MICROENCAPSULATION OF BETA-CAROTENE IN PEA PROTEIN WALL SYSTEM

By

Jiancheng Qi

A Thesis

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In Partial Fulfillment of the Requirements

For the Degree of

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FACULTY OF GRADUATE STUDIES

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

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ABSTRACT

Beta-carotene was microencapsulated in pea protein isolate wall system with and without maltodextrin using emulsification technology and spray drying. Effects of various factors including mass ratio of wall to core materials, and wall composition on microcapsule properties such as microstructure, particle size distribution, and moisture content, beta-carotene retention, and release time were determined. Uniform spherical microcapsules with smooth surfaces were observed from SEM image of the microcapsules. The average particle size ranged from 4.3 to 7.1 μm in radius determined by a computerized image analysis system. The half time of oxidation was extended 8 weeks. The wall to core ratio had significant effects ($P<0.05$) on drying yield, particle size, moisture content, and beta-carotene retention during storage of pea protein based, beta-carotene containing microcapsules. The wall composition (with different levels of maltodextrin content in the wall system) had significant effects ($P<0.05$) on particle size and beta-carotene retention during storage. The release of beta-carotene from swelling microcapsules could be controlled by altering the wall material with respect to the heat treatment of pea protein and to the composition of wall materials. The results confirmed that microencapsulation is an efficient method to preserve beta-carotene from oxidation. The results suggested that pea protein or pea protein combined with maltodextrin could be used as good microencapsulating agents for food ingredients, nutraceuticals, and pharmaceuticals.

Key Words: Microencapsulation; Beta-carotene; Pea protein isolate; Maltodextrin; Spray drying; Controlled release; Nutraceuticals

1.0 INTRODUCTION

Nutraceuticals is one of the fastest growing industries today and the future nutraceutical market is estimated significantly larger with continued growth. Millions of consumers have already been eating functional foods containing nutraceutical ingredients and have taken nutraceutical dietary supplements since calcium, fiber and fish oil sparked the beginnings of the nutraceutical era about two decades ago. Consumers seeking personal control over their own health are driving the fast growing nutraceutical industry. Health Canada (1998) defines nutraceutical as 'a product isolated or purified from foods, and generally sold in medicinal forms not usually associated with food and demonstrated to have a physiological benefit or provide protection against chronic disease'. Today, some important areas in nutraceutical development include the establishment of a regulatory system for nutraceuticals, clinical research, nutraceutical production, and nutraceutical delivery technology (DeFelice, 1998).

Microencapsulation can be described as micron-size packages, composed of a polymer wall (coat or shell), with the active ingredient referred as the core or nucleus. The active ingredients may be a food additive, medicament, or other specialty material. As compared with conventional packages that facilitate storage, transport, handling or presentation, microcapsules are generally employed to enhance material performance or create new applications (Arshady, 1993). Microencapsulation has found broad application in the pharmaceutical, health, food, paper and cosmetic industries. Microencapsulation techniques can produce a controlled release delivery system with the wall designed to permit controlled release of the encapsulated material under desired conditions (Reineccius, 1995).

Controlled release has been widely used in pharmaceuticals. It is designed to deliver active substances in a predetermined, predictable, and reproducible fashion. The practical purpose of polymeric drug delivery systems include extending the period of effective therapeutic dose, avoiding or minimizing excess concentration above the therapeutic requirement, decreasing the need for an expensive drug, protecting certain tissues which could be damaged easily after dose, and targeting diseased tissue while avoiding toxic effect and sub-therapeutic drug administration (Berner and Dihn, 1992). As nutraceuticals are also referred as medical foods, the needs for controlled release of nutraceuticals are obvious.

The abilities of predicting and controlling the release rate of active substances from formulated products are critical issues in pharmaceutical, nutraceutical, and food industries. The functionality and structural properties of active substances can be protected by delivery carrier materials in the form of microencapsulation systems. The mass transfer coefficient is a characteristic constant which represents both the transfer and the penetration rates. Multiple mechanisms involving mass transfer phenomena as well as multiple chemical interactions and repulsion and physical hindrance may determine the mass transfer coefficient.

Biopolymers including proteins can be used as a wall material and matrix to incorporate the core active agents such as nutraceuticals and pharmaceuticals, and provide chemical, physical and microbiological stable environments to the active core materials. The protein fraction extracted from field peas is a valuable ingredient, which can be purified to yield a protein concentrate or isolate with a high nutritional value as well as functional properties such as the emulsifying property and foam forming ability

(Tian *et al.*, 1999; Choi and Han, 2001). These functional properties suggest that pea protein can be utilized to construct microencapsulation systems for food ingredients, nutraceuticals, pharmaceuticals and other bioactive agents. This may open a new field for the utilization of the pea protein.

The common microencapsulation methods include spray drying, spray chilling and cooling, coacervation, fluidized bed coating and drying, the use of liposomes, suspension, extrusion, and inclusion complexation, etc. (Risch, 1995). Among them, spray drying is the most economical and widely used method in the food and pharmaceutical industry (Wagner and Warthesen, 1995).

Vitamin A deficiency is the most common dietary deficiency in the world (Simon, 1990). Since beta-carotene acts as a pro-vitamin A and anti-cancer compound (Desobry *et al.*, 1998), it is considered an important nutraceutical or pharmaceutical. However, beta-carotene is very unstable and highly oxidizable (Desobry *et al.*, 1998). The preservation of beta-carotene in food and pharmaceutical systems is essential to ensure a stable amount of vitamin A during storage and application. Microencapsulation of beta-carotene may provide a protective network to isolate beta-carotene from environmental conditions but will still allow it to be released under use conditions, and may also provide the controlled release of beta-carotene.

The objectives of this research were: 1) to develop a beta-carotene containing microencapsulation system using pea protein as wall material; 2) to determine the effect of wall composition and wall to core ratio on the microencapsulation, and to control the release rate of the beta-carotene by altering pea protein wall material through heat treatment or mixing with maltodextrin to change the wall composition; 3) to determine

the protection of the beta-carotene microcapsules against oxidative degradation; and 4) to model core release phenomena mathematically and determine the release rate based on a pea protein based microcapsule system.

Successful results of this project can not only provide an efficient delivery means for nutraceuticals, but also create new market and high value applications of pea proteins. Therefore, this research will yield significant benefit to the nutraceutical and food industries.

2.0 LITERATURE REVIEW

2.1 Introduction

Over the last two decades, great expansion has been taking place in the research of nutraceuticals worldwide, and the same trend can be seen in the research of microencapsulation of food ingredients. However, research on the application of microencapsulation technology in the area of nutraceuticals is limited. The purpose of this concise review is to provide exposition of all past work on the area that related to this present study, including microencapsulation of food additives, nutraceuticals, and pharmaceuticals; controlled release of active ingredients; preservation of beta-carotene; and the functionality of pea proteins.

2.2 The Function and Protection of Beta-Carotene

Of the many nutraceuticals that fortify functional foods and dietary supplements, vitamins, along with minerals, boast both a long history of usage and government dietary recommendation. Vitamin A has several important functions in the body. The need to improve vitamin A consumption in many parts of the world is well documented. Vitamin A deficiency is a primary cause of blindness in 500,000 children per year worldwide, and several million have less severe health problems associated with vitamin deficiency (Abraham and Kakuda, 2003; Combs, Jr., 1998; Hall, 1995; Simon, 1990).

Beta-carotene is part of the carotenoid family, which includes beta-carotene, lycopene, lutein, zeaxanthin, etc. Carotenoids are one of the most important groups of natural pigments that are responsible for many of the yellow and orange colors of fruit and vegetables (Germann, 1994). Beta-carotene is most abundant in carrots, but is also

found in many other fruits and vegetables as well as animal products such as milk, meat, and eggs. Beta-carotene is known as provitamin A because it is one of the most important precursors of vitamin A in the human diet. Theoretically beta-carotene possesses 100% vitamin A activity. Figure 1 (Combs, Jr., 1998) illustrates that beta-carotene can be metabolized to yield vitamin A. Beta-carotene also has anti-oxidant properties and may help in preventing cancer and other diseases (Desobry *et al.*, 1997).

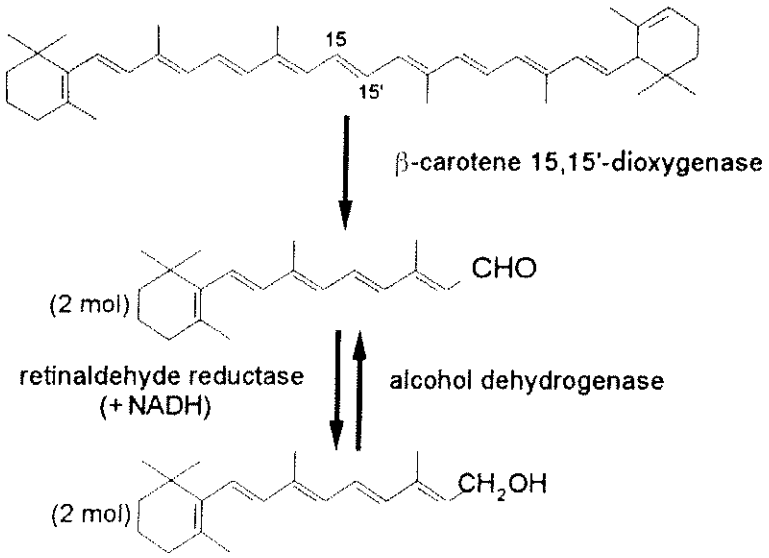

Figure 1. Bioconversion of provitamin A (beta-carotene) to yield vitamin A (retinal) (adapted from Combs, Jr., 1998)

Beta-carotene is very unstable because it is highly oxidizable (Desobry *et al.*, 1998; Mingguez-Mosquera and Jaren-Galan, 1995). The half reduction time of beta-carotene in aerated water is 15 – 30 min and 90% reduction time is 1 h - 3 h (Cruz *et al.*, 2001). Therefore, preservation of beta-carotene in food and pharmaceutical

systems is essential to ensure a stable amount of vitamin A during storage and application.

Wagner and Warthesen (1995) first reported a study on the stability of encapsulated beta-carotene from carrots. Maltodextrins of various DE (dextrose equivalent) as encapsulating agents for spray drying of carrot juice were evaluated and the effects of light exposure, temperature, and carrier level on the stability of spray dried carrot carotenes were determined. Maltodextrin of 36.5DE was superior to 4DE, 15DE, and 25DE in improving the retention beta-carotene in spray dried encapsulated carrot powder during storage, while all four maltodextrins improved the shelf life 70 - 220 times compared to carrot juice spray dried alone. Carotene retention could be increased by increasing the carrier level, decreasing storage temperature and packaging in an inert atmosphere. The degradation of beta-carotene followed first order reaction kinetics and an Arrhenius relationship.

Leach *et al.* (1998) reported on the spray drying of *dunaliella salina* to produce a beta-carotene rich powder. The beta-carotene in the resulting powder degraded rapidly in the presence of light and oxygen. A dramatic improvement in the beta-carotene stability of the resultant powders could be obtained by spray drying the cell concentrate with a microencapsulation polymer mix of maltodextrin and gum Arabic.

Desobry *et al.* (1997) investigated the effect of encapsulation methods to beta-carotene preservation. Pure beta-carotene was encapsulated in maltodextrin (DE 25) by three different drying processes: spray drying, freeze drying, and drum drying. Stability of encapsulated beta-carotene was studied at different levels of relative humidity and temperatures. The half-life time of beta-carotene at 25 °C was 6 weeks for

spray dried, 24 weeks for drum dried, and 6 weeks for freeze dried respectively. No significant influence of relative humidity was observed on the retention of beta-carotene. Oxidation followed first order kinetics with an initial fast first order reaction followed by a second much slower first order reaction period.

Desobry *et al.* (1998) also reviewed the recent studies concerning beta-carotene retention in carrots during processing and storage. They concluded that encapsulation is the best way to extend shelf life for carotene and spray drying is the most frequent process used to encapsulate carotene.

2.3 Development of Microencapsulation Technologies in the Food Industry

Microencapsulation has found broad application in the pharmaceutical, food, paper and cosmetic industries. While most of these applications are relatively recent developments, microencapsulation has been used in the food industry for more than 60 years. One of the earliest applications in food industry was the encapsulation of flavoring by spray drying in 1930's (Reineccius, 1995). In the past two decades, there has been increased interest in the research and applications of microencapsulated food additives, such as aroma and flavor compounds, fats, minerals, and vitamins.

Microencapsulation is simply the packaging of active substances inside microscopic capsules (Kirby, 1991). It is a technique whereby liquid droplets or solid particles are packed into continuous individual shells. The shell, also known as barrier wall, is designed to protect the encapsulated material, also know as core material, from factors that may cause its deterioration (Rosenberg *et al.*, 1990). By a different approach, the wall is designed to permit controlled release of the core material under desired

conditions (Rosenberg *et al.*, 1990). Microencapsulation provides not only a delivery system but also an approach to transform liquids into stable and free flowing powders which are easy to handle and incorporate into a dry system. Virtually any material that needs to be protected, isolated or slowly released can be encapsulated. In food systems, this includes acids, lipids, enzymes, microorganisms, flavors, sweeteners, vitamins, minerals, water, leavening agents, colorants, and salts (Risch, 1995).

Microencapsulation provides an excellent delivery system for food additives. The properties of microencapsulated food additives provide greater flexibility and control in food processing. When considering microencapsulation of food additives for a food application, the factors such as desired properties of food additives, wall materials selection, processing conditions of the foods, optimum concentration, release conditions, desired form of microcapsules, and production cost need to be considered. Researchers have been successful in microencapsulation of different kinds of food additives, and microencapsulation techniques have been applied commercially for delivery of food additives. New encapsulation technology will improve the number and quality of microencapsulated food additives (Risch, 1995). In this era of the fast development of nutraceuticals, the nutraceutical industry will highly benefit from microencapsulation technology.

2.3.1 Microencapsulation Techniques

Numerous techniques have been developed for microcapsule manufacture. The selection of a method depends on economics, sensitivity of core material, size of

microcapsule desired, physical and chemical properties of both core and wall materials, applications and the release mechanisms (Jackson and Lee, 1991a).

Encapsulation processes can be classified as physical processes and chemical processes (Thies, 1996). Physical processes for the microencapsulation of food additives include spray drying, extrusion, air suspension coating, spray chilling and cooling, co-crystallization, and multi-orifice centrifugal extrusion. Microencapsulation processes involving both physical and chemical techniques include coacervation/phase separation, liposome entrapment, and inclusion complexation. A chemical method for entrapping food additives is interfacial polymerization (Jackson and Lee, 1991a; Risch, 1995). The two most commonly used microencapsulation techniques in the food industry have been spray drying and air-suspension coating (Giese, 1993).

Spray drying is an established viable commercial method of forming microcapsules and it offers a relatively simple and less expensive microencapsulation technology that continues to develop (Re, 1998; Risch, 1995; Thies, 1996). It has primarily been used to encapsulate fragrances and flavors (Thies, 1996). The process of microencapsulation by spray drying involves two major unit operations, emulsification and dehydration. It is performed in three steps. First, a food additive is mixed with wall solution in a proper ratio. In the second step, an emulsifier is added and the mixture is homogenized to produce an oil-in-water emulsion. Finally, the emulsion is fed into a spray dryer where it is atomized through a nozzle or spinning wheel. Hot air flowing in either a co-current or counter-current direction contacts the atomized droplets and evaporates the water, producing a dried particle that is a wall material containing small droplets of food additive or core. The dried particles fall to the bottom of the dryer and

are collected (Risch, 1995). Choice of the spray drying conditions depends on the properties of emulsion and the characteristics of the product required. As low a temperature as possible during the drying operation and keeping the residence time to a minimum are considered beneficial.

Air-suspension coating or fluid bed encapsulation is generally used for encapsulating solid materials with hot melt or solvent-based coatings. Air-suspension coating is accomplished by suspending solid particles in an upward-moving stream of air, which is temperature and humidity controlled. Once this moving, fluid bed of particles has reached the prescribed temperature and is moving uniformly, it is sprayed from the top with a finely atomized liquid coating whose droplets are of smaller size than the substrate being coated. In the case of hot melts, the coating is hardened by solidification in cool air. In the case of solvent-based coatings, the coating is hardened by evaporation of the solvent in hot air. This technique allows a wide range of wall material to be used, both water-soluble and insoluble (DeZarn, 1995).

2.3.2 Emulsion for Microencapsulation

An emulsion is a mixture of at least two immiscible liquids, one being dispersed in the other in the form of fine droplets. Emulsion stability can be achieved by using emulsifiers that facilitate the formation of the emulsion by lowering the oil/water interfacial tension and imparting short-term stability by forming a protective film around the droplets of either the oil or water phase. Protein is a typical natural food emulsifier. Formation of an emulsion involves the disruption of one liquid phase into small droplets by vigorous agitation of the liquid mixture. Emulsification is achieved by passing the

mixture through a colloid mill, homogenizer or a high-speed mixer. Emulsification is an important step in microencapsulation as emulsification breaks the liquid core material into microdroplets. For adequate encapsulation the average droplet size should be in the range of 0.5 – 2 μm. The choice of emulsifiers in spray drying process is very important. Attention is given not only to stabilizing the emulsion prior to drying but also to maintaining adequate emulsion stability after rehydration. Obtaining an emulsion of low viscosity (at high solids) is important to the success of the process (Das and Kinsella, 1990).

2.3.3 Properties of Microcapsules

Microcapsules usually have a particle size range between 1 and 1000 μm. Products smaller than 1 μm are referred to as nanocapsules, and larger than 1000 μm are defined as macrocapsules. Commercial microcapsules typically have a diameter between 3 and 800 μm (Thies, 1996).

Depending on the manufacturing process, various types of microcapsule structure can be obtained as illustrated in Figure 2 (Deasy, 1984). They can be spherical, oblong or irregularly shaped, monolithic or aggregates, and may have single or multiple walls.

The properties of a microencapsulated system are largely dependent on its structure, i.e., microstructure affects the functionality, stability and flow-ability of microcapsules (Sheu and Rosenberg, 1998). The retention of core materials and the protection of these materials in a microencapsulated product are related to the porosity and degree of integrity of the microcapsules. The flow properties of microcapsule powders are linked to the structure and the outer topography of the particles. The

structure of the microcapsules depends on the characteristics both of core and wall materials, microencapsulation methods, processing conditions and many other factors.

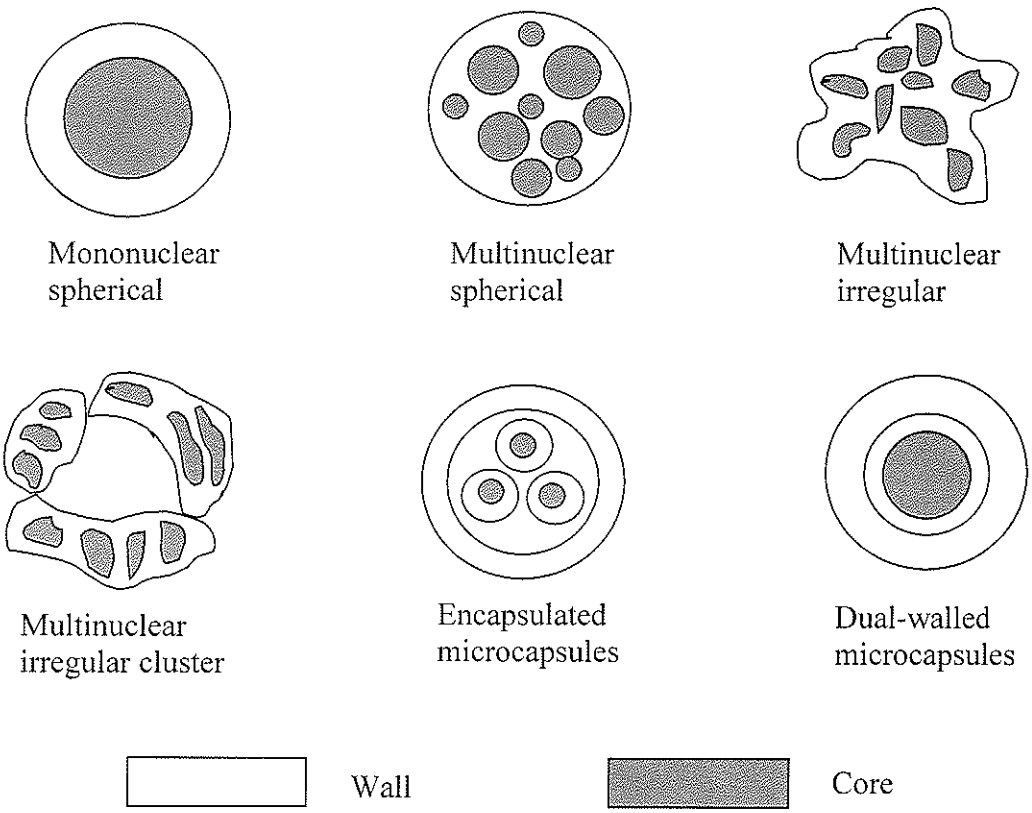

Figure 2. Typical structures of microcapsules (adapted from Deasy, 1984)

2.3.4 Wall Materials for Microencapsulation, and the Use of Proteins and Maltodextrins as Wall Materials

Stability and the properties of the microcapsules are influenced by the composition of the wall material (Moreau and Rosenberg, 1996). The choice of wall materials depends on the method of encapsulation employed and the mode of release desired. As for food additives, the primary requisite for the wall materials is food-grade edible materials. Other important considerations are that the matrix be relatively inert to the

core material as well as to the components of the food materials, and be a film former. Natural polymers, such as starch, protein, gums, are some of the most preferred wall materials. Some modified natural polymers, such as maltodextrin are also good wall materials. Some typical wall materials are presented in Table 1 (Jackson and Lee, 1991a).

Table 1. Types of wall materials used to produce microcapsules

Class of wall material	Specific types of wall materials
Gums	Gum arabic, agar, sodium alginate, carrageenan
Carbohydrates	Starch, dextrin, sucrose, corn syrup
Celluloses	Carboxymethylcellulose, methylcellulose, ethylcellulose, Nitrocellulose, acetylcellulose, cellulose acetate-phthalate, cellulose acetate-butylate-phthalate
Lipids	Wax, paraffin, tristearin, stearic acid, monoglycerides, diglycerides, beeswax, oils, fats, hardened oils
Inorganic materials	Calcium sulfate, silicates, clays
Proteins	Gluten, casein, gelatin, albumin

(Adapted from Jackson and Lee, 1991a)

Release of core materials is normally achieved by the dissolution of the wall material in water or by other means. A careful choice of wall material can lead to a controlled release of core material over a prescribed period of time.

Each of the encapsulation wall materials has its own strengths and weaknesses. A blend of several of these ingredients can give performance superior to using any one ingredient alone (Kenyon, 1995).

Food proteins are well identified as safe ingredients except for some that cause allergy problems. Various food proteins have film-forming properties before as well as after denaturation. This film-forming property is used to form the microencapsulation and entrapment systems. Whey protein, casein, soy protein, wheat gluten, corn zein, egg albumen, bovine serum proteins, gelatin and other proteins have these film-forming abilities. Usually, denaturation of these proteins produces stronger films and coating layers because of increased inter-molecular interactions. The physical properties of these protein coatings/films have been determined through research on physical strength, gas and moisture permeability and optical properties of protein coatings and films used with food and drugs. Because of the amphiphilic nature of proteins, the encapsulation or entrapment system can contain both hydrophobic and hydrophilic drugs or food ingredients and protect the incorporated active substances against physical leaching, chemical degradation and microbial spoilage (Magdssi and Vinetsky, 1996)

Maltodextrins are modified starches (hydrolyzed starches) which are used extensively in spray-dried microencapsulation of food ingredients (Kenyon, 1995). They are a good compromise between cost and effectiveness, blend in flavor, have low viscosity at high solid ratio and are available in different average molecular weights (different DE values). This allows for blending to create different wall densities, which provide protection against oxidation of the encapsulated ingredients (Desobry *et al.*, 1997).

2.3.5 Controlled Release Produced By Microencapsulation

Microencapsulation provides not only protection or stability but also controlled release for food additives. The reasons and functions of controlled release of food additives include (Kirby, 1991; Reineccius, 1995):

a. Time-specific release: a food additive in formulated food is released upon consumption but prevented from diffusion during the series of operation in food processing (e.g. flavors, nutrients).
b. Stage specific release: a food additive is released in a specific processing step, but protected in preceding steps (e.g. leavening agents, cross-linking agents).
c. Site-specific release: preservatives needed in given portion of food (e.g. surface) are prevented from diffusing to another portion (to avoid dilution).
d. Slow sustained release: needed in volatile flavors, acids and high intensity sweeteners in chewing gums.

The release of microencapsulated ingredients is complex and several mechanisms can occur. Generally, there are two possible release mechanisms for microencapsulated ingredients, disintegration of the particle or diffusion through the microcapsule wall (Nixon, 1984). Mechanisms of controlled release in food additives may include mechanical rupture of the capsule wall, dissolution of the wall, melting of the wall, diffusion through the wall, ablation (slow erosion of the shell), and biodegradation. For hydrophilic microcapsules, the most commonly used release methods are temperature and moisture release. For fat microcapsules, thermal release is normally used. There are also many other release methods can be used in food additive microcapsules, such as pH

control, addition of surfactant, enzymatic release, grinding, photo-release, etc. (Reineccius, 1995)

The needs of controlled release of food additives are obvious to food scientists or technologists. However, the reports on the studies of controlled release of food additives are very limited, and the mechanisms and applications of controlled release remain far from being fully exploited (Whorton and Reineccius, 1995).

2.3.6 The Release Kinetics and Mathematical Modeling of Mass Transfer Phenomena of Microencapsulated Food Additives

Regarding the studies on release kinetics and mathematical modeling of mass transfer phenomena, literature references are lacking for microencapsulated food ingredients and nutraceuticals, while more research has been conducted in the area of microencapsulated medicine and pharmacy. For example, Nixon (1984) reviewed release characteristics of microcapsules in the area of biomedical applications of microencapsulation, and Donbrow (1992) reviewed the relation of release profiles from ensembles to those of individual microcapsules and the influence of types of batch heterogeneity on release kinetics.

2.3.7 The Benefits and Applications of Microencapsulation for Food Additives

Commercial microencapsulated food additives are available for food industries including chewing gum, batter mixes and coatings, dairy products, baking, and ready meals. In the past two decades, research and application of microencapsulation of food additives mainly focused on: extended shelf life stability of flavors and aromas,

increased oxidative stability of nutrient oil and additives, improved protection of vitamins and minerals, thermal release of food additives, and isolation of food additives.

a. Flavors: microencapsulation of flavors has found considerable use in the food industry. The major need for encapsulation is to retard or to eliminate volatilization, and to minimize or to eliminate contact with atmospheric oxygen. Making liquid flavors behave like dry powders is another major use. The two major process used for the encapsulation of flavors are spray drying and extrusion. Reineccius (1991) found that both methods depend primarily on carbohydrates for the wall material. In the microencapsulation of flavors, maltodextrins, corn syrup solids, modified starch, and gum acacia each have strengths and weaknesses. Sheu and Rosenberg (1995) found that wall system consisting of whey protein isolate and high DE maltodextrins are effective for volatile microencapsulation by spray drying. Such wall systems provide high volatile retention levels and limit the proportion of solvent extractable core.

b. Acids: Acids are used as dough conditioners, taste adjuncts, and pH-lowering agents. When added directly to food, acids can cause undesirable flavor and color changes. Microencapsulation allows acids to be used at levels that were previously not feasible for food applications (Jackson and Lee, 1991a). Ascorbic acid was encapsulated with high efficiency inside liposomes for stabilization (Kirby *et al.*, 1991). According to Kirby *et al.* (1991), encapsulation of vitamin C gives significant improvements in shelf life, both in simple aqueous solution and especially in presence of common food components, which would normally lead

to its rapid degradation. Ascorbic acid encapsulated in solid form is already widely used in the food industry.

c. Minerals: minerals for food fortifications are microencapsulated to mask undesirable flavors, control release, enhance stability to temperature and moisture, and reduce reactions with other food ingredients (Jackson and Lee, 1991a). Iron fortification is difficult in food processing due to potential oxidized off-flavors, color changes and metallic flavors. Jackson and Lee (1991b) developed microencapsulated iron salts using milk fat as wall material, which could be used to fortify foods and ingredients containing a high level of moisture and fat. The microcapsule structure must be preserved during food preparation and then biodegrade on ingestion.

d. Lipids: microencapsulation facilitates the addition of liquid oils to dry ingredients and provide protection against oxidation and other deteriorative reactions. Flavor oils such as citrus, onion and garlic are the most frequently encapsulated lipids (Jackson and Lee, 1991a). Microencapsulated high-fat powders provide a functional alternative to the use of block fat in wheat dough and subsequently in bread. They are also easy to use and store (O'Brien *et al.*, 2000). Microencapsulation by spray drying of anhydrous milk fat in wall systems consisting of whey proteins provided anhydrous milk fat with effective protection against oxidation (Moreau and Rosenberg, 1996). The same study also indicated that wall systems consisting of whey protein isolate were characterized by good oxygen barrier properties, and microencapsulation in whey protein isolate provided maximum protection of anhydrous milk fat from oxidative degradation.

e. Pigments: due to the sensitivity of natural pigments to light, heat, oxygen, and pH, their stabilization and protection is highly desirable. Shahidi and Pegg (1991) conducted a study to investigate encapsulation for enhancing the storage stability of the pre-formed cooked cured-meat pigment (CCMP) and to examine the color characteristics of pigment-treated meat products. They found that CCMP was stabilized by storage under a nitric oxide atmosphere or microencapsulation in food-grade carbohydrates. Encapsulated pigment remained stable during an 18 month testing period under refrigeration. The color stability of the treated meat products was similar to their nitrite-cured analog. Thus, the presence of residual nitrite used in traditional curing of meat may not play an important role in color stability under extreme conditions.

2.4 The Function and Applications of Pea Proteins

Field pea (*Pisun sativum L.*) serves as an important protein source for human consumption and animal feeding. It is one of the most widely grown crops with increasing importance in the world market (Tian *et al.*, 1999, Owusu-Ansah and McCurdy, 1991). Field pea is rich in protein and contains a high level of lysine, which is an important essential amino acid in for balancing the nutrition in cereal-based diets (Gueguen, 1991). Therefore the protein extracted from field peas is potentially a source of functional food ingredient. The crude protein present in field peas is composed of several thousand specific proteins. Salt-extractable proteins (the globulins fraction) which is made up of two major proteins, legumin and vicilin, account for 65 - 80% of

proteins present in peas, and water-soluble proteins (the albumin fraction) generally account for 20 - 35% (Owusu-Ansah and McCurdy, 1991)

Owusu-Ansah and McCurdy (1991) reviewed various aspects of pea proteins, including chemistry, technology of production, nutritional properties, functional and sensory properties, and utilization. They found that attempts to improve the utilization of pea protein had generally involved a conventional approach, that is, incorporation of unmodified protein products in traditional food. This approach had generally been unsuccessful, as products with good nutritional quality but poor acceptability, had been produced, due to the green color or bitter flavor associated with products containing pea protein. Therefore, it was concluded that research efforts should be directed toward modifying pea protein to enhance its functional properties for specific food application and that analogous research and development on pea protein, as had been done for soy protein, would be necessary if any meaningful boost in pea protein utilization was envisaged.

Pea protein is normally extracted by either a dry process or a wet process. A dry process is based on physical separation by air classification techniques. The functional properties of pea protein obtained by a dry process are relatively poor, according to Gueguen (1991). There are two different approaches for a wet process, alkali solution extraction and salt solution extraction. In order to compare two different approaches of wet processing and to evaluate different properties of the proteins made by using different drying methods, Tian *et al.* (1999) conducted a research study on wet processing of pea protein extraction on pilot scale. The results showed that the proteins extracted with salt were purer and had a lighter color than those extracted with alkali

solution. On the other hand, freeze-dried proteins had a darker color than the spray-dried products. The protein extracted with salt and spray-dried had a relatively even size, while for spray-dried protein that was extracted with alkali solution, there was a wide range of fine particles. The results also showed that the use of the two different extracting solutions did not result in any chemical changes. Furthermore, the application of freeze drying or spray drying did not cause any chemical differences. The solubility test indicated that proteins extracted both with salt and alkali solution had similar solubility property, and the solubility of spray-dried and freeze-dried products was also similar. It was suggested that salt solution extraction was a good approach for the extraction of field pea proteins on a large scale. This method gave a product that was light in color and had enhanced functional properties. Ultrafiltration was an appropriate method to concentrate proteins. Spray drying was better than freeze-drying for pea protein production since it gave better protein properties and saved on time and energy (Tian *et al.*, 1999).

Laboratory and pilot plant processes were developed for producing pea protein isolate from field peas by Sumner *et al.* (1980). Sodium proteinate and isoelectric products containing up to 90% protein were obtained by alkaline extraction and precipitation at the isoelectric point. Drying was carried out by freeze, spray and drum processes. Chemical analysis, functional properties, color and flavor of the dried isolates compared favorably with their soy counterparts. Generally, the sodium proteinates exhibited more functionality than isoelectric isolates. Drum drying decreased the nitrogen solubility index and increased water absorption. Freeze drying and spray drying

resulted in isolates with the highest emulsification and water absorption values. Spray drying produced the best foaming, color and flavor properties.

Choi and Han (2001) investigated the physical and mechanical properties of pea protein based edible films. The pea protein concentrate film was strong and elastic, and possessed a good moisture barrier property and physical integrity. The characteristics of the pea protein based edible films were comparable with other edible protein films, such as soy protein and whey protein, in terms of tensile strength, elongation, moisture barrier property and water solubility. Choi and Han (2002) further found that the strength of pea protein film was increased by heat treatment at 90°C over 5 min due to protein denaturation. It was also found that heat denaturation reduced the water absorption rate of the films to 19 to 40% of the non-heated film.

2.5 Summary

Beta-carotene is an important nutraceutical and is added to foods to provide a source of vitamin A. Microencapsulation can be used to protect the beta-carotene against oxidation, to prevent reaction with other additives, and to provide color masking. Microencapsulation is a technique that allows liquid or solid substances to be covered by a barrier wall. It provides a delivery system with the following major benefits: 1) Increasing shelf life by preventing evaporation and oxidation; 2) Stabilization of sensitive components; 3) Masking of undesirable taste or flavor; 4) Improving processability and texture; 5) Preventing undesirable interactions; 6) Ease of handling; and 7) Permitting controlled release under desired conditions. Spray drying is one of the most commonly used microencapsulation technologies and the technology continues to

develop. No investigations have been reported on utilizing pea protein as wall material for microencapsulation.

3.0 MATERIALS AND METHODS

3.1 Materials

Synthetic beta-carotene (approx. 95%, Sigma Chemical Co. St. Louis, MO, USA) was chosen as a model nutraceutical. It was dissolved in 100% corn oil (Mazola corn oil, Bestfoods Canada Inc., Toronto, ON, Canada) to make a concentration of 1% beta-carotene liquid as a hydrophobic core.

Pea protein concentrate (PPC) extracted from yellow field peas (*Pisum sativum L.*) was supplied by Parrheim Foods (Portage la Prairie, MB, Canada) in a liquid form. It was processed by acidified protein curds from the soluble fraction of the wet milling of yellow field peas. The PPC contained 76.6% protein, 3% fat, 4% ash, and 10% non-protein soluble fractions (dry basis) (Choi and Han, 2001). The PPC solution was purified and freeze-dried to yield pea protein isolate (PPI) containing 83.7% protein (dry basis). The PPI was used as wall material with or without maltodextrin (Maltrin M040, DE = 40, Grain Processing Co. Muscatine, IA, US), which is a commonly used wall material.

3.2 Microencapsulation Method

PPI (with or without maltodextrin M040) solutions containing 15% or 20% (w/w) solids were prepared in deionized water. The PPI solution was heated at 100°C for 20 - 60 min to denature protein when heat treatment (heat denaturation) is necessary. The M040 content in solid wall material was 0, 25, 50, and 75% (w/w).

The core liquid (1% beta-carotene in corn oil) was emulsified into the wall solutions at wall to core ratio of 6 : 1, 6 : 2, 6 : 3, 6 : 4, and 6 : 6 (w : w). Emulsions

were prepared using a homogenizer (PowerGen 700, Fisher Scientific, Pittsburgh, PA, USA) operated at 13,500 rpm for 90 seconds with Generator 20 x 195 mm.

The emulsions were spray dried using a Portable spray dryer (Niro Portable, Niro Inc., Copenhagen, Denmark) equipped with a compressed air driven centrifugal wheel atomizer. The evaporation rate of the dryer was approximately 1 kg/h. The emulsions were atomized by the centrifugal atomizer operated at 25,000 rpm or 32,000 rpm with correspondent air pressure at 5.5 MPa or 6.0 MPa. Drying was carried out in the co-current mode. Inlet and outlet air temperatures were 155 ± 5 °C and 55 ± 5 °C, respectively. The temperatures as listed were limited by equipment capabilities.

The outline of the processes to produce pea protein based beta-carotene containing microcapsules is summarized in Figure 3.

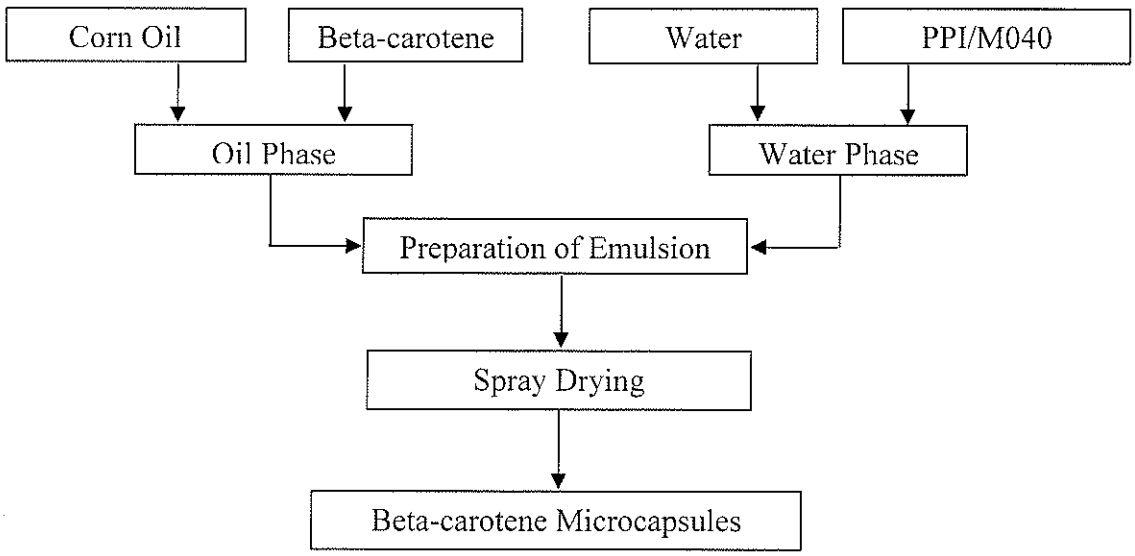

Figure 3. Flowchart of the processes to prepare spray dried pea protein based beta-carotene microcapsules

Figure 4 shows a schematic configuration of the co-current spray dryer used for microencapsulation of beta-carotene in this study.

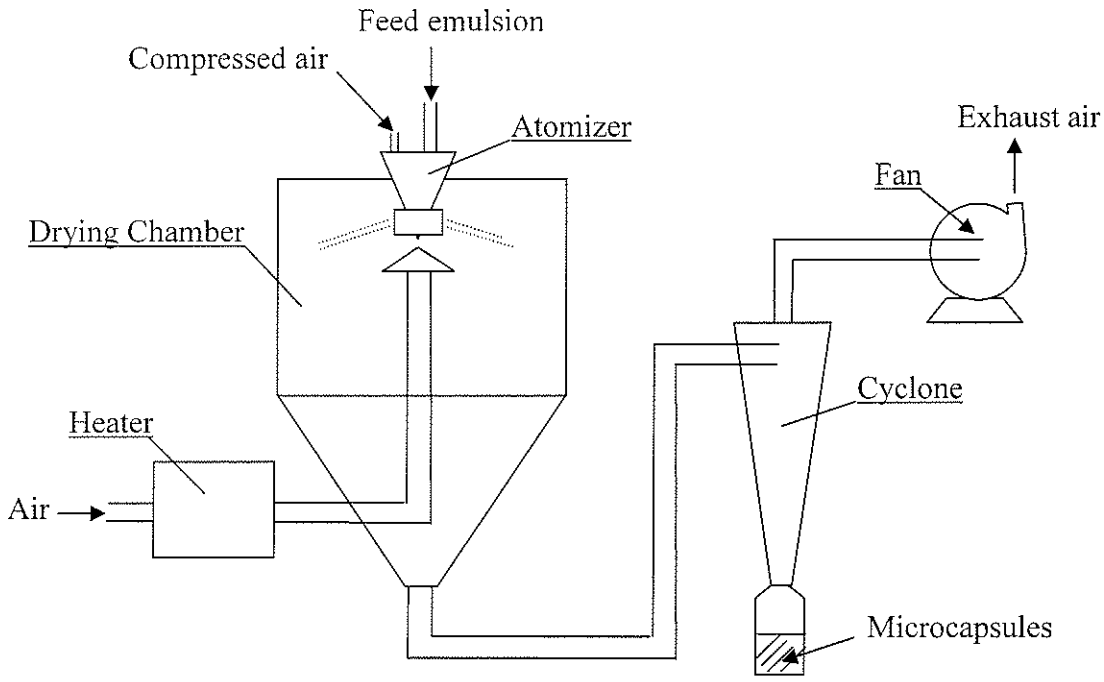

Figure 4. Co-current spray drying equipment

3.3 Analyses

3.3.1 Determination of Drying Yield

Microcapsule powder collected from the cyclone was combined with microcapsule powder collected from the chamber after dry cleaning. The drying yield was calculated according to equation (1):

$$\text{Drying yield} = \frac{T}{C + W} \times \% \qquad (1)$$

Where: T is the total weight of powder obtained, C is the total weight of the core

material and W is the total weight of the wall material.

3.3.2 Beta-Carotene Content

20-50 mg of microcapsule power was dispersed in 2.5 mL of water in a 125 mL Erlenmeyer flask. 25 mL of hexane was added to the microcapsule dispersion. The flask was sealed and stirred with a magnet stirrer at approx. 120 rpm for 30 min. After phase separation, the absorbance of hexane phase was measured at 450 nm using a spectrophotometer (Uitrospec 200 UV/Visible, Biochrom Ltd., England). Beta-carotene concentration was obtained by comparing sample absorbance to a standard curve of beta-carotene, 0 – 10.0 ppm (Figure 5) (Desobry *et al.,* 1997). Tests were conducted in triplicate.

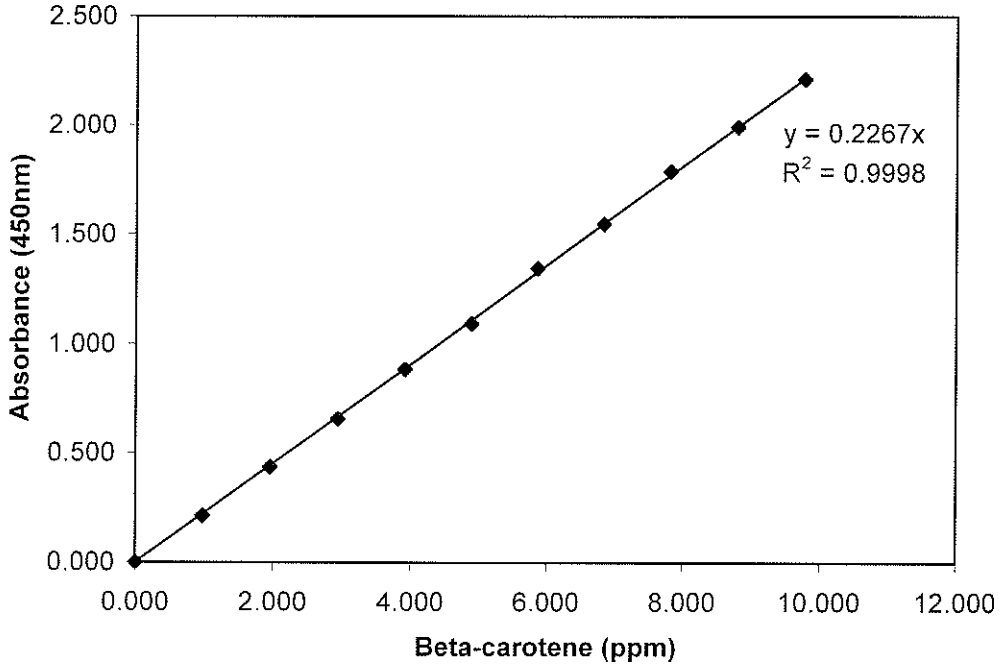

Figure 5. Standard curve of beta-carotene content

3.3.3 Microstructure Examination

The microstructure of the microcapsules was examined by scanning electron microscope (SEM) at the University of California (Advanced Instrumentation, the University of California, Davis, CA, USA), and by ESEM environmental scanning electron microscope (XL30, Philips, Holland) at the Department of Mechanical & Industrial Engineering, University of Manitoba, Canada, respectively. The specimen was prepared by razor fracturing in order to exam detailed outer and inner structure of the microcapsules (Rosenberg and Young, 1993). The specimen for SEM was gold-coated.

3.3.4 Particle Size

The average radius and the standard deviation of microcapsules were examined by an image analyzer (CUE-2 Image Analyzer, Olympus, Japan) (Method A). Approximately 5 mg of microcapsules was placed on a slide, and one drop of mineral oil (Sigma Chemical Co., MO, USA) was added. The sample was stirred with a needle and a cover slip was added. The sample was placed on the light microscope with magnification of 400 ×; image was acquired by a video camera. Each image contains 200 – 500 particles. After the image is acquired, it is enhanced to adjust contrast and signal to noise ratio, filtered to sharpen image detail (by smoothing and edge enhancement), thresholded to isolate the particles for measurements by the system. The size distribution, average radius and its standard deviation were then obtained by the CUE -2 image analysis system.

The average diameter and the standard deviation of microcapsules were also examined using an inverted phase contrast microscope (PCM, Nikon Diaphot) equipped

with a TV camera (Panasonic WV-1550) (Method B). Five images of each sample were taken, each image contains about 30 individual microcapsules, the diameter of each microcapsule was measure by using a micrometer, and the average diameter and the standard deviation were calculated.

3.3.5 Drying Loss of Beta-Carotene

The beta-carotene content (dry basis) of microcapsule samples for the zero time was analyzed within 2 hours after drying. The drying loss of beta-carotene was determined by comparing the zero time value with the theoretical value of beta-carotene content in the emulsion (dry basis). Tests were conducted in triplicate.

3.3.6 Determination of the Moisture Content of Microcapsules

Approximately 1 g of microcapsule sample was weighed in an aluminum weighing dish and dried in a laboratory oven at 100 ± 2 °C for 3 h. After the sample was cooled in a desiccator, the moisture content (% w/w) was calculated from the mass loss. Tests were conducted in triplicate.

3.4 Determination of Moisture Sorption Isotherms (MSI)

Eight saturated aqueous salt solutions were prepared and placed in the sealed chamber to obtain the relative humidity level as listed in Table 2. Aluminum dishes containing about 0.02 g of microcapsules were weighed. Aluminum dishes were then put into the desiccators at 22 ± 1 °C for 7 days. After the equilibrium state was reached, samples were weighed before and after drying at 100 ± 2 °C for 6 h. Moisture content

was calculated as the percentage of mass loss based on the original mass. The water activity (a_w) was measured by a_w meter (AW SPRINT, Novasina, TH-500, Swiss) (Yang and Paulson, 2000; Desobry *et al.*, 1997; Chinnan and Park, 1995). Tests were conducted in triplicate.

Table 2. Equilibrium relative humidity value for saturated aqueous salt solutions at 25°C

Saturated aqueous salt solution	Equilibrium relative humidity (%)
LiCl H_2O	12
CH_3COOK 1.5H_2O	22
$Mg(Cl)_2$	33
K_2CO_3	43
$Mg(NO_3)_2$ 6H_2O	53
$NaNO_3$	63
NaCl	75
KCl	85

3.5 Swelling Kinetics and Release Time Estimation

Approximately 5 mg of microcapsules was placed on a slide and mixed with two drops of water, the swelling release was observed through a research light microscope (Universal transmitted light microscope, Zeiss, Germany) at 160× of magnitude. A camera was attached to the microscope. The swelling and release time was recorded to measure the resistance of wall structure against water. Images of microcapsules in water against time were taken by the attached camera every 10 minutes to record a change in shape.

Swelling ratio at predetermined time (S_t) was calculated from the following equation (2) (Han *et al*, 2000):

$$S_t = \frac{r_t^3 - r_o^3}{r_o^3} \qquad (2)$$

Where, r_o and r_t are the radii of a microcapsule at time 0 and t, respectively.

The changes in swelling ratio with time were monitored by the following first-order kinetic equation (3) (Donbrow, 1992).

$$S_t = S_\infty (1 - e^{-kt}) \qquad (3)$$

Where, S_∞ is the maximum swelling ratio at $t = \infty$, and k is swelling rate.

To calculate k, the above equation was transformed to the following linear equation (4) in natural logarithm coordinates (Nixon, 1984).

$$\ln (1 - S_t/S_\infty) = - kt \qquad (4)$$

Experimental data of swelling ratio (S_t) with time was plotted and S_∞ was obtained where R^2 showed the best fit of $\ln(1 - S_t/S_\infty)$ to t. The swelling rate (k) was calculated from the regressing estimate of the slope.

3.6 Retention of Beta-Carotene at Shelf Storage Condition

Microcapsule samples for the zero time were analyzed within 2 hours after drying. For the storage test, samples were kept in plastic bottles without any attempt to remove the air and the lids were closed properly. They were stored at room temperature (22 ± 2 °C) with relative humidity (RH) of 20 - 60 %. The beta-carotene content in microcapsules was tested weekly for 7 - 8 weeks. Tests were conducted in triplicate.

3.7 Statistical Analysis

Two factors and three levels of treatment (2 × 3) factorial design was applied to examine the effect and interaction of the different wall to core ratio and different wall composition to the microencapsulation.

Data were analyzed as complete randomized designs using general linear models (GLM) procedure of SAS program (Version 8.1, SAS Institute Inc., Cary, NC, USA). Mean differences were compared using a Tukey test at 95% significance level ($P < 0.05$).

4. 0 RESULTS AND DISCUSSIONS

4.1 Drying Yield of Spray Drying

Figure 6 illustrates the effect of wall to core ratio to the drying yield when the PPI based beta-carotene containing microcapsules were prepared. It shows a trend that the yield increased when wall to core ratio decreased. However, when the wall to core ratio was too low, i.e. 6 : 4, the yield decreased substantially. That was due to the inadequate amount of wall material that could not encapsulate the entire core and caused the core material loss. The optimum wall to core ratio was 2 : 1 under the experimental conditions.

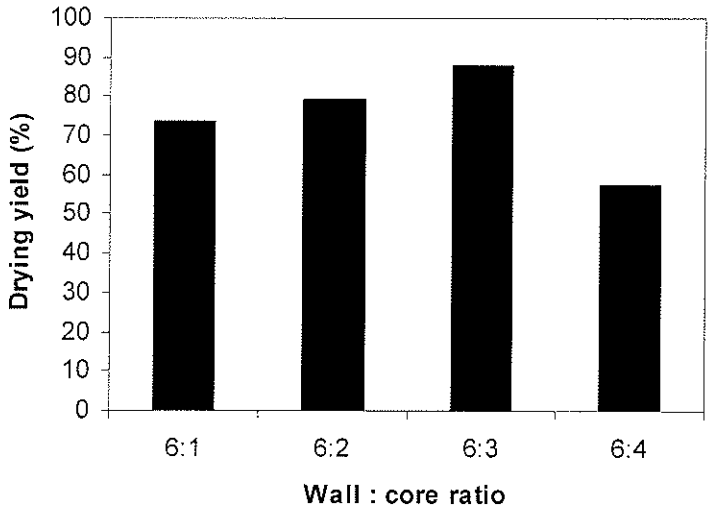

Figure 6. The effect of wall to core ratio on the drying yield. Bar presents one single sample

Figure 7 illustrates the relationship between maltodextrin (M040) content in the wall and the drying yield when the PPI based beta-carotene containing microcapsule

were prepared at wall to core ratio 2 : 1. The data indicates that M040 content had no effect on drying yield.

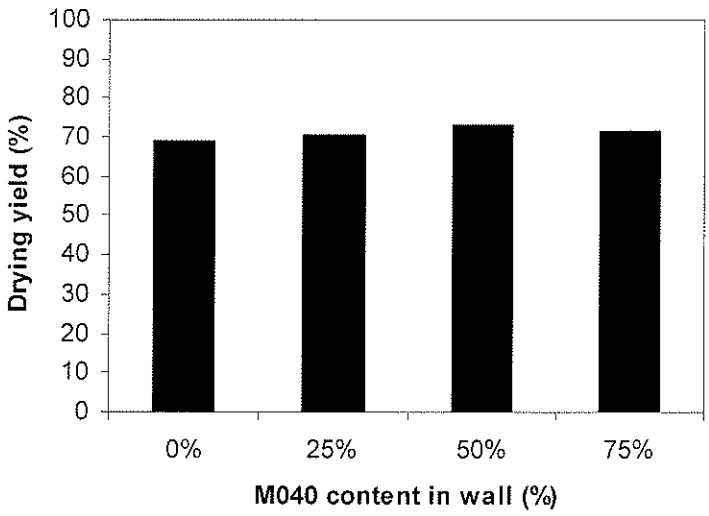

Figure 7. The effect of M040 content in wall on drying yield. Bar presents one single sample

Of all the spray drying experiments, an average drying yield of 73.3 ± 8.7% was obtained. During the spray drying process the microcapsule powder adhered to the wall of drying chamber (caking) and would represent an experimental loss of drying yield if not recovered and used in the drying yield calculation. In a commercial unit, design features would minimize powder caking to the drying chamber.

4.2 Moisture Content of Microcapsules

The average moisture content of the microcapsule powder obtained after spray drying under different conditions was 2.6 ± 0.6 %.

Figure 8 illustrates the effect of the wall to core ratio to the moisture content when the PPI based beta-carotene containing microcapsules were prepared. The data shows a trend that the moisture content decreased when wall to core ratio decreased.

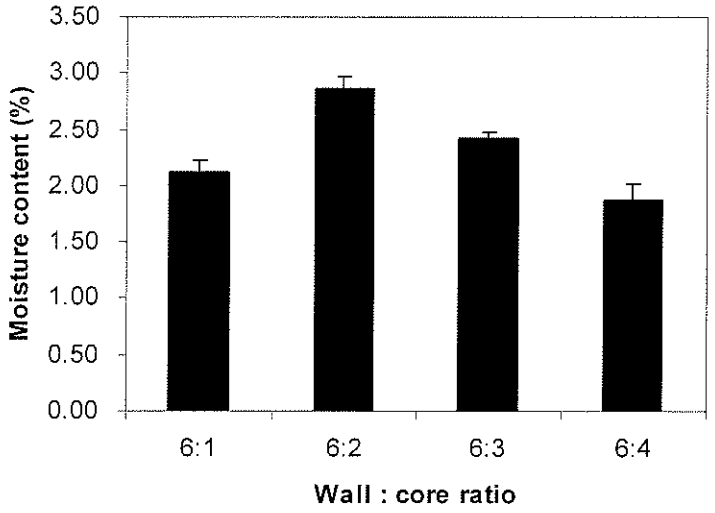

Figure 8. The effect of wall to core ratio on the microcapsule moisture content

Figure 9 illustrates the effect of maltodextrin content in wall on the moisture content when the PPI based beta-carotene containing microcapsule were prepared at wall to core ratio 2 : 1. An increase in the maltodextrin content could decrease the moisture content of the microcapsules.

Table 3 indicates that the heat treatment of PPI had no effect on the particle moisture content. The microcapsules obtained from either native or heat treated PPI wall, have moisture content of 3.8%, but a moisture content of 3.3% was obtained when 25% of M040 was blended into heat treated PPI as the wall.

Although factors such as wall system composition and the wall to core ratio have effects on the moisture content, other factors in the drying process, such as drying temperature and feed rate would also affect the moisture content of microcapsules.

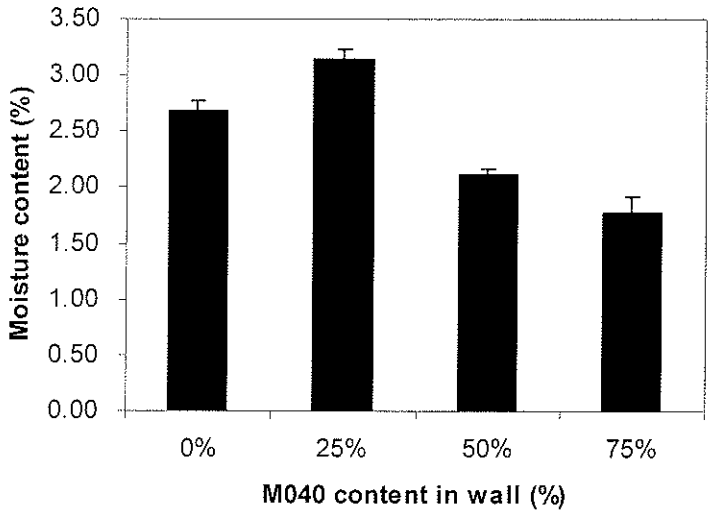

Figure 9. The effect of M040 content in wall on the microcapsule moisture content

Table 3. The effect of different wall systems on the moisture content of microcapsules

Wall system	Moisture content of microcapsules (%)
PPI (native)	3.8 ± 0.1
PPI (heat-treated)	3.8 ± 0.2
75% PPI (heat-treated) + 25% M040	3.3 ± 0.1

* Each value represents the mean and standard deviation of three measurements

4.3 Microstructure of Microcapsules

Figures 10, 11 and 12 illustrate the microstructure of PPI and M040 based, beta-carotene containing microcapsules. Overall, similar spherical capsules with a smooth surface were observed in all cases. The external structures of the microcapsules showed no cracks, indicating the formation of a continuous film in the wall. Few pores were observed in some cases (b in Figure 10, and c and d in Figure 11). Most microcapsules showed a dimple (surface dent) effect (a in Figure 10).

A cross section of the micrographs as seen in Figure 10 (c and d) shows that uniform thickness of wall structure was observed, indicating an integrated wall system was formed during spray drying.

Figure 11 indicates that with decreasing wall to core ratio, the surface dents appeared to be decreased but the numbers of pores of the microcapsules increased. The surface dents are undesirable as they adversely affect the flow ability and wet ability of the microcapsule powder because they lead to the formation of clumps or aggregates. Figure 11 (e and f) also shows that when the wall to core ratio was too low, i.e., 6 : 4, the inadequate amount of wall material could not encapsulate all of the core. Therefore excess core material (beta-carotene oil solution) was observed.

Figure 12 (c, d, e, and f) shows that by increasing the content of M040 in wall material, the surface dents of the microcapsules decrease. Therefore, incorporation of M040 at a proper ratio may provide means to limit or minimize the presence of surface dents.

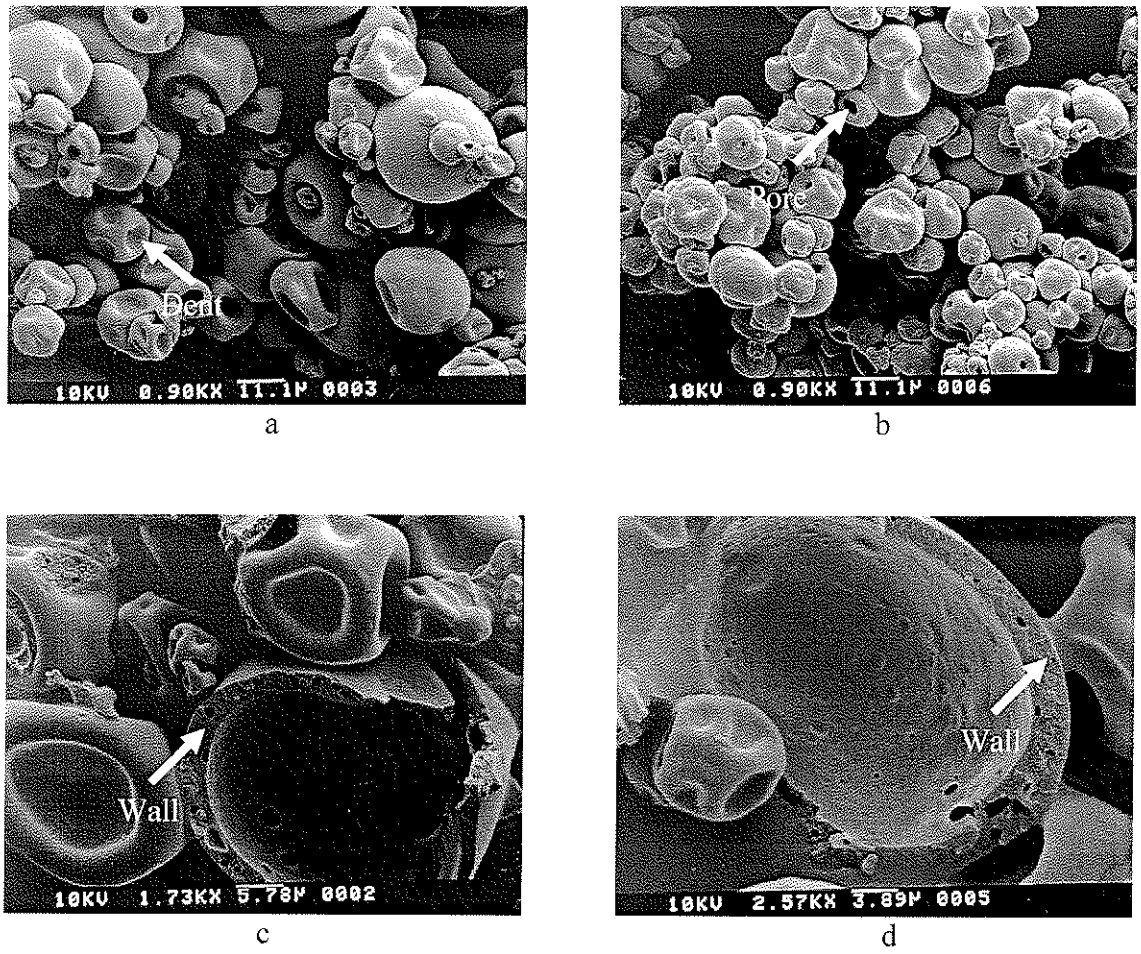

Figure 10. SEM Micrographs of PPI based, beta-carotene containing microcapsules prepared by spray drying. a) wall : core = 8 : 1; b) wall : core = 4 : 1; c) cross section, typical inner and outer microstructure of wall system, wall : core = 8 : 1; d) cross section, typical inner and outer microstructure of wall system, wall : core = 4 : 1

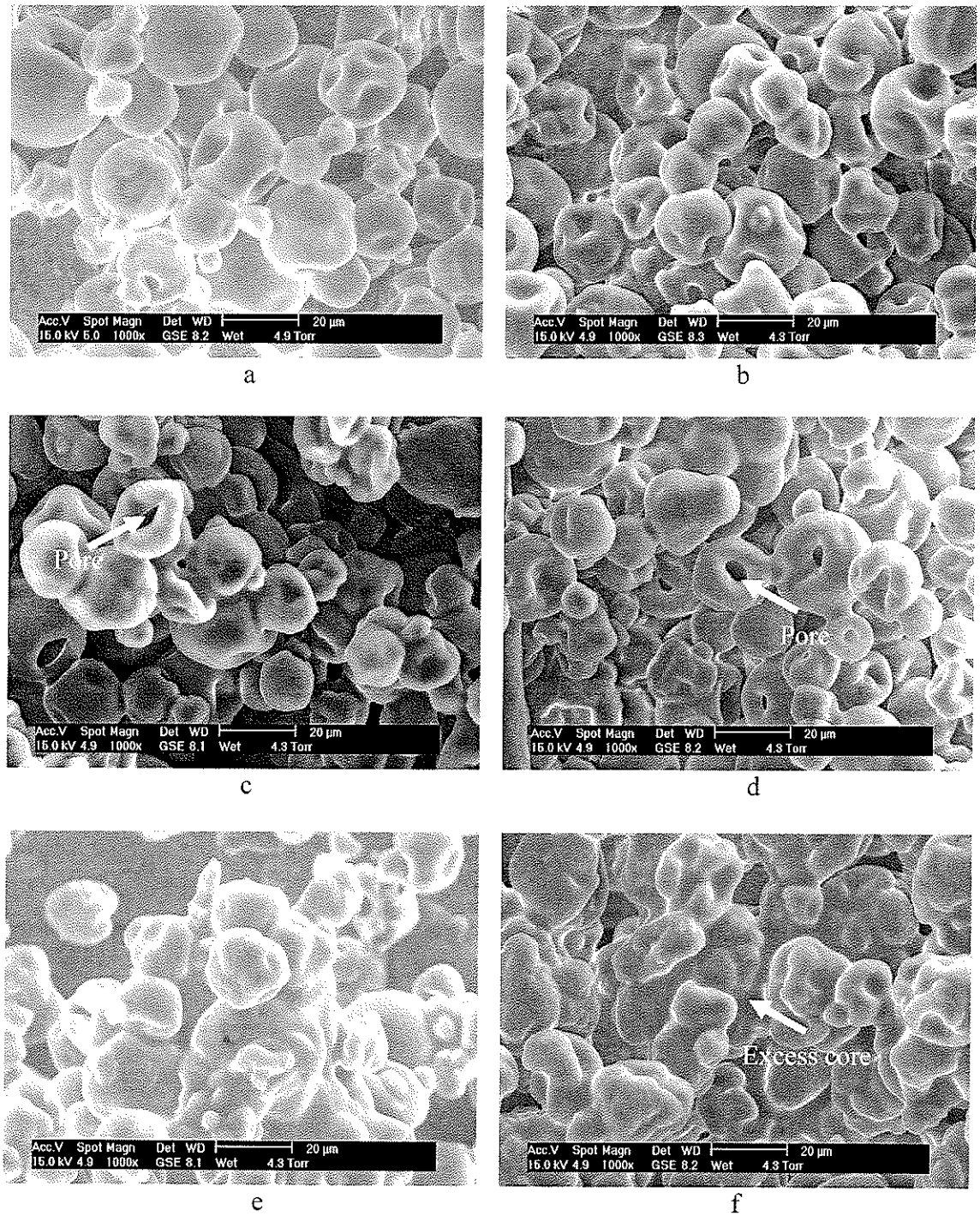

Figure 11. ESEM micrographs of PPI based, beta-carotene containing microcapsules prepared by spray drying, atomizer of spray dryer at 2800 rpm, PPI : M040 = 1 : 0. a) wall : core = 6 : 1; b) wall : core = 6 : 2; c) wall : core = 6 : 3; d) wall :core = 6 : 3; e) wall : core = 6 : 4; f) wall : core = 6 : 4

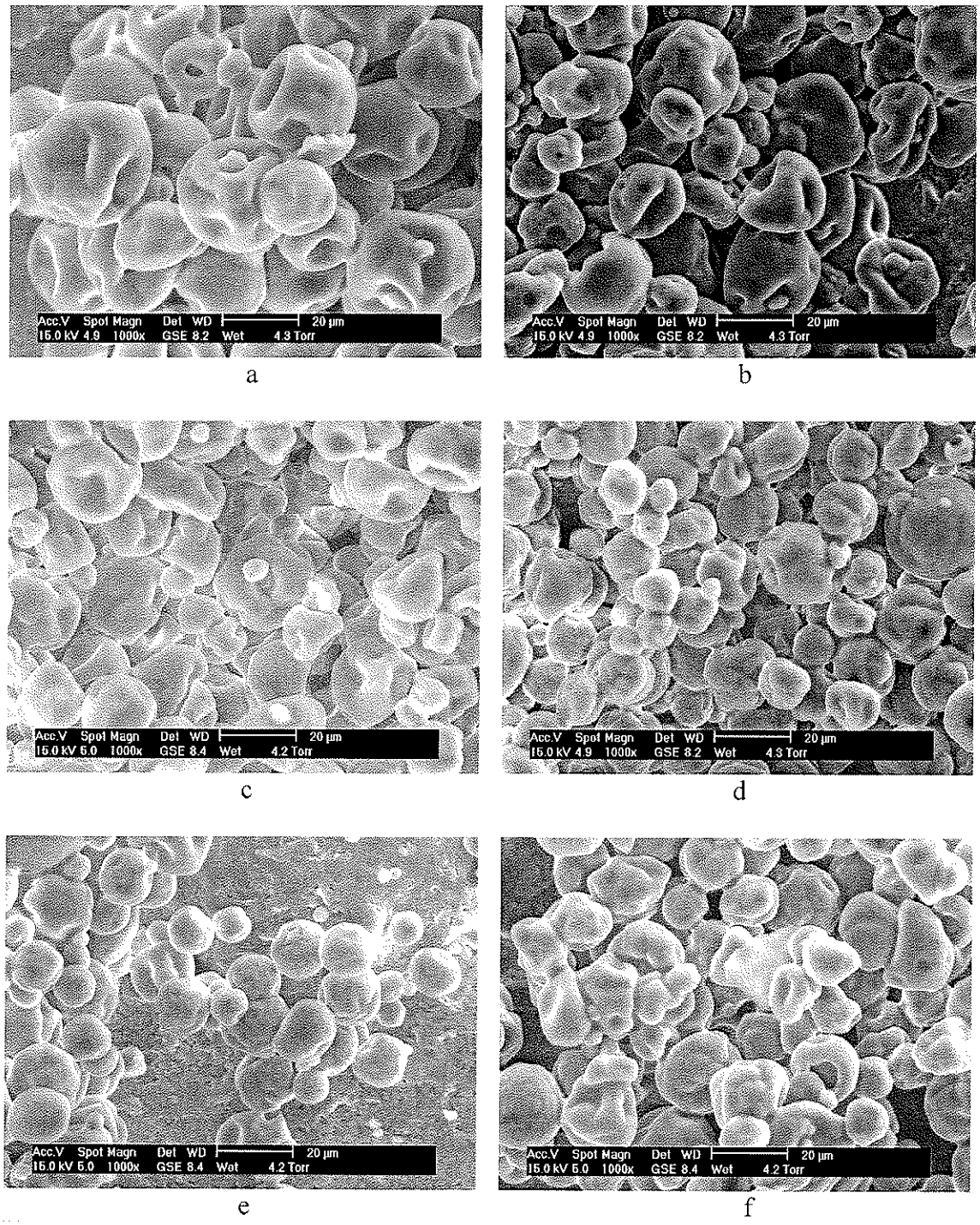

Figure 12. ESEM micrographs of PPI and M040 based, beta-carotene containing microcapsules prepared by spray drying, wall : core = 2 : 1. a) atomizer of spray dryer at 2800 rpm, PPI : M040 = 1 : 0; b) atomizer of spray dryer at 2800 rpm, PPI : M040 = 1 : 1, c) atomizer of spray dryer at 3200 rpm, PPI : M040 = 3 : 1; d) atomizer of spray dryer at 3200rpm, PPI : M040 =1 : 1; e) atomizer of spray dryer at 3200 rpm, PPI : M040 = 1 : 3; f) atomizer of spray dryer at 3200 rpm, PPI : M040 = 1 : 3

Microcapsules obtained were mostly in matrix type (shell and matrix) and a few in reservoir type (shell and core) structure, as illustrated in Figure 13. Both structures are capable of acting as a core-entrapped reservoir and can provide a controlled release profile.

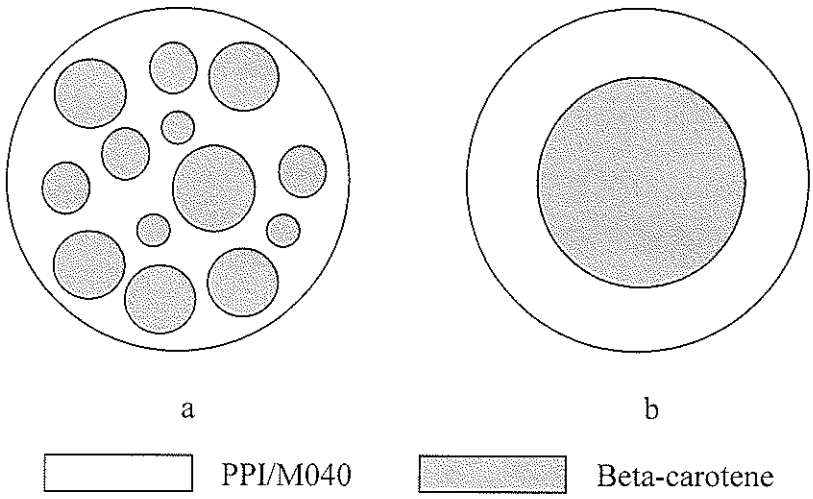

Figure 13. Schematic diagrams of typical microcapsule structures obtained by spray drying. a) shell and matrix (polynulear) (discrete beta-carotene core regions surrounded by a continuous shell); b) shell and core (continuous beta-carotene core surrounded by a continuous shell)

4.4 Particle Size of Microcapsules

The average particle size of the microcapsules was 4.3-7.1 μm in radius.

Many factors can influence the microcapsule powder properties. Table 4 shows the effect of wall to core ratio on the average size and standard deviation of microcapsules. Increasing the amount of wall material generated larger particle size. Particle size

distribution results indicate that the standard deviation of microcapsule size was approximately half of the average size. The wall to core ratio did not affect the standard deviation to average size ratio of microcapsules. Therefore, the wall to core ratio only relates to microcapsule size, not to the precision of process or size distribution.

Table 4. The effect of wall to core ratio on particle size of microcapsules

Wall : core	Particle size in radius (μm)
2 : 1	4.9 ± 2.4
3 : 1	5.5 ± 2.6
6 : 1	6.0 ± 3.0

* The particle size was measured by Method A
** Each value represents the mean and standard deviation

Levels of maltodextrin content in the wall could also influence the microcapsule powder properties. Table 5 shows the effects of maltodextrin (M040) content in wall on the average size and standard deviation of microcapsules. Increasing the amount of maltodextrin content in wall generated smaller particle size. Particles size distribution results indicate that the standard deviation of microcapsule size was about half of the average size. The maltodextrin content in wall did not affect the standard deviation to average size ratio of microcapsules. Therefore, the maltodextrin content in wall relates to microcapsule size, not to the precision of process or size distribution.

Table 5. The effect of maltodextrin content in wall on particle size of microcapsules

M040 content in wall (%)	Particle size in radius (μm)
0	7.1 ± 4.1
25	5.2 ± 2.3
50	4.7 ± 2.4
75	4.3 ± 2.2

* The particle size was measured by Method A
** Each value represents the mean and standard deviation

Table 6 indicates that increasing the rotate speed of the atomizer from 2800 to 3200 rpm on the spray dryer decreases the particle size of PPI based beta-carotene containing microcapsules. Therefore the particle size can be adjusted by changing the rotate speed of the atomizer. The ideal particle size of beta-carotene containing microcapsules for this study however, has not been determined.

Table 6. The effect of atomizer speed on particle size of microcapsules

Atomizer speed (rpm)	Particle size in radius (μm)
2800	5.9 ± 3.1
3200	4.6 ± 2.2

* The particle size was measured by Method A
** Each value represents the mean and standard deviation

4.5 Moisture Sorption Isotherm (MSI) of Microcapsules

Both pea protein and maltodextrin are hydrophilic in nature. They are both sensitive to the moisture in the environment. The moisture in the wall material affects the physical properties and barrier properties of the microcapsules. The moisture content of microcapsules also depends on the relative humidity of the atmosphere in which they are stored.

The results as shown in Figure 14 and 15 indicate that similar sorption isotherm shapes were obtained, which were typical sigmoid shape. In all cases, the equilibrium moisture content increased suddenly at water activity greater than 0.55 (relative humidity greater than 50 %).

Figure 14 compares moisture sorption isotherms of microcapsules with PPI of different level of heat treatment (denaturation) at 100 °C. Longer holding time increased the PPI denaturation level. The results showed that the PPI with a higher denaturation level (e.g.100 °C for 60 min) absorbed more moisture at a given water activity. In other words, the water holding capacity (or hygroscopicity) of denatured PPI was higher than native PPI. High water holding capacity could be a good characteristic of wall material for the beta-carotene retention in the microcapsule, as water may act as an oxygen barrier.

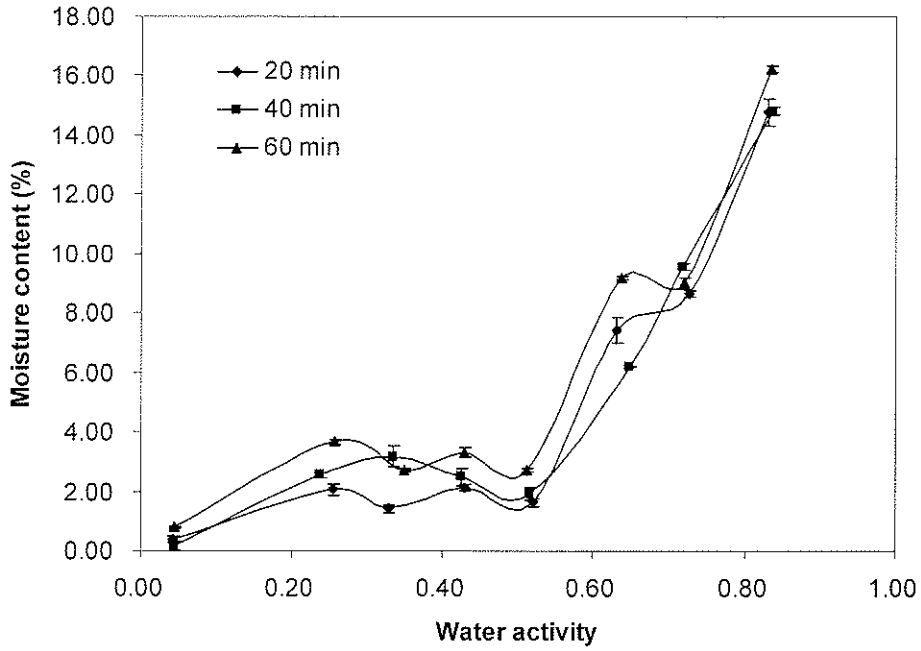

Figure 14. Moisture sorption isotherms of microcapsules at 22 °C. PPI wall materials were heat treated at 100 °C for 20, 40, and 60 min

Figure 15 compares moisture sorption isotherms of microcapsules with different wall composition. The wall with higher M040 content absorbed less moisture at a given water activity. Therefore, the water holding capacity of the wall with higher content of maltodextrin was lower than those with lower or no maltodextrin content. Again, high water holding capacity could be a good characteristic of wall material for the beta-carotene retention in the microcapsule, as water may act as an oxygen barrier.

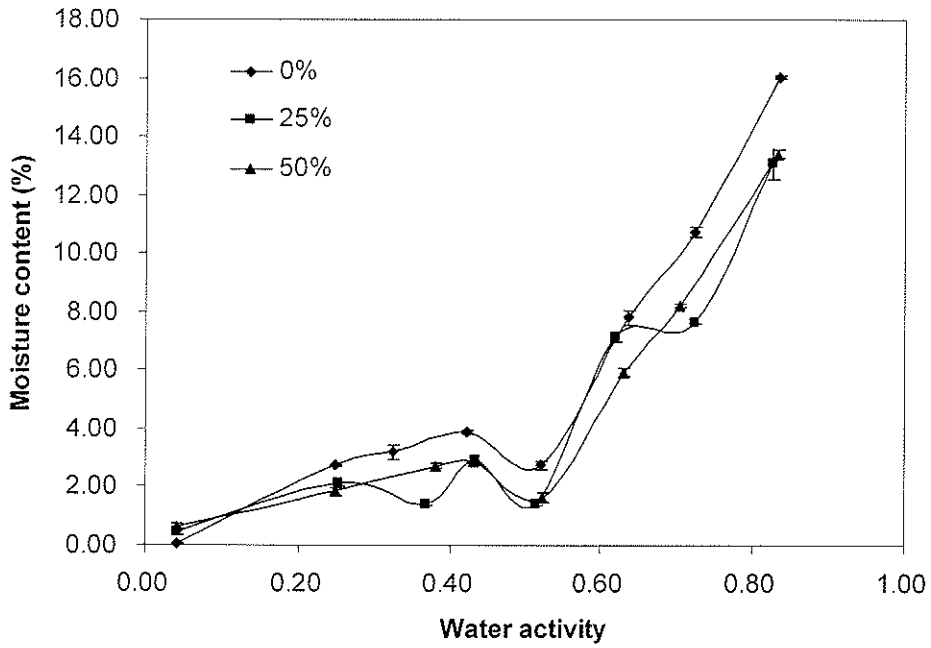

Figure 15. Moisture sorption isotherms of microcapsules at 22 °C. Wall materials contained 0, 25, and 50% of M040

4.6 Drying Loss and Retention of Beta-Carotene during Shelf Storage

The drying loss and retention of beta-carotene during storage was compared for the different wall to core ratio, different M040 content in wall, and different PPI heat treatment, respectively.

Drying loss of beta-carotene was determined from the beta-carotene content at 0 week of storage, which was measured right after spray drying.

Both Figure 16 and 17 obtained from separate experiments illustrate that different wall to core ratio can influence the beta-carotene retention during storage. The difference in the wall to core ratio can initially influence the drying loss of beta-carotene (Figure 17). Increasing the amount of wall component decreased the beta-carotene amount in microcapsules just after drying. After 4 weeks of storage, the beta-carotene in

the higher wall to core ratio, such as 6 : 1 in Figure 16 and 3 : 1 in Figure 17, decreased faster than those of lower wall to core ratio. Lower wall to core ratio was more efficient in terms of the storage stability of beta-carotene. Under the experimental conditions, the best beta-carotene retention obtained was 58% after 7 weeks with wall to core ratio of 1 : 1.

Generally, it is considered that the wall material must be thick or dense enough in order to form a good oxygen barrier. Nevertheless, spray drying provides a very larger surface area which could enhance oxidation. The result suggested that the higher wall content (higher wall to core ratio) might contribute to a smaller core droplets in the wall matrix, therefore, the surface area of the encapsulated beta-carotene was much larger than those in lower wall to core ratio.

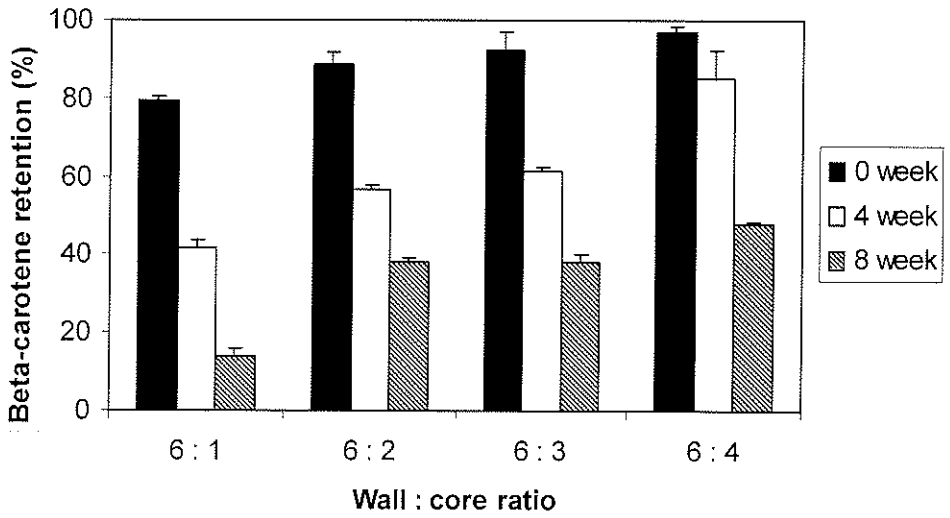

Figure 16. The effect of wall to core ratio on the drying loss and retention of beta-carotene at week 4 and 8 during storage

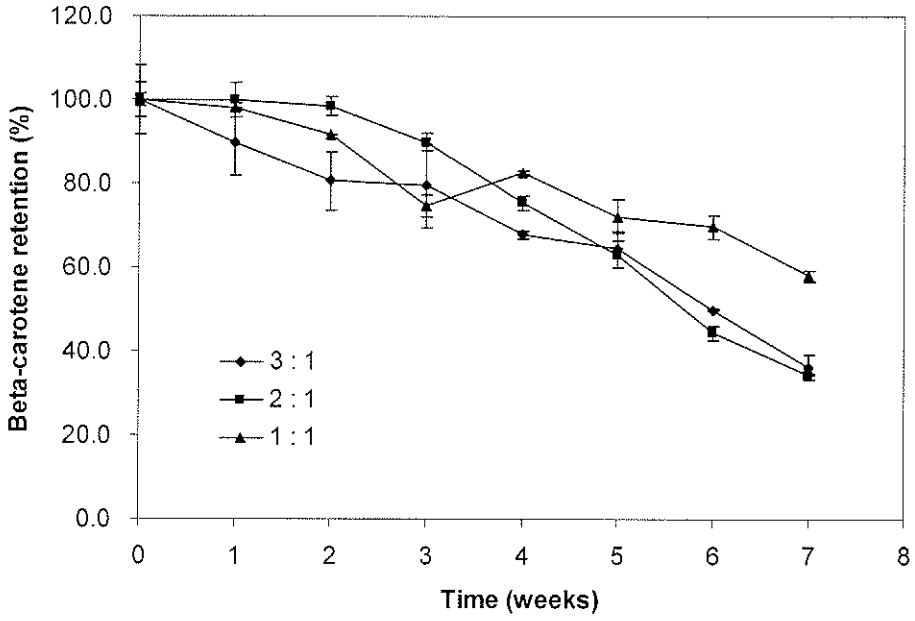

Figure 17. The effect of wall to core ratio (wall : core = 3 : 1, 2 : 1, and 1 : 1) on the retention of beta-carotene during shelf storage

Figure 18 shows that the effect of M040 content in wall material on the drying loss of beta-carotene is not significant. Data shown in Figure 18 and 19 obtained from separated experiments however indicate that different wall composition could influence the beta-carotene retention during storage. Increasing the amount of M040 content in wall decreased beta-carotene stability. Under the experimental conditions, the best beta-carotene retention obtained was 59.2 % after 7 weeks with wall containing PPI only.

The results suggest that PPI has a superior oxygen barrier property compared to maltodextrin and would therefore be the choice wall constituent.

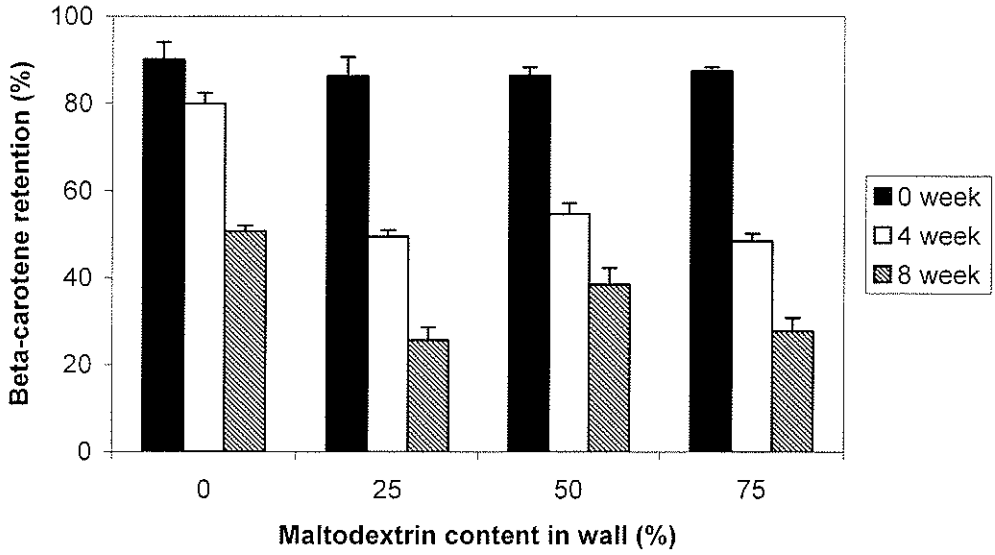

Figure 18. The effect of M040 content in wall on the drying loss and retention of beta-carotene at week 4 and 8 during shelf storage

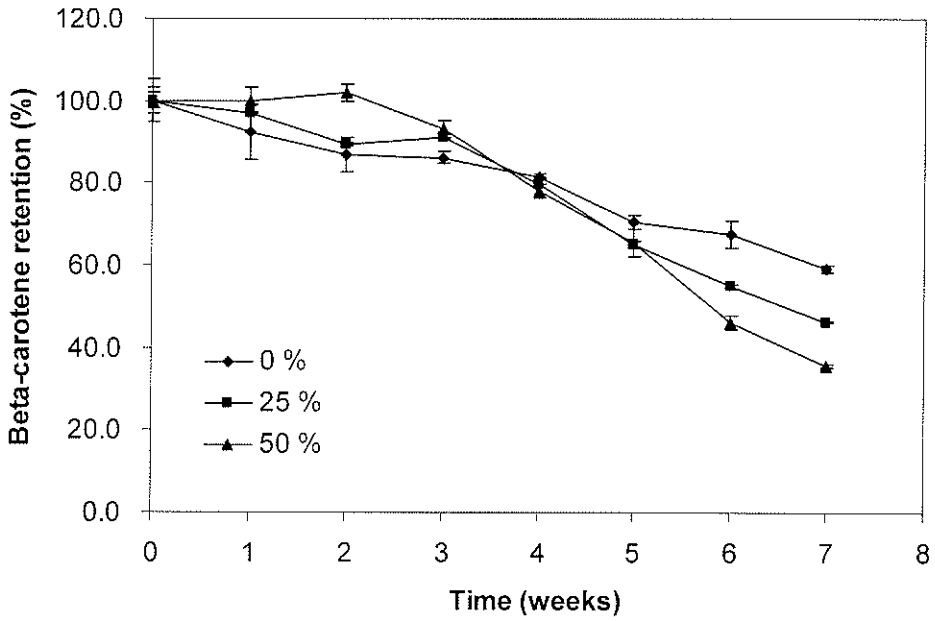

Figure 19. The effect of wall composition (M040 content at the level of 0, 25, and 50%) on the retention of beta-carotene during shelf storage

Figure 20 illustrates that the heat treatment of PPI wall material could improve the retention of beta-carotene during shelf storage. Results indicated that increasing the heat treatment time of PPI wall solution increased efficiency of the storage stability of beta-carotene. Under the experimental conditions, the best beta-carotene retention obtained was 80.9 % after 7 weeks with PPI wall solution which was heated at 100°C for 60 min. The results suggest that increasing the denaturation level of PPI may have improved its property as an oxygen barrier. Therefore, it is necessary to denature pea protein by heat treatment, e.g., to heat the PPI wall solution at 100°C for 60 min before preparing the emulsion for microencapsulation, in order to obtain desirable beta-carotene retention.

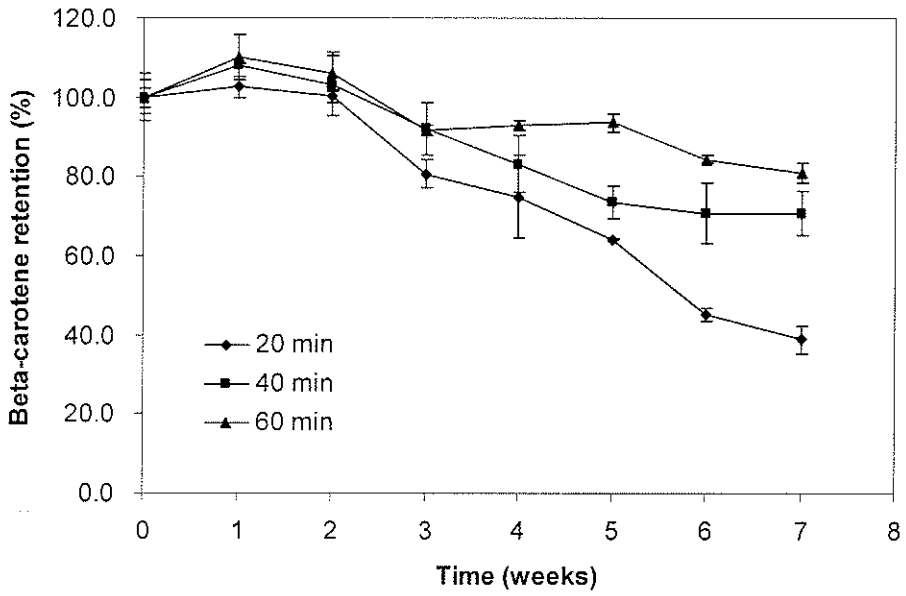

Figure 20. The effect of heat treatment time of PPI wall material (15% PPI solution was heated at 100°C for 20, 40 and 60 min) on the retention of beta-carotene during shelf storage

4.7 Swelling Characteristics of Microcapsules in Water and the Release of Beta-Carotene

Figure 21 compares the swelling release characteristics of beta-carotene from the microcapsules of different wall systems when they are placed in water. In all cases, swelling took place after 10 min. For microcapsules with native PPI wall system, the wall was fully collapsed and core fully released after 60 min as evidenced in Figure 21 (a) , similar to the microcapsules with 75% heat treated PPI + 25% M040 wall system (Figure 21, c). However, for microcapsules with 100% heat treated PPI wall system, the full release of the core did not take place until after 60 min (Figure 21, b).

Microcapsules with native PPI had the highest swelling ratio (Figure 22) indicating higher release rate of beta-carotene and the microcapsules with heat treated PPI had the lowest swelling ratio indicating lower release rate of beta-carotene when they were put in water. The swelling ratio of microcapsules with mixed wall material (75% heat treated PPI + 25% M040) was in between. The results suggest that the release rate can be controlled by changing the wall composition.

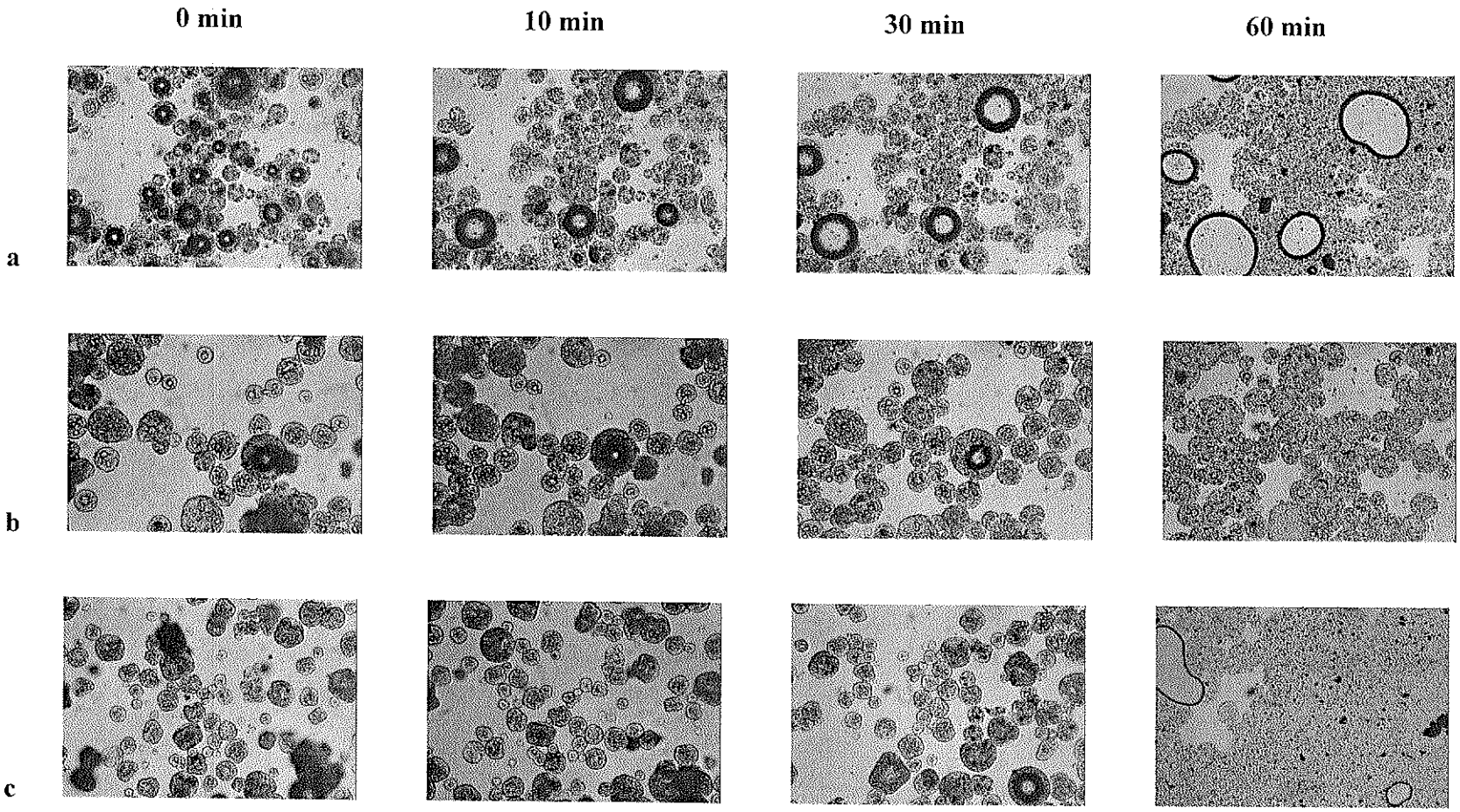

Figure 21. Comparison of the different water swelling release rate of beta-carotene microcapsules with different wall composition. a) native PPI wall at 0, 10, 30, and 60 min; b) heat-treated PPI wall at 0, 10, 30, and 60 min; c) 75% heat-treated PPI + 25% M040 wall at 0, 10, 30, and 60 min

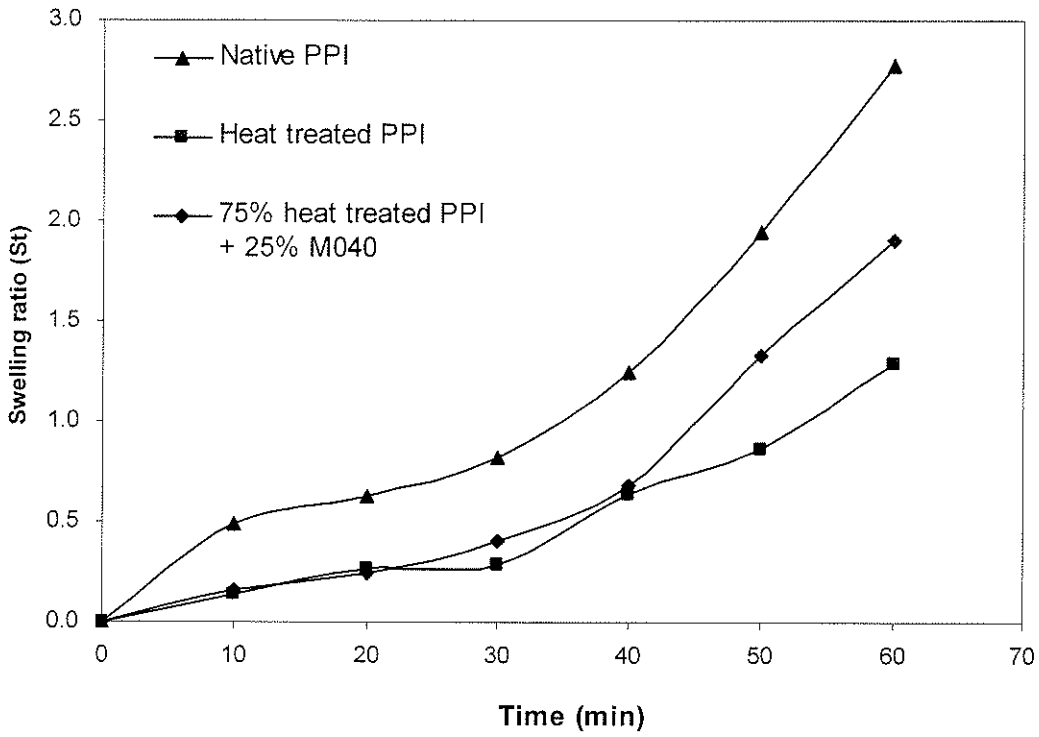

Figure 22. Swelling ratio curve of beta-carotene containing microcapsules with native PPI, heat-treated PPI, and 75% heat-treated PPI + 25% M040 wall system

Table 7 illustrates that different full release time and release rates could be obtained by heat treatment of PPI or by altering the wall composition, i.e. by changing the maltodextrin content in wall. The heat treated (denatured) PPI provides a slower swelling rate and longer release time than non-heated native PPI, possibly due to the formation of intermolecular disulfide bonds and lower water solubility after denaturation.

The swelling rate (k) (Table 7) was calculated from the regressing estimate of the slope by plotting the experimental data of swelling ratio (S_t) with time as shown in

Figure 23. For the three different wall systems, swelling followed first order kinetics with an initial slower first order reaction (before 40 min) followed by a second faster first order reaction period (after 40 min). For each wall system, the swelling rate increased substantially after 40 min in water, possibly due to the integrity and elasticity of wall became weaker at 40 min.

Table 7. The effect of different wall systems on full release time and swelling rate

Wall system	Full release time (min)	Swelling rate k (min^{-1}) before 40 min	Swelling rate k (min^{-1}) after 40 min
PPI (native)	50 ± 5	0.013	0.103
PPI (heat-treated)	90 ± 5	0.005	0.016
75% PPI (heat-treated) + 25% M040	60 ± 5	0.006	0.038

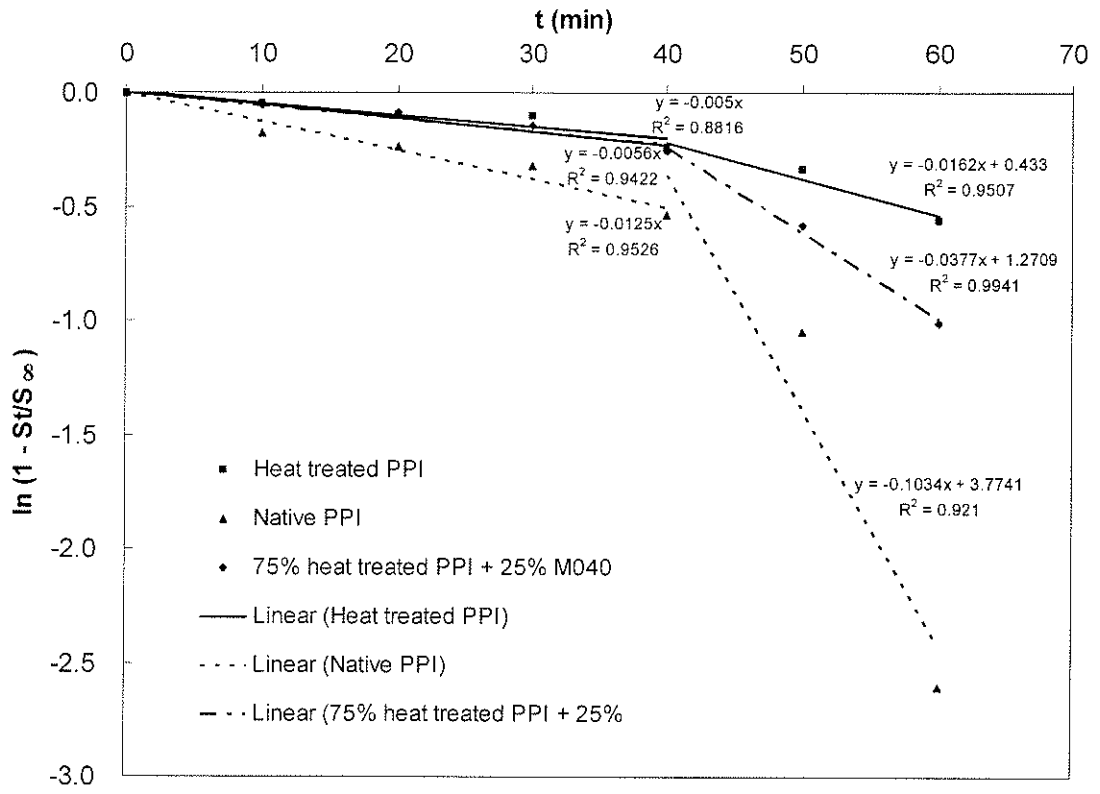

Figure 23. Determination of swelling rate (k) of different wall systems

4.8 Statistical Validation of the Effects of Wall to Core Ratio and Different Wall Composition on Microencapsulation

Experimental design is usually utilized for formulation optimization purposes. By this method, few experiments allowed the selection of the optimum conditions of product manufacture in a multivariable environment (Magenheim and Bentia, 1996). Major factors that affect the microcapsule properties, beta-carotene retention, and release rate include: nature and different composition of wall material; wall and core ratio; emulsion preparation condition; and spray drying condition. The composition of wall material and wall and core ratio was the focus of this study.

The effects of wall to core ratio and different wall composition on drying yield, moisture content, particle size, and beta-carotene retention of pea protein based beta-carotene containing microcapsules are summarized in Table 8 and 9.

Table 8 confirms that wall to core ratio had significant effects on drying yield, moisture content, particle size, and beta-carotene retention during storage of PPI based, beta-carotene containing microcapsules. Under the experimental conditions, by decreasing the wall to core ratio from 3 : 1 to 1 : 1, the drying yield increased from 54.0 to 72.4%, Moisture content decreased from 1.9 to 0.9%, particle size decreased from 21.9 to 19.4 μm, and beta-carotene retention at week 7 increased from 21.7 to 57.3%, respectively.

Table 8. Drying yield, moisture content, particle size, beta-carotene retention of pea protein based beta-carotene containing microcapsules with different wall to core ratio

Wall : core (w : w)	3 : 1	2 : 1	1 : 1
Drying yield (%)	54.0 ± 9.4a	66.4 ± 7.3ab	72.4 ± 4.3b
Moisture (%)	1.9 ± 0.3a	1.6 ± 0.5b	0.9 ± 0.2c
Particle size (μm)	21.8 ± 1.6a	18.4 ± 2.8b	19.4 ± 3.2b
Beta-carotene retention @ week 7 (%)	21.7 ± 12.3a	51.9 ± 14.8b	57.3 ± 10.8c

* Means with the same letter in the same row are not significantly different
* The particle size in diameter was measured by Method B

Table 9 confirms that wall composition (with different levels of maltodextrin content) had significant effects on particle size and beta-carotene retention during storage, but no significant effects on drying yield and moisture content of PPI based,

beta-carotene containing microcapsules. Under the experimental conditions, by increasing the M040 content in wall system from 0 to 50%, the drying yield decreased from 70.7 to 60.5%, particle size decreased from 21.1 to 17.8 μm, respectively.

Table 9. Drying yield, moisture content, particle size, beta-carotene retention of pea protein based beta-carotene containing microcapsules with different M040 content in wall composition

M040 content (w/w)	0 %	25%	50%
Drying yield (%)	70.7 ± 6.5a	61.6 ± 6.8a	60.5 ± 15.6a
Moisture (%)	1.4 ± 0.4a	1.5 ± 0.6a	1.6 ± 0.6a
Particle size (μm)	21.1 ± 2.9a	20.7 ± 1.7a	17.8 ± 2.9b
Beta-carotene retention @ week 7 (%)	42.1 ± 25.4a	43.9 ± 22.2ab	45.1 ± 13.2b

*Means with the same letter in the same row are not significantly different
* The particle size in diameter was measured by Method B

5.0. CONCLUSIONS

A pea protein based beta-carotene containing microencapsulation system was developed in this study. It can be concluded that:

1. Pea protein isolate (PPI) or PPI combined with maltodextrin (M040) are good microencapsulation agents. The application of pea protein as wall material for microencapsulation of nutraceuticals may exploit new utilization of pea protein as a value-added pea product.
2. The wall to core ratio has significant effects ($P<0.05$) on drying yield, particle size, moisture content, and beta-carotene retention of pea protein based beta-carotene containing microcapsules. The wall composition (with different levels of maltodextrin content in the wall system) had significant effects ($P<0.05$) on particle size and beta-carotene retention. The release rate of microencapsulated beta-carotene could be controlled by altering pea protein wall material through heat treatment or mixing with maltodextrin to change the wall composition.
3. Microencapsulation with pea protein isolate based wall material with or without maltodextrin provides efficient protection against oxidative degradation of the beta-carotene. The half time of beta-carotene oxidation was extended 8 weeks. The best beta-carotene retention was 80.9 % after 7 weeks using heat treated PPI as wall material.
4. A mathematic model of release kinetics was tested on pea protein based microcapsule system, and it can be used to determine the swelling release rate.

6.0. RECOMMENDATIONS

The following recommendations may be appropriate for further studies on pea protein based, beta-carotene containing microencapsulation, based on this current preliminary study:

1. Further optimizing the emulsification and microencapsulation conditions, such as drying conditions to improve the properties of microcapsules and retention of beta-carotene.
2. Optimizing the controlled release rate by manipulating the PPI based wall system in order to yield the desirable results required by functional food or nutraceutical applications.
3. Further determine the physical properties and microstructure characterization of the PPI based microcapsules, such as porosity and degree of integrity.
4. Further modeling mass transfer phenomena to determine the release rate mathematically and determine the mass transfer coefficient.

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