MICROENCAPSULATION OF VITAMIN C AND GALLIC ACID IN WHEY PROTEIN CONCENTRATE BY SPRAY AND FREEZE DRYING - CHARACTERIZATION AND DEGRADATION KINETICS

by

SHRIPAD RATNAKAR TANDALE

(Under the Direction of Manjeet Singh Chinnan)

ABSTRACT

Vitamin C and gallic acid were chosen as model antioxidants to be used as core material and whey protein concentrate (WPC) was selected as wall materials for microencapsulation. Microencapsulation methods used were spray and freeze drying. Vitamin C: WPC (w/w) ratios and gallic acid: WPC ratios (w/w) were 1:4.0, 1:5.3, 1:8.0, and 1:16.0 for both spray and freeze drying process. 1:4.0 ratio resulted in highest microencapsulation efficiency for both vitamin C and gallic acid and therefore chosen for accelerated storage study. The various factors selected for accelerated storage were temperature (25 and 45 ˚C), relative humidity (22, 44, 66, 85% RH), UV light and dark, type of microencapsulation method (freeze and spray drying). Maximum degradation was found at intermediate humidity level (66%) in vitamin C as well as gallic acid powder. Microcapsules produced were characterized using scanning electron microscopy (SEM) and particle size analysis (PSD). SEM revealed outer morphology and structure of capsules and PSD determined average diameters and modal distribution.

INDEX WORDS: Microencapsulation, freeze drying, spray drying, scanning electron microscopy, particle size distribution, storage study, vitamin C, gallic acid.

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DEDICATION

To

Lord Ganesha, Mom, Dad and my brother Aniruaddha!

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CHAPTER 1

INTRODUCTION

Functional foods

The recent advancements in food and nutritional science support the concept that diet plays a significant role in improved state of well-being, prevention and mitigation of certain diseases and important part of healthy lifestyle. There is an increasing trend of consumers' preference for functional food products because of variety of health benefits offered by the functional foods and growing awareness among the consumers about functional foods. It is estimated that functional food market in US alone is $ 30 billion and is growing at 20% annually. Functional foods include many nutritive compounds which offer some kind of health benefit/s to consumers. There are various functional food ingredients such as antioxidants, phytochemicals, vitamin A, β-carotene, ascorbic acid (vitamin C), vitamin D, vitamin E, soy isoflavones, omega 3 and omega 6 fatty acids particularly in fish oil, minerals such as calcium and iron, lutein, lycopene, co-enzyme Q_{10}, and probiotics. These functional food ingredients are added in various food products in order to impart specific health benefit that consumer desires.

Need of microencapsulation

The incorporation of these compounds into food products has many challenges. These functional food ingredients are susceptible to degradation due to environmental stress conditions like temperature, humidity, light, oxygen and its interaction with other food ingredients and therefore they loose their bioactivity and their nutritive value is compromised.

Microencapsulation is used as a tool in order to protect these sensitive bioactive compounds from degradation. Microencapsulation has the ability to facilitate protected and targeted nutrition in a number of processed food products. It is fast becoming the most successful delivery systems that enable food ingredient companies to tap into fast growing functional foods market.

Additionally, there is a need to any off flavors of these nutrients. Consumers expect great tasting products which are nutritious and seek for food products that offer convenience, good taste, flavor and texture in addition to nutritive value. Due to increasing preference of functional foods which accounts for substantial proportion of the global nutrition market, food companies are looking at various ways to incorporate health promoting compounds in their products to deliver some kind of health benefits. Microencapsulation technology is used by the food scientist to address formulation challenges and to deliver these nutrients at specific sites under controlled fashion.

Vitamin C

Vitamin C is having numerous health benefits and widely used in the food industry as anti-oxidant, vitamin supplement and as a preservative. It has various biological, pharmaceutical and dermatological functions. In United States, vitamin C market is segmented into cosmetic formulations, dietary supplement, animal feed fortification and food and beverage. The seven key manufacturers of vitamin C generated $ 151.7 million in 2005. Vitamin C is also called as L-ascorbic acid. This vitamin is synthesized by mammals but not by humans and therefore must be supplied in the diet. One of the principal biochemical reactions of vitamin C is to destroy toxic free radicals (hydroxyl and perhydrolxyl) Thus when terminating free radicals, L-ascorbic acid is converted into dehydroascorbic acid, which is again recycled into L-ascorbic acid by reductase enzyme and co-factors. Vitamin C is considered as an important anti-oxidant in

neutralizing these free radicals. Free radicals cause oxidative stress, involved in various degenerative diseases such as cancer, cardiovascular diseases, cataracts and Alzheimer's disease. Ascorbic acid is very reactive and is readily destroyed during food processing. Ascorbic acid rapidly oxidizes in the presence of copper and iron ions, at neutral and above pH, temperature, humidity and light conditions. Microencapsulation should aid in preventing interaction between metal ions such as iron with ascorbic acid and will improve bioavailability of ascorbic acid without compromising fortification with minerals.

Gallic acid

Muscadine grapes and blueberries have been reported to contain rich sources of polyphenolic compounds (gallic acid, p-hydroxybenzoic acid, caffeic acid, ferulic acid, and ellagic acid) and flavonoids (catechin, epicatechin, myricetin, quercetin, and kaempferol). These compounds, due to its free radical scavenging action, possess variety of health benefits such as protecting low density lipoprotein, preventing platelet aggregation and improving neuronal and cognitive functions in elderly due to age related dementia. We choose gallic acid as one of the model compound to microencapsulate considering high costs of other compounds and which will serve as model compound for encapsulating polyphenolic muscadine extract to produce free flowing, non-sticky powder to be marketed as dietary supplements and other food applications.

Spray drying is one of the most common methods of choice for microencapsulation. It is widely used for encapsulating food flavors. Advantages of spray drying include its low cost, wide choice of encapsulating material, easy scale up, good quality of microcapsules produced. Freeze drying is comparatively costly method but the high value of nutraceutical extract and ability to encapsulate heat sensitive compounds could justify such costs. Also high retention of volatile compounds favors the freeze drying process in certain applications. The completion of

this project could generate useful information which could be used to generate pilot scale production of microencapsulated muscadine extract powder and suitable storage conditions.

The objectives of the project were to

1) To microencapsulate vitamin C and gallic acid as model anti-oxidants by spray and freeze drying methods; to compare protective effectiveness of each method in terms of protection to anti-oxidant core.
2) To characterize the microcapsules produced by spray and freeze drying methods using scanning electron microscopy (SEM) and particle size analysis using Malvern Mastersizer.
3) To investigate degradation kinetics of microencapsulated gallic acid and vitamin C using accelerated shelf life testing and to generate information which will serve to produce microencapsulated muscadine grapes extracts as well as blueberry extracts which could be potentially used in various food applications.

CHAPTER 2

LITERATURE REVIEW

Health continues to be the number one driver of food industry and worldwide consumers are increasingly aware of the health benefits certain types of food provide. This is evidenced by growing nutraceuticals and functional foods market. There are numerous evidences suggesting the health promoting properties of polyunsaturated lipids, omega-3 fatty acids, phytochemicals, vitamins etc. In order to benefit the population from the health benefits these nutraceuticals and health promoting compounds are providing, these need to be fortified in the food. However, the major challenges in incorporating these compounds in food matrix are their susceptibility towards oxidative deterioration, these ingredients have unpleasant odor and it may affect the organoleptic and sensory properties of the foods in which they are incorporated.

Microencapsulation is the process of coating tiny droplets of liquids, solids or gases (core material) by thin film (coating) which protects the core material until it is subsequently released (Sheu and Rosenberg 1995). Microencapsulation is also aimed at controlling mass transfer between core material and the surrounding environment (Dziezak 1988; Jackson and Lee 1991; Arshady 1993; Shahidi and Han 1993). Permeability of gases (oxygen) and volatiles is also controlled through the wall system. The core can be released in a controlled fashion depending on the conditions required. Microencapsulation is an important technique for food and flavor industries for packaging flavors and sensitive ingredients to protect from heat, light and oxygen. Demand for microencapsulation within the ingredients industry has grown significantly in recent

years. The demand for encapsulation technologies is increasing at around 10% annually with new markets and opportunities opening up every year. (Source: www.foodnavigator.com).

L-Ascorbic acid as an anti-oxidant

L-ascorbic acid is also known as L-threo-2-hexenono-1, 4-lactone. Vitamin C or L-ascorbic acid is known to prevent scurvy. One of the major biochemical reactions of L-ascorbic acid is to destroy toxic free radicals (hydroxyl and perhydroxyl) in body resulting from metabolic products of oxygen. The mixture of L-ascorbic acid and its oxidation product is considered as 'redox buffer' (Sapper and others 1982). While quenching free radicals, L-ascorbic acid is converted into dehydroascorbic acid which is then recycled to L-ascorbic acid by reductase enzyme and cofactors. Besides redox function in cells, other physiological actions of ascorbic acid are related to compound's chelation with metals and complexing with proteins (Gorman and Clydesdale 1983; Fleming and Bensch 1983). One of the industrial methods of producing L-ascorbic acid was first devised by Reichstein and Grussner (Crawford and Crawford 1980). The second industrial method of production of ascorbic acid is based on production of key intermediate L-xylo-2-hexenonic acid (2-keto-L-gluconic acid) by single recombinant bacterium (Anderson and others 1985) or step-wise fermentation using two organisms.

L-ascorbic acid is one of pair of enantiomers having 2-hexenono-1, 4-lactone structure. The other pair is D and L-isoascorbic acid which are also referred to as D and L erythorbic acid. Based on L-ascorbic acid with 100% Vitamin C activity, other three stereoisomers namely, L-isoascorbic acid, D-isoascorbic acid and D-ascorbic acid have 0-5% activity (Hay and others 1985). D-isoascorbic acid can be substituted for L-ascorbic acid in most functional uses in foods. But the bread improving function of L-ascorbic acid is strereospecific. Also use of D-ascorbic acid in foods is regulated due to its interference with bioavailability of L-ascorbic acid (Hornig

and others 1974). Ascorbic acid is widely used in various types of foods such as canned or bottled fruits, vegetables, beer, wine and other beverages to prevent the development of oxidative off-flavors and off-colors, especially during pasteurization (Bauernfeind 1982). Here, oxygen in the headspace is reduced to water by ascorbate. According to Kirby and others (1991), the significant loss of this vitamin is resulted during processing and storage of foods in the presence of oxygen and metallic ions such as iron and copper. In order to preserve the Vitamin C in foods, chemically modified form of ascorbic acid is added to foods to resist oxidation. L-ascorbate 2 phosphate is at least 10-20 times more resistant to oxidation than L-ascorbic acid (Sieb 1985). The commercial forms of ascorbic acid available to the food industry are L-ascorbic acid and its sodium and calcium salts and 6-palmitae ester.

One electron reduction by L-ascorbic acid

The most important chemical property of L-ascorbic acid is its ease of oxidation by either one or two electron transfer. When one electron is transferred in rate limiting reaction, the first product formed is L-ascorbic acid radical, which is also called as monodehydroascorbic acid radical. L-ascorbic acid is a strong acid with pK0.45. The free radicals that oxidize lipids and other readily oxidized compounds in foods and tissues are terminated by reduction with L-ascorbate radical. Thus the oxidative rancidity in lipids is initiated when free radical attacks lipid molecule to give alkyl radical. This initiation step is terminated by L-ascrobyl-6-palmitate. Thus ascorbyl palmitate and L-ascorbic acid acts as typical anti-oxidants and prevent peroxide formation in lipids and delay the onset of chain reaction that causes deterioration of vegetable oils, animal fats, fish, milk, Vitamin A, carotenoids (Cort 1982). As an anti-oxidant L-ascorbic acid reacts with superoxide radical, perhydroxyl radical, hydroxyl radical and singlet oxygen. Ascorbyl palmitate is more effective anti-oxidant when used in combination with tocopherol

(Cort 1982). Tocopherol first reacts with free radicals and Ascorbyl palmitate then reduces tocopherol radical to regenerate tocopherol and give ascorbate radical (Wayner 1986). The regeneration of tocopherol persists until all ascorbate is consumed.

Two-electron reduction of dioxygen by L-ascorbic acid

The reaction of L-ascorbate with dioxygen in the presence of transition metal ions is very important reaction. The transition metals such as cupric and ferric ions catalyze the autoxidation by joining L-ascorbate and oxygen in ternary complex (Martell 1982). The two Π-electrons from L-ascorbate in the complex are proposed to shift to oxygen through transition metal ions. The complex dissociates into dehydroascorbic acid, hydrogen peroxide and metal ion. In most foods dehydroascorbic acid is rapidly hydrolyzed to 2, 3-diketogluconic acid, this reaction being non-reversible results in loss of vitamin C in foods.

The metal catalyzed reaction between oxygen and L-ascorbic acid increases when acidity is decreased from pH 1.5 to 3.5. Autoxidative loss of L-ascorbic acid accelerates above pH 7.0. A variety of metal chelating agents are known to retard the autoxidation of L-ascorbate such as ethylenediamine tetraacetate (EDTA), oxalate, citrate, phosphates, uric acid, sugars, acidic polysaccharides and flavonoids. Proteins, histidines may also retard autoxidation. Possible complexing between proteins and L-ascorbic acid is known from activation of myrosinase with L-ascorbic acid (Fleming and Bensch 1983).

Gallic acid as model anti-oxidant

Rabbiteye blueberry (*Vaccinium ashei*), common in Georgia, is a rich source of various health promoting compounds like phenolic acids (gallic acid, p-hydroxybenzoic acid, caffeic acid, ferulic acid, and ellagic acid) and flavonoids (catechin, epicatechin, myricetin, quercetin, and kaempferol) (Sellapan and Akoh 2002). These compounds neutralize free radicals associated

with number of degenerative diseases such as cancer, cardiovascular diseases, cataracts and Alzheimer's. However, these compounds are sensitive to degradation with exposure to environmental conditions; such as temperature, oxygen, light and relative humidity. Rabbiteye blueberry contains 259 mg/100 gm of gallic acid (a major phenolic acid). Intermediate extract of muscadine grape contains sugars which makes it difficult to handle and may stick on the walls of the spray dryer. High molecular weight compounds like hydrocolloids such as modified starch, gum Arabic or proteins such as whey proteins will serve as coating material and make it non-sticky powder. We chose gallic acid as model core material for microencapsulation considering very high cost of other pure compounds. Two major protein groups of bovine milk are whey protein and casein. Whey is derived as natural byproduct of cheese-making process. In addition to proteins, the raw form of whey contains fat, lactose and other substances. The raw form is processed to produce protein rich whey protein concentrate (WPC) and whey protein isolate (WPI). Whey proteins have been reported to have excellent encapsulation properties and are superior to those of commonly used ingredients. These studies have indicated that whey proteins were very effective in encapsulating volatile, non-volatile, polar and non-polar compounds (Moreau and Rosenberg 1996, 1998, 1999; Sheu and Rosenberg 1995; Young, Sarda and Rosenberg 1993). Microencapsulation of gallic acid will help in understanding the protective effect offered to the similar phenolic compounds under various environmental stress conditions.

Selection of wall material

The first step in encapsulating food ingredients the selection of suitable coating material which is commonly called as shell material, wall material, carrier or encapsulation matrix.

These coating materials are film forming substances which are selected from various natural and synthetic polymers depending upon the core material to be encapsulated and desired characteristics in the microcapsule.

Ideal coating material should have the following characteristics (Shahidi and Han 1993).

- Good rheological properties. It should possess low viscosity at high solids concentration to facilitate in encapsulating higher load of core (Reineccius 1988).
- The ability to emulsify the core and stabilize the emulsion.
- Solubility in solvent e.g. water, ethanol.
- Ability to seal and hold the active material within its structure during processing and storage.
- It should be non-reactive to the core during high temperatures in processing as well as during the storage.
- Should be non-toxic and generally recognized as safe (GRAS).
- Complete release of the core under desired conditions.
- Ability to protect maximum protection to core material against environmental condition (light, heat, humidity etc) from oxidation, reactivity to food components etc.
- Should produce desired capsule solubility properties and active material release properties.
- It should be economical and stable in supply.

The ideal carrier/shell material should have good emulsifying properties, should be able to form good film, have low viscosity (Reineccius 1988). At 35-40% range, should exhibit low hygroscopicity and should release the coated material in controlled manner or when desired, should be low in cost, bland in taste, stable in supply and should not interact with the core

material and be non toxic. Since no single coating material can meet all of the criteria listed above, in practice, combinations of coating materials are used or modifiers such as oxygen scavengers, antioxidants, chelating agents and surfactants are added. Chemical modification of the existing coating materials is also considered.

Maltodextrin and glucose syrup solids

Maltodextrin and glucose syrup solids have excellent oxidation protection but they exhibit poor retention of volatiles (Qi and Hedges 1995). It is considered that since maltodextrins and corn syrup solids have no emulsification properties, they produce coarse emulsion that results in poor flavor retention during drying (Reineccius and Risch 1986). The major shortcoming of maltodextrins is virtual lack of emulsifying capacity and low retention of volatile compounds (Reineccius 1991). Maltodextrins and corn syrup solids offer varying degree of protection to the core material from oxidation depending on DE of hydrolyzed starch. The product with highest DE is extremely stable and would have shelf life of years without using anti-oxidants. High DE maltodextrin protected encapsulated orange peel oil against oxidation, thus effect of DE on the functionality of wall system is observed (Anandaraman and Reineccius 1986). It has been suggested that higher DE systems are less permeable to oxygen and therefore, offer better protection to encapsulated ingredient (Reineccius 1991). The mono and disaccharides are very inexpensive and are used in blends with chemically modified starch and are used in blends with chemically modified starch or gum acacia. These are used to reduce cost or improved protection against oxidation.

Modified Starch

Modified starches which are used in encapsulation are chemically modified to incorporate lipophilic groups into their molecules. The modified starch is produced by adding octenyl

succinate at 0.02 degree substitution. This results in modified starch with excellent emulsification properties. Modified starch provides excellent retention of volatiles during spray drying. Additional benefit of modified starch since it is substantially less viscous than gum acacia is that it can be used at higher infeed solids level upto 50% whereas gum acacia cannot be used at higher than 35%. The high solids level reduces the loss of encapsulated ingredients and increases spray dryer output. Modified starch also has excellent emulsion stability. Solution of gum acacia produced the average emulsion of about 3 μm and modified starch gave droplets of about < 2 μm. The emulsions made with modified starch were more stable than with gum acacia.

Cyclodextrins

Cyclodextrins are cyclic molecules that are produced enzymatically from starch. Cyclodextrins have the ability to entrap guest molecules within its ringed structures. Cyclodextrins started its application in food industry in 1970s when Japan and Hungary began its use. They are produced from starch by selected microorganisms such as *Bacillus macerans* and *B. circulans*. These organisms contain cyclodextrin glycotransferase (CGTase) that converts partially hydrolyzed starch into cyclic dextrins containing six (α), seven (β), or eight (γ) glucose monomers. These monomers are connected to each other in a doublenut shaped ring, giving cyclodextrin a molecular structure that is relatively rigid and a hollow cavity of specific diameter and volume. The polar hydroxyl groups are oriented to outside thus imparting hydrophilic character and internal cavity is hydrophobic in nature because of its high electron density due to hydrogen and glycosidic oxygen oriented towards interior of cavity. Thus due to hydrophobic nature of the cavity the molecules of suitable size, shape and hydrophobicity are able to interact noncovalently with cyclodextrins to form inclusion complexes. β-cyclodextrins are able to form inclusion complexes with flavor substances with molecular mass between 80 and 250. Linder (1982)

reported that molecules of nearly all natural spices and flavors fit into this range. Cyclodextrin complexes protect the ingredients from oxidation, light induced reactions, thermal decomposition and evaporation loss. Spices that have been complexed with cyclodextrins have shown controlled release. Thermal stability was also increased when fats were added to them. Cyclodextrins have been found to preserve the flavors of cookies, vegetable pastes, biscuits, citrus fruits, Japanese onions, garlic and celery.

Sucrose

Sucrose is used as carrier material because of following properties

- Quick dissolution in water, producing clear turbidity free solution.
- Heat stability and non-hygroscopicity
- Indefinite shelf life under ambient condition
- Low cost

Sucrose along with maltodextrin is used as main coating material used in extrusion encapsulation process. Mixtures of sucrose ad maltodextrins are commonly used coating for extrusion encapsulation. It is also used for encapsulation of flavors with process of co-crystallization. Structure of sucrose is modified from single perfect crystal to microsized, irregular, agglomerated form before co-crystallization happens. This modified structure has an increased void space and surface area and provides a porous bed for the incorporation of active material.

Chitosan

Chitosan is the principle product of the alkaline hydrolysis of chitin, main constituent of exoskeleton of crustaceans such as crabs. Complex coacervate capsule formation takes place between chitosan, a cationic polyglucosoamine and carageenan or alginic acid which is anionic in nature. The gelling properties of chitosan allows wide range of application in food and

pharmaceuticals in gel entrapment of biochemicals, plant embryos, whole cells, microorganisms or algae. This entrapment allows microencapsulation and controlled release of flavors, nutrients and drugs. Polycationic chitosan molecules can be incorporated with oppositely charged polymers to form coacervate having good mechanical strength. The permeability of these coacervate capsules can be controlled by altering type of chitosan and counterion.

Cellulose

Cellulose is a main constituent of plant cell walls and it has been used as dietary fiber. Cellulose has also been investigated for food preservation and as an edible film for food coating has been studied. The permeability of cellulose can be changed by combination with other coating materials (Vodjani and Torres 1990). It was found that methyl and Hydroxypropyl methylcellulose mixed with lauric, palmitic, stearic and arachidic acids significantly lowered permeation rate compared to cellulose ether films containing no fatty acids. Cellulose is used in encapsulation of water soluble food ingredients like sweeteners and acids. Cellulose is also used for encapsulation of enzymes and cells (Poncelet 1989).

Gums

Hydrocolloids are gums which are long chain polymers that dissolve or disperse in water to give thickening or viscosity building effect. Gums are generally used as texturing ingredients. They are also used for stabilization of emulsion, suspension of particulates, controlling crystallization and inhibition of syneresis. Various sources of gums include plant materials such as seaweed, seeds and tree exudates; others are products of microbial synthesis, and also chemically modified natural polysaccharides.

Gum arabic

Gum arabic is used as an encapsulating material because of its low viscosity, solubility and emulsification properties. And its good retention of volatile compounds makes it suitable for many encapsulating methods. Also wall material is ideally suited for encapsulation of liquid droplets because of its role as surface active agent and drying matrix. The application of gum arabic within food industry is limited because gum arabic is more expensive than maltodextrin (Kenyon 1995**;** Shiga and others 2001) and its availability and cost are subject to fluctuations. As an alternative mixtures of gum arabic and maltodextrin have been tried and it has been promising for use in high solid carriers, yielding acceptable viscosities in studies of microencapsulation of cardamom oil by spray drying (Sankarikutty and others 1988). When a mixture of ethyl propionate, ethyl butyrate, orange oil, cinnamic aldehyde and benzaldehyde was encapsulated in a blend of gum arabic and maltodextrin a general trend of increase in retention of core was observed when gum arabic fraction was increased (Reineccius 1991). Spray dried particles formed by mixtures of maltodextrin with gum arabic are typically 10-200 μm in size and retention of volatile depends on number of variables such as inlet temperature of spray dryer, the emulsion concentration and viscosity and proportion of gum arabic to maltodextrin (Williams and Phillips, 2000). Apintanapong and Noomhorm (2003) used different ratios of gum arabic and maltodextrin to investigate the appropriate wall materials for encapsulation using spray drying. These authors showed that the best quality microcapsules were obtained by using 70:30 combination of gum arabic and maltodextrin gave best quality results.

Alginates

Alginates are made up of D-mannuronic acid and L-gluconic acid. Both the ratio of mannuronic acid to gluconic acid and the structure of polymer determine the solution properties of the

alginate. It has been reported that water-soluble alginate was capable of forming encapsulated liquid capsules. Viscous high fat foods can also be encapsulated with Ca-alginate.

Lecithins

Pure lecithin is surface active substance and W/O (water-in-oil) emulsifier. Lecithin vesicles have recently been used for encapsulation of food enzymes because of the formation of lecithin capsules can be achieved under relatively low temperatures. With lecithin vesicles it was found that the encapsulation efficiency was best when the pH was close to enzyme's isoelectric point.

Proteins

Whey protein isolate (W.P.I.) has been shown to provide a good barrier against oxidation for microencapsulated orange oil (Kim and Morr 1996). In international market, whey proteins are available as whey protein isolate (95-96% protein) or whey protein concentrate (WPC-50, WPC-70) powders. Whey proteins in combination with carbohydrates have been used as carrier material in encapsulation of volatile material in encapsulation of volatile material (Young and others, 1993b; Sheu and Rosenberg 1995). In this combination whey proteins served as emulsifying agents and carbohydrate (maltodextrin and corn syrup solids) formed the matrix structure (Sheu and Rosenberg 1998). Whey proteins have been found to exhibit excellent encapsulation properties and are superior to commonly used agents (Young, Sarda and Rosenberg 1993). These studies indicated that whey proteins have been found to be very effective in encapsulating volatile, non-volatile, polar as well as non-polar, liquid and crystalline compounds for food and pharmaceutical applications and both water soluble and water insoluble microcapsules can be prepared. Wall matrices consisting of whey protein isolate (W.P.I.) were extremely effective in protecting encapsulated lipids against oxidation and exhibited encapsulation properties superior to whey protein concentrate (W.P.C.). Encapsulation of lipids

in carbohydrate based wall materials has benefit of pH-independent high solubility and excellent drying properties of these materials. Even though these carbohydrate based wall materials are inexpensive, they offer relatively poor protection against the oxidation. Whey protein concentrates have been shown to contain residual lipids and phospholipids that compromise the functionality of the whey proteins as encapsulating agents. The presence of these materials represents a pro-oxidative factor that accelerates the oxidative deterioration of the encapsulated material (lipids, aroma, vitamins, etc). In addressing these issues, we have focused on developing microcapsule prototypes with wall systems consisting of WPI and inexpensive carbohydrates. This approach can reduce the cost per unit mass of microcapsules while maintaining the unique functionality of whey proteins delivered by WPI. We have developed microcapsules consisting of anhydrous milk fat encapsulated in a blend of WPI and dextrins. The oxidative stability at accelerated condition was excellent. This approach in developing affordable, yet highly functional microcapsules may open opportunities for commercialization of our technology.

Other proteins

Protein based material such as polypeptone, soy protein or gelatin derivatives form stable emulsion with volatile compounds. Limitations include their solubilities in cold water, potential to react carbonyls and their high cost. Addition of gelatin (1%) into maltodextrin and gum arabic carrier increased the retention of ethyl butyrate in spray drying and provided better controlled release ability. This suggests that gelatin would promote the formation of crust on the surface of droplet (Yoshii and others 2001). The sodium caseinate offers physical and functional characteristics required to encapsulate oil materials (Hogan and others 2001). Also mixture of casein and carbohydrate such as maltodextrin and corn syrup solids, may offer potential as a cost

effective, functional, core encapsulating material. Sodium caseinate has also shown to provide effective wall material for retention of orange oil (Kim and Morr, 1996).

Various types of microencapsulated ingredients

Vitamins

The rationale behind microencapsulating vitamins is either extending the shelf life by protecting them from oxidation or by preventing reactions with other components of food systems such as iron, copper etc. Both lipid soluble (e.g. Vitamin A, β-carotene, Vitamin D, Vitamin E and K) or water soluble vitamin (e.g. L-ascorbic acid) can be encapsulated. Ascorbic acid is added to various food products as anti-oxidants or as vitamin supplement (Kirby and others 1991). Vitamin C (L-Ascorbic Acid) is added in various types of foods as vitamin supplement and to fortify foods in order to increase its nutritive value. However, according to Kirby and others (1991) functional properties of vitamin C is limited by its reactivity and low stability in the presence of air, oxygen, humidity, light and metallic ions such as iron and copper. The oxidation is accelerated at neutral to alkaline pH. Losses of ascorbic acid can also occur enzymatically. Attempts have been made to encapsulate vitamin C in tripolyphosphate cross-linked chitosan microspheres by spray drying (Desai and Park 2005). Their objective was to develop the oral delivery route and to characterize microspheres using scanning electron microscope (SEM), transmission electron microscope (TEM), X-ray diffraction (XRD), Fourier Transform infrared (FTIR) etc. Chitosan was chosen because it has been biodegradable compound of low toxicity and is degraded by the microflora available in colon. Uddin, Hawlader and Zhu (2001) investigated the characteristics of ascorbic acid microcapsules using carboxymethylcellulose, gelatin, β-cyclodextrin, various combinations of starch and carboxymethylcellulose, gum, β-cyclodextrin. Release ratio is defined as the ratio of ascorbic acid released to the solution to its

initial encapsulated weight. The results showed that there were significant differences in the initial release of ascorbic acid for three different coating materials i.e. ethyl cellulose (7, 45 and 100 cps). Effect of plasticizer on the release rate of ascorbic acid was also studied. Uddin and coworkers (2001) observed that presence of plastisizer (triethyl citrate) decreased the release rate of ascorbic acid from the microcapsules. It was attributed to the incorporation of plasticizer improved the flexibility of the film and formed a less porous network. Plastisizer exerts its effect by interposing between the polymer chains, hence reducing cohesion between the polymers. For w/w system, the product consisted of homogenously blended matrix of the polymer entrapping the core. For spray dried microcapsules using various encapsulating wall materials like gelatin, carboxymethylcellulose (CMC), β-cyclodextrin and various ratios of starch and CMC, b-cyclodextrin, gum, the loss of vitamin C was within 2% range. In storage studies, it was found that starch and β-cyclodextrin had improved active content. The storage studies were conducted at 38 ˚C and 84% relative humidity. Lipid soluble vitamins such as Vitamin A, β-carotene and vitamin D, E or K are easier to encapsulate than water soluble ingredients. A spay drying is method of choice for encapsulation of oil based compounds such as flavors, vitamins and fatty acids. Riboflavin, thiamin and niacin are partially destroyed during processing and cooking of pasta products. Studies on unprotected vs. encapsulated thiamine, riboflavin and niacin in cooked enriched spaghetti showed the concentration of three B vitamins tested were higher in cooked pasta that contained encapsulated vitamins (Berglung, Dick and Dreher 1987). Stability of vitamin A in skim milk was found to have been substantially increased by encapsulation in gelatin. Loss of vitamin in fortified milk was in gelatin. Loss of vitamin in fortified milk powder was minimal after storage at 28˚C for 40 weeks (DeMan and Wygerde 1986). Vitamin A

palmitate was encapsulated in modified gelatin film and degradation under test condition was significantly reduced by microencapsulation.

Minerals

Fe is one of the most nutritionally important mineral and its deficiency affects about one-third of the population including developed nations. The best way to overcome this problem is through food fortification of Fe. However, in fortification, its bioavailability is negatively affected by interactions with food ingredients like tannins, phytates and polyphenol. Additionally, Fe catalyses oxidative processes in fatty acids, vitamins and amino acids and affects sensory characteristics and nutritional value. Microencapsulation is used to prevent these reactions and improve nutritional and sensory characteristics including increasing bioavailability. $FeSO_4$ or ferrous lactate is readily soluble in water compared to ferrous fumarate. Fe bioavailability study on enriched milk was done with $FeSO_4$ encapsulated in lecithin liposome (Boccio and others 1997; Uicich and others 1999). Heat treatment and storage for 6 months did not result in decreased Fe bioavailability of Fe fortified milk. Calcium fortification as been attempted in soy milk. Soy milk contains much less calcium (12mg/100gm soy milk) compared with cow's milk (120 mg/100 g of cow's milk). Attempts to fortify soy milk with Ca salts have been unsuccessful because this process causes soybean proteins to coagulate and precipitate (Weingarten and others 1983). By encapsulating Ca salts (calcium lactate) in lecithin liposome it was possible to fortify 100 gm soy milk with upto 110 mg Ca, equivalent to cow's milk. Soy milk remained stable at 4 ˚C for 1 week.

Enzymes

The enzymes are used in food system to alter the properties of food system in a favorable way. The stability and functionality of enzymes are enhanced by use of microencapsulation since

enzymes are complex biomolecules which are vulnerable to inactivation by other components or food systems. By properly selecting wall materials we can choose when, where and how it can react with intended substrate. Microcapsules can be made to accumulate to specific location within food altering its surface properties and thus influence its textural properties. In multistage process, we can control release time of enzyme depending on whether we want to release it earlier or later at specific time. A variety of other stabilizing agents such as buffers, chelating agents or anti-oxidants can be included to protect them from antagonistic effects. Penetrating ions can be removed by buffers, chelating agents. Thermostabilizers such as sugars will protect from extreme processing conditions such as dehydration or freezing. Control of cheese ripening using encapsulated enzymes has been successful (Magee 1979; Magee and Others 1981).

Microorganisms

Encapsulation of bacterial cell has been successfully attempted in milk fat wall material by Kim and Olson (1989). The bacteria using methionine to produce methanethiol and other sulfur compounds contribute to cheddar cheese flavor of low fat cheese product. The advantages of using microencapsulated bacteria over encapsulated isolated cheese enzymes are stability of enzymes in intact cells is greater than that of extracts. Also, the production of the enzymes by cells can be manipulated by controlling substrate concentration in microcapsules. Thus, microencapsulated enzymes are useful in reducing the ripening time of blue cheese. Probiotics are living organisms which when ingested have numerous beneficial effects on the equilibrium and functions of human intestinal microflora. Probiotics have been defined as ‘live organisms which transit gastro-intestinal tract and in doing so benefit the health of the consumer (Tannock and others 2000). Probiotics have been reported to play therapeutic role by modulating immunity, lowering cholesterol, improving lactose tolerance and preventing certain cancers.

Probiotics survival is affected by many factors such as poor survival in intestinal pH, hydrogen peroxide formation, oxygen toxicity and post-acidification during storage, storage temperatures, stability in dried or frozen form, compatibility with traditional culture, poor growth in milk. The materials used as excipients are gentle and non-toxic. The most common material is sodium alginate as it offers advantages such as non-toxicity, forms gentle matrices with calcium chloride to trap sensitive bacteria, viability of bacteria during storage and release of entrapped bacteria as gels are solubilized by sequestering calcium ions and releasing entrapped cells (Sheu and Marshall 1993; Shah and Ravula 2000). Several other gelling agents used as excipients are pectate, kappa-carageenan, locust bean gum, gellan gum and agar.

Flavors

Flavors play very important role in consumer satisfaction. Flavors improve the product quality and acceptability; improve sensory characteristics and influence purchasing and consumption of food products. Food processing and storage conditions, packaging materials and ingredients in foods often cause modification in overall flavor either by reducing aroma compound intensity or producing off-flavor compounds (Lubbers and others 1998). Encapsulation can be employed to retain the aroma in food product during storage, protect the flavor from undesirable interactions with other food ingredients, minimize flavor-flavor interactions, protect against light induced degradation and oxidation, increase in product shelf life and achieve controlled delivery at the desired site and time (Reneccius 1991; Tari and Singhal, 2002). Retention of flavors is governed by chemical nature of flavors such as molecular weight, chemical functionality, polarity, relative volatility, properties of encapsulating material and various parameters of encapsulating technique used. The design of appropriate encapsulation system requires physico-chemical understandings of the mechanisms by which flavor compounds are encapsulated, stored and released under right

stimulus (Chang and others 1988; Whorton 1995; Whorton and Reineccius 1995; Goubet and others 1998). Examples of commonly used encapsulated flavors are citrus oils, mint oil, onion and garlic oils, spice oleoresins and whole spices. Citrus oils are especially vulnerable to oxidation due to sites of unsaturation in their mono and sesquiterpenoid structures. Oxidative deterioration results in painty or turpentine like off-flavors. Encapsulated citrus oil in maltodextrin matrix has better stability than unprotected oil (Anandaraman and Reineccius 1986). Many volatile liquids can be encapsulated and subsequently dried to form free flowing powders with minimal loss of activity. Flavors encapsulated by inclusion complexation in β-cyclodextrin were protected against volatilization and degradation by oxidation. The storage stability of flavors encapsulated in β-cyclodextrin under non-stress conditions at room temperature showed that molecular encapsulation provided almost perfect preservation for up to 10 years. For encapsulation of flavor compounds, carrier material should not be reactive to flavor compound, should possess low viscosity at high concentration, allow complete elimination of solvent in any process requiring a phase of desolvatation, give maximum protection to active ingredient, and ensure good-emulsion stabilization properties and effective redispersion behavior in order to release the flavor at time and place desired.

Different methods of microencapsulation

Spray Drying

Among many encapsulating techniques, spray drying is the most common to produce flavor powders in few seconds. More than 90% of the encapsulated flavors are produced by this method (Reifsteck and Jeon 2000). Encapsulation by spray drying has been widely used in the food industry from late 1950s to provide flavor oils protection against the oxidation and also it is

particularly easy to convert the flavors which are liquid at room temperature to free flowing powders. Following are the major steps in spray drying process.

Preparation of emulsion/aqueous carrier phase

This is the first step in the spray drying of microencapsulated flavors. It is important to use particular infeed solid levels which are optimum for each carrier. The infeed solids level is the most significant factor in retention of the flavor during spray drying process (Reineccius 1991). Increasing the solid levels up to the point that the additional solids are no longer soluble benefits the flavor retention by decreasing the required drying time to form high solids surface film around the drying droplets. When droplet surface reaches 10% moisture, flavor molecules cannot diffuse through this surface film, whereas relatively smaller water molecule continue to diffuse through this surface film and are lost to drying air (Bomben 1973). High infeed solid level facilitates the formation of this semi permeable membrane quickly and helps in flavor retention. Insoluble carrier solids do not create barrier to the diffusion of the flavor molecules and do not help in retention of flavor compounds. Thus for each carrier material there is an optimum infeed solid level. The most important factor in determination of retention of volatiles during drying is the infeed solids content. High infeed solids increase retention during drying primarily by reducing the time required to form the semipermeable membrane at the drying surface of the droplet. Earlier it was suggested to use the highest infeed solids possible, but later from subsequent experiments it was found that there is optimum infeed solid content for the drying of flavoring material (Reineccius and Bangs 1982). There could be two reasons for optimum solids infeed level. The constant ratio of infeed solids and carrier solids is used, after adding more material exceeds carrier solubility. Although it is possible to pump and atomize at this solids content, the undissolved carrier does not provide any encapsulating effect and poorer retention is

observed after drying process. Also there is the effect of solids content on the viscosity of infeed material. Excessive infeed solids viscosity can delay the formation of the particle which will results in the volatile losses during drying. After carrier material is solubilized, the core material is added. Generally, 20% of core load is selected. Higher solid levels generally result in unacceptable high losses of flavors in the dryer. Compared to 10% flavor loading when 25% flavor loading was used, 33 to 50% of flavor was retained during drying. Brenner (1976) obtained the patent for a process which can produce high load spray dried flavorings. He found that high surface oil and poor flavor retention is the result of particle shrinkage and cracking during drying process. Brenner and others (1976) used combination of polysaccharides (e.g. gum Arabic, starch derivatives and dextrinized and hydrolyzed starches) and polyhydroxy compounds (e.g. sugar alcohols, lactones monoethers and acetals) to prepare carrier material which can remain plastic during the drying process (Brenner and others U.S. Patent 3, 971, 852, 1976).

Homogenization of the dispersion

Following the addition of emulsifier, the heated dispersion is homogenized before spray drying. Risch and Reineccius (1988) have demonstrated that there is direct relationship between degree of homogenization and retention of orange peel oil during spray drying.

Atomization of the infeed emulsion

Atomization parameters have significant effect on the particle size distribution of the resultant powder Chang and others (1988) showed that there is optimum particle size for flavor retention However, Bomben (1973) showed that particle size is insignificant if high infeed solids were used. Although particle size has not significant effect on flavor retention, larger particle sizes are preferred since smaller particles are difficult to disperse and float on the liquid surface.

Large particles can be obtained by using large orifice size/low atomization pressure (in case of pressure nozzle) and high infeed solids, high infeed viscosity, low wheel speed (centrifugal atomizer).

Dehydration of Atomized particles

The atomized particles assume spherical shape as they pass through the gaseous medium. The evaporation takes place very rapidly as the droplet comes in contact with hot air. The core temperature is kept below 100˚C despite the high temperatures used in the experiment because of rapid evaporation of the coating. Thus the major advantage of spray drying method is its ability to handle heat labile materials.

Spray cooling/ chilling

Spray cooling and spray chilling are two encapsulation processes that are similar to spray drying. Both involve dispersing the core material in liquid coating material and spraying through heated nozzle in controlled environment. The major difference lies in the temperature of the air in the drying chamber and the type of shell material used. Spray drying uses the heated air in volatilizing the solvent from coating dispersion, in spray cooling/chilling air used is at ambient or refrigerated temperature considerably below the solidification point of a molten fat or wax coating. In spray chilling there is no mass transfer, evaporation from the atomized droplets, i.e. evaporation from the atomized droplets; hence these solidify almost perfect spheres which result in free flowing powders without the dents on the surface. The shell material is selected from a variety of vegetable oils with lower melting points between 32 to 42˚C. Coating material with lower melting point than this also can be used and it requires specialized handling and storage conditions (Taylor 1983). In spray chilling, material to be encapsulated is mixed with the carrier and atomized by cooled or chilled air instead of heated air in spray drying (Risch 1995). The

carrier material is usually vegetable oil when spray cooling (45 to 122˚C) is used or hydrogenated vegetable oil in case of spray chilling (32 to 42˚C). Spray chilling is used to encapsulate solid food additives such as ferrous sulfate, acidulants, vitamins and solid flavors and solid flavors as well as for sensitive materials. The product formed is in the form of fine beads which are water soluble but they release their content at melting point. These spray-chilled products are used in bakery products, dry soup mixes and foods with high fat contents (Blenford 1986).

Freeze drying/ lyophilization

Freeze drying method is also called as lyophilization. Freeze drying method is suitable for the heat sensitive compounds which are unstable in aqueous solutions and volatile flavors. The complete dehydration takes place at low temperature and low pressure conditions and therefore it has high retention of volatile compounds. Studies by Flink and Karel (1970), Thijssen and co-workers (1972) showed that retention of volatile compounds during freeze drying is dependent on the composition of wall and core material. The retention was found to be higher when low molecular weight carbohydrates were used and total soluble solids were increased to 20%. Freeze drying method was used by Kopelman and others (1977) for the production of citrus aroma powders which can be used as natural flavor ingredient in soft drink dry mixes. In this process corn syrup and sugars (mono and disaccharide) were added in the aroma solutions at 25% (w/w) and subjected to freeze drying. Buffo and Reineccius (2001) compared spray drying, tray drying, drum drying and freeze drying to encapsulate cold pressed orange oil and reported that freeze drying process gives most desirable properties. Minemoto and others (1997) compared oxidation of menthyl linoleate when encapsulated in gum arabic by hot air drying and freeze drying. Menthyl linoleate encapsulated by freeze drying was more slowly oxidized at any

relative humidity. Heinzelmann and Franke (1999) showed that the production of dried microencapsulated fish oil by freezing and subsequent freeze drying resulted in product with good resistance to oxidation. Freeze drying is a simple technique except the long dehydration period (20 hour). Also the cost of this process is 50 times higher than spray drying (Desorby and others 1997) and storage & transport of particles produced is extremely expensive (Jacquot and Pernetti 2003).

Fluidized bed coating

Fluidized bed technology was first developed by D.E. Wurster in 1950s and therefore also called as "Wurster process". Fluidized bed technology is very efficient way of applying uniform layer of shell material on solid particles. This is a very versatile technology in the sense that any kind of shell material (e.g. polysaccharides, proteins, emulsifiers, fats, complex formulations and enteric coating) can be used as coating material. Therefore, controlled release possibilities are considerably more versatile with fluidized bed technology than any other technology. This technique is applicable for hot-melt coatings such as hydrogenated vegetable oil, fatty acids, emulsifiers, waxes or solvent based coatings such as starches, gums and maltodextrins. For hot melts, cool air is used to harden the carrier; whereas, for solvent based coatings, hot air is used to evaporate the solvent. Hot melt ingredients release active core material by increase in temperature or physical breakage of the capsule, whereas water based coatings release their content when water is added. There are three different fluidized-bed coating methods: 1) top spray; 2) bottom-spray; and 3) tangential spray. In top spray method, the coating solution is sprayed through top and air is passed from bottom through the bed of core particles to suspend and coat them. The coated particles travel through coating zone into expansion chamber and then fall back into product container and continue cycling throughout the process. Top spray method

is used to coat particles as small as 100 μm. But Thiel and Nguyen (1984) have demonstrated the possibility of encapsulating very fine particles (2-5 μm). In top spray configuration, by controlling distance the droplet travels before contacting substrate is impossible and coating imperfections can occur. In bottom spray, both air and coating solution is passed through the bottom. The advantage of this method is path of droplets towards core particles is extremely short and premature droplet evaporation is almost absent. Additional advantages include, coating solution can spread out at lower viscosity producing very dense film with superior physical strength. A fascinating advancement has been reported by Matsuda and others (2001) for the fluidization and coating of very fine particles in a fluidized bed. In this process, gravitational force is multiplied through use of rotating perforated drum that contains the particle. The airflow is applied tangentially to the rotation of the drum as compensation to the gravity force (upto 37 g) of normal gravitational force. This ensures complete fluidization of the particles. This technique is used to encapsulate nutritional substances such as vitamin C, B vitamins, ferrous sulfate, ferrous fumarate, sodium ascorbate, potassium chloride and a variety of vitamin/mineral premixes. These are used as nutritional supplements. In bakery products, the above mentioned technique has been used to encapsulate leavening ingredients, vitamin C, acetic acid, lactic acid, potassium sorbate, sorbic acid, calcium propionate and salt. In meat industry, several food acids have been fluid-bed encapsulated to develop color and flavor system. Encapsulated acids are used to achieve a reproducible pH in cured meat products and shorten processing time. Fluid-bed encapsulated salt is used in meats to prevent development of rancidity, as well as premature set due to myofibrilar settings.

Extrusion

Encapsulation of food ingredients by extrusion is relatively new process compared to spray drying. It is relatively low temperature encapsulation method which involves forcing of core material dispersed in molten carbohydrate mass through a series of dies into a bath of dehydrating liquid (e.g. alcohol). Upon contacting the liquid, the coating material that forms encapsulating matrix hardens to entrap the core material. The extruded filaments are separated from the liquid bath, dried to mitigate hygroscopicity (anticaking agent such as calcium triphosphate can be added). The first patent for flavor encapsulation was awarded to Swisher in 1957. The primary benefit claimed in the patent was maintenance of fresh flavor of citrus oil which can easily oxidize during storage to produce off-flavors. This involved addition of citrus oil in molten solution of sucrose and dextrose, cooling solution to form hard slab like rock candy and then grinding the solid to desired size. Beck (1972) made some improvement in the process in which he replaced the high DE corn syrup solids with low DE maltodextrin and combined 55% sucrose with 41% maltodextrin (10 to 13 DE). This low DE maltodextrin/sucrose matrix was considerably less hygroscopic than Swisher. Barnes and Steinke were awarded patent for their improvement in the process in which they replaced sucrose by modified starch which possess good emulsifying properties and also due to its lipophilic characteristic would absorb flavor oil in the matrix. Barnes and Steinke (1987) claimed that modified starch would increase the loading capacity of the flavors upto 40%. Additional benefits include total replacement of sucrose with modified starch and product that was sugar-free. Also sucrose inverts into glucose and fructose at low pH and high temperature. This will result in product becoming more hygroscopic and will readily participate in nonenzymatic browning. The extrusion process is particularly useful for heat-sensitive compounds and has been used to encapsulate flavors,

vitamin C and colorants. It offers outstanding protection to oxidation of flavors. The accelerated shelf life testing done by Swisher (1957) on encapsulated orange oil containing antioxidant resulted in one year of shelf life. Westing and others (1988) showed that shelf life of single fold orange peel oil was in excess of four years. This is much superior to any other flavor encapsulation process in commercial use.

Coacervation

Coacervation is also called as phase separation which was developed first by National Cash Register Co., Dayton, USA for production of carbonless copy paper. Coacervation involves separation of liquid phase of coating from polymeric solution and wrapping of that phase as uniform layer around the suspended core particle. It consists of three steps which are carried under continuous agitation. **a)**. Formation of three immiscible chemical phases. This can be done by one of these two methods. 1) Direct addition- coating insoluble waxes, immiscible polymer solution and insoluble liquid polymer is added to liquid manufacturing solution. 2) *In-situ* separation- Monomer is dissolved in liquid vehicle and subsequently polymerized at the interface. **b)**. Deposition of coating-deposition of liquid polymer coating around core material can occur if the coating polymer is sorbed at the interface formed between core material and liquid vehicle phase. **c)**. Solidification of the coating: Solidification of coating takes place by thermal, cross-linking or desolvation technique. Coacervation can also be divided into nonaqueous phase separation and aqueous phase separation. In aqueous phase separation, hydrophilic coating material like gelatin, gum arabic is used for coating water-insoluble material such as citrus oil, vegetable oil and vitamin A where the core material can be released by pressure, hot water or chemical reaction. In non-aqueous phase separation, hydrophobic coating is used whereas core can be water soluble or insoluble. This process is investigated for

encapsulation of solid food additives like ferrous sulfate. Coacervation has limited use in food industry for flavor encapsulation because of evaporation of volatiles, dissolution of active compound into the processing solvent and oxidation of product since residual core materials sometimes cling to exterior of capsule (Floros and others 1992). Also, limited suitable encapsulated materials that are food approved are available for coacervation.

Co-crystallization

Co-crystallization is relatively simple and economical process compared with other flavor encapsulation processes. Chen and others (1988) described number of products that can be encapsulated such as fruit juices, essential oils, flavors and brown sugar. In this process spontaneous crystallization of supersaturated sucrose syrup is achieved at high temperature (above 120˚C) and low moisture (95-97 ˚Brix) and aroma compounds can be added at the time of spontaneous crystallization (Bhandari and others 1998). The crystal structures of sucrose can be modified to incorporate flavors either by inclusion within the crystals or by entrapment. This serves to enhance flavor stability (Mullin 1972; Chen and others 1988). The granular product has low hygroscopicity and good flowability and dispersion properties (LaBell 1991; Quellet and others 2001). During the process, some heat sensitive compound may get degraded (Bhandari and others 1998). Beistain and others (1996) encapsulated orange peel oil using co-crystallization method. The authors demonstrated that co-crystallization product retained as much volatile oil as spray dried and extruded products. Addition of strong anti-oxidant was necessary to retard the development of oxidized flavors during storage.

Inclusion complexation or molecular inclusion

Cyclodextrins are enzymatically modified starch molecules which are made by the action of cyclodextrin glucosyltransferase on starch in which the ends are joined to form circular molecule with α (1-4) linkage. The inclusion complexes are defined as the result of interaction of compounds in which guest molecule fits and is surrounded by lattice of other (Godshall 1997). Structure of β-cyclodextrin is such that inner cavity is hydrophobic and is torus shaped and its molecular dimension allows total or partial inclusion of range of aroma compounds. The outer surface is having hydrophilic nature. Pagington (1986) and Bhandari and others (1999) have listed several methods for complexing β-cyclodextrin with flavor compounds. The following methods are commonly used for complexing.

1. Stirring or shaking cyclodextrin with flavors in aqueous solution and filtering off precipitated complex.
2. Blending solid cyclodextrin with guest molecule in powerful mixer and bubbling flavors as vapors through the solution of cyclodextrin.
3. Kneading the flavor substances with cyclodextrin-water paste.

Cyclodextrins are relatively expensive product and around $ 6.00 per kg (Gouin 2004). Therefore, the application is found in the area of high value added and specialty flavor chemicals (Uhlemann and others 2002).

Microencapsulation efficiency

Microencapsulation efficiency (MEE) is defined as the proportion of the core that could not be extracted by the solvent under test conditions. (Young and others 1993). Jimenez and others (2004) microencapsulated conjugated linoleic acid using whey protein concentrate as wall material and defined microencapsulation efficiency as below

MEE= (total oil – extractable oil) X 100 / total oil.

Desai and Park (2005) reported the microencapsulation efficiency 45.05 to 58.30 % for vitamin C encapsulated in tripolyphosphate cross linked chitosan microspheres. It was observed that as the amount of cross linking agent increased, the encapsulation efficiency decreased. This was attributed to the surface irregularities observed of chitosan microspheres as the amount of cross linking agent was increased. It was hypothesized that damaged microspheres with surface irregularities, fragmentation or holes are likely to cause the loss of substantial amount of vitamin C during the spray encapsulation process.

The shelf life of the spray dried microcapsule

Spray dried encapsulated flavor have the shelf life of 6 months or more depending on the type of the core material/flavor compound used, carrier material used, absence of the surface oil (which oxidizes rapidly), moisture content of the powder, powder surface properties and moisture absorption during storage (Bhandari and D'Arcy 1996). The porosity of the dried particle is also proposed to be the major determinant of the shelf life of the spray dried flavor microcapsule (Reineccius 1988). A large portion of the dry flavorings produced in the industry includes some citrus oil. These citrus oils are prone to oxidation during storage. The industry requires at least one year of shelf life. In order to improve upon shelf life, the encapsulated flavor must be protected from oxidation. This brings up consideration for the presence of antioxidants (natural), trace metals, entrained air and oxygen barrier properties of final spray dried particle. Anandaraman and Reineccius (1986) have shown that there is a very strong protective effect of higher dextrose equivalent (DE) starch hydrolysates (corn syrup solids) against oxidative deterioration of spray dried orange peel oil. Many authors suggest that glass transition (Tg) plays a major role in determination of oxidative stability of foods (Bhandari and others 1999).

Physical Properties of spray dried particles

Particle size and shape

Particle size is the result of the selection of the operating parameters chosen for atomization. Spherical particle size is desirable because of better aroma retention (spherical particle has least surface to volume ratio and therefore least exposure to the surface with maximum core is encapsulated in spherical particle). Highest bulk densities and best flowability is obtained in spherical particles. For wheel atomization, particle size is determined by the speed of the atomizer and the physical properties of the emulsion (e.g. viscosity and solids concentration). In case of nozzle atomization, particle size is determined by the nozzle orifice and the spray pressure (or air flow for the two fluid atomizer. A high pressure and small orifice will result in smaller particles and low pressure and larger particle. Particle size can be influenced by the operating temperatures also. High drying rates (High inlet temperature and low ΔT, i.e., temperature difference between inlet and exit temperatures) will result in slightly larger particles compared to slow drying. This is because fast drying does not allow the particles to shrink compared to slow drying. Also particles dry quickly if solid contents are high and are expected to produce larger particles and cannot shrink as much (Masters 1991). Zakarian and King have shown that if both volatile loss and rate of drying are diffusion controlled, volatile retention should be independent of particle size. It is desirable to produce larger particles to facilitate rehydration. The smaller particles tend to disperse very poorly, especially in cold water and instead form lumps on liquid surface. Larger particles can be obtained by controlling dryer operating temperatures (e.g. high infeed viscosity and solids, low pressure large-orifice if using a pressure spray atomizer or low wheel speed if using centrifugal atomizer) or using agglomeration techniques.

Absolute and bulk densities

Absolute density is defined as the weight of a given particle volume of powder while bulk density is defined as the weight of a given total (bed) volume of powder. Absolute density can be determined by the He pycnometry and takes into account only the volume of particles that is not permeable to the displacing gas. Helium displacement methods have been used to determine absolute densities for many years. Some materials such as modified starches foam excessively during hydration and blending. This entrapped air is retained during drying and thus results in a powder of low absolute density. Degassing of the infeed material conversely increases the absolute density. The absolute density is influenced by the amount of air in the infeed matrix or included during atomization process, steam formation within the particle during drying and the rate of drying. These factors can be influenced by the dryer air temperature, particle size, atomization conditions and infeed matrix (material, entrapped gas and solids content) (Verhey 1972). The smaller particles are usually denser than larger particle. Higher inlet temperatures and lower ΔT result in lower density powder since rapid drying of the particle and forming the particle structure before much water has evaporated (Verhey 1972). The bulk density is primarily determined by placing a given weight of powder in a graduated cylinder and then vibrating or shaking the cylinder until the powder bed reaches a constant volume. The rate and intensity of agitation is important in influencing this value. Bulk density is important in packaging and shipping considerations. Absolute density, particle shape and size influences bulk density. Particle geometry also determines the bulk density. Spherical particles fit the best in the pack and have highest bulk density if other factors are equal. The particle density is decreased by steam formation in the drying droplet thereby expanding the particle whose dimensions become fixed as drying continues (Verhey 1972).

Flowability

Flowability is important since if powder does not flow well then manufacturing process becomes problematic. Flowability is measured by pouring a fixed weight of volume through a selected funnel configuration. The powder is allowed to flow through the funnel and the angle of powder pile above horizontal (angle of response) is measured. Smaller the angle better is the flowability. The more common method is measuring the amount of time required for the powder to flow through funnel. However, the values obtained are extremely dependent on experimental set-up (e.g. the funnel diameter and/or height above a table). Flowability is influenced by particle geometry (spherical particles flow best), density (high density gives better flow ability) and surface oil. The particles with high surface oil become sticky and do not flow well. Flowability can be improved by using silica free flowing agents (<2% of weight of product).

Dispersability

Dispersability is primarily influenced by the particle size, density and carrier matrix used. Small and low density particle are difficult to disperse. Particle size can be increased through instantizing processes. This can be done by rewetting the powder in a fludized bed, allowing the powder to agglomerate and then again drying it. Gum acacia are particularly difficult to disperse and therefore agglomerated forms of gum acacia is available. Ingredients difficult to disperse are preblended with other ingredients such as sugar or salt to improve dispersability. The appearance of spray dried microencapsulated color (e.g. colorings, many spice oils, or other coloring materials) is greatly influenced by the particle size. The small particles produced by spray drying method have light color. Large or agglomerated particles are darker and richer in color. Thus, the particle size, agglomeration influences the appearance of the powder.

Structural Strength

Structural strength assumes importance when dry blending powder with salt or sugar or when the active material inside the microcapsule is liquid or soft paste. The dry blending process is very abrasive and will crack or damage powders high in liquid actives or those that are low in density. The industry has accepted spray dried powder containing 20% flavor and if one uses (>40% flavor), the particle loses structural integrity during blending and fracture. This results in exposure of flavor to oxygen which results in rapid degradation during storage. Thus it may be necessary to produce high density low load powder in case the flavor is susceptible to oxidation.

Characterization of microcapsules

Varieties of methods are available to characterize the microcapsules. These include electron microscopy (EM), radio tracers, fluorescence quenching, ultrasonic absorption, electron spin resonance (ESR) spectroscopy, Nuclear magnetic resonance spectroscopy (NMR) can be used to characterize the microcapsules. Each of these techniques have characteristic advantages.

Electron Microscopy (EM)

Electron Microscopy can reveal the average size distribution of microcapsules. Negative staining and freeze fracture are the methods most commonly used, especially liposome structures. (Dreamer and Uster 1983). However, other properties, such as the permeability and stability of microcapsule cannot be studied by this technique.

Scanning Electron Microscopy (SEM)

The protection to the core material provided by the wall material depends on the flow properties of the inner and outer microstructures of the microcapsule and the organization of the core material within the microcapsule. These characteristics of microcapsules can be studied by SEM in secondary electron imaging (SEI) with great depth of field and sufficiently high resolution

(Rosenberg and others 1985). Rosenberg and others (1985) developed new embedding and microtoming technique that enables the study of inner structure of fractured microcapsule. The SEM technique was improved by Rosenberg and others (1984) for studying the effects of wall composition and drying conditions for inner and outer structure of spray dried microcapsules. Thus, it can be observed how the core material is organized in solid wall matrix, about the existence of one or more internal voids in the microcapsule, indentation and caps on exterior of microcapsule, and how the microstructure features are affected by the solid's concentration in the sprayed emulsion, temperature of feed and drying air.

Electron Spin Resonance (ESR) Spectroscopy

The permeability of liposomes has been studied using ESR spectroscopy in conjunction with water-soluble electron paramagnetic resonance probe, trimethyl-4-amino-2, 2, 6, 6-tetramethyl-1-oxy-piperidine (CAT_1) (Kim and others 1991). Release of CAT_1 from liposomes can be measured by adding the spin label quenching agent potassium ferricyanide to the suspension and monitoring the reduction of ESR signal strength. The movement of CAT_1 and ferricyanide ions across the liposomes membrane could be monitored by increasing the temperature to the phase transition point. The ESR signal light at Tc is smaller than that below Tc because of the reduction of CAT by ferricyanide ion. Thus, ESR spectra can be used to test the stability and permeability of liposomes. However, this method is only intended for the testing of liposomes in research laboratories and will not be useful for quality control of processed food due to toxicity of most spin labels.

Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy uses electromagnetic radiation in the radio frequency range to induce transitions between nuclear spin energy levels in the presence of strong magnetic field. When

nuclear spin relaxes back to their equilibrium state, voltage is induced in a detection coil; this voltage is NMR or 'free induction decay' signal. NMR relaxation techniques provide rapid sensitive and nondestructive means of obtaining detailed information about molecular dynamics and intermolecular interactions in complex systems like foods. The NMR spectroscopy is faster and more suitable to characterize microcapsules like liposomes than fluorescence method. Recently, such technique has been used to study many complex food proteins (Baianu IC and others 1982; Myers-Betts PA and others 1990) and starches (Yakubu PI and others 1990).

Fluorescence Quenching

A number of flurophore-modified starch lipids have been used to label liposomes and to study liposome stability and liposome-cell interactions. One effective method of assessing liposome properties uses the carboxyfluorescein self-quenching method (Hagins 1978).

Fluorescence self-quenching is simple and effective means of testing liposome characteristics such as stability and integrity. The fluorescence quenching is based on the loss of fluorescence efficiency due to probe-probe interactions when fluorophore molecule is present at high concentration. Carboxyfluorescein is a highly water-soluble derivative of fluorescein that can be trapped within liposomes at various concentrations. The molecule does not fluoresce significantly when trapped within liposomes at high concentration because of the fluorescence quenching phenomenon (Kim and others 1991).

Table 2.1: Methods used to reduce the loss of L-ascorbic acid in foods (Liao and others 1988).

Principle	Example
1. Limit Oxygen	Deaerate by vacuum, boil or purge with Nitrogen
	Add glucose oxidase plus catalase
	Add sulfite
	Add yeast
2. Limit Cu(ii) and Fe(iii)	Avoid brass, bronze, monel, steel and iron
	Chelate with citrate, phosphates, EDTA, protein
3. Limit complexing between metal ion and L-ascorbic acid	Lower water activity
	Lower pH
	Coat L-ascorbic acid or use derivative substituted at O_2
	Add selected proteins

Table 2.2: Coating materials for encapsulating food ingredients

Carbohydrates	Starch, maltodextrin, corn syrup, dextran, sucrose, cyclodextrins
Cellulose	Carboxy methylcellulose, methylcellulose, ethylcellulose, nitrocellulose, acetylcellulose, cellulose acetate-phthalate
Gums	Gum acacia, agar, sodium alginate, carageenan
Lipids	Wax, paraffin, beeswax, tristearic acid, diglycerides, monoglycerides, oils, fats, hardened oils
Proteins	Whey protein concentrate, whey protein isolate, sodium caseinate, gelatin, albumin, peptides

Table 2.3: Strengths and weaknesses of some of the food grade materials with good encapsulating properties

Material	Strength	Weakness
A/B- gelatin	Very good emulsion, safe source	Non-kosher, BSE (B-gelatin)
Fish gelatin	Excellent emulsion, Kosher	Expensive, availability (?)
Casein/whey protein	Very good emulsion, Kosher	Instabilities at low pH, allergenic potential
Soya protein, gluten zein	Very good emulsion, Kosher, animal-free, safe source, cheap	Allergenic potential, GMO
Modified starches	Very good emulsion, kosher, cheap	Sometimes varying quality, not universally usable owing to regulatory situation.
Gum acacia	Good emulsion, very good inclusion of volatiles	Varying quality, price depending on availability
Sucrose, maltodextrin, dextrin	Very good oxygen barrier, cheap	No/ limited emulsion stabilization
Lecithin	Generally usable in all foods	GMO

Table 2.4: Methods of microencapsulation (Thies 2001)

Chemical Process	**Mechanical process**
Complex Coacervation	Spray Drying
Polymer/polymer incompatibility	Spray Chilling
Interfacial Polymerization	Fluidized Bed
In situ polymerization	Electrostatic deposition
In-liquid drying	Centrifugal Extrusion
Thermal and ionic gelation in liquid media	Spinning disk or rotational suspension separation
Desolvation in liquid media	Polymerization at liquid/gas or solid/gas interface, Pressure extrusion, Hot-melt extrusion

Table 2.5: Different variables affecting fluidized-bed operation (Desai and Others 2005).

Sr. No.	Variables
1	Process conditions 1. Inlet air temperature 2. Inlet air velocity 3. Spray rate 4. Solution temperature 5. Solution dry matter content 6. Atomization pressure
2	Ambient air conditions 1. Temperature 2. Relative humidity
3	Thermodynamic 1. Outlet air temperature 2. Outlet air relative humidity

Table 2.6: Parameters affecting the release rate of core material

Coating Properties	Density, crystallanity, orientation, solubility, plasticizer level, cross-linking, pretreatments
Capsule Properties	Size, wall thickness, configuration, conformity, coating layers, post treatment
Experimental parameters	Temperature, pH, moisture, solvent, mechanical action, partial pressure differential (inside and outside coating)

Table 2.7: Different types of microencapsulated food ingredients and application of microencapsulation (Desai and others 2005)

No	Category of Food Ingredient	Examples	Preferred mode of encapsulation	Applications
1	Acidulants	Lactic acid, glucono-∂-lactone, Vit. C, Acetic acid, potassium sorbate, sorbic acid and calcium propionate.	Fluidized-bed coating, Extrusion	1. Used to assist in the development of color and flavor in meat emulsion and dry sausage products 2. Baking industry use stable acid and baking soda in wet and dry mixes to control the release of CO2 during processing.
2	Flavoring agents	Citrus oil, mint oils, onion oils, garlic oils and spice oleoresins	Inclusion complexation extrusion, centrifugal extrusion, spray-drying.	1. To transform liquid flavorings into stable and free flowing powders, which are easier to handle & incorporate in food products.
3	Sweeteners	Sugar, nutritive or artificial sugars and aspartame	Co-crystallization, fluidized bed coating	1. Reduce hygroscopicity, improve flowability and prolong sweetness perception.
4	Colorants	Annato, β-carotene and turmeric	Extrusion	1. Encapsulated colors easier to handle and offer improved solubility, stability to oxidation and control over stratification from dry blends.
5	Lipids	Fish Oil, linoleic acid, rice bran oil and sardine oil.	Spray-drying Freeze drying, vacuum drying	1. To prevent oxidative degradation during processing and storage
6	Vitamins and Minerals	Fat soluble- Vit. A, D, E and K. Lipid soluble: Vit C, Vit B_1, B_2, B_6, B_{12}, Niacin, folic acid and Choline (Vit B_4)	Coacervation, Inclusion Complexation, spray-drying, liposome entrapment	1. To reduce off-flavors. 2. To permit time release of nutrients 3. Enhance stability in extreme temperature and moisture 4. Reduce each nutrient interaction with other ingredients
7	Enzymes and Microorganisms	Lipase, Invertase, *Penicillium roquefortii*, *Brevibacterium linens*.	Coacervation, Spray-drying, liposome entrapment	1. To improve stability, 2. To reduce ripening time 3. Improve survivability

Table 2.8: Physical properties of L-ascorbic acid (Liao and others 1988)

Appearance: White colorless crystals

M.P. : 190-2 ˚C

Optical Activity: $[\alpha]_D^{25}$ + 21˚ (c 1.0, water)

$[\alpha]_D^{25}$ + 48˚ (c 1.0, methanol)

Solubility: g/100 ml

Water	33	Ethanol	2
Propylene glycol	5	Glycerol	1
95% ethanol	3.3	Lipid Solvents	insoluble

UV

pH 2: λ_{max} 243 nm, ε mM 10.0

pH 7: λ_{max} 265 nm ε mM 16.5

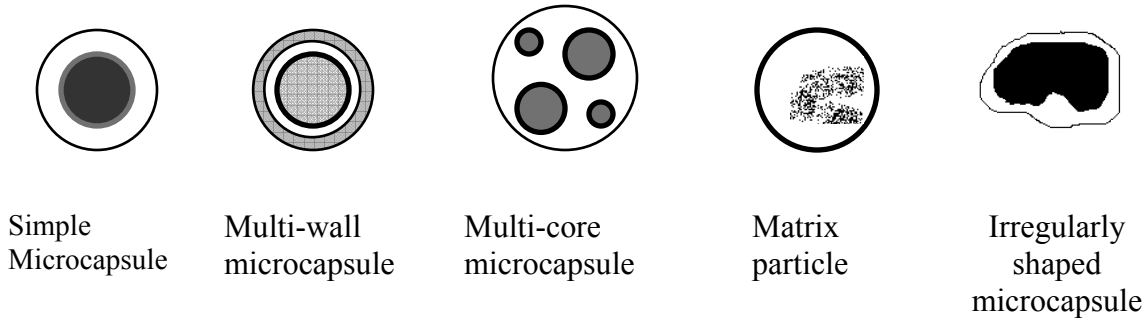


Figure 2.1: Microencapsulation – particle morphology (Zeoli and others 1983)

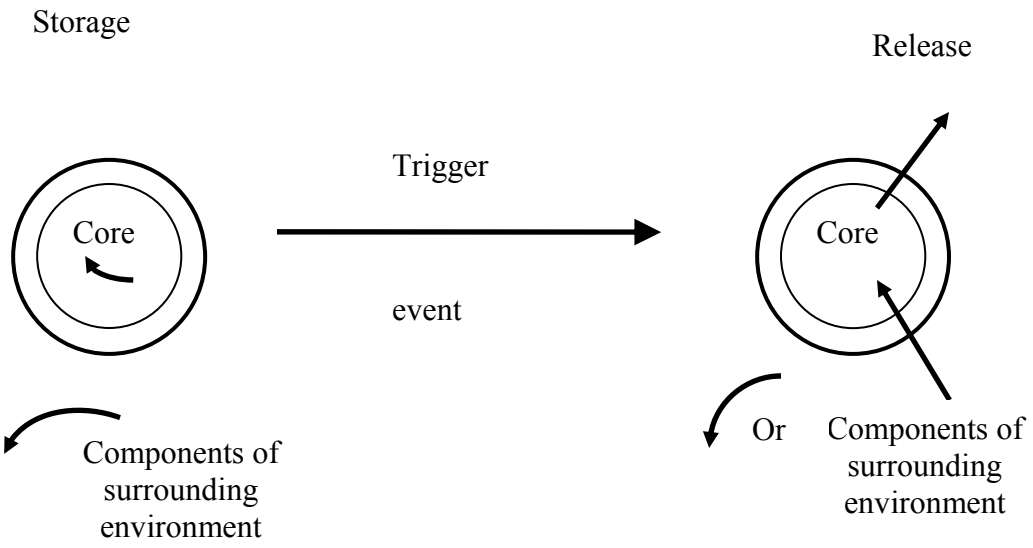


Figure 2.2: Schematic diagram of ideal storage and release behavior (Thies 2001)

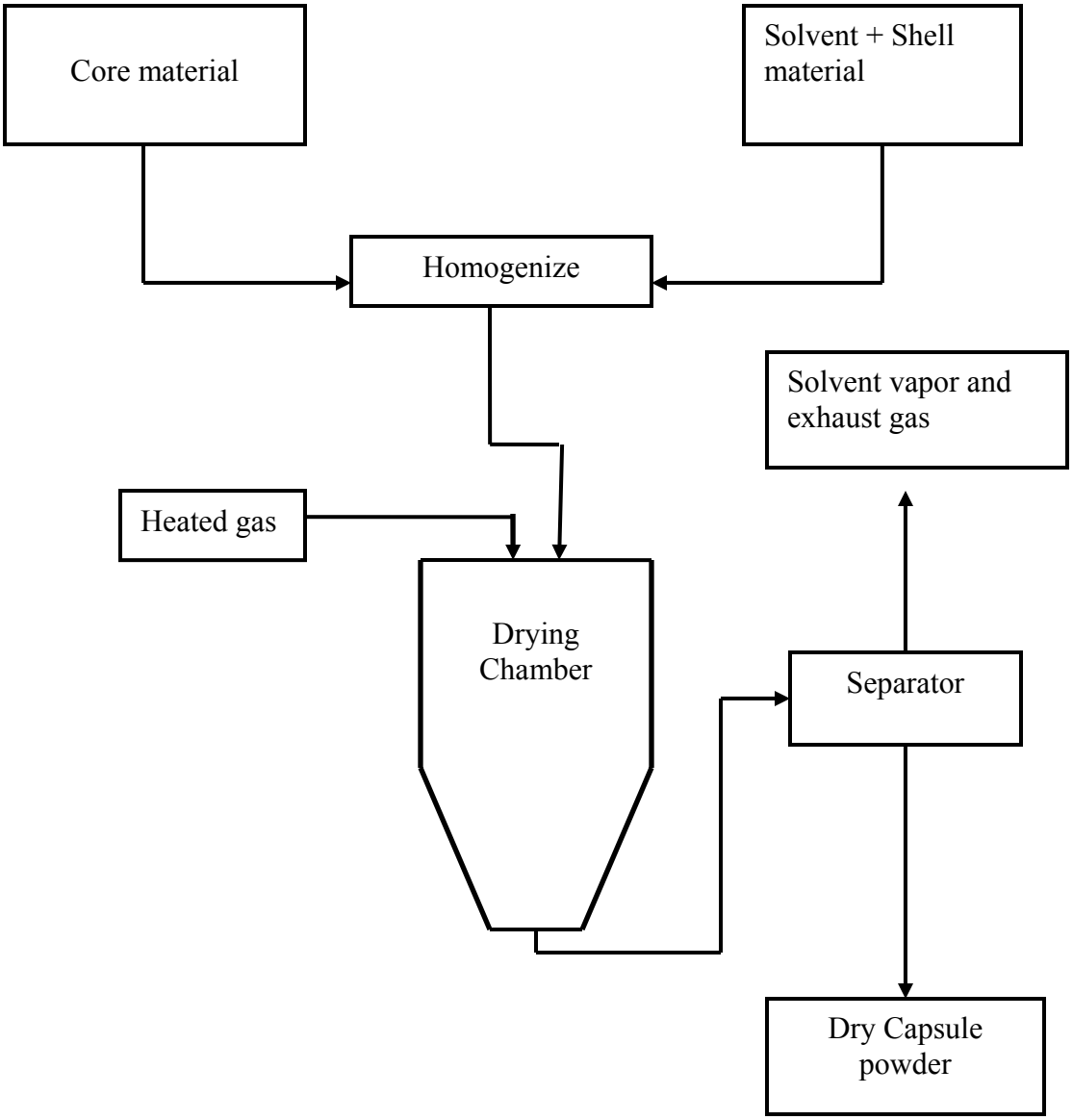


Figure 2.3: Schematic diagram of spray-dry encapsulation process (Shahidi and others 1993).

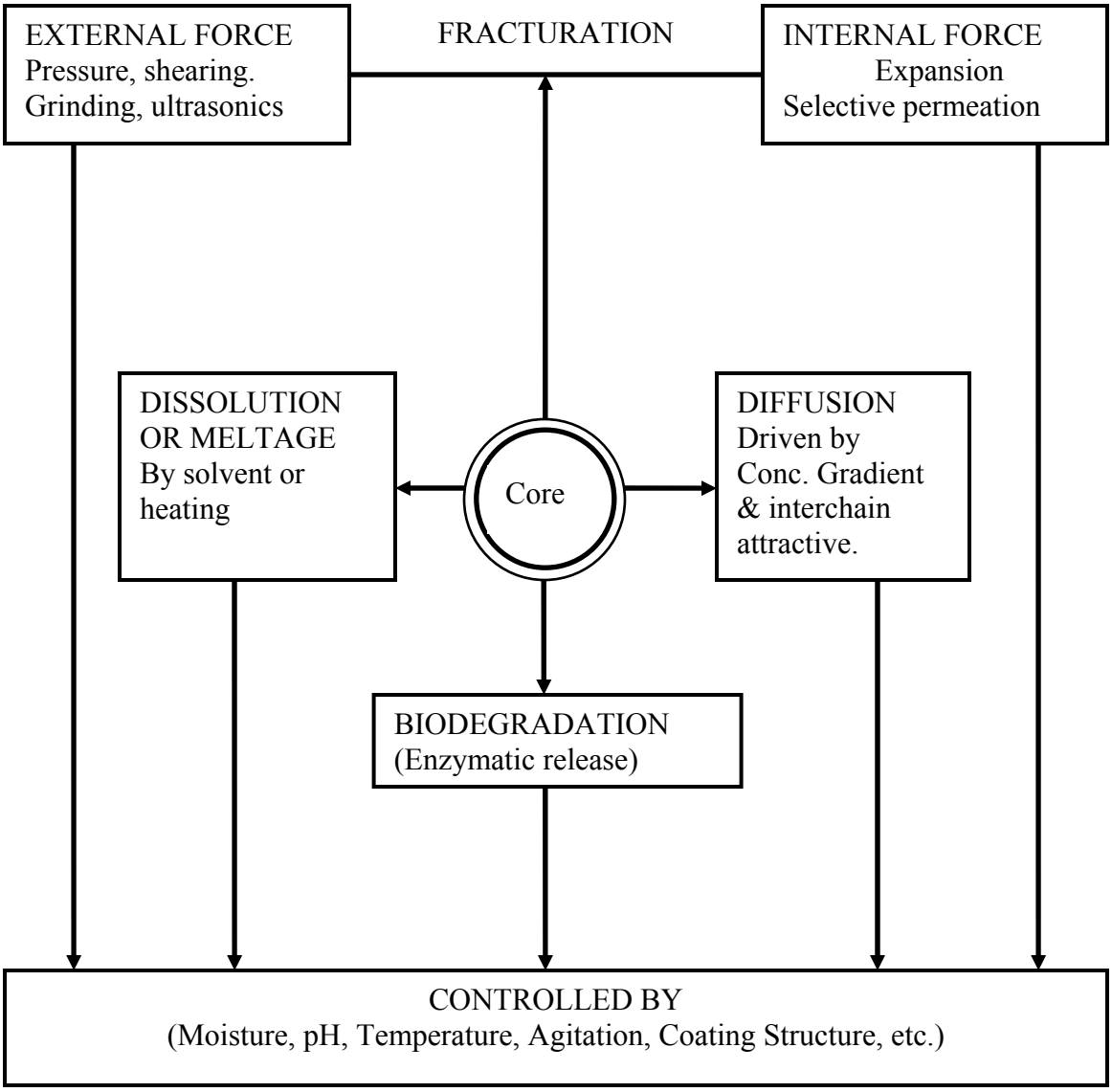
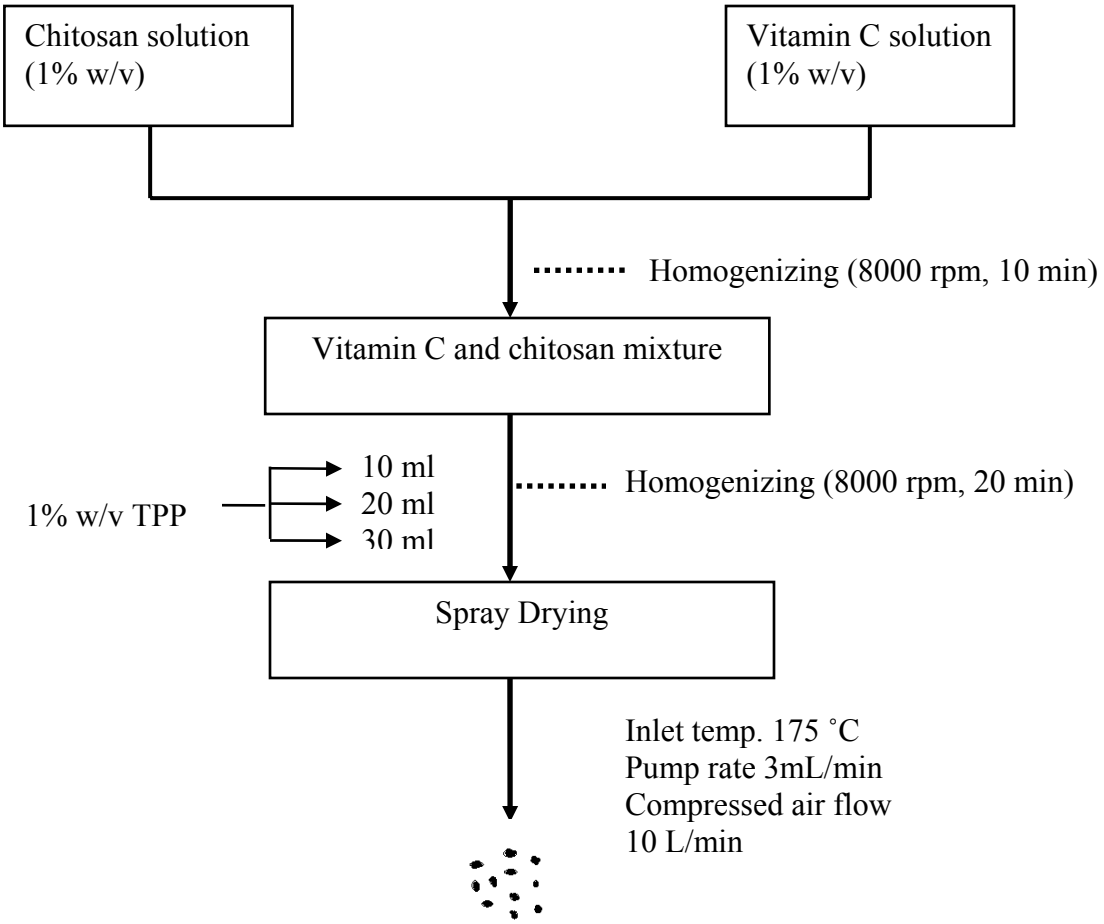


Figure 2.4: Controlled release mechanism of encapsulated food ingredients (Shahidi and others 1993)



Vitamin C encapsulated chitosan microspheres

Figure 2.5: Procedure for preparation of vitamin C encapsulated chitosan microspheres by spray drying (Desai and others 2005)

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CHAPTER 3

MICROENCAPSULATION OF VITAMIN C IN WHEY PROTEIN CONCENTRATE (WPC-80) USING SPRAY AND FREEZE DRYING METHOD

[1] S. R. Tandale, M. S. Chinnan, and R. D. Phillips. To be submitted to the *Journal of Food Science.*

ABSTRACT

Microencapsulation of ascorbic acid was carried out using spray drying and freeze drying method in which whey protein concentrate (WPC) was used as coating material. Ascorbic acid: WPC (w/w) ratio of 1:4.0, 1:5.3, 1:8.0 and 1:16.0 was selected to determine microencapsulation efficiency which showed 1:4 ratio had highest efficiency for both spray dried (96.35%) and freeze dried process (98.23%) processes. 1:4 ratio was selected for further analysis. Morphology of microcapsules was examined by scanning electron microscope (SEM) which revealed good sphericity with some outer pores in spray dried microencapsulated ascorbic acid powder. Freeze dried powder was found to be irregularly shaped. Some agglomeration of small capsules was observed in spray dried powder. Particle size analysis was conducted using the Mastersizer which revealed multi-modal distribution (4.17-36.54 μm) in spray dried powder with average diameter of 17.46 μm. Freeze dried powder had single peak distribution (3.33-8.18 μm) with average diameter of 11.51 μm. Accelerated shelf life testing was carried out under various environmental conditions, temperature (25 and 45 ˚C), Humidity (22, 44, 66 and 85% RH), Light (UV light and dark), and 100% oxygen in sealed container for 7 weeks. Freeze dried microencapsulated vitamin C powder provided better protection from degradation compared to spray dried powder at all the RH conditions. This could be attributed to the difference in bulk density. Freeze dried powder was considerably denser compared to spray dried powder and thus minimizing the area of exposure to environmental degradation conditions.

INDEX WORDS: Microencapsulation, spray drying, freeze drying, ascorbic acid, stability, and microencapsulation efficiency.

INTRODUCTION

Microencapsulation is defined as the process of coating solids, liquids and gaseous materials in microscopic, sealed microcapsules which release their active contents at controlled rate under specific stimuli (Dziezak 1988, Risch 1995). These microcapsules can range from several microns to millimeters and are usually spherical in shape. However, their shape is largely influenced by the structure of the original core material as well as drying conditions. One of the many objectives of microencapsulation is protecting active core content from various degradation factors that can cause its oxidation or deterioration such as temperature, humidity, light, oxygen and its interaction with various minerals such as copper, iron, and zinc. Microencapsulation is also used to prevent off-flavors produced by vitamins, minerals, taste masking certain food ingredients, preventing interaction among various food ingredients, improve the stability and handling of colors, improve flavor and texture of food product. In short, microencapsulation gives flexibility in terms of using food ingredients in food formulation in the hands of food scientist who can come up with innovative food products using this technology.

Spray drying is the most common method used for encapsulation in the food industry for variety of reasons; such as economics, flexibility of the process, ready availability of equipment, wide choice of carrier material, good retention of volatile material, good stability of finished product and large scale production in continuous mode (Reinecccius 1989). The advantage of spray drying method is that heat-labile (low boiling point) and heat sensitive materials can also be encapsulated because of the lower temperature that the core material reaches (Dziezak 1988). Microencapsulation by spray drying process involves formation of emulsion or suspension of coating and core material, followed by atomization and spraying of the mixture in a hot chamber.

Ascorbic acid is used as vitamin supplement in many food products. The commercial forms of ascorbic acid used in the food industry are L-ascorbic acid, its sodium and calcium salts and 6 palmitate ester. One of the important biochemical reactions of L-ascorbic acid is to destroy free radicals (hydroxyl and perhydroxyl). In this role, L-ascorbic acid and its oxidation product dehydroascorbic acid acts as 'redox buffer' (Sapper 1982). When terminating free radicals, L-ascorbic acid is converted to dehydroascorbic acid which is then recycled to L-ascorbic acid by reductase enzyme and cofactors. Since ascorbyl palmitate and L-ascorbic acid reacts with anti-oxidants, they prevent peroxide formation in lipids and delay onset of chain reactions that causes deterioration of vegetable oils, animal fat, fish, margarine, milk, vitamin A and carotenoids (Cort 1982). For certain applications, excess amounts of vitamin C is added prior to packaging in order to remove the free oxygen and to replenish losses anticipated during storage. However, this can increase the risk of undesirable interactions with other components.

Microencapsulated ascorbic acid could be used to prevent its interaction with other ferrous and copper ions which can cause its oxidation. It can be incorporated in powder form in cake mixes, pudding, gelatin dessert, chewing gum, milk powder and breakfast cereals. Microencapsulated vitamin C can be incorporated in the dry form to prevent browning in potato chips by Milliard Reaction, the adverse effects of this reaction are food darkening, development of off-flavors, and reduced bioavailability of certain amino acids especially lysine.

The objective of this study was to develop microencapsulated ascorbic acid to protect it from degradation and to be used as anti-oxidant in the foods to increase the shelf life of foods. Another objective was Microencapsulated ascorbic acid was produced by using two methods freeze and spray drying and characterization of microcapsules was done using scanning electron

microscopy (SEM), particle size analysis, loading efficiencies and storage stability studies under various environmental conditions.

MATERIALS AND METHODS

Materials

Vitamin C (L-ascorbic acid) was purchased from Sigma Aldrich (St. Louis, MO). Whey protein concentrate-80 (WPC) as AVONLAC 180^{TM} was graciously provided by Glanbia Foods (East Gooding, ID, USA). According to certificate of analysis, composition of WPC was protein (dry basis) – 79.3%, Moisture – 3.7%, Fat - 8.9%. All other chemicals used were of analytical grade. Deionized water was used throughout the study.

Microencapsulation of ascorbic acid by spray drying

Eighty grams of WPC was dissolved in 400 ml deionized water at room temperature (22 ˚C) and kept for hydrating overnight at 4˚C. Following day, 5, 10, 15 and 20 gram of L-ascorbic acid was measured and dissolved in 20, 45, 70 and 95 ml of deionized water using magnetic stirring for 20 minutes under dark condition. The ascorbic acid was readily dissolved in water. These ascorbic acid solutions were dissolved in four WPC solutions prepared a day earlier. The four weight ratio of ascorbic acid: WPC was 1:4, 1:5.3, 1:8, 1:16 (w/w) and the total solids level was maintained at 20.2% (w/v) in all four weight ratios. Homogenization of the wall solutions was carried out using LabTek homogenizer (Omni International Inc., Gainesville, VA) at 8000 rpm for 20 minutes for all the four solutions. The solutions were covered with aluminum foils at all the time and homogenization was carried out in subdued light condition. The spray drying of feed solution was carried out using pilot scale spray dryer (Anhydro Inc., Olympia Fields, IL, USA). The inlet and outlet temperature were 180 and 92 ˚C respectively, and atomization speed was

kept at 30,000 RPM (60% of maximum 50,000 RPM). The feed flow rates were 37, 42, 47 and 52 ml/min for 1:16.0, 1:8.0, 1:5.3 and 1:4.0, respectively. The powder was collected in opaque plastic bottles and kept in refrigerator at 4 ˚C until further analysis.

Microencapsulation of ascorbic acid by freeze drying

The spray drying procedure was followed until the homogenization of the ascorbic acid (AA) solution in whey protein concentrate (WPC). The AA: WPC weight ratios were as 1:4.0, 1:5.3, 1:8.0 and 1:16.0. Here too, total solids level were maintained at 20.2% (w/v). After homogenization, the solution was poured in plastic trays and was covered with aluminum foils for protection from light. Homogenization was conducted under subdued light condition and these trays were kept for freezing for 24 hours. The freeze drying was carried out using Virtis Genesis SQ freeze dryer (Gardiner, NY, USA). It was recommended (from manual) that knowing eutectic point will conserve time and energy and produce highest quality freeze dried product. The freeze dried product was frozen below -20 ˚C. Care was taken that homogenously blended matrix was completely frozen in plastic trays before loading them in freeze dryer shelves. The thermocouples were inserted in frozen matrix to monitor the changes in the temperature. The shelf temperature was allowed to reach below -40 ˚C before turning on condenser. Once the condenser temperature dropped below -40 ˚C, the vacuum was turned on until it indicated vacuum 100 millitors. Following that the freeze drying was started and the product was periodically monitored. The trays were covered with aluminum foils to protect from light conditions and some holes were made in the foil to allow water vapor to escape from the trays. After freeze drying was completed, the samples were ground using mortar and pastel under subdued light conditions and were stored in opaque plastic bottles and kept in refrigerated conditions at 4 ˚C until further analysis.

Scanning electron microscopy

Morphology of microcapsules was observed using variable pressure scanning electron microscope Zeiss 1450 EP (Carl Zeiss MicroImaging Inc. One Zeiss Drive, Thornwood, NY). using methodology described by Sheu (1998). The powders were fixed on 10 mm stubs with double sided adhesive tape and then were made electrically conductive by coating in vacuum with gold using SPI-module sputter coater for 60 s. (15.3 nm). The SEM pictures were taken at excitation voltage of 15kV and 100, 200 and 500 X magnification for freeze dried powder and 100, 200 and 573 X magnification for spray dried powder.

Particle size analysis

Particle size analysis was done using Malvern Mastersizer S laser diffraction system with QSpec small volume sample dispersion unit operating at 3000 rpm (Malvern Instruments, Worcestershire, U.K.). Mastersizer type used was S 300 RF with particle size range between 0.5 to 900 μm. The Mastersizer S uses the Mie theory of light diffraction for particle size measurement which assumes an equivalent sphere size of particles and performs particle size calculations on the fact that angle of light diffraction is inversely proportional to particle size. (Rawle 2002). To establish standard operating procedure (SOP) for particle size measurements, 3 replicate samples were used for both spray dried and freeze dried microencapsulated ascorbic acid powder. The 10% (w/w) solution of freeze or spray dried powder was prepared in deionized water and allowed to hydrate for 10 minutes and stirred before applying to dispersion unit of the Mastersizer. The raw measurement data obtained from the Mastersizer consisted of particle size distribution (PSD, d_i = 0.05-865 μm) of the respective sample expressed as volumetric diameter of particles. Additionally, particle size percentiles, volumetric mean diameter, summary statistics

of PSD was calculated using the Mastersizer data acquisition software package (Mastersizer S-long Bed v2.19).

Microencapsulation efficiency

In order to determine microencapsulation efficiency (ME), 200 mg of microencapsulated powder was measured and dissolved in 100 ml of 0.1N HCl. This solution was further diluted 100 times and measured at 244 nm wavelength (λ max of vitamin C in 0.1N HCl) using Hewlett Packard 8451A diode array spectrophotometer (Avondale, PA, USA). For determination of vitamin C concentration equivalent amount of WPC absorbance at 244 nm was subtracted from total absorbance of microencapsulated vitamin C. Experiments were performed in triplicate and ME was determined based on the formula below (Desai and Park 2005).

$$\text{ME (\%)} = \frac{\text{Calculated vitamin C concentration}}{\text{Theoretical vitamin C concentration}} \text{ X } 100$$

Accelerated shelf life testing

Vitamin C: WPC (w/w) ratio of 1:4 was selected for accelerated shelf life studies due to high microencapsulation efficiency obtained in spray dried (96.35%) and freeze dried (98.75%) powders. Microencapsulated ascorbic acid was spread in small cups and care was taken that the layer of powder did not exceed more than a few millimeters to avoid uneven exposure to humidity and UV light conditions. Two wooden chambers were constructed for storage at 25 and 45 ˚C and each wooden chamber had two shelves. The upper top shelf was fitted with black light which emitted UV light confined to top shelf. Dark condition was maintained in bottom shelf. Both the shelves were covered with opaque black plastic covers to avoid outside light exposure. The small cups were covered with transparent loosely knitted cloth to allow the passage of UV

light and moisture but which will prevent cross-contamination between samples. Saturated salt solutions of potassium acetate (22.5% RH), potassium carbonate (44.7% RH), sodium nitrite (65% RH) and potassium chloride (85% RH) were prepared and transferred to transparent glass containers. The RH inside the glass containers was monitored using temperature-humidity data loggers. Once saturated salt solutions reached the optimum RH levels, sample cups containing freeze dried and spray dried microcapsules were kept over the circular disc mounted on stand which would allow free circulation of humid air over the saturated salt solutions. Out of two wooden chambers, one was maintained at 25 ˚C and the other at 45 ˚C. Eight glass containers were kept in 25 ˚C; four containers with four different humidity conditions (22.5, 44.7, 65, 85% RH) in UV light and remaining four in dark conditions. Remaining eight glass containers were kept at 45 ˚C under similar light and dark conditions. The 'zero' week concentration of vitamin C was the beginning concentration at the start of storage conditions. 100 mg each of spray and freeze dried powder was measured and dissolved in 100 ml 0.1N hydrochloric acid using magnetic stir bars for 20 minutes (1gm/L). The subdued light conditions were maintained during the assay. The vitamin C concentration was measured by absorbance at 244 nm (λ max of vitamin C in 0.1 N HCl) after 10 times dilutions. Special arrangement was made which would maintain the constant oxygen environment inside the glass containers. On the glass cover, a small aperture was made and rubber septas were glued over that aperture which allowed a metal needle to pass through the septas so that glass containers could be filled with oxygen. Septas were again sealed with the glue to prevent oxygen to escape. Digital headspace analyzer (Check-mate 9900, Topac Inc., Hingham, MA, USA) was used to monitor residual oxygen concentration inside glass containers. The samples were withdrawn every week from glass containers to

determine oxidative degradation and glass containers were filled with 100% oxygen concentration before placing back to storage conditions.

RESULTS AND DISCUSSIONS

Scanning electron microscopy

The objective behind using scanning electron microscopy was a) to observe the degree to which microcapsules are well formed; b) examine the porosity, size, shape, outer topography to reveal the surface dents and shrinkage, tendency to agglomerate and c) presence of extraneous matter, if any. The morphology of the microcapsules was observed using scanning electron microscope (SEM) for both freeze dried as well as spray dried microcapsule. However, here we should remember that in preparing freeze dried microcapsules, the freeze dried matrix (homogeneously blended whey protein concentrate and ascorbic acid) was subjected to mortal and pastel to obtain the free flowing powder which was used for analytical purposes. Freeze dried microcapsules were observed at 100, 200, 500 X and spray dried microcapsules were observed at 100, 200 and 573 X magnification. Freeze dried microcapsule particles, as expected, were irregular shaped. The edges of the capsules are considerably sharper. In fig. 3.2 (A), (B) and (C), we can see the slab like structure of irregularly shaped microcapsule having thickness of 20 μm. Spray dried microcapsules were having good sphericity as we can observe in fig.3.2 (C), (D) and (E). Some of the microcapsules were broken, thus increasing the area exposed to surrounding air. However, there were many spherical shaped microcapsules compared to broken microcapsule in the spray dried sample. Some of the smaller capsules of spray dried microcapsules showed the tendency of agglomeration which explains less free flowing characteristic of spray dried microcapsules.

Particle size distribution

The cumulative percentage size distribution of spray and freeze dried samples are also shown in fig. 3.3 (A) and (B) respectively. The particle size distribution of both spray dried and freeze dried samples are shown in the fig. 3.4 (A) and (B). In spray dried microencapsulated ascorbic acid powder, the mean particle size diameter was 17.5 μm and the particle size ranged from 1.3 to 83.2 μm. In freeze dried microcapsules, the mean diameter was 11.5 μm and the particle size ranged from 1.3 to 77.2 μm. Spray dried microcapsules showed multi-modal distribution with first mode from 3.8-11.0μm and second mode from 11.0-36.5μm. In freeze dried microencapsulated powder; particle size distribution showed single mode distribution with main mode in intermediate diameter range 3.5 – 6.5 μm. Narrower particle size distribution results in more homogenous particles which can ensure better stability and accurate release of the core material (Finotelli and Leao 2005). The larger particles observed under SEM compared to particle size distribution may be attributed to the some dissolution of in water while measuring the particle size in Malvern Mastersizer. Ethanol as a solvent could yield more accurate information about the particle sizes. Finotelli and Leao (2005) obtained ascorbic acid microcapsules with Capsule ^{TM} from National Starch, maltodextrin and combination of Capsule and maltodextrin but the method used was spray drying. Capsule is octenyl succinate substituted starch also called as modified starch. In particle size analysis, multi-modal distribution of particles was observed in all the microcapsules. According to Trindade and Grosso (2000), mean diameter of ascorbic acid microcapsule using gum arabic coating was 8 μm and starch containing 1% and 2% gelatin was 20.5 and 17.7 μm, respectively. This is similar to our average particle size of 17.5 μm in spray dried powder although the coating material was different. Trindade and others (2000) also found that ascorbic acid in gum arabic had multimodal distribution with range

from 0.3 to 90 μm. This also is similar to our spray dried powder range of 1.3 to 83.2 μm. It seems that spray drying method of microencapsulation probably produce multi-modal distribution (Finotelli and others 2005; Trindade and Grosso 2000).

Microencapsulation efficiency

Microencapsulation efficiency is an important criterion for successful microencapsulation. It indicates the loss of the core during the process. The retention of core material during microencapsulation by spray drying is affected (among other things) by the properties and composition of the emulsion and by spray drying conditions. An ideal microencapsulation process should not result in any losses of encapsulated core during the process. Effects of process conditions and compositional aspects on core retention during microencapsulation by spray drying have been discussed pertaining to volatiles and essential oils (Rosenberg, Kopelman and Talmon 1990). High drying rates that lead to rapid crust formation around drying droplets can lead to high core retention. Losses can occur before the formation of dry crust. The microencapsulation efficiencies (ME) of spray freeze dried vitamin C powders are shown in table 3.1 and 3.2. The ME of 96.25% in spray drying condition for 1:4 ratio indicates that there was not much loss during spray drying and some loss could be attributed to loss of vitamin C before formation of the crust due to the drying temperatures. There is possibility that vitamin C migrating to the surface and undergoing some degradation. In freeze dried powder, microencapsulation efficiency of 98.75% indicates the low temperature maintained in freeze drying resulted in better protection of vitamin C. Lee and others (2004) have reported similar ME of 1:5 for L-ascorbic acid: polyacylglycerol monostearate which resulted in 94.2% ME and efficiency decreased by increasing the coating to core ratio of 10:1, 15:1 and 20:1 (polyacylglycerol: L-ascorbic acid) as 86%, 85% and 80%, respectively.

Accelerated shelf life testing

Ascorbic acid is added to foods primarily for two reasons; as vitamin supplement to increase recommended dietary allowance and to protect food from oxidative deterioration. However, its use in food is compromised due to its instability and high reactivity with transitional metals ions such as copper and iron (Liao and Seib, 1988). Other environmental factors that can compromise stability of vitamin C are temperature, pH value, UV rays, X-rays and humidity. Oxidation is accelerated at neutral and above pH (Csuros and Petro, 1955). The degradation of vitamin C can occur in foods by variety of mechanisms. Copper and iron acts as catalyst in oxidation of vitamin C. Enzymatic destruction can occur by enzymes such as ascorbate oxidase and ascorbate peroxidase (Pollard and Timberlake 1971). Losses can also occur by anaerobic mechanism in strongly acidic conditions, in the presence of fructose and derivatives (Heulin 1953) and by amino acids (Kurata 1973).

Number of metal chelating agents are known to prevent autoxidation of L-ascorbate including ethylenediamine tetraacetate (EDTA), oxalate citrate, phosphates, histidines, uric acid, sugars, acidic polysaccharides and flavonoids. Agents that complex with L-ascorbic acid such as protein, EDTA and histidine may retard oxidation of L-ascorbic acid (Fleming and Bensch, 1983). Therefore, we thought of choosing whey protein concentrate as coating material. In our study we wanted to examine the effect of temperature, UV light, water activity and the type of drying method on protective effect of ascorbic acid in whey protein concentrate. Therefore, we selected two temperatures levels: 25 and 45 ˚C; UV light and dark condition; four relative humidity levels: 22, 44, 66 and 85 %; and two dying methods: freeze and spray drying. Microencapsulation efficiency was determined by total amount of ascorbic acid present after spray and freeze drying process. The microencapsulation efficiency was 96.35 in spray dried

powder and 98.23% in freeze dried powders. Finotelli (2005) obtained 100% retention of ascorbic acid by spray drying in capsul and maltodextrin. Trindade (2000) reported ascorbic acid recovery after spray drying as 99.7 and 97.6% for starch containing 1 and 2% gelatin and 98.8 for gum arabic containing 30% ascorbic acid. Esposito (2002) used Eudragit® for microencapsulation of ascorbic acid who reported microencapsulation efficiencies between 98-100%. Eudragit are methylacrylate copolymers which are inert and freely soluble in organic solvents. This is pH dependent enteric polymer composed of methacrylic acid-methyl ester copolymers soluble at pH 6. The pH of the stomach is 7.5 and therefore is used for delivery of ascorbic acid in this lower part of intestine (Esposito 2002). Since drying time in spray drying is short between 5-30 s, it is not significant for the degradation of vitamin C. Also, during spray drying process, the droplet temperature is maintained at low temperature due cooling effect of evaporation resulting in lesser degree of degradation (Dziezak 1988). In 100 mg of spray dried powder (1:4 ratio), the beginning concentration was 19.26 mg and in 100 mg of freeze dried powder (1:4 ratio), the beginning concentration was 19.75 mg of ascorbic acid. This was the beginning concentration at 0 week of storage. During the accelerated storage studies, microencapsulated vitamin C powder in whey protein coating material exhibited color changes specific to relative humidity levels after 2 weeks of storage. The color of the microencapsulated powder ranged from dark pink, light brown to dark brown. At 22 % RH initially light pink color changed into dark pink color as the storage time progressed. Maximum dark brown color was observed at 85% RH followed by 65% RH. At 44% RH, light brown color was observed. This may be attributed to series of chemical reactions that may be taking place. The first initial product of oxidation of L-ascorbic acid in the presence of dioxygen in water is dehydroascorbic acid and hydrogen peroxide. In aqueous solution dehydroascorbic acid exists primarily in the

bicyclic structure having 6, 3-hemiketal ring and C2-gen-diol group as opposed to its diketo form. Streker degradation reaction between dehydroascorbic acid and an amino acid give aldehyde and scorbamic acid. However, dehydroascorbic acid can form brown pigment in the absence of amino acids too. In Streker degradation, initial product is Schiff base that decarboxylates and then undergoes hydride shift to give a second Schiff base or substituted imine intermediate. This substituted imine hydrolyses to release aldehyde and scorbamic acid. This amino reductone, L-scorbamic acid (SCA) is presumed to be an important intermediate in the formation of an assortment of colored compounds (Hayashi 1983, 1980). This SCA reacts with molecule of dehydroascorbic acid to give red pigment; this is chain reaction. Thus, we can explain why pink color was observed at 22% RH and subsequently dark brown pigment was observed at higher 85% RH. This was further substantiated by higher degradation found at higher humidity than lower humidity levels. Dehydroascorbic acid can be quantitatively reduced back to L-ascorbic acid by hydrogen sulfide, dothiothreitol, reduced glutathione and stannous ions plus thiourea, among others. In practical applications, this dehydroascorbic acid is involved in browning of citrus juices and some dehydrated foods (Kurata 1973). Favorable reaction between protein and dehydroascorbic acid has been proposed to explain the improvement of bread when ascorbic acid is added to bread formula. This dehydroascorbic acid acts as cross linking agent between gluten and thus increasing the elasticity of dough. One of the potential application of microencapsulated ascorbic acid is incorporation in bread formulation in which required concentration of ascorbic acid can be used for cross linking between gluten to improve the elasticity of dough and at the same time some concentration could be preserved by microencapsulation thereby increased bioavailability of vitamin C in addition to improved bread texture.

We can observe from the degradation plots (fig. 3.5 to fig. 3.8) the different trends in degradation due to various factors. Humidity plays significant role in the degradation kinetics. At 25 ˚C, we can see lowest degradation at 22% RH in both freeze and spray dried samples and under light and dark conditions. However, at 45 ˚C there is lowest degradation in 44% RH in both freeze dried and spray dried samples and both light and dark conditions. Therefore, higher temperature may be causing more degradation at 22% compared to 44% RH. Also there is interesting trend in 66% and 85% RH. At 25˚C, greater degradation is observed in 66% RH compared to 22%, 44% and 85% RH in spray dried vitamin C samples in both light and dark conditions. But at 45 ˚C maximum degradation is found in 85% RH in both light and dark conditions. Serris and Biliaderis (2001) reported that degradation kinetics of beetroot pigments consisting of betalain was highest at intermediate moisture content (a_w = 0.64) for all the matrices (pullulan and two maltodextrin samples). The reaction rates (K) and half-life period ($T_{1/2}$) for encapsulated beetroot pigments were compared at 0.23, 0.43, 0.64, 0.75 and 0.84 a_w levels and at 30, 40 and 50˚C. The maximum reaction rate (k) was observed at 0.64 a_w. However, reaction rates (K) at 0.23 and 0.44 a_w levels were lower than 0.75 and 0.84 a_w. The reaction rates (K) increased with increase in temperature. This trend can be explained by the fact that the degradation reaction is controlled by the mobility of the reactants. This type of behavior has been exhibited in many systems according to Von Elbe (1987). Saguy (1984) has reported that with increasing a_w, there is increase in oxygen concentration and this in turn increases degradation rates since oxygen accelerates the reaction. In dry environment, water is strongly bound to surface polar sites and is generally not available for any kind of reaction. This fact explains the lower reaction rates observed at a_w of 0.23 and 0.43. At high Aw, reactant dilution effect have been claimed to be predominant (Von Elbe 1987). As a result, reaction rate may reach maximum

at intermediate a_w and then decline with further increase in a_w. Thus, Serris and Biliaderis (2001) reported maximum reaction rates at a_w = 0.64 for all three temperatures. Although, our core material is vitamin C, similar trends of degradation kinetics have been observed in our studies.

Comparing spray dried powder with freeze dried powder, freeze dried powder provided better protection to microencapsulated vitamin C at all the RH's studied. The bulk density of freeze dried powder was about 4 times higher compared to spray dried powder. Higher the density, lesser the surface area exposed compared to lower density powder. This may explain the higher degradation in spray dried powder compared to freeze dried powder. Cai and Corke (2000) reported that in spray dried Amaranthus betacyanin extracts, the bulk density of pigment powder decreased with increase in spray drying temperature. Although higher drying rate was obtained at higher drying temperature, higher ratio of surface-to-volume for the spray dried powder caused lower bulk density of the powder. The lower the bulk density, the more occluded air within the powders and therefore, greater the possibility of oxidative degradation of pigments and reduced storage stability.

Among all the samples, 70.4 % of active vitamin C content was preserved in freeze dried microencapsulated vitamin C powder stored at 25 ˚C, dark and 22% RH conditions and at these conditions 70.4 % of retention was maximum among all the storage conditions. This proves the ideal condition of storage and preferred method of drying as freeze drying. Trindade and Grosso (2000) have found that pure ascorbic acid did not find much decrease in concentration. However, at 45 C and 90.7 % RH the encapsulated ascorbic acid showed great losses in ascorbic acid concentration. The explanation for lower stability in uncovered form than crystalline form was due to dissolved ascorbic acid distributed itself in layers around starch granules during spray drying process thus establishing the larger surface area than pure unencapsulated ascorbic acid

thus more susceptible to degradation from oxygen. Kirby (1991) has found that in the absence of other potential reactants or antagonists, ascorbic acid is significantly more stable in concentrated form. Ascorbic acid in free solution exposed to normal condition of dissolved oxygen and stored under excess head space air and dark conditions, was completely disappeared from solution after storage for 18 days at 4 ˚C at the starting concentration of 1.15 mM. At 22 ˚C and no light conditions, the same amount was lost after only 6 days. When liposome encapsulated ascorbate was suspended in appropriate buffers under identical conditions, the retention of ascorbate at both 4 and 22 ˚C in dark were significantly increased. At 4 ˚C, more than 50% ascorbate was retained after 50 days of storage; whereas about 50% was lost at room temperature. Thus, Kirby (1991) has successfully shown that when in free solution form, ascorbic acid could be substantially protected from degradation by microencapsulating in liposomes. Kirby (1991) has also studied the effects of various antagonists such as ascorbic acid oxidase, copper ions and lysine on degradation of liposome encapsulated ascorbic acid in various buffer solutions. Free ascorbate in the presence of 10 ppm copper ions, oxidized rapidly and was completely gone after 30 hours. However, loss of ascorbate in liposomes was considerably reduced. After 50 days, level of ascorbate remaining in liposomes were only 18% less than liposomes stored in the absence of copper ions which proved the protective effect of microencapsulation. Lysine which is one of the amino acids acts as antagonist on ascorbic acid. This amino acid resulted in accelerated loss in free solution. At 7.0 pH and 4 ˚C the ascorbate disappeared completely within 10 days. At 22 ˚C in free solution in the presence of lysine, ascorbate disappeared completely within 5 days and brown coloration was observed. However, microencapsulation within liposomes gave almost complete protection and after 40 days the level of ascorbic acid was less than 5% lower compared to the solution in which lysine was absent. This is important from our

studies since whey protein contains lysine as one of the amino acids and the relative rapid loss of vitamin C could be explained due to the presence of lysine as one of the amino acid.

CONCLUSIONS

The results from this study demonstrate that it is possible to successfully microencapsulate vitamin C using WPC as wall material. The maximum retention of vitamin C after 7 weeks of storage was found under 25 ˚C, dark and 22% RH conditions whereas 70.4 % of original concentration was retained. Compared to spray dried powder, freeze dried powder protected the core material at all 22, 44, 66 and 85% RH; light and dark; 25 and 45 ˚C. Thus, freeze dried powder was superior under all storage conditions compared to spray dried powder. Thus, results from this study could be used to prepare microencapsulated muscadine and blueberry extracts which could be utilized for variety of food applications. Scanning electron microscopy revealed production of well formed microcapsules with fewer dents is possible by spray drying process. Freeze dried microcapsules were irregularly shaped but it did not affect the retention of the vitamin C. In fact, freeze dried powder produced powder with high bulk density. Maximum microencapsulation efficiency was found at 1:4 (vitamin C: WPC) w/w ratio for both spray and freeze dried powder.

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Table 3.1: Microencapsulation efficiency of spray dried vitamin C

Vitamin C (g)	WPC (g)	Vitamin C: WPC ratio (w/w)	Microencapsulation efficiency (%)
5	80	1:16.0	75.41[c]
10	80	1:8.0	85.71[b]
15	80	1:5.3	84.61[b]
20	80	1:4.0	96.35[a]

[a,b,c] Values followed by different letters in the same column are significant (α=0.05)

Table 3.2: Microencapsulation efficiency of freeze dried vitamin C

Vitamin C (g)	WPC (g)	Vitamin C: WPC ratio (w/w)	Microencapsulation efficiency (%)
5	80	1:16.0	79.11 [b]
10	80	1:8.0	80.40 [b]
15	80	1:5.3	85.97 [b]
20	80	1:4.0	98.23 [a]

[a, b, c] Values followed by different letters in the same column are significant (α=0.05)

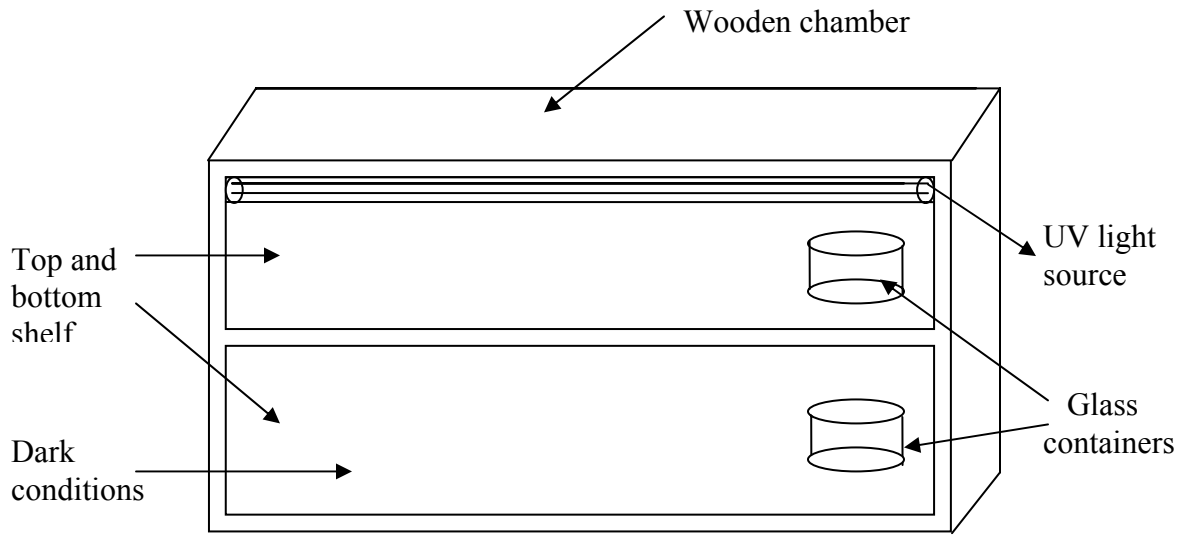


Figure 3.1: Wooden chambers fitted with black light constructed for storage of microencapsulated powder

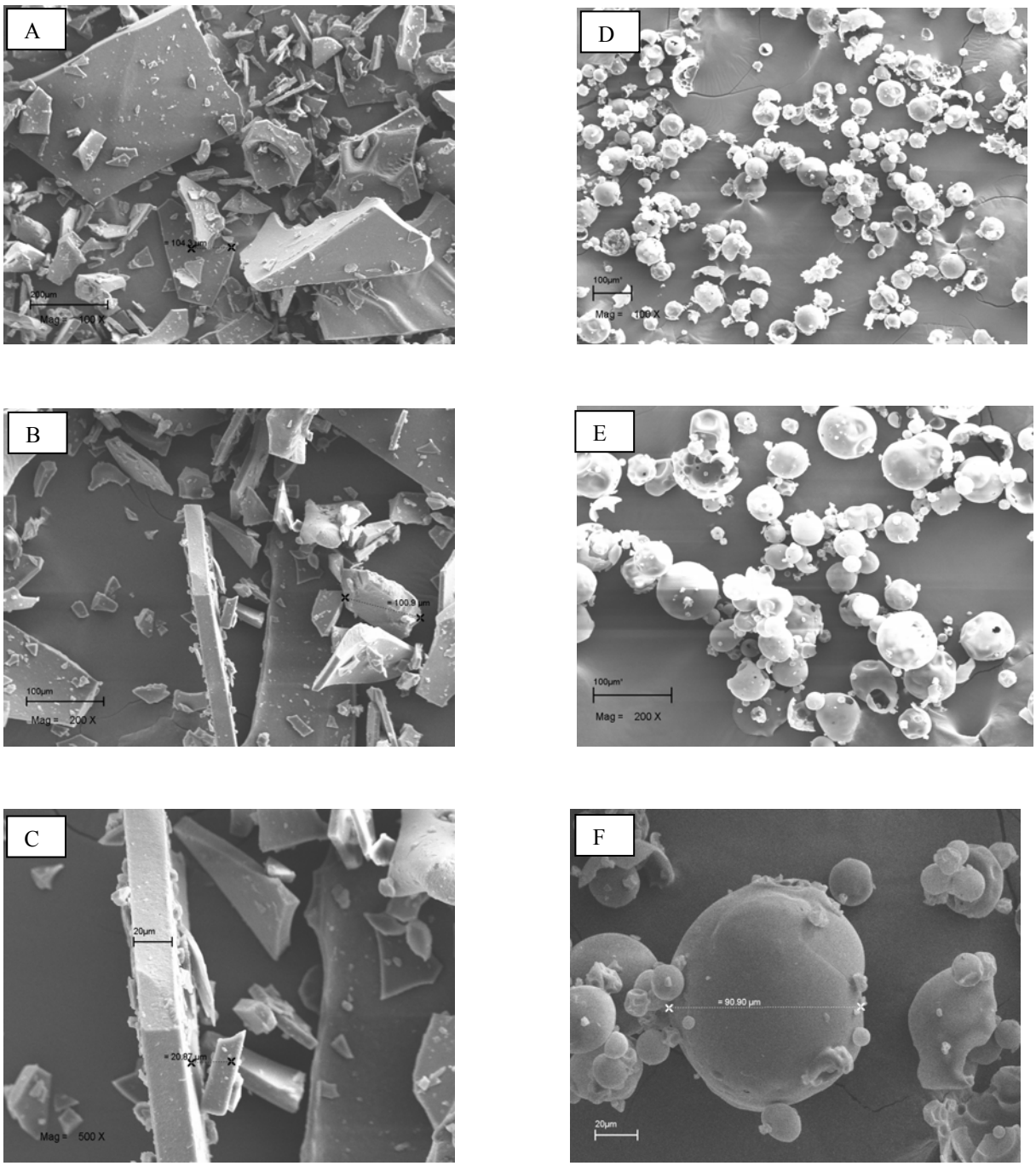
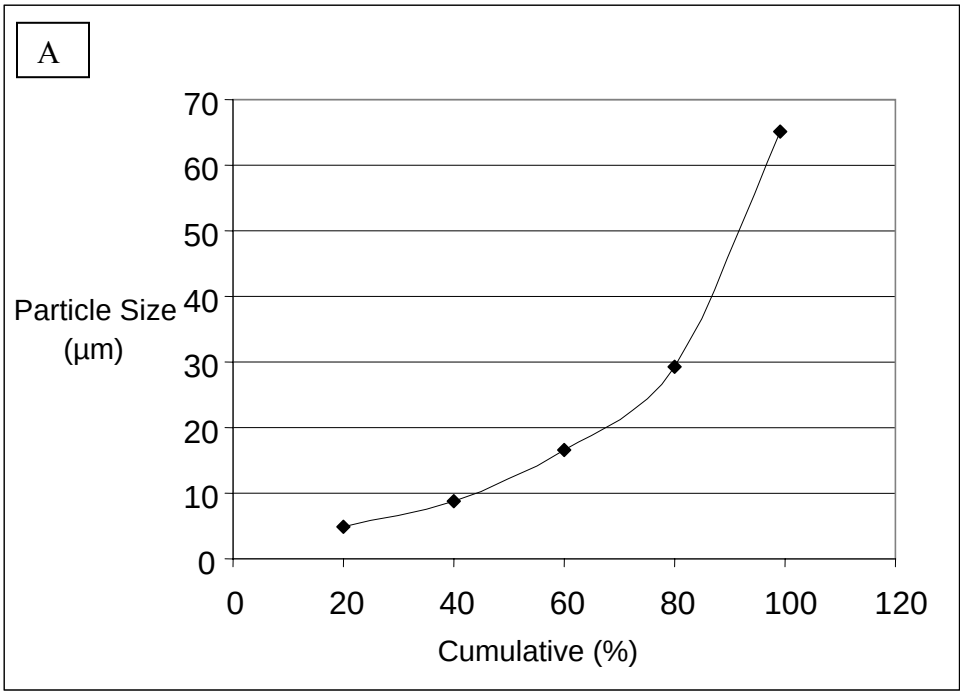


Figure 3.2: SEM micrograph of freeze dried microencapsulated vitamin C (vitamin C: WPC ratio of 1:4) with magnifications of (A) 100X (B) 200 X (C) 500 X.
SEM micrographs of spray dried microencapsulated vitamin C (vitamin C: WPC ratio of 1:4) with magnifications of (D) 100X (E) 200X and (E) 573 X.



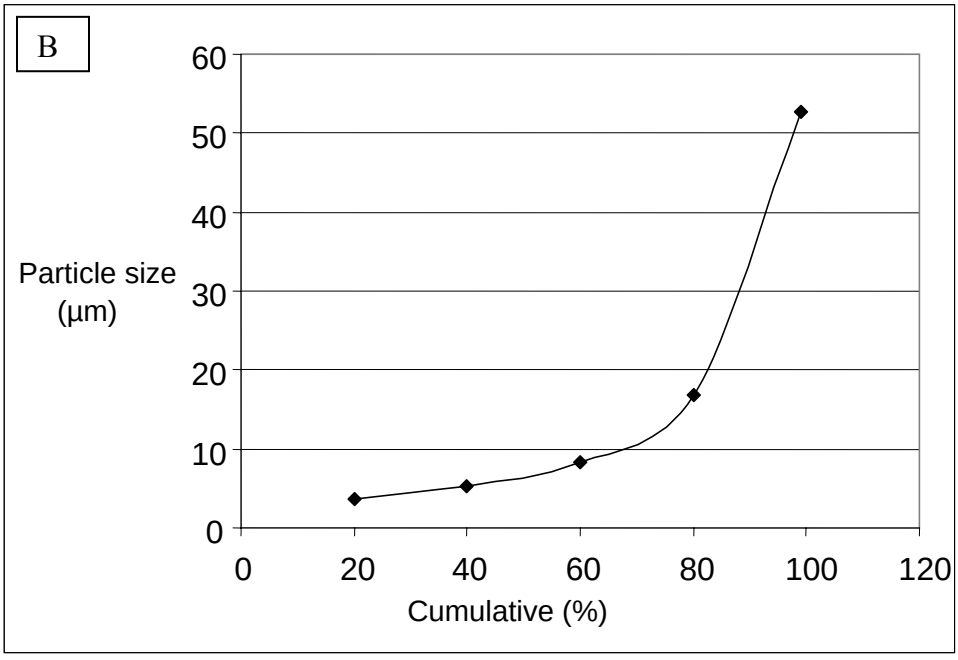


Figure 3.3: Cumulative percentage particle size distribution of (A) spray dried microencapsulated vitamin C powder (B) freeze dried microencapsulated vitamin C powder.

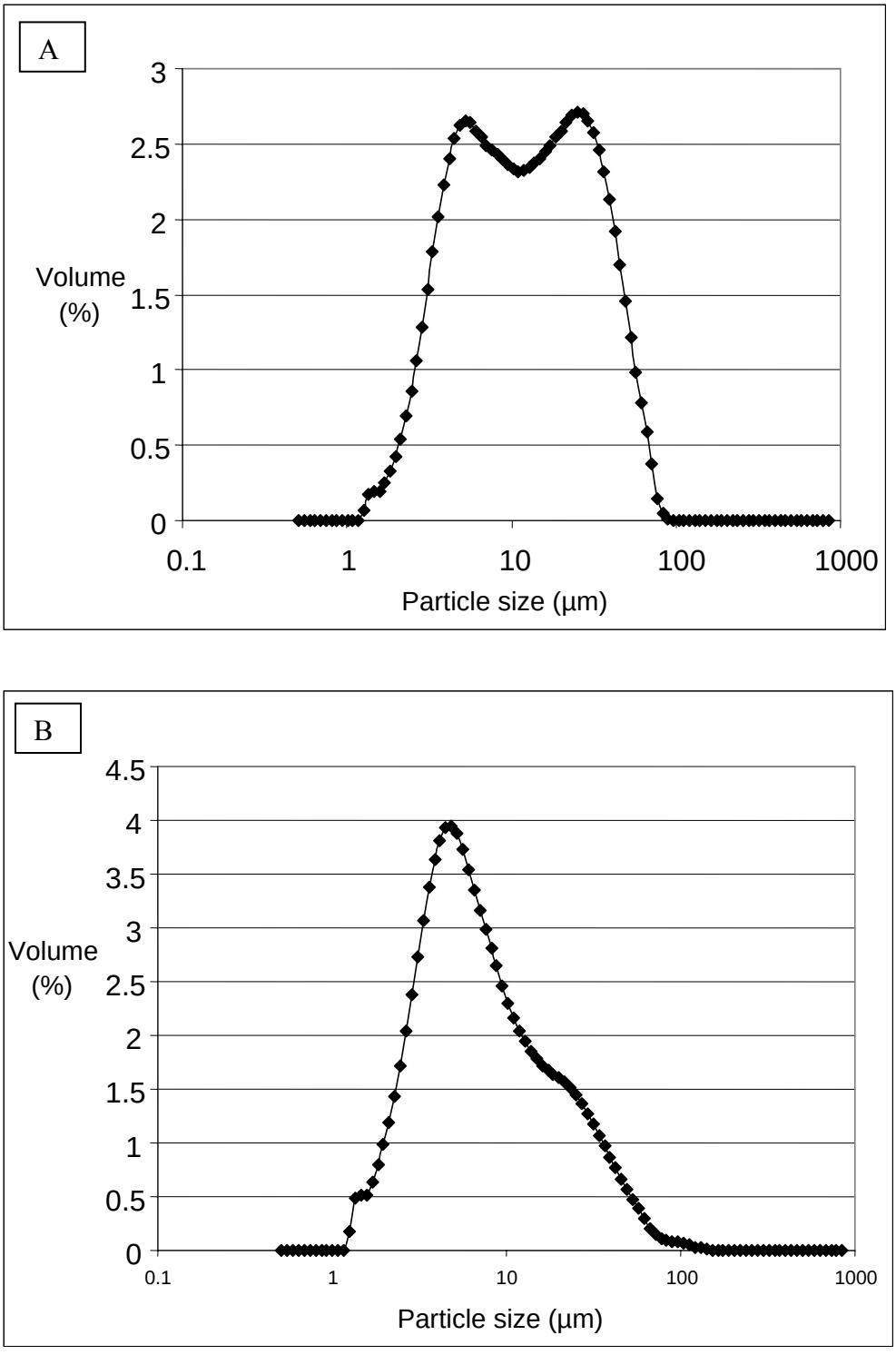


Figure 3.4: Particle size distribution of (A) spray dried microencapsulated vitamin C powder (B) freeze dried microencapsulated vitamin C powder

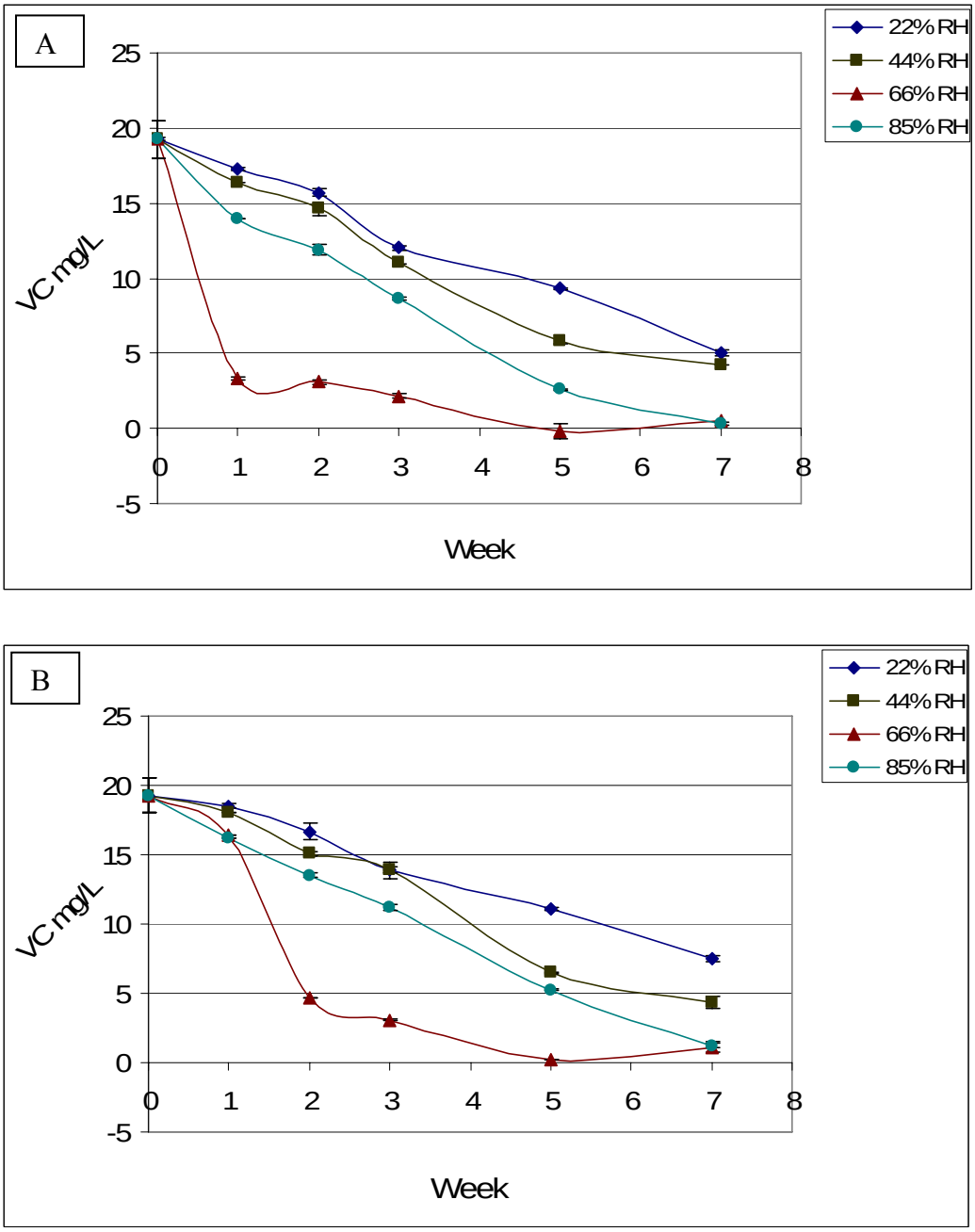


Figure 3.5: Degradation kinetics of spray dried microencapsulated vitamin C stored at 25˚C and (A) UV Light and (B) Dark condition (VC denotes vitamin C)

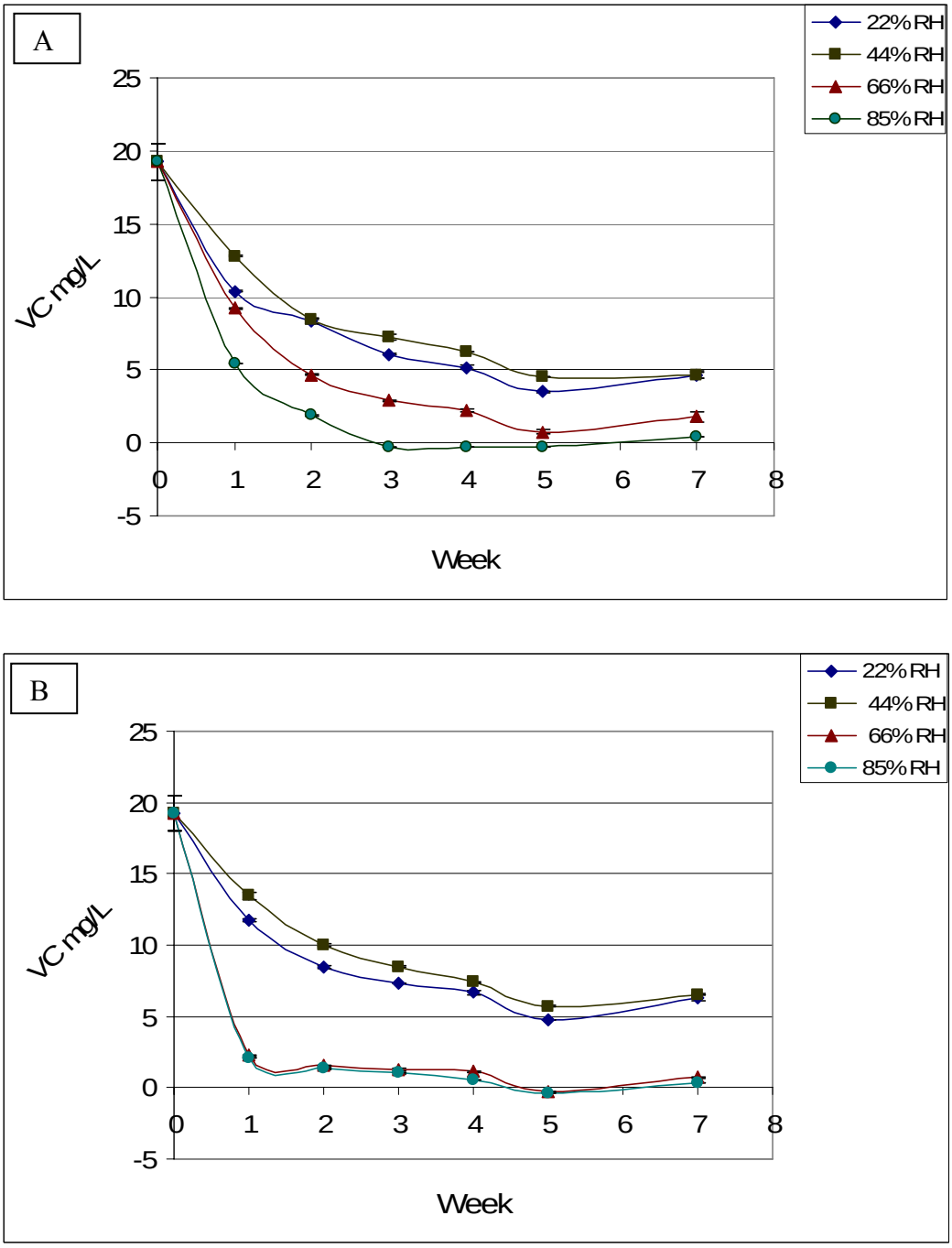


Figure 3.6: Degradation kinetics of spray dried microencapsulated vitamin C stored at 45 ˚C and (A) UV Light (B) Dark condition (VC denotes vitamin C)

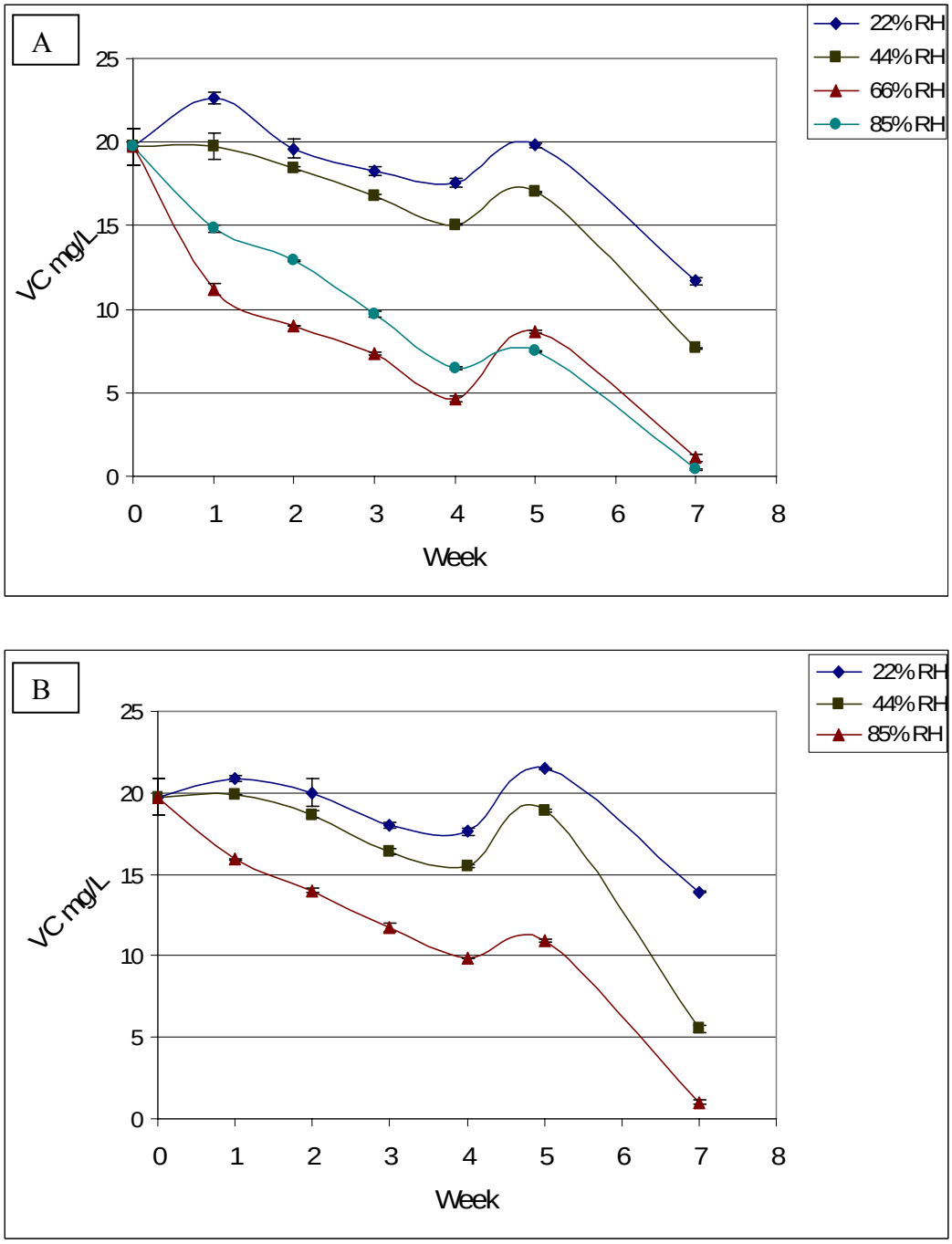


Figure 3.7: Degradation kinetics of freeze dried microencapsulated vitamin C stored at 25 ˚C and (A) UV light and (B) dark condition (VC denotes vitamin C)

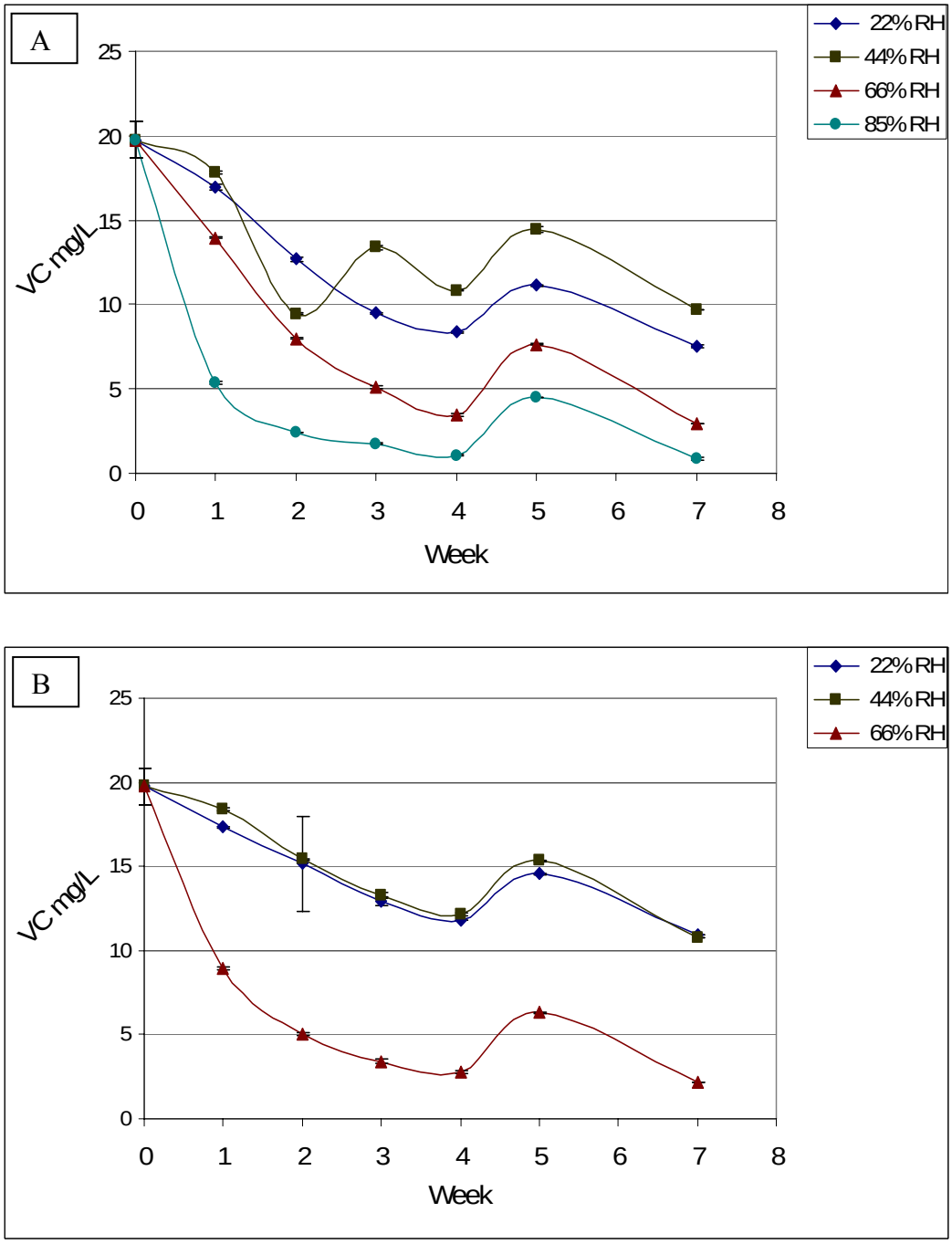


Figure 3.8: Degradation kinetics of freeze dried microencapsulated vitamin C stored at 45 ˚C (A) UV light (B) dark condition (VC denotes vitamin C)

CHAPTER 4

MICROENCAPSULATION OF GALLIC ACID IN WHEY PROTEIN CONCENTRATE (WPC) USING SPRAY AND FREEZE DRYING METHODS

[1] S. R. Tandale, M. S. Chinnan, and R. D. Phillips. To be submitted to the *Journal of Food Science.*

ABSTRACT

Gallic acid as a model core was selected and microencapsulation of gallic acid was carried out with whey protein concentrate (WPC) as wall material, using spray drying and freeze drying methods. Gallic acid: WPC (w/w) ratio of 1:4.0, 1:5.3, 1:8.0 and 1:16.0 was selected to determine microencapsulation efficiency which showed 1:4 ratio had highest efficiency for both spray dried (99.39%) and freeze dried (99.54%) processes. Therefore, 1:4 ratio was further selected for analysis. Morphology of spray and freeze dried microcapsules was examined by scanning electron microscope (SEM). Spray dried gallic acid microcapsules were well formed, spherical in shape with few capsules having visible surface dents. Freeze dried powder was found be irregularly shaped. Some agglomeration of small capsules was seen in spray dried powder. Particle size analysis was carried out which revealed for spray dried powder, single-mode distribution with particle size range (1.4-104.2 μm) with average diameter of 29.9 μm was observed. Freeze dried powder had multi-modal distribution with particle size range (1.4-255.7μm) with average diameter of 38.8 μm. Accelerated shelf life testing was carried out under various environmental conditions, temperature (25 and 45 ˚C), humidity (22, 44, 66 and 85% RH), light (UV light and dark), and 100% oxygen in sealed container for 8 weeks. The maximum degradation of gallic acid was observed at intermediate 66% RH in both spray and freeze dried microcapsules.

INDEX WORDS: Microencapsulation, spray drying, freeze drying, gallic acid, stability, microencapsulation efficiency.

INTRODUCTION

Microencapsulation is defined as the technology of packaging solids, liquids and gaseous materials in miniature, sealed microcapsules which releases their active contents at controlled rate under right stimulus (Dziezak 1988, Risch 1995). The structure formed by the microencapsulating agent around microencapsulated material (core) is called wall or coating material. These microcapsules can range from several microns to millimeters and they are ideally spherical in shape but their shape is heavily influenced by the structure of the original unencapsulated material as well as drying conditions. The shape of the microcapsules could be single core within single wall with spherical or irregular shape, single core within multiple walls or numerous cores within same wall. One of the many objectives of microencapsulation is protecting active core content from various factors that can cause its oxidation or deterioration such as temperature, humidity, light, oxygen and its interaction with various minerals such as copper, iron, and zinc. Other important objectives of microencapsulation are to prevent the off-flavors produced by vitamins, minerals and bioactive compounds, taste masking certain food ingredients, preventing interaction among various food ingredients, prevent nutritional loss, transform liquids into easily handled solid ingredients, and improve flavor and texture of food product (Balassa and Fanger 1971).

Spray drying is the most common method used for encapsulation in the food industry for variety of reasons such as the process is economical and flexible, ready availability of equipment, wide choice of carrier material, good retention of volatile material, good stability of finished product and large scale production in continuous mode (Reinecccius 1989). The advantage of spray drying method is heat-labile (low boiling point) and heat sensitive materials can also be encapsulated because of the lower temperature that the core material reaches

(Dziezak 1988). Microencapsulation process involves formation of emulsion or suspension of coating and core material, followed by atomization and spraying of the mixture in hot chamber. Freeze drying technique, which is also called as lyophilization, is useful for drying thermo-sensitive compounds. Buffo & Reineccius (2001) have compared spray drying, tray drying and freeze drying to encapsulate cold-pressed orange oil Valencia with gum arabic and modified food starch and concluded that freeze drying process gave most desirable properties compared to spray dried powder. Minemoto (1997) compared oxidation of menthyl linoleate when encapsulated in gum arabic by hot air drying and freeze drying and showed that freeze drying was better than hot air drying in terms of protection from oxidation. Heizelmann & Franke (1999) reported that microencapsulated fish oil by freeze drying offered good resistance to oxidation. Freeze drying is less attractive than spray drying because costs are about 50 times higher compared to spray drying (Desorby 1997). However, nutraceutical powder produced from muscadine grapes which is high value added product could utilize this technique to produce microencapsulated powder which could result in better protection from oxidation compared to spray drying.

Rabbiteye blueberry (*Vaccinium ashei*), common in Georgia, is rich source of various health promoting compounds like phenolic acids (gallic acid, p-hydroxybenzoic acid, caffeic acid, ferulic acid, and ellagic acid) and flavonoids (catechin, epicatechin, myricetin, quercetin, and kaempferol) (Sellapan and Akoh 2002). These compounds neutralize free radicals associated with number of degenerative diseases such as cancer, cardiovascular diseases, cataracts and Alzheimer's. However, these compounds are sensitive to degradation with exposure to environmental conditions; such as temperature, oxygen, light and relative humidity. Rabbiteye blueberry contains 259 mg/100 gm of gallic acid (a major phenolic acid). Intermediate extract of

muscadine grape contains sugars which makes it difficult to handle and may stick on the walls of the spray dryer. High molecular weight hydrocolloids such as modified starch, gum arabic or proteins such as whey proteins could be used to make non-sticky nutraceutical powder. We chose gallic acid as model core material for microencapsulation considering very high cost of other pure compounds. Two major protein groups of bovine milk are whey protein and casein. Whey is derived as natural byproduct of cheese-making process. In addition to proteins, the raw form of whey contains fat, lactose and other substances. The raw form is processed to produce protein rich whey protein concentrate (WPC) and whey protein isolate (WPI). Whey proteins have been reported to have excellent encapsulation properties and are superior to those of commonly used ingredients. These studies have indicated that whey proteins are very effective in encapsulating volatile, non-volatile, polar and non-polar compounds (Moreau and Rosenberg 1996, 1998, 1999; Sheu and Rosenberg 1995; Young, Sarda and Rosenberg 1993). The microencapsulation of gallic acid will help in understanding the protective effect offered to the similar phenolic compounds under various environmental stress conditions.

The objective of this study was to microencapsulate gallic acid to protect it from degradation and generate data which will serve as a model for developing nutraceutical powder from muscadine grapes. Microencapsulated gallic acid was produced by using two methods: freeze drying and spray drying and characterization of microcapsules was done using scanning electron microscopy (SEM), particle size analysis, loading efficiencies and storage stability studies under various environmental conditions.

MATERIALS AND METHODS

Materials

Gallic acid and sodium bicarbonate and Folin-Ciocalteu reagent were purchased from Sigma Aldrich (St. Louis, MO, USA). Whey protein concentrate-80 (WPC) as AVONLAC 180^{TM} was graciously provided by Glanbia Foods Inc. (East Gooding, ID, USA). As per certificate of analysis, composition of WPC was protein (dry basis) – 79.3%, moisture – 3.7% and fat - 8.9%. All other chemicals used were analytical grade and deionized water was used throughout experiment.

Microencapsulation of gallic acid by spray drying

Four batches of 100 gram of WPC dissolved in 500 ml deionized water at room temperature (22 ˚C) were kept for hydrating overnight at 4 ˚C. Following day, 6.25, 12.5, 18.75 and 25 gram of gallic acid was dispersed in 20, 45, 70 and 95 ml of deionized water and temperature was increased upto 80 ˚C along with magnetic stirring for 20 minutes to completely dissolve gallic acid crystals for better dispersion of gallic acid in WPC solution. These gallic acid solutions were dispersed in WPC solutions which were kept for soaking earlier day. Four ratios of gallic acid: WPC (w/w) were 1:4.0, 1:5.3, 1:8.0, 1:16.0 and the total solids level were maintained at 17.85, 18.26, 18.75 and 19.31 % (w/v) respectively. Homogenization of the solutions was carried out using LabTek (Omni International Inc., Gainesville, VA) homogenizer at 8000 rpm for 20 minutes for all the four solutions. Prior to homogenization, nitrogen was flushed through the mixing jar for 10 minutes to avoid contact with oxygen while homogenization at high speed. The solutions were covered with aluminum foils at all the time and homogenization was carried out under subdued light conditions. The spray drying of feed solution was conducted using pilot scale spray dryer (Anhydro Inc., Olympia Fields, IL, USA).

The dryer had evaporation capacity of 7.5 kg/hr and chamber diameter was 1 meter. Drying was carried out in co-current mode. The inlet and outlet temperature were 180 and 91 ˚C respectively, and atomization speed was kept at 30,000 RPM (60% of maximum 50,000 RPM). The feed flow rates were 65, 58 ml/min for 1:16.0 and 1:8.0 gallic acid: WPC ratio respectively and 47 ml/min for 1:5.3 and 1:4.0 ratio of gallic acid: WPC ratios. The microcapsules were collected at the bottom of cyclone and were stored in plastic jars protected from light and stored at 4 ˚C until further analysis.

Microencapsulation of gallic acid by freeze drying

For freeze drying of the gallic acid microcapsules, the procedure is same till the homogenization of the gallic acid solution in WPC. The weight ratios gallic acid: WPC (w/w) were 1:4.0, 1:5.3, 1:8.0 and 1:16.0 and total solids maintained were 17.85, 18.26, 18.75 and 19.31% (w/v) respectively. After homogenization, the solutions were poured in plastic trays and covered with aluminum foils for protection from light. Homogenization was carried out under subdued light condition and these trays were kept for freezing at -20 ˚C for 24 hours. The freeze drying was carried out using Virtis Genesis SQ Super ES freeze dryer (Gardiner, NY, USA). It was recommended (from manual) that knowing eutectic point will conserve time and energy and produce highest quality freeze dried product. The care was taken that homogenously blended matrix was well frozen in plastic trays before loading in freeze dryer shelves. The thermocouples were inserted in frozen matrix to monitor the changes in the temperature. The shelf temperature was allowed to reach below -40 ˚C before turning on condenser. Once the condenser temperature dropped below -40 ˚C, the vacuum switch was turned on until vacuum reached 100 millitor. After that, the freeze drying was started and the product was periodically monitored. The trays were covered with aluminum foils to protect from light conditions and some holes on foils were

made to facilitate escape of water vapors. Once the freeze drying was completed, the microencapsulated mass in trays was subjected to grinding by mortar and pastel to form freeze dried powder. These microcapsules were stored in plastic jars and kept in refrigerator at 4 ˚C until further analysis.

Scanning electron microscopy

Morphology of microcapsules was observed using variable pressure scanning electron microscope Zeiss 1450 EP (Carl Zeiss Micro Imaging Inc. One Zeiss Drive, Thornwood, NY). using methodology described by Sheu (1998). The powders were fixed on 10 mm stubs with double sided adhesive tape and then were made electrically conductive by coating in vacuum with gold using SPI-module sputter coater for 60 s. (15.3 nm). The SEM pictures were taken at excitation voltage of 15kV and 100, 200 and 500 X magnification for freeze dried powder and 200, 500 and 573 X magnification for spray dried powder.

Particle size analysis

Particle size analysis was done using Malvern Mastersizer S laser diffraction system with Q Spec small volume sample dispersion unit operating at 3000 rpm (Malvern Instruments, Worcestershire, U.K.). The Mastersizer type used was S 300 RF with particle size range between 0.5 to 900 μm. The Mastersizer S uses the Mie theory of light diffraction for particle size measurement which assumes an equivalent sphere size of particles and performs particle size calculations based on the fact that angle of light diffraction is inversely proportional to particle size (Rawle 2002). To establish standard operating procedure (SOP) for particle size measurements, 3 replicate samples were used for both spray dried and freeze dried microencapsulated gallic acid powder. The 10% (w/v) solutions of freeze and spray dried powder were prepared in deionized water and allowed to hydrate for 10 minutes and stirred before

applying to dispersion unit of the Mastersizer. The raw measurement data obtained from the Mastersizer consisted of particle size distribution (PSD, d_i = 0.05-865 μm) of the respective samples expressed as volumetric diameter of particles. Additionally, particle size percentiles, volumetric mean diameter, summary statistics of PSD was calculated using the Mastersizer data acquisition software package (Mastersizer S-long Bed v2.19).

Microencapsulation efficiency

Microencapsulation efficiency (ME) was determined by Folin-Ciocalteu method (fig 4.1) and the absorbance was compared against standard solution of gallic acid at λ_{max}= 766 nm using Hewlett Packard 8451A diode array spectrophotometer (Avondale, PA, USA) Two hundred mg of microencapsulated gallic acid powder was measured and dissolved in 100 ml of deionized water using magnetic stir bars for 20 minutes. Out of this solution, 0.2 ml was measured in test tube and 0.8 ml of deionized water was mixed in test tube. Five ml of 0.2 N Folin-Ciocalteu reagent (2N of Folin-Ciocalteu reagent is diluted to 0.2 N) and 4.0 ml of sodium carbonate solution (7.5 gm in 100 ml) was added to the solution and blue coloration was allowed to develop by keeping solution under dark condition for 2 hours. The concentration of gallic acid was determined by comparing the values with standard curve generated by equivalent amount of gallic acid. Experiments were performed in triplicate and ME was determined based on following formula.

$$\text{ME (\%)} = \frac{\text{Calculated gallic acid concentration}}{\text{Theoretical gallic acid concentration}} \times 100$$

Accelerated shelf life testing

Gallic acid: WPC (w/w) ratio of 1:4 was selected for accelerated shelf life studies due to high microencapsulation efficiency obtained in spray dried (99.39%) and freeze dried (99.52%) powders. Microencapsulated gallic acid was spread in sample cups and care was taken that the layer of powder did not to exceed few millimeters so that uneven exposure to humidity and UV light conditions could be avoided. Two wooden chambers were constructed for investigating degradation kinetics at 25 and 45 ˚C. Each wooden chamber had two shelves. The upper top shelf was fitted with black light which emitted UV light confined to top shelf. Both the shelves were covered with opaque black plastic covers to avoid outside light exposure. The figure is shown in chapter 3 (Fig. 3.1). The sample cups were covered with transparent loosely knitted cloth to allow the passage of UV light and moisture but which will prevent cross-contamination between samples. Saturated salt solutions of potassium acetate (22.5% RH), potassium carbonate (44.7% RH), sodium nitrite (65% RH) and potassium chloride (85% RH) were prepared and kept in glass containers to test the RH inside the glass containers using temperature-humidity data loggers. Once saturated salt solutions reached the optimum relative humidity levels, sample cups containing freeze and spray dried microcapsules were kept in sample plastic cups over the circular disc mounted on stand which would allow free circulation of humid air over the saturated salt solutions. Out of two wooden chambers, one was maintained at 25 ˚C and the other at 45 ˚C. Eight glass containers were kept in 25 ˚C and the remaining eight at 45˚C. In 25 ˚C chamber, four containers with four different humidity conditions (22.5, 44.7, 65, 85% RH) in UV light and remaining four in dark conditions. ‘The zero week’ gallic acid concentration in both freeze dried and spray dried sample was determined using spectrophotometer. Fifty mg of spray dried and freeze dried gallic acid microcapsules was measured and dissolved in 100 ml of

deionized water using magnetic stirrer bars for 20 minutes. The gallic acid released by the microcapsules was determined using Folin-Ciocalteu method as described in Figure 4.1. Duplicate measurements were taken. The experiment was carried out in subdued light condition. The gallic acid absorbance was measured at 766 nm using Hewlett Packard 8451A diode array spectrophotometer (Avondale, PA, USA) and compared with standard solutions of freshly prepared gallic acid. The concentration was determined using standard curve. Special arrangement was made which would maintain the constant oxygen environment inside the glass containers. On the glass cover, small aperture was made and rubber septas were glued over that aperture which would allow metal needle to pass through the septas so that glass containers could be filled with oxygen. Septas were again sealed with the glue to prevent oxygen to escape. Digital headspace analyzer of type Check-mate 9900 (Topac Inc., Hingham, MA, USA) was used to monitor residual oxygen concentration inside glass containers. The samples were withdrawn every week from glass containers to determine oxidative degradation and glass containers were filled with 100% oxygen concentration before placing back to storage conditions.

RESULTS AND DISCUSSIONS

Scanning electron microscopy

The objective behind using scanning electron microscopy is to observe the degree to which microcapsules are well formed, check the porosity, size, shape, outer topography which reveals the surface dents and shrinkage, tendency to agglomerate and the presence of extraneous matter, if any. The morphology of microcapsules was conducted using scanning electron microscope (SEM) for both freeze dried and spray dried microcapsule. However, here we should remember that in preparing freeze dried microcapsules, the freeze dried slabs (obtained in freeze

dried trays of homogeneously blended whey protein concentrate and ascorbic acid) were subjected to mortal and pastel to obtain the free flowing powder which was used for analytical purposes. 100X, 200X and 500X magnifications of freeze dried micro capsules were obtained and 200X, 500X and 573X magnifications were obtained for spray dried microcapsules. Freeze dried microcapsule particles exhibited irregular shape and gallic acid was part of whey protein concentrate in matrix. The freeze dried microcapsules showed larger particle size than spray dried microcapsules. The edges of the microcapsules are considerably sharper in freeze dried microcapsules compared to spray dried microcapsules. In figure 4.2 (A), (B) and (C), we can see the slab like structure of irregularly shaped microcapsule having thickness of 20 μm. SEM micrographs of spray dried microcapsules are shown in fig. 4.2 (C), (D) and (E). Spray dried microcapsules were spherical in shape. Some of the microcapsules were broken increasing the surface area exposed to surrounding air. However, there were many spherical intact microcapsules compared to broken microcapsule in spray dried powder. Some of the smaller capsules of spray dried microcapsules showed the tendency of agglomeration which explains lesser degree of free flowing characteristic of spray dried microcapsules compared to freeze dried powder.

Particle size distribution

The cumulative percentage size distribution of spray and freeze dried samples are shown in fig. 4.3 (A) and (B) respectively. The particle size distribution of spray and freeze dried samples are shown in the fig. 4.4 (A) and (B) respectively. In spray dried microencapsulated gallic acid powder; the mean particle size diameter was 29.9 μm with particle size ranging from 1.4 to 104.2 μm. In freeze dried microcapsules; the mean diameter was 38.85 μm and particle size ranged from 1.4 to 255.7 μm.

Spray dried microcapsules showed single mode distribution with first mode from 2.9 to 53.1 μm. In freeze dried microencapsulated powder, particle size distribution showed multi-modal distribution with first mode from 3.8 to 11.0 μm and the second mode from 66.5 to 130.4 μm. Narrower the particle size distribution, more homogenous particles are which can ensure better stability and accurate release of the core (Finotelli and Leao 2005).

Microencapsulation efficiency

Microencapsulation efficiency (ME) is important criteria for successful microencapsulation. It indicates the loss of the core during the process of microencapsulation. The retention of core material during microencapsulation by spray drying is affected (among other things) by the properties and composition of the suspension/emulsion and by spray drying conditions. An ideal microencapsulation process should not result in any loss of encapsulated core during the process of drying. Effects of process conditions and compositional aspects on core retention during microencapsulation by spray drying have been discussed pertaining to volatiles and essential oils (Rosenberg, Kopelman and Talmon 1990). High drying rates that lead to rapid crust formation around drying droplets can lead to high core retention. Losses can occur before the formation of dry crust. In our study, gallic acid becomes part of the matrix and is not volatile material. The microencapsulation efficiency of 99.39% in spray drying condition for 1:4 ratio indicates that there was little or no loss during spray drying and also gallic acid is non-volatile compound. The ME of spray and freeze dried powders are shown in table 4.1 and 4.2 respectively. In freeze dried powder, microencapsulation efficiency of 99.52% for 1:4 ratio indicates the low temperature maintained in freeze drying did not result in loss of gallic acid. The process of making freeze dried powder by mortal and pastle could have uncovered some of the gallic acid crystals and that could explain extra losses during freeze drying process. Young and

others (1993) have reported ME ranging from 92.5 to 99.2% for 25% fat loading and using various wall material type and wall solution concentration using spray drying technique. The milk fat being non-volatile material, these efficiencies are similar to reported by us.

Accelerated shelf life testing

Georgia-produced muscadine grapes and rabbiteye blueberries contain an array of polyphenolic antioxidants including hydroxylbenzoic and hydroxycinnamic acid derivatives as well as flavonoids such as catechin, epicatechin, quercetin, myricetin, kaempferol and gallic acid. These plant antioxidants counteract the subcellular-, cellular- and systemic-level oxidative stress in humans and other health promoting benefits including prevention of cancer, cardiovascular disease, cataracts, rheumatoid diseases and diabetes. Biswas and Phillips (2006) proposed a scheme by which these health-promoting compounds could be isolated and produced commercially as nutraceutical powders. However, these phenolic antioxidants are labile to oxidation as their name and function imply. Factors which affect the color and stability of anthocyanins include structure and concentration, pH, temperature, light, presence of copigments, self association, metallic ions, enzymes, oxygen, ascorbic acid, sugar and their degradation products, proteins and sulphur dioxide (Francis 1989; Mazza and Miniati 1993; Rodriguez-Saona, Giusti, & Wrolstad, 1999). For this reason it is important that they be protected from accidental degradation until they are needed. Before studying the complex mixtures resulting form extraction of blueberries or muscadine, gallic acid which is one of the phenolic compounds was chosen as core compound considering the high costs of other pure compounds. In our experiments, we proposed to examine the effect of temperature, UV light, relative humidity and the type of drying method on protective effect of gallic acid in whey protein concentrate. Two temperatures levels 25 and 45 ˚C; UV light and dark condition; four

relative humidity (RH) levels 22, 44, 66 and 85 % and two dying method i.e. freeze and spray drying were selected for shelf life investigation. Microencapsulation efficiency (ME) was determined by total gallic acid present after spray and freeze drying process. For 1:4 (gallic acid: whey protein concentrate) ratio, ME was 99.38% in spray dried powder and 99.54% in freeze dried powder. This ratio was selected for accelerated storage study.

Degradation kinetics of spray dried microencapsulated gallic acid have been shown in fig. 4.5 and 4.6 while freeze dried microencapsulated gallic acid in fig. 4.7 and 4.8. Humidity plays significant role in the degradation of gallic acid. Maximum degradation of gallic acid was found at intermediate 66% RH in both spray dried and freeze dried gallic acid, in both light and dark and both 25 and 45˚C. The only exception was freeze dried microencapsulated gallic acid at 25 ˚C and dark condition; at this condition more degradation was found in 85% RH compared to 66% RH. Thus, the trend shows intermediate RH 66% plays role in higher degradation of gallic acid. In freeze dried microencapsulated gallic acid for 66% RH, at 25 ˚C and UV light, the initial gallic acid concentration of 99.39 mg/L was reduced to 79.39 mg/L and 25 ˚C and in dark it reduced to 89.12 mg/L. At 45 ˚C, the freeze dried gallic acid at 66% RH and UV light conditions reduced to 77.2 mg/L and in dark conditions it reduced to 85.12 mg/L from initial concentration of 99.39 mg/L. This also shows higher degradation at higher temperature (fig. 4.7 and 4.8.). Gallic acid degradation phenomenon is more pronounced at spray dried powder kept at 45 ˚C temperature. In 25 ˚C, 66% RH and UV light condition spray dried microencapsulated gallic acid concentration at the end of 8^{th} week, reduced to 67.8 mg/L and in dark condition it reduced to 66.4 mg/L from the initial concentration of 99.39 mg/L (fig. 4.5 A and B) whereas at 45 ˚C the concentration at the end of 8^{th} week the concentration reduced to the level of 50.3 and 36.5 mg/L

for UV light and dark condition respectively (fig. 4.6 A and B). Thus, we can say that freeze dried product better protected gallic acid from degradation at 66% RH.

Serris and Biliaderis (2001) reported that degradation kinetics of beetroot pigments consisting of betalain was highest at intermediate moisture content (a_w = 0.64) for all the matrices (pullulan and two maltodextrin samples). The reaction rates (K) and half-life period ($T_{1/2}$) for encapsulated beetroot pigments were compared at 0.23, 0.43, 0.64, 0.75 and 0.84 water activity (a_w) levels and at 30, 40 and 50 ˚C. The maximum reaction rate (k) was observed at 0.64 a_w. However, reaction rates (K) at 0.23 and 0.44 a_w levels were lower than 0.75 and 0.84 a_w. The reaction rates (K) increased with increase in temperature. This trend can be explained by the fact that the degradation reaction is controlled by the mobility of the reactants. This type of behavior has been exhibited in many systems according to Von Elbe (1987). Saguy and Cohen (1983) have reported that with increasing a_w there is increase in oxygen concentration and this in turn increases degradation rates since oxygen accelerates the reaction. In dry environment, water is strongly bound to surface polar sites and is generally not available for any kind of reaction. This fact explains the lower reaction rates observed at a_w of 0.23 and 0.43. At high a_w, reactant dilution effect have been claimed to be predominant (Von Elbe 1987). As a result, reaction rate may reach maximum at intermediate a_w and then decline with further increase in a_w. Thus, Serris and Biliaderis (2001) reported maximum reaction rates at a_w= 0.64 for all three temperatures.

There is increasing oxygen concentration with increase in water activity level. Thus it reaches maximum at a_w= 0.64. But at a_w above 0.64 the reactant dilution rates have been predominant. Therefore. at a_w= 0.64, maximum degradation rates have been observed. Although, our core material is gallic acid, similar trends of degradation kinetics have been observed in our studies.

In addition, we have also found higher degradation rate of microencapsulated gallic acid at intermediate moisture levels. Cai and Corke (2000) reported that in spray dried Amaranthus betacyanin extracts, the bulk density of pigment powder decreased with increase in spray drying temperature. Although higher drying rate was obtained at higher drying temperature, higher ratio of surface-to-volume for the spray dried powder caused lower bulk density of the powder. The lower the bulk density, the more occluded air within the powders and therefore, greater the possibility of oxidative degradation of pigments and reduced storage stability. Chou and Breene (1972) also observed the spray dried product had high specific surface area and carotene surface concentration on surface causes very rapid oxidation. Desorby and Labuza (1998) observed that freeze drying of β-carotene using maltodextrin provided better protection when compared to spray dried powder. They also observed the better protection by drum dried maltodextrin which was assumed to reduce the surface of exchange between oxygen in air and carotene. This proves to minimize the contact between oxygen and core and better protection offered to freeze dried microencapsulated β- carotene compared to spray dried microencapsulated β-carotene. Also according to Nelson and Labuza (1992), matrix-carotenoid complex is critical and the right wall material is needed to have oxygen impermeable matrix in glassy state below the monolayer moisture. Nelson and Labuza (1992) reported limonene oxidation was inhibited below the monolayer moisture because of a very impermeable glassy layer of the matrix material. In our studies, at the end of 5^{th} week of storage, spray dried microencapsulated gallic acid powder was clumped together and the capsule walls were fused together at 66% and particularly at 85% relative humidity (RH). In addition to that at the end of 5^{th} week, the dark brown coloration was observed in microencapsulated gallic acid and color was lighter at 85% RH. At 22 and 44% RH, the color of microcapsule was still white. This again substantiates, qualitatively, higher

degradation found at intermediate humidity level. Beristain and others (2003) have shown the effect of caking and stickiness on retention of spray dried microencapsulated orange peel oil using mesquite gum as wall material. They observed surface caking temperature and advance caking temperature decreased with increase in storage water activity. Beristain and others (2003) have also compared the retention of orange oil as function of different water activity. High retention of oil was obtained upto a_w of 0.743 but oil retention dropped sharply in Aw range of 0.743-0.972 as the dissolution of polymer wall. Total loss was recorded at 0.972 where the structure was completely lost. They also observed the samples stored at water activity upto 0.654 and 0.628 showed change from glassy state to rubbery state. Since we have obtained higher degradation rates at 66% RH, particularly in spray dried gallic acid powder and marked degradation at 45˚C temperature as we can see from Fig. 4.8 and 4.9., it would be interesting to see if we can explain this phenomenon using glass transition theory.

Glass transition theory is increasingly applied to the biological materials due to limitations of water activity since water activity theory does not indicate the state of water present and how it is bound to substate (Rahman and Labuza 1999). The glass transition theory has been proposed in diffusion process, texture and structure, stickiness, pore formation, oxidation, enzymatic and non-enzymatic browning, enzymes and vitamin inactivation, oxidation and denaturation of the protein (Rahman 2006). All these phenomenon are important in predicting the shelf life of the core material and the shelf stability of the active core inside the microcapsule. Recently, it has been stated that glass transition greatly influences food stability, as the water in the concentrated phase becomes kinetically immobilized and therefore does not participate in reactions (Rahman 1999). Glass transition is the nature of second-order time temperature dependent transition, which is characterized by discontinuity in physical,

mechanical, electrical, thermal properties of material. As far as oxidation is concerned, it has been observed that oxidation of unsaturated lipids entrapped in sugar based matrices is affected by physical changes such as collapse or crystallization occurring above glass transition (Shimada and others 1991). Thus, it is necessary to store the microencapsulated powder in dry, free flowing form at low temperatures to minimize degradation.

CONCLUSIONS

The microencapsulation of gallic acid was successfully carried out using whey protein as coating material using spray dying and freeze drying as methods of microencapsulation. The scanning electron micrographs revealed the morphology of the capsules and particle size analysis helped in understanding the powder characteristics and size distribution. The accelerated storage study carried could be potentially useful in production and storage of microencapsulated nutraceutical powder which could be used in various food applications such as dietary supplements or meat products to determine its antimicrobial properties. Microencapsulated powder could also be used as an anti-oxidant in variety of foods.

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Table 4.1: Microencapsulation efficiency for spray dried microencapsulated gallic acid

Gallic acid (g)	WPC (g)	Vitamin C: WPC ratio (w/w)	Microencapsulation efficiency (%)
6.25	100	1:16.0	82.15[b]
12.5	100	1:8.0	83.16[b]
18.75	100	1:5.3	96.58[a]
25	100	1:4.0	99.38[a]

[a, b, c] Values followed by different letters in the same column are significant (α=0.05)

Table 4.2: Microencapsulation efficiency for freeze dried microencapsulated gallic acid

Gallic acid (g)	WPC (g)	Vitamin C: WPC ratio (w/w)	Microencapsulation efficiency (%)
6.25	100	1:16.0	96.14^{ab}
12.5	100	1:8.0	90.36^{bc}
18.75	100	1:5.3	89.01^{c}
25	100	1:4.0	99.54^{a}

[a, b, c] Values followed by different letters in the same column are significant (α=0.05)

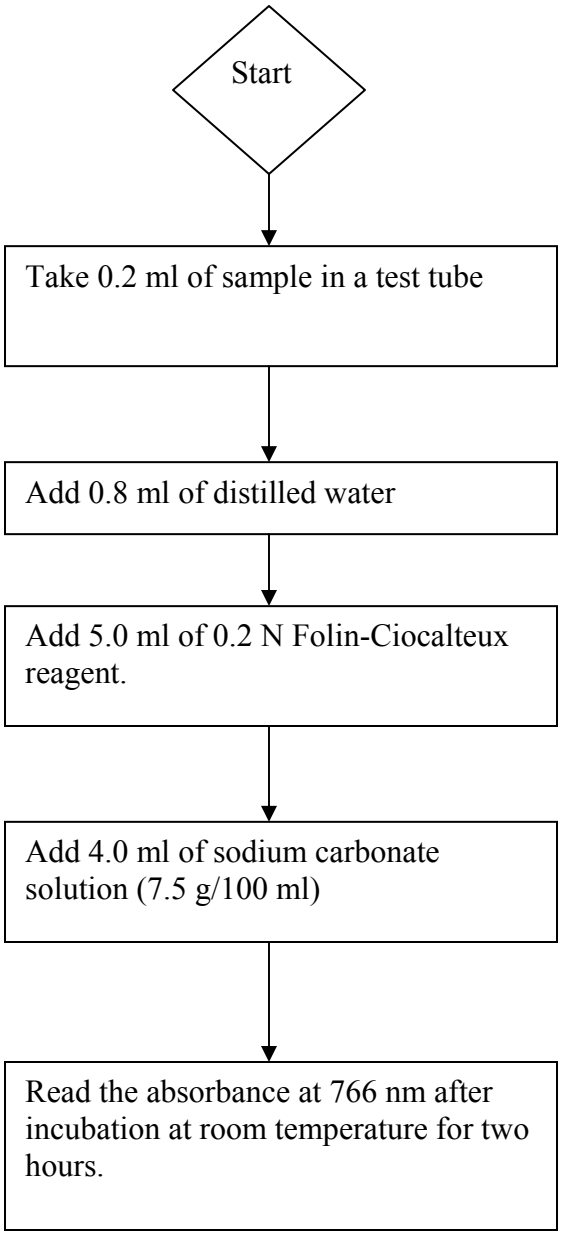


Figure 4.1: Folin-Ciocalteux Method of determination of total phenolics

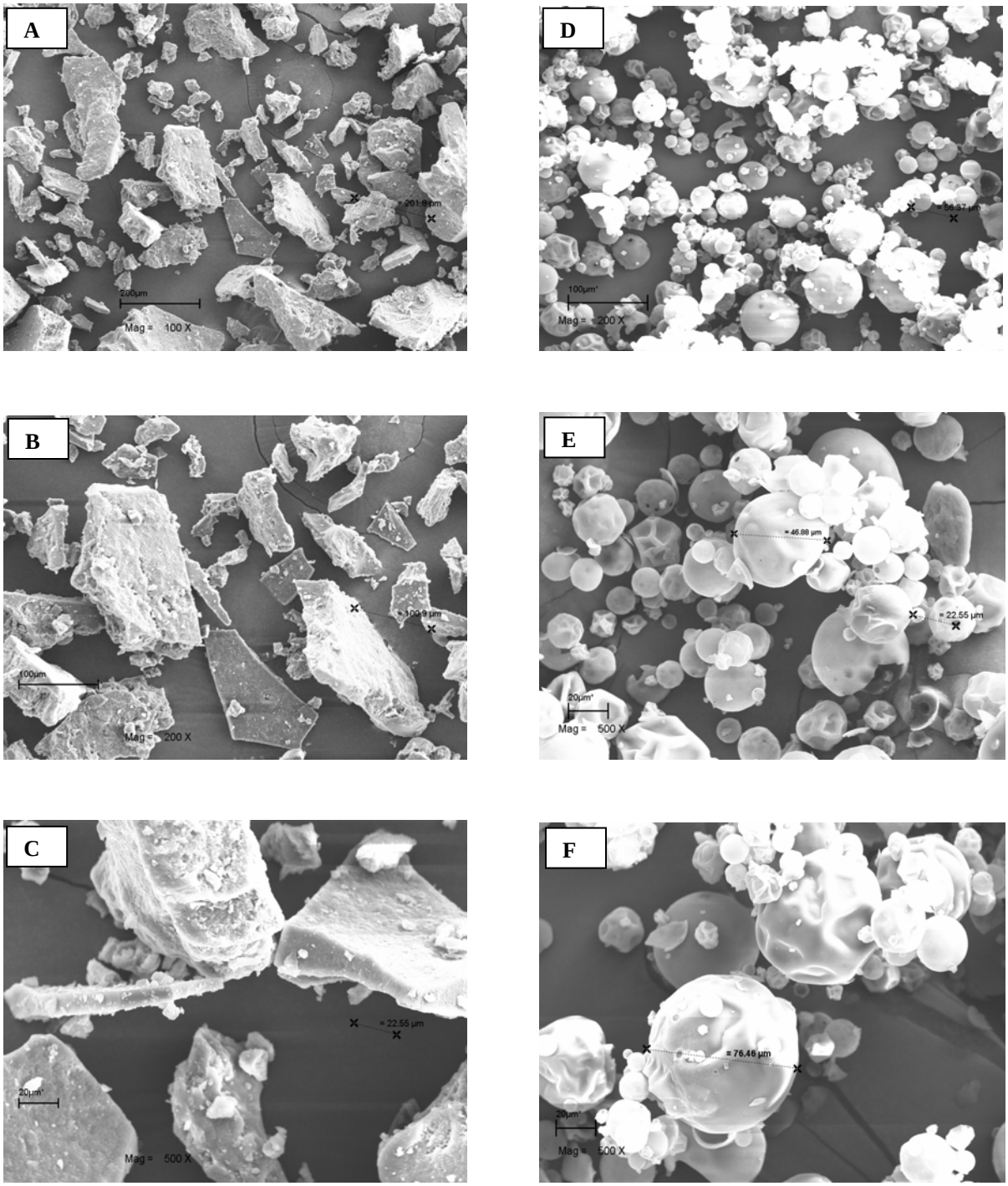
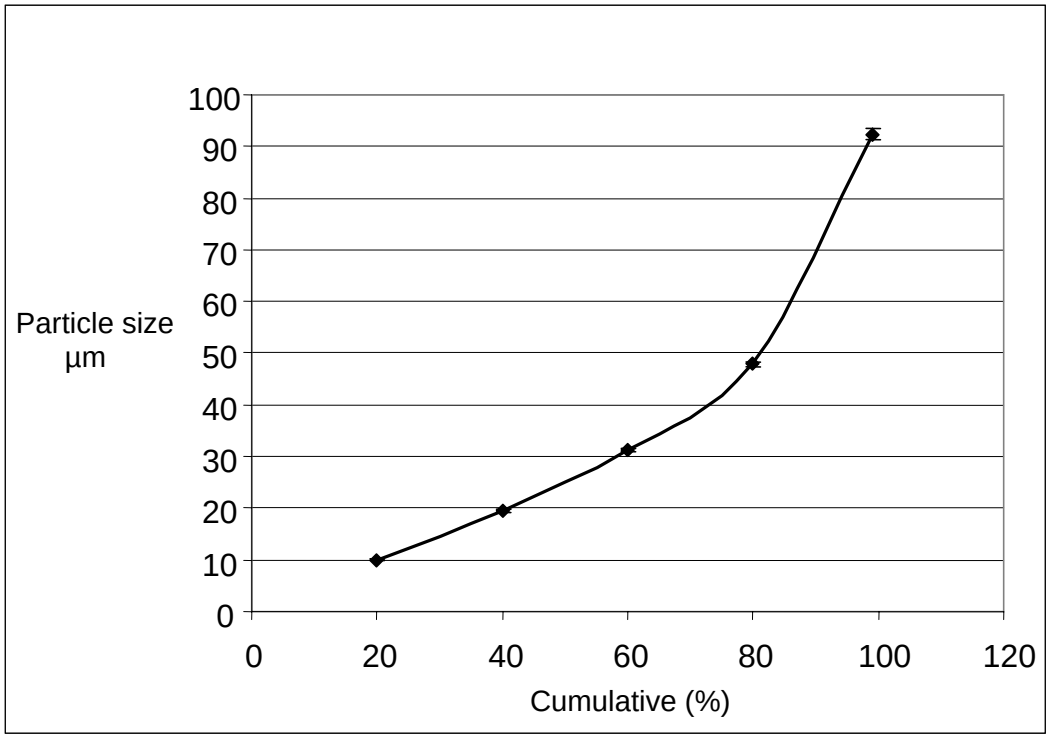


Figure 4.2: SEM micrograph of freeze dried microencapsulated gallic acid with gallic acid: WPC ratio of 1:4 with magnifications of (A) 100X (B) 200 X and (C) 500 X
SEM micrographs of spray dried microencapsulated gallic acid with gallic acid: WPC ratio of 1:4 with magnifications of (D) 100X (E) 200X and (F) 500X



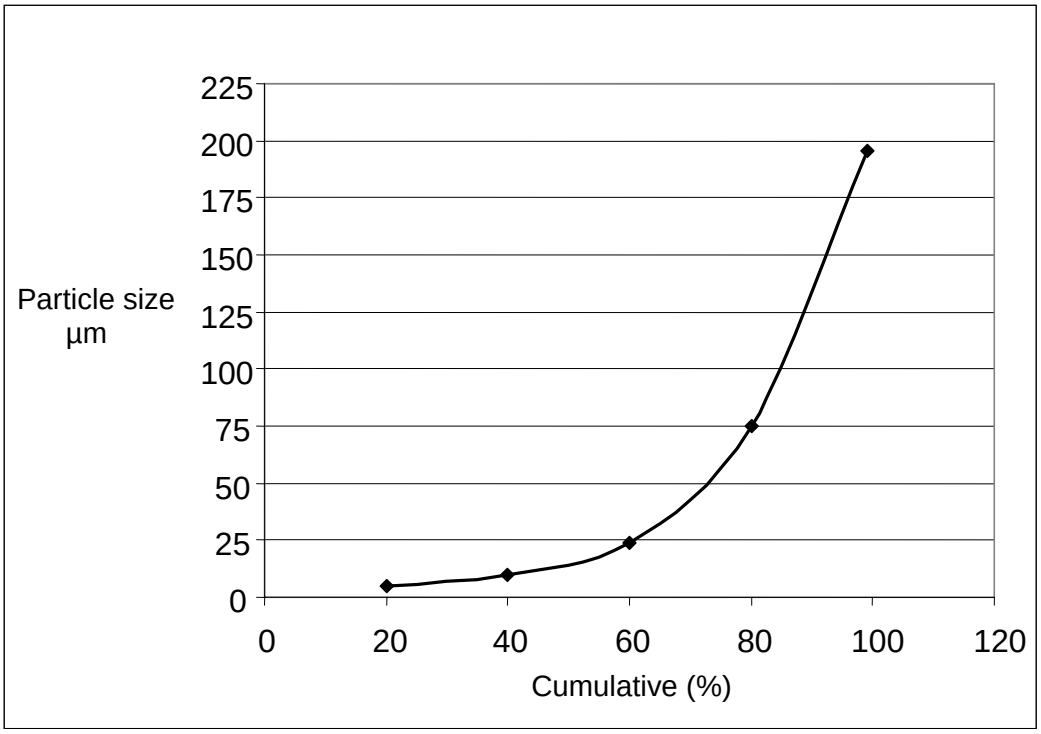
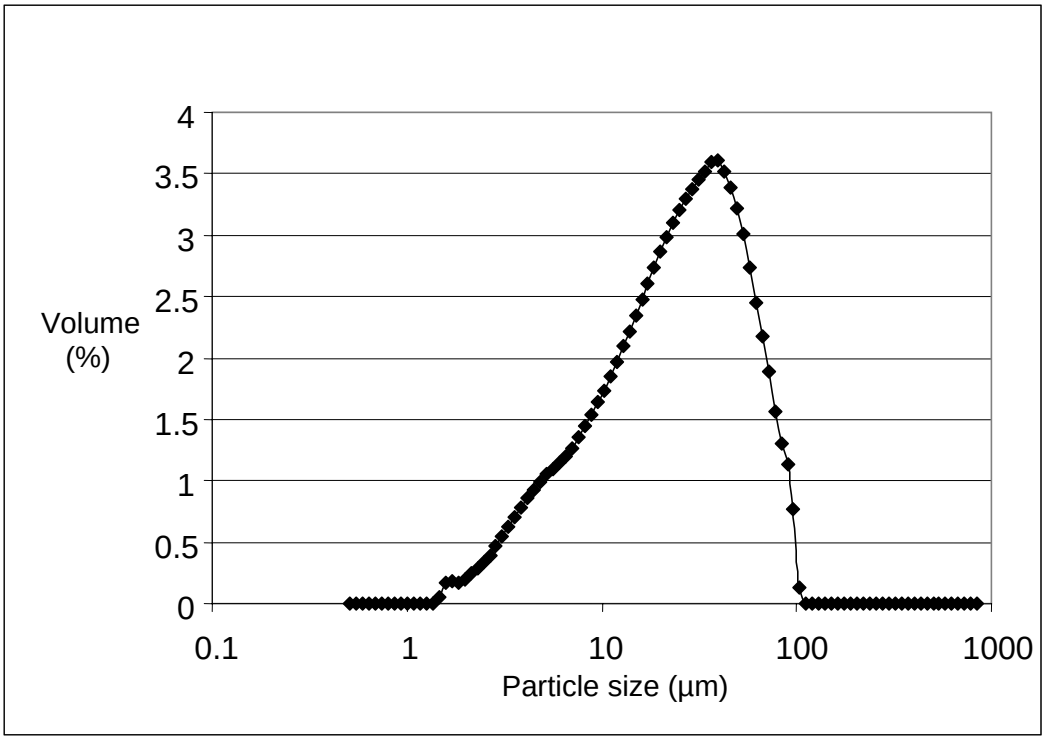


Figure 4.3: Cumulative percentage particle size distribution of (A) spray dried (B) freeze dried microencapsulated gallic acid powder



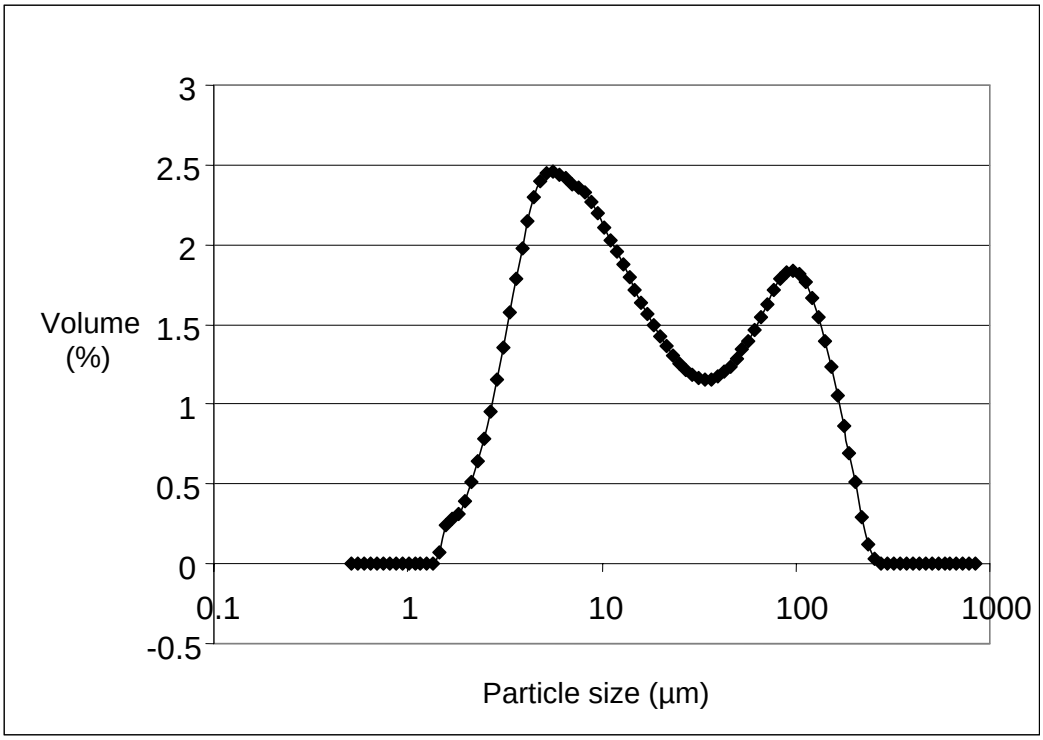
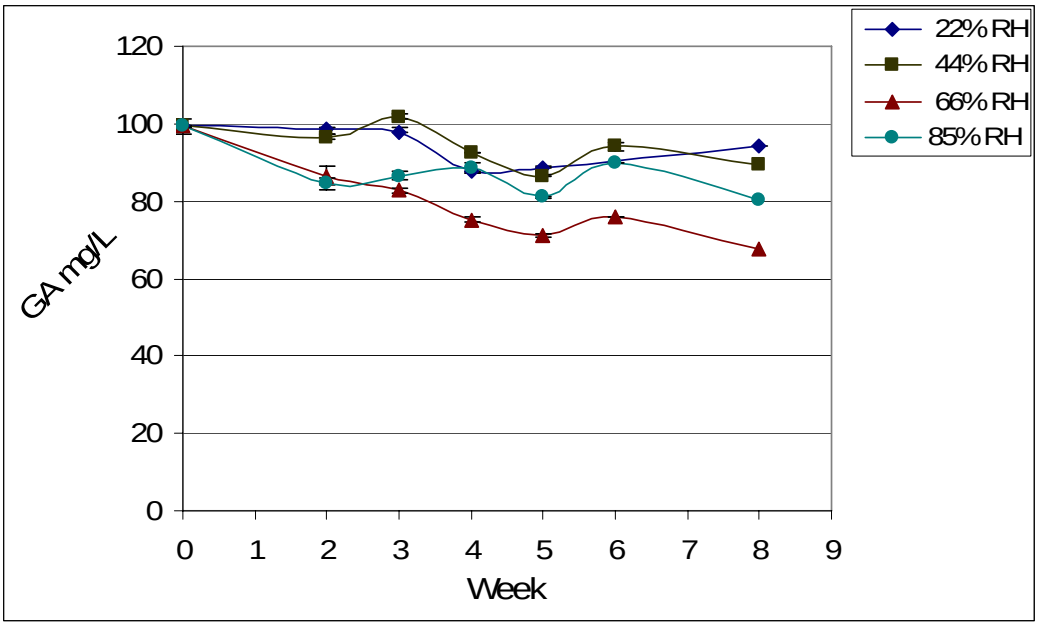


Figure 4.4: Particle size distribution of (A) spray dried (B) freeze dried, microencapsulated gallic acid powder



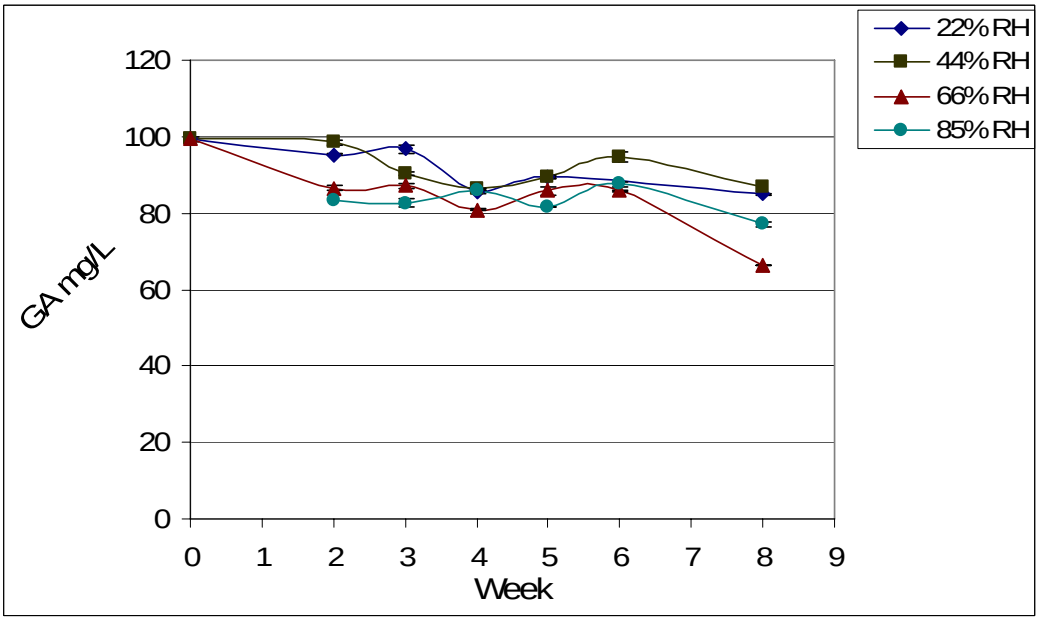


Figure 4.5: Degradation kinetics of spray dried microencapsulated gallic acid stored at 25 ˚C and (A) UV Light (B) dark conditions (G.A. denotes gallic acid)

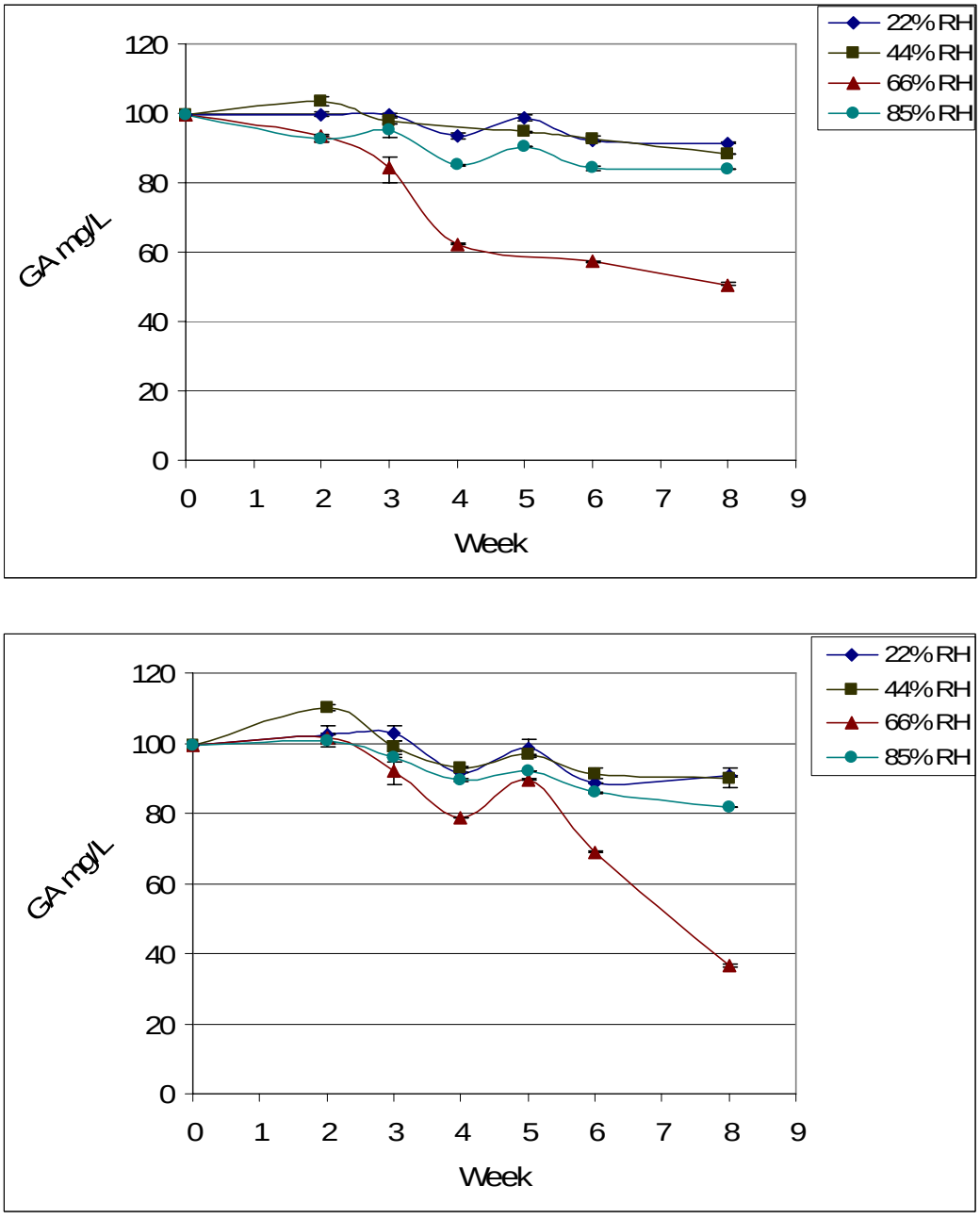


Figure 4.6: Degradation kinetics of spray dried microencapsulated gallic acid stored at 45 ˚C and (A) UV Light (B) dark condition (G.A. denotes gallic acid)

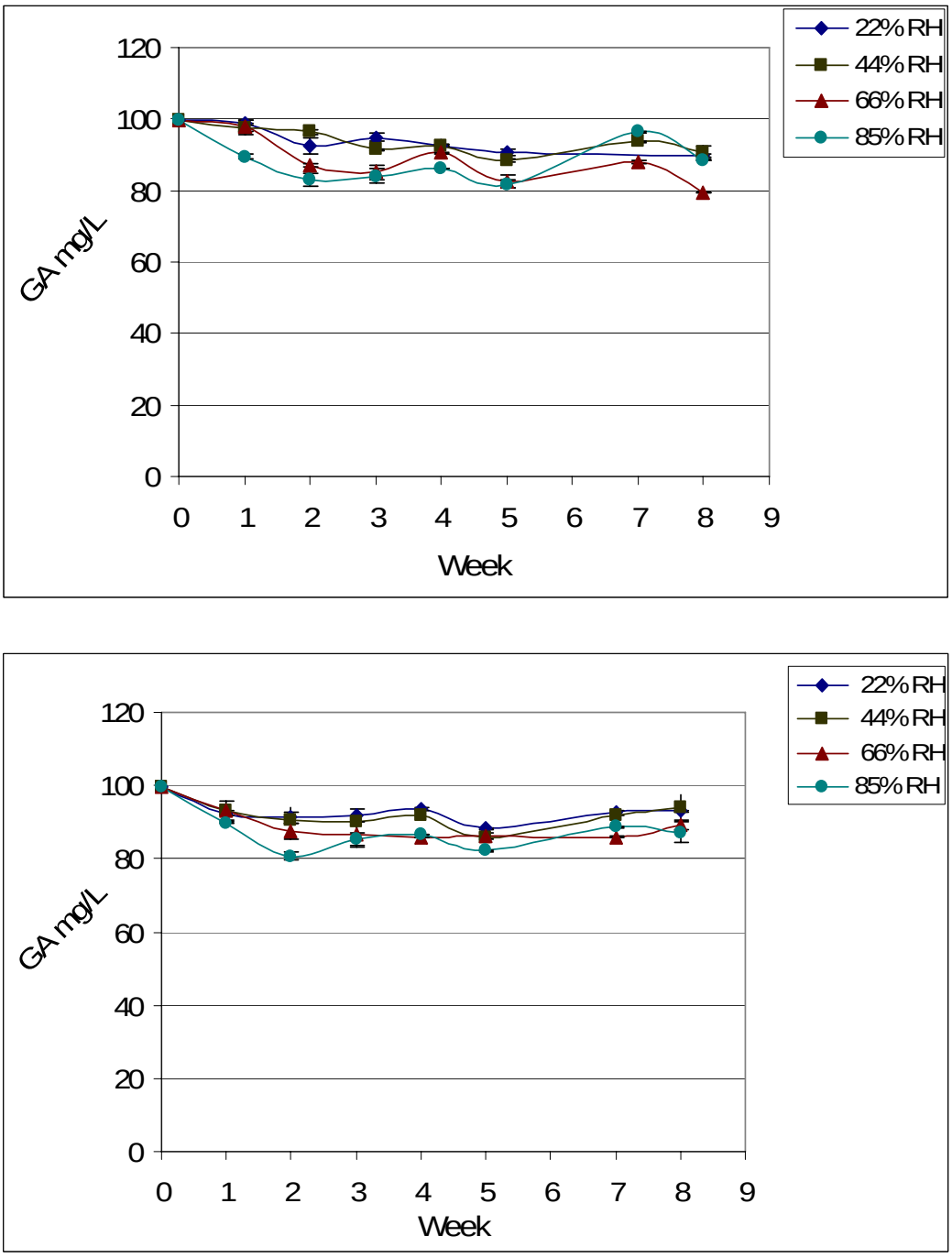


Figure 4.7: Degradation kinetics of freeze dried microencapsulated gallic acid stored at 25 ˚C (A) UV Light and (B) dark condition (G.A. denotes gallic acid)

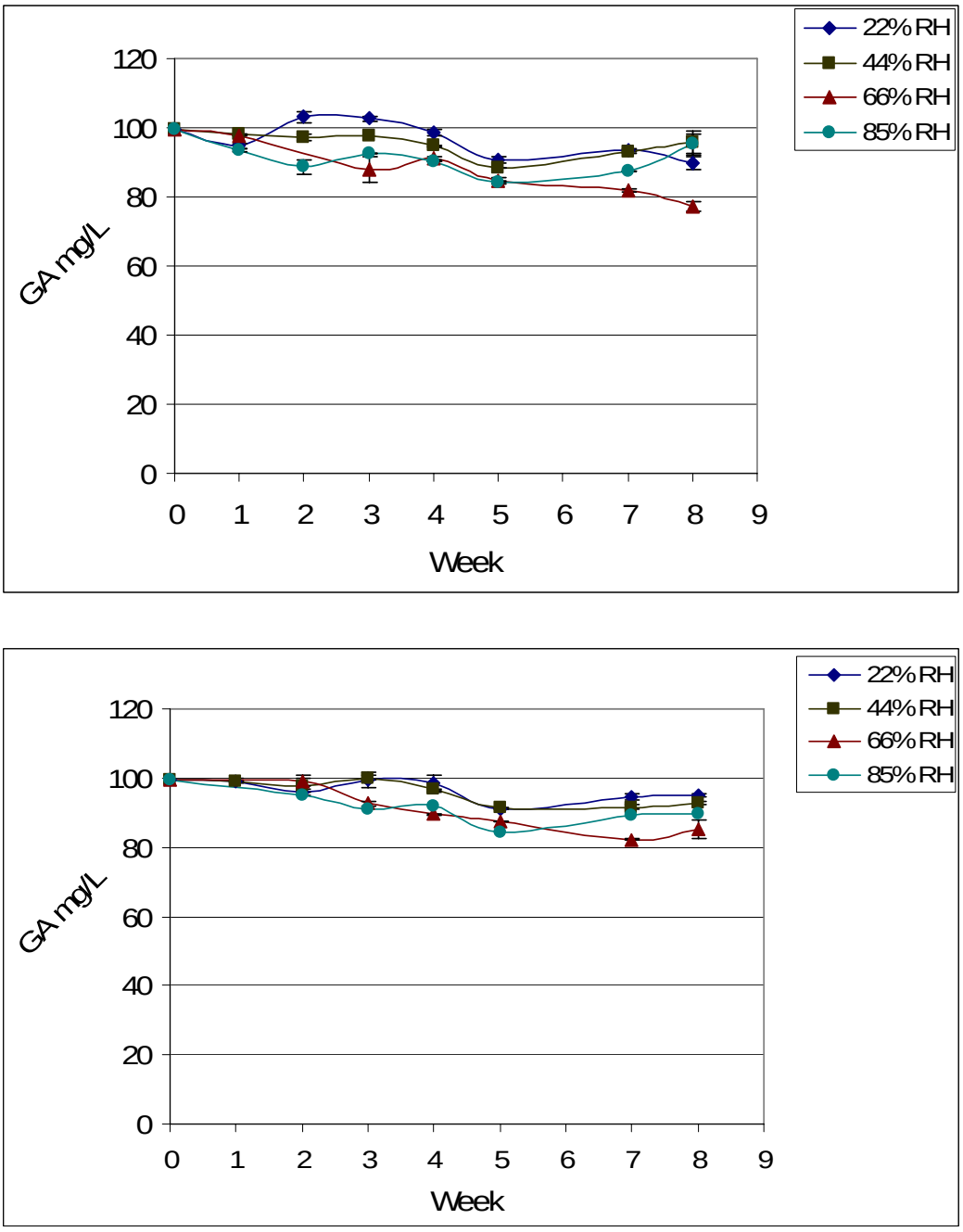


Figure 4.8: Degradation kinetics of freeze dried microencapsulated gallic acid stored at 45 ˚C and (A) UV light and (B) dark condition (G.A. denotes gallic acid)

CHAPTER 5

SUMMARY AND CONCLUSIONS

Many researchers have reported that muscadine grapes contain a variety of health promoting phenolic compounds which may be used to produce nutraceutical powder. The potential commercial applications of this powder is dietary supplements, use as antioxidants in foods, antimicrobial agent and natural colorants. Potency of these compounds is compromised due to their susceptibility to oxidation due to their reactivity to various environmental factors such as temperature, humidity and light.

Gallic acid, one of the phenolic compounds found in muscadine grape and grape seed extract, was selected as model anti-oxidant to microencapsulate in order to test protective effectiveness of microencapsulation from various environmental degradation factors. Vitamin C was another anti-oxidant selected as model due to its high reactivity and susceptibility to oxidation. Many researchers have demonstrated good film forming and oxygen barrier properties of whey protein concentrate (WPC). Microencapsulation of gallic acid was carried out using WPC as wall material by spray and freeze drying method. The gallic acid: WPC (w/w) ratios of 1:4.0. 1:5.3, 1:8.0 and 1:16.0 were selected for both spray and freeze drying method. Maximum microencapsulation efficiency (MEE) was found in 1:4 ratio for spray dried (99.38%) and freeze dried (99.54%) powders. Similarly vitamin C was microencapsulated in spray dried and freeze dried powders. Vitamin C: WPC (w/w) ratios of 1:4.0, 1:5.3, 1:8.0 and 1:16.0 were selected to investigate ratios resulting in highest MEE. Maximum MEE was found in 1:4 ratio for both spray dried (96.35%) and freeze dried (98.23%) powders. The microcapsules were characterized by

scanning electron microscope (SEM) and particle size analysis using Malvern Mastersizer. The objective behind the SEM was to a) examine degree to which microcapsules are well formed b) to observe outer morphology (presence of pores, surface cracking and dents), c) tendency of agglomeration and presence of extraneous matters, if any. SEM micrographs revealed that spray dried vitamin C microcapsules were spherical in shape and some capsules were broken. There were few pores formed indicating effectiveness of drying process. Some small capsules showed tendency to agglomerate which may interfere with free flowing properties. Freeze dried microcapsules were irregularly shaped but the edges were considerably sharper. Vitamin C was part of the matrix of WPC. SEM micrographs of gallic acid revealed similar structure to that of vitamin C microcapsules. However, gallic acid microcapsules of both freeze and spray dried were considerably larger than vitamin C microcapsules. Spray dried microcapsules were well formed spherical in shape. Very few capsules were broken and small number showed visible shrinkage indicating effectiveness of microcapsule formation and drying conditions. Particle size distribution revealed the average size, range of the microcapsule size and the modal distribution of the microcapsules. Spray dried microencapsulated vitamin C (SDVC) and freeze dried microencapsulated vitamin C (FDVC) had particle size range between 1.3 – 83.2 μm and 1.3 – 77.2 respectively. SDVC and FDVC showed mean particle size diameter 17.5 and 11.5 μm respectively. SDVC showed multi-modal distribution while SDVC showed single-modal distribution. Spray dried gallic acid (SDGA) microcapsule had particle size range of 1.4 - 104.2 μm and mean particle size of 29.9 μm. Freeze dried gallic acid microcapsules (FDGA) had particle size range of 1.4 to 255.7 μm and mean particle size diameter of 38.85 μm. SDGA showed single modal distribution while FDGA showed multi-modal distribution.

Microencapsulated vitamin C and gallic acid were subjected to accelerated storage condition to investigate the degradation kinetics of these anti-oxidants. The various factors selected were temperature (25 and 45 ˚C), RH (22, 44, 66 and 85%), UV light and dark, type of drying method (spray and freeze drying). It was observed that humidity played significant role in degradation kinetics. For spray and freeze dried microencapsulated gallic acid at 25 ˚C, maximum degradation was found at 66% RH and at 45 ˚C maximum degradation was observed at 85% RH. Compared to spray dried powder, freeze dried powder provided better protection to vitamin C at all the RH conditions. This was attributed to the difference in the bulk density between spray dried and freeze dried powder. The spray dried powder was considerably less dense (about 4 times) compared to freeze dried powder which increased surface area exposed to environmental condition and therefore caused greater degradation. In microencapsulated gallic acid (spray and freeze dried), maximum degradation was found at 66% RH in both UV light and dark condition and at both 25 and 45 ˚C. The only exception was freeze dried gallic acid at 25 ˚C and dark condition where maximum degradation was found at 85% RH.

Similar trend of degradation was found by some of the researchers. It was reported that by increasing $a_{w,}$ there is an increase in oxygen concentration, this in turn increases degradation rate since oxygen accelerates the degradation rate. In dry environment, water is strongly bound to polar sites and does not participate in reaction which explains lower degradation rates at 22 and 44% RH compared to 66 and 85% RH.

The results showed that it should be possible to microencapsulate muscadine grape extract based on the information generated from this project. Since the phenolic compounds in muscadine grapes are similar to gallic acid, it can be expected that those compounds will also show similar

trend in degradation kinetics. We can also get specific information about best storage conditions at which these microencapsulated nutraceutical powder should be stored.