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Starch-Pectin Matrices for Encapsulation of Ascorbic Acid

Yiwei Liu
University of Nebraska-Lincoln, lll9876@msn.com

STARCH-PECTIN MATRICES FOR ENCAPSULATION OF ASCORBIC ACID

by

Yiwei Liu

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Yiwei Liu, M.S.

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Advisor: Wajira S. Ratnayake

Starch and pectin are two food-grade carbohydrates widely utilized in the food industry. Starch and pectin polymers have been investigated in encapsulating functional food ingredients and in pharmaceutical applications. Resistance to enzyme hydrolysis, differential solubilities, depending on the pH, and the ability to 'protect' unstable molecules are considered some of the beneficial properties of starch and pectin polymers, for encapsulation applications. Food ingredients could be delivered in a controlled manner to a specific target by encapsulating in micro-scale particles, *i. e.*, microencapsulation. Two studies were conducted to investigate the ability of selected starch-pectin blends in microencapsulating ascorbic acid (vitamin C) by spray-drying. The first study investigated the properties of heat-treated resistant starch and pectin based microparticles; blends of 50% amylose and 70% amylose, and type 4 resistant (RS 4) starches, were used with high methoxyl pectin at selected ratios. The second study investigated the properties of gelatinized regular starch and pectin based microparticles that were prepared by spray drying with a three-fluid nozzle, in encapsulating ascorbic acid. The type of starch, as well as starch-pectin ratio influenced both physical and functional properties of the microparticles.

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ABBREVIATIONS

50% Amy	50% amylose corn starch
70% Amy	70% amylose corn starch
AACC	American Association of Cereal Chemists
ACS	American Chemical Society
CRD	Completely randomized design
DSC	Differential scanning calorimetry
HM	High methoxyl
LM	Low methoxyl
HSD	Honestly significant difference
RS4	Type 4 resistant starch
SEM	Scanning electron microscopy
T_c	Conclusion temperature
T_o	Onset temperature
T_p	Peak temperature
ΔH	Transition enthalpy (DSC)

INTRODUCTION

Microencapsulation technologies have been first introduced to the food industry approximately over 50 years ago (Swisher 1957). Encapsulation is used in order to improve the quality of food products, and to develop novel functional foods (Desai and Park 2005b; Shahidi and Han 1993). Spray drying is the most widely used encapsulation technique in the food industry. The process is cost effective and efficient, and offers one-step continuous production of dry microparticles (Gharsallaoui et al. 2007).

Starches could be used as food grade wall materials for microencapsulation. Native starches have the advantages of low cost and being readily available (Zuidam and Nedović 2009). Resistant starches, which have resistance to enzymatic digestion, are frequently used in the encapsulation of dietary bioactives, primarily due to their ability to avoid degradation in upper gastrointestinal tract and provide prolonged and/or targeted release of active ingredients (Beneke et al. 2009; Topping et al. 2008). Granular starches can be considered micoparticles for the delivery of active ingredients, and heat-induced swelling of starch granules increases the encapsulation efficiency (Eden et al. 1989; Tomasik and Schilling 1998). Amylose, the linear polysaccharide component of starch, has the ability to form inclusion complex with certain guest molecules; a property that has been utilized for encapsulation, especially in the encapsulation of flavor compounds (Conde-Petit et al. 2006; Rutschmann et al. 1989).

Pectin is a plant based polysaccharide, which has long been used in the food industry. The strong film forming and binding abilities have made pectin an ideal wall material for encapsulation applications (Liu et al. 2007). Pectin is also an effective emulsion stabilizer that is desirable for spray drying (Drusch 2007). Pectin is not readily

digested by human digestive enzymes, and, therefore, is used to increase transit time or to obtain site specific delivery of bioactive ingredients (Wong et al. 2011). Pectin, with a degree of esterification (DE) greater than 50%, is known as high methoxyl (HM) pectin. HM pectin gels are stable at acidic (pH < 4.0) pH, but dissolve at pH 7.0 or above. HM pectin is often used in pH-dependent delivery systems, due to its pH-sensitive behavior (Liu et al. 2003).

Ascorbic acid, also known as vitamin C, is an important bioactive ingredient in maintaining good health (Nishikimi and Yagi 1991). It is also added to foods as an antioxidant, in order to protect product quality (Righetto and Netto 2006). Ascorbic acid is highly unstable, due to its high antioxidant activity and high water solubility (Steskova et al. 2006). Microencapsulation has been used to improve the stability of ascorbic acid (Uddin et al. 2001). Encapsulation of ascorbic acid as a functional dietary ingredient has drawn more attention in recent years (Desai and Park 2005a). Ascorbic acid absorption is most efficient in lower gastrointestinal tract (Malo and Wilson 2000), and therefore, pH-dependent delivery systems have been developed to promote its bioavailability, by utilizing the changes in pH in different gastrointestinal segments (Alishahi et al. 2011; Esposito et al. 2002).

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OBJECTIVES AND HYPOTHESES

Overall objective:

To develop and evaluate the properties of starch-pectin based matrices for encapsulation of ascorbic acid.

Specific objectives:

1. To create and evaluate the properties of microparticles using swollen resistant starches and high methoxyl pectin, for the encapsulation of ascorbic acid.

Hypothesis:

The type of resistant starch and/or the starch:pectin ratio influence the physicochemical and functional properties of microparticles.

2. To develop microparticles, from gelatinized regular starch and high methoxyl pectin, for encapsulation of ascorbic acid.

Hypothesis:

The ratio of regular corn starch and pectin influences the physicochemical and functional properties of microparticles.

CHAPER 1. LITERATURE REVIEW

1.1 Microencapsulation

Microencapsulation refers to a process of coating or entrapping solid, liquid, or gaseous materials into another material or system (Thies 2005). The capsules assembled are called microcapsules or microparticles, and have diameters between 1 and 1000 μm. The encapsulating material is usually described as the wall, shell, matrix, membrane, or carrier; the encapsulated material can be described as the core, fill, active agent, or nucleus. These terms are used interchangeably to identify wall and core materials, respectively. Microparticles may be present in various forms, including the simplest single wall-single core sphere, single wall with irregular shaped core, multi-wall, multi-core forms, and even matrix form, where the core material is distributed throughout the microparticle (Augustin et al. 2001; Gibbs et al. 1999; Gouin 2004; Risch 1995; Thies 2005). Although the first large scale commercial application of microencapsulation was on carbonless copy paper (Green 1957), the use of microencapsulation in the food industry emerged at almost the same time in the flavor industry (Swisher 1957).

Microencapsulation is a multi-disciplinary process, making it somewhat difficult to provide a complete description. However, microencapsulation applications in the food industry can be classified into several categories of objectives (Desai and Park 2005b; Dziezak 1988; Shahidi and Han 1993):

1. To reduce the direct contact of core material to undesired factors (*e.g.*, light, moisture, oxygen, and incompatible components) within the same product.

2. To mask the odor, color, or taste of the core material.

3. To produce a uniform dilution of the core material when only very small quantities are used, with large quantities of other material.

4. To obtain better handling of the core material, usually by altering its physical characteristics (*e.g.*, converting liquid into solid).

5. To control the release of the core material to:

a. prevent undesired migration within the product,

b. manipulate the rate of evaporation/transfer,

c. delay the release until the right stimulus or trigger is present/applied.

1.2 Microencapsulation techniques

Microencapsulation techniques frequently used for food applications include spray drying, spray chilling/cooling, coacervation, extrusion, fluidized bed coating, and liposome entrapment. The advantages and disadvantages of these techniques are summarized in Table 1.1. Selection of the proper technique is based on the physicochemical properties of the wall and core materials, as well as the desired functional properties of the microparticles (Augustin et al. 2001). Limitations on selecting encapsulation techniques are mainly cost issues and the lack of available food grade wall materials (Gibbs et al. 1999).

1.2.1 Spray drying

Spray drying is the most widely used method in the food industry, among the commonly used encapsulation techniques. Being one of the oldest microencapsulation methods, spray drying is both cost effective and efficient, and offers one-step continuous

production of dry powders. The ease of preparation and reproducibility also make spray drying a very good technique for encapsulation. The spray drying process involves preparation of a liquid feed (solution, emulsion, or suspension), atomization of the feed through a nozzle, and formation of dry particles by evaporation in the drying chamber. Operating conditions are critical in achieving optimal microencapsulation. Spray drying parameters including inlet temperature, feed flow rate, outlet temperature, and feed solid concentration are considered critical, and their effects vary with core material and wall material (Gharsallaoui et al. 2007; Masters 1979; Reineccius 1988; Rosenberg et al. 1990; Zbicinski et al. 2002).

1.3 Wall material selection

A major limitation in microencapsulation is the limited number of wall material choices available, especially for spray drying (Gouin 2004; Thies 2005). Commonly used wall materials in spray drying have been reviewed by Gharsallaoui *et al* (2007). The spray drying process requires a liquid feed, and therefore, wall materials generally need to have sufficient solubility, film forming ability, emulsifying ability, and low viscosities at high concentrations (Reineccius 1988; Sheu and Rosenberg 1998). The ability of wall material to form a fine and dense network during particle drying is also important (Matsuno and Adachi 1993). Estimation of activation energy, which is the energy required to evaporate a mass of moisture from the material during drying, has been reported to further distinguish the characteristics of wall materials (Pérez-Alonso et al. 2003).

Apart from the prerequisites of the spray drying process, selection of wall materials is carried out based on the characteristics of the core material and the expected properties of assembled microparticles. Economic considerations may also limit the selection of certain wall materials. Many published studies have made the selections of wall materials using empirical knowledge, and decisions on combinations and processing conditions are generally made on a trial and error basis (Zuidam and Nedović 2009). Wall materials are often used in combination, as there is hardly any situation that a single wall material alone meets all the requirements (Forssell 2004; Hogan et al. 2001). Adding materials with lower costs (*e.g.*, carbohydrates) into expensive materials (*e.g.*, gums) also reduces the final product cost (McNamee et al. 2001). Risks of toxicity can be minimized by using naturally occurring dietary polysaccharides (Wong et al. 2011), which is especially important for food applications.

1.3.1 Starch

Starch is the second most abundant polysaccharide in nature (Bastioli 2005). The two major components of starch are amylose, a linear macromolecule consisting of (1-4) linked α-glucopyranosyl units, and amylopectin, a larger branched macromolecule consisting of (1-4) linked α-glucopyranosyl with α(1-6) branch points. Regular starches contain 20~30% amylose and 70~80% amylopectin, while high amylose starches contain ≥ 50% amylose (BeMiller and Whistler 2009). Amylose, when extracted in hot aqueous solution, occurs in an unstable random coil form (Hayashi et al. 1981). In the absence of complexing agents, amylose molecules gradually retrograde as the solution is allowed to cool down, resulting in a double helix form of associated chains (Miles et al. 1984; Miles

et al. 1985; Morris 1990). In the presence of complexing molecules, however, amylose molecules tend to interact with guest molecules and form inclusion (single helical) complexes (Takeo et al. 1973). For example, the formation of the blue complex of amylose with iodine has been used for amylose quantification (Rundle et al. 1944). Helices of amylose inclusion complexes may have variable glucose units (usually from six to eight) per turn, depending on the size of cross section of the complexing agent (Jane 2009). Amylopectin is also able to retrograde or interact with guest molecules, such as lipids, but the complex formations are less effective and usually considered negligible. Only outer branches of amylopectin molecules are capable of participating in such interactions. Outer branches, on average, have the shortest chain length, many of them are not sufficiently long to form double helices (Eliasson 2006; Eliasson and Ljunger 1988; Gudmundsson and Eliasson 1990). Yet extensive branching of amylopectin offers very high binding capacities when guest molecules are present at high concentrations (Rutschmann and Solms 1990).

Native starches have the advantages of low cost, low viscosities at high concentrations, ease of drying, and being comparatively readily available (Kenyon 1995; Zuidam and Nedović 2009). Yet native starches lack surface active properties that are required to provide film forming ability and cohesiveness in the microencapsulation process (Gennadios 2002; Loh and Hubbard 2002), so they are often modified by methods such as cross-linking and oxidization (Wurzburg 1986), or used with emulsion stabilizers such as proteins and gums (Gharsallaoui et al. 2007; Young et al. 1993). Starches with resistance to enzymatic digestion are also used in encapsulating dietary bioactives, considering their potential to prevent degradation in the upper gastrointestinal

tract and to provide prolonged or targeted release (Beneke et al. 2009; Topping et al. 2008). Resistant starches (RS) frequently used in microencapsulation include high amylose starches and chemically modified starches (*i.e.*, type 4 resistant starch, 'RS4') (Bie et al. 2010; Desai and Park 2005a; Dimantov et al. 2004; Fang et al. 2008; Levy and Andry 1990).

Starches could be used as encapsulating wall material in a granular state, when core materials are capable of interacting with starches through sorption onto the granule surface or into intergranular spaces (capillaries) (Tomasik and Schilling 1998). Swelling of starch granules by heat treatment increases the encapsulation efficiency using a sorption mechanism (Eden et al. 1989), and the swelling can be carried out under mild conditions (Korus et al. 2003). Self-assembly of microcapsules has been reported to occur between pregelatinized starch granules and pesticides when they were blended with enough water and allowed to agglomerate (Trimnell and Shasha 1988). The amylose content of native starches is believed to be a major contributor in reducing the release rate of encapsulated active materials (Wing et al. 1988).

Inclusion complexation has also been extensively utilized for encapsulation. In a typical inclusion complex, the guest molecule, which is called a ligand, binds non-covalently into the helical cavity of an amylose chain (Rutschmann et al. 1989). Many flavor compounds are well studied ligands that complex readily with amylose (Conde-Petit et al. 2006). Amylose complexes can thus be used for controlled release of volatile flavors, and the stability of encapsulated flavors has been suggested to increase with the chain length of amylose (Wulff et al. 2005). Since complexed amylose is less susceptible to enzymatic digestion compared to its uncomplexed form, it has the potential for

controlled delivery in the gastrointestinal tract (Hanna and Lelievre 1975; Putseys et al. 2010). Encapsulation based on inclusion complexes, however, is often limited to batch processing (Lesmes et al. 2008).

1.3.2 Pectin

Pectin refers to a group of complex polysaccharides located in plant cell walls that provide rigidity and adhesion of cells (Van Buren 1991). They are essentially polymers of both α(1-4) linked D-galacturonic acid units (homogalacturonan regions) and alternating α(1-4) linked D-galacturonic acid and α(1-2) linked rhamnose units (rhamnogalacturonan regions) (Thakur et al. 1997). Pectin molecules usually contain branched (hairy), heavily methyl-esterified blocks, and unbranched (smooth), non-esterified blocks (Jarvis 1984). Pectins are classified according to their degree of esterification (DE), which is defined as the percent of methoxylated carboxyl groups (Van Buren 1991). Pectins with DE > 50% are high methoxyl (HM) pectins, while those with DE < 50% are low methoxyl (LM) pectins (BeMiller 1986). HM and LM pectins form gels with different mechanisms, which is their major difference in functionality (Sriamornsak 2003). HM pectin form gels through hydrogen bonding between free carboxyl groups and hydrophobic interactions between methyl esters (Oakenfull 1991). Therefore, the gelling of HM pectin requires an acidic pH. LM pectins gel in the presence of divalent cations (*e.g.*, Ca^{2+}) that act as bridges between pairs of carboxyl groups. This mechanism is described as the “egg box” model (Grant et al. 1973). When pH increases over neutral values, both demethylation and β-elimination occur, leading to pectin degradation. HM pectins are relatively more

sensitive to high pH, and lose gel stability more rapidly (Renard and Thibault 1996; Rolin 1993).

Pectin has properties desirable for microencapsulation by spray drying, especially producing stable emulsions at low concentrations (Drusch 2007). This is critical for encapsulation of hydrophobic ingredients (Ré 1998). Due to the relatively lower cost, pectin can be used as a substitute for expensive wall materials, such as proteins and gum Arabic (Drusch 2007). The gelling ability of pectin makes it an ideal agent in making biodegradable hydrogel beads, films and coatings for microencapsulation purposes (Burey et al. 2008; Humblet-Hua et al. 2011; Liu et al. 2007; Tharanathan 2003).

HM pectin has been frequently used in pH-dependent delivery systems, since pH variations have significant impact on HM pectin stability and hydration rate (Liu et al. 2003; Yao et al. 1996). HM pectins have poor water solubility at pH < 4.0, but dissolve at pH 7.0 or above (Jain et al. 2009). Mura et al. (2003) compared the solubilities of HM pectin, LM pectin and amidated LM pectin, at acidic pH (1.1), and reported HM pectin to be the least water soluble. Known as a dietary fiber, pectins are not digested by human digestive enzymes, so they have been used to increase transit time or to obtain site specific delivery of sensitive ingredients (Wong et al. 2011).

1.4 Release mechanism

Delivery systems are designed to enable controlled release of the core material, in addition to providing protection. That means that the release is started or greatly enhanced once a “trigger” is applied. Various types of release triggers, including temperature, pH, mechanical force, osmosis, enzyme, and microbial fermentation, have

been investigated and adopted, but the most common release activation mechanism in the food industry is solvent activated release (also termed hydration for water soluble ingredients), which is often accompanied by other release triggers (Pegg and Shahidi 2007). There is an increasing trend of developing pH-sensitive delivery systems, based on carbohydrates, as many polysaccharides are polyelectrolytes and exhibit pH-responsive swelling (Liu 2008).

1.5 Ascorbic acid

Ascorbic acid, also known as vitamin C, is an important food ingredient added for mainly two reasons: 1) as a dietary supplement or nutrient for health benefits; 2) as an antioxidant to protect food quality (Ashurst 2005; Nishikimi and Yagi 1991; Righetto and Netto 2006). Ascorbic acid is occasionally used as an acidulant, but it is less effective than other organic acids (Furia 1972). A certain level of ascorbic acid intake is important to maintain good health; as it is essential for bone collagen formation, immune system modulation, and as an antioxidant. Ascorbic acid has been suggested to reduce risks of cardiovascular disease, atherosclerosis, type 2 diabetes, and many types of cancer (Block 1991; Padh 1991; Wintergerst et al. 2006).

The highly unstable nature of ascorbic acid limits its use in certain food applications. The antioxidant activity of ascorbic acid renders it easily degraded into inactive forms during processing and storage. It is even used to indicate severity of food processing conditions. Ascorbic acid is prone to leaching in the presence of moisture, due to its high water solubility. Leaching is a major cause of vitamin C activity loss in processed foods (Ghosh et al. 2012; Steskova et al. 2006; Yuan and Chen 1998).

Studies on microencapsulation of ascorbic acid have been reported since the early 2000s, predominantly by means of spray drying (Alishahi et al. 2011b; Desai and Park 2005a; Esposito et al. 2002; Finotelli and Rocha-Leão 2005; Trindade and Grosso 2000; Uddin et al. 2001; Wijaya et al. 2011). Analysis results have suggested possible applications in taste masking, increasing retention during storage, and improving bioavailability. Encapsulation of ascorbic acid for purposes of dietary supplementation is gaining more attention (Desai and Park 2005b; Schrooyen et al. 2001).

By avoiding losses of ascorbic acid in the upper gastrointestinal tract, especially in the stomach, its bioavailability can be greatly improved, since ascorbic acid absorption is most efficient in the distal ileum (Malo and Wilson 2000; Rock et al. 1996). The variation in pH value in different gastrointestinal segments has been exploited for targeted delivery, and pH-sensitive microparticles have been created (Sinha and Kumria 2001). Esposito et al. (2002) have encapsulated ascorbic acid into Eudragit, a synthetic pH-sensitive polymer, which remains insoluble at acidic pH, but dissolves at pH 6.0 or above. Alishahi et al. (2011a) developed an ascorbic acid delivery system based on chitosan. Chitosan exhibits pH-responsive swelling, which protects the encapsulated ascorbic acid at low pH, but promotes release of ascorbic acid at physiological pH (7.4).

1.6 Physicochemical characterization of microparticles

Characterization of microparticles, *i.e.* analysis of physicochemical and functional properties, is critical in understanding behaviors of microparticles under required conditions (Zhang et al. 2010). It is also used to evaluate the delivery system and processing parameters.

1.6.1 Particle size distribution

The average size and uniformity (polydispersity) of microparticles have a critical impact on release kinetics of encapsulated ingredients, as microparticles with the same size tend to have the same release rate (Ramos 2011; Rhine et al. 1980; Wilkins 1999). The size of microparticles influences release behaviors of core materials through basically two mechanisms, according to Berkland et al. (2004): 1) Release rates increase with decreasing particle size, due to the increased surface area to volume ratio. 2) Smaller microparticles harden faster during particle formation, and thus they trap quickly highly water soluble core materials, which tend to migrate outward during particle formation. Smaller microparticles, therefore, have a more uniform core material distribution, leading to slower release rates. One of the most commonly used particle sizing methods is laser diffraction. Laser diffraction is highly efficient and repeatable compared to other methods. Besides, a volume size distribution can be generated directly, which is preferred in many industrial applications (Merkus 2009).

1.6.2 Morphology of microparticles

A specific particle morphological feature (shape, internal structure, surface porosity, *etc*.) is often preferred for a specific encapsulation application (Mittal 2013). Impacts of processing are also revealed by changes in microparticle morphology (Yang et al. 2001). Morphology of microparticles has a direct impact on the release behavior of encapsulated ingredients. Klose et al. (2006) reported that the release rates of bioactive compounds from porous poly(lactic-co-glycolic acid) (PLGA) microparticles were higher than smooth particles with the same size, as the increasing porosity increased core

material mobility. High porosity can lead to initial burst release, which is undesirable for controlled delivery systems (Yeo and Park 2004). Optical and electron microscopy are both frequently used in characterizing microencapsulation products. Microscopy provides information on sample morphology as well as general size distribution. SEM is usually used to observe the intricate details of surface morphology, such as pores and indentations, and internal structures by viewing cross-sections (Zhang et al. 2010). Optical microscopy is often used for dynamic observations such as study of colloidal microsphere swelling (Crocker and Grier 1996).

1.6.3 Encapsulation efficiency

Encapsulation efficiency, also known as loading efficiency, is a numerical measure of the amount of incorporated core material in microparticles, which is generally expressed as the percentage of core material encapsulated relative to the amount of initially added core material (Liu et al. 2008). Encapsulation efficiency is one of the critical properties of microencapsulation that have important influence on subsequent applications. Microparticles with high encapsulation efficiencies are often desired as less carrier materials are required, and less net amount of encapsulated products could deliver the required amount of core material (Lu et al. 2011). Steps for determining ascorbic acid encapsulation efficiency include completely dissolving microparticles, determining ascorbic acid concentration in the solution, and comparing with the calculated theoretical ascorbic acid concentration. The dissolution methods vary with wall material composition of microparticles. Most determinations of ascorbic acid concentration rely on either HPLC with UV detection (Alishahi et al. 2011b; Liu and Park 2010) or direct

spectrophotometry at a wavelength of 265nm (Desai and Park 2005a; Marsanasco et al. 2011).

1.6.4 *In vitro* release profile

The *In vitro* release test is one of the most important analyses to assure the functionality of an encapsulated ingredient (Wise 2000). The release test provides an estimate of the behavior of microparticles in actual applications, by using similar environmental conditions (Rathbone and Butler 2011). Another important goal of the release test is to evaluate the sensitivity of the designed release mechanism; release tests under different conditions are compared. Results of release tests are commonly known as release profiles, where cumulative concentration or percentage release of the core ingredient is plotted against time, and, based on such profiles, decisions are made on whether the release pattern meets the expectation or not (Zhang et al. 2010).

1.7 Conclusions

Microencapsulation by spray drying has become an important technique in developing novel applications of bioactive ingredients, with the increasing interest in functional food products. Polysaccharides are especially preferred in dietary controlled delivery systems, among which starches and pectin have gained much attention for being versatile wall materials and possessing potential health benefits. Microencapsulation of ascorbic acid, a water soluble vitamin, is a relatively new area, compared to encapsulation of lipophilic ingredients. The sensitive nature of ascorbic acid makes the control of its release critical in achieving high bioavailability. Characterization of

microparticles is necessary to obtain information on the physicochemical and functional properties of microparticles, in order to evaluate the design of the delivery system and predict end-user applications. Despite the fact that establishing a polysaccharide delivery system for ascorbic acid is challenging, it is presumable from published studies that controlled release of ascorbic acid could be accomplished by developing a carbohydrate polymer based matrix particle system.

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Table 1.1. Comparison of microencapsulation techniques.

Technique	Advantage	Disadvantage	Reference
Spray drying	Cost effective and well established	Limited suitable wall materials	(Gouin 2004)
Spray chilling/cooling	Inexpensive	Special handling/storage conditions required	(Taylor 1983)
Coacervation	High encapsulation capacity	Expensive	(Gouin 2004)
Extrusion	High retention and stability	Limited suitable wall materials	
Fluidized bed	Wide range of suitable wall materials	Only apply to solid particle coating	(Augustin et al. 2001)
Liposome entrapment	Stable at high water activity	Difficult to scale up	(Gouin 2004)

CHAPER 2. ENCAPSULATION OF ASCORBIC ACID IN HEAT TREATED RESISTANT STARCH-PECTIN BASED MICROPARTICLES

Abstract

Microparticles were prepared, using granular starch and pectin, for encapsulation of ascorbic acid. Three types of starch, 50% amylose corn starch, 70% amylose corn starch and type 4 resistant starch (RS4) were used in combination with high methoxyl pectin at selected ratios (2:1, 1:1, and 1:2). Microparticles were prepared by spray drying using a two-fluid nozzle. Particle size distributions were influenced by starch-pectin ratio. The largest particles were obtained with the highest starch ratio (2:1). Scanning electron microscopy showed all microparticles with similar surface indentations. The lowest encapsulation efficiencies were obtained with a starch-pectin ratio of 2:1, while there were no significant differences among different starches when starch-pectin ratios were the same. Ascorbic acid release profiles at pH 1.2 and 7.0 revealed pH-dependent behavior of the microparticles. Ascorbic acid release was influenced by both the type of starch and starch-pectin ratio.

2.1 Introduction

Granular starches have been used in the food industry for controlled release of flavor compounds (Madene et al. 2006). Boutboul et al. (2002) studied the retention of four aroma compounds (1-hexanol, octanal, ethyl hexanoate and *d*-limonene) in native and modified corn starches. The results showed increased retention with increased polarity of the aroma compound (*i.e.*, 1-hexanol > octanal > ethyl hexanoate > *d*-

limonene), regardless of the starch type. Pre-treatments, such as swelling the starch granules, facilitate increased encapsulation capacities in starch based delivery systems. Swollen starch granules can be considered microparticles for controlled delivery (Lii et al. 2003). Korus et al. (2003) obtained up to 30% (w/w) retention of fragrance molecules in swollen potato starches; whereas untreated starches yielded less than 10% (w/w) retention (Polaczek et al. 2000). Formation of complexes also takes place during starch based microencapsulation. The molecules of active ingredients bind non-covalently into the helical cavity of amylose, forming inclusion complexes (Rutschmann et al. 1989). According to Wulff et al. (2005), amylose-flavor complexation was successful in encapsulating low boiling point flavors, as the encapsulated flavors were well retained in normal dry foods stored at room temperature. Ades et al. (2012) tested the encapsulation ability of corn starches with various amylose contents, using menthone, menthol, and limonene, and reported increased complexation efficiency (low levels of free aroma) with increased amylose content. Starches are also known to trap guest molecules through sorption onto the granule surface or into intergranular spaces, which have been utilized for delivering bioactive ingredients (Buttery et al. 1999; Tomasik and Schilling 1998).

The spray drying encapsulation process requires uniform, homogeneous liquid feed (solution, dispersion, or emulsion) (Cai and Corke 2000; Ré 1998). A minimum amount of surface active agent is usually used to reduce surface tension of emulsion droplets, and form a homogeneous mixture (Etchells and Meyer 2004; Langevin 2006). Native starches, as well as many starch derivatives, lack surface active properties, which can be improved by combining with emulsion stabilizers, such as hydrocolloid polysaccharides. Blends of gum Arabic and maltodextrin have been successfully used in

spray drying of flavors, such as cardamom oleoresin and ethyl butyrate (Krishnan et al. 2005; Yoshii et al. 2001). The presence of emulsifiers allows spray drying with higher solids levels compared to using low surface-activity carbohydrates alone (Young et al. 1993). Zhao and Whistler (1994) reported a simple method for creating starch carriers for flavor oil: Raw starch granules were immersed in peppermint oil, and then coated with polysaccharide binding agents, resulting in a final product with up to 48% encapsulation efficiency.

Pectins are effective emulsifiers, at low concentrations, due to their strong gelling abilities. Formation of pectin gel networks stabilizes the aqueous phase, by increasing the viscosity (Drusch 2007; Paraskevopoulou and Kiosseoglou 2013; Thakur et al. 1997). Therefore, pectin is desired as a wall matrix component when a homogenous liquid mixture of wall matrix is required (Gharsallaoui et al. 2007). The film forming abilities of pectins are exploited in producing coatings for encapsulation purposes (Humblet-Hua et al. 2011; Liu et al. 2007; Tharanathan 2003).

Pectins with a degree of esterification (DE) higher than 50% are considered high methoxyl pectins (BeMiller 1986). High methoxyl pectin requires an acidic pH for gelling and loses its stability at neutral or higher pH, which makes it a natural pH-sensitive polymer (Renard and Thibault 1996; Rolin 1993).

This study was conducted to evaluate a delivery system, prepared with granular starch and pectin blends as matrix material, for microencapsulation of ascorbic acid. Microparticles were created by spray drying with a two-fluid nozzle. Selected types of resistant starches, 50% and 70% amylose corn starches, and type 4 resistant starch (RS4), were used with high methoxyl pectin at starch-pectin ratios of 2:1, 1:1, and 1:2, in order

to investigate the effect of starch-pectin composition on the properties of the delivery system.

2.2 Materials and methods

2.2.1 Materials

High methoxyl pectin (TIC Pretested® Pectin 1400) was obtained from TIC Gums (White Marsh, MD, USA). 50% amylose corn starch (AmyloGel™ 03001, Cargill Corn Milling, Cedar Rapids, IA, USA), 70% amylose corn starch (Hylon® VII, National Starch, Bridgewater, NJ, USA), and type 4 resistant starch (RS4) (Fibersym® RW, MGP Ingredients, Atchison, KS, USA) were obtained from commercial sources. Ascorbic acid (NOW Foods, Bloomingdale, IL, USA) was purchased from a commercial source. All other chemicals and solvents used for the experiments were of ACS certified grade.

2.2.2 Thermal properties of raw starches

Thermal properties of raw starches (50% amylose, 70% amylose, and RS4 starches) were determined using differential scanning calorimetry (DSC) as outlined by Ratnayake et al. (2009). Approximately 10 mg of a starch sample was hermetically sealed with excess distilled water (55 μL) in a DSC pan, and equilibrated at room temperature for 2 hours. The sample was then scanned against a blank (empty pan) using a Perkin Elmer Pyris 1 DSC system (Perkin-Elmer Co., Norwalk, CT) from 25°C to 135°C at a scanning rate of 10°C/min. The onset (T_o), peak (T_p), and conclusion (T_c) temperatures were collected and analyzed with the Pyris software (Version 3.50, Perkin-Elmer Co.). The instrument was calibrated using an indium reference.

2.2.3 Swelling factor of raw starches

Swelling factors of raw starches (50% amylose, 70% amylose, and RS4 starches) were determined using a blue dextran dye exclusion method (Tester and Morrison 1990). A starch sample (0.200 g) and 5 mL distilled water were added to a 15 mL centrifuge tube and incubated in a shaking water bath (Model 2876, Thermo Scientific, Marietta, OH, USA), at predetermined temperatures for 30 minutes with constant shaking at 60 rpm. The tube was then cooled to 20°C in an ice bath, and 0.5 mL blue dextran solution (5 mg/mL) was added and mixed by inverting the tube 10 times manually. The tube was then centrifuged at 1,500 g for 5 minutes at 20°C, in a Sorvall Legend XTR centrifuge (Thermo Scientific, Osterode, Germany). The absorbance of the supernatant (A_S), at 620 nm, was measured in a BioMate 3S UV-Vis spectrophotometer (Fisher Scientific, Madison, WI, USA). Control samples, without starches, were prepared and treated in an identical manner, and absorbances of control supernatants (A_C) at 620 nm were measured. Moisture contents of starch samples were determined using AACC Approved Method 44-15A (AACC 2000). Swelling factors (SF) of starch samples were calculated using the equation below:

$$SF = 1 + \{(7{,}700/W)[(A_S - A_C)/A_S \quad \text{Equation (2.1)}$$

Where W is the starch weight (g, dry basis).

2.2.4 Preparation of microparticles

2.2.4.1 Preparation of feed solutions

Three starches, 50% amylose corn starch, 70% amylose corn starch, and RS4, were used; each starch was used in combination with high methoxyl pectin at starch-

pectin ratios of 2:1, 1:1, and 1:2. The wall matrix solution was prepared with 20g (dry basis) of starch-pectin blend and 1L of distilled water, while maintaining a total 2% (w/w, wet basis) concentration of starch and pectin. Starch was added and dispersed in distilled water first, the heated to a temperature 5°C below the T_o of each starch. The dispersion was kept at that temperature for 30 minutes in a water bath, and cooled to room temperature in ice bath. Pectin was then added and the mixture was homogenized at 10,000 rpm for 2 minutes, using a VirTishear homogenizer (Model 225318, The Virtis Company, Inc., Gardiner, NY, USA). Then 5.00 g of ascorbic acid was added and the mixture was further homogenized at 10,000 rpm for 3 minutes.

2.2.4.2 Spray drying with two-fluid nozzle

Microparticles were prepared using a bench top mini spray dryer (B-290, Buchi Labortechnik AG, Switzerland), equipped with a two-fluid (gas/liquid) 0.7 mm nozzle (Model 044698). The two-fluid nozzle utilizes high-velocity compressed air to atomize the liquid feed, and is able to atomize feeds with high viscosities (Cal and Sollohub 2010). Spray drying parameters were set as follows: Inlet temperature 105°C, aspirator rate 85%, feed flow rate 4.5 mL/min, and nozzle cleaner level 5. Microparticles were collected and stored at -20°C until analysis.

2.2.5 Analysis of physical properties of microparticles

2.2.5.1 Particle size distribution

Particle size analyses were performed using a Malvern Mastersizer 3000 laser diffraction particle size analyzer, equipped with an Aero S dry powder disperser

(Malvern instruments Ltd, Malvern, Worcestershire, UK). Microparticles were delivered into the disperser cell within the obscuration limit of 0.1 to 20%. Measurement parameters used were: Refractive index of 1.53, density of 1.5 g/cm^3, absorption index of 0.10, air pressure 1 bar, and feed rate of 50%. Data were collected and analyzed using Malvern software (Version 2.20, Malvern instruments Ltd, Malvern, UK).

2.2.5.2 Surface morphology

The morphologies of dry microparticles were observed using scanning electron microscopy, as described by Ratnayake and Jackson (2007). Microparticles were mounted on metal stubs and coated with a gold-palladium alloy using a Hummer sputter coating system (Anatech Ltd., Union City, CA, USA). Coated microparticles were observed with a Hitachi S-3000N variable pressure scanning electron microscope (Hitachi Science Systems, Tokyo, Japan) at an acceleration potential of 25kV. Pictures were recorded by an image capturing software (Version 10-16-2266, Hitachi High-Technologies, Pleasanton, CA, USA). Spray dried heat treated granules of the three starches were also observed in an identical manner for comparison.

2.2.6 Evaluation of functional properties of microparticles

2.2.6.1 Encapsulation efficiency

Microparticles (0.400 g) were weighed into a clean 50 mL plastic centrifuge tube. Microparticles were washed by adding 10 mL of ethanol, then manually inverting the tube 10 times. The tube was then centrifuged at 1,500g for 5 minutes at 20°C, and the supernatant was discarded. The remaining microparticles were mixed with 40 mL of

phosphate buffer (pH 6.0), and the mixture was sonicated for 1 hour, to ensure complete dispersion, using a Vibra-Cell VC300 sonicator (Sonics & Materials, Inc., Newtown, CT, USA) at output 10 and duty cycle 50%. The supernatant was recovered by centrifuging at 3,000 g for 15 minutes at 20°C.

The concentration of ascorbic acid was determined using a colorimetric method (Jagota and Dani 1982) as follows: An aliquot (1 mL) of supernatant was first diluted to 25 mL, and then 1 mL of diluted supernatant and 1 mL of 0.2 N Folin-Ciocalteu reagent were mixed and diluted to 10 mL. The dilution was held for 30 minutes for color development. Absorbance of developed color at 760 nm was measured in a BioMate 3S UV-Vis spectrophotometer (Fisher Scientific, Madison, WI, USA), using plastic cuvettes. Concentration of ascorbic acid was then determined against a standard curve prepared with a serial dilution of ascorbic acid. Encapsulation efficiency was calculated according to the equation below (Desai and Park 2005):

$$\text{Encapsulation efficiency} = \frac{A}{A_0} \times 100\% \quad \text{Equation (2.2)}$$

Where A is measured ascorbic acid concentration (μg/mL); A_0 is calculated theoretical ascorbic acid concentration (μg/mL).

2.2.6.2 *In vitro* release profiles

The *in vitro* release tests of microparticles were performed under selected conditions in chloride buffer (pH 1.2) and phosphate buffered saline (pH 7.0). Eleven 15 mL centrifuge tubes, each containing 0.100 g microparticles and 10 mL buffer, were set on a multi-tube rotator (Model 4632Q, Thermo Scientific, Waltham, MA, USA). One tube was analyzed at 30 minute intervals during the first 5 hours, then one tube was

analyzed at 7 hours. The supernatant was recovered by centrifuging at 3,000g for 15 minutes at 20°C, and the concentration of released ascorbic acid was determined using the same colorimetric procedure described above.

2.2.7 Statistical analysis

The study was conducted using a completely randomized design (CRD). Formulations of wall matrices are shown in Table 2.1. Microparticles of each combination were prepared in triplicate. Analyses of variance were performed and mean separations were performed by the Tukey-Kramer HSD test at $p < 0.05$ significance level using JMP software (Version 10.0.2, SAS Institute Inc., Cary, NC, USA).

2.3 Results and discussion

2.3.1 Properties of raw starches

2.3.1.1 Thermal properties

Phase transition parameters, onset (T_o), peak (T_p), and conclusion (T_c) temperatures, range, and enthalpy (ΔH) of the three starches are shown in Table 2.2. Previous studies have shown that swelling of starch granules is accompanied by leaching of polysaccharides, especially amylose – the linear polymer (Tester and Morrison 1990). Swelling increases as the temperature approaches T_o. The molecules leached below T_o are mainly low molecular weight α-glucans; leaching of large molecular weight amylopectin occurs after the temperature reaches T_o (Banks et al. 1959; Tester and Morrison 1990). Yeh and Li (1996) reported that loss of starch granular integrity starts mainly from T_o. Treatments at 5°C below T_o have been reported to produce the same

swelling effects as T_o (Li and Yeh 2001; Tester and Morrison 1990). Therefore, temperatures approximately 5°C below the onset temperatures of each starch were used for the swelling treatment, in order to retain structural integrity of starch granules.

2.3.1.2 Swelling factor

The swelling factor analysis method uses blue dextran dye exclusion to indirectly estimate the degree of starch granule swelling. Blue dextran is a high molecular weight (2,000,000) polysaccharide that cannot penetrate swollen starch granules. The concentration of blue dextran in the solution increases, as water is absorbed by swelling of starch granules. Absorption of water increases the absorbance of blue dextran solution at 620nm. The difference in absorbance, therefore, can be used to calculate the degree of starch swelling. Swelling factors of the three starches at treatment temperatures are shown in Table 2.3. 50% amylose starch had a higher swelling factor than 70% amylose starch, despite their treatment at the same temperature. Previous studies have shown an inverse relationship of degree of swelling and amylose content for starches from the same botanical source (Sasaki and Matsuki 1998; Zavareze et al. 2010). Hydrogen bonds between double helices of glucans, which stabilize starch structure, are disrupted during swelling and replaced by hydrogen bonds with water. Amylose, with a higher proportion of longer chains, forms longer double helices that require higher energy to break, compared to amylopectin. Therefore, an increase in amylose content reduces the ease of granular swelling (Tester and Karkalas 1996; Yuan et al. 1993). The higher amylose content of 70% amylose starch resulted in a lower degree of swelling, compared to 50% amylose starch. The RS4 used in this study (Fibersym® RW) is a cross-linked wheat

starch. The cross-linking treatment restricts the swelling of starch granules, by stabilizing amylose and amylopectin (Jane et al. 1992; Liu et al. 1999). Therefore, RS4 had a lower swelling factor (6.62), compared to an unmodified wheat starch treated at the same temperature (~ 8 at 71°C) (Tester and Morrison 1990).

2.3.2 Physical properties of microparticles

2.3.2.1 Particle size distributions

Microparticles prepared with 50% and 70% amylose starches displayed size distributions with a single peak, while microparticles prepared with RS4 displayed bi-modal distributions (Figure 2.1). Volume weighted size distribution percentiles of microparticles are given in Table 2.4. Microparticles with the highest starch proportion (2:1) had the largest particle sizes, although differences were not significant for microparticles containing 70% amylose starch. Size distribution peaks of microparticles prepared with 50% and 70% amylose starches overlapped with the size distribution peaks of raw 50% and 70% amylose starches, respectively; the distribution peaks with larger size of microparticles prepared with RS4 overlapped with the distribution peak of raw RS4 (data not shown). Therefore, most of the resistant starch granules could have retained the granular integrity after processing. Finotelli and Rocha-Leão (2005) reported similar observations with spray dried microparticles containing starch and maltodextrin; the largest microparticles were produced using the highest starch proportion. Studies have shown that high amylose starches are more resistant to mechanical damage than regular and waxy starches, due to the higher crystallinity (Bettge et al. 2000; Morrison et al. 1994). The cross-linking process increases the granular hardness of starch (Liu et al.

1999). Therefore, RS4 has higher hardness compared to native wheat starch. Pectin, once dispersed into aqueous solution (≤ 2% w/w, wet basis), can be readily atomized into a fine mist (Chen et al. 2005). The resistant starches used in this study could have remained in a granular state after spray drying, which resulted in a larger particle size with higher starch proportion in the wall matrix.

2.3.2.2 Surface morphology

All prepared microparticles displayed similar surface indentations (Figure 2.2). Desai and Park (2005) reported similar surface indentations on spray dried microparticles of 70% amylose corn starch and pectin. Spray dried microparticles made with pectin and maltodextrin (Sansone et al. 2011) and with pectin and pea protein (Aberkane et al. 2013) also displayed similar surface indentations. However, spray dried microparticles produced from a pectin-glucose blend had only slight surface wrinkles (Drusch 2007), and microparticles made using solely pectin, had a spherical shape and smooth surface (Lee et al. 2004). Solutions of large polymers, such as maltodextrin and proteins, have much lower water diffusivity than simple sugars (glucose). Droplets made by low water diffusivity polymer matrices tend to shrink during fast drying, in order to increase the surface available for moisture evaporation, and reduce the diffusion path (Adhikari et al. 2003; Adhikari et al. 2002). Fu et al. (2012) reported that spray drying of swollen starch granules resulted in considerable volume shrinkage, leading to indentations on the surface. The starches, pre-treated with temperatures below T_o (Fu et al. 2012; Figure 2.2 – 1, 2, and 3), showed similar surface indentations after spray drying. Therefore, the

distinct surface indentations could have been created primarily by rapid evaporation of moisture from swollen starch granules during spray drying.

2.3.3 Functional properties of microparticles

2.3.3.1 Encapsulation efficiency

Encapsulation efficiency measures the percentage of core material loaded in microparticles, and is an important property of microencapsulated particles, as higher encapsulation efficiency means less wall material is required for processing microparticles (Liu et al. 2008; Lu et al. 2011). Microparticles with high starch proportions had correspondingly low encapsulation efficiencies, and *vice-versa*, regardless of the starch type used (Table 2.5). No significant difference ($p > 0.05$) in encapsulation efficiency was found among three starches, when the starch-pectin ratios were the same. The results suggested that the starch-pectin ratio, rather than the starch type, influenced ascorbic acid encapsulation efficiency. According to Sansone et al. (2011), a pectin concentration lower than 1% in the feed solution is unable to form well coated droplets, resulting in the loss of core material during spray drying. The pectin concentration in the feed solution with starch-pectin ratio of 2:1 was less than 1% (Table 2.1), which could explain the lowest ascorbic acid retention after spray drying.

2.3.3.2 *In vitro* release profiles

Ascorbic acid release profiles of prepared microparticles, under pH 1.2 and 7.0, are shown in Figure 2.3. All microparticles had lower ascorbic acid release after 3.5 hours at pH 1.2, compared to pH 7.0. Microparticles made with all matrix formulations

released essentially all encapsulated ascorbic acid after 7 hours at pH 7.0; microparticles made with 50% amylose starch had the lowest ascorbic acid releases after 7 hours at pH 1.2 (Table 2.6). The pH sensitivity of a starch-pectin encapsulation matrix was mainly caused by the presence of HM pectin, according to Dimantov et al. (2004). At acidic pH values, HM pectin gel is stabilized by hydrogen bonding between free carboxyl groups; at neutral and higher pH values, the percentage of free carboxyl groups is decreased, therefore HM pectin becomes unstable and more easily hydrated, which accelerates the release of encapsulated material (Liu et al. 2003; Oakenfull 1991; Yao et al. 1996). The most significant difference in ascorbic acid release, under the same pH condition, was caused by the starch type. Bhatnagar and Hanna (1994) reported that the degree of expansion of high amylose starch, after extrusion cooking, was inversely related to the percentage of amylose available to form inclusion complex. The higher degree of granular swelling of 50% amylose starch could have led to a higher complexation capacity than 70% amylose starch. Therefore, the ascorbic acid release rate was lower from microparticles with 50% amylose starch, as the increased interaction with amylose reduces the release of core material (Wing et al. 1988). Formation of inclusion complexes has comparable effects with cross-linking, on limiting the swelling of starch granules (Gelders et al. 2006). The ascorbic acid retention ability of RS4 could have been limited by cross-linking, which resulted in higher ascorbic acid release, compared to microparticles prepared with 50% amylose starch.

2.4 Conclusions

The microparticle matrices developed using swollen resistant starch-pectin can be used to successfully encapsulate ascorbic acid. The sizes of microparticles tend to increase with increased starch proportion, regardless of starch type. Similar surface indentations were observed on all microparticles, which is typical for starch based microparticles prepared by spray drying. Ascorbic acid encapsulation efficiency was influenced by starch-pectin ratio, while the type of starch had no significant impact. Release of ascorbic acid from microparticles showed significantly different behaviors under acidic (pH 1.2) and neutral (pH 7.0) conditions. The release rates were mainly influenced by the starch type in the microparticles. The lowest percentage releases at 7 hours, at pH 1.2 were obtained with microparticles containing 50% amylose starch.

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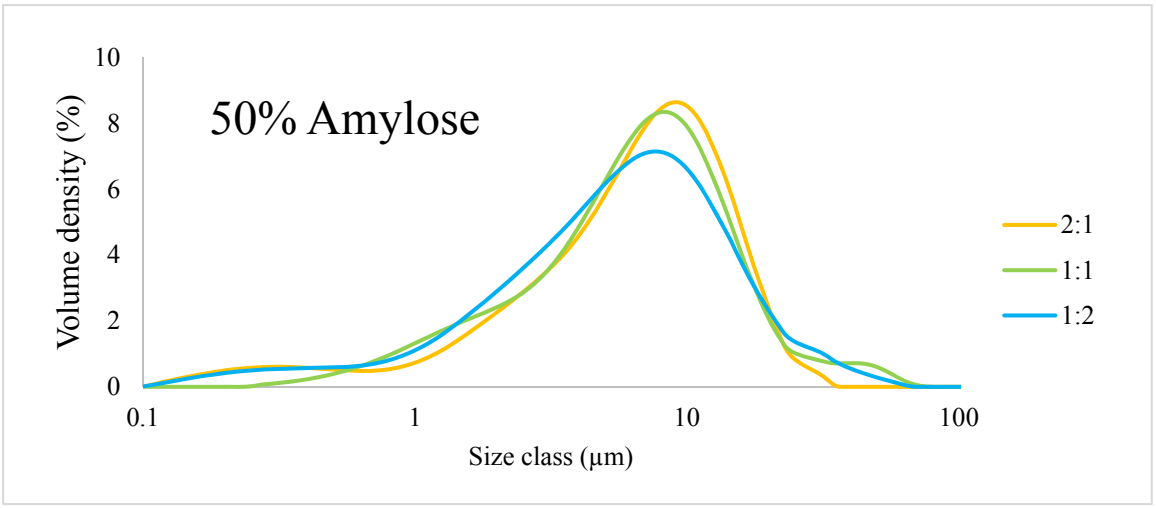

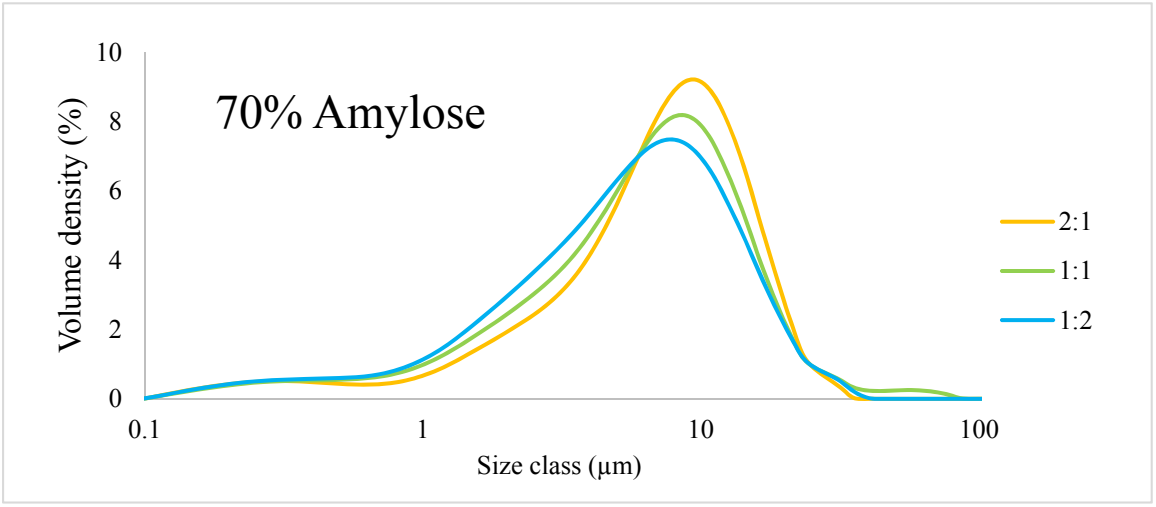

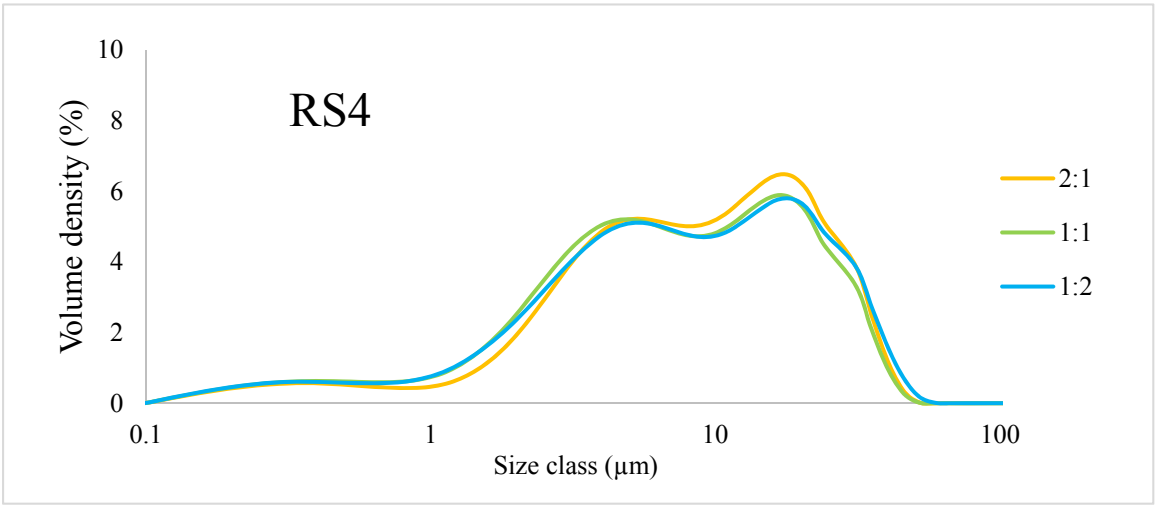

Figure 2.1. Size distribution profiles of microparticles. Starch-pectin ratios are given in corresponding legends.

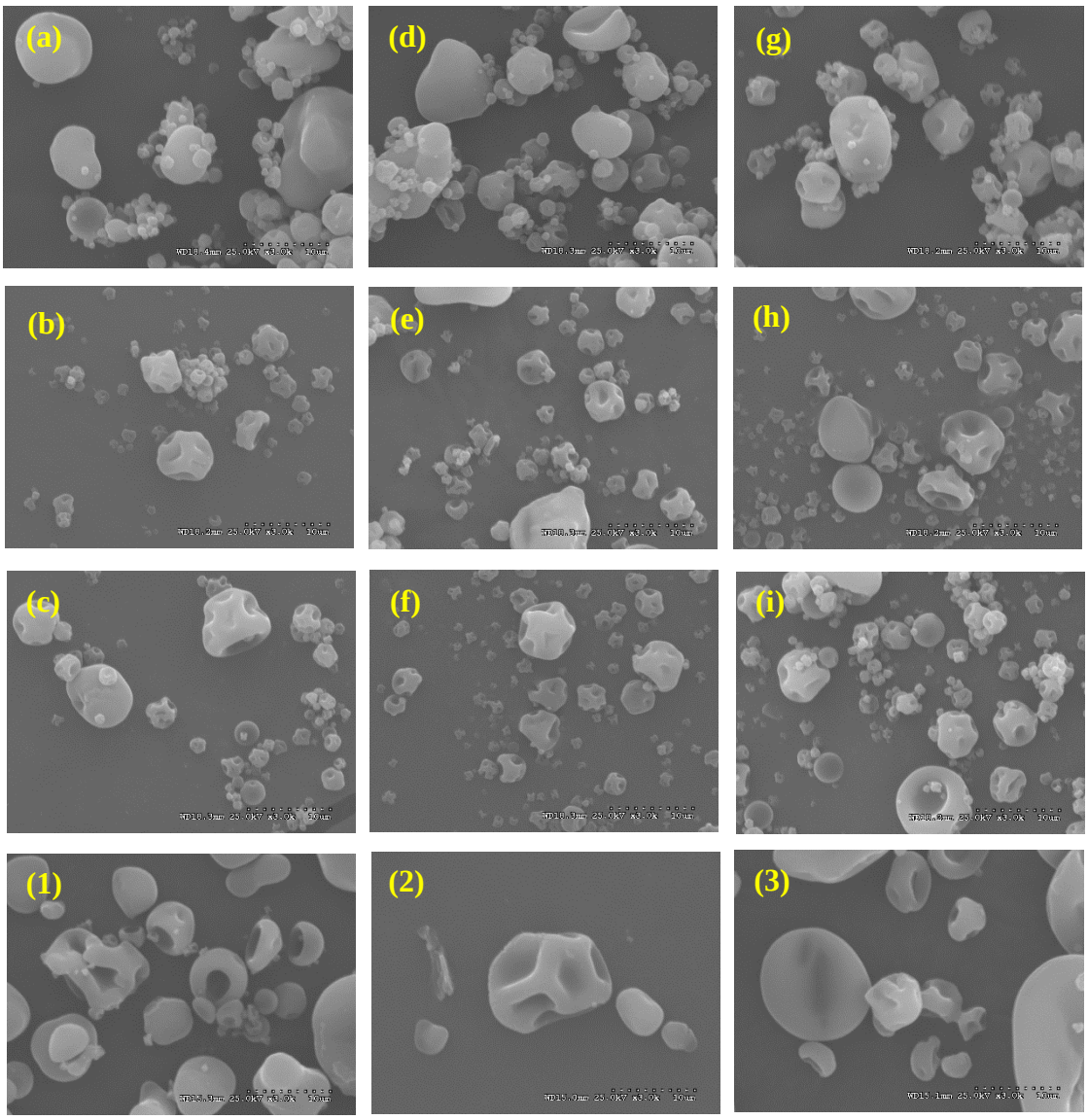

Figure 2.2. SEM images of microparticles prepared with 50% amylose starch-pectin at ratios of (a) 2:1, (b) 1:1, and (c) 1:2; with 70% amylose starch-pectin at ratios of (d) 2:1, (e) 1:1, and (f) 1:2; with RS4-pectin at ratios of (g) 2:1, (h) 1:1, and (i) 1:2, and spray dried heat treated granules of (1) 50% amylose starch, (2) 70% amylose starch, and (3) RS4. Images were captured at 3000x magnification.

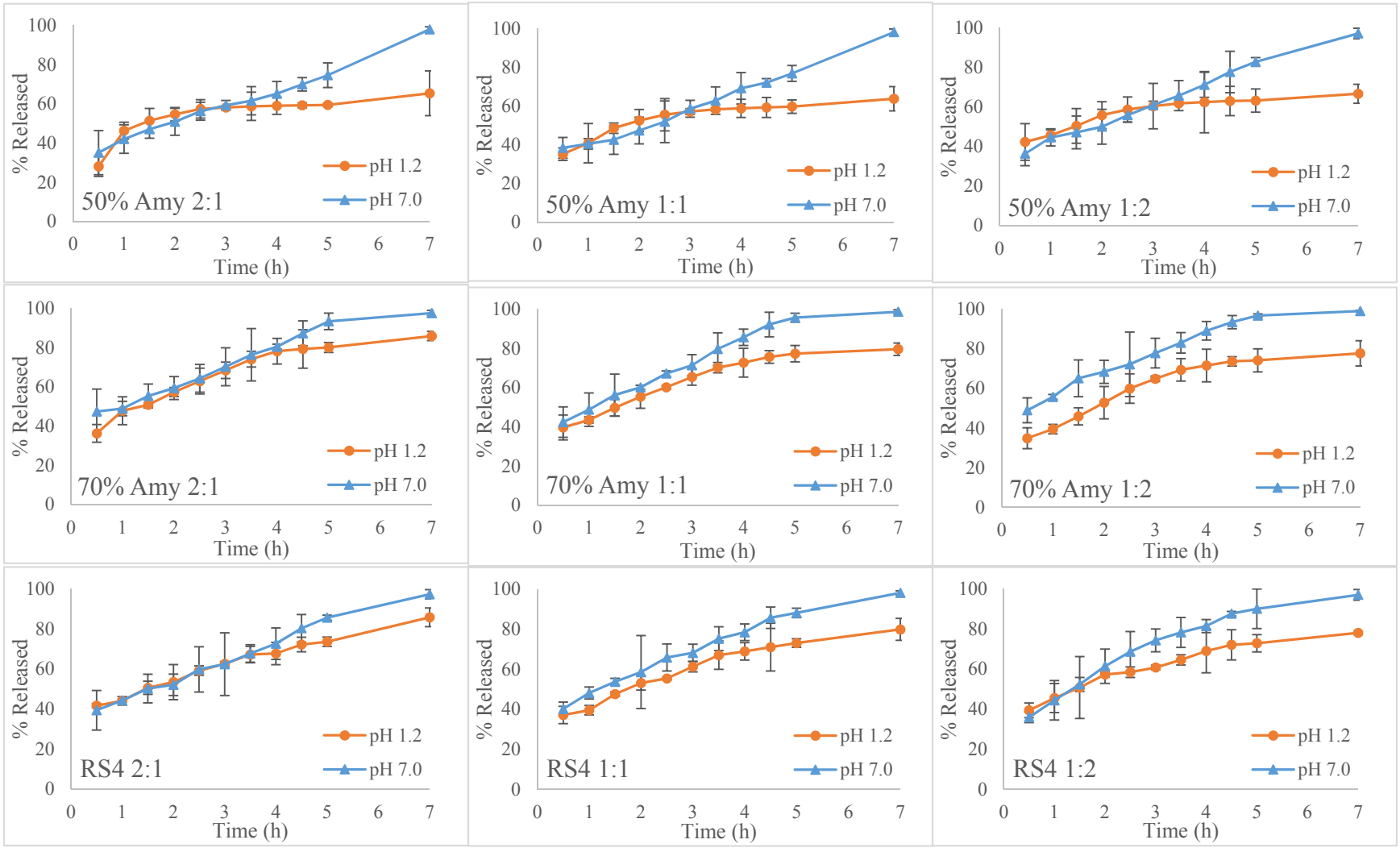

Figure 2.3. Release profiles of ascorbic acid from microparticles at selected pH levels.

Table 2.1. Starch & pectin compositions of wall material formulations*

Starch:pectin ratio	Starch (g)	Pectin (g)
2:1	13.33	6.67
1:1	10.00	10.00
1:2	6.67	13.33

*Dispersed in 1000 mL of distilled water.

Table 2.2. DSC phase transition parameters of starches used for encapsulation[1]

Transition parameter	Starch type		
	50% amylose	70% amylose	RS4
Onset temperature, T_o (°C)	70.41 ± 0.21[b]	70.77 ± 0.34[b]	76.64 ± 0.28[a]
Peak temperature, T_p (°C)	75.75 ± 0.23[c]	93.23 ± 0.25[a]	79.75 ± 0.34[b]
Conclusion temperature, T_c (°C)	88.66 ± 1.05[b]	104.37 ± 0.16[a]	84.69 ± 0.33[c]
Range, $T_c - T_o$ (°C)	18.24 ± 1.26[b]	33.60 ± 0.49[a]	8.04 ± 0.10[c]
Enthalpy, ΔH (J/g)	5.54 ± 1.03[b]	10.09 ± 1.43[a]	8.88 ± 0.42[a]

[1]Means followed by the same superscript, within the same row, are not significantly different ($p > 0.05$).

Table 2.3. Swelling factors of raw starches

Starch	Swelling factor[1]
50% amylose[2]	3.28 ± 0.26[b]
70% amylose[2]	1.78 ± 0.06[c]
RS4[3]	6.62 ± 0.13[a]

[1]Means followed by the same superscript are not significantly different ($p > 0.05$).

[2]Tested at 65°C.

[3]Tested at 71°C.

Table 2.4. Size distributions of microparticles[1]

Starch	Starch:pectin ratio	Dv[2] 10 (μm)	Dv 50 (μm)	Dv 90 (μm)	Span[3]
50% amylose	2:1	2.29 ± 0.74[b]	8.69 ± 1.78[ab]	24.43 ± 1.50[ab]	2.63 ± 0.67[abc]
	1:1	1.87 ± 0.47[b]	9.95 ± 4.98[ab]	16.10 ± 0.70[cd]	1.67 ± 0.72[c]
	1:2	1.54 ± 0.15[b]	6.37 ± 0.16[b]	17.53 ± 0.63[c]	2.51 ± 0.07[abc]
70% amylose	2:1	1.81 ± 0.10[b]	7.72 ± 0.24[ab]	16.13 ± 0.55[cd]	1.85 ± 0.03[bc]
	1:1	1.67 ± 0.16[b]	7.04 ± 0.12[ab]	15.90 ± 0.40[cd]	2.02 ± 0.08[abc]
	1:2	1.49 ± 0.10[b]	6.49 ± 0.27[b]	14.80 ± 0.87[d]	2.06 ± 0.22[abc]
RS4	2:1	3.29 ± 0.40[a]	14.44 ± 5.65[a]	25.07 ± 0.31[a]	1.70 ± 0.75[c]
	1:1	1.57 ± 0.01[b]	7.61 ± 0.35[ab]	24.47 ± 0.75[ab]	3.02 ± 0.21[ab]
	1:2	1.49 ± 0.02[b]	6.63 ± 0.53[b]	22.67 ± 0.23[b]	3.21 ± 0.28[a]

[1]Means followed by the same superscript, within the same column are not significantly different ($p > 0.05$).

[2]Dv = volume weighted size distribution percentile.

[3]Span = (Dv90 – Dv10)/Dv50

Table 2.5. Encapsulation efficiencies of microparticles[1]

Starch:pectin ratio	Starch type		
	50% amylose	70% amylose	RS4
2:1	35.45 ± 4.79^{c}	31.27 ± 3.57^{c}	35.65 ± 4.91^{c}
1:1	50.06 ± 4.35^{b}	53.72 ± 2.16^{b}	59.91 ± 2.03^{ab}
1:2	65.29 ± 3.37^{a}	56.86 ± 5.69^{ab}	58.58 ± 3.76^{ab}

[1]Means followed by the same superscript are not significantly different ($p > 0.05$). Values are calculated using equation 2.2.

Table 2.6. Cumulative ascorbic acid released by microparticles after 7 hours[1]

Starch	Starch:pectin ratio	% Release under selected pH conditions[2]	
		pH = 1.2	pH = 7.0
50% amylose	2:1	65.37 ± 11.43[d]	97.96 ± 1.33[a]
	1:1	63.75 ± 6.25[d]	98.07 ± 1.67[a]
	1:2	66.58 ± 4.78[d]	97.02 ± 2.67[a]
70% amylose	2:1	85.95 ± 2.33[b]	97.65 ± 1.37[a]
	1:1	79.56 ± 3.15[c]	98.66 ± 1.05[a]
	1:2	77.69 ± 6.37[c]	99.03 ± 0.33[a]
RS4	2:1	85.75 ± 4.66[b]	97.25 ± 2.33[a]
	1:1	79.89 ± 5.46[c]	98.12 ± 1.03[a]
	1:2	78.03 ± 0.79[c]	96.99 ± 2.67[a]

[1]Means followed by the same superscript are not significantly different ($p > 0.05$).

[2]Temperature = 20°C

CHAPER 3. ENCAPSULATION OF ASCORBIC ACID IN GELATINIZED STARCH-PECTIN MICROPARTICLES BY SPRAY DRYING WITH THREE-FLUID NOZZLE

Abstract

Microparticles, prepared using gelatinized regular corn starch and high methoxyl pectin, were developed for microencapsulation of ascorbic acid. Selected starch-pectin ratios (2:1, 1:1, and 1:2) were used to investigate the effect of starch-pectin composition on the properties of microparticles. Microparticles were prepared by spray drying with a three-fluid nozzle. Microparticles with the highest starch ratio (2:1) had the largest size distribution span, but there were no significant surface morphological differences among the particles prepared from the three ratios. Ascorbic acid encapsulation efficiency increased with the starch proportion. All microparticles displayed higher percentage ascorbic acid releases at 7 hours at pH 7.0, compared to pH 1.2. Microparticles having the highest pectin ratio (1:2) were the most sensitive to pH variations.

3.1 Introduction

Starches and pectins are often used as wall materials for microencapsulation by spray drying, because they possess physical properties that favor the spray drying process (Gharsallaoui et al. 2007). Native starches have advantages such as low cost, low viscosities at high concentrations, ease of drying, and being readily available (Kenyon 1995; Zuidam and Nedović 2009). Pectins are effective emulsion stabilizers, and are effective at low concentrations (Drusch 2007).

The amylose released from starch granules during gelatinization is capable of forming inclusion complexes, by binding core material (*i.e.*, guest molecules) into the helical cavities of amylose chains (Rutschmann et al. 1989). Inclusion complexes with amylose have been successfully used for flavor encapsulation (Madene et al. 2006; Wulff et al. 2005). Amylose becomes resistant to enzymatic digestion once it forms inclusion complexes with guest molecules, such as lipids (Hanna and Lelievre 1975), which has the potential for controlled delivery in the gastrointestinal tract (Putseys et al. 2010).

Pectins have strong film forming and binding abilities that are desired for encapsulation applications (Liu et al. 2007), especially as coatings for core-shell forms of encapsulation (Shahidi and Han 1993). High methoxyl pectins exhibit pH-sensitive behaviors, which is frequently utilized for designing pH-triggered delivery systems (Liu et al. 2003). Spray drying with a three-fluid nozzle allows formation of microparticles with a defined core-shell construction (Sunderland et al. 2013).

The three-fluid (gas/liquid/liquid) nozzle design consists of a center passage for the inner feed, a peripheral passage for the outer feed, and an outermost passage for the atomizing gas; the two liquid feeds are not in contact with each other until they reach the nozzle exit. This unique design avoids unwanted mixing between microparticle components, and enables “coating” of one liquid by another liquid (Kondo et al. 2014).

This study was conducted to investigate the properties of a starch-pectin based delivery system for microencapsulation of ascorbic acid. Spray drying, with a three-fluid nozzle, was used to prepare ascorbic acid loaded microparticles. Selected ratios of starch and pectin were used to prepare microparticles, in order to understand the impact of

starch and pectin composition on the physical and functional properties of ascorbic acid encapsulated microparticles.

3.2 Materials and methods

3.2.1 Materials

High methoxyl pectin (TIC Pretested® Pectin 1400) was obtained from TIC Gums (White Marsh, MD, USA). Regular corn starch (Cargill Gel™ 03401) was obtained from from Cargill Corn Milling (Cedar Rapids, IA, USA). Ascorbic acid was obtained from NOW Foods (Bloomingdale, IL, USA). All other chemicals and solvents used for the experiments were of ACS certified grade.

3.2.2 Preparation of microparticles

Microparticles were prepared from two feed solutions by spray drying with a three-fluid nozzle, as outlined in Figure 3.1.

3.2.2.1 Preparation of feed solutions

The inner feed solution was prepared by the following procedure: A starch water dispersion was heated to boiling, at a rate of ~ 1.5°C/min, on a Corning PC-320 stirrer/hotplate (Corning Inc., New York, NY), with a stirring speed of 600 rpm. The starch dispersion was maintained at a temperature of > 95°C for 30 minutes, then cooled to 20°C in ice bath, while the temperature was monitored by a thermocouple. Ascorbic acid was added at 0.5% (w/w, wet basis) concentration and mixed by homogenizing at 10,000 rpm for 3 minutes, using a VirTishear homogenizer (Model 225318, The Virtis

Company, Inc., Gardiner, NY, USA). Then 0.5% (w/w, wet basis) pectin was also added as a stabilizing agent and mixed by homogenizing at 10,000 rpm for 2 minutes.

The outer feed solution was a pectin solution created by homogenizing dry powdered high methoxyl pectin with distilled water at 10,000 rpm for 3 minutes.

The concentrations of starch in inner feed solutions and pectin in outer feed solutions were adjusted to obtain starch-pectin ratios of 2:1, 1:1, and 1:2, while total 80% (w/w, dry basis) content of starch and pectin in microparticles was maintained.

3.2.2.2 Spray drying with three-fluid nozzle

Microparticles were prepared from the feed solutions in a bench top mini spray dryer (B-290, Buchi Labortechnik AG, Switzerland), with a three-fluid (gas/liquid/liquid) nozzle (Model 046555, 2.0 mm outer nozzle tip and 0.7 mm inner nozzle tip). The inner feed solution was pumped through the needle tip at a flow rate of 1.5 mL/min; outer feed solution was pumped through the outer nozzle tip at a flow rate of 3 mL/min. Spray drying parameters were: Inlet temperature 105°C, aspirator rate 85%, and atomizing gas flow 473 L/h. Microparticles were collected and stored at -20°C until analysis.

3.2.3 Analysis of physical properties

3.2.3.1 Particle size analysis

Particle size analyses were performed using a Malvern Mastersizer 3000 laser diffraction particle size analyzer, equipped with an Aero S dry powder disperser (Malvern instruments Ltd, Malvern, Worcestershire, UK). Microparticles were delivered into the disperser cell within the obscuration limit of 0.1 to 20%. Measurement

parameters were: Refractive index of 1.53, density of 1.5 g/cm^3, absorption index of 0.10, air pressure 1 bar, and feed rate of 50%. Data were collected and analyzed using Malvern software (Version 2.20, Malvern instruments Ltd, Malvern, UK).

3.2.3.2 Surface morphology

The surface morphology of dry microparticles were observed using scanning electron microscopy, as described by Ratnayake and Jackson (2007). Microparticles were mounted on metal stubs and coated with gold-palladium alloy using a Hummer sputter coating system (Anatech Ltd., Union City, CA, USA). Coated microparticles were observed with a Hitachi S4700 field emission scanning electron microscope (Hitachi Science Systems, Tokyo, Japan) at an acceleration potential of 10kV. Pictures were recorded by an image capturing software (Hitachi High-Technologies, Pleasanton, CA, USA).

3.2.4 Analysis of functional properties

3.2.4.1 Encapsulation efficiency

Microparticles (~ 0.400 g) were weighed into a clean 50 mL plastic centrifuge tube. Microparticles were washed by adding 10 mL of ethanol, and manually inverting the tube 10 times. The tube was then centrifuged at 1,500g for 5 minutes at 20°C, in a Sorvall Legend XTR centrifuge (Thermo Scientific, Asheville, NC, USA), and the supernatant was discarded. The remaining microparticles were mixed with 40 mL of phosphate buffered saline (pH 6.0), and completely dispersed by sonicating the mixture for 1 hour using a Vibra-Cell VC300 sonicator (Sonics & Materials, Inc., Newtown, CT,

USA) at output 10 and duty cycle 50%. The supernatant was recovered by centrifuging at 3,000 g for 15 minutes at 20°C. The concentration of ascorbic acid was determined using a colorimetric method (Jagota and Dani 1982) as follows: An aliquot (1 mL) of supernatant was first diluted to 25 mL, and then 1 mL of diluted supernatant and 1 mL of 0.2 N Folin-Ciocalteu reagent was mixed and diluted to 10 mL. The dilution was set for 30 minutes for color development. Absorbance of developed color at 760 nm was measured in a BioMate 3S UV-Vis spectrophotometer (Fisher Scientific, Madison, WI, USA), using plastic cuvettes. Concentration of ascorbic acid was then determined against a standard curve prepared with a serial dilution of ascorbic acid. Encapsulation efficiency was calculated according to the equation below (Desai and Park 2005):

$$\text{Encapsulation efficiency} = \frac{A}{A_0} \times 100\% \quad \text{Equation (3.1)}$$

Where A is calculated ascorbic acid concentration (μg/mL); A_0 is theoretical ascorbic acid concentration (μg/mL).

3.2.4.2 *In vitro* release profile

The *in vitro* release tests of microparticles were performed under selected conditions in chloride buffer (pH 1.2) and phosphate buffered saline (pH 7.0). Eleven 15 mL centrifuge tubes, each containing ~ 0.100 g microparticles and 10 mL buffer, were set on a multi-tube rotator (Thermo Scientific, Waltham, MA, USA). One tube was analyzed at 30 minute intervals during the first 5 hours, then one tube was analyzed at 7 hours. The supernatant was recovered by centrifuging at 3,000g for 15 minutes at 20°C, and the concentration of released ascorbic acid was determined using the same colorimetric method as described above.

3.2.5 Statistical analysis

The study was conducted using completely randomized design (CRD). Microparticles were prepared with starch/pectin ratios of 2:1, 1:1, and 1:2, and each ratio was prepared in triplicates. Analyses of variance were performed and mean separations were performed by Tukey-Kramer HSD test at $p < 0.05$ significance level, using JMP software (Version 10.0.2, SAS Institute Inc., Cary, NC, USA).

3.3 Results and discussion

3.3.1 Physical properties of microparticles

3.3.1.1 Particle size distributions

All microparticles showed uni-modal size distribution patterns, with a peak in size range 4 to 6 μm, as shown in Figure 3.2. Volume weighted size distribution percentiles of microparticles are given in Table 3.1. Microparticles with the highest starch proportion (2:1) had the largest size distribution span, while no significant differences were found between starch-pectin ratios 1:1 and 1:2. Microparticles with starch-pectin ratio of 2:1 also had more large particles, as indicated by the highest Dv90 size. Microparticles with narrow distributions have higher homogeneity in size (Gaumet et al. 2008).

Berkland et al. (2004) proposed two mechanisms for how microparticle size influences core ingredient release: 1) Release rates increase with decreasing particle size, due to the increased surface area to volume ratio. 2) Smaller microparticles harden faster during particle formation, and thus they trap quickly highly water soluble core materials, which tend to migrate outward during particle formation. Smaller microparticles then have more uniform core material distribution, leading to slower release rates. Therefore,

microparticles with the same size are more likely to have same release rates, and a narrow size distribution indicates a more uniform release pattern.

3.3.1.2 Surface morphology

SEM images confirmed the observations made on prepared microparticles, by particle size distribution analysis. Microparticles made with starch-pectin ratio 2:1 were less uniform in size, compared with the other two ratios. Essentially similar surface indentations were observed on microparticles prepared with all three starch/pectin ratios (Figure 3.3), which is typical of microparticles created by spray drying (Tonon et al. 2011). The development of surface indentations on microparticles during spray drying can be explained according to the mechanism proposed by Adhikari et al. (2003). The low moisture diffusion rate of the polymeric wall matrix resulted in very high moisture gradients between the droplets and the drying air. The droplets tend to reduce the diffusion path by shrinking to increase the surface area, leading to the distinct surface indentations on dried microparticles. All microparticles had undamaged surfaces with no visible pores or cracks, which is known to provide better core material protection (Bertolini et al. 2001).

3.3.2 Functional properties of microparticles

3.3.2.1 Encapsulation efficiency

Encapsulation efficiency provides an estimation of the percentage of core material recovered after the encapsulation process. An important aspect of successful microencapsulation is to achieve high encapsulation efficiency. High encapsulation

efficiency permits minimum use of core materials (Jafari et al. 2008). Encapsulation efficiencies of prepared microparticles for this study are shown in Table 3.2. Microparticles with all three starch-pectin ratios were prepared using an identical process. Therefore, it could be speculated that the differences in encapsulation efficiencies were a direct result of the variation in starch-pectin ratios. Ascorbic acid encapsulation efficiency increased with increasing starch proportion. Mongenot et al. (2000) reported that the encapsulation efficiency of cheese aroma was higher in a matrix containing starch, compared to a matrix containing maltodextrin. The interaction between amylose and aroma molecules increased the retention of core material. The amylose content of the microparticles increased with increasing starch-pectin ratio. Therefore, more ascorbic acid was retained in microparticles with higher starch-pectin ratios.

3.3.2.2 *In vitro* release profiles

All microparticles exhibited similar ascorbic acid release patterns under the same pH conditions. (Figure 3.4). Microparticles had nearly 100% released concentrations at 7 hours at pH 7.0, while the released concentrations at 7 hours at pH 1.2 were significantly lower (Table 3.3). There were no significant differences in final released concentration at pH 7.0, while microparticles with starch-pectin ratio of 1:2 had the lowest release at pH 1.2. Therefore, ascorbic acid release behaviors of microparticles with the highest pectin ratio (1:2) were the most sensitive to pH variations. Dimantov et al. (2004) studied the variations in dissolution behaviors of high amylose corn starch-high methoxyl pectin coatings on glass slides, under acidic (pH 1.6) and neutral (pH 7.0) conditions, and indicated an increased difference in dissolution rates, between the two pH conditions,

with increased pectin content. The number of free carboxyl groups in high methoxyl pectin is reduced as pH increases, resulting in a loss of hydrogen bonding and a consequent unstable gel (Oakenfull 1991). The weaker pectin outer layer at pH 7.0, therefore, induces faster release of ascorbic acid.

3.4 Conclusions

Ascorbic acid loaded microparticles were created from gelatinized regular starch coated with pectin, by spray drying with a three-fluid nozzle. The starch-pectin ratio had an impact on the size distributions of microparticles, but essentially similar surface morphological features were observed on microparticles with all three starch/pectin ratios. Ascorbic encapsulation efficiency was dependent on the starch-pectin ratio, and higher encapsulation efficiencies were obtained with higher starch ratios. All microparticles displayed higher ascorbic acid release over time at pH 7.0 compared to pH 1.2, while the most significant difference in release behavior between the two pH conditions was observed in microparticles made with starch-pectin ratio 1:2. The release profiles suggested that a starch-pectin based system could be used for pH-triggered ascorbic acid delivery.

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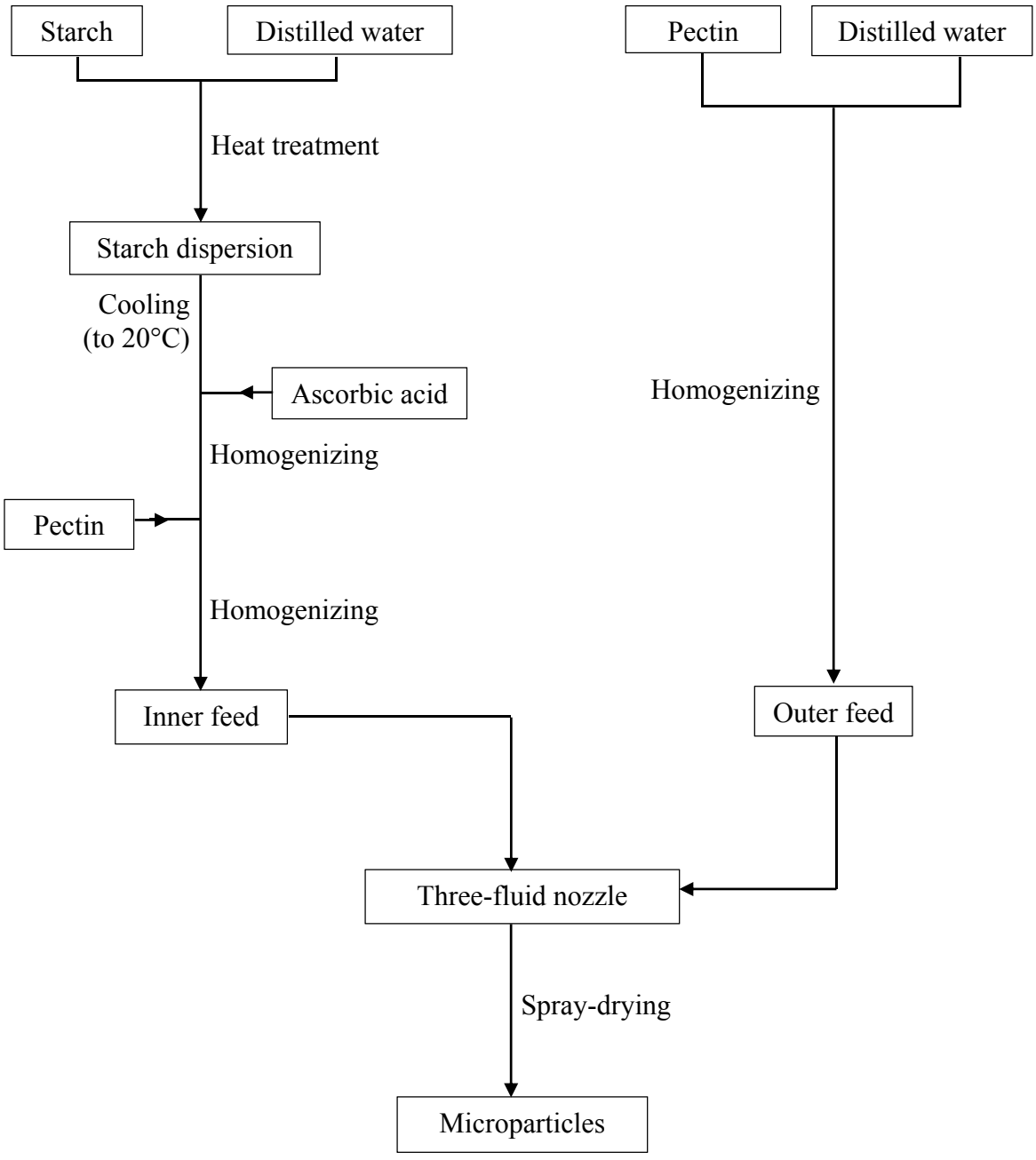

Figure 3.1. Preparation of microparticles.

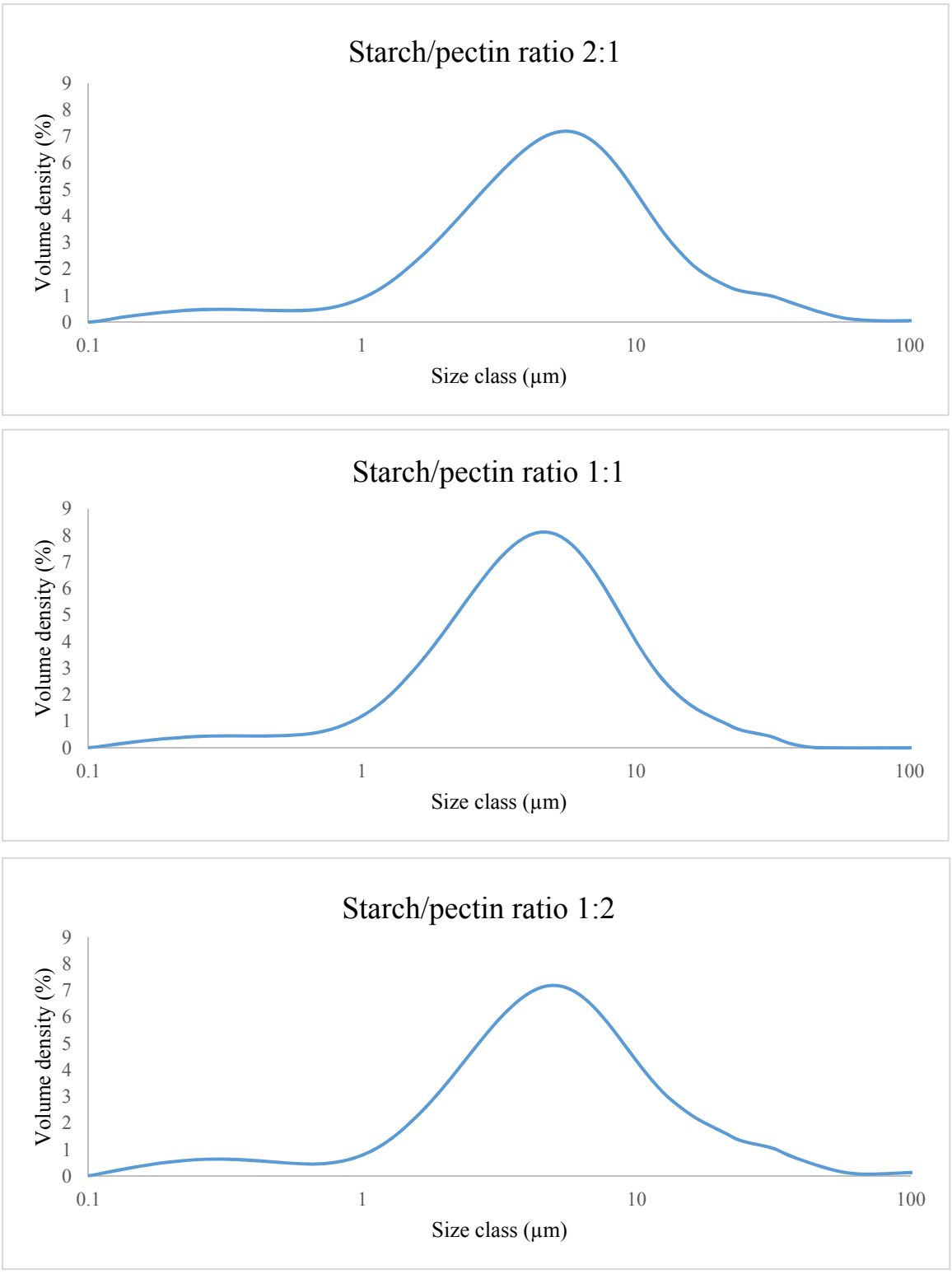

Figure 3.2. Size distribution profiles of microparticles.

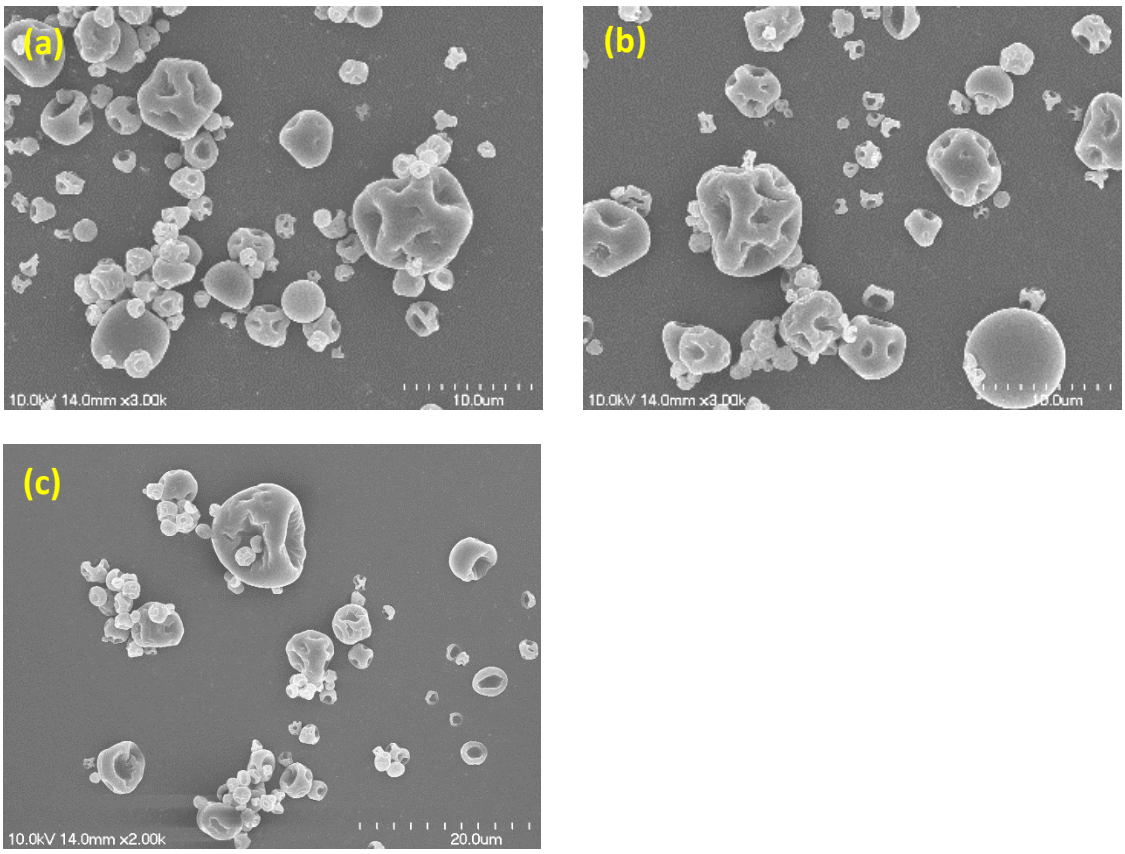

Figure 3.3. SEM images of microparticles prepared with starch-pectin ratios at (a) 1:2, (b) 1:1, and (c) 2:1 (Magnification = 3000x).

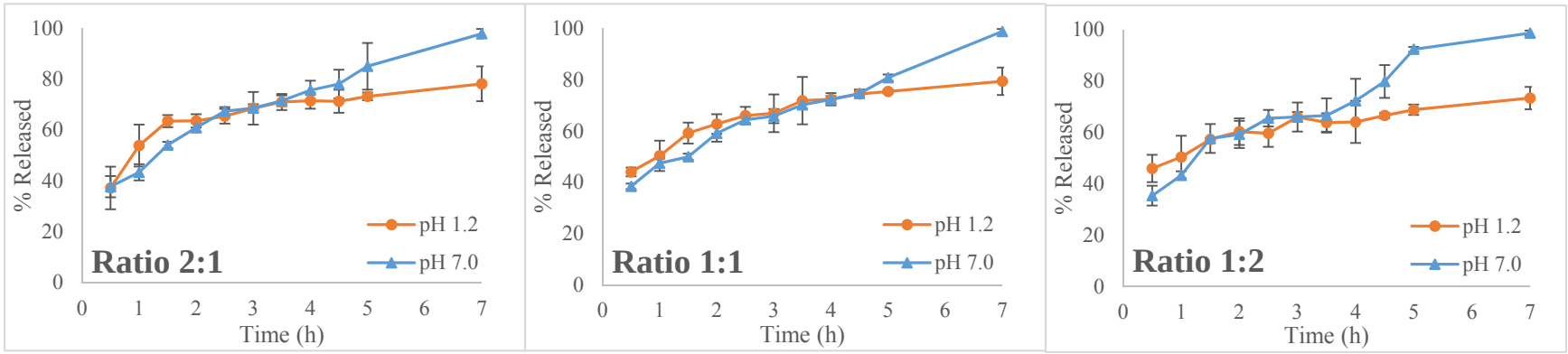

Figure 3.4. Release profiles of ascorbic acid at selected pH levels.

Table 3.1. Size distributions of microparticles[1]

Starch:pectin Ratio	Dv[2] 10 (μm)	Dv 50 (μm)	Dv 90 (μm)	Span[3]
2:1	1.59 ± 0.04[a]	5.59 ± 0.41[a]	25.37 ± 1.65[a]	4.26 ± 0.27[a]
1:1	1.45 ± 0.05[a]	4.51 ± 0.29[b]	11.50 ± 1.68[b]	2.21 ± 0.23[b]
1:2	1.49 ± 0.27[a]	5.41 ± 0.89[a]	13.73 ± 1.79[b]	2.30 ± 0.47[b]

[1]Means followed by the same superscript, within the same column are not significantly different ($p > 0.05$).

[2]Dv = volume weighted size distribution percentile.

[3]Span = (Dv90 – Dv10)/Dv50

Table 3.2. Encapsulation efficiencies of microparticles[1]

Starch:pectin Ratio	Encapsulation efficiency (%)
2:1	77.06 ± 0.65^{a}
1:1	73.17 ± 1.19^{b}
1:2	66.68 ± 1.20^{c}

[1]Means followed by the same superscript are not significantly different ($p > 0.05$).

Table 3.3. Total ascorbic acid released by microparticles after 7 hours at selected pH levels[1]

Starch:pectin ratio	% Ascorbic acid released	
	pH = 1.2	pH = 7.0
2:1	78.15 ± 6.84[b]	97.85 ± 1.85[a]
1:1	79.35 ± 5.33[b]	98.76 ± 0.99[a]
1:2	73.36 ± 4.37[c]	98.66 ± 1.03[a]

[1]Means followed by the same superscript are not significantly different ($p > 0.05$).

OVERALL SUMMARY

Starch and pectin are both commonly used food grade encapsulation wall materials for spray drying. Resistant starches and pectin are regarded as dietary fibers, since they are not digested by human digestive enzymes. Ascorbic acid, also known as vitamin C, is an important antioxidant that is highly unstable, which has limited its direct incorporation into food products and its bioavailability as a dietary supplement.

The two studies reported in this thesis investigated selected formulations for starch-pectin based microparticles, prepared by spray drying, for the encapsulation of ascorbic acid. The physical properties (particle size distribution and surface morphology) as well as the functional properties (ascorbic acid encapsulation efficiency and *in vitro* release profile) of the microparticles were evaluated.

The first study evaluated the physical and functional properties of heat treated resistant starch and high methoxyl pectin based, ascorbic acid loaded microparticles. The second study evaluated the physical and functional properties of gelatinized regular starch and high methoxyl pectin based, ascorbic acid loaded microparticles, which were prepared by spray drying with a novel three-fluid nozzle.

The results suggested that the size distributions of starch-pectin based microparticles were impacted by both the type of starch and starch-pectin ratio. Encapsulation efficiency was primarily controlled by the starch content in the encapsulation matrix. The prepared microparticles exhibited pH-dependent *in vitro* ascorbic acid release behaviors, as a result of the presence of high methoxyl pectin in the wall matrix. Processing with three-fluid nozzle produced microparticles with higher encapsulation efficiencies, compared to the conventional two-fluid nozzle.

The starch-pectin based microparticles, prepared in these studies, could be considered prototypes for *in vivo* delivery systems that utilize the pH variations as release triggers for ascorbic acid.