MICROENCAPSULATION OF FOLIC ACID USING HYDROLYZED COLLAGEN BY CONVENTIONAL AND ULTRASONIC SPRAY DRYING METHOD

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by

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DAYANG NORLAILA HAJI LATIP

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CCD	Central Composite design
COA	Certificate of analysis
Cc	Commercial fish scale hydrolyzed collagen
Cs	Spray dried fish scale hydrolyzed collagen
CLSM	Confocal laser scanning microscopy
CV	Coefficient of variation
DSC	Differential Scanning Calorimeter
EA	Emulsifying activity
EE	Encapsulation efficiency
ES	Emulsifying stability
Esd	Encapsulated folic acid using conventional spray dryer
Eus	Encapsulated folic acid using ultrasonic spray dryer
FTIR	Fourier Transform Infrared Spectrophotometer
HPLC	High Performance Liquid Chromatography
MEE	Microencapsulation Efficiency
RSM	Response Surface Methodology
SEM	Scanning Electron Microscopy
WHC	Water Holding Capacity

LIST OF SYMBOLS

℃	Degree celcius
%	Percentage
mg	Milligram
g	Gram
ml	Milliliter

PEMIKROKAPSULAN ASID FOLIK DENGAN KAEDAH PENGERINGAN SEMBURAN KONVENSIONAL DAN ULTRASONIK

ABSTRAK

Terbaru, beberapa kajian telah menunjukkan trend yang baik untuk mencari alternatif bagi menggantikan bahan dinding polimer sintetik kepada polimer semulajadi. Walaubagaimanapun, tiada penyelidikan yang dijalankan menggunakan sisik ikan kolagen hidrolis komersial, Cc sebagai agen pengkapsulan. Oleh itu, matlamat kajian ini adalah untuk menganalisis ciri-ciri fizikokimia dan sifat-sifat Cc, sebagai agen salutan. Kaedah gerak balas permukaan telah digunakan untuk optimumkan kajian menggunakan teknik semburan kering. Fizikokimia dan fungsi analisis telah dijalankan terhadap sisik ikan kolagen hidrolis komersial sebelum pengeringan (Cc) dan selepas pengeringan (Cs). Dua faktor telah dioptimumkan termasuk suhu dalaman dan kadar pengaliran, keputusan menunjukkan 138 °C dan 7 %. Asid amino analisis menunjukkan kedua-dua sampel Cc dan Cs, mempunyai glycine sebagai komponen utama asid amino diikuti oleh proline dan hydroxyproline. Analisis asid amino menunjukkan kedua-dua sampel mengandungi glycine sebagai komponen asid amino yang utama diikuti oleh proline dan hydroxyproline dengan masing-masing mencatatkan 362.78±6.35, 109.32±4.80, 105.73±7.84 untuk Cc. Manakala, Cs menunjukkan nilai yang sedikit rendah dengan glycine 348.68±2.44, proline 116.21±2.16, dan hydroxyproline 101.72±0.83. FT-IR menunjukkan perubahan terhadap kadar intensiti serta nombor gelombang untuk amide A, amide B, amide I, II, III antara kedua-dua sampel. Analisis terhadap permukaan morfologi oleh SEM memvisualkan partikel Cs kecil dan mengecut berbanding Cc. Analisis

DSC menunjukkan peralihan haba yang tinggi pada 107.87 °C untuk Cc sementara Cs menujukkan pada 101.04 C°. Selanjutnya, analisis kadar kelarutan serta potensi zeta membuktikan keputusan yang selari dimana titik isoelektrik adalah pada pH 4. Tiada signifikan trend diperhatikan pada aktiviti mengemulsi, EA, and kestabilan mengemulsi, ES. Nilai EA untuk Cc dan Cs masing-masing adalah 61.11% dan 59.67%, sementara ES menunjukkan penurunan yang perlahan berkadaran dengan masa untuk kedua-dua sampel. Keseluruhan pemerhatian menunjukkan bahawa sisik ikan komersial kolagen mempunyai potensi untuk dijadikan sebagai bahan dinding kerana kebolehannya untuk kekal stabil pada kondisi yang ekstrem seperti suhu yang ekstrem serta kondisi pH yang melampau. Pemikrokapsulan adalah alat yang berguna untuk melindungi bahan sensitif atau bahan-bahan dari persekitaran yang sukar. Terdapat banyak kaedah telah diterbitkan untuk menjalankan proses pemikrokapsulan itu. Walau bagaimanapun pemilihan bahan dinding yang sesuai juga menyumbang dalam kejayaan tersebut. Objektif-objektif dalam kajian ini adalah untuk mengoptimumkan proses semburan kering iaitu dari segi kepekatan, suhu dalaman dan kadar pengaliran terhadap pengkapsulan asid folik dan untuk menyiasat potensi sisik ikan kolagen hidrolis komersial sebagai bahan dinding untuk pemikrokapsulan asid folik dengan menggunakan teknik semburan kering yang konvensional serta teknik semburan kering ultrasonik. Teknik semburan kering yang konvensional (Esd) serta teknik semburan kering ultrasonik (Eus) digunakan untuk menghasilkan pemikrokapsulan asid folik oleh sisik ikan kolagen hidrolis komersial. Kajian morfologi telah dilakukan dengan menggunakan SEM mikrofotografi dan analisis CLSM. Kedua-duanya menunjukkan hasil yang boleh diterima di mana Esd menunjukkan bentuk yang tidak teratur berbanding Eus mempunyai permukaan yang licin serta bentuk yang bulat. Imej CLSM menunjukkan kedua-dua teknik berjaya

menghasilkan satu bentuk matriks pengkapsulan. Walaubagaimanapun, kadar keupayaan pemikrokapsulan oleh Esd agak rendah berbanding Eus dengan keupayaan pemikrokapsulan adalah 50 % dan 85 %. Taburan saiz partikel menunjukkan Esd mempunyai kadar taburan yang luas dengan corak polidisperse sementara Eus mempunyai taburan saiz yang sempit dengan corak monodisperse. Halaju tinggi yang digunakan oleh semburan kering konvensional memberi kesan terhadap morfologi partikel. Analisis FTIR menunjukkan interaksi yang baik di antara asid folik sebagai agen teras dan sisik ikan kolagen hidrolis komersial sebagai bahan dinding. Oleh itu, keseluruhan keputusan menunjukkan pengkapsulan menggunakan kaedah semburan kering ultrasonik menunjukkan keputusan yang lebih bagus berbanding semburan kering konvensional dan juga dapat dirumuskan sisik ikan kolagen hidrolis komersial menunjukkan potensi sebagai agen pengkapsulan kepada asid folik.

MICROENCAPSULATION OF FOLIC ACID USING HYDROLYZED COLLAGEN BY CONVENTIONAL AND ULTRASONIC SPRAY DRYING METHOD

ABSTRACT

Recently, several studies have shown great trends to find good alternatives to replace synthetic polymer wall material with natural polymer. However, none of the study has utilized commercial fish scale hydrolyzed collagen, Cc, as a coating agent Therefore, the aim of this study is to analyze physicochemical properties and functional properties of Cc, as a coating agent. Response surface methodology was employed to optimize the experiment using the spray dryer method. Physicochemical and functional properties analysis was done on the commercial fish scale hydrolyzed collagen before drying (Cc) and after drying (Cs). Two factors have been optimized, including inlet temperature and flow rate, the results showed 138 °C and 7 % respectively. Amino acid analysis showed both samples have glycine as the amino acid primary components followed by proline and hydroxyproline with 362.78±6.35, 109.32±4.80, 105.73±7.84 respectively for Cc. Meanwhile, Cs shows slightly low value with glycine 348.68±2.44, proline 116.21±2.16, and hydroxyproline 101.72±0.83. FT-IR indicates some changes on the intensity and wavenumber of amide A, amide B, amide I, II, III between both samples. Analysis on the surface morphology by the SEM visualized Cs have much smaller and more shrinkage particles compared to Cc. DSC analysis showed high thermal transition temperature at 107.87 C° for Cc while Cs resulted at 101.04 C°. Furthermore, solubility analysis and zeta potential analysis proved a parallel result with respect to pH where the

isoelectric point was at pH 4. No significant trends were observed in both emulsifying activity, EA, and emulsifying stability, ES. EA value for Cc and Cs were 61.11% and 59.67% respectively, whilst ES were slowly decreased by the time for both samples. The overall observation showed that commercial fish scale hydrolyzed collagen has the potential to be used as wall material due to their ability to remain stable even under extreme conditions such as extreme temperature and extreme pH conditions. Microencapsulation is a useful tool to protect sensitive substances or materials from the environment. There are many methods that have been published to conduct the microencapsulation process. However the selection of suitable wall materials also contributed to their successfulness. The objectives of this study were to optimize the spray-drying process in terms of concentration, inlet temperatures and the flow rate for encapsulation of folic acid and to investigate the potential of commercial fish scale hydrolyzed collagen as a wall material to microencapsulate folic acid by using conventional spray drying and ultrasonic spray drying techniques. Conventional spray drying method (Esd) and ultrasonic spray drying method (Eus) were utilized to produce microencapsulation of folic acid by commercial fish scale hydrolyzed collagen. Morphological studies were done by using SEM microphotography and CLSM analysis. Both showed tolerable results where Esd visualized irregular shape compared to Esd which obtained smooth surface with a spherical shape. CLSM images showed both techniques managed to produce a matrix form of encapsulation. However, microencapsulation efficiency of Esd was much lower compared to Eus, their microencapsulation efficiency were in 50% and 85% respectively. In particle size distribution analysis, Esd has broader size distribution with polydisperse pattern, meanwhile Eus has narrower size distribution with monodisperse pattern. High velocities applied by conventional spray dryer affected

the morphology of the particle. FTIR analysis of Esd and Eus showed slight changes in the intensity of the functional group and the fingerprints structure compared to control. Thus, overall results showed encapsulation by ultrasonic spray dryer gave better results compared to conventional spray dryer, it can be concluded that commercial fish scale hydrolyzed collagen possesses high potential to be used as encapsulation agents for folic acid.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Microencapsulation is a technology that is widely applied in the field of food industries, cosmetics industries, agrochemicals, medicals as well as in pharmaceutical productions to control drug release in time, and in physiological environments (Dalmoro et al., 2013; de Azeredo, 2005; Silva et al., 2014). Mazloom and Farhadyar, (2014) defined microencapsulation as a technique to surround small particles or droplets with coating material to transform it into useful products. This technology is considered as the primary least cost packaging approach for active ingredients that provides full protection, masks off flavors, delivers and controls the release of nutrients (Leclercq et al., 2009; Onwulata, 2012).

Several methods have been applied in the microencapsulation process including, spray drying, freeze drying, spray cooling, spray chilling, molecular inclusion, coacervation, and liposome entrapment (Gharsallaoui & Chambin, 2007). In food industries, spray drying is the most common method used for encapsulation. Although many techniques have been developed for microencapsulation purposes, spray dryer technology is still preferable due to this technology is well established, inexpensive, easily accessible, easy to handle and straightforward (Gouin, 2004; Mazloom & Farhadyar, 2014).

However, some drawbacks of using this technique are; the temperature is not tolerable for encapsulation of some heat sensitive biomaterials such as vitamins, enzymes and oil (Klaypradit & Huang, 2008; Maa et al., 1998). Limited availability

of suitable wall materials is also a disadvantage of microencapsulation by using spray drying technology (Gouin, 2004). The high temperature applied during the drying process accelerates oxidation and produces harmful effect to the samples. To overcome the cons, Nizori et al., (2012) suggested that optimization of the feed temperature, air inlet temperature and air outlet temperature is a need for spray drying process.

Moreover, according to Dalmoro et al., (2013), ultrasonic-assisted atomization is a new technique to produce microencapsulated systems by a mechanical approach. The conventional atomizing nozzles apply pressure and high-velocity motion to shear a fluid into small drops. The ultrasonic atomizer relies only on the low- ultrasonic vibrational energy for atomization (Klaypradit & Huang, 2008). Advantages of using this technique are lower energy required, producing more homogenized emulsion droplets with ultrafine particles, less emulsifier utilization compared to other mechanical methods (Cascone et al., 2012; Mazloom & Farhadyar, 2014). The main application of ultrasonic atomization is to use of ultrasound energy to break up the drugs or carrier mixtures into microparticles (Park & Yeo, 2004; Rodriguez et al., 1999).

Selection of suitable coating materials, the emulsion characteristics, preparation of emulsions with core and coating materials, as well as drying process are primary factors that determine the effectiveness of encapsulation (de Vos et al., 2010; Parada & Aguilera, 2007; Graves & Weiss 1992). A previous report by Cascone et al., (2012) related the effectiveness of microencapsulation to the nature of the drugs, composition of the biopolymer, production method, and features of the obtained microparticles. Jafari et al., (2008) reported the selection of wall material combinations and types of encapsulating agents affect both the emulsion

characteristics and the particle properties after drying and during storage. A good encapsulating agent should include desirable characteristics like, high solubility, low viscosity, good emulsifying properties, blend in test, and gelling characteristics. Recently, few researchers led to look for new alternative wall materials or to produce new combinations of coating material (Charve & Reineccius, 2009).

Recently, increasing numbers of researchers shows their interest in exploring the suitability of collagen hydrolysates as a coating material. Several studies that utilized collagen hydrolysate as coating material, including the use of a protein hydrolysate emulsifier to produce microcapsules by (Traubel, Ehlert, Nehen, & Weisser, 2002), while Lee et al., (2009), produced two new drug carrier systems, namely collagen–alginate composite gel and collagen microspheres embedded in alginate gel for improving the performance of Glial cell line-derived neurotrophic factor (GDNF) – secreting HEK293 cells. Furthermore, Depypere et al., (2009) investigated the potential of porcine collagen hydrolysate as coating material and Ocak et al., (2011) reported the application of collagen hydrolysate as a wall material in the simple coacervation process.

According to Bui and Small, (2012) and Hau et al., (2008) water soluble vitamins such as ascorbic acid (AA), thiamin, riboflavin, vitamin B6 and folic acid will degrade during food processing process. Folic acid is a synthetic, and oxidised form of folate, a broad group of compounds with vitamin functionality which is used in supplements and added to food because of its stability and high bioavailability (Bakhshi et al., 2012; Tomiuk et al., 2012), but folic acid cannot be synthesized by humans and, thus, it must be ingested through the diet (Lopera et al., 2009). As mentioned before, folic acid undergoes degradation reactions when it is exposed to moisture, light, temperature, acid or alkaline medium and oxygen atmosphere during

processing or storage. Therefore, the encapsulation of this bioactive ingredient within inert matrices is an optional to improve its stability, to maintain its bioactivity within the food product and prevent it from degradation during commercialization Garcia-Perez et al., 2012).

Many studies have been done to improve the stability of folic acid such as the use ethyl cellulose microcapsules to protect and control the release of folic acid (Prasertmanakit, Praphairaksit, Chiangthong, & Muangsin, 2009), encapsulation of folic acid by electrohydrodynamic technology (Bakhshi et al., 2012), folic acid in Sodium Alginate-Pectin-Poly(Ethylene Oxide) (Alborzi, 2012), folic acid in food hydrocolloids (Pérez-Masiá et al., 2015).

1.2 Problem statement

The type of wall material has great influence in physicochemical properties and stability of microcapsule that is produced. However, some coating materials are not resistant to extreme conditions, have high molecular weight with low emulsifying properties and hardly solubilize in solvent that will influence their ability to be a good coating agent. Hence, commercial fish scale hydrolysed collagen was selected due to it have potential that can be utilised as a good coating agent.

Folic acid is sensitive material that can be destroyed when expose to several conditions such as acidic or alkaline environment, high temperature, light, pH and so on. Thus, by encapsulated folic acid in wall material will provide protections to avoid them from being damaged.

1.3 Objectives

1. To observe the influence of spray dryer on the physicochemical and functional properties of commercial fish scale hydrolyzed collagen.
2. To investigate the potential of commercial fish scale hydrolyzed collagen as a wall material to microencapsulate folic acid by using commercial spray dryer and ultrasonic spray dryer techniques.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction in microencapsulation

Some food materials, including vitamins, minerals, flavor, oil, are very sensitive to certain environments such as, high/ low temperature, light, pH, acidic or alkaline. Due to the sensitiveness give some difficulties to handle it especially during processing and storage. Thus, to avoid them from being degraded or destroyed, special protections need to be concerned. In food industry, microencapsulation is a common technique to protect these so call sensitive-environment-materials

Microencapsulation is a process of packaging solid, liquid, and gaseous materials in microscopic particle or small capsules for maximum protection, as targeted delivery and to controlled release of nutrients (Agnihotri et al., 2012; Champagne & Fustier, 2007 ; Leclercq et al., 2009).

The microencapsulation technology has been used by the food industry for more than 60 years (Desai & Jin Park, 2005). The history for this process had begun in the late 1930s where encapsulation was applied in a cleaner substitute for carbon paper and carbon ribbons as sought by the business machines industry. In 1950s, microencapsulation technique was used in production of paper and ribbons that contained dyes in tiny gelatine capsules. Those dyes will released due to the impact by a typewriter key or the pressure of a pen or pencil (Alagusundaram et al., 2009). Starting in the early 1980s, food processing industry has emphasised to process foods in ways that may preserve the beneficial structures and nutrients for proper biological functions when consumed (Onwulata, 2012).

2.1.1 Reason for microencapsulation

Betoret et al., (2011) mentioned that microencapsulation techniques provide protection to the materials of biological interest for example; small molecules and protein such as enzymes and hormones, to cells of bacterial, yeast and animal origin. Generally, microencapsulation was based on the need to protect the active ingredient from adverse environments using the coating (matrix) material and hence it can be applied in functional delivery system (Onwulata, 2012).

In functional delivery system, efficacious delivery of bioactive food components is not only the major problem that occurs during passage through the gastrointestinal tract but the deleterious circumstances during storage of the product were also the main hazardous (Figure 2.1) (de Vos et al., 2010; Wilson & Shah, 2007). Hence, the matrix material creates a microenvironment in the capsule that able to control the interactions between the internal and the external part and promising approach to preserve their native properties over time (Borgogna et al., 2010; Gonnet et al., 2010).

Numbers of reason for microencapsulation can be listed. In some cases, microencapsulation helps to protect the core from surrounding environment; light, temperature, pH, oxygen, chemical and odour, to reduce evaporation of a volatile core, to promote easier handling properties of the core and to control released of drugs or pesticides (Agnihotri et al., 2012; Desai & Jin Park, 2005; Wilson & Shah, 2007).

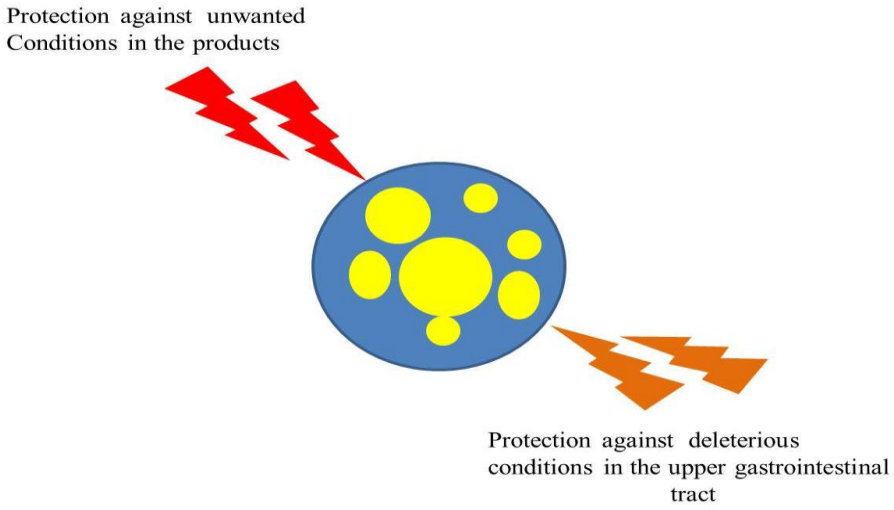

Figure 2.1: Capsules have to protect bioactive food components against: i) unwanted conditions during processing and storage of products and ii) against deleterious conditions during passage through the gastrointestinal tract.

2.2 Type of microencapsulation

Basically, microparticles or microcapsules consist of two main components, namely core material and wall or shell material (Figure 2.2). Core material can be defined as specific material to be coated (usually an active ingredient) and it can be either liquid or solid in nature whilst, wall or shell material are components that surround or coated the core material and it may consist of one or more material (Agnihotri et al., 2012; Jyothi et al., 2010). According to Wilson and Shah (2007), generally a hydrophobic core is usually protected by a hydrophilic shell, and vice versa.

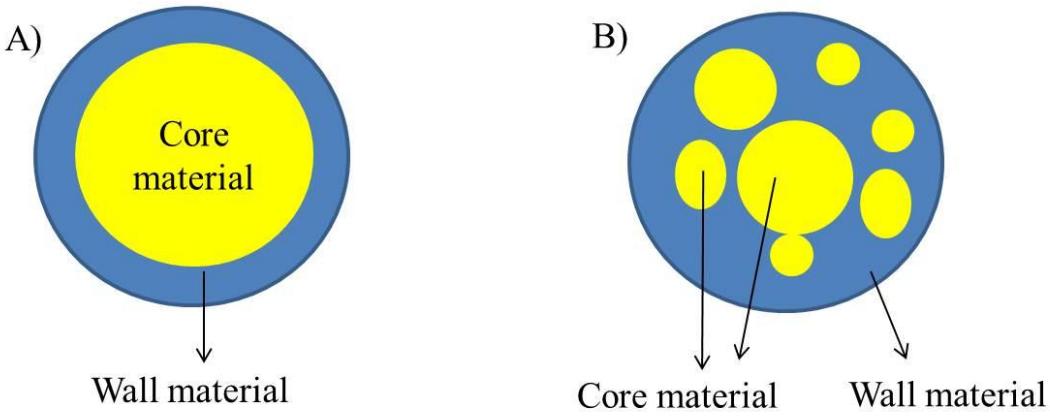

Figure 2.2: Schematic diagram of two representative types of microcapsules.

Capsules can be classified according to their size: macrocapsules (>5,000μm), microcapsules (0.2 to 5,000μm) and nanocapsules (<0.2μm). In terms of their shape and construction, capsules can be divided into two groups: microcapsules and microspheres. Microcapsules are the simplest structure where particles consisting of an inner core, containing the active substance, which is coated by a wall or membrane of uniform thickness. This microcapsule so called as a single-particle structure (Figure 2.3B). Mononuclear and polynuclear microcapsules can be differentiate by whether the core is divided. Commonly, polynuclear microcapsules have numerous core particles embedded in a continuous matrix of wall material (Figure 2.3C) (Desai & Jin Park, 2005; Favaro-trindade & Pinho, 2008).

In contrast, microspheres morphology is a matrix systems where the core contained small number of discrete droplets or particles that are dispersed uniformly in a polymer network. Microspheres might be in homogeneous or heterogeneous form depending on the core condition either in the molecular state (dissolved) or in the form of particles (suspended) (Figure 2.3D and Figure 2.3E). Those conditions are significantly impact the stability and release of the active ingredient (Gaonkar et al., 2014; Silva et al., 2014).

The term irregular is used for indented or wrinkled particles. However, irregular particles may still be spherical but not in perfect sphere shape (Figure 2.3A) (Vehring, 2008).

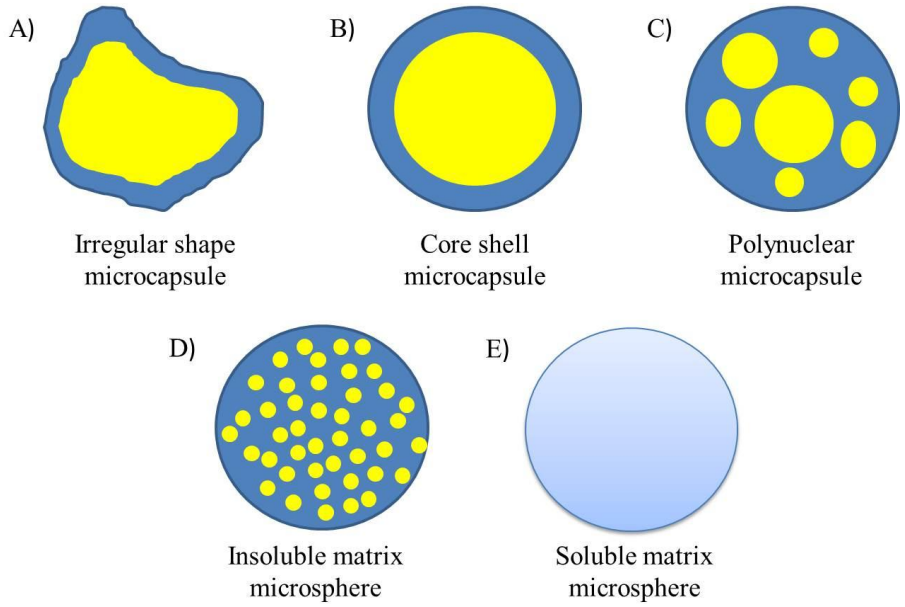

Figure 2.3: Schematic representation of microcapsules and microspheres morphology: A) irregular shape microcapsules, B) core shell microcapsules, C) polynuclear microcapsules, D) insoluble matrix microspheres and E) soluble matrix microspheres.

2.3 Process technology in microencapsulation

Variety of techniques have been applied for microencapsulation process, however, those techniques may broadly divided into two main categories; chemical methods and physical/ mechanical methods. The selection of the microencapsulation process is depends on the physical and chemical properties of core and wall materials and the intended application of final products.

Chemical microencapsulation methods are based on polymerisation or polycondensation mechanisms, in which starting materials are monomers or prepolymers. In these methods chemical reactions are also involved along with

microsphere formation and may be implemented in a variety of different ways. Interfacial method, dispersion method, emulsion polymerization and suspension polymerization were the optional methods for chemical microencapsulation. Among them, interfacial and in situ polymerisation processes received most scientific and industrial attention, and became an important alternative to coacervation microencapsulation processes (Boh & Frere, 2013; Dubey et al., 2009)

Mechanical techniques apply mechanical procedures for producing microspheres or microcapsules rather than well-defined physical or chemical approached (Li et al., 1988). These techniques are one of the most preferred types of mechanisms used in medical and biotechnological applications due to no chemical reaction were involved, and only used shape fabrication (Dubey et al., 2009). Whelehan and Marison (2011) explained on the principle of mechanical methods. Mechanical methods, such as cutting or vibration forces, were used to produce a droplet(s) from a polymer extruded through a nozzle (orifice). After done with the production, the droplets are immediately solidified to spheres or capsules by either physical technique such as, cooling or heating, or chemical means (gelation). Spray dryer, coacervation or phase separation, fluidized bed coating, supercritical fluid expansion and ultrasonic spray dryer can be categorized in mechanical techniques.

Table 2.1: Methods apply in microencapsulation.

Physical Method	Chemical Method
Fluidized bed	Interfacial polymerization
Spray drying	Solvent evaporation
Spray cooling/ chilling	Liposome
Extrusion	Coacervation
Coextrusion	In-situ polymerization
Spinning/ rotating disc	Phase separation
Molecular encapsulation	
Supercritical fluid expansion	

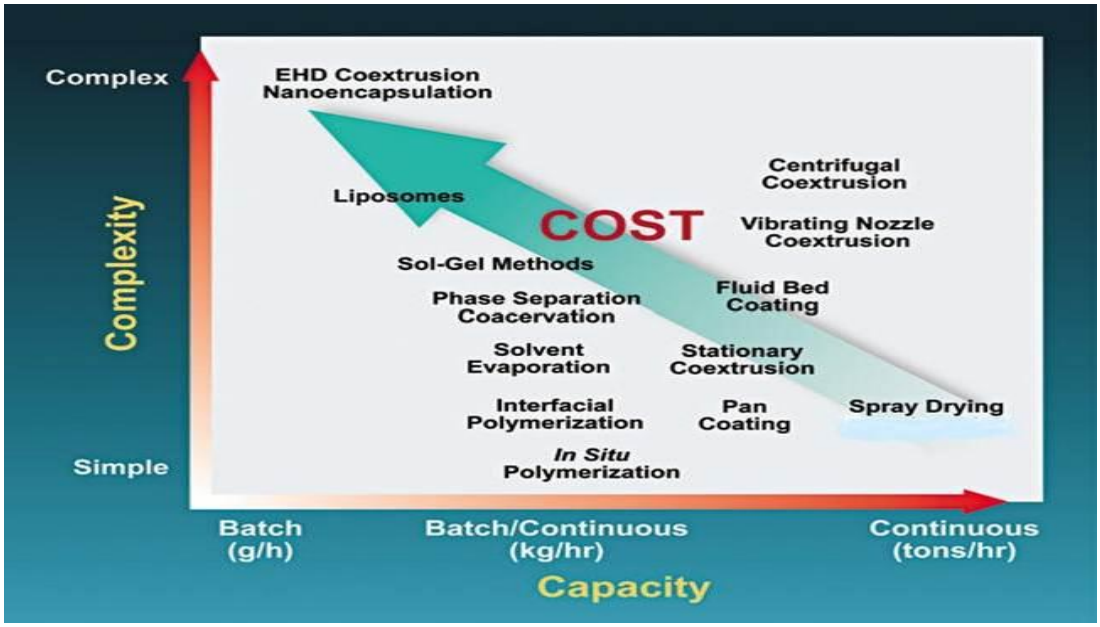

Figure 2.4: Different types of microencapsulation techniques according to ability of production capacity and complexity.
Source: (Gaonkar et al., 2014)

2.3.1 Spray dryer

Spray drying serves as a microencapsulation technique by conversion of suspension or solution (active material) into the dried particles (Agnihotri et al., 2012). This technique has been utilized in the food industry since the late 1950s to

protect flavour oils from degradation or oxidation and to convert liquids to powders (Gouin, 2004).

Spray drying is the most commonly techniques of encapsulation applied in the food industry, especially for vitamins, minerals, colourants, fat and oil flavour, aroma compounds, oleoresins and enzymes, because it offers advantages compared to conventional microencapsulation techniques (Poshadri & Kuna, 2010). Spray dryer offers simple and continuous process, economical, effective method for material protection and is most widely employed, particularly for flavours for which specialized equipment is not required (Poshadri & Kuna, 2010). Furthermore, spray drying may form two main types of structures in encapsulation process; single and multiple-core microcapsules (Onwulata, 2012).

According to Patel et al., (2009), spray dryer has some critical elements that need to be concerned, which is atomizer, air flow and drying chamber. Meanwhile, researched by Schwartzbach (2010) mentioned that three essential steps for basic spray drying; atomization, drying gas and droplet contact and lastly powder recovery. Briefly, during the spray drying process, solution feed was atomized to form the droplet before it contact with a hot gas. Once the droplets come into contact with the heated gas, the solvent in the droplets was evaporated and finally dry powdered products were produced (Afoakwah et al., 2012).

2.3.1.1 Spray dryer process

Material in the liquid state is sprayed into the drying chamber; low humidity hot gas is mixed with dispersed droplets. The spray of individual droplets is produced by the atomizer. Moisture in the form of vapour quickly evaporates from the suspended droplets due to simultaneous and fast heat and mass transfer process.

The conversion of the liquid droplet to the dried particle is accompanied by an approximate weight loss, 50 % loss of water and 25 % volume loss due to shrinkage. Droplets drying continue inside the drying chamber until desired particle characteristics are achieved. Separation of the dried particles from the drying gas and their subsequent collections take place in external equipment such as cyclone or filter house. Exhaust drying gas from the cyclone is discharged to the environment. Bag filter is installed to recover small particles from the exhaust drying gas in order to meet environmental laws for minimizing pollution (Figure 2.5).

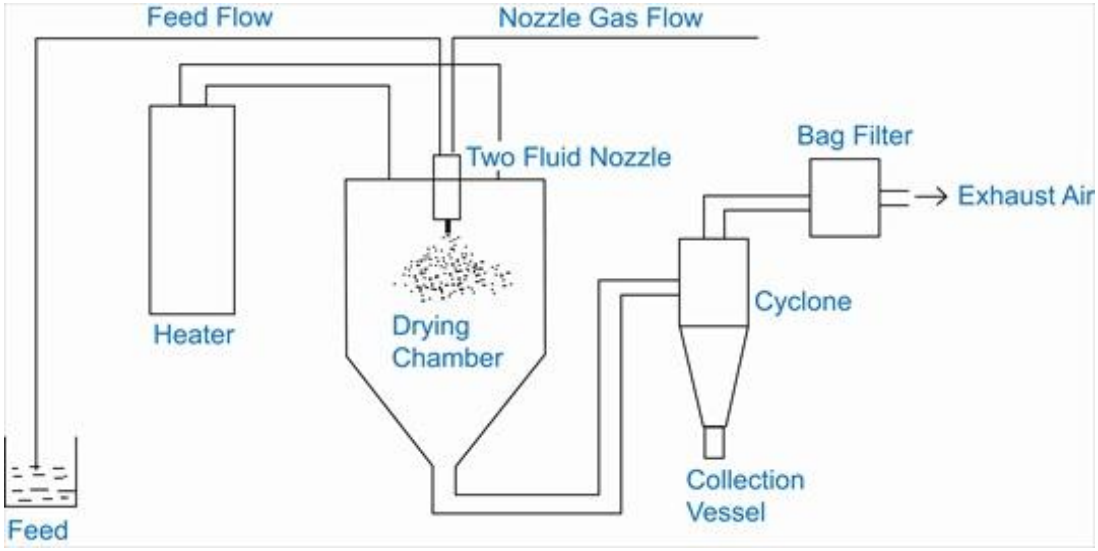

Figure 2.5: Design of the spray dryer.
Source:(Patel et al., 2009)

2.3.2 Ultrasonic atomization spray dryer

Recently, the application of power ultrasound has been explored for conventional hot air drying of different vegetables and fruits (Garcia-Perez et al., 2012; Mulet et al., 2003). Researched by Dalmoro et al., (2012) stated the ultrasonic atomization is achieved by several methods: by focusing high-frequency ultrasonic energy on the surface of a liquid in a bowl shaped transducer (0.4–10.0 MHz), by

feeding the fluid into the active zone of a whistle (8–30 kHz), or ultrasonically vibrating a surface over which the liquid flows (18–100 kHz). Study by Mason (2003) observed that ultrasonic spray technology was employed in industrial and research applications related to the electronics and biomedical fields, mainly for surface coating and liquid dispensing.

Conventional atomizing nozzles usually rely on pressure and high-velocity motion to shear a fluid into small drops, however, some drawbacks appeared including poor control over the mean droplet size, broad particle size distributions, and clogging risk (Dalmoro et al., 2013; Klaypradit & Huang, 2008). Differently, ultrasonic atomizers use only low ultrasonic vibrational energy; the ultrasound energy is transmitted with high efficiency to the liquid by a sonotrode for atomization (Dalmoro et al., 2013; Garcia-Perez et al., 2012; Klaypradit & Huang, 2008; Ramisetty et al., 2013). Thus, during drying the particles, ultrasound may intensify water removal without introducing a high amount of thermal energy (García-Pérez et al 2011).

Therefore, Albertini et al., (2005) proposed ultrasonic atomization as one of robust and innovative single-step procedure for microparticles preparation with scale-up potential. The main advantage of ultrasonic atomizer is the formation of droplets with a relatively uniform size distribution, which could lead to more homogeneous size distribution of microspheres and smaller particle sizes (Bittner & Kissel, 1999; Dalmoro et al., 2012; Jagannathan et al., 2011; Luz et al., 2007; Park & Yeo, 2004). Moreover, mechanical stress produced by vibration within ultrasonic processing is lower, thus, avoiding deactivation of bioactive substances, improved product quality, process improvement, and reduce the cost on a commercial scale (Dalmoro et al., 2013).

2.3.2.1 Ultrasonic atomization mechanism

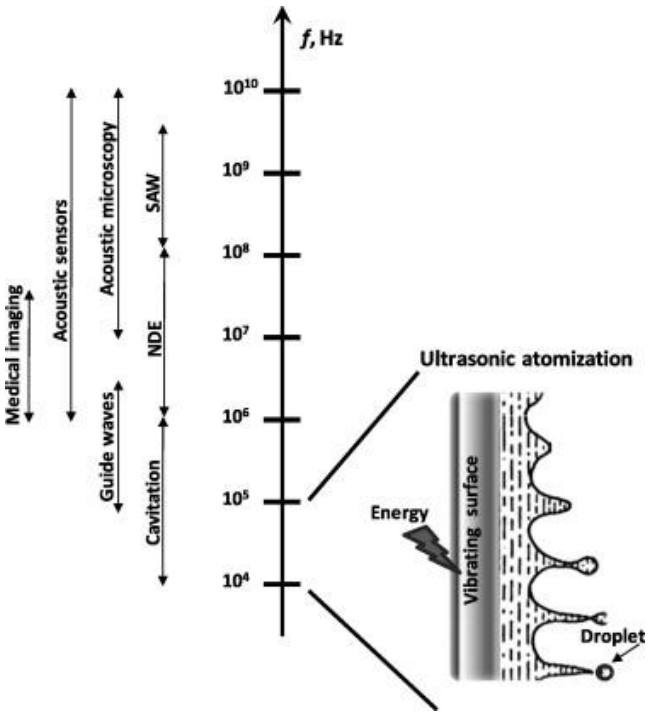

Figure 2.6: Ultrasonic frequency ranges, kind of applications, and ultrasonic atomization mechanism.
Source: (Dalmoro et al., 2013)

Ultrasonic atomization applied vibration energy or vibrating surface in order to form liquid threads and droplets (Figure 2.6). Cavitation and capillary wave mechanisms are the two rival theories in vibration energy (H. Liu, 1999; Mason & Lorimer, 2002; Ramisetty et al., 2013). According to Ramisetty et al., (2013) and Dalmoro et al., (2013), capillary wave hypothesis is so-called Taylor instability; atomization occurs when unstable oscillations split the peaks of surface capillary waves, composed by crests or peaks and troughs, away from the liquid bulk. Since the drops are produced from the peaks, their sizes are proportional to the wavelength.

Meanwhile, droplet formation for cavitation wave exist at both high-energy intensity and frequency which are generated by the ultrasound wave when it passes through the liquid medium (Figure 2.7) (Canselier et al., 2002; Dalmoro et al., 2013;

Hielscher, 2007; Ramisetty et al., 2013). Ashokkumar (2011) briefly explained, acoustic cavitation as ‘‘growth and collapse of micro-bubbles under an ultrasonic field’’. Sound waves dissipate part of the acoustical energy into heat energy while passing through the medium, (depending upon viscosity and conductivity of the medium) (Canselier et al., 2002).

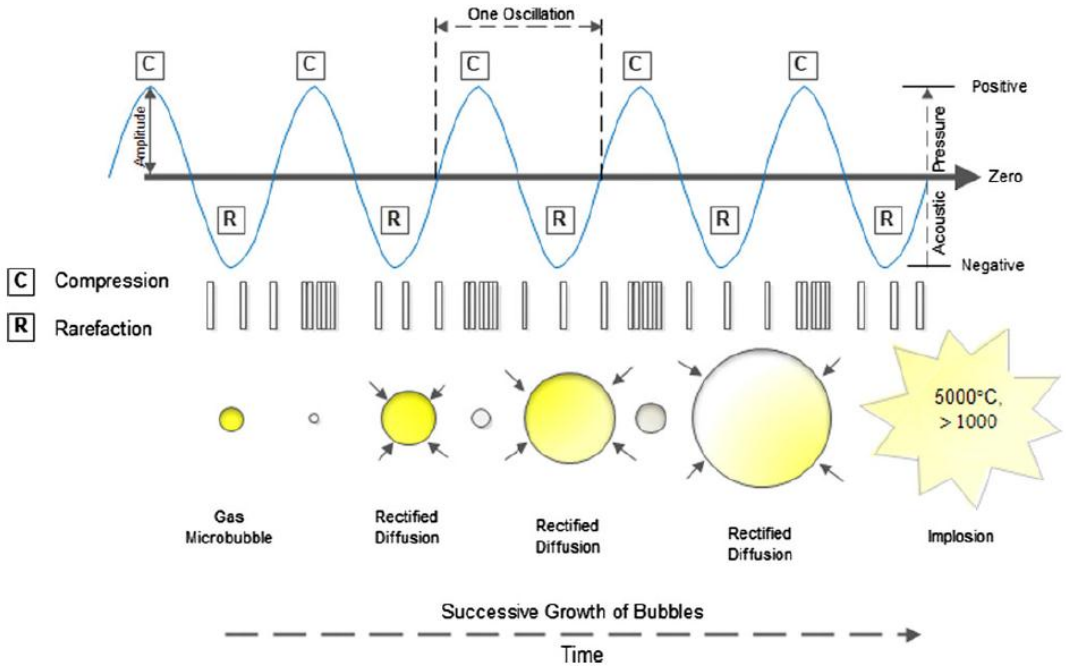

Figure 2.7: Growth and collapse of bubble in acoustic cavitation process.
Source: (Abbas, Hayat, Karangwa, Bashari, & Zhang, 2013)

2.3.3 Interfacial polymerization method

Interfacial polymerizations involves step polymerization between two highly reactive monomers, which are dissolved respectively in two immiscible phases, and those reaction occur at the interface of the two liquids (Karode et al., 1998; Nagavarma et al., 2012). In simple explanation, the polymer formed at the water-oil interface and precipitated to produce the nanocapsule shell. This technique is widely applied in production of synthetic fibres such as polyester, nylon and polyurethane (Thies, 2005). A multifunctional monomer (eg: acid chloride, isocyanate, or

combination of reactants) is dissolved in the core material, and this solution is dispersed in an aqueous phase that contain emulsifier (eg: lignosulfonate). A reactant to the monomer is added to the aqueous phase, and polymerization quickly ensues at the surfaces of the core droplets, forming the capsule walls (Figure 2.8) (Nagavarma et al., 2012).

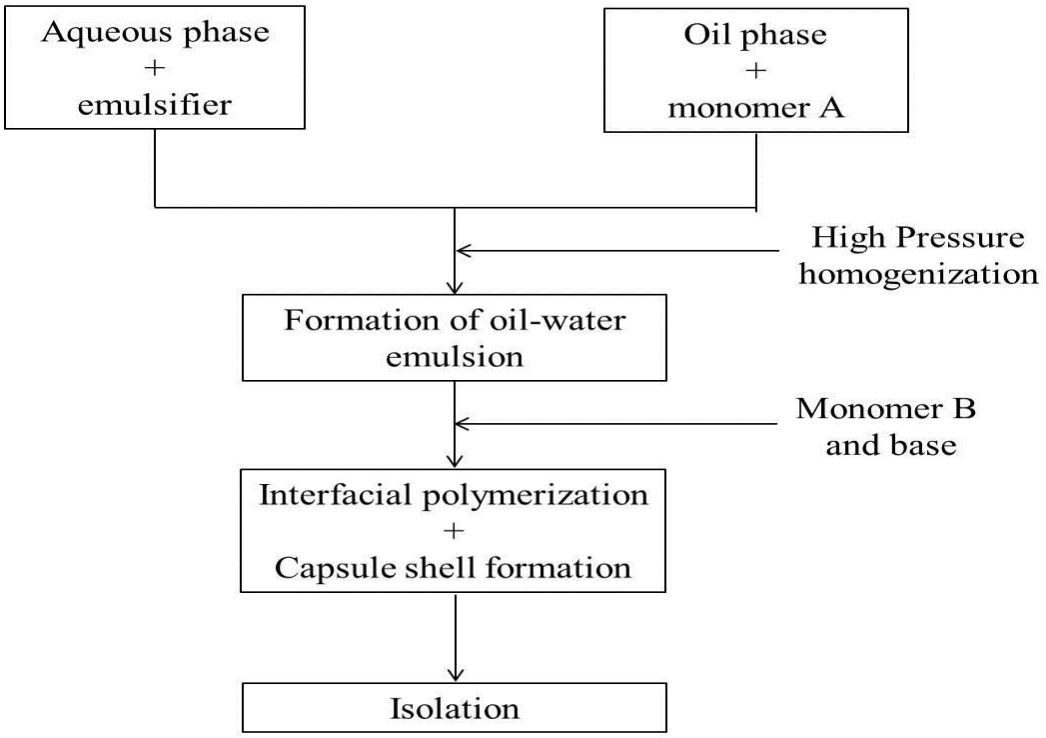

Figure 2.8: Flow chart of typical interfacial polymerization encapsulation process.

2.4 Materials for microencapsulation

2.4.1 Selection of wall materials for microencapsulation

The key success of microencapsulation technology is governed by the right selection of the wall material, the core release form and the encapsulation method (Silva et al., 2014). It is very critical to select suitable wall materials because it influences on the encapsulation efficiency and stability of the microcapsules. Huynh

et al., (2008) suggested by selecting appropriate encapsulating agents at a suitable concentration may help in optimize the efficiency and effectiveness of the microencapsulation process.

To be an ideal wall material, some characteristics are required such as, economic viability, not reactive with the core, provide maximum protection to the core from unwanted or adverse conditions, capability to seal and keep the core inside the capsule and lack of unpleasant taste (Gharsallaoui & Chambin, 2007; Nazzaro, Orlando, Fratianni, & Coppola, 2012). However, majority of wall materials cannot provide all desired characteristics, thus, it need to be mix with two or more materials. Such materials can be choose from a wide variety of natural sources and synthetic polymers (Favaro-trindade & Pinho, 2008).

Although numbers of food grade coating material can be conceptually used as a candidate for the microcapsule shell material, but only small number of different shell materials were utilized. This is due to the shell materials for food products are limited to materials that are approved by the US Food and Drug Administration (FDA) (Gaonkar et al., 2014). Table 2.2 listed some of the common wall materials used in the microencapsulation process and their applications.

In the last few years, the development of alternative, low cost and natural polymeric wall materials for microencapsulation have gained interest from researchers. (Elzatahry & Mohy Eldin, 2008; Ocak, 2012) suggested the use of natural and synthetic polymers in the formulations of wall material of the capsule. However, it is not recommendable to use synthetic polymers in the food industry since they may cause toxicity problems some of them do not undergo biodegradation at all. Thus, natural polymer has received considerable attention because it offers

environmental concerns and safety and more economical (Bazo et al., 2013; Haroun, 2010).

Table 2.2: Example of wall materials with their application.

Wall materials	Encapsulation process	Regulatory status	Applications
Gelatin	Spray drying	Edible	Vitamins
Gum arabic	Spray drying	Edible	Food flavors
Maltodextrin	Spray drying and desolvation	Edible	Food flavors
Gelatin-gum arabic	Complex coacervation	Nonedible	Carbonless paper
Ethycellulose	Polymer incompatibility	Edible	Oral pharmaceutical

2.4.1.1 Protein

Proteins not only widely used in the food industry but also in other fields including drug and nutrient delivery, due to their functional properties are suitable for encapsulation (Charve & Reineccius, 2009). According to Yeo and Park (2004), several strategies have been developed to improve protein microencapsulation, such as variations in formulation parameters, development of different polymeric systems, and modifications of the existing encapsulation methods. Unlike synthetic polymers, proteins exhibited lower toxicity and good degradability, which made them an attractive candidate in the food and pharmaceutical industry (Jahanshi & Babaei, 2012).

Proteins may be derived from the animal base or vegetable base and microencapsulation of protein are exploited depending on its sources. Soy proteins, wheat gluten, zein or corn protein, rice-bran protein, pea protein and cereal proteins

from vegetables can be classified in vegetables protein. Meanwhile, animal-derived proteins include gelatin, collagen hydrolysate, casein, whey, and egg albumin (Gaonkar et al., 2014; Nesterenko et al., 2013).

Trending in the use of vegetable protein represents the demands for renewable source, acceptability in natural food components and even healthy diets (F. Liu, Chen, & Tang, 2014). Soy protein isolated (SPI), has gained much attention compared to others, perhaps because of their easy availability. A study by Cho et al., (2007) showed that SPI is produced from defatted soy meal by alkali extraction followed by acid precipitation (pH 4.5) and has higher protein contents (490 %). Favaro-Trindade et al., (2010), Molina Ortiz et al., (2009) and Nesterenko et al., (2013) suggested the application of soy proteins as the encapsulating wall materials. Furthermore, SPI has good functional properties of encapsulation, such as emulsification, solubility, film-forming and water binding capacity, in addition to high nutritional value and generally recognized safe (GRAS) attributes. However, it still have several limitations, thus, modify the properties of this protein help to improve their effectiveness as wall materials in microencapsulation (F. Liu et al., 2014).

Ahmad and Benjakul, (2011) defined gelatin as protein obtained from collagen by heat denaturation. Gelatin is derived from the skins, hides, and bones of bovine and porcine. It has good functional properties, enhance the elasticity, consistency, and stability of food products, increase protein content and also may be used as an outer film to protect against drying, light, and oxygen (Montero & Gomez-Gullen, 2000). Additionally, due to its good properties such as emulsification, ability to film-formation, water-solubility, edibility and biodegradation, gelatin is a good choice as wall material for microencapsulation (Su

et al., 2008). Based on the research findings by Cheng et al., (2009), gelatin microcapsules were proved to be non-cytotoxic, hence it is safe to be use in food industry and pharmaceutical industry.

Collagen hydrolysate is a polypeptide composite obtained by enzymatic proteolysis of collagen with average molecular weight ranging from 3 to 6 kDa (Figure 2.9) (Peres et al., 2012). Collagen hydrolysate may derive from few sources including porcine, bovine and marine life. Numbers of studies have done on finding new sources for collagen hydrolysate including collagen hydrolysate derived from bovine limed split wastes (Zhang et al., 2006), Spanish mackerel skin (C.-F. Chi et al., 2014), fins, scales, skins, bones and swim bladder of bighead carp (D. Liu, et al., 2012), and chicken skin (Zeng et al., 2013). Collagen hydrolysate can be considered to be an use in functional food field due to its high nutritional value, high antihypertensive activity, and low antigenicity (Peres et al., 2012; Zhang, et al., 2006). Furthermore, more applications of collagen hydrolysate has been investigated, including as an anti-fatigue and antioxidant (Chi et al., 2014; Ding et al., 2011; Giménez et al., 2009), treatment of osteoarthritis and osteoporosis (Roland W. Moskowitz, 2000), neutraceutical (Z. Zhang et al., 2006) and functional food (Zague, 2008).

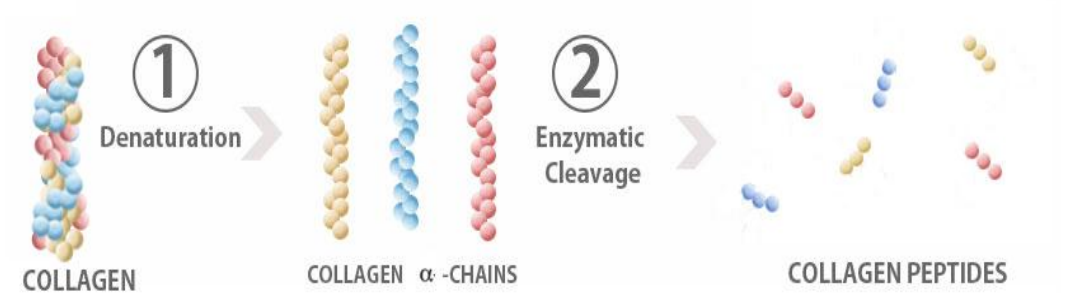

Figure 2.9: Synthesis of collagen hydrolysate.

2.4.1.2 Maltodextrin

Maltodextrin is a common wall material used in microencapsulation of food ingredients which produced by partial hydrolysis of starch with acid or enzymes (Gharsallaoui & Chambin, 2007; Goula & Adamopoulos, 2012). It has advantages properties such as relatively reasonable price, ability to provide good protection against oxidation, neutral taste and aroma and low viscosity at high solids concentrations (Fernandes, Borges, & Botrel, 2014). However, the greatest limitation of maltodextrin is its low emulsifying capacity and marginal retention of volatiles so, it is generally used in combination with other wall materials (Fernandes et al., 2014; Carneiro et al., 2013; Tontul & Topuz, 2014).

2.4.1.3 Gum Arabic

Gum arabic is highly branched complex with heteropolyelectrolytes formed principally by L-arabinose and D-galactose, and minor proportions of 4-O- methyl-D-glucuronate, and L-rhamnose with ratio 4:2:1:1 (Román-Guerrero et al., 2009). It contain small amount of protein (1.0 % to 2.0 %), that responsible for the emulsifying and film forming properties (Trejo-Espino et al., 2010). Gum Arabic is one of the most popular wall materials used in microencapsulation; especially in spray drying method. Presence of many desirable characteristics as a good encapsulating agent such as high solubility, low viscosity, good emulsifying properties, and protect volatiles against losses during processing and storage contributes on the rising of the demand for this product. Due to the limited in supply, as well as the increasing cost, leading for finding other alternative wall materials or may be used in combination with it (Charve & Reineccius, 2009).

2.5 Microencapsulation of vitamin for food applications

Inclusion of vitamins loaded particles in food matrix will produce functional food or nutraceutics. This type of functionality will imply the use of food-grade or GRAS (Generally Recognized As Safe) components for particles formulation (Gonnet et al., 2010). Recent studies by Bui and Small (2012) and Hau Fung Cheung et al., (2008) classified ascorbic acid, riboflavin, thiamine, Vitamin B_6 and folic acid as water soluble vitamins. Meanwhile, Amidzic et al., (2005) reported riboflavin, thiamine, nicotinic acid, pyridoxine, pantothenic acid, folic acid, biotin and cyanocobalamine were member of vitamin B even though their chemicals structures are not related.

2.6 Folic acid

Folic acid may be referred as pteroyl-L-glutamic acid, Vitamin M and vitamin B_9, is a water-soluble vitamin. Molecular formula for folic acid is $C_{19}H_{19}N_7O_6$ and their chemical structure composed of three primary structures, a hetero-bicyclic pteridine ring, *p*-aminobenzoic acid and glutamic acid (Figure 2.10). Under synthetic and oxidised form, folic acid known as folate which is used in healthy products such as supplements and was added to some of food products due to their essential value and high bioavailability (Stevanovic et al., 2008; Tomiuk et al., 2012). Unfortunately, folic acid is very sensitive to the light and may degraded in the presence of ultraviolet light (Li et al., 2011) and subject to loss during food processing (Bui et al., 2008). However, under suitable conditions, folic acid remains stable and effective (Y. O. Li et al., 2011).