premix membrane emulsification: Effect of interfacial composition.* Food Chemistry, 2015. **178**: p. 251-258.
108. Almajano, M.P., et al., *Effect of pH on the antimicrobial activity and oxidative stability of oil-in-water emulsions containing caffeic acid.* Journal of Food Science, 2007. **72**(5): p. C258-C263.
109. Almajano, M.P. and M.H. Gordon, *Synergistic effect of BSA on antioxidant activities in model food emulsions.* Journal of the American Oil Chemists Society, 2004. **81**(3): p. 275-280.
110. Barnaby, S.N., et al., *Fabrication of ellagic acid incorporated self-assembled peptide microtubes and their applications.* Colloids and Surfaces B-Biointerfaces, 2012. **95**: p. 154-161.
111. Shutava, T.G., et al., *Layer-by-Layer-Coated Gelatin Nanoparticles as a Vehicle for Delivery of Natural Polyphenols.* Acs Nano, 2009. **3**(7): p. 1877-1885.
112. George, M. and T.E. Abraham, *Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan - a review.* Journal of Controlled Release, 2006. **114**(1): p. 1-14.
113. Pawar, S.N. and K.J. Edgar, *Alginate derivatization: A review of chemistry, properties and applications.* Biomaterials, 2012. **33**(11): p. 3279-3305.
114. Goh, C.H., P.W.S. Heng, and L.W. Chan, *Alginates as a useful natural polymer for microencapsulation and therapeutic applications.* Carbohydrate Polymers, 2012. **88**(1): p. 1-12.
115. Lee, B.B., P. Ravindra, and E.S. Chan, *Size and Shape of Calcium Alginate Beads Produced by Extrusion Dripping.* Chemical Engineering & Technology, 2013. **36**(10): p. 1627-1642.
116. Burey, P., et al., *Hydrocolloid gel particles: formation, characterization, and application.* Critical reviews in food science and nutrition, 2008. **48**(5): p. 361-377.
117. Belscak-Cvitanovic, A., et al., *Improving the controlled delivery formulations of caffeine in alginate hydrogel beads combined with pectin, carrageenan, chitosan and psyllium.* Food Chemistry, 2015. **167**: p. 378-386.
118. Belscak-Cvitanovic, A., et al., *Protein-reinforced and chitosan-pectin coated alginate microparticles for delivery of flavan-3-ol antioxidants and caffeine from green tea extract.* Food Hydrocolloids, 2015. **51**: p. 361-374.

119. Deat-Laine, E., et al., *Development and in vitro characterization of insulin loaded whey protein and alginate microparticles.* International Journal of Pharmaceutics, 2012. **439**(1-2): p. 136-144.
120. Deat-Laine, E., et al., *Efficacy of Mucoadhesive Hydrogel Microparticles of Whey Protein and Alginate for Oral Insulin Delivery.* Pharmaceutical Research, 2013. **30**(3): p. 721-734.
121. Pereira, H.V.R., et al., *Legumes seeds protein isolates in the production of ascorbic acid microparticles.* Food Research International, 2009. **42**(1): p. 115-121.
122. Klemmer, K.J., et al., *Pea protein-based capsules for probiotic and prebiotic delivery.* International Journal of Food Science and Technology, 2011. **46**(11): p. 2248-2256.
123. Sarmento, B., et al., *Characterization of insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies.* Carbohydrate Polymers, 2006. **66**(1): p. 1-7.
124. Lawrie, G., et al., *Interactions between alginate and chitosan biopolymers characterized using FTIR and XPS.* Biomacromolecules, 2007. **8**(8): p. 2533-2541.
125. Kaewprachu, P. and S. Rawdkuen, *Mechanical and physico-chemical properties of biodegradable protein-based films: A comparative study.* Food and Applied Bioscience Journal, 2014. **2**(1): p. 1.
126. Ji, X., et al., *Fabrication of lanthanum-based phosphate binder using cross-linked alginate as a carrier.* RSC Advances, 2015. **5**(68): p. 55191-55200.
127. Sundarrajan, P., et al., *One pot synthesis and characterization of alginate stabilized semiconductor nanoparticles.* Bulletin of the Korean Chemical Society, 2012. **33**(10): p. 3218-3224.
128. Choonara, Y.E., et al., *Chemometric, physicomechanical and rheological analysis of the sol–gel dynamics and degree of crosslinking of glycosidic polymers.* Biomedical Materials, 2008. **3**(2): p. 025003.
129. Shaikh, R.P., et al., *The application of a crosslinked pectin-based wafer matrix for gradual buccal drug delivery.* Journal of Biomedical Materials Research Part B-Applied Biomaterials, 2012. **100B**(4): p. 1029-1043.
130. Pillay, V. and R. Fassihi, *In vitro release modulation from crosslinked pellets for site-specific drug delivery to the gastrointestinal tract - II. Physicochemical characterization of calcium-alginate, calcium-pectinate and calcium-alginate-pectinate pellets.* Journal of Controlled Release, 1999. **59**(2): p. 243-256.
131. Harilall, S.L., et al., *Development and in vivo evaluation of an implantable nano-enabled multipolymeric scaffold for the management of AIDS dementia complex (ADC).* International Journal of Pharmaceutics, 2015. **496**(2): p. 863-877.
132. Bawa, P., et al., *A Composite Polyelectrolytic Matrix for Controlled Oral Drug Delivery.* Aaps Pharmscitech, 2011. **12**(1): p. 227-238.
133. Zaveri, N.T., *Green tea and its polyphenolic catechins: Medicinal uses in cancer and noncancer applications.* Life Sciences, 2006. **78**(18): p. 2073-2080.
134. Sanna, V., et al., *Polymeric Nanoparticles Encapsulating White Tea Extract for Nutraceutical Application.* Journal of Agricultural and Food Chemistry, 2015. **63**(7): p. 2026-2032.
135. Dube, A., et al., *Effective use of reducing agents and nanoparticle encapsulation in stabilizing catechins in alkaline solution.* Food Chemistry, 2010. **122**(3): p. 662-667.

136. Yadav, R., et al., *ENCAPSULATION OF CATECHIN AND EPICATECHIN ON BSA NPS IMPROVED THEIR STABILITY AND ANTIOXIDANT POTENTIAL.* Excli Journal, 2014. **13**: p. 331-346.
137. Kim, E.S., J.S. Lee, and H.G. Lee, *Microencapsulation of catechin with high loading and encapsulation efficiencies using soaking methods.* Food Science and Biotechnology, 2015. **24**(5): p. 1735-1739.
138. Ananingsih, V.K., A. Sharma, and W.B. Zhou, *Green tea catechins during food processing and storage: A review on stability and detection.* Food Research International, 2013. **50**(2): p. 469-479.
139. Rashidinejad, A., et al., *Effects of catechin on the phenolic content and antioxidant properties of low-fat cheese.* International Journal of Food Science and Technology, 2013. **48**(12): p. 2448-2455.
140. Rupasinghe, H.P.V., et al., *Effect of baking on dietary fibre and phenolics of muffins incorporated with apple skin powder.* Food Chemistry, 2008. **107**(3): p. 1217-1224.
141. Lavelli, V., et al., *Formulation of a Dry Green Tea-Apple Product: Study on Antioxidant and Color Stability.* Journal of Food Science, 2010. **75**(2): p. C184-C190.
142. Goh, R., et al., *Green tea catechins reduced the glycaemic potential of bread: An in vitro digestibility study.* Food Chemistry, 2015. **180**: p. 203-210.
143. Mildner-Szkudlarz, S., et al., *White grape pomace as a source of dietary fibre and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits.* Journal of the Science of Food and Agriculture, 2013. **93**(2): p. 389-395.
144. Dube, A., J.A. Nicolazzo, and I. Larson, *Chitosan nanoparticles enhance the intestinal absorption of the green tea catechins (+)-catechin and (−)-epigallocatechin gallate.* European Journal of Pharmaceutical Sciences, 2010. **41**(2): p. 219-225.
145. Deladino, L., et al., *Encapsulation of natural antioxidants extracted from Ilex paraguariensis.* Carbohydrate Polymers, 2008. **71**(1): p. 126-134.
146. Cuevas-Valenzuela, J., et al., *Solubility of (+)-catechin in water and water-ethanol mixtures within the temperature range 277.6-331.2 K: Fundamental data to design polyphenol extraction processes.* Fluid Phase Equilibria, 2014. **382**: p. 279-285.
147. Routray, W., V. Orsat, and Y. Gariepy, *Effect of Different Drying Methods on the Microwave Extraction of Phenolic Components and Antioxidant Activity of Highbush Blueberry Leaves.* Drying Technology, 2014. **32**(16): p. 1888-1904.
148. Ramos-Tejada, M.M., et al., *Investigation of alumina/(+)-catechin system properties. Part I: a study of the system by FTIR-UV–Vis spectroscopy.* Colloids and Surfaces B: Biointerfaces, 2002. **24**(3–4): p. 297-308.
149. Devi, N. and D.K. Kakati, *Smart porous microparticles based on gelatin/sodium alginate polyelectrolyte complex.* Journal of Food Engineering, 2013. **117**(2): p. 193-204.
150. Soetaredjo, F.E., et al., *Catechin sublimation pressure and solubility in supercritical carbon dioxide.* Fluid Phase Equilibria, 2013. **358**: p. 220-225.
151. Liu, J., et al., *Synthesis, characterization and in vitro anti-diabetic activity of catechin grafted inulin.* International Journal of Biological Macromolecules, 2014. **64**: p. 76-83.
152. Gujar, J.G., et al., *EXPERIMENTAL AND MODELING STUDIES ON EXTRACTION OF CATECHIN HYDRATE AND EPICATECHIN FROM INDIAN GREEN TEA LEAVES.* Canadian Journal of Chemical Engineering, 2010. **88**(2): p. 232-240.

153. Otalora, M.C., et al., *Encapsulating betalains from Opuntia ficus-indica fruits by ionic gelation: Pigment chemical stability during storage of beads.* Food Chemistry, 2016. **202**: p. 373-382.
154. Aizpurua-Olaizola, O., et al., *Microencapsulation and storage stability of polyphenols from Vitis vinifera grape wastes.* Food Chemistry, 2016. **190**: p. 614-621.