MICROENCAPSULATION OF PHENOLIC COMPOUNDS EXTRACTED FROM SOUR CHERRY (*Prunus cerasus* L.) POMACE

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

MICROENCAPSULATION OF PHENOLIC COMPOUNDS EXTRACTED FROM SOUR CHERRY (*Prunus cerasus* L.) POMACE

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The main objective of the study was to encapsulate the phenolic compounds from sour cherry pulp in micro size, to investigate the physicochemical properties of capsules and their digestability in simulated gastric and intestinal fluid.

The effect of different coating materials, ultrasonication time and core to coating ratio on encapsulation of phenolic compounds from sour cherry pomace was investigated. Maltodextrin and gum Arabic were chosen as coating materials. Coating material was prepared with different maltodextrin:gum Arabic (MD:GA) ratios of 10:0, 8:2, 6:4 to make the total solid content 10%. In addition, two different core to coating ratios of 1:10 and 1:20 were used. Emulsions were prepared by homogenization through ultrasonication at 160 W power and 20 KHz frequency for different time periods (5-30 min). Then, the emulsions were freeze dried for 48 hours to obtain the microcapsules. Encapsulation efficiency, antioxidant activity, surface morphology, particle size, color, digestability and glass transition temperatures of the microcapsules were determined.

The microcapsules with a core to coating ratio of 1:20 were found to have higher encapsulation efficiencies (78.80-92.26%) than those with a core to coating ratio of 1:10 (69.38-77.83%). Increasing the gum Arabic ratio in the coating material increased encapsulation efficiency. Optimum conditions for encapsulation with the highest efficiency and the lowest particle size were sonication time of 22.5 min, MD:GA ratio of 8:2 and core to coating ratio of 1:20. Encapsulation was effective in preventing the release of the phenolic compounds in gastric fluid. On the other hand, phenolic compounds were released from the capsules into the intestinal fluid.

Keywords: microencapsulation, sour cherry pomace, ultrasonication, phenolic compounds, encapsulation efficiency

ÖZ

VİŞNE (*Prunus cerasus* L.) POSASINDAN ÖZÜTLENEN FENOLİK BİLEŞENLERİN MİKROENKAPSÜLASYONU

Çilek, Betül
Yüksek Lisans, Gıda Mühendisliği Bölümü
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Eylül 2012, 168 sayfa

Çalışmanın ana amacı vişne posasından özütlenen fenolik bileşenlerin mikrokapsüller oluşturularak kaplanması ve bu kapsüllerin fizikokimyasal özelliklerinin ve yapay mide ve bağırsak sıvılarında sindirilebilirliklerinin araştırılmasıdır.

Farklı kaplama maddelerinin, ultrason sürelerinin ve çekirdek:kaplama maddesi oranlarının fenolik bileşenlerin enkapsülasyonu üzerine etkileri araştırılmıştır. Maltodekstrin ve Arap zamkı kaplama maddesi olarak seçilmiştir. Toplam katı madde oranı %10 olacak şekilde kaplama maddesi

10:0, 8:2, 6:4 oranlarında maltodekstrin:Arap zamkı (MD:AZ) karışımlarından hazırlanmıştır. Ayrıca, iki farklı çekirdek:kaplama maddesi oranı olarak 1:10 ve 1:20 kullanılmıştır. Ultrason yöntemiyle homojenize edilen emülsiyonlar 160 W güçte ve 20 KHz frekansta farklı süreler (5-30 min) kullanılarak hazırlanmıştır. Mikrokapsülleri elde edebilmek için bu emülsiyonlar dondurmalı kurutucuda 48 saat boyunca kurutulmuştur. Mikrokapsüllerde enkapsülasyon verimi, antioksidan aktivitesi, yüzey morfolojisi, parçacık boyut analizi, renk, yapay mide ve bağırsak sıvılarında sindirilebilirlikleri ve camsı geçiş sıcaklıkları belirlenmiştir.

Çekirdek:kaplama maddesi oranı 1:20 olan mikrokapsüllerin kaplama verimlerinin (78.80-92.26%), çekirdek:kaplama maddesi oranları 1:10 olanlara göre (69.38-77.83%) daha yüksek olduğu bulunmuştur. Kaplama maddesinin içerisinde Arap zamkı oranının artırılması kaplama verimini artırmıştır. Enkapsülasyon işleminde en yüksek verim ve en düşük parçacık boyutunu sağlayan optimum koşullar, 22.5 dak ultrason süresi, 8:2 MD:AZ oranı ve 1:20 çekirdek:kaplama maddesi oranı olarak belirlenmiştir. Enkapsülasyon, fenolik bileşenlerin yapay mide sıvısında salımını engellemede etkili olmuştur. Buna karşın, kapsül içindeki fenolik bileşenler yapay bağırsak sıvısında açığa çıkabilmiştir.

Anahtar kelimeler: mikroenkapsülasyon, vişne posası, ultrasonikasyon, fenolik bileşenler, enkapsülasyon verimi

To my family

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INTRODUCTION

1.1 Phenolic compounds

Phenolic compounds are secondary metabolites that are synthesized by plants during normal development and in response to stress conditions as infection, stabbing, UV irradiation, herbivores and reactive oxygen species (Beckman, 2000; Dixon and Paiva, 1995; Nicholson and Hammerschmidt, 1992). They possess higher in vitro antioxidant capacity than other antioxidants and are the most stable and powerful types of dietary antioxidants like vitamins and carotenoids (Gardner et al., 2000; Lee et al., 2003). In this respect, they defend other compounds or tissues from damages caused by free radicals. In addition with their antioxidant property, phenolic compounds offer a wide range of physiological properties like anti-microbial, anti-inflammatory, anti-allergenic, anti-atherogenic, anti-thrombotic effects (Benavente- Garcia et al., 1997; Manach et al., 2004; Middleton et al., 2000; Puupponen- Pimiä et al., 2001). Moreover, the intake of phenolic compounds decrease the proclivity to several chronic diseases such as several types of cancer (Eberhardt et al., 2000, Kuntz et al., 1999; Veeriah et al., 2006), coronary, artery and cardiovascular diseases (Hercberg et al., 1999; Yardim-Akaydin et al., 2003).

In their general structure, phenolic compounds have an aromatic ring with a hydroxyl substituent and a functional residue. These compounds are classified into different groups according to the number of phenol rings that they contain and the structural elements that bind these rings to another as phenolic acids, flavonoids, lignans and stilbenes (Figure 1). Besides this diversity, most of the naturally occuring phenolic compounds are associated with various carbohydrates and organic acids and with each other (Manach et al., 2004; Balasundram et al., 2006).

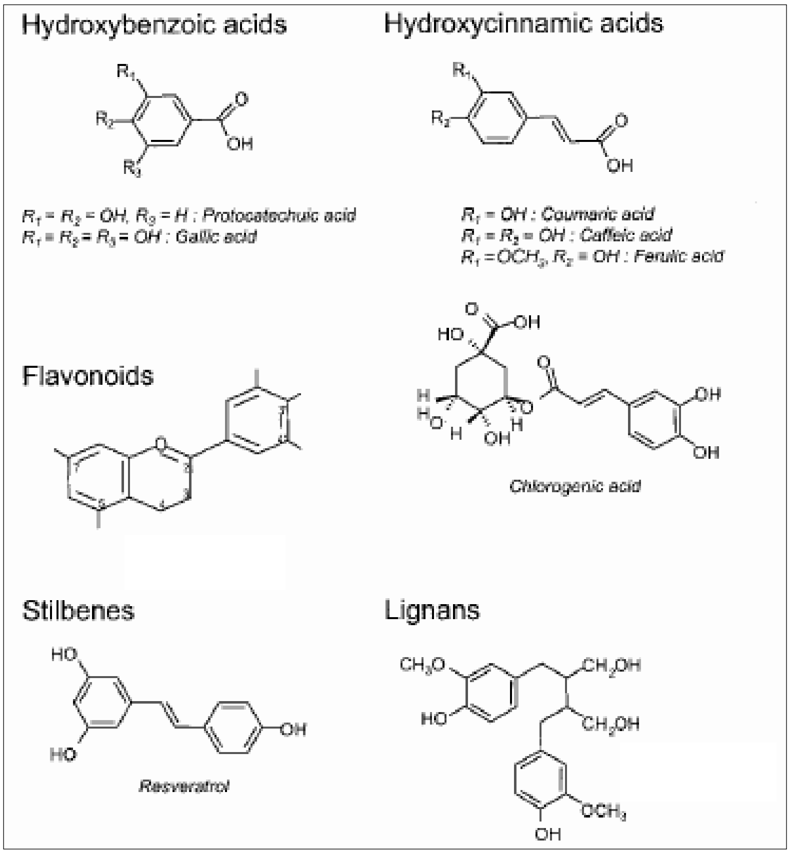

Figure 1.1 Chemical structure of phenolic compounds (Manach et al., 2004)

1.1.1 Phenolic acids

There are two subgroups in phenolic acids; hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids contain p-hydroxybenzoic, protocatechuic, gallic, syringic and vanillic acids (Balasundram et al., 2006). The content of former in edible plants is generally very low, with the exception of onions and black radish (Manach et al., 2004). In general, hydroxybenzoic acids are present as conjugates, although they can be detected as free acids in some fruits (e.g. gallic acid in persimmons) or after being released during fruit and vegetable processing. Gallic acid can also be conjugated as such, or as its dimer, trimer and tetramer (ellagic acid, tergallic acid and gallagic acid, respectively). The trimer and tetramer are comparatively scarce, but in hydrolyzable tannins, gallic and ellagic acids are esterified to glucose. Gallic acid is also esterified to condensed tannins, their monomers, some derived tannins (particularly in black tea) and quinic acid (theogallin) (Tomás-Barberán and Clifford, 2000).

Hydroxycinnamic acids are comprised of p-coumaric, caffeic, ferulic, and sinapic acids and more generally found in edible plants (Clifford, 1999). Although the crusts of ripe fruit have the highest concentrations, they are found in both free and esterified form in all parts of fruit. Concentrations generally diminish during ripening, but when the size of the fruit increases, total quantities also increase (Manach et al., 2004). Ferulic acid is the most plenteous phenolic acid determined in grains of cereal. It is covalently linked to plant cell walls. Chlorogenic acid is formed with the combination of caffeic and quinic acid and found at exceedingly high levels in coffee, also at lower levels in other foods and vegetables (Kroon and Williamson, 1999).

1.1.2 Flavonoids

Flavonoids are the largest group of phenolic compounds found in plants. There are 6 subgroups of flavonoids according to the type of heterocycle included: flavonols, flavones, flavanones, isoflavonoids (isoflavones), anthocyanidins and flavanols (cathechins & proanthocyanidins) (Figure 2) (Manach et al., 2004; Aherne and O'Brien, 2002).

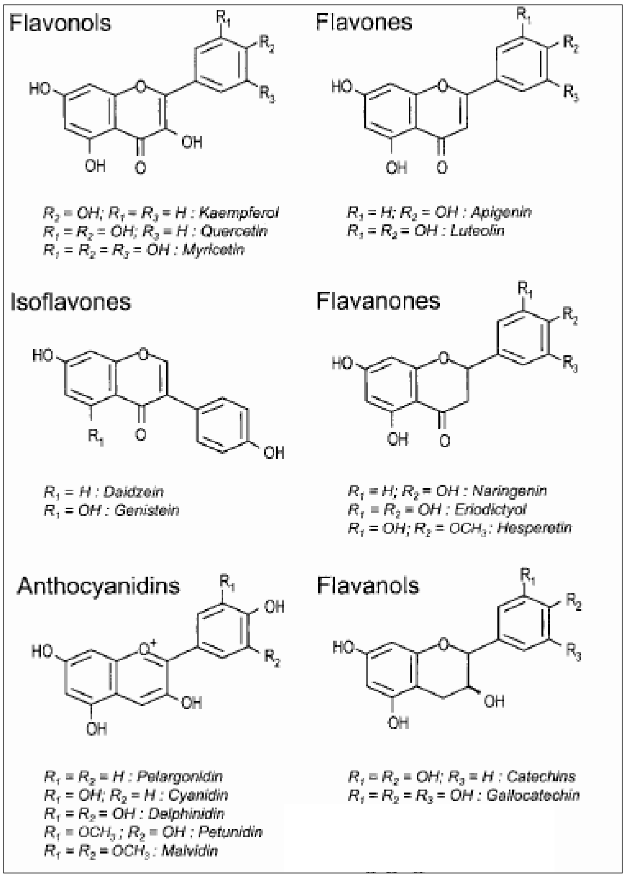

Figure 1.2 Chemical structure of flavonoids (Manach et al., 2004)

Quercetin, kaempferol, isorhamnetin and myricetin are the main exemplification of flavonols. Quercetin is found in different types of fruits and vegetables but onions have the highest concentration. Quercetin-3-rutinoside, quercetin-3-glucoside, quercetin-4′- glucoside and quercetin-3,4′-diglucoside are the some different glycosidic forms of quercetin that found in plants (Erlund, 2004; Aherne and O’Brien, 2002). Kaempferol is mostly found among fruits and leafy vegetables but also present in some legumes, root vegetables and herbs. lsorhamnetin appears in onions and pears. Myricetin is present mostly in berries, tea and maize. Flavonols and their glycosides are found preponderantly in the skin of fruits.

Flavones are not frequently found in fruits but are found generally in herbs (parsley, rosemary and thyme), grains, vegetables and their leaves. Common flavones are found in glycosylated form in plants which are apigenin and luteolin. Nobiletin, sinensetin, and tangeretin participate in taste which are nonglycosidic, highly methoxylated bitter citrus flavones. If flavones occur in high concentrations or are complexed with metal ions, they can also play a role in plant tissue color (Peterson and Dwyer, 1998).

Citrus fruits and juices are the main sources of flavanones that are usually found in glycosylated form. Narirutin (naringenin-7-rutinoside) and hesperidin (hesperetin-7-rutinoside) are present in oranges and mandarins where narirutin and naringin (naringenin-7-neohesperoside) are the major flavonoids in grapefruits. Tomatoes and tomato-based products contain low concentrations of naringenin (Erlund, 2004).

Because of structural similarity of isoflavonoids to estrogens and estrogenic activity, they are called as estrogenic flavonoids. They are found most often in the legume family. Soybeans are the major source of daidzein and genistein which are also found in clover sprouts, black beans and green split peas. Other isoflavonoids, biochanin A, coumesterol, and formononetin, have been found in lima beans, sunflower seeds, green beans, chick peas, split peas, clover sprouts and alfalfa sprouts (Peterson and Dwyer, 1998).

Flavanols are found not only in the monomer form (catechins) but also in the polymer form (proanthocyanidins). Catechin and epicatechin are the main flavanols in fruits, which occur in combined form as epigallo-catechin gallate and epicatechin gallate, combined with gallic acid in tea or as condensed tannin polymers in fruits, legumes, and grains. Catechins are often more plentiful in outer tissues. Catechins are oxidized to theaflavins and thearubigens in black tea (King and Young, 1999). Flavanols are not glycosylated in foods with the exception of other classes of flavonoids (Van der Sluis, 2005). Proanthocyanidins (also known as condensed tannins) are dimers, oligomers, and polymers of (+) catechins, (–) epicatechin and flavan-3-ols. They are flavor and inclemency determinants in teas, fruit juices and wines (Dixon et al., 2005). This inclemency alters during ripening and often vanishes when the fruit reaches its maturity. During the ripening of many types of fruit, the apparent reduction in tannin content is commonly seen probably because of the polymerization of tannins. Since proanthocyanidins have a wide range of structures and molecular weights, it is hard to estimate the content of proanthocyanidins in foods (Manach et al., 2004).

Anthocyanidins which are called anthocyanins naturally occur in the glycosylated form. Although anthocyanins are resistant to light, pH, and oxidation conditions, they are highly unstable. Their degradation is prevented by glycosylation and esterification with a number of organic acids (citric and malic acids) and phenolic acids. Moreover, stabilization of anthocyanins is achieved by the formation of complexes with other flavonoids (Manach et al., 2004). Those phenolic compounds are responsible for the red, blue or violet color of edible fruits, such as plums, many berries, apples and eggplant. The most common anthocyanidins include pelargonidin, cyanidin, delphinidin, and malvidin (Erlund, 2004). The color of anthocyanidins changes when pH is altered. They are usually red at low pH, become colorless (pH 4.5) with sulfur compound and then shift to blue when pH increases. Anthocyanins can form complexes with flavones and metal ions; namely, iron and magnesium in flowers. As the fruit approaches its ripeness, anthocyanin content usually increases (Peterson and Dwyer, 1998).

1.1.3 Lignans and stilbenes

Flaxseed is the richest dietary source of lignans, a type of phenylpropanoid. They consist of secoisolariciresinol and low quantities of matairesinol. Other cereals, grains, some vegetables and fruits generally contain trace amounts of these lignans. Edible plants comprise only low quantities of stilbenes (Manach et al., 2004). Soleas et al. (1997) reported low amounts of trihydroxystilbenes, cis- and trans- polydatin and cis- and trans- resveratrol in red and white wines.

1.2 Antioxidant properties of phenolic compounds

Normal metabolic processes or exogenous factors such as cigarette smoke, car exhaust fumes and oxidant gases, such as ozone, nitrogen dioxide and sulphur dioxide cause formation of free radicals such as superoxide ($O2^{\cdot}$), hydroxyl ($OH^{\cdot}$) and peroxyl ($RO2^{\cdot}$). They are known to attack and damage body cells because of their missing electron. Various chronic diseases, such as coronary heart diseases, cancer, atherosclerosis and aging are enhanced by such oxidative damages on nucleic acids, lipids and proteins. Antioxidants protect compounds or tissues from damage caused by oxygen or free radicals and prevent or decelerate those oxidation reactions. Therefore, they are health supporting substances (Chung et al., 2006; Kirkham and Rahman, 2006; Wong et al., 2006; Valentão et al., 2002). Lipid oxidative rancidity in food is also retarded by antioxidants, so they are deliberately added as food additives to enhance the quality of foods. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are synthetic phenolic antioxidants, are added to foods to prevent free radical damage to lipids. However, BHA and BHT are possible carcinogens and there is an increasing demand by the consumers to not use synthetic antioxidants (Valentão et al., 2002; Duh et al., 1999; Velioglu et al., 1998; Wang et al., 1998).

There are two major types of antioxidants. Firstly, “primary” (chain breaking, free radical scavengers) and “secondary” (deactivation of metals, inhibition of breakdown of lipid hydroperoxides, singlet oxygen quenching and regeneration of “primary” antioxidants) (Gordon, 1990). Natural and synthetic tocopherols, BHA, BHT, tertiary butyl hydroquinone (TBHQ), alkyl gallates are some examples of primary antioxidants. Secondary antioxidants include ethylenediaminetetraacetic acid (EDTA), citric acid, phosphoric acid, ascorbic acid (useful in canned or bottled products), ascorbyl palmitate (useful in fatty foods because of high solubility in fat phase) and erythrobic acid (isoascorbic acid) (Madhavi et al., 1995). Antioxidants are generally present as mixtures in plants and in various foods, so there is currently a great activation due to the synergistic interaction between antioxidants. Mixtures of antioxidants produce a more pronounced activity than the sum of the activities of the individual antioxidants when they are used separately (Duh, 1998). Primary antioxidants are often used in combination with other phenolic antioxidants, or with various metal chelating agents in order to have maximum efficiency like the usage of ascorbic acid with tocopherols (Doba et al., 1985).

1.3 Extraction of phenolic compounds

It is important to extract phenolic compounds from plants for the fabrication of phenol-rich products, which are highly used in the functional food production, pharmaceutical, nutraceutical and cosmetic industry.

Maceration, pressurized liquid extraction, microwave and ultrasound assisted extractions are some examples of different extraction types. Ethanol, methanol, acetone, water or their mixtures can be used as solvent; nevertheless, special care is needed when foods are processed. It must not have any toxicity or should not leave any dregs after utilization with respect to health and safety concerns (Hasbay-Adil et al, 2008).

Maceration is the classical method for the extraction of phenolic compounds. This technique is simply associated with the dissociation of soluble phenolic compounds by diffusion from the solid matrix using a solvent. The mechanism has two stages: initial and diffusion stage. In the initial stage, solid particles are swollen due to absorption of the solvent through the solid phase caused by osmotic forces and capillarity. In the second stage, phenolics diffuse from the solid phase into the medium (Saltmarsh et al. 2003).

In the application of pressurized liquid extraction (PLE), generally a solid sample is put into a stainless steel extraction cell with a solvent under high pressure (500-3000 psi) and above its boiling point (40-200ºC) for a short time (5-15 min) (Garcia-Salas et al, 2010). It was shown that PLE is an effective method for extraction of trans-resveratrol (a type of natural phenol) from grapes and by increasing the temperature process is accelerated (Pineiro et al., 2006).

Microwave assisted extraction (MAE) gives a chance to perform extractions in a small time and without light, which are effective factors for phenolic compounds to be destroyed. Recently, MAE, also called microwave-assisted process (MAP), has been used for extraction of organic compounds from food matrices and edible plants. It is a method, in which the microwave radiation energy is used to heat solvents rapidly and effectively. Desorption of chemicals from the matrix is enhanced because disruption of cell is promoted by internal superheating owing to water within the plant absorbs microwave energy. The study by Sutivisedsak et al. (2010) shows the usage of MAE in determination of the total phenolic contents of eight bean types.

There are a few studies in literature about MAE of phenolic compounds from foods. Štěrbová et al. (2004) combined microwave assisted isolation and solid phase purification procedures for the determination of phenolic compounds in plants (Cocksfoot (*Dactylis glomerata*), red fescue (*Festuca rubra L.*), sheep fescue (*Festuca ovina L.*), chess (*Bromus inermis* and *Bromus marginatus*), St. John’s wort (*Hypericum perforatum*), thyme (*Thymus vulgaris L.*) and blossom of a lime (*Tilia cordata*)) and showed that this technique generated a useful tool for isolation and purification of bioactive compounds. Achieveing highly efficient solid phase extraction clean up step and obtaining highly consistent results in microwave assisted extraction were the main advantages of the proposed procedure. Pan et al. (2003) showed a method related with MAE for the extraction of phenolic compounds from green tea leaves. The extraction efficiency of phenolic compounds after pre-leaching for 90 min at room temperature with MAE for 4 min were higher than those of extraction at room temperature for 20 h and ultrasonic extraction for 90 min at 20-80°C, respectively. The solvent was ethanol/water (50:50 v/v) for all extractions with the solid/liquid ratio of 1:20 g/mL. MAE was found to be more effective than the studied methods in terms of extraction time.

MAE is commonly applied for its time saving effect; however, special care must be taken when the usage of flammable solvents or with samples that contain constituents which couple strongly with microwave radiation. A rapid rise in temperature may occur and thereby result in potentially hazardous situations (Jáuregui and Galceran, 2001).

In ultrasound assisted extraction (UAE), the surface area between the solid and liquid phases is greater owing to the disruption of particles (Filgueiras et al, 2000). There are two types of application; namely, using probe system and bath set up. Both methods are suitable for extraction purposes (Rostagno et al., 2003). In the study of Vilkhu et al. (2008) UAE increased extraction efficiency of red grape from 11 to 35%. The phenolic compounds in strawberries was extracted by Herrera and Luque de Castro (2005) using ultrasound. In their study, UAE was found to produce less analyte degradation than other methods as solid–liquid, subcritical water (130°C, 10 min, solvent: water) and MAE (3 min, solvent: water and 1.2 M hydrochloric acid). Goli et al. (2005) compared solvent extraction (with 3 different solvent types; water, methanol and ethyl acetate) and UAE of phenolic compounds from pistachio hull. No significant difference ($p < 0.05$) was found between solvent extraction and UAE in terms of extraction efficiencies.

1.4 Microencapsulation

Microencapsulation is one of the techniques used for enhancing the shelf life and stability of food ingredients for over 60 years. It is described as a technique in which a bioactive compound is encapsulated by a biopolymer, so that it is protected from oxygen, light, water or other environmental conditions (Desai and Park, 2005). It can be performed to store phenolic compounds extracted from fruits or vegetables from which compounds can be released under specific conditions (Bakowska-Barczak and Kolodziejczyk, 2011; Saénz et al., 2009; Ferreira et al., 2007; Laine et al., 2008).

1.4.1 Emulsion preparation

For the encapsulation of bioactives in aqueous solutions, emulsion technology is usually implemented. As a matter of fact, it is a part of encapsulation process. Before microcapsules are obtained, emulsion is prepared. It is composed of core material, the substance that is wanted to be capsulated, and coating material dissolved in suitable solvent. After this technique is applied, it can be dried (by spray, freeze or roller drying) in order to obtain powders (Friberg et al., 2004).

Emulsions are generally obtained by two types of emulsification techniques such as low energy emulsification and high energy/pressure emulsification. Phase inversion temperature (PIT) or phase inversion composition is low energy emulsification methods. High energy emulsification methods are named as ultrasonication and microfluidization (Jafari et al., 2007a).

1.4.1.1 Ultrasonication

Different types of ultrasound devices are used to make emulsions. The main phenomenon responsible for ultrasonication is acoustic cavitations. Turbulence was generated by the occurrence of micro-size bubbles, and with pressure difference explosion of bubbles makes high levels of turbulence. Turbulence causes smaller particles to be obtained (Li and Fogler, 1978). Increasing sonication time caused formation of smaller emulsion droplets by increasing energy input. This can be explained by the increasing disruption with the droplet deformation (Jafari et al., 2007a).

It was shown that ultrasonication had an advantage over microfluidization because "over-processing" was not observed during ultrasonication. In microfluidization, there was a critical level above which the emulsions became over-processed. Ultrasonication produced emulsion droplets as small as microfluidized emulsions but had little effect on droplet size above the optimum level of time. Therefore, it is unnecessary to give extra ultrasound energy. Besides, ultrasonication was also better with respect to operation and cleaning (Jafari et al., 2007a).

Jafari et al. (2007b) studied encapsulation efficiency of oils and microemulsions obtained by two different methods; microfluidizer and ultrasound. It was found that emulsion size was increased whereas powder size was decreased when ultrasonication was used to prepare capsules.

Zambaux et al. (1999) investigated the influence of the sonication time on the activity of protein C. They used sonication technique for emulsification in order to obtain the protein C nanoparticles. It was indicated that when the sonication time decreased or when a vortex was used, the residual activity of protein C increased. It was stated that the emulsification operated with a vortex is less stable than by sonication, probably due to increase in contact area between protein and organic solvent.

Yeo and Park (2004) proposed a microencapsulation method by using an ultrasonic atomizer. In this method, lysozyme was succesfully encapsulated with no loss of functional integrity and releasing was found to fit zero-order kinetics for over 50 days. Yeo and Park (2004) stated that the proteins (core material) were not subjected to detrimentally strong mechanial stresses when the microcapsules formed by the collision between different species of liquid droplets.

Morlock et al. (1997) studied the microencapsulation of recombinant human erythropoietin (EPO) and found that the percent of total erythropoietin content of EPO aggregates increased comparing with high-speed (Ultra-turrax) mixing when ultrasonication was used to prepare water-in-oil emulsion.

1.4.1.2 Microfluidization

A microfluidizer is working on the principle of a pressure stream which is divided into two parts; each part of stream passes through an orifice and so transports the fluid to the crucial part of microfluidizer that is the interaction chamber (Jafari et al., 2006). In the interaction chamber of the microfluidizer, two channels coming from opposite sides collide with another. The stream is carried by a pump which is able to pressurize the internal compressed air up to approximately 150 MPa.

The results of Jafari et al. (2007a) showed that the emulsion temperature increased by increasing pressure or time. Although a cooling jacket was used at the exit of the valve, significant temperature rise was observed in the emulsion (Bouaouina et al., 2006). Another problem can be caused from the high-energy densities used during microfluidization because volume of emulsion passing through chamber is smaller than the emulsion volume in the emulsification zone (Jafari et al., 2007a).

1.4.2 Coating material type

Different kinds of encapsulating materials have been used for microencapsulation; namely, polysaccharides (starches, maltodextrins (MD), gum Arabic (GA) and corn syrups), lipids (mono and diglycerides) and proteins (casein, milk serum and gelatin) (Gibbs et al., 1999).

Lim et al. (2012) investigated the effects of different coating materials on the encapsulation efficiency and particle size of seed oil capsules. It was concluded that using GA and MD (DE 10) mixture as coating material had the smallest particle size among all other mixtures except sodium caseinate and maltodextrin mixture. In general, GA are known to have stabilizing and emulsifying effects on encapsulation (Krishnan et al., 2005).

Jafari et al. (2006) also used maltodextrin (DE 17) and modified starch (Hi-cap) to produce nano-emulsion of d-limonene to use it for nano-particle encapsulation. Sanchez et al. (2011) investigated the encapsulation of red wine polyphenols in the matrix of MD. They choose MD as coating material because of its ability to increase encapsulation stability. As a result, after storage at 38 ^{o}C, there was almost no change in total polyphenolic content at the end of 15th day.

Buffo and Reineccius (2000) studied the optimization of coating material type containing gum Arabic, modified starch and maltodextrin for the encapsulation of flavors. Similarly, Kanakdande et al. (2007) encapsulated cumin oleoresin by spray drying using GA, MD and modified starch and their blends as wall materials. According to their results usage of gum Arabic / MD / modified starch blend of 4/6:1/6:1/6 resulted in better protection when compared to protection provided by GA alone. Besides, in order to avoid the degradation of vitamin over the storage time, the bioactives compounds were encapsulated by spray drying with GA as the wall material (Romo-Hualde et al, 2012).

Soottitantawat et al. (2003), investigated the influence of emulsion size on the retention of volatile compounds by means of microencapsulation. Increasing emulsion droplet size decreased the retention of flavors. As the model flavors d-limonene, ethyl butyrate, and ethyl propionate and as the coating material gum Arabic (GA), soy bean water-soluble polysaccharides or modified starch blended with MD were used. In the encapsulation of d-limonene, blend of GA and MD showed the smallest powder size.

1.4.3 Drying of microemulsion

Freeze drying and spray drying are the common drying methods used to prepare microcapsules.

1.4.3.1 Freeze drying

Freeze-drying, also known as lyophilization or cryodesiccation, has been proven as the best method for drying thermosensitive substances. This method minimizes thermal degradation reactions. A recent review of Fang and Bhandari (2010) discussed about the technologies of polyphenol encapsulation, containing freeze drying. Since its utilization is costly, commercial application of freeze drying is restricted to very high value ingredients such as antioxidants (Augustin and Hemar, 2009).

Maltodextrin is the most utilized material in freeze drying for encapsulation stability. It is a powerful barrier against oxidation of core material and protective against undesired physical and chemical changes (Sanchez et al., 2011).

1.4.3.2 Spray drying

Spray drying is a low-cost process for microencapsulation and generally applied for encapsulation of fragrances, oils and flavors. In spray drying, dispersion of core particles in a polymer solution sprayed into a hot chamber (Fig. 3). Hardening is obtained as the solvent evaporates or by cooling if shell materials are made of fat or wax. Water-soluble polymers are commonly preferred as coating materials because solvent-borne systems may produce unpleasant odors and environmental problems. The cylindrical nozzle is used for spraying the wall material. As the particles pass through the nozzle area, the coating material encapsulates them. Air stream carries the solid capsules along until separation takes place in a cyclone. Until desired weight and thickness are obtained, spray drying is continued. Despite of its being a time consuming process, the multiplayer coating procedure helps to accelerate the capsulation and reduce the particle defects (Erikci, 2007).

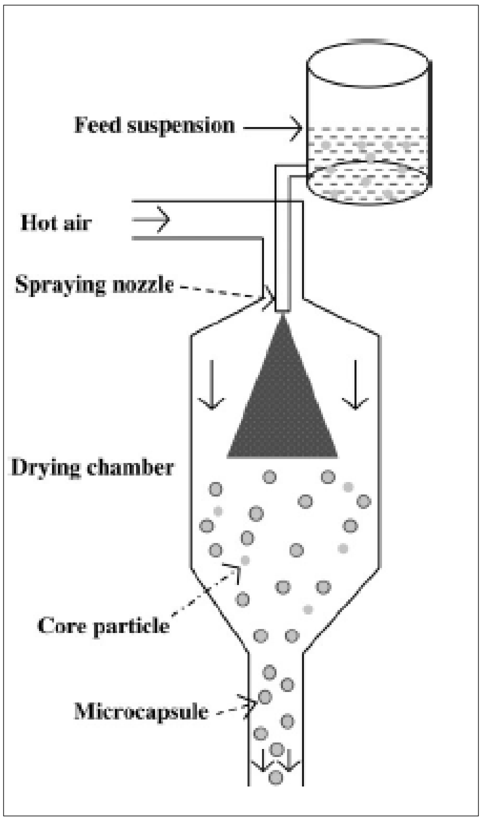

Figure 1.3 Schematic illustration of spray drying process for microencapsulation.

1.4.4 Microencapsulation of phenolic compounds

The utilization of polyphenol encapsulation method is implemented to prevent unwanted taste and odor of phenolic compounds; thus, being the best method that serves the purpose of extending their shelf life and usage areas.

Several morphologies can be developed for encapsulation, but two of them are commonly seen. The first type is in the shape of mononuclear capsules, having a single core restricted by a shell, while the second is aggregates, in which the core material embedded in a matrix (Schrooyen et al., 2001) (Figure 1.4). Applied process technologies and coating materials (as well as the core material) affect the specific shape of capsules.

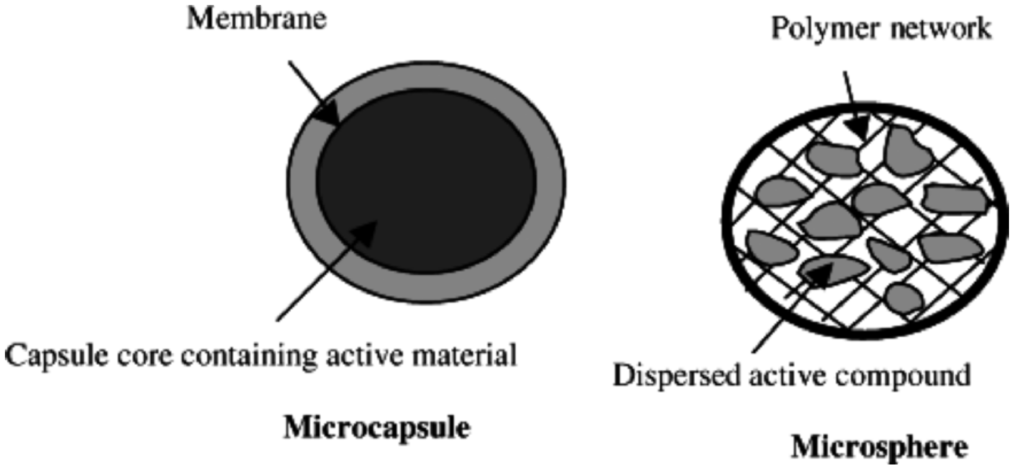

Figure 1.4 A schematic representation of encapsulated bioactives.

Microencapsulation of natural phenolic compounds extracted from different plants has been recently studied by various researchers (Bakowska-Barczak & Kolodziejczyk, 2011; Deladino et al., 2008; Nedovic et al., 2011 and Zheng et al., 2011).

Saénz et al. (2009) examined the microencapsulation of bioactive compounds extracted from cactus pear. After phenolic compounds were extracted, MD was added with the core to coating ratio of 3:1 and mixed with high speed homogenizer. Spray drier was used in order to obtain microcapsules. Encapsulation yield of microcapsules were determined as 80.79 %.

Ersus and Yurdagel (2007) studied microencapsulation of anthocyanin pigments obtained from black carrot. It was shown that higher air inlet temperatures of spray drying of black carrot anthocyanins resulted in more anthocyanin losses and the highest anthocyanin content was observed at the end of drying process when 20-21 DE maltodextrin was used as a coating material.

Sanchez et al. (2011) encapsulated Argentine red wine using MD (20% concentration). In order to get wine powder, the mixture was freeze-dried with almost no loss of total polyphenols. Wine powder, containing polyphenols in the amorphous glassy matrix of MD, was obtained in the research and total polyphenol content of wine powder was found to remain unchanged after 15th day of storage at 38°C.

Chandrasekar (2010) aimed to optimize extraction of phenolic antioxidants using microwave from apple pomace and to encapsulate the extract in cyclodextrins. Encapsulation of the extracted phenolics obtained under optimized conditions was performed with α-, β-, and 2-hydroxypropyl-β-cyclodextrin. The stability of capsules enhanced when β-cyclodextrin was used for encapsulation.

1.5 Sour cherry pomace

Sour cherries (*Prunus cerasus* L.) are widely used in food industry in the area of production of juices and juice drinks. According to crop production statistics declared by Turkish Statistical Institute, sour cherry production in Turkey is approximately 200,000 tons in 2010 (Turkish Statistical Institute, 2012). Since its cultivation in Turkey is substantially high, different kinds of sour and sweet cherries have been studied by several researchers (Khoo et al., 2011; Kim and Padilla-Zakour, 2004; Usenik et al., 2008). Sour cherry was shown to have high amounts of total phenolic, anthocyanin content and antioxidant capacity (Mulabagal et al., 2009; Pantelidis et al., 2007; Seeram et al., 2001).

Sour cherries can not be consumed as fruit in considerable amounts because of their acidulous taste. Mostly it is consumed as sour cherry juice. Production of sour cherry juice causes accumulation of huge amounts of sour cherry pomace. Thus, it would be beneficial to develop a novel approach in the utilization of these waste materials.

Generally, in literature phenolic content in the peel and flesh parts of the fruits have been analyzed separately. One of the rich sources of natural antioxidants has been declared as the peels of fruits. Wolfe et al. (2003) studied the total phenolic content and antioxidant activity of the flesh, peel and combination of peel and flesh parts of different kinds of apples commonly used in the production of applesauce. The total phenolic contents and the antioxidant activities of the peel extracts were found to have higher than the flesh. The high content of phenolics in the skin of the fruits makes industrial by-products as valuable sources for the production of natural antioxidants. Similar to fruits and vegetables, pomaces are rich in antioxidants (Khoo et al., 2011) such as polyphenols, anthocyanins (Mulabagal et al., 2009; Seeram et al., 2001) and flavonoids (including flavones, isoflavones, flavonones, catechin, and isocatechin) (Wang et al., 1997). There are many studies in the literature for the extraction of phenolic compounds from fruit and vegetable by-products such as mango, grape and cherry pomaces (Berardini et al., 2005; Gómez-Plaza et al., 2006 and Rødtjer et al., 2006).

Halvorsen et al. (2002) analysed the total antioxidant concentrations of various dietary plants, including various fruits, berries, vegetables, cereals, nuts and pulses, by the FRAP (ferric reducing antioxidant power). It was found that total antioxidant concentration in sour cherries was 5.53 mmol per 100g fresh weight which was one of the highest antioxidant capacities among the fruits analyzed such as berries, pomegranate, orange, plum, lemon, pineapple, grape, pineapple, kiwi, papaya, apricot, mango and banana.

Sour cherries contain anthocyanins as the major phenolics with high antioxidant activity. Recent studies have shown that anthocyanins from sour cherry revealed in vitro antioxidant activities comparable with commercial products, such as BHA and BHT, and superior to vitamin E at 2 mM concentration (Wang et al., 1999a). Wang et al. (1999b) specified the phenolic compounds as 5,7,4'-trihydroxyflavanone, 5,7, 4'-trihydroxyisoflavone, chlorogenic acid, 5,7,3',4'-tetrahydroxyflavonol-3- rhamnoside, 5,7,4'-trihydroxyflavonol-3-rutinoside, 5,7,4'-trihydroxy-3'- methoxyflavonol-3-rutinoside, 5,7,4'-trihydroxyisoflavone-7-glucoside, and 6,7- dimethoxy-

5,8,4'-trihydroxyflavone by the experiments of NMR. The antioxidant assays discovered that 6,7-dimethoxy-5,8,4'-trihydroxyflavone is the most active one, followed by quercetin 3-rhamnoside and genistein.

1.6 Objectives of the study

Utilization of industrial by-products for phenolic compound extraction have gained importance in recent years. In Turkey, growth of sour cherry is considerably high. However, their acidulous taste prevents the consumption of them as fruit in high amount. Mostly it is consumed as sour cherry juice and juice production causes accumulation of huge amount of waste pomace. Extraction of phenolic compounds from sour cherry will be very beneficial because of its considerably high phenolic content.

In addition, increasing demand of consumption of natural antioxidants strengthens the importance of the research in this field. Polyphenols extracted from the pomace could be used in the production of natural food additives. However, they have undesired taste and they are unstable during storage. The encapsulated phenolic compounds from sour cherry pomace could be efficiently used in food industry and could replace synthetic antioxidants used in the production of "functional foods", cosmetics and pharmaceuticals.

Encapsulation is an important method used to enhance the shelf life and stability of compounds and to prevent unwanted taste and odor of food ingredients like phenolics. There is no study in literature on microencapsulation of phenolic compounds from sour cherry pomace.

The objective of this study was to encapsulate phenolic compounds extracted from sour cherry pomace. In addition, the effects of coating material formulation, ultrasonication time and core to coating ratio on encapsulation efficiency, antioxidant activity, surface morphology, particle size, color, digestion in simulated gastric and intestinal fluid and glass transition temperatures were investigated.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Sour cherry (*Prunus cerasus* L.) pomace was obtained from Karmey fruit juice factory located in Karaman, Turkey. Cores, stems and other foreign materials were manually removed from pomace, which was then stored at -18 °C in low density polyethylene bags. Phenolic compounds obtained from pomace was used as the core material.

Maltodextrin (Dextrose Equivalent (DE) 4.0-7.0) and gum Arabic (acacia powder) supplied by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) were used as coating materials.

All other reagents used in this study (Gallic acid, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), potassium persulfate, sodium carbonate, DPPH$^{\cdot}$ (2,2-diphenyl-1-picrylhydrazyl), sodium hydroxide, hydrochloric acid, monobasic potassium phosphate, Folin-Ciocalteau's phenol reagent, trolox, barium chloride, ethanol, methanol, acetic acid, pepsin and pancreatin) were of analytical grade.

2.2 Extraction of phenolic compounds

Maceration was done in order to extract phenolic compounds from pomace. Sour cherry pomace of 20 g was weighed and mixed with 400 mL of ethanol:water (50:50 v/v) mixture. The extraction was performed in sealed 500 mL glass flasks in shaking water bath (GFL 1086, Burgwedel, Germany) at 30 ^{o}C with continuous shaking at 70 rpm for 24 hours. The extract was filtered two times. First aqueous part was seperated from the solid part by using filtering cloth. Then extract was vacuum filtered through a micro filter (Whatman 4, GE Healthcare UK Limited).

2.3 Phenolic powder preparation

The filtered extract was concentrated in vacuum evaporator (Heidolph Laborota 4000 efficient, Schwabach, Germany) at 40 ^{o}C until its volume was decreased to 1/13-1/14 times of its initial value. The concentrated extracts were frozen at -18 ^{o}C in beakers and then freeze dried (Christ, Alpha 1-2 LD plus, Osterode, Germany) at -52 ^{o}C for 48 hours below 0.1 mPa. Finally dried content was manually ground into a fine powder and kept in a freezer at -18 ^{o}C until capsulated and analyzed.

2.4 Encapsulation procedure

Phenolic powder was encapsulated with three different coating material types (maltodextrin : gum Arabic mixtures at ratios of 10:0, 8:2, 6:4) and two different core to coating ratios (1:10 and 1:20). Phenolic powder and coating material was mixed with high speed homogenizer and then ultrasonication was applied during different time periods (5, 10, 15, 20, 25 and 30 min). Finally, it was freeze-dried in order to obtain microcapsules.

2.4.1 Preparation of coating materials for the microcapsules

Maltodextrin (MD) (DE 4.0-7.0) and gum Arabic (GA) mixed with different ratios of 10:0, 8:2, 6:4 were used as coating materials. It was reported that compared to DE of 18.5, DE of 5.0-8.0 had not only higher encapsulation efficiency but also offered better protection for phenolic compounds during storage (Laine et al., 2008). Therefore, using MD with DE of 4.0-7.0 was preferred. MD were previously swollen in distilled water and kept overnight in a shaking water bath (70 rpm) at 27 ^{o}C to make the solutions of 10%, 16% and 12% (w/w) in concentration. GA solutions having total solid content of 4% and 8% (w/w) were prepared two hours prior to encapsulation procedure. The solutions were mixed with a magnetic stirrer (Heidolph MR 3001 K, Heidolph Instruments GmbH & Co, Schwabach, Germany) at 1250 rpm to obtain total solid content of 10% (w/w) with MD:GA ratios of 10:0, 8:2 and 6:4 in weight.

2.4.2 Preparation of the microcapsules

After coating solutions were prepared, phenolic powder and coating solution were mixed to obtain core to coating ratio of 1:10 and 1:20 by weight.

Phenolic powder of 2 g and 1 g were accurately weighed for core to coating ratio 1:10 and 1:20, respectively, and added into 20 g of required coating material (MD:GA 10:0, 8:2 or 6:4). The mixtures were homogenized using a high-speed homogenizer (IKA T25 digital Ultra-Turrax, Selangor, Malaysia) at 4000 rpm for 5 min. Then, mixtures were disrupted by ultrasonication at 160 W power, 20 KHz frequency and with 50% pulse (Sonic Ruptor 400, OMNI International the Homogenizer Company, GA, USA) for different time periods (5, 10, 15, 20, 25 and 30 min). A titanium probe with the diameter of 3.8 mm was used. During sonication, samples were placed in a waterbath at 4 ^{o}C to prevent overheating of emulsion. Then emulsions were dried in freeze drier for 48 hours. Finally the dried content was ground into a fine powder. Each experiment was duplicated.

2.5 Analysis of phenolic powder and encapsulated phenolic powder

2.5.1 Determination of total phenolic content

The total phenolic content (TPC) was determined by the method proposed by Beretta et al. (2005) with some modifications (Saénz et al., 2009). In this method, Folin-Ciocalteau reagent is reduced by sodium carbonate in the presence of phenolic substances which is determined by a color change.

100 mg of phenolic powder or encapsulated phenolic powder was accurately weighed and dissolved in 1 mL ethanol:water (50:50 v/v) or ethanol:acetic acid:water mixture (50:8:42 v/v), respectively. This mixture was agitated using a Vortex (ZX3, VELP Scientifica, Usmate, MB, Italy) for 1 min and

filtered through a micro filter (0.45 µm Gema Medical Filter, Spain). TPC was measured by the Folin–Ciocalteau method (Beretta et al., 2005).

500 µl diluted sample and 2.5 mL 0.2 N Folin-Ciocalteau (2N, SIGMA-ALDRICH F9252) reagent were put into a tube and vortexed. After keeping it in dark place for 5 minutes, 2 mL of 75 g/L sodium carbonate (SIGMA-ALDRICH S7795) solution was added to tubes and mixed again. Samples were kept in dark at room temperature (25°C) for 1 hour and then absorption at 760 nm was measured by using UV/VIS spectrometer T 70, (PG Instruments LTD, UK).

Calibration curve was prepared with different gallic acid concentrations (20, 40, 60, 80, 100 ppm) in ethanol:water (50:50, v/v) mixture, so total phenolic content of freeze dried phenolic powder was expressed as gallic acid equivalents (GAE) in milligrams per gram dry weight. Also, another calibration curve for ethanol:acetic acid:water mixture was prepared in the same manner with different concentrations of gallic acid (20, 40, 60, 80 and 100 ppm) for the determination of total phenolic content of microcapsules.

Calibration curves are given in Appendix A (Figure A.1 and A.2).

2.5.2 Surface phenolic content of capsules

For the determination of surface phenolic content, the same method of Folin-Ciocalteau was used. Microcapsules of 100 mg were treated with 1 mL of ethanol and methanol mixture (50:50 v/v). The contents of surface phenolic compounds were measured and quantified with the same method described in section 2.5.1.

Another calibration curve was prepared with different gallic acid concentrations (10, 20, 30, 40, 50 ppm) in ethanol:methanol (50:50 v/v) mixture. Thus surface phenolic content of encapsulated phenolic powder was expressed as GAE in milligrams per gram dry weight.

Calibration curve is given in Figure A.3.

2.5.3 Encapsulation efficiency

The encapsulation efficiency (EE) is the ratio of encapsulated phenolic content (EPC) to total phenolic content (TPC) that was the initial value that phenolic powder contained before encapsulation process. SPC represents the surface phenolic content found on the surface of the capsules. TPC of microcapsules must be the same as that of phenolic powder; therefore, total phenolic content of capsules were calculated by multiplying total phenolic content of powder with its weight (g) in sample and divided by total solid content. EE of microcapsules were calculated according to following equation:

$$EE\ (\%) = \frac{EPC}{TPC}\ x\ 100 = \frac{TPC-SPC}{TPC}\ x\ 100\ \% \qquad (1)$$

2.5.4 Total antioxidant activity with $DPPH^{\cdot}$ radical scavenging method

Total antioxidant activity (AA) was evaluated in accordance with the $DPPH^{\cdot}$ (2,2-Diphenyl-1-picrylhydrazyl) method described by Yen and Duh (1994) with some modifications. Substances which have antioxidant characteristics cause a color change of $DPPH^{\cdot}$ solution and with this method color change is determined spectrophotometrically. Since $DPPH^{\cdot}$ solution is very sensitive and degradation of $DPPH^{\cdot}$ occurs very quickly in the presence of light, it is always stored at dark.

Phenolic powder and microcapsules were accurately weighed as 100 mg and dissolved in 1 mL ethanol:acetic acid:water mixture (50:8:42 v/v). This emulsion was agitated using a Vortex (ZX3, VELP Scientifica, Usmate, MB, Italy) for 1 min. The liquid part of emulsion was drawn into syringe and filtered with a filter having pore size of 0.45 μm (Gema Medical Filter, Spain). Then samples were diluted. 3.9 mL of 25 ppm $DPPH^{\cdot}$ radical solution (2.5 mg $DPPH^{\cdot}$/ 100 mL MetOH) and 100 μl of methanol were mixed, and absorption at 517 nm was measured (A_1) by using UV/VIS spectrofotometer T 70 (PG Instruments LTD, UK) using methanol as blank. Diluted samples of 100 μl were mixed with 3.9 mL $DPPH^{\cdot}$ radical solution and allowed to wait in the dark at room temperature. For the samples, it was found that 1 hour waiting period was enough for the reaction of $DPPH^{\cdot}$ solution and gallic acid to be completed. After 1 hour, the absorptions of samples and their parallels were detected spectrometrically (A_2).

Different concentrations of $DPPH^{\cdot}$ in methanol with the highest concentration of 25 ppm $DPPH^{\cdot}$/L of methanol were used to prepare a calibration curve (Figure A.4). By using calibration curve, concentrations (C_1 and C_2) were found for A_1 and A_2, and then the results were calculated according to following equation:

$$AA(mg\ DPPH/g\ dry\ weight) = \frac{(C_1 - C_2)}{W_{sample}}\ x\ V\ x\ d \quad (2)$$

where C_1 is the concentration of DPPH$^{\cdot}$ immediately after the sample and DPPH$^{\cdot}$ solution was mixed, C_2 is the concentration of DPPH$^{\cdot}$ 1h after mixing, d is the dilution rate, V is the volume of extract in mL, W_{sample} is the amount of dry sample in g.

2.5.5 Total antioxidant activity with TEAC method

It has been recommended to carry out at least two different assays varying in their mechanisms of antioxidant action in order to evaluate the antioxidant potential of phenolic compounds (Schlesier et al., 2002). By reducing the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical, the TROLOX (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid, a water-soluble derivative of vitamin E) equivalent antioxidant capacity (TEAC) test directly yields the radical scavenging capacity of the respective compound.

Antioxidant activity was essentially measured with TEAC method as described by Miller et al. (1993) and Rice-Evans & Miller (1994) with some modifications (Tyrakowska et al., 1999). The ability of the antioxidant to scavenge the ABTS (+) radical cation relative to the Trolox is used to determine the TEAC value. This method was performed using UV/VIS spectrofotometer T 70, (PG Instruments LTD, UK) with the process explained by Cemeroglu, B. (2007) and Re et al., (1999). ABTS of 0.0384 g was weighed in volumetric flask (10 mL) and dissolved in distilled water. After adding 2 mL of 12.25 mM potassium persulfate solution, volume was completed to 10 mL with distilled water. The mixture was vortexed and kept in the dark place at room temperature during 12 - 16 h before use to achieve the stabilization of solution. The obtained solution was stable for at least 2 days when stored in the dark. Before each measurement, the ABTS solution was diluted with ethanol to an absorbance of 0.700 (± 0.020) at 734 nm which was recorded ($TEAC_0$). Absorbance ($TEAC_6$) of sample was determined exactly 6 min after the addition of 1.0 mL of diluted ABTS solution to 10 µl of diluted sample. The same procedure was performed by the addition of 20 µl and 30 µl of the same sample to ABTS solution. Each sample was measured in triplicates. The percentage inhibition of each sample for separate volume was calculated from the following formula:

$$Inhibition\ (\%) = \frac{TEAC_0 - TEAC_6}{TEAC_0}\ x\ 100\ \% \qquad (3)$$

Then, inhibition was used to plot a function of volume versus inhibition for each sample from which slope was calculated ($Slope_{sample}$). Calibration curve for TEAC analysis was prepared with Trolox standard solution. 0.0625 g Trolox was weighed and by adding 10 mL of ethanol 2.5 mM Trolox solution was obtained. The concentrations of 5 µM, 10 µM, 15 µM and 20 µM of standard solution was prepared with dilution by ethanol and used for calibration curve (Figure A.5). The results were expressed as mM TEAC/g dry weight and were calculated from the following equation:

$$AA(mM\ TEAC/g\ dry\ weight) = \frac{Slope_{sample}}{Slope_{cal}}\ x\ \frac{V_{solvent}}{W_{sample}}\ x\ d \qquad (4)$$

where, $Slope_{cal}$ is slope of the calibration curve, $V_{solvent}$ is volume of solvent added to the dry sample, W_{sample} is weight of dry sample (capsule or powder) and d is dilution rate.

2.5.6 Surface morphology analysis

Particle structures of the powder microcapsules were evaluated by scanning electron microscopy (SEM). JSM-6400 Electron Microscope (JEOL Ltd, Tokyo, Japan) equipped with NORAN System 6 X-ray Microanalysis System and Semafore Digitizer was used to monitor outer surface of microcapsules at 20kV. Samples were coated with gold/palladium by Hummel VII sputter, under vacuum before examination and SEM images were taken at 100x magnification.

2.5.7 Particle size analysis

In order to determine the size distribution of the microcapsules, particle size analyzer (Mastersizer 2000, Malvern Instruments, Worcestershire, UK) was used. All results of measurements were reported as averages of two repeats. The mean diameter of the microcapsules was expressed as the Sauter mean diameter, D32, representing a surface average diameter (Equation 5) associated with the smaller particles. The equipment determined the size of particles according to laser diffraction technique and particle size distributions were presented as volume percentage versus microcapsule diameter. The “span” or width of the microcapsules was calculated from the equation 6

(Elversson et al., 2003): Specific surface area of particles (m^2/g) was also calculated by the instrument.

$$D_{32} = \sum n_i d_i^3 / \sum n_i d_i^2 \tag{5}$$

$$Span = \frac{[d(v,90) - d(v,10)]}{d(v,50)} \tag{6}$$

where, n_i is number and d_i is diameter of particles; d(v,90), d(v,10), and d(v,50) are diameters at 90%, 10%, and 50% of cumulative volume, respectively. In other words, [d(v,90) – d(v,10)] is the range of the data and d(v,50) is the median diameter.

2.5.8 Color analysis

Color measurements were performed by using the CIE L* (whiteness/darkness), a*(redness/greenness), and b*(yellowness/blueness) color scale. Colors were determined with UV-2450 UV-VIS Spectrophotometer (Shimadzu Co, Kyoto, Japan) with three replications for each sample. Total color change (ΔE) was calculated from the following formula and barium chloride ($BaCl_2$) was selected as reference (L_0^*, a_0^*, b_0^*).

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \tag{7}$$

2.5.9 Digestion in simulated gastric fluid

Digestion of the microcapsules in the gastric fluid was simulated by an in vitro method. Firstly, simulated gastric fluid (SGF) was prepared by the method proposed by U.S. Pharmacopeia (USP 29, 2012). In order to prepare the SGF briefly, 2 g of sodium chloride was mixed with 3.2 g of pepsin from porcine stomach mucosa. Then 7 mL of hydrochloric acid was added. Finally, sufficient amount of distilled water was added to the solution in order to make it 1000 mL and pH of the SGF was brought to 1.2.

To simulate the digestion in gastric fluid, 1.4 mL of SGF was added into a 10 mL tube which contained 100 mg of encapsulated or free phenolic powder. Sealed tubes were placed in a shaking water bath for 2 h at 37 ± 0.5^{o}C with continuous shaking at 80 rpm. At the end of incubation, tubes were immediately cooled and the solutions were filtered. Then, filtered solutions were neutralized by the addition of 0.2 M NaOH solution. Samples were analyzed by Folin-Ciocalteau colorimetric method by measuring the absorbances at 760 nm. Results of the tests were expressed as the fraction of TPC released into the medium to initial TPC of phenolic powder or microcapsule. Since the solvent (SGF) used in this analysis contains organic molecules a new calibration curve was prepared accordingly (Figure A.6).

2.5.10 Digestion in simulated intestinal fluid

Digestion of the microcapsules in intestinal fluid was simulated by an in vitro method. Firstly, simulated intestinal fluid (SIF) was prepared by the method proposed by U.S. Pharmacopeia (USP 29, 2012). In order to prepare the SIF monobasic potassium phosphate (6.8 g) was dissolved in 250 mL of water. Then, 77 mL of 0.2 N sodium hydroxide and 500 mL of water was added and again mixed. After addition of 10.0 g of pancreatin, distilled water was added until the volume is 1000 mL and finally the pH of the resulting solution was adjusted to 6.8 ± 0.10 using 0.2 N sodium hydroxide and 0.2 N hydrochloric acid.

In order to simulate the digestion in the intestinal fluid, 2.4 mL of SGF was added into a 10 mL tube which contained 100 mg of encapsulated or free phenolic powder. Sealed tubes were placed in a waterbath with no shaking for 2 h at 36.6^{o}C. At the end of the incubation, tubes were immediately cooled and the solutions were filtered. First the pH was reduced to 1.2 by adding 100 µL of 3 M HCl to 2 mL filtrate in order to inactivate the enzyme (Zheng et al., 2010). After 15 min, 900 µL of 0.2 N NaOH was added to the mixture to bring the pH to 7.0 (neutralization). Samples were analyzed by Folin-Ciocalteau colorimetric method by determining spectrophotometrically the amount of phenolic compounds released (760 nm). Results of the tests were expressed as the fraction of TPC released into the medium of the initial TPC of phenolic powder or microcapsule. Since the medium (SIF) contained enzymes and other components, a special calibration curve was used(Figure A.7).

2.5.11 Determination of glass transition temperature

Glass transition temperatures (T_g) of MD, GA and microcapsules were determined by using TA-Q20 Model Differential Scanning Calorimeter (DSC - TA Instruments, New Castle, DE, USA). For analyses, 5 ± 0.5 mg of dry samples were weighed into pans and then the pans were hermetically sealed. An empty pan was used as a reference. The differential scanning calorimeter was heated at a rate of 5°C/min from -70 °C to 180°C. T_g was computed automatically using the analysis software supplied with the instrument.

2.6 Statistical analysis and optimization

The independent parameters affecting the dependent variables were MD:GA ratio, core to coating ratio and ultrasonication time. Analysis of variance (ANOVA) was used for the determination of differences between independent parameters. SAS software version 9.1 used (SAS Institute Inc., NC, USA) to conduct ANOVA. If significant differences were found, Duncan's Multiple Comparison Test was used for comparisons ($p \leq 0.05$). All the results represent the means of at least two replications.

The multiple regression equations and coefficients were determined by using MINITAB Release 14.1 (Minitab Inc. State College, PA, USA). The optimization of the encapsulation conditions of different coating materials and ultrasonication time was calculated by optimization feature of MATLAB Package (Version: 7.4.0.278, R2007a, The MathWorks Inc., Natick, MA, USA). The model was maximized for encapsulation efficiency and minimized for particle size of capsules.

CHAPTER 3

RESULTS AND DISCUSSION

In this research, it was aimed to investigate the effects of formulation, ultrasonication and core to coating ratio on efficiency of encapsulation. In addition, phenolic content, antioxidant activity, surface morphology, particle size, color and digestability of microcapsules in simulated gastric and intestinal fluid were determined.

3.1 Surface phenolic content, total phenolic content and encapsulation efficiency

Total phenolic content of phenolic powder was determined as 91.29 mg GAE/g dry weight, and total phenolic content of microcapsules having core to coating ratio of 1:10 and 1:20 were found as; 45.65 and 30.43 mg GAE/g dry weight, respectively.

Determination of surface phenolic content of capsules was needed in order to calculate the encapsulation efficiency. Less quantity of phenolic content on the surface of the capsule shows more efficient the encapsulation process is. The effect of core to coating ratio, ultrasonication time and coating material types on surface phenolic content are shown in Table 3.1. There was significant ($p \leq 0.05$) difference between the surface phenolic content of the samples having core to coating ratio of 1:10 and 1:20 (Table 3.1) (Table B.1).

Table 3.1 Surface phenolic content (SPC) and antioxidant activities of capsules having different MD:GA ratio, core to coating ratio (CCR) and ultrasonication time (UT).

CCR	MD:GA	UT	SPC (mg GAE/g dry wt)	TEAC (mmol TEAC/g dry wt)	$DPPH^{\cdot}$ (ppm $DPPH^{\cdot}$/g dry wt)
1:20	10:0	15	6.45±0.380b[*]	121.7±8.65b	2.04±0.052b
1:20	10:0	20	4.24±0.020b	113.7±3.22b	1.85±0.050b
1:20	10:0	25	4.20±0.055b	107.0±1.24b	2.04±0.150b
1:20	8:2	15	3.00±0.286bc	111.3±6.86b	1.87±0.084b
1:20	8:2	20	3.52±0.355bc	106.2±5.05b	1.87±0.020b
1:20	8:2	25	3.18±0.285bc	105.2±2.05b	1.73±0.160b
1:20	6:4	15	3.58±0.050c	110.1±1.33b	1.83±0.130b
1:20	6:4	20	2.36±0.185c	106.0±2.98b	1.78±0.015b
1:20	6:4	25	2.73±0.065c	101.5±1.43b	1.98±0.175b
1:10	10:0	15	13.60±1.060a	181.4±5.30a	2.90±0.050a
1:10	10:0	20	14.00±1.670a	170.4±10.28a	2.80±0.230a
1:10	8:2	15	12.20±1.120a	179.4±0.50a	2.83±0.195a
1:10	8:2	20	10.30±1.390a	165.5±4.92a	2.80±0.230a
1:10	6:4	15	11.70±1.300a	181.7±2.42a	2.85±0.050a
1:10	6:4	20	10.10±1.160a	162.6±13.24a	2.62±0.205a

[*] Means having different letters (a, b & c) within the same column are significantly different at $p \leq 0.05$.

As represented in Figure 3.1, the samples having core to coating ratio of 1:20 had higher efficiencies than those having core to coating ratio of 1:10. For different MD:GA ratios, efficiency of capsules having core to coating ratio of 1:20 changed between 78.80-92.26% while it was in between 69.38-77.83% for core to coating ratio of 1:10. Higher efficiency results were expected for capsules having core to coating ratio of 1:20 since better encapsulation could be performed by using more coating material relative to core material. In MD:GA ratio, increasing GA concentration from 10:0 to 6:4 resulted in lower surface phenolic content (Table 3.1). Thus, encapsulation efficiency of capsules increased with the increase in GA concentration (Figure 3.1). Adding GA to coating material or changing ultrasonication time had no significant ($p > 0.05$) effect on encapsulation efficiency for core to coating ratio of 1:10 (Table B.3). However, for core to coating ratio of 1:20, addition of GA to the coating material increased efficiency significantly ($p \leq 0.05$) (Table B.4). This can be explained by stabilizing and emulsifying effects of GA on encapsulation (Alftrén et al., 2012). GA has the ability of forming a dried matrix around core material which prevents contact of core material with air (Thevenet, 1988). The surface active characteristic of GA has increased its intended use as an encapsulation material for protection of chemically reactive and volatile compounds (Kaushik & Roos, 2007). However, there was no significant difference between MD:GA ratio of 8:2 and 6:4 in affecting encapsulation efficiency (Figure 3.1) (Table B.2-B.4).

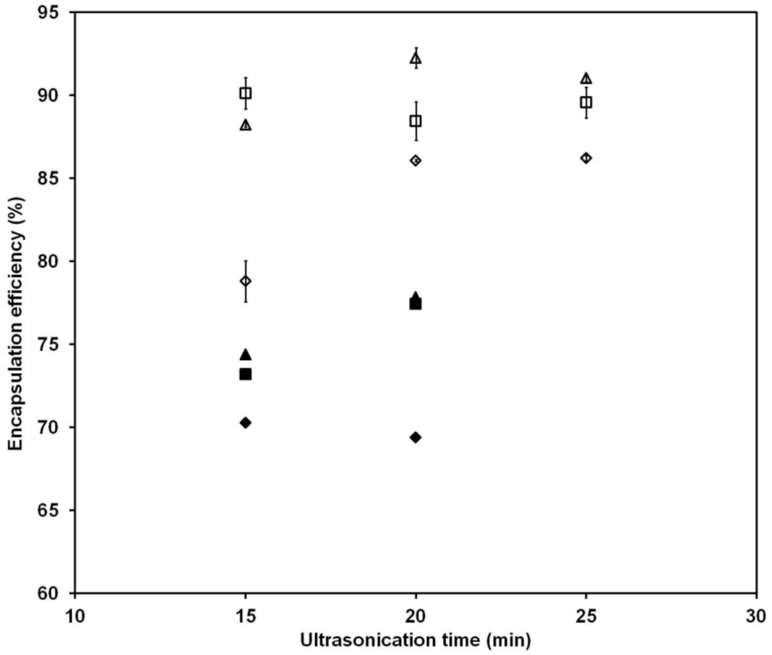

Figure 3.1 Effect of ultrasonication time on efficiency of microcapsules having different MD:GA ratio and core to coating ratio. (∆): 6:4 and 1:20a*, (□): 8:2 and 1:20a, (◊): 10:0 and 1:20b, (▲): 6:4 and 1:10c, (■): 8:2 and 1:10c, (◆): 10:0 and 1:10c. *Formulations having different letters (a, b & c) are significantly different at $p \leq 0.05$.

In order to examine the effects of ultrasonication time on surface phenolic content and efficiency for a longer time period (5-30 min), MD:GA ratio of 8:2 and core to coating ratio of 1:20 which gave the highest efficiency was chosen as a capsulation material (Figure 3.2). The inverse relationship between surface phenolic content and encapsulation efficiency can also be seen in Figure 3.2 and 3.3. Higher amount of phenolic compounds on the surface of microcapsules means encapsulation efficiency is lower. According to one-way ANOVA results (Table B.5), surface phenolic content was not significantly different for samples prepared by ultrasonication for 5 and 10 min but significantly higher than that of samples ultrasonicated for 15, 20 and 25 min. The higher surface phenolic content of capsules prepared using ultrasonication time of 5 and 10 min as compared to the ones prepared by using 15, 20 and 25 min may be due to the inefficiency of ultrasonication time on reduction of droplet size of emulsions. Particle size was shown to be related to the surface concentration of core material (Jafari et al., 2007b). It was shown that there was more unencapsulated oil at the surface of larger particles in the study of Jafari et al. (2007b). Large amount of core material at the surface led to lower encapsulation efficiency. This showed that encapsulation time was important to obtain capsules in smaller size.

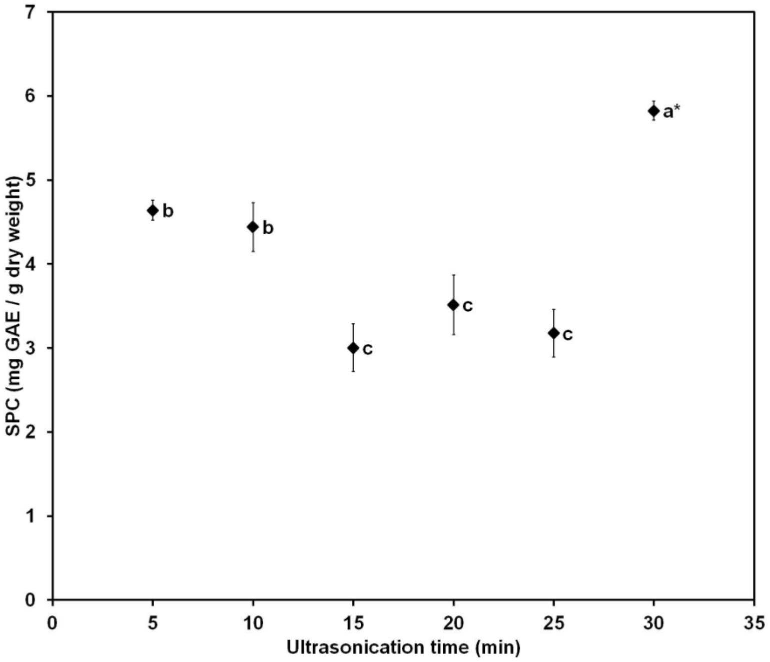

Figure 3.2 Effect of ultrasonication time on surface phenolic content of capsules having MD:GA ratio of 8:2 with core to coating ratio 1:20. *Means having different letters (a, b & c) are significantly different at $p \leq 0.05$.

When ultrasonication time of 15, 20 and 25 min were compared, no significant difference was determined in terms of surface phenolic content and efficiency (Table B.5 and 6). Additionally, not only for capsules prepared with MD:GA ratio of 8:2 and core to coating ratio of 1:20, but also for all the samples, ultrasonication time of 15, 20 and 25 min had no significant influence ($p > 0.05$) on surface phenolic content and efficiency (Figure 3.1, 3.2 and Table 3.1) (Table B.4 and 7). On the other hand, when 30 min of ultrasonication was applied, surface phenolic content increased ($p \leq 0.05$) and consequently encapsulation efficiency decreased significantly (Figure 3.2 and 3.3) (Table B.5 and 6). Higher ultrasonication time might have resulted in higher energy density which caused the degradation of phenolic compounds.

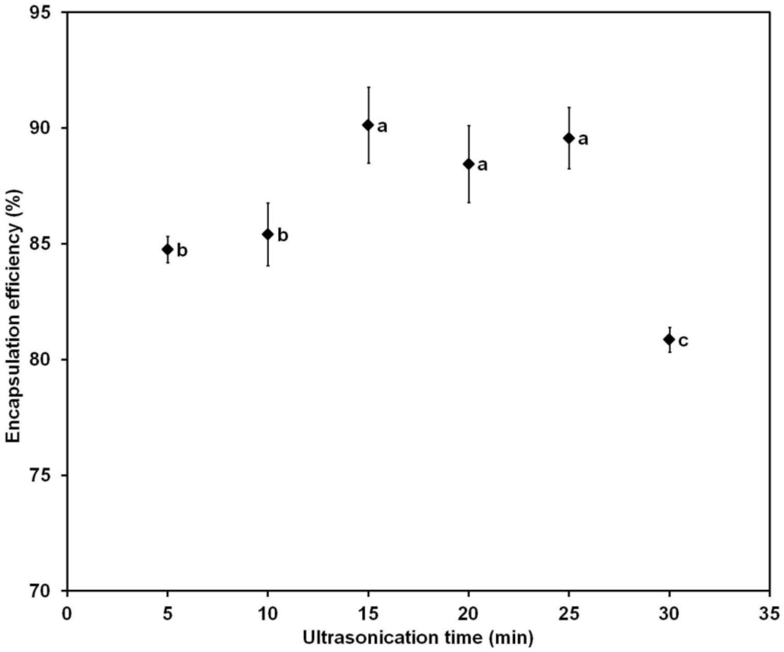

Figure 3.3 Effect of ultrasonication time on efficiency of capsules having MD:GA ratio of 8:2 with core to coating ratio 1:20. *Means having different letters (a, b, c) are significantly different at $p \leq 0.05$.

Consequently, the optimum ultrasonication time could be chosen as the 15, 20 or 25 min because there was no significant ($p > 0.05$) difference between them in terms of encapsulation efficiency (90.13%, 88.45% and 89.57%, respectively) (Table B.6). However, using efficiency results are not sufficient to decide about the optimum time of ultrasonication. The results of SEM and particle size analysis must also be taken into consideration.

3.2 Antioxidant activities with DPPH$^{\cdot}$ and TEAC method

In order to measure the antioxidant activity of phenolic powder and microcapsules, both DPPH$^{\cdot}$ and TEAC methods were implemented. Results of TEAC method were found to be correlated with the results of DPPH$^{\cdot}$ method with a correlation coefficient of 0.98.

Antioxidant activity of phenolic powder was determined as 7.09 ppm DPPH$^{\cdot}$/g dry weight and 458.25 mmol TEAC/g dry weight. As it can be seen in Table 3.1, core to coating ratio had a significant ($p \leq 0.05$) effect on antioxidant activity (Table B.8 and 9). The reason of this difference was that different amounts of phenolic powder was used (phenolic powder of 2 g and 1 g, respectively) for core to coating ratio of 1:10 and 1:20.

The related changes of antioxidant activities with ultrasonication time are also shown in Table 3.1. When the effect of ultrasonication time on antioxidant activity was examined, no significant ($p > 0.05$) differences were observed between different times (Table B.10-13).

Besides, no significant ($p > 0.05$) difference was found between the antioxidant activities of the samples with different encapsulation materials which were subjected to the same ultrasonication time (Table B.10-13).

3.3 Surface morphology analysis

SEM was used for surface morphology analysis. The SEM images of phenolic powder and microcapsules prepared with core to coating ratio of 1:10 were illustrated in Figure 3.4. It could be observed that phenolic powder and microcapsules were in irregular shape. The outer surface of the capsules with MD:GA 10:0, 8:2 and 6:4 were similar (Figure 3.4).

As can be seen in Figure 3.5, increasing sonication time of microcapsules prepared with core to coating ratio of 1:20 from 15 min to 20 min decreased particle sizes. Formulation had no effect on appearance of capsules. Furthermore, comparison of Figures 3.4 and 3.5 showed that particle size became smaller when core to coating ratio decreased from 1:10 to 1:20. Samples having core to coating ratio of 1:20 had higher amount of coating material which led to well-mixing and higher energy density compared to core to coating ratio of 1:10. As a result, samples having core to coating ratio of 1:20 had smaller particles.

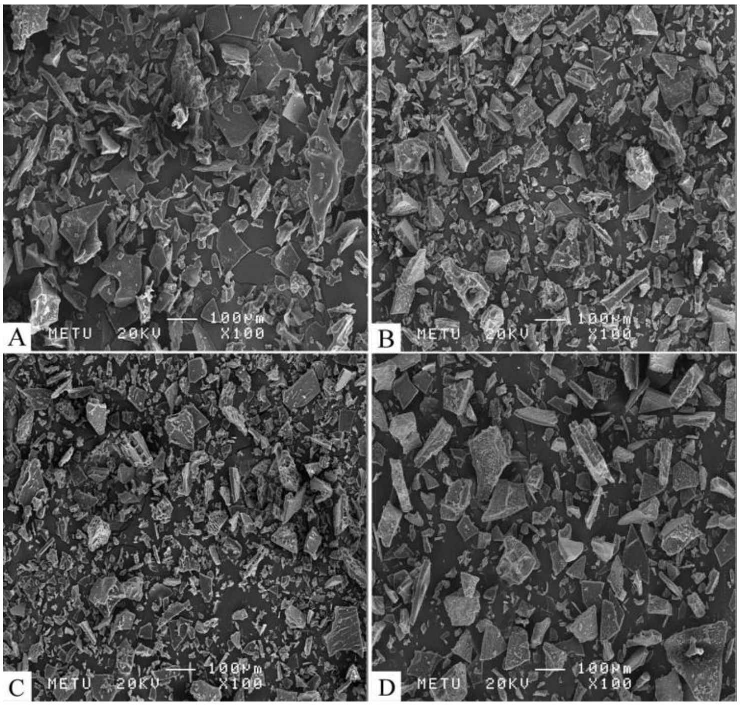

Figure 3.4 SEM images of phenolic powder (A), and microcapsules prepared with core to coating ratio of 1:10 and ultrasonication time of 20 min having different MD:GA ratios, 10:0 (B), 8:2 (C), 6:4 (D).

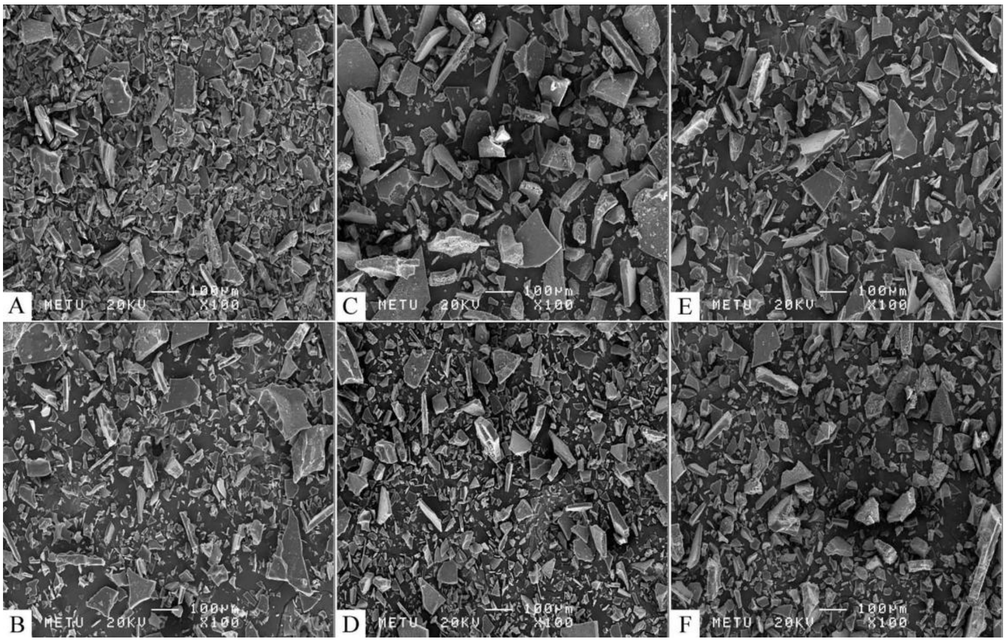

Figure 3.5 SEM images of microcapsules prepared with core to coating ratio of 1:20, different MD:GA ratios and ultrasonication times. (A): 10:0 15 min, (B): 10:0 20 min, (C): 8:2 15 min, (D): 8:2 20 min, (E): 6:4 15 min, (F): 6:4 20 min.

3.4 Particle size analysis

According to the results of SEM analysis, capsules prepared from emulsions using ultrasound for 20 min were smaller in size, as compared to capsules prepared from 15 min treated emulsions. Therefore, to investigate the effect of sonication time, particle size measurement was performed for emulsions treated for 20 and 25 min. In addition, capsules coated with core to coating ratio of 1:20 were selected to determine particle size since these capsules were smaller in size as compared to capsules prepared with core to coating ratio of 1:10. In order to compare the particle size of the samples, the results of Sauter mean diameter (D32), span and specific surface area (SSA) are used (Table 3.2).

Particle size analysis results (Table 3.2) indicated that sonication time of 20 and 25 min had no significant ($p > 0.05$) effect on D32 (Table B.14), specific surface area (Table B.15) and span (Table B.16). It can be explained by increasing energy density with sonication time that leads to formation of smaller particles and extra disruption; therefore, D32 slightly decreases. Delmas et al. (2011) demonstrated that the variation of particle size with ultrasound fitted exponential behavior. In other words, increasing ultrasonication time decreased particle size to a certain extent, and then particle size was stabilized. The particle size of phenolic powder which has not been ruptured under ultrasonication was larger than the size of samples which were under ultrasonication (Table 3.2 and Figure 3.6). Mechanism of ultrasonication at low frequency can be explained by acoustic cavitations (Li and Fogler, 1978). In other words, pressure fluctuations results in formation and subsequent collapse of micro-size bubbles and consequently high turbulence occurs. Turbulence causes smaller particles to be obtained.

Table 3.2 Particle size analysis results of phenolic powder (PP) and microcapsules having different MD:GA with core to coating ratio of 1:20.

	PP	MD:GA 10:0		MD:GA 8:2		MD:GA 6:4	
		20 min	25 min	20 min	25 min	20 min	25 min
D32 (µm)	5.78±0.015a*	1.65±0.072b	1.55±0.008b	1.61±0.124b	1.42±0.076b	1.51±0.101b	1.53±0.123b
SSA** (m^2/g)	1.22±0.180b	3.66±0.160a	3.87±0.020a	3.75±0.285a	4.23±0.220a	3.99±0.265a	3.95±0.315a
Span	3.29±0.020b	5.83±1.100a	6.37±0.005a	5.50±0.313a	5.38±0.943a	6.52±0.363a	6.24±0.133a

*Means having different (a & b) leters within the same row are significantly different at $p < 0.05$.

**SSA: Specific surface area.

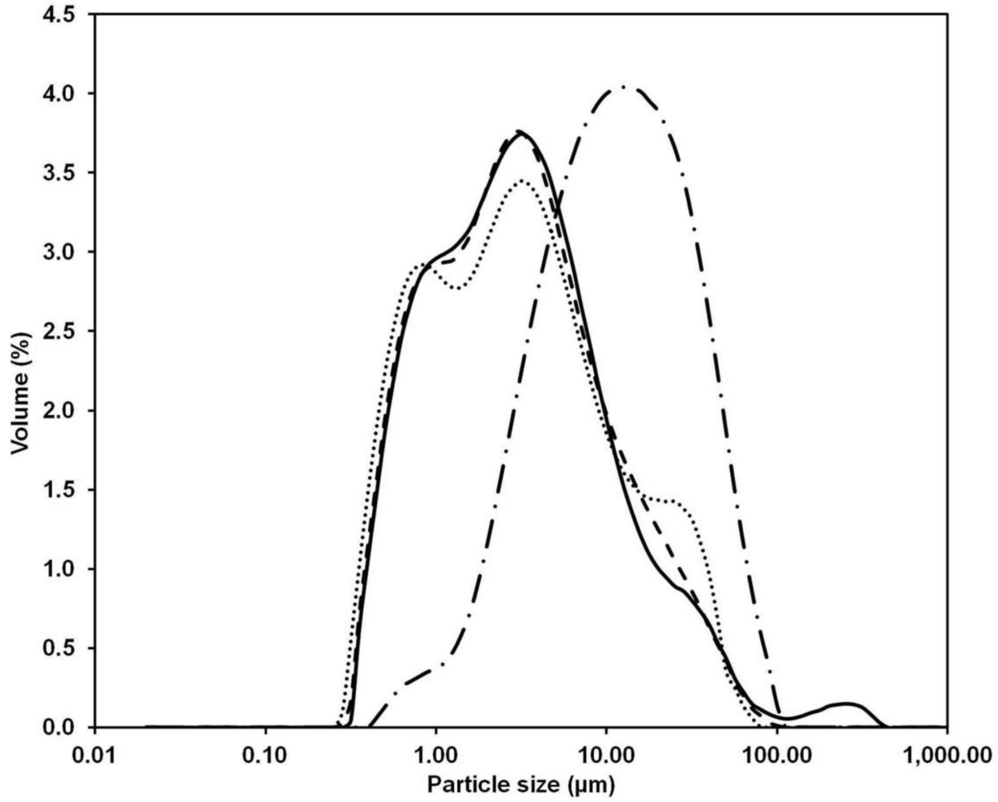

Figure 3.6 Particle size distribution of samples having different MD:GA ratio with core to coating ratio of 1: 20 and 20 min sonication. (—): 10:0, (— —): 8:2, (· · ·): 6:4, (— · —): phenolic powder.

Specific surface area of capsules was greater than that of the phenolic powder (Table 3.2) since, presence of smaller particles in emulsion increased specific surface area. Moreover, no significant difference ($p > 0.05$) between different MD: GA ratio in terms of particle size was observed according to the results of ANOVA (Table B.14-16). As can be seen in Figure 3.6, particle size distributions of different formulations were similar.

3.5 Color analysis

Color parameters of phenolic powder was determined as 34.46 for L*, 20.07 for a*, 11.67 for b*, 69.51 for ΔE* (Table 3.3). When color of phenolic powder was compared to the color of capsules, it was found that encapsulation increased lightness (L*) and decreased redness (a*) (Appendix E). This could be explained by the white color of MD and GA which were used as a coating material in capsules. Due to transparency of MD and GA solutions, the ratio of them inside the coating material did not contribute to significant changes ($p > 0.05$) in the color (Table B.17-20). Especially between L*, b* and ΔE* values of capsules with different formulations, no significant difference was observed (Table 3.3) (Table B.17, 19 and 20).

Table 3.3 Color results of phenolic powder and capsules having different MD:GA ratio, core to coating ratio (CCR) and ultrasonication time (UT).

CCR	MD:GA	UT	L*	a*	b*	ΔE*
Phenolic Powder			34.46±0.240c**	20.07±0.091a	11.67±0.034b	69.51±0.566a
1:20	10:0	15	48.74±0.170a	18.20±0.310c	13.52±0.030a	56.00±0.250c
1:20	10:0	20	50.08±0.500a	17.40±0.070c	13.35±0.000a	54.50±0.480c
1:20	10:0	25	51.36±0.230a	17.80±0.260d	13.84±0.240a	53.60±0.050c
1:20	8:2	15	52.81±1.100a	17.20±0.700c	13.52±0.095a	52.00±1.980c
1:20	8:2	20	50.40±0.270a	18.30±0.120c	14.05±0.055a	54.70±0.270c
1:20	8:2	25	50.18±2.240a	17.80±0.560d	14.05±0.215a	54.70±2.260c
1:20	6:4	15	49.34±2.695a	18.10±0.110c	13.40±0.520a	55.50±2.360c
1:20	6:4	20	49.63±0.030a	18.20±0.350c	13.91±0.400a	55.30±0.180c
1:20	6:4	25	47.63±1.130a	18.10±0.490d	13.45±0.905a	57.00±0.660c
1:10	10:0	15	45.48±0.205b	19.98±0.160b	13.59±0.190a	59.63±0.085b
1:10	10:0	20	47.09±0.050b	19.57±0.240b	13.60±0.200a	58.01±0.150b
1:10	8:2	15	45.31±0.040b	19.67±0.715b	13.63±0.420a	59.69±0.300b
1:10	8:2	20	46.98±2.350b	19.41±0.060b	13.63±0.230a	58.07±1.790b
1:10	6:4	15	46.67±2.800b	19.39±0.255b	13.70±0.645a	58.38±2.480b
1:10	6:4	20	46.44±0.505b	19.26±0.200b	13.42±0.175a	58.46±0.325b

**Means having different letters (a, b, c & d) within the same column are significantly different at $p \leq 0.05$.

The difference in color was observed when different core to coating ratios were used. The samples with core to coating ratio of 1:10 had higher a*(redness) values than the samples with core to coating ratio of 1:20. The reason of that was the higher intensity of red phenolic powder in the coating material when core to coating ratio was 1:10. The amount of phenolic powder used for the samples having core to coating ratio of 1:10 was twice as the phenolic powder used for those having core to coating ratio of 1:20. Therefore, capsules prepared with coating ratio of 1:10 had lower L* values as compared to the ones prepared with core to coating ratio of 1:20. When Table 3.3 was analyzed, it was clearly seen that the ultrasonication time was not so effective on color change.

3.6 Optimization of microencapsulation

Multiple regression was performed to express encapsulation efficiency and particle size as a function of MD:GA ratio and ultrasonication time. The model constants and coefficient of determination (R^2_{adj}) are given in Table 3.4 (Appendix C). For good fit models, R^2 value of at least 0.80 is recommended (Gan et al., 2007). The results in our study showed that models were adequate since they had satisfactory R^2 values.

Table 3.4 Model constants for capsules having core to coating ratio of 1:20.

Parameter	Equation	R^2_{adj}
Encapsulation efficiency	$Y_1 = 89.0 - 2.75\ MD{:}GA^{****} + 0.014\ UT^{ns} - 0.120\ MD{:}GA^{2\ ns} + 0.333\ MD{:}GA{*}UT^{ns}$	0.84
Particle size (D32)	$Y_2 = 1.46 + 0.0475\ MD{:}GA^{**} - 0.0350\ UT^{*} + 0.120\ MD{:}GA^{2****} - 0.0375\ MD{:}GA{*}UT^{*}$	0.94

^{ns} not significant, * significant at $p \leq 0.05$, ** significant at $p \leq 0.01$, **** significant at $p \leq 0.0001$.

A MATLAB program was written in order to maximize the encapsulation efficiency and minimize the particle size of the capsules to find the optimum point (Table C.3). Equations were determined using only the results of microcapsules having core to coating ratio of 1:20, since the encapsulation efficiency of them was significantly ($p \leq 0.05$) higher than that of the capsules having core to coating ratio of 1:10. For the encapsulation efficiency, MD:GA ratio was significantly important ($p \leq 0.0001$), as MD:GA ratio decreased encapsulation efficiency increased. On the other hand, for particle size equations both MD:GA ratio and ultrasonication time were significantly important ($p \leq 0.0001$ and $p \leq 0.05$). The optimum point was found as MD:GA ratio of 8:2 and ultrasonication time of 22.5 min.

3.7 Digestability in simulated gastric and intestinal fluid

Bioavailability of polyphenols is one of the major issues concerning the beneficial effects of phenolic compounds. It is described as the ratio of a digested nutrient that is accessible and can be used for body in normal physiological functions or for storage (Castenmiller et al., 1999). Bioavailability depends on the dietary source and the digestability of phenolic compounds, which is considerably different for phenolics. Even though in vivo experiments are necessary for such studies about digestability of phenolic compounds, in vitro methods are also beneficial in the determination of their stability under simulated gastric or small intestinal fluid digestion conditions. It has been shown that the in vitro methods can be successfully correlated with the results of human studies (clinical) and animal models (in vivo) despite their limitations (Bouayed et al., 2011).

In this study, the digestability of unencapsulated (free) phenolic powders was compared with the digestion of microcapsules prepared with core to coating ratio of 1:20 and sonication time of 20 min.

3.7.1 Digestability in simulated gastric fluid

The impact of digestability of microcapsules in the simulated gastric fluid (SGF) are shown in Table 3.5. The release of phenolic compounds from the phenolic powder were significantly higher than the release of phenolic compounds from capsules (Table B.21); the release from microcapsules can be considered as low. The low level release of phenolic compounds in SGF showed that the coating material type was gastric-insoluble material. Coating material acted as a barrier against the gastric medium. Thus, encapsulation had a significant effect on the retention of the phenolic compounds. The results of release of phenolic compounds from capsules in SGF in this study are in accordance with those of Zheng et al. (2011). They found that the release of bayberry polyphenols from microcapsules in SGF is between 12.35%-14.53%.

Table 3.5 Release of phenolic compounds from phenolic powder and microcapsules with core to coating ratio 1:20 and ultrasonication time of 20 min in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF).

Sample	Release (%)	
	SGF	SIF
Phenolic powder	26.79±0.760a*	47.07±2.191a
MD:GA 10:0	11.79±0.569b	34.30±2.097b
MD:GA 8:2	11.88±0.163b	31.74±2.569b

*Columns having different letters (a & b) are significantly different ($p \leq 0.05$).

3.7.2 Digestability in simulated intestinal fluid

Results showed that the release of phenolic compounds in SGF (pH 1.2) was lower than that in SIF (pH 6.8) (Table 3.5).The results are in accordance with those indicated by Sansone et al. (2011) who studied the dissolution/release test of flavonoid microparticles in SGF and SIF (Sansone et al., 2011) and found significantly higher amounts of phenolic compounds released from the uncoated phenolic powder as compared to that from the encapsulated phenolic powders. Moreover, they found that dissolution rate of the microparticles in SGF were lower than the dissolution rate in SIF as was obtained in this study. Statistical analysis showed that the release of phenolic powder in SGF or in SIF was significantly ($p \leq 0.05$) higher than that from the microcapsules (Table B.21 and B.22). This was explained by the low solubility of the coating materials at low pHs. Capsule material was reported to be digested easily and this resulted in effective capsule breakage at neutral pH values (Zheng et al., 2011). Seok et al. (2003) performed a similar study in order to determine the stability of isoflavone microencapsulated by polyglycerol monostearate in simulated gastrointestinal fluid in media with different pHs. They found that most of the isoflavones were released at pH 7 and 8. This study confirmed that dissolution of microcapsules in SIF was higher (pH 6.8).

Relatively small amount of phenolic compounds was released at low pH and their release increased in SIF. These results show that encapsulated phenolic compounds could be effectively absorbed in the small intestine. This behavior could be described by increase in water interaction, wettability and solubility of the microcapsules at the higher pH of the SIF (Sansone et al., 2011). Sansone et al. (2011) stated that encapsulation improved digestion stability of the phenolic compounds. In addition, the capsule provided an extra barrier to gastric fluid and caused to improve in release rate during intestinal digestion.

Thus, their digestion would be improved after oral administration by carrying flavonoids in gastroresistant polymers.

When Table 3.5 is analyzed, it could be seen that complete absorption of phenolic compounds was hardly possible. The reason for not reaching complete release of phenolics in SIF might be due to the loss of phenolic compounds during digestion. According to Perez-Vicente et al., (2002) the high loss of anthocyanins could be related to their degradation into other chemicals, oxidation, or changing into some colorless forms, which might hardly be detected under the present test conditions. The results of this study are in accordance with other studies where low bioavailability of anthocyanins in SIF was observed (Perez-Vicente et al., 2002; McDougall et al., 2005; Bouayed et al., 2011).

Although the human in vivo results can not be estimated directly from the results obtained with simulated in vitro digestions, this model is still beneficial for studying digestion of phenolic compounds. During the in vitro simulation of intestinal digestion, an increase in release of TPC isobserved after gastric digestion. The reason of this increment is the effect of pH. Coating material that was damaged at low pHs by pepsin digestion, could easily dissolve in the intestinal fluid, and absorption of high amounts of phenolic compounds could be achieved. It must not be forgotten that microencapsulation prevents the unwanted taste and odor of the phenolic compounds and increases their intake. When the intake of phenolics becomes higher, retention will definitely be higher regardless of the released fraction.

3.8 Glass transition temperature

The stability of a material is mainly controlled or determined by the glass transition temperature (T_g), since molecular mobility is extremely decreased due to the high viscosity of the matrix below T_g (Roos, 1995). During the storage of amorphous capsule under glass transition temperature, the capsule matrix stays in glassy-like state and it protects the encapsulated core material from numerous deteriorative changes (especially oxidation for phenolic compounds encapsulation) (Laine et al., 2008).

Bhandari et al. (1993) stated that the powders may have problems such as stickiness and high hygroscopicity due to the low glass transition temperatures. The researchers proposed that addition of some carier agents like gums and polymers could be a solution for this problem. These agents were shown to increase the glass transition temperatures, thus microcapsule stability was promoted and even controlled release of them were enhanced (Ré, 1998; Tonon et al., 2009).

The results of glass transition temperatures of gum Arabic, maltodextrin, microcapsules having core to coating ratio of 1:20, ultrasonication time of 20 min and different MD:GA ratios are shown in Table 3.6 (Figure D.1-5). Changing GA concentration in coating material made no significant change in T_g (Table B.23).

Table 3.6 Glass transition temperatures of coating materials and microcapsules with ultrasonication time (UT) and core to coating ratio (CCR).

Sample	UT (min)	CCR	Tg (°C)
Gum Arabic	-	-	75.00a*
Maltodextrin	-	-	59.32b
MD:GA 10:0	20	1:20	60.04b
MD:GA 8:2	20	1:20	57.47b
MD:GA 6:4	20	1:20	58.96b

*Means having different letters (a & b) within the same column are significantly different at $p \leq 0.05$.

Gum Arabic were shown to have higher T_g as compared to encapsulated phenolic powders (Table 3.6). This indicates that phenolic powder had low molecular mass components in it, which caused to decrease T_g of microcapsules (Laine et al., 2008). Additionally, organic acids have very low T_g values, so they decrease the T_g of amorphous material as in the study of Adhikari et al. (2003). They revealed that T_g of anhydrous citric acid (mainly found in fruits) can be as low as 12 ^{o}C.

As can be seen in Table 3.6, T_g of microcapsules are not significantly ($p >$ 0.05) different from T_g of maltodextrin. This shows that utilization of gum Arabic in coating material has no effect on changing T_g.

CHAPTER 4

CONCLUSION

In this study, phenolic compounds extracted from sour cherry pomace were successfully encapsulated by using ultrasonic treatment and coatings containing MD and GA.

When core to coating ratio was decreased from 1:10 to 1:20, significant decrease in red color and increase in lightness value were observed. In addition, the encapsulation efficiency and antioxidant activity increased while particle size of capsules decreased.

Among different MD:GA ratios, 8:2 was determined as the best coating material in terms of its higher encapsulation efficiency. Utilization of GA increased encapsulation efficiency but had no influence on antioxidant activity, glass transition temperature and color.

Ultrasonication time was found to affect encapsulation efficiency and particle size. Nevertheless, antioxidant activity and particle size were not affected with changing ultrasonication time between 15 to 25 min.

The digestion of phenolic compounds and capsules showed that encapsulation prevented the degradation of phenolics at low pH values. The coating material formed a good barrier, and caused to increase ingestion during intestinal absorption.

As a conclusion, microcapsules coated with MD:GA ratio of 8:2 with core to coating ratio of 1:20 and prepared by sonication for 22.5 min can be recommended to be incorporated into functional foods since the capsules prepared with these conditions had the highest encapsulation efficiency and the smallest particle size. However, further research on storage stability, thermal stability and bioavailability of capsules incorporated in foods is necessary before using microcapsules as food additives or nutraceuticals.

It can be recommended that two or more bioactive compounds can be combined with polyphenols in order to extend their antioxidant activity with the help of synergistic effect of phenolic compounds with others such as tocopherol or ascorbic acid. In addition, spray drying could be used instead of freeze drying for encapsulation process in order to obtain dry powders.

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APPENDIX A

CALIBRATION CURVES

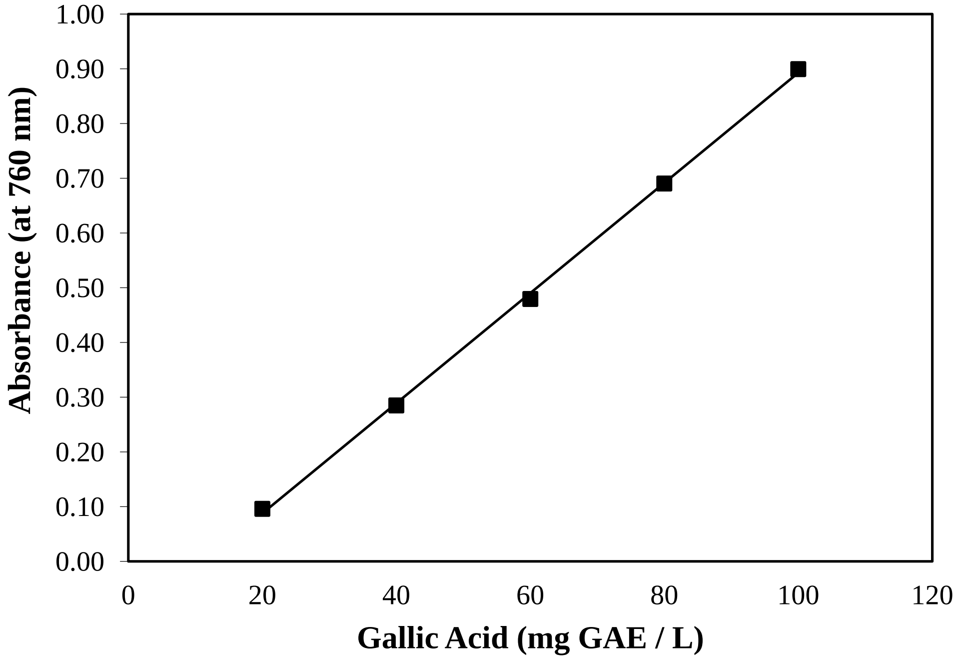

Figure A.1 Calibration curve prepared by gallic acid in ethanol:water mixture (50:50 v/v) for determination of total phenolic contents.

Absorbance (760 nm) = 0.0101 * (mg GAE / L) - 0.1137

$R^2 = 0.9994$

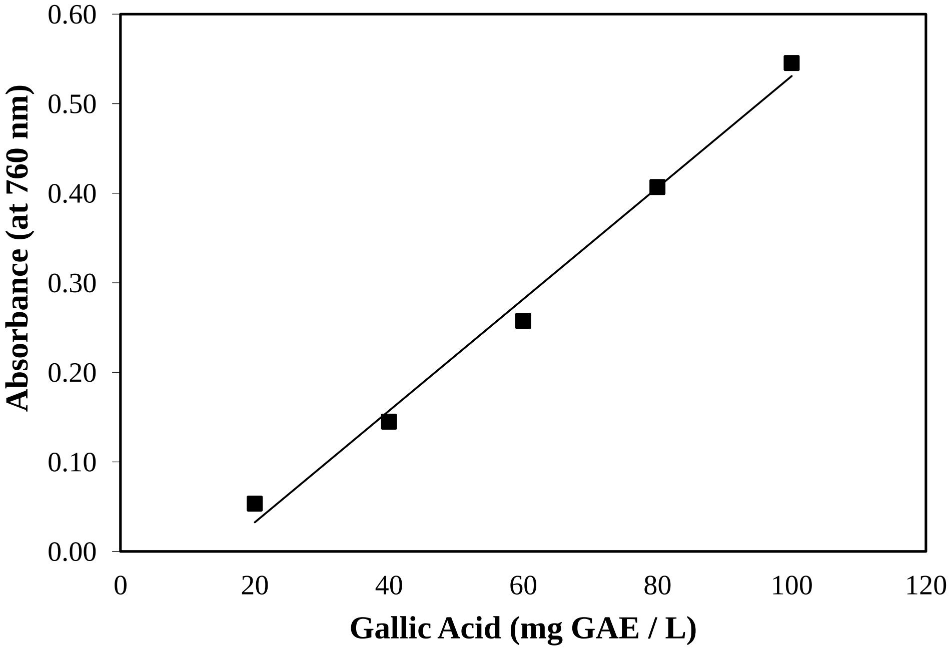

Figure A.2 Calibration curve prepared by gallic acid in ethanol:acetic acid:water mixture (50:8:42 v/v) for determination of total phenolic contents.

Absorbance (760 nm) = 0.0062 * (mg GAE / L) - 0.0921

$R^2 = 0.9911$

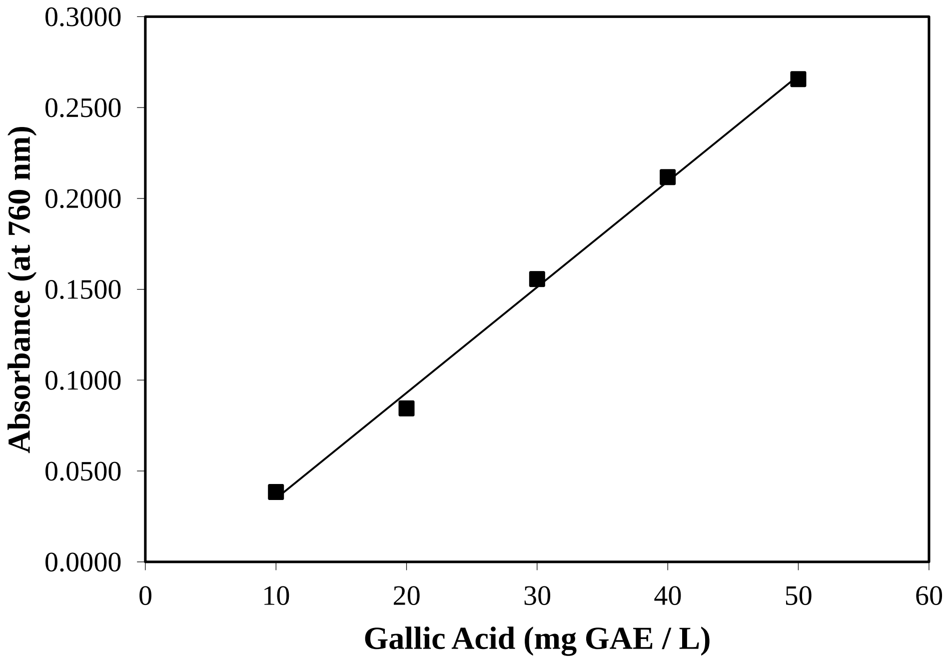

Figure A.3 Calibration curve prepared by gallic acid in ethanol:methanol mixture (50:50 v/v) for determination of surface phenolic contents of microcapsules.

Absorbance (760 nm) = 0.0058 * (mg GAE / L) - 0.0233

$R^2 = 0.9966$

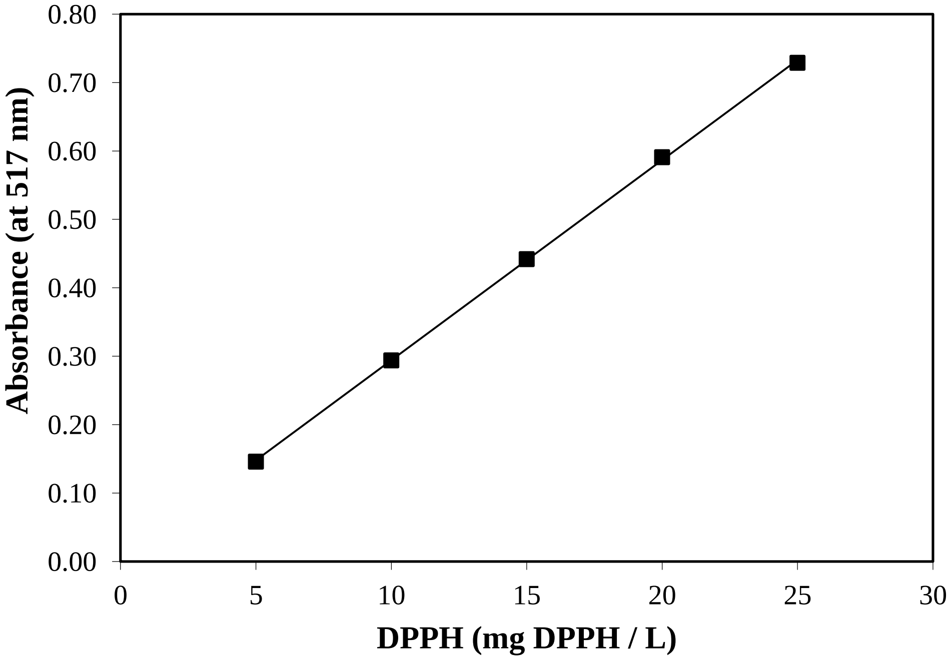

Figure A.4 Calibration curve prepared by DPPH$^{\cdot}$ radical in methanol for determination of antioxidant activity.

Absorbance (517 nm) = 0.0293 * (mg DPPH$^{\cdot}$ / L) + 0.0015

$R^2 = 0.9998$

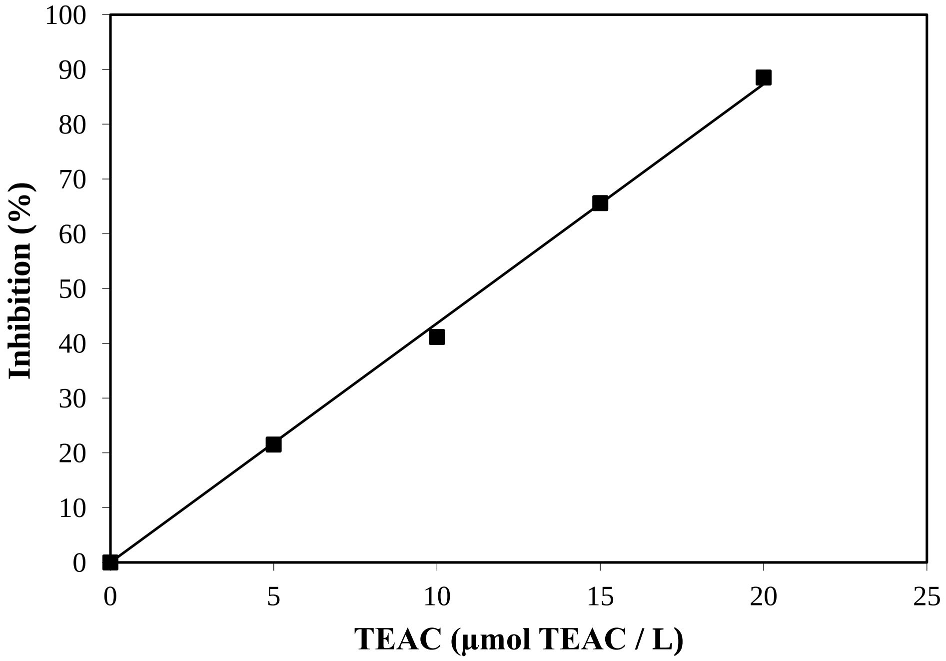

Figure A.5 Calibration curve prepared by Trolox standard solution in ethanol for determination of antioxidant activity.

Absorbance (734 nm) = 4.423 * (µmol TEAC / L) - 0.864

R^2 = 0.9987

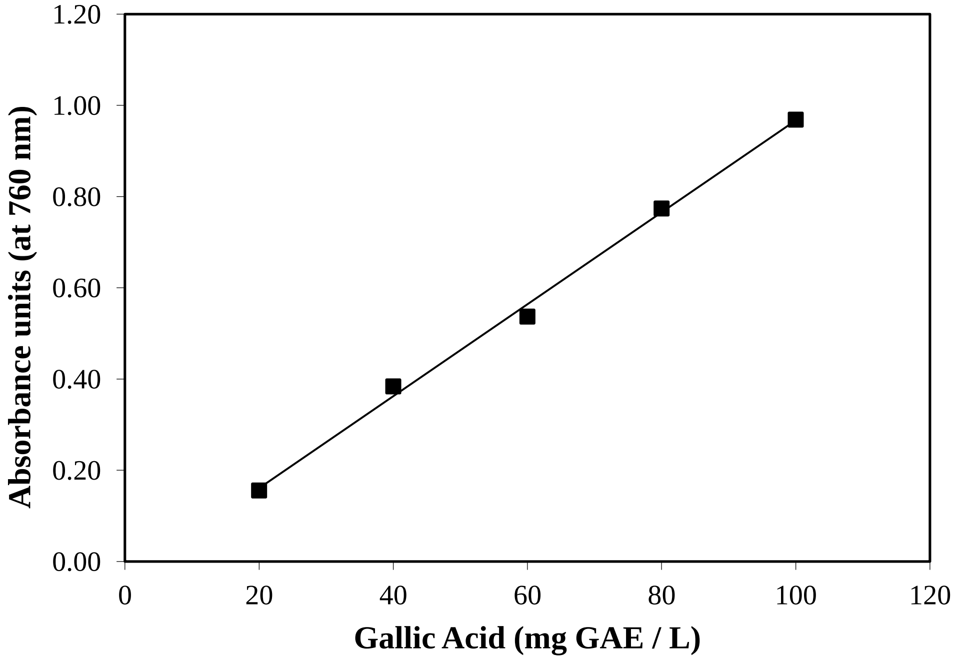

Figure A.6 Calibration curve prepared by gallic acid in SGF for determination of total phenolic contents of microcapsules.

Absorbance (760 nm) = 0.0101 * (mg GAE / L) - 0.041

$R^2 = 0.9968$

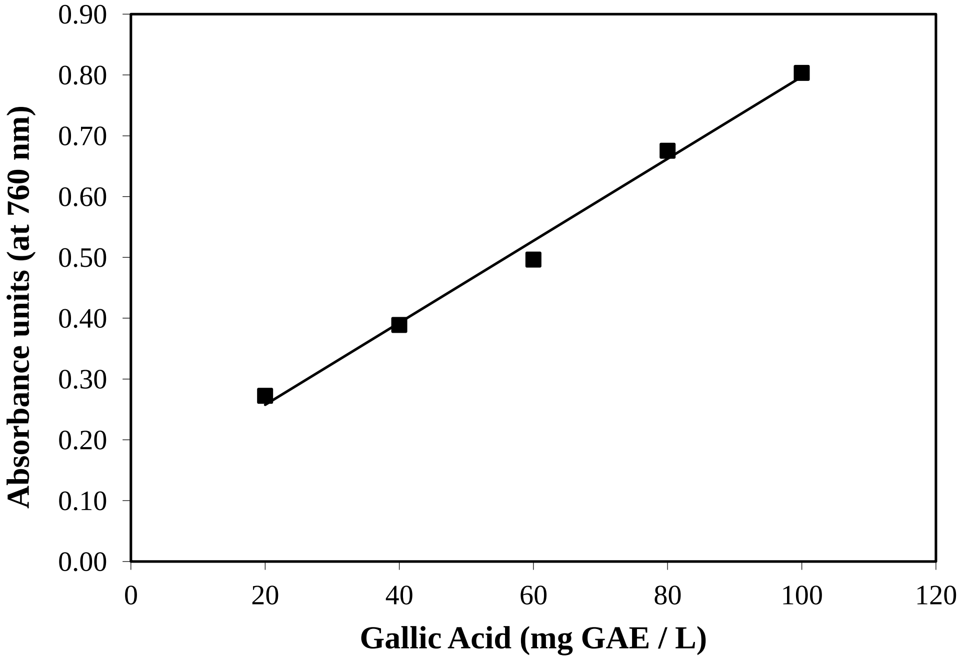

Figure A.7 Calibration curve prepared by gallic acid in SIF for determination of total phenolic contents of microcapsules

Absorbance (760 nm) = 0.007 * (mg GAE / L) + 0.1229

$R^2 = 0.9923$

APPENDIX B

STATISTICAL ANALYSES

Table B.1 Surface phenolic content of capsules

X1 Core to coating ratio (1, 1:10; 2, 1:20)
X2 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X3 UT (min)

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	6 8 10
X3	2	15 20

Number of Observations Read	12
Number of Observations Used	12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	217.3144833	54.3286208	104.37	<.0001
Error	7	3.6439167	0.5205595		
Corrected Total	11	220.9584000			

```
R-Square     Coeff Var      Root MSE        Y Mean

0.983509      9.109825      0.721498          7.920000


Source                DF       Type I SS     Mean Square    F Value    Pr > F

X1                     1     197.9656333     197.9656333     380.29    <.0001

X2                     2      16.3488500       8.1744250      15.70     0.0026

X3                     1       3.0000000       3.0000000       5.76     0.0474


Source                DF     Type III SS     Mean Square    F Value    Pr > F

X1                     1     197.9656333     197.9656333     380.29    <.0001

X2                     2      16.3488500       8.1744250      15.70     0.0026

X3                     1       3.0000000      3.0000000        5.76     0.0474
```

Duncan's Multiple Range Test for Y

```
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.


Alpha                        0.05

Error Degrees of Freedom        7

Error Mean Square         0.52056


Number of Means          2

Critical Range       .9850


Means with the same letter are not significantly different.
```

```
Duncan Grouping          Mean      N    X1
A                      11.9817     6    1
B                        3.8583    6    2
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
Alpha                     0.05
Error Degrees of Freedom        7
Error Mean Square         0.52056

Number of Means          2          3
Critical Range       1.206      1.254
```

Means with the same letter are not significantly different.

```
Duncan Grouping          Mean      N    X2
A                       9.5600     4    10
B                       7.2625     4    8
B                       6.9375     4    6
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05

Error Degrees of Freedom 7

Error Mean Square 0.52056

Number of Means 2

Critical Range .9850

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X3
A	8.4200	6	15
B	7.4200	6	20

Table B.2 Encapsulation efficiency of capsules

X1 core to coating ratio (1, 1:10; 2, 1:20)
X2 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X3 UT (min)

```
Class Level Information
Class      Levels   Values
X1            2     1 2
X2            3     6 8 10
X3            2     15 20

Number of Observations Read     12
Number of Observations Used     12

Dependent Variable: Y

Source             DF   Sum of Squares   Mean Square    F Value   Pr > F
Model               4   693.1234333      173.2808583    40.17     <.0001
Error               7   30.1936583       4.3133798
Corrected Total    11   723.3170917

R-Square    Coeff Var    Root MSE     Y Mean
0.958257    2.578705     2.076868     80.53917

Source             DF      Type I SS     Mean Square    F Value    Pr > F
X1                 1    552.5704083    552.5704083    128.11    <.0001
X2                 2    118.1670167     59.0835083     13.70    0.0038
X3                 1     22.3860083     22.3860083      5.19    0.0568
```

```
Source              DF     Type III SS     Mean Square    F Value    Pr > F
X1                   1     552.5704083     552.5704083     128.11    <.0001
X2                   2     118.1670167      59.0835083      13.70    0.0038
X3                   1      22.3860083      22.3860083       5.19    0.0568
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
Alpha                        0.05
Error Degrees of Freedom        7
Error Mean Square         4.31338

Number of Means         2
Critical Range      2.835
```

Means with the same letter are not significantly different.

```
Duncan Grouping        Mean     N    X1
A                    87.325     6    2
B                    73.753     6    1
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05

Error Degrees of Freedom 7

Error Mean Square 4.31338

Number of Means 2 3

Critical Range 3.472 3.611

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	83.180	4	6
A	82.308	4	8
B	76.130	4	10

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05

Error Degrees of Freedom 7

Error Mean Square 4.31338

Number of Means 2

Critical Range 2.835

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X3
A	81.905	6	20
A	79.173	6	15

Table B.3 Encapsulation efficiency of capsules having core to coating RATİO 1:10

```
X1 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X2 UT (min)

Class Level Information
Class        Levels    Values
X1               3    6 8 10
X2               2    15 20

Number of Observations Read          6
Number of Observations Used          6

Dependent Variable: Y

Source              DF      Sum of Squares      Mean Square    F Value    Pr > F
Model                3      54.57303333        18.19101111      4.79      0.1776
Error                2      7.59790000         3.79895000
Corrected Total      5      62.17093333

R-Square    Coeff Var    Root MSE       Y Mean
0.877790     2.642714     1.949090     73.75333

Source              DF      Type I SS       Mean Square     F Value    Pr > F
X1                   2     46.91163333     23.45581667      6.17       0.1394
X2                   1     7.66140000      7.66140000       2.02       0.2914

Source              DF      Type III SS       Mean Square     F Value    Pr > F
X1                   2       46.91163333     23.45581667      6.17       0.1394
X2                   1       7.66140000       7.66140000      2.02       0.2914
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 3.79895

Number of Means	2	3
Critical Range	8.386	8.013

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	76.110	2	6
A	75.325	2	8
A	69.825	2	10

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 3.79895

Number of Means 2

Critical Range 6.847

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	74.883	3	20
A	72.623	3	15

Table B.4 Encapsulation efficiency of capsules having core to coating ratio 1:20

```
X1 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X2 UT (min)

Class Level Information
Class        Levels    Values
X1              3    6 8 10
X2              3    15 20 25

Number of Observations Read          9
Number of Observations Used          9

Dependent Variable: Y

Source              DF       Sum of Squares     Mean Square    F Value    Pr > F
Model                4       101.7980444        25.4495111      4.08       0.1011
Error                4       24.9603778         6.2400944
Corrected Total      8       126.7584222

R-Square     Coeff Var      Root MSE        Y Mean
0.803087      2.842389      2.498018      87.88444


Source                  DF       Type I SS     Mean Square    F Value    Pr > F
X1                       2     80.77242222     40.38621111      6.47    0.0557
X2                       2     21.02562222     10.51281111      1.68    0.2946
```

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	80.77242222	40.38621111	6.47	0.0557
X2	2	21.02562222	10.51281111	1.68	0.2946

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	4
Error Mean Square	6.240094

Number of Means	2	3
Critical Range	5.663	5.787

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	90.517	3	6
A	89.443	3	8
B	83.693	3	10

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
Alpha                    0.05
Error Degrees of Freedom        4
Error Mean Square        6.240094

Number of Means          2          3
Critical Range       5.663      5.787


Means with the same letter are not significantly different.

Duncan Grouping        Mean      N    X2
A                      89.003     3    25
A                      88.927     3    20
A                      85.723     3    15
```

Table B.5 Surface phenolic content of capsules having core to coating ratio 1:20, MD:GA 8:2 and ultrasonication time of 20 min

```
X1 UT (min)

Class Level Information
Class        Levels    Values
X1               6     5 10 15 20 25 30

Number of Observations Read        13
Number of Observations Used        13

Dependent Variable: Y

Source                DF    Sum of Squares     Mean Square    F Value    Pr > F
Model                  5     12.67630641        2.53526128     15.72      0.0011
Error                  7      1.12921667        0.16131667
Corrected Total       12     13.80552308

R-Square     Coeff Var      Root MSE        Y Mean
0.918205      10.00259      0.401642         4.015385

Source              DF       Type I SS     Mean Square      F Value    Pr > F
X1                   5     12.67630641      2.53526128       15.72      0.0011

Source              DF     Type III SS     Mean Square    F Value    Pr > F
X1                   5     12.67630641      2.53526128       15.72      0.0011
```

Duncan's Multiple Range Test for Y

```
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.

Alpha                        0.05
Error Degrees of Freedom           7
Error Mean Square           0.161317
Harmonic Mean of Cell Sizes 2.117647

NOTE: Cell sizes are not equal.

Number of Means          2          3          4          5          6
Critical Range       .9229      .9597      .9793      .9904      .9965


Means with the same letter are not significantly different.

Duncan Grouping       Mean      N    X1
A                   5.8250      2    30
B                   4.6400      2    5
B                   4.4400      2    10
C                   3.5150      2    20
C                   3.1750      2    25
C                   3.0033      3    15
```

Table B.6 Encapsulation efficiency of capsules having core to coating ratio 1:20, MD:GA 8:2 and ultrasonication time of 20 min.

```
Class Level Information
Class      Levels      Values
X1         6           5 10 15 20 25 30

Number of Observations Read       13
Number of Observations Used       13

Dependent Variable: Y

Source             DF   Sum of Squares      Mean Square   F Value     Pr > F
Model               5    136.9851269         27.3970254    15.70       0.0011
Error               7    12.2161500          1.74516433
Corrected Total    12    149.2012769

R-Square    Coeff Var     Root MSE       Y Mean
0.918123     1.521889     1.321047     86.80308

Source                DF      Type I SS     Mean Square    F Value    Pr > F
X1                     5     136.9851269     27.3970254     15.70    0.0011

Source                DF     Type III SS     Mean Square    F Value    Pr > F
X1                     5     136.9851269     27.3970254     15.70    0.0011
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	7
Error Mean Square	1.745164
Harmonic Mean of Cell Sizes	2.117647

NOTE: Cell sizes are not equal.

Number of Means	2	3	4	5	6
Critical Range	3.036	3.157	3.221	3.257	3.278

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	90.130	3	15
A	89.565	2	25
A	88.450	2	20
B	85.405	2	10
B	84.750	2	5
C	80.855	2	30

Table B.7 Surface phenolic content of capsules having core to coating ratio 1:20

```
Class Level Information
Class        Levels   Values
X1                3   6 8 10
X2                3   15 20 25

Number of Observations Read          9
Number of Observations Used          9

Dependent Variable: Y

Source              DF    Sum of Squares    Mean Square   F Value    Pr > F
Model                4      9.29777778       2.32444444      3.96     0.1057
Error                4      2.34984444       0.58746111
Corrected Total      8     11.64762222

R-Square    Coeff Var     Root MSE       Y Mean
0.798255     20.74005     0.766460     3.695556

Source              DF      Type I SS    Mean Square   F Value    Pr > F
X1                   2     7.40948889     3.70474444      6.31    0.0580
X2                   2     1.88828889     0.94414444      1.61    0.3074

Source              DF    Type III SS    Mean Square   F Value    Pr > F
X1                   2     7.40948889     3.70474444      6.31    0.0580
X2                   2     1.88828889     0.94414444      1.61    0.3074
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
Alpha                      0.05
Error Degrees of Freedom      4
Error Mean Square      0.587461

Number of Means        2        3
Critical Range     1.738    1.776
```

Means with the same letter are not significantly different.

```
Duncan Grouping      Mean    N   X1
A                  4.9633    3   10
B    A             3.2333    3   8
B                  2.8900    3   6
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
Alpha                      0.05
Error Degrees of Freedom      4
Error Mean Square      0.587461
```

```
Number of Means        2        3
Critical Range     1.738    1.776


Means with the same letter are not significantly different.

Duncan Grouping      Mean    N   X2
A                  4.3433    3   15
A                  3.3733    3   20
A                  3.3700    3   25
```

Table B.8 Antioxidant activity (DPPH$^{\cdot}$) of capsules

X1 core to coating ratio (1, 1:10; 2, 1:20)
X2 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X3 UT (min)

```
Class Level Information
Class        Levels   Values
X1               2    1 2
X2               3    6 8 10
X3               2    15 20

Number of Observations Read        12
Number of Observations Used        12

Dependent Variable: Y

Source              DF    Sum of Squares    Mean Square    F Value    Pr > F
Model                4     2.56010000        0.64002500     217.57    <.0001
Error                7     0.02059167        0.00294167
Corrected Total     11     2.58069167

R-Square    Coeff Var     Root MSE       Y Mean
0.992021     2.313706     0.054237     2.344167

Source              DF       Type I SS     Mean Square    F Value    Pr > F
X1                   1     2.49340833     2.49340833     847.62     <.0001
X2                   2     0.04501667     0.02250833       7.65     0.0173
X3                   1     0.02167500     0.02167500       7.37     0.0300
```

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	2.49340833	2.49340833	847.62	<.0001
X2	2	0.04501667	0.02250833	7.65	0.0173
X3	1	0.02167500	0.02167500	7.37	0.0300

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05

Error Degrees of Freedom 7

Error Mean Square 0.002942

Number of Means 2

Critical Range .07404

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	2.80000	6	1
B	1.88833	6	2

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05

```
Error Degrees of Freedom        7
Error Mean Square        0.002942



Number of Means           2           3
Critical Range       .09068      .09430



Means with the same letter are not significantly different.

Duncan Grouping         Mean      N    X2
A                        2.42000      4    10
B    A                   2.34250      4    8
B                        2.27000      4    6
```

Duncan's Multiple Range Test for Y

```
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.

Alpha                    0.05
Error Degrees of Freedom        7
Error Mean Square        0.002942

Number of Means           2
Critical Range       .07404



Means with the same letter are not significantly different.
```

Duncan Grouping	Mean	N	X3
A	2.38667	6	15
B	2.30167	6	20

Table B.9 Antioxidant activity (TEAC) of capsules

X1 core to coating ratio (1, 1:10; 2, 1:20)
X2 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X3 UT (min)

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	6 8 10
X3	2	15 20

Number of Observations Read	12
Number of Observations Used	12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	12362.95417	1373.66157	887.66	0.0011
Error	2	3.09500	1.54750		
Corrected Total	11	12366.04917			

R-Square	Coeff Var	Root MSE	Y Mean
0.999750	0.877333	1.243986	141.7917

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	12204.94083	12204.94083	7886.88	0.0001
X2	2	18.56167	9.28083	6.00	0.1429
X3	1	65.80083	65.80083	42.52	0.0227
X1*X2	2	1.47167	0.73583	0.48	0.6777
X1*X3	1	46.80750	46.80750	30.25	0.0315
X2*X3	2	25.37167	12.68583	8.20	0.1087

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	12204.94083	12204.94083	7886.88	0.0001
X2	2	18.56167	9.28083	6.00	0.1429
X3	1	65.80083	65.80083	42.52	0.0227
X1*X2	2	1.47167	0.73583	0.48	0.6777
X1*X3	1	46.80750	46.80750	30.25	0.0315
X2*X3	2	25.37167	12.68583	8.20	0.1087

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	1.5475

Number of Means	2
Critical Range	3.090

```
Means with the same letter are not significantly different.

Duncan Grouping          Mean      N    X1

A                    173.6833      6    1

B                    109.9000      6    2
```

Duncan's Multiple Range Test for Y

```
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.

Alpha                        0.05
Error Degrees of Freedom        2
Error Mean Square          1.5475

Number of Means          2          3
Critical Range       3.785      3.616


Means with the same letter are not significantly different.

Duncan Grouping          Mean      N    X2

A                    143.5500      4    10

A                    140.9500      4    8

A                    140.8750      4    6
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	1.5475

Number of Means	2
Critical Range	3.090

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X3
A	144.1333	6	15
B	139.4500	6	20

Table B.10 Antioxidant activity (DPPH$^{\cdot}$) of capsules having core to coating ratio 1:10

```
X1 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X2 UT (min)

Class Level Information
Class       Levels    Values
X1               3    6 8 10
X2               2    15 20

Number of Observations Read          6
Number of Observations Used          6

Dependent Variable: Y

Source              DF      Sum of  Squares     Mean Square    F Value    Pr > F
Model                3     0.03550000           0.01183333       2.30      0.3176
Error                2     0.01030000           0.00515000
Corrected Total      5     0.04580000

R-Square     Coeff Var      Root MSE        Y Mean
0.775109      2.562982      0.071764      2.800000

Source                DF       Type I SS      Mean Square    F Value    Pr > F
X1                     2     0.01390000      0.00695000       1.35     0.4256
X2                     1     0.02160000      0.02160000       4.19     0.1771

Source                DF     Type III SS      Mean Square    F Value    Pr > F
X1                     2     0.01390000      0.00695000       1.35     0.4256
```

```
X2                    1      0.02160000      0.02160000      4.19    0.1771
Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.

Alpha                    0.05
Error Degrees of Freedom        2
Error Mean Square         0.00515

Number of Means          2          3
Critical Range       .3088      .2950


Means with the same letter are not significantly different.

Duncan Grouping          Mean      N    X1
A                     2.85000      2    10
A                     2.81500      2    8
A                     2.73500      2    6


Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.

Alpha                    0.05
Error Degrees of Freedom        2
Error Mean Square         0.00515
```

```
Number of Means          2
Critical Range       .2521


Means with the same letter are not significantly different.

Duncan Grouping          Mean      N    X2
A                     2.86000      3    15
A                     2.74000      3    20
```

Table B.11 Antioxidant activity (DPPH$^{\cdot}$) of capsules having core to coating ratio 1:20

X1 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X2 UT (min)

```
Class Level Information
Class        Levels    Values
X1                3    6 8 10
X2                2    15 20 25

Number of Observations Read          9
Number of Observations Used          9

Dependent Variable: Y

Source                DF    Sum of Squares    Mean Square    F Value    Pr > F
Model                  4      0.06111111        0.01527778        1.70    0.3108
Error                  4      0.03604444        0.00901111
Corrected Total        8      0.09715556

R-Square    Coeff Var     Root MSE       Y Mean
0.629003     5.002001     0.094927     1.897778

Source                DF      Type I SS     Mean Square    F Value    Pr > F
X1                     2    0.05575556     0.02787778       3.09    0.1542
X2                     2    0.00535556     0.00267778       0.30    0.7580

Source                DF    Type III SS     Mean Square    F Value    Pr > F
X1                     2    0.05575556     0.02787778       3.09    0.1542
```

```
X2                     2      0.00535556      0.00267778       0.30    0.7580
```

Duncan's Multiple Range Test for Y

```
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.

Alpha                    0.05
Error Degrees of Freedom        4
Error Mean Square       0.009011

Number of Means          2          3
Critical Range       .2152      .2199



Means with the same letter are not significantly different.

Duncan Grouping          Mean      N    X1
A                      2.00667      3    10
A                      1.86333      3    6
A                      1.82333      3    8
```

Duncan's Multiple Range Test for Y

```
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.

Alpha                    0.05
Error Degrees of Freedom        4
Error Mean Square       0.009011
```

Number of Means	2	3
Critical Range	.2152	.2199

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	1.91667	3	25
A	1.91333	3	15
A	1.86333	3	20

Table B.12 Antioxidant activity (TEAC) of capsules having core to coating ratio 1:10

X1 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X2 UT (min)

```
Class Level Information
Class        Levels    Values
X1                3    6 8 10
X2                2    15 20

Number of Observations Read          6
Number of Observations Used          6

Dependent Variable: Y

Source             DF       Sum of Squares     Mean Square    F Value    Pr > F
Model               3     126.6050000          42.2016667     6.14        0.1432
Error               2      13.7433333           6.8716667
Corrected Total     5     140.3483333

R-Square     Coeff Var      Root MSE        Y Mean
0.902077      1.509291      2.621386      173.6833

Source                  DF       Type I SS     Mean Square    F Value    Pr > F
X1                       2      14.8033333       7.4016667       1.08      0.4814
X2                       1     111.8016667     111.8016667      16.27      0.0563

Source                  DF     Type III SS     Mean Square    F Value    Pr > F
X1                       2      14.8033333       7.4016667       1.08      0.4814
```

```
X2                    1     111.8016667     111.8016667      16.27    0.0563
```

Duncan's Multiple Range Test for Y

```
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.

Alpha                      0.05
Error Degrees of Freedom        2
Error Mean Square        6.871667

Number of Means          2          3
Critical Range       11.28      10.78


Means with the same letter are not significantly different.

Duncan Grouping          Mean      N    X1
A                      175.900      2   10
A                      172.700      2   6
A                      172.450      2   8
```

Duncan's Multiple Range Test for Y

```
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.

Alpha                      0.05
Error Degrees of Freedom        2
Error Mean Square        6.871667
```

```
Number of Means          2
Critical Range       9.209


Means with the same letter are not significantly different.

Duncan Grouping          Mean      N    X2
A                     178.000      3    15
A                     169.367      3    20
```

Table B.13 Antioxidant activity (TEAC) of capsules having core to coating ratio 1:20

```
X1 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X2 UT (min)

Class Level Information
Class        Levels   Values
X1                3   6 8 10
X2                3   15 20 25

Number of Observations Read         9
Number of Observations Used         9


Dependent Variable: Y

Source             DF       Sum of Squares    Mean Square   F Value   Pr > F
Model               4    41.28666667         10.32166667      2.72      0.1775
Error               4    15.15333333         3.78833333
Corrected Tot       8    56.44000000

R-Square    Coeff Var     Root MSE       Y Mean
0.731514     1.793333     1.946364     108.5333

Source                 DF      Type I SS    Mean Square   F Value   Pr > F
X1                      2     6.86000000    3.43000000       0.91    0.4739
X2                      2    34.42666667    17.21333333      4.54    0.0934
```

```
Source                DF     Type III SS     Mean Square    F Value    Pr > F
X1                     2      6.86000000      3.43000000       0.91    0.4739
X2                     2     34.42666667     17.21333333       4.54    0.0934
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
Alpha                         0.05
Error Degrees of Freedom         4
Error Mean Square         3.788333

Number of Means          2          3
Critical Range       4.412      4.509
```

Means with the same letter are not significantly different.

```
Duncan Grouping        Mean      N    X1
A                   109.700      3    10
A                   108.300      3    8
A                   107.600      3    6
```

Duncan's Multiple Range Test for Y

```
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.

Alpha                     0.05
Error Degrees of Freedom       4
Error Mean Square       3.788333

Number of Means         2          3
Critical Range      4.412      4.509


Means with the same letter are not significantly different.

Duncan Grouping          Mean      N    X2
A                     110.267      3    15
A                     109.533      3    20
A                     105.800      3    25
```

Table B.14 D32 values of capsules having core to coating ratio 1:20

```
X1 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X2 UT (min)

Class Level Information
Class        Levels    Values
X1              3    6 8 10
X2              2    20 25

Number of Observations Read          6
Number of Observations Used          6

Dependent Variable: Y

Source              DF       Sum of Squares     Mean Square    F Value    Pr > F
Model                3      0.01990125           0.00663375          1.23    0.4781
Error                2      0.01079908           0.00539954
Corrected Total      5      0.03070033

R-Square     Coeff Var      Root MSE        Y Mean
0.648242      4.755063      0.073482      1.545333

Source                  DF       Type I SS     Mean Square    F Value    Pr > F
X1                       2      0.00810858      0.00405429      0.75    0.5711
X2                       1      0.01179267      0.01179267      2.18    0.2775

Source                  DF     Type III SS     Mean Square    F Value    Pr > F
X1                       2      0.00810858      0.00405429      0.75    0.5711
X2                       1      0.01179267      0.01179267      2.18    0.2775
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
Alpha                        0.05
Error Degrees of Freedom        2
Error Mean Square          0.0054

Number of Means         2        3
Critical Range      .3162    .3021
```

Means with the same letter are not significantly different.

```
Duncan Grouping        Mean     N   X1
A                   1.59725     2   10
A                   1.52175     2   6
A                   1.51700     2   8
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
Alpha                        0.05
Error Degrees of Freedom        2
Error Mean Square          0.0054
```

Number of Means	2
Critical Range	.2581

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	1.58967	3	20
A	1.50100	3	25

Table B.15 Specific surface area of capsules having core to coating ratio 1:20

X1 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)

X2 UT (min)

Class Level Information

Class	Levels	Values
X1	3	6 8 10
X2	2	20 25

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.13151250	0.04383750	1.27	0.4687
Error	2	0.06895833	0.03447917		
Corrected Total	5	0.20047083			

R-Square	Coeff Var	Root MSE	Y Mean
0.656018	4.754060	0.185686	3.905833

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	0.06000833	0.03000417	0.87	0.5347
X2	1	0.07150417	0.07150417	2.07	0.2865

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	0.06000833	0.03000417	0.87	0.5347
X2	1	0.07150417	0.07150417	2.07	0.2865

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.034479

Number of Means	2	3
Critical Range	.7989	.7633

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	3.9875	2	8
A	3.9650	2	6
A	3.7650	2	10

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.Alpha 0.05

Error Degrees of Freedom 2

Error Mean Square 0.034479

Number of Means 2

Critical Range .6523

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	4.0150	3	25
A	3.7967	3	20

Table B.16 Span of capsules having core to coating ratio 1:20

X1 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)

X2 UT (min)

Class Level Information

Class	Levels	Values
X1	3	6 8 10
X2	2	20 25

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.93502817	0.31167606	3.25	0.2439
Error	2	0.19156933	0.09578467		
Corrected Total	5	1.12659750			

R-Square	Coeff Var	Root MSE	Y Mean
0.829958	5.181066	0.309491	5.973500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	0.93122800	0.46561400	4.86	0.1706
X2	1	0.00380017	0.00380017	0.04	0.8605

```
Source              DF     Type III SS     Mean Square    F Value    Pr > F
X1                   2      0.93122800      0.46561400       4.86    0.1706
X2                   1      0.00380017      0.00380017       0.04    0.8605
```

Duncan's Multiple Range Test for Y

```
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.


Alpha                     0.05
Error Degrees of Freedom       2
Error Mean Square       0.095785


Number of Means          2          3
Critical Range       1.332      1.272



Means with the same letter are not significantly different.


Duncan Grouping      Mean      N    X1
A                  6.3805      2    6
A                  6.0995      2    10
A                  5.4405      2    8
```

Duncan's Multiple Range Test for Y

```
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.Alpha                      0.05
```

```
Error Degrees of Freedom        2
Error Mean Square        0.095785

Number of Means          2
Critical Range       1.087



Means with the same letter are not significantly different.

Duncan Grouping        Mean      N    X2
A                     5.9987      3    25
A                     5.9483      3    20
```

Table B.17 L* values of microcapsules

X1 core to coating ratio (1, 1:10; 2, 1:20)
X2 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X3 UT (min)

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	6 8 10
X3	3	15 20 25

Number of Observations Read	15
Number of Observations Used	15

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	53.41971167	10.68394233	5.93	0.0107
Error	9	16.22198167	1.80244241		
Corrected Total	14	69.64169333			

R-Square	Coeff Var	Root MSE	Y Mean
0.767065	2.765713	1.342551	48.54267

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	49.03272111	49.03272111	27.20	0.0006
X2	2	3.56449333	1.78224667	0.99	0.4091
X3	2	0.82249722	0.41124861	0.23	0.8005

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	44.19840833	44.19840833	24.52	0.0008
X2	2	3.56449333	1.78224667	0.99	0.4091
X3	2	0.82249722	0.41124861	0.23	0.8005

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	9
Error Mean Square	1.802442
Harmonic Mean of Cell Sizes	7.2

NOTE: Cell sizes are not equal.

Number of Means	2
Critical Range	1.601

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	50.0189	9	2
B	46.3283	6	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 9
Error Mean Square 1.802442

Number of Means	2	3
Critical Range	1.921	2.005

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	49.1360	5	8
A	48.5500	5	10
A	47.9420	5	6

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 9
Error Mean Square 1.802442
Harmonic Mean of Cell Sizes 4.5

```
NOTE: Cell sizes are not equal.

Number of Means       2       3
Critical Range    2.025   2.113


Means with the same letter are not significantly different.

Duncan Grouping        Mean    N   X3
A                   49.7233    3   25
A                   48.4367    6   20
A                   48.0583    6   15
```

Table B.18 a* values of microcapsules

X1 core to coating ratio (1, 1:10; 2, 1:20)
X2 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X3 UT (min)

```
Class        Levels    Values
X1                2    1 2
X2                3    6 8 10
X3                3    15 20 25

Number of Observations Read         15
Number of Observations Used         15

Dependent Variable: Y

Source               DF       Sum of Squares     Mean Square    F Value    Pr > F
Model                 5      9.82702667          1.96540533        12.26    0.0008
Error                 9      1.44334667          0.16037185
Corrected Total          14     11.27037333

R-Square     Coeff Var      Root MSE        Y Mean
0.871934      2.157830      0.400465      18.55867

Source                 DF       Type I SS     Mean Square    F Value    Pr > F
X1                      1      9.76144000      9.76144000      60.87    <.0001
X2                      2      0.05225333      0.02612667       0.16    0.8521
X3                      2      0.01333333      0.00666667       0.04    0.9595
```

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	8.13453333	8.13453333	50.72	<.0001
X2	2	0.05225333	0.02612667	0.16	0.8521
X3	2	0.01333333	0.00666667	0.04	0.9595

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	9
Error Mean Square	0.160372
Harmonic Mean of Cell Sizes	7.2

NOTE: Cell sizes are not equal.

Number of Means	2
Critical Range	.4774

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	19.5467	6	1
B	17.9000	9	2

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05

Error Degrees of Freedom 9

Error Mean Square 0.160372

Number of Means 2 3

Critical Range .5729 .5980

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	18.6100	5	6
A	18.5900	5	10
A	18.4760	5	8

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05

Error Degrees of Freedom 9

Error Mean Square 0.160372

Harmonic Mean of Cell Sizes

```
NOTE: Cell sizes are not equal.

Number of Means          2          3
Critical Range       .6039      .6304


Means with the same letter are not significantly different.

Duncan Grouping          Mean      N    X3
A                     18.7567      6    15
A                     18.6900      6    20
B                     17.9000      3    25
```

Table B.19 b* values of microcapsules

X1 core to coating ratio (1, 1:10; 2, 1:20)
X2 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X3 UT (min)

```
Class Level Information
Class        Levels    Values
X1                2    1 2
X2                3    6 8 10
X3                3    15 20 25

Number of Observations Read        15
Number of Observations Used        15

Dependent Variable: Y

Source                DF     Sum of Squares     Mean Square    F Value    Pr > F
Model                  5      0.23278000          0.04655600       0.87      0.5342
Error                  9      0.47898000          0.05322000
Corrected Total       14      0.71176000

R-Square     Coeff Var      Root MSE        Y Mean
0.327048      1.690814      0.230695      13.64400

Source                DF       Type I SS     Mean Square    F Value    Pr > F
X1                    1      0.02401000      0.02401000       0.45    0.5187
X2                    2      0.13072000      0.06536000       1.23    0.3376
X3                    2      0.07805000      0.03902500       0.73    0.5070
```

```
Source                DF    Type III SS    Mean Square    F Value    Pr > F
X1                    1     0.00270000     0.00270000     0.05       0.8268
X2                    2     0.13072000     0.06536000     1.23       0.3376
X3                    2     0.07805000     0.03902500     0.73       0.5070
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
Alpha                          0.05
Error Degrees of Freedom          9
Error Mean Square           0.05322
Harmonic Mean of Cell Sizes     7.2
```

NOTE: Cell sizes are not equal.

```
Number of Means        2
Critical Range     .2750
```

Means with the same letter are not significantly different.

```
Duncan Grouping        Mean     N    X1
A                   13.6767     9    2
A                   13.5950     6    1
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
Alpha                     0.05
Error Degrees of Freedom       9
Error Mean Square        0.05322

Number of Means          2          3
Critical Range       .3300      .3445



Means with the same letter are not significantly different.

Duncan Grouping        Mean      N    X2
A                    13.7760      5    8
A                    13.5800      5    10
A                    13.5760      5    6
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
Alpha                          0.05
Error Degrees of Freedom          9
Error Mean Square           0.05322
Harmonic Mean of Cell Sizes      4.5
```

```
NOTE: Cell sizes are not equal.

Number of Means          2          3
Critical Range       .3479      .3631


Means with the same letter are not significantly different.

Duncan Grouping         Mean      N    X3
A                    13.7800      3    25
A                    13.6600      6    20
A                    13.5600      6    15
```

Table B.20 ΔE* values of microcapsules

X1 core to coating ratio (1, 1:10; 2, 1:20)
X2 MD:GA (6, 6:4; 8, 8:2; 10, 10:0)
X3 UT (min)

```
Class Level Information
Class        Levels   Values
X1                2   1 2
X2                3   6 8 10
X3                3   15 20 25

Number of Observations Read        15
Number of Observations Used        15

Dependent Variable: Y

Source             DF      Sum of Squares    Mean Square   F Value   Pr > F
Model               5    58.40208000         11.68041600      6.78    0.0069
Error               9    15.51581333         1.72397926
Corrected Total    14    73.91789333

R-Square    Coeff Var     Root MSE       Y Mean
0.790094     2.329288     1.313004     56.36933

Source                DF      Type I SS     Mean Square    F Value    Pr > F
X1                     1    54.63127111    54.63127111      31.69    0.0003
X2                     2     3.00645333     1.50322667       0.87    0.4507
X3                     2     0.76435556     0.38217778       0.22    0.8054
```

```
Source              DF     Type III SS     Mean Square    F Value    Pr > F
X1                   1     48.96480000     48.96480000      28.40    0.0005
X2                   2      3.00645333      1.50322667       0.87    0.4507
X3                   2      0.76435556      0.38217778       0.22    0.8054
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
Alpha                          0.05
Error Degrees of Freedom          9
Error Mean Square          1.723979
Harmonic Mean of Cell Sizes     7.2
```

NOTE: Cell sizes are not equal.

```
Number of Means         2
Critical Range      1.565
```

Means with the same letter are not significantly different.

```
Duncan Grouping         Mean      N    X1
A                    58.7067      6    1
B                    54.8111      9    2
```

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	9
Error Mean Square	1.723979

Number of Means	2	3
Critical Range	1.878	1.961

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	56.9280	5	6
A	56.3480	5	10
A	55.8320	5	8

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	9
Error Mean Square	1.723979
Harmonic Mean of Cell Sizes	4.5

```
NOTE: Cell sizes are not equal.

Number of Means        2        3
Critical Range     1.980    2.067


Means with the same letter are not significantly different.

Duncan Grouping        Mean     N    X3
A                   56.8667     6    15
A                   56.5067     6    20
A                   55.1000     3    25
```

Table B.21 Retention of TPC in simulated gastric fluid

X1 type of sample (1, microcapsule with MD:GA 10:0; 2, microcapsule with MD:GA 8:2; 3, phenolic powder)

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read 6

Number of Observations Used 6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	298.2091000	149.1045500	242.04	0.0005
Error	3	1.8481000	0.6160333		
Corrected Total	5	300.0572000			

R-Square	Coeff Var	Root MSE	Y Mean
0.993841	4.666337	0.784878	16.82000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	298.2091000	149.1045500	242.04	0.0005

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	298.2091000	149.1045500	242.04	0.0005

Duncan's Multiple Range Test for Y

```
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.

Alpha                         0.05
Error Degrees of Freedom         3
Error Mean Square         0.616033

Number of Means        2        3
Critical Range     2.498    2.506


Means with the same letter are not significantly different.

Duncan Grouping          Mean    N   X1
A                     26.7900    2   3
B                     11.8750    2   2
B                     11.7950    2   1
```

Table B.22 Retention of TPC in simulated intestinal fluid

X1 type of sample (1, microcapsule with MD:GA 10:0; 2, microcapsule with MD:GA 8:2; 3, phenolic powder)

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	269.8082333	134.9041167	12.84	0.0338
Error	3	31.5287000	10.5095667		
Corrected Total	5	301.3369333			

R-Square	Coeff Var	Root MSE	Y Mean
0.895371	8.598301	3.241846	37.70333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	269.8082333	134.9041167	12.84	0.0338

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	269.8082333	134.9041167	12.84	0.0338

Duncan's Multiple Range Test for Y

```
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.


Alpha                      0.05
Error Degrees of Freedom        3
Error Mean Square        10.50957


Number of Means          2          3
Critical Range       10.32      10.35



Means with the same letter are not significantly different.


Duncan Grouping       Mean      N    X1
A                    47.070     2    3
B                    34.305     2    1
B                    31.735     2    2
```

Table B.23 Glass transition temperature of PP and microcapsules

X1 type of sample (0, GA; 6, microcapsule with MD:GA 6:4; 8, microcapsule with MD:GA 8:2; 10, microcapsule with MD:GA 10:0; 100, MD)

Class Level Information

Class	Levels	Values
X1	5	0 6 8 10 100

Number of Observations Read	10
Number of Observations Used	10

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	419.0810600	104.7702650	20.51	0.0027
Error	5	25.5451500	5.1090300		
Corrected Total	9	444.6262100			

R-Square	Coeff Var	Root MSE	Y Mean
0.942547	3.636463	2.260316	62.15700

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	4	419.0810600	104.7702650	20.51	0.0027

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	4	419.0810600	104.7702650	20.51	0.0027

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

```
Alpha                      0.05
Error Degrees of Freedom        5
Error Mean Square         5.10903

Number of Means          2          3          4          5
Critical Range       5.810      5.991      6.068      6.095



Means with the same letter are not significantly different.

Duncan Grouping        Mean      N    X1
A                      74.995    2    0
B                      60.045    2    10
B                      59.315    2    100
B                      58.960    2    6
B                      57.470    2    8
```

APPENDIX C

MODEL CONSTANTS AND MATLAB PROGRAM

Table C.1 Model constants of encapsulation efficiency equation

Predictor	Coef	SE Coef	T	P
Constant	89.0075	0.5031	176.91	0.000
MD:GA	-2.7474	0.3558	-7.72	0.000
UT	0.0142	0.2905	0.05	0.962
$MD:GA^2$	-0.1200	0.6162	-0.19	0.851
MD:GA*UT	0.3325	0.3558	0.93	0.381

S = 1.00627 R-Sq = 89.6% R-Sq(adj) = 83.7%

Table C.2 Model constants of particle size equation

Predictor	Coef	SE Coef	T	P
Constant	1.45500	0.00781	186.39	0.000
MD:GA	0.04750	0.01008	4.71	0.009
UT	-0.035000	0.007806	-4.48	0.011
MD:GA^2	0.1200	0.01104	10.87	0.000
MD:GA*UT	-0.03750	0.01008	-3.72	0.020

S = 0.0127475 R-Sq = 97.0% R-Sq(adj) = 93.9%

Table C.3 MATLAB program

```
x0=[0 0]';[x,fval,exitflag]=fmincon(@(x)f(x),x0,[],[],[-1 -1]',[1 1]');

function[y]=f(x)
lambda=1;
qmc=[0.12                                   -0.0375/2;-0.0375/2];
lmc=[0.0475                                             -0.035]';
cmc=1.46;
qFF=[-0.12                 0.333;0.333                       0];
lFF=[-2.75                                               0.014]';
cFF=89.0;
y=x'*qFF*x+x'*lFF+cFF+lambda*(x'*qmc*x+x'*lmc+cmc);
```

Codes for MATLAB program

MD:GA 6:4	-1
MD:GA 8:2	0
MD:GA 10:0	1
UT 20 min	-1
UT 25 min	1

x0 Value [0;0] Min:0 Max:0

APPENDIX D

DSC GRAPHS

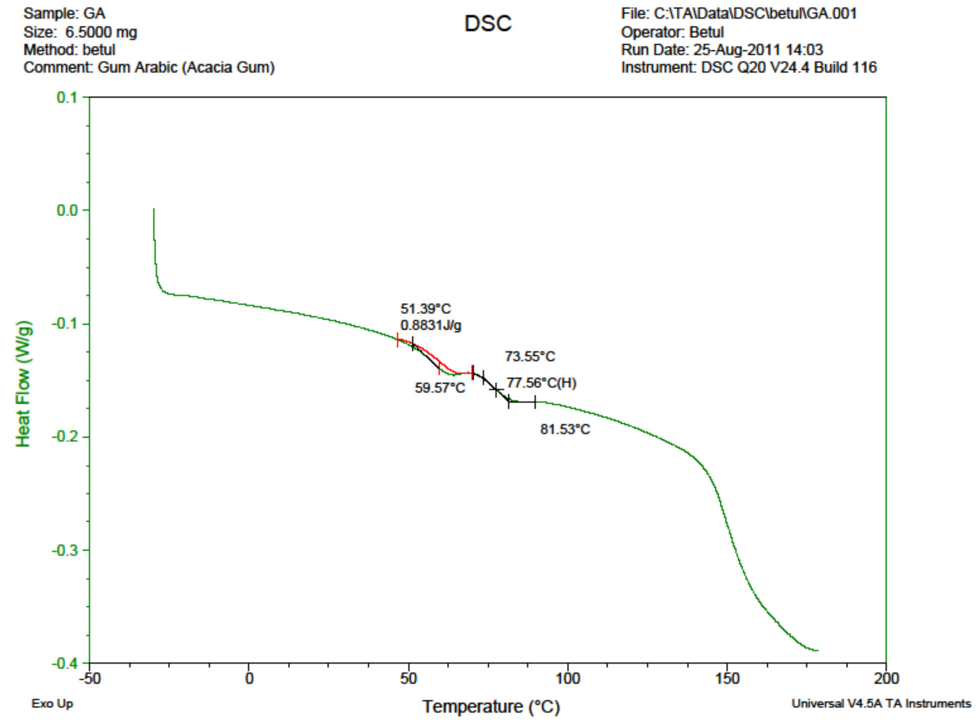

Figure D.1 T_g of gum Arabic

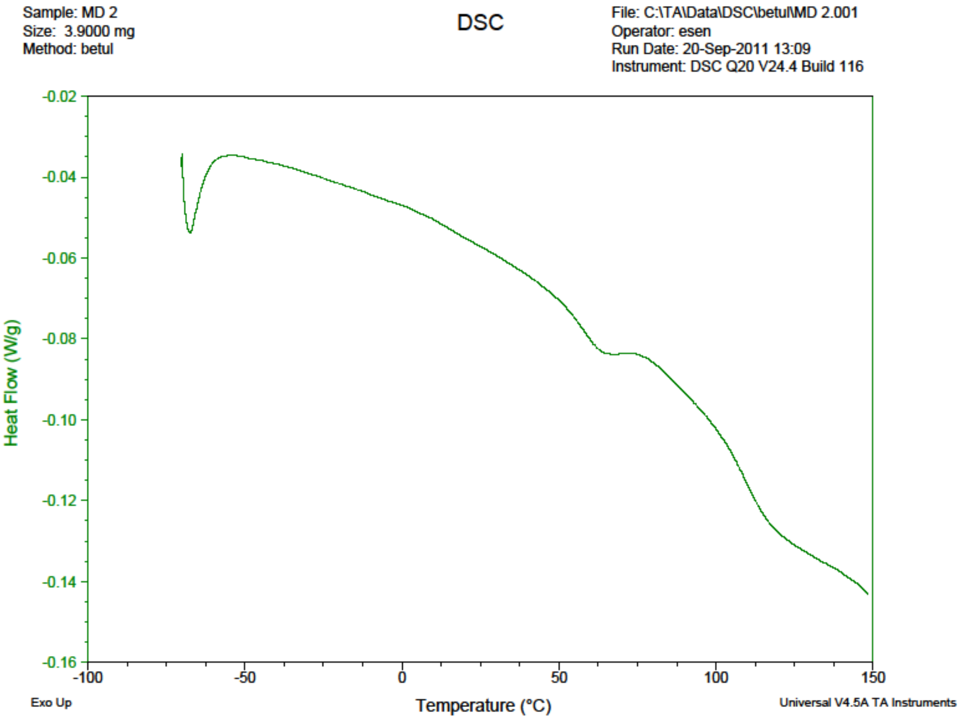

Figure D.2 Tg of maltodextrin

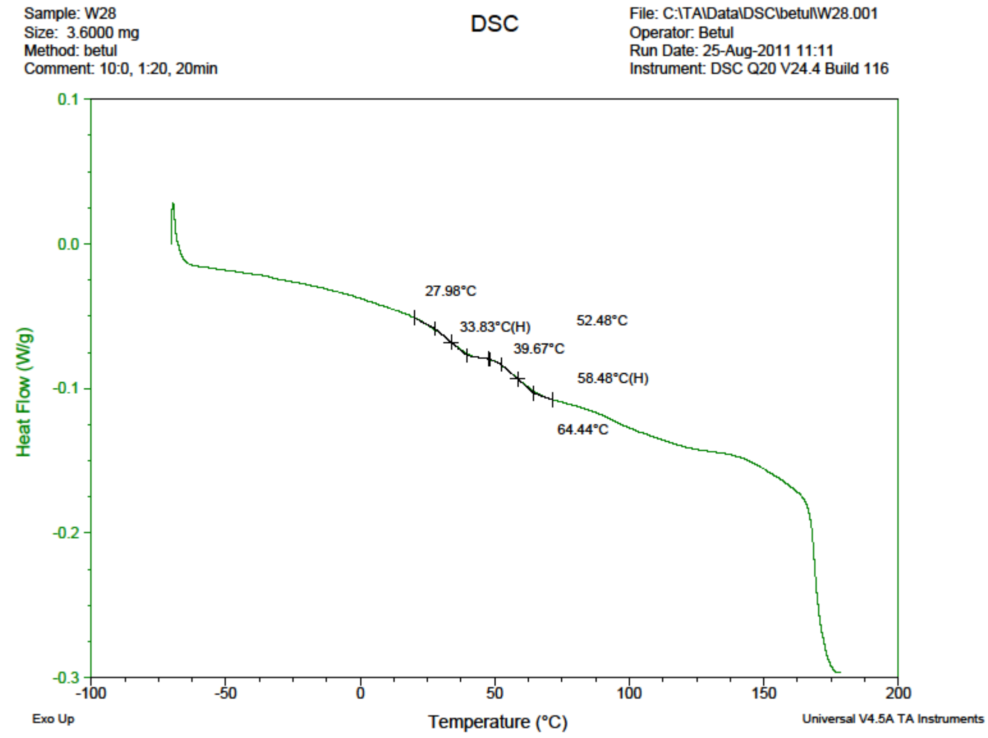

Figure D.3 Tg of capsules having MD:GA 10:0

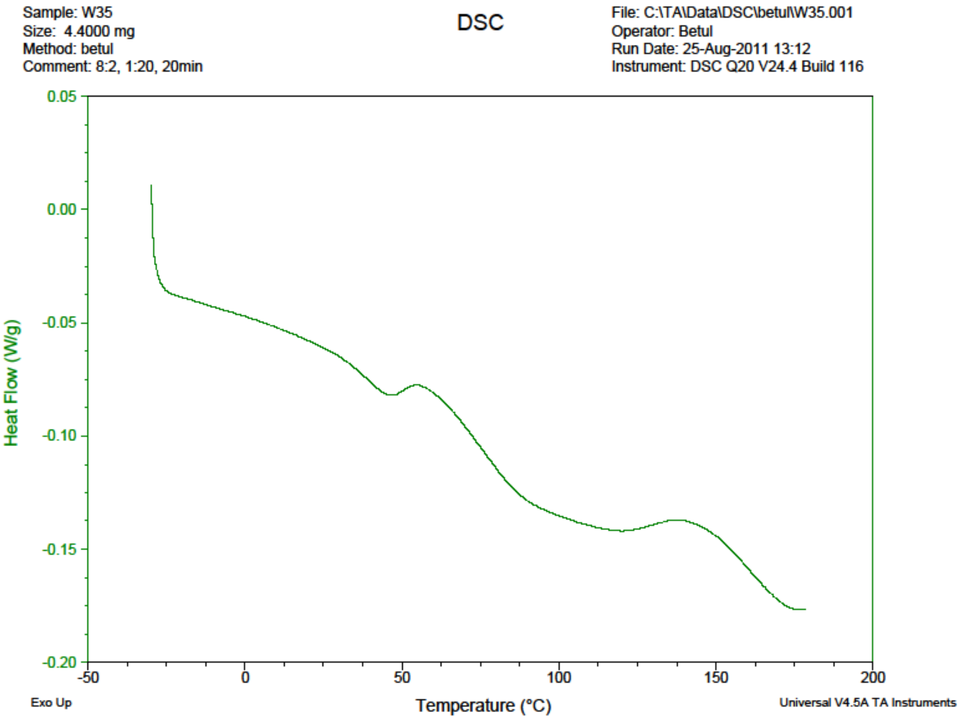

Figure D.3 T_g of capsules having MD:GA 8:2

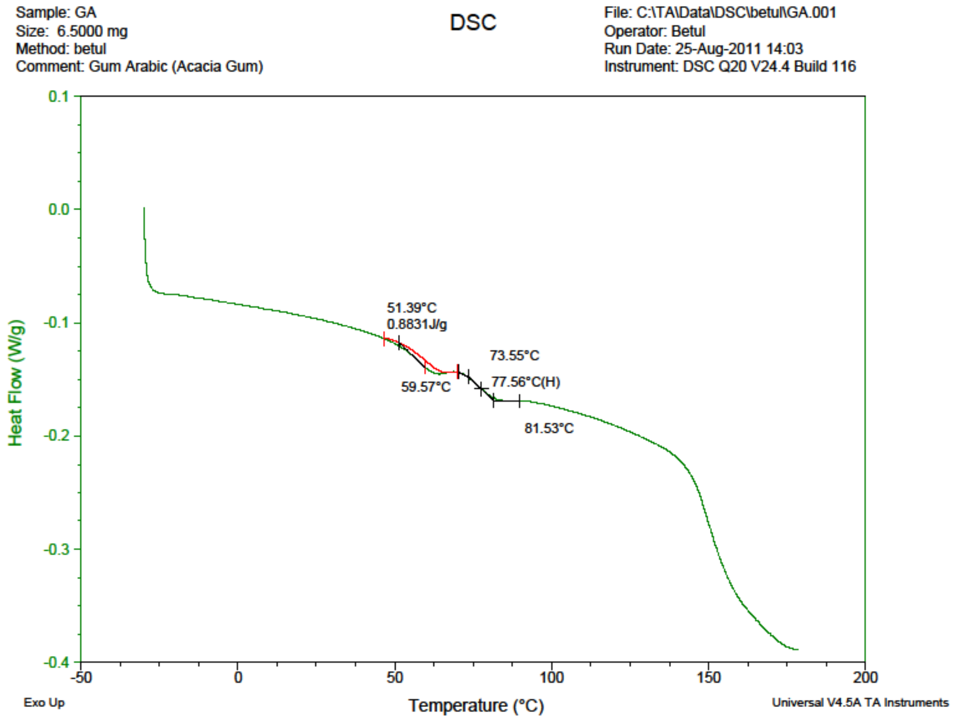

Figure D.4 T_g of capsules having MD:GA 6:4

APPENDIX E

PICTURES OF PHENOLIC POWDER AND CAPSULES

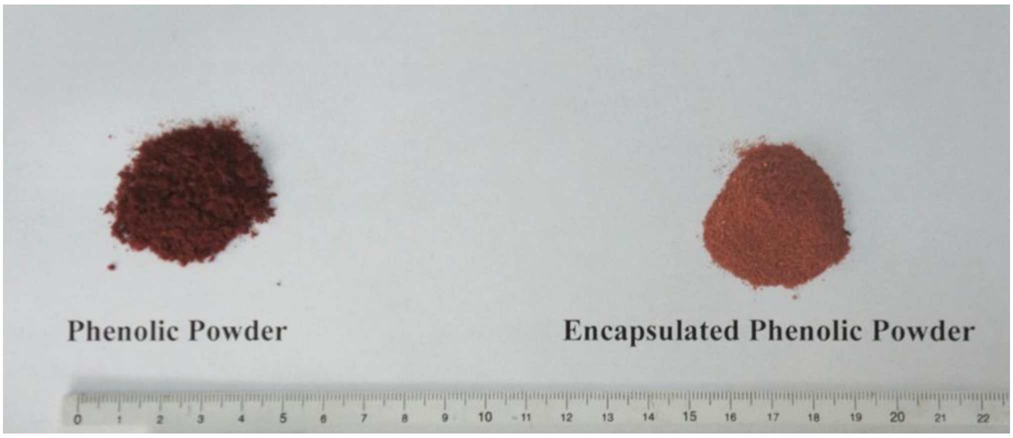

Figure E.1 Picture of phenolic powder and encapsulated phenolic powder