MICROENCAPSULATION OF MUSCADINE GRAPE EXTRACTS TO PRODUCE SHELF STABLE NUTRACEUTICAL POWDER

by

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(Under the Direction of Manjeet S. Chinnan)

ABSTRACT

Phenolic compounds found in muscadine pomace were extracted using enzyme treatment, bladder press and microfiltration operation. These extracts were microencapsulated using coating materials of gum arabic and whey protein and spray drying operation. The microcapsules were characterized using Scanning electron microscopy and particle size distribution. Microencapsulation yield of particles was 99.17% and 88.15% for particles coated with whey protein and gum arabic respectively. Microencapsulated and uncoated samples were kept at controlled environmental conditions. Uncoated powder samples changed color from purple to black while appearance changed from free flowing to rubbery. In coated samples, the color changed and appearance changed from free flowing to caking. Half lives were obtained from the storage of samples over 8 weeks and indicate that microencapsulation helps in protecting the antioxidants at 4 °C as compared to 25 °C. It was that found maximum degradation was found in following order pure compound>whey protein>gum arabic.

INDEX WORDS: Phenolic, whey protein, gum arabic, Microencapsulation, scanning electron microscopy, degradation.

MICROENCAPSULATION OF MUSCADINE GRAPE EXTRACTS TO PRODUCE SHELF STABLE NUTRACEUTICAL POWDER AND STORAGE STUDIES

by

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A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2008

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December 2008

ACKNOWLEDGEMENTS

I would like to express my appreciation to my major professor for his guidance, support, encouragement and critical assessment. He gave equal importance to work and work ethics which is important when you go and work for somebody else. I am also grateful to the members of my advisory committee Dr. Phillips and Dr. Kerr for their comments and inputs which made this research possible. I also acknowledge help of Glen Farrell in setting up the experiments.

The members of food engineering lab were really helpful: Shripad Tandale, for encouragement and words of advice, Hannah Smith, for help with anything whenever I asked for. I would like to thank other people in food science department Sue Ellen, Sandra, Larry Hitchcock for help with my work. I would like to thank Mr. Jerry Davis for his help with statistical data analysis.

To my friends Dharmendra Singh Pawar, Suvang, Neha for company. To my brother Nirmalya and family back in India for support and words of encouragement.

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CHAPTER 1

INTRODUCTION

The muscadine grape (*Vitis rotundeflora*) is a native to southeastern United States. Many modifications in the cultivar have made these grapes resistant to pests and have increased their phenolic content and yield. Muscadine grapes have significant amounts of resveratrol, a compound in French red and white wines that is being touted as an agent for lowering cholesterol levels and the risk of coronary heart disease. It has been reported that extracts from muscadine grapes (phenolics and anthocyanins) were responsible for a 50% decrease in cancer cell populations indicating that polyphenols from muscadine grapes may have anticancer properties (Akoh and others 2005). There is high level of gallic acid, catechin, epicatechin, ellagic acid, and resveratrol acid found in the skins and seeds of muscadine which gives it a high antioxidant capacity. Antioxidants prevent or reduce destructive oxidation reactions. They protect the cell components from the effect of free radicals which are formed as a result of cellular metabolism. These free radicals are harmful to the body and can damage cell components. Antioxidants scavenge these free radicals preventing destruction of cells and tissue. A number of components contribute to the antioxidant capacity of muscadine grapes such as vitamins, phenols, carotenoids and flavanols. As per Pastrana and others (2003) the maximum amount of phenolics in Muscadine grapes is found in the seeds and skins. Baydar and others (2006) used grape seed extracts from three varities and found them to be bacteriocidal against E. *coli* O157:H7, S *aureus* and A *hydrophila* which indicates a potential use for extracts. Baydar and others (2007) investigated grape extracts for prolonging shelf life of food grape extracts, they found these to be

alternative natural antioxidants to the synthetic antioxidants (BHA or BHT) used in food industry.

The functional and organic foods market in the USA is currently estimated to be around 100 billion dollars (Source: http://www.nutraingredients-usa.com/). This includes functional/fortified foods and beverages (38.6 bn $), vitamins, minerals, dietary supplements (21.7 bn $) and organic and natural foods. In 2007 demand for dietary supplements increased at the rate of 7 % whereas, demand for organic and natural foods and beverages increased at rate of 20 to 29%. As per report by Burdock and others (2006) the market for dietary supplements was 20.5 billion dollars.

Microencapsulation is a process in which very small pieces of the ingredients are packaged in small capsules to facilitate their release later on. The capsule size can range in size from microns to several millimeters. The ingredient release takes place by mechanical rupture of capsule cell wall, dissolution of the cell wall or diffusion from the cell wall. By controlled release of certain ingredients, manufacturers have the ability to: increase shelf life, enhance flavor and quality, deliver functional ingredients, provide ingredient stability, improve health and wellness, prevent ingredient interaction, improve the efficacy of active ingredients in pharmaceutical applications, act as or facilitate delivery systems. Microencapsulation of the functional ingredients here polyphenolic can help in protecting the antioxidants and help in their use in other foods as additives or it can be sold as a dietary supplement. Microencapsulation helps in creation of non sticky powders which are easy to handle and have longer shelf life than without microencapsulation. Consumers are looking at functional foods as a means of preventing or healing disease and creation of muscadine grape extract powder can help fill this market gap and create revenue for farmers from by products.

Georgia is a large producer of muscadine grapes which is used to make jams, jellies, juice, wine, etc. The press part of wine making is pomace and skin which has the maximum amount of polyphenolic antioxidants. The press fraction is 40% of the weight of the total fruit. There is an effort to develop products so that a producer can realize the benefits in terms of dollars at farm level. Figure 1.1 illustrates various types of products which can be processed from muscadine. The by-product of processing, pomace, can be used to make colorants, ingredients, dried fruit and nut mixtures and nutraceuticals. The aim of the project was to use muscadine grape pomace to make functional food additives (nutraceuticals) using microencapsulation.

The main objectives of this study were to 1) microfilter extracts obtained from muscadine pomace, 2) spray dry microfiltered extracts with and without microencapsulating agents, 3) characterize microencapsulated powders, and 4) examine degradation of nutraceuticals from microencapsulated powder made from muscadine grape extracts.

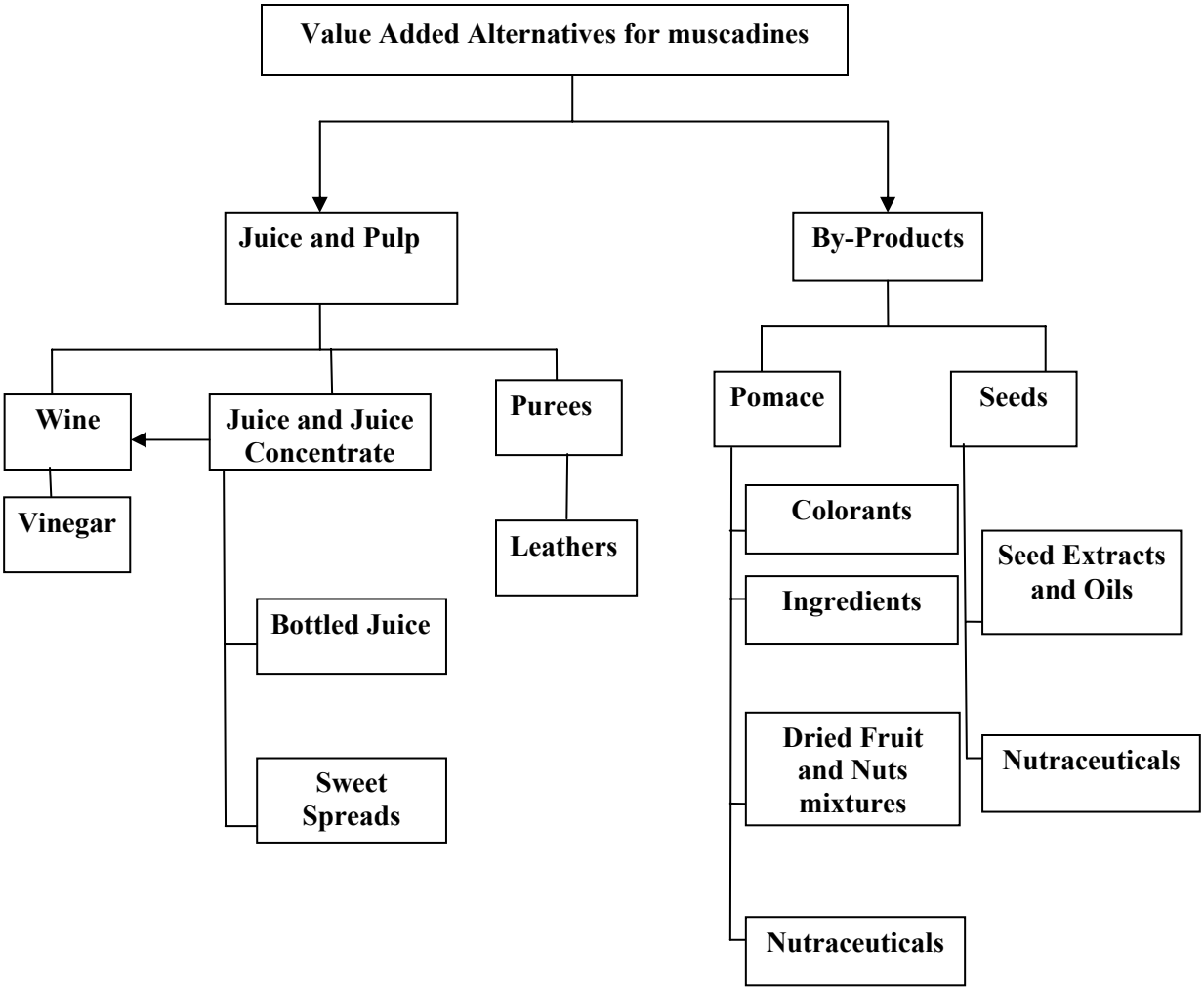

Fig 1.1: Choices for processed muscadine products (Morris and Brady 2004)

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CHAPTER 2

LITERATURE REVIEW

Muscadine grape

The muscadine grape (*Vitis rotundifolia* Michx) is found in the wild from Delaware to the Gulf of Mexico and westward to Missouri, Kansas, Oklahoma, and Texas. The plant is sensitive to temperature variations and is not grown in regions where temperatures frequently go below 10°F. There are many cultivars of muscadine grape such as Black Beauty, Black Fry, Darlene, Fry, Higgins, Jumbo, Scuppernong, Sugargate, Summit, Supreme, Sweet Jenny, Carlos, Cowart, Dixieland, Dixie Red, Fry Seedless, Magnolia, Nesbitt, Noble, Redgate, Regale, and Sterling (California Rare Fruit Growers, Inc 1999).

Vitis rotundofolia

The three American species of the subgenera Muscadinia are *V. rotundifolia* found in southern US, *V. munsoniana* found in central and southern Florida and *V. popenoei* in New Mexico. Muscadine grapes are best suited for the southern states from eastern Texas to the Atlantic seaboard. Vitis *rotundofolia* is commonly known as "muscadine". Muscadine grapes are found as small, loose clusters of large berries with a thick skin and dense pulp. The bronze skinned varieties are known as scuppernongs. Cultivars in the rotundifolia species with the most commercial importance are the Carlos, Noble and Magnolia. The muscadine grape is more disease and pest resistant as compared to vinifera. The advantage of the V. *labrusca* and V. *rotundifolia* are their resistance to fungal diseases and winter killing. The entire family of

phenols, phenolics, and tannins are referred to as polyphenols. Polyphenols or phenolic compounds are categorized into two major categories, flavonoids and nonflavonoids. Flavonoids are large polymer molecules involved with wine color and tannins. The various phenolic compounds found in muscadine grapes are: gallic acid, 4 hydroxy benzoic acid, catechin, caffeic acid, epicatechin, p-coumaric acid, ferulic acid, ellagic acid, resveratrol, myricitin, quercetin, and kaempferol. The highest concentration of phenolic compounds found in muscadine grapes are gallic acid and Catechin (Table 2.1). The phenolics in muscadine grapes are shown in Table 2.1. On the average, approximately 65% of grape polyphenols are found in the seeds; 22% in the stems; 12% in the skins and only 1% in the pulp (Vine 2002). The weight of various muscadine grape parts for several common cultivars is shown in Table 2.2. The polyphenol content of seeds may range from 5 to 8% by weight (Polyphenolics Inc 2003).

Biological Importance of Wine and Grape Components

In 1991, 60 minutes CBS News presented a scientific report entitled "The French Paradox" It showed epidemiological data that compared dietary intakes and disease incidences in various countries including Britain, France, United States and others. Renaud and De Lorgeril (1992) reported that red wine consumption offered a degree of protection from atherosclerosis and cardiovascular diseases in the French population. The report revealed that according to the MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) data, the French population had lower death rates related to atherosclerosis. The death rates from the 13 coronary vascular diseases (CVD) were much lower in the French population in spite of having dietary food consumption patterns similar to that of many developed nations that had high incidences of CVD. Coronary heart disease can be defined as atherosclerosis of the coronary arteries. Atherosclerosis results in abnormally thickened regions called plaques on the vascular wall. As

plaques develop, they narrow the arteries and decrease blood supply, which causes damage to the heart and brain. A clot may form on the abnormal surface of the plaque region and block the artery. This may eventually lead to a heart attack or stroke (Stanley and Mazier 1999). Scientists postulated that the polyphenolic compounds found in red wine acted as a source of dietary antioxidants that reduced the risk associated with the development of CVD (Kinsella and others 1993). Studies have shown that high dietary intakes of cholesterol and saturated fats are directly associated with risk factors related to the development of coronary heart disease (German and Walzem 2000; De Lorimier 2000).

Numerous epidemiological studies have documented that a reduced risk of coronary heart disease usually accompanies regular consumption of a moderate level of ethanol. It has also been recently reported that ethanol consumption reduces the risk of development of adult onset or Non Insulin Dependent Diabetes Mellitus (NIDDM) (Bisson and others 1995). In recent years, understanding the "French Paradox" has stimulated new research interest to investigate whether polyphenolic antioxidants may offer protective effects beyond the cardiovascular system, and whether polyphenols from other botanical sources may similarly offer beneficial effects to human health. Studies with resveratrol, an important component of grape polyphenols, also show protective effects on neuron cell death induced by ethanol and other oxidative agents (Sun and others 2002).

Functional Foods

Functional foods is a term that is usually applied to foods that have been modified or combined in order to enhance the health benefits but may include any food that naturally possesses components with demonstrable pharmacologic activity. The concept of functional foods differs in different countries for historical, cultural and regulatory reasons. These foods

provide functions in addition to its nutritional value and help in enhancing the physiological and cognitive functions of the human body. It is assumed that a functional food should be consumed in a way a natural food ingredient is consumed in order for it to be considered a functional food ingredient. If the ingredients are incorporated into pills, sachets, or other dosage forms they are considered as dietary supplements or nutraceuticals. The International Life Sciences Institute of North America (ILSI NA) defines such foods as those that provide a health benefit beyond basic nutrition through the presence of physiologically active food components. Health Canada considers functional foods as "similar in appearance to a conventional food, consumed as part of the usual diet, with demonstrated physiological benefits, and/ or to reduce the risk of chronic disease beyond nutritional functions." The Institute of Medicine of the US National Academy of Sciences defines it as "those in which the concentrations of one or more ingredients has been manipulated or modified to enhance their contribution to a healthful diet". There is an increasing awareness of connection between diet and health and along with soaring health care costs; both consumers and governments have great interest in capitalizing the benefits of functional foods for health promotion. Though there is no standard definition of functional food the guidelines state that it is a food which provides health benefits beyond that provided by basic nutrition (Galland 2005). The various terms associated with functional foods and their definitions are listed in Table 2.3. There is no universally accepted definition of functional foods but considering their market value and bioactivity they could be placed between clinical foods and pharmaceutical products as shown in fig 2.1 (Korhonen 2002).

Microencapsulation

Microencapsulation has been employed by the food industry and food scientists to incorporate food ingredients in food systems for more than 60 years. Microencapsulation is

defined as the technology of packaging solids, liquids, or gases in miniature, sealed capsules that can release their contents at controlled rates under specific conditions (Shahidi and Han 1993). Shahidi and others (1993) said that the miniature packages called microcapsules may range in diameter from a few micrometers to several millimeters.

The architecture of microencapsulation is generally divided into several arbitrary and overlapping classifications (fig 2.2). One such is matrix encapsulation in which the wall is surrounded by a structure of uniform thickness. There is a single core in each matrix wall. The other is multicore encapsulation where a number of cores are encapsulated in the same matrix.

Microencapsulation is essential in the delivery of essential flavor oils (Porzio MA 2007). For example: a dried herb ingredient that retains the color and flavor character of the fresh herb can be prepared using a patented encapsulation process; a fruit-flavored dry drink mix requires incorporating and stabilizing the volatile flavor chemical acetaldehyde as a "freshness" top note; an injectable flavor oil with controlled-release properties for application in marinades usually requires encapsulation in the form of a cross-linked complex coacervate that forms a membrane to surround and protect the liquid flavor droplet; stable flavored vinegars to complement a product line of flavor oils; a stable lemon-flavor powder for use in cake and pudding mixes can be delivered in the form of β-cyclodextrin and lemon oil; thermally stable flavors; and flavor masking .

Microencapsulation Techniques

There are several alternatives by which microencapsulation can be achieved which is shown in Table 2.4; a brief description of each of these techniques is described below.

Spray Drying

Spray drying encapsulation technology has been used in food industry since late 1950 to provide protection to oils against oxidation. Spray drying is the most commonly used encapsulating technique in the food industry because it is economical; flexible; and produces particles of good quality (Desai and Park 2005). In this method the material for encapsulation is homogenized with the carrier material. The mixture is then fed into the spray dryer and atomized with a nozzle or a spinning disc (fig 2.3). Water is evaporated by the hot air contacting the atomized material. The microcapsules are then collected after they fall to the bottom of the drier. The picture of spray dryer used in the study is shown in fig 2.4. The advantages and disadvantages of spray drying are shown in Table 2.5 and spray drying parameters are shown in Table 2.6 respectively.

Spray Cooling or Spray Chilling

Spray chilling or spray cooling is a variation of the normal spray drying process. In spray cooling the coating material is some form of vegetable oil or its derivative. However a wide range of other encapsulating materials may be used like fat and stearin with melting points of 45-122°C (Desai and Park 2005). In spray-chilling the coating material is typically a hydrogenated or fractionated vegetable oil with a melting point in the range of 32-42 °C (GEA Process Engineering Inc).

Spray cooling finds applications in the chemical, food, and pharmaceutical industries. It is a most convenient method of transforming melted feedstocks into free-flowing particulates of controlled particle size.

Fluidized Bed Coating

Fluidized bed coating is being increasingly used by the food industry for the encapsulation of the functional food ingredients. This process provides a wide variety of encapsulated versions of food ingredients and additives. In this method solid particles are suspended in a temperature and humidity controlled chamber of high-velocity air where the coating material is atomized (Desai and others 2005). This technique is available for hot melt coatings like hydrogenated vegetable oil, stearines, fatty acids and emulsifiers and waxes, or solvent based coatings such as starches, gums, and maltodextrins.

Fluidized bed coating was developed by W.E. Wurster and hence the term "Wurster process". The different methods for fluidized bed coating are: 1) top spray, 2) bottom spray, and 3) tangential spray.

Fluidized bed technique is used to encapsulate nutritional substances such as vitamin C, B vitamins, ferrous sulfate, ferrous fumarate, sodium ascorbate, potassium chloride, and a variety of vitamin/mineral premixes. These encapsulated products are used as nutritional supplements. In the case of bakery products, it is also used to encapsulate the leavening ingredients, as well as vitamin C, acetic acid, lactic acid, potassium sorbate, sorbic acid, calcium propionate, and salt. In the meat industry several food acids have been fluid-bed encapsulated to develop color and flavor systems. They are also used to achieve a reproducible pH in cured meat products and to shorten their processing time. Fluid-bed encapsulated salt is used in meats to prevent development of rancidity, as well as premature set due to myofibrilar binding (Dewettinck and Huyghebaert 1999).

Extrusion

Extrusion, as it is applied to flavor encapsulation, is a relatively low temperature entrapping method, which involves forcing a core material in a molten carbohydrate mass through a series of dies into a bath of dehydrating liquid. The pressure and temperatures used are <100 psi and 115°C, respectively. The coating material hardens on contacting the liquids, forming an encapsulating matrix to entrap the core material. Then the extruded filaments are separated from the liquid bath, dried, and sized (Desai and others 2005).

The primary benefit of this method is that the material is completely surrounded by the wall material (true encapsulation), and any residual oil or core material is removed from the surface in an alcohol bath. This method provides excellent stability to oxidation and prolongs the shelf life of the product by 1-2 years without any substantial quality degradation. This method may be also classified as a glass encapsulation system or a controlled-release system, depending on the polymeric substances used (Desai and others 2005).

Centrifugal Extrusion

Centrifugal extrusion is a liquid co-extrusion process utilizing nozzles consisting of concentric orifices located on the outer circumference of a rotating cylinder (head). The encapsulating cylinder or head consists of a concentric feed tube through which coating and core materials are pumped separately to the many nozzles mounted on the outer surface of the device. While the core material passes through the center tube, coating material passes through the outer tube. The entire device is attached to a rotating device such that the head rotates around its vertical axis. Centrifugal force impels the rod outward, causing it to break into tiny particles. By the action of surface tension the coating material envelopes the core material. Centrifugal

extrusion has been used to encapsulate flavorings, seasoning, and vitamins (Desai and others 2005).

Lyophilization

Lyophilization or freeze drying is a process which has been utilized for the dehydration of freeze dried aromas. It has a disadvantage in that it requires a long time for freeze drying (up to 20 hrs). Freeze drying is a costly operation but can be for encapsulating high value ingredients. Heinzelmann and Franke (1999) showed that the production of dried microencapsulated fish oil by freezing and subsequent freeze drying resulted in product with good resistance to oxidation. The retention of volatile components during freeze drying is dependent on the nature of the system.

Coacervation

Coacervation consists of separation of a liquid phase of coating material from a polymeric solution followed by the coating of that phase as a uniform layer around suspended core particles. The coating is then solidified. The batch type process consists of three stages:

1. Formation of a three-immiscible chemical phase
2. Deposition of the coating
3. Solidification of the coating

A large number of coating materials have been evaluated for coacervation like gelatin/gum acacia system, gliadin, heparin/gelatin, carrageenan, chitosan, soy protein, polyvinyl alcohol, gelatin/carboxymethycellulose, β-lactoglobulin/gum acacia, and guar gum/dextran (Desai and others 2005). The coacervation method has some drawbacks in that it is expensive and complex and the cross linking of the wall material usually involves glutaraldehyde which

must be carefully controlled and used per the country's legislation. The problems related to harmful chemical cross-linkers could be solved by using enzymatic cross-linkers.

Centrifugal Suspension Separation

Centrifugal suspension is a more recent microencapsulation process which involves mixing the core and the wall materials and then adding to a rotating disk. It is used for foods that are sensitive or readily absorb moisture like aspartame, vitamins, or methionine (Desai and others 2005).

Co-crystallization

Co-crystallization is a new method which utilizes sucrose as a coating material for encapsulation of the core materials. The sucrose syrup is concentrated to a supersaturated state and then maintained at a high enough temperature to prevent crystallization. A predetermined amount of core is then added to the syrup and then agitation is continued vigorously for the sucrose mixture to crystallize. As the syrup reaches the temperature at which transformation and crystallization takes place, a large amount of heat is liberated. Agitation is continued in order to promote and extend transformation/crystallization until the agglomerates are discharged from the vessel. The encapsulated products are then dried to the desired moisture and screened to a uniform size, which are required to control the rate of nucleation and crystallization as well as the thermal balance during various phases (Desai and others 2005).

The advantage of this technique is that it can be used to achieve particle drying. Products offer direct tableting characteristics because of their agglomerated structure and therefore offer significant advantages to the candy and the pharmaceutical industries.

Liposome Entrapment

Liposomes consist of an aqueous phase that is completely surrounded by a phospholipid-based membrane. When phospholipids such as lecithin are dispersed in an aqueous phase, the liposomes form spontaneously. Liposomes have been used for the delivery of vaccines, hormones, enzymes and vitamins. As per study carried out by Kheader and others (2000), liposome entrapped enzymes could be used for flavor entrapment in cheese and accelerated flavor development to prevent the problems associated with use of free enzymes. The main issues for liposome encapsulation for food industry are the scaling up of the microencapsulation process at an acceptable cost in use levels and delivery form of the liposome encapsulated ingredients.

Inclusion Complexation

Molecular inclusion is another means of achieving encapsulation. It takes place at a molecular level and β-cyclodextrin is used as the encapsulating medium. β-cyclodextrin is a cyclic derivative of starch made up of seven glucopyranose units. They are partially prepared from starch by an enzymatic process. The outer part of β-cyclodextrin is hydrophilic while the interior part is hydrophobic. The guest molecules can be entrapped in the apolar internal cavity which is 0.65nm in diameter which permits inclusion of essential oil compounds and can take one or more flavor volatile molecules (Desai and others 2005). There are three methods of inclusion complexation using β-cyclodextrin, flavor molecule and water. It has been found that the one using less water leads to better entrapment of flavor molecules.

Coating Materials

There are a variety of coating materials available in the market for coating of flavors, light sensitive materials, antioxidants against degradation by environment. Various materials

commonly used for coating functional food additives is given in Table 2.7. Here are a few criteria for selecting the ideal coating materials listed by Shahidi and Han (1993).

- Functionality that the encapsulated ingredients provide to the final product.
- The type of coating material to be selected from the large variety available.
- Processing conditions the encapsulated ingredient must survive before releasing its contents.
- The optimum concentration of the active material in the microcapsule.
- Mechanism by which the ingredient be released from the microcapsule.
- The particle size, density, and stability requirements for the encapsulated ingredients.
- The cost constraints of the encapsulated ingredients.

There is a direct relationship between homogenization degree and retention of oil during spray drying. Therefore, it appears advantageous to efficiently homogenize the feed material. Water soluble materials may be encapsulated by homogenization. There is no clear boundary between the core and the coating here and we have a homogenous blended matrix of the material. Following is a brief description of some of the commonly used coating materials.

Gum Arabic

Gum Arabic, a natural gum also called gum acacia, is a substance obtained from two sub-Saharan species of the acacia tree, *Acacia senegal* and *Acacia seyal*. It is used primarily in the food industry as a stabilizer, but has had more varied uses in the past, including viscosity control in inks. Analytical data for gum obtained from acacia Senegal is in Table 2.8 (Verbeken and others 2003). Its E number (E numbers are number codes for food additives and are usually found on food labels throughout the European Union) is E-414.

The gum produced by the trees in question reseals the plant's bark in the event of damage - a process called gummosis. Gum arabic is a complex mixture of saccharides and glycoproteins, which gives it one of its most useful properties: it is completely edible. As compared to other chemicals Acacia Gum is resistant to various physico-chemical conditions (especially acidic conditions). This makes it a good choice for microencapsulation of muscadine grape juice having an acidic pH. It was used for microencapsulation of camu-camu (Myrciaria dubia) juice by Taxi and others (2003) by spray drying, using gum arabic as the coating material, the optimum conditions for juice yield and Vitamin C retention were established as 15% wall material; air entry temperature of 150°C; resulting in a yield of more than 24% and vitamin C retention of 6%. Gum arabic used in food has to be in accordance with FDA code of federal regulations as shown in Table 2.9 (Verbeken and others 2003). The structure of gum arabic is shown in fig 2.5. Gum arabic is composed of several simple sugars, galactose, arabinose, rhamnose, and glucuronic acids as well as a protein fraction which play an important role in its stability. Gum arabic is an effective encapsulating agent because of its high water solubility, low viscosity and emulsification properties. It is used in soup and dessert mixes.

Pectin

Pectins are groups of polysaccharides occurring in the cell walls and intercellular layers of all land plants. Native pectin is a mixture of polysaccharides, with the major component a polymer of α-D-galacturonic acid, mainly as the methyl ester and often with some acetyl groups on the hydroxyls at C-2, although some may be on the C-3 hydroxyls. The chemical structure of pectin is shown in fig 2.6. Aqueous solutions of commercial pectin of 2-3% concentration may be easily prepared in warm or hot water. Pectin along with glucose syrup was used to microencapsulate fish oil rich in polyunsaturated fatty acids (Drusch 2006). Physicochemical

parameters like particle morphology, particle size and extractable fat generally represent good microencapsulation efficiency and therefore indicate a good oxidative stability.

Starch

Starch is a carbohydrate that is found in the stems, leaves, fruits, roots of higher plants. Starch functions as the main storage or reserve form of carbohydrate and is second only to cellulose as a reserve form of carbohydrate. Starch is isolated commercially from cereal grain seeds, roots and tubers and stems and pith.

Structure and architecture

Starch which is a polymer of glucose is an alpha-glucan predominantly containing alpha-1, 4-glucosidic linkages with a relatively small amount of alpha-1, 6-glucosidic linkages forming branch points. Two major compounds present in starch are amylase and amylopectin. The structure of starch is shown in fig 2.7.

Microencapsulation

Starch based wall material is commonly for extrusion and spray drying. Starch cannot be used alone for microencapsulation due to its lack of emulsifying capacity except in case of encapsulating water soluble substances. Starch along with other wall materials such as protein is used for encapsulating vitamins, flavors and oils (Forssell and others 2004).

Proteins

Proteins play several important roles in biological and food systems. Some of these include biocatalysts (enzymes), structural components of cells and organs (e.g., collagen, keratin, elastin, etc.), contractile proteins (actin, myosin, tubulin), hormones (insulin, growth factor, etc.), transport proteins (serum albumin, transferrin, hemoglobin), metal chelation (phosvitin, ferritin), antibodies (immunoglobulin's), protective proteins (toxins, and allergens), and storage proteins

(seed proteins, casein micelles, egg albumen) as nitrogen and energy source for embryos (Damodaran and Paraf 1997).

Proteins are the major constituents of the food and they perform several critical functions in food that include thickening, gelation, emulsification, foaming, texturization, water binding, adhesion and cohesion, and lipid and flavor binding and retention. The properties of proteins are also affected by their structural states in the foods. Proteins are made up of amino acids - essential and non essential amino acids. It is essential to take the essential amino acids in our diet as the body cannot synthesize them like isoleucine, leucine, lysine, etc. Proteins have many other functions in the body such as enzymatic catalysts, used as transport molecules and storage molecules, needed for mechanical support (skin and bone containing collagen- a fibrous protein).

Whey proteins

Whey from the cheese industry is obtained in two stages: 1) Soft whey which is obtained from the rennet coagulation of milk 2) Acid whey obtained from fresh soft cheese production. Whey proteins consist of a mixture of several diverse proteins which are responsible for its diverse functional properties. The main proteins are ß-lactoglobulin and α-lactalbumin which are 70% of the proteins and are responsible for the hydration, gelling and surface active properties (emulsifying and foaming properties) of the whey protein ingredients. The uses of whey proteins in food industry are listed in Table 2.10.

Whey Protein Isolate+Lactose

Whey protein isolate (WPI) or soy protein isolate (SPI) in combination with dried glucose syrup (DGS) were tested for stabilization of microencapsulated spray-dried emulsions containing tuna oil, palm stearin, or a tuna oil-palm stearin blend (Augustin and others 2006). Pre-emulsions containing heated (100°C/30 min) protein-DGS mixtures and oils at oil/protein

ratios of 0.75:1 to 4.5:1 were homogenized at two passes (35+10 or 18+8 MPa) and spray-dried to produce 20-60% oil powders. Powder containing Palm Stearin was more stable to oxidation than powder containing 1:1 ratio of palm stearin and tuna oil or tuna oil only. Heated WPI-DGS formulations were superior to corresponding formulations made up of SPI-DGS, producing spray-dried powders with higher micro encapsulation efficiency and superior oxidative stability.

Beristain and others (2004) microencapsulated conjugated linoleic acid (CLA) as free acid using whey protein concentrate (WPC) as a wall material. The encapsulation efficiency was 89.6% and surface oil concentration was 1.77 g/100 g of sample. Microcapsules stored at a_w=0.743-0.898 had good stability against oxidation so WPC is considered as an effective microencapsulating agent.

Analytical testing

Quantification of polyphenolics

There are numerous spectrophotometric methods available for quantification of polyphenolic compounds. These methods are based on different principles and are specific for different functional groups.

Spectrophotometric assays

The Folin-Denis Assay is the most widely used assay for total phenolic. The principle of this assay is reduction of phosphomolybdic-phosphotungstic acid (Folin Denis) to a blue colored compound by polyphenol compounds. Most of the researchers have used Folin-Ciocalteaux method (Singleton 1965) which is based on the same principle as above. The Folin-Ciocalteau reagent gives greater color than Folin-Denis reagent with all phenols and particularly the less responsive ones, but it also gave slightly less color in proportion to that from Folin-Denis for the possibly interfering reductants ascorbic acid, ferrous ion, and sulfur dioxide. These effects are attributed to the relatively high content of active oxidant in the Folin-Ciocalteau preparation

which promotes the more complete oxidation measurement of the slowly reacting phenols. The interfering substances are less limited to alkaline reaction conditions and are more easily oxidized than some phenols, which probably explain their disproportionate reaction with the weaker Folin-Denis reagent.

Giusti (2001) used the pH differential method to determine total anthocyanins. The principle of this method is dependent on the structural changes of anthocyanins due to pH change. It is observed that colored oxonium and colorless hemiketal form dominate at pH of 1.0 and 4.5, respectively. The absorbance of the solution is measured at two different wavelengths (510 and 700nm). The monomeric anthocyanin pigments is calculated as follows

$$Absdiff = [A_{510} - A_{700}]_{pH1.0} - [A_{510} - A_{700}]_{pH4.5}$$

Monomeric anthocyanins pigment (mg/L) = (A × MW × DF × 1000)/(e × 1)

Where MW = molecular weight of cyanidin–3 glucoside (449.2).

e = molar absorptivity (26,900)

DF = dilution factor

A = Absdiff

The other methods for determination of antioxidants are FRAP (Ferric Reducing Antioxidant Power), ORAC (Oxygen Radical Absorbance Capacity), TEAC (Trolox Equivalent Antioxidant Capacity), HPLC (High Performance Liquid Chromatography).

Microencapsulation Efficiency

Microencapsulation efficiency is defined as the proportion of core that could not be extracted by the solvent under test conditions (Young and others 1993).. The retention of core material and final content of core in the dry microcapsules is of great practical significance and

are therefore important determinants when wall materials or microencapsulation processes are being evaluated.

$$\text{Microencapsulation efficiency} = \frac{\text{Total Phenol-Extractable Phenol}}{\text{Total Phenol}} \text{ X } 100$$

Among the most important variables affecting the retention of the core are the choice and concentration of wall solids, the mass ratio of wall to core, physiochemical properties of wall and core components, and physical properties of the emulsion prior to drying, and drying conditions. High drying rates that lead to a rapid formation of crust around the drying droplets favor high retention as long as no structural damage is introduced.

Physical properties of spray dried particles

Particle size and shape

Particle size strongly influences the appearance, flowability, dispersability of a powder and is an important issue in ready to use food powders. The agglomeration step during spray drying facilitates in having a powder with better reconstitution and dispersion. Two kinds of spray dryers are commonly used, one is a nozzle atomizer and other is disc atomizer. In nozzle atomization particle size is determined by the nozzle orifice and the spray pressure as well as the physical properties of the matrix to be dried like viscosity and solids concentration. For spray dryers using a disc atomizer, particle size is determined by atomizer speed and physical properties of matrix to be dried (viscosity and solids concentration). Particle size is also influenced by the drying temperatures (Reineccius 2004). Fast drying results in setting up a structure early on and does not allow particles to shrink as we observe in slow drying. If the infeed solids level is high then particles dry quickly. It is advisable to have operating conditions

setup so as to obtain spherical particles because they have better aroma retention, high bulk densities and best flow ability.

Absolute and bulk densities

Absolute density is defined as the weight of a given particle volume of powder while bulk density is defined as the weight of a given total volume of powder. Absolute density is determined by He pycnometry and considers volume of particles not permeable to displacing gas. Bulk density may be determined by taking a given weight of powder, filling it in graduated cylinder and tapping it a fixed number of times or until it reaches a fixed volume (Reineccius 2004). Absolute density is primarily influenced by amount of entrapped air in the powder matrix during atomization process, steam formation in particle during drying, drying air temperature, particle size, atomization conditions and infeed matrix. Higher feed solids content increases particle absolute density. Bulk density is important in packaging and shipping as it is an indication of how much product will fill in a package and determines the packing and shipping cost.

Flowability

Flowability is an important characteristic in the processing and packing operation as it effects powder performance. For measuring flowability one can allow the powder to flow through the funnel and measure the angle of the powder pile above horizontal also known as the angle of repose (Reineccius 2004). A smaller angle of repose is desirable to have better flowability.

Dispersability

Dispersability is important in the manufacture or final reconstitution of powder. Generally dispersability is primarily influenced by particle size, density and the carrier matrix;

low density powder particles are difficult to disperse (Reineccius 2004). The problem of dispersability can be overcome by instantizing the process whereby you rewet the powder in the fluidized bed and allow the powder to agglomerate and redry.

Structural Strength

Structural strength assumes importance when dry blending of powders with salt or sugar or when the active material of a microcapsule is a liquid or soft paste. The dry blending process is very abrasive and will crack or damage powders high in liquid actives or those that are low in density (Reineccius 2004). Usually it is advisable to have 20% core when spray drying flavor ingredients as higher core ratios usually lead to loss of flavor and the particle may lose structural strength and break (Reineccius 2004).

Filtration

Filtration is the process of separating two or more components in a fluid stream or passing a mixture of a fluid containing suspended or dissolved solids through a porous medium that entraps a portion of the solids in its matrix or retains them on its surface (dead-ended filtration) or rejects the solids and allows them to continue along with a circulating feed stream (cross-flow filtration) (LCI Corporation 2006).

Often the filtration process is used to clarify or purify the material passing through the barrier and other times the filter barrier is used to collect or concentrate the material that does not pass through; the recovery of enzymes or the washing of modified starch in wet corn milling are good examples.

Membrane Filtration

Membrane separation systems are used for a wide variety of applications in a broad cross-section of industries, including power, waste treatment, textile, grain, food and beverage, pharmaceutical, biotechnology and many others.

The membrane's function is to separate or reject particles of various sizes. Depending upon the intended use, membranes may be produced in many different configurations: cartridges, plate and frame devices, tubes, disks, etc. Commonly used membrane substrate materials include polymeric compounds such as polysulfone, TFE fluorocarbon, polyamide, polyethylene and polyacrylonitrile. Cellulose acetate is also used, as are certain ceramics, carbon and stainless steel.

Scepter Membrane system

The Scepter system has a porous stainless steel tube of 1 inch diameter at its heart. These tubes may be used singly for small scale applications or for pilot systems. Scepter's tubular filtration technology, combined with the process compatibility, stainless steel construction, allows processing of a wide variety of streams, including dirty and/ or hostile fluids, over a broad range of chemical conditions, pressures and temperatures. When used in cross-flow mode, the rugged large-diameter tubes allow for high velocity in the tubes even with very dirty (having high solids content) process fluids. This maximizes "sweeping" action at the membrane surface and minimizes foulant buildup (LCI Corporation 2006). The large diameter tubes also give the Scepter cross-flow systems the ability to handle extremely viscous process feeds (up to 100,000 centipoises), and complex mixtures of solutes and suspended particles (up to 50%, depending on the fluid).

Scales of Membrane Filtration

Depending on their performance characteristics, particularly their nominal pore size, membranes used in cross-flow filtration devices are placed in one of the four categories; microfiltration (MF), ultra filtration (UF), nanofiltration (NF) or reverse osmosis (RO). Because of the barrier surface formed by the rejected material, referred to as a polarized layer, boundary layer, or gel layer and the "parallel to pore" flow, cross-flow filters often reject particles smaller than the nominal opening in the filter media (LCI Corporation 2006).

Microfiltration

Microfiltration (MF) is used to separate suspended solids from dissolved substances in a process stream, or to concentrate fine colloidal suspensions. Microfiltration membranes are the most open of the four membrane filtration categories. Generally MF membranes separate or reject particles from about 0.05-0.1 micron to about 1 micron. On a molecular weight basis, these membranes can separate/reject macromolecules in the 100,000 to 500,000 MW ranges. Water, dissolved solids, and macromolecules generally less than 100,000 MW can pass through the membrane (LCI Corporation 2006).

The separation mechanism of microfiltration is commonly attributed to geometry; i.e., passage through the membrane is a function of particle size relative to opening or pore dimensions of the membrane.

Cross-flow filtration devices using MF membranes usually operate at low trans-membrane pressure (the pressure difference from one side of the membrane and the other), 0.7 to 3.4 bar (10 to 50 psig), to limit flow through the membrane. Increasing flow initially increases flow through the membrane; however, because of mass transfer phenomena, higher pressures do not result in increasing permeate flow. It is possible to influence this plateau by intensifying the

sweeping action by increasing the cross-flow velocity. The microfiltration unit being used rejects particles anywhere in the range of 0.05-0.1 micron range.

There are three basic configurations used in commercial applications of cross-flow microfiltration: single-pass, batch and feed and bleed (fig 2.8).

I. ***Single –Pass Operation***

In the single-pass configuration, the feed is pumped through the membrane unit and the retentate is collected or fed to a subsequent processing step. This is the simplest process configuration, and it can be used for both batch and continuous processing (Zemen and Zydney (1996). The single-pass system is used extensively for the reverse osmosis where the products permeate (pure water) and retentate which is of low economic value is easily disposed of.

II. ***Batch***

In batch filtration process, the entire retentate stream is recycled back to a single large feed tank, allowing the permeate removal per pass to be dramatically smaller than in a single-pass system. The concentration of the retained solutes/particles in the feed tank increases with time as permeate is removed, causing a continual decline in filtrate flux due to the increase in the bulk solute concentration (Zemen and Zydney 1996). This processing protocol will be used in current operation.

III. ***Feed-and-Bleed***

The feed-and-bleed configuration is used in almost all large-scale continuous membrane processes. Part of the retentate stream is recycled back to the unit to obtain the desired flow rate, Q, and thus the desired mass transfer coefficient, k0. The rest of the retentate is continuously fed to any subsequent processing steps or to an appropriate holding tank. The membrane area required for feed-and-bleed configuration is about three times that required for batch filtration.

The very large membrane required for feed-and-bleed configuration is due to the fact that the entire system operates at the final Retentate concentration, corresponding to the lowest filtrate flux, throughout the filtration. In contrast, the batch filtration begins at a relatively low bulk solute concentration, and thus at a relatively high filtrate flux, and only attains the final bulk concentration towards the end of the process (Zemen and Zydney 1996).

Extraction Processes and Technologies for Phenolics from Plant Material

During the extraction process, the solvent used for extraction is mixed with the plant material. The extraction is completed by addition of solvent to the sample and this extract has to be completely dried to powder form. After the solvent has been added SFE (Supercritical fluid Extraction) can be used to obtain the powder without final drying or spray drying can be used. There are various methods for extraction and concentration of polyphenolic compounds from plant material (Nawaz and others 2001) such as:

1. Solvent Extraction
   a. Hexane and methanol
   b. Ethanol and benzene
   c. Hot water
   d. Water and ethanol
   e. Water and methanol
   f. Ethyl acetate and water
   g. Sulfur dioxide and water
2. Microwave-assisted extraction
3. Polyphenolic Concentration and Purification by Membrane Technology
4. Polyphenol Concentration by Evaporation and Drying

Membrane extraction of polyphenolics

Zaid and others (2006) carried out nanofiltration on apple juice concentrate using size 1 and 0.25 kDa molecular weight cut-off (MWCO) spiral wound membranes. For the 1 kDa MWCO membrane, the concentration of polyphenolic on the retentate side increased by a factor of up to 4 and the sugar concentration increased by 1.5 times. When nanofiltration was performed on permeate collected using 0.25 kDa membrane the concentration of phenolic compounds increased by a factor of 2 on retentate side. This indicates that membrane separation is an efficient and cost effective technology to separate phenolics from fruit juice to be used as a functional ingredient.

Nawaz and others (2006) used a solvent extraction method utilizing 50% ethanol and 50% water as solvent when ultrafiltration was used for the extraction of polyphenols from grape seeds. With the concentration step of UF the procedure led to high extraction rates, shorter extraction time and significant labor savings.

Velic and others (2007) used different solid-liquid ratio, four particle sizes and different temperatures to check extraction kinetics of polyphenols from grape seeds. Temperature, solid–liquid ratio and milling degree had a positive influence on extraction rate and extent of extraction. The amount of total polyphenols extracted was 1.47-6.68% per dry matter of grape seeds in 200 minutes.

Kammerer and others (2005) used enzymatic hydrolysis of grape skins, using pectinolytic and cellulotic enzymes, enzyme-substrate ratio and time temperature regime. It was observed that the extraction of polyphenols was increased by treatment of pomace with hot water and cell wall degrading enzymes.

Rektor and others (2004) used membrane filtration for must preservation and concentration. They used microfiltration and reverse osmosis on white and red grape juice samples which gave a concentrate with high sugar content.

Spigno and others (2007) performed extraction of polyphenolics from grape marc with different concentrations of solvent (ethanol), different temperatures and different extraction temperatures. They found that phenols content increased for water content of ethanol from 10 to 30%, remained constant from 30 to 60% water and started decreasing after 50%. The extraction rate at 60°C was higher compared to 45°C.

Vaillant and others (2005) clarified melon juice from fruits discarded by exporters and concentrated it using osmotic evaporation (OE). This integrated membrane process helped in obtaining two valuable products: a clarified concentrate of melon juice which had not undergone any thermal treatment and a retentate rich in provitamin A.

Usually ethanol and water as solvents are considered to be safe for human consumption. The present work will use water as solvent and enzyme (pectinase) for extraction of polyphenolic compounds from grape pomace. It will use membrane filtration for concentration of phenolics from muscadine pomace.

Sample Storage for Shelf Life Determination

Shelf life is a multifaceted property that is important to the food processors and manufacturers as well as the consumers. Shelf life is defined as (IFST 1993) "the period of time during which the food product will

a. Remain safe.
b. Be certain to retain its desired sensory, chemical, physical, microbiological and functional characteristics.

c. Where appropriate, comply with any label declaration of nutrition data, when stored under the recommended conditions".

In physical chemistry, chemical kinetics or reaction kinetics is the study of reaction rates in a chemical/biochemical reaction. Analyzing the influence of different reaction conditions on the reaction rate gives information about the reaction mechanism and the transition state of a chemical reaction and thus can depict quality changes in foods.

Kinetics deals with experimental determination of reaction rates from which a rate law and reaction rate constant are derived. Essential rate laws exist for zero order reactions (for which reaction rates are independent of initial concentration), first order reactions, second order reactions and can be derived for others through calculus. In consecutive reactions, the rate-determining step often determines the kinetics. In consecutive first order reactions, a steady state approximation can simplify the rate law. The activation energy for a reaction is experimentally determined through the Arrhenius equation and the Eyring equation. The main factors that influence the reaction rate include: the physical state (solid, liquid, gas) of the reactants, the concentrations of the reactants, the temperature at which the reaction occurs, and whether or not any catalysts (enzymes) are present in the reaction.

The rate of food quality change may be expressed as a combination of composition and environmental factors (Saguy and Karel 1980):

$DQ/dt = F(C_i, E_j)$

where Ci are compositional factors such as concentration of reactive compounds, inorganic catalysts, enzymes, reaction inhibitors, pH, water activity and microbial activity and Ej are environmental factors like temperature, relative humidity, total and partial pressure of gases, light and mechanical stress.

Kinetic studies on the degradation of beetroot pigment encapsulated in three different polymer matrices (pullulan and two maltodextrin samples) were carried out by Serris and Biliaderis (2001) under different water activity and storage temperatures. The highest amount of rate constants for degradation of pigments were observed at intermediate water activity level (a_w=0.64) for all matrices and all three storage temperatures studied. The reaction rates (K) increased with increase in temperature. This trend can be explained by the fact that the degradation reaction is controlled by the mobility of the reactants. This was also observed by Tandale (2007) which led to selecting a_w of 0.64 instead of using multiple water activities. The photodegradation of phenol was studied by Maleki and others (2006) in a batch reactor configuration illuminated with a 400 W medium pressure mercury lamp. The effects of parameters such as pH, kinetic constants and initial phenol concentration on the photolytic degradation and toxicity assay were studied. The experimental results showed that lower pH and lower concentration of phenols favor the phenol degradation. The disappearance of phenol in each case approximately followed first-order kinetics with the apparent first-order decay constant increasing with decreasing solute concentration. Kowska and others (2004) studied the effects of UV irradiation, temperature and storage on the stability of anthocyanins copigment complexes. They reported an increase in copigmentation with copigment content, and a decrease in the stability of copigment complexes (greater than heating at 80 C). Direct sunlight has a negative effect on copigment complexes. These studies indicated that phenol is degraded by light exposure which led to choosing of UV light conditions in experiment.

The majority of reactions that have been studied are pseudo-zero order or pseudo-first order reactions (Labuza 1984). Some examples of zero and first order reactions are shown in the Table 2.11. To determine the reaction order one assumes different values of m (0, 1 or other)

and tries out a graphical or a least square linear fit to the corresponding equations (Table 2.12) of the experimental data. If the experiment has been carried out to at least 50% conversion or preferably 75%, it is easy to determine which reaction order and equation gives the best fit, either graphically or using statistical goodness. The coefficient of determination (R^2) is often a useful criterion. Once the apparent order of the quality deterioration reaction has been decided, further statistical analysis and statistical evaluation of the parameter k, the rate constant is required, to get an estimate of the error in the determination of k (Labuza and Saguy 1997). If a linear regression is used to estimate the parameters, their 95% estimation can be obtained using the student t distribution.

Table 2.1: Phenolic in muscadine grapes (milligrams per 100 g of fresh whole fruit)[a]
(Bonilla and others 2003)

Cultivar	ellagic acid	myricetin	quercetin	kaempferol	resveratrol	epicatechin	catechin	gallic acid	% skin	% seeds	% pulp
bronze											
Carlos	6.4	6.3	0.4	0.1	0.1	71.8	86.1	0.6	32.3	6	61.6
Early Fry	7	5.8	0.6	0.1	0.1	32.4	19	0.1	35.7	2	62.3
Fry	5.7	1.8	1.1	0.4	0.1	33.1	6.4	0.1	43.3	1.8	54.9
Summit	5.4	4.2	1.8	1.4	0.1	6.9	5.4	0.1	45.8	1.5	52.7
Late Fry	9.9	5.6	0.4	0.1	ndb	74	19.9	0.4	46.7	3.9	49.4
av	6.8	4.7	0.9	0.4	0.1	43.6	27.4	0.3	40.8	1.9	56.2
SD[c]	1.8	1.8	0.6	0.6	0	28.7	33.5	0.2	6.4	1.8	5.6
purple											
Paulk	6	0.7	0.7	0.2	ndb	30.4	5.8	0.2	40.7	1.8	57.5
Cowart	7.4	2.2	0.3	0.1	0.1	60.3	17.7	0.3	34.2	5.1	60.7
Supreme	3	1	1.4	0.1	0.1	17.1	5.1	nd	47.8	1.1	51.1
Ison	8.7	2.8	0.5	0.2	0.1	30.9	19.2	0.3	39.1	3.5	57.3
Noble	6.8	2.2	0.2	0.2	0.1	66.6	30.7	1.1	46.2	9.2	44.6
av	6.4	1.8	0.6	0.2	0.1	41.1	15.7	0.4	41.6	4.1	54.2
SD	2.1	0.9	0.5	0.1	0	21.3	10.6	0.4	5.5	3.2	6.4

[a] Values are the average of triplicates. [b] Not detected. [c] Standard deviation.

Table 2.2: Dry matter of muscadine grape fruits and fruit parts (Grams per Gram of Fresh Weight)[a] (Bonilla and others 2003)

Cultivar	Skin	Seed	Pulp	Whole Fruit
Carlos	0.179	0.532	0.137	0.174
Early Fry	0.159	0.562	0.149	0.161
Fry	0.139	0.523	0.144	0.148
Summit	0.165	0.571	0.166	0.18
Late Fry	0.161	0.516	0.152	0.162
Paulk	0.163	0.578	0.146	0.159
Cowart	0.149	0.531	0.121	0.139
Supreme	0.169	0.514	0.137	0.186
Ison	0.182	0.559	0.157	0.184
Noble	0.135	0.596	0.122	0.151

[a] Values are the average of triplicates.

Table 2.3: Definitions of functional foods

Functional Food	Definitions
Edible Plants and Phytochemicals	The consumptions of fruits, vegetables, cereal grains, nuts, seeds are known to enhance the health of human beings and are most wide range of functional foods.
Probiotics and Prebiotics	Probiotics are live microbes that exert health benefits when ingested in sufficient quantities. Prebiotics are nondigestable food ingredients that stimulate the growth or modify the metabolic activity of intestinal tract bacteria that have the potential to improve the health of their human host.
Immune Modulators	Several substances produced by animals and fungi are seen to function as immune modulators. Eg. fish oils, mushrooms, flax seed meal.
Designer Foods	They are the combination of numerous ingredients to achieve a specific set of goals, rather than efforts to uncover the potential health benefits of a single food source. Eg. DHA in infant formulas and sport drinks.

Table 2.4: Various microencapsulation techniques and processes involved in each technique (Desai and others 2005)

Sr. No	Microencapsulation technique	Major steps in encapsulation
1	Spray-drying	a. Preparation of the dispersion b. Homogenization of the dispersion c. Atomization of the in feed dispersion d. Dehydration of the atomized particles
2	Spray-cooling	a. Preparation of the dispersion b. Homogenization of the dispersion c. Atomization of the in feed dispersion
3	Spray-chilling	a. Preparation of the dispersion b. Homogenization of the dispersion c. Atomization of the in feed dispersion
4	Fluidized-bed coating	a. Preparation of coating solution b. Fluidization of core particles. c. Coating of core particles
5	Extrusion	a. Preparation of molten coating solution b. Dispersion of core into molten polymer c. Cooling or passing of core-coat mixture through dehydrating liquid
6	Centrifugal extrusion	a. Preparation of core solution b. Preparation of coating material solution c. Co-extrusion of core and coat solution through nozzles
7	Lyophilization	a. Mixing of core in a coating solution b. Freeze-drying of the mixture
8	Coacervation	a. Formation of a three-immiscible chemical phases b. Deposition of the coating c. Solidification of the coating
9	Centrifugal suspension separation	a. Mixing of core in a coating material b. Pour the mixture over a rotating disc to obtain encapsulated tiny particles c. Drying
10	Co crystallization	a. Preparation of supersaturated sucrose solution b. Adding of core into supersaturated solution c. Emission of substantial heat after solution reaches the sucrose crystallization temperature
11	Liposome entrapment	a. Micro fluidization b. Ultrasonication c. Reverse-phase evaporation
12	Inclusion complexation	Preparation of complexes by mixing or grinding or spray-drying

Table 2.5: Advantages and disadvantages of spray drying

Advantages	**Disadvantages**
Well-established technology	Only water-soluble shell materials with a low to moderate viscosity
Production of large amount of capsules	20-30% core loading
Many shell materials approved for Food use	Oxidation of un encapsulated oil
Variety of particle sizes	Loss of low boiling compounds
Useful for heat sensitive food ingredients	A necessary supplementary agglomeration
Excellent dispersibility of the microcapsules in aqueous media	

Table 2.6: Spray drying parameters

Parameter	**Value**
Inlet temperature	170-190^{0}C
Outlet temperature	90-95^{0}C
Air pressure	4 kg/cm^3
Orifice diameter	2 mm
Atomizer speed, variable up to	50,000 rpm
Feed concentration	20% Total solids

Table 2.7: Coating materials for microencapsulation of functional food additives

Category	Coating materials	Widely used methods
Carbohydrate	Starch, maltodextrins, chitosan, corn syrup solids, dextran, modified starch, lactose, cyclodextrins	Spray and freeze-drying, extrusion, coacervation, inclusion complexation
Cellulose	Carboxymethylcellulose, methyl cellulose, ethyl cellulose, cellulose acetate-phthalate, celluloseacetate-butylate-phthalate	Coacervation, spray-drying, and edible films
Gum	Guar gum, agar, sodium alginate, carrageenan	Spray-drying, syringe method (gel beads)
Lipids	Wax, paraffin, beeswax, diacylglycerols, oils, fats	Emulsion, liposome's, film formation
Protein	Gluten, casein, whey protein isolate, whey protein concentrate, gelatin, albumin, peptides	Emulsion, spray-drying

Table 2.8: Analytical data for the gum obtained from Acacia Senegal (Idris and others 1998)

Parameter	**Range**
Moisture content (%)	12.5-16.0
Specific rotation	-32.7º to -27.0 º
Nitrogen (%)	0.22-0.39
Protein (%)	1.5-2.6
Galactose (%)	39-42
Arabinose (%)	24-27
Rhamnose (%)	12-16
Glucuronic acid (%)	15-16
Equivalent mass (Da)	1,118-1,238

Table 2.9: Maximum usage levels (%) of gum arabic permitted in accordance with the FDA Code of federal regulations (title 21)

Food (as served)	Percentage	Function
Beverages and beverage bases	2.0	Emulsifier and emulsifier salt, flavoring agent/adjuvant, formulation aid, stabilizer/thickener.
Chewing gum	5.6	Flavoring agent/adjuvant, humectant, surface-finishing agent
Confections and frostings	12.4	Formulation aid, stabilizer/thickener, surface-finishing agent
Dairy products analogues	1.3	Formulation aid, stabilizer/thickener
Fats and oils	1.5	Formulation aid, stabilizer/thickener
Gelatins, puddings, and fillings	2.5	Emulsifier, emulsifier salt, formulation aid, stabilizer/thickener
Hard candy and cough drops	46.5	Flavoring agent/adjuvant, formulation aid
Nuts and nut products	8.3	Formulation aid, surface-finishing agent
Quiescently frozen confectionery	6.0	Formulation aid, stabilizer/thickener
Snack foods	4.0	Emulsifier, emulsifier salt, Formulation aid
Soft candy	85.0	Emulsifier, emulsifier salt, firming agent, flavoring agent/adjuvant, formulation aid, humectant, stabilizer/thickener, surface-finishing agent
Other food categories	1.0	Emulsifier, emulsifier salt, flavoring agent/adjuvant, formulation aid, stabilizer/thickener, surface-finishing agent, texturizer

Table 2.10: Uses of whey proteins in human foods

Industrial applications	**Functional properties expected**	**Proteins used**
Bread making	Water holding	WPC or WPC + Caseinate
Biscuit manufacturing	Fat dispersibility	WPI
Breakfast cereals	Emulsion stabilization, Overrun of foam, Gelling properties, Browning, Aroma enhancement	WPI, co precipitates Whey
Pasta	Binding and texturing effect Browning	Co precipitates
Confectionary	Emulsion manufacturing	WPC + hydrolyzed caseinates
Chocolate	Overrun of foam	WPC
confectionary	Browning, aroma, antioxidizing effect	Whey , Co precipitates
Ice cream	Emulsion stability, overrun of foams, gelling properties	WPC + caseinates and total milk proteins
Meat products Delicatessen Meat	Emulsion making Water holding (creamy and smooth texture) Adhesive or binding properties	WPC, WPI alone or in mixture with caseinate
Sauces	Emulsion stability	WPC + caseinates + egg yolk
Soups,Ready-to-eat food	Water holding	WPC + caseinates + whole egg
Milk products(cheese, yogurts, “light” butter)	Emulsion stability, Water holding, Gelling properties	Caseinates, WPC + caseinates, WPI
Alcoholic beverages	Cream stabilization, Cloudy aspect	WPC + caseinates, WPC or WPI
Nutritional uses	Protein intake, Enteral nutrition	Whey, WPC, or WPI, WPC hydrolysates

Table 2.11: Examples of zero and first order reactions

▪ Zero order	Non enzymatic browning
	Overall quality loss of frozen foods
▪ First order	Vitamin loss
	Microbial growth/death
	Oxidative color loss
	Texture loss in food processing

Table 2.12: Quality function form and half life times for different order reactions

Apparent reaction order	**Quality function Q(A)t**	**Half life time $t_{1/2}$**
0	A0-At	A0/ (2k0)
1	ln (A0/At)	$\ln 2/k_1$
2	1/A0-1/At	$1/ (k_2 A_0)$
A_0^{1-m} (m#1)	$1/m-1(A_t^{1-m}-A_0^{1-m})$	$2^{m-1}-1/k_m (m-1)$

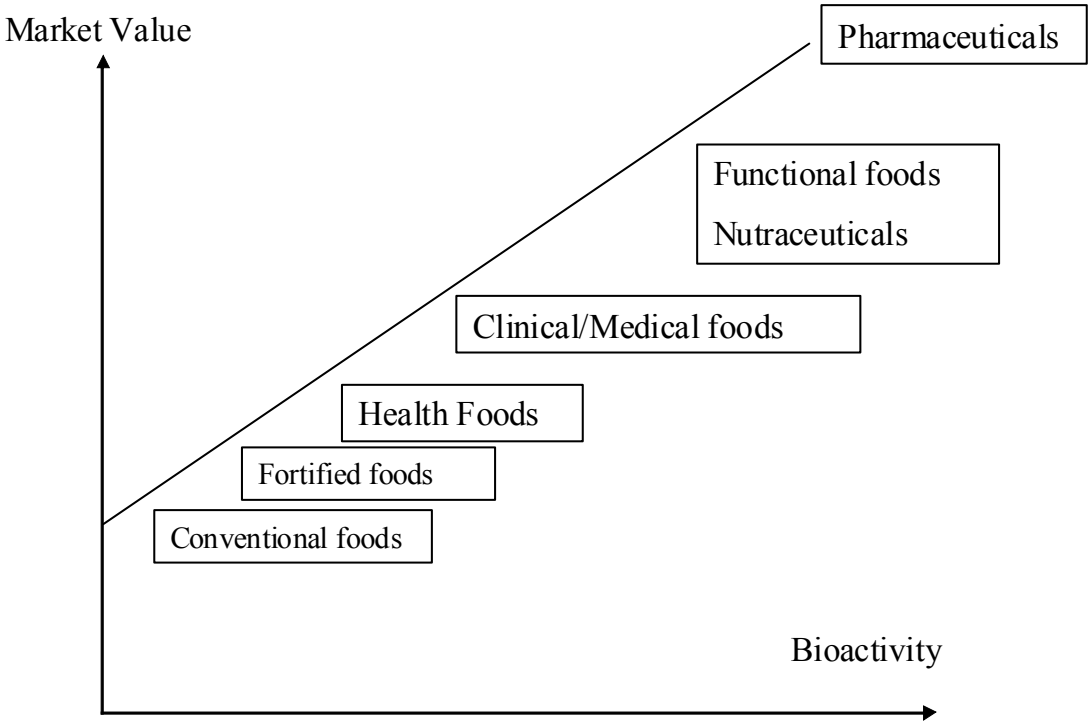


Figure 2.1: Positioning of food categories and pharmaceuticals in relation to bioactivity and market value (Korhonen 2002)

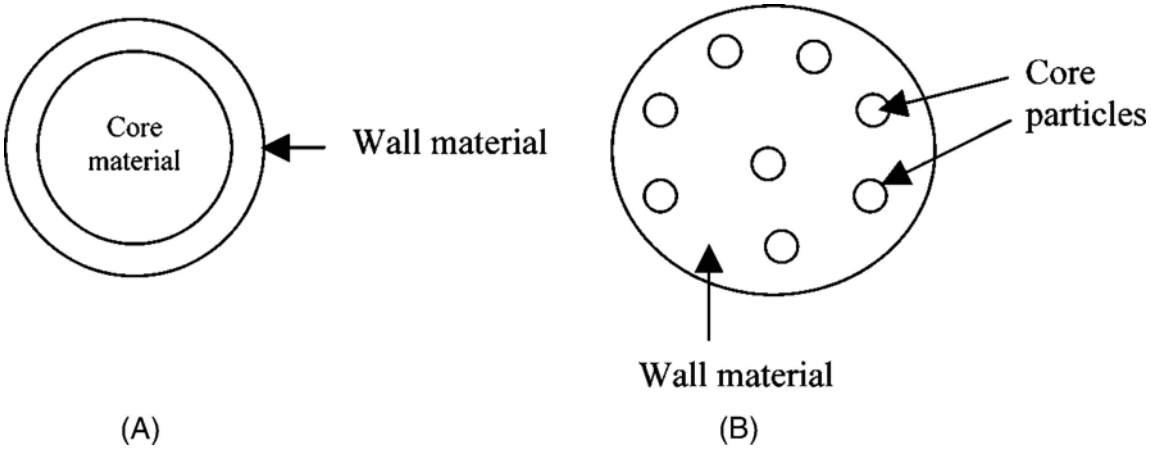


Figure 2.2a: Schematic representation of two types of microcapsules (Desai and Park 2005)

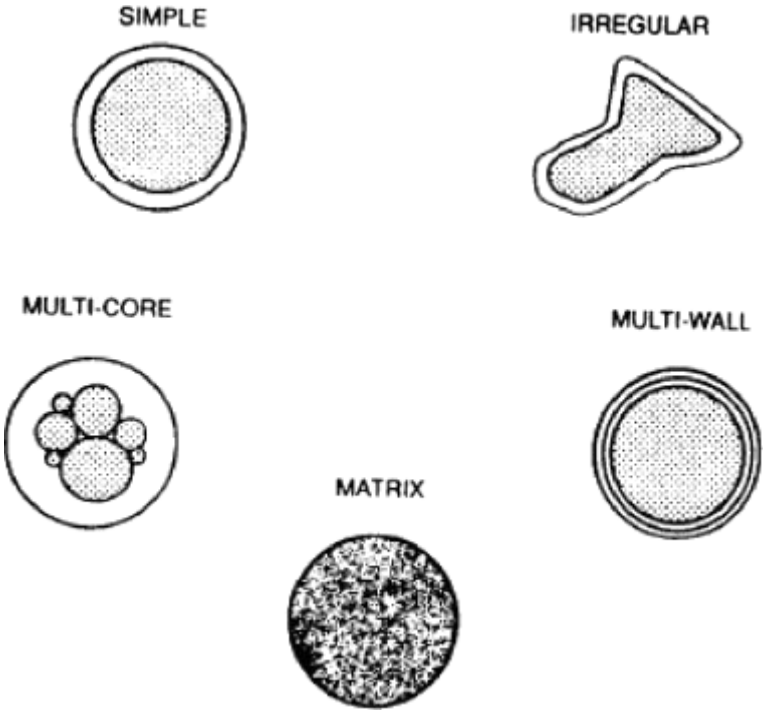


Figure 2.2b: Various forms of microcapsules (Gharsallaoui and others 2007)

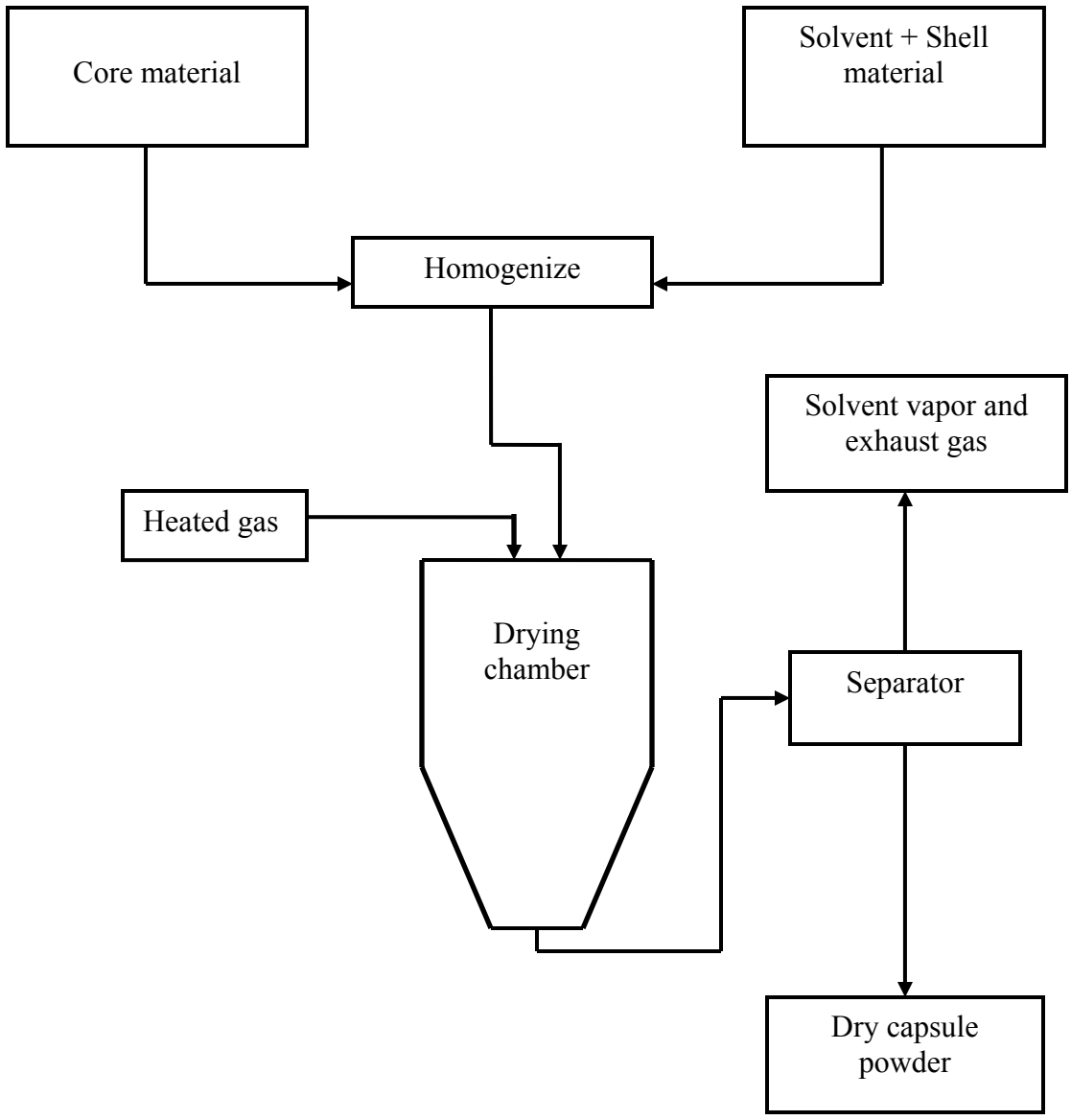


Figure 2.3: Schematic diagram of a spray-drying encapsulation process (Shahidi and others 1993)

Figure 2.4: Picture of spray dryer

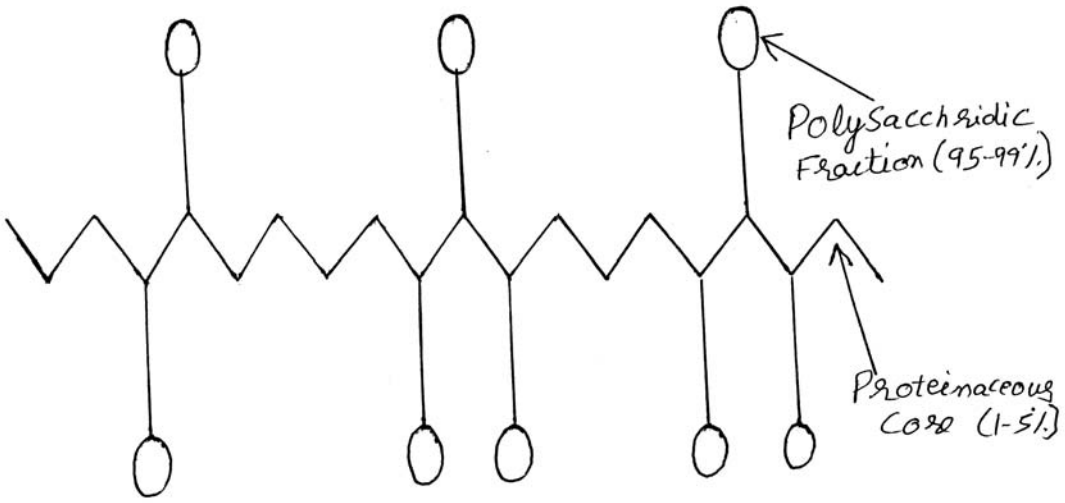


Figure 2.5: Chemical structure of gum arabic

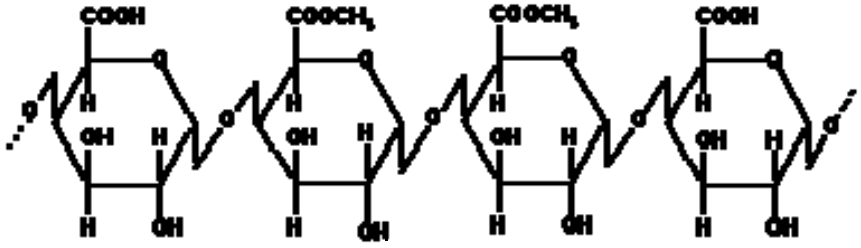
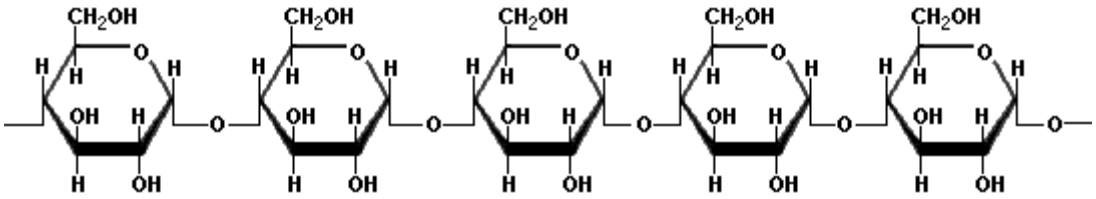
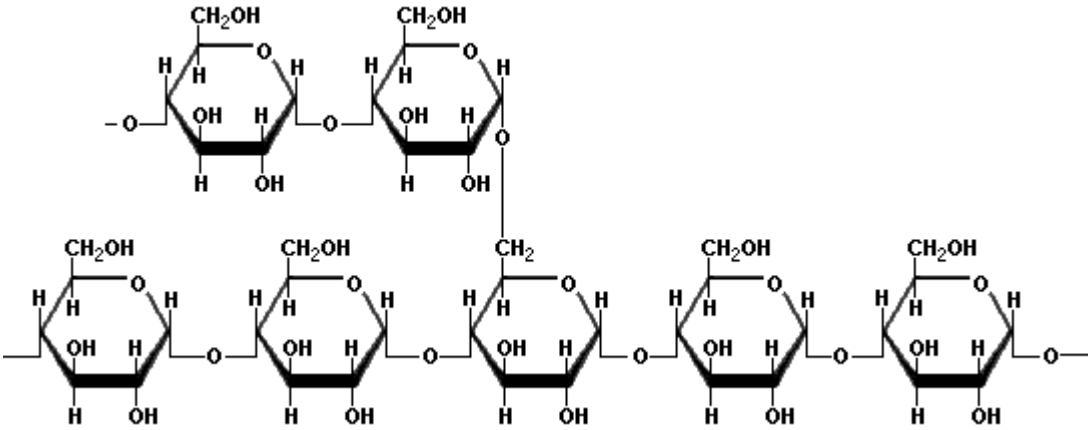


Figure 2.6: Chemical structure of pectin
(Source: http://food.oregonstate.edu/images/learni/55.gif)



(a)



(b)

Figure 2.7: Structures involved in starch (*a*) Linear chain of alpha-1, 4-glucan
(*b*) Alpha-1, 6 branch point

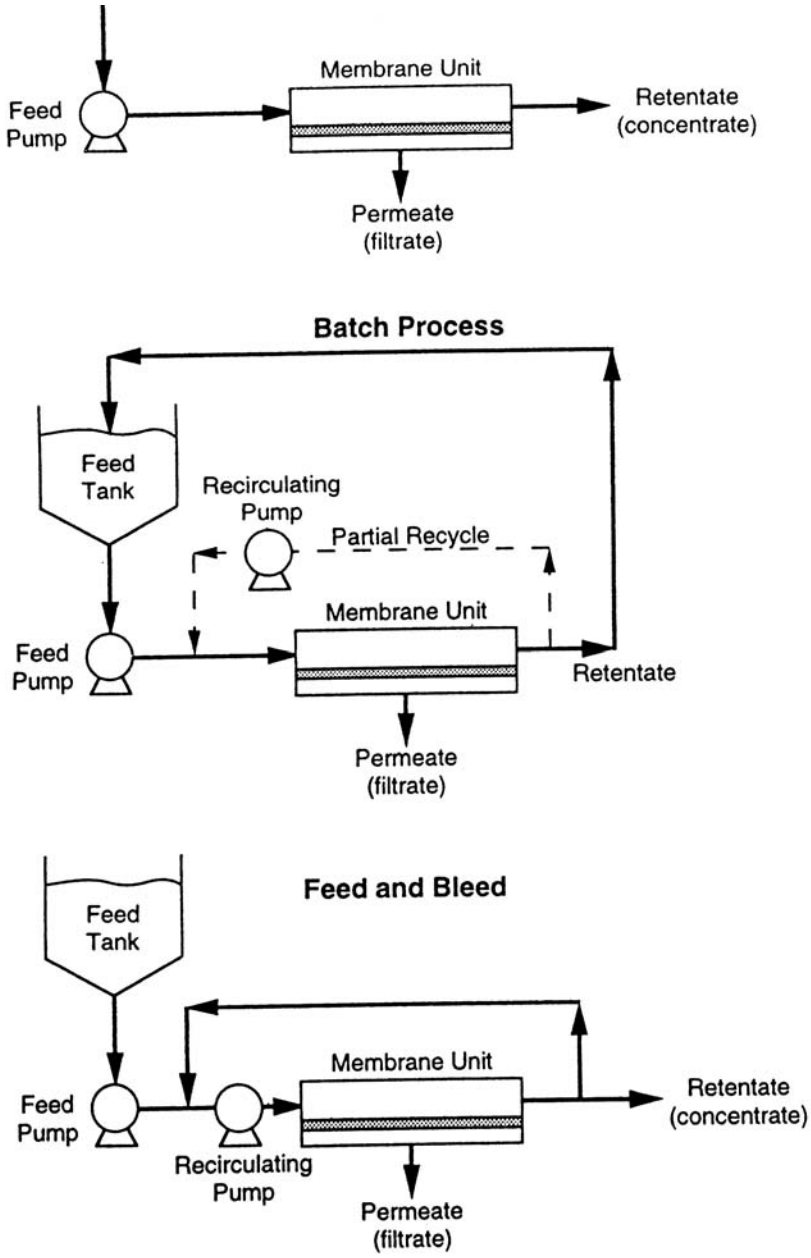


Figure 2.8: Composition of single-pass, batch, and feed-and-bleed configurations for cross-flow filtration. The dashed line in the middle panel represents an optional partial recycle stream in the batch configuration. (Zemen and Zydney 1996)

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CHAPTER 3

MICROFILTRATION OF MUSCADINE GRAPE POMACE AND PREPARATION OF SPRAY DRIED POWDER

Ray N., Chinnan M.S., and Phillips R.D. To be submitted in *Journal of Food Science*

ABSTRACT

Muscadine grapes are an excellent source of antioxidants which are mainly located in the skin and seeds (pomace) and aim of this research is to make muscadine powders which are rich in antioxidants. The study evaluated effect of microfiltration with or without bladder press. The total phenols increased from 7.25-7.93 to 8.33-8.92 and 6.25-7.98 to 7.01-8.73 g gallic acid/kg pomace with and without bladder press. The total anthocyanins decreased from 1.95-2.05 to 1.71-1.80 and 1.46-1.65 to 1.26-1.41 g cyanidine-3 glucoside equivalents/kg pomace with and without bladder press, respectively. It was found that flux rate did not change for constant cross flow velocity. It is also found that microfilter, bladder press and enzyme treatment is significant to extraction of total anthocyanins while microfilter is significant to extraction of total phenols.

Key words: Microfiltration, muscadine, pomace, spray drying, extracts, total phenols, total anthocyanins.

INTRODUCTION

Muscadine grapes are native to southeast US where they grow in abundance because of the warm and humid climate. The color of fruit ranges from greenish bronze to bronze, pinkish red, purple, and almost black (California Rare Fruit Growers, Inc 1999). Georgia has about 1,200 acres of commercial muscadine grapes (Georgia Faces 2004).

The major phenols found in muscadine are ellagic acid, kaempferol, myricetin, and quercetin. The seeds have highest antioxidant capacity compared to other fruit parts (Pastrana and Bonilla 2003). Muscadines have about 40% of their weight in the skin and seeds (Morris and Brady 2004). Thus for processing operations about half of fruit weight is lost as press fraction. The use of this press fraction (pomace) could have a big impact on muscadine wine and juice industry. The pomace can be used to produce colorants, nutraceuticals additives, seed extracts and oil and as ingredients for food industry. It can be used for production of ethanol or for the recovery of organic acids such as tartarates, malates, and citric acid (Schieber and others 2001).

Grape seed is a source of high quality cooking oil (Hang 1988). Grape seed oil has about 10 milligrams of vitamin E, slightly more than sunflower or safflower oil which are also high in vitamin E. Pigments extracted from grape seed oils are also receiving considerable attention as food ingredients. Depending on usage level, these pigments have potential to both color products and increase nutraceuticals content of foods containing them (Katz 2004). The grape extracts show wide range of biological activities which include antibacterial, antiviral, anti-inflammatory, anticarcinogenic, and lipid peroxidation (Bagchi and others 2000).

Membrane filtration has been used since a long time for filtration of materials or recovering of phenols or other valuable components from the wastes of food processing. Here are a few examples of the same as below.

Nanofiltration was carried out on apple juice retentate using membrane of sizes 0.1 and 0.25 kDa molecular weight cut-off (MWCO) and it was found that by using 1 kDa membrane concentration of phenolics increase by 4 times on retentate (Zaid and others 2006). When using 0.25kDa membrane on the collected permeate concentration of phenolics increased by two times on retentate side. A solvent extraction method utilizing 50% ethanol and 50% water as solvent and ultrafiltration (UF) was used for extraction of polyphenols from grape seeds (Nawaz and others 2006) and found that UF procedure led to high extraction rates, shorter extraction times and significant labor savings. For extraction of polyphenol from grape seeds Bucic and others (2007) used different solid-liquid ratio, four particle sizes and different temperatures and found that temperature, solid–liquid ratio and milling degree had a positive influence on extraction rate and extent of extraction and could extract as much as 1.47-6.68% of total polyphenol per dry matter of grape seeds in 200 minutes. To extract polyphenols from grape skins Kammerer and others (2005) used enzymatic hydrolysis of grape skins, using pectinolytic and cellulosic enzymes, different enzyme-substrate ratio and time temperature regime and observed that extraction of polyphenols was increased by treatment of pomace with hot water and cell wall degrading enzymes. Membrane filtration was used for must preservation and concentration using microfiltration and reverse osmosis on white and red grape juice samples (Rektor and others 2004) and obtained a retentate with high sugar and anthocyanins content indicating good membrane retention. During extraction of polyphenols from grape marc with different concentrations of solvent (ethanol), it was found that polyphenols content increased for water

content of ethanol from 10 to 30 % remained constant from 30 to 60% water and started decreasing after 50%. It was also observed that there was more extraction at 60°C as compared to 45°C. All the above studies indicate that grape pomace can be used for extracting polyphenol and making nutraceuticals out of them. These nutraceuticals can be added to food or used as an ingredient in food. There are various factors affecting the removal of polyphenol from solvent like solvent type, particle size, temperature and time of extraction, solvent to solid ratio, solvent pressure. The objectives of this study are: 1) Use muscadine pomace to extract polyphenols using microfiltration 2) To investigate effects of microfiltration process on extraction of polyphenols and prepare spray dried powders from it.

MATERIALS AND METHODS

Materials

Muscadine pomace of Supreme variety was obtained as gift from Paulk Vineyards (Wray, GA).

Chemicals

Gallic acid, Folin Ciocalteau reagent, ascorbic acid, sodium carbonate, potassium iodide, sodium acetate was obtained from Sigma Aldrich (St. Louis, MO).

Grinding

Grinding of muscadine pomace was done by Urschel mill (fig 3.1A) (Urschel Comitrol Processor Model 1700, Urschel Laboratories Inc., Valparaiso, IN). The ratio of solid to liquid was kept at 1:3 (1 part of pomace: 3 parts of water). Fifty five kg of pomace was used while using bladder press (fig 3.1B) (RLS Equipment Corp., Egg Harbor City, NJ) and microfilter (fig 3.2) and thirty kg of pomace was used while using only microfilter (LCI Corp., Charlotte, NC). The microfilter being used in Sceptor membrane system had two microfilter tubes of 5 feet

length each. The tubes are made of Stainless steel 316L with TiO2 coating on it. While grinding pomace (55 kg), weight of water (55kg) added was same as quantity of pomace taken to ease in grinding operation. The remaining quantity of water (110 kg) was added while heating pomace. The ground muscadine pomace was mixed with ascorbic acid (0.2%) to prevent oxidation. It was ground two more times by passing it through Urschel mill. There are two sets of blades (teeth) which move in opposite direction and help in crushing pomace to fine size. The teeth count could be kept either at 120, 140, or 200. Attempts were made to grind pomace with all teeth sizes and it was found that Urschel mill could not grind pomace at 200 teeth count, and could grind at 120 and 140 teeth count. In this study the teeth count was kept at 140. More deionized water was added during regrinding pomace second and third time. Grinding grape seeds also helps in extraction kinetics of polyphenol and solvent has better access to grape seed polyphenol (Bonilla and others 1999; Nawaz and others 2005).

Aqueous Extraction

Ground muscadine pomace (60 kg) was transferred to an extraction tank (fig 3.3) of capacity 55 gallon and 60 liters of deionized water was added to it. The extraction tank was then covered with a lid coupled with a stirrer (Stir-pak Model 50002- 02 Stirrer Controller Cole-Parmer Instrument Co., Vernon Hills, IL). The tank was heated by a gas burner until the temperature reached 60°C (Biswas 2007). The photograph of extraction tank is given in fig 3.3. The extraction was done at 60° C for 1 h with added Pectinex BE enzyme ® (30ml). The extracts were then collected into buckets before filtration. The weights of extracts were noted. A flowchart of extraction procedure is shown in fig 3.4.

While doing extraction using bladder press (weight of sample taken= 55 kg) the extracts were taken from extraction tank as in last process and passed through bladder press subjected to

pressure of 3 kg/cm^2. The collected filtrate was passed through a second bag filter of 50 micron pore size and then passed through microfilter. The press cake obtained from bladder press was weighed and discarded and extract was used for microfiltration.

Microfiltration of Muscadine Grape Pomace

Microfiltration of muscadine grape pomace was done by either of the two processing steps

a) Passing it through microfilter

b) Passing it through bladder press and then microfilter

Each process was repeated two times. The temperature and pH of sample was checked every fifteen minutes from both permeate and retentate sides during microfiltration.

Muscadine grape pomace was micro filtered without bladder press or after using bladder press and operated at constant low trans membrane pressure (TMP), constant cross flow velocity. Various parameters can be modified to optimize flux across the membrane, transmembrane pressure is of significant importance. Transmembrane pressure is the average of pressure difference between the retentate and permeate sides. The transmembrane pressure can be controlled by backpressure valve and the pump flow rate. The cross flow velocity was maintained at 68.13-75.7 L/min; inlet pressure at 150 psi, outlet pressure at 130 psi; and the trans membrane pressure at 35 psi. The flux varied between 0.32-0.35 L/min at cross flow velocity of 68.89 L/min. Samples were taken from permeate and retentate sides to monitor changes in total phenolics, total anthocyanins, pH and temperature.

Spray Drying

The aqueous extracts obtained from microfilter operation were spray dried using pilot scale spray drier (Anhydro Inc., Olympia Fields, IL). The inlet temperature was set at 180 °C

and outlet 90 °C. The system was initially stabilized with deionized water before running the extracts. The flow rate was set at 47-53 ml/minute. The atomizer was set to run at 70 percent of its rated capacity (50,000 RPM). The spray dried powder was collected in bottles and kept in amber colored glass bottles which were flushed with nitrogen gas before being stored in freezer at -20 °C.

Moisture Determination of Pomace

Moisture analysis of pomace was done by vacuum oven method at 70 °C and 25mm Hg for 8h (AOAC 934.06).

Total Solids Determination of Extract

An isotemp oven model (Lindberg Blue M, Asheville, NC,) was used for determination of total solids. Aluminum dishes were dried for 24 hr at 85°C, placed in dessicator and weighed. The samples (five to ten grams) were taken and placed in an aluminum dish and left for 24 h at 85 °C in oven. The samples were placed in dessicator and weighed using Fisher Scientific A-250 analytical scale (Chamul 1990). The weight difference was used to calculate total solids.

Soluble Solids (°Brix)

A Palette digital handheld "Pocket" refractometer (Atago Co, Ltd Tokyo) was used to measure percent soluble solids. The refractometer was calibrated using distilled water (0° Brix) prior to making sample measurements. The soluble solids (° Brix) was measured for one drop of juice at room temperature (22°C) and performed in duplicate.

Titratable Acidity

Twenty milliliters of juice was placed in a 50-ml Erlenmeyer flask and phenolphthalein (three drops of a 5 % phenolpthaniel solution) was added as an indicator. The juice sample was titrated against an aqueous 0.1 N NaOH solution till final endpoint of 8.2 on pH meter.

Titratable acidity (TA) was expressed as percent tartaric acid using the formula below in which mill equivalent factor used for tartaric acid was 0.075 (Liu 1999).

$$\textbf{TA (\%)} = \frac{\text{ml of NaOH x normality of NaOH x meq. wt acid x 100}}{\text{ml of sample}}$$

pH

The pH of extract was determined by a pH tester (Oakton pH Testr 3, Singapore) every fifteen minutes.

Cleaning

Cleaning was done by first flushing unit with hot water. Then sodium hydroxide was circulated after adjusting pH to 12.0 at 70-75°C for 30 minutes followed by flushing it with water at 70-75°C. Again aqueous sodium hydroxide was circulated in system and flushed with water at 70-75°C. Permeate and retentate sides were checked with a pH paper to keep pH of 7.0.

Total phenol

Total phenol was estimated colorimetrically using Folin-Ciocalteau method (Singleton and Rossi 1965). A sample aliquot of 200µL was added to 800µL of deionized water, 5 ml of Folin Ciocalteau reagent and 4 ml of saturated sodium carbonate solution (75g/L). The absorbance was measured at 765 nm with a Hewlett Packard 8451A diode array spectrophotometer (Avondale, PA) after incubation for two hours at room temperature (~25 °C). Quantification was based on standard curve generated with 50, 100, 200, 300, 400, 600, 800 and 1000 mg/L of gallic acid. The final concentration of phenols was calculated based on total volume of extract and initial weight of pomace; and expressed as mg/kg dry weight. The experiment was conducted under yellow light.

Total anthocyanin

Total anthocyanin was measured by pH differential method (Giusti and Wrolstad 2001) using two buffer systems - potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). Extracts were diluted with water to give absorbance in range of 0.1 - 1.2 at 510 nm. Aliquots of 0.2 ml of diluted extracts were mixed with 1.8 ml of each buffer, vortexed and read against a blank at 510 and 700 nm with a Hewlett Packard 8451A diode array spectrophotometer (Avondale, PA).

Absorbance was calculated as

$$A = [A_{510\,nm} - A_{700\,nm}]_{pH\ 1.0} - [A_{510\,nm} - A_{700\,nm}]_{pH\ 4.5}$$

Monomeric anthocyanin pigment concentration in the extract was calculated as cyanidine-3 glucoside

$$\text{Monomeric anthocyanin pigment (mg/L)} = A \times MW \times DF \times 1000 / (e \times l)$$

Where A = absorbance, MW = molecular weight (449.2), DF = dilution factor, e = molar absortivity (29,600), l = path length (1 cm).

The final concentration of total anthocyanins was calculated based on total volume of extract and weight of starting fruit, and expressed as mg/kg dry weight.

Statistical analysis

The statistical analysis software (SAS® Proprietary Software, 2003-2005), Release 9.1 for Windows (SAS® Institute Inc., Cary, NC) was used for data analysis. The Proc GLM procedure was used to check change in total phenol and total anthocyanins due to bladder press, filter, repetition and interaction of bladder press and filter at 90 and 95% level of significance (α=0.1 and α=0.05).

Calculations

The weights of samples were taken after each processing step. However volume was not known at all processing steps. To determine the volume lab tests were done to determine the volumes at each corresponding steps. After determining densities at different steps the values were then applied to actual tests to determine volumes.

RESULTS AND DISCUSSION

Total Soluble Solids

The total soluble solids increased from 2.4 to 2.8 on permeate side and 2.4 to 3.7% on retentate side during microfiltration.

pH

The pH of the slurry decreased from 3.7 (before heating) to 3.35 after enzyme treatment. The lowering of pH helps in extraction of phenols from cell walls.

Microfiltration with Bladder Press

The total phenols changed from 1.78-2.19 to 3.33-3.50 g gallic acid equivalents/L and upto 2.77-3.16 g gallic acid equivalents/L in the retentate and permeate sides (fig 3.5). The total anthocyanin content changed from 0.42-0.56 to 0.64-0.75 g cyanidine-3 glucoside equivalents/L and upto 0.45-0.56 g cyanidine-3 glucoside equivalents/L on the retentate and permeate sides as shown in fig 3.6. The total anthocyanin content increased on retentate side whereas concentrations of total phenol increased on both permeate and retentate sides (fig 3.5 and 3.6). The concentration of total phenol decreased from 7.25 -7.93 (enzyme treatment) to 6.35-6.48 g gallic acid equivalents/kg pomace after passing through bladder press. After microfiltration it increased from 6.35-6.48 to 8.33-8.92 g gallic acid equivalents/kg pomace. The concentration of total anthocyanin decreased from 1.95-2.05 to 1.63-1.67 g cyaniding-3 glucoside after bladder

press and increased to 1.78-1.80 g cyanidine-3 glucoside equivalents/kg pomace after microfiltration (Table 3.2 and fig 3.10). While using microfilter, flux change in permeate was not observed over time due to high cross flow velocity and as a result there was no caking of membrane. It may also be due to the fact that the flow-rate was not maintained constant for a long time to observe changes in flux-rate (Table 3.3 and 3.4).

Microfiltration without Bladder Press

The total phenol in retentate changed from 1.87-2.27 to 2.73-3.56 g gallic acid equivalents/L and upto 1.79-1.85 g gallic acid equivalents/L on retentate and permeate sides (fig 3.7). The total anthocyanin content changed from 0.274-0.310 to 0.427-0.506 g cyanidine-3 glucoside equivalents/L and upto 0.302-0.315 g cyanidine-3 glucoside equivalents/L on permeate and retentate sides (fig 3.8). There was an increase in concentration of anthocyanins on permeate side whereas there was a decrease on the retentate side. There was an increase in concentration of phenols on both retentate and permeate sides (fig 3.7 and 3.8). The concentration of total phenols increased from 6.28-7.98 g gallic acid equivalents/kg pomace after enzyme treatment to 7.0-8.92 g gallic acid equivalents/kg pomace after passing through the bladder press. The concentration of total anthocyanins decreased from 1.43-1.65 to 1.26-1.44 g cyanidine-3 glucoside/kg pomace after microfiltration (Table 3.2 and fig 3.10). When bladder press was not used the microfiltration operation could not be continued beyond two hours and fifteen minutes because of high total solids in retentate as the particle size was too large and trans membrane pressure approached the maximum operating pressure. Here we were also unable to see flux changes because of sudden stopping of operation. According to the manufacturer membrane could handle viscous solids up to 50 percent whereas in our study the feed had total solids

concentration of 12.5 per cent. It may be advisable to dilute feed to 10 times instead of 4 times as done in this experimental setup.

The concentration of total phenol and total anthocyanins is 1.5 times on concentrate side as compared to permeate (after microfiltration). It was observed that weight of total phenol and total anthocyanin on permeate side was 80 percent and remaining 20 percent on the retentate side. We could use some membranes like UF having appropriate pore sizes for concentration of permeate obtained to get higher concentration of total phenol and total anthocyanin. We may also use low temperature flash evaporation for removal of excess water and then spray dry extracts to get powder which is more concentrated.

Since there was no heat exchanger to control temperature during microfiltration, an increase was seen in concentration of total phenol with temperature as opposed to a plateau which should be observed as reported by Bucic and others (2007) when operating at different extraction temperatures.

Grape seed polyphenols are sensitive to light, oxygen, acid and alkaline conditons but are less sensitive to heat (Shi and others 2005). As per study carried out by Spigno and others (2007), there was more extraction of phenols at 60°C as compared to 45°C. This explains the increase in extraction of phenols with temperature increase and increase in concentration of polyphenols with temperature rise. There are four kinds of bonds existing between proteins and polyphenols: hydrogen, hydrophobic, ionic and covalent bonds (Shi and others 2005). More than 33 % of dry weight of proteins is polyphenolic compounds bound by hydrogen bond (Shi and others 2005). Some of these bonds are broken during microfiltration which lead to release of phenols from the cellulose and protein matrix.

There are various factors which could contribute to degradation of total anthocyanins like structural effects, concentration effects, pH, temperature, oxygen, light, enzymes, ascorbic acid and sugars. The degradation rate of anthocyanins increase during processing and storage as temperature rises (Maccarone and others 1985). Temperature rise in pH 2-4 induces loss of glycosyl moieties of anthocyanins, by hydrolysis of the glycosidic bond (Adams 2006). This leads to further loss of anthocyanins color, since aglycones are much less stable than their glycosidic forms. The presence of oxygen, together with elevated temperature, was most detrimental combination of the many factors which were tested against color deterioration of different berry juices and isolated anthocyanins (Nebesky and others 1949). Sugars as well as their degradation products are known to decrease anthocyanins stability (Meschter 1953; Thakur and Arya 1989). Of typical sugar degradation products, furfural accelerated anthocyanins pigment deterioration more prominently than hydroxyl-methylfurfural (Meschter 1953). The reactions of anthocyanins with both degradation products of sugars and ascorbic acid yield in the formation of brown pigment polymers (Krifi and others 2000).

Statistical analysis

It was found that microfilter; bladder press and enzyme treatment had significant effect on yield of total anthocyanins at 0.10 level of significance. It was also found that the use of microfilter was significant to extraction of total phenols at 0.05 level of significance but there was no significant effect with use of bladder press (fig 3.9 and 3.10).

CONCLUSIONS

Extraction of muscadine grape pomace was carried out using enzyme treatment, bladder press and microfilter. It was found that use of microfilter was significant to extraction of phenols and use of enzyme treatment, bladder press and microfilter was significant to extraction of total

anthocyanins. These results can be used in designing a system of continuous extraction of phenols using a series of filters kept in parallel.

Table 3.1 Composition of grape extract after enzyme treatment

Parameter	**Value**
pH	3.56
Soluble Solids °Brix	2.20
Titratable acidity (Tartaric acid)/ g/100ml	0.37

Table 3.2 Change in total phenols and total anthocyanins during processing of muscadine pomace

Processing Step	**With Bladder Press**				**Without Bladder Press**			
	Rep 1		**Rep 2**		**Rep 1**		**Rep 2**	
	TP (g/kg)	**TA (g/kg)**	**TP (g/kg)**	**TA (g/kg)**	**TP (g/kg)**	**TA (g/kg)**	**TP (g/kg)**	**TA (g/kg)**
Enzyme trt	7.93	2.049	7.25	1.95	7.98	1.65	6.28	1.46
After BP	6.48	1.675	6.35	1.63	NA	NA	NA	NA
After MF	8.92	1.706	8.33	1.80	8.73	1.41	7.01	1.26

where, TP= Total Phenols; TA= Total Anthocyanins; MF= Microfiltration; trt= treatment.

Table 3.3 Experimental data for change in total phenolics, total anthocyanins in aqueous extracts of muscadine pomace during microfiltration without bladder press

Time	Total Solids (%)		Total Phenol (mg/L)		Anthocyanin (mg/L)		Pressure (Psi)				Flowrate (L/min)		Freq	Temperature (°C)	
Min	Ret	Per	Ret	Per	Ret	Per	Inlet	Outlet	Permeate	TMP	Ret	Per	%	Ret	Per
15	1.93	1.53	2821.56	2618.89	566.74	566.74	150	140	110	35	66.24		27.1	39	10
30							150	140	110	35	66.24	0.25	27.1	40	10
45							150	145	110	35	46.94		19.6	41.7	19
60	1.94	1.56	2835.04	2645	648.74	533.75	150	140	110	37.5	47.32	0.21	19.6	41.8	19.1
75							150	140	110	35	46.56		19.6	41.8	18.1
90							150	140	110	35	47.32	0.2	19.6	42	17.6
105							150	140	110	35	47.32		19.7	42	17.3
120	1.98	1.51	2761.1	2705.49	639.44	505.41	150	140	110	35	47.32		19.7	42.7	16.4
135							150	140	110	35	48.45		19.7	43.4	15.4
150							150	140	110	35	66.24	0.27	27.1	44.3	16.3
165							150	140	110	35	66.24		27.1	45.2	17.8
180	2.04	1.51	2896.97	2685.07	657.82	503.54	150	140	110	35	66.24	0.28	27.1	46.3	18
195							150	140	110	35	66.24		27.7	47.7	17.4
210							150	140	110	35	67.76	0.29	28	49.3	17.6
225							150	140	110	35	68.14		28	50.4	11.3
240	2.1	1.56	3045.67	2737.57	727.77	513.89	150	140	110	35	68.52		28	51.9	11.4
255							150	140	110	35	68.52		28	53.3	10.6
270							150	140	110	35	68.89	0.33	28	54.9	10.1
285							150	140	110	35	68.89		28	60.3	8.1
300	2.21	1.57	3018.57	2884.44	683.83	522.44	150	140	110	35	68.89	0.32	28	61.3	8.4
315							150	140	110	35	68.89		28	62.4	9.1
330							150	140	110	35	68.89	0.32	28	63.1	9.4
345							150	140	110	35	68.89		28	64.1	9.4
360	2.32	1.63	3003.78	2914.27	711.875	523.9475	150	140	110	35	68.89		28	64.4	9.3

375							150	140	110	35	68.89		28	63.8	9.7
390							150	140	110	35	68.89	0.3	28	61.7	10.1
405							150	140	110	35	68.89		28	61.7	10.1
420	2.58	1.72	3228.61	3161.79	724.495	553.225	150	140	110	35	68.89	0.35	28	61.4	10.5
435							150	140	110	35	68.89		28	61.4	10.5
450							150	140	110	35	68.89		28	64.5	10.5
465							150	140	110	35	68.89		28	64.5	10.3
480	3.06	1.82	3507.85	3172.68	757.585	564.4075	150	140	110	35	68.89	0.33	28	66	9.5

Where, Phen=Phenols, Ret=Retentate, TMP= Trans membrane pressure, Freq= Frequency.

Table 3.4 Experimental data for change in total phenolics, total anthocyanins in aqueous extracts of muscadine pomace during microfiltration without bladder press

Time	Total Solids (%)		Total Phenol (mg/L)		Anthocyanin (mg/L)		Pressure (Psi)				Flowrate (L/min)		Freq	Temperature (°C)	
Min	Ret	Per	Ret	Per	Ret	Per	Inlet	Outlet	Permeate	TMP	Ret	Per	%	Ret	Per
0	1.74	9.51	2344.23	2116.23	548.38	346.34	150	135	110	32.5	66.24	0.22	31.5	54	
15	1.74	8.38	2934.38	2155.96	525.03	399	150	132	110	31	66.24	0.35	30.7	52.8	
30							150	125	110	27.5	66.24		30.7	50.4	*
45	2.6	2.3	3136.7	1853.25	507.75	272.44	150	125	110	27.5	66.24	0.38	30.7	62	
60							150	110	100	30	66.24		30.8	69	
75	1.90	17.97	3044.1	1854.39	490.82	295.59	150	110	100	30	66.24	0.4	30.8		
90							150	110	100	30	66.24		30.8	52	
105	1.95	16.4	3343.2	1843.18	492.07	306.57	150	110	100	30	66.24		30.8		
120							150	110	100	30	66.24	0.35	30.8	60.2	
135	1.97	17.69	3563.27	1856.91	506.42	314.79	150	110	100	30	66.24		30.8	60.2	

* Taken in buckets and kept in cold store at 2° C.

Where, Min= Minutes, Per= Permeate, Ret= Retentate, Freq= Frequency, Temp= Temperature, TMP= Transmembrane pressure.

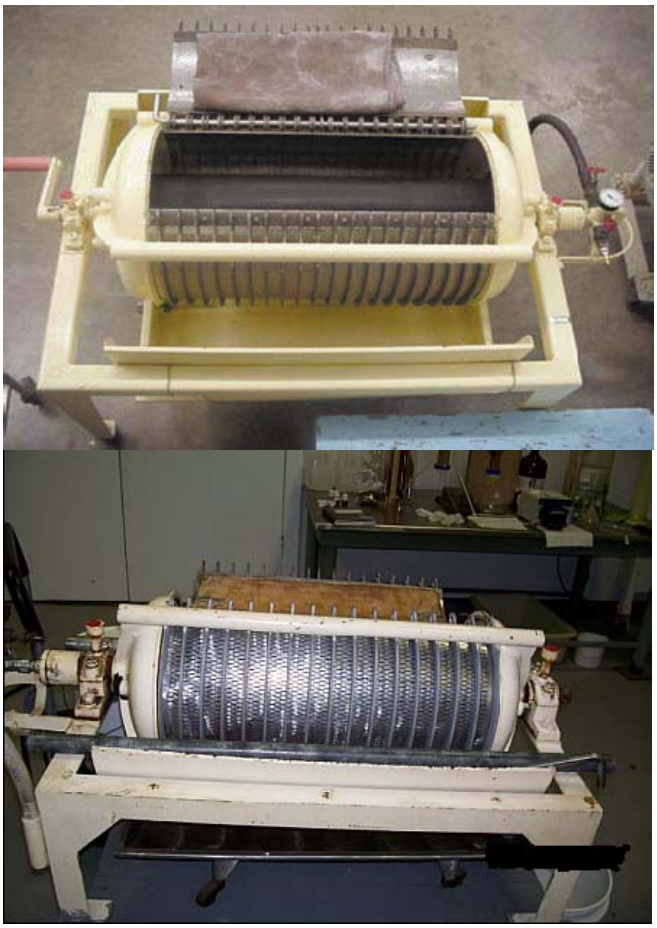


Figure 3.1 A) Urschel mill B) Bladder Press (Top and side view)

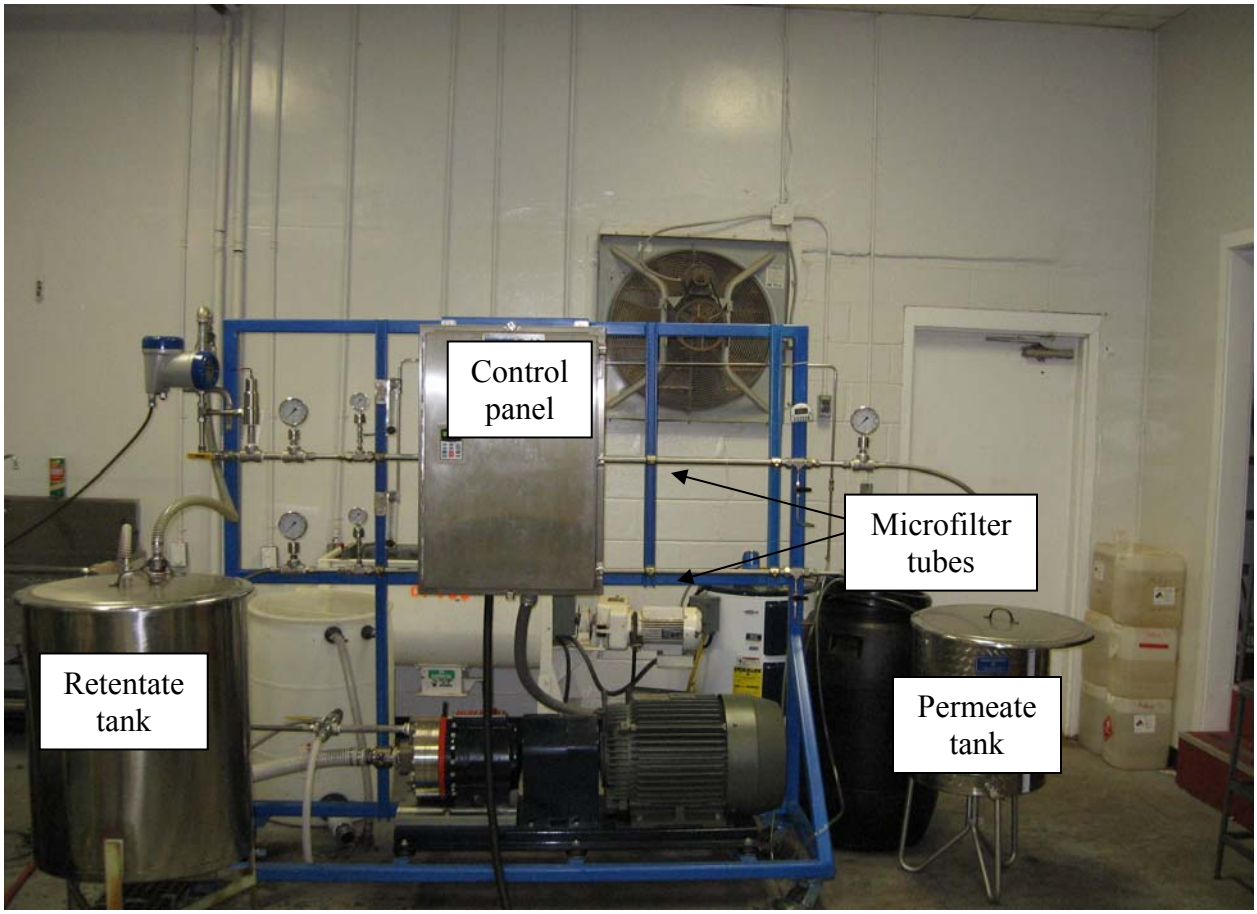


Figure 3.2 Photograph of microfiltration unit with its parts

Fig 3.3 Extraction tank with gas fired heater

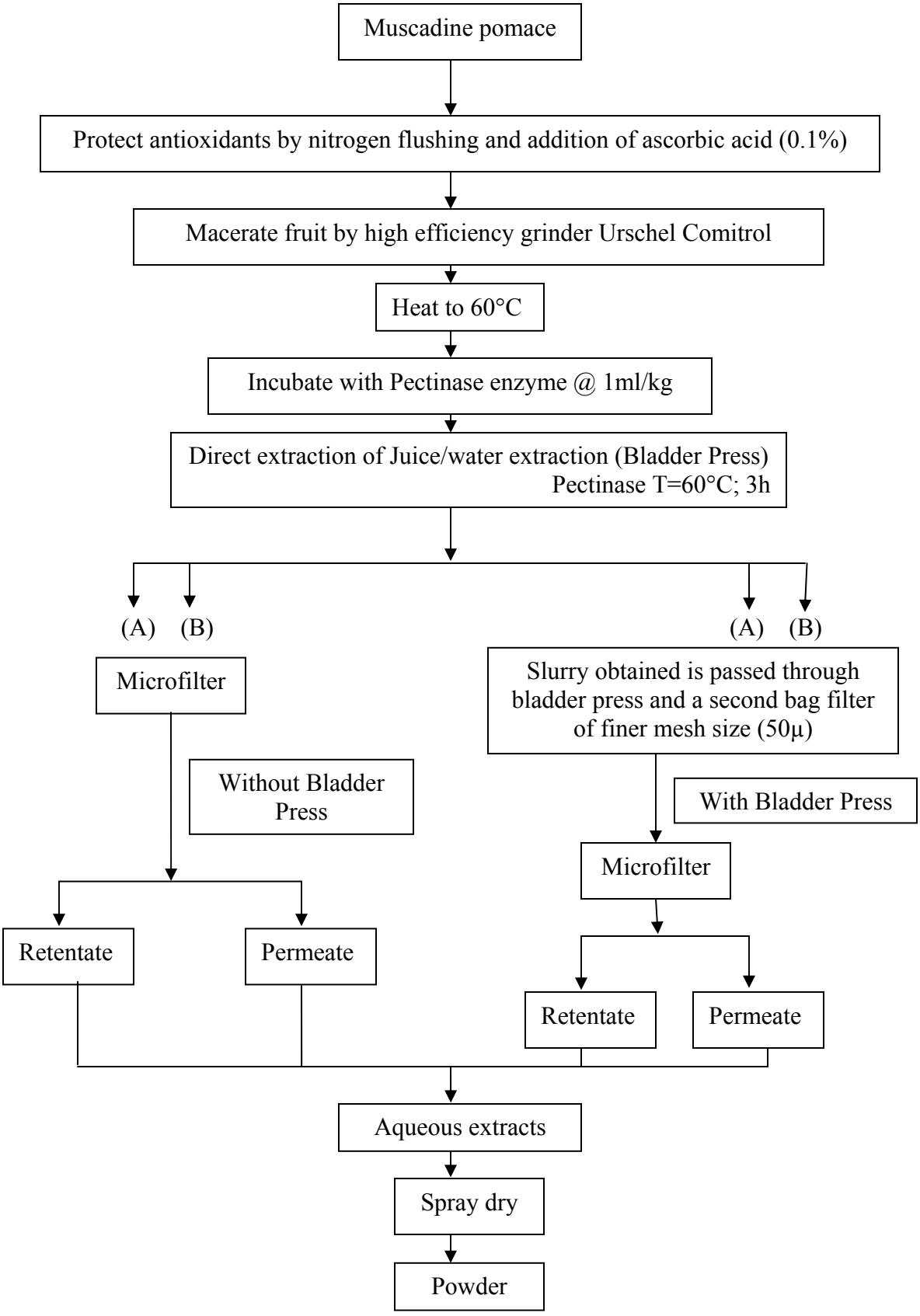


Figure 3.4 Experimental schemes for making grape extract from muscadine pomace

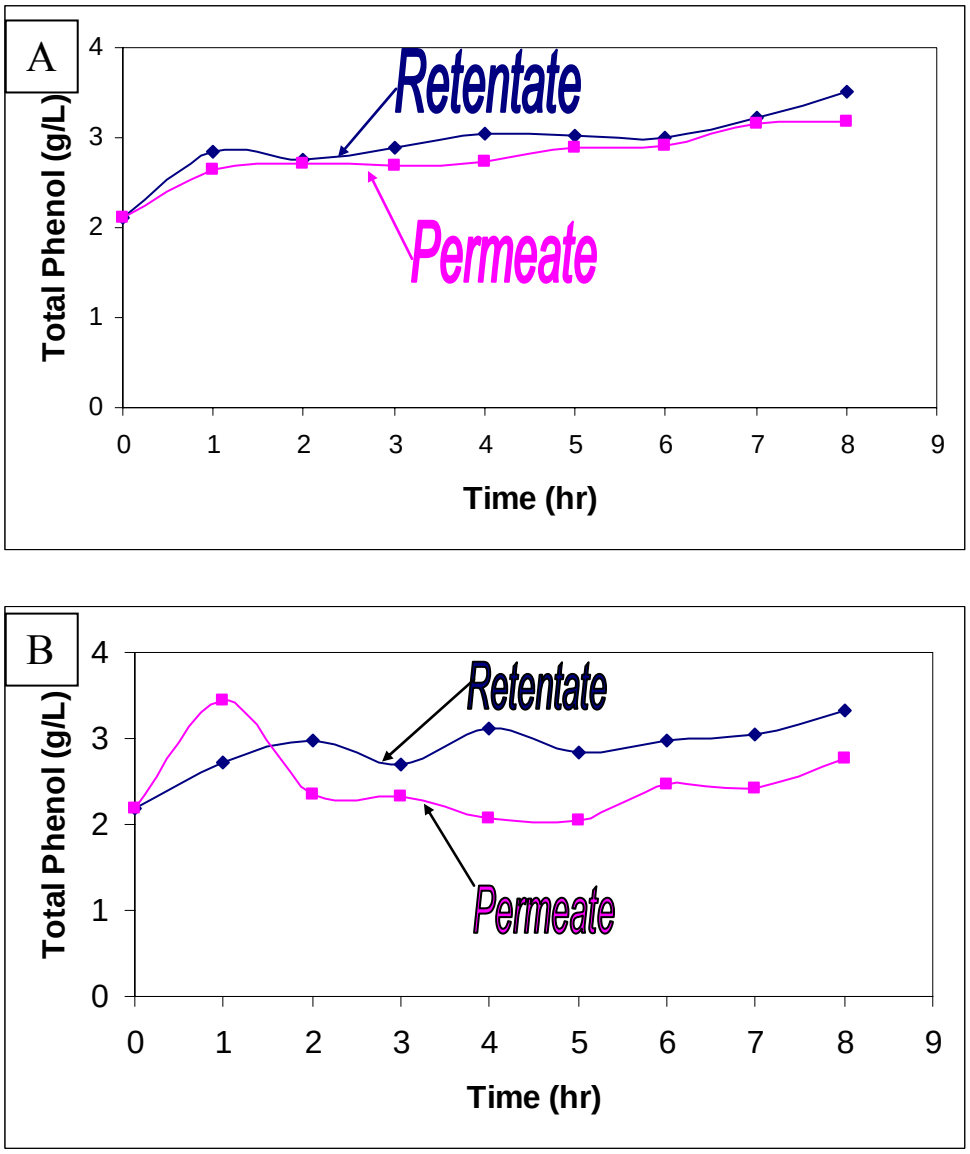


Figure 3.5 Change in total phenol with time during microfiltration with bladder press [(A) Rep 1 and (B) Rep 2]

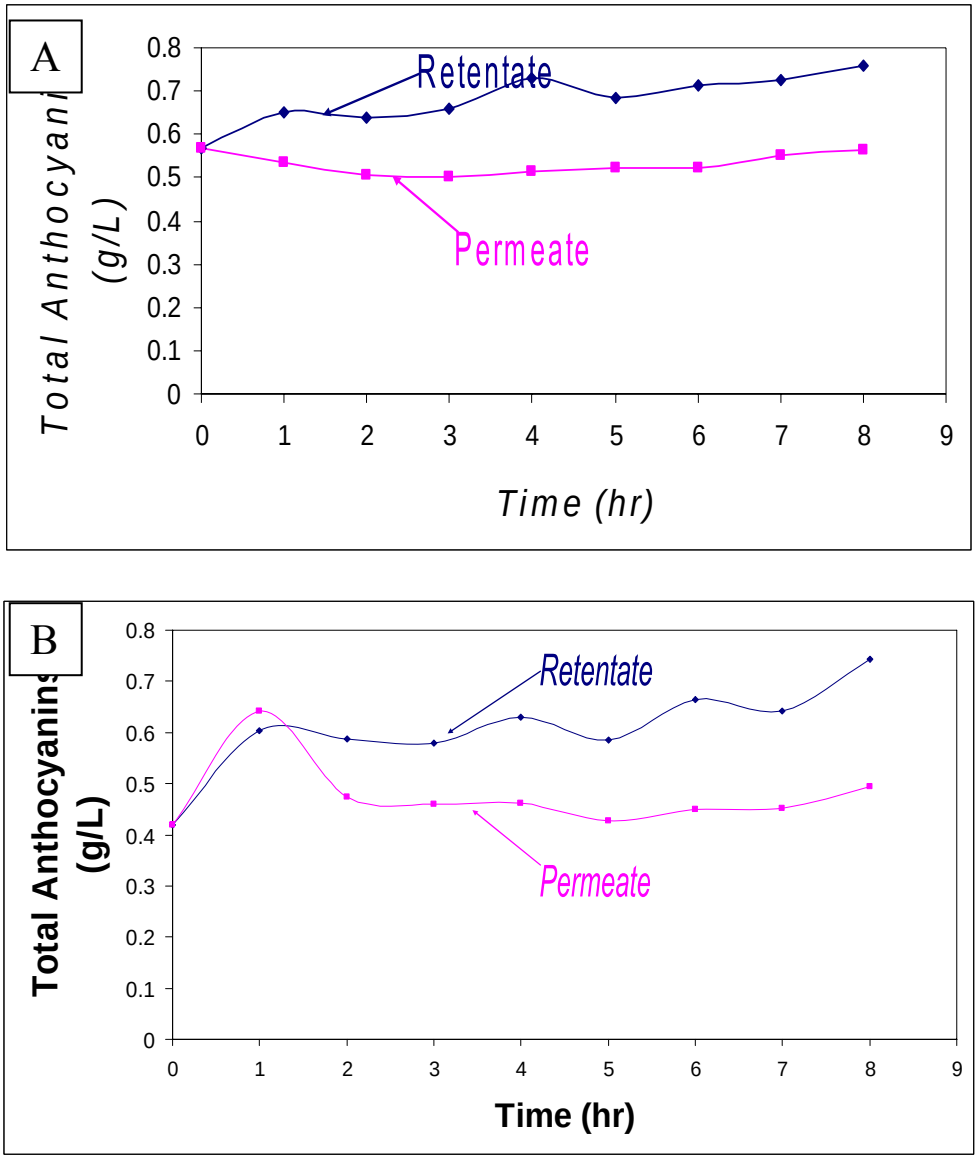


Figure 3.6 Change in total anthocyanins with time during microfiltration with bladder press [(A) Rep 1 and (B) Rep 2]

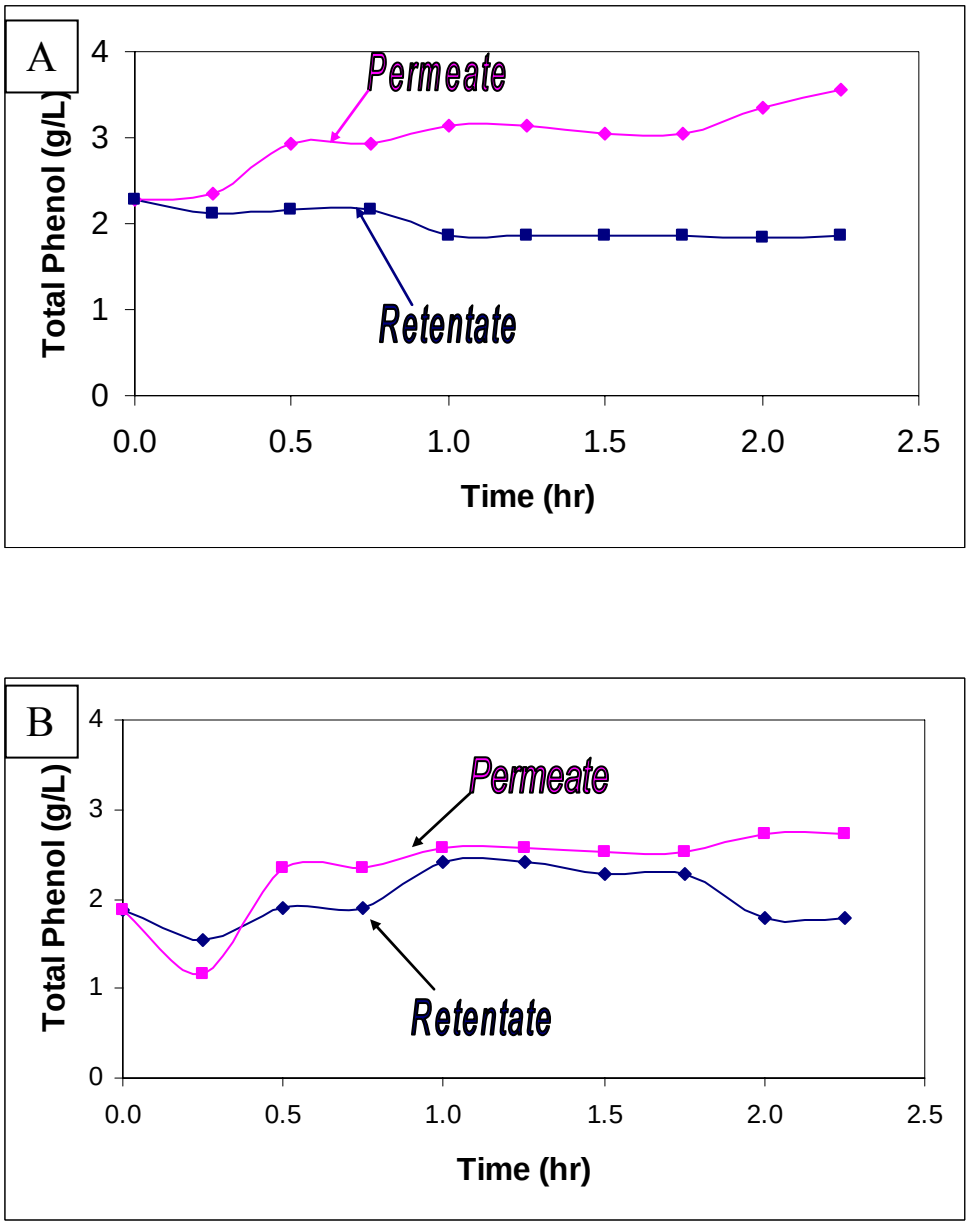


Figure 3.7 Change in total phenol with time during microfiltration without bladder press [(A) Rep 1 and (B) Rep 2]

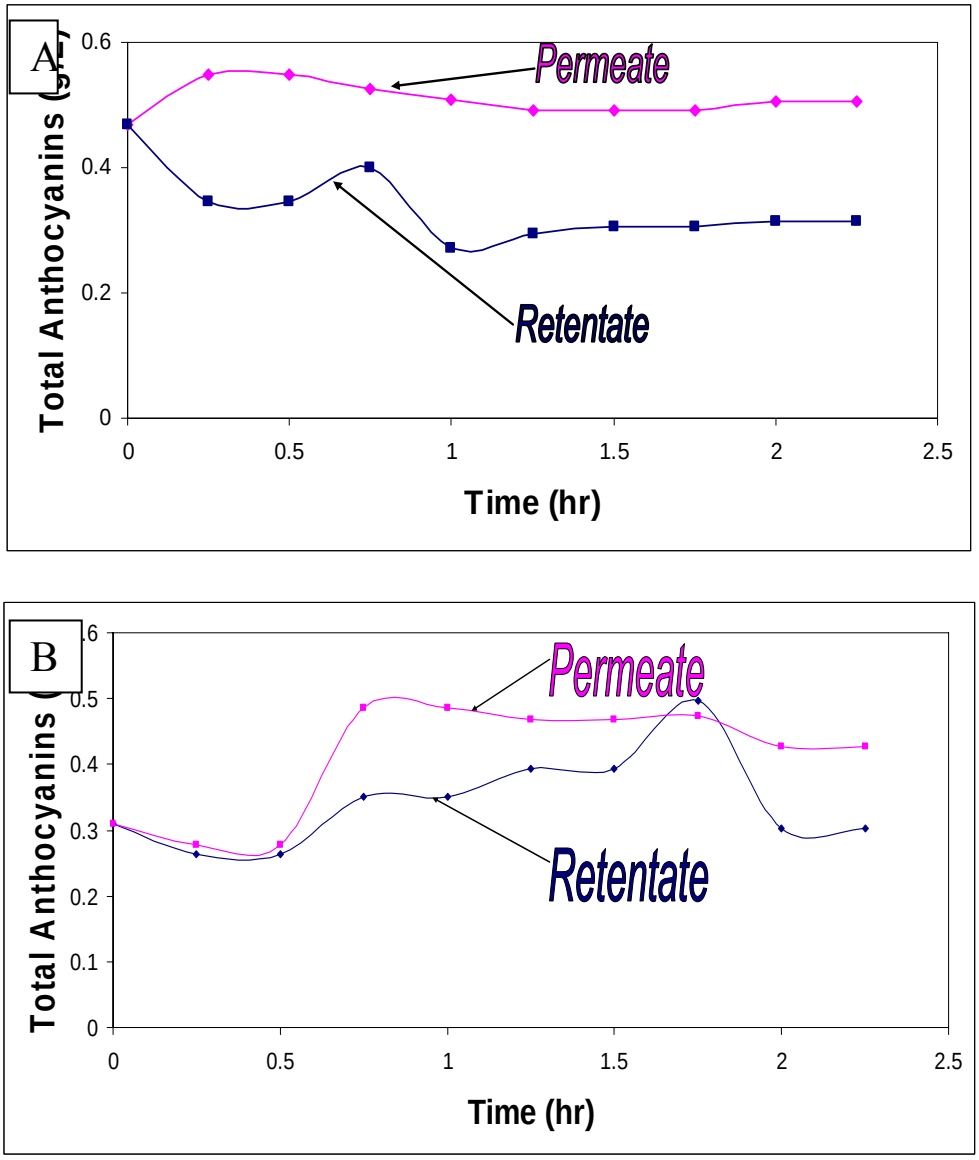
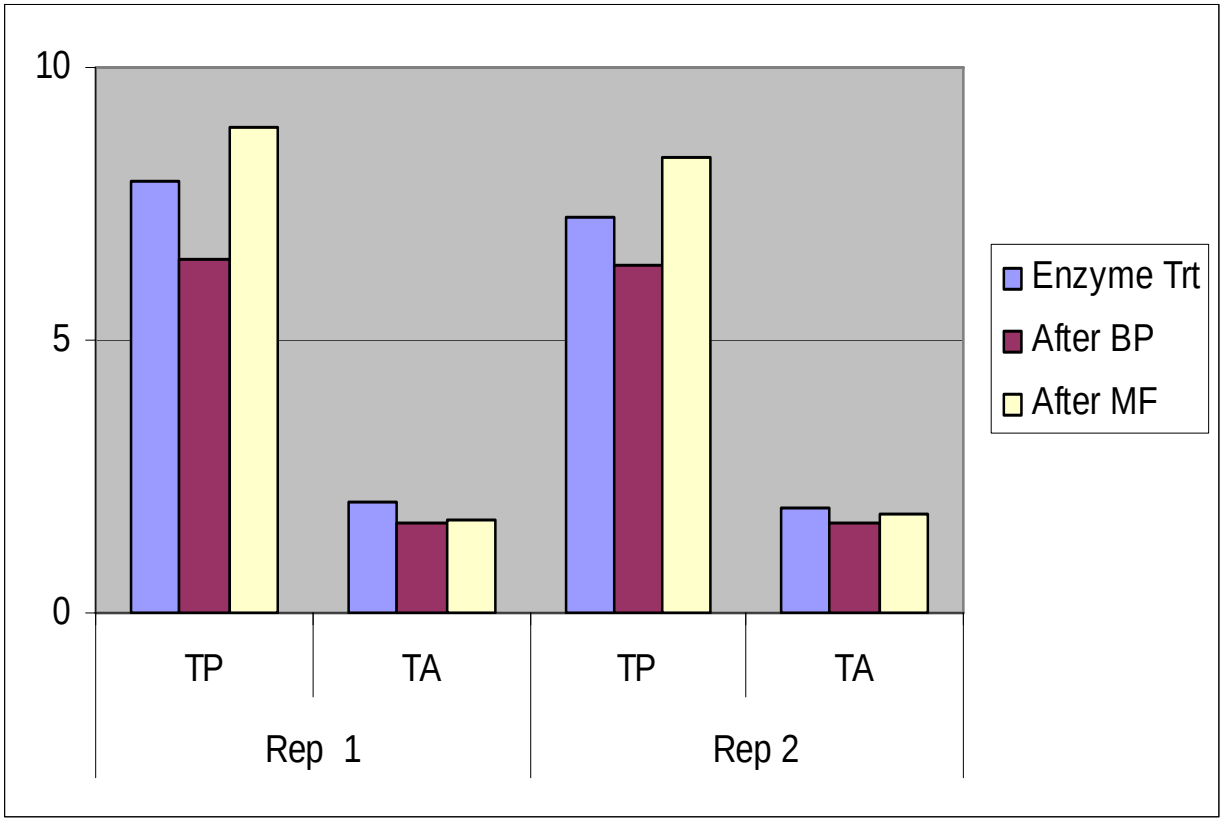
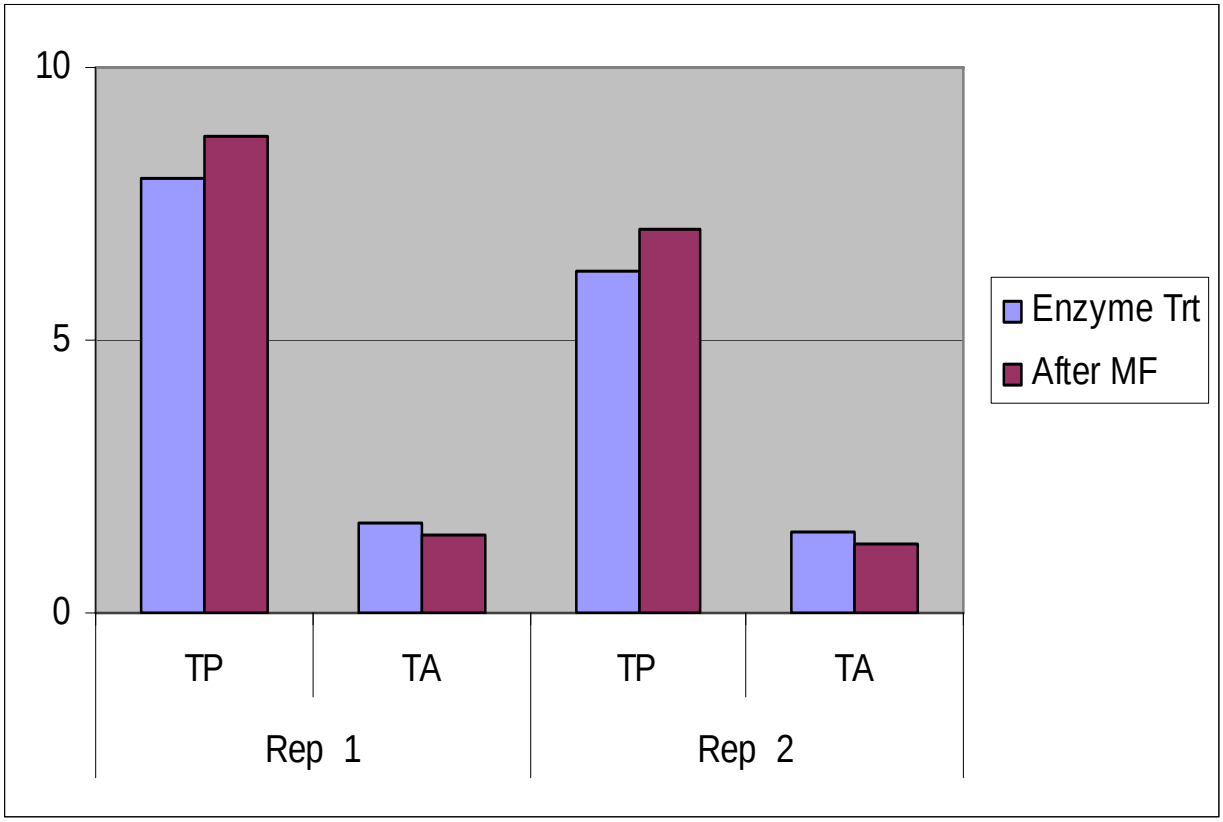


Figure 3.8 Change in total anthocyanins with time during microfiltration without bladder press [(A) Rep 1 and (B) Rep 2]



Where BP= Bladder Press, MF= Microfiltration

Figure 3.9 Change in concentration of total phenols, TP (g/kg) and total anthocyanins, TA (g/kg) at different processing steps with bladder press



Where BP= Bladder Press, MF= Microfiltration

Figure 3.10 Change in concentration of total phenols, TP (g/kg) and total anthocyanins, TA (g/kg) at different processing steps without bladder press

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CHAPTER 4

MICROENCAPSULATION OF GRAPE EXTRACTS WITH WHEY PROTEIN CONCENTRATE AND GUM ARABIC USING SPRAY DRYING AND STORAGE STUDIES ON RESULTING MICROCAPSULES

Ray N., Chinnan M. S., and Phillips R.D. To be submitted in *Journal of Food Science*

ABSTRACT

Muscadine pomace is rich source of polyphenols which are known to have lot of health benefits including protection against oxidation of high density lipids, while helping to lower low density lipids, anti-ulcer, anti-carcinogenic and anti mutagenic activities.

These extracts are high in total phenol but unstable due to oxidation and so were microencapsulated. Microencapsulation also protects the phenols in powder by making them free flowing and preventing stickiness. Morphology of microcapsules was studied with scanning electron microscope (SEM) and particle size using Mastersizer. Accelerated shelf life testing was carried out under various environmental conditions of temperature (4 and 25 ˚C), humidity 64%, UV light and dark condition, coating materials (gum arabic and whey protein) and one uncoated sample, bladder press and no bladder press in sealed containers for 8 weeks. It was observed that maximum degradation was observed in uncoated sample followed by whey protein and gum arabic coating.

Key words: Muscadine pomace, accelerated shelf life testing, SEM, whey protein, gum arabic

INTRODUCTION

Microencapsulation is defined as the technology of packaging solids, liquids, or gases in miniature, sealed capsules that can release their contents at controlled rates under specific conditions (Dziezak 1988). Shahidi and others (1993) reported that the miniature packages called microcapsules may range in diameter from few micrometers to several millimeters. The microcapsules have ability to preserve a substance in a finely divided state and release it as required. Their structure depends to a large extent on wall and core materials and drying conditions. Microencapsulation is also used to prevent off-flavors produced by vitamins and minerals, taste masking of certain food ingredients, prevent interaction among various food ingredients, improve the stability and handling of colors, improve flavor and texture of food products. There are many other advantages to microencapsulation of food such as protection of core material from environmental conditions such as light, oxygen and water, to decrease the transfer rate of core to environment, to control the release of core material or dilute the core material as required, etc. Microcapsule offers the manufacturer a choice to protect the sensitive food compounds, ensure against nutritional loss, utilize sensitive ingredients, incorporate unusual or time-release mechanisms into formulation, mask or preserve flavors and aromas and transform liquids to solid ingredients that are easier to handle. The properties that the encapsulated ingredients provide give the food technologist greater choice in developing new food ingredients that are not only more nutritious but meet the expectations of today's consumer.

Spray drying is the most commonly used encapsulating technique in the food industry because of cost considerations and flexibility (Desai and Park 2005). In this method the material for encapsulation is homogenized with carrier material. The mixture is then fed into a spray

dryer and atomized with a nozzle or a spinning disc. Water is evaporated by hot air contacting atomized material. The microcapsules are then collected after they fall to the bottom of drier.

Muscadine grapes are native to southeast United States. Hot and humid climate are favorable for their growth. The fruits are round with a tough skin surrounding it. Muscadine grapes and seeds are rich source of polyphenolic compounds (Yilmaz and others 2004; Bonilla and others 2003).

Byproducts of grape industries are produced in large quantities every year and are economically profitable but difficult to dispose off. Various by products are produced from grape pomace such as colorants, ingredients for the food and beverage industry, dried fruit and nut mixtures, nutraceuticals, grape seed oil and grape seed powder. The spray dried extracts prepared after microfiltration are hygroscopic because they contain sugars and are not shelf stable as they absorb moisture and degrade. The problem of stickiness is also caused by very low glass transition temperatures (Tg) of glucose, fructose, tartaric acid, citric acid, and other small molecular weight sugars. It is therefore essential to add high molecular weight compounds having high Tg so as to have successful drying under practical conditions (Adhikari and others 2005). Microencapsulation also provides a protective coating to the spray dried product and prevents it from oxidation.

The current extracts from fruits high in phenolic compounds available in the market are highly pure and expensive at the same time. The aim of this project is to have a low cost alternative to long extraction and purification procedures for total phenol and anthocyanin from muscadine pomace by coating the extracts obtained with two materials and examine their effect on storage stability of total phenol. The specific objectives of this experiment are 1) To develop a low cost alternative to long extraction and purification procedures for extraction of total phenol

which can be utilized by the producers at producer level. 2) To prepare microencapsulated grape extract powder using two coating materials (gum arabic and whey protein) and without coating, characterize microcapsules using SEM and particle size analysis. 3) To evaluate degradation of total phenol over time by storing at different environmental storage conditions.

MATERIALS AND METHODS

Samples

Grape extract samples were produced following the extraction procedure in fig 4.1. P1 and P2 were permeate samples produced without bladder press. P3 and P4 were permeate samples produced with bladder press. C3 and C4 were retentate samples produced with bladder press. These samples were microencapsulated with gum arabic (GA) and whey protein concentrate (WP) and then subjected to storage study.

Chemicals

Gallic acid, sodium bicarbonate, TCA (Trichloro acetic acid) and Folin-Ciocalteau reagent were purchased from Sigma Aldrich (St. Louis, MO, USA). Ethanol (95%) and isopropyl alcohol (99.9%) were obtained from Central Research Store at UGA. Whey protein concentrate-80 (WP) was obtained from Glanbia Foods (East Gooding, ID, USA) whereas gum arabic was provided by TIC gums (Belcamp, MD). Composition of WP was protein (dry basis) – 79.3%, moisture – 3.7%, fat - 8.9% while composition of gum arabic was carbohydrate (dry basis) - 85%, moisture -10%, protein-2%.

Microencapsulation of Grape Extract with Gum Arabic and Whey Protein Concentrate by Spray Drying

Eighty grams of whey protein was hydrated for one hour in 320 ml of grape extract. The total solids of solution was kept at 20% w/v. The solution was homogenized using a high speed shear stirrer LabTek (Omni International Inc, Gainesville, VA) at 8000 rpm for 10 minutes. The

procedure was repeated using gum arabic as coating material. The solution was covered with an aluminum foil at all times and homogenization was carried out under yellow light conditions. Solution of grape extract without any coating material was also taken for spray drying. The three solutions were spray dried using spray dryer (Anhydro Inc., Olympia Fields, IL) at a feed rate of 57-65 ml/min, inlet air temperature 190°C, outlet air temperature 90°C, atomizer speed of 30,000 rpm (60% of maximum 50,000rpm). The powders obtained from spray drier were then stored in amber colored air tight glass containers flushed with nitrogen gas and stored in freezer at -20°C until further use.

Particle Size Distribution (PSD)

Particle size analysis was done using Malvern Mastersizer S laser diffraction system with QSpec small volume sample dispersion unit operating at 2300 rpm (Malvern Instruments, Worcestershire, U.K.). Mastersizer type used was S 300 RF with particle size range between 0.5 to 900 μm. The Mastersizer S uses the mie theory of light diffraction for particle size measurement which assumes an equivalent sphere size of particles and performs particle size calculations on the fact that angle of light diffraction is inversely proportional to particle size (Rawle 2002). The 10% (w/w) solution of spray dried powder was prepared in deionized water and allowed to hydrate for 10 minutes and stirred before applying to dispersion unit of the mastersizer. The powders were further suspended in ethanol and isopropanol using the above protocol to determine particle size distribution due to medium changes. Before proceeding with determination of particle size distribution, we checked the percentage of particles retained by suspending sample in different mediums. The raw measurement data obtained from the mastersizer consisted of particle size distribution PSD (d_i = 0.05-865 μm) of the respective sample expressed as volumetric mean diameter of particles. Additionally, particle size

percentiles, summary statistics of PSD was calculated using the mastersizer data acquisition software package (Mastersizer S long Bed v2.19). Triplicate samples were evaluated for each type of spray-dried powder. Since the results obtained for Isopropyl alcohol and ethyl alcohol were similar, we have represented the results for ethyl alcohol.

Scanning Electron Microscope (SEM)

Morphology of microcapsules was observed using scanning electron microscope LEO 982 (FE-SEM, Carl Zeiss SMT Inc. One Corporation Way, Peabody, MA 1960) using methodology described by Sheu and Rosenberg (1998). The powders were fixed on 10 mm stubs with double sided adhesive tape which were made electrically conductive by coating under vacuum with gold using SPI-module sputter coater for 60 seconds (15.3 nm; it is thickness of gold coating). Digital images were obtained in the slow scan mode, working distance of 4mm, excitation voltage of 3kV, resolution of 1024*768 pixels and magnification varying from 50 to 50,000X.

Microencapsulation Yield (MEE)

In order to determine MEE of microencapsulated whey protein powder, 100mg of microencapsulated (whey protein) grape extract powder was taken and dissolved in 15ml of deionized water. Subsequently, 10ml of 33% trichloro acetic acid solution was added to it for precipitating proteins. This solution was centrifuged in an induction drive centrifuge (Beckman J2-21M, Fullerton, CA) at 14000 RPM for 15 minutes. The supernatant was taken in 25ml volumetric flask and made upto mark with deionized water (Georgetti and others 2007). In order to determine MEE of microencapsulated gum arabic powder, 100mg of microencapsulated (gum arabic) grape extract powder was taken and 1.0 ml of water was added to it and stirred for 5 minutes. Further 20 ml of 80% ethyl alcohol was added and stirred for 15 minutes. The ethanol

solution was centrifuged at 14000 rpm for 20 minutes and supernatant fractions collected in 25ml of volumetric flask where volume was made up to mark with deionized water (Zhang and others 2007; Ersus and Yurdagel 2007). Supernatant fraction was taken from volumetric flask and analyzed for microencapsulation yield using the Folin Ciocalteau method and the absorbance was compared against standard solution of gallic acid at λ_{max}= 766 nm using Hewlett Packard 8451A diode array spectrophotometer (Avondale, PA, USA). For determination of total phenol concentration, equivalent amount of gum arabic absorbance at 766 nm was subtracted from total absorbance of microencapsulated gum arabic. Experiments were done in duplicate and MEE was determined based on formula given below.

$$\text{Microencapsulation efficiency} = \frac{\text{Total Phenol- Extractable Phenol}}{\text{Total Phenol}} \text{ X } 100$$

Accelerated Shelf Life Testing

Microencapsulated grape extract coated with whey protein concentrate and gum arabic were spread in small sample cups and care was taken to see that the layer of powder did not exceed few millimeters so as to avoid uneven exposure to humidity and ultraviolet (UV) light conditions. Two wooden chambers were constructed for storage at 4 and 25 ˚C and each wooden chamber had two shelves (fig 4.2). The upper top shelf was fitted with black light which emitted UV light confined to top shelf. The UV light intensity was measured by UVX Radiometer (Upland, CA, USA). The UV light intensity of the lights was 83$\mu W/cm^2$. We checked the UV light intensity emitted by direct Sun and it was 65 $\mu W/cm^2$. The UV light intensity after it passed through the glass lid was 16.5 $\mu W/cm^2$ and when it passed the semi permeable cloths cover the intensity was 10.7 $\mu W/cm^2$ .Dark conditions were maintained in bottom shelf. Both the shelves were covered with opaque black plastic covers to avoid outside light exposure. The small sample

cups were covered with transparent loosely knitted cloth to allow the passage of UV light and moisture but which would prevent cross-contamination between samples (fig 4.3). Saturated salt solutions of sodium bromide, sodium nitrite were prepared and transferred to transparent glass display jars where they would generate water activity of 0.64 at 4 and 25 °C (http://tinyurl.com/6res2e), respectively. The RH inside the glass display jars was monitored using temperature-humidity data loggers. The top of display jar was sealed with airtight lid. Once saturated salt solutions reached the optimum RH levels, sample cups containing spray-dried microcapsules were kept inside. There were three powder types 1) Pure grape extract sample, 2) microencapsulated grape extract with whey protein and 3) microencapsulated grape extract with gum arabic. Again there were three sample types in each powder types. P1 and P2 were samples of permeate without bladder press, P3 and P4 were samples of permeate with bladder press and C3 and C4 were retentate samples with bladder press. The concentration of total phenol on day one is considered as zero week sample. Samples were taken out every week for 8 weeks, from glass containers to determine the change in total phenol content and returned to the glass containers.

Total Phenol

Total phenol was estimated colorimetrically using the Folin-Ciocalteau method (Singleton and Rossi 1965). A sample aliquot of 200µL was added to 800 µL of deionized water, 5 mL of Folin-Ciocalteau reagent, and 4 mL of saturated sodium carbonate solution (75g/L) and mixed in vortex stirrer (Fisher vortex Genie 3, Fisher Scientific, Pittsburgh, PA). The absorbance was measured at 765nm with a Hewlett Packard 8451A diode array spectrophotometer (Avondale, PA) after incubation for two hours at room temperature (~25°C). Quantification was based on the standard curve generated with 0, 50, 100, 200, 300, 400, 600,

800 and 1000 mg/L of gallic acid. The final concentration of total phenol in extract samples was calculated as mg/L. The experiment was conducted under yellow light.

Statistical analysis

Linear regression analysis was used to obtain the degradation rate constants (k) for all the samples studied. The standard errors of the rate constant k ($s_{k,}$ standard errors of the slope) were calculated using (SAS Proprietary Software, 2003-2005), Release 9.1 for Windows (SAS Institute Inc., Cary, NC) and for determining the correlation coefficients.

RESULTS AND DISCUSSION

Particle Size Distribution

The particle size distribution of spray dried powder particles was carried out in solvents ethyl alcohol and water (Table 4.1). To determine the solubility in different solvents the particles were dissolved in ethyl alcohol and isopropyl alcohol. One gram of sample was taken and dispersed in 50 ml of solvent (deionized water, 50% ethanol, 95% ethyl alcohol, 99% isopropyl alcohol); stirred for 5 minutes and kept undisturbed for 15 minutes before being filtered through Whatman filter paper no 42. The filter paper has a cutoff size of 2.5 µm. The weight of particles retained after passing through the filter paper was recorded. It was observed that with deionized water 2.03% of particles were retained, with 50% ethyl alcohol 7.05% of particles were retained and with 95% ethyl alcohol and 99% isopropyl alcohol 89% of the particles were retained. This indicated that the particle size using ethanol and isopropyl alcohol were substantially different than that obtained with water. The mean particle size ranged from 1.04µm to 92.70µm when using water as dispersant; ranged from 23.14µm to 92.70µm when using ethyl alcohol as dispersant. The particle size distribution of particles in ethyl alcohol and water and cumulative percentage of particles is shown in fig 4.4 and 4.5 respectively. While

measuring size of particles made from whey protein using water and ethyl alcohol as dispersant the mean size was around 15µm and 50 µm, respectively. While measuring size of particles made from gum arabic using water and ethyl alcohol as dispersant the mean particle size was around 15µm and 40µm, respectively. The change in the particle size is attributed to change in solubility of particles in different solvents. It also indicates that it is better to use a dispersant other than water for particle size distribution as particles got solublized in water resulting in erroneous results for particle size distribution.

Scanning Electron Microscopy

Particles made without any wall material were more solid and compact compared to particles with wall material (fig 4.6). The outer wall material affected the morphology and structure of the spray-dried microcapsules. It was observed that the structure was more spherical with whey protein compared to gum arabic (fig 4.7 and 4.8). Smaller capsules exhibited deep surface dents indicating solidification of the walls prior to onset of expansion (Sheu and Rosenberg 1998). The wall of microcapsule made of gum arabic is more homogenous, compact and thick as compared to whey protein where the wall is thin and has some voids in it (fig 4.7 and 4.8). Further (fig 4.8D, 4.8E and 4.8F) it is seen that there are voids in the microcapsule wall (whey protein) and pores on the wall surface which are absent in fig 4.7B, 4.7C and 4.7D microcapsule wall (gum arabic), respectively.

The micrographs of microcapsules also show that indentation of surface is more prevalent in smaller particles than larger ones indicating that solidification of walls occurred prior to expansion of microcapsules (fig 4.7A, 4.7C and 4.8C). It can also be attributed to drying rate. High drying rates, associated with small particles usually lead to rapid wall formation and preventing occurrence of dent smoothening.

Spray dried particles are usually hollow particles. Figure 4.7B, 4.7D, and 4.8D indicate that there is a vacuole in the microcapsule center which occupies most of microcapsule volume. Vacuole formation happens from a shrinking process that occurs after case hardening of the outer surface followed by expansion of air bubbles trapped inside the droplet.

Spray-dried microcapsules with wall material consisting of polysaccharides exhibit considerable surface indentations and the formation of indentations has been attributed to the effects of wall composition, atomization and drying parameters, uneven shrinkage at early stages of drying, and to the effect of a surface tension-driven viscous flow (Sheu and Rosenberg 1998). The thermal expansion of air or water vapor inside the drying particles also known as "ballooning" associated with high drying rates can smooth out dents to a large extent. The rate at which the dent smoothes out is dependent on the drying rate and viscoelastic properties of the wall matrix. Results from fig 4.7 and 4.8 indicate that surface indentation was more prevalent in small particles than in large ones.

Microencapsulation Efficiency (MEE)

Microencapsulation efficiency (MEE) represents the degree of retention of the core, the active ingredient within the wall material. The most useful microencapsulation process would lead to a least loss of the core material from the wall material and is a useful determination in selecting the wall material. Among the most important variables affecting the retention of the core are the choice and concentration of wall solids, the mass ratio of wall to core, physiochemical properties of wall and core components, and physical properties of the emulsion prior to drying, and drying conditions. High drying rates that lead to a fast formation of crust around the drying droplets favor high retention as long as no structural damage is introduced. Microencapsulation efficiency (MEE) was determined by extracting total phenols after spray

drying process. The microencapsulation yield of grape extract with whey protein and gum arabic was 99.15% and 88.15%, respectively.

Accelerated Shelf Life Testing

In our study, we examined the effects of temperature (4 and 25 °C); light conditions (UV light and dark condition); coating materials (gum arabic and whey protein concentrate) vs. no coating material; effect of processing method (with bladder press vs. without bladder press) on the degradation kinetics of total phenol. Degradation kinetics of total phenol in pure grape extract powder, grape extract powder microencapsulated with gum arabic, and grape extract powder microencapsulated with whey protein is shown in fig 4.9-4.10, 4.11- 4.12, 4.13- 4.14, and Table 4.2-4.3 respectively.

From fig.4.9 it is noted that the concentration of total phenol in permeate (P1) decreases from 634.59mg/L to 574.92mg/L at 4 °C. It is observed from fig. 4.10 that concentration of total phenol in permeate (P1) decreases from 634.59mg/L to 462.59 mg/L at 25 °C. It is also seen from fig 4.10 that the concentration of total phenol in retentate (C3) decreases from 606.21 to 356.59mg/L under dark conditions and from 606.21 to 294.28mg/L under light conditions at 25 °C. This shows that there is more degradation at 25 °C compared to 4 °C and more in retentate over permeate. As per study carried out by Chang and others (2006) on total phenol stability in Hawthorn fruits and drink, they found that phenolic were stable at 4 °C but were relatively unstable at higher temperatures (23 °C and 40 °C) with varying rates of degradation. At room temperature of 23 °C there was degradation of 50% and 30% in epicatechin and procyanidin B2 after storage for period of 6 months.

In spray dried grape extract samples there is decrease in total phenol content from week 1 to week 8. The color of spray dried powder changes from purple to black at the end of week 8.

The structure of powder changed from free flowing to glassy to rubbery observed in both the powders kept at 4 and 25 °C. It is observed from fig. 4.11 that concentration of total phenol in permeate (P1) increased from 44.14 to 49.36mg/L and in retentate (C3) from 57.0 8 to 81.51 mg/L at 4 °C. It is observed from fig. 4.12 that concentrations of total phenol in permeate (P1) increased from 44.14 to 46.03 mg/L and in retentate (C3) from 57.08 to 64.20 mg/L at 25 °C. Singleton and Rossi (1965) reported that phenols react with protein and carbohydrates which gives a higher total phenol reading than is expected. Kinetic studies on the degradation of beetroot pigment encapsulated in three different polymer matrices (pullulan and two maltodextrin samples) were carried out by Serris and Biliaderis (2001) under different water activity and three different storage temperatures. The highest amount of rate constants for degradation of pigments were observed at intermediate water activity level (a_w=0.64) for all matrices and all three storage temperatures studied. The reaction rates (K) increased with increase in temperature. This trend can be explained by the fact that the degradation reaction is controlled by the mobility of the reactants. This was also observed by Tandale (2007) which led to selecting a_w of 0.64 instead of using multiple water activities. In powders encapsulated with gum arabic where there is an increase or no change in the total phenol concentration at 4 and 25 °C. It can be related to the protein fraction of gum arabic reacting with phenols forming polymers and giving higher readings. The anthocyanin contents of encapsulated black carrot powder which were encapsulated with three different coating materials was decreased by 33% at end of 64 days storage period at 25 °C (Ersus and Yurdagel 2007). They reported that at 50 °C stability of polar phenolic compounds was reduced to 72% over the initial value. In study carried out on immature acerola juice (Righetto and Netto 2005) microencapsulation using maltodextrins DE25, gum arabic, or a mixture of both in different proportions; the Tg of gum

arabic varied from 62 (a_w =0.33) to 42.6 °C (a_w =0.54), respectively. Tg decreased to 1.84-8.05 °C (a_w=0.54) when juice from immature acerola was added. This is observed in microencapsulated gum arabic powders made from grape extract in present study where the powders were caking both at 4 °C and 25 °C which indicated that powder was near Tg and therefore did not have significant changes in phenolic content over time. The powder color had changed from pink to red over period of 8 weeks.

It is observed from fig. 4.13 that concentration of total phenol in permeate (P1) decreased from 44.47 mg/L to 41.97 mg/L at 4 °C while on retentate (C3) it decreased from 59.31 mg/L to 51.50 mg/L at 4 °C. It is observed from fig. 4.14 that concentration of total phenol in permeate (P1) decreased from 44.47 mg/L to 28.74 mg/L at 25 °C while on retentate (C3) side it decreased from 59.31 to 26.48 mg/L at 25 °C. Thus there is a decrease in total phenol with increase in temperature and more decrease is observed in retentate over permeate side. Cai and Corke (2000) reported that in spray dried Amaranthus betacyanin extracts, the bulk density of pigment powder decreased with increase in spray drying temperature. Although higher drying rate was obtained at higher drying temperature, higher ratio of surface-to-volume for the spray dried powder caused lower bulk density of the powder. The lower the bulk density, the more occluded air within the powders and therefore, greater the possibility of oxidative degradation of pigments and reduced storage stability. Also we observed (fig 4.8D, 4.8E and 4.8F) that there are voids on the microcapsule and pores in microcapsule wall which are sources of oxidation. The color of the sample was faint pink initially which turned to nearly white at the end of eight weeks of storage.

Statistical analysis

The results of linear regression shows that samples (whey protein and no coating) follow zero order reaction. Samples encapsulated with gum arabic seem to have increase in concentration with storage time and do not follow any reaction order. The rate constants and the half lives (table 4.2 and 4.3) of samples with whey protein coating and without any coating show change with changes in temperature but do not show any change with change in light and dark conditions. The half lives at 25 °C is one third to one fifth to that at 4 °C. It is found that (table 4.2) samples coated with whey protein which have a half life of 25 to 66 g/ml /week at 4 °C have a half life of 8 to 10 weeks at 25 °C. It is found that samples coated with whey protein have a rate constant of 0.3 to 3.7 g/ml /week; samples without coating have a rate constant from 7.0 to 45.0 g/ml /week depending on temperature. This indicates that it is beneficial to have a coating of whey protein or gum arabic for protecting the phenolic antioxidants in the extract powder which would degrade faster without any coating. The samples should be stored in closed airtight containers at refrigerated temperature to have increased shelf life.

CONCLUSIONS

Microencpsulation of muscadine grape extract was carried out with two coating materials whey protein and gum arabic. Particle size distribution was carried out on the encapsulated particles and particle size was determined using scanning electron microscopy. Accelerated shelf life testing was carried out by exposing the encapsulated and uncoated samples to various environmental conditions. It was found that maximum degradation was observed in uncoated samples followed by whey protein and gum arabic. The samples were shown to follow zero order reaction and their half life was also determined using linear regression. The results of the

present study indicate that it is better to encapsulate samples with whey protein and gum Arabic to have increase in shelf life.

ACKNOWLEDGEMENTS

The authors would like to thank Glanbia Foods Inc. for graciously providing whey protein concentrate and TIC gums for providing gum arabic. The authors would like to thank Mr. Glenn D. Farrell for his technical help and Jerry Davis for Statistical analysis. We would also like to thank Dr. John Shields for his help preparing scanning micrographs.

Table 4.1 Particle size distribution of spray dried powder particles (in different dispersants)

Sample Type**	**Volume mean diameter of Particles* in Dispersing Medium (μm)**	
	Water	**Ethyl Alcohol**
P1	18.02	75.38
P3	92.70	92.45
C3	1.04	23.14
WP P1	15.99	56.22
WP P3	14.80	50.20
WP C3	15.54	48.11
GA P1	20.82	43.18
GA P3	16.62	36.82
GA C3	3.50	39.22

**Average of triplicates*

**P1 and P3 - permeate, C3 - retentate, WP - whey protein, GA- gum arabic.

Table 4.2 Degradation reaction rates (k), standard errors of the slope (s_k), half life periods ($T_{1/2}$) and correlation coefficients (r^2) for encapsulated whey protein samples stored at different temperatures, light and dark conditions and 0.64 water activity

Sample	Parameters	Temperature			
		4 °C		25°C	
		Dark	Light	Dark	Light
P1	$k \pm s_k$	0.27± 0.12	0.40 ± 0.08	2.64 ± 0.15	2.28 ± 0.45
	$T_{1/2}$	38.61	51.65	8.28	9.23
	r^2	0.42	0.81	0.98	0.81
P2	$k \pm s_k$	0.07 ± 0.28	0.39 ± 0.16	2.40 ± 0.43	2.69 ± 0.11
	$T_{1/2}$	-314.6	56	9.11	8.15
	r^2	0.01	0.47	0.84	0.99
P3	$k \pm s_k$	0.136 ± 0.35	0.29 ± 0.13	3.02 ± 0.40	2.73 ± 0.15
	$T_{1/2}$	145.26	66.47	6.58	7.26
	r^2	0.02	0.44	0.9	0.98
P4	$k \pm s_k$	0.34 ± 0.08	0.36 ± 0.15	2.08 ± 0.19	2.12 ± 0.01
	$T_{1/2}$	44.33	41.93	7.35	7.21
	r^2	0.72	0.47	0.95	0.99
C3	$k \pm s_k$	0.93 ± 0.11	0.91 ± 0.12	3.33 ± 0.11	3.73 ± 0.11
	$T_{1/2}$	31.63	32.25	8.89	7.93
	r^2	0.91	0.91	0.99	0.99
C4	$k \pm s_k$	0.99 ± 0.39	0.33 ± 0.39	2.37 ± 0.22	3.21 ± 0.20
	$T_{1/2}$	25.59	75.26	10.72	7.92
	r^2	0.51	0.11	0.95	0.97

Where P1,P2,P3 and P4 - permeate, C3 and C4- retentate, WP - whey protein, GA- gum arabic.

Table 4.3 Degradation reaction rates (k), standard errors of the slope (s_k), half life periods ($T_{1/2}$) and correlation coefficients (r^2) for samples without coating stored at different temperatures, light and dark conditions and 0.64 water activity

Sample	Parameters	Temperature			
		4 °C		25°C	
		Dark	Light	Dark	Light
P1	$k \pm s_k$	7.45 ± 0.39	8.61 ± 0.82	21.50 ± 1.44	25.31 ± 13.63
	$T_{1/2}$	42.50	36.80	14.57	12.53
	r^2	0.99	0.99	0.77	0.99
P2	$k \pm s_k$	13.41 ± 0.94	7.06 ± 1.27	22.94 ± 4.75	26.32 ± 12.84
	$T_{1/2}$	23.12	43.88	13.51	11.77
	r^2	0.99	0.96	0.80	0.96
P3	$k \pm s_k$	11.96 ± 0.56	7.07 ± 0.005	23.87 ± 7.32	25.33 ± 9.61
	$T_{1/2}$	18.74	31.7	9.38	8.84
	r^2	0.99	1.00	0.88	0.91
P4	$k \pm s_k$	7.37 ± 1.76	2.30 ± 3.15	24.65 ± 9.52	27.63 ± 3.03
	$T_{1/2}$	30.21	96.59	9.03	8.05
	r^2	0.94	0.35	0.98	0.87
C3	$k \pm s_k$	10.58 ± 0.41	11.68 ± 0.32	31.20 ± 10.56	40.19 ± 8.88
	$T_{1/2}$	28.62	25.95	9.71	7.54
	r^2	0.99	0.99	0.95	0.89
C4	$k \pm s_k$	18.61 ± 0.70	18.64 ± 0.70	37.81 ± 11.90	45.39 ± 10.10
	$T_{1/2}$	17.63	17.63	8.69	7.24
	r^2	0.99	0.97	0.95	0.91

Where P1,P2,P3 and P4 - permeate, C3 and C4 - retentate, WP - whey protein, GA- gum arabic.

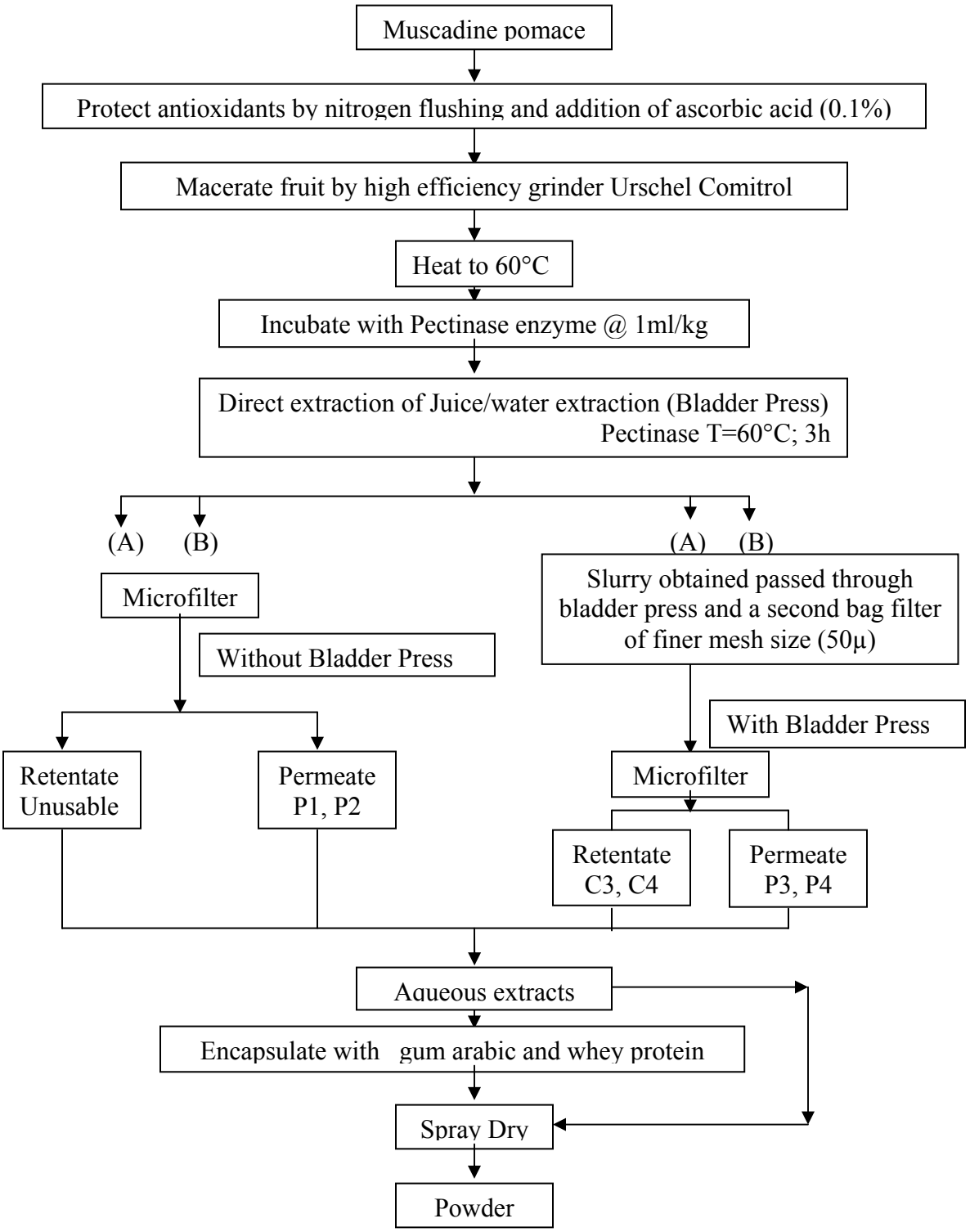

Figure 4.1 Experimental schemes for making microencapsulated grape extract powder using gum arabic and whey protein and without any coating

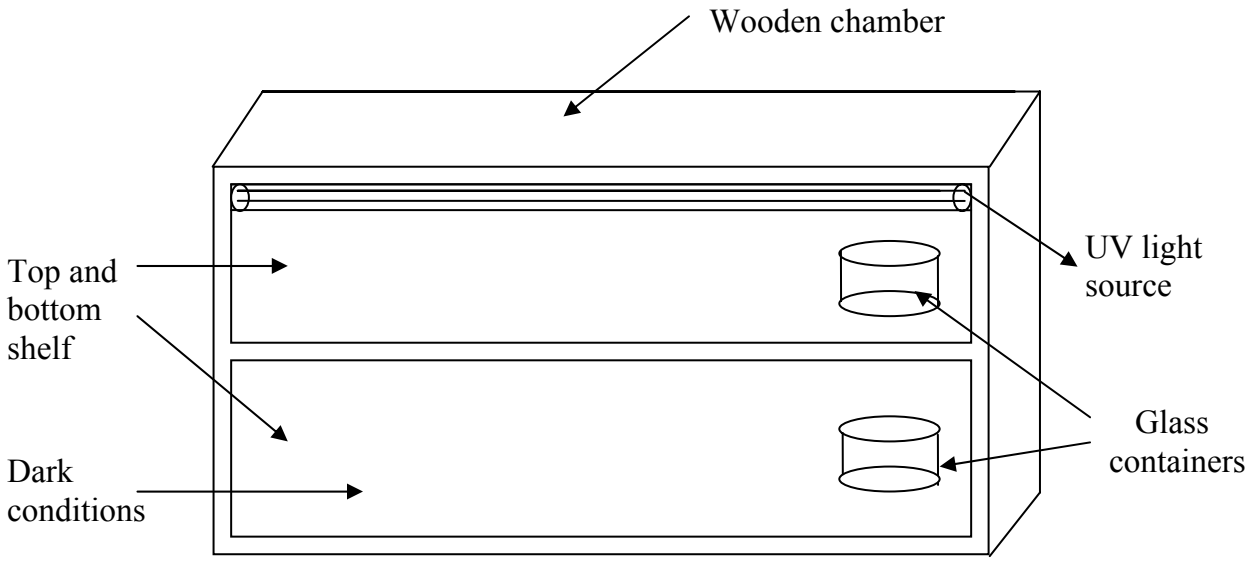

Figure 4.2 Wooden chambers fitted with black light to see effect on storage of powder

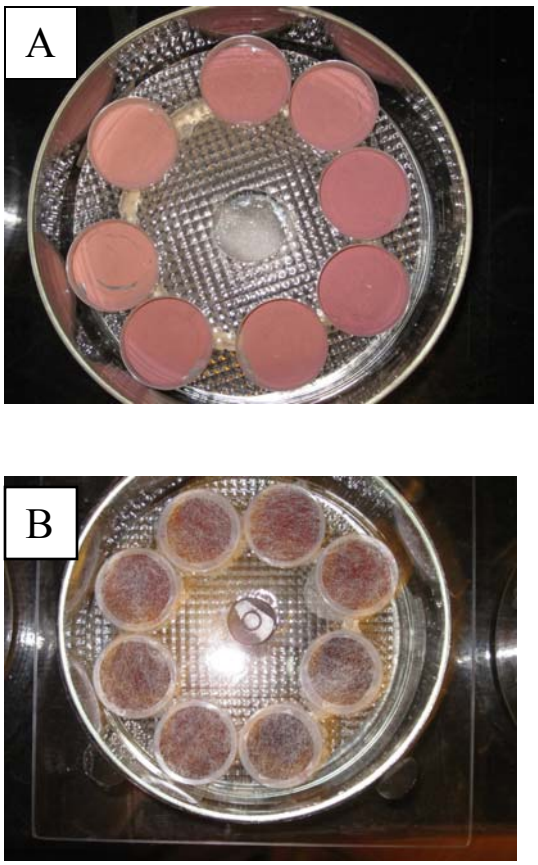

Figure 4.3 Photograph of spray dried powder in glass display jars (A) No lid (B) Lid and semi permeable cloths cover

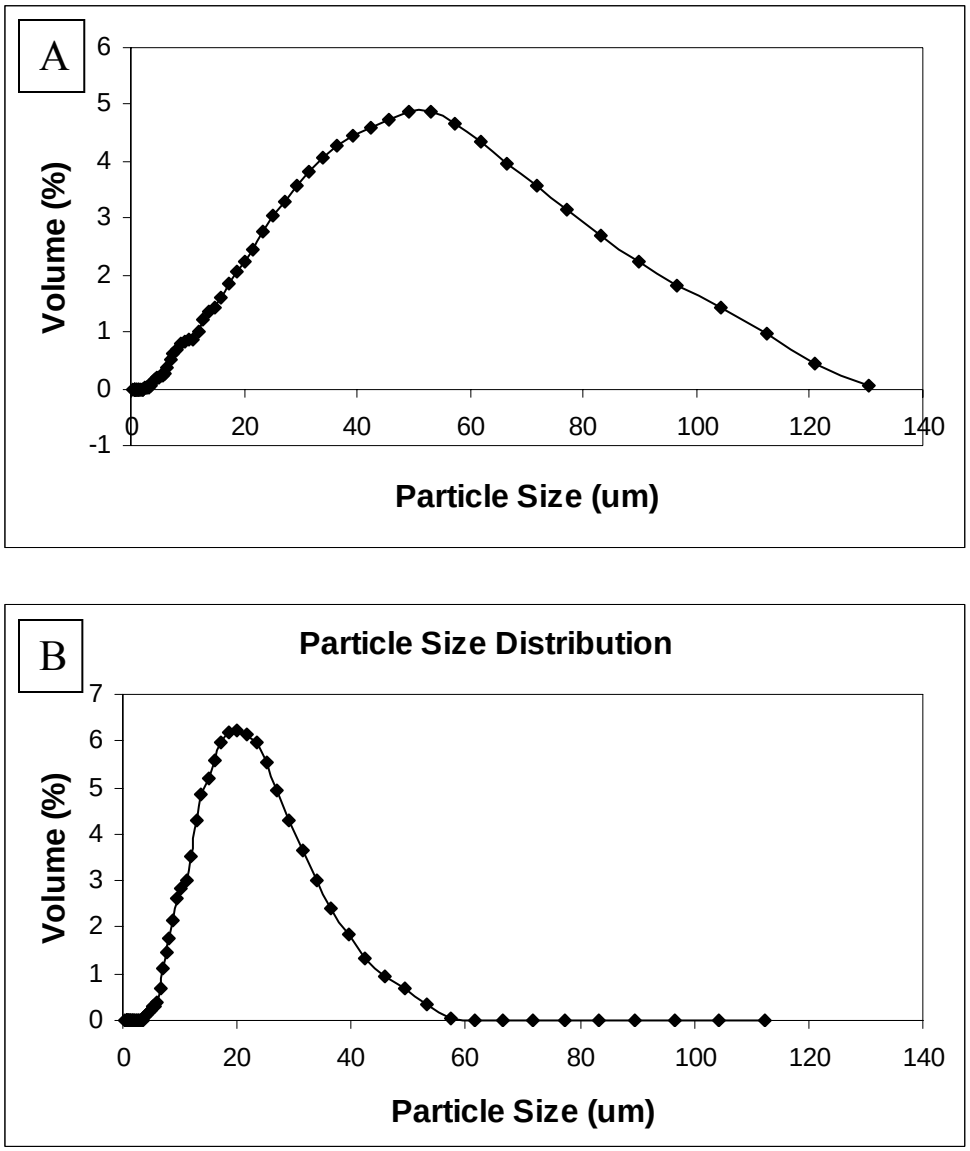

Figure 4.4 Particle size distributions of spray dried powder particles with medium [(A) Ethyl alcohol, (B) Water]

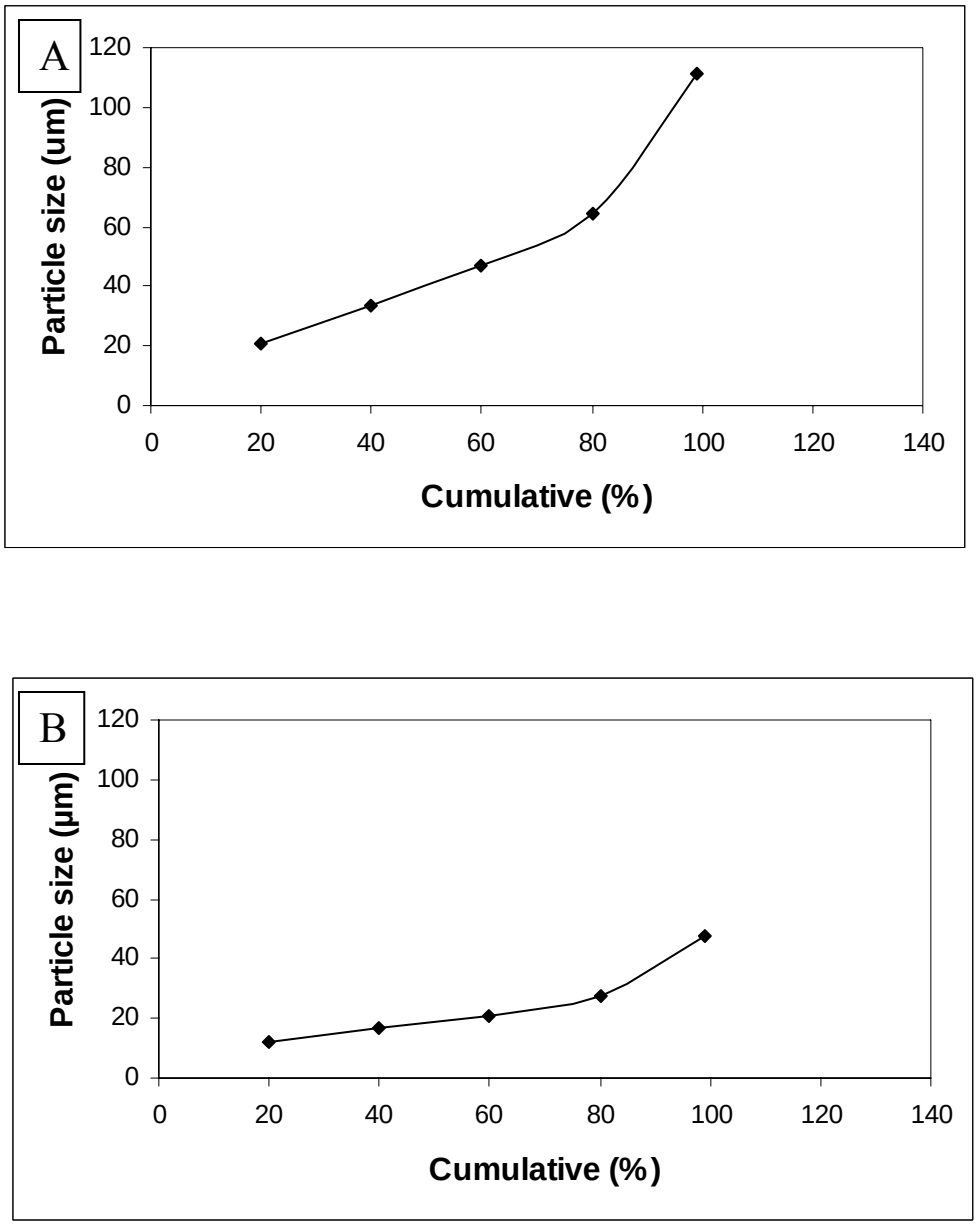

Figure 4.5 Cumulative percentage particle size distribution in medium [(A) Ethyl Alcohol, (B) Water]

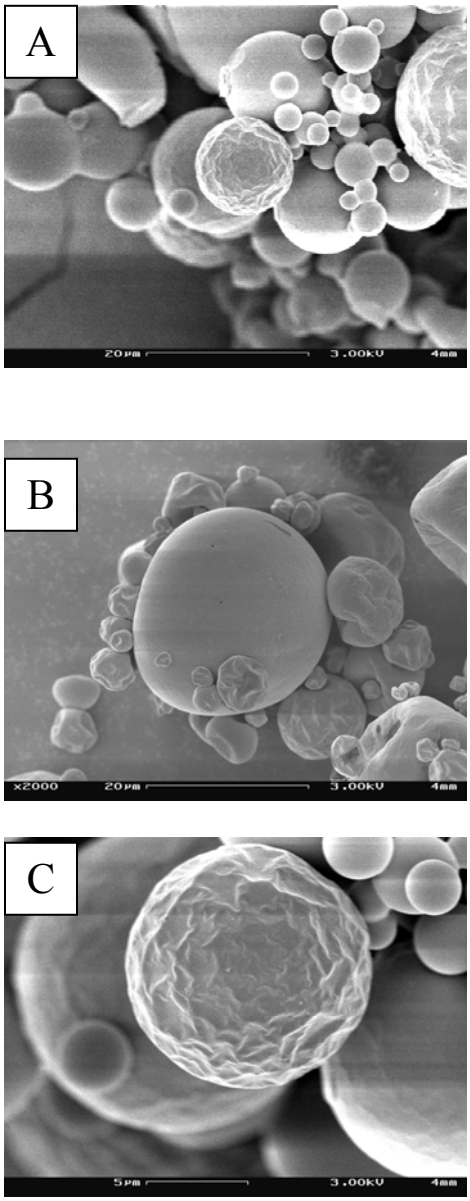

Figure 4.6 SEM micrograph of grape extract powder particles without microencapsulation with magnifications of (A) 1000X (B) 2000X and (C) 3000 X

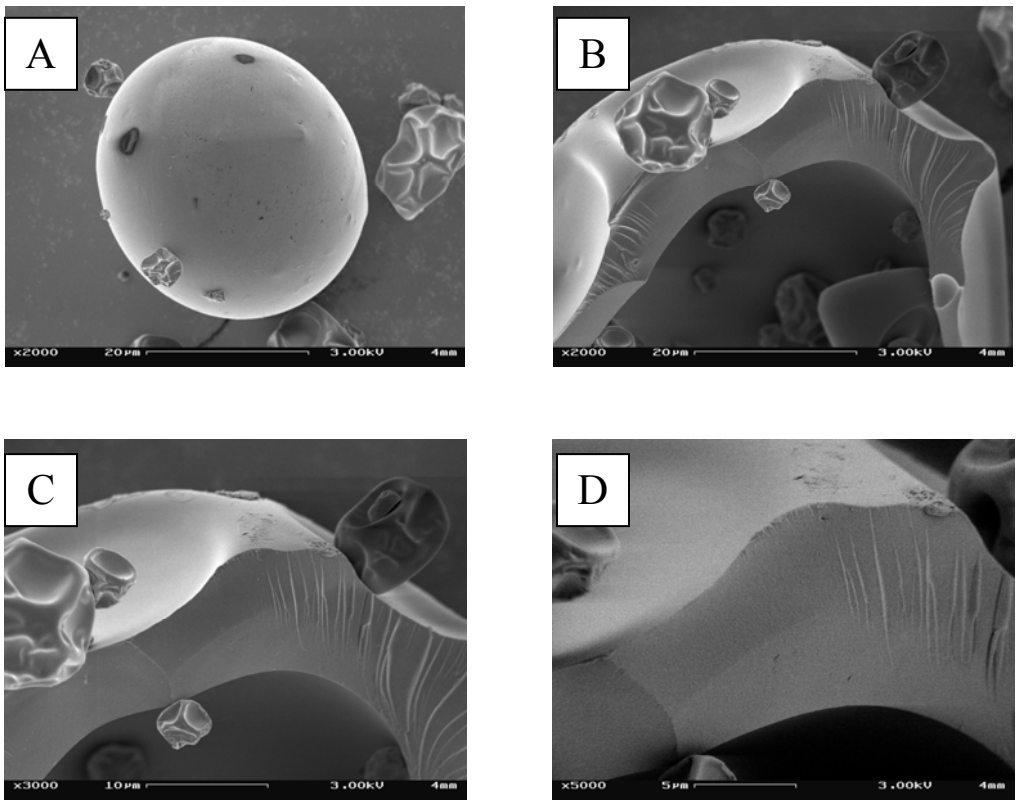

Figure 4.7 SEM micrographs of grape extract microencapsulated with gum arabic coating with magnifications of (A) 2000X (B) Cut out of shell of gum arabic microcapsule with magnification 2000X (C) Magnified view of cut out of shell of microcapsule 3000X and (D) Magnified view of the cut out of shell wall of microcapsule 5000X

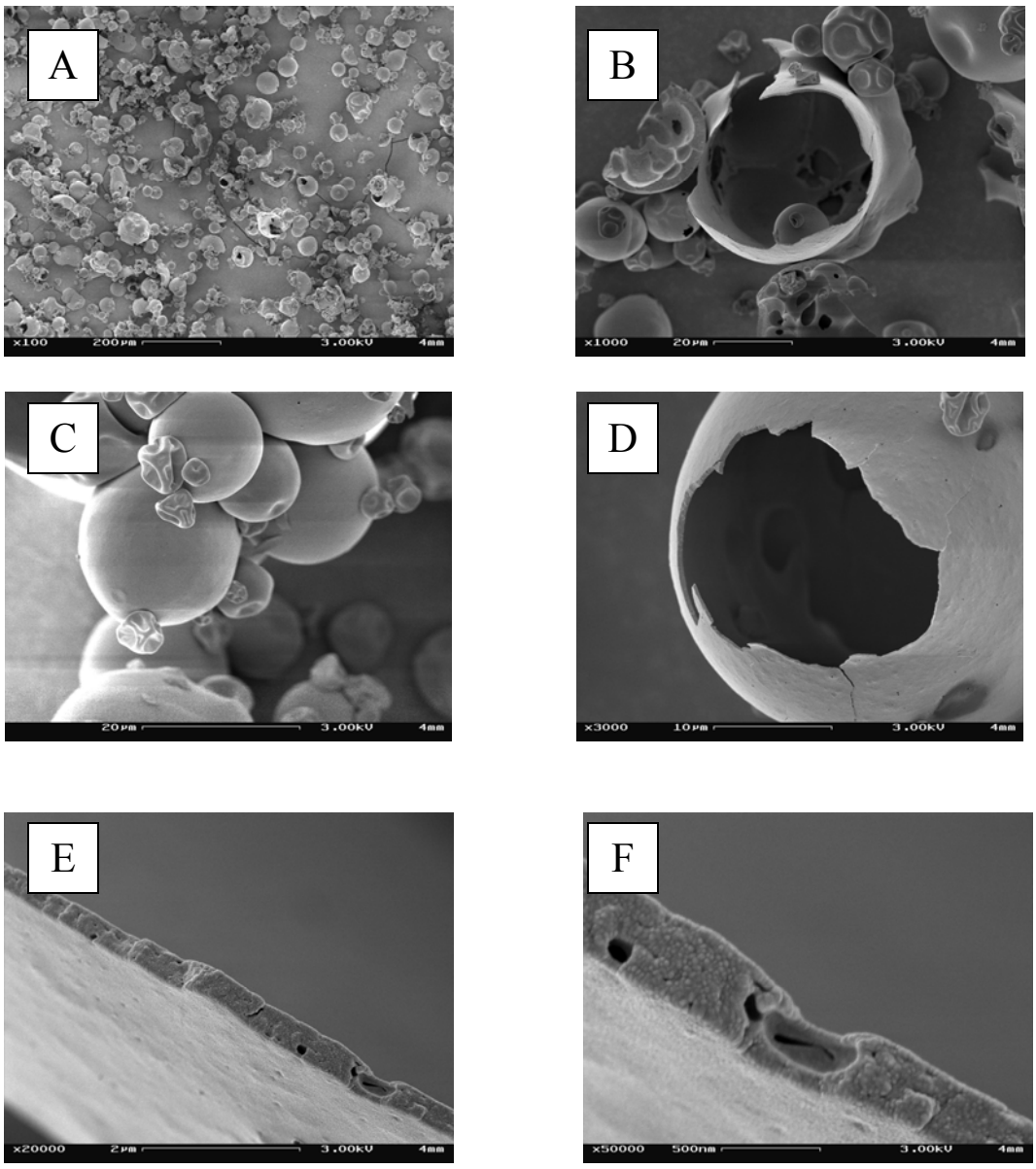

Figure 4.8 SEM micrographs of grape extract microencapsulated with whey protein coating with magnifications of (A) 100X (B) 1000X (C) 2000X (D) Broken hollow part of microcapsule 3000X (E) Magnified view of cut out of shell shown in fig 4.8D 20,000X and (F) Magnified view of fig 4.8 E 50,000X.

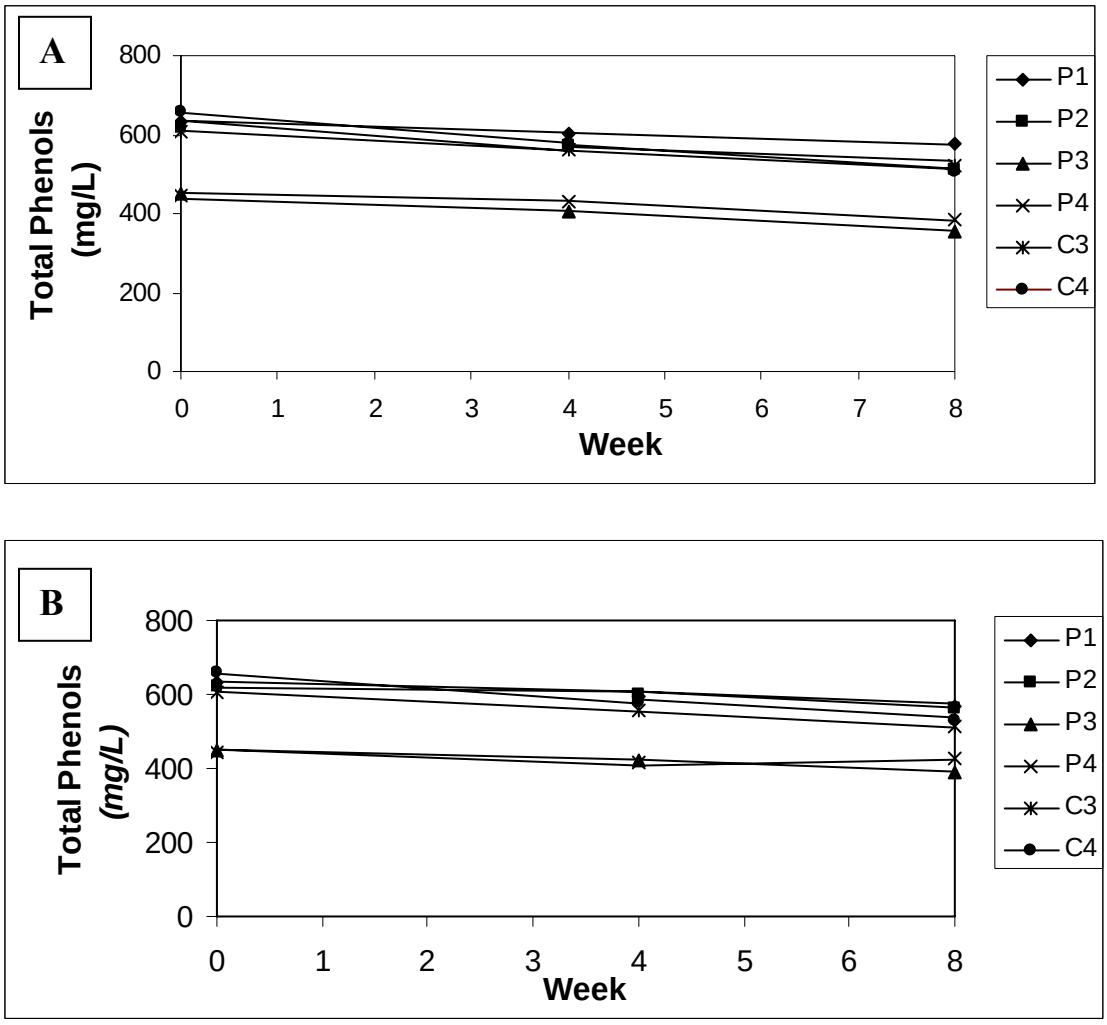

Figure 4.9 Degradation kinetics of total phenol in powder at 4 °C (A) Dark and (B) UV light conditions. Where P1,P2,P3 and P4 - permeate, C3 and C4 - retentate, WP - whey protein, GA- gum arabic.

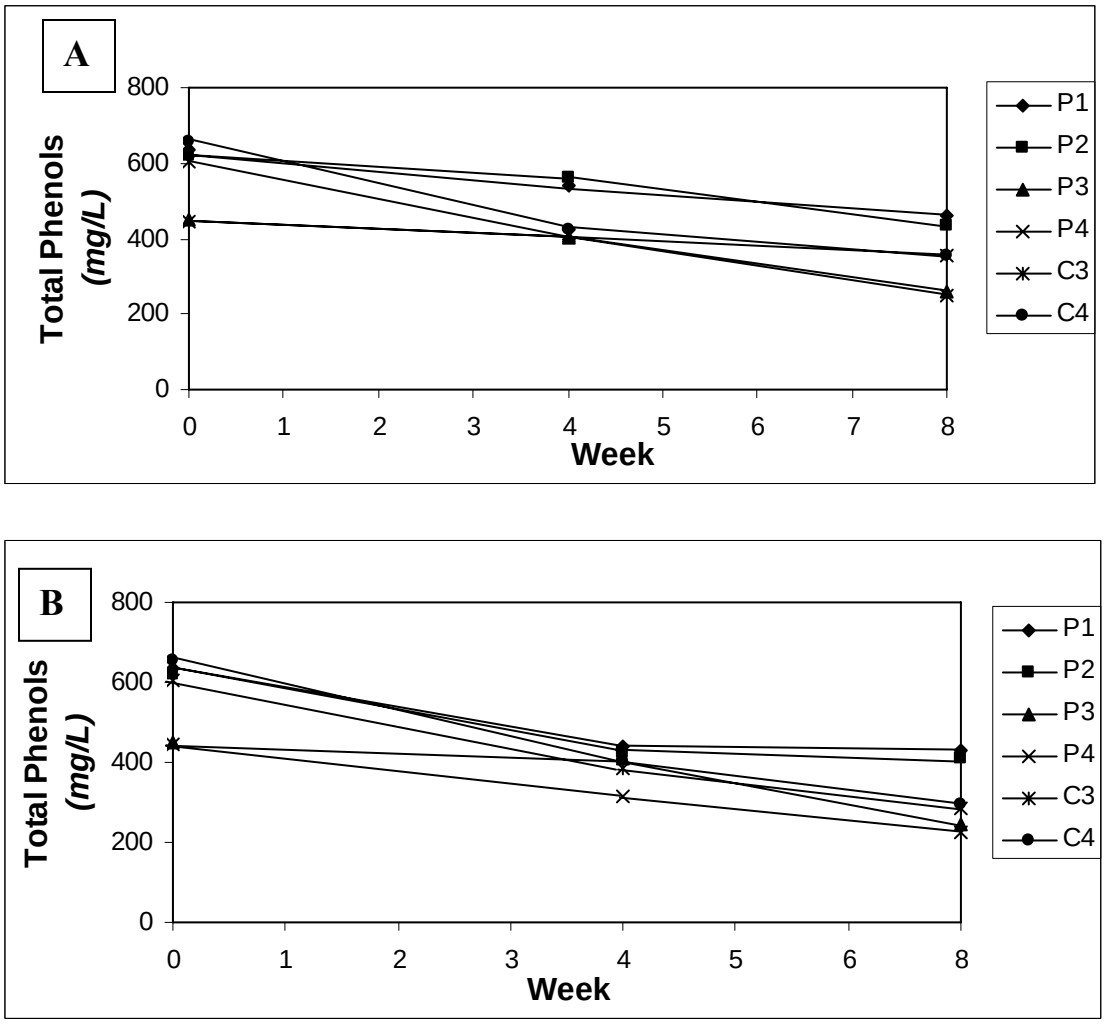

Figure 4.10 Degradation kinetics of total phenol in powder at 25 °C (A) Dark and (B) UV light conditions. Where P1,P2,P3 and P4 - permeate, C3 and C4- retentate, WP - whey protein, GA- gum arabic.

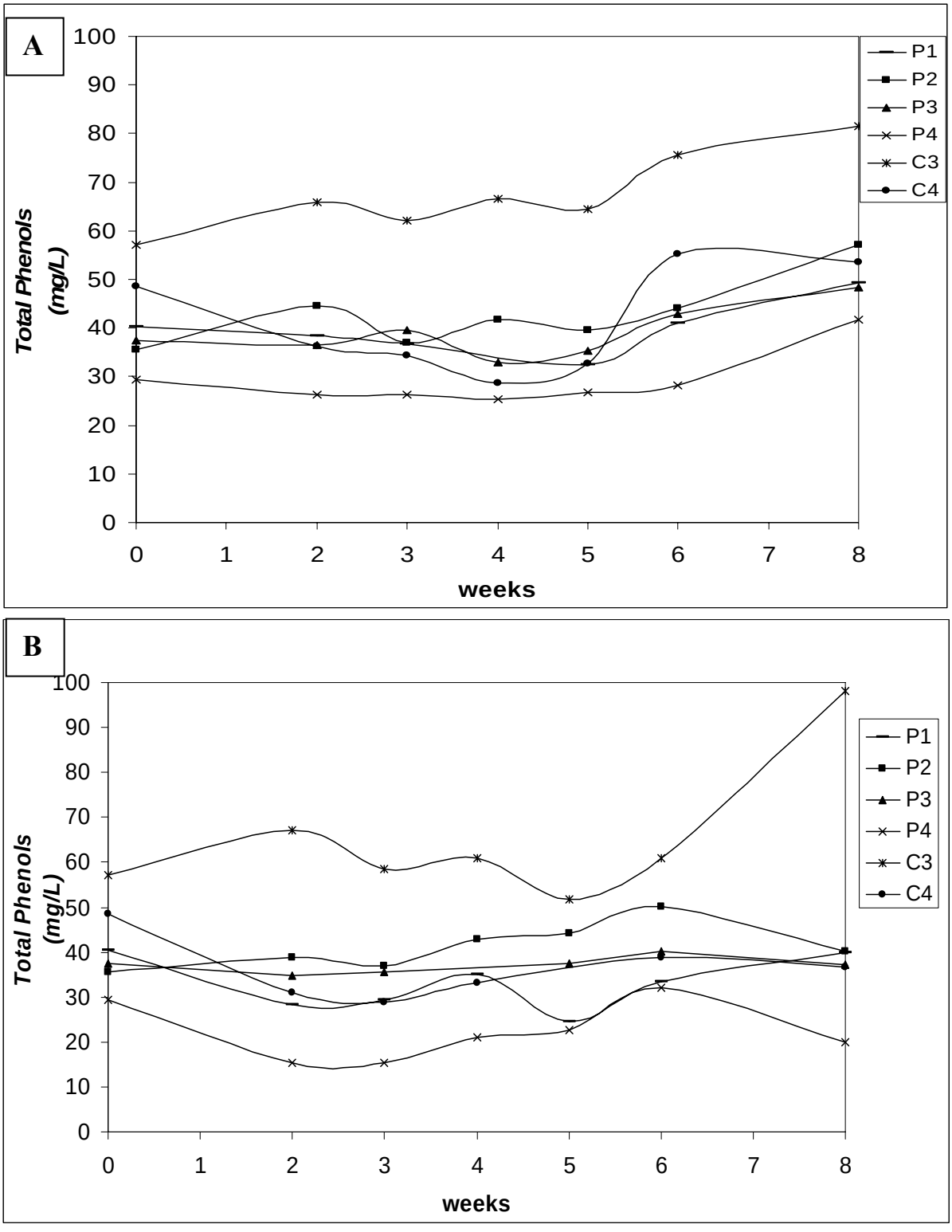

Figure 4.11 Degradation kinetics of total phenol microencapsulated in gum arabic at 4 °C (A) Dark and (B) UV light conditions. Where P1,P2,P3 and P4 - permeate, C3 and C4 - retentate, WP - whey protein, GA- gum arabic.

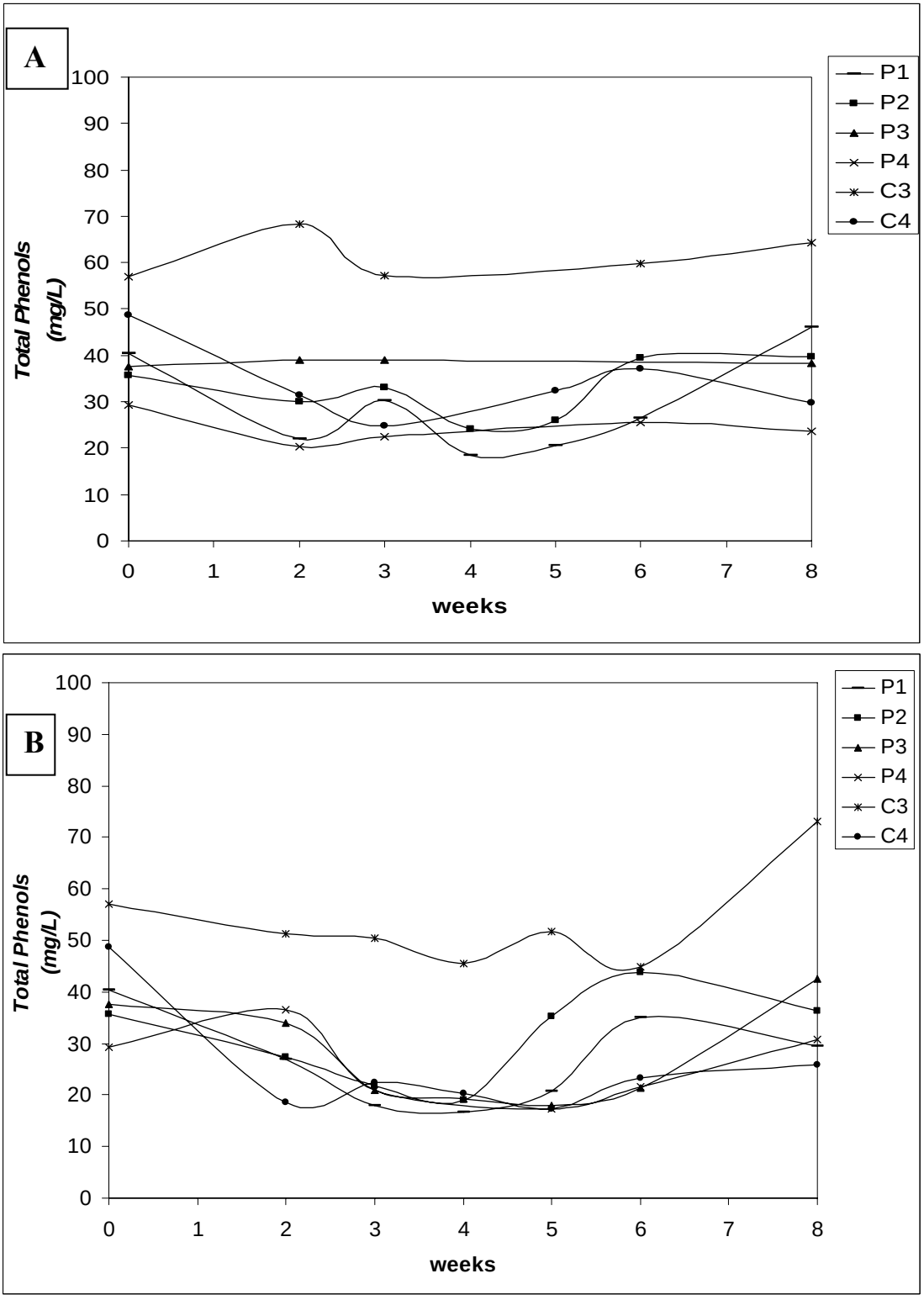

Figure 4.12 Degradation kinetics of total phenol microencapsulated in gum arabic at 25 °C (A) Dark and (B) UV light conditions. Where P1,P2,P3 and P4 - permeate, C3 and C4 - retentate, WP - whey protein, GA- gum arabic.

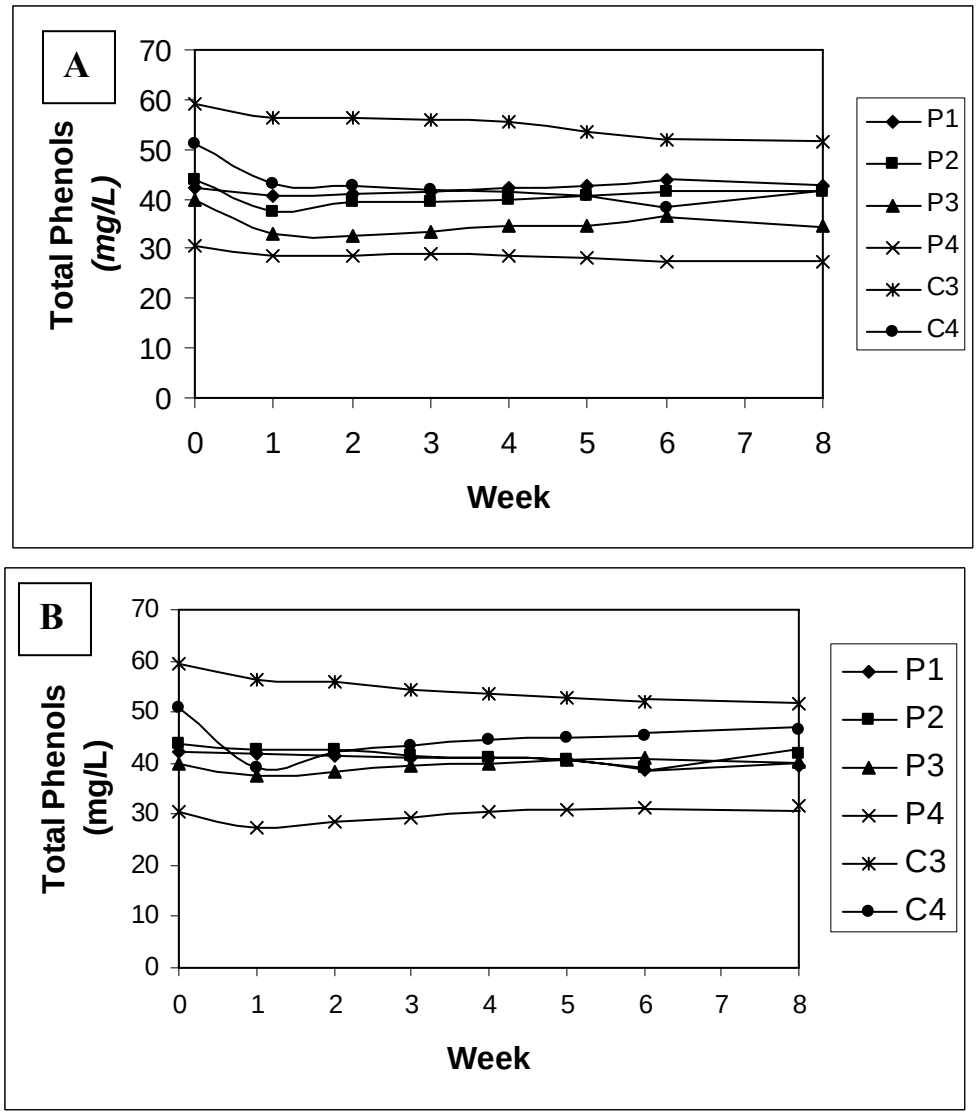

Figure 4.13 Degradation kinetics of total phenol microencapsulated in whey protein at 4 °C (A) Dark and (B) UV light conditions. Where P1,P2,P3 and P4 - permeate, C3 and C4 - retentate, WP - whey protein, GA- gum arabic.

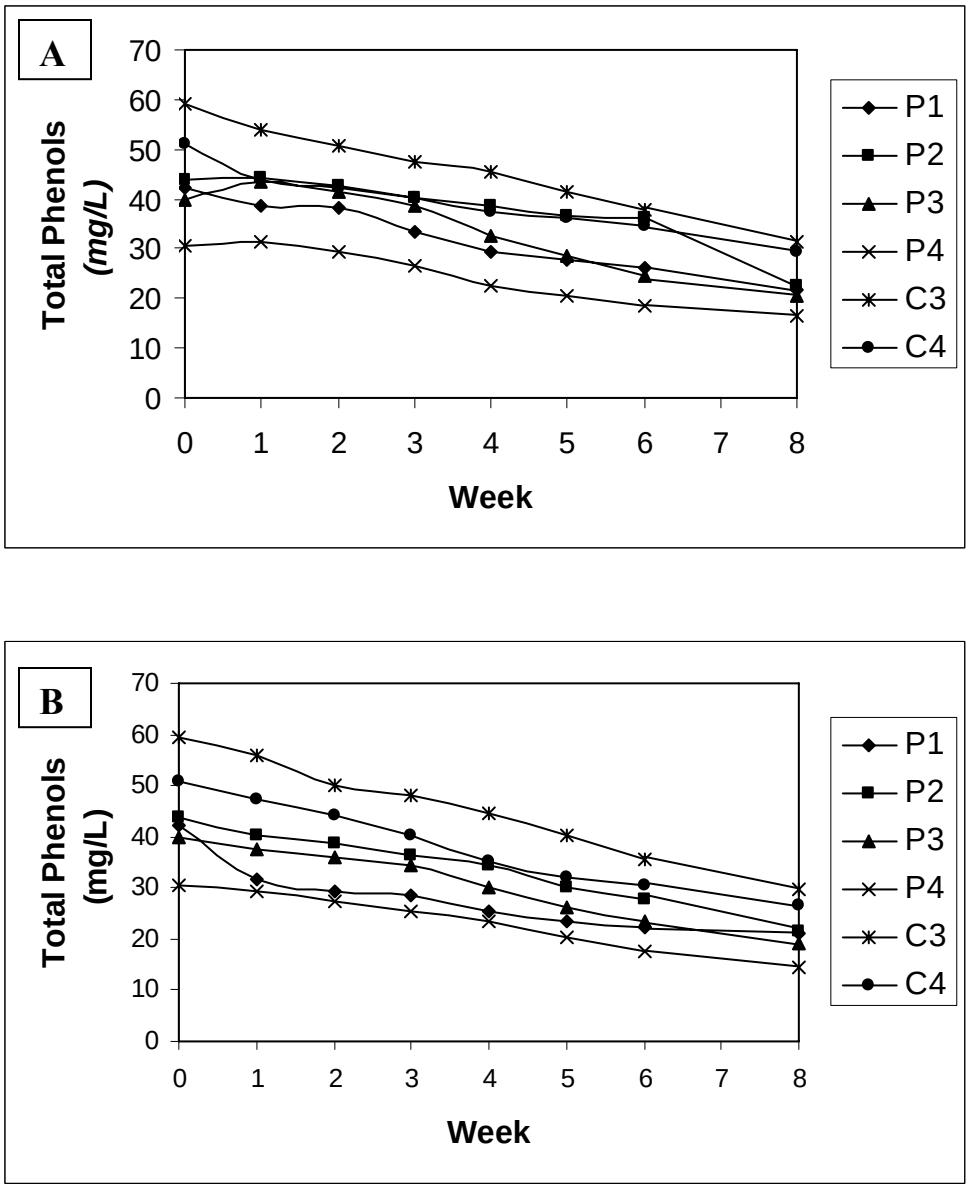

Figure 4.14 Degradation kinetics of total phenol microencapsulated in whey protein at 25 °C (A) Dark and (B) UV light conditions. Where P1,P2,P3 and P4 - permeate, C3 and C4 - retentate, WP - whey protein, GA- gum arabic.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

Muscadine grapes are rich source of phenolics compounds which have excellent health benefits like antiulcer, anti-carcinogenic, anti-ageing and also help in reducing the quantity of HDL (high density lipids) while increasing the content of LDLS (low density lipids).

Microfiltration was carried out to examine the effects of microfiltration in extracting phenolics compounds and preparing spray dried powders. Extraction was carried out by heating muscadine grape pomace at 60 degree C for one hour with enzyme pectinase followed by filtration first with bladder press and then microfilter. It was observed that the concentration of total phenols increased after microfiltration (with or without bladder press) while the concentration of total anthocyanins decreased during the same time. It was found that microfilter; bladder press and enzyme treatment had significant effect on yield of total anthocyanins. It was also found that the use of microfilter was significant to extraction of total phenols but there was no significant effect with use of bladder press. It was found that there is increase in concentration of phenols with temperature increase instead of observing a plateau after a period of time.

Since grape extracts are high in phenolic compounds, it can be used to produce powders which are high in nutraceutical content. However these compounds are prone to oxidation and rapid degradation; microencapsulation was carried out to investigate the protective effects of microencapsulation on the encapsulated phenolic compounds. Grape extract powder was prepared using grape extract, microencapsulated with whey protein and

gum arabic; which were characterized using particle size distribution and SEM (Scanning Electron Microscopy) analysis. It was seen that the outer wall material affected the structure and morphology of microcapsules. The wall of microcapsules made of gum arabic was more compact, homogenous, thick and had no pores on the wall surface as compared to whey protein where the wall was thin and had more pores on the wall surface. The micrographs of the capsules showed that greater indentation on particle walls for smaller size particles compared to large ones indicating solidification occurred prior to expansion of the walls. The particle size distribution varied considerably when using water and ethanol as dispersants during particle size measurements. The mean particle size for particles encapsulated with whey protein in water was 15 μm whereas the same particle size was 50 μm dispersed in alcohol. The microencapsulation yield of the particles ranged from 99.15% for whey protein and 88.15% for gum arabic.

The other aim of the project was to examine the effectiveness of microencapsulation by coating with two materials gum arabic and whey protein and evaluate the degradation of total phenol over time by storing at two temperatures (4 and 25°C), light and dark conditions and 0.64 water activity. It was found that maximum degradation occurred in the uncoated samples followed by samples coated with whey protein and gum arabic, respectively. The color of uncoated powder changed from purple to black whereas the appearance of powder changed from free flowing to rubbery. In samples coated with whey protein the color of powder changed from light pink to white at the end of eight weeks of storage whereas the appearance changed from free flowing to caking. In samples coated with gum arabic the color of samples changed from pink to red at the end of four weeks whereas the appearance of powder changed from free flowing to caking. The concentration of total phenols decreased from 634.59 mg/L to 462.59 mg/L at 25 °C (uncoated sample). It was also observed that concentration of P1 (extract with

bladder press) increased from 44.14 to 46.04 mg/L at 25 °C in sample coated with gum arabic. It was observed that concentration of P1 decreased from 44.47 to 38.74 mg/L at 25 °C in sample coated with whey protein.

The half life of the samples was determined using linear regression. The samples followed zero order rate of reaction. The half life of the samples was 32 to 52 weeks for samples coated with whey protein coating stored at 4 °C while it was 7 to 11 weeks at 25 °C. The half life of samples without any coating material was 18-44 weeks at 4 °C while it was 7 to 14 weeks at 25 °C. This indicates that it is better to store the particles at 4 °C as compared to 25 °C. Samples coated with gum arabic did not follow any trend and so half life could not be determined for these samples.

The results of this study indicate that it is better to store the product at 4 °C as compared to 25 °C. Microencapsulating the grape extracts definitely has benefits such as it protects the structure and color of powder. It also helps in reducing the degradation rate. The half life obtained using whey protein coating was 32 to 52 weeks at 4 °C at 0.64 water activity compared to 18 to 44 weeks without any coating material. If we store the product in a controlled environment such as air tight containers and low temperatures we should be able to obtain better shelf life. It is therefore recommended to microencapsulate the grape extract using whey protein to have longer shelf life for grape extract powder.